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

**Short title: Characterization of *Biomphalaria/Schistosoma* immunobiological interactions**

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## 26 **Abstract**

27 Selective pressures between hosts and their parasites can result in reciprocal evolution or  
28 adaptation of specific life history traits. Local adaptation of resident hosts and parasites should  
29 lead to increase parasite infectivity/virulence (higher compatibility) when infecting hosts from  
30 the same location (in sympatry) than from a foreign location (in allopatry). Analysis of  
31 geographic variations in compatibility phenotypes is the most common proxy used to infer local  
32 adaptation. However, in some cases, allopatric host-parasite systems demonstrate similar or  
33 greater compatibility than in sympatry. In such cases, the potential for local adaptation remains  
34 unclear. Here, we study the interaction between *Schistosoma* and its vector snail *Biomphalaria*  
35 in which such discrepancy in local versus foreign compatibility phenotype has been reported.  
36 Herein, we aim at bridging this gap of knowledge by comparing life history traits (immune  
37 cellular response, host mortality, and parasite growth) and molecular responses in highly  
38 compatible sympatric and allopatric *Schistosoma/Biomphalaria* interactions originating from  
39 different geographic localities (Brazil, Venezuela and Burundi). We found that despite  
40 displaying similar prevalence phenotypes, sympatric schistosomes triggered a rapid immune  
41 suppression (dual-RNAseq analyses) in the snails within 24h post infection, whereas infection  
42 by allopatric schistosomes (regardless of the species) was associated with immune cell  
43 proliferation and triggered a non-specific generalized immune response after 96h. We observed  
44 that, sympatric schistosomes grow more rapidly. Finally, we identify miRNAs differentially  
45 expressed by *Schistosoma mansoni* that target host immune genes and could be responsible for  
46 hijacking the host immune response during the sympatric interaction. We show that despite  
47 having similar prevalence phenotypes, sympatric and allopatric snail-*Schistosoma* interactions  
48 displayed strong differences in their immunobiological molecular dialogue. Understanding the  
49 mechanisms allowing parasites to adapt rapidly and efficiently to new hosts is critical to control  
50 disease emergence and risks of Schistosomiasis outbreaks.

51

## 52 **Author summary**

53 Schistosomiasis, the second most widespread human parasitic disease after malaria, is caused  
54 by helminth parasites of the genus *Schistosoma*. More than 200 million people in 74 countries  
55 suffer from the pathological, and societal consequences of this disease. To complete its life  
56 cycle, the parasite requires an intermediate host, a freshwater snail of the genus *Biomphalaria*  
57 for its transmission. Given the limited options for treating *Schistosoma mansoni* infections in  
58 humans, much research has focused on developing methods to control transmission by its

59 intermediate snail host. *Biomphalaria glabrata*. Comparative studies have shown that infection  
60 of the snail triggers complex cellular and humoral immune responses resulting in significant  
61 variations in parasite infectivity and snail susceptibility, known as the so-called polymorphism  
62 of compatibility. However, studies have mostly focused on characterizing the  
63 immunobiological mechanisms in sympatric interactions. Herein we used a combination of  
64 molecular and phenotypic approaches to compare the effect of infection in various sympatric  
65 and allopatric evolutionary contexts, allowing us to better understand the mechanisms of host-  
66 parasite local adaptation. Learning more about the immunobiological interactions between *B.*  
67 *glabrata* and *S. mansoni* could have important socioeconomic and public health impacts by  
68 changing the way we attempt to eradicate parasitic diseases and prevent or control  
69 schistosomiasis in the field.

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73

## 74 Introduction

75 Schistosomiasis is the second most widespread human parasitic disease after malaria and affects over  
76 200 million people worldwide [1]. *Schistosoma mansoni* (Platyhelminthes, Lophotrochozoa) causes  
77 intestinal schistosomiasis. *Schistosoma* needs a fresh water snail acting as its first intermediate host to  
78 undergo part of its life cycle before infecting humans. Patently infected snails support the continuous  
79 production of thousands of cercariae, infective for humans. Vector snails are central actors of the parasite  
80 transmission and obvious targets for schistosomiasis control that deserve more attention. It is therefore  
81 necessary to understand snail-parasite immunobiological interactions and to characterize the molecular  
82 mechanisms of successful snails and *Schistosoma* interactions.

83 The compatibility of numerous strains of *Biomphalaria glabrata* and *Schistosoma sp.* has been  
84 extensively tested, revealing that (i) different *B. glabrata* laboratory strains (or isolates) show various  
85 degrees of susceptibility to *S. mansoni* infection and (ii) different strains of *S. mansoni* display different  
86 levels of infectivity towards a particular strain of snail host [2-6]. Compatibility is defined as the ability  
87 for the miracidia to infect snail and become a living primary sporocyst in snail tissue. Incompatibility  
88 refers to miracidia that are recognized by the snail immune system and encapsulated and killed by the  
89 hemocytes (the snail immune cells). Thus, the success or failure of the infection of *B. glabrata* by *S.*  
90 *mansoni* reflects a complex interplay between the host's defense mechanisms and the parasite's infective  
91 strategies, based on a complex phenotype-to-phenotype or matching-phenotype model [2-4, 7-9]. In the  
92 past 15 years, the molecular basis of this compatibility polymorphism has been investigated at the  
93 genomic [10-12], transcriptomic [8, 13-17], proteomic/biochemical [18-23] and epigenomic levels [24-  
94 29]. These studies have revealed that various molecules and pathways involved in immune recognition  
95 (snail immune receptors versus parasite antigens), immune effector/anti-effector systems, and immune  
96 regulation/activation participate in a complex interplay that governs the match or mismatch of host and  
97 parasite phenotypes [30]. This complex phenotype-by-phenotype interaction or compatibility  
98 polymorphism varies between populations and individuals resulting in a "multi-parasite susceptibility"  
99 or "multi-host infectivity" phenotypes [4] that reflect between-population variations in parasite  
100 infectivity/virulence and host defense/resistance [31, 32].

101 Most of the time, interaction in *B. glabrata/Schistosoma* models has been investigated by comparing, (i)  
102 sympatric/compatible and (ii) allopatric/incompatible host-parasite associations. The general  
103 assumption is that the parasites thanks to their shorter generation times, larger population sizes and  
104 higher reproductive outputs, are ahead in the co-evolutionary race against their host and are therefore  
105 more likely to locally adapt and perform better when infecting local hosts [33, 34], than allopatric hosts  
106 [34-37]. However, in many instances, Schistosomes are highly compatible to hosts from other localities,  
107 showing the same or even greater infection success when exposed to allopatric hosts. Thus they do not  
108 fulfil the "local versus foreign" main criterion of the local adaptation between a host and its parasite [5,  
109 38-40]. Very few studies have investigated the molecular basis of allopatric compatible interactions  
110 from the perspective of both side of the interaction, the host and the parasite [41, 42].

111 Hence, in order to bridge this gap, we herein study sympatric/allopatric interactions displaying similar  
112 compatibilities using an integrative approach that links the underlying molecular mechanisms to the  
113 resulting phenotypes, based on comparative molecular approaches on both host snails and *Schistosoma*  
114 parasites. We characterize the underlying cellular and molecular mechanisms of the interaction between  
115 South American snail strains (from Recife Brazil and Guacara Venezuela) and three different highly  
116 compatible parasite isolates: (i) the sympatric strains of *S. mansoni* from Recife Brazil, (ii) the allopatric  
117 *S. mansoni* from Guacara Venezuela (narrow geographic scale), and (iii) the allopatric *S. rodhaini* from  
118 Burundi Africa (large geographic and phylogenetic scales).

119 Our results clearly show that even though the compatibility phenotypes among these strains is similar,  
120 a very different immunobiological dialogue is taking place between *B. glabrata* vector snails and their  
121 sympatric or allopatric *Schistosoma* parasites at the cellular and molecular levels.

122

## 123 **Results**

### 124 A RNAseq approach of host immune response in sympatric and allopatric infections

125 The *B. glabrata* transcriptome was analyzed using the previously described RNAseq pipeline developed  
126 in our laboratory [8, 43, 44]. Of the 159,711 transcripts of the *BgBRE* transcriptome, 3,865 (2.4%) were  
127 differentially represented in all sympatric and allopatric conditions compared to naive snails (Table 1,

128 S1 Fig). We performed automatic Blast2GO annotation, discarded the non-annotated transcripts, and  
129 retained 1,017 annotated transcripts (26.3% of the differentially expressed (DE) transcripts, S1 Fig). In  
130 the following analysis, we focused on the 336 transcripts known to have immune-related functions  
131 (8.7% of DE transcripts, S1 Fig).

132 Of these immune related transcripts, 189, 180, and 164 DE transcripts were identified in the BB  
133 (BgBRE/SmBRE, sympatric), BV (BgBRE/SmVEN, allopatric), and BR (BgBRE/Srod, allopatric)  
134 interactions, respectively (Fig 1A). Among those, 40 transcripts were consistently differentially  
135 expressed in response to infection (Fig 1A). They also displayed similar expression profile (Fig 1B,  
136 cluster 1).

137 Most (74.1%) of the transcripts differentially expressed in response to infection by the sympatric parasite  
138 (BB) were not differentially expressed in response to either one of the two other parasites. Most  
139 importantly, all of the sympatric-specific transcripts were under-represented at 24 h post-infection, and  
140 74.6% of these transcripts were differentially expressed exclusively at this time point (Fig 1B, cluster  
141 5), suggesting a parasite-induced immunosuppression.

142 In contrast, very similar transcript expression patterns were observed in response to infection by the two  
143 different species of allopatric parasites: *S. mansoni* (BV) and *S. rodhaini* (BR) and most of the variations  
144 in gene expression occurred 96h after infection. Of the 108 transcripts consistently differentially  
145 expressed in allopatric response (Fig 1A; Fig 1B, cluster 3), 98.1% were differentially expressed at 96  
146 h post-infection, and 28.2% were more abundant following infection (Fig 1B, cluster 3). Transcripts  
147 differentially expressed exclusively in response to SmVEN or Srod were group in Clusters 6 (28  
148 transcripts) and 7 (11 transcripts), respectively. In response to SmVEN ( BV, Fig 1B, cluster 6), 96.5%  
149 of the transcripts were differentially abundant 96 h after infection (22% over-represented) and in  
150 response to Srod (BR, Fig 1B, cluster 7), 100% of the transcripts were differentially abundant 96 h after  
151 infection (82% over-represented).

152

153

154

155

156 We explored the function of DE transcripts in response to the three different parasites. We initially  
157 distributed the relevant differentially expressed immune transcripts into three groups: (i) immune  
158 recognition molecules, (ii) immune effectors, and (iii) immune signalling molecules (Fig 1C, S2 Table),  
159 that were then subdivided into functional categories (Fig 2). When we compared the percentage of each  
160 immunological group in the sympatric and allopatric interactions, no specific functional subset was  
161 particularly repressed in the BB sympatric interaction (Fig 1C; Fig 2). The same immune functions were  
162 affected in response to infections by sympatric or allopatric parasites but different immune transcripts  
163 (grey and black diamond in Fig 2) showed differential regulation following infections (Fig 2).  
164 The differentially regulated transcripts belonging to the three immunological groups (Fig 2) were largely  
165 involved in immune cellular responses, cell adhesion, extra cellular matrix component, cell migration,  
166 cell differentiation and cell proliferation. These functions were consistently reduced at the 24h time  
167 point in sympatric interaction (76%), whereas many transcripts involved in the same molecular  
168 processes were over-represented in allopatric interactions (39%) (Fig 2).

169

## 170 Immune cellular responses in the sympatric and allopatric contexts

171 Hemocytes, the snail immune cells, participate directly in the immune response against the parasites,  
172 and immune cell activation under an immunological challenge can translate into cell proliferation and/or  
173 cell morphology modifications. Thus, cell proliferation was quantified using *in vitro* (Fig 3) and *in vivo*  
174 (Fig 4) EdU nuclear labelling. EdU is a nucleoside analogue of thymine incorporated into DNA during  
175 DNA synthesis. Its incorporation reflects the mitotic activity of hemocytes.

176 *In vitro* labelling was used on circulating hemocytes recovered from BgBRE 24h after infection with  
177 SmBRE and SmVEN to compare the proportion of mitotic circulating hemocytes in sympatric and  
178 allopatric interaction, respectively (Fig 3A). Quantification of Edu-positive hemocytes using confocal  
179 microscopy showed that 24h after infection, hemocyte proliferation was 3 times more important  
180 following infection of BgBRE by SmVEN (5.2% of proliferative cells in BV) than SmBRE (2.6% of  
181 proliferative cells in BB) (Fisher exact test two-tailed  $p = 7.6 \times 10^{-6}$ ) (Fig 3B). Moreover, this result  
182 demonstrates for the first time that “circulating” hemocytes are able to proliferate following *Schistosoma*  
183 infections.



184

185 Hemocyte proliferation 24h after infection was then further assessed using flow cytometry after *in-vivo*  
186 EdU-labelling (Fig 4A, B).

187 Here, we performed the same experiments using another *Biomphalaria glabrata* strain, BgVEN as the  
188 host and SmVEN and SmBRE as the sympatric and the allopatric parasite, respectively (Fig 4B). The  
189 rate of proliferating cells was significantly higher in allopatric than sympatric interaction in both BgBRE  
190 and BgVEN (BgBRE Mann Whitney U test:  $U=36$ ;  $z = -2.8$ ;  $p = 0.0022$ ; BgVEN, Mann Whitney U  
191 test:  $U=36$ ;  $z=-2.8$ ;  $p = 0.0022$ ). In BgBRE, allopatric interaction (BV) was associated with 4.2% of  
192 proliferative cells whereas sympatric interaction resulted in 1.8% of proliferative cells (Fig 4A). In

193 BgVEN, allopatric interaction (BgVEN/SmBRE, VB) was associated with 6.8% of proliferative cells  
194 whereas sympatric interaction (BgVEN/SmVEN, VV) resulted in 2.0% of proliferative cells (Fig 4B).

195 At 96 h after infection, there were fewer proliferating cells: the percentage of proliferating hemocytes  
196 in sympatric BB and VV interactions were similar to the non-infected controls (BB, 1%, Mann Whitney  
197 U test:  $U=17$ ;  $z=-0.27$ ;  $p = 0.3936$ ; VV, 0.1%, Mann Whitney U test:  $U=2$ ;  $z=2.48$ ;  $p = 0.013$ ), while  
198 remaining somewhat higher in both allopatric interactions (BV, 2.3%, Mann Whitney U test:  $U=0$ ;  
199  $z=2.65$ ;  $p = 0.009$ ; .VB, 2.7%, Mann Whitney U test:  $U=36$ ;  $z=2.8$ ;  $p = 0.0022$ ). These results confirm  
200 that the reduced cell proliferation is associated with sympatric interaction regardless of the strain used..

201 The morphology of hemocytes (size and granularity) from non-infected and infected *B. glabrata* snails  
202 (BgBRE and BgVEN) in sympatric and allopatric interactions with the parasites SmBRE and SmVEN  
203 was observed using flow cytometry (Fig 4C and 4D). Morphology and heterogeneity of circulating  
204 hemocytes varied similarly in BgBRE and BgVEN snails (Fig 4C and 4D). In non-infected snails, the  
205 content of circulating hemocytes was very heterogeneous, but represented a single population with  
206 continuous gradient of size and granularity typical of *B. glabrata* hemocytes (Fig 4C and 4D) [45].

207 Hemocyte population heterogeneity changed quickly after infection. In allopatric interactions, 24 h after  
208 infection (Fig 4C, BV24, and 4D, VB24) two populations could be distinguished: a population P1  
209 (corresponding to that seen in non-infected snails) and a population P2 (a new population). P2 cells  
210 exhibited increased granularity, retained a high degree of size variability, and showed a mitotic activity,  
211 as indicated by EdU labeling (Fig 4C and 4D, red dots). This profile was transitory, as the P2 population

212 had disappeared 96 h after infection (Fig 4C, BV96, and 4D, VB96). Altogether, these results show that,  
213 upon infection, the snail circulating immune cells exhibit a particular population dynamic with transient  
214 increase of the mitotic activity associated with morphology modifications. Moreover, this cellular  
215 response appears to be inhibited by sympatric parasites.

216

## 217 *Schistosoma* growth and development in *Biomphalaria* tissues

### 218 Parasite development

219 To investigate the development of *S. mansoni* in *B. glabrata* tissues, we examined the fate of sporocyst  
220 in sympatric and allopatric compatible interactions using a histological approach, for this we used 3  
221 snails per conditions. For both interactions, miracidia were able to penetrate, transform into primary  
222 sporocysts (SpI), and develop. At 24 h after infection, we observed a significant difference (Mann  
223 Whitney U test:  $U=40$ ;  $z=4.33$ ;  $p = 1.42 \times 10^{-6}$ ) in the size of sporocyst from sympatric parasites (11,838  
224  $\mu\text{m}^2$  average size on 9 parasites) versus allopatric parasites (7,402  $\mu\text{m}^2$  average size on 8 parasites) (Fig  
225 5). A small difference in sporocyst size is still observed at 96 h after infection but without being  
226 significant (41,413  $\mu\text{m}^2$  on 7 parasites for sympatric and 36,920  $\mu\text{m}^2$  on 10 parasites for allopatric, Mann  
227 Whitney U test:  $U=280$ ;  $z=-1.31$ ;  $p = 0.1917$ ) (Fig 5). These results show that during the early events  
228 following infection, the allopatric parasites develop more slowly than sympatric one's; thereafter,  
229 allopatric parasites seemed to catch up quickly, resulting in no significant difference in size observed at  
230 96 h post-infection (Fig 5).

231

### 232 Parasite transcript expression analysis

233 We used dual RNAseq data to identify transcripts expressed by SmBRE, SmVEN and Srod during their  
234 intra-molluscal development in BgBRE. The parasite RNAseq data at 24 h after infection, revealed five  
235 clusters of DE transcripts from the sympatric (SmBRE) and the allopatric (SmVEN, Srod) parasite  
236 responses (Fig 6). Cluster 1 corresponds to transcripts highly expressed and cluster 5 weakly expressed  
237 for all parasite strains. Cluster 2 represents transcripts over-expressed in SmBRE versus SmVEN and  
238 Srod. Cluster 3 contained transcripts over-expressed in SmBRE and SmVEN versus Srod and cluster 4  
239 SmBRE and Srod versus SmVEN. In all clusters, the transcript expression levels in SmBRE sympatric

240 parasite are always greater than for the other allopatric parasites. Blast2GO annotation was successful  
241 for 70% of the 351 transcripts identified in the five clusters described above (S3 Table). According to  
242 the global Gene Ontology (GO): 70% of the annotated genes were involved in general metabolism and  
243 growth, translation processes, regulation of cellular processes and RNA biosynthesis; 25% were  
244 involved in molecular transport or cell organization; and 5% were involved in organism defence or  
245 response to stimuli. In all these clusters, we identified 6 parasite gene products been involved in parasite  
246 modulation or suppression of snail immunity. These molecules correspond to heat shock proteins (Fig  
247 6, clusters 1 and 2) [27]; glutathione-S-transferase, NADH dehydrogenase subunit, and calreticulin (Fig  
248 6, cluster 2) [20, 46, 47]; Alpha-2-macroglobulin (Fig 6, cluster 4) [48]; von willebrand factor type EGF  
249 with pentraxin domain (Fig 6, cluster 5) [49] (see S3 Table).

250 Interestingly, allopatric parasites did not over express any transcripts that could have  
251 immunosuppression function or impair the activation of the immune response (Fig 6 and S3 Table).  
252 Furthermore, a variant of a glycerol-3-phosphate acyl-transferase (*Schisto\_mansoni*.Chr\_3.5623) is  
253 highly over expressed in SmVEN and Srod compared to SmBRE (cluster 1, S3 Table). This molecule  
254 is known to participate in the biosynthesis of phosphatidic acid, itself involved in macrophage activation  
255 and regulation of inflammatory signalling [50, 51].

256

### 257 Parasite microRNAs analysis

258 The microRNAs (miRNAs) are known as non-coding small RNA (<24nt) highlighted to regulate gene  
259 expressions. As we identified strong differences in the transcriptional responses between sympatric and  
260 allopatric interactions, we can hypothesized that the processes of transcriptional or post-transcriptional  
261 regulations may be deeply affected. In this respect, we investigated *in-silico*, the potential presence of  
262 *Schistosoma mansoni* miRNAs (sma-mir) in our parasite RNAseq data. At 24 h post-infections, we  
263 identified 54 miRNA precursors from miRBase with high quality alignment scores against the different  
264 RNAseq read libraries (naïve BgBRE, BB24, BV24, BR24). To avoid cross-species misidentifications,  
265 we selected precursors that were exclusively identified in infected and never identified in uninfected  
266 snails (naive BgBRE). Eleven miRNA precursors corresponding to *Schistosoma mansoni* were  
267 identified (Fig 7A). Nine of the parasite miRNA precursors were specific to the Brazil-infected libraries

268 (BB24); two were specific of the Venezuela-infected libraries (BV24); and one was shared across the  
269 three infected conditions (BB24, BV24 and BR24). Although we identify 49 miRNA precursor  
270 sequences specific to *S. mansoni* (Fig 7B), we decided to select only miRNAs covered by 100%  
271 nucleotide similarity that allowed to predict 11 miRNAs in mature (eg. sma-mir-2d-3p, sma-mir-190-  
272 3p) or precursor (sma-mir-8431) forms. Then, in order to identify candidate sequences that could  
273 represent putative miRNA targets, we used the Miranda tool (S4 Table). Only RNA-RNA interactions  
274 that showed good scores for pairing (>140) and enthalpy (<15 Kcal) were considered. The number of  
275 targets pertaining to the differentially expressed immune-related transcripts identified in Fig 1 that were  
276 found for the identified miRNAs ranged from 2 targets for sma-mir-8456, to 50 targets for sma-mir-2d  
277 of the differentially expressed immune-related transcripts.

278 The miRNAs identified under the sympatric condition (SmbRE) were predicted to potentially target  
279 43.5% of the differentially represented immune-related transcripts identified in the RNAseq experiment  
280 (Fig 1B, Fig 7) whereas 6.8% and 5.1% were targeted in allopatric conditions, SmVEN and Srod,  
281 respectively with fewer available miRNA as well (Fig 1B, Fig 7). The lack of such potential weapon to  
282 target host immune system in allopatric compared to the sympatric strain may explain the absence of  
283 immunosuppression observed in allopatric conditions. Otherwise, we did a focus on miRNAs that were  
284 shared between sympatric and allopatric interactions to try to understand the similar prevalence observed  
285 between sympatric and allopatric infections. Like so, we identified one miRNA: sma-miR-190-3p (Fig  
286 7 and S4 Table). This miRNA was predicted to bind 17 different targets among which, we identified  
287 different variants of the Fibrinogen Related Protein (FREP) family and a cytotoxic/cytolytic humoral  
288 factor the biomphalysin. To go further, we look at the expression of those candidates following infection.  
289 If FREP transcripts were down regulated in sympatric interaction, it is not always the case in allopatry.  
290 However, interestingly all biomphalysin transcripts were under-represented in sympatric and allopatric  
291 interactions. Altogether, these data suggest that the parasites might hijack the host immune response  
292 using dedicated miRNAs as the sma-miR-190-3p described in the present study.

293

294 Survival of snail following infection

295 To examine the potential impact of allopatric or sympatric parasites on snail survival, we investigated  
296 the mortality rates of infected snails over 4 months. The survival rate was significantly higher for non-  
297 infected snails compared to infected snails (sympatric interaction Kaplan-Meier Log Rank test  $p = 1.39$   
298  $\times 10^{-5}$  and allopatric interaction  $p = 0.0005$ ). However, there was no significant difference in the mortality  
299 rates of snails subjected to sympatric versus allopatric interactions: at the end of the experiment, the  
300 survival rates were 72% and 65% for the allopatric and sympatric interactions, respectively (Kaplan-  
301 Meier Log Rank test  $p = 0.243$ ) (S2 Fig).

302

## 303 Discussion

304 In the natural environment, it is assumed that the parasitic genes responsible for infectivity will evolve  
305 alongside the host defence genes, resulting in adaptation of the interactions between local host and  
306 parasite populations [52, 53]. In this context, local/sympatric parasites were expected to display a greater  
307 infectiveness, reproductive success, and virulence in host populations compared to foreign/allopatric  
308 parasites [36, 37, 54, 55]. However, in some cases this rule may be contradicted, as certain allopatric  
309 parasite-host interactions have been reported to be significantly more compatibles than the  
310 corresponding sympatric combinations [56, 57], it appears that certain *Biomphalaria/Schistosoma*  
311 interactions do not fulfil at the local adaptation between the host and the parasite, in which the sympatric  
312 parasite is expected to perform better than the allopatric one [36, 37, 54, 55].

313 Using field data, Morand et al. (1996) [38], Prugnolle et al. (2006) [5] and Mutuku et al. (2014) [39]  
314 showed that although sympatric parasite-host combinations of schistosomes and snails do tend to be  
315 more compatible, exceptions exist wherein particular allopatric combinations are equally or significantly  
316 more compatibles. Similar results were obtained when comparing the interactions of Brazilian and  
317 Guadeloupean snails versus *Schistosoma* infections [41]. The authors found that allopatric  
318 Guadeloupean parasites were not able to infect Brazilian snails; but Brazilian parasites were able to  
319 infect the allopatric Guadeloupean snails. Furthermore, this work demonstrated the presence of local  
320 adaptation between reactive oxygen species (ROS) and ROS scavengers in this system [41]. Based on  
321 these observations, we propose that it would be important to develop integrative analysis to depict and

322 understand the precise molecular crosstalk (immunobiological interactions) occurring in such highly  
323 compatible sympatric and allopatric systems. Thus, dual-comparative approaches were used herein to  
324 simultaneously analyze the responses of *Biomphalaria* snails and *Schistosoma* parasites into sympatric  
325 or allopatric interactions displaying similar compatibilities.

326 The present RNAseq analysis demonstrated that in sympatric interaction (BB) a huge  
327 immunosuppression occurs. Twenty-four hours after the infection, the three immunological processes:  
328 (i) immune recognition, (ii) effector and (iii) signaling pathways (Fig 1 and 2) were down regulated.  
329 Conversely, in allopatric interactions (BV and BR), host immune response was activated after 96 hours  
330 (Fig 1 and 2). Differentially regulated transcripts mostly belong to immune cellular activation,  
331 migration, proliferation, or differentiation (Fig 2). An EdU labelling was used to detect proliferation and  
332 confirmed that hemocyte proliferation is inhibited during interaction with two different strains from  
333 Brazil and Venezuela (Fig 3, 4A and 4B). In addition, we discovered that a new subpopulation of  
334 proliferating hemocytes (named P2), exclusively differentiate 24h following allopatric infections (Fig  
335 4C and 4D). P2 was EdU-positive and characterized by an increased in granularity, indicating that the  
336 new P2 cell subtype could proliferate (Fig 4). However, in absence of specific hemocyte markers, it is  
337 difficult to analyze precisely which hemocyte morphotype are proliferating (Fig 4C, D). The P2  
338 subpopulation would thus originates from either a morphological change in an existing subset  
339 (correlating potentially with a decline in the P1 population), or represents cells that are migrating from  
340 tissues or hematopoietic organ to reach the hemolymph. Indeed, P2 population reflects newly  
341 proliferating cells that present higher EdU positive cells than the P1 population (Fig 4C, D). Further  
342 investigations will be necessary to conclude on the origin of P2 population.

343 In *Biomphalaria* snails, we know 3 main hemocyte morphotypes, the blast-like cells, the type I  
344 hyalinocytes and the granulocytes [58]. Based on the flow cytometry and Edu labelling approaches we  
345 can supposed that bigger and granular cells (granulocytes and hyalinocytes) are the ones that  
346 proliferates. This is demonstrated in S3 Fig in which Edu labelling was observed for hyalinocytes and  
347 granulocytes but never for blast-like cells (S3 Fig). These results seem to demonstrate a differentiation  
348 or sub-functionalization in hemocyte subtypes following infection.

349 This differentiation or sub-functionalization is different comparing sympatric and allopatric interactions,  
350 i.e., hemocyte proliferation decreased more rapidly in sympatric rather than in allopatric interactions  
351 (Fig 3 and 4), P2 population observed solely in allopatric interactions (Fig 4). Using reciprocal sympatric  
352 and allopatric interactions, we demonstrate that the cellular or molecular phenotype observed refers to  
353 potential co-evolution or adaptation rather to a simple host or parasite strain effect (Fig 3 and 4).

354 The strong immunosuppression observed within 24h of infection by a sympatric parasite, and the  
355 inhibition of hemocyte proliferation can certainly explain the differences in the growth of sympatric and  
356 allopatric parasites. Indeed, we observed a significant difference in sporocyst size 24h after infection  
357 (Fig 5), with sympatric sporocysts that were one-third bigger than allopatric sporocysts. But, 96h after  
358 infection, there was no more significant size difference between sympatric and allopatric parasites (Fig  
359 5). This difference in size between the sympatric and the allopatric parasites at the beginning of the  
360 interaction can be explained by several hypotheses, (i) a delay in development of the allopatric parasite  
361 due to the necessity to circumvent the host immune response, (ii) the intrinsic ontogenesis or  
362 morphogenesis of post-miracidial intramolluscan stages that can be longer for allopatric SmVEN  
363 parasite compared to sympatric SmbRE parasite, finally (iii) the miracidial binding and penetration into  
364 the tissues of the host may take longer for the allopatric parasite than for the sympatric parasite. The  
365 consequences of this delay in terms of secondary sporocyst development, number of cercariae produced,  
366 or cercariae infectivity and pathogenicity for the vertebrate host, will deserve further investigation to  
367 conclude about a potential fitness cost between sympatric and allopatric parasites.

368 To find new clues as to how sympatric parasites immunosuppress the host or circumvent the host  
369 immune system, we used a dual-RNAseq approach to compare transcripts expression of the sympatric  
370 and allopatric parasite intra-molluscal stages (Fig 6). As the histological differences were solely  
371 observed at 24h after infection, we used dual-RNAseq to investigate the parasite expression patterns at  
372 the same time point of infection. Most of the parasite transcripts belonged to the processes of nucleotide  
373 metabolism, transcription, translation and cell differentiation, development, and growth. We also  
374 identified some transcripts with GO terms or functions related to immuno-modulation or immuno-  
375 suppression (Fig 6 and S4 Table). Nearly all of the identified transcripts were over-represented in the  
376 sympatric interaction compared to the allopatric ones. Our results therefore suggest that the installation,

377 development and growth of the parasite occurred much more rapidly in the BgBRE/SmBRE  
378 combination, as sympatric parasites seemed to interfere more efficiently with the host immune system.  
379 However, RNAseq data did not give any clear information about how allopatric parasites succeed in  
380 circumventing the host immune system. We thus next examined the generated dual-RNAseq libraries in  
381 an effort to identify whether sympatric and/or allopatric schistosomes could hijack the host immune  
382 system using microRNAs. To begin testing this hypothesis, we confronted the dual-RNAseq data to the  
383 *Schistosoma mansoni* subset of miRBase to identify the presence of parasite microRNAs (pmiRNAs) in  
384 our datasets. Even if we don't know whether pmiRNAs were present in contact with the host immune  
385 system or simply endogenic, this in-silico exploration may ask the question to a potential molecular  
386 discussion between metazoan organisms in a host-parasite system, based on nucleic acid weapons.  
387 miRNAs are known to regulate numerous biological processes, including key immune response genes  
388 [59, 60]. Recent work has demonstrated that circulating small non-coding RNAs from parasites have  
389 hijack roles against host metabolism, notably in the interaction of schistosomes with their vertebrate  
390 hosts [61-63]. Such non-coding RNAs could act as exogenous miRNAs to interfere with or circumvent  
391 the host immune system. In the present study, 24h after infection, several differentially expressed  
392 pmiRNAs were identified. We predicted targets of such pmiRNAs in the *Biomphalaria* immune  
393 reference transcriptome and found that they may target 43.5% of the differentially regulated immune  
394 transcripts identified in the RNAseq approach (Fig 7). In contrast, far fewer correspondences were  
395 identified for the allopatric interactions (Fig 7). The higher proportion of targeted genes in the sympatric  
396 interaction may be responsible for the observed efficient immunosuppression. If confirmed, such  
397 mechanism would reveal a specific co-evolution or adaptation in the transcriptional regulation between  
398 sympatric host and parasite. However, even if more host immune genes appeared to be targeted in the  
399 sympatric combination compared to the allopatric one's (Fig 7), both sympatric and allopatric  
400 interactions displayed the same ability to succeed to infect the host. This similarity in compatibility  
401 phenotype between sympatric and allopatric parasites could potentially results from their ability to target  
402 host immune weapons or host genes that regulate innate cellular response using miRNAs. A unique  
403 miRNA was found in all allopatric and sympatric parasites, sma-miR-190-3p. It is predicted to bind  
404 various targets including Fibrinogen Related Protein (FREP) and biomphalysin. The FREP family



405 members are known as pathogen recognition receptors [64, 65] and FREP knockdown is associated with  
406 an increase of snail compatibility toward *Schistosoma* infections [66, 67]. The biomphalysins belong to  
407 beta pore forming toxins and are key humoral factors of biomphalaria snails involved in  
408 cytotoxic/cytolytic activities against *Schistosoma* parasites with the ability to bind miracidia and  
409 sporocyst surfaces [68, 69]. Moreover, transcription of these molecules was mostly reduced in sympatric  
410 and allopatric interactions (figs. 1 and 2) supporting the hypothesis that sma-miR-190-3p or other  
411 pmiRNA members could play an essential role in parasite compatibility. Parasites expressing such  
412 miRNAs would thus be considered as highly virulent parasites with strong infecting capabilities. By  
413 producing dedicated miRNAs, the parasites were potentially able to regulate transcriptional, post-  
414 transcriptional, translational and protein stability processes that might help them to subvert the snail's  
415 immune defences. Even if these results are particularly interesting, a dedicated small RNAs (<30nt)  
416 sequencing is now mandatory to validate or not the miRNA molecular cross talk occurring between  
417 Schistosome larval stages and their snail intermediate hosts as it has been shown for the interaction with  
418 their vertebrate definitive hosts.

419 Compatibility reflects the outcome of complex immunobiological interactions and depends on: (i) the  
420 ability of the snail immune system to recognize and kill the parasite; and (ii) the ability of the parasite  
421 to circumvent or evade the host immune response [20, 46, 70]. Based on the present observations, we  
422 propose that sympatric and allopatric interactions trigger totally different responses. In the sympatric  
423 interaction, the parasite is able to induce a host immunosuppression within the first day of infection  
424 enabling it to quickly infect the host and readily begins its development. In the allopatric interaction, the  
425 parasite is not able to quickly neutralize the host immune system, and as a consequence the parasite is  
426 recognized by host defense system that mounts a potent immune response. In allopatric parasite, the  
427 disruption of the activation of their developmental program during the first day of infection could results  
428 from the need to resist to the snail immune system. However, they seemed to be able to quickly protect  
429 themselves against the host immune response and develop normally in snail tissues as soon as 96h post-  
430 infection. Thereafter, in the medium- or long-term, there are no observable differences in the prevalence,  
431 intensity, or snail survival comparing sympatric and allopatric interactions (S1 Table, S2 Fig).

432 Thus, we show that despite having similar prevalence phenotypes, sympatric and allopatric snail-  
433 *Schistosoma* interactions displayed a very different immunobiological dialogue at the molecular level.  
434 Intriguingly, these different immunobiological interactions seem to have no repercussions upon parasite  
435 growth at longer term or to host survival. As differences at the molecular level do not correspond  
436 apparently to any ecologically meaningful changes in term of fitness, it is not straightforward to  
437 demonstrate local adaptation in such systems. However, we do not know if fitness costs could affect  
438 other biological traits in sympatric and allopatric interactions, as for example secondary sporocysts  
439 production and growth, number of cercariae shedding, or cercariae infectivity and pathogenicity towards  
440 the vertebrate host. Demonstrating local adaptation would thus appear extremely complex and would  
441 indeed deserve further investigation. It is hard to draw the line as to when local adaptation is or is not  
442 present. However, our results argue that the differences find at the molecular level may ultimately  
443 contribute to the evolution of local adaptation at an ecological level.

444 Nevertheless, the ability for allopatric pathogens to adapt rapidly and efficiently to new hosts could have  
445 critical consequences on disease emergence and risk of schistosomiasis outbreaks.

446 Past events of allopatric parasites reaching new areas of transmission, even in large-geographic scale  
447 dispersion, have been largely documented. The most famous example being the schistosomiasis  
448 colonization of South America since the slave trade of the 16<sup>th</sup>-19<sup>th</sup> Centuries [71, 72]. *Schistosoma*  
449 originated in Asia, reached Africa 12 to 19 million years ago (MYA), and gave rise to all *Schistosoma*  
450 species known in Africa [72]. *S. mansoni* diverged from *S. rodhaini* around 2.8MYA [71, 73], and  
451 thereafter, 400 to 500 years ago, colonized South America [71, 72]. This colonization of South America  
452 by *S. mansoni* from Africa was rendered possible by the presence of the snail host: *Biomphalaria*  
453 *glabrata*. All African species of *Biomphalaria* are monophyletic and seem to have originated from  
454 paraphyletic South American clade [74-76]. The ancestor of *B. glabrata* appears to have colonized  
455 Africa 1 to 5 MYA, giving rise to all 12 species of *Biomphalaria* known today in Africa [77]. In South  
456 America and Caribbean Island, *S. mansoni* infects *B. glabrata*; in Africa, it infects mostly *B. pfeifferi*  
457 and *B. alexandrina*. We found that South American *S. mansoni* parasites are highly compatible with  
458 their sympatric South American snail hosts, whereas African *S. mansoni* parasites display low  
459 compatibility phenotype with South American snail hosts (S1 Table). Interestingly, the South American

460 parasites did not lose their compatibility for African snail hosts; i.e., the prevalences are similar to  
461 African parasites when confronted to African snails (S1 Table). The recent African origin of South  
462 American *Schistosoma* parasites (introduction in South America 400 to 500 years ago) may explain why  
463 they have not diverged sufficiently in South America to lose their compatibility for African snail hosts.  
464 In this case, the transfer of allopatric parasites from Africa to South American snail hosts have be  
465 successful and result in the emergence of schistosomiasis in South America.  
466 More recently another case of compatible allopatric parasite emergence have been observed when  
467 schistosomiasis have reach Europe [78, 79]. Here, humans infected in Senegal have imported a hybrid  
468 between *Schistosoma haematobium* and *Schistosoma bovis* into Corsica. In this case urogenital  
469 schistosomiasis could be introduced and easily and rapidly spread into this novel area of south Corsica  
470 because *Bulinus truncatus* the vector snail of *S. haematobium* was endemic in the Corsica Cavu River  
471 [78, 79]. However, this allopatric African hybrid parasite was able to adapt efficiently to the Corsican  
472 new *B. truncatus* host. If parasite hybridization can potentially have a putative role in increasing the  
473 colonization potential of such *S. haematobium*, it would be particularly interesting to analyze and depict  
474 the molecular support of such allopatric interactions to predict the potential risk of schistosomiasis  
475 outbreaks in other European areas, or other potential transmission foci.

476

477         If we hope to draw conclusions regarding the existence of emerging or outbreak risks, we need  
478 to develop integrative approaches to explore fine-scale patterns of host-parasite interactions. We must  
479 consider the spatial scale at which comparisons are conducted, the patterns of disease occurrence, the  
480 population genetics, and the involvement of physiological, immunological, and molecular processes.  
481 Studying the relevant factors at the relevant timing would be of critical importance in terms of  
482 schistosomiasis control. Understanding further, how these allopatric parasites efficiently infect host  
483 snails would be mandatory to identify markers and develop new tools to predict or to quantify risks of  
484 schistosomiasis outbreaks. Now it would be particularly relevant to go back to the field to see how  
485 translatable are our results in a more dynamic field situations with genetically diverse hosts and parasites  
486 witch evolved under complex abiotic and biotic interactions, with newly encountered allopatric hosts  
487 and potentially on quite different spatial scales. For this we have a wonderful playground in Corsica.

## 489 **Materials and Methods**

### 490 Ethics statement

491 Our laboratory holds permit # A66040 for experiments on animals from both the French  
492 Ministry of Agriculture and Fisheries, and the French Ministry of National Education, Research,  
493 and Technology. The housing, breeding and animal care of the utilized animals followed the  
494 ethical requirements of our country. The researchers also possess an official certificate for  
495 animal experimentation from both French ministries (Decree # 87–848, October 19, 1987).  
496 Animal experimentation followed the guidelines of the French CNRS. The different protocols  
497 used in this study had been approved by the French veterinary agency from the DRAAF  
498 Languedoc-Roussillon (Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt),  
499 Montpellier, France (authorization # 007083).

500

### 501 Biological materials

502 The two studied strains of *S. mansoni* (the Brazilian (SmBRE) or the Venezuelan (SmVEN) strains) and  
503 the strain of *S. rodhaini* (Srod) had been maintained in the laboratory using Swiss OF1 mice (Charles  
504 River Laboratories, France) as the definitive host. Two snail strains of *Biomphalaria glabrata* were used  
505 in this study: the albino Brazilian strain, (BgBRE) and the Venezuelan strain, (BgVEN). All host and  
506 parasite strains of each different geographical origin were recovered in their native locality and parasite  
507 strains were maintain in the laboratory always on their sympatric snail hosts to maintain the same  
508 selective pressure and sympatric adaptation on parasite. We housed snails in tanks filled with pond water  
509 at 25°C with a 12:12 hour light:dark cycle and supplied ad libitum with fresh lettuce. The Brazilian  
510 strain originates from the locality of Recife (east Brazil, recovered in the field in 1975), the Venezuelan  
511 strains of snail and parasite were recovered from the locality of Guacara (north Venezuela, recovered in  
512 the field in 1975) and the African species *Schistosoma rodhaini* originates from Burundi and was  
513 obtained from the British Museum National History (recovered in 1984). These *Schistosoma*  
514 isolates/species have been selected because they exhibited similar infectivity toward BgBRE or BgVEN  
515 strains (see prevalence and intensity in S1 Table). These high compatibilities were followed-up by the  
516 cercariae emissions. For all these interactions we observed comparable cercariae shedding (S1 Table).

517 Prevalence of SmBRE and SmVEN for the African vector snail *Biomphalaria pfeifferi* from Senegal  
518 (BpSEN), and prevalence of the corresponding parasite SmSEN on South American snails were also  
519 tested (S1 Table).

520

## 521 RNAseq experimental protocol

522 In order to investigate the molecular response of snails against sympatric and allopatric parasites, a  
523 global comparative transcriptomic approach was conducted. One hundred and twenty BgBRE snails  
524 were infected with SmBRE, SmVEN or Srod. Each snail was individually exposed for 12 h to 10  
525 miracidia in 5mL of pond water. For each experimental infection, 30 snails were recovered at 24h and  
526 96h after infection. Pools of 30 snails were composed of 10 juvenile snails (shell diameter from 3 to 5  
527 mm), 10 mature adult snails (shell diameter from 7 to 9 mm) and 10 old adult snails (shell diameter from  
528 11 to 13 mm). The samples were named as follows: BB24, BB96 for BgBRE infected with SmBRE;  
529 BV24, BV96 for BgBRE infected with SmVEN; and BR24, BR96 for BgBRE infected with Srod. We  
530 realised 2 pools of 30 uninfected BgBRE snails (pool of immature, mature and old snails) named Bre1  
531 and Bre2, that were used as control conditions for all downstream comparative analyses.

### 532 - Whole-snail RNA extraction and sequencing

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

### 536 - De novo transcriptome assembly

537 *De novo* transcriptome assembly, using all time points, was performed using an in-house pipeline  
538 created with the Velvet (1.2.02), Oases (v0.2.04), and CD-HIT-EST (v4.5.4) programs. The assembly  
539 of the consensus reference transcriptome was optimized using various parameters, including k-mer  
540 length, insert length and expected coverage, as previously described [43, 44]. A *de novo* transcriptome  
541 was created and contained 159,711 transcripts.

### 542 - Differential expression analysis

543 High-quality reads (Phred score >29) were aligned to the *de novo* transcriptome using Bowtie2 (v2.0.2),

544 which was run locally on a Galaxy server. To compare the host responses during the sympatric or  
545 allopatric interactions, we used the DESeq2 (v2.12) was used to analyse the differential transcript  
546 representation between *BgBRE* control strains (uninfected *BgBRE1* and *BgBRE2*) to the sympatric and  
547 allopatric conditions (p-value < 0.1) [44]. A Venn diagram was generated using the Venny 2.1 software  
548 to highlight which differentially expressed transcripts were specific or common to the different  
549 interactions. A heatmap was obtained using the log2 Fold Change with Hierarchical Ascending  
550 Clustering (HAC) and Pearson correlation (uncentered) as applied by the Cluster (v3.0) and Java  
551 TreeView (v1.1.6r4) softwares packages. The differentially represented transcripts were functionally  
552 classified using a BlastX analysis with the cut-off set to e-value < 1e<sup>-3</sup> (NCBI dataset; thanks to the  
553 Roscoff Data center Cluster, UPMC) and gene ontology was assigned using an automatic annotation,  
554 implemented in Blast2GO (v3.0.8) (S2 Table). We identified potential immune transcripts involved in  
555 snail immunity based on functional domains predictions and literature searches.

556

## 557 *Schistosoma* intra-molluscal stage transcriptome analysis: Dual RNA-seq

558 A dual RNA-seq approach was conducted to gain in a broader understanding of sympatric and allopatric  
559 host/parasite interactions.

### 560 - Schistosome read selection

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

### 568 - Gene analysis

569 The above-selected *Schistosoma* reads were mapped against the concatenate genome to identify intra-  
570 molluscal stage-specific *Schistosoma* genes. In order to select the relevant genes, the reads mapped in  
571 all experimental conditions were summed. Solely genes with a minimal sum of 10 reads were kept for

572 the analysis. A heatmap was generated to analyse *Schistosoma* gene expression patterns using  
573 Hierarchical Ascending Clustering (HAC) with Pearson correlation (uncentered) as applied by the  
574 Cluster (v3.0) and Java TreeView (v1.1.6r4) software packages. Functional annotation of the genes was  
575 assigned using BlastX with the cut-off set to e-value  $< 1e^{-3}$  (NCBI dataset, local cluster) and gene  
576 ontology was performed using Blast2GO (v4.0.7) (S3 Table).

577

## 578 Innate immune cellular response analysis: microscopy and flow cytometry

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

### 587 - Hemocyte proliferation analysis: microscopy

588 Microscopic inspection of hemocyte proliferation was conducted using 12 infected BgBRE (6  
589 BgBRExSmBRE and 6 BgBRExSmVEN) and 3 uninfected BgBRE snails. The hemocytes of 3 snails  
590 (biological replicates) were counted for each condition at 24h and 96h after infection. The proliferation  
591 of circulating hemocytes was studied by using a Click-iT EdU Alexa Fluor 488 Flow Imaging Kit  
592 (Molecular Probes). At each time point, circulating hemocytes were recovered by direct puncture after  
593 foot retraction and 1mM of EdU solution was added to the hemolymph. Three hours later, the amount  
594 of EdU incorporated by the circulating hemocytes was visualized *in-vitro* after fixation of the cells and  
595 performing a covalent coupling of Alexa Fluor 488 to the EdU residues through a click chemistry reaction  
596 following manufacturer indications, then nuclei of hemocytes were counterstained with DAPI (Biotum)  
597 staining, and the sample was analysed on a confocal microscope using a Zeiss LSM 700, with 4 lasers  
598 (405, 488, 555 and 633 nm). Positive cells were counted and between-sample differences in the

599 percentage of proliferation were tested using a Fisher exact test, with significance accepted at p-  
600 value<0.05.

#### 601 - Hemocyte proliferation and population profiles analysis: flow cytometry

602 Qualitative changes in hemocyte populations following infection by sympatric or allopatric parasites  
603 were studied using a flow cytometry approach. For this 72 infected BgBRE or BgVEN (36 infected by  
604 SmBRE and 36 infected by SmVEN) and 18 uninfected BgBRE or BgVEN snails were used. Six  
605 biological replicates (pools of 3 snails per replicate) were used for each condition. Flow cytometry was  
606 used to profile and assess the proliferation of circulating hemocytes using Click-iTEdUAlexa Fluor 647  
607 labelling (Molecular Probes). At each time point, 1mM of EdU solution was injected into pericardial  
608 cavity of each snail. Three hours later six replicates of 3 snails were collected, and the hemolymph was  
609 extracted from the head-foot according to standard procedures [80]. The hemolymph was pooled from  
610 the three snails, and 100 µl were subjected to analysis with the above-listed kit, according to the  
611 manufacturer's instructions. The percentage of proliferative cells was calculated by flow cytometry.

612 The hemocytes were profiled along the course of infection using Side Scatter Chanel (SSC) to estimate  
613 cell granularity and Forward Scatter Chanel (FSC) to estimate cell size. The cell repartition along these  
614 two parameters enables to identify cell sub-populations. The flow cytometry was performed using a  
615 FACS Canto from BD Biosciences (RIO Imaging Platform, Montpellier, France). For each sample,  
616 10,000 events were counted. The results were analyzed with the FlowJo V 10.0.8 software. Between-  
617 group differences in the percent of proliferation were tested using the Mann-Whitney U-test, with  
618 significance accepted at p-value<0.05.

619

#### 620 **Histological procedures**

621 A histological approach was conducted in order to investigate differences in miracidia to sporocyst  
622 development, while comparing sympatric and allopatric parasite growth, development and maturation  
623 into snail tissues. BgBRE snails were infected as described above with either 10 mi of SmBRE  
624 (sympatric) (n = 6 snails) or 10 mi of SmVEN (allopatric) parasite (n = 6 snails). At each time point, 24  
625 and 96 h after infection, three snails were fixed in Halmi's fixative (4.5% mercuric chloride, 0.5%  
626 sodium chloride, 2% trichloroacetic acid, 20% formol, 4% acetic acid and 10% picric acid-saturated



627 aqueous solution). Embedding in paraffin and transverse histological sections (3-  $\mu$  m) were performed  
628 using the RHEM platform (Montpellier, France) facilities. The slides were stained using Heidenhain's  
629 azan trichromatic staining solution as follows: (i) serial re-hydration was performed in toluene followed  
630 by 95%, 70%, and 30% ethanol and then distilled water; (ii) coloration was performed using azocarmine  
631 G (70% ethanol, 1% aniline, 1% acetic alcohol, distilled water, 5% phosphotungstic acid, distilled water,  
632 Heidenhain's azan) and (iii) serial dehydration was performed using 95% ethanol, absolute ethanol, and  
633 toluene. The preparations were then mounted with Entellan (Sigma Life Science, St. Louis Missouri,  
634 USA) and subjected to microscopic examination. When a parasite is observed in snail tissue, the parasite  
635 size was measured using the imaging analysis software ImageJ (v2.0.0) for each adjacent histological  
636 section in which the parasite is observed. The contour of the parasite is detailed very precisely using  
637 ImageJ and the pixel number is reported on a size scale analyzed in the same manner to calculate parasite  
638 size. Size is expressed as parasite surface in  $\mu\text{m}^2$  as the mean of the 3 bigger parasite sections recorded.  
639 At 24h, n = 9 sympatric and n = 8 allopatric parasites were measured and at 96h, n = 7 sympatric and n  
640 = 10 allopatric parasites were measured. The size differences between sympatric and allopatric parasite  
641 groups were tested using the Mann-Whitney U-test with statistical significance accepted at a p-value <  
642 0.05.

643

#### 644 In-silico characterization of *Schistosoma* miRNAs

645 Parasites may communicate or interfere with their host using different strategies based mainly on  
646 excreted/secreted products released into hemolymph. In this context, miRNAs appeared as the most  
647 relevant mean of communication that can be used by parasites. To test for such hypothesis *S. mansoni*  
648 miRNAs were analyzed *in-silico* by comparing the relevant miRNA database (miRBase) to our RNAseq  
649 libraries generated at the 24h following sympatric or allopatric infections. *S. mansoni* precursor  
650 sequences were downloaded from miRBase (<http://www.mirbase.org>, 03/09/2017), and high-quality  
651 reads from naive (BgBRE) and 24 h post-infection samples (BB24, BV24, BR24) were aligned against  
652 a *S. mansoni* sub-database of miRBase, as previously described [81]. The identified precursors were  
653 confirmed by alignment of high-scoring reads onto precursor and mature miRNAs from miRBase.

654 Solely reads with 100% identity were retained for analysis. The localization of each read against miRNA  
655 sequence allowed us to identify either the precursor or just the mature miRNA. Precursors found under  
656 both naive and infected conditions were excluded to retain exclusively the miRNAs present in samples  
657 from infected snails and avoid cross-species contamination because of the potential conserved features  
658 of miRNAs from *B. glabrata* and *S. mansoni*.

659 Putative miRNA targets were predicted from among the differentially represented immune-related  
660 transcripts (figure 1) using Miranda tools (using parameters: Miranda input\_miRinput\_Transcriptome -  
661 out results.txt -quiet -sc 140 -en -15) [82]. Because mature miRNAs may exist in two forms depending  
662 on which strand (5'-3') of the precursor stem-loop is matured the predicted interactions could involve  
663 the 5' and/or 3' forms, as noted. The results were extracted using the awk tool, listed in S4 Table, and  
664 used to generate a Venn diagram. To confirm the ability of a selected pre-miRNA to produce the stem-  
665 loop necessary to produce the mature form, the secondary structures of precursor were predicted using  
666 RNA structure Web tool (<http://rna.urmc.rochester.edu/RNAstructureWeb>, 03/09/2017) using default  
667 parameters.

668

## 669 Snail survival analysis

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

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680

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687 **References:**

- 688 1. WHO. Schistosomiasis Fact Sheet No 115 World Health Organisation. 2010.
- 689 2. Basch PF. Intermediate host specificity in *Schistosoma mansoni*. *Experimental*  
690 *Parasitology*. 1976;39(1):150-69.
- 691 3. Theron A, Pages JR, Rognon A. *Schistosoma mansoni*: distribution patterns of miracidia  
692 among *Biomphalaria glabrata* snail as related to host susceptibility and sporocyst regulatory  
693 processes. *Experimental Parasitology*. 1997;85(1):1-9.
- 694 4. Theron A, Rognon A, Gourbal B, Mitta G. Multi-parasite host susceptibility and multi-  
695 host parasite infectivity: a new approach of the *Biomphalaria glabrata*/*Schistosoma mansoni*  
696 compatibility polymorphism. *Infection Genetics and Evolution*. 2014;26:80-8.
- 697 5. Prugnolle F, de Meeus T, Pointier JP, Durand P, Rognon A, Theron A. Geographical  
698 variations in infectivity and susceptibility in the host-parasite system *Schistosoma*  
699 *mansoni*/*Biomphalaria glabrata*: no evidence for local adaptation. *Parasitology*. 2006;133(Pt  
700 3):313-9.
- 701 6. Richards CS. Genetic factors in susceptibility of *Biomphalaria glabrata* for different  
702 strains of *Schistosoma mansoni*. *Parasitology*. 1975;70(2):231-41.
- 703 7. Mitta G, Adema CM, Gourbal B, Loker ES, Theron A. Compatibility polymorphism in  
704 snail/schistosome interactions: From field to theory to molecular mechanisms. *Dev Comp*  
705 *Immunol*. 2012;37(1):1-8.
- 706 8. Galinier R, Roger E, Mone Y, Duval D, Portet A, Pinaud S, et al. A multistrain approach  
707 to studying the mechanisms underlying compatibility in the interaction between *Biomphalaria*  
708 *glabrata* and *Schistosoma mansoni*. *PLoS Negl Trop Dis*. 2017;11(3):e0005398.
- 709 9. Mitta G, Gourbal B, Grunau C, Knight M, Bridger JM, Theron A. The Compatibility  
710 Between *Biomphalaria glabrata* Snails and *Schistosoma mansoni*: An Increasingly Complex  
711 Puzzle. *Adv Parasitol*. 2017;97:111-45.
- 712 10. Bonner KM, Bayne CJ, Larson MK, Blouin MS. Effects of Cu/Zn superoxide dismutase  
713 (sod1) genotype and genetic background on growth, reproduction and defense in  
714 *Biomphalaria glabrata*. *PLoS Negl Trop Dis*. 2012;6(6):e1701.
- 715 11. Goodall CP, Bender RC, Brooks JK, Bayne CJ. *Biomphalaria glabrata* cytosolic  
716 copper/zinc superoxide dismutase (SOD1) gene: association of SOD1 alleles with  
717 resistance/susceptibility to *Schistosoma mansoni*. *Mol Biochem Parasitol*. 2006;147(2):207-  
718 10.
- 719 12. Tennessen JA, Theron A, Marine M, Yeh JY, Rognon A, Blouin MS. Hyperdiverse gene  
720 cluster in snail host conveys resistance to human schistosome parasites. *PLoS genetics*.  
721 2015;11(3):e1005067.
- 722 13. Ittiprasert W, Miller A, Myers J, Nene V, El-Sayed NM, Knight M. Identification of  
723 immediate response genes dominantly expressed in juvenile resistant and susceptible  
724 *Biomphalaria glabrata* snails upon exposure to *Schistosoma mansoni*. *Molecular and*  
725 *Biochemical Parasitology*. 2010;169(1):27-39.
- 726 14. Lockyer AE, Emery AM, Kane RA, Walker AJ, Mayer CD, Mitta G, et al. Early differential  
727 gene expression in haemocytes from resistant and susceptible *Biomphalaria glabrata* strains  
728 in response to *Schistosoma mansoni*. *PloS one*. 2012;7(12):e51102.
- 729 15. Lockyer AE, Noble LR, Rollinson D, Jones CS. *Schistosoma mansoni*: resistant specific  
730 infection-induced gene expression in *Biomphalaria glabrata* identified by fluorescent-based  
731 differential display. *Experimental Parasitology*. 2004;107(1-2):97-104.
- 732 16. Lockyer AE, Spinks JN, Walker AJ, Kane RA, Noble LR, Rollinson D, et al. *Biomphalaria*  
733 *glabrata* transcriptome: identification of cell-signalling, transcriptional control and immune-

734 related genes from open reading frame expressed sequence tags (ORESTES). *Developmental*  
735 *and Comparative Immunology*. 2007;31(8):763-82.

736 17. Roger E, Grunau C, Pierce RJ, Hirai H, Gourbal B, Galinier R, et al. Controlled Chaos of  
737 Polymorphic Mucins in a Metazoan Parasite (*Schistosoma mansoni*) Interacting with Its  
738 Invertebrate Host (*Biomphalaria glabrata*). *PLoS Negl Trop Dis*. 2008;2(11):e330.

739 18. Galinier R, Portela J, Mone Y, Allienne JF, Henri H, Delbecq S, et al. Biomphalysin, a New  
740 beta Pore-forming Toxin Involved in *Biomphalaria glabrata* Immune Defense against  
741 *Schistosoma mansoni*. *PLoS Pathog*. 2013;9(3):e1003216.

742 19. Hahn UK, Bender RC, Bayne CJ. Involvement of nitric oxide in killing of *Schistosoma*  
743 *mansoni* sporocysts by hemocytes from resistant *Biomphalaria glabrata*. *Journal of*  
744 *Parasitology*. 2001;87(4):778-85.

745 20. Mone Y, Gourbal B, Duval D, Du Pasquier L, Kieffer-Jaquinod S, Mitta G. A large  
746 repertoire of parasite epitopes matched by a large repertoire of host immune receptors in an  
747 invertebrate host/parasite model. *PLoS Negl Trop Dis*. 2010;4(9).

748 21. Myers J, Ittiprasert W, Raghavan N, Miller A, Knight M. Differences in cysteine protease  
749 activity in *Schistosoma mansoni*-resistant and -susceptible *Biomphalaria glabrata* and  
750 characterization of the hepatopancreas cathepsin B Full-length cDNA. *Journal of Parasitology*.  
751 2008;94(3):659-68.

752 22. Pila EA, Gordy MA, Phillips VK, Kabore AL, Rudko SP, Hanington PC. Endogenous growth  
753 factor stimulation of hemocyte proliferation induces resistance to *Schistosoma mansoni*  
754 challenge in the snail host. *Proc Natl Acad Sci U S A*. 2016;113(19):5305-10.

755 23. Roger E, Mitta G, Mone Y, Bouchut A, Rognon A, Grunau C, et al. Molecular  
756 determinants of compatibility polymorphism in the *Biomphalaria glabrata*/*Schistosoma*  
757 *mansoni* model: New candidates identified by a global comparative proteomics approach. *Mol*  
758 *Biochem Parasitol*. 2008;157(2):205-16.

759 24. Bridger JM, Arican-Gotkas HD, Foster HA, Godwin LS, Harvey A, Kill IR, et al. The non-  
760 random repositioning of whole chromosomes and individual gene loci in interphase nuclei and  
761 its relevance in disease, infection, aging, and cancer. *Adv Exp Med Biol*. 2014;773:263-79.

762 25. Ittiprasert W, Knight M. Reversing the resistance phenotype of the *Biomphalaria*  
763 *glabrata* snail host *Schistosoma mansoni* infection by temperature modulation. *PLoS*  
764 *pathogens*. 2012;8(4):e1002677.

765 26. Ittiprasert W, Nene R, Miller A, Raghavan N, Lewis F, Hodgson J, et al. *Schistosoma*  
766 *mansoni* infection of juvenile *Biomphalaria glabrata* induces a differential stress response  
767 between resistant and susceptible snails. *Experimental Parasitology*. 2009;123(3):203-11.

768 27. Knight M, Elhelu O, Smith M, Haugen B, Miller A, Raghavan N, et al. Susceptibility of  
769 Snails to Infection with Schistosomes is influenced by Temperature and Expression of Heat  
770 Shock Proteins. *Epidemiology*. 2015;5(2):pii: 189. Epub.

771 28. Nelson MK, Cruz BC, Buena KL, Nguyen H, Sullivan JT. Effects of abnormal temperature  
772 and starvation on the internal defense system of the schistosome-transmitting snail  
773 *Biomphalaria glabrata*. *J Invertebr Pathol*. 2016.

774 29. Perrin C, Lepesant JM, Roger E, Duval D, Fneich S, Thuillier V, et al. *Schistosoma*  
775 *mansoni* mucin gene (SmPoMuc) expression: epigenetic control to shape adaptation to a new  
776 host. *PLoS pathogens*. 2012;9(8):e1003571.

777 30. Portet A, Pinaud S, Tetreau G, Galinier R, Cosseau C, Duval D, et al. Integrated multi-  
778 omic analyses in *Biomphalaria-Schistosoma* dialogue reveal the immunobiological significance  
779 of FREP-SmPoMuc interaction. *Developmental and comparative immunology*. 2017;doi:  
780 10.1016/j.dci.2017.02.025.

- 781 31. Osnas EE, Hurtado PJ, Dobson AP. Evolution of pathogen virulence across space during  
782 an epidemic. *Am Nat.* 2015;185(3):332-42.
- 783 32. Tack AJ, Thrall PH, Barrett LG, Burdon JJ, Laine AL. Variation in infectivity and  
784 aggressiveness in space and time in wild host-pathogen systems: causes and consequences. *J*  
785 *Evol Biol.* 2012;25(10):1918-36.
- 786 33. Frank SA. Models of parasite virulence. *The Quarterly review of biology.* 1996;71(1):37-  
787 78.
- 788 34. Kawecki T, Ebert D. Conceptual issues in local adaptation. *Ecology letters.* 2004;7:1225-  
789 41.
- 790 35. Kalbe M, Eizaguirre C, Scharsack JP, Jakobsen PJ. Reciprocal cross infection of  
791 sticklebacks with the diphylobothriidean cestode *Schistocephalus solidus* reveals consistent  
792 population differences in parasite growth and host resistance. *Parasites & vectors.*  
793 2016;9(1):130.
- 794 36. Ebert D. Virulence and local adaptation of horizontally transmitted parasite. *Science.*  
795 1994;265(5393):1084-6.
- 796 37. Lively CM. Adaptation by a Parasitic Trematode to Local Populations of Its Snail Host.  
797 *Evolution.* 1989;43(8)(1663-1671).
- 798 38. Morand S, Manning SD, Woolhouse ME. Parasite-host coevolution and geographic  
799 patterns of parasite infectivity and host susceptibility. *Proc Biol Sci.* 1996;263(1366):119-28.
- 800 39. Mutuku MW, Dweni CK, Mwangi M, Kinuthia JM, Mwangi IN, Maina GM, et al. Field-  
801 derived *Schistosoma mansoni* and *Biomphalaria pfeifferi* in Kenya: a compatible association  
802 characterized by lack of strong local adaptation, and presence of some snails able to  
803 persistently produce cercariae for over a year. *Parasites & vectors.* 2014;7:533.
- 804 40. Osnas EE, Lively CM. Parasite dose, prevalence of infection and local adaptation in a  
805 host-parasite system. *Parasitology.* 2004;128(Pt 2):223-8.
- 806 41. Mone Y, Ribou AC, Cosseau C, Duval D, Theron A, Mitta G, et al. An example of  
807 molecular co-evolution: reactive oxygen species (ROS) and ROS scavenger levels in  
808 *Schistosoma mansoni*/*Biomphalaria glabrata* interactions. *International journal for*  
809 *parasitology.* 2011;41(7):721-30.
- 810 42. Penczykowski RM, Laine AL, Koskella B. Understanding the ecology and evolution of  
811 host-parasite interactions across scales. *Evolutionary applications.* 2015;9(1):37-52.
- 812 43. Dheilly NM, Duval D, Mouahid G, Emans R, Allienne JF, Galinier R, et al. A family of  
813 variable immunoglobulin and lectin domain containing molecules in the snail *Biomphalaria*  
814 *glabrata*. *Dev Comp Immunol.* 2015;48(1):234-43.
- 815 44. Pinaud S, Portela J, Duval D, Nowacki FC, Olive MA, Allienne JF, et al. A Shift from  
816 Cellular to Humoral Responses Contributes to Innate Immune Memory in the Vector Snail  
817 *Biomphalaria glabrata*. *PLoS pathogens.* 2016;12(1):e1005361.
- 818 45. Baeza Garcia A, Pierce RJ, Gourbal B, Werkmeister E, Colinet D, Reichhart JM, et al.  
819 Involvement of the cytokine MIF in the snail host immune response to the parasite  
820 *Schistosoma mansoni*. *PLoS pathogens.* 2010;6(9):e1001115.
- 821 46. Guillou F, Roger E, Mone Y, Rognon A, Grunau C, Theron A, et al. Excretory-secretory  
822 proteome of larval *Schistosoma mansoni* and *Echinostoma caproni*, two parasites of  
823 *Biomphalaria glabrata*. *Molecular and biochemical parasitology.* 2007.
- 824 47. Raghavan M, Wijeyesakere SJ, Peters LR, Del Cid N. Calreticulin in the immune system:  
825 ins and outs. *Trends in immunology.* 2013;34(1):13-21.
- 826 48. Ryu SJ, Park SC. Targeting major vault protein in senescence-associated apoptosis  
827 resistance. *Expert Opinion on Therapeutic Targets.* 2009;13(4):479-84.

- 828 49. Gilges D, Vinit MA, Callebaut I, Coulombel L, Cacheux V, Romeo PH, et al. Polydom: a  
829 secreted protein with pentraxin, complement control protein, epidermal growth factor and  
830 von Willebrand factor A domains. *Biochemical Journal*. 2000;352 Pt 1:49-59.
- 831 50. Bohdanowicz M, Schlam D, Hermansson M, Rizzuti D, Fairn GD, Ueyama T, et al.  
832 Phosphatidic acid is required for the constitutive ruffling and macropinocytosis of phagocytes.  
833 *Molecular Biology of the Cell*. 2013;24(11):1700-12, S1-7.
- 834 51. Grkovich A, Dennis EA. Phosphatidic acid phosphohydrolase in the regulation of  
835 inflammatory signaling. *Advances in Enzyme Regulation*. 2009;49(1):114-20.
- 836 52. Dybdahl MF, Storfer A. Parasite local adaptation: Red Queen versus suicide king.  
837 *TRENDS in Ecology and Evolution*. 2003;18(10):523-30.
- 838 53. Osnas EE, Lively CM. Immune response to sympatric and allopatric parasites in a snail-  
839 trematode interaction. *Frontiers in Zoology*. 2005;2:8.
- 840 54. Ebert D. Experimental evolution of parasites. *Science*. 1998;282(5393):1432-5.
- 841 55. Minchella DJ. Host life-history variation in response to parasitism. *Parasitology*.  
842 1985;90:205-16.
- 843 56. Greischar MA, Koskella B. A synthesis of experimental work on parasite local  
844 adaptation. *Ecology letters*. 2007;10(5):418-34.
- 845 57. Morand S, Manning SD, Woolhouse ME. Parasite-host coevolution and geographic  
846 patterns of parasite infectivity and host susceptibility. *Proceedings of the Royal Society B*.  
847 1996;263(1366):119-28.
- 848 58. Cavalcanti MG, Filho FC, Mendonca AM, Duarte GR, Barbosa CC, De Castro CM, et al.  
849 Morphological characterization of hemocytes from *Biomphalaria glabrata* and *Biomphalaria*  
850 *straminea*. *Micron*. 2012;43(2-3):285-91.
- 851 59. Li S, Shen L, Sun L, Xu J, Jin P, Chen L, et al. Small RNA-Seq analysis reveals microRNA-  
852 regulation of the Imd pathway during *Escherichia coli* infection in *Drosophila*. *Dev Comp*  
853 *Immunol*. 2017;70:80-7.
- 854 60. Zawada AM, Zhang L, Emrich IE, Rogacev KS, Krezdorn N, Rotter B, et al. MicroRNA  
855 profiling of human intermediate monocytes. *Immunobiology*. 2017;222(3):587-96.
- 856 61. Meningher T, Lerman G, Regev-Rudzki N, Gold D, Ben-Dov IZ, Sidi Y, et al. Schistosomal  
857 MicroRNAs Isolated From Extracellular Vesicles in Sera of Infected Patients: A New Tool for  
858 Diagnosis and Follow-up of Human Schistosomiasis. *Journal of Infectious Diseases*.  
859 2017;215(3):378-86.
- 860 62. Nowacki FC, Swain MT, Klychnikov OI, Niazi U, Ivens A, Quintana JF, et al. Protein and  
861 small non-coding RNA-enriched extracellular vesicles are released by the pathogenic blood  
862 fluke *Schistosoma mansoni*. *Journal of Extracellular Vesicles*. 2015;4:28665.
- 863 63. Walzer KA, Chi JT. Trans-kingdom small RNA transfer during host-pathogen  
864 interactions: The case of *P. falciparum* and erythrocytes. *RNA Biology*. 2017:1-8.
- 865 64. Adema CM, Hertel LA, Miller RD, Loker ES. A family of fibrinogen-related proteins that  
866 precipitates parasite-derived molecules is produced by an invertebrate after infection. *Proc*  
867 *Natl Acad Sci U S A*. 1997;94(16):8691-6.
- 868 65. Zhang H, Song L, Li C, Zhao J, Wang H, Gao Q, et al. Molecular cloning and  
869 characterization of a thioester-containing protein from Zhikong scallop *Chlamys farreri*.  
870 *Molecular Immunology*. 2007;44(14):3492-500.
- 871 66. Hanington PC, Forys MA, Loker ES. A somatically diversified defense factor, FREP3, is a  
872 determinant of snail resistance to schistosome infection. *PLoS Negl Trop Dis*. 2012;6(3):e1591.

- 873 67. Jiang Y, Loker ES, Zhang SM. In vivo and in vitro knockdown of FREP2 gene expression  
874 in the snail *Biomphalaria glabrata* using RNA interference. *Dev Comp Immunol.*  
875 2006;30(10):855-66.
- 876 68. Galinier R, Portela J, Mone Y, Allienne JF, Henri H, Delbecq S, et al. Biomphalysin, a new  
877 beta pore-forming toxin involved in *Biomphalaria glabrata* immune defense against  
878 *Schistosoma mansoni*. *PLoS pathogens.* 2013;9(3):e1003216.
- 879 69. Tetreau G, Pinaud S, Portet A, Galinier R, Gourbal B, Duval D. Specific Pathogen  
880 Recognition by Multiple Innate Immune Sensors in an Invertebrate. *Front Immunol.*  
881 2017;8:1249.
- 882 70. Van Der Knaap WPW, Loker ES. Immune mechanisms in trematod-snail interactions.  
883 *Parasitology Today.* 1990;6(6):175-82.
- 884 71. Crellen T, Allan F, David S, Durrant C, Huckvale T, Holroyd N, et al. Whole genome  
885 resequencing of the human parasite *Schistosoma mansoni* reveals population history and  
886 effects of selection. *Scientific reports.* 2016;6:20954.
- 887 72. Lockyer AE, Olson PD, Ostergaard P, Rollinson D, Johnston DA, Attwood SW, et al. The  
888 phylogeny of the Schistosomatidae based on three genes with emphasis on the  
889 interrelationships of *Schistosoma* Weinland, 1858. *Parasitology.* 2003;126(Pt 3):203-24.
- 890 73. Morgan JA, Dejong RJ, Adeoye GO, Ansa ED, Barbosa CS, Bremond P, et al. Origin and  
891 diversification of the human parasite *Schistosoma mansoni*. *Mol Ecol.* 2005;14(12):3889-902.
- 892 74. Campbell G, Jones CS, Lockyer AE, Hughes S, Brown D, Noble LR, et al. Molecular  
893 evidence supports an african affinity of the neotropical freshwater gastropod, *Biomphalaria*  
894 *glabrata*, say 1818, an intermediate host for *Schistosoma mansoni*. *Proceedings of the Royal*  
895 *Society B.* 2000;267(1460):2351-8.
- 896 75. DeJong RJ, Morgan JA, Paraense WL, Pointier JP, Amarista M, Ayeh-Kumi PF, et al.  
897 Evolutionary relationships and biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with  
898 implications regarding its role as host of the human bloodfluke, *Schistosoma mansoni*.  
899 *Molecular biology and evolution.* 2001;18(12):2225-39.
- 900 76. Theron A. Les schistosomes et leurs hôtes : apport des marqueurs moléculaires à la  
901 connaissance de leur phylogéographie, épidémiologie et écologie. *Bulletin de la Société*  
902 *Zoologique de France.* 2005;130 205-18.
- 903 77. Morgan JA, Dejong RJ, Snyder SD, Mkoji GM, Loker ES. *Schistosoma mansoni* and  
904 *Biomphalaria*: past history and future trends. *Parasitology.* 2001;123 Suppl:S211-28.
- 905 78. Boissier J, Grech-Angelini S, Webster BL, Allienne JF, Huyse T, Mas-Coma S, et al.  
906 Outbreak of urogenital schistosomiasis in Corsica (France): an epidemiological case study. *The*  
907 *Lancet Infectious Diseases.* 2016;16(8):971-9.
- 908 79. Boissier J, Mone H, Mitta G, Bargues MD, Molyneux D, Mas-Coma S. Schistosomiasis  
909 reaches Europe. *The Lancet Infectious Diseases.* 2015;15(7):757-8.
- 910 80. Sminia T, Barendsen L. A comparative morphological and enzyme histochemical study  
911 on blood cells of the freshwater snails, *Lymnaea stagnalis*, *Biomphalaria glabrata*, and *Bulinus*  
912 *truncatus*. *Journal of Morphology.* 1980;165:31-9.
- 913 81. Zhao X, Yu H, Kong L, Liu S, Li Q. High throughput sequencing of small RNAs  
914 transcriptomes in two *Crassostrea* oysters identifies microRNAs involved in osmotic stress  
915 response. *Scientific reports.* 2016;6:22687.
- 916 82. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. MicroRNA targets in  
917 *Drosophila*. *Genome biology.* 2003;5(1):R1.
- 918  
919



920 Supporting Information Legends

921

922 S1 Fig: Clustering of all differentially represented transcripts

923 Clustering of differentially represented transcripts. Heatmap representing the profiles of the  
924 1,895 differentially represented immune-related transcripts in the BB, BV, or BR interactions  
925 along the time course of infection (at 24 and 96 h). Each transcript is represented once and each  
926 line represents one transcript. Colors: yellow, over-represented transcripts; purple, under-  
927 represented transcripts; and black, unchanged relative to levels in control naïve snails.

928

929 S2 Fig: Mortality of *B. glabrata* snails after infections

930 The survival rates of *B. glabrata* subjected to infection by different *S. mansoni* strains were  
931 observed over 14 weeks. Kaplan Meier graphs were generated using xlstat, and the log-rank  
932 test ( $p < 0.05$ ) was used to test for significant between-group differences. Colored curves  
933 indicate the mortality rates of naïve snails (yellow) ( $n=60$ ), snails infected by the sympatric  
934 parasite (BB, BgBRE/SmBRE, blue) ( $n=50$ ), and snails infected by the allopatric parasite (BV,  
935 BgBRE/SmVEN, green) ( $n=50$ ). The difference in mortality between naïve and infected snails  
936 was significant ( $p<0.05$ ), whereas that between the two infected conditions was not ( $p = 0.243$ ).

937

938 S3 Fig: Blast-like cells are non-proliferative cells

939 *In vitro* EdU labeling of hemocytes collected for *in vitro* analysis. Confocal microscopy of  
940 EdU-labeled hemocytes from snails subjected to the allopatric interaction BgBRE/SmVEN at  
941 24 h post-infection (BV24). Pictures corresponded to the merge of DAPI labelling (blue); EdU  
942 labelling (green) and phase contrast pictures. The white arrows indicate the Blast-like cells.  
943 Blast-like cells were never labelled by EdU, indicating that these cells are not proliferative  
944 when circulating in the hemolymph. Three individual snails were used for each condition.  
945 Green label: EdU-positive cells; and blue label: DAPI. Magnification x63.

946

947 S1 Table: *Biomphalaria* and *Schistosoma* compatibility between African and South-American  
948 strains

949 The prevalence (P %: percentage of snail infected) and intensity (I: number of parasites per  
950 infected host) of infection are presented for each experimental infection. The indicated values  
951 correspond to 10 miracidia. Each pairwise combination of *Biomphalaria glabrata* (BgBRE,  
952 BgVEN), *Biomphalaria pfeifferi* from Senegal (BpSEN) and *Schistosoma mansoni* (SmBRE,  
953 SmVEN, SmSEN) or *Schistosoma rodhaini* (Srod) were tested for compatibility. The  
954 observation of cercariae shedding is also indicated. Cercariae shedding have been observed  
955 between 35 and 38 days after miracidial infections NA: non-available data.

956

957 S2 Table: List of differentially represented transcripts in RNAseq clusters.

958 Quality reads (Phred score  $>29$ ) were aligned on the transcriptome assembly using the C++  
959 script Bowtie2 (v2.0.2) (255 score) running thanks local engine using Galaxy Project server  
960 (Giardine, Riemer et al. 2005). The DESeq2 software (Love, Huber et al. 2014)  
961 (v2.12;<http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html>)  
962 (defaults settings) allows for quantifying the differential gene expression with comparing two biological  
963 duplicates from uninfected snails sample (Bre1 and Bre2) against infected samples  
964 ( $Pvalue<0.1$ ). For each cluster transcript ID, Blast2GO annotation and Log2FC results were  
965 indicated.

966

967 S3 Table: List of transcripts express by *Schistosoma* within *Biomphalaria glabrata* tissues  
968 highlight in RNAseq clusters.

969 The *Biomphalaria* (v1) and *Schistosoma* (v5.2) genome have been concatenate to mapped the  
970 RNAseq reads of each experimental condition. Only quality reads (Phred score >29) were  
971 aligned to the concatenate genomes using Bowtie2 (v2.0.2), which run locally on the Galaxy  
972 project server (Giardine, Riemer et al. 2005). The reads that mapped only once are conserved.  
973 Elimination of reads which mapped on *Biomphalaria* genome and only the reads that mapped  
974 on *Schistosoma* genome are kept.

975

976 S4 Table: miRNAs precursor identified in *Biomphalaria glabrata* RNAseq data.

977

978

979

## 980 Figure Legends

### 981 Fig 1: Dual-RNAseq of *Biomphalaria* immune-related transcripts

982 Among the differentially represented transcripts, Blast2GO functional annotation allowed us to identify 336  
983 transcripts that appeared to be related to the *Biomphalaria* immune response. Abbreviations and colors: blue BB,  
984 sympatric interaction between BgBRE and SmBRE; green BV, allopatric interaction between BgBRE and  
985 SmVEN; and red BR, allopatric interaction between BgBRE and Srod. For each interaction 40 whole-snails are  
986 used, 20 pooled at 24h and 20 at 96h post-infection. A) Venn diagram showing the relationships among the immune  
987 transcripts found to be differentially expressed in the sympatric and allopatric interactions. B) Clustering of  
988 differentially represented immune transcripts. Heatmap representing the profiles of the 336 differentially  
989 represented immune-related transcripts in the BB, BV, or BR interactions along the kinetic of infection (at 24 and  
990 96 h). Each transcript is represented once and each line represents one transcript. Colors: yellow, over-represented  
991 transcripts; purple, under-represented transcripts; and black, unchanged relative to levels in control naïve snails.  
992 C) Pie chart showing the distribution of the selected immune-related transcripts across three immunological  
993 processes: immune recognition (pink), immune effectors (brown), and immune signaling (blue). For each category  
994 and interaction, the respective proportion of transcripts and the direction of the effect (over- or underexpression)  
995 are indicated.

996

### 997 Fig 2: Differentially represented immune-related transcripts in sympatric and allopatric interactions.

998 Cumulative expression [Log2FC (fold change) from DESeq2 analysis] of the immune-related transcripts identified  
999 as being differentially represented following sympatric or allopatric infection. Transcripts were grouped into the  
1000 three immunological groups described in Fig 1, and from there into functional categories. The yellow histograms  
1001 correspond to cumulatively over-represented transcripts, while the purple histograms show under-represented  
1002 transcripts. The black (over-represented) and gray (under-represented) diamonds correspond to the number of  
1003 transcripts analyzed in each functional category. Abbreviations: BB, BgBRE/SmBRE interaction; BV,  
1004 BgBRE/SmVEN interaction; and BR, BgBRE/Srod interaction. A. Immune transcript expression at 24 h post-  
1005 infection. B. Immune transcript expression at 96 h post-infection.

1006

### 1007 Fig 3: Microscopic analyse of snail hemocyte proliferations

1008 *In vitro* EdU labeling of hemocytes was conducted for sympatric and allopatric interactions A) Hemocytes were  
1009 collected at 24 h post-infection for *in vitro* analysis. Confocal microscopy of EdU-labeled hemocytes from snails  
1010 subjected to the allopatric BV interaction (BgBRE/SmVEN). Colors: blue/DAPI; green/EdU; white/phase  
1011 contrast. B) Microscopic counting of EdU-labeled hemocytes from naïve control snails (BgBRE) (n=1,811) and  
1012 those subjected to the sympatric interaction (BB: BgBRE/SmBRE) (n=2,064) or an allopatric interaction (BV:  
1013 BgBRE/SmVEN) (n=1,366) recovered from 3 individual snails by condition. Colors: green, EdU-positive cells;  
1014 and blue, EdU-negative cells. Between-group differences in the percentage of proliferation were tested using a  
1015 Fisher exact test, with statistical significance accepted at p<0.05. The “a” indicates a significant difference between  
1016 the naïve and infective conditions, while “b” indicates a significant difference between the infective conditions.

1017

### 1018 Fig 4: Flow cytometry analyse of the hemocyte response in sympatric and allopatric interactions

1019 A) Flow cytometry was used to count *in vivo* EdU-labeled hemocytes at 24 h and 96 h after infection in sympatric  
1020 and allopatric interactions. A total number of hemocytes of n=10,000 were recovered for 6 biological replicates of  
1021 3 snails. Control naïve snails (BgBRE, yellow) were compared to those subjected to the sympatric interaction (BB,  
1022 BgBRE/SmBRE, blue) or an allopatric interaction (BV, BgBRE/SmVEN, green).

1023 B) The experiment described in A was repeated using the BgVEN snail strain. Control naïve snails (BgVEN,  
1024 yellow) were compared to those subjected to the sympatric interaction (VV, BgVEN/SmVEN, green) or an  
1025 allopatric interaction (VB, BgVEN/SmBRE, blue).

1026 C) FSC (forward-scattered light, representing cell size) and SSC (side-scattered light, representing cell granularity)  
1027 circulating hemocyte patterns in BgBRE snails under the naïve condition (yellow) or 24 h and 96 h after infections  
1028 in sympatry (BB24/96, BgBRE/SmBRE, blue) or allopatry (BV24/96, BgBRE/SmVEN, green). D) FSC and SSC  
1029 circulating hemocyte patterns in BgVEN snails under the naïve condition (yellow) or 24 h and 96 h after infections  
1030 in sympatry (VV24/96, BgVEN/SmVEN, blue) or allopatry (VB24/96, BgVEN/SmBRE, green). The red dots  
1031 correspond to EdU-positive hemocytes.

1032 Between-group differences in the percentage of proliferation were tested using the Mann-Whitney U-test, with  
1033 statistical significance accepted at p<0.05. The “a” indicates a significant difference between the naïve and  
1034 infective condition, “b” indicates a significant difference between the infective conditions at 24h, and “c” indicates  
1035 a significant difference between the infective conditions at 96h.

1036

### 1037 Fig 5: Development of parasites into snail tissues

1038 A histological approach was used to monitor parasite size along the course of snail infection. The sympatric  
1039 interaction (BgBRE x SmBRE) is shown in blue, and the allopatric interaction (BgBRE x SmVEN) is shown in

1040 green. For each experimental interaction, the parasite sizes were quantified at 24 and 96 h after infection. Morpho-  
1041 anatomical aspects of the parasite are depicted to highlight a potential difference in parasite survival. N=7 to  
1042 10sporocysts were used as indicated in the figure. Between-group parasite size differences were assessed using  
1043 the Mann-Whitney U-test, with significance accepted at  $p < 0.05$  (indicated by “a” on the histograms).  
1044

1045 Fig 6: Clustering of intra-molluscal Schistosoma expression patterns

1046 RNAseq library mapping enabled us to identify 351 genes expressed by *Schistosoma* parasites in *Biomphalaria*  
1047 snail tissues. Colors: blue, *S. mansoni* Brazil (SmBRE); green, *S. mansoni* Venezuela (SmVEN); and red, *S.*  
1048 *rodhaini* (Srod). The heatmap represents the profiles of the 351 genes expressed by the different parasites at 24 h  
1049 after infection. Each transcript is represented once and each line represents one transcript. The expression level is  
1050 highlighted by the different shades of blue.  
1051

1052 Fig 7: In-silico identification of parasite miRNAs

1053 miRNAs were assessed using libraries obtained from naïve snails and snails infected for 24 h under the various  
1054 interaction conditions (BB24, BgBRE x SmBRE; BV24, BgBRE x SmVEN; BR24, BgBRE x Srod). A) Table  
1055 highlighting the precursor miRNAs that may have targets among the immune-related snail transcripts selected in  
1056 the present work. They include eight precursors specifically recovered in BB24, two in BV24, and one shared  
1057 across the three infected conditions. The total numbers of potential targets in each condition are indicated. B) Venn  
1058 diagram showing the potential targets according to the sympatric or allopatric interactions. Shown is an example  
1059 miRNA stem-loop precursor that presents the highest number of potential host targets.  
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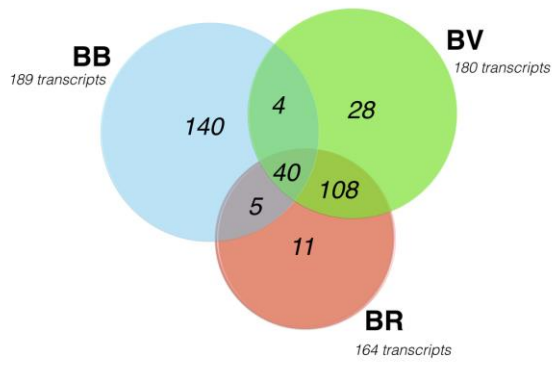
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**Table 1:** Number of transcripts in each step of transcriptomic analysis

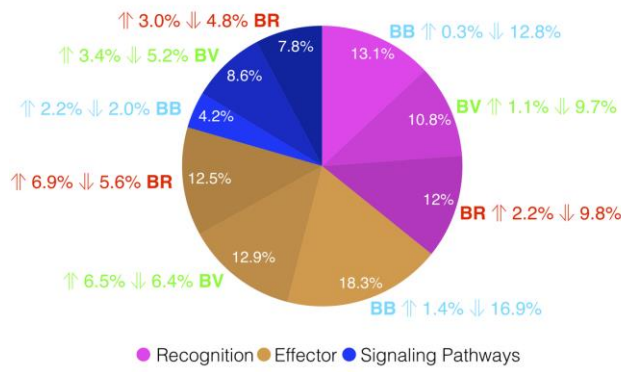
<b>Analysis of transcriptomic data</b>		<b>Analyses of Blast2GO</b>	<b>Analyses of annotations</b>	
Full Transcriptome	Differentially expressed	Informative annotation	Immune Transcripts	Non Immune Transcripts
159,711	3,865	1,017	336	681

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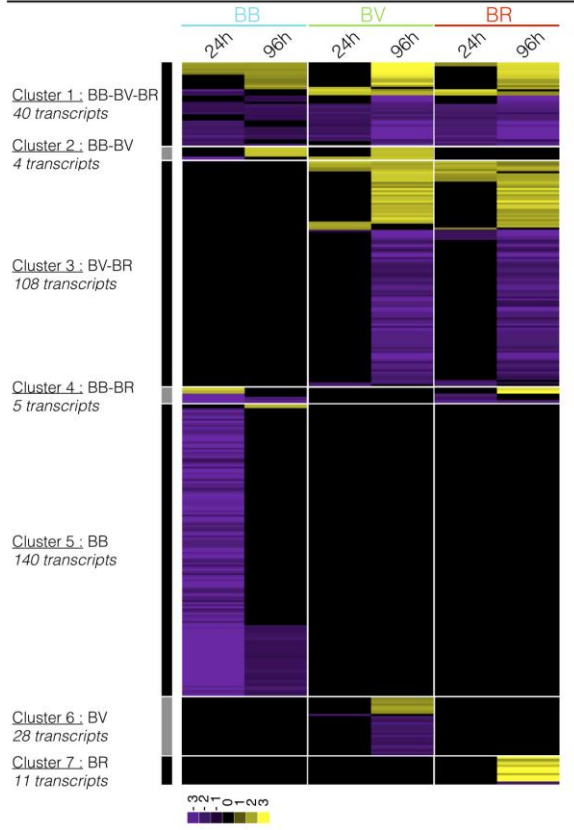
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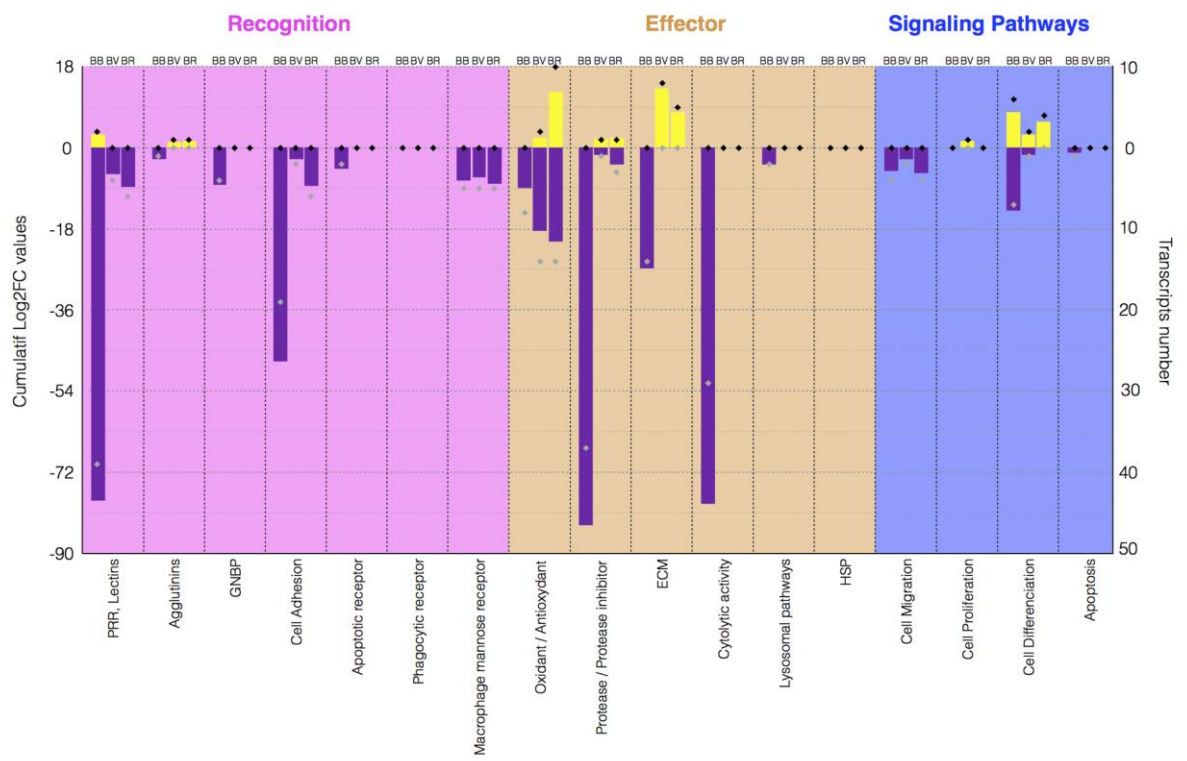
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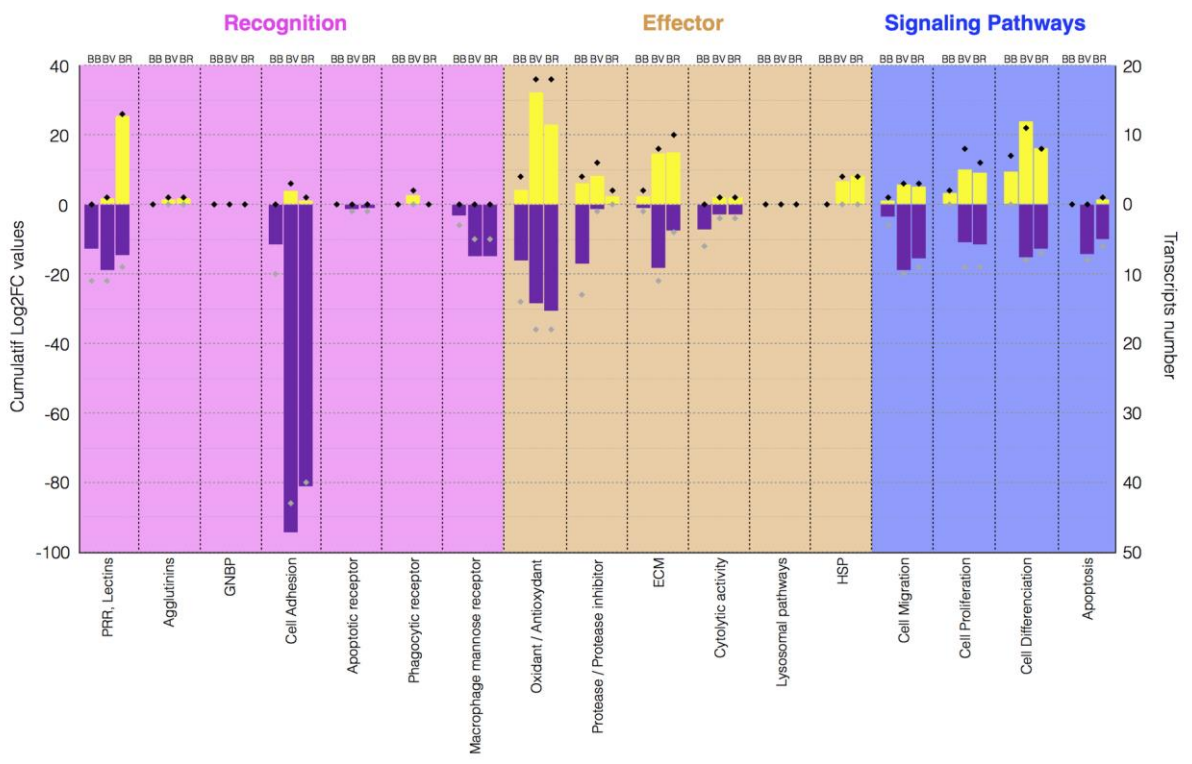
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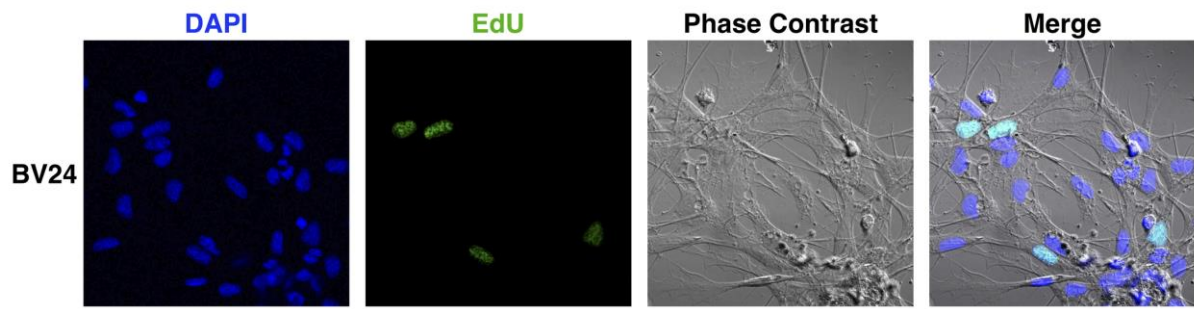


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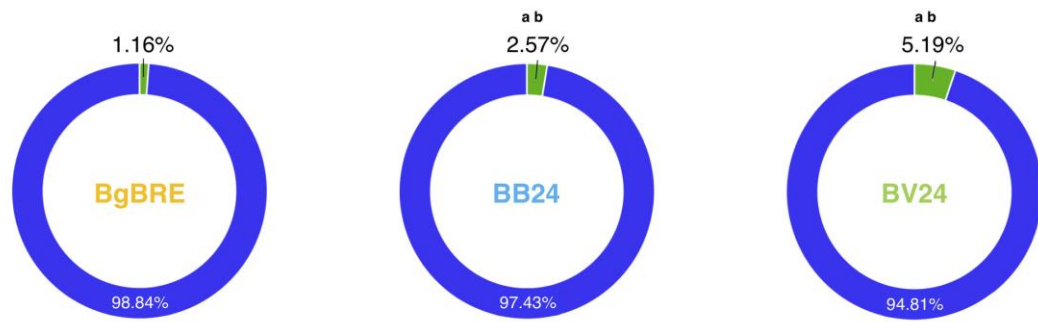


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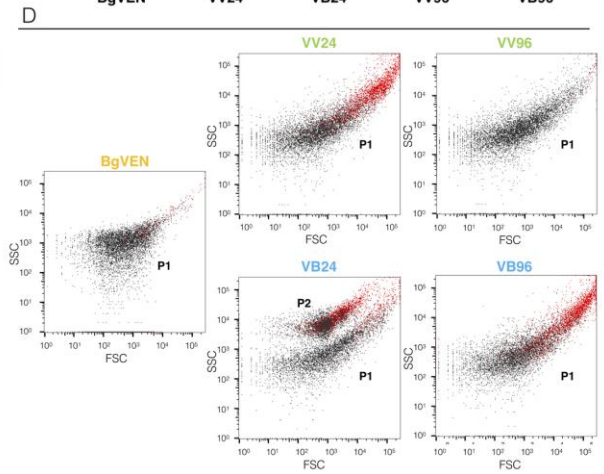
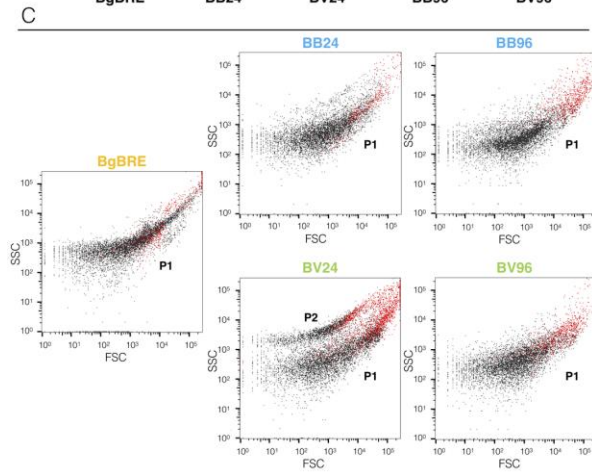
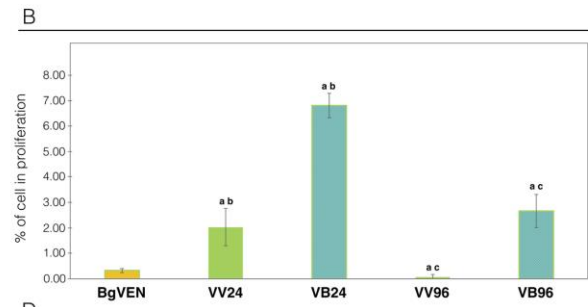
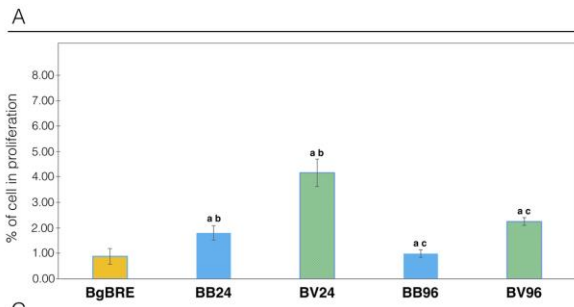


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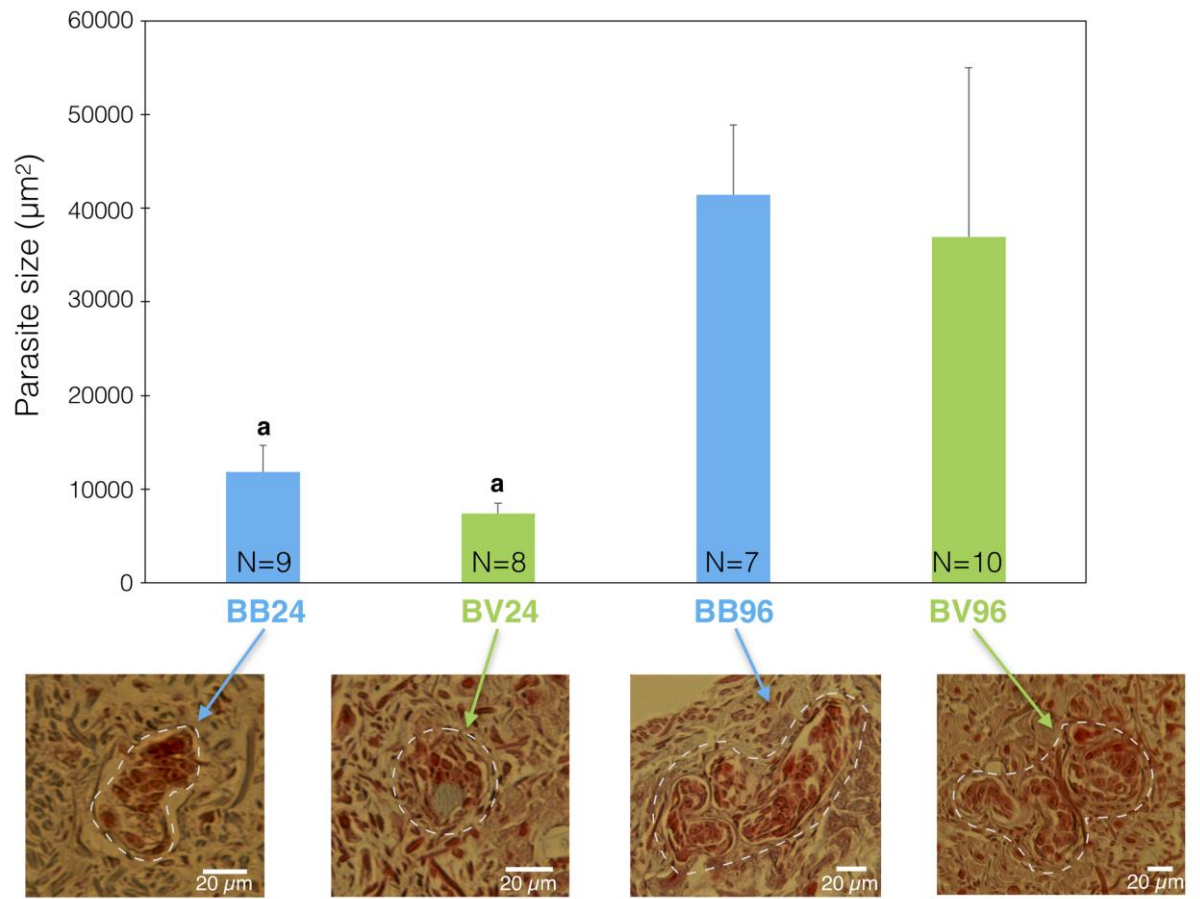


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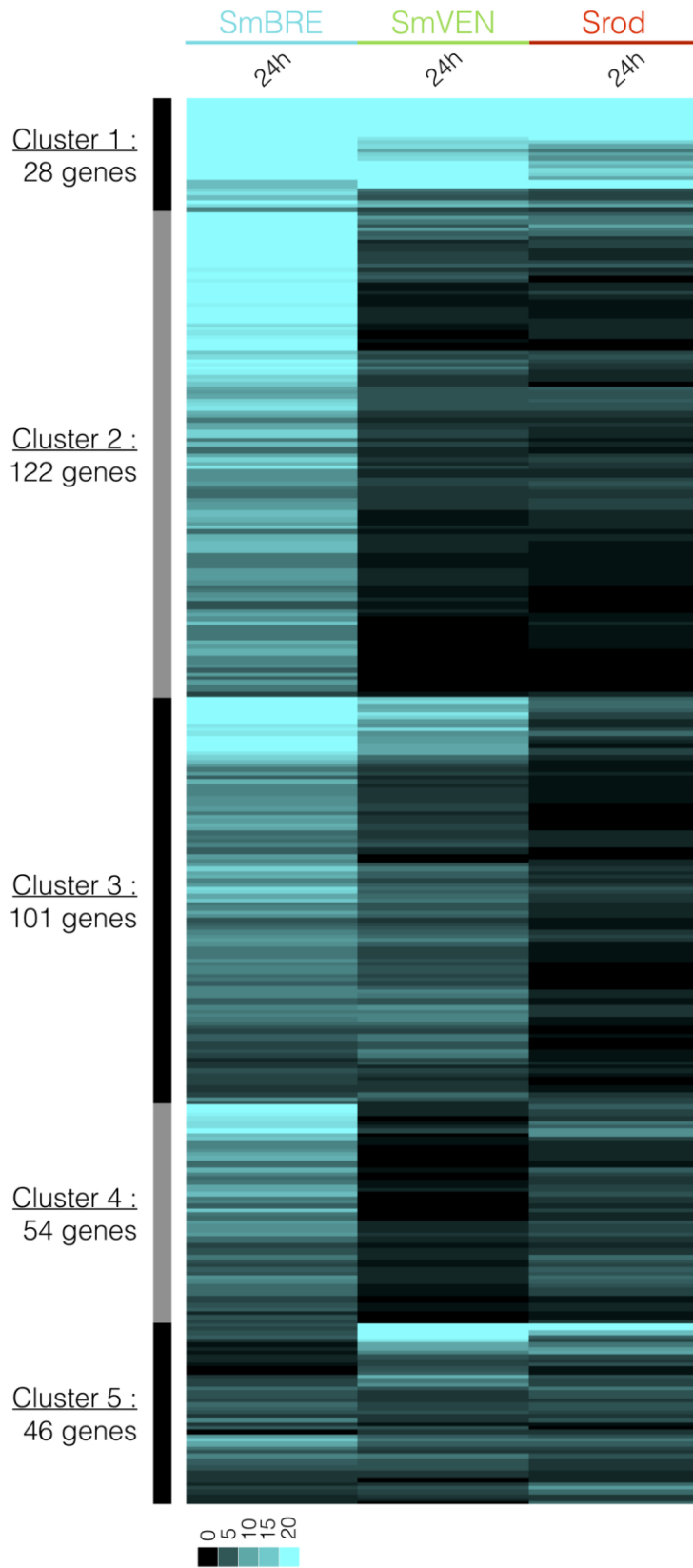




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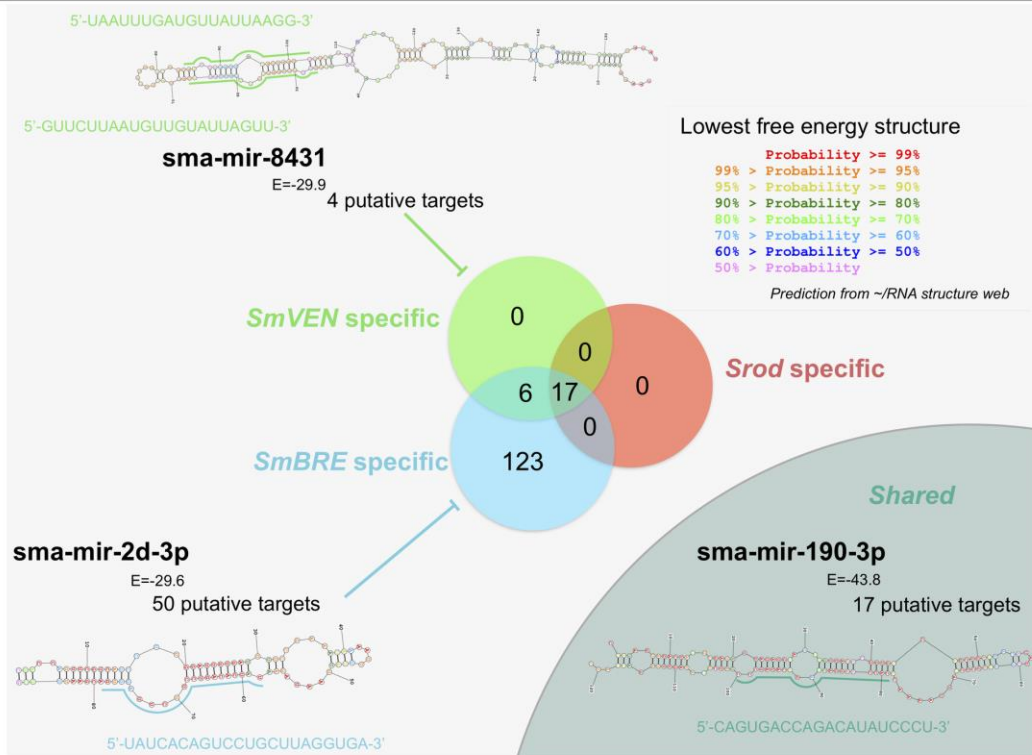


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A

Precursor	BgBRE duplicates		BB24	BV24	BR24	Targets	SmBRE specific	SmVEN specific	Shared
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			
<b>sma-mir-2d</b>	0	0	1	0	0	50			
sma-mir-3492	0	0	1	0	0	10			
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			
<b>sma-mir-8431</b>	0	0	0	1	0	4			
<b>sma-mir-190</b>	0	0	1	1	1	17			
<b>Sum of potential Bg targets</b>	<b>0</b>	<b>0</b>	<b>154</b>	<b>23</b>	<b>17</b>				

B



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1089