Host–parasite population genetics: a cross-sectional comparison of *Bulinus globosus* and *Schistosoma haematobium*

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**SUMMARY**

The genetic population structures of the freshwater snail *Bulinus globosus* and its trematode parasite *Schistosoma haematobium* from 8 river sites in the Zimbabwean highveld were compared using randomly amplified DNA (RAPD) markers. There was significant variability between snail populations collected at different sites, but schistosome populations only showed differentiation at a wider geographical scale (between 2 non-connected river systems). For snails, genetic distance was better correlated with proximity along rivers than absolute geographical separation. In contrast, schistosome genetic distance was better correlated with absolute geographical separation than proximity along rivers. These results are consistent with different dispersal mechanisms for snails and schistosomes and the implications for host–parasite coevolution are discussed.

Key words: gene flow, geographical variation, RAPDs, coevolution, snail, schistosome.

**INTRODUCTION**

Hosts are anticipated to be an important influence on the population structure of their parasites. For instance, Price (1980) predicted that parasite populations should show a high degree of genetic structure with little gene flow between populations, as a result of adaptation to local host populations. High parasite gene flow will tend to counteract local adaptation (Slatkin, 1987) but could, on the other hand, also spread adaptive traits (Thompson, 1994) and restore variation that is lost by frequent extinction of local populations in a metapopulation (Frank, 1991).

Snail–schistosome interactions are an excellent model system in which to investigate such evolutionary predictions. Geographical variation in host–parasite compatibility is a widely reported feature of snail–schistosome interactions (Manning, Woolhouse & Ndamba, 1995) and reciprocal cross-infection studies have identified local adaptation of parasite infectivity/host susceptibility between river systems (Manning et al. 1995; Mukaratirwa et al. 1996). Schistosomes, however, also spend much of their life-cycle within highly mobile definitive hosts, which too may be predicted to influence their population structure.

Direct comparisons of genetic population structure of both host and parasite from the same sites are thus far limited to other snail–trematode systems (e.g. Richards & Shade, 1987; Richards, Knight & Lewis, 1992; Dybdahl & Lively, 1996). As a consequence, the interactions between *Bulinus globosus* and *Schistosoma haematobium* are only poorly understood (Preston & Southgate, 1994). Here we used the randomly amplified DNA (RAPD) technique to cross-sectionally study the genetic population structure of this host–parasite system across 2 river systems in the Zimbabwean highveld.

**MATERIALS AND METHODS**

The study site was located in north-east Zimbabwe (17° 03′ 28″–17° 13′ 26″ S and 30° 58′ 39″–31° 10′ 10″ E) in the Chiweshe highveld. Sampling sites were located along 2 major river systems (Fig. 1). Snails were collected from 8 sites, 5 on and around the Ruya river and 3 on and around the Sawi river. The 8 sites were chosen such that their direct
geographical separation across land was in some cases very different from their proximity along the river. Geographical distances between collection sites ranged from 1 km (Kw–Mz: see Fig. 1 for labelling of sites) to 26 km (Ns–Z). The rivers from which the snails were collected were between 1 and 8 m wide and normally between 0·5 and 1·5 m deep. Some of the smaller rivers could fall partially dry during the dry season, creating small isolated ponds. The climate of the region is subtropical (Woolhouse & Chandiwana, 1990).

Eight snails were collected from each site, with the exception of the D population where only 4 snails could be found, during March 1996. Parasite cercarial stages were harvested by keeping the snails in darkness for 48 h and then exposing them, at 10 a.m., in vials containing 25 ml of dechlorinated water, for 2 h to a bright (100 W) overhead light source. Cercariae from each individual infected snail were used to infect an individual hamster, by allowing the animal's feet to paddle freely for 30 min in 100 ml of water containing approximately 220 cercariae. Following this period, before the hamsters showed signs of illness, they were killed with carbon dioxide. Adult schistosomes were recovered from hamsters using a modified vascular hepatic-portal perfusion technique (Smithers & Terry, 1965) and were then stored in 100% molecular grade ethanol. Wherever possible, 8 adult worms from each hamster (i.e. arising from each infected snail) were analysed. Worms from 3 hamsters were analysed from the Mz site (Mz1–Mz3), 2 from the Z site (Z1–Z2) and 1 from the remainder, with the exception of D and S where no infected snails were found. Thus there were 9 infected snails in total.

Snails were immediately relaxed in menthol (Thornton and Ross Ltd) in order to prevent bursting of the hepatopancreas and then preserved in molecular grade ethanol (Hayman Ltd) prior to genetic analysis. Genomic DNA was extracted from the tip of the head-foot region only of each snail (hence avoiding any potential contamination by schistosome stages present), and from entire individual worms, using a standard phenol–chloroform procedure modified to overcome the problems associated with DNA degradation from gastropod nucleases, as described by Vernon, Jones & Noble (1995).

PCR amplification conditions and reaction mixtures were as described by Hoffman et al. (1998). Thirty primers were sampled, and 10 oligonucleotide primers (each of 10 bp) were chosen (based on reproducibility) for B. globosus [R&D-9(AGCAGCGTGG); R&D12(ATGGATCCGC); R&D15(CTGGCGGGCTG); R&D17(GTGATCGCAG); R&D19(CATGCAGGCG) [R&D Technologies, Abingdon, UK]; OPR01(TGCGGGGCTCCT); OPR02(CACAGCTGCC); OPY3(ACAGCCTGCT); OPR4(CCCCGTAGCAC); OPR6(GTCTACGGCA) [Operon Technologies, Alameda, CA, USA]] and 6 for S. haematobium [R&D12, R&D19, OPR1, OPR2, OPR4, OPY20(AGCCGTTGGA)].
Amplification products were electrophoresed for 8–10 h on 1.4% agarose gels in 1× TBE buffer (0.089 M Tris-borate, 0.002 M EDTA) and stained with ethidium bromide (Sigma). The gels were photographed under ultraviolet light using Polaroid 667 film.

As controls, PCR reactions were run without primer, without DNA, or without Taq polymerase, and DNA samples were re-run in order to verify repeatability. Controls yielded no DNA bands and re-runs exhibited identical amplification products.

Pilot studies at a single site had suggested that variation of sample size from 8 to 100 individuals had little effect on estimates of population similarity (J. P. Webster, unpublished data). Thus we chose to study a relatively small number of individuals from multiple sites in order to investigate local patterns of gene flow. Sample sizes were kept constant throughout, and though smaller sizes will tend to overestimate population differentiation (Apostol et al. 1996), it is the comparison of host and parasite which is of interest in this study. Thus we feel that conclusions can be sensibly, though cautiously, drawn.

RAPDs were chosen because they provide an accessible and reliable tool to investigate molecular genetics, and have recently proved very successful when applied to other snail and schistosome systems (see Hoffman et al. (1998) for further details).

**Data analyses**

A total of 60 snails and 57 parasites were analysed. Bands for each were compared with a marker (Lambda DNA EcoRI HindIII Digest, Sigma) to ensure consistent scoring between gels and to measure band size. Faint, unclear bands and high molecular weight fragments (>2.5 kbp) were discounted from analysis. Bands of the same size were assumed to correspond to identical segments of genomic DNA. A binary data matrix was constructed based upon band present/absence, and a genetic distance matrix between each pair of individuals was calculated for both snail and parasite using the ‘R Package’ (Legendre & Vaudor, 1991). Two measures of relatedness, Euclidean squared distances and Jaccard coefficient of community or similarity (asymmetric binary coefficient excluding double-zeros; js) (Jaccard, 1908) were used in all analyses (1-j$s$ in AMOVA and clustering), but these gave similar results and thus only those based on Jaccard distances or similarities are presented.

Analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) was used to quantify the amount of variation between individuals from the same and differing collection sites, and between the two river systems. This is analogous to classical analysis of variance and is suitable for RAPD phenotypes (Gabrielsen et al. 1997; Huff, Peakall & Smouse, 1993). Analysis was conducted using WINAMOVA 1.55 (Excoffier et al. 1992) and significance levels of the variance components was based on 1000 permutations. Analyses were conducted separately for snails and parasites. Two separate nested analyses were conducted for the parasites, one emphasizing differences between the river systems and the other between sites, since the software was limited to 2 hierarchical levels. UPGMA non-hierarchical clustering was performed on individuals using ‘The R Package’ (Legendre & Vaudor, 1991). Cluster analysis is a family of related techniques for representing similarity or distance data in the form of an ultrametric tree (Sneath & Sokal, 1973). The tree is constructed by linking the least distant pairs of individuals or populations, followed by successively more distant individuals/populations, or groups of individuals/populations. When 2 individuals or populations are linked, they lose their individual identities and are subsequently referred to as a single cluster. Initially, each individual or population constitutes its own cluster. At each stage in the process, as the 2 clusters are merged into 1, the number of clusters declines by 1. The process is complete when the last 2 clusters are merged into a single cluster containing all of the original individuals or populations. The most commonly used clustering method is UPGMA (unweighted pair group method using arithmetic averages) in which the average distance of a new cluster to each of the existing clusters is taken to define branch length. Thus this analysis demonstrates visually the similarity of individuals or populations, with the length of branches showing the genetic distance separating clusters. Summary trees of population relationships were produced using S-PLUS.

Mantel correlograms were constructed separately to investigate individual relationships between genetic and geographical distances for hosts and parasites. The correlogram is a plot of genetic similarity as a function of geographical distance classes (Legendre & Fortin, 1989). Five distance classes were set up for each analysis based on distances between sites measured on a map. For *B. globosus*, the smallest distance class was set to less than 20 m to include all snails collected at the same site. Other distance classes were divided such that there were approximately the same number of distances in each. The *S. haematobium* distance classes were similar except that the smallest class was at the level of an individual snail and the second class within a geographical site. This was repeated using distance along the river system between collection sites (river flow distance) rather than straight-line geographical separation. In the river flow analysis, the highest distance class was awarded to comparisons between individuals from sites not connected along the rivers. The standardized Mantel
Table 1. AMOVA results for (A) Bulinus globosus and (B) Schistosoma haematobium.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>D.F.</th>
<th>Variance component</th>
<th>Total variance (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nested analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between river systems</td>
<td>0.53</td>
<td>6</td>
<td>0.002*</td>
<td>0.78</td>
<td>0.001</td>
</tr>
<tr>
<td>Among sites within river system</td>
<td>3.34</td>
<td>52</td>
<td>0.030</td>
<td>2.16</td>
<td>0.004</td>
</tr>
<tr>
<td>Among individuals within sites</td>
<td>9.57</td>
<td>184</td>
<td>0.184</td>
<td>79.2</td>
<td></td>
</tr>
<tr>
<td>Nested analysis 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between parasite populations of different river systems</td>
<td>0.74</td>
<td>1</td>
<td>0.001</td>
<td>8.14</td>
<td>0.001</td>
</tr>
<tr>
<td>Among individuals within parasites at the level of collection site</td>
<td>2.99</td>
<td>7</td>
<td>0.048</td>
<td>23.1</td>
<td>0.012</td>
</tr>
<tr>
<td>Among individuals within snails</td>
<td>6.79</td>
<td>48</td>
<td>0.142</td>
<td>68.8</td>
<td></td>
</tr>
<tr>
<td>Nested analysis 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between parasite populations of different sites</td>
<td>2.44</td>
<td>5</td>
<td>0.005</td>
<td>2.39</td>
<td>0.001</td>
</tr>
<tr>
<td>Among individuals within parasites at the level of collection site</td>
<td>1.29</td>
<td>3</td>
<td>0.049</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>Among individuals within snails</td>
<td>6.79</td>
<td>48</td>
<td>0.142</td>
<td>72.6</td>
<td></td>
</tr>
</tbody>
</table>

* Negative molecular variance components may sometimes occur for the higher hierarchical levels as these statistics are not computed as sums of squares but instead are extracted from a single equation involving means square deviations and lower level variance components (Excoffier et al. 1992). If the actual values are close to zero, their estimates may sometimes take small negative values. Variance component testing showed that this value was not significantly different from zero.

Results

A total of 234 bands were identified for the 60 snails, and of these 13 (6.5%) were monomorphic. A total of 40 bands were identified for the 57 S. haematobium parasites, of which 3 (7.5%) were monomorphic.

Analysis of molecular variation (Table 1A, B)

For B. globosus, the greatest genetic variation occurred among individuals within collection sites (79.2%). There were significant differences between populations at different sites (21.6% of total variation, \( P < 0.001 \)) but no apparent differentiation of the populations between the 2 river systems. The within population sums of squared deviation (SSD) was similar at all sites (1.11 < SSD < 1.49) with the exception of D (SSD = 0.50), but this may be expected because of the smaller sample size.

For S. haematobium, variation between individuals within single snail hosts accounted for 72.6% of the total variation, suggesting a high level of plurimicrocoidal snail infections in this region. Sixty-seven per cent of infected snails harboured a multiple infection, with a mean of 6.2 genotypes per snail. The division between parasites from the Ruya and Sawi river regions approached significance \( (P = 0.08) \). This \( P \) value was lower \( (P = 0.06) \) when the data were analysed using Euclidean distances (results not shown). No further differentiation of the parasites from hosts at different sites was detected.

Cluster analysis (Fig. 2)

UGPMA cluster analysis of individuals (results not shown) showed evidence of clustering of snails at the level of collection site, and of parasites at the level of the individual snail host, but considerable overlap occurred. The population dendrograms (Fig. 2) highlight the features seen in the AMOVA analyses. For S. haematobium, the Sawi river sites were well distinguished from those of the Ruya river system, which together formed 1 cluster. There was no evidence of parasite populations from snails collected at the same site within the Ruya river clustering more closely than with parasite populations at other sites in the Ruya. For B. globosus 2 of the 3 Sawi sites were also separated but the Ns population was...
Fig. 2. Average-linkage dendrograms for the (A) 8 Bulinus globosus geographical populations and the (B) 9 Schistosoma haematobium within-snail populations \([n = 64 \text{ (hosts)}; n = 57 \text{ (parasites)}] \).

Fig. 3. Mantel correlograms showing relationship between genetic distance and (A) geographical distance between Schistosoma haematobium individuals, (B) river flow distance between S. haematobium individuals, (C) geographical distance between Bulinus globosus individuals and (D) river flow distance between B. globosus individuals. Asterixes (*) show autocorrelations significant at Bonferroni corrected level \((P = 0.05/5 = 0.01)\).

Snail–schistosome population genetics

299

integrated with those of the Ruya. All parasite populations from the Ruya were quite similar genetically, in contrast to the host population where, for example, the Mz and Kw populations which were very close geographically but widely separated by river flow, were highly genetically divergent.

**Mantel tests for spatial autocorrelation (Fig. 3)**

The Mantel correlograms revealed clinal variation with distance in both parasite and host populations (Fig. 3). Absolute geographical distance was a better indicator of parasite genetic variation than river distance. This is evidenced by the higher number of geographical classes where autocorrelation was identified and the stronger trend from positive to negative autocorrelation within increasing distance class, reflecting clinal variation. Within-snail populations showed a positive autocorrelation while widely separated individuals showed a negative autocorrelation. At the between-snail/within-site and between neighbouring sites, however, there was no significant spatial autocorrelation. This supports the earlier analyses suggesting parasite population differentiation is occurring only between the 2 river systems (Ruya and Sawi). Parasite genetic diversity was only correlated with river flow distance at the within-snail level and between sites not connected by the river, where river flow distance is strongly correlated with absolute geographical distances. Conversely, host genetic distance shows clinal variation with river flow distance, Mantel statistics being highly significant in all distance classes. Host genetic variation showed some relation to absolute geographical separation, but this may be a result of covariation with river flow distance.

**Relationships of parasite and host population genetic structure**

There was wide variation in prevalence of patent infection between sites (0–23 %). There was, however, no correlation between snail genetic diversity and the percentage of patent infections at the collection time \((rs = -0.41; n = 7; P = 0.35)\).

**DISCUSSION**

Mutation, genetic drift due to limited population size and natural selection favouring adaptations to local environmental conditions will all lead to genetic differentiation of local populations, while the movement of gametes, individuals or populations (i.e. gene flow) will oppose that differentiation (Slatkin, 1987).

In this study, AMOVA, Mantel correlograms and clustering techniques all identified higher levels of
population structure in *B. globosus* than its trematode parasite, *S. haematobium*. Host populations from individual sites within a river system could be differentiated. In contrast, populations of *S. haematobium* from different sites within a river system were not differentiated, although populations from between the 2 river regions appeared to be. Schistosome population differentiation has not previously been reported (Dabo et al. 1997).

Increased population subdivision in the snails, as compared to their parasites, may reflect higher levels of genetic drift due to their ability to reconstitute populations from 1 or very few individuals. Strong seasonal variations are a feature of *B. globosus* habitats (Woolhouse & Chandiwana, 1990), producing patterns of alternating rapid population growth and extinctions. Genetic drift is a major force in subdivided populations experiencing seasonal variation in their size. Alternatively, our results may suggest that the levels of gene flow are higher in the parasite than the intermediate host populations, at least within the Ruya system. This might reflect the influence of migration of the highly mobile definitive hosts on the dynamics of the parasite populations. Other factors decreasing genetic subdivision of parasite populations (genetic structure) include persistent life-cycle stages in definitive hosts and low definitive host specificity (Nadler, 1995), both of which also apply to this system. In reality the population structure seen will be likely to reflect a combination of these factors.

The genetic similarity of *B. globosus* populations was closely related to their proximity along the river system and little related to their straight-line geographical separation. This may reflect the dispersal of snails by passive migration along the river (particularly during flooding) but could also reflect a systematic variation in environmental conditions. In contrast, the similarity of *S. haematobium* populations was more related to their straight-line geographical separation. This may suggest that definitive (probably human) host dispersal may be the most important mechanisms for the parasites, as could be predicted considering the higher mobility of, and increased duration of infection in, definitive hosts, and thus provides support for increased gene flow through migration in the parasite population explaining the lower population differentiation relative to that of its host seen in this study.

The population structures observed here may thus have implications for theoretical models of local adaptation of parasites and their hosts. Models predict that high parasite gene flow will counteract local adaptation if there is spatial variability but no temporal variability in the host population (Gandon & Van Zandt, 1998). However, when host co-evolves with parasite, the environment changes both in space and time and the role of migration is more complicated. Gandon et al. (1996) predicted that high parasite gene flow can actually promote the emergence of local adaptation, because gene flow introduces genetic variability on which selection can act. In their model, the migration rate of the parasite relative to that of the host, determines local adaptation. If the parasite migrates more than the host, it should become locally adapted. Conversely, if it migrates less than the host, the parasite will be locally maladapted. Our results, where parasite flow is inferred to be higher than that of the intermediate host, and where local adaptation has been observed using reciprocal cross-infection studies on snails from within the same region (Mukaratirwa et al. 1996) are in line with these predictions. Dybdahl & Lively (1996) considered that restoration of variation may be particularly important when selection leads to time-lagged oscillatory or chaotic cycles in allele frequencies since parasite alleles engaged in frequency dependent selection with host alleles can easily ‘overshoot’ and become fixed in local populations due to such time lags. Models of local adaptation developed for this system, where parasite infectivity and host susceptibility are defined by matching genotypes in a diploid system, predict dynamic polymorphisms where parasite allele frequencies track host frequencies but with a time lag (Morand, Manning & Woolhouse, 1996; Webster & Woolhouse, 1998). Thus high levels of gene flow may be beneficial for this parasite population.

A second important observation of this study is the high levels of within-snail diversity observed in *S. haematobium*, which suggests a very high frequency of plurimiracidial snail infections (two-thirds of the snails were multiply infected with a mean number of genotypes per infected snail of 6-2). Multiple infections have been seen to be common in a number of snail–schistosome systems (Woolhouse, Chandiwana & Bradley, 1990; Minchella, Sollenberger & Pereira de Sousa, 1995; but see Dabo et al. 1997). Indeed as the Chiweshe region of our study is endemic for urinary schistosomiasis, and transmission is thought to be high (Mukaratirwa et al. 1996), high levels of multiple infection may well be expected here. Such multiple genotype snail infections are important epidemiologically since they will increase the probability that humans acquire a dual sex infection and high genotypic overdispersion within intermediate hosts may facilitate maintenance of genetic diversity among adult intrapopulations (Minchella et al. 1995). Competition between genotypes within snail hosts may potentially also be important in the evolution of parasite virulence, as suggested by many recent mathematical models (e.g. Frank, 1996; Bull, 1994).

In summary, *B. globosus* in this study were more genetically structured than *S. haematobium* and local differentiation of populations separated by 1 to 20 kilometres was possible. Lower levels of population structure were apparent in the parasite, with popu-
loration differentiation only suggested over larger areas. Differences in dispersal methods and persistence of schistosomes in definitive hosts are suggested to be important.

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