Genetic diversity of schistosomes and snails: implications for control

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SUMMARY

Molecular approaches are providing new insights into the genetic diversity of schistosomes and their intermediate snail hosts. For instance, molecular tools based on the polymerase chain reaction are being developed for the diagnosis of schistosomiasis and the detection of prepatent schistosome infections in snails at transmission sites. Robust phylogenies of the different species of *Schistosoma*, *Bulinus* and *Biomphalaria* have been determined and novel methods are available to identify the different and cryptic taxa involved. Microsatellite analyses and mitochondrial DNA sequencing methods have been developed and are contributing to a better understanding of the genetic structure of both schistosome and snail populations. New sampling procedures to capture DNA of eggs and larval stages of schistosomes in field situations are facilitating more detailed and ethically advantageous studies on parasite heterogeneity. Knowledge of the genetic diversity of schistosome and snail populations adds a further dimension to the monitoring and surveillance of disease, and the implementation of new molecular-based approaches will be of increasing importance in helping to assess the impact of schistosomiasis control strategies.

Key words: Schistosomes, snails, MtDNA, microsatellites, diagnosis, population genetics, control, Africa.

INTRODUCTION

Together with other Neglected Tropical Diseases (NTDs), schistosomiasis control is now firmly placed on the global health agenda (Anon, 2007). National schistosomiasis control programmes in sub-Saharan Africa and elsewhere are already promoting or putting in place the widespread use of mass drug administration (MDA) of praziquantel at school and community levels in an effort to reduce the morbidity inflicted by this persistent parasitic disease. Within this growing and encouraging control scenario questions are likely to arise which will require a better understanding of the genetic diversity of both the parasites and their intermediate snail hosts. The selective pressures imposed by increasing levels of drug administration necessitate a clearer idea of population structure and genetics of the parasite to enable the prediction and monitoring of changes in, for example, drug susceptibility. Likewise, it is well recognised that transmission control may be a powerful addition to ongoing chemotherapy-based morbidity control (King *et al*. 2006) and this requires a sound knowledge of the habitats and snails involved in transmission. Better markers for identifying schistosomes and their intermediate snail hosts can contribute to the construction of accurate risk maps of transmission to help target control, especially valuable when resources are limited.

In addition to a perspective on control, observations on the genetic diversity of schistosomes can give insight into the overall biology of disease. For example, the possible association of parasite genotypes with different degrees of disease pathology is often mentioned, but requires further examination; the interactions between snail and parasite genotypes govern the spread and distribution of disease; the wide definitive host range of species such as *Schistosoma japonicum* and to a lesser extent *S. mansoni* may lead to the segregation of parasites more able to develop in one definitive host than another and the presence of reservoir hosts may complicate control strategies focused on the human population.

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Progress in molecular epidemiology of parasite infections in recent years has been rapid and what follows is a consideration of some selected topics relating to novel molecular techniques for diagnosis of schistosomiasis and for the examination of genetic variation primarily of *S. mansoni* and *S. haematobium* and their intermediate snail hosts. Emphasis is given to the ways in which these new approaches may be used to enhance the understanding of host and parasite biology and the implementation of control strategies.

SCHISTOSOME DNA DETECTION AND DIAGNOSIS

Routine diagnosis of schistosome infection in the field is likely to continue to revolve around traditional low-cost egg detection methods such as the Kato-Katz technique for stool samples and urine filtration techniques. However, new molecular detection methods will be of value for early diagnosis of infection (especially in travellers) and in transmission areas where prevalence and intensity of infection are low or have been diminished (such as in post-treatment situations), where standard egg detection by microscopy becomes difficult and less sensitive. Furthermore, new diagnostic tools may be of particular value for the detection of light infections in infants, as schistosomiasis in pre-school children appears to be more common than previously realized (Stothard and Gabrielli, 2007). Undoubtedly, accurate, rapid and high-throughput methods of diagnosis could well transform surveillance and monitoring programmes, especially if costs can be reduced and diagnostic services in endemic settings can be improved. Various promising polymerase chain reaction (PCR) tests for detection of schistosome infection have recently been developed although, as yet, they are not being used on a wide scale or included routinely in sentinel monitoring sites of ongoing schistosomiasis control programmes.

The sensitivity of a touchdown PCR assay for the detection of *S. mansoni* infection has been demonstrated by using the mouse model (Suzuki *et al.* 2006). Assays were conducted using serum samples post-infection without extracting DNA. The reaction was specific to *S. mansoni* DNA, which was detected in the sera at two weeks post-infection, six weeks before eggs were observed in faeces. In contrast, a standard enzyme-linked immunosorbent assay (ELISA) detected anti-worm antigen IgG and anti-soluble egg antigen IgG in the sera at six weeks post-infection. This touchdown method therefore clearly has potential for the early diagnosis of infection, particularly light infections. However, it should be noted that large numbers (200) of cercariae were used in these experiments.

PCR methods for parasite detection based on urine samples have also been developed (Sandoval *et al.* 2006*a*). A highly sensitive PCR approach that allows the genus- and species-specific amplification of the main *Schistosoma* species causing disease in humans plus *S. bovis* from cattle has been developed (Sandoval *et al.* 2006*b*). The technique is able to detect parasite DNA in urine samples from patients (and/or cattle hosts) with schistosomiasis. Sandoval *et al.* (2006*b*) recorded 94·4% sensitivity and 99·9% specificity when applying a genus-specific (*Schistosoma* spp.) primer pair, and 100% sensitivity and 98·9% specificity in a species-specific (*S. mansoni*) PCR.

Taking advantage of fluorescent labeling techniques, Lier *et al.* (2006) designed a novel real-time PCR (RtPCR) method for detection of *S. japonicum* in stool samples and evaluated different DNA extraction methods. PCR primer sequences were designed targeting the mitochondrial NADH dehydrogenase subunit I gene. Bovine serum albumin was added to the DNA extracts and SYBR Green was used for detection. The PCR method was evaluated with non-infected stool samples spiked with *S. japonicum* eggs. It demonstrated high sensitivity, even in samples containing a single egg. The PCR was specific for *S. japonicum* when tested against other *Schistosoma* species, *Trichuris trichiura*, hookworm and *Taenia* spp..

RtPCR has also been used for the accurate measurement of *S. mansoni* DNA, negating the need for detecting PCR products by gel electrophoresis (Gomes *et al.* 2006). Mulitiplex RtPCR to detect *S. mansoni* and *S. haematobium* DNA has been successfully used in northern Senegal. Primers were based on the cytochrome c oxidase gene, the method proved to be 100% sensitive and there was correlation between the RtPCR results and egg counts by microscopy (Ten Hove *et al.* 2008). Moreover, RtPCR assays open up the exciting possibility of quantifying *S. mansoni* DNA (and indirectly parasite burden) in a number of samples, such as snail tissue and in serum, urine and faeces from patients. It will be interesting to observe how detectable parasite DNA levels fluctuate with treatment, for example, to elucidate how long parasite DNA persists after treatment and, indeed, whether DNA levels relate at all to egg production and deposition in tissues as eggs progressively accumulate during the course of an infection.

Furthermore, this RtPCR technique could also be developed as a possible method for the detection of cercariae at transmission sites. Of perhaps even greater interest is the detection of parasites within snails collected at potential transmission sites. PCR of a highly repeated sequence identified in the genome of *S. mansoni* has been used to detect a single miracidium one day after penetration of the snail (Hamburger *et al.* 1998*a*, *b*). Amplification of a similar repeat (DRA1) in *S. haematobium* has been used to study the timing and number of prepatent and

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patent infections of snails from known transmission sites in Coastal Kenya (Hamburger *et al.* 2004). A similar analysis of *Bulinus globosus* from a known transmission site on the island of Zanzibar has shown high levels of pre-patent infections with only a few snails becoming patent and releasing cercariae (F. Allen, A. Dunn, J. Smith, J. R. Stothard and D. Rollinson, unpublished data) potentially indicating very high pre-patent mortality in these snail poplulations.

Intermediate snail host infections are routinely screened by observations of cercarial shedding; however, this method can be inaccurate. Melo *et al.* (2006) used a highly sensitive nested-PCR using *S. mansoni*-specific primers for the highly conserved gene encoding the small subunit rRNA, to detect *S. mansoni* infections in DNA from pooled *Biomphalaria* snails. This assay was found to be about three times more sensitive than the conventional method of light-induced cercariae shedding, so having potential for large-scale screening and monitoring of transmission in *S. mansoni* endemic areas (Melo *et al.* 2006).

Thus new PCR assays hold promise for diagnosis and possibly the quantification of human (and animal) infections as well as providing powerful tools for detecting and monitoring schistosome transmission. In the longer term, advances in parasite molecular biology and genetics offer ample scope for the development of sensitive, accurate and cost-effective diagnostic methods for assessing multiple parasitic infections. This would be particularly valuable in areas where treatment programmes for various other diseases are being integrated.

SCHISTOSOME MOLECULAR PHYLOGENETICS

An area where DNA data have been extremely useful is in the molecular characterization of species, leading to better resolution of the phylogeny of the *Schistosoma* genus and the family Schistosomatidae. Traditionally, *Schistosoma* species were identified by morphological characteristics, such as egg morphology and were arranged into four species groups; the *S. haematobium*, *S. mansoni*, *S. japonicum* and *S. indicum* groups (Rollinson and Southgate, 1987). More recently, molecular characterization of schistosomes now recognises 24 species within the *Schistosoma* genus (this includes 3 species of *Orientobilharzia*) which are better grouped into seven distinct lineages (B. Webster and T. Littlewood, unpublished data).

Since the development of genetic markers and DNA species identification, many interesting facts have come to light. For example: the splitting of *S. intercalatum*, a human parasite in West and Central Africa causing intestinal schistosomiasis, into two different species (*S. guineensis* and *S. intercalatum*); the confirmation of the relationships of the different schistosome groups with the validity of the *S. indicum* group being questioned; the inclusion of *Orientobilharzia* in the *Schistosoma* genus; the illustration that the *S. japonicum* group represents the ancestral position within the genus (see Agatsuma *et al.* 2002; Lockyer *et al.* 2003; Morgan *et al.* 2003; Webster, Southgate and Littlewood, 2006) and the realisation that *Griphobilharzia*, a parasite of crocodiles, is not actually a schistosome but a Spirorchiid, determining that schistosomes are exclusively parasites of endotherms (birds and mammals) (Brant and Loker, 2005).

Mitochondrial DNA (mtDNA) sequences have been used, usually in conjunction with nuclear nucleotide sequences, to resolve the phylogeny for the genus. The most up to date and robust phylogeny of the *Schistosoma* group based on the complete small and large nuclear subunit rRNA genes (18S and 28S) and partial mitochondrial *cox1* gene, incorporating 20 species of *Schistosoma* has been put forward by Webster *et al.* (2006). Whilst there appears to be general agreement concerning the topology of the tree with other molecular analyses (Lockyer *et al.* 2007), in this study, *S. margrebowiei* was resolved as the sister taxon to all others in the *S. haematobium* species group and *S. guineensis* was placed as sister species to both *S. bovis* and *S. curassoni*. The *S. haematobium* species group is the largest, containing eight species transmitted in Africa, of which many are of significant medical and veterinary importance. Many of the species are closely related and have the potential to hybridize both in the wild and experimentally in the laboratory, making the correct identification and recognition of these species very important, as different species and their hybrids may have different disease outcomes. The detection of hybridization between schistosomes requires a multi-locus approach, using both nuclear and mitochondrial DNA markers (Webster, Tchuente and Southgate, 2007) and is extremely important for understanding the evolution and epidemiology of the disease. This highlights the importance of developing both nuclear and mitochondrial diagnostics.

MITOCHONDRIAL DNA AND BARCODING

Completely characterized mitochondrial genomes for five species of schistosomes (*S. mansoni*, *S. japonicum*, *S. mekongi*, *S. haematobium* and *S. spindale*) have now been published, creating excellent opportunities to compare large amounts of sequence data from an evolutionary and phylogenetic perspective (Le, Blair and McManus, 2000, 2002*a*, *b*; Le *et al.* 2001; Littlewood *et al.* 2006). The 12 fast evolving protein coding genes and 2 rRNA subunits found within the mitochondrial genomes provide a suite of molecular markers for phylogenetic and population

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studies. One of the most striking observations has been the discovery of an extremely rare and novel mitochondrial gene order change within the genus, which reinforces the Asian origin of the genus and indeed highlights some key genetic differences between schistosome species. It is not yet known what effect (if any) this mt gene order change has on the biology of the parasites but it does provide a robust synapomorphy within the genus *Schistosoma*, helping further to resolve the phylogeny and historical biogeography of the genus (B. Webster and T. Littlewood, unpublished data). Fortunately, although the parasites are clearly distinct from a phylogenetic perspective, available treatments for schistosomiasis (namely the drug praziquantel) are effective against both Asian and African schistosomes.

Phylogeographical and population genetic studies have relied mainly on sequence analysis of the partial *cox1* mtDNA 'barcode' gene and the most detailed study to date concerns *S. mansoni*. Morgan *et al.* (2005) examined around 2500 bp of MtDNA from 143 parasites collected in 53 geographically widespread localities. Within-species diversity was high, with 85 groups being identified, which clustered into five distinct lineages with most variation associated with parasites from East Africa. The finding of only seven haplotypes in parasites from the New World with clear affinities to parasites from West Africa supported the hypothesis that *S. mansoni* was imported into the New World at the time of the slave trade from West Africa. Likewise, an apparent lack of *cox1* mtDNA genetic variability in parasites from Brazil, relative to their African counterparts assessed using the same technique, suggested a founder effect resulting from the establishment and spread of the parasite. Studies are ongoing using the partial *cox1* gene for schistosome population genetic studies to investigate the genetic diversity found within species and populations. It is hoped that these studies will provide insights into the genetic make-up within and between populations and to relate this to the epidemiology of the parasites. This may also enable us genetically to type specific schistosome strains or genotypes presenting particular phenotypes of interest.

The availability of complete mitochondrial genome data for schistosomes has also allowed an assessment of individual mt genes relative to one another for their potential information content (Zarowiecki, Huyse and Littlewood, 2007). This is a useful and rational approach, which allows the selection and optimization of suitable molecular markers. Interestingly, the authors conclude that *cox1* would not be the ideal marker for species identification (barcoding) or population studies. Instead they propose *cox3* and *nad5* for both phylogenetic and population studies of *Schistosoma* species.

RAPID DNA TYPING METHODS

When large numbers of individuals are to be screened for DNA sequence variation, it is recognised that conventional DNA cycle sequencing approaches can be both slow and costly. Rapid DNA typing methods, which use mutational screening technologies (Gasser, 1998) are required to identify genetic variants and, at the same time, also recognise invariant individuals. While sequencing is still necessary to characterize fully the mutational basis of the observed variation, putting forward only those individuals shown to vary helps to avoid unnecessary duplications and therefore streamline DNA sequencing costs. Single strand conformational polymorphism (SSCP) is a convenient mutational screening method used to compare PCR amplicons for sequence variation. SSCP profiles can be equally informative for cross matching against reference samples, for example, when used for detection of variation within populations of *S. japonicum* (Bogh *et al.* 1999). Kane *et al.* (2002) adapted the conventional SSCP protocol by using fluorescently labelled PCR primers in a Perkin Elmer ABI Prism 377 automated sequencer, which allows separate visualisation of both forward and negative DNA strands, thereby increasing discrimination of amplicons. Using male and female adult worms representative of *S. haematobium* group species, both *coxI* and ITS2 were amplified, with the finding that changes in SSCP profiles revealed variation at individual, isolate and species level comparisons. DNA sequencing confirmed that single point substitutions were detectable (Kane *et al.* 2002).

SSCP analysis of variation within ITS2 and *coxI* has proved very useful in a variety of settings. Foremost have been investigations of hybridization between *S. haematobium* and *S. guineensis* in Cameroon that have shown the presence of natural hybrids within humans in Loum over a 10-year period; whilst *S. guineensis* is no longer present, its genetic legacy still remains (Webster *et al.* 2005, 2007). This assay was also used to analyse *S. haematobium* populations in the middle Senegal River Basin (SRB) in relation to parasite genotypes and intermediate host compatibility (Southgate *et al.* 2000; Sene, Southgate and Vercruysse, 2004).

The unique SSCP profiles produced for different *S. haematobium* group species (Kane *et al.* 2002) also allows rapid screening of schistosome populations to identify sympatric infections such as *S. haematobium* and *S. bovis* which have the ability to infect the same *Bulinus* intermediate snail hosts. Other low cost PCR-based assays have also been developed as rapid diagnostic tests (RDT) to screen *S. haematobium* group populations for mixed species infections. In 2000, Barber and colleagues developed a nuclear RDT based on restriction fragment length polymorphism (RFLP) analysis of the ITS2 PCR

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product using two restriction enzymes that generated a species-specific banding pattern to distinguish *S. haematobium* from other *S. haematobium* group species, while Abbasi *et al.* (2007) differentiated *S. haematobium* from related species by PCR amplification of a nuclear inter-repeat sequence. As more DNA data become available for different species of *Schistosoma*, more sensitive and robust diagnostic assays can be developed. Recently Webster *et al.* (2009) developed a high throughput diagnostic multiplex PCR using the mtDNA barcoding region to detect and discriminate between *S. haematobium* and *S. bovis* in all lifecycle stages (miracidium, cercariae and adults). This technique uses one universal forward primer and two species-specific reverse primers, producing species-specific PCR fragment lengths for *S. haematobium* and *S. bovis*, allowing one to detect both PCR fragments in a mixture of the two species. This assay proved sensitive, cost-effective, rapid and robust, thereby providing a useful tool for large-scale screening of *S. haematobium* and *S. bovis* populations.

SNP PROFILING OF KEY BARCODE INFORMATION

In species and populations where DNA barcode variation has been already largely determined, to make a realistic contribution to field epidemiology it may be necessary to identify thousands of biological specimens taken directly from the field. Molecular processing will therefore need to be further scaled-up and, with this in mind, it would be prudent to focus typing efforts on the most pertinent aspects of the DNA barcode that allow differentiation between samples; for example, it may be that only several key point mutations, or single nucleotide polymorphisms (SNPs), need to be inspected to identify with confidence a known biological variant. Separation of individual *B. globosus* and *B. nasutus* snails, for example, can be achieved by inspection of only four SNPs within the *cox1* using SNaPshot^{TM} methods (Stothard *et al.* 2002*a*).

SNaPshot^{TM} is an excellent rapid point mutation typing method and takes advantage of two key principles (Rollinson, Stothard and Southgate, 2001). First, a custom made synthetic oligonucleotide is designed to abut to the variable SNP position, which, after performing a mini-sequencing reaction, becomes end-labelled with a fluorescent dideoxy nucleotide complimentary to the SNP variant. Second, as the synthetic oligonucleotides can be of variable length and sequence, they can be used in a multiplexed manner to type simultaneously several SNPs within the sample amplicons, or SNPs within several amplicons within the same PCR reaction. Thus, it is possible, within a single multiplexed PCR, to investigate SNPs which give resolutions at different levels, e.g. between species, populations and molecular variants. Such a rationalised intraspecific nomenclature for both schistosomes and snails could provide a basis for exploring snail-parasite compatibilities both in the laboratory and in the field.

MICROSATELLITE ANALYSIS AND POPULATION STRUCTURE

Microsatellite analysis has been seen as a promising method to acquire a more detailed characterization of genetic variation and schistosome population structure. One of the first attempts to isolate microsatellite markers for *S. mansoni* was that of Durand, Sire and Theron, (2000) who identified 11 polymorphic loci with the number of alleles ranging from two to eight. Similarly, Blair, Webster and Barker, (2001) identified 10 polymorphic microsatellite markers for *S. mansoni* that showed high variability both between individuals and populations.

Comparison of a laboratory strain (LE) and 10 field isolates of *S. mansoni* was carried out by Rodrigues *et al.* (2002), who examined six loci and found four to be polymorphic. The laboratory strain showed the lowest number of alleles per polymorphic locus while little variation was shown between the field isolates, suggesting a high degree of gene flow between them. Curtis, Sorensen and Minchella, (2002) also attempted to show how diversity is partitioned within natural populations from a single Brazilian village using allelic markers. Analyses with a set of seven microsatellite loci consistently revealed moderate genetic differentiation when village boroughs were used to define parasite subpopulations and greater subdivision when human hosts defined subpopulations. Thus, microsatellite analyses show a level of genetic diversity not apparent from the analysis of *cox1* mtDNA sequence data (Morgan *et al.* 2005).

More recently, Agola *et al.* (2006) examined seven populations of *S. mansoni* from Kenya using five microsatellite markers and detected a high level of genetic diversity and suggested that the strong genetic structure observed was a result of limited gene flow and large population sizes. An interesting hypothesis to emerge from this study was that limited gene flow between populations might hinder the spread of drug resistance.

Schistosome infection in the natural definitive rat host, *Rattus rattus*, in Guadeloupe has provided an excellent system to study the detailed population genetics and transmission of *S. mansoni*. Prugnolle *et al.* (2002) used seven microsatellite and seven random amplified polymorphic DNA (RAPD) markers to study population structure of *S. mansoni*. Interestingly, it was found that male genotypes were more randomly distributed among rats than female genotypes. Prugnolle *et al.* (2004) genotyped 71 pairs of adult *S. mansoni* worms sampled from naturally infected rats to investigate how male and female schistosomes paired according to their genetic

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relatedness and found a random association between males and females. Interesting observations have been made in Kenya concerning introgressive hybridization between *S. mansoni* and the rodent parasite *S. rodhaini*, which may impact on the epidemiology and control of schistosomiasis in this region (Steinauer *et al*. 2008*b*).

Significant progress has also been made with *S. japonicum* microsatellites. Shrivastava *et al*. (2003) isolated and characterized the 11 polymorphic *S. japonicum* microsatellite markers, and their subsequent application to field studies in the Philippines and China has revealed high levels of polymorphism between and within population samples. Chinese and Philippine strains appear to follow different lineages, with distinct branching between provinces in relation to geography and habitat (lake and marshland region *S. japonicum* genotypes are distinct from those obtained from mountainous regions), and even in relation to intermediate host Snails (smooth- versus ribbed-shelled *Oncomelania hupensis*) (Shrivastava *et al*. 2005). Further work has also used these microsatellites to elucidate the relative gene flow of *S. japonicum* between its potential definitive host species. Work to date within China has indicated that, whilst there is gene flow between parasites associated with bovines, humans, cats, dogs and goats, parasites associated with bovines appear to contribute the most towards parasite genotypes within humans in the marshland regions (Wang *et al*. 2006; Rudge *et al*. 2009) whilst rodents appear to contribute the most towards maintaining transmission and human infection in the highland regions (Rudge *et al*. 2009). In contrast, within the Philippines parasite genetic differentiation was not evident between the different areas examined, with highest transmission occurring between dogs and humans (Rudge *et al*. 2008). Such elucidation is essential for appropriate and sustainable surveillance and prevention, particularly in terms of characterizing which, if any, animal hosts are responsible for transmitting the parasites to humans. These animal hosts may serve as re-infection reservoirs after chemotherapy, and should be targeted for focused disease control through, where feasible (i.e. bovines and dogs) chemotherapy or vaccination.

A key challenge in all such population genetic studies of schistosomes is, however, that adult worms cannot be directly sampled from human or important animal/livestock infections because of their location in the blood vessels draining the intestine and/or the bladder. Many of the earlier studies of schistosome population genetics have therefore depended upon exposing laboratory mammals to cercariae from field isolates in order to obtain adult schistosomes. Laboratory passage inevitably introduces bottlenecks and selection biases such that isolates may not be representative of the genetic variation in the original population. Long-established laboratory schistosome strains have been reported to represent only 10–15% of the diversity present in field isolates, with many rare alleles notably absent. The ethically and biologically superior alternative to laboratory passage is therefore through the development of novel methodologies for the collection, storage and genetic analysis of miracidia and cercariae collected directly from natural infections in the field. Single-locus microsatellite genotyping of the naturally sampled larval stages of *S. japonicum* was carried out by Shrivastava *et al*. (2005), enabling unbiased population genetic studies of this multi-host zoonotic parasite. The methodology required samples to be frozen on collection. A further significant advance has been made recently by using Whatman FTA® cards, which allow the collection and analysis of a single miracidium (or cercaria) in field situations. Cards can be kept long-term at room temperature. By coupling this sampling technique with multiplex PCR analysis, it has been possible to carry out multilocus genotyping of single larval samples: up to seven microsatellite loci can be genotyped in a single miracidium (Gower *et al*. 2007). The utility of this assay was evaluated using seven *S. mansoni* population isolates collected from schoolchildren in the Hoima district of Uganda. The results demonstrated the reduced variability of laboratory reared adult worms compared to field collected larval samples. Interestingly, some heterozygote deficiency was observed in the field–collected samples but not in laboratory-derived samples.

Another important new high through-put technique to generate reliable multilocus microsatellite data from individual *S. mansoni* miracidia was described by Steinauer *et al*. (2008*a*). They showed that 21 loci can be amplified in four multiplexed PCR reactions and indeed that it would be possible to increase this to six PCR reactions. The technique was validated by using laboratory crosses of *S. mansoni* from Kenya and comparing parents and offspring. DNA analysis of miracidia collected from patients in Western Kenya was carried out and samples were shipped on ice to a laboratory where the PCR amplifications were performed. Techniques such as these create exciting possibilities for detailed molecular epidemiological studies, which will allow new insights into schistosome population size and structure.

Microsatellite analysis of natural schistosome populations in humans is therefore now technically possible and there are a number of interesting questions that can and should be addressed relating to genetic structuring of parasites within individuals and communities, as well as parasite population turnover in relation to age and immune status and external selection pressure. The neutral microsatellite markers developed by Gower *et al*. (2007)

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have been used to assess the impact of MDA programmes on *S. mansoni* population structure in two Tanzanian schools, reinforcing the importance of careful genetic monitoring of parasites subjected to control programmes in relation to outcomes, such as drug resistance (A. J. Norton, C. M. Gower, P. H. L. Lamberton, B. L. Webster, N. J. Lwambo, A. Fenwick and J. P. Webster, unpublished). Nine novel polymorphic microsatellite markers were isolated for the other important human schistosome in Africa, *S. haematobium* (Golan *et al*. 2008) suggesting high variability between individuals and between unrelated populations. Further work is ongoing to improve and add to these *S. haematobium* markers to enhance population studies of this prevalent parasite.

GENETIC VARIATION AND PATHOLOGY

Although not easily quantified, observations suggest that, in different endemic areas, pathology and associated morbidity of infection may differ (Boisier *et al*. 2001; Balen *et al*. 2006). The genetic background of the host is clearly important (Quinnell, 2003; Van de Vijver *et al*. 2006) but one might also expect genetic differences in the parasite to play some role. Brouwer *et al*. (2003) were interested in the fact that the disease outcome in persons infected with *S. haematobium* may range from mild symptoms to severe damage of the kidneys and/or bladder. They examined the extent of urinary pathology in infected children in Zimbabwe and the genetic diversity of isolated parasites using randomly amplified DNA (RAPD) markers. Their preliminary findings suggested that 3 of 13 identified parasite clusters were significantly over-represented in children with severe lesions. The fact that parasite genetic variation in *S. mansoni* influences infectivity and virulence has been shown in laboratory experiments using different strains of *S. mansoni* and *Biomphalaria glabrata* (Davies, Webster and Woolhouse, 2001). Interestingly, there was trade-off in parasite reproductive success between the intermediate and definitive hosts.

The recent developments in the methods used to sample parasite populations directly from the host, without the need for laboratory passaging, together with advances in DNA assays to genetically type schistosome populations and assess population genetic structure and diversity, will have important implications for studies on pathology related to parasite genetic diversity. Such methods will provide a genetic insight into the parasite population within the host and this can be recorded in association with any pathology seen. These methods will also allow population structure to be tracked within the host in relation to treatment; for example, it will be possible to see if the parasite population structure changes before or after treatment or if certain parasite genotypes are showing any sign of reduced drug susceptibility.

GENOME PROJECTS

The most recent draft of the *S. mansoni* genome was V3 released in July 2006 and can be downloaded from ftp://ftp.sanger.ac.uk/pub/pathogens/Schistosoma/mansoni/genome/Assembly-v3.0. The *S. japonicum* genome is available at http://lifecenter.sgst.cn/sj.do. Work has also started on the genome of *Biomphalaria glabrata* (see http://biology.unm.edu/biomphalaria-genome/index.html).

Wilson *et al*. (2007) have reviewed progress in schistosome genomics and post-genomics and highlighted prospects for advances in understanding schistosome biology. As yet the possibilities for comparative genomics are limited due to the few taxa being sequenced. However, with the advent of pyrotechnic sequencing methods and the development of mutation assays, detailed comparisons of parasite genotypes within species should soon be possible. Recent gene expression studies have extended earlier studies on stage-specific gene expression. For example, Jolly *et al*. (2007) used a genomic microarray made of 12 000, 45–50-mer oligonucleotides based on expressed sequence tags, to compare gene expression in three different developmental stages of the schistosome parasite. Of interest was the robust protein synthesis and programmed cell death in development of cercariae in the sporocyst stages, the importance of genes associated with energy production in cercariae and the range of gene expression of adult worms associated with their ability for long-term survival in the complex and hostile environment of the definitive host.

The challenge for the future in relation to schistosomiasis control is to mine the developing databases and use the new ''omic'' tools to promote fresh ideas for vaccine and drug targets.

SNAIL IDENTIFICATION AND PARASITE INTERACTIONS

The geographical distribution and transmission of schistosomiasis is closely linked to that of susceptible intermediate snail hosts, with local ecological conditions determining the occurrence of particular snail species. Water development programmes ranging from the construction of large dams, as seen in Senegal and Mali, to the creation of small irrigation ponds can influence snail distributions. Freshwater snails are extremely vulnerable to changes in environmental conditions, with rainfall and temperature being key factors in their distribution. Molecular analyses have been used extensively to complement morphological approaches to differentiate species and establish phylogenetic relationships for both *Bulinus* (Rollinson *et al*. 2001) and *Biomphalaria* (Morgan *et al*. 2001; Jørgensen, Kristensen and Stothard, 2007). Correct identification of morphologically similar snail species can help pinpoint

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schistosomiasis transmission, as seen on the island of Zanzibar, where molecular techniques are required to differentiate *Bulinus globosus*, a snail compatible with local strains of *S. haematobium*, and *B. nasutus*, which is refractory (Stothard *et al*. 2002*b*). A good local knowledge of snail distribution can help focus parasitological surveys and prioritize treatment campaigns. Development of a barcode database for the differentiation of both *Biomphalaria* and *Bulinus* across Africa is an ongoing requirement. Kane *et al*. (2008) showed high sequence diversity within cox1 of *Bulinus* spp. and suggested that a barcoding approach may offer the best method for characterization of populations and species within the genus from different geographical locations.

Whereas snail control remains a big challenge in the field, especially in Africa, laboratory studies are focusing on the mechanisms and genes associated with resistance to schistosome infection using the *S. mansoni* and *B. glabrata* model (see Zhang *et al*. 2004; Lockyer *et al*. 2007). The use of microarray technology will help to identify genes involved in the interactions of snails and parasites and a future task will be to investigate how these genes operate in natural populations. Nevertheless, exploring the molecular basis of molluscan defence to schistosomes may in the longer-term help to generate novel methods to reduce transmission by the manipulation of snail populations to increase resistance to schistosomes.

Genotyping schistosomes in relation to intermediate host susceptibility will enable us to investigate if certain genotypes within a species are more or less compatible with a particular snail and this can also be linked to the genotype of the snail itself. This may enable us to predict which parasite genotypes could be transmitted in different areas, thus facilitating the production of risk maps for the transmission of certain schistosome species.

CONCLUDING REMARKS

The challenge for the future is to refine and develop the molecular approaches which have been developed in the laboratory, and to implement them more widely in the field, in order to assist with monitoring and surveillance of schistosomiasis control programmes. New molecular tools can help to improve diagnosis of disease, to demarcate hot spots of transmission and to monitor the perturbations of schistosome and snail populations when subjected to increasing drug pressure or environmental changes.

The exponential development in genomics provides rapid advances in the methods and resources available for parasite research. This will enable researchers to investigate many areas that have in the past seemed inaccessible and will allow major advances in the understanding of the biology of these parasites, so leading the way to developing better and more robust control strategies which will alleviate the significant burden of schistosomiasis.

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