Chapter 5: Oligonucleotide Microarray Analysis of Gender-Associated Gene Expression in the Human Blood Fluke, *Schistosoma japonicum.*

5.1 Introduction

Parasite sequencing projects have proven to be a valuable resource for gene discovery but, unfortunately, most of the information resulting from such projects lack a description of gene function, that may only be assigned after the investigation of individual genes [111]. In order to understand this, several groups have used microarray technology to elucidate the roles of specific gene products in parasite biology [15, 111]. Schistosomes, unlike most other platyhelminths are unusual because they are dioecious instead of being hermaphroditic [156]. Their sex is genetically determined in the zygote at fertilisation and is propagated through the sporocyst stage in the molluscan intermediate host [156]. The sexually dimorphic state is an important feature of the biology of schistosomes, and here I describe the use of a custom made 60mer schistosome microarray (see Chapter 3) to profile the gender-associated differential gene expression between male and female adults of Chinese (SJC) and Philippine (SJP) strains of *S. japonicum* [4].

5.2 Materials and Methods

All Materials and Methods excluding the GEO submitted Materials and Methods have been presented in Chapter 4.2 but are briefly described below.

Oncomelania hupensis hupensis snails, infected with Chinese (Anhui) *S. japonicum* (SJC), were imported from the Institute of Parasitic Diseases, Shanghai, China. *O. hupensis quadrasi* snails, harbouring the Philippine (Sorsogon, Luzon) strain (SJP) were maintained at QIMR. Adult worms were perfused 6 weeks after challenge from infected mice. After perfusion, schistosomes were stored in liquid nitrogen until used. Total RNA was isolated from adult pairs or single sex schistosomes (100-200 worms) using published protocols [110]. A 500 ng aliquot of total RNA from each sample was used to create fluorophore-labelled cRNA using CY3c or CY5c. All Cyanine-labelled cRNA samples were analysed by the Bioanalyzer, after which, samples were measured at A*260*, A*550* and A*650* nm in a BioMate 3 Thermo Spectronic spectrometer to determine yield, concentration, amplification efficiency and abundance of Cyanine fluorophore. Once the concentration had been determined, 0.75 µg aliquots of CY3c and CY5c were combined in a fresh tube together and "10 X control targets" added. Sample fluorophore-labelled cRNA (target) was applied to a gasket slide that was pre-positioned in a hybridization chamber. The chamber was assembled and placed in a hybridization oven and incubated for 17 h at 60˚C rotating at 4-rpm. After hybridization the chamber was opened and the microarray slide was washed for one minute in a 6× sodium chloride sodium phosphate and ethylenediaminetetraacetic acid (SSPE) / N-Lauroylsarcosine solution. The slide was then washed for another minute in a 0.06 × SSPE/N-Lauroylsarcosine solution before

drying using a Wash 3 Solution [Stabilization and Drying Solution (active component acetonitrile)] [4].

Microarray slides were scanned by a Microarray Scanner (B version). The tiff images produced by the scanner were loaded into the image analysis program Feature Extraction 7.5.1 to produce standardised data for statistical analysis. Differential expression was analysed using Rosetta Resolver. Image files were automatically processed according to default settings contained in the XML design file that describes the microarray physical layout, content and statistical parameters for normalisation by both Linear and LOWESS to correct for systematic errors and to model technology-specific random errors. Two lists of differentially expressed schistosome sequences meeting a p-value threshold of $\leq$0.001 were generated; one for up-regulated probes, the other for down-regulated probes. Experiments included one or more dye swaps (replicates), of which any bias was removed by the linear and LOWESS normalisation to produce extensive lists of differentially expressed probes, which were further filtered through p-value ($\leq$0.001) and flag error exclusion. Considerable care was taken to ensure that the results were MIAME compliant [79] (Table 5.1) [4].

Table 5.1 MIAME compliant GEO submitted Materials and Methods

Experiment 5	**SJCM V SJCF**	
GEO Samples	GSM116387	GSM116388
Chip Barcode	251260210037	251260210039
Article URL:	http://dx.doi.org/ doi:10.1016/j.mcp.2006.02.002	
Experiment 6	**SJPM V SJPF**	
GEO Samples	GSM116393	GSM116394
Chip Barcode	251260210060	251260210061
Article URL:	http://dx.doi.org/ doi:10.1016/j.mcp.2006.02.002	

Key: SJP = *S. japonicum* Philippine, SJC = *S. japonicum* Chinese, M = Males, F = Females, V = versus.

All of the microarray material, methods and results were submitted to NCBI Gene Expression Omnibus. The GEO sample number relates to the accession display of the relevant microarray experiment which may be viewed online: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5234.

Gene expression patterns determined by microarray analysis were validated using real time PCR. Complementary DNA was synthesised from total RNA using a modified SuperScript™ III protocol with $p(dT)_{15}$ primers. Forward and reverse primers (Sigma-Aldrich) were designed from *S. japonicum* contigs (see Chapter 4, Table 4.2). Since the housekeeping genes GAPDH (section 6.3.1) or ribosomal 18s (section 4.3.5) were not suitable for these experiments; considerable care was taken to ensure that the cDNA was diluted to 1 µg/µl, quantified by a ND-1000 spectrophotometer (Nano Drop). All reactions were performed on a Rotor-Gene (3000) real time PCR and analysed by Rotor Gene 6 Software. In order to minimise indiscriminate binding of double-stranded DNA, which can produce readings in the "no template" controls, separate reverse transcription and PCR steps were used [125]. Parameters were set by determination of primer melting temperature and addition of a melt curve to show primer viability [4].

5.3 Results and Discussion

5.3.1 Gender-associated differential gene expression in SJP and SJC

When male and female adult SJP were profiled by microarray analysis, 1,163 male and 1,016 female probes were differentially expressed at a p value of ≤0.001; with SJC, 1,047 male and 897 female probes were differentially expressed at a p value of ≤0.001 (Fig. 5.1). This data greatly expands the previously published lists of differential expression between the sexes of schistosomes where studies have used either <600 cDNA [110] or <8000 different oligonucleotide probes [111] for *S. mansoni,* and <500 cDNA probes for *S. japonicum* [15]. Complete lists of all probes differentially expressed between male and female worms of SJC and SJP are presented in supplementary Tables 11 and 12.

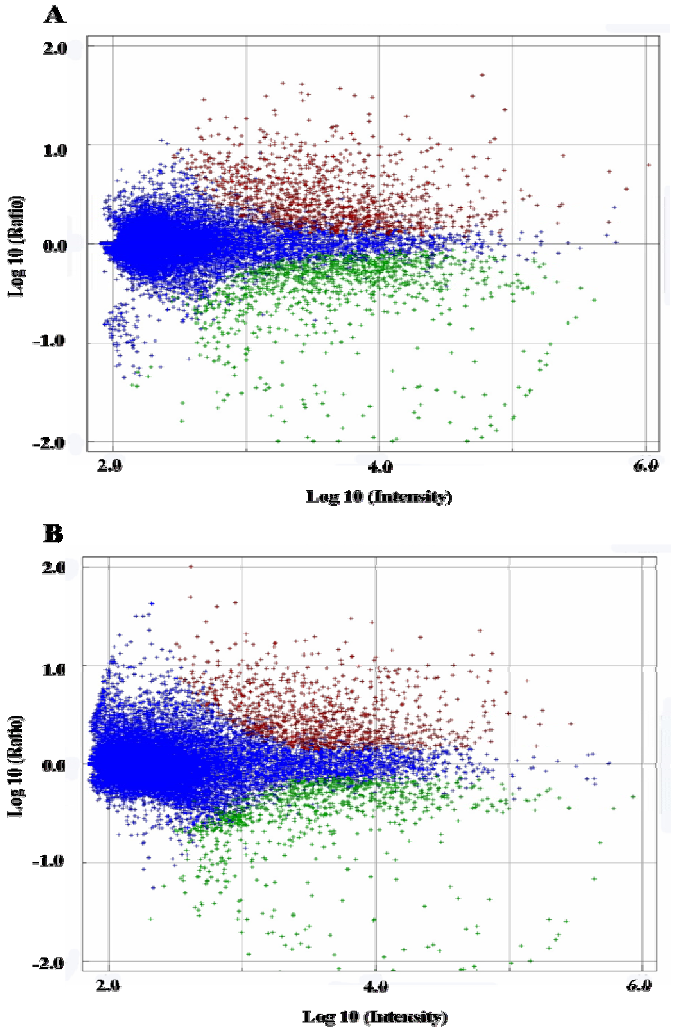

Figure 5.1 Scatter plots showing the distribution of fold changes between male and female *S. japonicum*. (**A**) Scatterplot for gender variation between male and female Philippine *S. japonicum* representing 1,163 up-regulated (male) compared to 1,016 down-regulated (female) probes. (**B**) Gender variation between male and female Chinese *S. japonicum* showing 1,047 up-regulated (male) compared to 897 down-regulated (female) probes. Blue (expanding from $\log_{10}$ Ratio [0.0]) indicates no differential expression (p-value $\leq$0.001). Up-regulated probes are shown in red while down-regulated probes are shown in green [4].

Examination of the lists (Supplementary Tables 11 and 12) showing gender-specific differential gene expression, indicated a number of genes present with

clear relevance to gender specific biological functions. Examples of established gender-specific genes reported here included an adult female-specific *S. mansoni* gene [homologue to p48 eggshell protein] (TC 7634) and male gender-specific genes encoding gynaecophoral canal sequences, Contig 6643, Contig 7087 and TC 10854 (Table 5.1). Both of these examples are not surprising as each are established components of well known gender-specific biological functions such as the female reproductive system or structures such as the gynaecophoral canal of the male which is used to hold the female parasite *in copula*. The probes that encode gynaecophoral canal genes are essential for the complete maturation of female adult worms; previous studies have shown that female schistosomes from single sex infections are sexually immature and stunted in size [157, 158].

Additional probes that were differentially expressed between the sexes of the Chinese and the Philippine strains of *S. japonicum* included Contigs 8717, 346, 8263 and 8054 (Table 5.2). The probe Contig 8717, up-regulated in SJC and SJP females, is homologous to methionine adenosyltransferase (MAT) at the protein level and contains a conserved S-adenosylmethionine synthetase (S-AdoMet_synt) region. It has been suggested that the over-expression of MAT in *Leishmania major* may contribute to its resistance to methotrexate, an antifolate drug used in the treatment of several diseases including cancer [159]. Antifolate drugs are also effective against malaria parasites, inhibiting reproduction, although resistance has also been reported [160]. Although other antimalarials such as artemether are effective in treating acute schistosomiasis [161], antifolates may not be effective against schistosomes worms.

Another probe up-regulated in female worms was Contig 346 [*S. mansoni* glutaminyl-tRNA synthetase (GlnRS)]. The enzyme GlnRS catalyses the ligation of glutamine to its cognate tRNA and has been associated with an increase of protein

synthesis in *S. mansoni* [162]. The increase of protein synthesis may be related to the up-regulation of translation regulator activity GO (Gene Ontology) associated female probes discussed further below. Two male associated probes, Contigs 8263 and 8054 showed a high sequence similarity to the heavy and light chain of myosin respectively. Myosins play an important role in the immune responses of infected hosts [163]. The myosin heavy chain is a multimeric protein (composed of several identical or different subunits) that is localised in the muscle tissue and is present in the cytoplasm of the majority of eukaryotic cells [163]. Fitzpatrick *et al*. [15] showed that the myosin light chain, which is associated with the heavy chain, is up-regulated in SJC males, presumedly related to the greater muscular development compared to female worms [26]. This result was confirmed here with the up-regulation of these two probes in male worms.

Table 5.2 Examples of differentially expressed gender-associated probes in adult Philippine and Chinese strains of *S. japonicum.**

Probe Name	Female worms compared to male worms across both strains Description/Annotation	Fold change SJP	 p-value	Fold change SJC	 p-value
Contig 4005	*C. elegans* cosmid F42C5, complete sequence	100	1.82E-35	243.2	0.00E+00
TC 8061	No associated annotation	94.94	0.00E+00	86.88	0.00E+00
Contig 8363	*T. halophila* lipid transfer protein 4 mRNA, complete cds	91.54	0.00E+00	66.99	4.42E-28
Contig 8161	*S. japonicum* clone ZZZ201 mRNA sequence	90.82	0.00E+00	39.22	0.00E+00
TC 17031	Similar to unnamed protein product (*M. musculus*), partial (88%)	90.6	0.00E+00	155.93	0.00E+00
Contig 7083	*Spiroplasma kunkelii* strain CR2-3x partial genome sequence	77.15	0.00E+00	114.79	0.00E+00
TC 17048	Weakly similar to Pur-gamma B-form (*H. sapiens*}, partial (9%)	74.71	0.00E+00	144.35	0.00E+00
Contig 5469	*Neurospora crassa* strain OR74A	73.29	0.00E+00	120.48	0.00E+00
Contig 3400	*H. sapiens* chromosome 1 clone RP5-1099E6, complete sequence	68.57	7.01E-45	68.9	0.00E+00
TC 17944	Weakly similar to ribosomal protein eL12 (*Artemia* sp.), partial (30%)	64.56	8.72E-35	79	0.00E+00
Contig 6725	Human DNA sequence from clone RP11-517P14, complete sequence	63.28	0.00E+00	53.31	4.17E-37
Contig 8717	*S. japonicum* 8C gene encoding an immunogenic miracidial antigen	12.23	0.00E+00	23.82	0.00E+00
TC 7634	Homologue to p48 eggshell protein (*S. mansoni*), partial (46%)	-	-	15.35	1.74E-11
Contig 346	*S. mansoni* glutaminyl-tRNA synthetase mRNA, complete cds	5.65	0.00E+00	1.72	7.15E-03
TC 14318	Dolichol phosphate mannose synthase (*S. mansoni*), complete	2.05	1.64E-07	2.04	4.64E-10

Table 5.2 Examples of differentially expressed gender-associated probes in adult Philippine and Chinese strains of *S. japonicum* (Continued)

Probe Name	Male worms compared to female worms across both strains Description/Annotation	Fold change SJP	 p-value	Fold change SJC	 p-value
TC 7927	Homologue to Tegument antigen (I(H)A) (Antigen SMA22.6) (A12)	50.4	1.70E-34	22.51	0.00E+00
Contig 1988	*P. zombamontana* ribosomal protein S16 (rps16) gene, partial	41.72	1.09E-30	12.3	1.44E-22
Contig 7688	*S. japonicum* dynein light chain 3 (DLC3) mRNA, complete cds	40.33	0.00E+00	17.39	0.00E+00
Contig 7639	Human DNA sequence from clone RP11-12I24, complete	36.67	0.00E+00	17.08	0.00E+00
Contig 8186	*S. japonicum* clone ZZZ469 mRNA sequence	33.17	0.00E+00	19.27	0.00E+00
Contig 5336	*S. japonicum* tetra span transmembrane protein Sj25 mRNA	31.69	0.00E+00	9.22	1.32E-20
Contig 5824	*H. sapiens* jun dimerisation protein gene, partial cds	31.16	0.00E+00	29.92	7.29E-38
Contig 7113	*Z. mays* PCO128289 mRNA sequence	30.54	0.00E+00	4.5	1.64E-09
Contig 8689	*S. japonicum* 22.6kDa membrane-associated antigen mRNA	30.49	4.05E-41	19.23	0.00E+00
Contig 7052	*C. elegans* cosmid C52B5, complete sequence	29.88	0.00E+00	27.38	0.00E+00
Contig 8263	*S. mansoni* myosin heavy chain (MYH) mRNA, complete cds	20.08	5.61E-45	7.47	5.00E-05
Contig 8054	*S. mansoni* myosin light chain mRNA, complete cds	10.6	0.00E+00	12.7	0.00E+00
Contig 6643	*S. japonicum* gynaecophoral canal protein mRNA, complete cds	8.61	0.00E+00	7.44	0.00E+00
Contig 7087	*S. japonicum* gynaecophoral canal protein mRNA, complete cds	5.82	9.18E-12	14.85	1.05E-23
TC 10854	Gynaecophoral canal protein (*S. mansoni*), partial (84%)	4.03	3.55E-05	7.12	1.56E-28

*Gender-specific probes (p-values $\leq$0.001) were selected on the basis of high fold change, low p-value and/or annotation. The symbol (-) indicates that the associated p-value was greater than 0.001 and consequently the fold change was not included.

Hoffmann *et al*. [110], using five independent cDNA microarray hybridizations (576 probes), were able to identify 12 female- and 4 male-associated *S. mansoni* genes using a 99% confidence interval (≤p-value 0.01). Fitzpatrick *et al*. [15] reported gender-associated gene expression in 457 cDNA probes of a population of Chinese strain *S. japonicum* from Anhui (22 female, 8 male) and Zhejiang (21 female, 7 male). In the current study, some of these genes were also up-regulated in adult female and male SJC and SJP (Table 5.3). Additional work by Fitzpatrick *et al*. [111] identified a further 117 and 80 genes unique for adult female and male *S. mansoni*, respectively, using 7,335 probes at a 90% confidence interval. Some correlation was possible (data not shown), but the gender-associated gene expression reported by Fitzpatrick *et al*. [111] was in a format that did not allow direct comparison to the genes present on the larger microarray used here.

Another approach to the molecular characterisation of the dimorphic nature of adult schistosomes is the proteomic identification of molecules differentially expressed between male and female worms. Cheng *et al.* [164] reported multiple unique spots for proteins expressed between male and female SJC using comparative two dimensional gel electrophoresis and mass spectrometry. Fifty excised spots were analysed, resulting in the identification of 11 female- and 16 male-associated proteins [164]. Of these 27 gender-associated proteins, 4 corresponded with the microarray analysis presented here, including TC 8655 [weakly similar to ABC transporter-like protein], TC 7403 [similar to 60S ribosomal protein L30], Contig 8651 [*S. japonicum* endothelial differentiation-related factor 1] and Contig 7452 [*H. sapiens* T cell receptor alpha delta locus]; no matching annotation of the remaining 23 proteins was evident with our microarray dataset.

Table 5.3 A comparison of the current microarray data with some previously reported gender-associated gene expression studies on schistosomes [15, 110].

Fitzpatrick *et al*. (2004)	**Current Comparisons between Male and Female SJP and SJC**		**Up-regulated in Females**		**Up-regulated in Males**	
Up-regulated Female *S. japonicum* (Anhui)	**Probe Name**	**Sequence Description**	**SJP**	**SJC**	**SJP**	**SJC**
Extracellular superoxide dismutase	Contig 208	*S. mansoni* superoxide dismutase	55.17	86.00	-	-
	TC 16781	Extracellular superoxide dismutase	-	38.30	-	-
	TC 12708	Weakly similar to Extracellular superoxide dismutase	-	-	**2.99**	**5.60**
Histidine-rich protein	TC 14408	Similar to Histidine-rich membrane protein	5.06	2.48	-	-
Eggshell protein precursor	TC 16680	Similar to eggshell precursor protein (*S. japonicum*)	10.95	-	-	-
Hoffmann *et al*. (2002)						
Up-regulated Female *S. mansoni*	**Probe Name**	**Sequence Description**	**SJP**	**SJC**	**SJP**	**SJC**
Similar to eggshell protein/chorion	TC 7634	Homologue to p48 eggshell protein	-	15.35	-	-
Similar to Rn 60 S ribosomal protein	TC 10534	Weakly similar to 60S ribosomal protein	4.21	3.40	-	-
	TC 7403	Similar to 60S ribosomal protein L30	4.09	2.44	-	-
Up-regulated Male *S. mansoni*	**Probe Name**	**Sequence Description**	**SJP**	**SJC**	**SJP**	**SJC**
Similar to Sj DLC3	Contig 7688	*S. japonicum* dynein light chain 3 (DLC3)	-	-	40.33	17.39
Sm Actin	Contig 1498	*S. mansoni* actin mRNA, complete cds	**1.46**	**2.10**	-	-
	Contig 6746	*S. mansoni* actin mRNA, complete cds	-	-	2.48	3.47
	Contig 8822	*S. japonicum* actin mRNA, complete cds	-	-	9.22	10.02
Sm Tropomyosin	TC 10539	Tropomyosin 1 (TMI) (Polypeptide 49)	**7.57**	**7.51**	-	-
	Contig 4092	*S. japonicum* tropomyosin mRNA, complete cds	-	-	5.53	4.96
	Contig 8706	*S. japonicum* tropomyosin mRNA, complete cds	-	-	5.54	7.62

KEY Contig = *S. japonicum*, TC = *S. mansoni* (TIGR), SJP = *S. japonicum* Philippine, SJC = *S. japonicum* Chinese, - = Not up-regulated

The results show SJP and SJC expression as relative "fold change" between male and female schistosomes (p-value $\leq$0.001). The left hand column shows gender-specific genes identified by Hoffmann *et al*. [110] and Fitzpatrick *et al*. [15]. Any differences shown between the microarray data and previous reported work may be due to inconsistencies in annotation or differential expression between species.

Liu *et al*. [165], using two-dimensional nano liquid chromatography mass spectrometry (2D-nano-LC-MS), identified 491/574 soluble proteins from female and male SJC worms, respectively. The proteins were identified from MS spectra searched against rabbit or mouse and the *S. japonicum* databases. Further to this, proteins with more than three-fold differences throughout the life cycle were selected. This selection, based on quantitative proteomics, highlighted 23 gender-specific proteins [165]. A subset of these highlighted proteins contained similar annotations to the contigs in the current microarray study (Table 5.4), assuming that the contigs are their precursors. The mRNA fold expression did not directly correlate with the protein expression frequency in scale (Table 5.3), but did show similar directional gene expression patterns to that obtained by Liu *et al*. [165]. It is known from gene expression studies that mRNA levels do not necessarily correlate with protein expression [166]. This may be due to the differentiation of proteins generated from a single gene as a result of post-transcriptional modification and differential gene splicing [167].

Table 5.4 A comparison of the current microarray data with proteomic data previously reported [165].

Liu *et al*. (2006)		Similar annotated microarray contigs
Female protein frequency	**Description**	**Up-regulated in female**
>10	Similar to dolichyl-diphosphooligosaccharide-protein glycosyltransferase	TC10667 (SJC 1.79, SJP 2.58)
5-10	Extracellular superoxide dismutase	TC16781 (SJC 38.29)
Male protein frequency	**Description**	**Up-regulated in male**
3-4	Gynaecophoral canal protein	Contig6643 (SJC 7.44, SJP 8.61)
		Contig7087 (SJC 14.84, SJP 5.82)
		TC10854 (SJC 7.12, SJP 4.03)

KEY SJC = *S. japonicum* Chinese, SJP = *S. japonicum* Philippine

All *S. japonicum* proteins shown were similar in annotation to microarray contigs. The microarray contig results show SJP or SJC expression as relative "fold change" between male and female schistosomes (p-value $\leq$0.001). The left hand columns show gender-specific genes with more than 3-fold differences throughout the schistosome life cycle identified by Liu *et al*. [165].

5.3.2 Gene-ontological comparison of male and female adult worms of *S. japonicum*

To better demonstrate the overall differences between the male and female *S. japonicum*, gender-associated transcripts were categorised by GO. There was a greater number of male up-regulated GO categorised transcripts but the majority did not show a definitive gender-association when compared to the females (Table 5.5). This analysis excluded the category 'Cell Differentiation' which was clearly male-associated, the definition of which includes "the process whereby relatively unspecialised cells, e.g. regenerative cells, acquire specialised structural and/or functional features that characterise the cells" (Table 4.6, Chapter 4). This may be demonstrated by one of the ontology defined 'cell differentiation' probes that was up-regulated in SJP and SJC male

worms, TC 7355 (Supplementary Table 13). The probe TC 7355 is defined as a *S. mansoni* Rac Guanosine 5′-triphosphatase (GTPase); Rho GTPases including Rac1 are the key regulators of cadherin-based cell-cell adhesion [168]. This GO may be associated with the maturation of the male worm following coupling with female [111], and may thus be a putative target for an anti-fecundity therapeutic as proposed for *S. mansoni* GTPase [168]. The majority of female-associated GO categorised probes that were up-regulated when compared to males in both strains were related to egg production. Examples included those with cell organisation and translation regulator activity. Other ontologies involved in metabolism, regulation of the cell and binding may be indirectly involved in the development of the schistosome for egg production [111]. It is noteworthy that in the study reported here, there were more structural molecule activity GO-associated probes in the females than males (Table 5.5), in direct contrast to the findings of Fitzpatrick *et al*. [111]. This may be related to the number of probes with a GO classification, and can only be clarified by further study and classification of the probes on the current microarray.

Table 5.5 The number of probes with associated gene ontology within male and female SJC and SJP gender associated results

Molecular function	Male	Female
Binding	47	58
Catalytic Activity	46	45
Chaperone Activity	1	4
Enzyme Regulator Activity	8	3
Motor Activity	1	0
Signal Transducer Activity	14	10
Structural Molecule Activity	8	20
Transcription Regulator Activity	4	4
Translation Regulator Activity	0	7
Transporter Activity	14	17

Biological Function	Male	Female
Behaviour	1	0
Cell Communication	25	21
Cell Cycle	14	12
Cell Differentiation	11	3
Cell Organisation	33	51
Cell Proliferation	4	2
Homeostasis	1	1
Membrane Fusion	19	13
Metabolism	54	75
Physiological Process	62	81
Regulation of the Cell	33	30
Transport	8	11

Probes that were not differentially expressed between male and female SJC or SJP at p-values $\geq$0.001 were not included.

5.3.3 Real time PCR validation of fold changes

In order to validate the cRNA microarray predictions detailed above, 9 probes were examined using real time PCR as an independent analysis of gene expression using the primers described in Chapter 4 (section 4.3.5). The real time PCR results shown were a

calculated mean of four replicates from two separate experiments. In gender-specific associations, all nine showed similar expression patterns to the predicted cRNA microarray hybridization results, thus showing the reliability of the filtering criteria and the microarray resource (Table 5.6).

Table 5.6 Real time PCR validation of microarray results based on fold change.

	Real Time PCR		**Microarray**		
SJP-sex	**Direction**	**Fold Change**	**Direction**	**Fold change**	**p-value**
Contig 5336	Male	20.95	Male	31.69	0.00E+00
Contig 8365	Female	146.4	Female	100	0.00E+00
Contig 8689	Male	127.35	Male	30.49	0.00E+00
Contig 3466	Female	1.1	Female	29.26	0.00E+00
Contig 7411	Female	3.15	Female	11.41	6.98E-10
Contig 8664	Female	3.05	Female	7.31	8.79E-09
Contig 2733	Female	2.25	Female	1.92	3.42E-17
Contig 204	Female	1.65	Female	1.54	1.59E-05
Contig 4489	Male	28.8	Male	12.55	7.06E-06

The real time PCR results for both Philippine (SJP) and Chinese *S. japonicum* are means of four replicates from two separate experiments. The results in italics indicate real time PCR results that did not agree with the microarray data. The right hand column shows p-values for correlating microarray fold change.

5.3.4 Conclusions

This expression data reported in this chapter has expanded significantly the list of gender-associated genes in adult *S. japonicum* reflecting the quality of the microarray resource used. The analyses identified, with a confidence interval of 99.9%, 2,179 probes that were differentially expressed between male and female SJP, and a further 1,944 probes that were differentially expressed between male and female SJC. Some of these and other probes are further elaborated on in Chapter 6. The novel gender-associated genes identified here provide a stepping stone for understanding the complexities of sexual differentiation, maturation and development of the adult worms in human schistosomiasis. Additionally this information may prove valuable for identifying novel intervention and diagnostic targets against the schistosomes.