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Population genetics of African *Schistosoma* species

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Abstract :

Blood flukes within the genus Schistosoma (schistosomes) are responsible for the major disease, schistosomiasis, in tropical and sub-tropical areas. This disease is predominantly present on the African continent with more than 85% of the human cases. Schistosomes are also parasites of veterinary importance infecting livestock and wildlife. Schistosoma population genetic structure and diversity are important characteristics that may reflect variations in selection pressures such as those induced by host (mammalian and snail) environments, habitat change, migration and also treatment/control interventions, all of which also shape speciation and evolution of the whole Schistosoma genus. Investigations intoschistosome population genetic structure, diversity and evolution has been an area of important debate and research. Supported by advancesin molecular techniques with capabilitiesfor multi-locus genetic analyses for single larvae schistosome geneticinvestigations have greatly progressed in the last decade. This paper aims to review the genetic studies of both animal and human infecting schistosome. Population genetic structures are reviewed at different spatial scales: local, regional or continental (i.e. phylogeography). Within species genetic diversities are discussed compared and the compounding factors discussed, including the effect of mass drug administration. Finally, the ability for intra-species hybridisation questions species integrities and poses many questions in relation to the natural epidemiology of co-endemic species. Here we review molecularly confirmed hybridisation events (in relation to human disease) and discuss the possible impact for ongoing and future control and elimination.

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Highlights

► Literature on population genetic structure and genetic diversity of African schistosomes is synthesised. ► The effect of Mass Drug Administration on parasite population genetic structure shows that MDA alone is not sufficient. ► More efforts must be done on parasite genetics on animal reservoir hosts and on animal schistosomes. ► Hybridization of schistosomes are important to consider for future disease control.

Keywords : *Schistosoma*, Population structure, Genetic diversity, Phylogeography, Hybrids

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1. Introduction

Schistosomes are parasitic trematodes of mammals that are transmitted by freshwater intermediate molluscan hosts. A total of 23described *Schistosoma* species belong tothe *Schistosoma* genus (Boissier et al., 2019) (Figure 1). Among trematodes, this genus is particularly well studied because six species are of medical importance. Schistosomes cause schistosomiasis or bilharziasis, a chronic neglected tropical disease (NTD). It is the most prevalent NTD in sub-Saharan Africa (Colley et al., 2014; Lai et al., 2015; McManus et al., 2018) and a major public health problem in several tropical and subtropical countries. The

number of schistosomiasis cases in the world is considered to be greater than 250 million, mostly in sub Saharan Africa and the DALYs index ("Disability-Adjusted Life Years") was estimated at 1.4 (Hotez et al., 2014).

Schistosoma species have a two host life-cycle including a mammalian definitive host and a snail intermediate host. A unique feature of schistosomes is that they have separate sexes (i.e. gonochoric) whereas trematodes are typically hermaphroditic (Beltran and Boissier, 2008, 2010; Mone and Boissier, 2004). Within the mammalian hosts, highly sexually dimorophic male and female adult worms pair in the veins of either the peri-vesical vascular system (*S. haematobium*) or in the mesenteric system(most other species). This sexual reproduction ensures genetic mixing and the emergence of novel combinations of genes in each offspring. This allows schistosome populations to potentially adapt to selection pressures, both natural and anthropogenic, with natural selection leading to highly successful and diverse populations. The mammalian host spectrum varies greatly according to the species concerned. For instance,some species only infect humans (e.g. *S. guineensis*), others have a narrow host spectrum (e.g. *S. mansoni* infects humans, rodents and non-human primates) while *S. japonicum* is able to infect many mammalian species from variousgroups including Cetartiodactyla, primates, Rodentia, Carnivora, Insectivora or Lagomorpha (Pitchford, 1977), and is considered to be highly zoonotic. Multi host versus restricted host specificities appears to be evolutionary, with the multi host species being more ancestral. Host specificity will also affect population genetics, increasing diversity where multiple hosts are involved versus reduced diversity where schistosomes are more specific.

Eggs from adult worms are releasedvia the urine (*S. haematobium*) or the faeces (other species) and hatch in freshwater systems, releasing the highly motile larva: the miracidium. This larva actively searches for a specific/compatible planorbid host. As with all trematodes the intermediate host spectrum is often narrowwith clear species-specific compatibilities, which governs geographical species distributions. Within the snail,asexual reproduction occurs with the clonal production of the free swimming larvae: the cercariaewhich once released from the snail actively search for their mammal definitive hosts, penetrating the skin and infecting their host. This clonal replication is highly productive with a single miracidia resulting in many thousands of cercariae produced over many weeks. The tight compatibilities between individual snails and their schistosomes leads to high selection pressures and reduced population variation, however the prolific cercarial production ensures ongoing transmission. It is clear that schistosomes have evolved unique life-history traits to maintain high-levels of

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transmission whilst maintaining populations diversity through alternate asexual and sexual life-cycle stages.

Molecular epidemiological studiesprovide valuable insightsinto the genetic variation of *Schistosoma* species and populations in time and space. This may reflect variations inselection pressures such as host (mammalian and snail) environments, habitat change, emergence in new areas and also treatment / control interventions, all of which will also shape speciation and evolution. Several population genetic studies have shown their importance in the identification of reservoir hosts, the potentiality/likelihood of drug resistance development and spread, the understanding of the recruitment pattern in each host and the genetic structure and gene flow in the context of disease control. This review aims to review several of these studies, highlights how dynamic both human and animal *Schistosoma* species are in terms of their ability to adapt and evolve. Certainly, evidence shows that *Schistosoma* species adapt quickly to new environments and challenges to ensure their survival, a trait that should not be underestimated in global efforts for control, elimination and eventual eradication.

2. Nested Russian doll genetic structuration

For *Schistosoma* spp., different levels of nested populations can be defined as i) all the parasites(worms, sporocysts, excreted miracidia or emitted cercariae) from an individual host (i.e. infrapopulation) ii) all the parasites from a communityiii) all the villages from a region and iv) all the regionsof thearea of repartition of a given species. Sinceschistosometransmission is restricted to freshwaterbodies, the 'community' scale could be equivalent to a small number of transmission sites. The extent of gene flow between these populations will determine the degree of population structure at each level. Here we define three levels of spatial scales. The first level,usually referredto as the'local scale',includes the study of subpopulations between hosts or spatial populations distanced from each otherby 100's of metres to 10's of kilometres (Table 1). The second level, usually referred to as the 'regional scale', includes analysesof parasite subpopulations far from each other by 10's to 100's of kilometres (Table 1). The differentiation between these two first scales can be blurry. The third level, usually referred to as 'phylogeography', analyses parasites subpopulations far from each other by more than 100's of kilometres (i.e. between and within countries) and give insights on the evolutionary history of the species.

A. Local scale

For schistosomes or trematodes in general, an infrapopulation (i.e. all individuals from a same host individual) cannot be considered a single population because it depends on a continuous recruitment of new individuals during re-infection and notthrough intra-host natality. As for any population genetic study on schistosomes, the miracidial populations are used as a proxy of genetic diversity of parental populations locked inside the mammal host. Moreover, the fact that the parasite asexually reproduces inside the snail, creating clonal populations, means that different definitive hosts may be infected by genetically identical schistosomes. Theoretically, the structuration of the infrapopulation and the differentiation between infrapopulations is a balance between factors having tendencies to homogenize the population, and factors having tendencies to differentiate the parasite infrapopulations (Théron et al. 2004). At a local scale, it seems that the majority of these factors are expected to favour the homogenization of the population; (i) snails are not highly mobile within and between sites supporting infections of different definitive hosts with the same clonal schistosomes, (ii) definitive hosts are infected through a repeated light exposure mechanism (i.e. trickle infection process), (iii) mammalian hosts are mobile and long lived and (iv) genetic exchange due to sexual reproduction of adult worms, with the ability to change partners. Opposite to this (i) the rapid turn-over of infected snails and (iii) the acquired immunity of the definitive host, could lead to infrapopulation discriminations. Finally, an expected consequence for parasites in general is a deviation from Hardy-Weinberg equilibrium at the global scale but not within each host. This could be explained by a Wahlund effect with a non-random mating of parasites between hosts and random mating within each host.

The first empirical study aiming to analyse the infrapopulation genetic structure of schistosomes concern adult *Schistosoma mansoni* worms recovered from naturally infected *Rattus rattus* on the island of Guadeloupe (Sire et al., 2001). At that time no method was available for the molecular analysis of individual larval stages (samples were subject to passage through laboratory rodents) and the genetic marker used was non-allelic (e.g. Random Polymorphic DNA marker). The main finding was that *S. mansoni*exhibited high genetic diversity and this diversity occurs on average more within than between hosts (Sire et al., 2001). Despite this, a significant degree of differentiation was also observed between the schistosome infrapopulations(Sire et al., 2001). The first study that investigated*S. mansoni* from humans also used adult wormsresulting fromsuccessive passage through experimental

snail and rodent hosts using miracidia excreted by Brazilian patients (Thiele et al., 2008). This passage system presents obvious sampling bias, bottlenecks and/or selection pressures(Gower et al., 2007). Despite this, the study showed both an important genetic diversity within the host and that the genetic variation within the infrapopulations accounted for the majority of the variance observed(Thiele et al., 2008). Later, the development of methods to collect, store and to amplify multiple (>10) microsatellite markers from a single larval stage has greatly advanced our knowledgeof schistosome population genetics(Gower et al., 2007). The microsatellite analysis of individual miracidia collected from infected children in Kenya showed significant Fst values both between patients (ranging from 0.44% to 3.98%) and between schools (ranging from 0.16% to 3.92%)(Agola et al., 2009). At this scale (<7 km between villages), the genetic structure wasmore similar between patients than between sites suggesting high levels of gene flow between infrapopulations. Similar results were obtainedfrom Uganda and Senegal, from analysis of both mitochondrial cytochrome c oxidase (cox1)or nuclear microsatellite DNA regions, respectively (Betson et al., 2013; Van den Broeck et al., 2014). In one village located on the shore of Lake Albert (Uganda), no genetic differentiation was observed between infrapopulations and very weak differences (accounting for only 3.5% of variation) were observed within another village on the shore of Lake Victoria (Uganda)(Betson et al., 2013). The authors explained this difference by the fact that the shore of Lake Victoria is more convoluted than that of Lake Albert, providing micro-environments for transmission. People living in the village associated with Lake Victoriaappeared to acquire their infections from different micro-foci, hosting different parasite infrapopulation (Betson et al., 2013). In Senegal, hierarchical analyses of genetic structure revealed no spatial-temporal structure in *S. mansoni* from three communities located on the Western shores of Lake Guiers(Van den Broeck et al., 2014). Although significant, parasite genetic differentiation was found to be low between the three communities located 4 to 22 km from eachother (F_{ST} = 0.003), and between infrapopulations within each community (F_{ST} = 0.004)(Van den Broeck et al., 2014). Genetic differentiation between infrapopulations from all three communities ranged between -0.027 and 0.067 (Table 1), and the large majority of F_{ST}-values were not significant after Bonferroni correction, suggesting relatively high connectivity among the three communities (Van den Broeck et al., 2014).Most studies on population structure have focused on *S. mansoni*while only two studies have focused on *S. haematobium*at a local scale (Boon et al., 2019; Gower et al., 2011). These studies are in accordance with results already observed for *S. mansoni*. Gower et al (2011)show low genetic differentiation between two schools separated by 7 km (Fst=0.004) and among children

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within the schools (Fst=0.001) and these variations accounted for only 0.35% and 0.1%, respectively. The 99.5% remaining variation wasattributable to the variation within each child. Boon et al. (2019) analysed the genetic structure of *S.haematobium*10 villages in the North of Senegal. Weak genetic structuration was observed from 7 villages clustering in a 45 km range. A tendency for isolation by distance was noticed for two other villages further awayfrom this cluster. To conclude all these studies,related to human hosts, suggest that few barriers to gene flow between populations exist at the local scale. Finally, similar results have been obtained in the single study on *Schistosoma bovis* (Djuikwo-Teukeng et al., 2019). This study showed that most variation isobserved within individual animals (98.09% and 88.83% for microsatellites and cox1 markers, respectively) rather than between animals.

B. Regional scale

At a regional scale the population structure mainly depends on the movement of human or animal hosts between different suitable transmission sites. At this scale a strong genetic population structure is expected. It is expected that at this scale little gene flow exists and that the genetic variation is more determined by the effective population size,rather than by gene flow between sites. This restriction in gene flow is reinforced by the fact that schistosomiasis is primarily transmitted by children who do not move muchbetween regionscompared to adults. The snail-miracidium compatibility could also restrict the gene flow between different sites(Mitta et al., 2017), due to fine scale snail-schistosome strain compatibilities.

The first empirical study on *S. mansoni* at the regional scale, analysed worms recovered from infected laboratory mice after exposure to cercariae from naturally infected molluscs (Agola et al., 2006). This approach will reduce the number of genotypes represented within the given population and in turn artificially increase the differentiation between the populations. The maximum Fst was 0.273 for two villages distancedby 175 km. Using the same sampling approach (i.e. genotyping adult worms after passage through a laboratory host), similar strong genetic structure was been observed in Uganda between lake Victoria and lake Albert (Stothard et al., 2009). Thislatter studies used a DNA barcoding approach and showed two distinct lineages corresponding to the two lakes (Stothard et al., 2009). Using miracidia collected from infected patients both low andhigh Fst values were also obtained in Ethiopia(See Table1) (Aemero et al., 2015). In 2015, Van den Broeck and colleagues reconstructed the disease outbreak of intestinal schistosomiasis in Northwest Senegal (Van

den Broeck et al., 2015). Parasites were sampled over a period of 14 years in seven villages located 4 to 65 km from eachother. They found that most parasites belonged to the same genetic population, with relatively low genetic differentiation between most villages, suggesting relatively high gene flow in the region (Table 4 in Van den Broeck et al. 2015). An interesting observation was that *S. mansoni* parasites from Mbodjene in the Lampsar region were significantly differentiated from most other samples in the vicinity of Lake Guiers and the Senegal River. Likewise, the *B. pfeifferi* population sampled close to this locality was genetically different from the other two populations near the Lampsar River and the populations around Lake Guiers(Campbell et al., 2010). This correspondence between host and parasite geographic structure suggests that the genetic composition of the intermediate snail hosts could be an important factor determining establishment success of *S. mansoni* in a certain region(Van den Broeck et al., 2015). At the molecular level, this could comply with the hypothesis of a matching phenotype model where the interactions between parasite antigens and host immune receptors during the early stages of the infection determine the success or failure of the infection(Mitta et al., 2012). Van den Broeck and colleagues (2015) also estimated genetic differentiation between the eight villages in North-West Senegal, one village in South-East Senegal and one village in Western Mali. At this scale, almost all pairwise F_{ST} estimates were significant after Bonferroni correction ($p < 0.001$). F_{ST} estimates between samples from the same year (2007) ranged between 0.032 and 0.066 when compared between sites from North-West Senegal and Western Mali (located 1200 km from eachother), and between 0.014 and 0.069 between sites from North-West Senegal and South-East Senegal (located 600 km from eachother) (Table 1). One noticeable exception of this general pattern was observed for*S. bovis* (Djuikwo-Teukeng et al., 2019). Indeed, at very large distances (175-672 km between sampled sites) no evidence for inter- or intrapopulation structuring was observed. Because the parasites were recovered from naturally infected cows, there was no possible bias due toselection within experimental snail or rodent hosts. The absence of genetic structure for *S. bovis* at a country scale (i.e. Cameroon) was explained by a large effective population size and/or movement of the definitive hosts within the country (Djuikwo-Teukeng et al., 2019). More studies on genetic diversity and population genetic structuring of animal schistosomes are needed to infer the role of the definitive hosts on parasite gene flow.

Within all the studies reviewed the interpopulation similarity indices showed that the populations do not follow a pattern of isolation by distance (Table 1) but instead they exhibit

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an island model of gene flow. This could be due to several factors such as restricted gene flow between sites, sib-transmission or due to local adaptations.

C. Phylogeography

A total of 13 *Schistosoma* species are described in Africa, four of which are associated with humans: *S. mansoni*, *S. haematobium*, *S. guineensis* and *S. intercalatum* (Webster et al., 2006) with the others being pathogens primarily of other animals. *Schistosoma mansoni* and *S. haematobium* are the two most prevalent species in Africa with important impacts on human health and *de facto* they have received considerable research interest. Conversely, our knowledge on the distribution and the evolutionary history of the other human-associated *Schistosoma* species (*S. intercalatum* and *S. guineensis*) is very limited (e.g. Webster et al., 2006; Tchuem Tchuenté et al., 2003) and there are currently no genetic population studies on these species. Animal *Schistosoma* species also receive very little attention and their phylogeography is still under documented. Yet, some animal *Schistosoma* species such as *S. bovis* have been recently studied, mainly at a local scale, mainly because they have been shown to interbreed with human associated species raising the possibility for the emergence of zoonoses (Leger and Webster, 2016; Catalano et al., 2018; see sections below). However, we still lack a global vision on the distribution and the phylogeography of most *Schistosoma* species at the continental scale. Below we review our knowledge on the phylogeography of the two best studied species, *S. mansoni* and *S. haematobium*and also the evolutionary history of *S. bovis*.

Schistosoma mansoni is by far the best studied species at variousgeographical scales, from local to global (even intercontinental) scales. Globally, *S. mansoni* displays high levels of genetic diversity at both the mitochondrial and nuclear level, which at a global scale is geographically well structured (Van den Broeck et al., 2015; Gower et al., 2013; Morgan et al., 2005, Webster et al., 2013); but see Crellen et al., 2016). Conversely, although *S. mansoni* can infect several molluscs including, among others, *Biomphalaria pfeifferi*, *B. sudanica* and *B. choanonphala* and a range of vertebrate definitive hosts; humans, rodents and non-human primates, no genetic signature is associated with host use (Lawton et al., 2011; Webster et al., 2013). Lawton and collaborators argue that this lack of structure by host results from continuous gene flow between parasites using different hosts hence arguing the need to considera 'One Health' approach for *S. mansoni* control. Second, populations from Eastern

Africa are far more diverse than Western populations (Gower et al., 2013; Morgan et al., 2005; Webster et al., 2013). This pattern has led several authors to hypothesise that *S. mansoni* has emerged in the former region and migrated toward Western Africa (Lawton et al., 2011; Morgan et al., 2005; Webster et al., 2013). More recent genomic evidence suggests that *S. mansoni* emerged in Eastern Africa around 126,5 KYA and that populations were confined to this area over a long period until around 7 KYA (Crellen et al., 2016). Populations have then expanded and further divergence events occurred giving rise to populations now present in Cameroon sixthousand years ago (KYA) and more recently in Senegal 1.5 KYA (Crellen et al., 2016). If confirmed, this evolutionary scenario might at least partly explain global geographical patterns of genetic structure and the gradual decrease of genetic diversity from Eastern to Western Africa. One exception concerning the globally observed geographical structure of *S. mansoni* populations concern those established in Coastal Kenya and Zambia (Webster et al., 2013). These two closely related populations genetically cluster together and display high genetic divergence compared to other West-African populations (Webster et al., 2013). Based on this former study and based on further genomic evidence that Coastal Kenya is genetically distinct from other East African populations, Crellen et al. (2016) argue that Zambia and Coastal Kenya share a common evolutionary history probably due to important human movements between these two countries during the 19th century. These results suggest that some cryptic diverging lineages of *S. mansoni* that emerged under the influence of human history could occur in other regions of Africa and that the overall genetic diversity of this species might therefore still be underestimated.

Molecular studies on *S. haematobium* are scarce at the global scale and mainly based on the analysis of mt DNA regions, few nuclear DNA regions (e.g. *ITS*) and more recently microsatellites (Webster et al., 2015). Although useful to identify potential hybrids, the rDNA *ITS* region has been found to be monomorphic and cannot be used for reconstructing genetic relationships between populations at any scale (Webster et al., 2012). Despite these few studies and the poor resolution of the routinely used genetic markers, some common patterns emerged regarding the genetic diversity, genetic structure and more recently the genome-wide evolutionary history of *S. haematobium* populations. The first major characteristic of *S. haematobium* is that this species displays very low genetic diversity compared to *S. mansoni* (Gower et al., 2013; Webster et al., 2012) but see Glenn et al. (2013). Despite such low genetic diversity, two major clades have been identified based on their mitochondrial haplotypes including populations from Eastern Africa on one hand and Western Africa on the other hand (Webster et al., 2012). The Eastern clade also includes populations established in

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some Indian Ocean islands (e.g. Madagascar, Mauritius). Interestingly, one mitotype (H1) is overrepresented at a global scale (Webster et al., 2012). This pattern has been interpreted as the result of a recent and important population bottleneck before recent expansion of this parasite species across Africa (Webster et al., 2012). However, contrary to *S. mansoni*, Webster et al. (2012) did not find the differences in genetic diversity between Eastern and Western populations. Gower et al. (2013) found important genetic differentiation among populations according to an Isolation-by-distance pattern although such patterns have been called into question (Glenn et al., 2013). Results from the latter study might at least partly be explained by the sampling of a highly diverging population in South Africa which increases genetic differentiation indexes computed between geographically close populations.

Although no genetic data exists at a global continental scale for the other *Schistosoma* species, some authors attempted to draw general phylogeographic patterns of some species based on their geographical distribution, the evolutionary history and the compatibility to their respective host snails. For instance, based on the comparison of the geographical distribution of *S. bovis* and that of their potential snail host species, combined with experimental assessments of compatibility between different *S. bovis* populations and different snail species, authors found important diversity among *S. bovis* populations and clear differences between populations from southern Mediterranean zones and southern Saharan zones (another distinct population being identified in southern Europe) (Mone et al., 1999). Based on these results the authors proposed a three-step evolutionary scenario for *S. bovis.* First this species emerged from (Western) South Sahara and extended its distribution to the South, East and West of Africa. Second and more recently this species reached the Mediterranean zone together with an important reduction of host spectrum. Finally, *S. bovis* colonised the Iberian Peninsula most likely through dispersion events over the Arabian Peninsula first and next around the Mediterranean basin. So far, no genetic data exists to confirm this scenario. We expect that with the recent interest for hybridization events between *S. bovis* with other *Schistosoma* species, together with the development of genomic resources for this species in particular, we will soon better understand the evolutionary history of this broadly distributed parasite species across Africa (and southern Europe).

3. Factors affecting schistosome genetic diversity

Parasite genetic diversity is expected to be balanced by the clonality within the mollusc host and by the fact that snails are usually infected by only a few genotypes (Theron et al., 2004). Opposite to this, the definitive host mobility, the rapid turnover of infected snails, the snail-parasite compatibility, the long lifespan of the definitive host and the genotype dependent host re-infection (Beltran et al., 2011), should all increase the parasite genetic diversity within the definitive host. In this context, some authors have proposed that the definitive host is a 'genetic mixing bowl' for the parasite (Curtis and Minchella, 2000). The 'genetic mixing bowl' hypothesis was validated by several studies. The classical indexes used to infer population diversity such as heterozygosity or allelic richness are always very high for *S. mansoni* or *S. haematobium* infrapopulations (Agola et al., 2009; Gower et al., 2011; Sire et al., 2001; Steinauer et al., 2009; Thiele et al., 2008; Van den Broeck et al., 2014). Using relatedness indices Steinaueur et al. (2009) showed that 94.44% of the schistosomes coming from a single patient was unrelated (Steinauer et al., 2009). Similarly, Aemero et al. (2015) using sibship analysis showed that the estimated number of unique *S. mansoni* worm pairs within a single human host is between 66% and 92% of the entire worm infrapopulation (Aemero et al., 2015). This parasite genetic diversity could also be modulated by intrinsic factors such as sex of the parasite or the species concerned, or by extrinsic factors such as the sex of the host, the age of the host, the infection intensity or the drug treatment.

A. A sex-specific population genetic structure

Sexual dimorphism is a remarkable trait in schistosomes (Mone and Boissier, 2004). The muscular male holds the thin female within its gynaecophoric canal. Considering this sexual dimorphism it has been shown that the male elicits a stronger immune response compared to females (Boissier et al., 2003). After repeated light exposure on experimentally infected mice

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it has been shown that male parasites induced a genotype-dependant negative effect, via the host immune system, on subsequent male infections (Dumont et al., 2007). Females alone seem unable to induce this protective effect. The same authors have demonstrated that the protective effect is genotype dependent and correlates with genetic dissimilarity between the male schistosomes (i.e. the smaller the genetic distance between the initial and secondary infecting schistosomes, the lower the infectivity rate of secondary infecting males) (Beltran et al., 2011). Comparative 2D proteomic analysis on different male clones revealed differences in antigens known to be involved in the induction of the immune system (Beltran et al., 2011). This gender difference in host-parasite interaction may be at the origin of a sex-specific population structure found in naturally infected rats (Prugnolle et al., 2002). These last authors have shown that the female parasites were more genetically similar than the males in infected rats (Prugnolle et al., 2002). This pattern could be the result of the gender differences previously demonstrated in experimental approaches and the successive trickle infection process that will in term lead to more dissimilar males compared to females in a single host. However, this sex-specific pattern has never been investigated for parasites recovered from humans.

B. Differences of genetic diversity between species

Important differences in genetic diversity indices have been observed according to the schistosome species. Several studies have shown that *S. mansoni* is much more diverse than *S. haematobium* (Gower et al., 2013; Sady et al., 2015; Webster et al., 2013a; Webster et al., 2012). At the African continental scale, the haplotype diversity (h) and the nucleotide diversity (Π) of the cox1 gene were 0.94 ± 0.0067 and 0.02553 (Webster et al., 2013a) and 0.36 ± 0.014 and 0.00434 (Webster et al., 2012) for *S. mansoni* and *S. haematobium*, respectively. The haplotype diversity is also higher for *S. bovis* (0.961 ± 0.012), even at a small population size than for *S. haematobium* (Djuikwo-Teukeng et al., 2019). The higher haplotype diversity of *S. bovis* compared to *S. haematobium* were confirmed by comparing microsatellite markers common to these two sister species (Djuikwo-Teukeng et al., 2019). However, if *S. bovis* is similar to *S. mansoni* concerning its haplotype diversity it differs concerning its nucleotide diversity (Π = 0.00602), which is more similar to *S. haematobium*. As a consequence, the Tajima's D* index did not differ from zero for *S. mansoni* or *S. haematobium* (Sady et al., 2015) but it is significantly negative for *S. bovis*, which could

constitute a signature of population demographic expansion (Djuikwo-Teukeng et al., 2019). This last study hypothesises that this difference in both nuclear and mitochondrial diversity could be the consequence of the absence of praziquantel treatment of infected animals compared to humans, imposing different bottlenecks/and selective pressures within these hosts accordingly.

C. The effect of the host age on parasite genetic diversity

The trickle infection model is expected to lead to more diverse parasite populations in older patients because they should be exposed to a wider variety of parasites(mixing bowl hypothesis). This expectation has been confirmed for *S. mansoni* populations in Senegal (Van den Broeck et al., 2014). The study showedsignificant correlations between inbreeding (negative correlation) or heterozygosity (positive correlation) and the age of the patients: the children being more infected by related parasites than adults. To date, this study is the only one showing this pattern. The explanation could be that the authors have sampled a variety of ages (between 4 and 50 years) and not only children, as in the majority of otherstudies(Agola et al., 2009; Gower et al., 2011). A more recent study failed to find a difference in parasite genetic diversity between mothers and young children (Betson et al., 2013). These authors proposed that the trickle dynamics is rapidly saturated and that two years old children could host the same parasite genetic diversity as their mothers(Betson et al., 2013). The differentfindings in these two studies could also relate to the difference in the genetic markersused. The first study used a more polymorphic and biparentally inherited genetic marker (microsatellite) compared to the second one that looked at haplotypic diversity of the maternal mitochondrial marker. It could also relate to the exposure and mobility of the hosts, the mothers (Betson et al., 2013) might be less mobile than males (Van den Broeck et al., 2014).

D. The effect of host sex on parasite genetic diversity

Because of behavioural or immunological differences, the sex of the host could also influence the parasite infrapopulation. For instance, it is commonly observed that boys are more heavily infected than girls due to difference in sexual roles in work, play and ultimately

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water contact (Angora et al., 2019; Kebede et al., 2018; Mohamed-Ali et al., 1999). This gender bias is also observed in travellerinfections(Nicolls et al., 2008). It has also been observed in mouse experimental models that females were more sensitive to schistosome infection than males due to physiological differences between sexes (Boissier et al., 2003; Nakazawa et al., 1997). These host gender differences in either behaviour and/or physiology are expected to shape the parasite genetic diversity. In natural*S. mansoni*infected rats, no difference was observed between male and females in the genetic composition of the parasite infrapopulation(Prugnolle et al., 2002). The same tendency has been observed for*S. mansoni*and*S. haematobium*from infected humans(Gower et al., 2011; Thiele et al., 2008; Van den Broeck et al., 2014). However, two recent studies showed that males have a higher schistosome genetic diversity than females(Faust et al., 2019; Kebede et al., 2020). This could reflect the differences in behaviour with young males having higher mobility and greater water contact compared to young females. One interesting outcome that differsbetween these two studies is that parasite genetic diversity increases only with male or female age Kebede et al (2020) or Faust et al. (2019), respectively. This confirms that human behaviour can shape the parasite genetic composition but also,that acquired immunity does not seem to limit this diversity because no decline in genetic diversity was observed (Faust et al., 2019).

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E. Infection intensity and schistosome genetic diversity

Schistosomiasis infection intensity is estimated by the number of eggs per gram of faeces for *S. mansoni* or per 10 mls of urine for *S. haematobium*. This intensity is dependent on several factors including the sex, age or the occupation of the host, the history of treatment, the transmission foci, the virulence of the parasite etc….Intuitively, we could expect a positive link between infection intensity and parasite genetic diversity. Using mitochondrial cox1 analysis, no correlation between *S. mansoni*infection intensity and either haplotype or nucleotide diversity was observed (Betson et al., 2013). Similar results wereobtained for*S. haematobium*(Gower et al., 2011). Based on microsatellite markers the authors showed that the allelic richness is the same whatever the intensity of infection, from low to very high(Gower et al., 2011). In this same study, the link between sibship analysis (measured as the number of unique parental genotypes) and infection intensity did not give consistent results. First, when comparing two villages, the higher the intensity of infection the less diverse the schistosome infrapopulations appear to be. Second, no difference in infection

intensity was observed between girls and boys but the number of unique parental genotypes was lower in girls than in boys. Third, counter-intuitively children with light infections had more diverse parasites than those with high infections. Finally, after controlling for several demographic parameters (village, gender andage) and co-infection status with *S. haematobium*, no link was observed between *S. mansoni*infection intensity and heterozygosity or allelic richness in Senegalese patients(Van den Broeck et al., 2014).

F. Effect of praziquantel treatment

Praziquantel (PZQ) is the drug of choice to treat schistosomiasis because of the absence of toxicity (Cioli and Pica-Mattoccia, 2003; Thetiot-Laurent et al., 2013), low cost (Fenwick, 2006)and the effectiveness against all *Schistosoma* species(Kumar and Gryseels, 1994). In 2002, the "Schistosomiasis Control Initiative" was founded with the aim for broad-scale elimination of schistosomiasis by regular (mass)treatment of school-aged children. This could exert an enormous selection pressure on parasite infrapopulations, which might, amongst others, increase the risk of drug resistance development(Doenhoff et al., 2002; Fenwick and Webster, 2006), although there is currently no conclusive evidence for PZQ-induced adaptive evolution.

Here, we consider the neutral evolution of *Schistosoma* populations in response to Mass Drug Administration (MDA) treatment programs. To this end, we explore the genetic concepts and tools that have enabled investigations into the possible impact of PZQ on the population genetic structure of schistosomes, and demographic changes over time to verify whether current programs actually decrease parasite transmission (and could ultimately lead to their elimination).

A theoretical framework to assess the impact of PZQ treatment on schistosome population genetics was outlined in (Huyse et al., 2013b). Within this framework it is assumed that praziquantel randomly kills worms in the treated individuals, leaving a random subset of worms from the infrapopulation after treatment. This will decrease the allelic richness and gene diversity of the treated infrapopulation, while observed heterozygosity is less sensitive to changes in the effective population size. Low effective population size will furthermore lead to a random loss and fixation of alleles (genetic drift) and thus an increase in genetic differentiation between the treated infrapopulations, and between the pre- and post-treatment infrapopulations. In addition, pre- and post-treatment infrapopulations could become

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genetically different due to re-infection of parasites from genetically differentiated source populations. In conclusion, the following population statistics are important to assess the impact of PZQ treatment on schistosome populations: allelic richness (AR), gene diversity (*D*), inbreeding coefficient (F_{IS}) and genetic differentiation of infrapopulations (F_{ST}). The inbreeding coefficient is included as it is also sensitive to changes in effective population size (see Huyse et al., 2013b).

Norton and colleagues were the first to investigate the impact of treatment on the population biology of schistosomes (Norton et al., 2010). Using seven microsatellite markers, they compared the genetic diversity and differentiation of *S. mansoni* populations in Tanzanian school aged children before (in April 2005) and twelve months after (in April 2006) the first MDA. The authors found a small but significant difference in AR and *D* as estimated per infrapopulation in 2005 and 2006, suggesting that parasite infrapopulations may have experienced genetic bottlenecks in response to PZQ treatment. However, this conclusion should be nuanced as the authors also reported a decline in AR in a control group of children that had not been previously treated as they were not yet of school age during baseline sampling (Norton et al., 2010), suggesting that factors other than preventive chemotherapy may have caused the decline in infrapopulation genetic diversity. This hypothesis is supported by the study of (Gower et al., 2017) that revisited the two Tanzanian schools in 2010 and genotyped miracidia using the same microsatellite markers as Norton et al. (2010). Children had received two (in 2005 and 2007) or four (in 2005, 2006, 2007 and 2009) rounds of treatment depending on the school. The authors observed a significant increase in infrapopulation AR and *D* in 2010 compared to baseline estimates in April 2005. The initial decline in 2006 (Norton et al., 2010) and then increase in 2010(Gower et al., 2017) may reflect temporal changes in parasite transmission dynamics, rather than being a direct consequence of PZQ treatment. Gower and colleagues (2017) also found a significant decline in worm burdens within an individual host as estimated with parentage analyses. Although the authors did not correct their estimates for family structure or the number of sampled miracidia (Steinauer et al., 2013), their observations could suggest that PZQ treatment reduced infrapopulation sizes, but not to an extent that it resulted in concomitant genetic bottlenecks of the entire parasite population. Both Norton et al. (2010) and Gower et al., (2017) found higher F_{ST} between years (within schools) compared to between schools (within years), an observation that may suggest high re-infection levels in the Tanzanian villages.

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These reports of a long-term decrease in genetic diversity(Norton et al., 2010) and number of adult worms per infrapopulation(Gower et al., 2017) in response to PZQ treatment could not be reproduced by other studies. In Senegal, 12 Senegalese children were followed over two years and samples were collected prior to treatment: once in April 2007 before the first double treatment (i.e. two PZQ treatments 3 weeks apart), once six months after the first double treatment and two years after baseline surveys with a total of five treatments(Huyse et al., 2013b). Although miracidial genotypes (based on nine microsatellite markers) could not be generated for all children at all timepoints, the authors found no change in *AR*, *D*, F_{IS} or F_{ST}. More-over, *K*-means clustering revealed 11 distinct genetic clusters, most of which were present in all children at all timepoints, suggesting that the genetic composition of parasite infrapopulations remained largely stable for two years despite a relatively large number of PZQ treatments (see Figure 1 in Huyse et al., 2013). This observation, together with the sustained high egg counts and high genetic diversity after several treatments is indicative of a surviving parasite population, that is tolerant to the administered PZQ dose. In Kenya, Lelo et al. (2014) followed parasite genetic diversity for four years (2008-2012) in 15 children that received four treatments during a MDA program, whereby samples were taken once a year prior to treatment (Lelo et al., 2014). One of the major strengths of this study is that it is the only African-based study that followed a relatively large cohort of children for four years (but see also (Blanton et al., 2011) for an example in Brazil), allowing comparable genetic statistics of the same infrapopulations before and after PZQ treatments (see Table 2 in Lelo et al. 2014). In addition, the authors performed a relatively deep miracidial sampling per child (average of 82miracidia per child), allowing robust parentage analyses and reducing the amount of bias introduced due to family structure (Steinauer et al., 2013). Contrary to expect-ations, Lelo et al. (2014) found a small but significant increase in AR and*D* after four years of MDA, but the number of full sibling families and effective number of breeders per infrapopulation did not change over time. In Uganda, Betson et al. (Betson et al., 2013) used mt cox1 data to compare the genetic diversity of *S. mansoni* before and 6 months after PZQ treatment of cohorts of pre-school aged children and mothers. Note that all participants were treated at baseline with PZQ, albendazole and artemether-lumefantrine, and that samples were taken from the same individual or from the same family. The authors found no decline in haplotype or nucleotide diversity. Finally, also in Uganda, Faust and colleagues (2019) performed one of the most extensively designed sampling studies (Faust et al., 2019). Children aged 6–12 years from three primary schools were sampled at 11 timepoints over two years, including one week, four weeks and six or 12 months after PZQ treatment. Most

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children received a total of four treatments. The major strength of this study is that the sampling scheme allowed examination of the parasite genetic diversity over short follow-ups after treatment (but see also (Blanton et al., 2011) for an example in Brazil). One of the main insights from this study is that AR only decreased in samples taken four weeks after treatment, suggesting that PZQ induced a genetic bottleneck, but this then recovered six months after treatment(Faust et al., 2019). The authors argued that high rates of gene flow in combination with refugia in snails and untreated individuals ensure rapid recovery of parasite genetic diversity. Sibship analysis furthermore demonstrated that adult worms can survive treatment but high gene flow and refugia prevent fixation of resistant/tolerant genotypes(Faust et al., 2019).All these observations together made the authors conclude that MDA alone is not sufficient for schistosomiasis elimination (Faust et al., 2019). Also,Lelo et al. (2014) concluded that MDA programs targetingschool-aged children only may have limited impact on parasite transmission at the community level. This was also the main finding of a simulation study that used an island model to investigate the impact of MDA on long-term parasite population dynamics(Van den Broeck et al., 2020). The study revealed that repeated treatments induced strong and lasting declines in parasite infrapopulation sizes, resulting in concomitant genetic bottlenecks. However, parasite genetic diversity recovered quickly in a few generations due to re-infection, and there was little or no impact of treatment on the genetic diversity of the component population when treatment coverage was 95% or lower (Van den Broeck et al., 2020). The authors concluded that community-wide distribution of praziquantel is essential to induce long-term effects on parasite population dynamics (Van den Broeck et al., 2020).

However, as also concluded in Barbosa et al (2016), more studies are needed to cover the enormous heterogeneity in transmission contexts in Africa before any firm conclusions can be made(Barbosa et al., 2016). But as it stands now, comparative analyses are complicated due to differences in sampling design, molecular markers and type of population genetic analysis used in the studies. Adopting standardized procedures is therefore key. Future studies may also need to distinguish infrapopulation from component population genetics. At the infrapopulation level, parentage analyses using genome-scale polymorphism data (e.g. (Shortt et al., 2020)) of large miracidial samples per child may become a powerful tool to assess the strength of the genetic bottleneck and dissect re-infection from persistent infections following treatment. At the component population level, genomic data of a few miracidial samples from a large number of hosts will provide major insights into the long-term regional effects of

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treatment programs, ultimately allowing the identification of the main factors/interventions that could help schistosomiasis elimination.

4. Schistosome genetic variation and disease outcome

Several experiments have been performed to study the influence of the parasite and/or hostsgenetic background on the host/parasite association. The work of André Theron's group is an example of how the genetic background of both the parasite and its mollusc host is important for successful infection (e.g. a phenomenon called "compatibility") (Mitta et al., 2017; Theron et al., 2014). Interestingly, experimental comparative studies have shown adaptative trade-offsaccording to the parasite strain (Davies et al., 2001; Le Clec'h et al., 2019). However, the influence of parasite genetic variations on virulence in term of human disease outcome is certainly an understudied aspect. Brouwer et al (2013) was first to find genetic differences, measured by randomly amplified polymorphic (RAPD) DNA regions, associated with pathology observed within*S. haematobium* infected children (Brouwer et al., 2003). No link was observed between heterozygosity and the severity of infection,however they found a positive association between genetically related clusters and pathology, measured as bladder or kidney pathology and infection intensity(Brouwer et al., 2003).In comparison this link was not observedin a study that investigated*S. haematobium*infections in Sudanese patients (Gasmelseed et al., 2014). However, this study used only one RAPD marker showing only 3 distinct genotypes clearly lacking thepower to concludethere is any link between genetic diversity and pathology. There is only one study that has used microsatellite markers to investigate any links between genetic diversity, prevalence and intensity of infection (Aemero et al., 2015). However, with only three studied sites no statistical tests could be validated. Only one study has clearly shown an association between genetic variation, in terms of microsatellite allelic variation, and human disease phenotype for *S. mansoni* (Huyse et al., 2018). The study showed a positive association between a specific microsatellite allele and infection intensity, while a negative association for another allele of the same locus was also associated(Huyse et al., 2018). Twenty one percent of the variation at

this locus was explained by infection intensity, host age and bladder morbidity (Huyse et al., 2018). This microsatellite allele is located near the cGMP-dependent protein kinase gene that is expressed in reproductive organs of adult schistosome worms and appears to be linked to egg production(Leutner et al., 2011; Leutner et al., 2013).

5. Hybridization

A. *Schistosoma* inter-species interactions and hybridization

It is clear that *Schistosoma* species are diverse and have evolved several genetically well-defined species that have distinct phenotypic traits. In Africa both biological (host specificities/preferences, relatedness and anatomical site of infection, inter-species dominance/competition) and ecological barriers (geographical distribution, intermediate snail host habitats, transmission site ecology) have certainly driven speciation within the genus and also prevent inter-species interactions and hybridisation. However, it is well known that given the opportunity species can interact and also inter-breed, facilitated by the dioecious sexual stage in the definitive host, and this has been clearly demonstrated through multiple experimental hybridization studies involving different *Schistosoma* species (reviewed in (Leger and Webster, 2017). This means that the presence or absence of reproductive isolating mechanisms cannot be used as a criterion for defining the different species. The success of inter-species interactions and the production of viable hybrid offspring is highly dependent of the species and also geographical strains involved, and there are also several biological features observed within different combinations. These include non-reciprocal crosses, species competition (particularly male worm competition) and dominance, parthenogenetic reproduction, hybrid vigour and also hybrid instability. These features are not uniform, are highly dynamic and probably play a crucial role in preventing prolific admixing in nature, particularly for species that co-infect the same definitive host. Relatedness also plays a key role in preventing genetic exchange and hybrid speciation (Figure 1, Table 2), with interactions between more distantly related species resulting in parthenogenetic reproduction or no viable hybrid offspring, compared to viable hybridisation between more closely related species. It does appear that under natural conditions interspecies genetic exchanges are limited by the existence of geographic separation and/or the existence of pre- or postzygotic

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reproductive isolating barriers. However, there is increasing evidence for natural hybridization between species possibly influenced by increased environmental changes, both manmade and natural, together with the movement of parasites within their definitive hosts into new areas enabling inter-species interactions. Additionally, our improved ability to characterise schistosome populations and identify hybrids, with the utility of molecular and genomic analyses, may also be uncovering hybridization and genetic introgression that have been occurring as part of the natural epidemiology of these species, but until recently remained undetected. The existence of genetic exchanges between species of schistosomes and the formation of natural hybrids is indeed a topical subject under investigation in several African foci. The relationship of changes in disease transmission and human/animal health with inter-species interactions, together with the potential for novel reservoirs of infection and zoonotic transmission are clearly important in terms of disease control and elimination. In the next sections we review and discuss the genetic evidence for some inter-species interactions between African *Schistosoma* species in their natural settings with a focus on those involving human species.

B. Molecular identification of hybrids

Modern genetic methods allow for a more accurate assessment of infections and also allow for the identification and deciphering of inter-species hybridization. Certain DNA regions are now routinely used for species and hybrid identification. These include a mitochondrial (mtDNA) DNA region (routinely *cox*1, although other gene regions have been used) to identify the maternal line. Species specific SNP's within nuclear ribosomal DNA regions (nrDNA) (routinelythe ITS1 and ITS2 rDNA regions are used however other markers are needed for certain species combinations)are used to provide the nuclear genetic profile of the species involved(Huyse et al., 2009; Pennance et al., 2020; Webster et al., 2019; Webster et al., 2012)(Figure 1). The occurrence of hybridisation eventsare often identified by the unexpected mitochondrial signatures presented by samples that do not relate to the infected hoststhat are being sampled e.g. *S. bovis* mt signatures for samples retrieved from human hosts. This then leads to more scrutiny of the nuclear profile of the samples with clear discordance between mt and nuclear signatures incriminating hybrid forms (Figure 1). It is acknowledged that this basic genetic profiling does not enable the identification of the hybrid generation or parental backcrosses however(Platt et al., 2019), itinfersthat inter-species

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genetic exchange has occurred successfully with the generation of hybrid offspring. Mixed nuclear profiles (copies from both parental species) do suggest earlier generation hybrids, and possibly F1 generations however, it still remains unknown as to the generational speed of genetic homogenization, and loss of signal from one of the parental species preventing accurate hybrid identification(Leger et al., 2020). This may also mean that hybrids are often missed by this form of identification and that they may be more prevalent than the data suggests. Whole genome studies are providing a more recent and valuable opportunity for investigating and understanding these hybridization events, and their outcomes, and should form an integral component of natural hybridization investigations going forward(Kincaid-Smith J et al., 2018; Platt et al., 2019). These enable the assessment of the genetic composition of hybrids across the whole genome allowing us to decipher different generations of hybrids and backcrossed / introgressed forms by the assessment of the different genomic levels from the two parental forms. From this we can determine if hybridization is active, rare and / or ancient and can also identify directional hybridization/introgression (Platt et al., 2019). This relates directly to any pre- and post-reproductive isolating barriers that may be in place that help maintain species integrities and prevent hybrid speciation (Kincaid-Smith J et al., 2018; Oey et al., 2019; Platt et al., 2019).

C. Natural *S. mansoni* group specieshybridisation (*S. mansoni* X *S. rodhaini*)

As described previously *S. mansoni* and *S. rodhaini*are closely related sister species and both have the ability to infect rodents. This provides the opportunity for these two species to encounter each other. *S. rodhaini*is highly specific to rodents and has also developed phenotypic traits supporting its transmission via nocturnal emergence patterns. *S. mansoni* is a human pathogen that has important zoonotic reservoirs including non-human primates and rodents(Standley et al., 2012b; Standley et al., 2012c).

Natural hybrids between *S. mansoni* and *S. rodhaini*have been identified in sympatric East African zones(Morgan et al., 2003; Steinauer et al., 2008b) (Table 2). *S. rodhaini*has a more focal distribution compared to *S. mansoni* and is currently only found transmitted in Rwanda, Kenya, Tanzania, Burundi, Uganda, Zaire and also there is a report from Nigeria. *S. mansoni* overlaps with *S. rodhaini* in several of these countries and natural hybrids have been identified in Tanzania and Kenya(Morgan et al., 2003; Steinauer et al., 2008b). Hybrids have only ever been found emitted from *Biomphalaria* snails with the molecular data concluded that no F1 hybrids were present with allappearing as introgressed forms. The data suggested a

relatively low hybridisation rate and thathybrid backcrossing was rapidly occurring. True hybridisation appeared episodic and strongly influenced by the transmission dynamics of the different species, particularly related to *S. rodhaini*with transmission and the opportunity to hybridise being highly seasonal. There also appeared to be strong directional introgression of genetic material from *S. rodhaini*into *S. mansoni.*

D. Natural *S. haematobium* group hybridisation

The *S. haematobium* group is the most diverse *Schistosoma*species group with nine species several of which are sympatric (Figure 1). Three closely related species are human pathogens providing opportunities for interactions and hybridisation. Several of the others also have opportunities to interact within their non-human hosts particularly bovids. Interestingly hybridisation within this group centres around the most widespread, prevalentand medically important species *S. haematobium.* Particularly its hybridisation with bovid species is attracting attention due to the zoonotic components that may be facilitating this or the potential risk of the emergence of zoonotic strains and the creation of zoonotic reservoirs(Leger et al., 2020).

1. S. haematobium and *S. guineensis*

Until 2003 *S. guineensis* and *S. intercalatum* were considered one species (*S. intercalatum*) but were split based on their mitochondrial divergence(Pagès et al., 2003; Webster et al., 2006).When *S. guineensis* and *S. intercalatum* were split into their separate species,*S. intercalatum* was recognised as being restricted to more central African regions, namely the Democratic Republic of Congo (DRC) whereas *S. guineensis* is found on South West coastal countries and Sao Tome(Figure 1). In the literature many of the earlier reports related to *S. guineensis* will refer to *S. intercalatum* and so for ease of understanding going forward, this will be described as *S. guineensis* (*intercalatum*).

It is apparent that *S. haematobium* and *S. guineensis* readily hybridise in nature. This is probably not surprising given the fact that they are sister species and human pathogens, however they do utilize different anatomical sites of infection (urogenital and intestinal respectively) probably reducing interactions. Evidence of hybridisation between *S. haematobium* and *S. guineensis* (*intercalatum*), has been reported in several areas of Cameroon (Loum, KindingNdjabi, Kumba, BarombiKotto(Ratard and Greer, 1991; Ratard et al., 1990; Southgate et al., 1976; Webster et al., 2003; Wright et al., 1974)predominately

based on polymorphic eggs excreted in the urine of patients and this was later backed up with molecular data obtained from preserved isolates (Webster et al., 2003, 2007). Molecular analyses, using mt*cox*1 and nrDNA ITS2 + IGS genetic markers, of samples collected from various time periods during the fluctuation of*S. haematobium* and *S. guineensis* (*intercalatum*)in Cameroon clearly showed the presence of *S. haematobium* X *S. guineensis* (*intercalatum*)hybrids, with a predominance of hybrids probably resulting from interactions between *S. haematobium* ♂'s with *S. guineensis* (*intercalatum*) ♀'s(Webster and Southgate, 2003; Webster et al., 2005; Webster et al., 2007). The data also clearly showed a decline in numbers of hybrids over time suggesting a replacement of *S. guineensis* (*intercalatum*)by *S. haematobium* through introgressive hybridization(Tchuem Tchuente et al., 2003; Webster et al., 2005).

The natural occurrence of *S. haematobium* X *S. guineensis* (*intercalatum*) hybridisation has also be reported in Gabon and also Benin (Mone et al., 2012; Zwingenberger et al., 1990). The Gabon account was based on egg morphology and remains unconfirmed. (Mone et al., 2012)molecularly identified*S. haematobium* X *S. guineensis*hybrid miracidia collected from human urine samples in Benin. All samples showed maternal mtDNA cox1 *S. haematobium* genetic profiles suggesting that these were not early generation hybrids but probably introgressed forms. Current foci for *S. guineensis* remain severely underreported due to their scarcity and lack of knowledge related to this species. However, foci do still exist in Central African/South Western regions and are likely in some areas to overlap with *S. haematobium*.

2. *S. haematobium and S. mattheei*

Many sympatric zones exist for these species however, compared to *S. haematobium* *S. mattheei* has a more restricted distribution to East African regions and typically below a latitude of 10º South (Figure 1). *S. mattheei* is considered to be a potential pathogen of humans although it is primarily a pathogen of bovids(Standley et al., 2012a). It still needs to be elucidated if *S. mattheei* actually infects humans or is only found in humans as part of mixed infections and/or in a hybrid form.

Many historical observations exist for*S. mattheei* shaped eggs being excreted by humans in South and South Eastern regions of Africa, where *S. haematobium* and *S. mattheei* are sympatric(reviewed in (Leger and Webster, 2017) (Table 2). At the time molecular methods could not be used and only observational data are available from these earlier studies. Wright and Ross (1980), followed up this earlier work and confirmed natural hybrids

between *S. haematobium* and *S. mattheei* using species-specific biochemical markers, with field isolates showing the same enzymatic patterns as laboratory produced hybrid offspring(Wright and Ross, 1980).

In the late 1980's Kruger (Kruger, 1987, 1988, 1990a; Kruger and Evans, 1990; Kruger and Hamilton-Attwell, 1988; Kruger et al., 1986)and colleagues further investigated*S. mattheei* X *S. haematobium*interactions,using both morphological and biochemical methods, identifying*S. mattheei* X *S. haematobium* hybrids from a patient from Nelspruit, in the north east of South Africa. Phenotypic differences suggested that the *S. mattheei* populations that were sympatric with *S. haematobium*, may have been infiltrated by genetic introgression from *S. haematobium* in these areas. However,it was evident that the proportion of hybrid forms was low and that parental backcrossing with introgression of *S. mattheei* genetic material into the *S. haematobium* populations was probable.

In 1994, De Bontand colleagues (De Bont et al., 1994), found evidence, based on egg morphology and biochemical markers, for suspected *S. haematobium-mattheei* hybrids in cattle however, they could not conclude if there were either *S. mattheei* x *S. haematobium* or *S. mattheei* x *S. leiperi* hybrids. Since then, very few studies have followed up on the occurrence of the natural hybridisation between *S. haematobium* and *S. mattheei*, but very recentlymolecular data (mt cox1, ITS and 18S nrDNA) has provided indisputable evidence for the occurrence of *S. haematobium-mattheei* in Malawi (Webster et al., 2019). The study was conducted due to the observations of atypical eggs (resembling *S. mattheei* and also *S. bovis*) excreted in the urine of children atspecific*S. haematobium* foci in Malawi. Additionally, very recently *S. haematobium-mattheei* mix / hybrid infections were alsoidentified, via the molecular analysis of serum,in a group of tourists who became infected in the north east of South Africa in 2016/2017(Cnops et al., 2020). As with the more historical accounts these more recent findings still point to asymmetric interactions highlighting the extreme biases observed in these hybridisation systems with pre- and postzygotic reproductive barriers in place to reduce prolific admixing.

3. *S. haematobium* and *S. bovis*

These two species are widespread across Africa and are sympatric in many countries. *S. bovis* has a slightly more restricted distribution primarily above a latitude of 10º South below which it is replaced by *S. mattheei.* Natural hybridisation between *S. haematobium* X *S. bovis*

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has become the most recent subject of several research groups mainly due to the identification of high numbers of hybrids excreted by humans in West African countriesand the possible implications for schistosomiasis control, in relation to zoonotic transmission (Table 2).

The first observations of natural hybridisation were made by Brémond*et al*. in 1993 who identified schistosomes excreted by humans in Niger, with allozyme profiles that were intermediate between *S. bovis* and *S. haematobium* or *S. curassoni*, but more sensitive markers were needed to discriminate *S. haematobium* from *S. curassoni*(Brémond et al., 1993). However, these findings did indicate that some sort of inter-species interaction had or was taking place. No further studies investigated these hybridisations in nature occurred until 2009 where(Huyse et al., 2009) using molecular methods (mt cox1 and nuclear ITS analysis) identified the high abundance and wide distribution of *S. haematobium* X *S. bovis* hybrids along the Senegal River Basin. These hybrids were found in multiple sites and were collected from both humans and snail hosts (*B. truncatus* and *B. globosus*). An expanded study was conducted in Senegal (both in the North and South of Senegal) in 2009-2010 (Webster et al., 2013), that also examined infections in domestic livestock and humans. It was found that high levels of hybrids occurred in the human population in areas of sympatry but no hybrids or *S. haematobium* were found in livestock. This clearly showed the biases within the hybrid system, with humans being the primary host for the hybrids withstrongcorrelation with urinary excretion of the hybrids. Further studies by (Boon et al., 2017; Boon et al., 2018)showed the heterogeneity of these hybrids and also the close association with co-infections with *S. mansoni*. In 2013, a surprise came with the outbreak of urogenital schistosomiasis on the Mediterranean Island of Corsica (France)(Boissier et al., 2015), with hybrids molecular identified within the schistosome populations excreted from several infected tourists(Boissier et al., 2016; Mone et al., 2015). It was also concluded, via molecular phylogenetic analyses that the parasites had been imported from West Africa and in particular Senegal, where there are high levels of migration between these French colonies(Boissier et al., 2016). Interestingly, a single egg excreted from one of the infected tourists showed a genetic profile suggesting a pure *S. bovis* origin (both nuclear ITS and mt cox1 DNA giving a *S. bovis* profile) presenting some evidence for potential *S. bovis* zoonotic infection however, more in-depth genomic analysis would be needed to determine the genetic make-up of this egg. Sporadic eggs like this have also been found in studies in Senegal and also suggest a pure *S. bovis* origin however, they are very rare and their viability needs to be determined. Whole genome analyses of miracidia from Senegal and Niger failed to identify F1 hybrids within the samples analysed and suggested that the hybrid populations are highly introgressed forms,

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with hybridisation being ancient and not active. Additionally, genetic microsatellite analyses of hybrids in Senegal and Niger have shown clear population differentiation between the schistosomes infecting humans (*S. haematobium* and the *S. haematobium-bovis* hybrids versus *S. bovis*) suggesting no or very limited active interactions/hybridisation and gene flow between the populations(Boon et al., 2019; Pennance et al., 2020). Further areas of hybridisation between *S. haematobium* and *S. bovis* have now been molecularly identified, including areas in Niger, Benin, Mali, Cote D'Ivoire and also Malawi(Leger et al., 2020; Pennance et al., 2020; Savassi et al., 2020; Soentjens et al., 2016; Tian-Bi et al., 2019). The Benin study (Savassi et al., 2020) indicated the possible occurrence of *S. haematobium-bovis* hybrids in cattle, identified by the shift in cercarial shedding patterns of the resultant progeny isolated from infected cattle together with atypical genetic profiles of the adult worms.It is clear from the data from multiple studies that this hybridisation is common but it is not random and there are specific biological and genetic influences taking place, which may also differ between foci. This raises several questions with regard to the impact of this hybridisation which, only further detailed epidemiological and in depth genetic/genomic studies can help unravel.

4. *S. haematobium* and *S. curassoni*

Interactions between these two species are rarer, mainly due to the more restricted distribution of*S. curassoni* to specific foci in a few West African countries with most transmission in Senegal(Leger et al., 2020; Leger and Webster, 2017; Webster et al., 2013b) (Table 2). In the early 1990's suspected hybrids between *S. haematobium* and *S. curassoni,* based on egg morphology and biochemical markers,were observed excreted by humans in Niger but the species involved could not decisively be determined as either *S. bovis* or *S. curassoni*(Brémond et al., 1993).More recent work in Senegal has providedevidence of *S. haematobium-curassoni* hybrids excreted via the urine of patients in sympatric areas of Senegal, although more in-depth genetic analysis would support our understanding of these interactions in these endemic zones(Webster et al., 2013b) (Table 2). Particularly the distinction between *S. bovis* and *S. curassoni* using traditional nuclear markers is not always robust with a single species-specific SNP in the ITS1 nrDNA that allows distinction between the two species (Pennance et al., 2020; Webster et al., 2013b), with the use of other nrDNA regions such as the 18S needed for further clarification. A very recent report by (Leger et al., 2020)showed no *S. haematobium* and *S. curassoni* interactions in sympatric zones in Senegal suggesting that interactions between these species are rare and unstable. More detailed studies

into the dynamics of these interactions and hybridisation events in different settings is certainly warranted as it is likely that differences occur at both micro and macro geographical scales (Stothard et al., 2020).

5. *S. haematobium and S. mansoni*

S. haematobium and *S. mansoni* represent two co-endemic pathogens that are both highly prevalent in humans but highly divergent from each other, a key postzygotic reproductive isolating barrier retaining these species integrities. There have been many reports of the possible interactions between these species in their human hosts based on atypical egg excretion, ectopic egg deposition and host morbidity assessments and competitive exclusion of *S. mansoni* by *S. haematobium* in some areas(Gouvras et al., 2013; Koukounari et al., 2010; Stothard et al., 2020) (Table 2). Lateral-spined eggs, with a typical *S. mansoni*morphology, have often been observed in urine samples and terminal-spined *S. haematobium* shapedeggs have been observed in stool samples in several co-endemic areas including Kenya, Egypt, Senegal, Malawi, Mali and Cote D'Ivoire. However, the occurrences of these atypical egg excretions in relation to heterospecific interactions are dynamic and are not observed in all co-endemic areas and in all patients. (Huyse et al., 2013a) conducted the first molecular analyses of eggs / miracidia using nrDNAITS and mt DNA *cox*1 markers and showed that the offspring contained genetic material from both parental species. This disputes parthenogenesis and suggests hybridisation has occurred between these two distantly related species however, the viability of the offspring to infect snails remains unknown. Additionally, a case of *S. haematobium-mansoni* hybrids were molecularly identified from atypical eggs excreted from a migrant boy who had migrated from Cote D'Ivoire, via West Africa to France, but again the viability of the offspring was not able to be tested(Le Govic et al., 2019). The impact of such inter-species interactions, even if they are not viable, on human health should not be dismissed as they are likely to cause increased pathology due to ectopic egg deposition and a higher abundance of eggs being deposited in organs and tissues.

6. Conclusion

Molecular epidemiological studies on the diverse range of *Schistosoma* specieshave provided valuable insights into transmission dynamics, population and species level diversity over time and space, and how selective pressures such as drug treatmentmay shape and change the schistosome populations. Results obtained in the last decade clearly show, and in

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particular for*S. mansoni* and *S. haematobium,* that whatever the schistosome species involved only few barriers to gene flow exist at a local and regional scale. However, despite this, species have maintained their integrity with natural selection processes making them highly adapted to their environments with successful and ongoing transmission. It is acknowledged that our knowledge on the genetic distribution and the evolutionary history of other human-associated and all animal-associated African *Schistosoma* species is currently very limited if not completely absent at a large geographical scale. It is thus crucial to gather more information on the population genetic structuring of these schistosomes including the recently identified hybrids in order to; i) infer the role of the definitive hosts on the parasite gene flow and ii) to better understand their relative importance in the epidemiology and evolution of African *Schistosoma* species, withparasite genetic variation known to be an important factor in explaining the variation in host disease phenotype.

Another important aspect in studying animal infecting schistosomes, potential zoonotic hybrids and animal reservoirs of infection is the role they may play in spreading resistance or virulence alleles among and between populations. Indeed, despite several years of vast MDA coverage in Africa and extensive treatment campaigns, many areas characterized as "persistent hotspots" fail to substantially decrease transmission, with on-going high levels of prevalence, intensity and associated human morbidity.

Studies focusing on the genetic structure of *Schistosoma* populations and their response to MDA suggest that rapid re-infection of parasites from genetically differentiated source populations occurs and that MDA alone is not sufficient to control the disease transmission (Van den Broeck et al., 2020). High levels of gene flow between parasite populations before and after treatment together with refugia may facilitate a rapid recovery of population genetic diversity with treatment having only a limited impact on parasite transmission at the community level. In addition, whether drug resistance or an increased genetic diversity brought about by inter-species/population genetic exchange, maybe influencing reduced drug efficacy in some foci needs to be investigated.

Broader and more fine-scale molecular epidemiological analyses of schistosome populations are warranted both to understand transmission and infection dynamics, but also in relation to treatmentresponses. Powerful genetic analysis tools, such as Whole-Genome Single Nucleotide Polymorphisms (SNP), now exist and are quickly being optimised to study natural *Schistosoma* populations and will provide greater insights into many aspects of schistosome biology, evolution, genetics, transmission dynamics, inter-species interactions

and resistance/versus re-infection dynamics, all of which will support control and elimination of this debilitating disease.

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Tables

Fst (range)	Species	Distances (Km) between site	Number of sites	Number of microsatellite markers used	IBD	Reference
Local scale						
0.0016-0.0392	Sm	0.8 - 8	4	9	NS	(Agola et al., 2009)
0.01-0.056	Sm	7-45	4	20	NS	(Steinauer et al., 2009)
0.0011-0.05	Sm	1-3	7	7	ND	(Curtis et al., 2002)
0.010-0.0564	Sm	7-40	4	20	NS	(Steinauer et al., 2009)
-0.027-0.067	Sm	4-22	3	9	ND	(Van den Broeck et al., 2014)
0.004	Sh	8	2	8	ND	(Gower et al., 2011)
Regional scale						
0.036-0.273	Sm	25-490	7	5	NS	(Agola et al., 2006)
0.0357-0.136	Sm	127-553	4	11	NS	(Aemero et al., 2015)
0.014-0.069	Sm	600-1200	10	9	ND	Van den Broeck et al. 2015
0.0002-0.0048	Sb	175-672	4	14	NS	(Djuikwo-Teukeng et al., 2019)

Table 1. Fst values at local or regional scales for schistosome populations analysed using microsatellite markers

NS : Not significant; ND : Not Done; IBD : Isolation By Distance. Sm = *Schistosoma mansoni;*

Sb = *S. bovis*; Sh = *S. haematobium*

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Years	Country	Species and Main Host	Host	Reference
2003-2008	**Tanzania** **Kenya**	*S. mansoni* *S. rodhaini*	Snails	(Morgan et al., 2003; Steinauer et al., 2008a; Steinauer et al., 2008b)
1974-2012	**Cameroon;** (Gabon); **Benin;** (Mali)	*S. haematobium* *S. guineensis*	Human	(Burchard and Kern, 1985; Mone et al., 2012; Ratard and Greer, 1991; Ratard et al., 1990; Southgate et al., 1976; Tchuem Tchuenté et al., 1997; Vercruysse et al., 1994; Webster et al., 2003; Webster et al., 2005; Wright et al., 1974; Zwingenberger et al., 1990)
1948-2019	(Zimbabwe); (South Africa); (Zambia); **Malawi;**	*S. haematobium* *S. mattheei*	Human Livestock	(Alves, 1948; De Bont et al., 1994; Kruger, 1987, 1988, 1990a, b; Kruger and Evans, 1990; Kruger and Hamilton-Attwell, 1988; Kruger et al., 1986; Le Roux, 1954; Pennance et al., 2020; Pitchford, 1959, 1961; Platt et al., 2019; Vercruysse et al., 1994; Webster et al., 2019; Wright and Ross, 1980)
1993-2020	**Senegal; France; Niger; Mali; Benin; Cote D'Ivoire; Malawi**	*S. haematobium* *S. bovis*	Human Snails (Livestock*)	(Boissier et al., 2016; Boon et al., 2017; Boon et al., 2019; Boon et al., 2018; Brémond et al., 1993; Catalano et al., 2018; Huyse et al., 2009; Leger et al., 2020; Mone et al., 2015; Ramalli et al., 2018; Rothe et al., 2020; Savassi et al., 2020; Soentjens et al., 2016; Tian-Bi et al., 2019; Webster et al., 2013b)
1993-2012	**Senegal;** (Niger)	*S. haematobium* *S. curassoni*	Human	(Brémond et al., 1993; Webster et al., 2013b)
2010-2020	**Senegal;** (Mali); (Kenya); **Cote D'Ivoire**	*S. haematobium* *S. mansoni*	Human	(Gouvras et al., 2013; Huyse et al., 2013a; Koukounari et al., 2010; Le Govic et al., 2019)

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Table 2. Details of the detected natural hybridisation between different *Schistosoma* species combinations in Africa. In bold are the cases that have been confirmed using molecular data. Other accounts shown in (), are from atypical/morphological observations and biochemical markers. *This is the suspected occurrence of *S. haematobium-bovis* hybrids in cattle based on cercarial circadium rhythms. The years are the first and last year of the reported hybridisation. The host in the definitive host that the hybrids / introgressed forms were recovered from.

Figure legend

Figure 1. A) Showing the interrelationships of the 23 *Schistosoma* species (adapted from Webster and Littlewood, 2012), the main definitive hosts and the geographical distribution. The African clades are shown in the red boxes and their fine scale African distribution shown by the coloured dots that correspond to the map, highlighting areas of sympatry and opportunities for inter-species interactions. B) Demonstrates the basic molecular profiling of *Schistosoma* hybrids. This shows maternal inheritance of the mitochondrial DNA from the maternal line and the mixed inheritance of the nuclear DNA, with the difficulty encountered with deciphering the hybrid generation or backcrossing. The sequence chromatograms show the pure and mixed signal in the nuclear data with * showing the double sequence chromatogram showing bi-parental inheritance of the nuclear DNA. Species specific SNP's within the nuclear ITS and 18S rDNA regions are used to identify hybrids, together with the maternal mitochondrial DNA.

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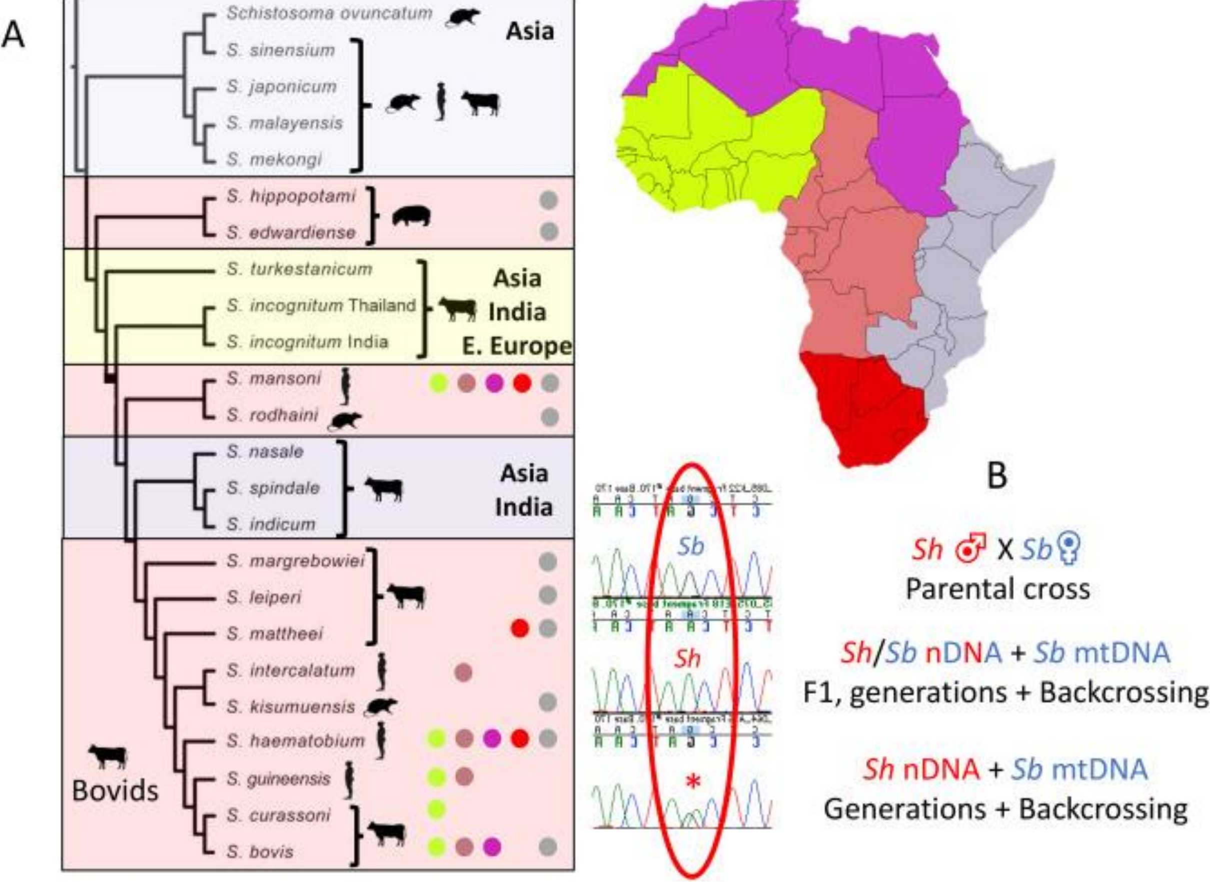

A
Schistosoma ovuncatum
S. sinensium
S. japonicum
S. malayensis
S. mekongi
Asia
S. hippopotami
S. edwardiense
S. turkestanicum
S. incognitum Thailand
S. incognitum India
Asia
India
E. Europe
S. mansoni
S. rodhaini
S. nasale
S. spindale
S. indicum
Asia
India
S. margrebowiei
S. leiperi
S. mattheei
S. intercalatum
S. kisumuensis
S. haematobium
S. guineensis
S. curassoni
S. bovis
Bovids
B
Sb
Sh
*
Sh ♂ X Sb ♀
Parental cross
Sh/Sb nDNA + Sb mtDNA
F1, generations + Backcrossing
Sh nDNA + Sb mtDNA
Generations + Backcrossing

Authors of the manuscript "**Genetics and evolution of African schistosomes"** declare that they have no competing interests.

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Highlights

. Literature on population genetic structure and genetic diversity of African schistosomes is synthesised

. The effect of Mass Drug Administration on parasite population genetic structure shows that MDA alone is not sufficient

. More efforts must be done on parasite genetics on animal reservoir hosts and on animal schistosomes

. Hybridization of schistosomes are important to consider for future disease control

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