SCHISTOSOMIASIS

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SCHISTOSOMIASIS

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Preface

Human schistosomiasis is a disease with a rich and well-documented past, and every expectation of an unfortunately long future. These infections were known to the ancient Egyptians and their transmission shows little evidence of slowing down, globally. The good news is that field applicable, and increasingly affordable, chemotherapy has been available for almost 25 years. Using chemotherapy and other means of control, some countries have decreased transmission and made excellent headway against morbidity. The bad news is that the public health problems caused by schistosomiasis are still with us, with the estimated number of cases of schistosomiasis, while shifting geographically, remaining approximately 200 million for the last 30 years. In fact, with the development of field usable ultrasound technology and meta-analyses performed on existing data, there is a new appreciation for the extent of non-lethal morbidity associated with these infections. While the percentage of individuals with severe hepatosplenic disease remains below 10%, recent reassessments of morbidity associated with schistosomiasis indicate that the prevelance of symptoms and the cost in diability-adjusted life years is much greater than was previously, commonly appreciated (Van der Werf, M. J., et al. 2003, *Acta Tropica* 86:125-139; Charles H. King, personnel communication). Strong impetus for addressing these issues is provided by the World Health Assembly's recently passed Resolution 54.19, which calls for efforts to reduce morbidity caused by schistosomiasis and soil-transmitted helminths in school-aged children, largely through chemotherapy campaigns.

World Health Assembly recognition of the public health problems caused by schistosome infections promotes efforts towards schistosomiasis morbidity control. However, for a long-term, permanent solution, an equally needed push is to encourage continued research by a community of

scientists, to learn more about multiple aspects of the parasites and disease. Unfortunately, the number of students and scholars who train in schistosomiasis, as is the case for most helminthic diseases, is decreasing. For continued progress, the field needs a critical mass of researchers investigating everything from the basic biology of the parasite to the best means by which to apply suitable public health interventions. Solid research begets useful public health tools and advances in the laboratory, even if not directly applicable to field work, provide benefits towards an overall understanding of the disease and its control. In addition, many discoveries made through the study of schistosomiasis have had profound scientific impact on other fields of biomedical research.

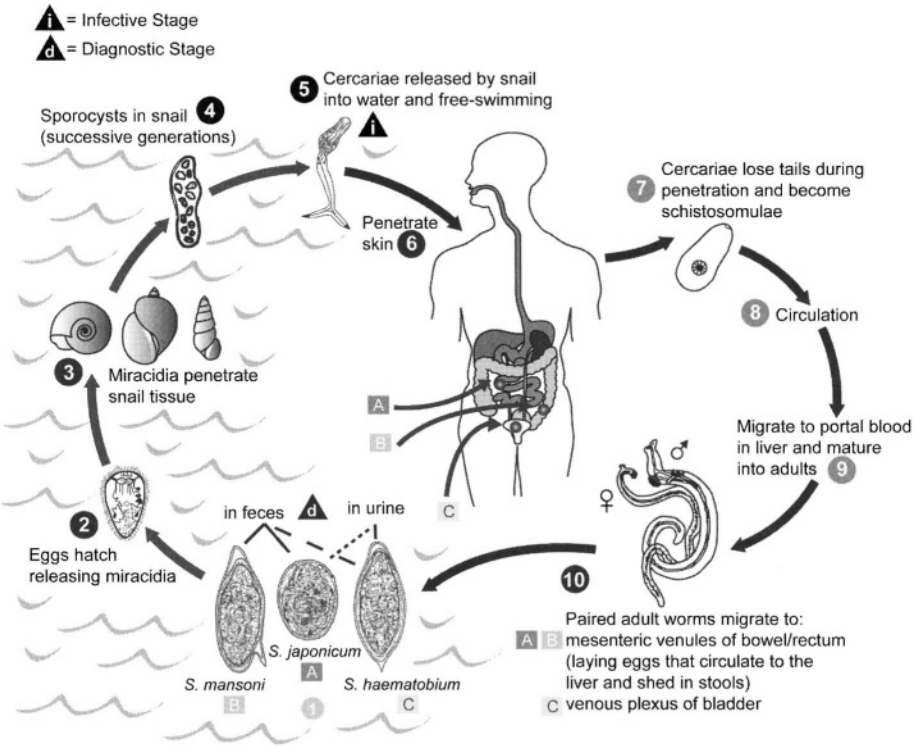
The goal of this book is to provide the reader with insights into the active research and programs currently related to schistosomiasis, and to use these insights as a way to project forward into the next 10-15 years of work on schistosomiasis, spanning the spectrum from research to public health interventions. The charge given to each of the authors of the chapters in the book has been to think and write freely about their work, with an emphasis on how it fits into current thinking, and where it might take us in the future. Through this we hope to bring heightened focus, and raise expectations, related to schistosomiasis, especially among trainees in the world of biomedicine and public health. A secondary goal of this volume is that it will initiate conversations among those working across the research-to-control spectrum on schistosomiasis about the future of their field, and by doing so lead to constructive efforts to identify and address the most critical questions and challenges related to schistosomiasis.

In the tradition of David Rollinson and Andrew Simpson (*The Biology of Schistosomes,* Academic Press, 1988), we organized this volume in an approach that takes the reader from genes to latrines. The first 4 chapters address schistosome phylogenetics, gene expression, and the overall genome, including information on exciting new tools for addressing questions that have long been inaccessible to schistosomologists. The next 3 chapters explore the host-schistosome interaction at the larval to adult worm interface and addresses aspects important for vaccine development as well as how differential gene expression as detected by DNA microarrays may be utilized to develop tools for detection and control of infection or pathology. The following 3 chapters explore the development of the host immune response to eggs, granuloma formation and factors affecting the development and regulation of immunopathology. The next 4 chapters address the public health concerns associated with schistosomiasis, including morbidity control, host genetics, treatment and proposals for improved partnerships. The volume concludes with a chapter addressing the schisms that sometimes exist along the spectrum from basic research programs to the implementation of control schemes, and a proposal to make these differences

benefit patients and researchers rather than succumb to base temptations to compete for resources to no one's benefit.

Like many of the diseases featured in the World Class Parasites series, the prospects for dramatic advances in schistosomiasis coincide with a seemingly shrinking pool of both human and material resources. Our hope is to point out that these challenges are not insurmountable, and are in fact exciting, and that the various disciplines employed in the study of schistosomiasis can and should be shaped by the needs identified by other disciplines. The most meaningful progress will occur as the laboratory better understands the needs in the field and the field better understands the capabilities of the laboratory. It is our desire that this volume contributes to the conversation in a useful, collegial manner.

W. Evan Secor and Daniel G. Colley



Schistosome Life Cycle. Eggs are eliminated with feces or urine . Under optimal conditions the eggs hatch and release miracidia , which swim and penetrate specific snail intermediate hosts . The stages in the snail include 2 generations of sporocysts and the production of cercariae . Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host , and shed their forked tail, becoming schistosomulae . The schistosomulae migrate through several tissues and stages to their residence in the veins (,). Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species . For instance, *S. japonicum* is more frequently located in the superior mesenteric veins draining the small intestine **A**, whereas *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine **B**. *S. haematobium* most often occurs in the venous plexus of bladder **C**, but it can also be found in the rectal venules. The females (size 7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine *(S. mansoni* and *S. japonicum)* and of the bladder and ureters *(S. haematobium),* and are eliminated with feces or urine, respectively . (from the DPDx website of CDC's Division of Parasitic Diseases: http://www.dpd.cdc.gov/dpdx)

Chapter 1

SCHISTOSOMES AND THEIR SNAIL HOSTS

The Present and Future of Reconstructing Their Past

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Key words: evolution, phylogenetics, host shifts, Platyhelminthes, Schistosomatidae

1. INTRODUCTION: PARASITES EXTRAORDINAIRE

The schistosomes are "World Class" parasites in every sense of the expression. They are among the most common and debilitating infectious agents of humans and their domestic animals – they still quietly infect an estimated 200 million people, especially children, and 165 million head of livestock (DeBont and Vercruysse, 1998; Chitsulo et al., 2000). The schistosomes have a geographic distribution that encompasses much of the world and their intrinsically fascinating biology poses questions of fundamental significance to both basic and applied biologists around the globe. Although many of the mysteries pertaining to the evolutionary history of this important parasite group will no doubt remain permanently shrouded in the past, there are today several new insights regarding their history that have emerged from molecular phylogenetic studies, and it is these that we wish to highlight below. We also attempt to identify particular studies that remain to be done. The fundamental premise is that by understanding their past, we can better comprehend all aspects of contemporary schistosome biology, including their likely response to ongoing environmental changes.

2. WHERE DO SCHISTOSOMES FIT IN THE DIGENEAN FAMILY TREE?

The 13 genera and approximately 100 species of the family Schistosomatidae are parasites of crocodiles (one known species), birds and mammals. Among the 18,000 or so species of digenetic trematodes (Phylum Platyhelminthes, Class Trematoda, Subclass Digenea), the schistosomes along with the families Spirorchiidae (in turtles) and Sanguinicolidae (in fish) are odd because they have two host life cycles (a snail intermediate host and a vertebrate definitive host) that feature direct penetration of the skin of the definitive host by cercariae. They do not have the metacercaria stage and the three host life cycle that is typical of most digeneans. Representatives of these three families are also known as "blood flukes" because the adults usually live in the vascular systems of their hosts. Most species of schistosomes live in the venous system, but at least two species colonize the arterial system (McCully et al, 1967; Ulmer and Vande Vusse, 1970). Perhaps because of their vascular habitats, the tegument of adult worms of all three families is bounded by a double lipid bilayer, whereas most other flukes possess a single bilayer (McLaren and Hockley, 1977). The schistosomes also have separate sexes (are dioecious), in sharp contrast to the spirorchiids, sanguinicolids and almost all remaining digeneans that are monoecious (Platt and Brooks (1997).

Given their peculiar properties, are schistosomes modern-day descendents of digeneans that diverged early in the evolution of the group and that have retained primitive features? Or do they represent more recent offshoots that have lost primitive features and adopted peculiar features in response to life in a specialized environment? The most recent and complete phylogenetic analyses of digeneans based on SSU and partial LSU rDNA data (Fig. 1-1A) suggest that schistosomes (and other blood flukes) are indeed part of a lineage that diverged early in the evolution of digeneans, but their two-host life cycles probably are indicative of secondary loss of the third host rather than of the primitive life cycle type in digeneans (Olson et al., 2003; Cribb et al., 2003). If we assume this figure represents the true relationships among the blood flukes, then the sister group of the schistosomes are the Spirorchiidae, the turtle blood flukes (see also Blair et al., 2001), a close relationship further suggested by life cycle and morphological studies (Carmichael, 1984; Brooks et al., 1985; Combes, 1990).

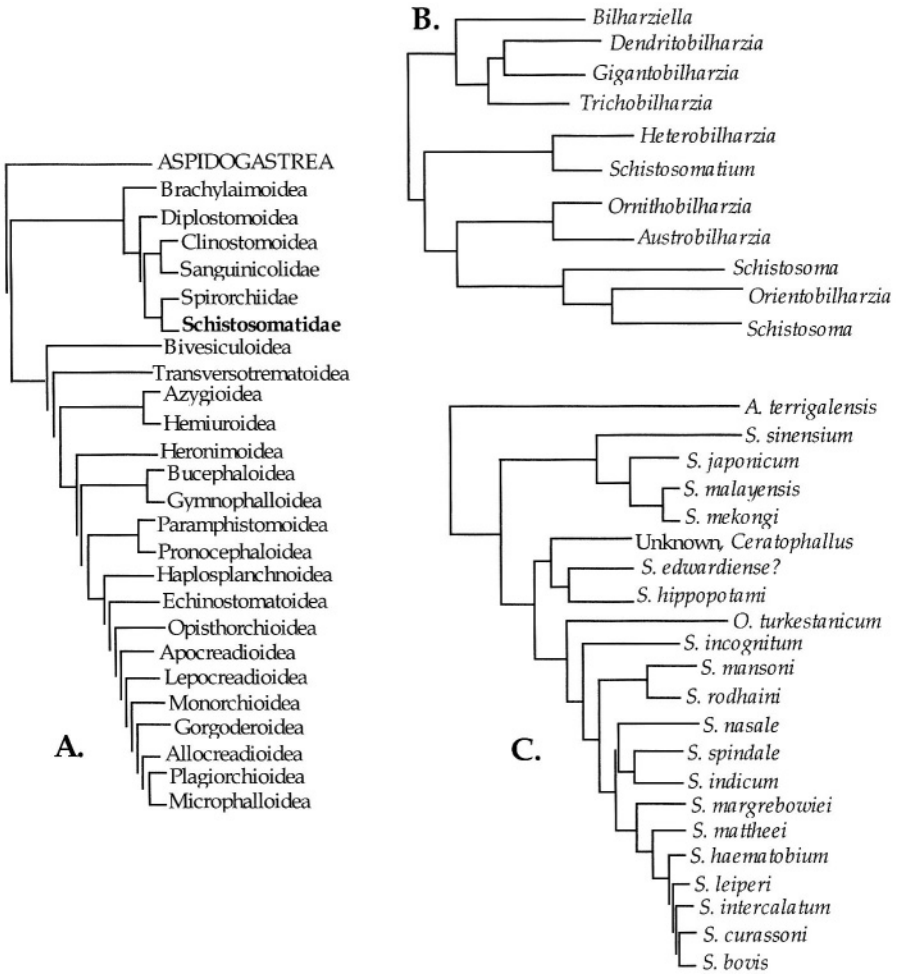

Figure 1-1. A. A summary of hypothesized relationships and higher classification of digenetic trematodes showing the relatively basal position of the schistosomes (Family Schislosomatidae), and that the turtle blood flukes (Family Spirorchiidae) comprise the likely sister group of the schistosomes. The phylogeny was estimated using the complete small subunit ribosomal RNA and partial large subunit ribosomal RNA gene sequences, based on data from Olson et al. (2003) and modified from Figure 3 of Cribb et al. (2003). B. A summary of relationships among the 10 genera of schistosomes for which sequence data are available, based on partial small and partial large subunit rRNA genes, ITS1, 5.8S, ITS2, mitochondrial cytochrome oxidase I and partial mitochondrial small subunit rRNA gene sequences, modified from Snyder and Loker (2000) and Lockyer et al. (2003). C. Relationships among the species of *Schistosoma,* using the same sequences identified in panel B, after Lockyer et al. (2003) and Morgan et al. (2003).

3. WHAT WAS THE PRIMORDIAL SCHISTOSOME LIKE?

The discovery by Platt et al. (1991) of the peculiar schistosome *Griphobilharzia amoena* from freshwater crocodiles in Australia provides a tantalizing glimpse into the schistosomes' past. This is the only species of schistosome known from other than a bird or mammal. It is also peculiar in that male and female worms seem to pair while still immature, with the female enclosed completely within a gynecophoric chamber of uncertain origin in the male (Platt et al., 1991). These authors raise the possibility that both sexes derive from a single cercaria. Unfortunately, we lack additional important information about this species. Although it is assumed that *G. amoena* must occupy a basal position within the Schistosomatidae, we lack any sequence data that could provide an independent corroboration of *G. amoena*'s phylogenetic position. This would also help us understand if the primordial schistosome was a parasite of crocodilians or other exothermic vertebrates, or if this family has exclusively colonized endotherms.

4. WHAT ARE THE FAMILY TIES OF *SCHISTOSOMA?*

We now have at least some sequence data for representatives of 10 of the 13 genera of schistosomes. We presently lack sequence information for *Griphobilharzia amoena,* the two known schistosome species from elephants *(Bivitellobilharzia)* and for *Macrobilharzia,* from cormorants and anhingas. So, another tangible objective for the future is to acquire sequence data for these genera so that a more complete and definitive phylogeny of the Schistosomatidae can be compiled. Regarding intrafamilial relationships, one reasonable hypothesis would be that the schistosomes are split into two major clades, one avian and one mammalian, but the results of two independent molecular studies (Snyder and Loker, 2000; Lockyer et al., 2003) suggest this is not the case (Fig. 1-1B). There is a basal clade comprised exclusively of four genera of avian schistosomes, but the remaining large clade can be subdivided into three smaller groups, with the North American mammalian schistosomes *Heterobilharzia* and *Schistosomatium* separated from the remaining mammalian schistosomes by the avian genera *Ornithobilharzia* and *Austrobilharzia.* The genus *Schistosoma,* which includes all the species that parasitize humans, is a derived lineage within the family.

Several studies have agreed that the one species thus far examined of *Orientobilharzia,* a genus of Asian mammalian schistosomes, nests within *Schistosoma* (Snyder and Loker, 2000; Zhang et al., 2001; Lockyer et al., 2003). Do the other two species of *Ornithobilharzia* also nest within *Schistosoma* in molecular phylogenies? Also, it would be particularly interesting to know how the elephant schistosomes fit into the picture. Do they represent a separate offshoot in the family or are they close relatives to one of the other lineages of schistosomes infecting mammals?

5. WHAT ARE THE RELATIONSHIPS AMONG SPECIES WITHIN *SCHISTOSOMA?*

Traditionally, the 20 species of *Schistosoma* have been informally divided into four species groups (Table 1-1), with the groups based on geographic area of origin, egg shape and snail host. The *S. japonicum* group is exclusively Asian in its distribution, and its members are transmitted by pomatiopsid caenogastropod snails. Most representatives possess round eggs with rudimentary, recessed or absent spines. The *S. mansoni* group is known, or considered, to be transmitted by the planorbid snail genus *Biomphalaria,* and is primarily African in its distribution although *S. mansoni* occurs in both southwest Asia and South America. The *S. indicum* group is also Asian, is comprised of four species with eggs of variable shape and its members infect planorbid or lymnaeid pulmonate gastropods. The *S. haematobium* group with seven species is the largest of the four traditional species groups, is almost exclusively transmitted by the planorbid genus *Bulinus* and is mostly confined to Africa.

Table 1-1. The traditionally recognized species groups of *Schistosoma*:

S. japonicum group	*S. mansoni* group	*S. indicum* group	*S. haematobium* group
japonicum	*mansoni*	*indicum*	*haematobium*
mekongi	*rodhaini*	*nasale*	*intercalatum*
malayensis	*hippopotami*	*spindale*	*bovis*
sinensium	*edwardiense*	*incognitum*	*mattheei*
ovuncatum			*curassoni*
			margrebowiei
			leiperi

Do these four species groups accurately delineate relationships among species within *Schistosoma,* as assessed independently by molecular phylogenetics? A perusal of the hypothesized relationships among *Schistosoma* species as depicted in Figure 1-1C suggests that the answer is

partially "yes" and partially "no". First, regarding the traditional *S. japonicum* group, several phylogenetic analyses (Snyder and Loker, 2000; Zhang et al., 2001; Agatsuma et al., 2001; Attwood et al., 2002; Lockyer et al., 2003) consistently affirm that the Asian pomatiopsid-transmitted species cluster together and that this lineage occupies a basal position in the genus. As noted by Agatsuma et al. (2001), and as recognized by other Asian schistosome workers (Attwood et al., 2002), *S. sinensium* and its close relative *S. ovuncatum* (and possibly other as yet undescribed species) are genetically distinct from the remaining species of the *S. japonicum* group. So, the Asian pomatiopsid-transmitted species (the traditional *S. japonicum* group) form a well-defined lineage that is comprised of two sublineages, *S. japonicum* and its allies and *S. sinensium* and its allies. Phylogenetic studies have also upheld the *Bulinus*-transmitted *S. haematobium* group, the species of which consistently cluster together and in this case occupy a more derived position in the tree.

The other two traditionally recognized species groups do not fare so well. For the *S. indicum* group, the three *Indoplanorbis*-transmitted species do in fact cluster together suggesting they comprise a natural group. Their close relationship to the *S. haematobium* group is of interest because their respective snail hosts, *Indoplanorbis* and *Bulinus,* are also close relatives. The fourth species of the *S. indicum* group, *S. incognitum,* occupies a much more basal position in the tree (Agatsuma et al., 2002; Lockyer et al., 2003). Interestingly, its placement on the tree is close to *Orientobilharzia turkestanicum,* a species with which it shares an Asian distribution and use of lymnaeid snail hosts.

The four species of the traditional *S. mansoni* group do not comprise a natural group and is essentially reduced to just two species (Fig. 1-1C), *S. mansoni* and *S. rodhaini.* The other two species, *S. hippopotami* and *S. edwardiense,* both parasites of the hippopotamus, and a third as yet undescribed species fall into a much more basal clade in the tree (Déspres et al., 1995; Morgan et al., 2003). Members of this newly recognized group are also distinguished by the presence of a long tail stem in the cercaria stage.

This same tree suggests humans or their immediate ancestors have been colonized by schistosomes on at least three and possibly as many as five separate occasions. Two members of the *S. japonicum* group infect humans, one of them being *S. japonicum* which is a relative generalist capable of infecting a broad range of mammals. The second species, *S. mekongi,* commonly infects dogs and humans. *S. mansoni* is today predominantly a human schistosome though it can and does infect rodents and its origins are likely as a rodent parasite that subsequently adapted to humans (Combes, 1990). *S. haematobium* and its close relative *S. intercalatum* probably originated in ungulates and separately colonized humans (Combes, 1990).

The former species with its distinctive site of egg deposition in the urinary system is the most human-adapted of all the *Schistosoma* species. We can conclude from the broad pattern of human use on the *Schistosoma* family tree that schistosomes readily colonize humans and that human-borne schistosomes are today faring very well. By virtue of being long-lived, large-bodied, mobile, water-loving and slow to develop immunity, humans provide a stable and productive environment in which the worms can propagate and be disseminated to their snail hosts.

6. WHERE DID *SCHISTOSOMA* COME FROM?

Africa currently supports a large number of successful *Schistosoma* species so it is not unreasonable to postulate that the genus originated there, and subsequently spread to other continents (Davis, 1980, 1992). However, molecular phylogenetic studies consistently retrieve a basal position of the exclusively Asian *S. japonicum* group in the *Schistosoma* tree, suggestive of an Asian origin (Snyder and Loker, 2000; Zhang et al., 2001; Morgan et al., 2001; Agatsuma et al., 2001, 2002; Attwood et al., 2002; Lockyer et al., 2003). Remarkably, the order of the genes in the mitochondrial genome for members of the lineage containing *S. japonicum* are like those of other flatworms, whereas the mitochondrial gene order for primarily African species such as *S. mansoni* and *S. haematobium* are different, further suggesting the African *Schistosoma* species lie in a more derived position (Le et al., 2001; Lockyer et al., 2003). Studies of C-banding chromosomal patterns by Hirai et al. (2000) also are concordant with the view that *S. japonicum* represents a basal position within the genus. Thus although many of the extant species of *Schistosoma* are African and the genus assumes its greatest medical and veterinary significance there, three lines of evidence suggest its origins lie in Asia.

The relatively basal lineage recently identified by Morgan et al. (2003) is comprised of species only known to be in Africa and their snail hosts are all genera that are found exclusively, or largely, within sub-Saharan Africa. Although this would strongly imply the origin of this clade is African, the fact that hippos once radiated extensively into Eurasia might suggest that the parasites were acquired there and brought back to Africa. In general as one ascends the topology of the tree, species that are Asian tend to alternate with groups that are African suggesting there has been a complicated series of movements of *Schistosoma* between continents. One example of the complexities involved is provided by the *S. indicum* group. Did members of this group arise in Africa and later colonize Asia (Barker and Blair, 1996), or were they originally in Asia and give rise to African species of the *S.*

haematobium group? Given that the clade basal to the *S. indicum* group is comprised only of African members, we would argue for the former scenario.

7. WHAT DO THE SNAILS HAVE TO TELL US?

Insofar as schistosomes are totally dependent on snails for completion of their life cycles, and their present day distributions are in general more limited by their snail hosts than their vertebrate hosts, it is important to gain some understanding of the histories and relationships among the snails that serve as intermediate hosts. Within *Schistosoma,* the five species of the basal *S. japonicum* group are all transmitted by operculate snails of the family Pomatiopsidae whereas the remaining species are transmitted by basommatophoran pulmonate snails of two families, the Lymnaeidae and the Planorbidae. It is clear that pomatiopsids and the two pulmonate families are not close relatives (Blair et al., 2001) so unless we are missing a great many representatives of extinct *Schistosoma* that lived in some of the intermediate snail groups, one of the defining events early in the evolution of *Schistosoma* must have been a dramatic snail host shift. If pomatiopsid-transmitted *Schistosoma* are indeed basal, then the shift would have been from pomatiopsid to basommatophoran snail. Once in basommatophorans, to account for the known host usage patterns, the parasites must have jumped to new snail hosts on several different occasions. The lineage containing the hippo parasites perhaps best exemplifies this trend as each of the three species develops in a different genus of snail host: *S. hippopotami* in *Bulinus,* putative *S. edwardiense* in *Biomphalaria,* and the third undescribed species in *Ceratophallus,* a snail genus not previously known to support *Schistosoma* sporocyst development. Furthermore, these snails are not particularly close relatives: *Bulinus* is basal and *Biomphalaria* derived within the Planorbidae (Morgan et al., 2002). So we are left with a bit of a paradox: although present-day schistosomes are unquestionably specific with respect to the snail hosts they currently use (for example, *S. mansoni* can only develop in *Biomphalaria* and *S. haematobium* can only develop in *Bulinus*), their history is replete with examples of host shifts. We don't understand how such shifts occurred.

The potential importance of understanding the evolutionary history of the snail hosts is exemplified by *Biomphalaria.* Whereas *Biomphalaria* was once considered to have been present in Africa before its separation from South America some 70 million years ago, recent phylogenetic studies (see references in DeJong et al., 2001), backed up by studies of the fossil record, suggest that *Biomphalaria* originated in South America and secondarily

colonized Africa, within the past five million years. This in turn implies that *S. mansoni* and its sister *species S. rodhaini,* both of which are *Biomphalaria*-transmitted*,* could not have existed as we know them today before *Biomphalaria's* introduction to Africa. Based purely on this reasoning, the origin of the *Biomphalaria*-transmitted human parasite *S. mansoni* must have been within this time frame as well. This provides a new point of view to anyone attempting to reconstruct how schistosomes may have interacted with evolving hominids in Africa. There is still much to do with respect to unraveling snail phylogenies. As just one example, *Biomphalaria salinarum,* a species indigenous to southwest Africa, has yet to be investigated with modern methods. Such a study would help us to understand the history of *Biomphalaria* in Africa.

8. SOME FINAL THOUGHTS – WHAT'S PAST IS PROLOGUE

Ongoing efforts to reconstruct the evolutionary history of schistosomes and their snail hosts have provided some notable new insights about basic schistosome biology – their relatively basal status among all digeneans, a better understanding of relationships among *Schistosoma* species, the facility with which schistosomes have shifted snail hosts over the millennia, new views about where *Schistosoma* originated and the possible ages of some of the prominent schistosomes of humans. As noted above, much exciting work remains to be done. Having solid hypotheses regarding their evolutionary past will help us understand some of the momentous changes lying ahead with respect to schistosomes. The wide scope of the Gates Foundation-funded schistosomiasis control initiative will no doubt impact the evolution of schistosomes possibly in ways quite unforeseen. Human-mediated environmental impacts will continue to greatly affect the distribution of snails that serve as hosts. The HIV pandemic, by creating immunosuppressed and extraordinarily susceptible hosts, might also influence the course of schistosome evolution (Combes and Jourdane, 1991). In each of these cases, knowing where the schistosomes have been should help us figure out where they are going.

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Chapter 2

SCHISTOSOME RETROTRANSPOSONS

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1. INTRODUCTION

Eukaryotic genomes generally contain substantial amounts of repetitive sequences, many of which are mobile genetic elements (e.g., Lander *et al.,* 2001; Holt *et al.,* 2002). These mobile sequences have played fundamental roles in the evolution of the human and other eukaryotic genomes (Charlesworth *et al.,* 1994; Deininger and Batzer, 2002), and are among the most powerful endogenous human mutagens (Kazazian, 1999; Dewannieux *et al.,* 2003). Although less is known about the schistosome genome, recent findings suggest that up to half of the entire schistosome genome may be comprised of repetitive sequences, and much of this repetitive complement will be comprised of mobile genetic elements (see Brindley *et al.,* 2003). Here we review a series of mobile genetic elements from the schistosome genome, focusing on schistosome retrotransposable sequences. The identity, structure, phylogenetic relationships, and contribution of these elements to genome size in schistosomes are described, and we address their probable role in schistosome evolution and potential utility in introducing transgenes into schistosomes and other applications.

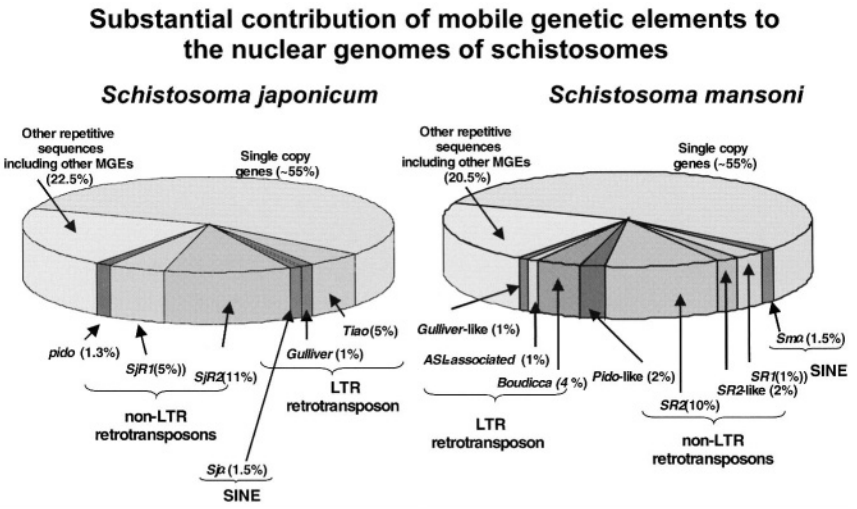

Figure 2-1. Predicted contribution of mobile genetic elements to the nuclear genomes of *Schistosoma japonicum* and *S. mansoni* are represented in pie charts, predicted from published and gene database sources (Adapted from Brindley et al., 2003, with permission).

2. THE SCHISTOSOME GENOME

Schistosomes have a comparatively large genome, estimated at ~270 megabase pairs for the haploid genome of *Schistosoma mansoni,* arrayed on seven pairs of autosomes and one pair of sex chromosomes (Simpson *et al.,* 1982). For comparison, the schistosome genome is about the same size as that of the puffer fish, *Fugu rubripes,* two to three times the size of that of the angiosperm, *Arabidopsis thaliana,* or the free-living nematode, *Caenorhabditis elegans,* ten times the size of the *Plasmodium falciparum* genome, and about one tenth the size of the human genome. The other major schistosome species parasitizing humans probably have a genome of similar size to that of *S. mansoni,* based on their karyotypes (Hirai *et al.,* 2000). Though none of the schistosome genomes have been sequenced in their entirety, several hundred thousand schistosome expressed sequence tags (ESTs) and genome survey sequences have been lodged in GenBank, probably covering the entire transcriptome and indicating that there are ~14,000 genes in *S. mansoni* (Verjovski-Almeida *et al.,* 2003; Hu *et al.,* 2003). The mobile genetic elements (MGEs) of the schistosome genome include SINE-like elements, non-long terminal repeat (non-LTR)

retrotransposons and LTR retrotransposons (Figs. 2-1-2-4), and appear to make up at least one quarter of the schistosome genome.

3. CATEGORIES OF TRANSPOSABLE ELEMENTS AND MODES OF TRANSPOSITION

MGEs are grouped in two major categories, Class I and Class II (Finnegan, 1992). Class I elements transpose through a RNA intermediate whereas Class II elements transpose directly as DNA. Class I comprises (a) the long terminal repeat (LTR) retrotransposons and the retroviruses, (b) the non-LTR retrotransposons, and (c) the short interspersed nuclear elements (SINEs). Class I elements occur in taxa as diverse as fungi and mammals, and are mobilized by replicative processes that generate numerous daughter copies and facilitate insertion into the host genome, thereby directly expanding the size of the host genome. Class II elements are termed 'transposons', and include groups from prokaryotes and eukaryotes.

LTR retrotransposons resemble retroviruses in their structure and intracellular life cycles. These elements are typically 5 - 10 kb in length. Their general structure consists of two open reading frames (ORFs) flanked by long direct terminal repeats of ~200-600 bp in length (Fig. 2-2). Some, such as *gypsy* from *Drosophila melanogaster, Osvaldo* from *Drosophila buzzatii,* and *Tas* from *Ascaris lumbricoides,* include a third ORF, *env,* encoding the envelope protein characteristic of retroviruses (Fig. 2-2). Retroviruses probably evolved from LTR retrotransposons, mediated by the acquisition of envelope proteins that facilitated extracellular existence and horizontal transmission between cells and species (Malik *et al.,* 2000). The LTRs play a pivotal role in initiating transcription and in transposition. The first ORF, *gag,* encodes a polyprotein precursor that is later processed to yield the structural proteins making up the virion core. Of these, the nucleocapsid protein associates directly with the RNA, and exhibits a characteristic cysteine/histidine motif, which appears to function as a zinc finger domain. The second ORF, *pol,* encodes a polyprotein with discrete protease, reverse transcriptase (RT), RNaseH, and integrase enzyme domains. The *pol* domain order varies between the two major *gypsy/Ty3* and *Copia/Ty1* clades of LTR retrotransposons. In retroviruses and LTR retrotransposons with an *env* gene, the envelope protein associates with the cell membrane, which envelops the virion core, allowing the viral particle to bud off from the host cell. Envelope facilitates infection via attachment to specific cell surface receptors. Thus, in addition to vertical transmission in the germ line, LTR retrotransposons with *envelope* genes are capable of extracellular existence and horizontal transmission.

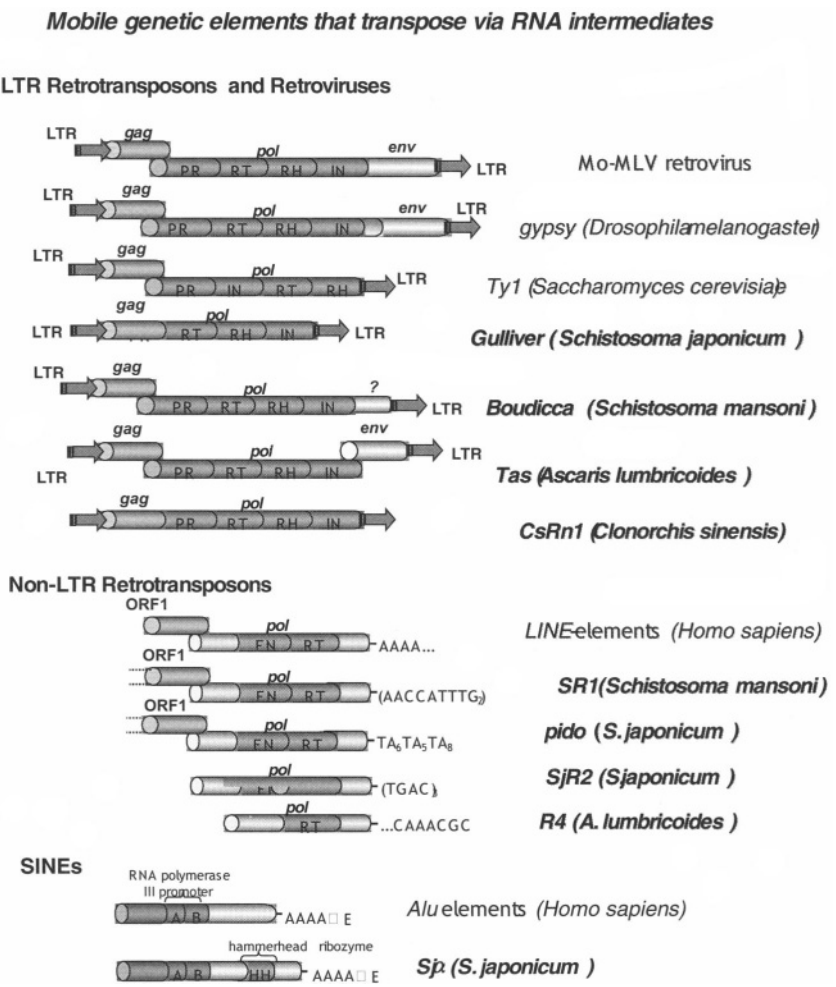

Figure 2-2. Schematic representation of the structure of representative retrotransposable elements from schistosomes and other parasitic helminths (denoted in bold text) and other hosts. Abbreviations: gag, group associated antigen; pol, polyprotein; env, envelope; PR, protease, RT, reverse transcriptase, RH, RNaseH, IN, integrase; EN, endonuclease; LTR, long terminal repeat (Adapted from Brindley et al., 2003, with permission).

Non-LTR retrotransposons are usually ~ 4 - 6 kb in length, generally have two ORFs, often have A-rich 3'-termini, and are transmitted vertically. The Long Interspersed Nuclear Elements (LINEs) of humans are well known members. Full length LINE1 is bicistronic: the product of the first ORF has RNA binding function, although this is not well characterized, whereas the second ORF encodes a polyprotein with RT and apurinic endonuclease (APE) activities. Of 11 clades of non-LTR retrotransposons recognized,