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Extensive genetic variation revealed in adjacent populations of the schistosome intermediate host *Biomphalaria pfeifferi* from a single river system

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Random amplified polymorphic DNA (RAPD) analysis was used to study genetic variation among schistosome hosts of the species *Biomphalaria pfeifferi*. The analysed snails came from 15 sites along a 6-km stretch of a Zimbabwean river, providing data on genetic–geographical relationships over, probably, the smallest scale yet studied for any snail intermediate host species.

Only 6% of the DNA fragments were common to all snails, and snail populations as geographically close as 100 m were genetically distinct. The most genetically polymorphic snail populations were those collected within and downstream from human water-contact sites.

Species of freshwater snail belonging to the genus *Biomphalaria* are intermediate hosts for *Schistosoma mansoni*, the causative agent of intestinal schistosomiasis. Extreme fluctuations in the size of *Biomphalaria* populations, due to floods, drought or molluscicide treatment, may contribute to the establishment of spatial and temporal genetic heterogeneity in these snails (Woolhouse, 1992). As proposed by Michelson and DuBois (1978), such genetic heterogeneity may be partly responsible for the geographical differences seen in snail susceptibility to schistosome infection.

The development of random amplified polymorphic DNA (RAPD) analysis by Williams *et al.* (1990) has provided an accessible and reliable tool to investigate molecular genetics. RAPD analyses have recently been shown to be capable of discriminating between closely related snail intermediate host species in the laboratory and between field strains collected from different countries. The snails investigated in this way include *Biomphalaria glabrata* (Vidigal *et al.*, 1994; Larson *et al.*, 1996) and several *Bulinus* species (*Bu. globosus*, *Bu. senegalensis*, *Bu. forskalii*, *Bu. camarunensis*,

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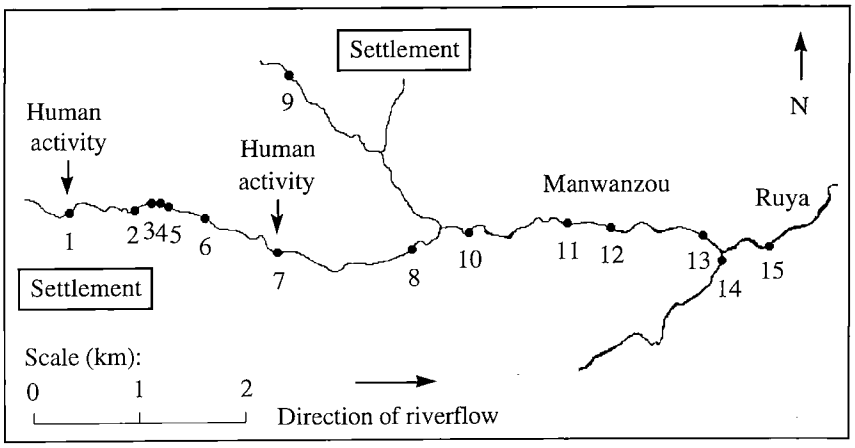

Fig. 1. Location of the 15 sites, on the Manwanzou and Ruya rivers, at which snails were collected for RAPD analysis. The two principal human water-contact sites were numbered 1 and 7.

Bu. nasatus and *Bu. umblicatus*; Langand *et al.*, 1993; Rollinson *et al.*, 1996; Stothard and Rollinson, 1996). Demonstration of strain variation within a country (Langand *et al.*, 1993) or within a snail population (Vidigal *et al.*, 1994) has proved elusive. Stothard and Rollinson (1996) proposed that the apparently low level of genetic variability seen within snail populations was the result of the high levels of self-fertilization characteristic of the snail species investigated.

The subjects of the present study were snails, of a previously neglected intermediate host species (*Biomphalaria pfeifferi*), collected directly from field locations in the Zimbabwean highveld. The present study followed that of Webster *et al.* (1996), in which genetic differences were described between snail populations as close together as 4.5 km in the field. In contrast to the related studies on other snail species, the aim of the present study was to investigate genetic–geographical relationships at very fine scales, down to as little as 100 m, from within a single river system.

ANIMALS AND METHODS

At least eight snails were collected from each of 15 sites in the Zimbabwean highveld (17°05′S, 31°05′E) during August 1995. Two populations were sampled from the river Ruya and 13 from its tributary, the Manwanzou (Fig. 1). The Manwanzou is mostly narrow and shallow, being no more than 10 m wide and 2 m deep and is, for most of the year, slow flowing; at the time of sampling the upper reaches were dry and the populations labelled 1–6 were collected from pools. Populations 1 and 7 were collected from popular community washing sites; *Schistosoma mansoni* is locally prevalent in the area of these sites.

The collected snails were immediately relaxed in menthol, to prevent bursting of the hepatopancreas, and then preserved in molecular-grade ethanol 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 any schistosome stages present), using a standard phenol–chloroform procedure modified to overcome the problems associated with DNA degradation from gastropod nucleases, as described by Vernon *et al.* (1995). The amplification conditions and reaction mixtures for the PCR were as described by Okamura *et al.* (1993), except that 10–100 ng DNA were used for each reaction. Amplification occurred in a total volume of 20 μl, overlaid with min-

eral oil, in a DNA thermal cycler (Perkin Elmer) for 45 cycles, each of 10 s at 94°C, 1 min at 36°C and 2 min at 72°C and using the fastest possible transition times (1°C/s) between the different temperature phases. Twelve arbitrarily selected oligonucleotide primers (each of 10 bp) were used. Eight of these were supplied by Ingenious R&D Technologies (Abingdon, U.K.) [R&D08 (CGCAGCGTTC), R&D09 (AGCAGCGTGG), R&D10 (TAGCAGCGGG), R&D12 (ATGGATCCGC), R&D15 (CTGGCGGCTG), R&D17 (GTGATCGCAG), R&D18 (AATCGGGCTG), and R&D19 (CATGCAGGCG)] and four by Operon Technologies (Alameda, CA) [OPR01 (TGCGGGTCCT), OPR03 (ACACAGAGGG), OPR04 (CCCGTAGCAC), and OPR05 (GACCTAGTGG)]. Amplification products were electrophoresed for 8–10 h on 1.4% agarose gels in 1 × TNE buffer (0.089 M Tris–borate, 0.002 M EDTA) and stained with ethidium bromide. The gels were photographed under ultraviolet light using Polaroid 667 film.

As controls, PCR reactions were run without primer, without snail DNA or without Taq polymerase, and snail DNA samples were re-run in order to verify repeatability.

Data Analysis

Bands were compared with a marker (lambda DNA EcoR I Hind III 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 the analysis.

For comparisons between samples, bands of the same size were assumed to correspond to identical segments of genomic DNA. A binary data matrix was constructed based upon band presence/absence and used to quantify genetic differences between individuals. For this, Jaccard's similarity coefficients (% scaled) were calculated using software compiled by Legendre and Vaudor (1991):

$$\mathcal{J}_{1,2} = 100a/(a + b + c)$$

where a is the number of shared bands between individuals 1 and 2, b is the number of bands present in individual 1 but absent from individual 2, and c is the number of bands present in individual 2 but absent from individual 1. Intra-population genetic diversities were estimated as the mean (S.E.) Jaccard's distances among snails comprising each population, and these means were then compared using two-tailed Mann–Whitney U-tests and commercial statistical software (SPSS; SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Each primer generated amplification products with characteristic banding profiles, comprising both monomorphic bands common amongst snails (species- or strain-specific markers) and several polymorphic bands (Fig. 2). Using the 12 primers, 126 bands were scored, of which only 6% were common to every individual snail. Controls yielded no DNA bands and re-runs exhibited identical amplification products to those seen previously.

The distribution of variation among the snails of the single river system investigated was noteworthy. Whilst some populations exhibited low intra- or inter-population variation, particularly the two sites (14 and 15) in the deep Ruya, a few populations were genetically highly diverse. The most diverse snail populations were not all continuous in geographical distribution, being interspersed with populations exhibiting low variability (Figs 1 and 3). Moreover, populations 3, 4 and 5, which lie within 100 m of one another, were genetically very different. Genetic variation therefore occurred on a very small geographical scale within a single river system. Whether the snails collected along the Manwanzou came from pools or the open river did not appear to determine intra-population variation *per se* ($P = 0.086$). However, the two sites frequented by the local community for washing, sanitation and recreational purposes (sites 1 and 7; Figs 1 and 3) had snail populations with the highest and third highest diversities ($P = 0.042$). Likewise, diversity was very high

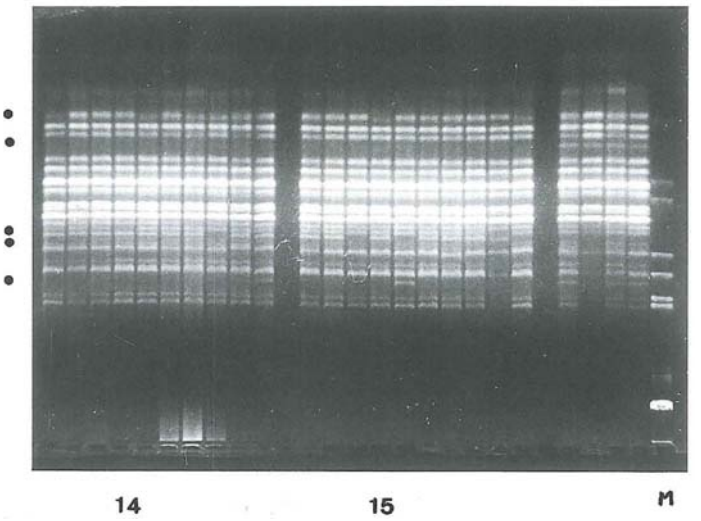

Fig. 2. RAPD–PCR products, from snail populations 14 and 15, created using primer R&D10, and the DNA markers (M). In the gels shown, 10 individuals from each population were run, in addition to a further block of four re-run individuals from various populations (to check for repeatability). (•), Polymorphic bands.

in the two sites immediately downstream of these human water-contact points (sites 2 and 8; Figs 1 and 3).

The proportion of amplified DNA bands common to all the *Bi. pfeifferi* collected in the present study (6%) is similar to that observed for Brazilian isolates of *Bi. glabrata* ($< 10\%$; Vidigal *et al.*, 1994). However, it is very much less than that observed by Langand *et al.* (1993) in snails of the genus *Bulinus* from Cameroon, Equatorial Guinea and Ivory Coast (57%–74%), or by Webster *et al.* (1996) in other Zimbabwean *Bi. pfeifferi* (87%). What is perhaps most remarkable about the variation described here is the geographical scale involved. All the present sites were within a 6-km stretch of river, whereas previous studies spanned at least tens of kilometres (Webster *et al.*, 1996) and often one or more countries (Langand *et al.*, 1993; Vidigal *et al.*, 1994). Surprisingly, Langand *et al.* (1993) failed to differentiate between geographical strains (of *Bu. forskalli*) collected from within the same country, despite also using the RAPD technique.

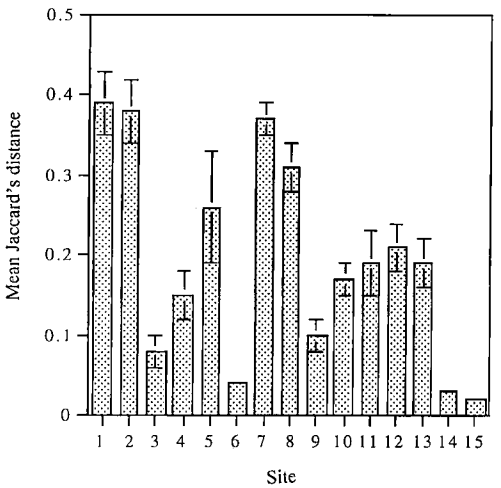

Fig. 3. Jaccard's distances for the snail populations from each collection site: the larger the distance, the more dissimilar the snails. Vertical lines indicate S.E.

The present data may support the idea that very local phenomena can drive genetic differentiation in *Bi. pfeifferi*. In addition to the spatial genetic heterogeneity predicted from the metapopulation biology of freshwater snails (Woolhouse and Chandiwana, 1989; Vernon and Taylor, 1996; Webster *et al.*, 1996), the finding that the greatest snail diver-

sity seen in the present study occurred at or directly downstream from the two principal human water-contact sites investigated may indicate that a human factor could affect the genetics of *Bi. pfeifferi*.

Schistosomiasis is endemic in the study area and humans are likely to be a source of schistosome infection for snails in areas of water contact. Indeed, Woolhouse and Chandiwana (1989) demonstrated that, in a nearby Zimbabwean river system, the distributions of snails supporting schistosome infections were closely related to spatial patterns in human water contact, prevalence of snail infection being highest at contact sites and in adjacent regions. Because schistosome infections are often a cause of mortality in host snails (Woolhouse, 1989; Webster and Woolhouse, 1998) and hence a force for natural selection, they could have a significant effect upon the genetics of local snail populations. Associations between hosts and parasites tend to generate and maintain genetic polymorphism (May, 1985), as has been predicted in models of schistosome–snail interactions (Morand *et al.*, 1996). It is thus possible that increased schistosome infection in sites frequented by humans could be responsible for elevating genetic diversity in local snail populations.

The present results appear to be the first to demonstrate genetic variability between snail populations as close as 100 m apart. They refute the earlier proposals of limited intrapopulation variation amongst such wild intermediate hosts (Langand *et al.*, 1993; Vidigal *et al.*, 1994; Stothard and Rollinson, 1996) and raise interesting questions on the possible influences of local phenomena, including humans and sympatric parasite presence, on the genetics of snail intermediate hosts.

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