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Author for correspondence:
Eve Toulza
e-mail: eve.toulza@univ-perp.fr

Transcriptomic plasticity in hybrid schistosomes can contribute to their zoonotic potential

Nelia Luviano Aparicio[1,2,†], Eglantine Mathieu-Bégné[1,3,†], Julien Kincaid-Smith[4], Olivier Rey[1], Marion Picard[1], Cristian Chaparro[1], Jean-François Allienne[1], Anne Rognon[1], Bruno Polack[5], Isabelle Vallée[5], Myriam Thomas[5], Jerome Boissier[1] and Eve Toulza[1]

[1]IHPE, University Perpignan Via Domitia, CNRS, IFREMER, University Montpellier, Perpignan 66860, France
[2]Department of Medicine, Sylvester Comprehensive Cancer Center, Miami, FL 33136, USA
[3]Université d'Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France
[4]CBGP, IRD, CIRAD, INRAE, Institut Agro, Université de Montpellier, 34060 Montpellier, France
[5]Anses, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale, Maisons-Alfort, Montpellier 94700, France

NLA, 0000-0002-7929-902X; EM-B, 0000-0002-6846-5491; JB, 0000-0002-0793-3108; ET, 0000-0003-2049-2279

Hybrids between *Schistosoma haematobium* and *Schistosoma bovis* contribute to human and animal infections, highlighting complex interspecies interactions that facilitate schistosomiasis transmission. *Schistosoma bovis* infects multiple ruminant hosts, promoting cross-species transmission and increasing zoonotic risk. This study explores transcriptomic plasticity as a mechanism enabling hybrid schistosomes to adapt to different definitive hosts. We analysed two contexts: (1) introgressed *S. haematobium* × *S. bovis* hybrids, which exhibited higher virulence in sheep than parental *S. bovis*; and (2) *S. bovis* infecting different mammalian hosts. Introgression, the transfer of genetic material between species through hybridization and repeated backcrossing, was associated with 366 differentially expressed genes (4% of coding genes) between introgressed hybrids and *S. bovis* in sheep. Additionally, *S. bovis* showed host-dependent transcriptomic changes, with 30% of genes differentially expressed between infections in hamsters and sheep. Enriched biological processes shared across introgression and host adaptation included nuclear mRNA catabolism and inner mitochondrial membrane organization, indicating increased gene expression plasticity and metabolic adaptation to environmental stress. These findings suggest that transcriptomic plasticity enhances the adaptability of *S. bovis* and hybrid worms, increasing their zoonotic potential. This raises concerns for schistosomiasis control, as such plasticity could expand transmission capacity and complicate intervention strategies.

This article is part of the Science+ meeting issue 'Parasite evolution and impact in action: exploring the importance and control of hybrid schistosomes'.

1. Introduction

Hybridization can significantly influence the ecology and evolution of host–parasite interactions. The viable offspring resulting from hybrids between two species might also backcross with one of their parental species. If these backcrossed offspring continue to reproduce with the same parental species, this can result over time in the lasting transfer of DNA from one of the species into the other, a process known as introgressive hybridization or

[†]Nelia Luviano Aparicio and Eglantine Mathieu-Bégné are joint first authors.

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introgression [1]. Introgression can promote gene flow between two species, increasing the genetic variability and fitness of one or the other species and facilitating adaptation to new environments. Genomic studies have revealed that introgression is far from exceptional, one in every 10 mammalian species carries the imprint of past or ongoing hybridization, and comparable proportions are reported for birds, fishes and many plant clades [2,3]. However, hybridization studies in parasites remain comparatively rare and underexplored. Both host and parasite introgressive hybridization have been shown to impact parasite virulence, transmission, and host specificity. However, research on hybridization has primarily focused on how hybrid hosts are affected by parasites, treating parasites as a selective force in host evolution. This underscores the importance of considering that parasites also undergo their own evolutionary dynamics [4].

In this work, we focus on schistosome parasites, which serve as an excellent model for studying introgression. Schistosomes are parasitic blood flukes that develop and grow in the vascular system of mammal definitive hosts. They are responsible for causing bilharzia, or schistosomiasis, a neglected tropical disease and the second most important parasitic disease after malaria. There are over 230 million people requiring treatment, of which the majority live in Africa [5]. Schistosomiasis is a waterborne disease. Schistosome parasites first infect at the miracidial stage an intermediate mollusc host in which the parasite asexually multiplies. Cercariae are then released and can infect the mammal definitive host when this later has contact with water. Sexual reproduction occurs in the definitive host and, depending on the schistosome species, eggs are released through urine or faeces in water so that the cycle can continue [6].

While numerous schistosome species exist, their barriers are often maintained through differences in ecology, host specificity and evolutionary history [7]. However, hybridization between closely related species can happen when parasites infect the same host [8]. For instance, hybrids between *Schistosoma haematobium* and *Schistosoma bovis* have been detected in humans across several African countries, including Benin, Ivory Coast, Mali, Niger and Senegal [9–11]. These hybrids have also been found in cattle in Benin, which evidences that these hybrids are present in livestock of sub-Saharan Africa [12]. Furthermore, *S. bovis*, as well as hybrids between *S. bovis* and *Schistosoma curassoni*, have been observed in sheep in the field [13]. By conducting laboratory infections of hybrids between *S. bovis* and *S. haematobium* in sheep, we can gain a better understanding of the potential for hybridization in this host and its phenotypic and epidemiological implications [14].

Schistosoma bovis infects numerous ruminant species, including cows and sheep [15,16], and can also use some rodents as definitive hosts, including the hamster (*Mesocricetus auratus*), which is commonly used as definitive host to maintain the parasite under laboratory conditions [17]. Previous studies have examined gene expression in *S. bovis* × *S. haematobium* hybrids, but these have been limited to infections in hamsters [18]. It remains unclear whether similar patterns occur in natural hosts or what changes in the parasite's transcriptomic machinery might facilitate long-term host adaptation in backcrossed individuals. This has significant implications given that most experimental research on schistosomes relies on host species rarely encountered in the field.

Both *S. haematobium* and *S. bovis* are phylogenetically close and share freshwater snails of the genus *Bulinus* as their intermediate host [19]. The study of the molecular variation of *S. haematobium* in Africa brought to light genomic signatures that correspond to species other than *S. haematobium*, suggesting the occurrence of introgression events [20]. Indeed, it was demonstrated that most *S. haematobium* African populations show signatures of genomic introgression from *S. bovis* [21]. Moreover, hybrid ancestry with *S. bovis* has been detected in *S. haematobium* Nigerian strains, revealing selection and fixation of an *S. bovis*-derived *invadolysin* gene variant [22].

In the case of *S. haematobium* × *S. bovis* hybrids, introgressive hybridization may enable the rapid acquisition of new traits, potentially facilitating adaptation to novel hosts and environments. RNA expression analysis of these hybrids has shown over-expression of processes linked to heterosis (improved biological traits in hybrids compared with parents), particularly in females, which may be associated with increased reproductive potential [18].

Schistosoma haematobium—like multiple *Schistosoma* species—possesses eight chromosomes, including seven autosomes and one pair of ZW sex chromosomes, where males are homogametic (ZZ) and females are heterogametic (ZW). The genome comprises 9431 gene models, and 2138 are found in the sex chromosomes [20,23]. The *S. bovis* genome also includes eight chromosomes, 9589 gene models, of which 2195 are on the Z chromosome without distinction of PAR and Z-specific regions [24]. Moreover, *S. haematobium* and *S. bovis* share 98% of their genes as orthologues [20]. Regarding sex-linked loci, comparative transcriptional analyses between male and female adult stages in *S. haematobium* have identified 1512 transcripts significantly upregulated in females. These genes are over-represented on chromosomes 1 and 3, as well as on the largest unplaced scaffold, suggesting potential sex-linked gene expression patterns [20].

Epigenetic mechanisms, particularly histone post-translational modifications, regulate gene expression in schistosomes, driving pathogenesis and offering therapeutic targets. During development, H3K27me3 marks are dynamically regulated between cercarial, schistosomula and adult stages to enable transcriptional changes [25], while also influencing sex determination and sexual differentiation [26]. Histone marks H3K9me3 and H3K9ac vary between susceptible and resistant strains, affecting host compatibility genes like the *Schistosoma mansoni* mucin gene (*SmPoMuc*), a mediator of compatibility between the parasite and its snail host [27]. Additionally, microRNAs (miRNAs) contribute to developmental regulation and sex-specific expression [28–30], with 13 miRNAs showing sex-biased patterns in *S. mansoni* [31], including bantam and miR-31, which are more abundant in females and linked to ovary formation in *Schistosoma japonicum* [32]. Another important molecular mechanism through which schistosomes escape immune detection during invasion and development is glycan mimicry, which consists of displaying surface molecules with glycosylation patterns similar to those in the host snail's plasma and haemocytes [33]. More recent research has revealed that host glycan-binding proteins (GBPs), including C-type lectin receptors and galectins, interact with helminth glycans to influence immune responses. This has led to the concept of 'glycan gimmickry', where parasites actively deploy specific glycans to engage host GBPs and enhance their survival chances [34].

The zoonotic potential of these introgressed hybrids warrants attention in epidemiological fieldwork, including diagnostics and treatment strategies, as they may display enhanced virulence, and a broader host range compared with their parental species [7]. This was confirmed by our recent study which found that introgressed *S. haematobium* × *S. bovis* worms can infect sheep, whereas pure *S. haematobium* parasites cannot [35]. This suggests that schistosomes possess a molecular mechanism that allows host change, particularly when hybridized with another species [36], raising public health concerns about their ability to exploit new hosts and regions [37,38]. Despite this, there is limited knowledge about the molecular machinery allowing parasites to successfully infect alternative definitive host species, and this knowledge exists mostly for parasitic insects [36,39], but is absent in other phyla, such as Platyhelminthes.

In this study, we analysed the transcriptomes of introgressed *S. haematobium* × *S. bovis* worms, which displayed increased number of worms in infected sheep [35], and investigated gene expression changes associated with host change in *S. bovis*. The goal was to uncover biological processes that might enhance the zoonotic potential of both *S. haematobium* × *S. bovis* hybrids and *S. bovis*. We hypothesize that (1) introgressed *S. haematobium* × *S. bovis* parasites, exhibiting higher numbers of worms in sheep than parental *S. bovis*, undergo significant transcriptomic changes; (2) host change in *S. bovis* is accompanied by substantial transcriptomic plasticity that facilitates adaptation to different host species; and (3) the transcriptomic changes associated with both introgression and host change involve common biological pathways.

2. Material and methods

(a) Ethics approval

Sheep infections with *S. bovis*, *S. haematobium* and hybrid cercariae were conducted in the Veterinary School, Ecole Nationale Vétérinaire d'Alfort in Maisons-Alfort, France. The animal facility possesses agreement E940462 delivered by the French Ministry of Higher Education, Research and Innovation. The sheep experiment was registered by the French Ministry of Higher Education, Research and Innovation, and approved by an independent ethics committee (APAFIS no. 23786-2020012416159143 v2).

Worms were collected and frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

(b) Parasite and host strains

The *S. haematobium* strain originated from infected patients (with ethical clearance) in the southeast part of Cameroon (Barombi Kotto lake), was isolated in 2015 and was maintained in the IHPE laboratory using *Bulinus truncatus* and the golden hamster (*Mesocricetus auratus*) as intermediate and definitive hosts. The *S. bovis* strain originated from the Spanish Laboratory of Parasitology of the Institute of Natural Resources and Agrobiology in Salamanca and was maintained in the IHPE laboratory using *Bulinus truncatus. truncatus* and *Planorbarius metidjensis* as intermediate hosts and *M. auratus* as definitive host.

(c) Experimental infection of the targeted definitive hosts

Hamster infections with the different parasite strains, including the pure *S. bovis* strain, the F1 (female *S. haematobium* × male *S. bovis*) and F1′ (female *S. bovis* × male *S. haematobium*) hybrids, and the backcross to *S. bovis*, were conducted as previously described [18].

The experimental infections in sheep with the different parasite strains were conducted at the Maisons-Alfort laboratory as previously described [35]. Briefly, cercariae from the different laboratory parasite strains were used to infect three series of sheep for each parasite strain: parental *S. bovis* and *S. haematobium* strains, F1 (and F1′) parasites, and backcrossed parasites (table 1).

Hamsters and sheep were infected following a natural percutaneous immersion method. Three control sheep were exposed to the pure strains: *S. bovis* and *S. haematobium*. The same number of cercariae (1500) was used to infect each sheep and 300 cercariae for hamsters. For each parasite line, a minimum of 12 snails exposed to five miracidia produced the cercariae for sheep and hamsters' infection. Sheep infection was done under anaesthesia (ketamine and diazepam) at the level of a foreleg previously wetted with warm water. Sheep euthanasia was performed by deep anaesthesia with a combination of xylazine and ketamine, followed by bleeding the animals to collect blood. Sheep autopsies were conducted under the supervision of a European College of Veterinary Pathologists board member. All viscera were inspected, and the vessels of the small intestine, caecum, colon, rectum and bladder were examined by transillumination with a strong light.

Adult male and female schistosomes were recovered from experimental infections of sheep and hamsters for each cross type. The goal was to collect adult parasites resulting from experimental infections as a primary resource for subsequent transcriptomic analyses. In sheep, the worms were isolated by opening the vein with a scalpel and carefully extracting the live schistosomes using forceps. In hamsters, worms were recovered through portal vein perfusion. Male and female worms were carefully separated to avoid cross-sex contamination and pooled into groups of 12 individuals per condition and sex.

In total, 24 samples were obtained from the four different crosses analysed from sheep, six pools of 12 worms per cross (three males and three females) for pure *S. bovis*, F1, F1′ and backcross *S. bovis*. *Schistosoma haematobium* was unable to infect sheep and we did not recover enough female and male worms for the *S. haematobium* backcrosses; therefore, these two conditions were not included in the present study. F1 and F1′ are used to distinguish between the two reciprocal first-generation hybrids. F1 denotes

Table 1. Crosses and backcrosses between *Schistosoma bovis* and *Schistosoma haematobium* used in this work (only adult parents are represented), with *Bulinus truncatus* and the golden hamster or sheep as intermediate and definitive hosts, respectively.

male parent	female parent	cross type	host
Schistosoma bovis	*Schistosoma bovis*	pure *S. bovis*	hamster and sheep
		♂ 3 pools of 12 individuals	
		♀ 3 pools of 12 individuals	
Schistosoma haematobium	*Schistosoma haematobium*	pure *S. haematobium*	unable to infect sheep
Schistosoma bovis	*Schistosoma haematobium*	F1 hybrid (♀ *S. haematobium* × ♂ *S. bovis*)	sheep
		♂ 3 pools of 12 individuals	
		♀ 3 pools of 12 individuals	
Schistosoma haematobium	*Schistosoma bovis*	F1′ hybrid (♀ *S. bovis* × ♂ *S. haematobium*)	sheep
		♂ 3 pools of 12 individuals	
		♀ 3 pools of 12 individuals	
Schistosoma bovis	F1′ hybrid	backcross *S. bovis*	sheep
		♂ 3 pools of 12 individuals	
		♀ 3 pools of 12 individuals	

the cross of a male *S. bovis* with a female *S. haematobium*, and F1′ denotes the cross of a male *S. haematobium* with a female *S. bovis*.

The six *S. bovis* samples infecting hamsters have been previously sequenced and published [35]. The RNA sequencing data from *S. bovis* infecting hamster that were used to analyse transcriptome modifications associated with host change were recovered from the Bioproject PRJNA49163, for which we used six samples from the homospecific pairing, three males and three females (Biosamples: SAMN10081090, SAMN10081089, SAMN10081088, SAMN10081087, SAMN10081086 and SAMN10081085).

(d) RNA extraction and transcriptome sequencing procedure

Total RNA extractions were performed using the Qiagen RNeasy Mini kit. Briefly, pools of 12 frozen adult worms in 2 ml microtubes were ground with glass beads (Sigma, G4649), using a blue RNase free pestle in the case of females, and by a different method for male worms (owing to larger size), using zirconium beads and the Retsch MM400 cryobrush (2 pulses at 30 Hz for 15 s). After extraction following the manufacturer's protocol, total RNA was eluted in 44 µl of RNase-free water. DNase treatment was then performed using the Thermo Fisher Scientific Turbo DNA-free kit.

Quality and concentration were assessed by spectrophotometry with the Agilent 2100 Bioanalyzer system and using the Agilent RNA 6000 Nano kit. cDNA library construction and sequencing were performed by the Bio-Environment sequencing platform at the University of Perpignan, Via Domitia, France. The NEBNext Ultra II directional RNA library preparation kit was used following the manufacturer's protocol on 300 ng of total RNA per sample. Sequencing was performed using 75 bp single-end reads on an Illumina NextSeq 550 instrument.

(e) Transcriptomic analyses

Bioinformatic analyses were performed on the Galaxy server instance of the IHPE (Interactions Hôtes-Pathogènes-Environnements) laboratory [40–42]. Raw reads were subjected to quality assessment using FastQC v. 0.72 [43]. We trimmed reads based on quality (Phred quality score threshold <20) using Trim Galore v. 0.6.3 [44]. Processed reads were mapped to the *S. haematobium* reference genome v. 3 since it has a better genomic resolution than the *S. bovis* genome [20] using RNA STAR mapper v. 2.7.8 [45].

Counting of reads per transcript was done using the HTSeq count tool (v. 0.9.1) [46] and using the gene transfer file from *S. haematobium* as reference transcriptome (schistosoma_haematobium.PRJNA78265.WBPS18.annotations.gff3 downloaded from WormBase Parasite, https://parasite.wormbase.org/index.html). Differential expression analyses were done with the R package DESeq2 [47], low counts were filtered (<10) and genes differentially expressed were identified by setting an adjusted *p*-value <0.05 and $\log_2$FC (Fold Change) >1. We performed principal components analysis (PCA) for all the samples to identify clusters between samples. To identify gene expression modifications associated with hybridization, we compared the gene expression of parental *S. bovis* with those of the first-generation hybrids (F1 and F1′). To identify gene expression modifications associated with introgression, we compared the gene expression of parental *S. bovis* with those of the backcrosses between male F1 × female *S. bovis* that displayed maximal infection rates in sheep [35]. Since the differences between sexes were stronger than those between genetic introgression levels (§3), we conducted these analyses for each sex separately. To address specifically which modifications in gene expression are associated with host change, we compared the transcriptome between the same *S. bovis* strain infecting the hamster and the sheep and accounting for a possible sex effect. To this aim, we compared three pools of

12 males, and three females collected from each of the two definitive hosts (i.e. hamster and sheep). One female *S. bovis* collected from sheep was discarded from the analysis as it was identified as an outlier. For all pairwise comparisons, we specified which samples to compare using the 'contrast' parameter in the DESeq2 software. This allowed us to analyse the differences in gene expression between the selected samples. It is worth mentioning that we could not analyse *S. haematobium* worms because they were not capable of infecting sheep.

The reference transcriptome assembly was converted from GTF to FASTA format using gffread (v. 2.2.1.3), with the reference genome provided as input [48]. This reference transcriptome in FASTA format was used as input in Orson, a Nextflow-based workflow for transcriptome functional annotation developed by the Bioinformatics Core Facility of Ifremer, the French National Institute for Ocean Science, and configured on the Datarmor supercomputer (https://gitlab.ifremer.fr/bioinfo/workflows/orson). Merged XML file output from the Orson workflow was then used as input in the OmicsBox bioinformatics software v. 1.3.11 (OmicsBox, 2019) to execute gene ontology (GO) mapping and GO annotation.

GO enrichment analysis of differentially expressed genes (DEGs) was performed using the R package RGBOA, available on GitHub (GO-MWU, https://github.com/z0on/GO_MWU). We used as input a comma-separated values (csv) file containing all genes with binary values (1 when the gene was significantly over- or under-expressed and otherwise 0), and we used the Fisher's exact test mode; we selected enriched biological processes with a false discovery rate (FDR) <0.05 as used in another study [49]. When the Fisher test did not reveal significant enrichment of GO terms, we selected the top 10 genes over- and under-expressed for functional investigation. The software REVIGO was used to remove redundant GO terms from the long lists obtained in the GO enrichment analysis and to illustrate representative enriched GO terms in a dot plot [50].

3. Results

(a) Transcriptomic analysis in hybrids and backcrossed schistosomes compared with parental *Schistosoma bovis*

On average, we obtained 27 570 576 ± 2 267 934 raw reads per sample after sequencing, and 27 283 136 ± 2 620 705 reads per sample after quality trimming. On average, 83 ± 3.5% of the reads mapped to the reference genome of *S. haematobium* (V3) which consists of 9431 protein-coding genes; the percentage of mapping per sample is provided in electronic supplementary material, table S1. No significant differences in mapping percentage was observed depending on the species/hybrid line ($p = 0.5$, Kruskal–Wallis test), highlighting the high similarity between the two genomes, at least in the coding regions. Based on their overall gene expression patterns, parasites first clustered according to their sex along the first PCA axis, which represented 48% of the overall transcriptomic variance among individuals, and then according to their introgression level along the second PCA axis (representing 14% of the variance) (figure 1A).

As sex was the first driver of transcriptomic differences, we next analysed males and females separately. In females, we identified 76 DEGs between *S. bovis* and first-generation hybrids from the F1 cross (♀ *S. haematobium* × ♂ S. *bovis*), 35 over-expressed and 41 under-expressed in *S. bovis* compared with F1. For the comparison between *S. bovis* and the F1′ cross (♀ *S. bovis* × ♂ *S. haematobium*), we found 28 DEGs, 21 over-expressed and 7 under-expressed (electronic supplementary material, S2, tables S1 and S2).

When comparing female *S. bovis* with females of the backcross with *S. bovis*, we identified 40 DEGs, with 20 genes being over-expressed and 20 genes under-expressed in the backcross relative to the pure *S. bovis* females.

Concerning the comparison between males, we found 38 DEGs between *S. bovis* and F1, 18 over-expressed and 20 under-expressed. In the comparison between males *S. bovis* and F1′, we found 85 DEGs, 68 over-expressed and 17 under-expressed (electronic supplementary material, S2, tables S3 and S4). In the males comparison between S. *bovis* and backcross *S. bovis*, we identified 366 DEGs; 260 were over-expressed and 106 under-expressed in the backcross relative to pure *S. bovis* males (electronic supplementary material, S2, tables S5 and S6).

Over all the transcripts identified in these DEG analyses, we found several transcripts related to domains of retrotransposons. This represented more than 60% of the DEGs in first-generation hybrid males (F1 and F1′) compared with pure *S. bovis* males (figure 1B). Similarly, more than 50% of DEGs were related to retrotransposable elements in males from F1 strains backcrossed with *S. bovis* (figure 1B). Despite fewer DEGs being observed between female parasites from the three strains differing in introgression levels (i.e. pure *S. bovis*, F1/F1′ and backcross), similar percentages of retrotransposable elements were observed among the overall DEGs (i.e. 45% in backcrossed females, 54% in F1′ females), although fewer in the F1 females (20%) (figure 1B). The complete annotations of all the differential expressed genes are provided in electronic supplementary material, S2, table S7.

(b) Transcriptional plasticity of *Schistosoma bovis* infecting two different definitive hosts

We explored the transcriptional plasticity associated with host change in the parasite *S. bovis* and the biological processes in the responsive genes that are involved. On average, 80 ± 7.4% of the reads mapped to the reference genome of *S. haematobium* (V3); the percentage of mapping per sample is provided in electronic supplementary material, S1, table S1.

The PCA showed that, based on their whole-gene expression patterns, parasites clearly clustered according to their definitive hosts (i.e. hamster versus sheep) along the first axis and according to their sex along the second axis; these two axes captured 77% (respectively, 52 and 25%) of the overall variability between samples' transcriptomes (figure 2).

In females, we identified 2488 DEGs between parasites infecting sheep and those infecting hamster hosts, representing 26% of the total genes. Of these, 1070 were over-expressed and 1418 were under-expressed in parasites from sheep. In males,

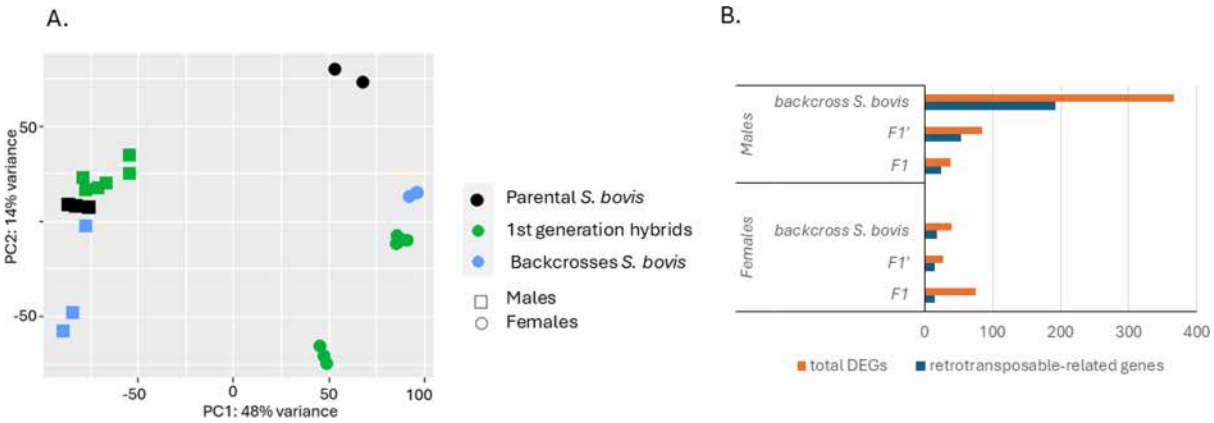

Figure 1. (A) Principal components analysis (PCA) plot showing the distribution of transcriptomes of schistosomes of the two sexes with different levels of genetic introgression. Each dot in the plot represents a sample, and the different colours represent the different levels of genetic introgression. The form indicates the sex, with squares representing males and circles representing females. (B) Number of genes differentially expressed related to retrotransposable elements across all comparisons with *Schistosoma bovis*. DEGs, differentially expressed genes.

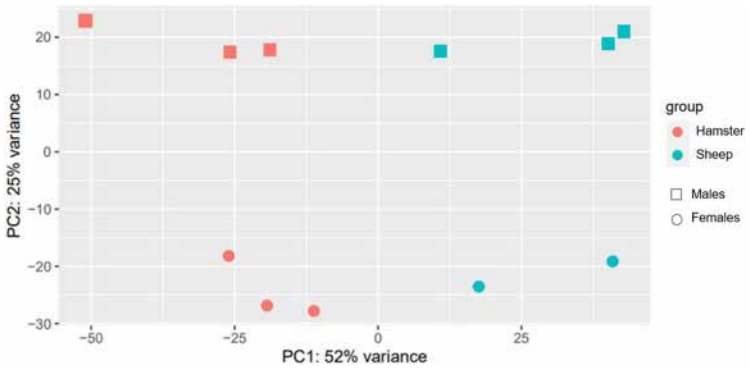

Figure 2. Principal components analysis (PCA) plot of male (squares) and female (circles) *Schistosoma bovis* worms collected from sheep and hamsters.

we found 2891 DEGs (30% of the total genes), with 1192 over-expressed and 1699 under-expressed in parasites from sheep compared with those from hamsters.

(c) Gene ontology enrichment analysis of introgressed worms and *Schistosoma bovis* from different hosts

(i) Backcross *Schistosoma bovis* versus parental *Schistosoma bovis*

To investigate the biological processes associated with gene expression differences during introgression, we performed GO enrichment analysis on the differentially expressed genes between males of *S. bovis* and introgressed backcross worms (♂ F1 × ♀ *S. bovis*). These analyses focused on comparisons between parental *S. bovis* and the *S. bovis* backcross exhibiting the highest number of worms in infected sheep reported in our previously published work [35], with significant processes identified based on an FDR-adjusted p-value <0.05.

In males from the backcross (F1 × *S. bovis*), over-expressed genes were significantly enriched for processes related to nuclear-transcribed mRNA catabolism, inner mitochondrial membrane organization, and response to endoplasmic reticulum stress. Conversely, under-expressed genes were enriched for processes including sensory perception, fucosylation, cilium/flagellum-dependent cell motility, neuropeptide signalling pathway, non-motile cilium assembly, microtubule-based movement, oligosaccharide biosynthesis and glucosylceramide metabolism (figure 3, first column).

In males from the backcross (F1 × *S. bovis*), over-expressed genes were significantly enriched in processes related to nuclear-transcribed mRNA catabolism, inner mitochondrial membrane organization and endoplasmic reticulum stress response. In contrast, under-expressed genes were associated with sensory perception, fucosylation, cilium- and flagellum-dependent cell motility, neuropeptide signalling, non-motile cilium assembly, microtubule-based movement, oligosaccharide biosynthesis, and glucosylceramide metabolism (figure 3, first column).

(ii) *Schistosoma bovis* infecting sheep versus *Schistosoma bovis* infecting hamster

Schistosoma bovis males infecting sheep, compared with those infecting hamsters, showed over-expressed genes related to processes such as action potential, sensory perception and microtubule-based movement. Under-expressed genes in this comparison were significantly associated with nuclear-transcribed mRNA catabolism, nonsense-mediated decay, response to endoplasmic reticulum stress, protein targeting, protein folding, protein deubiquitination, oxidative phosphorylation, and endoplasmic reticulum calcium ion homeostasis (figure 3, middle column).

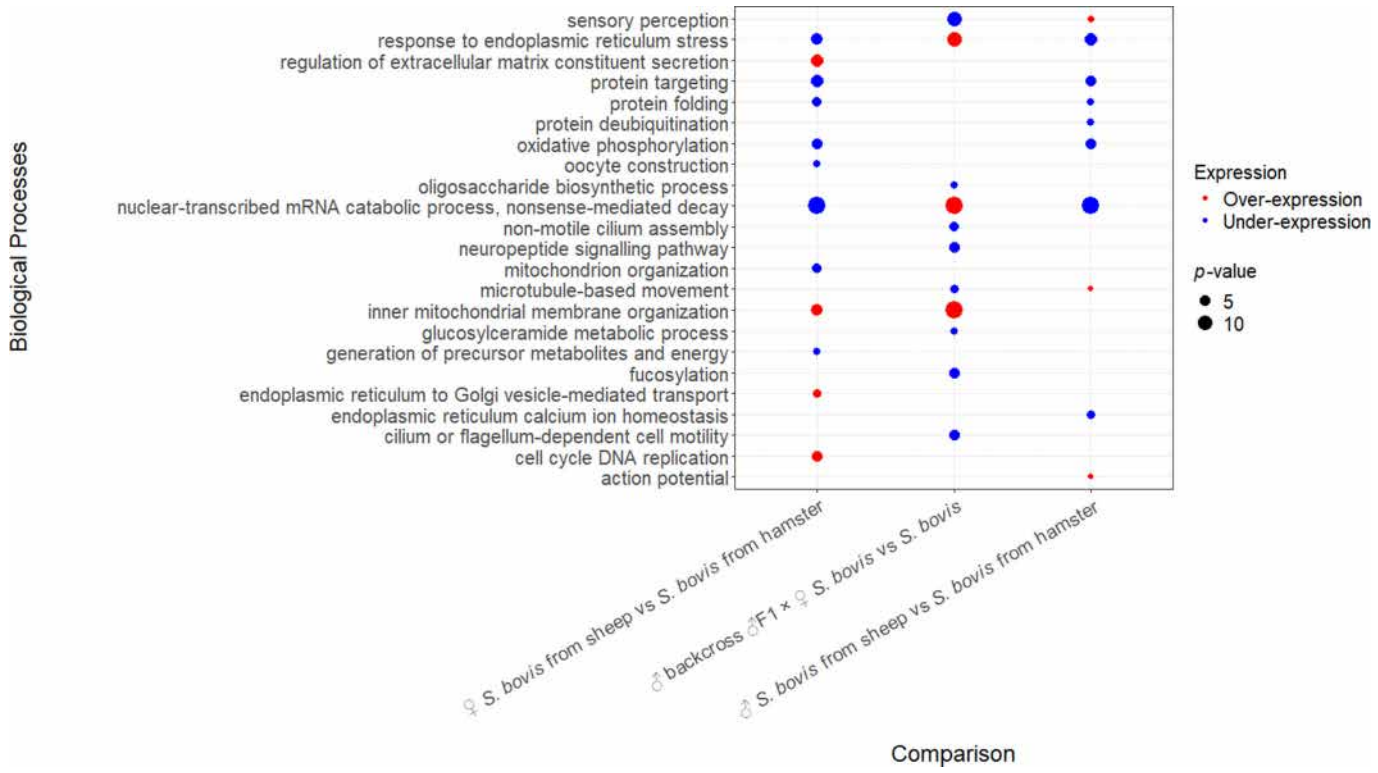

Figure 3. Dot plot illustrating the representative biological processes (*y*-axis) across experimental comparisons (*x*-axis). Dot size indicates the transformed significance level (*p*-value converted to percentages for improved visualization), with smaller percentages denoting greater significance. Dot colour represents differential gene expression levels. The *x*-axis reflects specific experimental comparisons, highlighting the biological processes shared across conditions.

Schistosoma bovis females infecting sheep, compared with those infecting hamsters, showed over-expressed genes related to regulation of extracellular matrix constituent secretion, cell cycle, DNA replication, inner mitochondrial membrane organization, and endoplasmic reticulum to Golgi vesicle-mediated transport. In contrast, under-expressed genes were enriched in nuclear-transcribed mRNA catabolism, nonsense-mediated decay, mitochondrion organization, protein targeting and folding, response to endoplasmic reticulum stress, oxidative phosphorylation, oocyte construction, and generation of precursor metabolites and energy (figure 3, third column).

4. Discussion

In this study, we investigated the differential gene expression in schistosome hybrids (*S. haematobium* × *S. bovis*) and backcrossed worms compared with parental *S. bovis*. We also examined the transcriptomic changes in *S. bovis* infecting sheep compared with those infecting hamsters. Our findings offer valuable insights into the transcriptomic plasticity that may facilitate the ability of *S. bovis* and *S. haematobium* × *S. bovis* hybrids to exploit diverse host environments. Collectively, these findings underscore the role of transcriptomic plasticity in shaping the evolutionary trajectories of schistosome hybrids. This plasticity allows the parasites to navigate physiological and immunological challenges, expand their host range, and increase their zoonotic potential.

(a) Differential expression in first-generation hybrids

In first-generation hybrids, fewer than 1% of the genes are differentially expressed compared with pure *S. bovis* parasites. These results are consistent with our previous results, indicating that the most prevalent profile of gene expression in hybrids is at intermediate level between the expression of each parental strain [18]. This highlights the high genomic compatibility between *S. bovis* and *S. haematobium*, which arises from their shared evolutionary history and limited divergence.

Differential gene expression analyses between first-generation hybrids and parental *S. bovis* revealed that DEGs were associated with retrotransposable elements. Retrotransposable elements were also found to be differentially expressed in hybrids of *S. bovis* and *S. haematobium* infecting hamsters, suggesting that these elements are mobilized independently of the host species [18]. While transposable elements are not annotated in *S. bovis* or *S. haematobium* genomes, we found sequences homologous to retrotransposable elements previously identified [7,51]. We found several transcripts coding for the retropepsin-like (K02A2.6-like) domain, which is only present in invertebrate retrotransposons and has been reported in *S. mansoni* as part of a polyprotein that also contains a reverse transcriptase, a ribonuclease H and an integrase [52], which were also found among the DEGs in most of the comparisons analysed, mostly between first-generation male hybrids and males of *S. bovis*. In addition, we found four protein domains of retrotransposons, Exo_endo_phos_2, RVT_1, Peptidas_A17 and rve. The percentage of DEGs linked to transposon activity was found to vary between 20% in the comparison of female *S. bovis* and F1 hybrids, but it went up

to 70% when comparing male *S. bovis* and F1 hybrids. Moreover, we also found that DEGs in males that were shared across the different sheep crosses were all related to retrotransposable elements.

Our results support the hypothesis that hybrid offspring experienced a genomic shock due to the combination of two genome species, which results in genomic rearrangements such as transposition and chromosomal reorganization [18,53–55]. These retrotransposons have the potential to influence the genetic identity and genome size. They can also impact regulatory networks and gene expression, and lead to genome rearrangements, ultimately providing an important source of phenotypic and genetic diversity available for ecological and evolutionary processes [56,57].

In conclusion, these results suggest that even rare hybridization events—such as those likely occurring between *S. bovis* and *S. haematobium* [24]—can lead to significant genomic innovations. These events can facilitate the introgression of specific genomic segments from one species into another and mobilize transposable elements within this new lineage.

(b) Differential expression found in introgression

We found 366 DEGs, representing 4% of the total coding genes (9431), when comparing the transcriptome of the hybrid strain backcrossed with *S. bovis* (males F1 × females *S. bovis*) that showed an increased number of worms infecting sheep relative to the transcriptome of parental *S. bovis*. This is a higher number of DEGs than found in first-generation hybrids compared with the *S. bovis* parental strain. Thus, even though backcrossed parasites display a genetic background that is getting closer to that of the parental *S. bovis* strain compared with F1 hybrids, their transcriptome continues to drift from that of the parental strain. Our previous research [35] reported an increased number of worms in sheep infected with introgressed individuals compared with sheep infected with the parental *S. bovis* and the F1 hybrids. Our work contributes to understanding the potential molecular basis of this phenomenon. Nevertheless, we emphasize the need for caution in directly attributing the higher infection levels to transcriptomic variations without additional experimental validation.

In the over-expressed genes in the backcrosses versus the parental strains, a prominent enrichment for processes related to nuclear-transcribed mRNA catabolism indicates a potential increase in RNA surveillance and turnover. This could reflect regulatory mechanisms for fine-tuning gene expression in response to genetic introgression [58].

The biological processes enriched among the genes under-expressed in males from backcross F1 × *S. bovis* compared with males of the *S. bovis* parental strain were sensory perception and neuropeptide signalling pathway. This suggests that schistosomes from the backcrossed strain might have altered sensory perception and neuronal signalling, potentially impacting their interactions with the host. Notably, processes associated with ciliary function and flagellum-dependent cell motility are also enriched. Cilia are essential organelles that play a critical role in sensing environmental cues and facilitating movement [59]. The under-expression of genes involved in these processes could have implications for the parasite's ability to navigate and interact with the host vascular environment.

Schistosome adult worms have a variety of sensory structures that allow them to respond to changes in levels of chemicals and nutrients in the host vascular system. These include various chemoreceptors and membrane channels. We found G protein-coupled receptors that have previously been identified in *S. haematobium* and *S. mansoni* [60] and in *S. bovis* and *S. haematobium* hybrids infecting hamster [35]. These receptors are responsible for detecting many extracellular signals and transducing them to the G proteins, which then communicate with various downstream effectors such as key molecules involved in developmental and neuromuscular functions [61]. Transcripts encoding neurotransmitter–sodium-symporter family proteins were also detected. These symporters regulate neurotransmitter uptake, potentially influencing parasite behaviour that facilitates the interaction between males and females originating from different species [18,62].

(c) Host-change-related transcriptional plasticity of the parasite *Schistosoma bovis*

The change of host can trigger a high remodelling of gene expression in parasites, and there are few works showing that parasite species have shown plasticity at the molecular level that allows them to adapt rapidly to a new host [37,63,64]. We found an intense remodelling of gene expression associated with host change, with 30% of the genes differentially expressed between male *S. bovis* from sheep and hamster and 26% in females. The sheep represents a completely different habitat for schistosome parasites compared with the hamster, particularly in terms of space, metabolic processes and blood flow dynamics. These differences can significantly influence the parasites' life cycle, development and interaction with the host's immune system.

Changes in the transcriptomes of males and females show similar patterns in terms of quantity, with 2488 DEGs in males compared with 2891 in females. Both groups exhibit comparable percentages of over-expressed and under-expressed genes. However, the underlying biological processes differ between the sexes.

In males, we observed that over-expressed genes in *S. bovis* infecting sheep, compared with those infecting hamsters, were significantly enriched in processes such as action potential, sensory perception, microtubule-based movement, mating, postsynapse organization and reflex. Notably, action potential, sensory perception, postsynapse organization and reflex are interconnected within the same pathway, where the nervous system transmits an action potential from sensory neurons in response to external stimuli, triggering a behavioural response. Previous studies have shown that neuronal processes are involved in male–female worm interactions [65]. These biological processes are also critical to colonize and navigate inside the mesenteric system of the definitive host [66].

Oxidative phosphorylation, protein targeting and protein folding were found to be enriched in under-expressed genes in both male and female *S. bovis* infecting sheep. These processes, which are associated with metabolism and energy acquisition,

have also been observed in other parasites using alternative host species [67]. This suggests that parasites may downregulate these functions to compensate for a less efficient use of energy from the host [37]. A reduction in the expression of genes associated with metabolic pathways could indicate that the parasite requires less energy to survive in a host that is relatively efficient in providing nutrients, or it may reflect a host-related constraint that limits energy utilization. However, paired females are generally retained by the males within their gynaecophoric canal, meaning they do not need substantial energy to sustain themselves in the blood vessels. Therefore, it is more likely that the observed downregulation in metabolic pathways is a consequence, with sheep providing a more stable and supportive environment for the development of *S. bovis* females and males.

(d) Global transcriptomic changes

The number of DEGs between *S. bovis* infecting sheep compared with those infecting hamsters was much higher (30% of the genes) than the number of DEGs we found in introgressed worms (4% of the genes). This suggests that host change induces a higher transcriptomic response in *S. bovis* than an interspecific hybridization event with *S. haematobium*. Host change often requires adaptations at multiple levels, such as coping with altered blood flow, nutrient availability and host immune responses. These challenges likely drive the extensive transcriptomic reprogramming observed in *S. bovis* when transitioning from a rodent (hamster) to a ruminant (sheep) host, as parasites must rapidly remodel their gene expression to adjust to new biochemical and structural environments. In contrast, the process of introgression, while significant, involves integrating genetic material from closely related species with shared evolutionary histories, such as *S. haematobium* and *S. bovis*. This genetic similarity may limit the need for extensive transcriptomic rewiring, as the hybrids inherit physiological adaptations already compatible to the host environment.

(e) Biological processes involved in introgression and host change

We found five common biological processes for which changes in gene expression were associated with the introgression process and the host change. These include the nuclear-transcribed mRNA catabolic process, inner mitochondrial membrane organization, microtubule-based movement, response to endoplasmic reticulum stress and sensory perception. These biological processes have a wide impact on gene function which can allow them to cope with environmental changes.

The common enriched biological processes suggest a set of adaptations of *S. bovis* and introgressed worms that enhance its survival and infectivity across different hosts, contributing to a higher zoonotic potential. Furthermore, the processes observed in hybrid and introgressed worms from sheep are highly similar to those seen in hybrids obtained from hamsters [18]. This suggests that there are 'universal' mechanisms that enable hybridization regardless of the host species. We hypothesized that the enrichment of nuclear-transcribed mRNA catabolic processes enables efficient and flexible gene expression and translation, which can help adapt to other hosts [68]. The energy required to cope with environmental stress is provided by the enrichment of the inner mitochondrial membrane organization process [69]. For motility and migration within the host, the enrichment of the movement of microtubules is indispensable [70]. The enrichment of the response to endoplasmic reticulum stress may help the parasite evade the host's immune system [71], while the enhancement of sensory perception could facilitate host recognition, avoid immune responses and reach optimal location for mating [72].

5. Conclusion

Together, our findings highlight how experimental infection in a natural host and transcriptomic analysis offer a powerful lens to explore the molecular underpinning of schistosome adaptation. By dissecting gene expression changes linked to hybridization, introgression and host change, we uncover core biological processes that enable parasites to adapt to new environments and that potentially contribute to their zoonotic potential.

Further investigation into the role of the biological processes highlighted in this work, and the associated molecular functions, will be essential to understand the full implications of schistosome worms' transcriptomic plasticity during introgressive hybridization and host change, and how it affects their zoonotic potential. We also acknowledge that further experimental studies to quantify parasite egg output and characterize host pathology would provide valuable and complementary insights to the molecular findings.

Understanding the factors contributing to the zoonotic potential of *S. bovis* and its hybrids with *S. haematobium* is crucial for developing more effective prevention and control strategies for urogenital schistosomiasis. Intervention efforts have focused on treating infected individuals and controlling snail populations, primarily targeting species that infect humans [73,74]. However, the presence of introgressed schistosomes, which can display altered transmission dynamics, drug resistance and host preferences, calls for a reevaluation of these strategies [9]. Transcriptomic plasticity in these hybrid parasites not only enhances their zoonotic potential but also complicates control measures by facilitating their survival in both human and potential animal reservoirs. Therefore, a deeper understanding of transcriptomic and other molecular changes in these hybrids is essential for addressing their zoonotic spread and for the development of more targeted intervention strategies [75].

Ethics. Sheep infections with *S. bovis*, *S. haematobium* and hybrid cercariae were conducted in the Veterinary School Ecole Nationale Vétérinaire d'Alfort in Maisons-Alfort, France. The animal facility possesses agreement E940462 delivered by the French Ministry of Higher Education,

Research and Innovation. The sheep experiment was registered by the French Ministry of Higher Education, Research and Innovation, and approved by an independent ethics committee (APAFIS no. 23786-2020012416159143 v2).

Data accessibility. Sequencing data from *Schistosoma bovis*, hybrids and introgressed worms infecting sheep are available at the NCBISRA under the BioProject accession number PRJNA1199762.

Supplementary material is available online [76].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. N.L.A.: formal analysis, investigation, methodology, writing—original draft; E.M.-B.: formal analysis, investigation, methodology, writing—original draft; J.K.-S.: investigation, methodology, writing—review and editing; O.R.: investigation, methodology, writing—review and editing; M.P.: formal analysis, writing—review and editing; C.C.: data curation, formal analysis, methodology; J.-F.A.: methodology; A.R.: methodology; B.P.: methodology; I.V.: methodology; M.T.: methodology; J.B.: conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, writing—review and editing; E.T.: conceptualization, formal analysis, investigation, methodology, project administration, resources, supervision, validation, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed herein.

Conflict of interest declaration. We declare we have no competing interests.

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