1. INTRODUCTION

1.1 Schistosome Background

1.1.1 Schistosome Origins and Discovery

Schistosomes are metazoan parasites, trematode worms (Morgan, Dejong, Snyder, Mkoji *et al*., 2001) that live within the blood vessels of man and his livestock. Schistosomiasis, the disease that results from infection with schistosomes, is characterised by diarrhoea, anaemia, abdominal pain and sometimes death (Rollinson & Simpson, 1987).

The life cycle of *Schistosoma mansoni*, the species of interest in this project, requires a human (definitive) host as well as freshwater snails (intermediate host) of the *Biomphalaria* genus (El-Ansary & Al-Daihan, 2006).

Schistosoma haematobium, the first schistosome to be described, was discovered in the veins of a man at autopsy in Cairo by the German surgeon Theodor Bilharz in 1851. It was not until 1913 when Miyairi and Suzuki showed that *Schistosoma japonicum* developed in the hydrobid snail *Oncomelania hupensis nosophora* that elucidation of the schistosome life cycle was made (Rollinson & Simpson, 1987).

Molecular phylogenetic studies suggest that schistosoma originated in Asia, and that a pulmonate transmitted progenitor colonized Africa giving rise to terminal spined as well as lateral spined egg species groups, the latter containing *S. mansoni* (Morgan *et al.*, 2001).

S. mansoni appeared after the Trans-Atlantic dispersal of *Biomphalaria* from the Neotropics to Africa, which occurred 2-3 million years ago, according to the present African fossil record. It became more prevalent in tropical Africa, entering the new world with the slave trade (Morgan *et al.*, 2001).

It has been reported (Chitsulo, Engels, Montresor & Saviolo, 2000) that across 74 countries, approximately 200 million people are infected with schistosomes, of which 20 million suffer severe illness, and 120 million are symptomatic. Around 600 million people are at risk of acquiring this infection (Fallon *et al.*, cited in El-Ansary and Al-Daihan, 2006).

1.1.2 Interactions Between Schistosomiasis and Other Diseases

There is increasing evidence that schistosome infections impact the transmission and etiology of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) (Kjetland, Ndhlovu, Gomo, Mduluza *et al.*, 2006), tuberculosis (Elias, Akuffo, Thors, Pawlowski *et al.*, 2005), and malaria (McManus & Loukas, 2008; Vennervald, Booth, Butterworth, Kariuki *et al.*, 2005)

Due to geographic overlap in distribution and the role of immune responses in both diseases, the interaction between schistosomiasis and HIV/AIDS is receiving increasing attention as described by Karanja (2002) (see McManus and Loukas, 2008). Susceptibility to schistosome infection and egg excretion may be influenced by HIV infection which results in low $CD4^+$ T cell counts, therefore in developing countries schistosomiasis imposes high socioeconomic burdens (McManus & Loukas, 2008).

1.1.3 Species of Schistosome

Five species of schistosome, *Schistosoma mansoni, S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. haematobium* infect humans, although a number of animal specific species occasionally accidently infect humans (e.g. *S. margrebowiei* or *S. bovis).* Well described associations with chronic hepatic and intestinal fibrosis have been described for the first 4 species, while *S. haematobium* infections cause fibrosis, and calcification of the urinary tract (McManus & Loukas, 2008).

1.1.4 *Schistosoma mansoni*

S. mansoni is the most intensively studied of the phylum platyhelminthes, with around three-to-four times the number of papers published annually, compared to any other platyhelminth species, reflecting its importance as a laboratory model for studying schistosomiasis (Morgan *et al.,* 2001).

Some basic information is known on the genome of *S. mansoni*, and where gaps in current knowledge exist information can be inferred from the data of a related species. It has a haploid chromosome number of 18, its karyotype is relatively conservative, and there is no evidence of polyploidy. Snyder *et al.*, (2001) suggested (see Morgan *et al.,* 2001) that it's likely the first complete genome sequence obtained for a flatworm will be that of *S. mansoni*.

The scrutiny given to this organism is well deserved as *S. mansoni* is the most widely distributed of all schistosomes infecting humans. It's widespread distribution is permitted by the broad geographic range of susceptible species of

the freshwater snail genus *Biomphalaria* that serves as obligatory hosts for its larval stages (Morgan *et al.*, 2001). Several *Biomphalaria* spp. can serve as its intermediate host. In Africa *S. mansoni* is known to be transmitted by *Biomphalaria alexandria, B. pfeifferi, B. camerunensis* and a few other lesser known species (Malek, 1981).

The most important intermediate host in the western hemisphere is *B. glabrata,* with *B. comphalaria tenagophila* and *B. straminea* playing a more restricted role in transmission (Knight, Ongele & Lewis, 2000).

1.1.5 Distribution of *Schistosome mansoni* & *Biomphalaria* Host Species

S. mansoni is transmitted by *Biomphalaria* snails in sub-Saharan Africa, northeast Brazil, Surinam, Venezuela, the Caribbean, lower and middle Egypt, and the Arabic peninsula (McManus & Loukas, 2008), as shown in Figure 1.

1.2 *Schistosoma mansoni* Lifecycle

Schistosomes are dioecious, unlike most other trematodes. The life cycle of *S. mansoni,* depicted in Figure 2, starts when eggs are excreted in the faeces into a fresh water system, remaining viable for up to 7 days. The eggs release miracidium on contact with the water.

Egg hatching is promoted by low osmolarity and bright sunlight, so that the miracidial stage within the eggs can emerge to find and invade the intermediate host, *Biomphalaria glabrata,* for asexual reproduction to occur (Pearce, 2005).

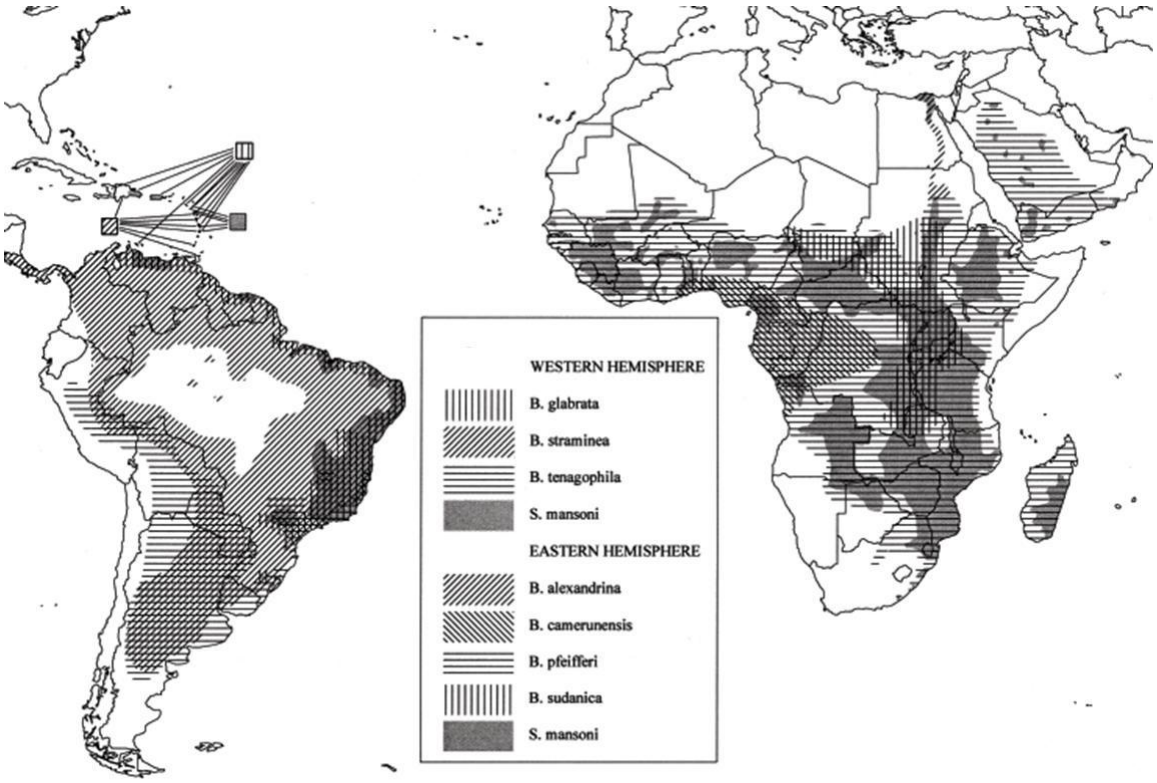

Figure 1: Distributions of *Schistosoma mansoni* and *Biomphalaria* host species of major medical importance. Distributions indicate the main areas of occurrence; continuity of distribution is not implied and there may be significant discontinuities within these areas (Morgan *et al.*, 2001).

Guided by light and chemical stimuli it searches for the intermediate host (Gryseels, Polman, Clerinx & Kestens, 2006). After penetrating the snail the miracidia transform into mother sporocysts which, via asexual reproduction, give rise to multiple daughter sporocysts, and then cercariae (El-Ansary & Al-Daihan, 2006). The cerariae have embryonic suckers and a characteristic bifurcated tail (Gryseels *et al.*, 2006). The cercariae exit the snail and, as a free-swimming larval stage, seek out a human host (El-Ansary & Al-Daihan, 2006).

Sommro *et al.*, (2005) followed the development of miracidia within the snail tissues (see El-Ansary and Al-Daihan, 2006) proving after successful penetration, the miracidia transform to mother sporocysts. These can be observed

on the second day, increasing in size during the infection. They are generally found in the head/foot regions of the host within 7–9 days. Mother sporocysts contained daughter sporocysts by day 11, few are elongated and almost mature, and by day 25 daughter sporocysts were found throughout the tissues around the gonad. More amoebocytes were present than usual, with higher concentrations around the parasite, and metasporocysts (i.e. daughter sporocysts containing developing cercariae) occupied all the available space between the digestive gland and the gonads. A huge number of fully developed cercariae had moved into the large blood vessels and were on their way to leave the snail on day 55 (El-Ansary & Al-Daihan, 2006).

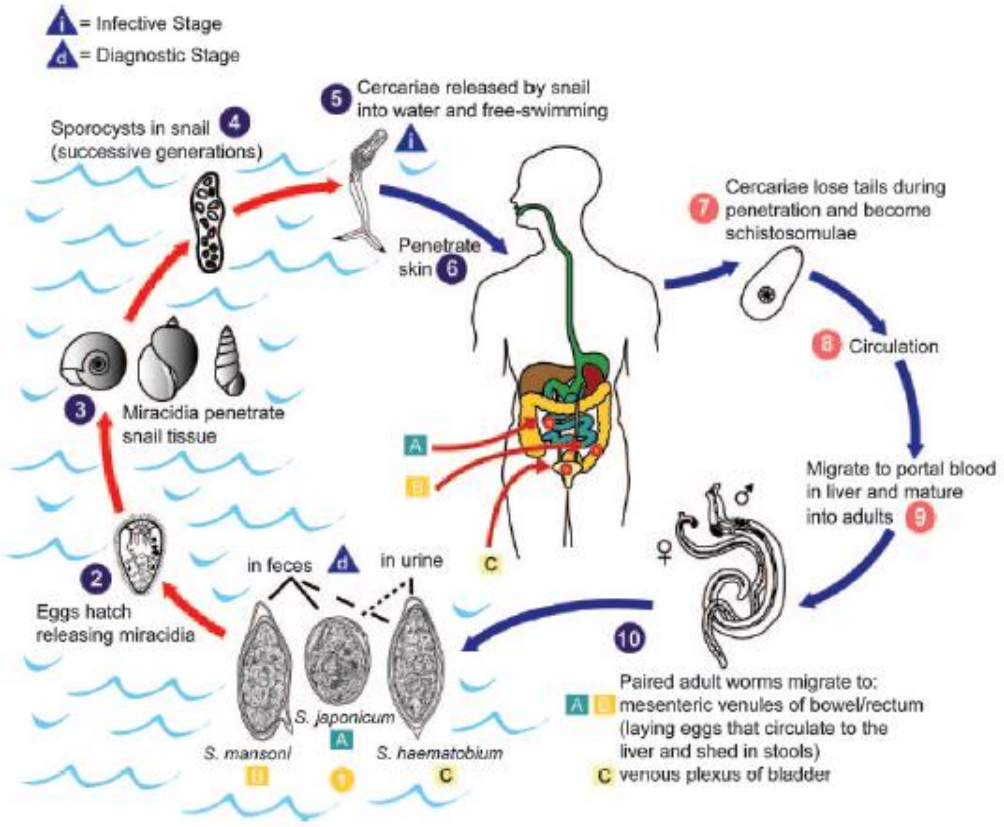

Figure 2: Life cycle of *S. mansoni*, *S. japonicum*, and *S. haematobium* Laboratory Identification of Parasites of Public Health Concern (2008).

Four to six weeks later the cerariae start leaving the snail, as a free swimming larval stage, and spin around in the water for up to 72 hours seeking the skin of a suitable definitive host using glycogen energy stores (Gryseels *et al.*, 2006). Rhythmic swimming helps achieve this, which is increased in moving shadows, turbulent waters and exposure to human skin substances, all three of which are human typical wading behaviours (Rollinson & Simpson, 1987). Light provokes cerarial shedding, mainly occurring during daytime. A single snail, infected by one miracidium, can shed thousands of cercariae every day for months (Gryseels *et al.*, 2006).

Upon finding a host, the cercariae utilize an elastase proteolytic enzyme produced in the head region to penetrate the skin of the host (McManus & Loukas, 2008). Their bifurcated tails are shed, and they enter capillaries and lymphatic vessels migrating via the lungs to the liver where they transform into young worms or schistosomulae.

After several days the schistosomula migrate to the portal venous system, where they mature, taking 4-6 weeks, and unite. Migration of worm pairs to their ultimate vascular bed, the superior mesenteric veins, occurs where they live as permanently embraced couples (McManus & Loukas, 2008).

The adults have a cylindrical body of 7 to 20 mm in length featuring two terminal suckers, a complex tegument, a blind digestive tract, and reproductive organs. The male's body forms a groove, or gynaecophoric channel or canal, in which it holds the longer and thinner female (Ross, Bartley, Sleigh, Olds *et al.*, 2002). Schistosomes feed on blood and globulins through anaerobic glycolysis. The debris is regurgitated in the host's blood (Gryseels *et al.*, 2006).

Egg production commences 4 to 6 weeks after infection and continues for the life of the worm, which averages at 3-5 years, but can be up to 15 years in the definitive host. The *S. mansoni* females produce hundreds of eggs per day, which are deposited in the vein lumen (McManus & Loukas, 2008). Theoretically the reproduction potential of a single schistosome pair is up to 600 billion schistosomes (Gryseels *et al.,* 2006). A ciliated miracidium larva, that helps the eggs to migrate into the intestine, by secreting proteolytic enzymes, is contained in each ovum (Gryseels *et al.,* 2006).

Eggs are shed in the faeces completing the life cycle. After two generations of primary and then daughter sporocysts within the snail, asexually produced cercariae are released (McManus & Loukas, 2008).

1.3 *Schistosome* Pathology, Diagnosis and Global Burden

The pathology and disease associated with schistosomiasis is not due to the adult worms, but is caused by the deposition in mucosae and tissues (the liver and intestines in particular) of eggs and the subsequent T – cell - dependent immune response of the host (McManus & Loukas, 2008). A range of molecules, such as proteolytic enzymes (Cheever, Hoffman & Wynn, 2000), are secreted by trapped eggs resulting in a marked $CD4^+$ T cell programmed granulomatous inflammation involving monocytes, eosinophils and lymphocytes analogous to a delayed type hypersensitivity. With intestinal schistosomes severe hepatic periportal (Symmer's) fibrosis occurs as granulomas are also characterized by collagen deposition. The deposition of connective tissue elements in affected

tissues attribute credit to much of the morbidity and mortality associated with this disease (McManus & Loukas, 2008).

Lambertucci, 1993 (cited in Gryseels *et al.*, 2006) described acute schistosomiasis (Katayma fever) as a feverish syndrome, occurring a few weeks to months after primary infection. It is a systemic hypersensitivity reaction against the migrating schistosomulae that affects mainly individuals with long term infections in poor rural areas.

Microscopic demonstration of eggs in the excreta is the diagnostic standard. Due to their shape, typical terminal or lateral spine, eggs are easy to detect and identify, as are living miracidium, in fresh sample, with mobile cilia and pulsating excretory cells (Engels, Nahimana & Gryseels, 1996).

Schistosomiasis's influence on public health and the priority of control measures have been long debated as it is highly prevalent, but the associated morbidity is low and variable. An attempt to quantify and rank health problems according to Disability Adjusted Life Years (DALY) was made by the Global Burden of Disease Study (Lopez, Mathers, Ezzati, Jamison *et al.*, 2006). Disease specific prevalence, disability weights and mortality are used to calculate this index. Schistosomiasis currently has an annual mortality of 14000 deaths per year and is attributed a disability weight of 0.06 (Gryseels *et al.*, 2006). The total number of DALY lost to schistosomiasis is estimated at 1.532 million per year, of which 77% are in sub- Saharan Africa, based on the generally accepted number of 200 million infected people worldwide. Therefore schistosomiasis accounts for 0.1% of the total world global burden of disease and 0.4% of that in sub-Saharan Africa (Gryseels *et al.*, 2006).

1.4 *Schistosome* Treatment and Control

1.4.1 Treatment

Fenwick *et al.,* 2003 (cited in Gryseels *et al.,* 2006) says safe and simple drugs became available for the treatment of schistosomiasis in the 1970's, whereas earlier treatments had severe and sometimes lethal side effects (Booth, Guyatt, Li & Tanner, 1996).

The most widely used drug Praziquantel, an acylated quinoline-pyrazine, is active against all schistosome species. Acting within an hour of ingestion by paralysing the worms and damaging the tegument (Gryseels *et al.,* 2006), it has no effect on eggs and immature worms. There are mild side effects including vomiting, nausea, abdominal pain and malaise. Acute colic with bleeding diarrhoea can occur shortly after treatment in heavy infections, thought to be provoked by massive worm shift and antigen release (Stelma, Talla, Sow, Kongs *et al.,* 1995).

Oxamniquine only acts on *S. mansoni,* where it is as effective as praziquantel but can provoke more pronounced side effects, most notably drowsiness, sleep induction and epileptic seizures (Gryseels *et al.,* 2006).

1.4.2 Drug Resistance

There is evidence for the presence of *S. mansoni* isolates that are relatively unresponsive to praziquantel, in the field situations of both Senegal and Egypt, although the underlying basis for this reason seems to be different in each

case (Morgan *et al.,* 2001). The low cure rates observed in northern Senegal could be explained by very intense transmission, re-infection, maturing prepatent infections and possibly the epidemic nature of the focus (Gryseels *et al.,* 2006).

Cioli *et al.,* 1993 (cited in Gryseels *et al.,* 2006) reported resistance to hycanthone and oxamniquine can develop in animals as well as in the field, although resistance to these drugs has never spread beyond local foci.

In animals, under drug pressure, praziquantel tolerant schistosome strains can quite easily be selected. Cioli *et al.,* (1993) argues caution is needed as is highlighted by the catastrophic experience in cattle, with widespread resistance to anthelmintics due to systematic mass treatment.

Praziquantel resistance has not yet become pervasive and praziquantel resistant worms are still susceptible to oxamniquine (Conceicao, Argento & Correa, 2001).

1.4.3 Control

The ultimate goal for control strategies is elimination of transmission, with specific measures (such as chemotherapy and snail control) developed in association with nonspecific measures (aimed at the general improvement of sanitary and health conditions and the provision of safe water supplies) (Lardans & Dissous, 1998).

S. mansoni control using praziquantel and oxamniquine is difficult to sustain although it has reduced global prevalence (Morgan *et al*., 2001). In the absence of behavioural or ecological changes, it has little durable affect on transmission therefore to ensure sustainability and progression to the more

demanding stages of infection and transmission control, careful long term planning is needed (Gryseels *et al.,* 2006).

Although the linchpin of current control programmes after over 20 years of experience, it is generally agreed that chemotherapy does have some limitations (Utzinger, Zhou, Chen & Bergquist, 2005). Mass treatment does not prevent re-infection which occurs swiftly in exposed populations in most areas of endemicity. The prevalence returns to its baseline level within a period of 6 to 8 months following chemotherapy.

The decreased susceptibility to praziquantel mentioned above is raising concerns about its future efficiency in control efforts (Chitsulo, Montresor & Savioli, 1998).

As the intermediate mollusc hosts play a major role in schistosome transmission, snail control strategies are considered a priority for reduction of transmission (Lardans & Dissous, 1998). Control is usually achieved with molluscicidal chemicals, although environmental and biological control measures are used in some situations.

Using molluscicides, toxic chemicals, for snail control is expensive and logistically complex. For efficient application and detailed eodemiological and malacological surveillance, substantial material and human resources are needed. Regular and long term treatment is necessary, as snail populations can be greatly reduced but rarely eliminated. Ecological and economic concerns are raised by the toxicity for other aquatic organisms including fish (Gryseels *et al.,* 2006).

Biological competitors and physical measures are not easy to put into practice (Gryseels *et al.,* 2006). It has been proven to be efficient where feasible to implement environmental management including elimination of natural water

bodies such as marshes and ponds, and regulation of human settlement in areas with significant risk (Lardans & Dissous, 1998).

Different biological control agents have been used to develop alternative methods of snail control. Predators, mainly fish have sometimes been used proving successful in islands to limit snail populations (Pointier & Guyard, 1992).

Widely used in control trials, snail pathogens particularly dominant trematodes (echinostomatidae), with the ability to sterilize snails, have shown an efficiency lower than that of molluscicide in field application (Lardans & Dissous, 1998).

Other promising ways of displacing target snail populations are the introduction of snail competitors (Madsen, 1990) or to compete for parasites by a possible decoy effect (Combes & Mone, 1987).

In principle behavioural changes, sanitation, and safe water supply can eliminate schistosomes. Knowledge about the disease and healthcare seeking can be improved by educational programmes, although without other options for water contact behaviour can be difficult to change (Gryseels *et al.*, 2006; Morgan *et al.*, 2001).

As an adjunct to chemotherapy, vaccine strategies represent an essential component for the future control of schistosomiasis. Development of a vaccine is possible as suggested by improving understanding of the immune response to schistosome infection, in both humans an animal models (McManus & Loukas, 2008). There is considerable optimism that consolidated international efforts to generate antischistosome vaccines will prove successful.

1.5 *Biomphalaria glabrata*

1.5.1 Resistance to Schistosomes

Strains of *B. glabrata* differ in their ability to suppress *S. mansoni* infection. Some strains are susceptible to *S. mansoni,* allowing parasite development and reproduction, while others demonstrate a resistant phenotype and are able to suppress the infection (Humphries & Yoshino, 2008). Interactions between parasite and snail genes influence the outcome of infection, with the most compatible relationships occurring where considerable co-evolution occurred (Knight, Ongele & Lewis, 2000). Resistance is defined as the inherent ability of organisms to prevent establishment or development of a given species or strain of compatible parasite (Theron & Coustau, 2005).

1.5.2 Matched/Mismatched

Compatibility between *B. glabrata* and *S. mansoni* is a highly relative concept. It has been suggested by Theron and Coustau (2005) that compatibility is tested independently for each individual host and miracidium, and that failure or success of infection depends on 'matched'/'mismatched' status of the host and parasite phenotypes, rather than on snail susceptibility/resistance status.

Unsuccessful infection reflects existence of snail resistance, while successful infection reflects existence the susceptible status of the host snail, within compatible snail-trematode combination.

1.5.3 Dominance and Heritability of Resistance

The heritable character of susceptibility was established by Newton (1953) cited in *Knight et al.,* (2000). The mendelian nature of the adult resistant phenotype, with resistance dominant was established by Richards (1973) cited in Knight *et al*. (2000).

1.5.4 Exploiting Resistance to *S. mansoni* for Control of *B. glabrata*

Snail parasite interaction studies are important in understanding the mechanisms by which resistant snails are able to resist parasite infection, and it is expected that unraveling both host and parasite gene expression might eventually lead to novel methods of disease control. Such understanding may present opportunity through chemical or genetic manipulations of the intermediate host to break the cycle of human and snail infection by schistosomes.

A better understanding of the molecular make up of the invertebrate host, at the very least, should reveal important information on genetic variation and other issues in the epidemiology of these diseases (Knight *et al.*, 2000).

Control of schistosomes by supplanting parasite susceptible snails with a resistant variant in the field was initially proposed by Hubendick (1958) cited in Knight *et al.,* (2000). This 'genetic control technique' sets out to interrupt the life cycle of schistosomes by increasing the proportion of genetically resistant intermediate host snails. Natural selection will increase the proportion of alleles for resistance if the susceptible snails suffer a loss of reproductive fitness compared to their resistant counterparts. This may not always be so as reported

by Webster & Woolhouse (1999), when observations of numbers of offspring produced by artificially selected lines suggested lower fertility among resistant snails. The resistant snails produce fewer offspring than susceptible lines, even though the prevalence of schistosomiasis was significantly higher in the susceptible lines.

1.5.5 Biological Mechanisms of Resistance

Haemocytes (circulating phagocytic blood cells) migrate towards the early developing sporocysts and bind to them in a multilayered cellular encapsulation response in resistant hosts, leading to destruction and clearance of the parasite (Knight *et al*., 2000). Such responses lead eventually to parasite death, usually within 24 hours post infection (Loker, Bayne, Buckley & Kruse, 1982).

Using an *in vitro* cellular cytotoxicity assay it was demonstrated that haemocyte derived reactive oxygen and nitrogen species specifically H_2O_2 and NO, respectively, were responsible for the schistosomicidal activity (Hahn, Bender & Bayne, 2001 *a, b*)

It has also been shown that selected carbohydrates were capable of triggering *B. glabrata* haemocyte H_2O_2 production *in vitro* (Hahn *et al.,* 2000 cited in Humphries and Yoshino, 2008) suggesting an association between carbohydrate reactive cell receptors and oxidative/nitrative responses (it is assumed that H_2O_2 production is tightly regulated although specific molecules remain largely unknown) (Humphries & Yoshino, 2008).

It is assumed the overall haemocytic response to sporocysts depends on a well developed signal transduction system as receptors and intracellular signaling pathways are likely to regulate haemocyte migration sporocyst detection and encapsulation, however specific signaling pathway regulating such phenomena remain undetermined (Humphries & Yoshino, 2006).

Numerous studies have described the possible role of snail proteins (lectins, alpha-macroglobulin proteinase, interleukin-1, tumour necrosis factor) involved in resistance, by examining alterations in cellular (haemocyte) or humeral (haemolymph) components following parasite exposure (Knight *et al.*, 2000).

A snail homologue of beta integrin, a protein that may have direct biological relevance to parasite recognition or cell adhesion pathways was reported by Davids, Wu & Yoshino (1999), and a fibrogen related protein (FREP) has been reported (Adema, Hertel, Miller & Loker, 1997). It has since been demonstrated that a family of *Biomphalaria* FREPs undergo processes of recombinational diversification leading to the concominant existence of a great diversity of FREPs within a single individual (Zhang *et al.*, 2004) cited in (Theron & Coustau, 2005). Their function has not been clarified although evidence suggests they are capable of binding molecules of foreign origin such as *S. mansoni* surface epitopes (Hertel, Adema & Loker., 2005 cited in Theron & Coustau, 2005). Carbohydrate binding molecules such as FREPs, interacting with *S. mansoni* carbohydrate ligands could determine the ‘matched’ or ‘mismatched’ status of *B. glabrata – S. mansoni* combination.

1.6 Proteomics

The term ‘proteome’ was first used to describe the protein compliment of a genome in 1995 (Wasinger, Cordwell, Cerpapoljak, Yan *et al.*, 1995), although it is now usually refined to describe the protein complement of an organ, tissue or cell. Proteomics is functional genomics at the protein level (Blackstock & Weir, 1999). It is proteins, not genes, that are responsible for an organisms phenotype. The genome does not directly code for the correct functioning of proteins, due to post translational modifications (Barrett, Brophy & Hamilton, 2005).

A study comparing message data derived from differential gene expression with corresponding 2D-gel protein-expression measurements showed that correlation between mRNA and the cognate protein is poor (Bergh & Arckens, 2008), so Walter suggests for accurate quantitative work, it may be preferable to work at the protein level (Blackstock & Weir, 1999). Proteomics provides both a qualitative and quantitative approach.

Two dimensional gel electrophoresis (2DE), separating proteins for visualisation, and mass spectrometry (MS), for protein identification, are most commonly used for proteome analysis (Gygi, Corthals, Zhang, Rochon *et al.*, 2000).

O’Farrell (O'Farrell, 1975) and Klose (Klose, 1975) demonstrated it was possible to separate proteins based on their molecular weights (MW) and isoelectric points (p*I*, The pH at which a particular protein carries no electrical charge) by electrophoresis on polyacrylamide gels, and now two dimensional

polyacrylamide gel electrophoresis (2D PAGE) is the most efficient way of separating complex protein mixtures.

Coomassie blue and silver staining allows protein visualisation and quantification, although this is not the same as identification (Blackstock & Weir, 1999). MS is the method of choice for protein identification and characterisation of post translational modifications. As the MW of proteins is insufficiently discriminating, most protein identification approaches rely on trypsin for proteolysis of the separated protein, and analysis of the resulting peptides. Trypsin cleaves proteins exclusively at the C terminal end of lysine or arginine residues (proving the next amino acid is not proline).

Ionization methods are used such as Matrix-Assisted Laser-Desorption-Ionisation-Time-of-Flight (MALDI-ToF) mass spectrometry which is usually used for peptide mass fingerprinting (PMF) (Henzel, Billeci, Stults, Wong *et al.*, 1999) cited in (Blackstock & Weir, 1999). Peptide masses derived from an in-gel proteolytic digestion are measured and searched against a computer-generated list, formed from the simulated digestion of a protein database using the same enzyme (Blackstock & Weir, 1999). When full length sequences are not available, as in the case of *B. glabrata*, problems can arise in identification of the proteins.

1.7 *Biomphalaria glabrata* Hepatopancreas: Previous Studies

Previous work has demonstrated there are significant differences in the number and expression levels of proteins between the proteomes of the hepatopancreas, the organ of *B. glabrata* where sporocysts reproduce asexually

and release cercariae (Negrão-Corrêa, Pereira, Rosa, Martins *et al.*, 2007), of susceptible and resistant gastropods (Baker unpublished, 2008; Murray unpublished, 2008).

2DE was used, to separate proteins by their p*I* in the first dimension and MW in the second dimension, then average gels created allowing susceptible and resistant protein sample to be compared and contrasted.

Proteins found to be up- or down-regulated by a factor of less than two, were suggested to show little variation and could be involved in other biological functions which are necessary in both susceptible and resistant snails, while proteins found to be up-regulated or down-regulated twofold or more (and therefore likely to be biologically significant) were examined more closely (Barrett *et al.*, 2005). Table 1 shows the main gel report data obtained.

Table 1: Table displaying essential numerical data obtained from gel report (Murray unpublished, 2008).

Total Number of Protein Spots on Average Gel from Susceptible Snails	672
Total Number of Protein Spots on Average Gel from Resistant Snails	764
Number of Matched Spots Up Regulated > Twofold in Gel from Susceptible Snails Compared to Gel from Resistant Snails	65
Number of Matched Spots Down Regulated > Twofold in Gel from Susceptible Snails Compared to Gel from Resistant Snails	75
Number of Unique (Unmatched) Spots on Average Gel from Susceptible Snails	437

It is likely that some of these changes will be immune related, although no specific proteins were identified. Some clues were provided into putative identifications of immune related *B. glabrata* proteins however their true identification remains to be determined empirically (Baker unpublished, 2008).

This project aims to move this work forward by identifying specific spots by MS and bioinformatics– thus providing more accurate results and resulting in a better understanding of the components of the proteome of the hepatopancreas, involved in the immune responses mounted by resistant snails compared to susceptible snails against the digenean *S. mansoni* parasite.

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