



Applying evolutionary genetics to schistosome epidemiology

Michelle L. Steinauer^{a,*}, Michael S. Blouin^b, Charles D. Criscione^c

^a Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, 105 Magruder Hall, Corvallis, OR 97331, United States

^b Department of Zoology, College of Science, Oregon State University, United States

^c Department of Biology, Texas A&M University, United States

ARTICLE INFO

Article history:

Received 28 August 2009

Received in revised form 9 February 2010

Accepted 15 February 2010

Available online 20 February 2010

Keywords:

Molecular Epidemiology
Schistosoma
Parasite
Pathogen
Genetic structure
Population genetics
Disease ecology
Evolutionary epidemiology
Hybridization

ABSTRACT

We review how molecular markers and evolutionary analysis have been applied to the study of schistosome parasites, important pathogens that infect over 200 million people worldwide. Topics reviewed include phylogenetics and biogeography, hybridization, infection within snails, mating systems, and genetic structure. Some interesting generalizations include that schistosome species hybridize frequently and have switched definitive hosts repeatedly in evolutionary time. We show that molecular markers can be used to infer epidemiologically relevant processes such as spatial variation in transmission, or to reveal complex patterns of mate choice. Analysis of genetic structure data shows that transmission foci can be structured by watershed boundaries, habitat types, and host species. We also discuss sampling and analytical problems that arise when using larvae to estimate genetic parameters of adult schistosome populations. Finally, we review pitfalls in methodologies such as genotyping very small individuals, statistical methods for identifying clonemates or for identifying sibling groups, and estimating allele frequencies from pooled egg samples.

© 2010 Elsevier B.V. All rights reserved.

Contents

1. Introduction	433
2. Phylogenetics, historical biogeography, and phylogeography	434
2.1. Hybridization	434
2.2. Diversity within snails and host sharing	435
2.3. Mating systems	435
2.4. Genetic structure	436
2.5. Sampling issues	437
2.5.1. Technical issues and possible solutions	438
3. Conclusions	441
Acknowledgements	441
References	441

1. Introduction

Evolutionary principles are increasingly recognized as critical tools in the epidemiological research of many pathogens (Tibayrenc, 1998; Conway, 2007; Nesse and Stearns, 2008; Restif, 2009), including schistosomes (Webster et al., 2008). Schistosomiasis is a neglected parasitic disease caused by at least 8 species

of blood flukes in the genus *Schistosoma*. Schistosomes infect over 200 million people across Africa, Asia, the Middle East, South America, and parts of the Caribbean (Engels et al., 2002; Chitsulo et al., 2004; Steinmann et al., 2006). As with many other parasites, the small body size, site of infection within the human host, and complex life cycle of schistosomes (obligate outcrossing and asexual reproduction in each generation, Fig. 1), inhibit the direct observation of many population processes that are critical for understanding the epidemiology of schistosomiasis (de Meeûs et al., 2007). Molecular genetic data, evolutionary theory, and analytical tools provide a means to infer parasite population

* Corresponding author.

E-mail address: Michelle.steinauer@oregonstate.edu (M.L. Steinauer).

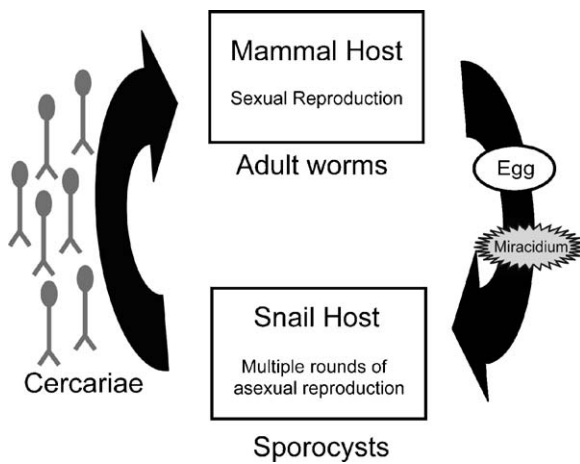


Fig. 1. Life cycle of schistosome parasites. Each generation undergoes sexual reproduction (obligate outcrossing) in a mammal host and asexual reproduction within a snail host. Adult worms reproduce sexually in the veins (mesenteric or bladder plexus) of their mammal host and excrete eggs that are eliminated with feces or urine of the host. Eggs hatch in freshwater and release a free swimming miracidium. The miracidium penetrates a snail host, develops into a sporocyst and undergoes several rounds of asexual reproduction that result in the generation of numerous cercariae that emerge from the snail, enter the water, and penetrate the skin of their mammal host. Thus, it is possible for mammal hosts to obtain multiple individuals that are essentially genetically identical or “clones”.

processes such as patterns of dispersal, mating systems, and population growth/decline, all of which have important implications for epidemiology (Nadler, 1995; Criscione et al., 2005; de Meeüs et al., 2007). Here, we provide a review of largely field-based studies that employ molecular markers to elucidate the ecology, evolution, and epidemiology of schistosomes including the following topics: Phylogenetics and biogeography, hybridization, infection within snails, mating systems, and genetic structure. We draw special attention to particular questions or methods that are of epidemiological relevance and will prove imperative in combating schistosomiasis. Much of this review focuses on techniques for uncovering genetic structure. The intravascular habitat of the adult worms makes sampling them from humans unethical. Therefore sampling relies on collecting immature stages from human fecal samples or from snails (life cycle, Fig. 1), and using them to estimate genetic parameters of adult populations. This methodology can lead to sampling biases and yield artifactual results. Thus, part of this review is devoted to understanding and attempting to resolve this issue so that these artifacts can be avoided in future studies.

2. Phylogenetics, historical biogeography, and phylogeography

Phylogenetic and phylogeographic studies can identify historically and evolutionary independent groups of populations and can identify natural or anthropogenic colonization routes (Bermingham and Moritz, 1998; Avise, 2000). Interspecies phylogenetic analyses of the genus *Schistosoma* suggest an Asian origin of *Schistosoma* due to the present day location of the basal group, the *S. japonicum* clade (Snyder and Loker, 2000; Attwood, 2001; Zhang et al., 2001; Attwood et al., 2002; Lockyer et al., 2003; Morgan et al., 2003a). This clade is hypothesized to have radiated from the Himalayas east and south throughout Asia during the Cenozoic via newly created freshwater habitats created by tectonic uplift (Attwood, 2001; Attwood et al., 2002). *Schistosoma* is hypothesized to have colonized Africa from Asia, and then recolonized Asia possibly several times, perhaps through the movement of animals through land corridors that connected the continents during lowered sea levels during the

Miocene (Barker and Blair, 1996; Morgan et al., 2003a; Attwood et al., 2007).

Data on the interspecies relationships and their host associations also illuminate their potential to colonize new locations or host species. Phylogenetic studies indicate that despite their specialized adaptations to host species, schistosomes have colonized distantly related snail and vertebrate hosts (Blair et al., 2001a; Lockyer et al., 2003; Brant and Loker, 2005). There are at least three independent origins of human colonization that possibly originated from different mammalian hosts (Lockyer et al., 2003; Webster et al., 2006). Host use is more conserved at the intermediate host level. The genus *Schistosoma* has radiated within 3 families of snails: the *S. japonicum* clade infects snails in the family Pomatiopsidae, *Orientobilharzia* and *S. incognitum* infect Lymnaeidae, and all others infect Planorbidae (Lockyer et al., 2003).

An extensive intraspecific phylogeographical study exists for the human pathogen *S. mansoni* (Morgan et al., 2005). This study reveals two important aspects of *S. mansoni* transmission. First, neutral genetic diversity is associated with geography and not snail host use. There are several divergent clades across Africa, with the highest diversity located in East Africa. Second, the data indicate a recent New World colonization (likely during the slave trade) that originated from multiple West African localities. Successful colonization was aided by the presence of viable snail hosts, namely *Biomphalaria glabrata*, the sister taxon to African species of *Biomphalaria* that host *S. mansoni* (DeJong et al., 2001). These studies highlight how host and parasite phylogeny and phylogeography can give important insight into the invasion success of introduced parasites. DNA sequence, biogeographic, and host use data are also useful for testing or confirming the ranges of schistosome species (Attwood et al., 2008), and for uncovering cryptic diversity or new species (Kane et al., 2003; Brant et al., 2006; Hanelt et al., 2009a).

2.1. Hybridization

Hybridization of pathogen species is of epidemiological importance because it could potentially lead to the formation of new hybrid pathogens and also gene flow across species boundaries, termed “genome introgression”. Hybridization can lead to the homogenization of species, the extinction of species, or adaptive evolution of either species as they acquire novel genes from the foreign gene pool. Laboratory crosses have shown that most closely related schistosomes have the ability to hybridize successfully and produce fertile offspring (LeRoux, 1954; Taylor, 1970; Jourdan and Southgate, 1992; Southgate et al., 1998). Molecular data have shown that hybridization occurs naturally between the following species: *S. mattheei* and *S. haematobium* (Wright and Ross, 1980; Kruger and Evans, 1990), *S. haematobium* and *S. guineensis* (Southgate et al., 1982; Webster et al., 2003), *S. bovis* and *S. curraoni* (Rollinson et al., 1990), and *S. mansoni* and *S. rodhaini* (Morgan et al., 2003b; Steinauer et al., 2008b). Of these hybrid combinations, it is known that hybrids of *S. haematobium* and *S. mattheei* infect humans (Wright and Ross, 1980; Kruger and Evans, 1990; Webster et al., 2003).

Hybridization has the potential to have large effects on the evolution of schistosome populations. For example, hybridization is hypothesized to be driving the decline of the native *S. guineensis* after the introduction of *S. haematobium* in West Africa. Because *S. guineensis* is rarer and less competitive than *S. haematobium*, it is much more likely to hybridize with *S. haematobium* than mate with conspecifics (Tchuem Tchuenté et al., 1997, 2003; Southgate et al., 1998). Genetic introgression can also influence the evolution of a species. The introduction of foreign genes into a species pool could lead to novel changes that influence the biology or disease

characteristics of human pathogenic schistosomes. Genetic introgression has been shown to occur between *S. mansoni* and *S. rodhaini* and is directional so the parasite of humans, *S. mansoni*, obtains genetic material from the parasite of rodents, *S. rodhaini* (Steinauer et al., 2008b). To date, only neutrally evolving genes have been investigated in the context of introgression, but it would be of great interest to determine whether functional genes are being shared among species and if this can lead to adaptive changes in pathogens. Despite their epidemiological importance, hybrid zones of schistosomes have yet to be fully characterized. The demographic effects of hybridization, the amount of gene flow between species and adaptive introgression can all be investigated with the use of molecular markers and newly developed analytical tools.

2.2. Diversity within snails and host sharing

The application of molecular methods to samples of cercariae collected from snails has predominantly focused on three objectives: (1) species identification to determine species prevalence among snails (Hamburger et al., 2004), (2) individual identification to identify the number of miracidia that infected each snail (Minchella et al., 1995; Dabo et al., 1997; Eppert et al., 2002; Steinauer et al., 2008c), and (3) examination of genetic structure among geographic areas (e.g. Agola et al., 2006; Steinauer et al., 2009). Species identification methods provide an effective means to identify prepatent infections and to monitor potential transmission sites (King et al., 2006). These data reveal seasonal and spatial patterns of transmission that may not be detected by only examining human populations. For example, samples of cercariae from snails were used to show that low efficacy of drug treatment in humans corresponded with high transmission from snails. These data suggested that high rates of new parasite recruitment were an alternative and likely explanation to treatment failure (Black et al., 2009). Another method of collecting transmission related data is to sample cercariae directly from water (Muhoho et al., 1997; Aoki et al., 2003; Hertel et al., 2004). Although traditional cercariometry studies have fallen out of vogue, they have the potential to detect the spatial partitioning of cercariae and cercarial diversity within transmission zones.

Molecular markers can, of course, also be used to distinguish individual parasite genotypes. For example, by sampling cercariae from snails or water, one could ask whether related parasites (or identical multilocus genotypes, termed “MLGs”) tend to be transmitted together on a small spatial scale. For example, if a definitive host deposits its parasites’ offspring into one area, it is conceivable that related parasites may stay in close proximity when snails shed. If such clumped transmission (*cf.* Criscione and Blouin, 2006) is maintained through the final hosts, local scale genetic structure will emerge. If clumped transmission is extreme, it can result in inbreeding and can reduce the effective sizes of infrapopulations (all the individuals of a parasite species in a host at one time; Bush et al., 1997). On the other hand, if snails acquire multiple infections by unrelated parasites, and they are transmitted together to the same definitive hosts, outcrossing rates would be increased (Minchella et al., 1995). Steinauer et al. (2009) have investigated the relatedness structure of individuals of *S. mansoni* that shared snail hosts in a natural lake population in Kenya. Individuals that shared a host were not more or less related than expected by the background levels of relatedness.

The rate of multiple infections within snails varies among transmission sites. Reports on natural populations of *S. mansoni*, *S. haematobium*, and *S. rodhaini* indicate that miracidia are overdispersed within snails and that the number of genotypically unique miracidia per snail ranges from 1 to 9 with means ranging from 1.14 to 3.28 among populations (reviewed in Steinauer et al.,

2008c). As prevalence increases, aggregation within hosts becomes reduced and schistosome distribution among snails becomes more random rather than overdispersed (Eppert et al., 2002). The number of MLGs per definitive and intermediate host is also informative about the transmission process. For example, Theron et al. (2004) found that individual snails in Guadeloupe carried an average of 1.14 unique MLGs, while individual rats carried an average of 34. Thus, rats are infected by cercariae from about 30 snails.

Understanding the rate of multiple infections within snails is also interesting because competition among parasites could affect parasite transmission. For example, laboratory experiments have shown within-snail competition between genetic strains of *S. mansoni* (Gower and Webster, 2005). In a natural population of *S. mansoni* in Kenya, multiple infections produced fewer cercariae per capita than single infections. However, competition within snails was not influenced by either relatedness or sex of the coinfecting individuals (Steinauer et al., 2009). Concomitant immunity in snails is another important aspect of transmission. Although there is some evidence for concomitant immunity from laboratory infections (Sire et al., 1998), it is difficult to distinguish from parasite competition. As these studies illustrate, host sharing may play an important role in disease transmission, but its role is poorly understood in natural populations.

2.3. Mating systems

Mating systems affect levels of inbreeding and the opportunity for kin selection. Thus mating systems can affect the distribution of genetic variation within and among populations (Charlesworth, 2003) and play a role in virulence evolution (Frank, 1996). Mating systems themselves may be under selection and can lead to sexual selection on traits that increase mating success. Molecular markers and parentage analyses are powerful tools for uncovering mating patterns in internal parasites and can help determine who is mating with whom and their reproductive success. Most human-infecting schistosomes are thought to be monogamous because the male typically mates with a single female at a time and sequesters her within his gynecophoral canal (LoVerde et al., 2004; Beltran and Boissier, 2008), although polygyny (a single male with multiple females in its gynecophoral canal) has been reported (Steinauer, 2009). Although schistosomes typically mate with one individual at a time, they are known to change mates, and there is also evidence for mate choice and mate competition (Tchuem Tchuente et al., 1995; Beltran et al., 2008a; Steinauer, 2009). Three studies used microsatellite markers to estimate the relatedness between mates. In one study using laboratory strains, females changed mates more commonly if a male more genetically different than her current mate was introduced, suggesting female choice for unrelated or genetically distant mates (Beltran et al., 2008a). However, in a natural population of rat hosts, males and females paired randomly with regard to estimated relatedness (Prugnolle et al., 2004b). Also, in an experimental study, estimated relatedness of mates was not correlated with reproductive success, as might be expected if female choice for genetically different mates conferred a fitness advantage (Steinauer, 2009). The same study found that larger males produced more offspring than smaller males even though males of all sizes sequestered mates. Interpreted in the context of sexual selection, this finding supports the hypothesis that larger males compete for higher quality females and/or that females may be able to compete and choose large males. Because schistosomes can be raised in the laboratory, they might be an interesting model for studying sexual selection in parasites. For example, because parasites interact with the immune systems of their hosts, they could be choosing mates for genetic benefits for their offspring. This would be an interesting

new example of 'good genes'-based sexual selection. More importantly, it is possible that understanding more about what drives mate choice and reproduction in schistosomes could lead to ways to reduce their reproductive output and thus reduce disease burden.

Little attention has been given to the impact that the asexual reproductive stage (Fig. 1) might have on schistosome mating systems. First, the presence of clones (identical MLGs) in a definitive host may affect genetic sex ratios because the sex ratio in terms of number of unique male and female MLGs may be quite different than the sex ratio observed from the total number of males and females in a host. The evolution of sex ratio in schistosomes is interesting because biased sex ratios increase sexual competition (May and Woolhouse, 1993; Morand et al., 1993; Morand and Müller-Graf, 2000) and reduce the effective number of breeders per definitive host (Criscione and Blouin, 2005). A second way in which clones can influence mating systems is that even if worm pairs remain monogamous throughout their lifetime, the occurrence of multiple individuals of the same clone can result in genetic half-sibships (Fig. 2). In other words, clonality leads to genetic polygamy. Third, high variance in the number of copies of each clone may translate into a large variation in reproductive success among MLGs. High variance in reproductive success increases the opportunity for selection (Arnold and Wade, 1984) and is another way (besides affecting sex ratio) in which clonality can reduce the effective number of breeders per infrapopulation (Criscione and Blouin, 2005). Finally, the presence of clones could conceivably alter mate competition if clonemates can recognize each other.

2.4. Genetic structure

Analyses of genetic structure use patterns of neutral genetic diversity to reveal how populations are structured across geography, and within and among hosts on a local scale. These techniques can provide information about pathogen transmission that is difficult to ascertain otherwise (Criscione et al., 2005; Archie et al., 2009). Various markers have been developed for studying genetic structure in schistosomes, and applications of those markers have been reviewed recently (Jarne and Théron, 2001; de Meeûs et al., 2007; Gentile and Oliveira, 2008). Here we focus on recent studies using microsatellite loci, which are now abundant for a few schistosome species.

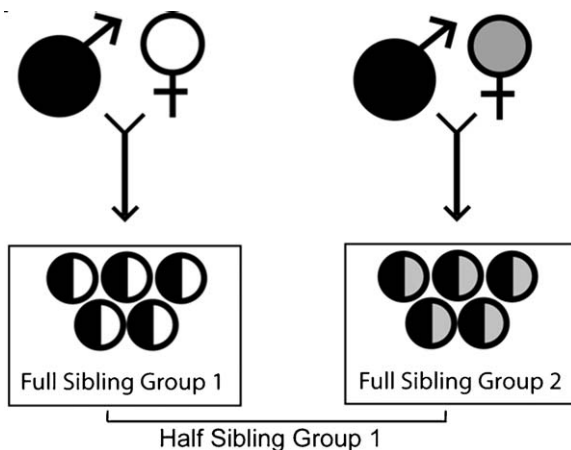


Fig. 2. An example of the family structure present in a sample of miracidia from a fecal sample. Adult males and females mate and produce offspring which are related to each other as full siblings. If the males shaded black are clonemates and each mates with a different female, then the two sibships will be related as paternal half siblings.

Different studies on *S. mansoni* have found what appear to be different patterns of subdivision in different geographic regions. In a large study in a village of Brazil, Virgem das Graças (60 km²), where schistosomes are primarily transmitted in stream habitats, schistosomes were significantly substructured among individual patients and among households (separated by 1 m to 6 km). However, this subdivision explained only a small proportion of the total genetic variation across the region and subdivision was not correlated with distance among households (Thiele et al., 2008). These data suggest substantial gene flow among schistosomes throughout the region. Contrasting results were found from schistosomes from another rural village, Melquiades (100 km²), in the same state of Brazil, where schistosomes are also transmitted in stream habitats. In this region, schistosomes showed much higher differentiation over similar geographic scales (hamlets separated by <1–3 km), and differentiation was associated with distance (Curtis et al., 2002). These data suggest more localized transmission cycles.

In Kenya, boundaries of watersheds and water bodies restrict gene flow of *S. mansoni* and help define transmission foci. This species shows strong genetic structure across regions that encompass different watersheds in the east, west, and southwest portions of the country (Agola et al., 2006). Within one watershed of Kenya, Lake Victoria, *S. mansoni* shows subdivision among water bodies (streams, marshes, and the lake), but no subdivision was detected within the Kenyan portion of the lake that comprises a surface area of about 1800 km² and supports an estimated 4.5 million people (Steinauer et al., 2009). These data suggest that in the absence of water body boundaries, gene flow can occur across large geographic distances. Structure has also been detected among schistosome infrapopulations (i.e. among individual hosts within a host population). In a rice farming irrigation region called Mwea, in the Kirinyaga District of central Kenya, samples of miracidia from school children showed significant, but low levels of pairwise subdivision among the infrapopulations of each child and among four schools that were 2–7 km apart (Agola et al., 2009). Thus, it appears that the geographic scale of differentiation identified can vary widely among different studies on *S. mansoni*. The interesting question will now be to explore what biological differences among sampled populations and also what issues of sampling are responsible for those different results.

One intriguing finding from a non-human focus of *S. mansoni* is sex specific genetic structure among hosts. In a population that cycles through rats in Guadeloupe (French West Indies) male schistosomes appeared to be more randomly dispersed among individual rats than females (Prugnolle et al., 2002; Prugnolle et al., 2004a). The authors suggested several possible explanations, including sex-specific interactions with the host immune system.

Schistosoma japonicum is a zoonotic pathogen and can infect more than 40 species of mammals (He et al., 2001). Thus, it is interesting to ask if humans and other mammals transmit the parasites to each other or if separate transmission cycles are maintained. Studies suggest that both types of transmission cycles may exist. For example, in the Philippines, the parasites of humans and dogs are not genetically differentiated (Rudge et al., 2008). In China, transmission may vary among regions in that parasites from different host species appear differentiated in some locations, but not in others (Rudge et al., 2009). *Schistosoma japonicum* also shows strong geographic structuring between China and the Philippines, and some structure among villages within 7 provinces within mainland China. Structure corresponded to habitat type (hilly or marshy lowland regions), which also corresponds to snail host morphotype (Shrivastava et al., 2005b). Similar habitat and/or snail host based structure also was detected in a smaller scale study of 10 villages within the Anhui province, China (139,000 km²) that are separated on a scale of <5–125 km (Rudge

et al., 2009). *Schistosoma japonicum* is arguably the most pathogenic and difficult to control schistosome. Therefore, it will be important to determine the factors that drive host specific transmission cycles and those that allow this disease to function as a zoonosis with many reservoir hosts. The latter presents a much more difficult epidemiological problem.

Genetic structure analyses are powerful tools for inferring epidemiological and evolutionary processes in schistosomes. They indicate that transmission foci are structured by watershed boundaries, habitat types, and host species. However, several sampling and methodological issues need to be acknowledged and addressed before these techniques can be used to their full potential with schistosomes. Below we discuss some of these issues with the goal of identifying sampling biases and solutions to avoid them.

2.5. Sampling issues

Here we discuss estimating parameters of adult schistosome populations such as allele and genotype frequencies, linkage disequilibrium (LD) and departures from Hardy-Weinberg equilibrium (HWE) within populations, or genetic structure across geographic sites or among infrapopulations within geographic sites. In the following, we refer to the adult parasites in one host as an infrapopulation, and to all the adult parasites in all hosts in a host population as the component population (Bush et al., 1997).

Unfortunately, the life history of schistosomes makes it difficult to know if sampling was adequate to estimate genetic parameters of adult worm populations. The first issue is whether the population of interest has been sampled *randomly*. The second is adequate statistical and genetic sampling (Holsinger and Weir, 2009). ‘Statistical sampling’ error results from studying a finite number of individuals per population or a finite number of populations from a larger metapopulation to which one wants to make inference (same as intralocus sampling error in Waples, 1998; Holsinger and Weir, 2009). ‘Genetic sampling’ results from using a limited number of loci to make inference to the genome as a whole (same as interlocus variance in Waples, 1998). Unlinked loci have largely independent evolutionary histories, so parametric F_{st} can vary substantially among loci (Chakraborty and Leimar, 1987; Waples, 1998). We do not focus on genetic sampling variance, but readers should keep this in mind when comparing studies that are based on a small number of loci (e.g. fewer than ten). Furthermore, each set of populations has its own unique evolutionary history, thus one should be cautious about extrapolating results to other sets of populations (another form of genetic sampling; Holsinger and Weir, 2009).

Say one wants to estimate F_{st} among geographic sites (i.e. among component populations). The first major hurdle is how to sample hosts (infrapopulations) in order to obtain a *random sample of adult worms* from the component population. The schistosomes in different demographic groups of humans may represent different subpopulations because infection risk can vary based on factors such as age, occupation, gender, socioeconomic status, and previous exposure. For example, schistosome populations may differ between children and adults because of their behavioral differences, the long life span of schistosomes (Fulford et al., 1995), and concomitant immunity (Smithers and Terry, 1967; Dean, 1983). Thus, deciding which humans to sample in order to obtain a random sample of the component population of adult worms may be difficult.

The second major hurdle is our inability to sample adult worms because they live in the vasculature of human hosts. Most studies resort to sampling eggs/miracidia obtained from human feces. The problem here is that we do not know to what extent a snapshot sample of eggs represents the adult worms in that host. First, it is

unknown how many adults were in each sampled host. Thus, we have unknown statistical sampling error from the adult component population. Second, if the sampled miracidia are derived from a few of the adults there will also be sampling bias in that the alleles from some adults are over-represented. So eggs from a few infrapopulations could give parameter estimates that are very different from those of the adult component population. Furthermore, the large sample sizes possible with egg collections could cause one to be wrong with high statistical confidence. These pitfalls of sampling juveniles were pointed out decades ago in the fish literature (Allendorf and Phelps, 1981; Waples, 1998). The extent of the problem in schistosomes is not yet clear, but it has been largely ignored (Criscione et al., 2005). Below we expand on the issue, discuss how likely it is to be a problem, and suggest potential solutions.

The basic problem is that although one can obtain thousands of eggs/miracidia from a single host, it is very difficult to know what effective number of adult breeders (N_b) produced that sample (Criscione and Blouin, 2005). To begin with, the total number of worms per host is highly skewed in most parasites (Crofton, 1971; Shaw et al., 1998). So you often do not know if your sample of eggs came from a host infected with a few adult worms or hundreds. Secondly, the N_b represented in your sample of eggs can be much smaller than the total number of adult worms in the sampled host. N_b is equal to the actual number of adults (census number, N_c) only when the number of offspring per adult, k , has a Poisson distribution (i.e. the variance, V_k equals the mean, \bar{k}) (Hedrick, 2005). The distribution of reproductive output among individuals in natural populations is usually highly skewed, causing $V_k/\bar{k} > 1$, and thus N_b to be smaller than N_c (e.g. Table 10.2 in Frankham et al., 2002). A highly skewed distribution of reproductive success seems likely in schistosomes for several reasons. For example, the immune system of the host might act more strongly on some schistosome genotypes, and thus greatly inflate the variance in egg production among pairs. Certain microhabitats within the host may be more conducive to egg production and excretion than others because the eggs must travel from venules, through tissues, and into the lumen of the digestive tract or bladder to be excreted. So worms in certain locales may contribute disproportionately to eggs in the feces. The presence of multiple clonemates within infrapopulations could also skew the distribution of reproductive success among MLGs. Furthermore, egg production for individual worms could be temporally variable and thus a single ‘snapshot’ sample from a host might not be representative of the long-term output from that host.

Thus, even though there may be many adults per definitive host, if reproduction is skewed so that a small number of breeders are contributing the majority of the offspring in a sample (i.e. that sample has a small N_b), a false conclusion of large differentiation among infrapopulations could be made simply as a consequence of sampling offspring rather than the adults that produced them (Fig. 3). If few infrapopulations are sampled per component population, one could observe an inflated F_{st} among component populations (or among host species, or whatever the larger unit of comparison). Additionally, a sample having small N_b will contain large numbers of full and/or half-siblings. Thus, sampling miracidia from a few infrapopulations that have low N_b could also cause one to wrongly conclude that there is substantial LD and deviations from HWE in the population, when in fact the adult population is in equilibrium.

Typical values of N_b/N_c for infrapopulations from humans are currently unknown. Steinauer et al. (2009) provide the only data available to date on the distribution of family sizes within individual hosts. In that study, mice were experimentally infected with schistosomes. Eggs trapped in the liver were collected and miracidia were hatched and genotyped. Parentage analyses were

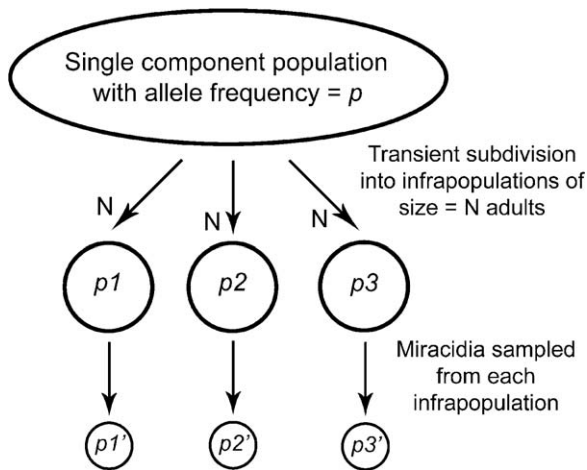


Fig. 3. Sources of sampling variance that can lead to apparent genetic structure among hosts (infrapopulations), or among component populations (e.g. geographic locations) if few hosts are sampled per component population. Each generation, eggs are passed into the environment and the resulting larvae randomly re-infect hosts creating an essentially random mating component population (cf. Criscione and Blouin, 2006). Reproducing adults (breeders) are subdivided into definitive host infrapopulations of say N breeders per host. If one were to measure the allele frequencies of the infrapopulations, the variance in allele frequencies among them would be $V_p = p(1-p)/2N$, where p is the frequency of the allele in the component population. In this example, p_1 – p_3 are the allele frequencies in the adults in each infrapopulation. p_1' – p_3' are the allele frequencies in samples of the offspring of those adults (eggs or miracidia). The variance among offspring samples, V_p' , is now calculated by replacing N with the effective number of breeders per host, N_b . Because N_b is typically much smaller than N , the variance among offspring samples can be much greater than the variance among infrapopulation samples ($V_p' > V_p$). Thus, when sampling offspring, a small N_b could cause one to conclude that there is large differentiation among infrapopulations, even though the adults were sampled from a panmictic population. If few infrapopulations are sampled per component population, F_{st} could be inflated among component populations. Passing miracidia through snails and mice and then genotyping adults could add additional components of variance owing to drift and to host-induced selection among parasite families.

used to assign miracidia to their parents collected from those mice in order to determine the reproductive success of each adult. Reproduction was over-dispersed among pairs within a host so that V_k/\bar{k} ranged from 7.24 to 7.41 per host giving N_b/N_c ratios of approximately 0.24 (Eq. (6.8b) in Hedrick, 2005). These data come from a single experimental infection in mice, and further work is necessary to determine if one would obtain similar values from natural infrapopulations from humans. Nevertheless, values of V_k/\bar{k} in the range of 5–20 are typical for wildlife populations (e.g. Table 1 in Frankham et al., 2002), so the above estimate of $V_k/\bar{k} \approx 7$ seems realistic.

So how can one adequately estimate F_{st} among component populations from samples of eggs? Again, one must understand the biology of transmission in order to sample hosts in a way such that the component population of adult worms is sampled randomly. Given that, one must then sample from a large enough sample of hosts to insure adequate statistical sampling of adult worms from their egg sample proxies. Preliminary work to estimate the among-host component of variance would help in rational design of a sampling scheme that balances the number of hosts sampled versus number of eggs genotyped per host. One should then estimate the genetic variance between geographic regions after removing the among-infrapopulation variance (see Rudge et al., 2008). An alternate approach would be to identify sibling groups within each sample of eggs and then substitute in the reconstructed genotypes of their parents. Or one could adjust allele frequencies so that each family is equally represented within each infrapopulation. Although these sibship-based approaches would give more accurate allele frequency estimates, they require

substantially more work (see further discussion of sibship methods below).

Another approach to estimating parameters of the adult population has been to sample cercariae from snails. However, this approach also raises issues of statistical sampling and of non-random sampling because of transient temporal and spatial variation in allele frequencies. Schistosomes are relatively short lived in snails compared to humans. If infection of snails is sporadic, rather than constant, a sample of cercariae may actually have originated from a small number of definitive hosts (and perhaps from only a few of their adult parasites, depending on the timing of egg output and the N_b of infrapopulations). A similar phenomenon, known as ‘sweepstakes recruitment’ is well known in the marine genetics literature (Hedgecock, 1994; Li and Hedgecock, 1998). Under sweepstakes recruitment there can be a large population of long-lived adults, but successful recruitment of larvae is a rare and unpredictable event. For example, in a population of thousands of adult oysters, the larvae in a single settlement event can be the offspring of just a handful of adults. Thus, if one samples only larvae, one might see a large F_{st} among geographic sites. But samples of adults, which consist of many cohorts that recruited over time, would show $F_{st} = 0$. Because schistosomes also have long-lived adults and presumably type III survivorship (high offspring mortality) in an uncertain environment, sweepstakes recruitment on a local scale seems plausible for schistosomes.

If a similar sweepstakes phenomenon is characteristic of schistosome populations, then how one samples snails on a microgeographic scale (when, where and how many snails per site) could greatly influence conclusions about the adult population of parasites. Yet the low prevalence of infected snails in most regions (often <5%) (Dabo et al., 1997; Sire et al., 1999; Kloos et al., 2001; Steinauer et al., 2008c) can make it very difficult to sample randomly across the geographic region of interest. For example, if one succumbs to the temptation to sample snails intensively from a microhabitat having high prevalence, allele frequencies estimated for the region as a whole could be highly biased if a transient parasite hotspot is not representative of the adult population.

Finally, we end this section by emphasizing that we discussed potential pitfalls of sampling juveniles in the context of estimating certain parameters of the adult population (such as F_{st} among component populations). However, if the goal is to address other question, say mating systems or non-random patterns of transmission (i.e., how genetic variation is disseminated from definitive hosts) in a landscape, then sampling eggs or cercariae would be very appropriate (e.g. see the Sections 2.2 and 2.3 above). The best way to sample depends on the question being asked.

2.5.1. Technical issues and possible solutions

2.5.1.1. Limited genomic DNA. Because the larval stages of schistosomes are small (miracidia $<200 \mu\text{m} \times 70 \mu\text{m}$; body of cercariae $<250 \times 100 \mu\text{m}$), researchers often passage field-collected miracidia through laboratory raised snails and mammals to obtain adults for genotyping. This approach can have several drawbacks. It is time consuming and expensive to maintain large populations within laboratory animals and their use can present ethical issues. For answering certain questions, one might need to identify adult clonemates that may have arisen during the asexual reproductive (snail) stage, and then collapse them into single MLGs prior to downstream analyses (discussed further below). A more critical issue is the loss of genetic diversity and spurious changes in allele frequencies that could result from selection and genetic drift during laboratory passage (Stohler et al., 2004; Shrivastava et al., 2005a). The number of snails used limits the number of genotypes that can be passed on to the next host. Variation among sporocysts

in production of cercariae will reduce the effective number of unique MLGs that are available to infect the rodent host. Rodents, in turn, are expensive and can carry only tens to a few hundred adults at most. Thus, even a single generation of laboratory passage could impose a severe genetic bottleneck on the sampled individuals. Selection among parasite genotypes via host immune systems could add an additional non-random component to which genotypes make it to adulthood. Such selection seems especially likely when passaging field-collected parasites through novel hosts (e.g. lab strains of snails, rodent hosts for miracidia collected from humans). For example, we know that in laboratory settings different cercarial clones vary greatly in their infectivity to mice (Steinauer unpublished data; J. Boissier, personal communication). Of course selection should not cause deterministic changes in the frequencies of neutral genetic markers unless those markers happen to be closely linked to a locus under selection. Selection simply adds an additional non-random component of variance in family size (e.g. among clones or among siblings from the original sample), which will further reduce the effective size of the passaged population. Thus drift and selection during laboratory passage could, for example, cause one to conclude that two field-collected samples of miracidia differ in allele frequencies when in fact they originated from the same parental gene pool.

Recent advances have led to techniques that allow genotyping of multiple microsatellite loci from single eggs or miracidia (Shrivastava et al., 2005a; Sorensen et al., 2006; Gower et al., 2007; Beltran et al., 2008b; Steinauer et al., 2008a), thereby eliminating the need to passage worms through hosts. These techniques not only reduce the use of animals and laboratory effort, but they also allow for a greater sample size to be analyzed. However, one of the greatest challenges in genotyping individual miracidia has been the limited amount of template available, which can lead to high rates of genotyping errors. Error is an important consideration for all studies involving microsatellite genotyping because it can lead to false interpretation of the data (Taberlet et al., 1996; Pompanon et al., 2005). Allelic dropout, the loss of one allele in a heterozygote, is particularly problematic with low quantities of template and when there is a large length difference among alleles (Taberlet et al., 1996). Often, the longer alleles are not detected because of PCR amplification bias for the shorter alleles; however, poor template quantity can lead to the loss of either allele (Walsh et al., 1992). Allelic dropout appears as a heterozygote deficiency in the population, which can lead to erroneous conclusions about population structure, mating systems, parentage, or even selection (Wattier et al., 1998). Methods aimed at uncovering genotyping errors have been developed and can be applied to investigate potential problematic loci or samples (e.g. Bonin et al., 2004; Van Oosterhout et al., 2004; Dewoody et al., 2006; Johnson and Haydon, 2007).

Whole genome amplification was recently evaluated as a means to increase the amount of DNA template from a single miracidium (Valentim et al., 2009). Genotyping error rate induced by this method was very low (0.45%) (measured 56 microsatellite loci evenly dispersed across the genome of *S. mansoni*; Criscione et al., 2009). This method will be useful because hundreds of molecular markers can now be genotyped from a single miracidium, and because individuals can be genotyped repeatedly to assess genotyping error rates in field studies.

2.5.1.2. Identifying clonemates. For some analyses one may wish to collapse clonemates into single MLGs (e.g. clones can cause LD among markers and large deviations from HWE; Théron et al., 2004; Prugnolle et al., 2005). For example, because clonal reproduction takes place after sexual reproduction, F_{IS} and LD in adults will only reflect mating patterns in the previous generation if one collapses clonemates. Thus, such deviations from equi-

ilibrium can be the biologically interesting phenomenon one wants to study or they can be a sampling nuisance—it depends on the question and the stage of the life cycle to which one wants to make inference. Given one has chosen to collapse clonemates, there are two complications with doing this in practice. First, one has to decide whether individuals that have the same MLG are actually clonemates or have the same MLG by chance alone. Software packages calculate the probability that identical MLGs arose through sexual rather than asexual reproduction, given the background allele frequencies (Stenberg et al., 2003; Meirmans and Van Tienderen, 2004; Arnaud-Haond and Belkhir, 2007). However, these methods do not work well if only a few loci were sampled, the population is characterized by low diversity, or one does not have an adequate source from which to estimate population allele frequencies.

The second complication for identifying clone mates is the presence of nearly identical MLG's (niMLG). niMLGs are individuals that have identical genotypes at all but (usually) one of a large number of loci (Yin et al., 2008). niMLGs can arise among true clonemates if there is a high mutation rate during the asexual phase in the snail. This phenomenon has been reported for *S. japonicum* (Yin et al., 2008), in which one miracidium apparently produced up to 9 different MLG (based on 17 microsatellite loci examined) after asexual reproduction within a snail. Diversity among clonemates has also been reported for intramolluscan stages of *S. mansoni* at the W1 locus, a repetitive element on the sex chromosomes (Grevelding, 1999; Bayne and Grevelding, 2003). Further work is necessary to determine if mutation rates are high for all schistosome species and if certain loci are more prone to mutation.

2.5.1.3. Identifying and accounting for sibship structure within a sample. Above we discussed the problems that can arise if larval samples descend from a small effective number of breeders. In that situation, the sample should contain groups of siblings. Several methods can be used to identify family structure within a sample of individuals. Large LD and deviations from HWE should clue one into the possibility of family structure in a dataset. The presence of relatives in a sample can also be inferred from a skewed distribution of pairwise relatedness estimates (e.g., as implemented in the program IDENTIX; Belkhir et al., 2002). If one has an *a priori* expectation that the sample has a simple kin structure consisting of just full siblings (or full and half siblings) and unrelated individuals (e.g. miracidia from a fecal sample), one can partition those individuals into full sibling and half sibling groups in the absence of parental genotypes (reviewed in Blouin, 2003). Examples of software packages include Colony v. 2, (Wang, 2004); Pedigree 2.2, (Herbinger, 2005); Parentage, (Emery et al., 2001), and KINALYZER (Ashley et al., 2009). However, pedigree reconstruction is still an active area of research, and there is as yet little consensus on the best method for every situation (Butler et al., 2004; Ashley et al., 2008). Note that the performance of these methods can be very sensitive to the number of loci used and the underlying family structure (distribution of family sizes, presence of maternal and paternal half siblings, number of clones present, presence of higher-order relatives), and that different methods can give different results. Thus, the accuracy of any particular family reconstruction can be difficult to estimate.

Assuming one can successfully reconstruct sib groups, two general approaches could be used to eliminate the problem of siblings in a dataset in order to make inference to the adult population. First, siblings can be removed leaving a single individual from each family in the dataset. An alternative and more difficult approach is to reconstruct the adult genotypes based on the offspring genotypes via rules of Mendelian inheritance and then replace the sibship with the genotypes of their parents (Banks

et al., 2000; Criscione et al., 2005). For each set of parents, the genotype at each locus can be inferred; however it will not be known which genotype belongs to which parent and thus the entire MLG cannot be reconstructed. However, complete MLGs of parents can be reconstructed when a common parent mates with two or more partners to produce half sibships (Jones, 2005). This situation might arise commonly in schistosomes when clonemates breed with mates that are genetically different (Fig. 2). Parental genotype reconstruction is ideal in many ways because it allows the adult genotypes to be used in genetic analyses. However, a large number of offspring genotypes must be sampled in order to accurately reconstruct parental genotypes and to obtain enough parental genotypes for analysis.

2.5.1.4. Genotyping aggregates of individuals. An alternative approach for obtaining allele frequency estimates from an inrapopulation is to genotype aggregates or pools of DNA from several individual eggs or miracidia derived from a single human patient (Silva et al., 2006; Blank et al., 2009; Hanelt et al., 2009b). The aggregate alleles are then determined by the number of bands or peaks after PCR amplification and their relative frequencies by the intensity or size of the band or peak.

Silva et al. (2006) and Blank et al. (2009) have investigated this method by comparing PCR amplifications from individually extracted adult worms to pools of known concentrations of extracted DNA from those same adults. Their results showed strong concordance between genotype profiles and the known DNA concentrations of the artificial pools of DNA. However, their pools included a low number of individuals (<16) with few alleles (<6) at a total of 7 loci. Thus, it is not known whether the same accuracy would be true for DNA extracted from a pooled sample of eggs from feces (in which DNA from each individual is not equalized), or when using highly polymorphic loci. Hanelt et al. (2009b) showed that allele frequencies correlated strongly between pools of miracidia (bulked DNA extraction) and individual miracidia collected from the same naturally infected human patient. However, error rates were significant, with an average of 8% of alleles lost and also false alleles detected in pools (Hanelt et al., 2009b). In part, errors were due to amplification bias and inadvertent scoring of stutter bands in chromatograms or gels (Hanelt et al., 2009b). In a pool, amplification bias for shorter alleles not only causes the apparent loss of the large alleles, but also skews the estimated allele frequencies because they are estimated according to amplification intensity (Hanelt et al., 2009b). Additionally, the chromatograms or gels derived from pools can be difficult to score because any microsatellite stutter peak (band) or electrophoresis generated artifact has to be considered an allele because they cannot be easily differentiated from the true alleles in

diverse samples (Hanelt et al., 2009b). These types of error are locus dependent and also potentially sample dependent as error generally increases with the diversity present (Hanelt et al., 2009b). Therefore, validation for each locus with diverse samples and from individually extracted egg/miracidia DNA that is subsequently pooled needs to be performed.

Another drawback to genotyping aggregates of offspring is that the inferences that can be made from the data are limited because only inrapopulation level data can be obtained rather than individual MLGs within the population. Thus, there is no ability to test for LD and deviations from HWE (Silva et al., 2006) or to assess family structure. The utility of this technique clearly relies on the question being addressed and how much error is acceptable. The utility of pooling lies in its efficiency in collecting data, but both error rates and the type of information needed must be carefully considered before this methodology is applied. Here we only discussed scoring microsatellite loci in pooled samples. However, pooling could be particularly useful once biomarkers are developed for genetically based traits such as virulence, host specificity, or drug resistance. In those cases one may wish to simply score presence/absence of a marker, or to track allele frequency changes (as in monitoring response to selection for drug resistance). For those applications pooling could be a very efficient approach.

2.5.1.5. Development of microsatellite loci. One additional technical issue is worthy of mention. Microsatellite markers are becoming more abundant for schistosome species that infect humans including 9 for *S. haematobium* (Golan et al., 2008), 28 for *S. japonicum* (Shrivastava et al., 2003; Yin et al., 2008), and 303 of *S. mansoni* (Durand et al., 2000; Blair et al., 2001b; Curtis et al., 2001; Rodrigues et al., 2002a; Rodrigues et al., 2002b; Silva et al., 2006; Criscione et al., 2009). An additional 8 markers for *S. haematobium* have been released to GenBank (EU887233–40) and an accompanying manuscript will follow (C.J. Schiff, Johns Hopkins Center for Global Health, personal communication). In *S. mansoni* 243 of microsatellites have been mapped across the genome including 23 that demarcate a Z chromosome specific region that does not recombine with the W chromosome (Criscione et al., 2009). These markers will be a powerful tool in field based studies. However, as independent laboratories have designed markers via various methods, there has been overlap in primer design for identical loci (examples in *S. mansoni* given in Table 1, other schistosome species have not been examined for marker redundancy). Our count reveals 11 redundant loci. In some cases, markers identified as separate loci, but really are a single locus, have been used in the same study. For example, sms6-1 (AF330104), sms7-1 (AF330105), and sms9-1 (AF330106) of Blair et al. (2001b) are the same locus with different primers and were used by Webster et al. (2007) as

Table 1

Redundant microsatellite markers reported for *Schistosoma mansoni*. These loci are the same but often are associated with different primer sequences.

Original marker	GenBank accession no.	Original reference	Redundant marker
SMD25	AF202965	Durand et al. (2000)	Supercontig_0000170 ^a
AI068335	AI068335	Durand et al. (2000)	CA11-1 ^b
L46951	L46951	Durand et al. (2000)	SmBr6 ^c
SMIMP25	X77211	Durand et al. (2000)	SmBr3 ^c
sms6-1	AF330104	Blair et al. (2001)	sms7-1 ^b , sms9-1 ^b
SMDA28	AF325695	Curtis et al. (2001)	13TAGA ^d , SmBr10 ^e
SMD43	AF325697	Curtis et al. (2001)	SmBr15 ^e
SMC1	AF325694	Curtis et al. (2001)	SMMS16 ^f , SmBr9 ^e
27AAT	BH795456	Rodrigues et al. (2002b)	SmBr7 ^e

^a Criscione et al. (2009).

^b Blair et al. (2001b).

^c Rodrigues et al. (2002a).

^d Rodrigues et al. (2002b).

^e Rodrigues et al. (2007).

^f Silva et al. (2006).

independent loci. Thus, researchers are advised to search for matches in databases prior to selecting markers.

3. Conclusions

Molecular methods and principles of evolution and ecology have revealed many aspects of the epidemiology of schistosomes. Phylogenetics and biogeography have given a historical perspective on the origin of schistosomes and revealed their ability to colonize new locations and new hosts. Molecular methods have also uncovered the complexities of the mating systems of schistosomes and that interspecific hybrid zones occur naturally. The consequences of these hybrid zones have yet to fully be explored. Molecular markers have been used to describe the transmission dynamics of schistosomes. Populations can be structured at local scales according to watershed boundaries, topography, and host species but more data is needed to understand how these causative factors differentially influence populations. Also co-infections within snails appear to be random with regard to relatedness suggesting that aggregation of relatives does not strongly influence population structure in snails.

One obvious hurdle to molecular epidemiological studies of schistosomes is sampling and using the appropriate methodology for samples collected. Their intravascular habitat in human hosts and also their asexual reproduction within the intermediate host, make sampling a challenge. Alternative methods have been developed; however, we have pointed out several pitfalls to these methods that may yield spurious results. We have also given recommendations of how to identify sampling artifacts and methods to remove them. Our hope is to not only spark interest in the molecular epidemiology of schistosomes and other pathogens, but also increase the awareness of how sampling methodology can produce artifactual results. The development of new analytical tools and ongoing genome projects will undoubtedly lead to new and exciting research in schistosome epidemiology, but methodological hurdles must be overcome before this field reaches its full potential.

Acknowledgement

Partial funding for MLS was provided by U.S. National Institutes of Health Grant AI044913.

References

- Agola, E.L., Steinauer, M.L., Mburu, D.N., Mungai, B.N., Mwangi, I.N., Magoma, G.N., Loker, E.S., Mkoji, G.M., 2009. Genetic diversity and population structure of *Schistosoma mansoni* within human infra-populations in Mwea, central Kenya assessed by microsatellite markers. *Acta Trop.* 111, 219–225.
- Agola, L.E., Mburu, D.N., DeJong, R.J., Mungai, B.N., Muluvi, G.M., Njagi, E.N.M., Loker, E.S., Mkoji, G.M., 2006. Microsatellite typing reveals strong genetic structure of *Schistosoma mansoni* from localities in Kenya. *Infect. Genet. Evol.* 6, 484–490.
- Allendorf, F.W., Phelps, S.R., 1981. Use of allele frequencies to describe population genetic structure. *Can. J. Fish. Aquat. Sci.* 38, 1507–1514.
- Aoki, Y., Sato, K., Muhoho, N.D., Noda, S., Kimura, E., 2003. Cercariometry for detection of transmission sites for schistosomiasis. *Parasitol. Int.* 52, 403–408.
- Archie, E.A., Luikart, G., Ezenwa, V.O., 2009. Infecting epidemiology with genetics: a new frontier in disease ecology. *Trends Ecol. Evol.* 24, 21–30.
- Arnaud-Haond, S., Belkhir, K., 2007. Genclone: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Mol. Ecol. Notes* 7, 15–17.
- Arnold, S.J., Wade, M.J., 1984. On the measurement of natural and sexual selection: theory. *Evolution* 38, 709–719.
- Ashley, M.V., Berger-Wolf, T.Y., Caballero, I.C., Chaovalitwongse, W.A., DasGupta, B., Sheikh, S.I., 2008. Full sibling reconstruction in wild populations from microsatellite genetic markers. In: Russe, A.S. (Ed.), *Computational Biology: New Research*. Nova Science Publishers, Hauppauge, NY, pp. 231–258.
- Ashley, M.V., Caballero, I.C., Chaovalitwongse, W.A., Dasgupta, B., Govindan, P., Sheikh, S.I., Berger-Wolf, T.Y., 2009. KINALYZER, a computer program for reconstructing sibling groups. *Mol. Ecol. Resour.* 9, 1127–1131.
- Attwood, S.W., 2001. Schistosomiasis in the Mekong region: Epidemiology and phylogeography. *Adv. Parasitol.* 50, 87–152.
- Attwood, S.W., Upatham, E.S., Meng, X.H., Qiu, D.C., Southgate, V.R., 2002. The phylogeography of Asian *Schistosoma* (Trematoda: Schistosomatidae). *Parasitology* 125, 99–112.
- Attwood, S.W., Fatih, F.A., Mondal, M.M.H., Alim, M.A., Fadjari, S., Rajapakse, R.P.V.J., Rollinson, D., 2007. A DNA sequence-based study of the *Schistosoma indicum* (Trematoda: Digenea) group: population phylogeny, taxonomy and historical biogeography. *Parasitology* 134, 2009–2020.
- Attwood, S.W., Fatih, F.A., Campbell, I., Upatham, E.S., 2008. The distribution of Mekong schistosomiasis, past and future: Preliminary indications from an analysis of genetic variation in the intermediate host. *Parasitol. Int.* 57, 256–270.
- Avise, J.C., 2000. *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge.
- Banks, M.A., Rashbrook, V.K., Calavetta, M.J., Dean, C.A., Hedgecock, D., 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. *Can. J. Fish. Aquat. Sci.* 57, 915–927.
- Barker, S.C., Blair, D., 1996. Molecular phylogeny of *Schistosoma* species supports traditional groupings within the genus. *J. Parasitol.* 82, 292–298.
- Bayne, C.J., Greveling, C.G., 2003. Cloning of *Schistosoma mansoni* sporocysts in vitro and detection of genetic heterogeneity among individuals within clones. *J. Parasitol.* 89, 1056–1060.
- Belkhir, K., Castric, V., Bonhomme, F., 2002. Identix, a software to test for relatedness in a population using permutation methods. *Mol. Ecol. Notes* 2, 611–614.
- Beltran, S., Boissier, J., 2008. Schistosome monogamy: Who, how, and why? *Trends Parasitol.* 24, 386–391.
- Beltran, S., Cézilly, F., Boissier, J., 2008a. Genetic dissimilarity between mates, but not male heterozygosity influences divorce in schistosomes. *PLoS One* 3, 1–6.
- Beltran, S., Galinier, R., Allienne, J.F., Boissier, J., 2008b. Cheap, rapid and efficient DNA extraction method to perform multilocus microsatellite genotyping on all *Schistosoma mansoni* stages. *Mem. Inst. Oswaldo Cruz* 103, 501–503.
- Bermingham, E., Moritz, C., 1998. Comparative phylogeography: concepts and applications. *Mol. Ecol.* 7, 367–369.
- Black, C.L., Steinauer, M.L., Mwinzi, P.N.M., Secor, W.E., Karanja, D.M.S., Colley, D.G., 2009. Impact of intense, longitudinal retreatment with praziquantel on cure rates of schistosomiasis mansoni in a cohort of occupationally exposed adults in western Kenya. *Trop. Med. Int. Health* 14, 1–8.
- Blair, D., Davis, G.M., Wu, B., 2001a. Evolutionary relationships between trematodes and snails emphasizing schistosomes and paragonimids. *Parasitology* 123, S229–S243.
- Blair, L., Webster, J.P., Barker, G.C., 2001b. Isolation and characterization of polymorphic microsatellite markers in *Schistosoma mansoni* from Africa. *Mol. Ecol. Notes* 1, 93–95.
- Blank, W.A., Reis, E.R., Thiong'o, F.W., Braghioroli, J.F., Santos, J.M., Melo, P.R.S., Guimarães, I.C.S., Silva, L.K., Carmo, T.M.A., Reis, M.F., Blanton, R.E., 2009. Analysis of *Schistosoma mansoni* population structure using total fecal egg sampling. *J. Parasitol.* 95, 881–889.
- Blouin, M.S., 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends Ecol. Evol.* 18, 503–511.
- Bonin, A., Bellemain, E., Eidesen, P.B., Pompanon, F., Brochmann, C., Taberlet, P., 2004. How to track and assess genotyping errors in population genetics studies. *Mol. Ecol.* 13, 3261–3273.
- Brant, S.V., Loker, E.S., 2005. Can specialized pathogens colonize distantly related hosts? Schistosome evolution as a case study. *PLoS Pathogens* 1, 167–169.
- Brant, S.V., Morgan, J.A.T., Mkoji, G.M., Snyder, S.D., Rajapakse, R.P.V.J., Loker, E.S., 2006. An approach to revealing blood fluke life cycles, taxonomy, and diversity: Provision of key reference data including DNA sequence from single life cycle stages. *J. Parasitol.* 92, 77–88.
- Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* 83, 575–583.
- Butler, K., Field, C., Herbinger, C.M., Smith, B.R., 2004. Accuracy, efficiency and robustness of four algorithms allowing full sibship reconstruction from DNA marker data. *Mol. Ecol.* 13, 1589–1600.
- Chakraborty, R., Leimar, O., 1987. Genetic variation within a subdivided population. In: Ryman, N., Utter, F. (Eds.), *Population Genetics and Fishery Management*. Washington Sea Grant Publications/University of Washington Press, Seattle, pp. 89–120.
- Charlesworth, D., 2003. Effects of inbreeding on the genetic diversity of populations. *Philos. Trans. R. Soc. B Biol. Sci.* 358, 1051–1070.
- Chitsulo, L., Loverde, R., Engels, D., Barakat, R., Colley, D., Cioli, D., Engels, D., Feldmeier, H., Loverde, P., Olds, G.R., Oura, J., Rabello, A., Savioli, L., Traore, M., Vennervald, B., Schistosomiasis, T.R.G., 2004. Schistosomiasis. *Nat. Rev. Microbiol.* 2, 12–13.
- Conway, D.J., 2007. Molecular epidemiology of malaria. *Clin. Microbiol. Rev.* 20, 188–204.
- Criscione, C.D., Blouin, M.S., 2005. Effective sizes of macroparasite populations: a conceptual model. *Trends Parasitol.* 21, 212–217.
- Criscione, C.D., Poulin, R., Blouin, M.S., 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. *Mol. Ecol.* 14, 2247–2257.
- Criscione, C.D., Blouin, M.S., 2006. Minimal selfing, few clones, and no among-host genetic structure in a hermaphroditic parasite with asexual larval propagation. *Evolution* 60, 553–562.
- Criscione, C.D., Valentim, C.L.L., Hirai, H., LoVerde, P.T., Anderson, T.J.C., 2009. Genomic linkage map of the human blood fluke *Schistosoma mansoni*. *Genome Biol.* 10, R71.

- Crofton, H.D., 1971. A quantitative approach to parasitism. *Parasitology* 62, 179–193.
- Curtis, J., Sorensen, R.E., Page, L.K., Minchella, D.J., 2001. Microsatellite loci in the human blood fluke *Schistosoma mansoni* and their utility for other schistosome species. *Mol. Ecol. Notes* 1, 143–145.
- Curtis, J., Sorensen, R.E., Minchella, D.J., 2002. Schistosome genetic diversity: the implications of population structure as detected with microsatellite markers. *Parasitology* 125, S51–S59.
- Dabo, A., Durand, P., Morand, S., Diakite, M., Langand, J., ImbertEstabiet, D., Doumbo, O., Jourdan, J., 1997. Distribution and genetic diversity of *Schistosoma haematobium* within its bulinid intermediate hosts in Mali. *Acta Trop.* 66, 15–26.
- de Meeûs, T., McCoy, K.D., Prugnolle, F., Chevillon, C., Durand, P., Hurtrez-Boussès, S., Renaud, F., 2007. Population genetics and molecular epidemiology or how to “débuser la bête”. *Infect. Genet. Evol.* 7, 308–332.
- Dean, C.A., 1983. *Schistosoma* and related genera: acquired resistance in mice. *Exp. Parasitol.* 55, 1–104.
- DeJong, R.J., Morgan, J.A.T., Paraense, W.L., Pointier, J.P., Amarista, M., Ayeh-Kumi, P.F.K., Babiker, A., Barbosa, C.S., Brémond, P., Canese, A.P., de Souza, C.P., Dominguez, C., File, S., Gutierrez, A., Incani, R.N., Kawano, T., Kazibwe, F., Kpikpi, J., Lwambo, N.J.S., Mimpfundi, R., Njikou, F., Poda, J.N., Sene, M., Velasquez, L.E., Yong, M., Adema, C.M., Hofkin, B.V., Mkoji, G.M., Loker, E.S., 2001. Evolutionary relationships and biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with implications regarding its role as host of the human bloodfluke, *Schistosoma mansoni*. *Mol. Biol. Evol.* 18, 2225–2239.
- Dewdney, J.A., Nason, J.D., Hipkins, V.D., 2006. Mitigating scoring errors in microsatellite data from wild populations. *Mol. Ecol. Notes* 6, 951–957.
- Durand, P., Sire, C., Théron, A., 2000. Isolation of microsatellite markers in the digenetic trematode *Schistosoma mansoni* from Guadeloupe island. *Mol. Ecol.* 9, 997–998.
- Emery, A.M., Wilson, I.J., Craig, S., Boyle, P.R., Noble, L.R., 2001. Assignment of paternity groups without access to parental genotypes: multiple mating and developmental plasticity in squid. *Mol. Ecol.* 10, 1265–1278.
- Engels, D., Chitsulo, L., Montresor, A., Savioli, L., 2002. The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Trop.* 82, 139–146.
- Eppert, A., Lewis, F.A., Grzywacz, C., Coura-Filho, P., Caldas, I., Minchella, D.J., 2002. Distribution of schistosome infections in molluscan hosts at different levels of parasite prevalence. *J. Parasitol.* 88, 232–236.
- Frank, S.A., 1996. Models of parasite virulence. *Q. Rev. Biol.* 71, 37–78.
- Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge.
- Fulford, A.J.C., Butterworth, A.E., Ouma, J.H., Sturrock, R.F., 1995. A statistical approach to schistosome population dynamics and estimation of the life span of *Schistosoma mansoni* in man. *Parasitology* 110, 307–316.
- Gentile, R., Oliveira, G., 2008. Brazilian studies on the genetics of *Schistosoma mansoni*. *Acta Trop.* 108, 175–178.
- Golan, R., Gower, C.M., Emery, A.M., Rollinson, D., Webster, J.P., 2008. Isolation and characterization of the first polymorphic microsatellite markers for *Schistosoma haematobium* and their application in multiplex reactions of larval stages. *Mol. Ecol. Resour.* 8, 647–649.
- Gower, C.M., Webster, J.P., 2005. Intraspecific competition and the evolution of virulence in a parasitic trematode. *Evolution* 59, 544–553.
- Gower, C.M., Shrivastava, J., Lamberton, P.H.L., Rollinson, D., Webster, B.L., Emery, A., Kabatereine, N.B., Webster, J.P., 2007. Development and application of an ethically and epidemiologically advantageous assay for the multi-locus microsatellite analysis of *Schistosoma mansoni*. *Parasitology* 134, 523–536.
- Grevelding, C.G., 1999. Genomic instability in *Schistosoma mansoni*. *Mol. Biochem. Parasitol.* 101, 207–216.
- Hamburger, J., Hoffman, O., Kariuki, H.C., Muchiri, E.M., Ouma, J.H., Koech, D.K., Sturrock, R.F., King, C.H., 2004. Large-scale, polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya: A new tool for studying the dynamics of snail infection. *Am. J. Trop. Med. Hyg.* 71, 765–773.
- Hanelt, B., Brant, S.V., Steinauer, M.L., Maina, G.M., Kinuthia, J.M., Ndungu, I.N., Agola, E.L., Mutuku, M.W., Mkoji, G.M., Loker, E.S., 2009a. *Schistosoma kisu-muensis* n. sp. (Digenea: Schistosomatidae) from murid rodents in the Lake Victoria Basin, Kenya and its phylogenetic position within the *S. haematobium* species group. *Parasitology* 39, 1353–1362.
- Hanelt, B., Steinauer, M.L., Mwangi, I.N., Maina, G.M., Agola, L.E., Mkoji, G.M., Loker, E.S., 2009b. A new approach to characterize populations of *Schistosoma mansoni* from humans: development and assessment of microsatellite analysis of pooled miracidia. *Trop. Med. Int. Health* 14, 322–331.
- He, Y.-X., Salafsky, B., Ramaswamy, K., 2001. Host-parasite relationships of *Schistosoma japonicum* in mammalian hosts. *Trends Parasitol.* 17, 320–324.
- Hedgecock, D., 1994. Does variance in reproductive success limit effective population size of marine organisms? In: Beaumont, A. (Ed.), *Genetics and Evolution of Aquatic Organisms*. Chapman & Hall, London, pp. 122–134.
- Hedrick, P.W., 2005. *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury.
- Herbinger, C.M., 2005. PEDIGREE 2.2., <http://herbinger.biology.dal.ca:5080/Pedigree>.
- Hertel, J., Kedves, K., Hassan, A.H.M., Haberl, B., Haas, W., 2004. Detection of *Schistosoma mansoni* cercariae in plankton samples. *Acta Trop.* 91, 43–46.
- Holsinger, K.E., Weir, B.S., 2009. Genetics in geographically structured populations: defining, estimating and interpreting F_{ST} . *Nat. Rev. Genet.* 10, 639–650.
- Jarne, P., Théron, A., 2001. Genetic structure in natural populations of flukes and snails: a practical approach and review. *Parasitology* 123, S27–S40.
- Johnson, P.C.D., Haydon, D.T., 2007. Maximum-likelihood estimation of allelic dropout and false allele error rates from microsatellite genotypes in the absence of reference data. *Genetics* 175, 827–842.
- Jones, A.G., 2005. GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents. *Mol. Ecol. Notes* 5, 708–711.
- Jourdan, J., Southgate, V., 1992. Genetic exchanges and sexual interactions between species of the genus *Schistosoma*. *Res. Rev. Parasitol.* 52, 21–26.
- Kane, R.A., Southgate, V.R., Rollinson, D., Littlewood, D.T.J., Lockyer, A.E., Pages, J.R., Tchuem Tchuenté, L.A., Jourdan, J., 2003. A phylogeny based on three mitochondrial genes supports the division of *Schistosoma intercalatum* into two separate species. *Parasitology* 127, 131–137.
- King, C.H., Sturrock, R.F., Kariuki, H.C., Hamburger, J., 2006. Transmission control for schistosomiasis—why it matters now. *Trends Parasitol.* 22, 575–582.
- Kloos, H., de Souza, C., Gazzinelli, A., Soares, B.S., Temba, P.D., Bethony, J., Page, K., Grzywacz, C., Lewis, F., Minchella, D., LoVerde, P., Oliveira, R.C., 2001. The distribution of *Biomphalaria* spp. in different habitats in relation to physical, biological, water contact and cognitive factors in a rural area in Minas Gerais, Brazil. *Mem. Inst. Oswaldo Cruz* 96, 57–66.
- Kruger, F.J., Evans, A.C., 1990. Do all human urinary infections with *Schistosoma matthei* represent hybridization between *Schistosoma haematobium* and *Schistosoma matthei*? *J. Helminthol.* 64, 330–332.
- LeRoux, P.L., 1954. Hybridization of *Schistosoma mansoni* and *S. rodhaini*. *Trans. R. Soc. Trop. Med. Hyg.* 48, 3–4.
- Li, G., Hedgecock, D., 1998. Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. *Can. J. Fish. Aquat. Sci.* 55, 1025–1033.
- Lockyer, A.E., Olson, P.D., Ostergaard, P., Rollinson, D., Johnston, D.A., Attwood, S.W., Southgate, V.R., Horak, P., Snyder, S.D., Le, T.H., Agatsuma, T., McManus, D.P., Carmichael, A.C., Naem, S., Littlewood, D.T.J., 2003. The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* Weinland, 1858. *Parasitology* 126, 203–224.
- LoVerde, P.T., Niles, E.G., Osman, A., Wu, W.J., 2004. *Schistosoma mansoni* male-female interactions. *Can. J. Zool.* 82, 357–374.
- May, R.M., Woolhouse, M.E.J., 1993. Biased sex-ratios and parasite mating probabilities. *Parasitology* 107, 287–295.
- Meirmans, P.G., Van Tienderen, P.H., 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol. Ecol. Notes* 4, 792–794.
- Minchella, D.J., Sollenberger, K.M., de Souza, C.P., 1995. Distribution of schistosome genetic diversity within molluscan intermediate hosts. *Parasitology* 111, 217–220.
- Morand, S., Pointier, J.P., Borel, G., Théron, A., 1993. Pairing probability of schistosomes related to their distribution among the host population. *Ecology* 74, 2444–2449.
- Morand, S., Müller-Graf, C.D.M., 2000. Muscles or testes? Comparative evidence for sexual competition among dioecious blood parasites (Schistosomatidae) of vertebrates. *Parasitology* 120, 45–56.
- Morgan, J.A.T., DeJong, R.J., Kazibwe, F., Mkoji, G.M., Loker, E.S., 2003a. A newly identified lineage of *Schistosoma*. *Int. J. Parasitol.* 33, 977–985.
- Morgan, J.A.T., DeJong, R.J., Lwambo, N.J.S., Mungai, B.N., Mkoji, G.M., Loker, E.S., 2003b. First report of a natural hybrid between *Schistosoma mansoni* and *S. rodhaini*. *J. Parasitol.* 89, 416–418.
- Morgan, J.A.T., DeJong, R.J., Adeoye, G.O., Ansa, E.D.O., Barbosa, C.S., Brémond, P., Cesari, I.M., Charbonnel, N., Correa, L.R., Coulibaly, G., D’Andrea, P.S., De Souza, C.P., Doenhoff, M.J., File, S., Idris, M.A., Incani, R.N., Jarne, P., Karanja, D.M.S., Kazibwe, F., Kpikpi, J., Lwambo, N.J.S., Mabaye, A., Magalhães, L.A., Makundi, V., Mone, H., Mouahid, G., Muchemi, G.M., Mungai, B.N., Sene, M., Southgate, V., Tchuem Tchuenté, L.A., Théron, A., Yousif, F., Zanotti-Magalhães, E.M., Mkoji, G.M., Loker, E.S., 2005. Origin and diversification of the human parasite *Schistosoma mansoni*. *Mol. Ecol.* 14, 3889–3902.
- Muhoho, N.D., Katsumata, T., Kimura, E., Migwi, D.K., Mutua, W.R., Kiliku, F.M., Habe, S., Aoki, Y., 1997. Cercarial density in the river of an endemic area of schistosomiasis haematobia in Kenya. *Am. J. Trop. Med. Hyg.* 57, 162–167.
- Nadler, S.A., 1995. Microevolution and the genetic structure of parasite populations. *J. Parasitol.* 81, 395–403.
- Nesse, R.M., Stearns, S.C., 2008. The great opportunity: evolutionary applications to medicine and public health. *Evol. Appl.* 1, 28–48.
- Pompanon, F., Bonin, A., Bellemain, E., Taberlet, P., 2005. Genotyping errors: causes, consequences and solutions. *Nat. Rev. Genet.* 6, 847–859.
- Prugnolle, F., de Meeûs, T., Durand, P., Sire, C., Théron, A., 2002. Sex-specific genetic structure in *Schistosoma mansoni*: evolutionary and epidemiological implications. *Mol. Ecol.* 11, 1231–1238.
- Prugnolle, F., Choisy, M., Théron, A., Durand, P., de Meeûs, T., 2004a. Sex-specific correlation between heterozygosity and clone size in the trematode *Schistosoma mansoni*. *Mol. Ecol.* 13, 2859–2864.
- Prugnolle, F., Théron, A., Durand, P., de Meeûs, T., 2004b. Test of pangamy by genetic analysis of *Schistosoma mansoni* pairs within its natural murine host in Guadeloupe. *J. Parasitol.* 90, 507–509.
- Prugnolle, F., Roze, D., Théron, A., de Meeûs, T., 2005. F-statistics under alternation of sexual and asexual reproduction: a model and data from schistosomes (platyhelminth parasites). *Mol. Ecol.* 14, 1355–1365.
- Restif, O., 2009. Evolutionary epidemiology 20 years on: challenges and prospects. *Infect. Genet. Evol.* 9, 108–123.
- Rodrigues, N.B., Coura, P., de Souza, C.P., Passos, L.K.J., Dias-Neto, E., Romanha, A.J., 2002a. Populational structure of *Schistosoma mansoni* assessed by DNA microsatellites. *Int. J. Parasitol.* 32, 843–851.

- Rodrigues, N.B., LoVerde, P.T., Romanha, A.J., Oliveira, G., 2002b. Characterization of new *Schistosoma mansoni* microsatellite loci in sequences obtained from public DNA databases and microsatellite enriched genomic libraries. *Mem. Inst. Oswaldo Cruz* 97, 71–75.
- Rodrigues, N.B., Silva, M.R., Pucci, M.M., Minchella, D.J., Sorensen, R., LoVerde, P.T., Romanha, A.J., Oliveria, G., 2007. Microsatellite-enriched genomic libraries as a source of polymorphic loci for *Schistosoma mansoni*. *Mol. Ecol. Notes* 7, 263–265.
- Rollinson, D., Southgate, V.R., Vercruysee, J., Moore, P.J., 1990. Observations on natural and experimental interactions between *S. bovis* and *S. curraioni* from west Africa. *Acta Trop.* 47, 101–114.
- Rudge, J.W., Carabin, H., Balolong, E., Tallo, V., Shrivastava, J., Lu, D.B., Basanez, M.G., Olveda, R., McGarvey, S.T., Webster, J.P., 2008. Population genetics of *Schistosoma japonicum* within the Philippines suggest high levels of transmission between humans and dogs. *PLoS Neg. Trop. Dis.* 2, e340.
- Rudge, J.W., Lu, D.B., Fang, G.R., Wang, T.P., Basáñez, M.G., Webster, J.P., 2009. Parasite genetic differentiation by habitat type and host species: molecular epidemiology of *Schistosoma japonicum* in hilly marshland areas of Anhui Province, China. *Mol. Ecol.* 18, 2134–2147.
- Shaw, D.J., Grenfell, B.T., Dobson, A.P., 1998. Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* 117, 597–610.
- Shrivastava, J., Barker, G.C., Johansen, M.V., Xiaonong, Z., Aligui, G.D., McGarvey, S.T., Webster, J.P., 2003. Isolation and characterization of polymorphic DNA microsatellite markers from *Schistosoma japonicum*. *Mol. Ecol. Notes* 3, 406–408.
- Shrivastava, J., Gower, C.M., Balolong, E., Wang, T.P., Qian, B.Z., Webster, J.P., 2005a. Population genetics of multi-host parasites: The case for molecular epidemiological studies of *Schistosoma japonicum* using larval stages from naturally infected hosts. *Parasitology* 131, 617–626.
- Shrivastava, J., Qian, B.Z., McVean, G., Webster, J.P., 2005b. An insight into the genetic variation of *Schistosoma japonicum* in mainland China using DNA microsatellite markers. *Mol. Ecol.* 14, 839–849.
- Silva, L., Liu, S., Blanton, R.E., 2006. Microsatellite analysis of pooled *Schistosoma mansoni* DNA: an approach for studies of parasite populations. *Parasitology* 132, 331–338.
- Sire, C., Rognon, A., Théron, A., 1998. Failure of *Schistosoma mansoni* to reinfect *Biomphalaria glabrata* snails: acquired humoral resistance or intra-specific larval antagonism? *Parasitology* 117, 117–122.
- Sire, C., Durand, P., Pointier, J.P., Théron, A., 1999. Genetic diversity and recruitment pattern of *Schistosoma mansoni* in a *Biomphalaria glabrata* snail population: a field study using random-amplified polymorphic DNA markers. *J. Parasitol.* 85, 436–441.
- Smithers, S.R., Terry, R.J., 1967. Resistance to experimental infection with *Schistosoma mansoni* in rhesus monkeys induced by the transfer of adult worms. *Trans. R. Soc. Trop. Med. Hyg.* 61, 517–533.
- Snyder, S.D., Loker, E.S., 2000. Evolutionary relationships among the schistosomatidae (Platyhelminthes: Digenea) and an Asian origin for *Schistosoma*. *J. Parasitol.* 86, 283–288.
- Sorensen, R.E., Rodrigues, N.B., Oliveira, G., Romanha, A.J., Minchella, D.J., 2006. Genetic filtering and optimal sampling of *Schistosoma mansoni* populations. *Parasitology* 133, 443–451.
- Southgate, V.R., Rollinson, D., Ross, G.C., Knowles, R.J., 1982. Mating behavior in mixed infections of *Schistosoma haematobium* and *Schistosoma intercalatum*. *J. Nat. Hist.* 16, 491–496.
- Southgate, V.R., Jourdan, J., Tchuem Tchuente, L.A., 1998. Recent studies on the reproductive biology of the schistosomes and their relevance to speciation in the Digenea. *Int. J. Parasitol.* 28, 1159–1172.
- Steinauer, M.L., Agola, L.E., Mwangi, I.N., Mkoji, G.M., Loker, E.S., 2008a. Molecular epidemiology of *Schistosoma mansoni*: a robust, high-throughput method to assess multiple microsatellite markers from individual miracidia. *Infect. Genet. Evol.* 8, 68–73.
- Steinauer, M.L., Hanelt, B., Mwangi, I.N., Maina, G.M., Agola, E.L., Kinuthia, J.M., Mutuku, M.W., Mungai, B.N., Wilson, W.D., Mkoji, G.M., Loker, E.S., 2008b. Introgressive hybridization of human and rodent schistosome parasites in western Kenya. *Mol. Ecol.* 17, 5062–5074.
- Steinauer, M.L., Mwangi, I.N., Maina, G.M., Kinuthia, J.M., Mutuku, M.W., Agola, L.E., Mungai, B.N., Mkoji, G.M., Loker, E.S., 2008c. Interactions between natural populations of human and rodent schistosomes in the Lake Victoria region of Kenya: a molecular epidemiological approach. *PLoS Neg. Trop. Dis.* 2, 1–11.
- Steinauer, M.L., 2009. The sex lives of parasites: Investigating the mating system and mechanisms of sexual selection of the human pathogen *Schistosoma mansoni*. *Int. J. Parasitol.* 39, 1157–1163.
- Steinauer, M.L., Hanelt, B., Agola, L.E., Mkoji, G.M., Loker, E.S., 2009. Genetic structure of *Schistosoma mansoni* in western Kenya: the effects of geography and host sharing. *Int. J. Parasitol.* 39, 1353–1362.
- Steinmann, P., Keiser, J., Bos, R., Tanner, M., Utzinger, J., 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect. Dis.* 6, 411–425.
- Stenberg, P., Lundmark, M., Saura, A., 2003. MLGsim: a program for detecting clones using a simulation approach. *Mol. Ecol. Notes* 3, 329–331.
- Stohler, R.A., Curtis, J., Minchella, D.J., 2004. A comparison of microsatellite polymorphism and heterozygosity among field and laboratory populations of *Schistosoma mansoni*. *Int. J. Parasitol.* 34, 595–601.
- Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P., Bouvet, J., 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucl. Acids Res.* 24, 3189–3194.
- Taylor, M.G., 1970. Hybridization experiments on five species of African schistosomes. *J. Helminthol.* 44, 253–314.
- Tchuem Tchuente, L.A., Southgate, V.R., Imbertestablet, D., Jourdan, J., 1995. Change of mate and mating competition between males of *Schistosoma intercalatum* and *Schistosoma mansoni*. *Parasitology* 110, 45–52.
- Tchuem Tchuente, L.A., Southgate, V.R., Njiokou, F., Njine, T., Kouemni, L.E., Jourdan, J., 1997. The evolution of schistosomiasis in Loum, Cameroon: replacement of *Schistosoma intercalatum* by *S. haematobium* through introgressive hybridization. *Trans. R. Soc. Trop. Med. Hyg.* 91, 664–665.
- Tchuem Tchuente, L.A., Southgate, V.R., Jourdan, J., Webster, B.L., Vercruysee, J., 2003. *Schistosoma intercalatum*: an endangered species in Cameroon? *Trends Parasitol.* 19, 389–393.
- Théron, A., Sire, C., Rognon, A., Prugnolle, F., Durand, P., 2004. Molecular ecology of *Schistosoma mansoni* transmission inferred from the genetic composition of larval and adult infrapopulations within intermediate and definitive hosts. *Parasitology* 129, 571–585.
- Thiele, E.A., Sorensen, R.E., Gazzinelli, A., Minchella, D.J., 2008. Genetic diversity and population structure of *Schistosoma mansoni* in a Brazilian village. *Int. J. Parasitol.* 38, 389–399.
- Tibayrenc, M., 1998. Genetic epidemiology of parasitic protozoa and other infectious agents: the need for an integrated approach. *Int. J. Parasitol.* 28, 85–104.
- Valentim, C.L.L., LoVerde, P.T., Anderson, T.J.C., Criscione, C.D., 2009. Efficient genotyping of *Schistosoma mansoni* miracidia following whole genome amplification. *Mol. Biochem. Parasitol.* 166, 81–84.
- Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4, 535–538.
- Walsh, P.S., Erlich, H.A., Higuchi, R., 1992. Preferential PCR amplification of alleles: mechanisms and solutions. *Genome Res.* 1, 241–250.
- Wang, J.L., 2004. Sibship reconstruction from genetic data with typing errors. *Genetics* 166, 1963–1979.
- Waples, R.S., 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *J. Hered.* 89, 438–450.
- Wattier, R., Engel, C.R., Saumitou-Laprade, P., Valero, M., 1998. Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). *Mol. Ecol.* 7, 1569–1573.
- Webster, B.L., Southgate, V.R., Tchuem Tchuente, L.A., 2003. Isoenzyme analysis of *Schistosoma haematobium*, *S. intercalatum* and their hybrids and occurrences of natural hybridization in Cameroon. *J. Helminthol.* 77, 269–274.
- Webster, B.L., Southgate, V.R., Littlewood, D.T.J., 2006. A revision of the interrelationships of *Schistosoma* including the recently described *Schistosoma guineensis*. *Int. J. Parasitol.* 36, 947–955.
- Webster, J.P., Shrivastava, J., Johnson, P.J., Blair, L., 2007. Is host-schistosome coevolution going anywhere? *BMC Evol. Biol.* 7, 91.
- Webster, J.P., Gower, C.M., Norton, A.J., 2008. Evolutionary concepts in predicting and evaluating the impact of mass chemotherapy schistosomiasis control programmes on parasites and their hosts. *Evol. Appl.* 1, 66–83.
- Wright, C.A., Ross, G.C., 1980. Hybrids between *Schistosoma haematobium* and *S. matthei* and their identification by isoelectric-focusing of enzymes. *Trans. R. Soc. Trop. Med. Hyg.* 74, 326–332.
- Yin, M.B., Hu, W., Mo, X.J., Wang, S.Y., Brindley, P.J., McManus, D.P., Davis, G.M., Feng, Z., Blair, D., 2008. Multiple near-identical genotypes of *Schistosoma japonicum* can occur in snails and have implications for population-genetic analyses. *Int. J. Parasitol.* 38, 1681–1691.
- Zhang, G.J., Verneau, O., Qiu, C.P., Jourdan, J., Xia, M.Y., 2001. An African or Asian evolutionary origin for human schistosomes? *Comptes Rendus Acad. Sci. Ser. III Sci. Vie Life Sci.* 324, 1001–1010.