Sympatric and allopatric evolutionary contexts shape differential immune response in
*Biomphalaria / Schistosoma* interaction.

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Abstract

Selective pressures between hosts and their parasites can result in reciprocal evolution or adaptation of specific life history traits. Local adaptation of resident hosts and parasites should lead to host-parasite systems performing better in sympatry when compared to allopatry. Between-population variations in parasite infectivity/virulence and host defence/resistance, referred to as compatibility phenotype, were often the proxy used to analyse sympatric or allopatric adaptation. Nevertheless, some reported cases exist where allopatric host-parasite systems demonstrate compatibility phenotypes similar or greater than the one observed in sympatry. In these cases, the role of local adaptation is worth considering.

Here, we study the interaction between Schistosoma and its vector snail Biomphalaria in which such a discrepancy in local versus foreign compatibility phenotype has been observed. Herein, we developed an integrative approach to investigate sympatric and allopatric interaction processes and link the underlying molecular mechanisms to the resulting phenotypes. Using comparative “omics” approaches joined to analysis of life history traits (immune cellular response, mortality, prevalence and compatibility) we tried to bridge the gap of knowledge that exists for connecting local adaptation observations to molecular phenotypes in Schistosoma/Biomphalaria interactions.

We found that despite displaying similar prevalence phenotypes, parasite infection triggered an immune suppression in snails living in sympatry, while it activated an immune response for those living in allopatry. Dual-comparative molecular analyses revealed that parasite infection causes immune suppression in sympatry. miRNAs were used to hijack the host's immune response, allowing sympatric parasites to initiate their developmental program earlier and more efficiently.

We show that despite having similar prevalence phenotypes, sympatric and allopatric snail-Schistosoma interactions displayed a strongly different immunobiological molecular dialogue. The ability of allopatric pathogens to adapt rapidly and efficiently to new hosts could have critical consequences on disease emergence and risk of schistosomiasis outbreaks. These observations would have important consequences in term of schistosomiasis disease control.

Keywords: sympatry, allopatry, host-parasite compatibility, RNA-seq, immunity
Introduction

Schistosomiasis is the second most widespread human parasitic disease after malaria and affects more than 200 million people worldwide (1). *Schistosoma mansoni* (Platyhelminthes, Lophotrochozoa) causes intestinal schistosomiasis. *Schistosoma* needs a mollusc (belonging to *Biomphalaria*, *Bulinus* or *Planorbarius* genus) to act as its first intermediate host, where it undergoes part of its life cycle before infecting humans. Patently infected snails support the continuous production of thousands of cercariae, the free-living human-infective stage of the parasite, until dying. As such, vector snails appear to be a central actor in the parasite transmission and thus have deserved greater attention as a potential target for schistosomiasis control. To achieve this objective, it is necessary to understand snail-parasite immunobiological interactions and characterize the molecular mechanisms by which snails and *Schistosoma* interact.

*Biomphalaria/Schistosoma* compatibility is based on a complex phenotype-to-phenotype or matching-phenotype model (2-7). 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. In the past 15 years, the molecular basis of this compatibility polymorphism has been investigated at the genomic level (8-10), the transcriptomic level (6, 11-15), the proteomic level (16-23) and the epigenomic level (24-29). These studies have revealed that various molecules and pathways involved in immune recognition (snail immune receptors versus parasite antigens), immune effector/anti-effector systems and immune regulation/activation participate in a complex interplay that governs the match or mismatch of host and parasite phenotypes (30). This complex phenotype-by-phenotype interaction (compatibility polymorphism) varies between populations and individuals resulting in “multi-parasite susceptibility” or “multi-host infectivity” phenotypes (5) that reflect between-population variations in parasite infectivity/virulence and host defence/resistance (31, 32).

In *B. glabrata/Schistosoma sp.* models, numerous sympatric and allopatric combinations have been tested: (i) different *B. glabrata* laboratory strains (or isolates) show various degrees of susceptibility to *S. mansoni* infection, and (ii) different strains of *S. mansoni* display different levels of infectivity toward a particular strain of snail host (2, 4, 5, 33, 34). In most cases, interaction in *B.*
glabrata/Schistosoma models has been investigated by comparing, (i) sympatric/compatible and (ii) allopatric/incompatible host-parasite associations. Indeed, the general assumption is that the parasites, which have a shorter generation time, larger population sizes and higher reproductive outputs, are ahead in the co-evolutionary conflict against their host and are therefore more likely to locally adapt to them (35, 36). This is exactly what is stated by the “host-parasite local adaptation hypothesis”, in which the sympatric parasite is supposed to perform better than the allopatric one (36-39). However, it has been observed that compatibility phenotypes between Biomphalaria and Schistosoma were the same comparing sympatric and allopatric combinations or even greater for allopatric parasites, and thus did not fulfil the “local versus foreign” criterion of the “host-parasite local adaptation hypothesis” (33, 40, 41). Such compatibility phenotypes have been mostly investigated in terms of parasite infectivity or pathogenicity toward snail hosts, but to our knowledge very few studies have investigated the molecular basis of allopatric compatible interactions from the perspective of both sides of the interaction, the host and parasite (42, 43).

Hence, in order to bridge this gap, we herein study sympatric/allopatric interactions displaying similar compatibilities using an integrative approach that links the underlying molecular mechanisms to the resulting phenotypes, based on comparative molecular approaches on both host snails and Schistosoma parasites. We set out to analyse the compatibility between South American snail strains (from Recife Brazil and Guacara Venezuela) in combination with three different parasite isolates that displayed nearly the same compatibility phenotype: (i) S. mansoni Recife Brazil, (ii) S. mansoni Guacara Venezuela (both sympatric and allopatric, narrow geographic scale) and (iii) S. rodhaini from Burundi Africa (allopatric, large geographic and phylogenetic scales). Thus, even if compatibility phenotypes (prevalence/intensity) are similar, the characterization of the underlying molecular mechanisms demonstrates a very different immunobiological dialogue between B. glabrata vector snails and their sympatric or allopatric Schistosoma parasites at the cellular and molecular scales.

To the best of our knowledge very few studies have investigated the molecular basis of local adaptation in host/parasite interactions (44, 45). Here we used a powerful global approach to identify the differentially regulated transcripts in snails of a single B. glabrata strain under infection by three sympatric or allopatric compatible strains or species of the parasite, Schistosoma sp.
Moreover, our results may be of critical importance in terms of schistosomiasis control. Understanding the mechanisms whereby allopatric parasites efficiently infect host snails, would be essential to develop new tools to predict or quantify risks of schistosomiasis disease outbreaks (46).

Materials and Methods

Ethical statements

Our laboratory holds permit # A66040 for experiments on animals, which was obtained from the French Ministry of Agriculture and Fisheries and the French Ministry of National Education, Research, and Technology. The housing, breeding and care of the utilized animals followed the ethical requirements of our country. The experimenter possesses an official certificate for animal experimentation from both of the above-listed French ministries (Decree # 87–848, October 19, 1987). The various protocols used in this study have been approved by the French veterinary agency of the DRAAF Languedoc-Roussillon (Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt), Montpellier, France (authorization # 007083).

Biological materials

Two snail strains of Biomphalaria glabrata were used in this study: the albino Brazilian strain, (BgBRE) and the Venezuelan strain, (BgVEN). They were exposed to infection by a Brazilian (SmBRE) or Venezuelan (SmVEN) strain of Schistosoma mansoni, or by another Schistosoma species, S. rodhaini (Srod). All host and parasite strains of each different geographical origin were recovered in their native locality and maintained in the laboratory always on their sympatric snail hosts to maintain reciprocal selective pressures and reciprocal sympatric strain adaptation. We housed snails in tanks filled with pond water at 25°C with a 12:12 hour light:dark cycle and supplied ad libitum with fresh lettuce. The Brazilian strain originates from the locality of Recife (east Brazil), the Venezuelan strains of snail and parasite were recovered from the locality of Guacara (north Venezuela) and the African species Schistosoma rodhaini originates from Burundi and was obtained from the London British Museum. These Schistosoma isolates/species have been selected because they exhibited similar infectivity toward BgBRE or BgVEN strains (see prevalence and intensity in
supplementary table S1). Prevalence of SmBRE and SmVEN for the African vector snail
*Biomphalaria pfeifferi* from Senegal (BpSEN), and prevalence of the corresponding parasite SmSEN
on South American snails were also tested (supplementary table S1).

**RNAseq experimental protocol**

In order to investigate the molecular response of snails against sympatric and allopatric parasites, a
global comparative transcriptomic approach was conducted. One hundred and twenty BgBRE snails
were infected with SmBRE, SmVEN or Srod. Each snail was individually exposed for 12 h to 10
miracidia in 5mL of pond water. For each experimental infection, 20 snails were recovered at 24 and
96 h after infection. The samples were named as follows: BB24, BB96 for BgBRE infected with
SmBRE; BV24, BV96 for BgBRE infected with SmVEN; and BR24, BR96 for BgBRE infected with
Srod. Two pools of 30 uninfected BgBRE snails (Bre1 and Bre2) were used as controls.

- **Whole-snail RNA extraction and sequencing**

Total RNA was extracted using TRIZOL® (Sigma Life Science, USA) according to the
manufacturer’s instructions. Sequencing was performed using paired-end 72-bp read lengths on
Illumina Genome Analyzer II (MGX-Montpellier GenomiX, Montpellier, France).

- **De novo transcriptome assembly**

*De novo* transcriptome assembly, using all time points, was performed using an in-house pipeline
created with the Velvet (1.2.02), Oases (v0.2.04) and CD-HIT-EST (v4.5.4) programs. The assembly
of the consensus reference transcriptome was optimized using various parameters, including k-mer
length, insert length and expected coverage, as previously described (47, 48). A de novo transcriptome
was created that contained 159,711 transcripts.

- **Differential expression analysis**

High-quality reads (Phred score >29) were aligned to the de novo transcriptome using Bowtie2
(v2.0.2), which was run locally on a Galaxy server. To compare the host responses during the
sympatric or allopatric interactions, we used the DESeq2 (v2.12) software to analyse the differential
transcript representation between *BgBRE* control strains (uninfected BgBRE1 and BgBRE2) to the
sympatric and allopatric conditions (p-value < 0.1) (48). A Venn diagram was generated using the
Venny 2.1 software to highlight which differentially expressed transcripts were specific or common to the different interactions. A heatmap was then created to analyse transcript expression patterns, using log2 Fold Change with Hierarchical Ascending Clustering (HAC) and Pearson correlation (uncentered) as applied by the Cluster (v3.0) and Java TreeView (v1.1.6r4) software packages. The differentially represented transcripts were functionally classified using a BlastX analysis with the cut-off set to e-value < 1e^-3 (NCBI dataset; thanks to the Roscoff Data center Cluster, UPMC) and gene ontology was assigned using an automatic annotation, Blast2GO (v3.0.8) (supplementary table S2). Based on automatic annotation and functional domains and according to the literature we identified the potential immune transcripts involved in snail immunity.

Schistosoma intra-molluscal stage transcriptome analysis: Dual RNA-seq

Host response against pathogens constitutes solely one side of the molecular dialogue occurring in host/parasite interactions. To analyse all the complexity of such dialogue, the parasite response into host tissue also needs to be investigated. Herein a dual RNA-seq approach was thus conducted to gain a broader understanding of sympatric and allopatric host/parasite interactions.

- Schistosome read selection

The Biomphalaria (v1) and Schistosoma (v5.2) genomes have been concatenated (https://www.vectorbase.org/organisms/biomphalaria-glabrata; http://www.sanger.ac.uk/resources/downloads/helminths/schistosoma-mansoni.html). High-quality reads (Phred score > 29) were mapped against these concatenated genomes using Bowtie2 (v2.0.2), which was run locally on the Galaxy project server. The reads that mapped only once and exclusively to the Schistosoma genome were collected as corresponding to Schistosoma reads; reads that mapped to the Biomphalaria genome or more than once to either genomes were removed from the analysis.

- Gene analysis

The above-selected Schistosoma reads were mapped against the concatenate genome to identify intra-molluscal stage-specific Schistosoma genes. In order to select the relevant genes, the reads mapped in all experimental conditions were summed. Only genes with a minimal sum of 10 reads were kept for the analysis. A heatmap was generated to analyse Schistosoma gene expression patterns using
Hierarchical Ascending Clustering (HAC) with Pearson correlation (uncentered) as applied by the Cluster (v3.0) and Java TreeView (v1.1.6r4) software packages. Functional annotation of the genes was assigned using BlastX with the cut-off set to e-value < 1e^-3 (NCBI dataset, local cluster) and gene ontology was performed using Blast2GO (v4.0.7) (supplementary table S3).

Innate immune cellular response analysis: microscopy and flow cytometry

Hemocytes appeared as the main cells supporting the Biomphalaria snail immune response. Thus, to go further in the description of snail response against parasites, quantitative and qualitative changes in hemocyte populations were investigated. For this purpose, BgBRE and BgVEN snails were used. Snails were infected as described above, using either SmBRE or SmVEN parasites. For each experimental infection, snails were recovered at 24 and 96 h after infection and designated as follows: BB24 and BB96 for BgBRE infected with SmBRE; BV24 and BV96 for BgBRE infected with SmVEN; VV24 and VV96 for BgVEN infected with SmVEN; and VB24 and VB96 for BgVEN infected with SmBRE. Snails of each strain, BgBRE and BgVEN, were recovered and used as controls.

Hemocyte proliferation analysis: microscopy

Microscopic inspection of hemocyte proliferation was conducted using 12 infected BgBRE (6 BgBRExSmBRE and 6 BgBRExSmVEN) and 3 uninfected BgBRE snails. The hemocytes of 3 snails (biological replicates) were counted for each condition at 24 h and 96 h after infection. The proliferation of circulating hemocytes was studied by using a Click-iT EdU Alexa Fluor 488 Flow Imaging Kit (Molecular Probes). At each time point, circulating hemocytes were recovered by direct puncture after foot retraction and 1mM of EdU solution was added to the hemolymph. Three hours later, the amount of EdU incorporated by the circulating hemocytes was visualized in vitro after fixation of the cells and performing a covalent coupling of Alexa Fluor 488 to the EdU residues through a click chemistry reaction flowing manufacturer indications. Next, nuclei of hemocytes were counterstained with DAPI (Biotum) staining, and the sample was analysed on a confocal microscope using a Zeiss LSM 700, with 4 lasers (405, 488, 555 and 633 nm). Positive cells were counted and
between-sample differences in the percentage of proliferation were tested using a Fisher exact test, with significance accepted at p-value < 0.05.

**- Hemocyte proliferation and population profiles analysis: flow cytometry**

Qualitative changes in hemocyte populations following infection by sympatric or allopatric parasites were studied using a flow cytometry approach. For this, 72 infected BgBRE or BgVEN (36 infected by SmBRE and 36 infected by SmVEN) and 18 uninfected BgBRE or BgVEN snails were used. Six biological replicates (pools of three snails per replicate) were performed for each condition. Flow cytometry was used to profile and assess the proliferation of circulating hemocytes using Click-iTEdU Alexa Fluor 647 labelling (Molecular Probes). At each time point, 1mM of EdU solution was injected into pericardial cavity of each snail. Three hours later six replicates of three snails were collected, and the hemolymph was extracted from the head-foot according to standard procedures (49). The hemolymph was pooled from the three snails, and 100 µl were subjected to analysis with the above listed kit, according to the manufacturer’s instructions. The percentage of proliferative cells was calculated by flow cytometry.

The hemocytes were profiled along the course of infection using Side Scatter Chanel (SSC) to estimate cell granularity and Forward Scatter Chanel (FSC) to estimate cell size. The cell repartition along these two parameters enables the identification of cell subpopulations. The flow cytometry was performed using a FACS Canto from BD Biosciences (RIO Imaging Platform, Montpellier, France). For each sample, 10,000 events were counted. The results were analysed with the FlowJo V 10.0.8 software. Between-group differences in the percent of proliferation were tested using the Mann-Whitney U-test, with significance accepted at p-value<0.05.

**Histological procedures**

A histological approach was conducted in order to investigate differences in miracidia to sporocyst development, while comparing sympatric and allopatric parasite growth, development and maturation into snail tissues. BgBRE snails (n=8 per group) were infected as described above with either SmBRE (sympatric) or SmVEN (allopatric) parasites. At 24 and 96 h after infection, two snails were fixed in Halmi’s fixative (4.5% mercuric chloride, 0.5% sodium chloride, 2% trichloroacetic acid, 20% formol,
4% acetic acid and 10% picric acid-saturated aqueous solution). Both, embedding in paraffin and transverse histological sections (3-μm) were performed using the RHEM platform (Montpellier, France) facilities. The slides were stained using Heidenhain’s azan trichromatic staining solution as follows: (i) serial re-hydration was performed in toluene followed by 95%, 70% and 30% ethanol and then distilled water; (ii) coloration was performed using azocarmine G (70% ethanol, 1% aniline, 1% acetic alcohol, distilled water, 5% phosphotungstic acid, distilled water, Heidenhain’s azan) and (iii) serial dehydration was performed using 95% ethanol, absolute ethanol, and toluene. The preparations were then mounted with Entellan (Sigma Life Science, St. Louis Missouri, USA) and subjected to microscopic examination. The parasite sizes were measured with the ImageJ software (v2.0.0), and between-group size differences were tested using the Mann-Whitney U-test with statistical significance accepted at a p-value<0.05 based on the mean of the three largest serial cuts of each sporocyst in sympatric or allopatric conditions.

In-silico characterization of Schistosoma miRNAs

Parasites may communicate or interfere with their host using different strategies based mainly on excreted/secreted products released into hemolymph. In this context, miRNAs appeared as the most relevant means of communication that can be used by parasites. To test for such a hypothesis S. mansoni miRNAs were analysed in-silico by comparing the relevant miRNA database (miRBase) to our RNAseq libraries generated at the 24 h following sympatric or allopatric infections. S. mansoni precursor sequences were downloaded from miRBase (http://www.mirbase.org, 03/09/2017), and high-quality reads from naive (BgBRE) and 24 h post-infection samples (BB24, BV24, BR24) were aligned against a S. mansoni sub-database of miRBase, as previously described (50). The identified precursors were confirmed by alignment of high-scoring reads onto precursor and mature miRNAs from miRBase. Only reads with 100% identity were retained for analysis. The localization of each read against miRNA sequence allowed us to identify either the precursor or just the mature miRNA. Precursors found under both naive and infected conditions were excluded to retain exclusively the miRNAs present in samples from infected snails and avoid cross-species contamination because of the potential conserved features of miRNAs from B. glabrata and S. mansoni.
Putative miRNA targets were predicted from among the differentially represented immune-related transcripts (Fig. 1) using Miranda tools (using parameters: Miranda input_miRinput_Transcriptome -out results.txt -quiet -sc 140 -en 15) (51). Because mature miRNAs may exist in two forms depending on which strand (5'-3') of the precursor stem-loop is matured the predicted interactions could involve the 5' and/or 3' forms, as noted. The results were extracted using the awk tool, listed in supplementary files (Supplementary Table 4), and used to generate a Venn diagram. To confirm the ability of a selected pre-miRNA to produce the stem-loop necessary to produce the mature form, the secondary structures of precursor were predicted using a RNA structure Web tool (http://rna.urmc.rochester.edu/RNAstructureWeb, 03/09/2017) using default parameters.

**Snail survival analysis**

Allopatric or sympatric parasites could have different levels of virulence or impacts on their host that could impair snail survival. To test for such discrepancy we investigated the mortality rates of infected snails over the course of sympatric or allopatric infections. One hundred and sixty BgBRE snails were infected as described above with SmBRE or SmVEN strains (n = 50), and 60 non-infected BgBRE snails were retained as controls. The numbers of dead snails were compiled weekly for 14 weeks. A Kaplan-Meier estimator was used to estimate the survival function from lifetime data. Survival curves were generated using the xlstats Mac software and the log-rank test was applied with significance accepted at p < 0.05.

**Results**

*A whole-snail transcriptomic approach for investigating the molecular basis of the innate immune response in the sympatric and allopatric contexts*

The global transcript representation was analysed using the previously described RNAseq pipeline developed in our laboratory (6, 47, 48). The BgBRE snail host had the same prevalence and intensity phenotypes when exposed to SmBRE, SmVEN or Srod parasites (see Supplementary Table 1). Of the 159,711 transcripts of the BgBRE transcriptome, 3,865 (2.4%) were differentially represented in all sympatric and allopatric conditions compared to naive snails (Table 1, Supplementary Fig. 1). We
performed automatic Blast2GO annotation, discarded the non-annotated transcripts, and retained 1,017 annotated transcripts (26.3% of the differentially represented transcripts). Finally, as we were interested in the innate immune response of the snail to the sympatric and allopatric parasites, we focused on transcripts known to have immune-related functions. Of the annotated differentially expressed transcripts, 336 were identified as being immune-related; this subset corresponded to 8.7% of all differentially represented transcripts. The heatmap generated for all differentially represented transcripts is presented in Supplementary Fig. 1.

Venn diagrams were used to highlight the relationships of the transcripts that were differentially represented in the B. glabrata and Schistosoma sp. interactions. Overall, 189, 180 and 164 transcripts were differentially represented in the BB (BgBRE/SmBRE, sympatric), BV (BgBRE/SmVEN, allopatric) and BR (BgBRE/Srod, allopatric) interactions, respectively (Fig. 1A). Of the selected transcripts, 40 were shared by the BB, BV and BR interactions (Fig. 1A), and displayed globally the same differentially expressed pattern of expression (Fig. 1B, cluster 1). These transcripts may correspond to the snail’s basal immune response against Schistosoma parasites.

Of the 140 transcripts exclusive to the sympatric (BB) interaction, which represented 74.1% of the immune transcripts found to be differentially represented in this interaction (Fig. 1A), 100% were underrepresented at 24 h post-infection, and 74.6% of these transcripts were observed exclusively at this time point (Fig. 1B, cluster 5).

Very similar transcript expression patterns were observed for the two allopatric interactions (BV and BR). Clusters 6 and 7, comprising only 28 and 11 transcripts, were exclusive to BV and BR, respectively. In BV interaction (Fig. 1B, cluster 6), at 96 h after infection 96.5% of the transcripts were expressed: 22% over-represented. In BR interaction (Fig. 1B, cluster 7), 100% of the transcripts were expressed at 96 h after infection: 82% overrepresented. Of the 108 transcripts shared by BV and BR (cluster 3, representing 60% and 65.9 % of the transcripts, respectively) (Fig. 1A; Fig. 1B, cluster 3), 98.1% were expressed at 96 h post-infection: 28.2% over-represented (Fig. 1B, cluster 3).

Altogether, these results show that the host immune-related transcriptomic response differed widely between the sympatric and allopatric interactions. All of the differentially represented transcripts were under expressed at 24 h after infection in sympatry, while the differentially
represented transcripts in allopatry were mostly over expressed at 96 h after infection. Moreover, the sympatric interaction was associated with a strong immunosuppression (Fig. 1B, cluster 5), whereas allopatry yielded an activation of the immune response (Fig. 1B, cluster 3).

We decided to explore the function of differentially regulated transcripts between the sympatric and allopatric interactions in order to investigate if these differentially regulated transcripts belong to different functional categories. For that, we performed an automatic annotation with Blast2GO (Supplementary Table 2). Based on automatic annotation and according to the literature we distributed the relevant differentially expressed immune transcripts into three groups: (i) immune recognition molecules, (ii) immune effectors and (iii) immune signalling molecules (Fig. 1C). Then these three groups were subdivided into functional categories (Fig. 2). In the BB sympatric interaction, immune recognition molecules, immune effectors and immune signalling pathways correspond to 13.1%, 18.3% and 4.2%, respectively (sums of all relevant transcripts at all infection time points). Among the transcripts distributed into these groups, 89.0% were underrepresented. In the BV allopatric interaction, the three groups corresponded respectively to 10.8%, 12.9% and 8.6% and among them 65.9% were underrepresented. Finally, in the BR allopatric interaction, they corresponded to 12%, 12.5% and 7.8% and 62.5% of them were underrepresented. When we compared the percentage of each immunological group in the sympatric and allopatric interactions, no specific functional subset was particularly repressed in the BB sympatric interaction (Fig. 1C; Fig. 2).

In fact, most of the time, the same immune functions were affected in sympatric and allopatric infections but different immune transcripts (grey and black diamond in Fig. 2) showed differential regulation following infections (Fig. 2).

The differentially regulated transcripts belonging to the three immunological groups (Fig. 2) were largely involved in immune cellular responses, cell adhesion, extracellular matrix component, cell migration, cell differentiation and cell proliferation. All of these functions were under-represented in sympatric interaction (76%), whereas many were over-represented in allopatric ones (39%) at 24 h after infections (Fig. 2).

*Immune cellular responses in the sympatric and allopatric contexts*
As the transcriptomic approach indicated that multiple immunobiological functions seem to be altered in the host response during the interaction with the parasites, we next investigated whether differences in immune responses in sympatric versus allopatric infections could be seen at the cellular level. Hemocytes, the snail immune cells, participate directly in the immune response against the parasites, and immune cell activation under an immunological challenge can translate into cell proliferation and/or cell morphology modifications.

Thus, to investigate such immune cell activation, cell proliferation was quantified using EdU nuclear labelling (Fig. 3). EdU is a nucleoside analogue of thymine incorporated into DNA during DNA synthesis. This incorporation reflects the mitotic activity of hemocytes. As the two tested allopatric interactions appeared extremely similar at the transcriptomic level, we compared only the BV (allopatric) interaction and BB (sympatric) interaction for further analyses. Circulating hemocytes recovered in snail hemolymph were EdU-labelled in vitro to identify mitotic circulating hemocytes (Fig. 3A). EdU-positive hemocytes were quantified using confocal microscopy and showed that hemocyte proliferation was induced threefold following Schistosoma infection in BV compared to BB (Fig. 3B). Twenty-four hours after infection, proliferative circulating cells increase significantly (Mann Whitney U test: $U=36; z = -2.8; p = 0.0022$) by more than twofold between the allopatric and sympatric interactions, 4.2% compared to 1.8% respectively. At 96 h after infection, there were fewer proliferating cells: the percentage of proliferating hemocytes was similar in sympatric BB interaction compared to the control (1%, Mann Whitney U test: $U = 17; z = -0.27; p = 0.3936$), while remaining somewhat higher in the allopatric BV interaction (2.3%, Mann Whitney U test: $U = 0; z = 2.65; p = 0.009$).

To confirm that our observations were related to the type of interaction rather than to a strain effect, we performed the same experiments using another Biomphalaria glabrata strain, BgVEN as the
host and SmVEN and SmBRE as the sympatric and the allopatric parasite, respectively (Fig. 4B). The rate of proliferating hemocytes increased significantly (Mann Whitney U test: U = 36; z = -2.8; p = 0.0022) by more than threefold between the allopatric (VB: BgVEN/SmBRE) and the sympatric (VV: BgVEN/SmVEN) interactions at 24 h after infection (6.8% compared to 2.0% respectively). At 96 h after infection, both rates were decreased, albeit more for VV (0.1%, Mann Whitney U test: U = 2; z = 2.48; p = 0.013) than for VB (2.7%, Mann Whitney U test: U = 36; z = 2.8; p = 0.0022). These results similar to those observed for BgBRE interactions, confirmed that the effects observed at the cellular level were related to the type of interactions (sympatric versus allopatric) rather than to the host or parasite strains used.

In addition to the mitotic activity, hemocyte morphology was estimated using flow cytometry by measuring size and granularity. We analysed the circulating hemocyte populations in noninfected versus infected snails in two B. glabrata snail strains (BgBRE and BgVEN) in sympatric and allopatric interactions with parasites (Fig. 4C and 4D). Morphology and heterogeneity of circulating hemocytes varied in a similar manner between BgBRE and BgVEN snails subjected to infection (Fig. 4C and 4D) and we observed infection-related differences in cell morphology. In noninfected snails, the content of circulating hemocytes was very heterogeneous but appeared to represent a single population that exhibited a continuous gradient of size and granularity (Fig. 4C and 4D), which is typical of B. glabrata hemocytes (52). However, hemocyte population heterogeneity changed quickly after infection. In allopatric interactions, 24 h after infection (Fig. 4C, BV24, and 4D, VB24) two populations could be distinguished: a population P1 (corresponding to that seen in noninfected snails) and a population P2 (a new population). P2 cells exhibited increased granularity, retained a high degree of size variability, and showed a mitotic activity, as indicated by EdU labelling (Fig. 4C and 4D, red dots). This profile was transitory, with the P2 population disappearing by 96 h after infection (Fig. 4C, BV96, and 4D, VB96). Altogether, these results show that, upon infection, the snail circulating immune cells exhibit a particular population dynamics with transient increase of the mitotic activity associated with morphology modifications that are likely to be involved in the host defence response against the parasite during the interaction. Moreover, this response of the immune cells appears to be exacerbated in the allopatric interaction when compared to the sympatric interaction.
Schistosoma growth and development in Biomphalaria tissues

**Parasite development**

To investigate the development of *S. mansoni* in *B. glabrata* tissues, we examined the fate of sporocyst in sympatric and allopatric compatible interactions using a histological approach. For both interactions, miracidia were able to penetrate, transform into primary sporocysts (SpI) and develop. At 24 h after infection, we observed a significant difference (Mann Whitney U test: U = 40; z = 4.33; p = 1.42 e10^-6) in the size of sporocyst from sympatric parasites (11,838 µm² average size) versus allopatric parasites (7,402 µm² average size) (Fig. 5). This difference was no longer seen 96 h after infection (41,413 µm² and 36,920 µm², respectively, Mann Whitney U test: U = 280; z = -1.31; p = 0.1917) (Fig. 5). These results show that during the early events following infection, the parasites develop more slowly in the allopatric compared to the sympatric interaction; thereafter, however, the allopatric parasites catch up quickly, such that no difference is seen at 96 h post-infection (Fig. 5).

**Parasite transcript expression analysis**

In order to investigate the biological responses of the parasites within their host, dual RNAseq analyses allowed us to identify regulated transcripts from *Schistosoma* parasites during their intramolluscal development. The parasite RNAseq data at 24 h after infection revealed five clusters of differentially expressed genes from the sympatric (SmBRE) and the allopatric (SmVEN, Srod) parasite responses (Fig. 6). Cluster 1 corresponds to genes highly expressed and cluster 5 weakly expressed for all parasite strains. Cluster 2 represents genes overexpressed in SmBRE versus SmVEN and Srod. Cluster 3 contained genes overexpressed in SmBRE and SmVEN versus Srod and cluster 4 SmBRE and Srod versus SmVEN. In all clusters, the gene expression levels in SmBRE sympatric parasite are always greater than in the other allopatric parasites. Blast2GO annotation helps in characterizing 70% of the 351 genes shown in the five clusters (Supplementary Table 3). According to the global Gene Ontology (GO): 70% of the annotated genes were involved in general metabolism and growth, translation processes, regulation of cellular processes and RNA biosynthesis; 25% were involved in molecular transport or cell organization; and 5% were involved in organism defence or
response to stimuli. In all these clusters, we identified six parasite gene products that had been involved in parasite modulation or suppression of snail immunity. These molecules correspond to heat shock proteins (Fig. 6, Clusters 1 and 2) (27); glutathione-S-transferase, NADH dehydrogenase subunit, and calreticulin (Fig. 6, Cluster 2) (20, 53, 54); Alpha-2-macroglobulin (Fig. 6, Cluster 4) (55); von Willebrand factor type EGF with pentraxin domain (Fig. 6, Cluster 5) (56) (see Supplementary Table 3). These particular genes could be involved in the strategy used by the parasite to ensure immunosuppression and favour its development and growth within host tissues.

In allopatric interactions, an activation of the immune response is observed. Interestingly, allopatric parasites did not overexpress any transcripts that could have immunosuppressive function or impair the activation of the immune response (Fig. 6 and Supplementary Table 3). Furthermore, a variant of a glycerol-3-phosphate acyl-transferase (Schisto_manson.Chr_3.5623) is highly overexpressed in SmVEN and Srod compared to SmBRE (Cluster 1 Supplementary Table 3). This molecule is known to participate in the biosynthesis of phosphatidic acid, itself involved in macrophage activation and regulation of inflammatory signalling (57, 58). This would thus indirectly enhance the cellular immune response of Biomphalaria glabrata against those allopatric parasites.

Parasite microRNAs analysis

The microRNAs (miRNAs) are known as noncoding small RNA (<24nt) able to regulate gene expressions. As we identified deep modifications of transcriptional or posttranscriptional activities between sympatric and allopatric interactions, we investigated, in silico, the potential presence of Schistosoma mansoni miRNAs in our parasite RNAseq data. At 24 h post infections, we identified 54 miRNA precursors from miRBase with high-quality alignment scores against the different RNAseq read libraries (naïve BgBRE, BB24, BV24, BR24). To avoid cross-species misidentifications, we selected precursors that were exclusively identified in infected and never identified in uninfected snails (naive BgBRE). Eleven miRNA precursors corresponding to Schistosoma mansoni were identified (Fig. 7A). Eight of the parasite miRNA precursors were specific to the Brazil-infected libraries (BB24); two were specific of the Venezuela-infected libraries (BV24); and one was shared across the three infected conditions (BB24, BV24 and BR24). Although we identified 49 miRNA
precursor sequences specific to *S. mansoni* (Fig. 7B), we decided to select only miRNAs covered by 100% nucleotide similarity that allowed us to predict 11 miRNAs in mature (e.g., sma-mir-2d-3p, sma-mir-190-3p) or precursor (sma-mir-8431) forms. Then, in order to identify candidate sequences that could represent putative miRNA targets, we used the Miranda tool (Supplementary Table 4). Only RNA-RNA interactions that showed good scores for pairing (> 140) and enthalpy (< 15 Kcal) were considered. The number of targets pertaining to the differentially expressed immune-related transcripts identified in Fig. 1 that were found for the identified miRNAs ranged from two targets for sma-mir-8456, to 50 targets for sma-mir-2d of the differentially expressed immune-related transcripts identified in Fig. 1.

The miRNAs identified under the sympatric condition (SmBRE) were predicted to target 43.5% of the differentially represented immune-related transcripts identified in the RNAseq data (Fig. 1B, Fig. 7) whereas 6.8% and 5.1% were targeted in allopatric conditions, SmVEN and Srod, respectively (Fig. 1B, Fig. 7). To explain the similar prevalence between sympatric and allopatric infections we focus our attention on miRNAs that were shared between sympatric and allopatric interactions and we identified one miRNA: sma-miR-190-3p (Fig. 7 and Supplementary Table 4). This miRNA was predicted to bind 17 different targets among which we identified different variants of the fibrinogen-related protein (FREP) family and a cytotoxic/cytolytic humoral factor the biomphalysin. To go further, we look at the expression of those candidates following infection. If FREP transcripts were downregulated in sympatric interactions, it is not always the case in allopatry. However, interestingly all biomphalysin transcripts were underrepresented in sympatric and allopatric interactions. Altogether, these data suggest that the parasites might hijack the host immune response using dedicated miRNAs.

**Survival of snail following infection**

To examine the potential impact of allopatric or sympatric parasites on snail survival, we investigated the mortality rates of infected snails over 4 months. The survival rate was significantly higher for noninfected snails compared to infected snails (sympatric interaction Kaplan-Meier Log Rank test p = 1.39 e10^-5 and allopatric interaction p = 0.0005). However, there was no significant difference in the

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mortality rates of snails subjected to sympatric versus allopatric interactions: at the end of the experiment, the survival rates were 72% and 65% for the allopatric and sympatric interactions, respectively (Kaplan-Meier Log Rank test $p = 0.243$) (Supplementary Fig. 2).

Discussion

In the natural environment, it is assumed that the parasitic genes responsible for infectivity will evolve alongside the host defence genes, resulting in adaptation of the interactions between local host and parasite populations (59, 60). In this context, local/sympatric parasites were expected to display greater infectiveness, reproductive success and virulence in host populations compared to foreign/allopatric parasites (38, 39, 61, 62). However, in some cases this rule may be contradicted, as certain allopatric parasite-host interactions have been reported to be significantly more compatible than the corresponding sympatric combinations (40, 63). It appears that certain Biomphalaria/Schistosoma interactions (64, 65) do not fulfil the “host-parasite local adaptation hypothesis” (38, 39, 61, 62), in which the sympatric parasite is expected to perform better than the allopatric one.

Using field data, Morand et al. (1996) and Prugnolle et al. (2006) showed that although sympatric parasite-host combinations of schistosomes and snails do tend to be more compatible, exceptions exist wherein particular allopatric combinations are equally or significantly more compatible. Similar results were obtained when comparing the interactions of Brazilian and Guadeloupean snails versus Schistosoma infections (42). The authors found that allopatric Guadeloupean parasites were not able to infect Brazilian snails; but Brazilian parasites were able to infect the allopatric Guadeloupean snails.

Furthermore, this work demonstrated the presence of local adaptation between reactive oxygen species (ROS) and ROS scavengers in this system (42). Based on these observations, we propose that it would be important to develop integrative analysis to depict and understand the precise molecular crosstalk (immunobiological interactions) occurring in such highly compatible sympatric and allopatric systems. Thus, dual-comparative approaches were used herein to simultaneously analyse the responses of Biomphalaria snails and Schistosoma parasites into sympatric or allopatric interactions displaying similar compatibilities.
The present RNAseq analysis demonstrated that in sympatric interaction (BB) a huge immunosuppression occurs. Twenty-four hours after the infection, the three immunological processes were downregulated: (i) immune recognition, (ii) effector and (iii) signalling pathways (Fig. 1 and 2).

Conversely, in allopatric interactions (BV and BR), host immune response was activated (Fig. 1 and 2). Differentially regulated transcripts mostly belong to immune cellular activation, migration, proliferation or differentiation (Fig. 2). An EdU-labelling was used to detect proliferation and to confirm that more hemocyte proliferation was observed in allopatry compared to sympatry (Fig. 3, 4A and 4B). Using flow cytometry, we demonstrated that a new hemocyte subpopulation (named P2) was observed exclusively following allopatric infection at 24 h (Fig. 4C and 4D). P2 was EdU-positive and characterized by an increase in granularity, indicating that the new P2 cell subtype could proliferate.

In Biomphalaria snails, we know three main hemocyte morphotypes: the Blast-like cells, the type I hyalinocytes and the granulocytes (66). However, in absence of specific hemocyte markers, it is impossible to analyse which hemocyte morphotypes are proliferating in our flow cytometry results (Figs. 4C, 4D). The P2 subpopulation would thus originate from either a morphological change in an existing subset (correlating potentially with a decline in the P1 population), or represent cells that are migrating from tissues or a hematopoietic organ to reach the hemolymph. Indeed the P2 population reflects newly proliferated cells that present higher EdU positive cells than the P1 population (Fig. 4C, 4D). Further investigations will be necessary to determine the origin of the P2 population. However, hemocyte proliferation decreased more rapidly in sympatric versus allopatric interactions (Fig. 3 and 4). By comparing different sympatric and allopatric interactions, we demonstrated that the cellular or molecular phenotype observed may refers to reciprocal evolution or adaptation rather to a simple host or parasite strain effect.

The strong immunosuppression observed in sympatric interaction compared to the immune cell activation in allopatry certainly results in differences in the capabilities of sympatric and allopatric parasites to infect and grow in such hosts. This hypothesis was confirmed by our histological analysis of sporocyst development in snail tissues. We observed a significant difference in sporocyst size 24 h after infection (Fig. 5), when sympatric sporocysts were one-third bigger than allopatric sporocysts. At 96 h after infection, in contrast, there was no size difference between sympatric and allopatric
parasites (Fig. 5). This indicates that the allopatric parasites seem to overcome this early delay in development. However, the consequences of this delay for duration of cercariae development, number of cercariae produced, cercariae infectivity and pathogenicity in vertebrate host, will deserve further investigation to determine the potential fitness cost between sympatric and allopatric parasites.

To go further and understand how parasites immunosuppress the host or circumvent the host immune system, we used a dual-RNAseq approach to investigate transcripts expression of the sympatric and allopatric parasite intra-molluscal stages (Fig. 6). As the histological differences were solely observed at 24 h after infection, we used dual-RNAseq to investigate the parasite expression patterns at the same time point of infection. Most of the parasite transcripts belonged to the processes of nucleotide metabolism, transcription, translation and cell differentiation, development and growth. We also identified some transcripts with GO terms or functions related to immunomodulation or immunosuppression (Fig. 6 and Supplementary Table 4). Nearly all of the identified transcripts were overrepresented in the sympatric interaction compared to the allopatric interactions. Our results therefore suggest that the installation, development and growth of the parasite occurred much more rapidly in the sympatric combination, as sympatric parasites seemed to interfere more efficiently with the host immune system. However, RNAseq data did not give any clear information about how allopatric parasites succeed in circumventing the host immune system. We thus next examined the generated dual-RNAseq libraries in an effort to identify whether sympatric and/or allopatric schistosomes could hijack the host immune system using microRNAs. To achieve this goal, we confronted the dual-RNAseq data to the Schistosoma mansoni subset of miRBase to identify the presence of microRNAs (miRNAs) in our datasets. miRNAs are known to regulate numerous biological processes, including key immune response genes (67, 68). Recent work has demonstrated that circulating small noncoding RNAs from parasites have hijack roles against host metabolism, especially in the interaction of schistosomes with their vertebrate hosts (69-71). Such noncoding RNAs could act as exogenous miRNAs to interfere with or circumvent the host immune system. At 24 h after infection, several differentially expressed parasite miRNAs were identified. We investigated the potential role played by these miRNAs on host immune response. We identified some predicted targets of such schistosome miRNAs in the Biomphalaria immune reference transcriptome. We found
that schistosome miRNAs identified in the snails 24 h after sympatric infection may target 43.5% of the differentially regulated immune transcripts (Fig. 7). In contrast, far fewer correspondences were identified for the allopatric interactions (Fig. 7). The higher proportion of targeted genes in the sympatric interaction may result in the observed efficient immunosuppression, demonstrating a specific co-evolution or adaptation in the transcriptional regulation between sympatric host and parasite. However, even if more host immune genes appeared to be targeted in the sympatric combination compared to the allopatric combination (Fig. 7), both sympatric and allopatric interactions displayed the same phenotype of compatibility. In the allopatric condition, despite the activation of the snail immune response, the parasite is able to develop and succeed to infect the host. This similarity in compatibility phenotype between sympatric and allopatric parasites could potentially result from their ability to target host immune weapons or host genes that regulate the snail innate cellular response. In this context, we were expecting to find miRNAs shared between sympatric and allopatric parasites, that is, miRNAs that tend to weaken the immune system. A unique miRNA was found to be shared by allopatric and sympatric parasites, sma-miR-190-3p, which has been identified and predicted to bind different targets among FREPs and biomphalysin. The FREP family members are known as pathogen recognition receptors (64, 65)). Moreover, FREP knockdown was associated with an increase of snail compatibility toward Schistosoma infections (72, 73). Biomphalysin is a key humoral factor involved in cytotoxic/cytolytic activities against Schistosoma parasites with the ability to bind miracidia and sporocyst surfaces (17, 74). Moreover, the associated snail transcripts to these molecules are mostly down represented in sympatric and allopatric interactions. The miRNA sma-miR-190-3p is perhaps essential for parasite compatibility. Parasites expressing such miRNA would thus be considered as highly virulent parasites with strong infecting capabilities. By producing dedicated miRNAs, the parasites were potentially able to regulate transcriptional, post-transcriptional, translational and protein stability processes that might help them to subvert the snail’s immune defences.

Compatibility reflects the outcome of complex immunobiological interactions and depends on: (i) the ability of the snail immune system to recognize and kill the parasite; and (ii) the ability of the parasite to circumvent or evade the host immune response (20, 53, 75). Based on the present
observations, we propose that sympatric and allopatric interactions trigger totally different responses. In the sympatric interaction, the parasite is able to induce a host immunosuppression within the first day of infection, enabling it to infect quickly the host and readily begins its development. In the allopatric interaction, the parasite is not able to neutralize quickly the host immune system, and therefore the parasite is recognized by the host defence system, which mounts a potent immune response. The need for allopatric parasites to resist the immune system seems to disrupt the activation of their developmental program during the first day of infection. However, they seemed to be rapidly able to protect themselves against the host immune response and develop normally in snail tissues 96 h post-infection. Thereafter, in the medium- or long-term, there are no observable differences in the prevalence, intensity or snail survival comparing sympatric and allopatric interactions (Supplementary Table 1, Supplementary Fig. 2).

Thus, we show that despite having similar prevalence phenotypes, sympatric and allopatric snail-Schistosoma interactions displayed a very different immunobiological dialogue at the molecular level. Intriguingly, these different immunobiological interactions seem to have no repercussions upon parasite growth at longer term or to host survival. As differences at the molecular level do not correspond apparently to any ecologically meaningful changes in term of fitness, it is not straightforward to demonstrate local adaptation in such systems. However, we do not know if fitness costs could affect other biological traits in sympatric and allopatric interactions, for example, sporocyst II or cercariae production, or cercariae infectivity and pathogenicity towards the vertebrate host. Demonstrating local adaptation would thus appear extremely complex and would indeed deserve further investigation. It is hard to draw the line as to when local adaptation is or is not present. However, our results argue that the differences found at the molecular level may ultimately contribute to the evolution of local adaptation at an ecological level. Nevertheless, the ability for allopatric pathogens to adapt rapidly and efficiently to new hosts could have critical consequences on disease emergence and risk of schistosomiasis outbreaks.

Past events of allopatric parasites reaching new areas of transmission, even in a large geographic scale dispersion, have been largely documented, the most famous example being the schistosomiasis colonization of South America since the slave trade of the 16th through 19th centuries
Schistosoma originated in Asia, reached Africa 12 to 19 million years ago (MYA), and gave rise to all Schistosoma species known in Africa (77). *S. mansoni* diverged from *S. rodhaini* around 2.8 MYA (76, 78), and thereafter, 400 to 500 years ago, colonized South America (76, 77). This colonization of South America by *S. mansoni* from Africa was rendered possible by the presence of the snail host *Biomphalaria glabrata*. All African species of *Biomphalaria* are monophyletic and seem to have originated from paraphyletic South American clades (79-81). The ancestor of *B. glabrata* appears to have colonized Africa 1 to 5 MYA, giving rise to all 12 species of *Biomphalaria* known today in Africa (82). In South America and the Caribbean islands, *S. mansoni* infects *B. glabrata*; in Africa, it infects mostly *B. pfeifferi* and *B. alexandrina*. We found that South American *S. mansoni* parasites are highly compatible with their sympatric South American snail hosts, whereas African *S. mansoni* parasites display low compatibility phenotype with South American snail hosts (Supplementary Table 1). Interestingly, the South American parasites did not lose their compatibility for African snail hosts; that is, prevalence is similar to African parasites when confronted with African snails (Supplementary Table 1). The recent African origin of South American *Schistosoma* parasites (introduction in South America 400 to 500 years ago) may explain that they have not diverged sufficiently in South America to lose their compatibility for African snail hosts. In this case, the transfer of allopatric parasites from Africa to South American snail hosts have been successful and resulted in the emergence of schistosomiasis in South America.

More recently another case of compatible allopatric parasite emergence have been observed when schistosomiasis reached Europe (83, 84). Here, humans infected in Senegal have imported a hybrid between *Schistosoma haematobium* and *Schistosoma bovis* into Corsica. In this case, urogenital schistosomiasis could be introduced and easily and rapidly spread into this novel area of south Corsica because *Bulinus truncatus* the vector snail of *S. haematobium* was endemic in the Corsica Cavu River (83, 84). However, this allopatric African hybrid parasite was able to adapt efficiently to the new Corsican *B. truncatus* host. If parasite hybridization can potentially have a putative role in increasing the colonization potential of such *S. haematobium*, it would be particularly interesting to analyse and depict the molecular support of such allopatric interactions to predict the potential risk of schistosomiasis outbreaks in other European areas, or other potential transmission foci.
Conclusions

If we hope to draw conclusions regarding the existence of emerging or outbreak risks, we need to develop integrative approaches to explore fine-scale patterns of host-parasite interactions. We must consider the spatial scale at which comparisons are conducted, the patterns of disease occurrence, the population genetics, and the involvement of physiological, immunological and molecular processes. Studying the relevant factors at the relevant timing would be of critical importance in terms of schistosomiasis control. Understanding further, how these allopatric parasites efficiently infect host snails would be mandatory to identify markers and develop new tools to predict or to quantify risks of schistosomiasis outbreaks.

Acknowledgements

We thank Cécile Saint-Béat, Nathalie Arancibia and Anne Rognon for their work and diligence in helping generate some of the data described herein.
Declarations

Ethics approval and consent to participate
Our laboratory holds permit # A66040 for experiments on animals, which was obtained from the French Ministry of Agriculture and Fisheries and the French Ministry of National Education, Research, and Technology. The housing, breeding, and care of the utilized animals followed the ethical requirements of our country. The experimenter possesses an official certificate for animal experimentation from both of the above-listed French ministries (Decree # 87–848, October 19, 1987). The various protocols used in this study have been approved by the French veterinary agency of the DRAAF Languedoc-Roussillon (Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt), Montpellier, France (authorization # 007083).

Availability of data and material
The datasets generated and/or analysed during the current study are not publicly available due to ongoing analysis of transcriptome but are available from the corresponding author on reasonable request. Raw read fastq files were submitted to the Sequence Read Archive at NCBI (http://trace.ncbi.nlm.nih.gov/Traces/sra/) under the reference PRJNA213050. The reference transcriptome is publicly available from the transcriptomic database of Biomphalaria glabrata at the IHPE website (http://ihpe.univ-perp.fr/acces-aux-donnees/)

Competing interests
The authors declare that they have no competing interests.

Funding
This work was supported by the French National Agency for Research (ANR) through the Invimory project grant [ANR-13-JSV7-0009] to BG. The funding agency had no role in study design, collection, analysis or interpretation of data and in writing of the manuscript.

Authors' contributions
Conceived and designed the experiments: AP, SP, RG, DD, BG. Performed the experiments: AP, SP, CC, GMC, JFA. Analysed the data: AP, SP, CC, RG, GMC, DD, BG. AP, RG, BG led the manuscript writing. All authors participated in manuscript writing, editing and critical reviewing. All authors read and approved the final manuscript.

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**Figure Legends**

**FIGURE 1.** Dual-RNAseq of Biomphalaria immune-related transcripts. Among the differentially represented transcripts, Blast2GO functional annotation allowed us to identify 336 transcripts that appeared to be related to the *Biomphalaria* immune response. Abbreviations and colours: blue BB, sympatric interaction between BgBRE and SmBRE; green BV, allopatric interaction between BgBRE and SmVEN; and red BR, allopatric interaction between BgBRE and Srod. For each interaction 40 whole-snails are used, 20 pooled at 24 h and 20 at 96 h post-infection. A) Venn diagram showing the relationships among the immune transcripts found to be differentially expressed in the sympatric and allopatric interactions. B) Clustering of differentially represented immune transcripts. Heatmap representing the profiles of the 336 differentially represented immune-related transcripts in the BB, BV or BR interactions along the kinetic of infection (at 24 and 96 h). Each transcript is represented once, and each line represents one transcript. Colours: yellow, over-represented transcripts; purple, under-represented transcripts; and black, unchanged relative to levels in control naïve snails. C) Pie chart showing the distribution of the selected immune-related transcripts across three immunological processes: immune recognition (pink), immune effectors (brown) and immune signalling (blue). For each category and interaction, the respective proportion of transcripts and the direction of the effect (over- or under-expression) are indicated.

**FIGURE 2.** Differentially represented immune-related transcripts in sympatric and allopatric interactions. Cumulative expression [Log2FC (fold change) from DESeq2 analysis] of the immune-related transcripts identified as being differentially represented following sympatric or allopatric infection. Transcripts were grouped into the three immunological groups described in Fig. 1, and from there into functional categories. The yellow histograms correspond to cumulatively overrepresented transcripts, while the purple histograms show underrepresented transcripts. The black (overrepresented) and gray (underrepresented) diamonds correspond to the number of transcripts analysed in each functional category. Abbreviations: BB, BgBRE/SmBRE interaction; BV, BgBRE/SmVEN interaction; and BR, BgBRE/Srod interaction. A. Immune transcript expression at 24 h post-infection. B. Immune transcript expression at 96 h post-infection.

**FIGURE 3.** Microscopic analysis of snail hemocyte proliferations. In vitro EdU-labelling of hemocytes was conducted for sympatric and allopatric interactions. A) Hemocytes were collected at 24 h post-infection for in vitro analysis. Confocal microscopy of EdU-labeled hemocytes from snails subjected to the allopatric BV interaction (BgBRE/SmVEN). Colours: blue, DAPI; and green, EdU. B) Microscopic counting of EdU-labelled hemocytes from naïve control snails (BgBRE) (n = 1,811) and those subjected to the sympatric interaction (BB: BgBRE/SmBRE) (n=2,064) or an allopatric interaction (BV: BgBRE/SmVEN) (n = 1,366) recovered from three individual snails by condition. Colours: green, EdU-positive cells; and blue, EdU-negative cells. Between-group differences in the percentage of proliferation were tested using a Fisher exact test, with statistical significance accepted at p < 0.05. The “a” indicates a significant difference between the naïve and infective conditions, while “b” indicates a significant difference between the infective conditions.

**FIGURE 4.** Flow cytometry analysis of the hemocyte response in sympatric and allopatric interactions. A) Flow cytometry was used to count in vivo EdU-labelled hemocytes at 24 h and 96 h after infection in sympatric and allopatric interactions. A total number of hemocytes of n = 10,000 were recovered for six biological replicates of
three snails. Control naïve snails (BgBRE, yellow) were compared to those subjected to the sympatric interaction (BB, BgBRE/SmBRE, blue) or an allopatric interaction (BV, BgBRE/SmVEN, green). B) The experiment described in A was repeated using the BgVEN snail strain. Control naïve snails (BgVEN, yellow) were compared to those subjected to the sympatric interaction (VV, BgVEN/SmVEN, green) or an allopatric interaction (VB, BgVEN/SmBRE, blue). C) FSC (forward-scattered light, representing cell size) and SSC (side-scattered light, representing cell granularity) circulating hemocyte patterns in BgBRE snails under the naïve condition (yellow) or 24 h and 96 h after infections in sympatry (BB24/96, BgBRE/SmBRE, blue) or allopatry (BV24/96, BgBRE/SmVEN, green). D) FSC and SSC circulating hemocyte patterns in BgVEN snails under the naïve condition (yellow) or 24 h and 96 h after infections in sympatry (VV24/96, BgVEN/SmVEN, blue) or allopatry (VB24/96, BgVEN/SmBRE, green). The red dots correspond to EdU-positive hemocytes. Between-group differences in the percentage of proliferation were tested using the Mann-Whitney U-test, with statistical significance accepted at p < 0.05. The “a” indicates a significant difference between the naïve and infective condition, “b” indicates a significant difference between the infective conditions at 24h, and “c” indicates a significant difference between the infective conditions at 96h.

**FIGURE 5.** Development of parasites into snail tissues. A histological approach was used to monitor parasite size along the course of snail infection. The sympatric interaction (BgBRE x SmBRE) is shown in blue, and the allopatric interaction (BgBRE x SmVEN) is shown in green. For each experimental interaction, the parasite sizes were quantified at 24 and 96 h after infection. Morpho-anatomical aspects of the parasite are depicted to highlight a potential difference in parasite survival. N = 7 to 10 sporocystes were used. Between-group parasite size differences were assessed using the Mann-Whitney U-test, with significance accepted at p < 0.05 (indicated by “a” on the histograms).

**FIGURE 6.** Clustering of intra-molluscal Schistosoma expression patterns. RNAseq library mapping enabled us to identify 351 genes expressed by Schistosoma parasites in Biomphalaria snail tissues. Colours: blue, S. mansoni Brazil (SmBRE); green, S. mansoni Venezuela (SmVEN); and red, S. rodhaini (Srod). The heatmap represents the profiles of the 351 genes expressed by the different parasites at 24 h after infection. Each transcript is represented once, and each line represents one transcript. The expression level is highlighted by the different shades of blue.

**FIGURE 7.** In silico identification of parasite miRNAs. miRNAs were assessed using libraries obtained from naïve snails and snails infected for 24 h under the various interaction conditions (BB24, BgBRE x SmBRE; BV24, BgBRE x SmVEN; BR24, BgBRE x Srod). A) Table highlighting the precursor miRNAs that may have targets among the immune-related snail transcripts selected in the present work. They include eight precursors specifically recovered in BB24, two in BV24, and one shared across the three infected conditions. The total numbers of potential targets in each condition are indicated. B) Venn diagram showing the potential targets according to the sympatric or allopatric interactions. Shown is an example miRNA stem-loop precursor that presents the highest number of potential host targets.

**SUPPLEMENTARY FIGURE 1.** Clustering of all differential represented transcripts. Clustering of differentially represented transcripts. Heatmap representing the profiles of the 1,895 differentially represented immune-related transcripts in the BB, BV or BR interactions along the time course of infection (at 24 and 96 h).
Each transcript is represented once, and each line represents one transcript. Colours: yellow, overrepresented transcripts; purple, underrepresented transcripts; and black, unchanged relative to levels in control naïve snails.

**SUPPLEMENTARY FIGURE 2.** Mortality of *B. glabrata* snails after infections. The survival rates of *B. glabrata* subjected to infection by different *S. mansoni* strains were observed over 14 weeks. Kaplan Meier graphs were generated using xlstat, and the log-rank test ($p < 0.05$) was used to test for significant between-group differences. Coloured curves indicate the mortality rates of naïve snails (yellow) ($n = 60$), snails infected by the sympatric parasite (BB, BgBRE/SmBRE, blue) ($n = 50$), and snails infected by the allopatric parasite (BV, BgBRE/SmVEN, green) ($n=50$). The difference in mortality between naïve and infected snails was significant ($p < 0.05$), whereas that between the two infected conditions was not ($p = 0.243$).
TABLE 1. Number of transcripts in each step of transcriptomic analysis

| Analysis of transcriptomic data | Analyses of Blast2GO | Analyses of annotations |
|---------------------------------|----------------------|-------------------------|
| Full transcriptome              | Differentially expressed | Informative annotation | Immune transcripts | Non-immune transcripts |
| 159,711                         | 3,865                 | 1,017                   | 336                | 681                   |
**SUPPLEMENTARY TABLE 1.** *Biomphalaria* and *Schistosoma* compatibility between African and South American strains

|                      | Biomphalaria |     |     |
|----------------------|--------------|-----|-----|
|                      | *gabra*      | *pfeiffer* |
|                      | BRE          | VEN | SEN |
| **Biomphalaria**     | **P %**      |     |     |
| **P **              | **100**      | **70** | **92** |
| **I **              | **3.5**      | **3.5** | **2.9** |
| **V**               | **100**      | **95** | **100** |
| **B**               | **3.2**      | **3.8** | **4** |
| **S**               | **27**       | **13** | **100** |
| **R**               | **1.3**      | **1** | **2.5** |
| **O**               | **80**       | **NA** | **NA** |
| **D**               | **2**        | **NA** | **NA** |

The prevalence (P %: percentage of snail infected) and intensity (I: number of parasites per infected host) of infection are presented for each experimental infection. The indicated values correspond to 20 miracidia. Each pairwise combination of *Biomphalaria* (BgBRE, BgVEN, BpSEN) and *Schistosoma mansoni* (SmBRE, SmVEN, SmSEN) or *Schistosoma Rodhaini* (Srod) were tested for compatibility. NA: non-available data.
Figure 1

A

B

C

Cluster 1: BB-BV BR
4 transcripts
Cluster 2: BB-BV
118 transcripts
Cluster 3: BV-BR
18 transcripts
Cluster 4: BB-BR
5 transcripts
Cluster 5: BB
140 transcripts
Cluster 6: BV
25 transcripts
Cluster 7: BR
11 transcripts

Recognition | Effector | Signaling Pathways
Figure 3

A

DAPI  EdU  Merge

BV24

B

1.16%  2.57%  5.19%

BgBRE  BB24  BV24

98.84%  97.43%  94.81%
Figure 4
Figure 5
Figure 6

Cluster 1: 28 genes
Cluster 2: 122 genes
Cluster 3: 101 genes
Cluster 4: 54 genes
Cluster 5: 46 genes

SmBRE  SmVEN  Srod
Figure 7

| Precursor     | BgBRE duplicates | BB24 | BV24 | BR24 | Targets |
|---------------|------------------|------|------|------|---------|
| sma-mir-8460  | 0 0 1 0 0 7      |      |      |      |         |
| sma-mir-8463  | 0 0 1 0 0 9      |      |      |      |         |
| sma-mir-2a    | 0 0 1 0 0 20     |      |      |      |         |
| sma-mir-3492  | 0 0 1 0 0 50     |      |      |      |         |
| sma-mir-8404  | 0 0 1 0 0 4      |      |      |      |         |
| sma-mir-8414  | 0 0 1 0 0 14     |      |      |      |         |
| sma-mir-8446  | 0 0 1 0 0 23     |      |      |      |         |
| sma-mir-8456  | 0 0 0 1 0 2      |      |      |      |         |
| sma-mir-8431  | 0 0 0 1 0 4      |      |      |      |         |
| sma-mir-190   | 0 0 1 1 1 17     |      |      |      |         |
| Sum of potential Bg targets | 0 0 154 23 17 |      |      |      |         |

A

B

Lowest free energy structure

45 putative targets

sma-mir-8431

E=29.9

SmVEn specific

0 0 6 17 0

SroD specific

SmBRE specific

123

Shared

sma-mir-2d-3p

E=29.8

50 putative targets

sma-mir-190-3p

E=43.8

17 putative targets

sma-mir-8446
Supplementary Figure 1

Cluster 1: Immune transcripts
336 transcripts

Cluster 2: Other functions transcripts
685 transcripts

Cluster 3: No annotated transcripts
674 transcripts
Supplementary Figure 2

- Survival Rate vs. Weeks
- Three categories: Naive, Sympatric interaction, Allopatric interaction
- Weeks range from 0 to 14
- Survival rates range from 0 to 1