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Frequency and mitotic heritability of epimutations in *Schistosoma mansoni*

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Abstract

Schistosoma mansoni is a parasitic platyhelminth responsible for intestinal bilharzia. It has a complex life cycle, infecting a freshwater snail of the *Biomphalaria* genus, and then a mammalian host. *S. mansoni* adapts rapidly to new (allopatric) strains of its intermediate host. To study the importance of epimutations in this process we infected sympatric and allopatric mollusk strains with parasite clones. ChIP-Seq was done on four histone modifications (H3K4me3, H3K27me3, H3K27ac and H4K20me1) in parallel with genome-wide DNA resequencing (i) on parasite larvae shed by the infected snails, and (ii) on adult worms that had developed from the larvae. No change in single nucleotide polymorphisms (SNP) and no mobilization of transposable elements (TE) was observed, but 58 – 105 copy number variations (CNV) within the parasite clones in different mollusks were detected. We also observed that the allopatric environment induces three types of chromatin structure changes: (i) host-induced changes on larvae epigenomes in 51 regions of the genome that are independent of the parasites' genetic background, (ii) spontaneous changes (not related to experimental condition or genotype of the parasite) at 64 locations, and (iii) 64 chromatin structure differences dependent on the parasite genotype. Up to 45 % of the spontaneous, but none of the host-induced chromatin structure changes were transmitted to adults. In our model, the environment induces epigenetic changes at specific loci but only spontaneous epimutations are mitotically heritable and have therefore the potential to contribute to transgenerational inheritance. We also show that CNV are the only source of genetic variation and occur at the same order of magnitude as epimutations.

Introduction

Adaptive evolution relies on the generation of heritable phenotypic variations. There is now an intense scientific discussion about the source of these variants (Laland *et al.* 2014). During the last couple of years, it has become clear that there are considerable non-genetic sources of heritable phenotypes (Jablonka *et al.* 1992; Jablonka & Lamb 2008; Danchin *et al.* 2011). In particular epigenetic modifications, that we define here tentatively as heritable changes in chromatin structure that can have an impact on gene expression, are in the center of a lively scientific debate (Kovalchuk 2011). Particularities of epimutations are (I) their metastability, *i.e.* changes in chromatin structure can be reversible and (II) their relations with the environment. In the genetic inheritance system, the environment acts solely as selective agent, but in the epigenetic system it could play a dual role, acting as an inducer of epimutations (Guerrero-Bosagna & Skinner 2011; Skinner *et al.* 2015) as well as applying selection on the resulting phenotypes. The question whether epimutations can generate new phenotypes is solved (Peaston & Whitelaw 2006; Nilsson & Skinner 2015). There are now many examples for phenotypes that are entirely or partially encoded by epialleles presenting changes in DNA methylation or histone modifications (Duncan *et al.* 2014). The role of the environment, however, is far from being comprehended. There is empirical support that epimutations are generated spontaneously, independently of environment but at a higher frequency than genetic mutations (van der Graaf *et al.* 2015). It was also argued that environmental stress could provoke adaptive and heritable changes through targeted épimutations. Others concluded that stress leads to an increase of the epimutation rate, thus representing an ephemeral, but inducible version of the genetic inheritance system, *i.e.* a way to induce reversible but possibly inheritable phenotypic changes (Jiang *et al.* 2014). The objective of the present study was to determine the frequency and nature of histone-based epimutations during a change of environment, and to measure their mitotic heritability. To address this question we used a parasite-host system. In these systems reciprocal selective forces, such as infectivity for the parasite and resistance for the host, are strong and adaptive evolution is expected to be fast (Thrall *et al.* 2007; Tellier *et al.* 2014).

The plathelminth *Schistosoma mansoni* is the causing agent of intestinal schistosomiasis, a human disease affecting more than 67 million people worldwide (Oliveira *et al.* 2004; King 2010). Its complex life cycle (**Supplementary Figure 1**) starts with eggs hatching in fresh water. A free-swimming larvae (miracidium) is released and will enter the tegument of the intermediate mollusk host of the *Biomphalaria* genus. Miracidia quickly transform into primary sporocysts, multiply asexually and differentiate into secondary sporocysts. They generate multiple cercariae, another free-swimming larvae, which leave the mollusk, infect the definitive mammalian host (rodent or primate), and transform into schistosomula. They follow a complex maturation and migration process in the mammalian body to reach the adult worm stage. Male and female worms form couples in the mesenteric veins, where eggs are released, go through the intestine wall and are excreted in the feces. *S. mansoni* can infect a wide range of intermediate and definitive host species (Martins 1957; DeJong *et al.* 2001). Changes in the intermediate host strain and species has a reproducible phenotypic impact on the parasite, and more specifically on its life history traits (sex-ratio, growth, prevalence and intensity of the infection) (Dias *et al.* 1988; Lepesant *et al.*

2013; Theron *et al.* 2014), but the underlying mechanisms are still unknown. Compatibility polymorphism is classically used to explain infectivity between *S. mansoni* and *B. glabrata* (Roger *et al.* 2008; Mitta *et al.* 2012). However, recent work has uncovered that epigenetic modifications could also play a role in the adaptation to new host strains (Knight *et al.* 2013). Knowing this, we were interested to find out what would be the impact of different host strains on the genome and epigenome of *S. mansoni*. In order to disentangle the impact of epimutations and mutations, we decided to work with genetically identical parasites and to let them develop in different snails. Miracidia, coming from sexual reproduction, are not clonal, and although it is relatively easy to infect snails with sibling miracidia with low genetic diversity between them, there will still be genetic diversity. To overcome this problem, we used sporocyst grafts (Jourdane & Theron 1980; Jourdane 1984). This technique allows, after a mono-miracidium infection, to extract secondary sporocysts and graft them into new mollusks. Secondary sporocysts revert to primary sporocysts once grafted, and then undergo the normal development until cercariae emission (Jourdane & Theron 1980; Jourdane 1984).

Here, we used a parasite strain with known genetic background (*SmBre* from Brazil; Clément *et al.* 2013) to infect a sympatric *Biomphalaria glabrata* strain (*BgBre* from Brazil) and an allopatric one (*BgGua* from Guadeloupe). Interactions between *SmBre* and the two mollusk strains have been previously characterized in great detail and the differences in life traits are known (Lepesant *et al.* 2013; Theron *et al.* 2014). Prevalence in the sympatric interaction (*SmBre* on *BgBre*) is 50-100%, and 30-85% in the allopatric interaction (*SmBre* on *BgGua*), depending on the number of miracidia used for infection (Theron *et al.* 2014). This shift in the environment does not represent a strong stress but has still measurable effects on the life history traits of the parasite (Lepesant *et al.* 2013). We sequenced the genomes of cercariae and adult parasites to search for somatic mutations, copy number variations and mobilization of transposable elements. DNA methylation is only present at a very low level in *S. mansoni* (Geyer *et al.* 2011), which allowed us to focused on chromatin marks based on histone modifications. We used chromatin immunoprecipitation followed by sequencing (ChIP-Seq). ChIP-Seq was done on cercariae emitted after grafts, but also on adult worms obtained by infecting mice with the same cercariae. The objective was to see if chromatin structure changes, potentially induced by development in different mollusks, are transmitted to the next developmental stage of the parasite. We selected four histone modifications (H3K4me3, H3K27ac, H3K27me3, H4K20me1), associated with different biological functions. H3K4me3 form narrow peaks (rarely larger than 2 kb wide) usually associated with the transcription start site (TSS) and promoters of transcriptionally competent genes (Berger 2007; Gu & Fire 2009; Kharchenko *et al.* 2010; Zentner & Henikoff 2013). H3K27me3 plays an important role in sequential expression and regulation of developmental genes, and is generally associated with repression of transcription (Kouzarides 2007; Trojer & Reinberg 2007; Bannister & Kouzarides 2011). Acetylation of H3K27 serves to discriminate active enhancers (where the mark is present) from poised ones (where the mark is absent) (Tie *et al.* 2009; Creighton *et al.* 2010; Liu *et al.* 2011; Shlyueva *et al.* 2014). It is described as a very punctate mark, similar to H3K4me3. The role of H4K20me1 is more controversial. Some authors report it being associated with distinct regions of silent chromatin (Sims *et al.* 2006; Vielle *et al.* 2012) in human and *C. elegans*, while others describe it as an essential mark for transcription activation (Wang *et al.* 2008; Li *et al.* 2011). It is

also involved in DNA repair and cell cycle (Jorgensen *et al.* 2013), and genome-wide increase of this mark impairs genome integrity (Schotta *et al.* 2008).

Our data show that changes in SNP and mobilization of TE do not contribute to genetic variation, but that CNV are relatively frequent. Roughly 10% of spontaneous CNV are mitotically heritable. In addition, we observed the occurrence of environment-directed (*i.e.* environment-dependent) and genotype-independent epimutations, as well as spontaneous epimutations (not related to experimental conditions or genotypes). However, while mitotic heritability of spontaneous epimutations can reach 45%, none of the environmentally targeted chromatin structure changes are transmitted to the adult worms. Our findings are therefore in favor of spontaneous epimutations as a possible source for heritable phenotypic variants that could be selected by the environment.

2. Materials and Method

The experimental procedure is schematically summarized in **Figure 1**.

Ethics statement

Housing, feeding and animal care followed the national ethical standards established in the writ of February 1st, 2013 (NOR: AGRG1238753A) setting the conditions for approval, planning and operation of establishments, breeders and suppliers of animals used for scientific purposes and controls. The French Ministère de l'Agriculture et de la Pêche and French Ministère de l'Éducation Nationale de la Recherche et de la Technologie provided permit A 66040 to our laboratory for experiments on animals and certificate for animal experimentation (authorization 007083, decree 87–848) for the experimenters.

Origin of the parasites and their hosts

The *Schistosoma mansoni* strain *SmBre* was originally sampled in Recife, Brazil in the 1960's. It was provided to our laboratory in 1975 by Pr. Y. Golvan (Faculté de Médecine de Paris – Saint Antoine) and has been since then maintained in its sympatric intermediate host strain *BgBre* of the mollusk *Biomphalaria glabrata* and *Mus musculus* (SWISS OF1) as definitive vertebrate host. *BgBre* (albino strain) is also from Recife and was also acquired in 1975. Albinism is of genetic origin and does not have an impact on the experimental design. The allopatric mollusk strain *BgGua* originates from the town of Dans Fond and arrived at our laboratory in 2005 (pers. comm. A. Théron). Mollusks were maintained as described in Boissier & Moné (2000).

Infection and sex determination

Thirty-two *B. glabrata* *BgBre* individuals were each infected with a single *SmBre* miracidium. Miracidia were freshly hatched from the liver of a single infected mouse. Miracidia hatching, as well as snails and mice infections, were performed as described in Boissier & Moné (2000). Mollusks were screened 30 days later for presence of parasites and cercariae shed by each positive *B. glabrata* were genotyped with sex markers (Portela *et al.* 2010). We chose to work only with female parasites, as it was previously shown that there is an increase in female cercariae production when *SmBre* develops in *BgGua* hosts (Lepesant *et al.* 2013), and also to avoid possible

160 bias caused by gender-specific epialleles. We selected two infected snails with a large number of
161 sporocysts to be donors for sporocyst grafts. We named the female genotypes of the parasites in
162 these two snails *Fa* and *Fb*.

163 *Sporocyst graft*

164 The grafts were done as detailed in Jourdan & Theron (1980) and Jourdan (1984). Large (10-12
165 mm) *BgBre* and *BgGua* mollusks were selected as receivers. They were anesthetized by incubating
166 4 hours in spring water mixed with sodium pentobarbital at a final concentration of 1.2 mg/mL.
167 Donor mollusks had their shells carefully removed with tweezers and their digestive gland (where
168 secondary sporocysts are located) was recovered. Using tweezers, explants of 1 mm³ containing 1-
169 3 sporocysts were prepared and grafted within an hour. Explants were grafted in the cephalopedal
170 sinus of receiver snails with a custom-made glass micro-needle attached to a 1 mL syringe. A small
171 incision of the tegument above the genital pore was done to reach the cephalopedal sinus.
172 Grafted snails were then kept in normal growing conditions. *Fa* sporocysts were grafted in 15
173 *BgGua* and 14 *BgBre*, and *Fb* sporocysts were transplanted in 17 *BgGua* and 15 *BgBre*.

174 *Parasite preparation*

175 Fifty days after transplantations, mollusks were screened under a stereomicroscope to look for
176 secondary sporocysts. Uninfected mollusks were discarded. Cercariae were collected from
177 infected snails 53 days after infection by gently pipetting from spring water, avoiding mucus and
178 feces, and sedimented on ice. Water was removed and cercariae from each snail were stored
179 separately at -80°C before being used for ChIP-Seq. As we were interested to see if epimutations
180 induced by the development in different snail strains would be maintained in adults, we also
181 infected mice with cercariae from some of the snails. For both *Fa* and *Fb* parasite genotype, we
182 selected *BgBre* and *BgGua* snails emitting the highest amount of cercariae (> 5,000) to use for
183 infection. For parasite genotype *Fa*, we had one *BgBre* and one *BgGua*, and for *Fb* we had two
184 snails of each strain. For each snail, three mice were infected with 300 cercariae each, as described
185 in Boissier & Moné (2000). Mice were sacrificed 42 days later by injection of sodium pentobarbital
186 and female adult worms were recovered by retrograde perfusions of the hepatic portal system
187 with citrate (7.5%) saline (8.5%) solution administrated through the left ventricle (Duvall & DeWitt
188 1967). Worms trapped in the liver or mesenteric system were collected after excising these
189 organs. Worms from each mouse were collected separately and stored at -80°C.

190

191 *Genomic DNA sequencing*

192 Cercariae emitted from a mollusk infected with a single miracidium are generally considered
193 genetically identical, but evidences exist that some genetic diversity can occur (Bayne &
194 Grevelding 2003). We could not exclude that sporocyst grafts and development in an allopatric
195 host were source of stress, which could activate mobilization of TE (Wijayawardena *et al.* 2015).
196 Knowing this, we performed DNA pool sequencing (Pool-Seq) on adult worms developed from
197 cercariae emitted after the grafts. We chose to sequence DNA of adults rather than cercariae
198 because current tools for the analysis of Pool-Seq data do not perform well on large numbers of
199 potentially different haploid genomes. It is not feasible to obtain sufficient amount of DNA for
200 sequencing from less than 1,000 cercariae while 10 adults are enough. We selected four pools of

12, 9, 11 and 17 Fb genotype female adult worms. The first two pools originated from two different sympatric (*BgBre*) snails, while the last two are from a single allopatric (*BgGua*) snail, but isolated from two different mice. DNA was extracted following Beltran *et al.* (2008) and sequencing was done as paired-end 125 bp reads on an Illumina HiSeq 2500 at the Montpellier GenomiX facility (<http://mgx.cnrs.fr>). Sequencing was designed to have a genomic coverage of at least 3X per individual per sample.

Chromatin immunoprecipitation and sequencing (ChIP-Seq)

Native ChIP was performed following the protocol developed for *Schistosoma mansoni* by Cosseau *et al.* (2009) (also available online at http://methdb.univ-perp.fr/cgrunau/methods/native_chip_sm.html) on cercariae and adult worms. We used at least 5,000 cercariae or 20 adult worms. Immunoprecipitation was performed using the following antibodies: H3K4me3 (Millipore, cat# 04-745 lot# NG1680351, 4 µL per reaction), H3K27ac (Abcam, cat# ab4729 lot# GR-150-367-2, 4 µL per reaction), H3K7me3 (Diagenode, cat# C15410069 lot# A1821D/2, 8 µL per reaction), and H4K20me1 (Abcam, cat# ab9051 lot# GR158874-1, 4 µL per reaction). For each sample, we used a control without antibody to assess unspecific background (bound fraction) and input (unbound fraction). Inputs were used for normalization in all subsequent bioinformatics analyses. Antibodies were carefully tested for specificity as described in Cosseau & Grunau (2011) and saturating quantities were used. We had earlier shown that the abovementioned catalogue/lot numbers for anti-H3K4me3 and anti-H3K27me3 can be used for ChIP-Seq (Roquis *et al.* 2015). For H3K27ac and H4K20me1, we performed all the validation steps described by Cosseau & Grunau (2011), including a chromatin titration to assess specificity of the antibodies. Titrations, using increasing amount of antibodies over the same quantity of *S. mansoni* chromatin, proved that antibodies were indeed specific and able to immunoprecipitate all target chromatin using 4 µL of the antibody for 5,000 cercariae or 20 adult female worms (**Supplementary Figure 2**).

ChIP products were sequenced as single-end 50 bp reads on an Illumina HiSeq 2500 at the McGill University and Génome Québec Innovation Centre (<http://gginnovationcenter.com/index.aspx>). Briefly, fragmented DNA was quantified using a 2100 Bioanalyzer (Agilent Technologies). Libraries were generated robotically with 10 ng of fragmented DNA (range 100-300 bp) using the Kapa HTP Library Preparation Kit (Kapa Biosystems) as per the manufacturer's recommendations, except that adapters and PCR primers were diluted 100-fold. The size selection step was done after the PCR step and the number of PCR cycles was increased by six. Adapters and PCR primers were purchased from Integrated DNA Technologies whereas size selection has been performed on a Pippin Prep instrument (SAGE Biosciences Inc). Libraries were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (D-Mark). Average size fragment was determined using a LabChip GX (PerkinElmer) instrument. Cluster formation on the flow cell was performed using the cBot instrument (Illumina) with four indexed libraries per lane. Sequencing, in the form of 50-cycle single end reads, was performed on a HiSeq 2000/2500 (Illumina) running HCS software version 2.2.38. Demultiplexed FASTQ files were generated by allowing up to one mismatch in the index.

Quality control, alignment and peak calling

All data treatment was carried out under a local galaxy instance (Goecks *et al.* 2010)

242 (<http://bioinfo.univ-perp.fr>). Read quality was verified using the FASTX-toolkit
243 (http://hannonlab.cshl.edu/fastx_toolkit/). All samples had a sanger quality score above 30 for
244 over 95% of their length.

245 Sequences were aligned to the *S. mansoni* reference genome (Protasio *et al.* 2012) with Bowtie2.1
246 (Langmead & Salzberg 2012) using parameters --end-to-end, --sensitive, --gbar 4 and filtered for
247 unique match with samtools (Li *et al.* 2009) (samtools view -Sh -q *quality value 40-42* -F 0x0004 - |
248 grep -v XS:i). SAM alignment files were converted into the bed format with pyicos (Althammer *et al.*
249 *et al.* 2011) and sorted with sortBed -i of the bedtools suite (Quinlan & Hall 2010). PCR duplicates
250 were removed using the remduplicates function from Pyicos (Althammer *et al.* 2011), allowing a
251 maximum of 4 duplicates per position. Random sampling using a custom Perl script was performed
252 on each library to generate an equal number of reads per library for peak calling. Although
253 random sampling is not mandatory, we found in previous work that it reduces false positives when
254 searching for histone modification differences between samples. We took 7 million reads for
255 H3K4me3, 23 millions for H3K27ac and H3K27me3, and 19 millions for H4K20me1. One of the
256 samples, Cercariae Fb Gua 8, had a higher amount of PCR duplicates than the other samples, and
257 we could only select 14 million reads for H3K27ac and 10 millions for H4K20me3. Ranger of
258 Peakranger v1.16 (Feng *et al.* 2011) with P-value cutoff 0.0001, FDR cutoff 0.01, Read extension
259 length 200, Smoothing bandwidth 99 and Delta 1 was used for peak identification. Input samples
260 were used as negative control (-c). Wiggle files were visualized under IGV (Thorvaldsdottir *et al.*
261 2013). Reads mapping to repetitive regions were aligned on the 3,145 repetitive consensus
262 sequences obtained from the same strain of *S. mansoni* (*SmBre*) in a previous study (Lepesant *et al.*
263 *et al.* 2012) (available at <http://methdb.univ-perp.fr/downloads/>) using Bowtie2.1 (Langmead &
264 Salzberg 2012) evoking parameters --end-to-end, --sensitive, --gbar 4. In order to obtain identical
265 effective library sizes for each sample, the same number of aligned reads was randomly sampled
266 for each of the ChIP experiments (H3K4me3 = 2.5 million, H3K27ac = 7 million, H3K27me3 = 7
267 million, H4K20me1 = 8 million). After the counts had been normalized, they were compared
268 between groups (allopatric vs sympatric, with three replicates; genotype Fa vs Fb, with two and
269 four replicates) and between individual samples using the DESeq package (Anders & Huber 2010)
270 of the R software. This package uses the negative binomial distribution to calculate the P-value for
271 each element and for each comparison between two samples and to find elements that present
272 significant differences in their enrichment levels.

273 *Chromatin structure profiles*

274 The analysis of the different histone modification combinations on the *S. mansoni* genome was
275 performed using EpiCSeq (Mammana & Chung 2015), using count normalization (*normalizecounts*)
276 and binsize of 500bp (--binsize 500). We tested 4 to 16 different possible chromatin states and
277 concluded that five states (--nstates 5) was the optimal number to avoid redundancy between
278 states. Profiles were done on cercariae and adults, each time using the three datasets from the
279 sympatric (BRE) condition as replicates for the bin count. In a similar way, we also compared
280 sympatric (BRE) and allopatric (GUA) chromatin profiles for adults and cercariae. Annotation files
281 used to generate chromatin profiles on genes, transcription start sites (TSS) and transcription end
282 site (TES) were derived from an earlier established transcriptome (Roquis *et al.* 2015), by keeping a
283 subset of genes longer than 5 kb (2,428 genes).

284 *Comparative epigenomic analysis*

285 For comparison of chromatin profiles of each mark, DiffBind v1.8.3 (Stark & Brown 2013) was used
286 with FDR of 0.1. Comparisons were done for each histone modification between allopatric and
287 sympatric conditions (3 replicates for each histone modification). Comparisons were also done for
288 genotype Fa (two replicates) vs Fb (four replicates), and for each individual sample (no replicate).
289 One library (cercariae Fb5 H3K4me3) was systematically excluded from these DiffBind analyses
290 because it had more than 20% of PCR duplicates, indicating probably a too few starting material,
291 thus reducing the number of replicates for this histone modification by one. Heatmaps were
292 generated evoking dba.plot Heatmap (sample, contrast=1, correlations=FALSE) and PCA was done
293 with dba.plotPCA(sample, contrast=1,th=.05). DiffBind (allopatric vs sympatric condition and
294 genotype Fa vs Fb with minOverlap = 2) will test a peak if it is identified in at least two of the six
295 total samples. It is the variance amongst all the replicates in each condition that drive the p-value
296 calculated for the comparison. For sample-by-sample comparison, non-parametric bootstrapping
297 or default value minOverlap = 1 was used for each sample. Both methods provided the same
298 results. Differentially modified chromatin regions were saved as BED files for further analysis.
299 Gene ontology enrichment analysis was done with BLAST2GO (Conesa *et al.* 2005). For analyses of
300 significant differences in histone isoform enrichment in repetitive sequences, normalized aligned
301 read counts were compared using the DESeq package of the R software (Anders & Huber 2010).

302 *Comparative genomic analysis*

303 For the detection of SNP frequency distortion, *i.e.* significant differences in allele frequency
304 between sympatric and allopatric conditions, we used paired-end data for genotype Fb and single-
305 end data corresponding to the ChIP-Seq input libraries for both Fa and Fb. All raw data were of
306 excellent quality and no quality filter was necessary. Reads were aligned with Bowtie 2 (Langmead
307 & Salzberg 2012) and default parameters. Unique reads were identified using the Bowtie 2 'XS:I'
308 flag and sorted with SamTools (Li *et al.* 2009). Alignment rates were between 54% and 59%, which
309 is consistent to what has been previously achieved on *S. mansoni* (Roquis *et al.* 2015) and can be
310 explained by the fact that we only align on unique sequences of the genome (47% of the genome
311 is composed of repetitive elements (Lepesant *et al.* 2012). To homogenize effective library size, 56
312 million aligned reads were randomly subsampled for the paired-end libraries and used for further
313 analysis. For single-end data, 24 million reads were subsampled. Pileup files were generated by
314 SamTools invoking -B -C 0 -d 250 -q 0 -Q 13. For the comparison of allele frequencies we used
315 PoPoolation2 (Kofler *et al.* 2011). Fisher's Exact Test was chosen to estimate the significance of
316 allele frequency differences in samples for which we had no replicates (Fa), and the Cochran-
317 Mantel-Haenszel (CMH) test was used to detect consistent allele frequency changes with
318 replicates (Fb). Pileup files were converted to sync file with parameter --min-qual 20. And Fisher
319 and CMH tests were used invoking --min-count 2 --min-coverage 10 --max-coverage 200 --
320 suppress-noninformative for single-end data and --min-count 12 for paired-end data. Copy-
321 number variation (CNV) are another source of genetic variability and we used CNV-seq (Xie &
322 Tammi 2009) to estimate the probability of CNV differences between the sympatric and the
323 allopatric conditions. We used default parameters (log2 = 0.6, pvalue = 0.001, minw = 4) with a
324 genome size of 364,541,798 bp. Results were converted into IGV or GWAS compatible formats and
325 visualized under IGV. Differences in genomes Fa and Fb were detected using FreeBayes v0.9.20

326 (Garrison & Marth 2012) using parameters --theta 0.001 --ploidy 2 -J -m 1 -q 0 -R 0 -Y 0 -e 1000 -F
327 0.2 -C 2 --min-alternate-qsum 0 -G 1 --min-coverage 20. Resulting VCF files were compared with
328 vcf-isec of the VCF tools (Danecek *et al.* 2011). CNV were searched for as described above.

329

330 The search for transposable element (TE) insertions was done by aligning the reads of the 4 Fb
331 paired-end pools using bwa-mem from the BWA software (Li & Durbin 2009) to the reference
332 genome of *S. mansoni*. Detection of the insertions and excision of elements was done using the
333 TEMP software (Zhuang *et al.* 2014) with the required repeat and annotation files, parameters -x
334 10 and -c 12 for the insertion detection, and default parameters for the absence (excision)
335 detection. The principle of this analysis is to use differences in mapping of forward and reverse
336 reads to detect TE insertion events. BED files were generated from the output of TEMP and the
337 files were sorted. In order to eliminate constitutive differences between the sequenced genomes
338 and the reference genome we subtracted the positions that were common to all four genomes
339 and visually inspected the remaining regions.

340

341 *Comparative Transcriptomics*

342 Parasites were used to infect sympatric and allopatric snail hosts for three generations as
343 described in Lepesant *et al.* (2013). For RNA extraction, cDNA generation and sequencing, and
344 comparative analysis with the TopHat2 and CuffLinks we followed Roquis *et al.* (2015). Briefly,
345 reads were aligned to the genome and exon-intron structures are established individually for each
346 sample. Cuffmerge was used to generate a consensus transcription annotation file in GFF format.
347 We then used htseq-count with the “union” option (Anders *et al.* 2015) to obtain read-counts for
348 each of the 18,844 loci (“genes”) identified by Cufflinks/Cuffmerge. A single count
349 (“pseudocount”) was added to each readcount to avoid division by 0 problems. DESeq (Anders &
350 Huber 2010) with default parameters was used for differential gene expression analysis. A gene
351 ontology enrichment analysis was done with BLAST2GO (Conesa *et al.* 2005) and prediction of
352 protein interaction networks was done with STRING10 (Szklarczyk *et al.* 2015).

353

354 *Results*

355 *There are no significant differences in SNP or TE mobilization between SmBre in sympatric and* 356 *allopatric condition*

357 SNP frequencies were evaluated using Fisher’s exact test (for Fa without biological replicates) and
358 the CMH test for Fb (with replicates). Both tests are robust but the CMH-test showed the highest
359 power to identify interesting loci (Kofler & Schlotterer 2014). Following others (Barsh *et al.* 2012,
360 Panagiotou *et al.* 2012, Kofler & Schlotterer 2014, Broer *et al.* 2013) we decided to establish a
361 significant threshold for p-values at $p = 1 \times 10^{-5}$. Despite this relatively relaxed threshold, we detect
362 no significant allele frequency distortion. We noted nevertheless that there are large blocks of 100
363 kb range in the genome (roughly one per chromosome) that show enrichment of non-significant
364 AF differences between the sympatric and allopatric condition in all replicates, whatever genotype
365 was used. We calculated nucleotide diversity between Fa and Fb using private SNP (*i.e.* with allele
366 frequency 1). We found 1,570 SNP differences (nucleotide diversity 4.3×10^{-6} based on the total

367 genome length of 364,541,798 bp) and 72 CNV between cercariae Fa and Fb emitted from the
368 sympatric host *BgBre*.

369
370 The analysis of transposable element insertion produced several thousand candidate regions
371 which showed differences in the number of supporting reads or the detection of one of the two
372 insertion borders with respect to the reference sequence. The elimination of the redundant
373 positions and those pointing to opposite insertion borders showed that the detected positions
374 were due to the difference between the sequenced genome and the reference genome. Once
375 these differences were eliminated the analysis showed that no neoinsertions of elements could be
376 detected.

377

378 *There are significant differences in CNV between sympatric and allopatric SmBre*

379

380 CNV-seq detected 58 - 105 CNV covering 0.1-0.2% of the genome in all comparisons (**Table 1a &**
381 **1b, Supplementary File 1**). Interestingly, some CNV covered or flanked the above-mentioned
382 blocks of non-significant AF differences. To answer the question whether mitotically heritable CNV
383 are systematically found in the allopatric condition, we compared these variants between Fa and
384 Fb. CNV between sympatric Fa and Fb were excluded since they could represent simply differences
385 to the reference genome. None of the regions *sensu stricto* fulfilled the condition to be a CNV in
386 cercariae allopatric vs sympatric in both Fa and Fb, and CNV in adults allopatric vs sympatric in
387 both Fa and Fb. Nevertheless, there is in one region (*Schisto_mansoni*.Chr_3:1,725,500-1,762,000)
388 with a clustering of environment dependent CNV. Three single exon genes are predicted in the
389 region (*Smp_192030*, *Smp_101720* and *Smp_201970*) for which we did, however, not find
390 transcripts in our RNA-Seq data. CNV between biological replicates in sympatric or allopatric
391 condition do not show significant differences (**Table 1a & 1b**). CNV locations are provided in the
392 supplementary material (**Supplementary File 1**).

393

394 Taken together, our data provide evidence that, upon exposure to the allopatric condition, point
395 mutations and TE mobilization are below the detection limit. CNV occurs at appreciable frequency,
396 but are not linked to a specific environmental condition (allopatric or sympatric). The frequency of
397 CNV is not different between the sympatric and allopatric hosts. Twelve to fifteen percent of
398 random CNV are maintained through development to adults. One region on chromosome 3 could
399 be a CNV hot-spot activated upon exposure to the allopatric environment.

400 In two cases (*Schisto_mansoni*.Chr_2:717900-817000 and *Schisto_mansoni*.Chr_1:58049000-
401 58059000), we found epimutations close to CNV, but not to SNP or transposable elements. After
402 having established the degree and type of genetic mutations we attempted to characterize the
403 epimutations.

404 *The epigenome of S. mansoni comes in five main chromatin colors*

405 We then performed ChIP-Seq experiments with the abovementioned antibodies for two
406 genotypes (Fa and Fb) of cercariae that had developed in sympatric or allopatric snail hosts, and of
407 the resulting female adults (a total of 60 libraries, details in **Supplementary File 2**). The analysis of
408 the different histone modification combinations on the *S. mansoni* genome shows five main states

(also called “colors”; Filion *et al.* 2010), in both cercariae (**Figure 2**) and adults (**Supplementary Figure 3**). Both profiles are qualitatively similar. The most frequent histone mark combination (state 3 on **Figure 2** and **Supplementary Figure 3**) is a combination of H3K27me3-H3K27ac-H4K20me1, which covers 60% of the genome in cercariae and only 46% in adults. This state has a low presence around the transcription start site of genes (TSS) but is strongly enriched at the transcription end site (TES). The second most frequent state (state 2) is characterized by an enrichment of H3K27ac and H3K20me1, covering 17% of the genome of cercariae and 24% of adults. It is enriched upstream of the TSS, decreases drastically in the first kb past the TSS before being enriched again in the gene body and decreases once more near the 3’end of the gene. The absence of any of the four histone marks (state 5) covers 14% of the genome in both developmental stages of the parasite. It is not very common in gene bodies, but is particularly depleted around TSS and TES. State 4, characterized by a stronger presence of H3K27me3, combined with a lower amount of H3K27ac and H4K20me1 is very lowly represented around genes. Outside of genic regions, it can be found covering 5% of cercariae and 14% of adult genomes. The last combination of marks (state 1) presents a very strong enrichment in H3K4me3 located right after the TSS (3% of cercariae and 2.3% of adult genomes). We also compared chromatin state between sympatric and allopatric cercariae, as well as adults, but we did not observe any large-scale chromatin structure profile differences (data not shown). It should be noted that except for H3K27ac and H3K27me3, that are mutually exclusive, our approach can neither confirm nor exclude that histone modifications occur at the same histone or same nucleosome. All we can say it that there is co-occurrence at the same genomic site.

430

431 *There are three types of chromatin changes upon exposure to allopatric hosts*

432 For comparison of chromatin changes in the sympatric and allopatric conditions, one would ideally
 433 use these five chromatin colors. Unfortunately, no algorithm exists yet to do so. Instead, we
 434 performed what was feasible, *i.e.* a comparative analysis histone mark by histone mark for the
 435 four modifications using peak-calling (PeakRanger) and differential enrichment analysis (DiffBind).
 436 For the cercaria we found in average 8,378 peaks for H3K4me3, 15,866 peaks for H3K27ac, 20,784
 437 peaks for H3K27me3, and 11,175 peaks for H4K20me1. In adults there were 11,967 peaks for
 438 H3K4me3, 9,1110 peaks for H3K27ac, 22,435 peaks for H3K27me3, and 17,403 peaks for
 439 H4K20me1 (**Supplementary File 3**).

440

441 Heatmaps generated by DiffBind provide a general overview of the chromatin landscape. They
 442 clearly show chromatin differences in cercariae clones exposed to the sympatric and allopatric
 443 host, and the resulting female adults (**Figure 3A** and **3B**). Principal component analysis, using
 444 genomic positions with differences on chromatin profiles, separated sympatric and allopatric
 445 samples extremely well, based on the number of differences in histone marks between samples
 446 (**Supplementary Figure 4A** and **4B**). We observed in cercariae three different types of chromatin
 447 changes depending on condition and genotype. In principle, to classify epimutations, one would fit
 448 a statistical model to test for a genotype effect, an environmental effect, and possibly the
 449 interaction, on peak presence and shape. In such cases one could detect a significant effect of (for
 450 instance) environment even if not all replicates showed the pattern consistently. To our

451 knowledge, such statistical model has not been developed and tested. We use here a simpler
452 approach and classified epimutations into **(1)** host-induced epimutations, **(2)** spontaneous
453 epimutations, and **(3)** genotype dependent epimutations. For convenience, we will also use the
454 term “targeted epimutation” or “induced epimutations” for type 1 epimutation, and “random
455 epimutations” for type 2. In other words, at a given developmental stage (cercariae or adults),
456 type 1 epimutations must be present in all samples of the allopatric condition and absent in the
457 sympatric (or vice versa). Type 2 epimutations are present in at least one sample of a condition or
458 a genotype, but must not be present in all samples of this condition or genotype. Type 3
459 epimutations are present in all samples of one genotype (independently of the environmental
460 condition) and absent in all samples of the other genotype. A potential caveat of this classification
461 method lies in the relatively low number of replicates, leading potentially to an overestimation of
462 type 1 epimutations. In addition, type 2 epimutations might not be stochastic in the strict sense.
463 For instance, they might also be environment or genotype-specific but with incomplete
464 “penetrance” among replicate samples.

465
466 For type 1 epimutations, using a FDR 0.1 (Diffbind’s default), we identified 131 differences for
467 H4K20me2, 592 for H3K4me3, 346 for H3K27ac and 203 for H3K27me3, present in all replicates
468 between sympatric and allopatric cercariae. All were verified by visual inspection, in which we
469 looked for background changes that DiffBind had probably mistakenly identified as real
470 differences. Basically, if a trained human eye (considering intensity, shape, and strength of
471 neighboring peaks) cannot detect the differences spotted by Diffbind, we considered it as a false
472 positive (see **Supplementary Figure 5** for examples). In general, validated epimutations
473 corresponded to a p-value $\leq 1 \times 10^{-5}$ for H3K4me3 and $\leq 1 \times 10^{-15}$ for the other marks. It reduced the
474 number of type 1 differences to a total of 51 genomic regions, among those all of them showed
475 changes for H3K4me3, 11 for H3K27ac, 2 for H3K27me3, and 3 for H4K20me1. Examples are
476 shown in **Figure 4A** and a detailed list of differences is available in **Supplementary File 4**.
477 Interestingly, epigenetic diversity is higher in samples from the allopatric condition (**Figure 3A** and
478 **Supplementary Figure 4A**). We found that 76,5% of induced epimutations are in transcribed
479 regions previously identified with RNA-Seq in cercariae (Roquis *et al.* 2015).

480
481 As outlined above we defined spontaneous epimutations (type 2) as a chromatin structure change
482 that occurs in one or more samples, but that is not consistently shared among replicates (**Figure**
483 **4B**). We detected 64 genomic locations where these random epimutations occurred, very often
484 with multiple changes in the same region: 60 changes in H3K4me3, 9 in H3K27ac, 6 in H3K27me3,
485 and 5 in H4K20me1. We identified that 79.7% of the differences were in transcribed regions
486 (details available in **Supplementary File 4**). We detected these epimutations by comparing
487 samples one by one on Diffbind results (**Supplementary File 5**), and verifying them through the
488 same visual inspection as for type 1 epimutations. We noted that for the two replicates of
489 genotype Fb in the allopatric condition, we systematically found several hundreds of random
490 epimutation for the four histone isoforms. We believe that this is due to a technical issue leading
491 to higher background in these ChIP-Seq libraries, which is consistent with our visual inspection.

492

493 We also looked for genotype related epimutations (type 3), *i.e.* chromatin structure changes that
494 are identical in both environmental conditions, but different when comparing cercariae of
495 genotype Fa and Fb (**Figure 4C**). After visual inspection (same as type 1 & 2), we observed a total
496 of 64 genomic locations with chromatin structure differences between the two genotypes, often
497 with two or more marks changing: 39 differences for H4K20me2, 41 for H3K4me3, 38 for H3K27ac
498 and 42 for H3K27me3. 73.4% of the changes between Fa and Fb are in transcribed genomic
499 regions. BED files are available as **Supplementary File 5**.

501 *Spontaneous epimutations, but not host-induced epimutations in cercariae are inherited to* 502 *adults*

503 By infecting mice with some of the cercariae collected from each sympatric and allopatric snail, we
504 produced adult worms and investigated if the different types of epimutations we had spotted in
505 cercariae were transmitted to the next developmental stage. None of the type 1 chromatin
506 structure changes, shared by all replicates, were maintained in adults. However, Diffbind
507 heatmaps (with the exception of H3K4me3) still show differences between adults developed from
508 cercariae that have experienced either the sympatric or allopatric condition (**Figure 3B**), regardless
509 of the genotype (Fa or Fb). With the exception of H3K27me3, PCA of adults separate adults by the
510 environmental condition they experienced as cercariae (**Supplementary Figure 4A**). While host-
511 induced epimutations were not inherited *sensu stricto* from cercariae to adults, the developmental
512 trajectory was altered, resulting in different chromatin profiles between adults from allopatric or
513 sympatric origin (**Figure 3A and 3B; Supplementary Figure 4A and 4B**).

514
515 Since we had replicates for genetically identical clones Fb, we investigated whether spontaneous
516 epimutation (type 2) would be transmitted to the corresponding adults. Indeed, 45% of the sites
517 with epimutations have the same chromatin structure in adults: 29 differences in H3K4me3, 3 in
518 H3K27ac, 5 in H3K27me3 but none in H4K20me1 were heritable *sensu stricto*, *i.e.* they had
519 occurred in the sporocysts and were maintained in cercariae and adults.

520
521 We found genotype-associated epimutations to be most mitotically heritable. Roughly 77% of the
522 genomic locations with epimutations between Fa and Fb cercariae were found to be identical in
523 adults. It corresponds to 31 changes in H3K4me3, 23 in H3K27ac, 24 in H3K27me3 and in 30
524 H4K20me1.

525
526 We then used the average number of peaks detected by PeakRanger (**Supplementary File 3**) to
527 calculate frequency and heritability of histonic epimutations. Environmentally triggered
528 epimutations (type 1) occur at a frequency of 4.2×10^{-3} (H3K4me3), 1.2×10^{-3} (H3K27ac), 8.9×10^{-5}
529 (H3K27me3), and 1.7×10^{-4} (H4K20me1). Random epimutations (type 2) are observed in the same
530 order of magnitude: 5×10^{-3} (H3K4me3), 1×10^{-3} (H3K27ac), 2.7×10^{-4} (H3K27me3), and 2.9×10^{-3}
531 (H4K20me1). As mentioned above, heritability of type 1 epimutations is 0 (reversibility = 1). In
532 contrast, heritability of type 2 epimutations reaches up to 0.83. In summary, frequency and
533 heritability of histonic epimutations depends on the chemical nature of the modification and their
534 type. Mean frequency is in the 10^{-3} order of magnitude.

535

536 Based on their locations, epimutations for the different histone marks were manually compiled
537 into a single table for further analysis (**Supplementary File 4**).
538

539 *Negligible chromatin structure changes occur around repetitive elements*

540 Mapping our ChIP-Seq library over the 3,145 identified repetitive sequences of *S. mansoni* genome
541 and analyzing the read counts between samples, we did not find any differences present in all
542 replicates of an environmental condition in cercariae or adults. Only when we compared
543 environmental conditions within a single genotype, or between genotypes in the same conditions,
544 we detected changes in 21 repetitive elements (17 of which being transposable elements). Only a
545 single histone mark was modified in these 21 repetitive elements. None of the histone marks were
546 systematically changed and we found no heritability of the changes from cercariae to adults
547 (**Supplementary File 6**).
548

549 *Chromatin structure changes are not associated with specific pathways or molecular/biological 550 functions*

551 After having established that chromatin architecture changes upon exposure to a different
552 environment and that this occurs primarily in regions of the genome that contain genes, we
553 wondered whether a phenotype was associated with these epimutations. GO enrichment analysis
554 was done for all the transcribed regions of the genome where chromatin structure changes were
555 detected (host-induced, spontaneous and genotype-associated epimutations). No specific
556 pathway or enrichment in molecular or biological functions was observed in any type of
557 comparison. STRING analysis showed that Smp_016630 (Trimeric G-protein alpha o subunit),
558 Smp_141980 (cAMP-specific 3,5-cyclic phosphodiesterase) and Smp_147260 (Ras-like) could
559 potentially interact in a signaling pathway. All three genes have environmentally triggered
560 complex epimutations (**Supplementary File 4**).
561

562 Next, we exposed miracidia for three generations to the allopatric host, extracted RNA from the
563 resulting cercariae and compared their RNA profile to cercariae produced in sympatric conditions.
564 We had earlier shown that transcription is stalled in cercariae (Roquis *et al.* 2015) and we
565 expected only small differences in RNA profiles. We compared the RNA levels of genes at or close
566 to the type 1 epimutations using the three generations as biological replicates (**Supplementary
567 File 7**). Differences in RNA levels of epimutated genes were moderate with a few exceptions:
568 Smp_130050 codes probably for a PDZ domain containing RING finger protein and its RNA is 3-5
569 fold overrepresented in allopatric cercariae. Smp_151290 codes for a putative multidrug and toxin
570 extrusion protein 2 and its RNA is 2.5 times enriched in cercariae from allopatric hosts.
571 Smp_015630 (Glutamate-gated chloride channel subunit) is threefold underrepresented in
572 allopatric cercariae.
573

574 We also identified genes with strong differences *i.e.* adjusted p-value < 0.01, and/or 10 fold
575 overrepresentation in one of the conditions (**Supplementary File 8**). For these genes we verified
576 chromatin structure in cercariae and adults. None of the genes were in the regions that we had
577 qualified as epimutated. However, it is noteworthy that 80% (50 of 62) of these genes do not

578 contain a canonical H3K4me3 peak at the TSS. This is two times higher than the genome average.
579 It could therefore be that with the antibody combination we used we did not capture the
580 chromatin structure of these genes. We also noticed subtle differences in the extent and position
581 of chromatin states 1 to 5 (**Supplementary Figure 6**). We would have considered this as random
582 noise but in some cases the chromatin state profile was faithfully inherited to the adults,
583 suggesting that these small differences are stable and might have an impact on gene expression.
584

585 Discussion

586
587 In this experiment we set out to investigate the impact of the environment on the genome and epigenome
588 of *S. mansoni* larvae. The environment could be (i) a selective agent, (ii) could induce specific mutations
589 and/or epimutations, and (iii) could change mutation and/or epimutation rate, or (iv) might have no effect.
590 The two *S. mansoni* genotypes we used in this study (Fa and Fb) come from the same inbred
591 population. Nucleotide diversity is 0.0004% based on SNP, but they show relatively strong
592 differences on the level of CNV (0.2% of the genome). We also found that they bear epigenetic
593 differences. Here, we considered genotype-associated epimutations as chromatin structure
594 changes present within a genotype in both allopatric and sympatric condition, but absent in the
595 other genotype. We observed 64 of these differences ($\text{FDR} \leq 0.1$, $p \leq 1 \times 10^{-5}$ and after visual
596 inspection). The majority (73.4%) of epimutations are in transcribed regions in cercariae, which is
597 similar to what we observed with spontaneous and induced epimutations. In at least two striking
598 cases that we investigated, genotype associated epimutations were linked to CNV. In the case of
599 clone Fa, there is an important CNV difference between cercariae emitted from sympatric and
600 allopatric snails, and could be a result of the environment, although without replicates for this
601 genotype we cannot assess it without doubt. It has been previously observed (Skinner *et al.* 2015)
602 that environmentally induced epimutations can lead to genetic mutation and CNV. We noticed
603 that the CNV were clustered (**Supplementary File 1**). Centromere positions on *S. mansoni* genome
604 are not mapped to the genome, and it is also possible that these CNV clusters are located in
605 pericentromeric regions, which are hotspots of segmental duplications and prone to generate CNV
606 (Makino *et al.* 2013). Apparition of these CNV could also possibly explain some of the genetic
607 heterogeneity between *S. mansoni* clonal sporocysts observed before (Bayne & Grevelding 2003).
608

609 We then looked at environmentally triggered epigenetic changes, *i.e.* epimutations that occur in
610 all the samples of a given mollusk condition. We found a total of 51 genomic regions, where at
611 least one histone mark is different in all three cercariae samples emitted from sympatric and
612 allopatric mollusks. Changes were often subtle (peaks of low intensities compared to neighbor
613 peaks). As we used whole organisms for ChIP-Seq, there is the possibility that these changes only
614 reflect a small subset of cells or tissues within cercariae. No gene pathway or gene ontology term
615 enrichments were uncovered in genes spanning induced epimutations, and none of these
616 epimutations were transmitted to adults. This type of ephemeral epigenetic change in response to
617 an environmental stimulus is reminiscent of what was found for instance in smokers and former
618 smokers where roughly a dozen CpG sites change their methylation status but revert progressively
619 to normal when smoking was abandoned (Wan *et al.* 2012; Elliott *et al.* 2014).
620

621 No major chromatin remodeling was observed in the 3,145 known repetitive sequences of the *S.*
622 *mansoni* genome, and although some of the epigenetic differences are located in transposable
623 elements, we did not detect new insertion sites. Transposable elements movement is known to
624 generate new genetic, epigenetic and phenotypic diversity in other works (Biemont 2010; Fedoroff
625 2012; Holoch & Moazed 2015), but they do not play a major role in this study.

626
627 Subsequently, we investigated the presence of spontaneous (type 2) epimutations through the
628 genome of cercariae. We first detected type 2 epimutations through comparison of replicates of
629 clone Fb. Sixty-four sites on the genome showed random epimutation, and 45% of them
630 maintained their epimutated chromatin structure at the adult stage. Our data suggest that a
631 change of the environment has an impact on epimutations since chromatin profiles of cercariae
632 emitted from *BgGua* (and the resulting adults) present more differences between samples (as
633 observed from the PCA on **Supplementary Figure 4A** and **4B**) than chromatin profiles of cercariae
634 and adults emitted from *BgBre*. We had shown before that *S. mansoni* worms exposed transiently
635 to hycanthone, an antelmintic drug, possess 57 days later a characteristic chromatin profile
636 different from unexposed worms (Roquis *et al.* 2013). At that time, we were not able to tell if the
637 drug directly induced the different profiles, or if it acted as a selection agent, killing most of the
638 parasites in the population, except those with a pre-existing distinct chromatin structure. In light
639 of our new findings we are now in favor of the hypothesis that random epimutations had occurred
640 prior to the drug exposure, and had produced in some individuals a resistant phenotype to the
641 treatment.

642
643 The average frequency of histone modification based epimutations in our system is about 10^{-3} .
644 This is one order of magnitude higher than what was reported for DNA methylation based
645 epimutations in CpG pairs (Schmitz *et al.* 2011; van der Graaf *et al.* 2015). In this issue, Kronholm &
646 Collins (2015) underline the importance of stability of epimutations for adaptive evolution. We
647 show that heritability (*i.e.* stability) depends strongly on the type of epimutation and that for most
648 of the detected epimutations reversion is actually above the threshold of 10^{-1} that Kronholm and
649 Collins define as the reversion rate that limits the adaptive effect of epimutations. In other words,
650 our data support the view that in *S. mansoni* epimutations do not contribute to adaptive walks,
651 unless they provide a strong fitness gain.

652
653 *Schistosoma mansoni* proves to be an interesting model for this kind of epigenetic studies, due to
654 its ecological/healthcare importance, the possibility to generate clones, and different
655 phenotypically distinct developmental stages allowing to observe mitotic inheritance of
656 epimutations. However, one of the caveats of this organism is its small size, making it impossible
657 at the moment to perform ChIP-Seq on specific tissues or individuals (we needed at least 5,000
658 cercariae for our experiments). It means that we can only observe epimutations that are present
659 in most of the cells and most individuals in the pools. Tissue-specific epimutations, as well as rare
660 epimutations within the clone population, cannot be detected. Conversely, this also means that
661 the chromatin structure changes we observed concern the majority of cells and individuals in our
662 pools, and hence are more likely to have a significant adaptive impact. We also keep in mind that
663 our sample size (three for each environmental condition and developmental stage) is small, and

664 more replicates, from the same and new genotypes, need to be done in order to strengthen our
665 findings.
666

667 Conclusion

668 In conclusion, our findings indicate that in *S. mansoni* sporocysts, there are two major sources of
669 mitotically heritable variation: CNV and spontaneous epimutations. This means that at least in our
670 biological model (i) epimutations occur at appreciably frequency and since they are
671 overrepresented in coding regions they can have an impact on gene expression, and (ii) that CNV
672 occur at the same order of magnitude as epimutations, potentially contributing as much as the
673 latter to phenotypic variation. We could not fully answer the question whether or not
674 environmental changes increase epimutations. That means that at least for *S. mansoni* theoretical
675 models in which epimutations serve to produce phenotypic variants that explore the fitness
676 landscape for invariant genotypes (e.g. Klironomos *et al.* 2013) are fully applicable but future
677 models must include CNV and must take into account the high reversion rate of histone-based
678 epimutations.
679

680 Acknowledgement and data availability

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682 helpful discussions. Data for ChIP-Seq, Pool-Seq and RNA-Seq are available as fastq files BioProject
683 Accession PRJNA236156 at the NCBI SRA. Chromatin profiles and transcripts can be visualized at
684 our GBrowse instance (Stein *et al.* 2002) (<http://genome.univ-perp.fr>). Funding was provided by
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686 program of the UMR5244/IHPE (<http://ihpe.univ-perp.fr/en/ihpe-transversal-epigenetics/>).
687

688 Figure legends

689

690 **Figure 1:** Experimental procedure for the sporocyst grafts. Brazilian mollusks (*BgBre*) were
691 infected with a single miracidium of the sympatric strain of *S. mansoni* (*SmBre*). Thirty days later,
692 secondary sporocysts were surgically removed from the infected mollusks and grafted in several
693 sympatric (*BgBre*) and guadeloupean allopatric (*BgGua*) mollusks. Emitted cercariae were
694 collected for chromatin immunoprecipitations followed by sequencing (ChIP-Seq) and infection of
695 mice to obtain adult worms. Adult worms were recovered for ChIP-Seq and paired-end DNA
696 sequencing. The experiment was performed twice, with two different genotypes of the parasite
697 (Fa and Fb). In Fb genotype, two replicates were done in sympatric and allopatric conditions.
698

699 **Figure 2:** Five different chromatin states (“colors”) as identified by EpiCSeq in cercariae. (A)
700 Composition in histone marks of the five chromatin states. Color code of the states is used in all
701 other parts of this figure. (B) Proportion of the genome covered by each state in cercariae.
702 Enrichment for each state around gene bodies (C), at transcription start site (TSS) (D) and
703 transcription end site (TES) (E) of cercariae. Enrichment profiles were done on 2,428 genes longer
704 than 5 kb. Results for adults are very similar and shown in **Supplementary Figure 2**.

705

706 **Figure 3:** Heatmaps for the four studied histone marks in cercariae (A) and adults (B). Only sites
707 with statistically significant differences (epimutations) were used. Red bars highlight conserved
708 changes. Red snail symbols represent sympatric host *B. glabrata* BgBre, blue snail symbols stand
709 for allopatric snail host *BgGua*. Cercariae sample GuaFb5 for H3K4me3 was excluded from analysis
710 (see methods).

711

712 **Figure 4:** Typical examples of epimutations. (A) environmentally dependent epimutations (green
713 frame) in H3K4me3 (blue profile) and H3K27ac (red profile): there are differences in all three
714 samples Fa and two replicates Fb between sympatric and allopatric cercariae. (B) Random
715 epimutations in H3K4me3, present in cercariae in three samples and transmitted to adult worms
716 in corresponding three samples. (C) genotype-associated epimutations in H3K4me3, H3K27ac,
717 H3K27me3 and H4K20me1 between genotypes Fa and Fb in sympatric condition. The track Ref.
718 transcriptome *S. mansoni* displays transcripts (exons as blocks and introns as arrows) deduced
719 from RNA-Seq data in cercariae.

720

721 **Supplementary Figure 1:** Life cycle of the parasite *Schistoma mansoni*. The endoparasites infect
722 two hosts, (1) a mollusk intermediate host (*Biomphalaria* genus) in which it multiplies asexually as
723 sporocysts and then cercariae, and (2) a vertebrate definitive host (primate or rodent) in which
724 adult worms reproduce sexually. Two types of free-swimming larvae, miracidia and cercariae, are
725 the infective form for the mollusk and vertebrate hosts, respectively. Eggs laid by adult worm
726 couples in the mesenteric vein of the vertebrate host can go through the intestine wall to be
727 excreted in the feces, and hatch in contact with freshwater.

728

729 **Supplementary Figure 2:** ChIP titration, using the same amount of *S. mansoni* chromatin
730 (equivalent to 20 adult worms) and an increasing amount of antibody. Percentage of input
731 recovery of chromatin was measured by qPCR on two housekeeping genes, alpha tubulin and 28S,
732 as described in (Cosseau *et al.* 2009). Titrations were done to measure the efficiency of
733 immunoprecipitation for antibody H3K27ac Abcam cat# ab4729 lot# GR-150-367-2 (A and B) and
734 H4K20me1 Abcam, cat# ab9051 lot# GR158874-1 (B and C).

735

736 **Supplementary Figure 3:** Five different chromatin states ("colors") as identified by EpiCseg in
737 adults. (A) Composition in histone marks of the five chromatin states. Color code of the states is
738 used in all other parts of this figure. (B) Proportion of the genome covered by each state in adults.
739 Enrichment for each state around gene bodies (C), at transcription start site (TSS) (D) and
740 transcription end site (TES) (E) of adult worms. Enrichment profiles were done on 2,428 genes
741 longer than 5 kb.

742

743 **Supplementary Figure 4:** Principal Component Analysis (PCA) for the four studied histone marks in
744 cercariae (A) and adults (B). Only sites with statistically significant differences (épimutations) were
745 used. Blue circles (Bre) represent cercariae and adults from sympatric conditions (*SmBre* in *BgBre*),
746 magenta (Gua) the allopatric conditions (*SmBre* in *BgGua*). Cercariae sample GuaFb5 for H3K4me3
747 was excluded from analysis (see methods).

748

749 **Supplementary Figure 5:** Examples of two regions (**A** and **B**) that were identified as epimutations
750 (displayed by the red boxes) by DiffBind but did not pass the visual inspection.

751

752 **Supplementary Figure 6:** Example of a region that was not identified as epimutated by DiffBind
753 but shows differences based on EpiCSeq chromatin “colors”, judged by visual inspection. The
754 associated gene is differentially expressed and chromatin states are transmitted to adults.

755

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967 **Data Accessibility**

968 RNA-Seq and ChIP-Seq rawdata are available as fastq files under study accession number
 969 SRP035609 (BioProject Accession number PRJNA236156) at the SRA of the NCBI.