Tissue Specific Profiling of Females of *Schistosoma japonicum* by Integrated Laser Microdissection Microscopy and Microarray Analysis

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**Abstract**

**Background:** The functions of many schistosome gene products remain to be characterized. A major step towards elucidating function of these genes would be in defining their sites of expression. This goal is rendered difficult to achieve by the generally small size of the parasites and the lack of a body cavity, which precludes analysis of transcriptional profiles of the tissues in isolation.

**Methodology/Principal Findings:** Here, we describe a combined laser microdissection microscopy (LMM) and microarray analysis approach to expedite tissue specific profiling and gene atlasing for tissues of adult female *Schistosoma japonicum*. This approach helps to solve the gene characterization “bottle-neck” brought about by acoelomy and the size of these parasites. Complementary RNA obtained after isolation from gastrodermis (parasite gut mucosa), vitelline glands and ovary by LMM were subjected to microarray analyses, resulting in identification of 147 genes upregulated in the gastrodermis, 4,149 genes in the ovary and 2,553 in the vitellaria.

**Conclusions:** This work will help to shed light on the molecular pathobiology of this debilitating human parasite and aid in the discovery of new targets for the development of anti-schistosome vaccines and drugs.

**Introduction**

Members of the genus *Schistosoma* are parasitic blood flukes responsible for the serious but neglected human disease of schistosomiasis [1,2]. In common with other platyhelminths, schistosomes exhibit acoelomy, the body plan characteristic of basal bilaterians whereby tissues are bound together by cells and matrices of the parenchyma in the absence of a body cavity. This body organization, together with the generally small size of adults and developing stages, has been a major hindrance for functional analyses of individual schistosome tissues and cells, because it has been impossible to isolate them. These problems are exacerbated by poor knowledge and limited annotations of many schistosome genes and the absence of basic knowledge of where, and when, in development the molecules are expressed. Localization methods incorporating immunocytochemistry and *in situ* hybridization have been at the vanguard of functional studies of schistosome proteins [3], but the prospect of obtaining robust, informative localization data of multiple genes expressed throughout the complex schistosome life cycle remains a daunting challenge.

Concerted international efforts have been directed at defining functional relevance of the predicted 14–16,000 schistosome genes to identify potential targets for drug and vaccine therapies [4,5]. Release of extensive schistosome ESTs (Expressed Sequence Tags) datasets and the anticipated publication of complete genomes for *Schistosoma mansoni* and *S. japonicum* [5,6,7] have provided new stimulus to achieving these goals. These datasets have enabled development of platforms for transcriptome and proteome analyses to explore gender, developmental and strain differences in schistosomes [8,9,10,11,12,13,14,15,16,17,18].

Here, we report on tissue-specific gene expression analysis of adult female *Schistosoma japonicum*, as a means to expedite functional characterization of schistosome gene products. Our approach incorporates methods of laser microdissection microscopy (LMM) to generate tissue-specific transcriptional extracts for subsequent microarray analysis. This work follows hypotheses [19,20] that LMM would prove an excellent means to expedite transcriptional typing of many schistosome tissues despite the acoelomate body plan of these parasites.

**Materials and Methods**

**Parasite isolation and sample preparation**

The use of mice in this study was approved under Project P288 by the Animal Ethics Committee of the Queensland Institute of Medical Research. *Schistosoma japonicum*-infected *Oncomelania hupen-
Author Summary

Schistosomes are parasitic worms responsible for important human diseases in tropical and developing nations. There is urgent need to develop new drugs and vaccines to augment current treatments for this disease. In recent years, concerted efforts by many laboratories have led to extensive genetic sequencing of the parasites, and the publication of genome sequence for two agents of schistosomiasis appears imminent. This genetic information has revealed many molecules expressed by the schistosome parasites for which no functional information is available. This lack of information extends to ignorance of where in the complex multicellular schistosome parasites the genes are expressed. We integrated two molecular and cellular techniques to address these knowledge gaps. We used laser microdissection microscopy to dissect small but highly important tissues involved in nutrition and reproduction from sections of female Schistosoma japonicum. From these dissected tissues we then used a broad molecular biology method to identify the multiple genes active in these tissues. Our approach has allowed us to formulate the basis of a “gene atlas” for schistosome parasites, defining the expression repertoire of specific tissues. The better understanding of the roles of tissues in parasite biology, especially in development, reproduction and interactions with its human hosts, should promote future investigations into pathogenesis and control of these significant parasites.

sis hupensis snails, collected from Anhui Province, China, were provided by the National Institute of Parasitic Diseases-CDC, Shanghai. Adult worm pairs were perfused 6 weeks post-challenge from infected ARC Swiss mice. Two batches of approximately 25 female worms, and the ovary and vitelline tissues from the stained females that were scraped by sterile scalpel blades from the slides into RNA extraction buffer (below) for analysis. The control samples therefore represent the transcriptional repertoire of entire females.

Total RNA isolation and hybridisation

Total RNA was isolated from the control and LMM samples using RNAsine-Micro kits (Ambion) kit using the manufacturer’s instructions and quantified using a Nano-Drop ND-1000 spectrophotometer (Thermo Scientific, USA). The quality of total RNA was assessed using a Bioanalyzer RNA Pico Lab Chip (Agilent) prior to storage at −80°C.

Microarray hybridisation and feature extraction

Full details of the design and construction of the schistosome microarray used have been reported [11]. In brief, the array was constructed from information based on the transcriptomes of adult S. japonicum and S. mansoni. The microarray consists of 19,222 target contiguous sequences (contigs) printed twice from two independent probe designs, and includes 12,166 probes derived from S. mansoni, and 7,056 probes derived from S. japonicum. An overview of the design and composition of the microarray is present in Table S1.

A 300 ng aliquot of total RNA from each sample was converted into complementary RNA was synthesized and labeled with the fluorophore Cyanine 3-CTP (CY3c) and hybridized according to the manufacturer’s instructions (Agilent Technologies -One-Color Microarray-Based Gene Expression Analysis). Microarray hybridisations were performed in duplicate for all samples. The yield, concentration, amplification efficiency and abundance of CY3c were measured at A260 and A550 by spectrophotometry.

Data analysis

Hybridized slides were scanned using an Agilent Microarray Scanner (B version) as tiff files and processed with the Feature Extraction 9.5.3.1 Image Analysis program (Agilent) to produce standardised data for statistical analysis. All microarray slides were assessed for background evenness by viewing the tiff image by Feature Extraction. Feature extracted data was analysed using GENESPRING (version 7.3.1; Agilent Technologies/Silicon Genetics, Redwood City, CA).

Microarray data were normalised using a normalisation scenario for “Agilent FE one-color” which including “Data Transformation: Set measurements less than 5.0 to 5.0”, “Per Chip: Normalize to 50th percentile” and “Per Gene: Normalize to median”. Data sets were further analysed using published procedures based on one-colour experiments [21]. The gProcessedSignal values determined in GENESPRING using Agilent’s Feature Extraction software including aspects of signal/noise ratio, spot morphology and homogeneity. Thus, gProcessedSignal represents signal after localised background subtraction and includes corrections for surface trends. Features were deemed Absent when the processed signal intensity was less than two fold the value of the processed signal error value. Features were deemed Marginal when the measured intensity was at a saturated value or if there was a substantial amount of variation in the signal intensity within the pixels of a particular feature. Features that were not Absent or Marginal were deemed Present. Data points were included only if Present or Present, Absent and probes or contigs retained if all data points were Present or Present, Absent.

Microarray data have been submitted to the Gene Expression Omnibus public database, under accession numbers GPL7160 and GSE12706.
Figure 1. Laser microdissection of *S. japonicum* tissues. (A) Longitudinal section through female, showing morphology of gastrodermis (G), ovary (O), and vitelline tissue (V) from toluidine blue-stained cryostat sections. Bar = 100 μm. (B) Gastrodermis. Bar = 100 μm (C) Vitellarium and (D) Ovary; before (left panels) and after (centre) LMM. For each tissue, the region of microdissected tissue is indicated with an arrow. The panel on the right shows quality of total RNA from the three microdissected tissues determined using a Bioanalyzer. The prominent 18S ribosomal RNA band is indicated by the arrowhead and is an indication of the fidelity of the total RNA. A distinct 28S band is never visible in total RNA fractions of schistosomes.

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Gene ontology analysis

Batch BlastX (6 frame translation protein homology) was performed at http://www.blast2go.de on all contigs. This presented a further overview of the gene ontologies that are modulated between tissue types in adult female S. japonicum (Figure S1 and Table S2). This information was used to supplement previously published GOs based on nucleotide sequence [11]. To gain a more complete overview of the GO categories that are modulated during the S. japonicum lifecycle we used the software ErmineJ to produce extended list of GOs associated with each of the microdissected tissue types [22].

Real time PCR validation of microarray data

A total of 9 gene sequences indentified as differentially expressed among the three S. japonicum tissues and whole worm control tissue were chosen for validation of microarray data using real time PCR as described [12]. The template for real time PCR was that obtained by microdissection. Forward and reverse primers (Sigma-Aldrich, Australia) were designed from the 10 contigs (Table S3). All total RNA samples were DNase treated (Promega, Australia) prior to synthesis of cDNA using a Quantitect Whole Transcriptome Kit (QIAGEN, Australia). All cDNA samples were diluted to a concentration of 5 ng/µl. Real time PCR was performed in a Gene Disc 100 ring (Corbett Research, Australia). A sequence from the NADH-ubiquinone reductase gene of S. japonicum was used for normalisation of data from all experiments. Each experiment was performed in duplicate, and the confidence threshold (CT) of the second set was normalised to the first set before evaluation. This was done by importing the standard curve of the first set to that of the second using Rotor Gene 6 software [12]. Microarray and real time PCR datasets were tested following Morley and colleagues [23]. Data were analysed using Graphpad Prism Version 5. Data from microarray and real time PCR populations were examined to ascertain if they fit normal distributions, using the D’Agostino and Pearson omnibus normality test and the Shapiro-Wilk normality test. Because both sets of data were not normally distributed, a Spearman correlation (Rho) was employed to test for correlation. The statistical analyses used an alpha value of 0.05.

Results/Discussion

We targeted three female tissues, namely, gastrodermis (absorptive gut lining) of the posterior halves of the worms, ovary, and the vitelline glands (= vitellaria, accessory glands of the female system that produce precursors for eggshell synthesis) (Figures 1 and 2). We chose these three tissues due to their relative abundance, clearly delimited structure and the important biological roles in schistosome development and reproductive biology.

In view of the closely knit organization of schistosome tissues, it was important to know whether the three tissues under investigation represented homogenous cell populations. Ultrastructural assessment indicated that the ovary and gastrodermis were homogenous (Figure 3). We had previously shown through ultrastructural studies incorporating a stereological analysis of the relative volumes of tissues in vitellogenic regions that although some parenchymal tissues intrude into the vitelline regions,

Figure 2. Microarray analysis of microdissected tissues of female S. japonicum. (A) A two-dimensional model of the arrangement of major organ systems of female schistosomes, showing relative location of the three tissues dissected here. (B) Venn diagram outlining the number of probes that were detected at 2 fold or greater in each tissue type and the degree of overlap in expression of those genes between and among tissues. (C–E) Lists of example genes enriched for each tissue. These lists are selected from full lists (Table S4).

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Figure 3. Ultrastructural morphology of microdissected tissues of female *S. japonicum*. (A) Ovary. Numerous oocytes with a high nuclear-cytoplasmic ratio are present as the sole cell type. (B). A thin cellular layer, incorporating myofibrils, is present as the limiting margin of the ovary. Arrow indicates margin of ovary. (C) Gastrodermis. The gastrodermis is a unilaminate syncytial layer forming the absorptive lining of the gut. (D). Vitelline cells. These accessory cells of the female reproductive system secrete egg-shell precursors and possibly yolk. Electron opaque lipid droplets are prominent features of this region. (E). Luminal surface of gastrodermis showing multiple stacked lamellae. Caption abbreviations; Gas = Gastrodermis epithelium, Lam = Lamellae, Lum = Lumen, My = Smooth muscle fibres, Oo = Oocyte, Vit = Vitelline cell.
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Vitellogenic regions are dominated by vitelline cells (vitellocytes) [24] which are highly synthetic cells. Thus, all tissue extracts represent homogenous or near homogenous samples.

For microarray analysis, unfixed frozen females were sectioned by cryostat onto membrane-coated slides, stained with toluidine blue and microdissected using a PALM laser catapult microscope (Figure 1). Total RNA integrity from microdissected samples was assessed (Figure 1) and shown to be of high fidelity. A distinct 28S band is never visible in total RNA fractions of schistosomes [25]. RNA was further processed for one-colour fluorophore-labelled cRNA synthesis and hybridization to a microarray representing the near complete transcriptome of adult schistosomes [11]. Of 38,444 probes (representing 19,222 contigs) on the chip, 8,454 (5,242 contigs) were retained after filtering (Table S4).

Principal component analysis (PCA) is a multi-dimension reduction method that allows the visual presentation of a complex data set, so that distances between plotted points represents the relative similarity of each datasets. Usually plotted in an X,Y,Z formation, each axis represents a distinct subset of data points, or in the current application, gene lists. Gene expression profiles of the three microdissected tissues and the control sample were analysed by PCA (Figure S2). The point of the control tissue was more similar to those of the gastrodermis and vitellaria, compared with the ovary. This observation is not surprising, for the former tissues are voluminous in female parasites and likely account for much of the female transcriptome.

Table 1. Examples of differentially expressed genes of the gastrodermis of S. japonicum normalised to signal intensity of the vitelline and ovary tissues.

| Systematic Name | Probe | Annotation Microarray | Protein Homology | Fold Change |
|-----------------|-------|-----------------------|------------------|-------------|
| Contig5007      | _1    | SJCHGC04509           | PV-fam-domain, with meprin domain | 230.9       |
| Contig517       | _899  | SJCHGC00284           | epididymal secretory protein e1    | 92.8        |
| Contig7606      | _687  | SJCHGC02336           | Cathepsin A       | 81.7        |
| Contig7602      | _446  | SJCHGC09134           | Lysosome-associated membrane glycoprotein (Lamp)/CD68 | 46.8        |
| Contig4694      | _436  | Schistosoma japonicum mRNA for calpain | Calpain | 33.2 |
| Contig1623      | _1100 | SJCHGC05100           | ABC transporter    | 31.3        |
| Contig5864      | _1109 | SJCHGC08182           | heme maturease [Tetrahymena pigmentosa] | 17.4        |
| Contig648       | _532  | SJCHGC01821           | Heme maturease     | 13.2        |
| Contig8540      | _456  | SJCHGC02844           | CD9 antigen/tetraspanin | 10.7        |
| Contig2584      | _847  | Caenorhabditis elegans cosmid C23H4 | Domain: Cystinosin/ERS1p repeat | 10.6        |
| Contig6609      | _1232 | SJCHGC09591           | Prostatic acid phosphatase/histidine acid phosphatase | 6.2        |
| Contig6992      | _996  | SJCHGC05833           | Phosphatidic acid phosphatase/chloroperoxidase, N-terminal | 5.8        |
| Contig3173      | _531  | SJCHGC09122           | Epidermal growth factor receptor | 4.4        |
| Contig4589      | _431  | SJCHGC04027           | Dynein heavy chain domain 3 | 4.3         |
| Contig5962      | _876  | Schistosoma japonicum mRNA for cathepsin B | Cathepsin B | 3.6        |
| Contig2623      | _2582 | Schistosoma mansoni mRNA for myosin heavy chain (MYH) | Myosin heavy chain | 3.4         |
| Contig7700      | _1006 | Schistosoma japonicum clone ZZD1392 | Tegumental protein 31.8 kDa [Clonorchis sinensis]Dynein light chain, type 1 and 2 | 3.2         |
| Contig5394      | _765  | SJCHGC02330           | novel transmembrane amino acid transporter protein | 2.9         |
| Contig8017      | _602  | SJCHGC06760           | Annexin a7        | 2.8         |
| Contig6015      | _909  | SJCHGC01645           | Alkaline-phosphatase-like | 2.5        |
| Contig6810      | _904  | SJCHGC05604           | Zinc/iron permease | 2.5        |
| Contig1093      | _646  | SJCHGC06386           | Permease for cytosine purines uracil thiamine allantoin | 2.0        |

A full list of genes, including systematic name and probe identification, expressed by the gastrodermis is shown in Table S4. Fold change refers to expression relative to ovary and vitellaria.

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| Systematic Name | Probe | Annotation Microarray | Protein Homology | Fold Change |
|-----------------|-------|-----------------------|------------------|-------------|
| Contig5637      | _571  | SJCHGC03728           | Unknown          | 336.6       |
| Contig6302      | _526  | SJCHGC04563           | Clathrin coat assembly protein ap19 | 135.2       |
| Contig3450      | _554  | Lymnaea stagnalis synaptotagmin I mRNA | synaptotagmin I | 63.5       |
| Contig1394      | _190  | SJCHGC06831           | Innexin          | 60.1       |
| Contig8876      | _2764 | SJCHGC06324           | Major egg antigen | 38.1       |
| Contig1301      | _18   | S’ end of clone FK0AAA23AE07 (strain 6–9), Anopheles gambiae | Geminin isoform ca_a | 37.5       |
| TC11333         | _1147 | Weakly similar to DNA mismatch repair protein MSH2 - African clawed frog | Mismatch repair protein | 32.6       |
| Contig6946      | _1090 | SJCHGC05810           | Serine threonine kinase cdc2 | 22.9       |
| TC8161          | _669  | Similar to DNA polymerase epsilon catalytic subunit A | DNA polymerase epsilon catalytic subunit | 16.8       |
| TC18876         | _4354 | SJCHGC08812           | DNA polymerase epsilon small subunit | 16.1       |
| Contig8250      | _2443 | Schistosoma japonicum preprocathepsin L mRNA, complete cds | Preprocathepsin L | 13.1       |
| Contig8644      | _1259 | SJCHGC01849           | DNA-damage repair protein drt111 precursor | 12.4       |
| TC17330         | _737  | SJCHGC05965           | Proliferating cell nuclear antigen | 11.7       |
| Contig2662      | _759  | SJCHGC05418           | Sentrin sumo-specific protease | 10.2       |
| Contig8918      | _1226 | Schistosoma japonicum clone ZZZ431 mRNA sequence | Egg protein cp422 | 10.1       |
| Contig6677      | _676  | SJCHGC04972           | Polycomb homologue | 9.1       |
| Contig3532      | _935  | SJCHGC02371           | Peter pan homolog | 8.8       |
| TC10915         | _564  | Similar to similar to GenBank Accession Number U00997 synaptobrevin in Aplysia californica, partial | Synaptobrevin | 8.7       |
| Contig7167      | _399  | Limulus polyphemus syntaxin 1C mRNA, complete cds/Hypothetical protein T26C11.2 (Caenorhabditis elegans) | Syntaxin 1a cg31136-pa | 8.0       |
| Contig6123      | _699  | Schistosoma mansoni Smad4 (Smad4) mRNA, complete cds | Smad4 | 7.2       |
| Contig6819      | _526  | Aquifex aeolicus VF5 section 109 of 109 of the complete genome | DNA repair endonuclease | 6.7       |
| Contig5796      | _486  | SJCHGC04715           | Major facilitator superfamily domain containing 8 | 6.6       |
| TC7109          | _2083 | SJCHGC02245           | enhancer of polycomb homolog 1 | 6.2       |
| Contig3374      | _1436 | Schistosoma mansoni Smad1 (Smad1) | Smad1 | 6.1       |
| TC13948         | _859  | Receptor tyrosine kinase (Schistosoma mansoni), partial (92%) | Tyrosine protein kinase | 6.1       |
| Contig5026      | _422  | SJCHGC06696           | DNA primase | 4.0       |
| TC9260          | _582  | Hypothetical protein (Plasmodium falciparum 3D7): PF14_0537 | DNA repair helicase | 2.3       |

A full list of genes, including systematic name and probe identification, expressed by the ovary is shown in Table S4. Fold change refers to expression relative to gastrodermis.

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cascade, membrane-associated molecular transporters, actin and associated molecular motors. A highly enriched gene of the gastrodermis, represented by Contig5007, is a hitherto uncharacterized gene with uncertain sequence identity, but which contains motifs with similarity to the meprin family of metalloproteinases, and an erythrocyte-binding protein of malaria parasites. This molecule potentially represents a novel class of proteases involved in haemoglobinolysis in these vascular parasites [26]. Surprisingly, cathepsin D, an early member of the proteinases involved in haemoglobinolysis cascade [27], was not enriched for the gastrodermis. Given its upstream role in this multi-enzyme cascade, membrane-associated molecular transporters, actin and associated molecular motors. A highly enriched gene of the gastrodermis, represented by Contig5007, is a hitherto uncharacterized gene with uncertain sequence identity, but which contains motifs with similarity to the meprin family of metalloproteinases, and an erythrocyte-binding protein of malaria parasites. This molecule potentially represents a novel class of proteases involved in haemoglobinolysis in these vascular parasites [26]. Surprisingly, cathepsin D, an early member of the proteinases involved in haemoglobinolysis cascade [27], was not enriched for the gastrodermis. Given its upstream role in this multi-enzyme cascade, membrane-associated molecular transporters, actin and associated molecular motors. A highly enriched gene of the gastrodermis, represented by Contig5007, is a hitherto uncharacterized gene with uncertain sequence identity, but which contains motifs with similarity to the meprin family of metalloproteinases, and an erythrocyte-binding protein of malaria parasites. This molecule potentially represents a novel class of proteases involved in haemoglobinolysis in these vascular parasites [26]. Surprisingly, cathepsin D, an early member of the proteinases involved in haemoglobinolysis cascade [27], was not enriched for the gastrodermis. Given its upstream role in this multi-enzyme cascade, membrane-associated molecular transporters, actin and associated molecular motors.

Table 3. Examples of differentially expressed genes of the vitelline glands, normalised to signal intensity of the gastrodermis.

| Systematic Name | Probe | Annotation Microarray | Protein Homology | Fold Change |
|-----------------|-------|-----------------------|------------------|-------------|
| Contig7083 _1167 | SICHGC03760 | Schistosoma japonicum clone ZZD46 mRNA sequence | protein serine threonine kinase | 8.8 |
| Contig5547 _760 | SICHGC01089 | Contig3752 _1130 SJCHGC03615 Tyrosine kinase 5 | 2.8 |
| Contig8039 _772 | SICHGC01089 | Contig4028 _618 SJCHC06704 Innexin | 3.0 |
| Contig6705 _319 | SICHGC04289 | Contig6208 _611 SJCHGC06498 Solute carrier family member 4 | 3.5 |
| Contig5142 _489 | Contig7264 _1186 TGF-beta Transforming growth factor-beta 3.3 | Contig6381 _463 Schistosoma japonicum clone ZZD128 mRNA sequence | 3.5 |
| Contig3412 _1137 | Contig8365 _797 Schistosoma japonicum clone ZZZ329 mRNA sequence | Schistosome venom allergen-like protein | 3.5 |
| Contig8876 _2764 | Contig7264 _1186 TGF-beta Transforming growth factor-beta 3.3 | Schistosome venom allergen-like protein | 3.5 |
| Contig4381 _463 | Contig6709 _725 SICHGC06813 Protein-4.1 G protein | 3.5 |
| Contig5541 _663 | Contig8457 _1851 SJCHGC01511 Selenoprotein w- eggshell precursor or fs800 | 4.0 |
| Contig6208 _611 | Contig6208 _611 SJCHGC06498 Solute carrier family member 4 | 4.0 |
| Contig7264 _1186 | Contig7264 _1186 TGF-beta Transforming growth factor-beta | 4.0 |
| Contig4028 _618 | Contig4028 _618 SJCHC06704 Innexin | 4.0 |
| Contig3752 _1130 | Contig3752 _1130 SJCHGC03615 Tyrosine kinase 5 | 4.0 |
| Contig5142 _489 | Contig5142 _489 SJCHGC04289 Solute carrier family member 7 (cationic amino acid system) | 4.0 |
| TC13948 _859 receptor tyrosine kinase (Schistosoma mansoni), partial | 4.0 |
| Contig1512 _665 | Contig1512 _665 Schistosoma mansoni immunophillin FKS06 binding protein FKB12 mRNA, complete cds | 4.0 |

A full list of genes, including systematic name and probe identification, expressed by the vitelline glands is shown in Table S4.

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Table S4) for vitellaria compared with the gastrodermis. Oocytes and vitellocytes, in platyhelminth evolution and ontogeny, are believed to be derived from common progenitor cells [31]. We decided, therefore, to determine whether the tissues have common expression identity that may reflect the common origin of the two tissues. Analysis of expression by Venn diagram indicated substantial overlap in expression between the two germinal tissues, but not with the gastrodermis (Figure 2B). Genes enriched for both cell types included egg-specific genes including major egg antigens and egg protein cp422. The former gene is also abundant in mature eggs of schistosomes [32]. Other genes enriched for the female reproductive tract included those encoding molecules for TGF-β and tyrosine kinase signalling pathways [33,34], different innexins (gap junction proteins of invertebrates), and a diversity of genes encoding molecules associated with DNA processing, replication, and transcription. With the exception of the egg-specific antigens, the upregulated genes common to the two tissues are involved in cell proliferation and intercellular signalling.

Genes enriched for the ovary (relative to the gastrodermis) included a number encoding proteins associated with cytokinesis, fertilization and coated pit-mediated endocytosis (Table 2). Oocytes express genes with identity to polycomb, enhancers of polycomb, and Peter pan homologues of vertebrates and ecdysozoans [35] (Table 2). Polycomb genes, not previously recognized for platyhelminths, repress Hox expression in embryogenesis leading to cellular and zonal differentiation in embryos. Discovery of genes involved in embryonic differentiation will provide new insight into developmental cascades in the complex multi-generational schistosome life-cycle, leading in turn to a better understanding of differentiation of the intraovular embryo, the stage primarily responsible for pathogenesis in schistosomiasis.

Expression analysis of vitellaria (Table 3, Figure 2, and Table S4) revealed enriched genes (relative to the gastrodermis) associated with egg-shell synthesis, as well as a range of membrane transporters with affinity for amino acids, metallo-ions and nucleotides. Eggshell precursors, egg-specific proteins and tyrosinases were upregulated as expected for this tissue that provides precursors for choriogenesis [30,36]. Numerous membrane-spanning transporters and genes encoding proteins for exocytosis were also enriched as were those associated with lipid metabolism. Some transcripts, annotated as containing signal peptides, did not contain abundant tyrosine residues, a prerequisite for eggshell precursors [37], possibly indicating that these molecules function in aspects other than shell formation. Given the essential role of vitellocytes in egg development and embryogenesis, functional characterization of these putative secreted proteins may enhance our understanding of the complexity of egg-shell synthesis and may help resolve long-standing questions about yolk function of vitellocytes [30].

The integration of microarray analysis of LMM-dissected tissues has provided the means to establish a gene expression atlasing strategy for S. japonicum, alleviating the technology hurdles imposed by the acoelomate nature of this platyhelminth and expediting localization of multiple genes. Tissue-specific expression profiling has been performed previously for cavitates invertebrates, incorporating LMM or gross dissection methods [38,39], but this approach demonstrates the feasibility for gene mapping in a platyhelminth, thus serving as an exemplar for similar studies of other basal bilaterians and small organisms. The localization data provided here serves as a novel resource to advance functional studies of many unannotated S. japonicum genes, thereby providing a valuable molecular platform to shed light on the complex
physiology and biochemistry of schistosomes, the pathogenesis of schistosomiasis, and to develop new treatments and effective interventions for its control.

Supporting Information

Figure S1 Major Gene Ontologies of genes represented at greater than or equal to a 2 fold in (a) gastrodermis (b) ovary and (c) vitelline tissues. The number of probes in each gene ontology is noted. Found at: doi:10.1371/journal.pntd.0000469.s001 (9.80 MB TIF)

Figure S2 Principal Component Analysis of the 7,623 flag filtered genes showing the gene expression profile of control, gastrodermis (gut), ovary and vitellaria of female S. japonicum.

Table S1 Complete lists of contiguous sequences listed in the custom designed schistosome microarray manufactured by Agilent Technologies used in this study. Column A (ProbeID): Unique identifier of probe on the microarray. Column B (Sequence): Nucleotide sequence of the 60mer probe. Column C (EST Sequence): Complete nucleotide sequence of the assembled EST contig. Column D (TargetID): Contig designation for either S. japonicum (Contig) or S. mansoni (TC). Column E (Accessions): Genbank accession number corresponding to the EST sequences. Column F (Description-Nucleotide): BLASTn annotation result based on nucleotide sequence. Column G (GeneSymbols): Designation of primary or secondary probe design to the corresponding contig. Column H (Protein Homology): BLASTX annotation result based on protein sequence. Column G (Gene Ontology): Gene Ontology number and description.

Table S2 Gene Ontology categories from 2 fold or higher differentially expressed gene detected in the three microdissected tissues. Found at: doi:10.1371/journal.pntd.0000469.s002 (0.08 MB TIF)

Table S3 Primer Sets for real time PCR validation of a subset of genes that were upregulated in the three microdissected tissues examined. Found at: doi:10.1371/journal.pntd.0000469.s003 (29.52 MB XLS)

Table S4 Complete list of differentially expressed genes, shown on separate sheets of an Microsoft Excel File. ALL DATA Sheet: Relative fold change of all contigs normalised to control. Fold change for gastrodermis is normalised to the signals from an average of ovary and vitelline and individually against the two germinal tissues. The fold change for vitellaria or ovary are normalised to the signal from gastrodermis. Legend: Systematic: Probe identifiers. A full list of probes is shown in Table S1. Protein Identifier: BLASTn annotation result based on nucleotide sequence. Normalised data and Flags are shown in separate columns for each tissue type. Fold changes relative to other tissues for the gastrodermis, ovary and vitellaria are shown in successive columns. Synonym: probe identity. Microarray Description: BLASTn annotation result based on nucleotide sequence. Abbreviations: P- Present; A-Absent; g-gastrodermis, o-ovary; v-vitellaria.

Table S5 Complete list of differentially expressed genes, shown on separate sheets of an Microsoft Excel File. ALL DATA Sheet: Relative fold change of all contigs normalised to control. Fold change for gastrodermis is normalised to the signals from an average of ovary and vitelline and individually against the two germinal tissues. The fold change for vitellaria or ovary are normalised to the signal from gastrodermis. Legend: Systematic: Probe identifiers. A full list of probes is shown in Table S1. Protein Identifier: BLASTn annotation result based on nucleotide sequence. Normalised data and Flags are shown in separate columns for each tissue type. Fold changes relative to other tissues for the gastrodermis, ovary and vitellaria are shown in successive columns. Synonym: probe identity. Microarray Description: BLASTn annotation result based on nucleotide sequence. Fold change of transcripts in tissues featured in sheet relative to other tissues. Synonym: probe identity. Microarray Description: BLASTn annotation result based on nucleotide sequence. Fold change of transcripts in tissues featured in sheet relative to other tissues. Synonym: probe identity. Microarray Description: BLASTn annotation result based on nucleotide sequence.

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Author Contributions

Conceived and designed the experiments: GNG MKJ. Performed the experiments: SN LM. Analyzed the data: GNG SN LM JM MKJ. Contributed reagents/materials/analysis tools: GNG MKJ. Wrote the paper: GNG DPM SN MKJ.

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