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The Compatibility Between *Biomphalaria glabrata* Snails and *Schistosoma mansoni*: An Increasingly Complex Puzzle

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ABSTRACT

This review reexamines the results obtained in recent decades regarding the compatibility polymorphism between the snail, *Biomphalaria glabrata*, and the pathogen, *Schistosoma mansoni*, which is one of the agents responsible for human schistosomiasis. Some results point to the snail's resistance as explaining the incompatibility, while others support a "matching hypothesis" between the snail's immune receptors and the schistosome's antigens. We propose here that the two hypotheses are not exclusive, and that the compatible/incompatible status of a particular host/parasite couple probably reflects the balance of multiple molecular determinants that support one hypothesis or the other. Because these genes are involved in a coevolutionary arms race, we also propose that the underlying mechanisms can vary. Finally, some recent results show that environmental factors could influence compatibility. Together, these results make the compatibility between *B. glabrata* and *S. mansoni* an increasingly complex puzzle.

We need to develop more integrative approaches in order to find targets that could potentially be manipulated to control the transmission of schistosomiasis.

1 INTRODUCTION

Schistosomes are the causative agents of schistosomiasis, one of the most important neglected human tropical diseases in the world (WHO, 2016; <http://www.who.int/mediacentre/factsheets/fs115/en/> accessed 2015/01/07). Schistosomes infect over 200 million people worldwide, causing both acute and chronic debilitating diseases (King, 2010; King et al., 2005). There is no effective vaccine against schistosomes, and treatment still relies on a single drug: praziquantel (Doenhoff et al., 2009). As praziquantel resistance can be easily selected in the laboratory (Fallon and Doenhoff, 1994) and mass treatment chemotherapy used now in the field show evidence of Schistosome reduced drug susceptibility (Melman et al., 2009), alternate control strategies are needed. Some such strategies seek to block the transmission of the disease at the level of the snail that acts as the intermediate host. We must understand the mechanisms through which snails and schistosomes interact, as they could offer valuable clues for developing new strategies aimed at disrupting the transmission of schistosomiasis. A community of investigators has concentrated on understanding the mechanisms of snail-trematode compatibility. The interaction between *Biomphalaria glabrata* and *Schistosoma mansoni* was chosen as a model system and has received concentrated research efforts over the past four decades. Indeed, most of the existing studies concerning snail-trematode compatibility were developed using these species. The genome of *S. mansoni* has been sequenced and annotated (Berriman et al., 2009), and the sequencing and annotation of the *B. glabrata* genome was recently submitted for publication (Adema C, personal communication).

It is largely accepted that the success or failure of the infection of *B. glabrata* by *S. mansoni* reflects a complex interplay between the host's defence mechanisms and the parasite's infective strategies. Moreover, within the interacting populations of snails and schistosomes, it appears that some *S. mansoni* parasites succeed in infecting *Biomphalaria* snails while others fail. To explain this phenomenon (called "compatibility polymorphism"), two alternative hypotheses have been proposed. The first is that an unsuccessful infection reflects the existence and success of snail resistance processes, whereas a successful infection reveals the susceptible status of the snail host (Webster and Davies, 2001). The second hypothesis is that the success or failure of an infection does not depend on the snail's susceptibility/resistance status, but rather on the matched or mismatched status of the host and parasite phenotypes (Theron and Coustau, 2005). In the latter scheme, all snails are potentially susceptible if they are exposed to a schistosome with a matching phenotype (Theron et al., 2008).

The present review will reexamine the studies performed in recent decades to test the compatibility between *B. glabrata* and *S. mansoni*. As described in the following sections, some results support the "resistance hypothesis" while others

support the “matching hypothesis.” We propose here that these two hypotheses are not exclusive, and that the compatible/incompatible status of a specific *B. glabrata*/*S. mansoni* interaction probably reflects a balance among multiple molecular determinants that fall into two different categories. The first category corresponds to the effector/antifeffector systems of the host and the parasite, and the involved molecular determinants tend to induce resistance processes. The second category corresponds to immune receptors and antigens, whose intraindividual diversifications and polymorphisms could favour the matched or mismatched status of host and parasite phenotypes. We propose that the compatibility between individual snails and schistosomes reflects the sum of these different determinants, which are variable between and within populations. Finally, the situation will be made more complex by environmental factors known to influence the compatibility between *B. glabrata* and *S. mansoni*. Indeed, two of these environmental factors, the water temperature or successive exposures of *B. glabrata* to schistosomes can change the phenotype of both partners, thereby altering their compatibility. This will be presented in the last two sections of this review.

2 THE GENETIC DETERMINISM OF THE COMPATIBILITY/INCOMPATIBILITY OF BIOMPHALARIA GLABRATA AND SCHISTOSOMA MANSONI

The low prevalence of snails with patent schistosome infection, which is usually observed in transmission foci (Anderson and May 1979; Sire et al., 1999), was first believed to be explained by the low probability of an encounter between the partners. This hypothesis cannot be excluded in the interaction between *B. glabrata* and *S. mansoni*. However, a molecular screening approach yielded interesting results regarding the interaction between *Schistosoma haematobium* and *Bulinus globosus* snails. This work showed that although patency was often very low (less than 4% shed cercariae), more than 40% of the snails were exposed to the parasite in the field (Allan et al., 2013). Thus, snails experienced high levels of parasitic exposure, but only a small proportion of infected snails reached the stage of cercarial shedding. Moreover, even if snails were penetrated by miracidia, the infections often failed to develop to patency (Allan et al., 2013). These results suggest that nonsusceptibility or incompatibility of a particular host and parasite combination is a major factor in the low prevalence observed in the field. This nonsusceptibility/incompatibility can also reflect a biochemically unfavourable intramolluscan environment for the parasite, which is termed “unsuitability” (Lie and Heyneman, 1977; Sullivan and Richards, 1981). However, the most frequently involved mechanism consists of the recognition, encapsulation and killing of the parasite by immune cells (haemocytes) of the snail (Fig. 1 A). Numerous infection-based experiments have been performed

between different homopatric and heteropatric isolates and lines of *B. glabrata* and *S. mansoni*, and the results have revealed high degrees of compatibility polymorphism within and between the populations (Richards and Shade, 1987; Theron et al., 2014). Using inbred lines of *B. glabrata*, researchers showed that compatibility has a genetic basis, with genes of both the snail and parasite affecting the outcome of an infection. For instance, the infection resistance seen in resistant adult snail stocks (e.g., BS-90, 13-16-R1, and 10-R2) is a dominant single-gene trait that is inherited in a simple Mendelian fashion (Richards et al., 1992; Spada et al., 2002). In contrast, resistance in juvenile snails is determined by five or six genes that each have multiple alleles (Ittiprasert et al., 2010; Richards and Merritt, 1972). Several studies have also shown that multigenic factors of the parasite influence compatibility (Kassim and Richards, 1979; Combes, 1985; Jourdane, 1982; Richards, 1975).

3 CROSSES AND GENETIC APPROACHES FOR IDENTIFYING COMPATIBILITY/INCOMPATIBILITY LINKED LOCI

Crosses between snail lines compatible (M-line or NMRI) and incompatible (BS-90) towards the same schistosome strain (PR1) have been used to investigate the genetic loci that govern the compatibility trait. Various DNA genotyping tools have been used to identify heritable markers related to the parasite-incompatible phenotype of adult snails (Knight et al., 1999). However, these sequences are repetitive in the snail genome, and thus further attempts to characterize the associated genes were not successful. A reverse genetic approach using linkage analysis of polymorphic expressed sequence tags (ESTs, particularly expressed simple sequence repeats, or eSSRs) and previously identified bi-allelic microsatellite markers (genomic SSRs or gSSRs) led to the identification of putative genomic locations for incompatibility gene loci (Ittiprasert et al., 2013). Moreover, studies in a different snail strain (13-16-R1) revealed that snail incompatibility could be associated with allelic variation in a linked cluster of redox genes that includes *sod1*, which encodes a cytosolic copper/zinc superoxide dismutase, a cytosolic enzyme that catalyses the conversion of superoxide anion to H_2O_2 (Blouin et al., 2013; Bonner et al., 2012; Goodall et al., 2006). Finally, a Restriction site associated DNA (RAD) genotyping approach was developed using experimentally evolved lines of Guadeloupean *B. glabrata* selected for their incompatibility (Tennessen et al., 2015). This work revealed association of the incompatibility phenotype with a <1-Mb region containing 15 coding genes; of them, seven encoded single-pass transmembrane proteins that display strikingly high nonsynonymous divergence among alleles. The presence of high linkage disequilibrium among the haplotypes across this genomic region revealed a significantly nonneutral pattern of evolution, suggesting that balancing selection maintains this diversity. The function of these genes is not yet known, but the authors proposed that they may contribute to parasite recognition (Tennessen et al., 2015). Future work is needed to functionally validate these hypotheses.

The current genomic characterization of *B. glabrata* and the availability of genetic and physical maps will facilitate such studies in the near future and should enable the identification of additional compatibility-associated genes in this snail species. In addition, the tools currently used or under development to allow gene knock-down in snails (e.g., RNAi or CRISPRcas9 technology) will be necessary to support the functional validation of the most promising candidates.

4 USE OF MOLECULAR COMPARATIVE APPROACHES ON COMPATIBLE AND INCOMPATIBLE STRAINS OF BIOMPHALARIA GLABRATA TO IDENTIFY CANDIDATE GENES

In addition to the genetic approaches reported above, advances have also been made towards the identification of putative genes associated with snail or schistosome compatibility by examining proteome and transcriptome between different snail or schistosome strains that differ in their compatibility phenotypes. In the past 15 years, numerous studies have used differential-display reverse-transcription polymerase chain reaction (PCR), suppression subtractive hybridization or cDNA microarray technologies to examine snails (Ittiprasert et al., 2010; Lockyer et al., 2012; Lockyer et al. 2004; Lockyer et al. 2007; Schneider and Zelck, 2001). Several such studies compared the transcripts expressed by different compatible (e.g., M-line and BB02) and incompatible (e.g., 13-16-R1 and BS-90) strains of *B. glabrata* infected with laboratory strains of *S. mansoni*. The authors focussed on different tissues (e.g., haemocytes, haematopoietic organs, brains, or whole snail tissues), snails at different life stages (juvenile or adults), and snails infected with or without *S. mansoni*. These studies revealed various genes encoding antimicrobial proteins, heat shock protein (HSP) 70, ferritin, protease, glycosidase, peroxidase, adhesion molecules, and molecules involved in cell signalling. However, the candidate genes were not subjected to any functional validation, meaning that their involvement in compatibility processes has not been conclusively demonstrated.

These studies, particularly those comparing the responses of compatible and incompatible snails, revealed that schistosome-incompatible snails recognize parasites and mount an appropriate defence response, whereas schistosome-compatible snails are unable to defend themselves against infection. This suggests that: (1) compatible snails lack the capacity to recognize and react to the parasite; and/or (2) the effector cells (haemocytes) of these compatible snails are rendered ineffective by active immunosuppression processes that occur during the early stages of infection. As a consequence, most of the genes identified by such studies are indirectly involved in the compatibility process, and were identified because incompatible snails are able to mount a cascade of efficient immune reactions to kill the parasite.

In parallel to the global transcriptomic approaches, more targeted approaches have been developed using different compatible and incompatible snail lines. Since haemocytes play an important role in the encapsulation process, the haemocyte content was evaluated for 52 inbred lines that displayed different compatible/incompatible phenotypes (Larson et al., 2014). The results revealed that snail lines harbouring higher numbers of circulating haemocytes were incompatible with *S. mansoni*, whereas lines characterized by lower haemocyte numbers showed variable phenotypes (compatible or incompatible). In the same work, the transcript levels of 18 putative defence-related genes were quantified in haemocytes isolated from the same lines. The compatible/incompatible phenotypes were found to correlate with differences in the transcript levels of two redox-relevant genes (a predicted phagocyte oxidase component and a peroxiredoxin) and an allograft inflammatory factor (Larson et al., 2014).

Another study focussed on hydrolytic enzymes known to play key roles in host-pathogen interactions. A comparative study was undertaken to examine the proteolytic enzyme activities and transcripts of susceptible and resistant snails (Myers et al., 2008). This work revealed between-strain differences in cysteine protease activity and a significant upregulation of cathepsin B transcripts in resistant (vs. susceptible) snails after parasite exposure. Recently the growth factors granulin (BgGRN) that drive proliferation of immune cells was evidenced as an essential factor for the proliferation and production of an adherent haemocyte subpopulation involved in *S. mansoni* killing. Additionally, susceptible *B. glabrata* snails can be rendered resistant to infection if they were previously treated with BgGRN (Pila et al., 2016). Finally, since hydrogen peroxide (H_2O_2) was shown to be involved in haemocytemediated sporocyst killing (Hahn et al., 2001), some authors hypothesized that Cu/Zn superoxide dismutase (SOD, a cytosolic enzyme that catalyses the conversion of superoxide anion to H_2O_2), could differ between resistant and susceptible snail strains. Indeed, studies showed that the amount of steady-state Cu/Zn SOD mRNA was constitutively higher in haemocytes from resistant snails. Moreover, this was directly correlated with a higher Cu/Zn SOD enzymatic activity (Goodall et al., 2004) and a higher capacity to produce H_2O_2 (Bender et al., 2005) in resistant snails. A causal relationship between *B. glabrata* SOD1 expression and susceptibility/resistance to *S. mansoni* was further supported by expressional analysis (Bender et al., 2007) and genetic linkage studies (Bonner et al., 2012; Goodall et al., 2006).

5 USE OF MOLECULAR COMPARATIVE APPROACHES ON STRAINS OF SCHISTOSOMA MANSONI AND THE DISCOVERY OF SCHISTOSOMA MANSONI POLYMORPHIC MUCINS

For *S. mansoni*, comparative approaches were developed using isolates that are compatible (C, Brazil) or incompatible (IC, Guadeloupe) towards the same snail strain (Brazil). In this system, newly penetrated parasites from the IC strain are contacted by host haemocytes within 1e2 h postinfection and entirely encapsulated by 8e12 h postinfection (Fig. 1A). In contrast, newly penetrated miracidia of the C strain are not encapsulated, and instead develop into primary sporocysts (Sp1) (Fig. 1B). These observations suggested that constitutive antigenic differences exist between the two schistosome strains. A comparative proteomics approach (Roger et al., 2008c) revealed that these *S. mansoni* strains mainly differed in their mucin-like glycoproteins. Mucins are generally known to play key roles in the host-parasite interplay (Buscaglia et al., 2006; Hicks et al., 2000; Rathore et al., 2005; Theodoropoulos et al., 2001). Further study of the identified mucin-like glycoproteins showed that they shared several features characteristic of mucins, including an N-terminal domain containing a variable number of tandem repeats and a conserved C-terminal domain (Roger et al., 2008a). Moreover, the proteins were: (1) only expressed by the larval schistosome stages that interact with the snail intermediate host; (2) produced and located in the apical gland of miracidia and sporocysts; (3) highly glycosylated and (4) highly polymorphic. Consequently, the authors called these proteins the *S. mansoni* polymorphic mucins (SmPoMucs). A detailed analysis of the high level intra- and interstrain variations of SmPoMucs revealed that their diversification is driven by a complex cascade of mechanisms involving recombinations between members of the SmPoMuc multigene family, posttranscriptional regulation events, and posttranslational modifications (Roger et al., 2008b). The consequence of this genetic variability is that a remarkably high degree of polymorphism arises from a limited set of genes, enabling each individual parasite to express a specific and unique pattern of SmPoMucs (Fig. 2). Taken together, these results suggested that SmPoMucs could be antigens that play a crucial role in the compatibility process.

To investigate this hypothesis and find the putative immune receptors responsible for interacting with SmPoMucs, coimmunoprecipitation (CoIP) experiments were developed (Mone et al., 2010). When antibodies raised against SmPoMucs were used in pull-down experiments, they were found to form molecular complexes with *B. glabrata* Fibrinogen Related Proteins (FREPs) (Adema et al., 1997). The FREPs are lectin-like haemolymph proteins that bind and precipitate soluble antigens derived from trematodes (Adema et al., 1997), making them perfect candidates to be among the snail molecular determinants of individual-level compatibility. A thorough study revealed that FREPs are highly polymorphic, and that somatic diversification generates unique FREP repertoires in individual *B. glabrata* (Zhang et al., 2004). A specific review dedicated to these molecules was recently published (Gordy et al., 2015).

Interestingly, the FREP/SmPoMuc-containing complex was found to have a third protein member: a thioester-containing protein (TEP) from *B. glabrata* (BgTEP, (Mone et al., 2010)). The presence of BgTEP in the complex was intriguing, as TEP family members play key roles in other invertebrate/pathogen interactions, especially in insects. For example, TEP1 is crucial for the phagocytosis of bacteria and the killing of *Plasmodium* parasites in the mosquito, *Anopheles gambiae*. TEP1 from the mosquito is secreted by haemocytes and cleaved in haemolymph into an active form that is called mature TEP1 or TEP1-cut (Fraiture et al., 2009). The C-terminal part of TEP1 binds to bacteria or the ookinete stage of malaria parasites through a thioester bond. The involvement of this complementlike molecule in the antiparasitic defence of mosquitoes has been discussed (Volohonsky et al., 2010). Precursor and phylogenetic analysis of BgTEP suggested that it shares features of invertebrate TEPs, particularly those known to be involved in antiparasitic defence and phagocytosis of microorganisms (Blandin and Levashina, 2004, 2007; Blandin et al., 2008; Stroschein-Stevenson et al., 2006). In addition, liquid chromatography tandem mass spectroscopy (LC-MS/MS) analysis of the interactome components identified peptides located in the C-terminal part of BgTEP, suggesting that it undergoes a thioester-dependent association with the two other members of the complex. BgTEP appears to occur in its activated form in the immune complex (Mone et al., 2010), suggesting that it may contribute to opsonization processes, as previously described for other members of the TEP family (Blandin et al., 2008). This hypothesis was further supported by the presence of an Alpha2 macroglobulin receptor binding domain (amino acids 1343-1427) in the C-terminal part of the BgTEP precursor (Mone et al., 2010). This domain is involved in the interactions with macrophage- and phagocyte-specific receptors (van Lookeren Campagne et al., 2007) and may participate in haemocyte recruitment and the encapsulation of incompatible parasites in snail tissues.

The importance of FREPs in the compatibility process was confirmed by specific knockdown of FREP 3 in *B. glabrata* BS-90 snails, which are totally resistant to a specific laboratory strain of *S. mansoni* (Hanington et al., 2012). The knockdown snails lost some (21.4%) of their resistance to *S. mansoni* infection, suggesting that FREP 3 functions in the recognition process, but it is not alone in its function. This was confirmed by two studies dedicated to characterizing the interactome between sporocyst/miracidial extracts from *S. mansoni* and plasma from *B. glabrata*. The first approach identified several snail lectins and parasite glycoproteins (Mone et al., 2010). The identified lectins included various FREPs, as well as a *B. glabrata* lectin that had sequence similarities with a secreted galactose-binding lectin previously described from another gastropod, *Helix pomatia* (Perez-Sanchez et al., 2006). In addition to these snail lectins, the interactome also contained several parasite glycosylated proteins that were identified as SmPoMucs, including the 23-kDa integral membrane protein, Sm23

(or tetraspanin), and the glycoprotein, K5 (Mone et al., 2010). The second approach used a combination of affinity chromatography and proteomic analyses to reveal that affinity-immobilized sporocyst surface glycoproteins and glycoproteins released during miracidial transformation selectively bind various plasma proteins (Yoshino T., personal communication). These proteins notably include members of several FREP subfamilies (e.g., FREPs 2, -3, -5, -7, and -12), as well as C-type lectins and dermatopontins.

Together, these results clearly support the idea that the diversified FREPs and their corresponding diversified antigens (e.g., SmPoMucs) may be involved in the compatibility polymorphism between *S. mansoni* and *B. glabrata*, but they are likely to be only a piece of the highly complex process through which the parasite is recognized, encapsulated and killed by an incompatible host. Other host immune receptors and carbohydrate components and/or glycoproteins from the parasite are likely to be involved.

6 OTHER PUTATIVE EFFECTOR AND/OR ANTIEFFECTOR SYSTEMS COULD PLAY ROLES IN COMPATIBILITY

Several studies seeking to characterize the response of *B. glabrata* to immune challenge by different elicitors, along with interactome studies, have identified additional molecular determinants that may contribute to compatibility.

After an immune challenge, different molecular effectors are activated and may help *B. glabrata* to fight infection. Some of them engage in a molecular battle with antieffector systems from the parasite, and the coevolution of these systems (while not always directly demonstrable at this point) is likely to influence the compatibility/incompatibility of a specific snail/ schistosome interaction.

Highly reactive chemical compounds derived from molecular oxygen, i.e., reactive oxygen species (ROS) are crucial to the snail's ability to defend itself against *S. mansoni* (Bayne et al., 2001). ROS produced by the haemocytes of *B. glabrata* seem to play a crucial role in killing of *S. mansoni*. *S. mansoni*, in turn, has adapted by developing several antioxidant systems. During the transformation of a miracidium to a mother sporocyst, the intramolluscan larval parasite expresses an array of antioxidant enzymes, such as glutathione-S-transferase, Cu/Zn SOD, glutathione peroxidase (GPx) and peroxiredoxins (Prxs) (Wu et al., 2009; Zelck and Von Janowsky, 2004; Guillou et al., 2007b; Mourao Mde et al., 2009; Vermeire et al., 2006). These enzymes are localized to the newly formed tegumental syncytium, where they support the survival of the parasite within the host (Vermeire and Yoshino, 2007; Mourao Mde et al., 2009). Interestingly, in response to miracidial penetration, the snail releases an oxidative burst that seems to be activated by antigen recognition. Consistent with this, mannose/galactose/fucose-containing antigens have been shown to rapidly induce the PI3K-dependent production of H₂O₂, which is the main ROS involved

in killing *S. mansoni* sporocysts (Hahn et al., 2000, 2001; Humphries and Yoshino, 2008). The parasite must use ROS scavengers to defend itself against ROS-induced oxidative damage. In this context, we could expect a coevolution between these two mechanisms. This hypothesis was tested by a study comparing host oxidant and parasite antioxidant capabilities between two *S. mansoni*/*B. glabrata* populations that coevolved independently (Mone et al., 2011). As expected, there was a clear correlation between the oxidant and antioxidant capabilities of the snail and schistosome. When a snail population (*B. glabrata* from Guadeloupe) displayed a low level of ROS production, its corresponding sympatric population of parasites displayed low antioxidant capabilities. In contrast, *B. glabrata* from Brazil displayed high level ROS production and their sympatric parasite displayed high antioxidant capabilities. In this system, it is notable that the schistosomes that displayed higher antioxidant capabilities (those from Brazil) were more prone to developing an infection under the allopatric combination, whereas the schistosomes from Guadeloupe were completely incompatible with snails from the Brazilian isolate (Mone et al., 2011). This result was corroborated by the findings of Chris Bayne's group in independent snail strains (Bender et al., 2005; Goodall et al., 2004). Thus, the ROS and ROS-scavenger-based mechanism appears to be an important pathway involved in the compatibility between *B. glabrata* and *S. mansoni*.

In addition to these ROS/ROS scavenger components, transcriptomic studies revealed that many extracellular components known to participate in cell/cell or cell/matrix interactions are induced after penetration of the parasite into snail tissues. The overexpressions of several proteins, including MFAP4 (microfibrillar associated protein), mucins, dermatopontins and matrilins, suggest that the extracellular matrix undergoes dynamic remodelling following parasitic penetration (Lockyer et al., 2012; Mitta et al., 2005). In addition, other studies identified modulations of *B. glabrata* cathepsin, elastase and Zn metalloproteinase expression pattern following parasitic infection (Ittiprasert et al., 2010; Lockyer et al., 2012), and host protease inhibitors (e.g., cystatin and serine protease inhibitors) were found to be upregulated in haemocytes after challenge with either schistosomes (Lockyer et al., 2012) or echinostomes (Mitta et al., 2005). These different induced factors may counteract parasitic proteases in order to maintain the abilities of haemocytes and/or defence factors to adhere to the surface of the parasite. This hypothesis was strengthened by *in situ* expression analysis of *B. glabrata* cystatin, which showed that *Bgcystatin* was expressed in haemocytes that aggregated around *Echinostoma caproni*, another trematode parasite of *B. glabrata* (Guillou et al., 2007a). Together, these results suggest that proteases and protease inhibitors of *B. glabrata* and *S. mansoni* may engage in an enzymatic battle that defines the compatibility of the interaction.

Several genes encoding putative antimicrobial proteins were found to be upregulated in response to trematode infection. Notably, genes encoding

members of the LBP/BPI family were highly upregulated, especially after echinostome challenge (Guillou et al., 2007a; Hanington et al., 2010).

One particular LBP/BPI protein was found to: (1) be expressed in the albumen gland (Guillou et al., 2007a); (2) represent the major protein of *B. S. mansoni* labrata egg masses (Hathaway et al., 2010); and (3) display both antibacterial activity and an unexpected activity against oomycetes (aquatic fungi) (Baron et al., 2013). The gene encoding putative macrophage expressed gene-1 (MPEG1), which is an antimicrobial member of the membrane attack complex/perforin protein superfamily (Bathige et al., 2014), was upregulated in haemocytes from snails challenged with an incompatible strain of *S. mansoni* (Ittiprasert et al., 2010). However, more detailed investigations are needed to define the putative antitrematode activities of LBP/BPI and MPEG1.

The previously cited characterization of the interactome between *S. mansoni* sporocyst extracts and plasma of *B. glabrata* (Mone et al., 2010) identified a very interesting protein: a relatively new putative cytolytic protein from *B. glabrata*, called biomphalysin (Mone et al., 2010). A functional characterization of biomphalysin (Galinier et al., 2013) revealed that it belongs to the b pore-forming toxin (b-PFT) superfamily, which includes amoeba virulence factors that trigger lysis by forming channels in the membranes of target cells. Biomphalysin is specifically expressed in haemocytes, and it does not show differential regulation following parasitic challenge. Recombinant biomphalysin had haemolytic activity, binds parasite membranes, and exerts high cytotoxicity toward *S. mansoni* sporocysts. Notably, this functional characterization of biomphalysin provided the first description of a gastropod immune effector protein involved in *S. mansoni* killing (Galinier et al., 2013).

For all the effectors discussed in this section, we could expect to see the same arms race observed between *B. glabrata*-produced ROS and the antioxidant capabilities of the schistosome (Mone et al., 2011). These factors could thus be involved in a coevolutionary battle between the snail's effector systems and the parasite's antieffector systems.

7 THE COMPATIBILITY POLYMORPHISM CAN BE EXPLAINED BY A COMBINATION OF MATCHING PHENOTYPE STATUS AND VIRULENCE/RESISTANCE PROCESSES

A key limitation of the compatibility studies mentioned above is that the data have been mainly obtained from one or several generations of laboratory-bred snails (some of which have been inbred) and/or schistosome strains passed through laboratory strains of intermediate and definitive hosts. Consequently, the data are likely to be poorly representative of the diversity present in the original populations.

To address these limitations, snail/schistosome compatibility has been investigated using miracidia and snails from the same geographic locality (Guadeloupe Island) (Theron et al., 2008). In this study, to retain a large fraction of the genetic diversity of the parasite, numerous naturally infected definitive hosts were used as sources of miracidia, and collected snails were exposed to different doses of these miracidia. Using this protocol, the infection rate increased with the parasite dose to reach 100% infection when 10 or 20 miracidia were used (Fig. 3). This result suggests that the probability of infection increases with the dose of miracidia simply because a larger fraction of the phenotypic diversity present in the parasite population is included. This indicates that all *B. glabrata* are potentially susceptible to *S. mansoni*, and they will develop a patent infection if they are confronted to enough phenotypically diverse miracidia. In such a scheme, the low natural prevalence of snails with patent schistosome infection observed in transmission foci (Anderson and May 1979; Sire et al., 1999) might then reflect the low probability that a schistosome phenotype encounters its corresponding compatible host phenotype, rather than providing evidence for a high level of resistance within host populations.

Snails and parasites from Guadeloupe Island were then used to establish laboratory strains, and compatibility was tested after one laboratory passage. In this transition from field to lab, compatibility dropped from 100% to around 50% (Fig. 3). This result suggests that the parasite was submitted to a more severe genetic bottleneck (Bech et al., 2010; Stohler et al., 2004; Theron et al., 2008) than the host (Campos et al., 2002; Mulvey and Vrijenhoek, 1981). After laboratory transition, the parasite strain retains a reduced set of phenotypes able to match only with a fraction of the phenotypes present in the host strain (Theron and Coustau, 2005). Consequently, even if miracidia doses are used, a large fraction of snails will never become infected (Fig. 2). However, these uninfected snails remain compatible with other schistosome isolates that have retained a different set of phenotypes able to match. Consistent with this hypothesis, the BS-90 strain of *B. glabrata* considered as resistant was exposed to several isolates of *S. mansoni* and it was found to be susceptible to a parasitic isolate from a Brazilian locality (Theron et al., 2014).

Histological studies corroborate the hypothesis developed in the previous paragraph. In sections of some snails exposed to 20 miracidia, it was observed both developing and encapsulated primary sporocysts within the tissues (Theron et al., 1997). This means that susceptibility is not a general feature of a snail. Indeed, the phenotype (compatible vs. incompatible) of a snail is a function of the phenotype of the entering parasite. Conversely, infectivity appears not to be a general feature of the parasite, it depends on the host phenotype it infects. Based on this concept of a phenotype-by-phenotype interaction (Basch, 1975), the various levels of compatibility observed in the field and laboratory will be highly

dependent on the phenotypic compositions of the hosts and parasites in interaction.

A matching-phenotypes model is believed to be based on a system of self/nonself recognition or mimicry molecules, and recent efforts have sought to identify the putative interacting molecular determinants. In this context *S. mansoni* antigens (SmPoMucs) and *Biomphalaria glabrata* immune receptors (FREPs) were discovered. These molecules are highly diversified (Roger et al., 2008b; Zhang et al., 2004) and they interact (Mone et al., 2010) to define the compatibility/incompatibility of the specific interaction of one snail with one schistosome. Specific knockdown of the most diversified FREP (FREP 3) demonstrated the importance of FREPs, as it increased (by more than 20%) the compatibility of a schistosome strain towards a snail strain known to be completely incompatible (BS-90). Although we cannot exclude the possibility that other FREPs could contribute to compatibility, this partial recovery of susceptibility could be explained by the involvement of other immune receptors (e.g., other lectins) plus other schistosome antigens (see above) that could collaborate with FREPs and SmPoMucs to define the match-mismatch status responsible for the compatibility status of a specific snail-schistosome interaction. We must note, however, that some of the results described in the previous section cannot be explained only by a matching phenotype hypothesis based on immune receptors and antigens. Indeed, some studies clearly showed that snail strains or populations can produce immune effectors, and that the production levels of some such effectors are clearly linked to an increase in snail resistance. For example, the ROS production ability of snails is clearly linked to their ability to resist schistosome infection. Snail strains that produce more ROS in their haemocytes or plasma are clearly less compatible (or more resistant) to schistosome strains (Hahn et al., 2001; Mone et al., 2011), even those living in allopatry (Fig. 4). Even without knowledge of the underlying mechanisms, this explanation could be generalized to isolates or strains of *B. glabrata* that are clearly less susceptible when confronted by a large number of isolates and strains of *S. mansoni* (Richards and Shade, 1987).

Given the diversity of snail immune receptors and effectors and the wide variety of parasitic antigens and antieffector systems that have come to light in the past 15 years, we could expect coevolutionary processes to have selected specific adaptations within different interacting populations (Fig. 4). Such coevolution was clearly demonstrated by a recent study in which infection experiments were performed with different populations of *B. glabrata* and *S. mansoni*, testing allopatric and sympatric combinations (Theron et al., 2014) (Fig. 5). In this study, Theron and collaborators have quantified the prevalence following infection by sympatric or allopatric strains of *S. mansoni* of four different geographical isolates of *B. glabrata*. The results showed that compatibility was generally higher when sympatric snails and schistosomes were used, but some schistosome

populations were more able to infect allopatric snails while some snail populations were less permissive to allopatric schistosomes (Fig. 5).

To conclude this section, we propose that the mechanism underlying snail/schistosome compatibility is a multiprotein process in which the weight of the different genes encoding these proteins seem to vary within and between populations. Beyond these genetic contributions to compatibility, several other parameters have been shown to influence the fate of an infection, including environmental parameters and the history of interaction between a snail and schistosomes. These two aspects are developed in Section 8.

8 A SNAIL'S HISTORY OF INTERACTION WITH A SCHISTOSOME CAN INFLUENCE A SUBSEQUENT INFECTION

Similar to vertebrates, invertebrates also are confronted by an environment filled with complex changing populations of pathogens. As would be expected given such dynamic interactions, invertebrates possess a wide range of immune receptors; among them, some diversify somatically (Dong et al. 2006, 2012; Zhang et al., 2004). These results and this unexpected level of specificity prompted researchers to question whether invertebrates may possess the second feature of adaptive immunity, i.e., immune memory, especially in long-lived species that might encounter the same pathogen several times during their lifespan. Indeed, accumulating evidence indicates that invertebrates, including *B. glabrata*, may be primed in a sustainable manner, leading to the failure of a secondary encounter with the same pathogen (Kurtz, 2004; Kurtz and Franz, 2003; Portela et al., 2013; Rodrigues et al., 2010; Sadd and Schmid-Hempel, 2006).

The first set of experiments demonstrating the existence of immune memory in *B. glabrata* was performed by Lie et al. on a trematode species belonging to the *Echinostoma* genus. The authors demonstrated that *B. glabrata* experimentally infected by *Echinostomes* were able to respond faster and neutralize a new infection when reexposed to the same pathogen (Lie and Heyneman, 1979; Lie et al., 1975, 1982). The first report of immune memory in the *B. glabrata*/*S. mansoni* interaction dates to the late 1990s, when a key study revealed that by 10 days after primo-infection of *B. glabrata* by schistosomes, snails were totally protected against secondary infections for the rest of their lifespans (Sire et al., 1998). More recently, the existence of this immune memory was confirmed using different combinations of snail and parasite populations, and the specificity of the priming was demonstrated using different homologous and heterologous primary and secondary infections (Portela et al., 2013). The protection was complete (100%) for homologous infections, whereas the protection against heterologous infections decreased with increasing genetic distance between the parasites used for the primary and secondary infections. Thus, these authors demonstrated the

presence of a genotype-dependent innate immune memory in *B. glabrata* (Portela et al., 2013).

Histological analysis has revealed that in primed snails, the parasites of a secondary challenge fail to develop into sporocysts and are killed by the host without any observable cellular response and encapsulation. A very recent study addressed the molecular mechanisms underlying this immune memory using transcriptomic and proteomic analyses of snail plasma (Pinaud et al., 2016). The results suggest that there is a shift from a cellular to humoral immune response during the development of immune memory in *B. glabrata*. After a primo-infection, further encounters with a parasite of a similar genotype will induce a humoral response involving multiple pattern recognition receptors (PRRs), including FREPs, and cytotoxic/cytolytic immune effectors (Pinaud et al., 2016). The same authors found that siRNA-mediated knock-down of FREPs reduced this immune memory by 15% (Pinaud et al., 2016). These findings indicate that although FREPs appear to be involved in *B. glabrata* immune memory, additional molecular determinants also contribute to the process.

These findings prompt us to speculate that the genotype-dependent immune memory of *B. glabrata* may be supported by a diverse repertoire of FREPs and other PRRs. As previously suggested (Dheilly et al., 2015), PRRs might serve as collaborative recognition factors that can be processed as homologous or heterologous multimers that subsequently act as immune recognition complexes to increase the host's recognition repertoire and initiate the antipathogen response. Upon recognition, the snail's system neutralizes the pathogen via the release of cytotoxic/cytolytic circulating humoral factors (Pinaud et al., 2016; Portela et al., 2013).

9 EPIGENETICS APPEAR TO MAKE THE SYSTEM EVEN MORE COMPLEX

9-1 Snail epigenetics : the stress pathway and plasticity in susceptibility of *Biomphalaria glabrata* to infection with *Schistosoma mansoni*

As the obligate intermediate host for schistosomes, the snail provides the internal milieu necessary for the larval development of the parasite. However, not every snail that encounters a parasite in the short-lived, free-swimming form (miracidium) that hatches from the egg can sustain their development. Over the past two decades, researchers have used this interesting difference in susceptibility to dissect the mechanism(s) responsible for shaping the outcome of the snail/schistosome interaction. By investigating laboratory-maintained and pedigreed *B. glabrata* snail stocks that are either resistant or susceptible to specific strains of *S. mansoni*, researchers have gained a deeper understanding of the molecular basis of the host pathogen-relationship. As mentioned, many factors influence the snail/schistosome interaction. However, advances in “omics”

science have enabled the recent identification of several transcripts that are differentially induced in resistant or susceptible snails during the early response to parasitic infection (reviewed in (Knight et al., 2014; Coustau et al., 2015).

Stress protein-encoding transcripts, including those encoding HSP70, HSP90, and the reverse transcriptase of the non-LTR retrotransposon, nimbus, are differentially expressed in resistant (BS-90) and susceptible (NMRI) snails (Ittiprasert et al., 2009; Ittiprasert and Knight, 2012). The transcription of these genes is upregulated within the first 2 h following miracidial penetration in NMRI snails, whereas this induction is delayed and reduced in BS-90 snails. Moreover, when heat shock was applied to BS-90 snails at a nonlethal temperature of 32C, triggering the induction of stress-encoding transcripts prior to infection, these resistant snails became susceptible to the parasite. Conversely, the pretreatment of susceptible NMRI snails with the HSP90 inhibitor, geldanamycin, blocked infection in these snails (Ittiprasert and Knight, 2012).

To investigate the significance of the snail's early parasite-induced stress response in relation to plasticity in susceptibility, the epigenome at the HSP70 locus was examined before and after exposure to either normal or irradiation-attenuated miracidia. Fluorescence in situ hybridization was used to localize this particular locus in interphase nuclei of *B. glabrata* cells from tissue culture, the Bge cell line (Odoemelam et al., 2009), and entire snails (Arican-Goktas et al., 2014). The results revealed that during the early stages of infection, schistosomes orchestrate reprogramming of the snail host genome: the HSP70 locus undergoes nonrandom relocalization within the nucleus, followed by transcription (Fig. 6). This so-called "spatial epigenetic change" (Bridger et al., 2014) occurred when snails were exposed to wildtype miracidia but not attenuated miracidia. Moreover, the HSP70 locus is hypomethylated at its CpG dinucleotide sites, concurrent with its transcriptional upregulation in susceptible snails (Knight et al., 2015). Changes in epigenetic marks at this locus are seen within the same timeframe (i.e., soon after infection, when the gene undergoes spatial relocation within the nucleus) in NIMR snails but not BS-90 snails. These findings indicate that schistosomes manipulate the genome of the snail host, presumably to support infection and productive parasitism.

Ongoing studies are focussing on the epigenetic modulations and schistosome susceptibility in resistant BS-90 snails cultured at the permissive temperature of 32C. Progeny produced at this temperature have been found to be susceptible to infection at 25C as early as the first filial generation. This suggests that we should perhaps be concerned about the effect of climate change on the transmission and spread of schistosomiasis into previously nonendemic regions. As an example of such a spread, the disease has reemerged in southern Europe, disturbingly due to a hybrid parasite (*S. haematobium*/*Schistosoma bovis*) known previously from Senegal (Webster et al., 2013; Mone et al., 2015).

In closing, it is clear from these data that the interplay that occurs at the interface of the *B. glabrata*/*S. mansoni* host-pathogen model is intriguing and deserves further investigation. For example, it is difficult to conceptualize how, within a short 2-h timeframe postexposure, invading miracidia can alter the genomic behaviour of a susceptible snail host (Fig. 7). We also need to identify the factor(s) that are released from wild-type (but not radiation-attenuated) miracidia to stimulate rapid epigenetic modulation and regulate stress-related genes to either support or block the parasite's development in the snail host. The future characterization and identification of such factors would suggest new research avenues aimed at blocking the transmission of schistosomiasis.

9.2 Schistosome Epigenetics

In all parasites in which the underlying mechanisms have been analysed sufficiently, there is evidence that variants are generated via a complex interplay of genetic determinants and epigenetic regulations (i.e., an ensemble of genetic and epigenetic information). One of the best-analysed examples is the expression of var genes in *Plasmodium falciparum*. This organism uses roughly 60 genes and their complex epigenetic modification (i.e., the displacement of chromosomes in the nucleus and concomitant histone modifications of individual genes) to produce phenotypic variations of adhesion proteins on the surfaces of the red blood cells that harbour the parasite (reviewed in (Ay et al., 2015)). This bars the adaptive immune system of vertebrates from clearing the infection, and the host never becomes resistant. Another classical example is the African trypanosome. In contrast to plasmodium, which hide within blood cells, trypanosomes swim with the bloodstream and are constantly exposed to attack by the vertebrate immune system. The genetic rearrangement of about 1200 surface-protein-encoding genes is the major source of diversity for this organism (reviewed in (Gunzl et al., 2015)), but the regulated expression of these diverse proteins relies on epigenetic mechanisms that are very similar to those observed in apicomplexans. The displacement of a chromosome to a transcriptionally permissive region of the nucleus, histone modifications and nucleosome replacement are used to express one gene at a time. Moreover, these systems are also used to switch expression as soon as the surface proteins come under attack from the immune system.

For *S. mansoni*, the situation is likely to differ from those experienced by the abovementioned parasites. Instead of escaping from the immune response, *S. mansoni* adults can modulate the immune system of their definitive (vertebrate) host. Despite extensive research efforts studying this phenomenon, however, its precise mechanism is still not clear, and we can only speculate on the potential involvement of epigenetics. One of the major pathogenic effects of *S. mansoni* in vertebrates is egg production. Eggshell formation, particularly expression of the

eggshell protein, Smp14, depends on nuclear receptors and coactivators with chromatin-modifying activities, such as the histone acetylation-modulating proteins, SmGCN5 and SmCBP1 (Carneiro et al., 2014). For the interaction of *S. mansoni* with the invertebrate host, in contrast, the importance of epigenetic control has been shown in Fig. 8. In contrast to apicomplexans and kinetoplastids, where phenotypic variants are generated consecutively to assure the success of infection within an individual host, schistosomes appear to apply epigenetically generated diversity on a population level. For the SmPoMucs, we showed that ratio of H3K9 methylation (a classical heterochromatin marker) to H3K9 acetylation (a euchromatin marker) correlated perfectly with the transcription level comparing compatible and incompatible strains of *S. mansoni* (SmBRE and SmGH2 respectively) (Perrin et al., 2012) (Fig. 8). When miracidia were treated with an epimutagenic agent, such as inhibitor of class II histone deacetylases, transcriptional polymorphisms increased and more SmPoMuc variants were observed in a given population of miracidia (Cosseau et al., 2010). Since the epigenetic status of SmPoMucs is faithfully transmitted through generations (Perrin et al., 2012) and epimutations are heritable in this species (Roquis et al., 2016), it is conceivable that SmPoMuc epimutations are maintained as long as they provide a strong fitness effect.

Changes in DNA methylation (Geyer et al., 2011) and noncoding RNAs (Oliveira et al., 2011) have also been shown or proposed to be involved in the infection success of *S. mansoni*. Chromosome architecture has not yet been studied in this context, but it might be a promising target. Since profound changes in histone modifications occur during the development of schistosoma (Roquis et al., 2015), histone-modifying enzymes are likely to represent new drug targets (Stolfa et al., 2014).

In summary, we expect that epigenetic control of genetic determinants in schistosomes is also a major component of virulence and host compatibility. Consequently, as seen for other parasites, hybridization or environmental stress might trigger epigenetic changes that allow infection of new snail hosts, potentially driving the spread of the disease to nonendemic areas.

10 CONCLUSION

The results obtained in last decade clearly show that the compatibility between *B. glabrata* and *S. mansoni* depends on: (1) the genetics of the snail and the schistosome; (2) the age of the snail; (3) the previous interactions of the snail with schistosomes; and (4) the environment (through epigenetic mechanisms) that could influence the compatible/incompatible phenotypes of both partners. This yields a complicated puzzle, and we need to develop novel approaches to address this high level of complexity. We propose that researchers should generate integrative approaches that combine comparative genomic, epigenomic, and

transcriptomic approaches under different environmental conditions. This should enable the identification of relevant candidate genes, environmental factors, and developmental factors, and the assessment of their relative contributions to the compatibility of snails and schistosomes. The function of these candidates should then be elucidated using CRISPR/Cas or RNAi methodologies. Given the variability of the mechanisms involved in compatibility, such studies must be undertaken on different snail and schistosome populations and strains. These ambitious approaches are absolutely necessary if we hope to identify the molecular pathways that are most likely to be good targets for therapeutic strategies aimed at blocking transmission through the snail intermediate host.

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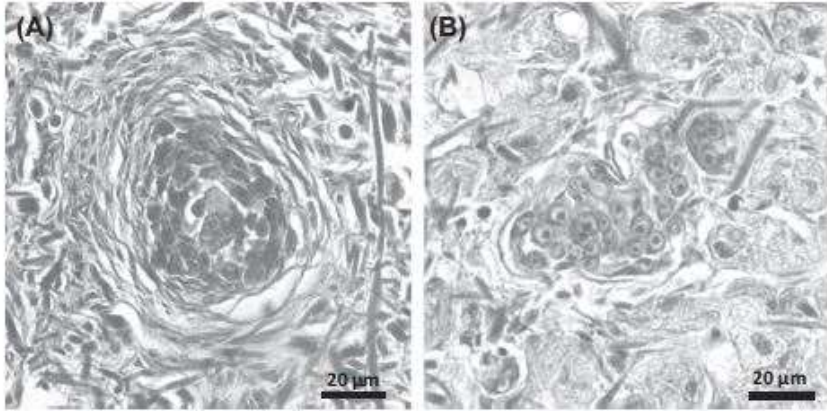
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Figure 1

Schistosoma mansoni that show incompatibility (A) or compatibility (B) with *Biomphalaria glabrata*. (A) In an incompatible combination, the parasite is recognized as nonself after penetration. It is then encapsulated and killed by haemocytes, and a multilayer haemocyte capsule surrounding a dead sporocyst can be observed. (B) In a compatible combination, the parasite is not recognized and develops normally in snail tissues. The primary sporocyst (Sp1) is seen growing in the head-foot of the snail after penetration.



Incompatible combination :
Sp1 encapsulated by hemocytes.

Compatible interaction:
growing Sp1.

Figure 2

Schistosoma mansoni polymorphic mucins (SmPoMucs) are highly diversified antigens. SmPoMuc diversification is controlled by multiple mechanisms at the genomic level, as well as during protein expression. Recombinations, epigenetic control of gene expression, trans-splicing, alternative or aberrant splicing, and posttranslational glycosylations account for the high level of diversification. The consequence is that each individual of a population harbours a specific pattern of SmPoMucs and their glycosylation. This specific antigenic pattern is probably a key component that helps define the compatibility status of a specific schistosome towards a specific mollusk.

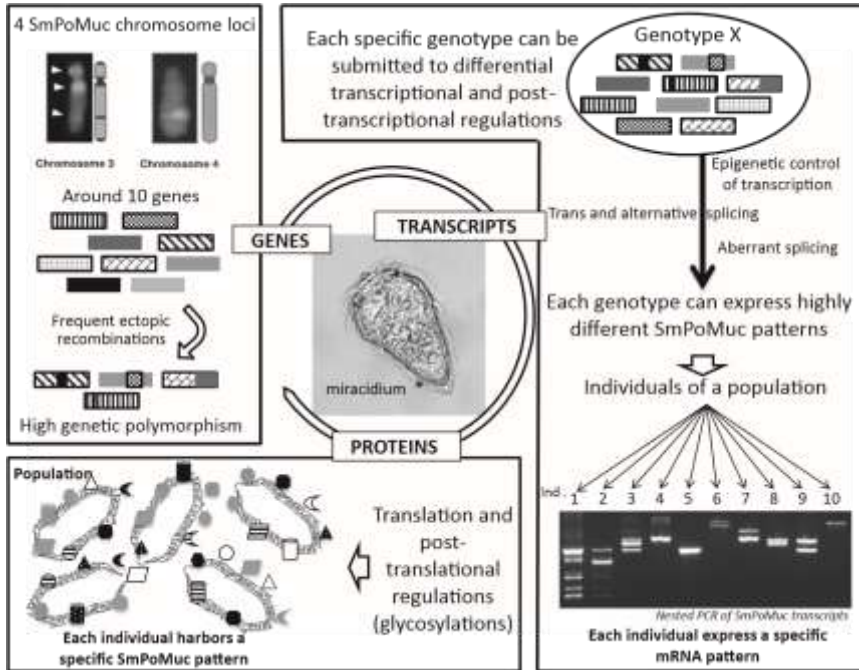


Figure 3

Compatibility of *Schistosoma mansoni*/*Biomphalaria glabrata* in the field versus the laboratory. Changes in levels of compatibility are seen among: (A) wildtype parasites and wild-type snails directly collected from the field; and (B) established laboratory strains derived from these populations. Individual snails were exposed to increasing doses of *S. mansoni* miracidia (Mi), and infection rates were enumerated. The observed reduction in compatibility (arrows) may be explained under the hypothesis of a matching phenotype model of interaction. The symbols represent phenotypic diversity in the compatibility between *B. glabrata* snails and *S. mansoni* parasites. Modified from Mitta, G., Adema, C.M., Gourbal, B., Loker, E.S., Theron, A., 2012. Compatibility polymorphism in snail/schistosome interactions: From field to theory to molecular mechanisms. *Dev. Comp. Immunol.* 37, 1e8.

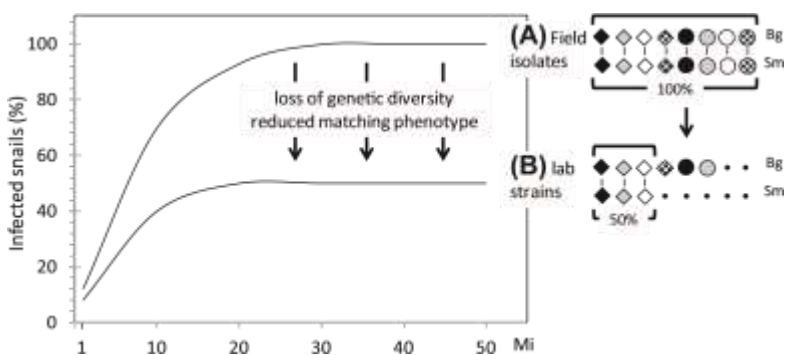


Figure 4

Compatibility is a multigenic process involving different immune receptors/ antigens (molecular pathways 1 and 2) and effectors/antieffectors (molecular pathways 3 and 4) from the snail and parasite. We postulate that the compatibility status of a specific host-parasite combination (A, B, N) depends on the investment of each individual in the different pathways. The matching phenotypes of the parasite's antigens/the snail's immune receptors and the snail's defence factors/the parasite's virulence factors have coevolved to define the compatibility status. Such a coevolutionary process would be expected to yield a wide variety of compatibility mechanisms within and between populations of snails and schistosomes.

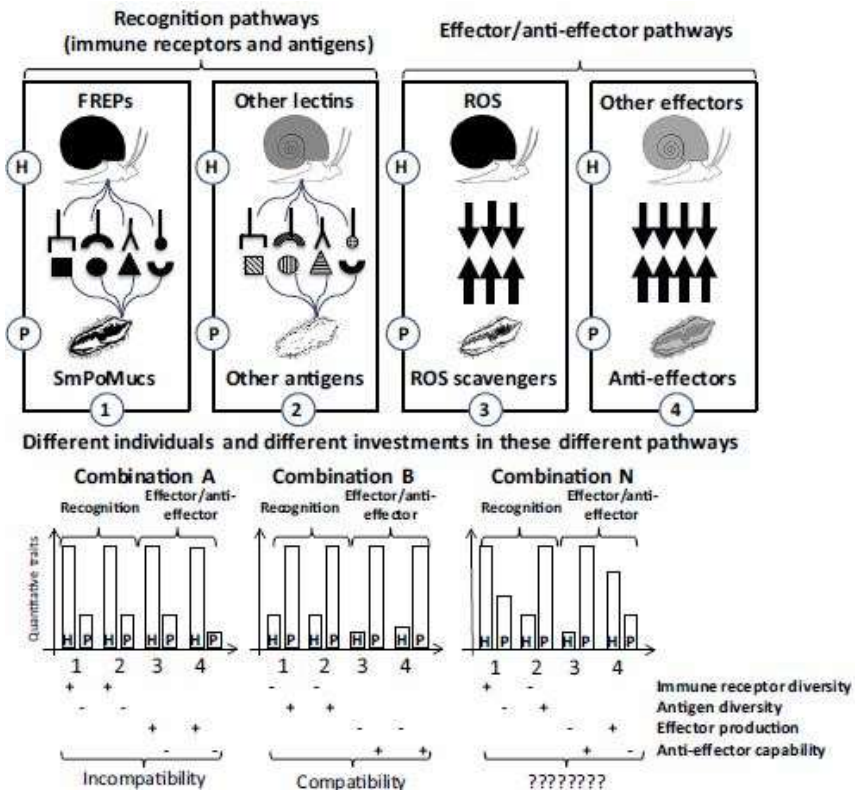
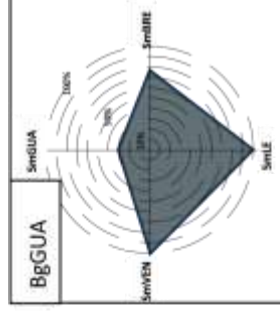
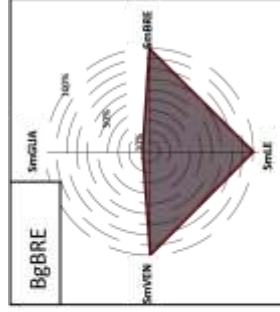
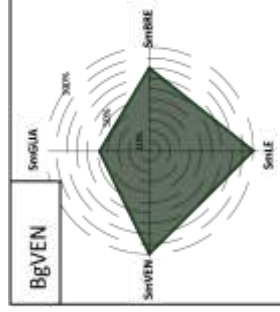
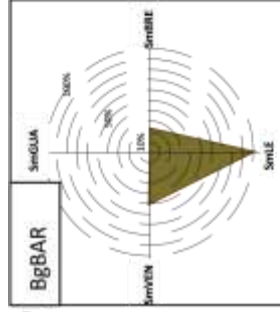


Figure 5

Radar graph representations of (A) the susceptibility phenotypes of four *Biomphalaria glabrata* isolates (BgBAR, BgVEN, BgBRE, and BgGUA) towards four *Schistosoma mansoni* isolates (SmLE, SmVEN, SmBRE, and SmGUA) and (B) the infectivity phenotypes of the same strains of *S. mansoni* toward the same isolates of *B. glabrata*. The presented infection rates (prevalence in %) correspond to an exposure dose of 20 miracidia/snail. The homopatric snail/parasite combinations are BgBAR/SmLE, BgVEN/SmVEN, BgBRE/SmBRE, and BgGUA/ SmGUA; these correspond to populations from Belo Horizonte (Brazil), Guaraca (Venezuela), Recife (Brazil) and Dans Fond (Guadeloupe island), respectively. Modified from Theron, A., Rognon, A., Gourbal, B., Mitta, G., 2014. Multiparasite host susceptibility and multihost parasite infectivity: a new approach of the *Biomphalaria glabrata*/*Schistosoma mansoni* compatibility polymorphism. *Infect. Genet. Evol.* 26, 80e88.

(A)



(B)

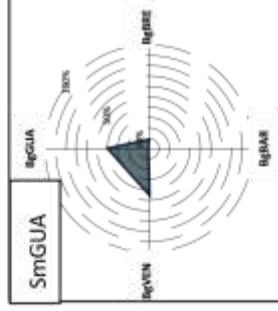
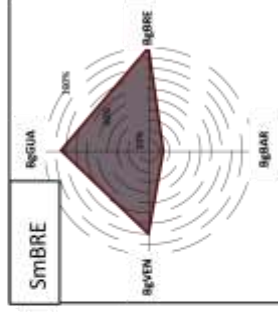
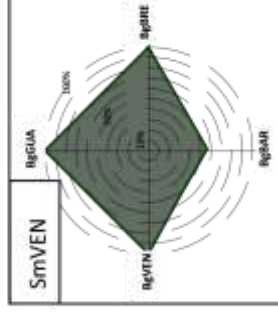
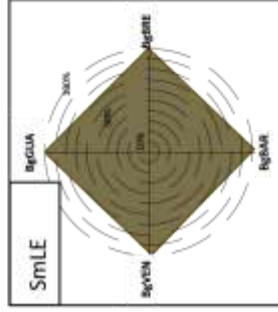


Figure 6

The HSP70 gene loci is relocated in susceptible *Biomphalaria glabrata* snail interphase nuclei 2 h after exposure to *Schistosoma mansoni* miracidia. Images A and B display HSP70 gene loci delineated in extracted and fixed nuclei by fluorescence in situ hybridization using specific labelled BAC probes. The stained gene loci appeared as stained dots in the nucleus. Over a period of 2 h the gene loci have relocated from an area more towards the nuclear edge to a nuclear location that is much more interior (panel C). This is measured in the snail nuclei by a bespoke erosion script, where the signal intensity of the gene foci are measured and normalised by the division of the intensity of the DNA signal (Arıcan-Goktas et al., 2014). This relocation is concomitant with the switching on of the HSP70 gene transcription at 2 h postinfection. The data are very similar to the situation in vitro when Bge cells are exposed to miracidia (Odoemelam et al., 2009). It should be noted that if the miracidia are irradiated to attenuate them the HSP70 gene loci neither relocate nor express transcript. Scale bar 5 μ m.

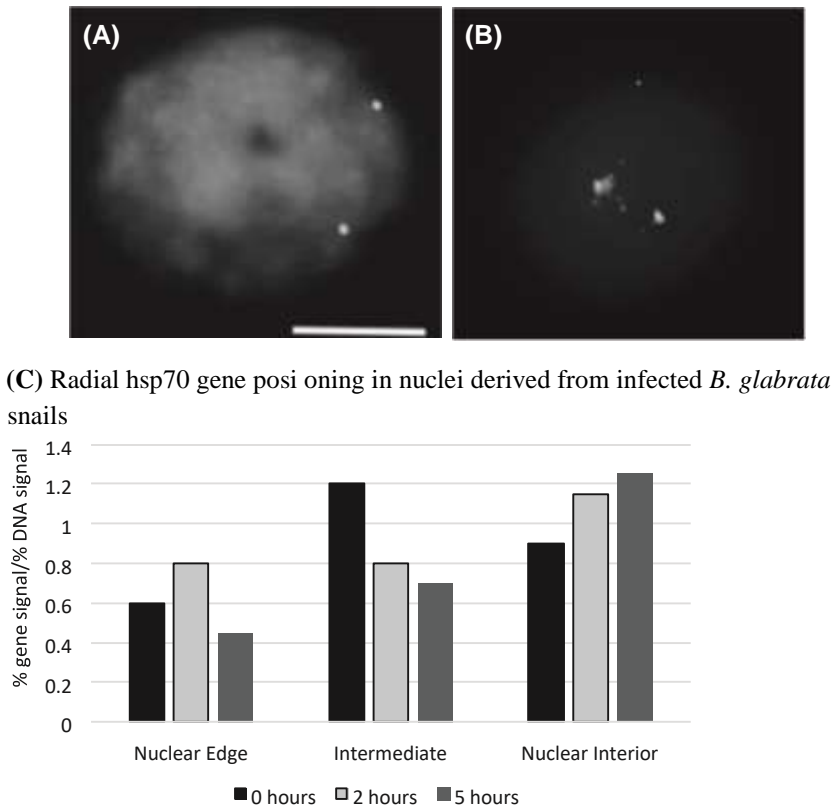


Figure 7

A model to describe the putative temporal events following exposure of Bge cells (a cell line derived from embryonic *Biomphalaria glabrata* explants), and susceptible *B. glabrata* snails (NIMR strain) to *Schistosoma mansoni* (NMRI strain) miracidia. The model shows a soluble ligand (pentagon), a stimulant, that is secreted by the parasite into the cell media or snail tissue. This stimulant is external to the cells both *in vitro* and *in vivo* and is shown as binding to receptors on the plasma membrane of cells after which cell signalling pathways send a signal to the nucleus that alters the epigenetics of specific genes, in this case hypomethylation of the HSP70 loci (black), which signals that these genes should be activated. This is followed by relocation of the gene loci to a new area of the nucleus, possibly to a transcription factory where transcripts are produced (dark grey) to be exported to the cytoplasm and translated into protein for the benefit of the parasite. This, we believe, all happens within the first few hours after an infection. Data for this model come from Odoemelam, E., Raghavan, N., Miller, A., Bridger, J.M., Knight, M., 2009. Revised karyotyping and gene mapping of the *Biomphalaria glabrata* embryonic (Bge) cell line. *Int. J. Parasitol.* 39, 675e681; Arican-Goktas, H.D., Ittiprasert, W., Bridger, J.M., Knight, M., 2014. Differential spatial repositioning of activated genes in *Biomphalaria glabrata* snails infected with *Schistosoma Mansoni*. *PLoS Negl. Trop. Dis.* 8, e3013; Knight, M., Elhelu, O., Smith, M., Haugen, B., Miller, A., Raghavan, N., Wellman, C., Cousin, C., Dixon, F., Mann, V., Rinaldi, G., Ittiprasert, W., Brindley, P.J., 2015. Susceptibility of snails to infection with schistosomes is influenced by temperature and expression of heat shock proteins. *Epidemiology* 5.

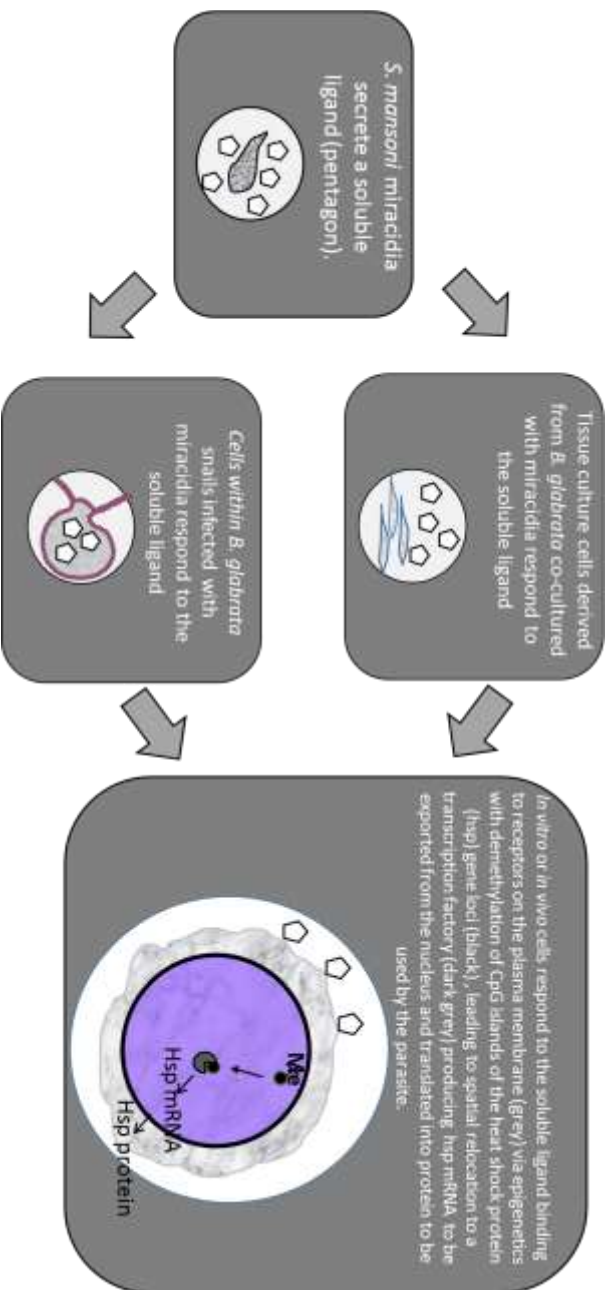


Figure 8

Transcription level and chromatin status of *Schistosoma mansoni* polymorphic mucins group 3.1 (r1r2) in two strains of *S. mansoni*: SmBRE and SmGH2 show differences in infection success towards a panel of *Biomphalaria glabrata* reference strains (Fig. 5). The level of transcription of a specific group of SmPoMucs is clearly different (left panel) and correlates with the chromatin status (right panel). Adapted from Perrin, C., Lepesant, J.M., Roger, E., Duval, D., Fneich, S., Thuillier, V., Alliene, J.F., Mitta, G., Grunau, C., Cosseau, C., 2012. *Schistosoma mansoni* mucin gene (SmPoMuc) expression: epigenetic control to shape adaptation to a new host. *PLoS Pathog.* 9, e1003571.

