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Genetic Diversity, Multiplicity of Infection and Population Structure of *Schistosoma mansoni* Isolates of Ethiopia within Human Hosts

A Dissertation Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology (Biomedical Sciences)

By

Mulugeta Aemero

October, 2011

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ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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Dedication

To

My beloved children: Amhasilassie Mulugeta, Yonas Mulugeta and Biniyam Mulugeta

&

My late best friend Akale Kirstos Taddesse whose untimely death had broken the hearts of many

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Abbreviations

5HT 5-hydroxytryptamine

A Allele

An Allelic richness

CD Cluster of differentiation

CI Confidence interval

DHEAS Dehydroepiandrosterone sulphate

DNA Deoxyribonucleic acid

EPG Egg per gram

GCP Gynaecophoral canal protein

GPx Glutathione peroxidase

H_E Expected heterozygosity

H_O Observed heterozygosity

IL Interleukin

INF Interferone

MHC Major histocompatibility complex

MNA Mean number of alleles

OR Odds ratio

PBWT Periportal thickening/Fibrosis

PCA Principal Component Analysis

PCR Polymerase Chain Reaction

Prx Peroxiredoxin

PZQ Praziquantel

q.s.p. (quantitié suffisante pour) quantity sufficient for

RAPD Randomly amplified DNA

SCHi intestinal schistosomiasis

SCHu urinary schistosomiasis

SmInAct *Schistosoma mansoni* Inhibin/Activin

SmTYR1/ SmTYR2 *Schistosoma mansoni* tyrosinase 1 and tyrosinase 2

SmTβRII *Schistosoma mansoni* TGF-β type II receptor

SNP Single nucleotide polymorphism

SPSS Statistical Package for the Social Sciences

TGF Transforming growth factor

WHO World Health Organization

Abstract

Schistosomiasis is a chronic parasitic disease, affecting over 200 million people and causing over 300,000 deaths per year mainly in sub-Saharan Africa. Strains from the same or different geographical locations have shown differences in egg production, infectivity, pathogenicity and susceptibility to chemotherapy. The objective of this study was to assess the dynamics, genetic polymorphism and structure of *Schistosoma mansoni* isolates from four endemic foci in Ethiopia. In a cross-sectional study involving 1,073 study participants from Kemissie (10°43'30''N, 039°04'20''E), Wondo Genet (07°05'35''N, 038°36'66''E), Ziway (07°56'37''N, 038°43'25''E), and Sille-Elgo (05°28'39''N, 037°26'02''E), stool specimens were collected and examined for *Schistosoma mansoni* infection using Kato method. Stool specimens were again collected from 91 positive individuals for molecular studies. The overall prevalence and intensity of *Schistosoma mansoni* infection from study subjects of Kemissie, Wondo Genet and Ziway was found to be 60.48% and 273 eggs per gram of stool, respectively. Out of 288 miracidia genotyped at a molecular level, 164 unique alleles were counted for all the 11 loci typed from Kemissie (127), Sille-Elgo (102), Wondo Genet (123) and Ziway (94). At a population level, the mean number of alleles per locus, allelic richness, expected heterozygosity in Hardy–Weinberg equilibrium and pairwise F_{ST} values ranged from 8.5 to 11.5, 3.46-20.8, 0.66–0.73 and 3.57–13.63%, respectively. The Bayesian structuration showed 67.5-87.3% genetic differentiation of the study populations. The PCA and the Bayesian STRUCTURE had shown four clusters of population. Generally, high level of genetic diversity and population differentiation characterized the *S. mansoni* isolates of Ethiopia. Genetic diversity and multiplicity of infection with *Schistosoma mansoni* isolates among and within individual subjects from the four endemic areas of Ethiopia at infrapopulation level also showed a value of 3.09 to 7.55, 1-1.96, 0.59–0.73 and 0.1763–0.4989, mean number of alleles per locus, allelic richness, expected heterozygosity in Hardy–Weinberg equilibrium and $F_{IS,}$ respectively. Mean estimated genetically unique adult worm pairs within hosts ranged from 66-92% revealing the occurrence of infection of a single host with multiple *S. mansoni* strains. The data also indicated the occurrence of inter- and intra-host genetic variations. Based on previous suggestions about the East African origin of *S. mansoni*, the present study enabled us to speculate that Ethiopia could be the probable country of origin for

schistosomes. However, definite conclusion requires further investigation using other genetic markers.

Key words: Ethiopia, *Schistosoma mansoni*, Microsatellite, Genetic diversity, Population structure

1. INTRODUCTION

Schistosomiasis is one of the chronic but neglected tropical diseases which is endemic in over 74 developing countries, affecting over 200 million people and causing over 300, 000 deaths per year mainly in sub-Saharan Africa (van der Werf *et al*., 2003) leading to the loss of 1·53 million disability-adjusted life years (Gryseels *et al*., 2006). It ranks second only to malaria among the parasitic diseases with regard to the number of people infected and those at risk. Typically, schistosomiasis is a disease affecting rural communities; particularly those dependent upon irrigation to support their agriculture (Steinmann *et al*., 2006). The problem of schistosomiasis became much more significant in the 19th century, when the combination of new irrigation projects and population increases led to a higher probability of exposure to the parasite. In particularly, more than 85% of the current global burden is concentrated mainly in the rural parts of sub-Saharan Africa (WHO, 2002; Utzinger & Keiser, 2004; Steinmann *et al*., 2006). Besides its public health impact, the disease has an adverse effect on socio-economic development in that it causes incapacity to work and premature death leading to decreased productivity and economic loss (King *et al*., 2005). Heavy infections contribute to anemia and can retard children's growth, physical activity, and cognitive function (Wang *et al*., 2004).

Infection occurs through contact with water containing the infective free-swimming cercariae. Cercariae utilize an elastase proteolytic enzyme produced in the head region to penetrate the skin of humans (van Velthuysen and Florquin, 2000). They shed their bifurcated tails and enter capillaries and lymphatic vessels *en route* to the lungs. After several days, the young worms, or schistosomula, migrate to the portal venous system, where they mature and unite. These worm pairs then migrate to their ultimate vascular bed, i.e., superior mesenteric veins in the case of *Schistosoma mansoni*, inferior mesenteric and superior hemorrhoidal veins in the case of *S. japonicum*, or the vesical plexus and veins draining the ureters in the case of *S. haematobium*. Egg production commences 4 to 6 weeks after infection and continues for the life of the worm. Eggs are deposited in the vein lumen (McManus and Loukas, 2008). The ensuing granulocytic response to the egg secretions leads to the destruction of the tissue, and the eggs are

discharged in the lumen of the gut or bladder, from where they are shed with urine and faeces (van Velthuysen and Florquin, 2000).

Schistosomes are known to survive in the host for long periods, despite the development of concomitant immunity. A key characteristic of schistosomiasis promoting this longevity is the development of several mechanisms by which the parasites evade or modulate the host's immunological attack (Loukas *et al*., 2001). Since parasites and hosts may co-exist for lengthy periods, this might indicate that some degrees of immunological tolerance or anergy become induced. It is because of the longevity of infection that the disease causes serious chronic morbidity rather than acute mortality. In addition to the longevity of individual infections, there is also persistence of infection due to repeated re-infection throughout the host life (Mduluza *et al*., 2001).

Current methods of control of schistosomiasis include mollusciciding, biological control of the intermediate snail hosts, the development of drugs to kill the adult worms, provision of clean water and health education. Since an effective vaccine against schistosomiasis is lacking, the emphasis today is placed on the drug praziquantel (PZQ) (Fenwick *et al*., 2006). Though there are challenges on the control of schistosomiasis due to change in climate, human migration, construction of new dams and irrigation schemes as well as hybridization of sister schistosomes in the snail vector, advances in genomics will have great advantage in understanding the host-parasite interaction and development of new control strategies including the development of effective drugs and vaccines.

Controlling schistosomiasis requires an understanding of evolutionary history, host-parasite interactions and epidemiology. Accurate species and strain identification is a key to successful epidemiological studies, monitoring and control. Increasingly, molecular-based methods are being developed and implemented for accurate, rapid and high throughput identification (Zarowiecki *et al*., 2007). Molecular approaches, microsatellite analyses and mitochondrial DNA sequencing, are providing new insights into the genetic diversity of schistosomes and their intermediate snail hosts. New sampling procedures to capture DNA of eggs and larval stages of schistosomes in field situations are facilitating more detailed and ethically advantageous studies on parasite heterogeneity. Knowledge of the genetic diversity of schistosome and snail populations adds a further dimension to the monitoring and surveillance of the disease, and the implementation of new molecular-based approaches will be of increasing importance in

helping to assess the impact of schistosomiasis control strategies (Rollinson *et al.*, 2009).

In snail hosts, the physiological transformation of schistosomes is established mainly by interfering with the two regulatory systems, the innate immunological defense and the neuroendocrine systems, by which they avoid immune-based clearance within the snail hosts (de Jong-Brink *et al.*, 2001). Within the tissues of the mammal, experimental evidence suggests that the parasite not only actively evades immune responses but also exploits the hormonal microenvironment to support schistosome establishment, growth, and reproduction (Escobedo *et al.*, 2005). In addition to this, the schistosome thrives through constant communication with the hosts by signaling mechanisms involving sensors, surface glycocalyx, surface membrane, and internal organs of the parasite (Kusel *et al.*, 2007).

Of a number of compounds with varying efficacy, only one drug, praziquantel, is currently effective against all species of schistosome, and it plays a key role in population-based disease-control programs in most endemic countries (Han *et al.*, 2009).

In Ethiopia two forms of human schistosomiasis occur, intestinal schistosomiasis caused by *Schistosoma mansoni* and transmitted by *Biomphalaria pfeifferi* and *B. sudanica,* and urinary schistosomiasis caused by *Schistosoma haematobium* and transmitted by *Bulinus abyssinicus* and *Bu. africanus* (Lo *et al.*, 1988). Most transmission sites and *S. mansoni* infections are in agricultural communities along streams between 1300 and 2000 m altitude infested with *Biomphalaria pfeifferi*. *S. mansoni* transmission above 2200 m and below 800 m is precluded in many parts of Ethiopia by low and high water temperatures, respectively. Endemic *S. haematobium* appears to be confined in its distribution to lowlands below 800 m altitude. The highly focal distribution of *S. haematobium* transmission is largely due to the nonsusceptibility of most bulinid snails to the Ethiopian strain of the parasite and low water temperatures in the highlands (Kloos *et al.*, 1988).

A community-based study involving 2,451 subjects (mean age, 18.8 ± 15.3 [SD] years) from four endemic sites in Ethiopia indicated an overall prevalence of 65.9% *Schistosoma mansoni* infection (Berhe *et al.*, 2007). According to Chitsulo and

colleagues (2000), it is estimated that 29.89 million people are at risk while 4 million people are infected with *S. mansoni* in Ethiopia.

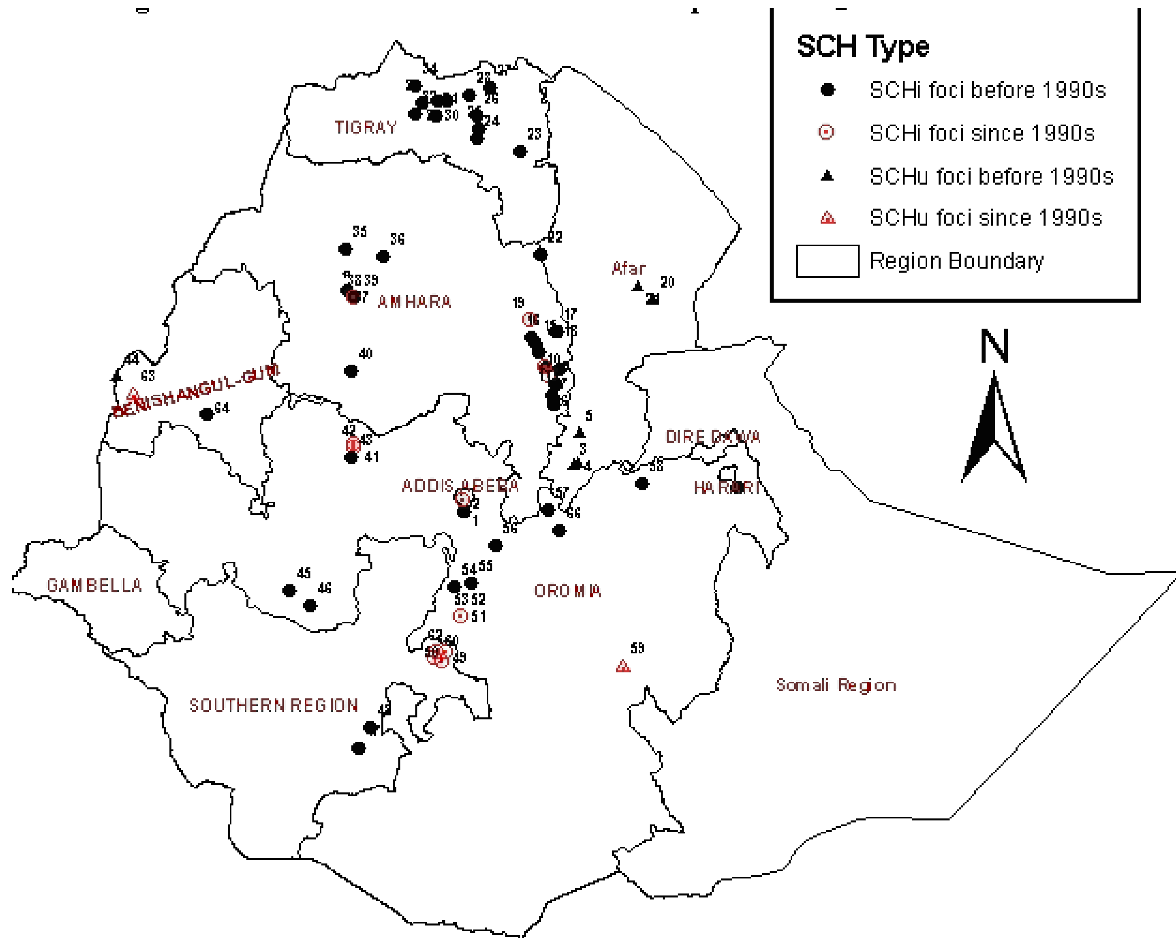

Figure 1. Schistosomiasis endemic foci in Ethiopia

(Source Erko, B. 2010)

Although different epidemiological surveys have been conducted by different researchers in Ethiopia, molecular characterization of *S. mansoni* is not conducted in the country. It is known that strains from the same or different geographical locations have shown differences in egg production, infectivity, pathogenicity and susceptibility to chemotherapy (Allen *et al.*, 2002). In particular, elucidating the distribution of parasite genetic diversity is critical to the understanding and predicting of disease epidemiology. One of the primary reasons for studying parasite population genetics is to understand demographic parameters, such as gene flow and population size, which are not readily observable using conventional ecological methods. These insights allow inferences regarding the patterns of parasite transmission and recruitment within the environment

(Thiele *et al*., 2008). Thus, based on this fact it is hypothesized that there will be genetic diversity of Ethiopian *S. mansoni* isolates that could have its own population structure which may also result variation in terms of infectivity and pathogenicity in different endemic areas of Ethiopia. Thus, the purpose of this study is to determine the genetic diversity, multiplicity of infection and population structure of *Schistosoma mansoni* isolates of the Ethiopian parasite.

1.2 Objectives of the Study

General Objective

The general objective of the study was to assess the dynamics, genetic polymorphism and structure of *Schistosoma mansoni* isolates from selected localities in Ethiopia

Specific Objectives

1. To assess the genetic polymorphism of *S. mansoni* in different endemic areas
2. To assess the intra-host genetic polymorphism of *S. mansoni*
3. To determine the genetic structure of *S. mansoni* isolates from the study areas
4. To evaluate and compare the intensity of *S. mansoni* infection in different geographical locations
5. To determine the level of schistosomiasis transmission in the study areas

1.3 Literature Review

Bilharzia, also later termed schistosomiasis, was named after Theodor Bilharz, a German pathologist who identified the worms in 1851 (McManus and Loukas, 2008). It is a primarily tropical parasitic disease caused by one or more of five types of flatworms or blood flukes known as schistosomes, belonging to the family Schistosomatidae. The family can be divided into three sub-families, the Schistosomatinae, Bilharziellinae and Gigantobilharziinae. Of the 12 genera within the family, seven are confined to birds and five to mammals. Nineteen species that parasitise humans, livestock and wild mammals are currently recognized in the genus *Schistosoma*. The species have been subdivided into four groups according to the morphological characteristics of their eggs and their life-cycle, including their intermediate host specificity (Southgate *et al*., 1998).

The *mansoni* group comprises the lateral-spined egg species developing in pulmonate mollusks of the genus *Biomphalaria*. It includes *Schistosoma mansoni, S. rodhaini, S. edwardiense* and *S. hippopotami.* The *haematobium* group corresponds to the terminal-spined egg species, which develop mainly in snails of the genus *Bulinus*. It includes *Schistosoma haematobium*, *S. bovis*, *S. intercalatum*, *S. mattheei* and *S. margrebowiei*. The *japonicum* group includes the vestigial-spined egg species, *Schistosoma japonicum*, *S. mekongi* and *S. malayensis*. The *indicum* group is formed of four species whose eggs, although of variable morphology, have a terminal spine; *Schistosoma indicum, S. spindale, S. nasale* and *S. incognitum*, are members that parasitise ungulates (Southgate *et al*., 1998).

The schistosome species that infect humans are *Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. haematobium*. The first four species have well described associations with chronic hepatic and intestinal fibrosis and their attendant consequences. *Schistosoma haematobium* infections cause fibrosis, stricturing, and calcification of the urinary tract (McManus and Loukas, 2008). Studies have agreed that the one species thus far examined of *Orientobilharzia,* a genus of Asian mammalian schistosomes, nests within *Schistosoma* (Snyder and Loker, 2000).

A number of animal-specific schistosome species (e.g., *S. bovis* or *S. margrebowiei*) may occasionally accidentally infect humans. The cercaria stage parasites of a large number of other non-human, particularly bird infecting schistosomes (e.g., *Trichobilharzia* sp.) may penetrate human skin but then die. These can give rise to an allergic condition called swimmer's itch, or cercarial dermatitis, a reaction caused by the release of antigens by the dying parasites in the skin (McManus and Loukas, 2008).

Schistosoma mansoni occurs in much of sub-Saharan Africa, northeast Brazil, Surinam, Venezuela, the Caribbean, lower and middle Egypt, and the Arabic peninsula. *S. haematobium* is present in much of sub-Saharan Africa, the Nile valley in Egypt and Sudan, the Maghreb, and the Arabian Peninsula. *S. japonicum* is endemic along the central lakes and River Yangtze in China, in Mindanao, Leyte, and some other islands in the Philippines, and in small pockets in Indonesia. *S. mekongi* occurs in the central Mekong Basin in Laos and Cambodia, and *S. intercalatum* is found in pockets in west and central Africa (Gryseels *et al*., 2006).

Schistosoma mansoni infects more than 83 million people in 54 countries, mostly in tropical Africa causing intestinal schistosomiasis which results in pathology, morbidity, and even death (Lambertucci *et al*., 2000). Based on estimate in sub-Saharan Africa, 54 million people are considered to be infected with *S. mansoni*, resulting in blood in stools in 4.4 million, hepatomegaly in 8.5 million, and an annual mortality of 130, 000 (van der Werf *et al*., 2003). Adult *S. mansoni* worms living in the veins surrounding the intestine produce eggs that either pass with the faeces to continue the lifecycle, or become entrapped in the tissues, provoking granulomatous reactions, fibro-obstructive disease in the liver and other organs and portal hypertension (Lambertucci *et al*., 2000).

Schistosomiasis is characterized by focal epidemiology and overdispersed population distribution, with higher infection rates in children than in adults (Gryseels *et al*., 2006). Although there are a number of diagnostic methods for the diagnosis of schistosome infections, quantitative Kato-Katz technique is preferred for the diagnosis of intestinal schistosome infection (Legesse & Erko, 2007). Treatment involves the administration of Praziquantel at a dose of 40mg/kg of body weight. Development of vaccine is still an ongoing research (Abdulla *et al*., 2007).

1.3.1 Anatomy and Morphology of Schistosomes

Adult schistosomes are white or grayish worms of 7-20 mm in length with cylindrical body that features two terminal suckers, a complex tegument, a blind digestive tract, and reproductive organs. Unlike other trematodes, schistosomes have separate sexes. The male's body forms a groove or gynaecophoric canal, in which it holds the longer and thinner female (Gryseels *et al*., 2006). The surface of the adult worm is covered by a living syncytial layer, the tegument, bounded by a complex multi laminate surface. This comprises a normal plasma membrane overlain by a secreted bilayer, the membranocalyx. The tegument is attached to underlying cell bodies by narrow cytoplasmic connections. The nuclei, ribosomes, endoplasmic reticulum, and Golgi apparatus are located in these cell bodies, and their vesicular products, the discoid bodies and multilaminate vesicles, traffic to the tegument syncytium via the connections (Braschi and Wilson, 2006). Adult females lay eggs. The eggs of schistosomes vary in their size and shape, their lateral or terminal spine. The *S. mansoni* egg measures approximately 140 by 61 µm and has a prominent lateral spine. The *S. haematobium* egg is approximately 150 by 62 µm with a prominent terminal spine. Both are ovoid. The *S. japonicum* egg is round, has a lateral spine that is often obscured, and is smaller than the other two types of eggs (60 by 100 µm) (Ross *et al*., 2002). The ova contain a characteristic ciliated miracidia that hatch and infect susceptible snails, which develop into a bifurcated cercariae having head and tail.

1.3.2 Development and Metabolism in Schistosomes

Schistosome parasites are multicellular eukaryotic organisms with a complex life cycle that involves mammalian and snail hosts (LoVerde *et al*., 2007). The life cycle is completed when the eggs passed in the faeces hatch, releasing miracidia that, in turn, infect specific freshwater snails (McManus and Loukas, 2008). After two generations of primary and then daughter sporocysts within the snail, asexually produced cercariae are released. Intramolluscan development of larval digenetic trematodes is a complex process involving initial infection of the snail host by the free-swimming miracidium, its subsequent transformation to a parasitic primary sporocyst stage, followed by asexual reproduction and release of secondary sporocysts or rediae, and finally the

eventual formation and release of cercariae, the next free-swimming stage in the life cycle (Wu *et al*., 2009). The cercariae start leaving the snail 4-6 weeks after infection and spin around in the water for up to 72 hours seeking the skin of a suitable definitive host. Cercaria shedding is stimulated by light and occurs mainly during the daytime. One snail, infected by one miracidium, can shed thousands of cercariae every day for months (Gryseels *et al*., 2006).

The vertebrate endoparasitic life cycle begins when schistosome cercariae penetrate the skin of a host. By about three hours after penetration, the trilaminate outer membrane of the cercariae is replaced by heptalaminate membrane. The new membrane is formed at about 30 minute after penetration from small membranous vacuoles which originate in subtegumental cells and pass into the tegument. Schistosome parasites stay in the skin and after two days enter the circulation and move to the lungs where they stay for further development. They transform into schistosomules and establish as an endoparasites (LoVerde *et al*., 2007). The parasites leave the lungs and move to the portal circulation of the liver where the male and female worms mate. These worm pairs then migrate to their ultimate vascular bed, i.e., superior mesenteric veins (*S. mansoni*), inferior mesenteric and superior hemorrhoidal veins (*S. japonicum*), or the vesical plexus and veins draining the ureters (*S. haematobium*). Egg production commences 4 to 6 weeks after infection and continues for the life of the worm in the definitive host (McManus and Loukas, 2008). This stage of the parasite life cycle takes place in a milieu of host signaling molecules. To continue its development it must take advantage of signals from the host (LoVerde *et al*., 2007).

Several schistosome genes are involved in transport, biosynthesis, cell adhesion and binding, cellular communication and signal transduction. In addition, analysis of the transcriptomes of *S. mansoni* and *S. japonicum* suggested the presence of genes associated with eukaryotic- and metazoan- specific processes, anterior-posterior axis differentiation, dorsoventral patterning, neural process, motility, sexual differentiation and maturation, longevity, parasitism, immune evasion and stress responsiveness (Brindley, 2005). Intriguingly, schistosomes also encode mammalian-like receptors for insulin, progesterone, cytokines and neuropeptides, suggesting that host hormones, or endogenous parasite homologues, might orchestrate parasite development and

maturation and that schistosomes modulate antiparasite immune responses through inhibitors, molecular mimicry and other evasion strategies (Brindley, 2005). The body wall of mammalian-parasitic schistosomes is a dynamic host-interactive layer involved in nutrition, immune evasion and modulation, excretion, osmoregulation, sensory reception and signal transduction (Jones *et al*., 2004).

During development in multicellular organisms, members of transforming growth factor-beta (TGF-β) superfamily play pivotal role. The TGF-β superfamily comprises a large number of structurally related polypeptide growth factors produced by diverse cell types, capable of regulating a vast array of cellular processes including cell proliferation, lineage determination, differentiation, motility, adhesion and apoptosis (reviewed in Loverde *et al*., 2007). To this effect, TGF-β signaling requires ligand binding to a specific surface receptor, TGF-β type II receptor.

Gynaecophoral canal protein (GCP) is a protein whose expression in male worms is limited to the gynaecophoric canal and is implicated in female reproductive maturation. *GCP* expression was found to be regulated by human TGF-β. Knocking down the expression of SmTβRII resulted in a concomitant reduction in the expression of *GCP,* providing evidence for the direct involvement of SmTβRII-mediated, host TGF-β–induced regulation of schistosome gene expression. This study implicates the TGF-β signaling pathway in worm pairing, a prerequisite for female egg production (Osman *et al*., 2006). In another study although *S. mansoni* TGF-β homolog, *S. mansoni* Inhibin/Activin (SmInAct) is expressed in adult male and female parasites, and in eggs, its expression is localized to the reproductive organs of female parasites indicating the role of this gene in egg production. A role for SmInAct in reproduction was supported by analyses of female parasites recovered from infertile infections, in which SmInAct protein was undetectable (Freitas *et al*., 2007).

Schistosomes feed on blood through anaerobic glycolysis (Gryseels *et al*., 2006). During development and egg production, adult intravascular schistosome parasites consume up to 33,000 red cells per hour and significant volumes of blood plasma with its rich source of host proteins (Delcroix *et al*., 2007). In a study conducted in Uganda, it had been indicated that anemia is likely to represent a valuable marker for morbidity caused by heavy infection with *S. mansoni*, provided that other likely causes, such as

hookworm, dietary iron intake, and malaria, are taken into consideration (Koukounari *et al*., 2006).

Despite possessing a mouth and functional gut, glucose is taken up by the parasites across their outer body surface or tegument. All cells appear to move glucose molecules across specific glucose transporter proteins (El-Ansary, 2003). Three different *S. mansoni* facilitated diffusion glucose transport proteins were identified on the basis of cloned cDNAs, designated, SGTP1, SGTP2and SGTP4. Western blot analysis shows that SGTP1 is present in all schistosome life stages, while SGTP4 is detected only in mammalian-stage parasites, where it appears upon cercarial infection (Skelly and Shoemaker, 1996). SGTP4 is undetected in cercariae, but noticeable 15–30 min after the initiation of cercarial transformation into the schistosomulum. Presumably, the rapid appearance of glucose transport proteins at the surface of the invading schistosomulum reflects the crucial importance of swift sugar uptake to replenish depleted reserves and ensure parasite maturation (Skelly *et al*., 1998).

Maturating and adult schistosomes obtain amino acids by catabolizing haemoglobin from ingested host erythrocytes for growth, development and reproduction. A variety of proteinases, such as cathepsin B (Sm31), cathepsin L1, cathepsin L2, cathepsin D, cathepsin C and legumain (Sm32) are believed to be involved in this process (El-Ansary, 2003). Schistosomes cannot synthesize cholesterol or fatty acids *de novo* and seem to obtain these nutrients from the host. Schistosomes use serum lipoproteins, blood-group glycolipids, histocompatibility antigens and other host molecules to circumvent deficits in their metabolic pathways and/or to deflect immune recognition (Brindley, 2005).

1.3.3 Reproduction and Cercarial Invasion in Schistosomes

In female schistosomes, an intimate and permanent association with the male is necessary for reproduction to occur. Virgin females that grow up in the absence of males differ considerably in size from those recovered from mixed infections in which they were paired with males and do not develop eggs. Similarly, when egg-laying female schistosomes are separated from their male partners and surgically reimplanted into the host, they stop laying eggs and begin to regress reproductively to the immature

state. However, if such regressed females are allowed to pair again with males, normal reproductive activity resumes even after months of regression. By contrast, reproductive development in the male is not significantly influenced by the presence of the female (Erasmus, 1973). This is because the reproductive development of the male is not dependent on signals from the female.

In schistosomes the females produce hundreds (African species) to thousands (oriental species) of eggs per day. Each ovum contains a ciliated miracidium larva which secret proteolytic enzymes that helps the eggs to migrate into the lumen of the bladder (*S. haematobium*) or the intestine (other species including *S. mansoni*) (Gryseels *et al*., 2006). Egg production is responsible for life cycle progression and host immunopathology during schistosomiasis. Fitzpatrick and colleagues (2007) characterized two *Schistosoma mansoni* products, tyrosinase 1 and tyrosinase 2 (SmTYR1/ SmTYR2) and show that their diphenol oxidase enzyme activities are critical for eggshell formation and production. The genes encoding these bi-functional enzymes (monophenol and diphenol oxidases) result from a duplication event that likely occurred before speciation and exist in the parasite's genome as multiple copies, which are linked and localized to chromosomes 4 and W (female-specific chromosome). SmTYR1/SmTYR2 transcription and diphenol oxidase action are developmentally regulated with most enzyme activity localized to the eggshell–producing cells contained within the vitellaria of adult female worms.

Schistosomes modulate the human immune system to survive in the blood for years and subvert it to facilitate egg migration to continue their lifecycle (Hoffmann and Dunne, 2004). *Schistosoma mansoni* worm pairs are not only able to produce hundreds of eggs daily in the immunologically hostile blood environment, but also provoke host immune responses that are essential for the passage of their eggs from the blood, through the host gut wall, to the environment outside. The eggs are excreted with the faeces and can stay viable for up to 7 days. On contact with water, the egg releases the miracidium. Low osmolarity and bright sunlight are then the stimuli that promote egg hatching, so that the miracidial stage within the eggs can emerge to find and invade the aquatic snail host (Pearce, 2005). The miracidium searches for the intermediate snail hosts (*Biomphalaria* species), guided by light and chemical stimuli. After penetrating the

snail, the miracidia multiply asexually into multicellular sporocysts and later into cercarial larvae with embryonic suckers and a characteristic bifurcated tail (Gryseels *et al*., 2006).

The penetration of cercariae through mammalian skin is a highly complex process that requires chemical signals from the host skin to initiate attachment and subsequent penetration. Each of the schistosome species studied so far shows its individual strategy of host-finding and host invasion, and this behavioral diversity seems to reflect adaptations to different ecological conditions within the habitats in which the parasites identify and invade their specific hosts. A particularly complex host-finding behavior is displayed by cercariae of *Schistosoma mansoni*. They show a high sensitivity and specificity primarily to chemical host signals during their host-finding (Haas *et al*., 2008). When *S. mansoni* cercariae have entered their host's active space, they swim towards the skin surface by responding to host-emitted fatty acids, L-arginine, and L-arginine containing peptides. The migration of the cercaria (developing into a schistosomulum) in the skin is influenced by ceramides, arginine and linoleic acid (Haas *et al*., 2008). The cercariae penetrate intact skin and through a series of morphological, membrane, biochemical, and antigenic changes transform into schistosomula (Stavitsky, 2004). In this cercarial transformation, the parasite switches from its immunesensitive to an immune-refractory state by completely replacing and remodeling the surface membrane of the tegumentary syncytium (Jones *et al*., 2004). The speed at which cercariae can penetrate skin and find a vascular portal varies considerably. A few cercariae can make this journey within five minutes. However, most cercariae wander for extended periods of time, some as long as 24 hr. After 24 hr, exhaustion of glycogen stores and acetabular secretions will make successful infection unlikely (McKerrow and Salter, 2002).

When entering the dermis, cercariae must find a small venule or a lymphatic vessel for gaining access to the vascular space. Migration through the extracellular matrix of the dermis is also assisted by proteolytic activity, and tunnels rimmed with degraded ends of fibrillar macromolecules, such as collagen and elastin, are present. Penetration into a small venule also requires migration through another basement membrane and disruption of the endothelial cell layer. In addition to acetabular gland secretions, the loss of tails and directed migration, cercariae lose their surface glycocalyx after

invasion. The glycocalyx is a complex carbohydrate coat, which protects the cercariae against osmotic shock in the aquatic environment before mammalian host entry (McKerrow and Salter, 2002). After days in subcutaneous tissue, the schistosomula travel to the lungs, where they undergo adaptations for intravascular migration. From the lung the schistosomula are distributed to all organs. Most schistosomula eventually reach the liver, where they attain sexual maturity and enter the portal venous system. The adult worms mate and then travel to the small mesenteric venules. The females release ova, which migrate through the venule wall, the lamina propria, and the gut epithelium into the lumen and ultimately to the outside environment with the faeces (Stavitsky, 2004) completing the lifecycle.

1.3.4 Adaptation of Schistosomes as Endoparasite

Parasites have been shown to employ a variety of mechanisms to enable them to live in the environment of the mammalian host. Host adaptation involves a range of interacting molecules and behavioral phenomena (Kusel *et al.*, 2009).

Schistosoma mansoni parasites reside in the circulatory system of their human host. In this aerobic environment, worms must have effective mechanisms to maintain cellular redox balance, including immune-generated oxygen radicals as well as those generated in the parasite during respiration and the breakdown and consumption of host hemoglobin. Addition of one electron to O_2 produces superoxide, which is rapidly dismutated to O_2 and H_2O_2 by superoxide dismutase. The H_2O_2 formed is itself able to diffuse and cause cellular damage and must be neutralized to prevent the formation of the more damaging hydroxyl radical. In many organisms, intracellular H_2O_2 is eliminated by catalase, glutathione peroxidase (GPx), and/or peroxiredoxin (Prx). Schistosomes have abundant superoxide dismutase but completely lack catalase and have relatively low levels of GPx, whose role may be to protect bio-membranes from oxidative damage (Sayed *et al.*, 2006).

The biogenic amine serotonin (5-hydroxytryptamine: 5HT) is a widely distributed neuroactive substance of vertebrates and invertebrates. Among parasitic flatworms, particularly in *Schistosoma mansoni*, 5HT is an important modulator of neuromuscular function and metabolism. Previous work has shown that schistosomes take up 5HT from host blood via a carrier mediated mechanism. This transport is thought to

contribute to the control of schistosome motility in the bloodstream and is essential for survival of the parasite (Patocka and Ribeiro, 2007). Moreover, transcripts for echicetin-like molecules that affect hemostasis and prevent thrombosis were found in adult *S. mansoni*. Adult worms also expressed apyrase (CD39/ATP-diphosphohydrolase), an enzyme involved in platelet aggregation and thromboregulation that is localized to the tegument, possibly indicating the capacity to inhibit platelet activation (Almeida *et al*., 2003).

Kusel and colleagues (2007) in their review summarized the possible functions of the glycocalyx on the adult schistosome surface as a means: (a) for the prevention of adhesion of erythrocytes and other cells, (b) to act as a sensor for the blood flow, (c) to transmit adhesion forces through the membrane when the sucker adheres to the endothelial cell of the portal vein, (d) to modulate the immune responses, (e) to mediate contact signals between male and female and perhaps between individuals, leading to quorum sensing, (f) to experience antibody binding, with or without C fixation, (g) for prevention of platelet aggregation, and(h) creation of a surface microenvironment.

The interaction of *Schistosoma mansoni* with its host's immune system is largely affected by multiple specific and non-specific evasion mechanisms employed by the parasite to reduce the host's immune reactivity. Fishelson (1995) had identified and studied four proteins that play role in immune evasion. (1) m28- A 28kDa membrane serine protease that cleaves iC3b and can thus restrict attack by effector cells utilizing complement receptors (especially CR3). (2) Smpi56-A 56kDa serine protease inhibitor binds covalently to m28 and to neutrophil's elastase and blocks their proteolytic activity. (3) P70-A 70kDa C3b binding protein. The postulated activity of P70 includes binding to C3b and blocking of complement activation of the C3 step. (4) SCIP-1-A 94kDa schistosome complement inhibitor shows antigenic and functional similarities to the human 18kDa complement inhibitor CD59. Like CD59, SCIP-1 binds to C8 and C9 and blocks formation of the complement membrane attack complex. Antibodies directed to human CD59 bind to schistosomula and potentiate their killing by complement.

1.3.5 Mechanism of *S. mansoni* Pathogenesis and Host Immune Interaction

Infection with *Schistosoma mansoni* results in progressive manifestations of clinical signs and symptoms. As reviewed by Gryseels *et al*. (2006), the acute pathology of *S. mansoni* results in Katayama fever in tourists (but not in chronically exposed population) which is a systemic hypersensitivity reaction against the migrating schistosomula, occurring a few weeks to months after a primary infection. The disease starts suddenly with fever, fatigue, myalgia, malaise, non productive cough, and eosinophilia. Schistosome eggs migrating through the intestinal wall provoke mucosal granulomatous inflammation, pseudopolyposis, microulcerations, and superficial bleeding. Most lesions are situated in the large bowel and rectum. The most common symptoms and signs are chronic or intermittent abdominal pain and discomfort, loss of appetite, and diarrhoea with or without blood whose frequency of symptoms is related to intensity of infection (Montor & Hall, 2007). Most chronic morbidity in schistosomiasis is not due to the adult worms but is related to the T-cell-dependent immune response of the host, which is directed against schistosome eggs trapped in tissues, mainly in the liver and intestines in the case of the intestinal forms (*S. japonicum* and *S. mansoni*) and in the bladder in the case of *S. haematobium*. The trapped eggs secrete a range of molecules leading to a marked $CD4^+$ T-cell programmed granulomatous inflammation involving eosinophils, monocytes, and lymphocytes, akin to a form of delayed type hypersensitivity (McManus and Loukas, 2008).

Fibrotic or chronic hepatic schistosomiasis develops years later in the course of infection, generally in young and middle-aged adults with long-standing intense infections and presumably, some form of immunogenetic predisposition. The disease results from a massive deposition of diffuse collagen deposits in the periportal spaces, leading to Symmer's pipestem fibrosis (Gryseels *et al*., 2006). This fibrosis leads in turn to progressive occlusion of the portal veins, portal hypertension, and splenomegaly. This fibrotic process takes 5-15 years, by which time the infection might no longer be present or detectable (Gryseels *et al*., 2006). Of the cytokines evaluated so far, IL-10 and IL-13 have demonstrated the strongest associations with the accumulation of fibrosis in humans (Montor & Hall, 2007).

During *Schistosoma mansoni* infection, to control inflammation associated with the presence of eggs in different locations inside the body, eggs inhibit toll like receptor mediated dendritic cell activation and activate innate and adaptive immune responses that result in the production of the anti-inflammatory cytokines, IL-4 and IL-10. These cytokines act together to regulate the development of potentially life-threatening inflammation that can be induced by conventional toll like receptor ligands and Th 1 cells (Pearce *et al*., 2004).

Studies have shown that host genetics is an important determinant of the intensity of infection and morbidity due to human helminths (Quinnell, 2003). Epidemiological studies of a number of parasite species have shown that the intensity of infection (worm burden) is a heritable phenotype. Human genome scans have identified a locus responsible for controlling *Schistosoma mansoni* infection intensity on chromosome 5q31-q33, and loci controlling *Ascaris lumbricoides* intensity on chromosomes 1 and 13, although the genes involved have not yet been identified. There is also evidence for genetic control of pathology due to *S. mansoni*, and linkage has been reported to a region containing the gene for the interferon-γ receptor 1 subunit (Quinnell, 2003). On the basis of different studies in man, it is accepted that a genetic predisposition to *Schistosoma mansoni*-associated pathology or to the capacity to mount protective responses exists and that the immune responses related with resistance to reinfection are of Th2 type (Khalife *et al*., 2000).

The immune response is intimately involved in the development of many of the pathological changes that accompany infection; infected individuals can have resistance to superinfection; and schistosomes survive for years in the host despite a strong immune response (Pearce & MacDonald, 2002). An unusual feature of schistosomiasis and other diseases caused by eukaryotic pathogens with complex life cycles is that the host is exposed to discrete life stages that express different subsets of genes. Thus during infection, the host is exposed sequentially to differing sets of antigens (Pearce *et al*., 2004). In the course of an infection, the immune response progresses through at least three phases. In the first 3-5 weeks, during which the host is exposed to migrating immature parasites, the dominant response is T helper 1 (Th1)-like. As the parasites mature, mate and begin to produce eggs at weeks 5-6, the response alters markedly; the Th1 component decreases and this is associated with the emergence of a strong Th2

response. This response is induced primarily by egg antigens (Pearce & MacDonald, 2002).

Schistosome eggs that do not successfully pass through the intestinal mucosa towards the lumen are usually carried by the portal vein blood flow to the liver until they stop inside small pre-sinusoidal vessels (Vennervald and Dunne, 2004).

Under normal conditions, $CD4^+$ T-cell responses are essential to prevent hepatocyte damage during *Schistosoma mansoni* infection (Pearce *et al*., 2004). Schistosome eggs secret molecules that are hepatotoxic (Dune *et al*., 1991; cited in Pearce *et al*., 2004) and Th-dependent antibodies along with Th cell-orchestrated granulomatous lesions that develop around tissue-trapped eggs cooperate to prevent these toxins from reaching surrounding hepatocytes (Deonhoff *et al*., 1981; cited in Pearce *et al*., 2004). Although macrophages, B lymphocytes, and dendritic cells all express major histocompatibility class II, it is dendritic cells that are responsible for initiating primarily Th cell responses. Dendritic cells proteolytically degrade acquired proteins in to peptides that will be complexed with MHC class II molecules at the surface of the cell. These peptide/MHC class II complexes are the ligands recognized by $CD4^+$ T-cell antigen receptors. T-cells activation by dendritic cells requires co-stimulatory signals in addition to those delivered through the T cell receptors. These signals are provided by molecules such as CD80 and CD86, which are expressed at the dendritic cells surface and ligate CD28 on T cells (Pearce and MacDonald, 2002). Up on initial activation, naïve Th cells enter the cycle and begin to produce the autocrine growth factor IL-2 plus one or more effector cytokines, such as interferon (IFN)-γ and IL-4 (Pearce *et al*., 2004).

Coincident with the development of the Th2 response, there are notable increases in plasma immunoglobulin (Ig) E levels and circulating eosinophil numbers, which reflect the production of interleukin (IL)-4 and IL-5, which are signature cytokines of Th2 cells. IL-4 and IL-5 help B cells to class switch Ig isotype production to IgE and act as a growth and survival factor for eosinophils, respectively (Mosman 1992; cited in Pearce *et al*., 2004). After about 3 months of mixed-sex infection, a significant diminution of the Th2 response is apparent, and this state of comparative hypo- responsiveness persists for the remainder of the infection. In contrast, in animals infected with single sex cercariae, where egg production does not occur, the worm antigen-specific Th1

response persists (Pearce *et al*. 2004). Studies concerning the effector mechanisms directed against *S. mansoni* have shown that antibodies are cytotoxic for schistosomula in the presence of effector cells, including eosinophils, macrophages and platelets (Khalife *et al*., 2000).

1.3.6 Genetic and Geographic Variation in *S. mansoni*

Genetic variations in schistosome populations can be expected to contribute to differences in infectivity, development and differentiation in intermediate and definitive hosts, drug sensitivity, pathogenicity, and immunogenicity (Han *et al*., 2009).

According to Han et al. 2009, availability of functional genomics data sets from genomic structure, transcriptomic, and proteomic profiles across different life-cycle developmental stages and genders, as well as the genetic manipulation of individual schistosome genes, should help clarify schistosome evolution, development, sexual dimorphism, parasite-host interactions, and immune evasion, as well as the molecular pathogenesis of schistosomiasis. These new methodologies and sequence data should also facilitate development of new intervention strategies, including candidate drug targets and vaccines, for schistosomiasis control.

Schistosome genome project was initiated by the World Health Organization in 1994 with the notion that the best prospects for identifying new targets for drugs, vaccines, and diagnostic development lie in schistosome gene discovery, development of chromosome maps, whole genome sequencing and genome analysis (LoVerde *et al*., 2004). The completion of the *S. mansoni* genome project had led to the post-genomic research developments Wilson *et al*., 2006).

In schistosomes, the **W** chromosome characterizes the heterogametic female-sex (**ZW**) whereas males are homogametic (**ZZ**) (Grevelding, 1999). For *S. mansoni*, the genetic information is contained on eight pairs of chromosomes, seven autosomal and one sex-determining pair. It has a haploid genome of 270 Mb. Chromosomes contain between 15 and 64 Mb of DNA and can be distinguished by differences in size, arm ratios, and amount of heterochromatin (C-banding pattern). It is estimated that the *S. mansoni*

genome contains between 15 000 and 25 000 genes. There are approximately 16,689 Expressed Sequence Tags obtained from diverse libraries representing different developmental stages of *S. mansoni* (LoVerde *et al*., 2004).

Using more than 2500 bp of mitochondrial DNA (mtDNA) from 143 *Schistosoma mansoni* parasites collected in 53 geographically widespread localities, Morgan & his colleagues (2005) had described the global phylogeography of *S. mansoni*. Considerable within-species mtDNA diversity was found, with 85 unique haplotypes grouping into five distinct lineages. Geographical separation, and not host use, appears to be the most important factor in the diversification of the parasite. East African specimens showed a remarkable amount of variation, comprising three clades and basal members of a fourth, strongly suggesting an East African origin for the parasite 0.30–0.43 million years ago, a period that follows the occurrence of the snail host.. Less but still substantial variation was found in the rest of Africa. A recent colonization of the New World is supported by finding only seven closely related New World haplotypes which have West African affinities (Morgan *et al*., 2005). A study of nine geographical isolates of *S. mansoni* (three from Egypt, three from Saudi Arabia and three from Puerto Rico) at the genotype level using random amplified polymorphic DNA showed that the Egyptian strain were closely related to Saudi strains but Puerto Rico strains clustered in different group. This suggested the presence of genetic diversity among different geographical strains of *S. mansoni* from different localities (Jamjoom, 2006).

Genetic heterogeneity of the parasite contributes to the observed phenotypic variation in this host–parasite interaction and might play a role in disease epidemiology. Current methods of DNA analysis allow for a quantitative assessment of this heterogeneity (Curtis & Minchella, 2000). A study on the distribution of schistosome genetic diversity within molluscan intermediate hosts conducted in Brazil has indicated that the genetic profile of *S. mansoni* individuals had revealed a diverse array of parasite genotypes among intermediate hosts. Moreover, snails were found harboring multiple miracidia. This degree of overdispersion combined with high levels of genetic variability facilitates multi-genotype transmission and helps maintain parasite genetic diversity (Minchella *et al*., 1995).

One important phenomenon of direct relevance to transmission is the degree of compatibility between local snails and schistosomes. The evidence from field study

suggests that absolute resistance is rare, and that the success or failure of an infection does not depend on the snail susceptibility/resistance status, but on the 'matched' or 'mismatched' status of the host and parasite phenotypes (Théron and Coustau, 2005). The basic idea here is that if a particular schistosome miracidium has a genetic constitution that appropriately matches a snail of a particular genetic constitution, a successful encounter will occur. If such a match does not occur, then the parasite will be recognized and destroyed. The low prevalence of infection noted following exposure to low doses of miracidia in natural snail–schistosome combinations suggests that such mismatches regularly occur in the field, and can play an important role in diminishing the number of patent infections achieved (Loker, 2005).

Intrasnail dynamics were investigated in the context of aggregation and kin selection theory to determine how relatedness and also sex influence host sharing and host exploitation. Cercarial production did not differ significantly between snails with one or two genotypes suggesting that mixed infections resulted in decreased individual fitness and provides a framework for reproductive competition. Co-infection patterns in snails were independent of parasite relatedness indicating that schistosomes were not aggregated according to their relatedness and that kin selection was not influencing host sharing (Steinauer *et al*., 2009).

Intra-host competition between parasite genotypes has been also predicted to be an important force shaping parasite ecology and evolution and has been extensively cited as a mechanism for the evolution of increased parasite virulence (Gower & Webster, 2005). Gower & Webster (2005) had compared within-host competitiveness between genetic strains of *Schistosoma mansoni* with high or low virulence to their intermediate snail host, *Biomphalaria glabrata*. Groups of snails were exposed to either one or the other of two parasite strains, or a mixed infection of both strains, and the resulting progeny were identified using a molecular marker. In two separate experiments investigating simultaneous and sequential infections, Gower and Webster demonstrated that the lifetime reproductive success of parasite strain of high virulence was reduced in the presence of a faster replicating parasite genotype of low virulence, regardless of whether it was in a majority or minority in the initial inoculum of the simultaneous exposure or of its relative position in the sequential exposure experiment. Thus, this demonstrated competition between parasite genotypes and asymmetry in competitive

success between parasite strains. Moreover, since the less virulent strain investigated had a competitive advantage, it is suggested that a high frequency of multiple infections could favor the evolution of less, rather than more, virulent parasites in this system.

Moreover, comparison of *Schistosoma mansoni* male and female performance and pathogenicity against *Biomphalaria glabrata* during the lifecycle had indicated the presence of variation in that male cercariae were significantly more infectious than female cercariae. Conversely, cercarial production and cercarial lifespan were significantly greater for females than for males; furthermore, females have a tendency to occur in molluscs of larger size (Boissier *et al*., 1999).

Hybridization of pathogen species is of epidemiological importance because it could potentially lead to the formation of new hybrid pathogens and also gene flow across species boundaries, termed ‘‘genome introgression’’. Hybridization can lead to the homogenization of species, the extinction of species, or adaptive evolution of either species as they acquire novel genes from the foreign gene pool (Steinauer *et al*., 2010).

Genetic introgression can also influence the evolution of a species. The introduction of foreign genes into a species pool could lead to novel changes that influence the biology or disease characteristics of human pathogenic schistosomes. Genetic introgression has been shown to occur between *S. mansoni* and *S. rodhaini* and is directional so the parasite of humans, *S. mansoni*, obtains genetic material from the parasite of rodents, *S. rodhaini* (Steinauer *et al*., 2008a). To date, only neutrally evolving genes have been investigated in the context of introgression, but it would be of great interest to determine whether functional genes are being shared among species and if this can lead to adaptive changes in pathogens. Despite their epidemiological importance, hybrid zones of schistosomes have yet to be fully characterized.

In nature, the distribution of schistosome species and their intermediate snail hosts, together with definitive host specificity are believed to restrict hybridization events from occurring. However, both natural (climate change) and anthropogenic changes (migration, deforestation, water development) can break down the ecological isolation barriers by facilitating the introduction of parasite species and strains into new areas, resulting in novel host-parasite and parasite – parasite interactions. Hybridization

between schistosome species can occur, but in most cases host specificity and ecology are thought to maintain species barriers. Huyse *et al.* (2009) had reported on the emergence of a new hybrid strain of schistosome found in northern Senegalese children, resulting from introgressive hybridization between a bovine and human parasite. This situation may have arisen due to the increased number of water contact sites commonly used by both cattle and people linked to recent major water development projects. Gene exchange following hybridization can lead to phenotypic innovations that can ultimately lead to the emergence of new diseases.

The demographic effects of hybridization, the amount of gene flow between species and adaptive introgression can all be investigated with the use of molecular markers and newly developed analytical tools (Steinauer *et al.*, 2010).
Long-term epidemiological studies in several natural host-parasite systems will allow for accurate modeling of infection and transmission dynamics. Estimates of parasite gene flow, derived from genetic analyses of the parasite sub-populations across many levels of the population structure hierarchy, have the potential to further our understanding of a number of disease processes: how super-infection (i.e. coinfection by multiple genotypes) may affect pathogenicity or the evolution of virulence, the genetic consequences of various control strategies for the parasite population, and the distribution of variation for genetic markers relevant to vaccine development throughout the range of this cosmopolitan parasite (Curtis *et al.*,2002).

Although successful interventions have greatly reduced the burden of disease in several regions (e.g. the Caribbean islands), there are many areas where *S. mansoni* prevalence remains high and concerns such as drug resistance and resurgence necessitate continued investigation. In particular, elucidating the distribution of parasite genetic diversity is critical to understanding and predicting disease epidemiology. One of the primary reasons for studying parasite population genetics is to understand demographic parameters, such as gene flow and population size, which are not readily observable using conventional ecological methods. These insights allow inferences regarding the patterns of parasite transmission and recruitment within the environment (Thiele *et al.*, 2008).

Schistosomes undergo sexual reproduction in human hosts and this presumably generates parasite genotypic diversity. Different schistosome genotypes may be brought together in space and time as: (1) male and female worms mate in the human host, (2) eggs representing the outcome of matings between different worm pairs are passed by defecation of the host and (3) larval stages coinfect the same snail host and simultaneously release cercariae available for infection of human hosts (Curtis *et al*., 2002).

1.3.7 Diagnosis, Treatment, Control and Vaccine Development for *S. mansoni*

Chronic infectious diseases are often easy to diagnose with regard to the detection of the infectious agent. In contrast, the evaluation of the intensity of the associated morbidity is difficult because of a lack of specific markers. For an efficient control of several chronic diseases, it seems appropriate not only to detect the presence of the infectious agent but also to diagnose the development of the related morbidity (Bonnard *et al*., 2004). Diagnosis is central to all aspects of schistosomiasis. Decisions on individual and community treatment, estimations on prognosis and assessment of morbidity, evaluation of chemotherapy and control measures all require the results from diagnostic tests. The microscopic examination of faeces can be used for diagnosis. Concentration methods, such as sedimentation in a glycerine solution or centrifugation in formolised ether are needed for detection of mild and light infections.

In the field, the fecal thick smear or Kato-Katz method is commonly used, because it allows quantification of the infections by egg counts, usually expressed as per gram of faeces (Feldmeier and Poggensee, 1993). Definitive diagnosis of *S. mansoni* infection requires the demonstration of eggs in feces. The Kato-Katz technique is currently the method of choice for parasitological diagnosis of *Schistosomiasis mansoni,* as it is relatively inexpensive and simple. However, it is observed that the sensitivity of parasitological methods diminishes when prevalence and intensity of infection are low, making these methods less appropriate for low-endemic areas and in post-treatment situations. In addition, parasitological methods are not sufficient for diagnosing recent infections in which worms have not yet started to produce eggs (the pre-patent period).

To address these shortcomings, antibody detection methods have been evaluated as adjuncts to fecal examinations. Comparative studies of parasitological and serological methods confirmed higher sensitivity of the latter (Doenhoff *et al*., 1993). The existence of cross-reactivity with other helminthic infections, however, and its low specificity after treatment due to the slow reduction of specific antibody levels, constitute great disadvantages of the immunodiagnostic methods (Pontes *et al*., 2002).

One possible solution to this problem could be the search for circulating antigens rather than antibodies. Several circulating antigens assays have been described in different laboratories (Barsoum *et al*., 1991; De Jonge *et al*., 1991). The high specificity is the main advantage that the detection of circulating antigens offers as compared with the antibodies determination and the disadvantages are low sensitivity in light infections and dependence on the production of monoclonal antibodies (Pontes *et al*., 2002).

Use of simple DNA extraction techniques and a rapid 2-step polymerase chain reaction (PCR) made possible to amplify *S. mansoni* DNA in human fecal and serum samples. The high sensitivity of the approach enabled the detection of the parasite DNA in fecal samples containing as few as 2.4 eggs per gram of feces, which makes it 10 times more sensitive than the Kato-Katz examination. The amplification reaction showed to be specific giving no cross-reaction with DNA from other helminths. The PCR assay may constitute a valuable alternative for the diagnosis of the *Schistosoma* sp. infection (Pontes *et al*., 2002). Ultrasound examination also effectively defines those with and without periportal thickening and can be used to detect the presence of Symmer's fibrosis. It allows investigators to determine the factors that favor development, progression, or regression of this complication (Cota *et al*., 2006).

Parasite population structure may influence transmission dynamics, host preference, and virulence, and all of these factors could significantly influence control strategies and drug design (Blank *et al*., 2009). Control of schistosomiasis is mainly achieved through repeated population based chemotherapy to reduce intensity of infection and thus morbidity (Gryseels *et al*., 2006). This method does not eliminate transmission and therefore requires regular drug treatment to be sustainable. However, with repeated selection pressure, there is a distinct probability that drug resistance will evolve in these organisms. The development of new tools and approaches to study the epidemiology

and evolution of these parasites is needed to monitor the impact of control programs and other environmental changes likely to affect schistosome distribution, transmission, and evolution (Steinauer *et al*., 2008b).

Praziquantel (PZQ), an acylated quinoline-pyrazine that is active against all schistosome species, is now the most widely used. It is the drug of choice for schistosomiasis and probably is the only highly effective drug currently available for treating schistosomiasis-infected individuals. It is mostly marketed as 600mg tablets, with a recommended standard regimen of 40mg/Kg bodyweight in a single dose (WHO, 2002). The mode of action of PZQ involves increasing the calcium uptake of the parasite, resulting in tegumental damage dislodging them from the mesenteric blood vessels to the liver, which is followed by the death and disintegration of the parasites. The subsequent granulomatous reaction that develops around the dead parasites causes the complete disintegration of the parasite within 2 weeks after treatment (Gnanasekar *et al*., 2009). Although there is not yet clear-cut evidence for the existence of PZQ-resistant schistosome strains, decreased susceptibility to the drug have been observed (Doenhoff and Pica-Mattoccia, 2006).

The parasite *Schistosoma* is known to exhibit variations among species, strains and genera in the levels of infectivity, pathogenicity and immunogenicity. These factors may differ among parasite populations according to the local epidemiological conditions. Diversity observed in *S. mansoni* from different geographical regions or within individuals of the same region can be determined by differences in the genotype of each parasite strain. Different natural populations of *S. mansoni* adapt to local conditions of the environment concerning not only abiotic parameters, but also the host species, and the characteristics of the host. Populations of the parasite may develop particular host-parasite interactions characteristic, which may result in different clinical histories of the disease. Therefore, control actions must consider local characteristics in order to produce efficient results for the reduction of transmission (Gentile and Oliveira, 2008).

A fresh broad-based schistosomiasis control initiative needs to be able to rapidly identify endemic regions, provide reliable estimates of populations at risk of the disease, facilitate optimal drug and health intervention delivery, and target the most

disadvantaged communities for provision of clean water and sanitation (Utzinger *et al*., 2003).

The endod (*Phytolacca dodecandra*)-based schistosomiasis mansoni control project was implemented in Ethiopia between 1994 and 1999. The aim was to develop an effective, cheap and sustainable method of controlling schistosomiasis. The study conducted by Abebe *et al*. (2005) indicates that endod is an effective schistosomiasis control agent, particularly when combined with chemotherapy. The spray method is the simplest and the most effective method for application of endod. Their data also indicate that endod soap, which had been delivered by a local agent and was linked to the basic laundry needs of the residents, was a reliable method. However, there is no currently on going control programme.

Schistosomes do not replicate within their mammalian hosts. Consequently, a non-sterilizing naturally or vaccine-acquired immunity could significantly decrease human pathology and disease transmission. Vaccination against schistosomes can be targeted towards the prevention of infection and/or to the reduction of parasite fecundity. A reduction in worm numbers is the "gold standard" for antischistosome vaccine development, with the migrating schistosomulum stage likely to be the major vaccine target of protective immune responses (Wilson and Coulson, 2006). However, as schistosome eggs are responsible for both pathology and transmission, a vaccine targeted at parasite fecundity and egg viability also appears entirely appropriate (McManus and Loukas, 2008). There are various types of vaccine candidates including, Sm14, Sm28, Sm29, Sm97, and Sm-TSP that are integral proteins of tegument and are tried by different researchers and showed different potency as reviewed by McManus and Loukas (2008).

2. MATERIALS AND METHODS

2.1 Prevalence and intensity of *Schistosoma mansoni* infection in different geographical areas

Planning and implementing intervention and control of disease activities are successful when they are based on the knowledge of their prevalence, intensity and level of morbidity. The current study was aimed at determining the transmission level and intensity of infection and genetic structuration of *Schistosoma mansoni* in selected endemic areas of Ethiopia.

2.1.1 Study areas and population

The study was conducted in four geographically apart *Schistosoma mansoni* endemic areas. For determination of prevalence and intensity of *Schistosoma mansoni* infection, stool specimens were collected in the villages of Shesha Kekele in Wondo Genet, Sille-Elgo in Arba Minch and Cheretee in Kemissie. The molecular epidemiology was determined by collecting stool specimens and hatching miracidia from *S. mansoni* positive subjects recruited from Shesha Kekele, Cheretee, Sille-Elgo, and Bochesa village (in Ziway) (Fig2).

Wondo Genet is found in western Arsi in Oromiya Regional State which is about 261Km south of Addis Ababa, located at 07°05'35"N, 038°36'66"E at an altitude of 1,755 meters above sea level. Chat (*Catha edulis*), sugarcane, enset, coffee, avocado and mango plantations are sources of income where irrigation is widely practiced. Ziway is found in eastern Oromiya Zone in the Oromiya Regional State about 164Km away from the capital Addis Ababa, located at 07°56'37"N, 038°43'25"E at an altitude of 1,642 meters above sea level. The community members of Bochessa are involved in fishery, on the shore of Lake Ziway, farming and irrigation activities for their livelihoods.

Sille-Elgo is found in Gofa Zone in the Southern Nations and Nationalities Regional State which is about 525Km away southwest of Addis Ababa located at about 05°28'39"N, 037°26'02"E at an altitude of 1,188 meters above sea level. The economic

activity is based on banana, avocado and mango plantations with other agricultural products.

Kemissie is a special woreda of Oromiya found in Administrative Zone of Amhara Regional State. The study site is situated within the Borkena River basin, approximately 305 km northeast of Addis Ababa located at 10°43'30"N, 039°04'20"E at an altitude of 1,450 meters above sea level. The people are involved in agricultural and livestock production. Irrigation is practiced for vegetables and Chat cultivation.

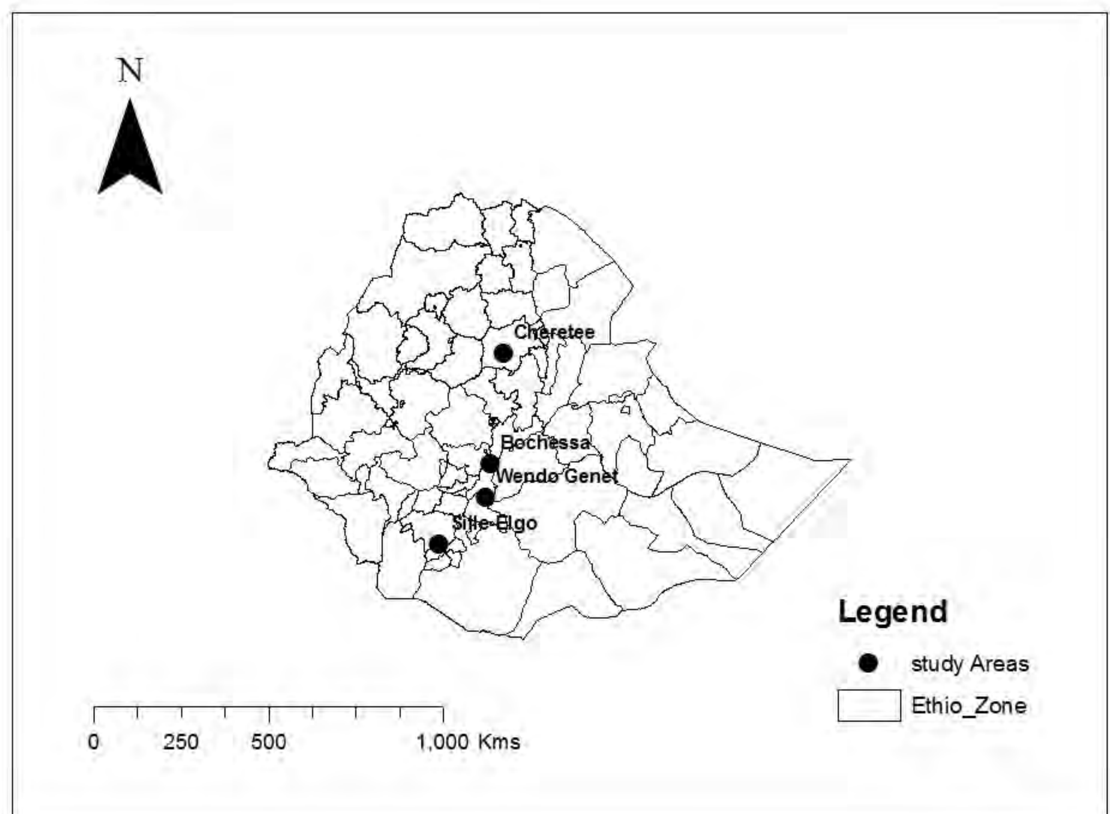

Figure 2. Map of study sites

2.1.2 Sample size determination

There is much scientific evidence that socio-demographic variables and contact with unsafe water are associated with schistosomiasis. In this study the prevalence and intensity of infection with *Schistosoma mansoni* was investigated in three different endemic areas. The sample size was estimated for each study site using Daniel's formula [$n=Z^2 P (1-P)/d^2$], where n is the sample size, Z is statistic for level of confidence, P is expected prevalence or proportion, and d is precision.

Sample size was determined based on the previous prevalence of *S. mansoni* infections, i.e., 55 % for Wondo Genet (Legesse *et al*., 2004), and 66% and 65% for Kemissie and Sille-Elgo (Berhe *et al*., 2007), respectively. Hence, the respective samples of 380, 345 and 350 were drawn for Wondo Genet, Kemissie and Sille-Elgo. An inclusion criterion to participate in the study was 5–60 years of age of both sexes. Exclusion criterion was age < 5 and > 60 years.

2.1.3 Stool specimen collection and examination

Small plastic sheets were distributed to voluntary study participants and sizable stool specimens were collected and examined using Kato-Katz method (41.7mg template) (WHO, 1991). Infection status was determined by the presence or absence of *Schistosoma mansoni* eggs and level of intensity was determined by egg count per gram (EPG) of the stool. Classes of intensity were determined as light (1 -99), moderate (100-399 eggs), and heavy (≥400 eggs). The EPG was determined by multiplying the number of eggs counted by a factor of 24 (WHO, 2002).

2.1.4 Data analysis

Data for both prevalence and intensity of infection was analyzed using Microsoft Excel 2007and SPSS version 16. Chi square test, odds ratio and ANOVA were used to describe statistical parameters. Data was presented in the form of tables and figures.

2.1.5 Ethical consideration

The study was ethically approved by the Institutional Research and Ethics Committee of Department of Biology and by the National Research and Review Committee. Informed consent was obtained from all adults. For school age children younger than 18, verbal consent was obtained from their parents or through health extension workers and school principals. In addition, the children also gave their assent. All study participants found positive for *S. mansoni* were treated with praziquantel at a dose of 40mg/Kg body weight.

2.2 Level of genetic diversity and multiplicity of *Schistosoma mansoni* infection

Different studies have shown the occurrence of infection in single intermediate and definite hosts with multiple genotypes of *Schistosoma mansoni* (Minchella *et al*., 1995; Theron *et al*., 2004). Thus, in order to determine the level of genetic diversity among the *Schistosoma mansoni* strains and the occurrence of infection with multiple genotypes of *S. mansoni* within a single human host stool specimen were collected from 16 *S. mansoni* positive subjects in Sille-Elgo, 30subjects in Wondo Genet, 30 subjects in Kemissie and15 subjects from Bochessa, totaling 91. The stool specimens were kept in 0.85% saline in vial and transported in ice box to the Medical Parasitology Laboratory of Aklilu Lemma Institute of Pathobiology to harvest miracidia.

2.2.1 Miracidia hatching

In order to stimulate hatching of miracidia, stool samples were homogenized with saline and sieved through tiered sieve of 425µm, 180µm and 140µm mesh size and kept for about 20 minutes in dark in order to allow the eggs to settle in the bottom of the flask. The supernatant was poured and the eggs were put in 250ml flask filled with aged water. The flasks were exposed to artificial light in order to initiate hatching. The flasks were covered with black carbon paper and aluminum foil. This induces the positive phototropic and negative geotropic characteristics of the miracidia which results in their accumulation on the top of the flask (Steinauer *et al*., 2008). From those specimens that hatched a total of 379 miracidia from 52 patients were collected. These included 81 miracidia from 7 individuals in Sille-Elgo, 88 miracidia from 19 individuals in Wondo Genet, 151 from 20 individuals of Kemissie, and 59 from 6 individuals in Ziway. The miracidia were transferred individually in 2µl of water using micropipette into eppendorf under a dissecting microscope. Single miracidium was put in eppendorf in 96% ethanol at -20°C until processed in the laboratory of Centre de Biologie et d'Ecologie Tropicale et Méditerranéenne, University of Perpignan Via Domitia, France.

2.2.2 DNA extraction

DNA extraction from *Schistosoma mansoni* miracidia was done following Beltran *et al*. (2008) protocol. In brief, before DNA extraction, miracidia were individually vacuum-dried for 15 min in a Speedvac evaporator. Next, 20 µl of NaOH (250 mM) was added to each tube. After a 15 min incubation period at 25°C, the tubes were heated in boiling water at 99°C for 2 min. Then, 10 µl HCl (250 mM), 5 µl of Tris-HCl (500 mM) and 5 µl Triton X-100 (2%) were added and a second heat shock in boiling water at 99°C for 2 min was performed. The products were put in room temperature until processed for Polymerase Chain Reaction (PCR).

Microsatellite analysis has been seen as a promising method to acquire a more detailed characterization of genetic variation and schistosome population structure. One of the first attempts to isolate microsatellite markers for *S. mansoni* was that of Durand *et al.* (2000), who identified 11 polymorphic loci with the number of alleles ranging from two to eight. Similarly, Blair, Webster and Barker, (2001) identified 10 polymorphic microsatellite markers for *S. man*soni that showed high variability both between individuals and populations. Thus, in the current study previously published 11 polymorphic microsatellite markers SMDA28, SMC1, SMDO11 and AF325698 (Curtis *et al*., 2001), R95529, SMD57, L46951 and SMD25 (Durand *et al*., 2000), SMBR16 and SMBR10 (Rodrigues *et al*., 2007) and SMS7 (Blair *et al.*, 2001) were used to determine the genetic variation and *Schistosoma mansoni* population structure of Ethiopian isolates. Details of the microsatellite loci used to genotype all the samples are presented in Table1. The relevant DNA fragments were amplified using PCR. All loci were tested pair-wise based on 4400 permutations and adjusting *P* value to 0.000227 and there was no linkage disequilibrium detected. Moreover, they are routinely used in the lab where this work had been conducted for they had confirmed that there is no linkage detected.

2.2.3 Polymerase Chain Reaction

To maximize efficiency and minimize costs, these PCRs were performed in three multiplexes. The PCR amplifications loci: R95529, SMC1, SMBR16, SMD57, SMDO11 are Multiplex1; SMDA28, SMS7, SMD28 are Multiplex2, and SMBR10,

L46951, SMD25 are Multiplex 3. The PCR reactions were carried out in a total volume of 20 µl containing 4 µl of 5X buffer (10 mM Tris-HCl, pH 9.0 at 25°C, 50 mM KCl, 0.1% Triton X-100), 0.2 µM of each oligonucleotide primer, 200 µM of each dNTP (Promega), 1 unit of GoTaq polymerase (Promega, Madison, Wisconsin), 1 µl of extracted DNA and DNase-free water q.s.p. 20 µl. The PCR programme consisted an initial denaturation phase at 95°C for 5 min, followed by 40 cycles at 95°C for 30 s, 57°C annealing temperature for 20 s, 72°C for 30 s, and a final extension at 72°C for 10 min in a thermocycler (Bio-Rad, Hercules, USA). For each marker, the forward PCR primer was 5' fluorescein labeled (Proligo, Cambridge, UK) allowing a precise analysis in an automated DNA sequencer. A mix of 40 µl sample loading solution (Beckman Coulter, Villepinte, France) and 0.1875 µl DNA size 400, a red labeled size standard (CEQTM DNA size standard kit, 400 Beckman Coulter), was prepared and 0.75 µl of the microsatellite PCR products were diluted in 39.25 µl sample loading solution. Mineral oil was dropped in each tube and electrophoresed using an automatic sequencer (CEQTM 8000, Beckman Coulter) with CEQTM 8000 sequence analysis software. The sizes of the alleles were calculated with the fragment analyzer package (Beltran *et al.*, 2008).

Table 1. Details of each locus used in the study

Locus	Accession No.	Primer sequence (5'–3')	Repeat motif	Ref.
SMDA28	AF325695	F: CATGATCTTAGCTCAGAGAGCC R: AGCCAGTATAGCGTTGATCATC	$(GATA)_{7\text{-}14}$	Curtis *et al*. 2001
SMS7	AF330105	F: TCCTCCTCTCTATTTTCTCTTTG R: ATTACGATTGCACAGATACTTTTG	$(CA)_{17}$	Blair *et al*. 2001
SMD28	AF202966	F: CATCACCATCAATCACTC R: TATTCACAGTAGTAGGCG	$(CAA)_5$	Durand *et al*. 2000
R95529	R95529	F : GTGATTGGGGTGATAAAG R : CATGTTTCTTCAGTGTCC	$(CAT)_{10}$	Durand *et al*. 2000
SMC1	AF325694	F:TGACGAGGTTGACCATAATTCTAC R: AACACAGATAAGAGCGTCATGG	$(AAT)_{6\text{-}12}$	Curtis *et al*. 2001
SMBR16	L04480	R: GGCCTGATACAATTCTCCGA F: TGTGACTTTGATGCCACTGA	$(TA)_{10}$	Rodrigues *et al*. 2007
SMD57	AF202967	F: TCCTTGATTCCACTGTTG R: GCAGTAATCCGAAAGATTAG	$(TA)_{22}(GA)_9$	Durand *et al*. 2000
SMDO11	AF325698	F: TGTTTAAGTCGTCGGTGCTG R: ACCCTGCCAGTTTAGCGTAG	$(GATA)_{20\text{-}37}$	Curtis *et al*. 2001
SMBR10	DQ448293	R: GTACATTTTATGTCAGTTAGCC F: CATGATCTTAGCTCAGAGAGC	$(GATA)_{10}$	Rodrigues *et al*. 2007
L46951	L46951	F:CAAACATATACATTGAATACAG R: TGAATTGATGAATGATTGAAG	$(GAA)_7$	Durand *et al*. 2000
SMD25	AF202965	F: GATTCCCAAGATTAATGCC R: GCCATTAGATAATGTACGTG	$(CA)_{10}$	Durand *et al*. 2000

2.2.4 Molecular data analysis

The results obtained in terms of allele count, allelic richness and heterozygosity were used for the analysis of genetic diversity of *Schistosoma mansoni* infection.

The mean number of alleles per locus (MNA) in each population and the observed heterozygosities were used as indicators of the genetic polymorphism within the populations under study. Differences both for the mean number of allele and the expected heterozygosity values were tested for samples of each population using Friedman nonparametric tests (Agola *et al*., 2009).

The MNA and allele frequencies were calculated using the program MICROSATELLITE TOOLKIT (Agola *et al*., 2006). Both the expected and observed heterozygosities were also calculated using MICROSATELLITE TOOLKIT and their statistical significance tested using the chi-square test at $\alpha' = 0.05$ (Agola *et al*., 2009). FSTAT was used to test for deviations from Hardy-Weinberg equilibrium using exact tests, testing the hypothesis that observed diploid genotypes are the product of random union of gametes. An exact test for linkage disequilibrium between pairs of loci was performed using the FSTAT. Mean estimates of F_{IS} (inbreeding coefficient) for each population and pairwise F_{ST} (between all population pairs) were also calculated following the method of Weir and Cockerham (1984). Deviation of F_{IS} and F_{ST} values from zero was tested using a permutation test. All F statistics were carried out using FSTAT 2.9.3.2 (Goudet, 2000).

The Colony software V1.1 (Wang, 2004), which takes into account the typing errors and mutations, has been developed and demonstrated to be able to accurately infer full- and half-sibships from the molecular data of a high error rate. This program implements a maximum likelihood method to assign individuals from a population into full-sib families nested within half-sib families (colonies). It adopts an iterative procedure for updating allele frequencies with reconstructed sibships taken into account, and uses efficient algorithms for calculating the likelihood function and searching for the maximum-likelihood configuration (Wang, 2004). Full and half-sibships were estimated considering the monogamous mating-system of schistosomes. Thus, in the current study

the colony software was used to determine the estimates of genetically unique adult worm pairs in each patient in order to infer multiplicity of infection in a single host.

In this study, schistosomes were defined at two hierarchical levels: 1) all miracidia from an individual patient designated as an infrapopulation (all individuals of a single parasite species in one host), and 2) miracidia from all the patients within a single study site treated as a population.

2.3 Genetic structure of *Schistosoma mansoni* isolates of Ethiopia

Population genetics deals with the variations of allele frequencies between and within populations. The fundamental prerequisite of any inference on the genetic structure of populations is the definition of populations themselves. Population determination is usually based upon geographical origin of samples or phenotypes. However, the genetic structure of populations is not always reflected in the geographical proximity of individuals. Populations that are not discretely distributed can nevertheless be genetically structured, due to unidentified barriers in gene flow (Evanno *et al*., 2005).

Population structure describes the extent to which a large population is subdivided into smaller distinct populations. The amount of population structure typically depends on the interaction of microevolutionary forces. Mutation creates novel alleles in subpopulations, selection increases the frequency of favorable alleles in the local environment, and genetic drift alters allele frequencies owing to stochastic fluctuations; each of these forces tends to make subpopulations less similar. Gene flow, the movement of gametes or migrant individuals between subpopulations, tends to blur the boundaries between subpopulations. Under some circumstances gene flow is a creative force in evolution, possibly distributing alleles throughout a population. At other times, gene flow prevents genetic differentiation by swamping local selection, preventing a group of individuals from adapting specifically to local conditions (Curtis and Minchella, 2000). Structure of parasite populations correlates with (i) host mobility, (ii) mode of reproduction of the parasite, (iii) complexity of the parasite life cycle, (iv) parasite infra-population size and (v) host specificity. The importance of these factors varies from one parasite species to the next. Therefore, a comparative approach with a phylogenetic perspective is crucial to disentangle the various processes that drive parasite diversification (Huyse *et al*., 2005).

The identification of genetically homogeneous groups of individuals is a long standing issue in population genetics. Bayesian algorithm implemented in the software STRUCTURE allows the identification of such groups. Population genetics deals with the variations of allele frequencies between and within populations. The most widely

used measures of population structure are Wright's *F* statistics (Wright 1931). To calculate these indices, one needs first to define groups of individuals and then to use their genotypes to compute variance in allele frequencies (Evanno *et al*., 2005).

A model-based method developed by Pritchard *et al*. (2000) and implemented in the software STRUCUTURE aims at delineating clusters of individuals on the basis of their genotypes at multiple loci using a Bayesian approach. The model accounts for the presence of Hardy–Weinberg or linkage disequilibrium by introducing population structure and attempts to find population groupings that (as far as possible) are not in disequilibrium. The estimated log probability of data for each value of *K* is given, allowing the estimation of the more likely number of clusters. A quantification of how likely each individual is to belong to each group is also given information that can be then used to assign individuals to populations.

Thus, genetic structure of a population is an important indicator at what level an infra-population and population is differentiated. This is measured using Wright's F-statistics, F_{ST}, employed to characterize the structures at the level of population and infra-populations (Wright, 1965).

Fixation index (F_{ST}) is a measure of population differentiation, genetic distance, based on genetic polymorphism data such as single-nucleotide polymorphisms (SNPs) or microsatellites. F_{ST} is simply the correlation of randomly chosen alleles within the same sub-population relative to that found in the entire population. It is often expressed as the proportion of genetic diversity due to allele frequency differences among populations (Holsinger *et al*., 2009). This comparison of genetic variability within and between populations is frequently used in the field of population genetics. The values range from 0 to 1. A zero value implies complete panmixies, where the two populations are interbreeding freely. A value of one would imply that the two populations are completely separate.

Thus, the genetic structure of *S. mansoni* isolates from the study areas was determined by F_{ST} using FSTAT software (Goudet, 2000). The Principal Component Analysis (PCA) is important in the determination of spatial structuration of a population. PCA was done using GENETIX 4.05 software. The Bayesian clustering analysis of

STRUCTURE version 2 (Pritchard *et al*., 2000; Falush *et al*., 2003) was utilized to test for subdivision of parasites into distinct genetic clusters.

3. RESULTS

3.1 Prevalence and intensity of *Schistosoma mansoni* infection in different geographical areas

Out of the study participants who voluntarily showed up, a total of 1073 individuals (528males and 545females) were found eligible for the parasitological stool examinations. The study participants included were in the age range of 5-60 years with mean age of 11 years in Wondo Genet, 22 years in Kemissie and 24 years in Sille-Elgo.

The parasitological findings are presented in Tables 2, 3 and 4. The prevalence of *Schistosoma mansoni* infection among the study participants in Kemissie, Wondo Genet and Sille-Elgo was 89.6%, 59.9%, and 31.6%, respectively.

In this study, *Ascaris lumbricoides* and *Trichuris trichiura* were also observed as major helminth parasites. In Wondo Genet, the prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* infections was 40.1% and 29.6%, respectively. The prevalence of *S. mansoni*, *Ascaris lumbricoides* and *Trichuris trichiura* mixed infections in Wondo Genet was 21.3%. In Kemissie the prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* infections was14.4% and 4.3%, respectively with 2.3% mixed infection. In Sille-Elgo the prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* infections was 5.8% and 9.5%, respectively with 0.58% mixed infection.

Table 2. Distribution of major helminth infection in the study areas with respect to sex, 2010

	No. Examined		*S. mansoni*		*A. lumbricoides*		*T. trichiura*		Positive for all three parasites (mixed infection)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Wondo Genet	177	207	116(65.5%)	114(55%)	64(36.1 %)	90(43.4%)	45(25.4%)	59(28.5%)	40(22.5%)	42(20.2%)
Kemissie	156	189	142(97.2%)	167(88.3%)	25(16%)	25(13.2%)	6(3.8%)	9(4.7%)	4(2.5%)	4(2.1%)
Sille-Elgo	195	149	64(32.8%)	45(30.2%)	8(4.1%)	12(8%)	9(4.6%	24(16.1%)	0(0%)	2(1.3%)

Among the study participants examined in Wondo Genet, 65.5% of the males and 55% of the females were positive for *S. mansoni*. The minimum and maximum egg per gram of feces count was 24 and 8472, respectively with a geometric mean of 252. In order to determine the significance of sex and age on infection rate and intensity level comparison of means and regression analysis was carried out. There was variation in the prevalence of infection among the different age categories (χ^2 = 43.596, 8DF, *P*= 0.000). There was significance association between sex and infection rate (χ^2 = 4.35, 1DF, *P*=0.037) (OR=1.551, 95%CI1.026, 2.346) as well as sex and intensity of infection (OR=1.55, 95%CI, 1.033, 1.446) (Table4). ANOVA indicated that there is significant association between age and intensity of *S. mansoni* infection (F=4.970, 8DF, *P*=0.000).

Table 3. Frequency of *S. mansoni* intensity of infection by age groups, 2010

AGE	Wondo Gent			Kemissie			Sille-Elgo		
	Light	Moderate	Heavy	Light	Moderate	Heavy	Light	Moderate	Heavy
5-9	39(32.5%)	31(25.8%)	50(41.6%)	20(30%)	23(33.3%)	26(37.6%)	10(41.6%)	9(37.5%)	5(20.8%)
10-14	30(30.9%)	30(30.9%)	37(38.1%)	11(16.9%)	17(26.1%)	37(56.9%)	16(61.5%)	7(26.9%)	3(11.5%)
15-19	0(0%)	3(100%)	0(0%)	4(11.1%)	10(27.7%)	22(61.1%)	10(71.4%)	4(28.5%)	0(0%)
20-24	2(66%)	1(34%)	0(0%)	5(26.3%)	4(21%)	10(52.6%)	10(76.9%)	1(7.6%)	2(15.3%)
25-29	2(66%)	0(0%)	1(34%)	3(15%)	10(50%)	7(35%)	8(72.7%)	3(26.3%)	0(0%)
30-34	0(0%)	1(50%)	1(50%)	6(33.3)	7(38.9)	5(27.8%)	5(71.4%)	2(28.6%)	0(0%)
35-39	0(0%)	0(0%)	0(0%)	4(21%)	10(52.6%)	5(26.3%)	4(100%)	0(0%)	0(0%)
40-44	1(100%)	0(0%)	0(0%)	11(36.6%)	14(46.7%)	5(16.7%)	2(100%)	0(0%)	0(0%)
45+	1(100%)	0(0%)	0(0%)	10(30.3%)	14(42.4%)	9(27.3%)	7(77.8%)	2(22.2%)	0(0%)
Total	75(32.6%)	66(28.6%)	89(38.6%)	74(23.9%)	109(35.3%)	126(40.7%)	72(65.5%)	28(25.5	10(9%)

In Kemissie 83.9% of males and 71.3% of females were found positive for *S. mansoni*. The minimum and maximum eggs per gram were 24 and 5208, respectively, the overall EPG being 346. There was no significant variation observed in infection rate with regard to sex and age. However, it was observed that intensity of infection is associated with age. Thus, odds ratio for 5-9years and 15-19 years of age group was (OR=1.517, 95%CI, 1.084, 2.123) and (OR=2.044, 95%CI, 1.299, 3.216), respectively.

In Sille-Elgo the proportion of *S. mansoni* positive males and females was 32.8% and 30.2%, respectively. The minimum and maximum egg per gram was 24 and 3960,

respectively, with an overall 91 EPG. There was no significant association between sex and infection rate. However, there was significant association between infection rate and age (χ^2 = 26.904, 8DF, P=0.001), (OR=2.889, 95%CI, 1.150, 7.259). It was also observed that there was variation in EPG count among the different age groups (F = 3.866, 8DF, P=0.000), (OR=7.457, 95%CI, 1.496, 3.718). In contrast to age there was no association observed between sex and EPG.

Table 4. Proportion of *S. mansoni* intensity of infection by sex, 2010

Sex	Wondo Genet			Kemissie			Sille-Elgo		
	Light	Moderate	Heavy	Light	Moderate	Heavy	Light	Moderate	Heavy
Male	18.6%	20.3%	26.5%	18.5%	33.3%	39.1%	23.5%	7.1%	2.5%
Female	20.2%	14.4%	20.2%	23.2%	31.2%	33.8%	17.4%	9.3%	3.3%
Total	19.5%	17.1%	23.1%	21.1%	32.1%	36.2%	20.9%	8.1%	2.9%

All of the three study sites combined, the overall prevalence of *Schistosoma mansoni* infection was 60.48%. The maximum EPG was 8472 with a geometric mean of 273. When subjects were compared within themselves based on sex 60.9% males and 59.8% of females were infected with *S. mansoni.* There was no association observed between sex and rate of infection (χ^2 =0.207, 1DF, P=0.649). However, age had impact on infection rate (χ^2 =51.663, 8DF, P=0.000). The odds ratio for the 5-9years of age compared with 15-19years and 20-24 years of age categories was (OR=2.262, 95%CI, 1.352, 3.784) (OR=2.524, 95%CI, 1.276, 4.989), respectively. ANOVA also indicated that age has significant influence on intensity of infection (F=8.357, 8DF, P=0.000). OR also showed association between age and intensity of infection (OR=1.156, 95%CI, 1.009, 1.326). However, there was no association observed between sex and intensity of infection.

3.2. Level of genetic diversity and multiplicity of *Schistosoma mansoni* infection

In studies of genetic variation in natural populations, the first step was to quantify the genetic variation present. While it is of value to know the variety of schistosomes that exist within a defined area, it would be even more advantageous to know how genotypes are distributed within and among parasite populations, and the epidemiological factors that determine these distributions (Curtis *et al*., 2002).

Cost and good PCR products are important limitation factors in molecular work. Thus, in order to minimize cost and depending on good PCR results, out of the 379 miracidia collected from 52 patients, it was only 288 miracidia from forty one patients (9 from Kemissie, 19 from Wondo Genet, 7 from Sille-Elgo and 6 from Ziway) that were genotyped for the 11 loci and analyzed. The chromatograms below indicate how heterozygosity was detected using the three multiplexes in the Beckman Coulter analyzer output (Figures 3-5). The red color peaks are the size standards used as reference for the detection of polymorphic loci. Those peaks with blue and green color were used to determine whether the allele was homozygote (one peak) or heterozygote (two peaks) or stutter (small peaks formed as a result of DNA artifacts due to a halted polymerase activity) at a particular locus.

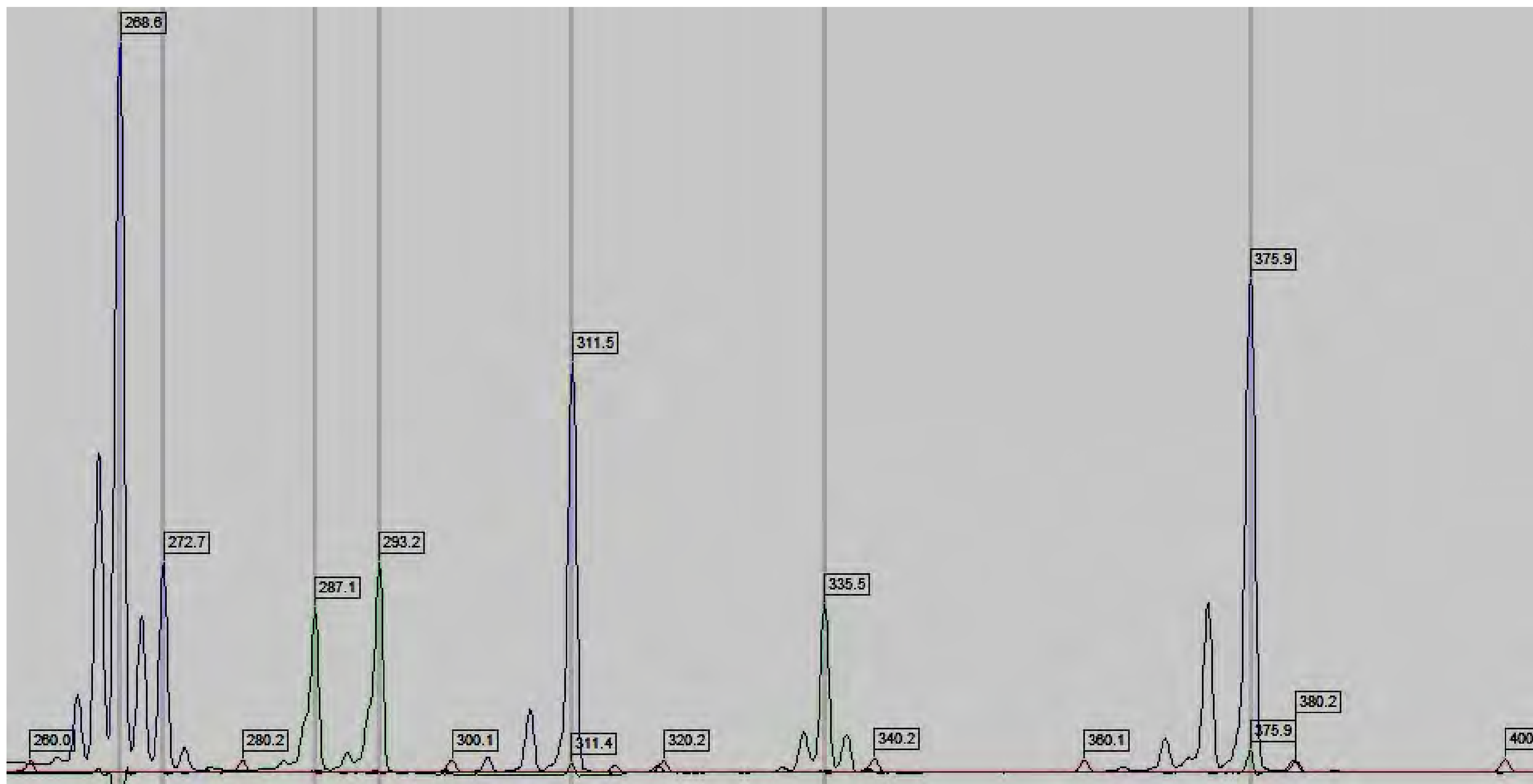

Figure 3. Chromatogram of Multiplex 1

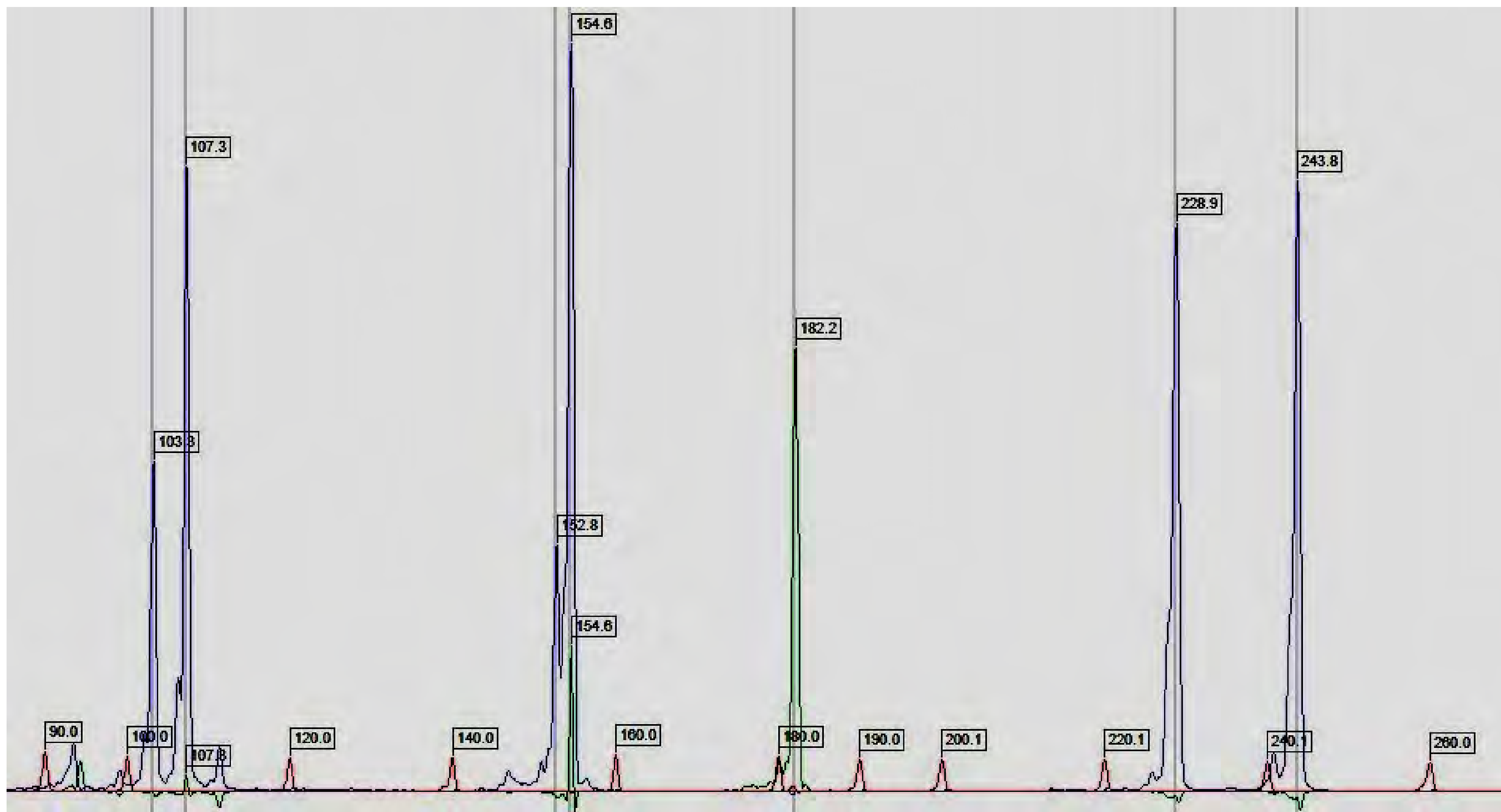

Figure 4. Chromatogram of Multiplex 2

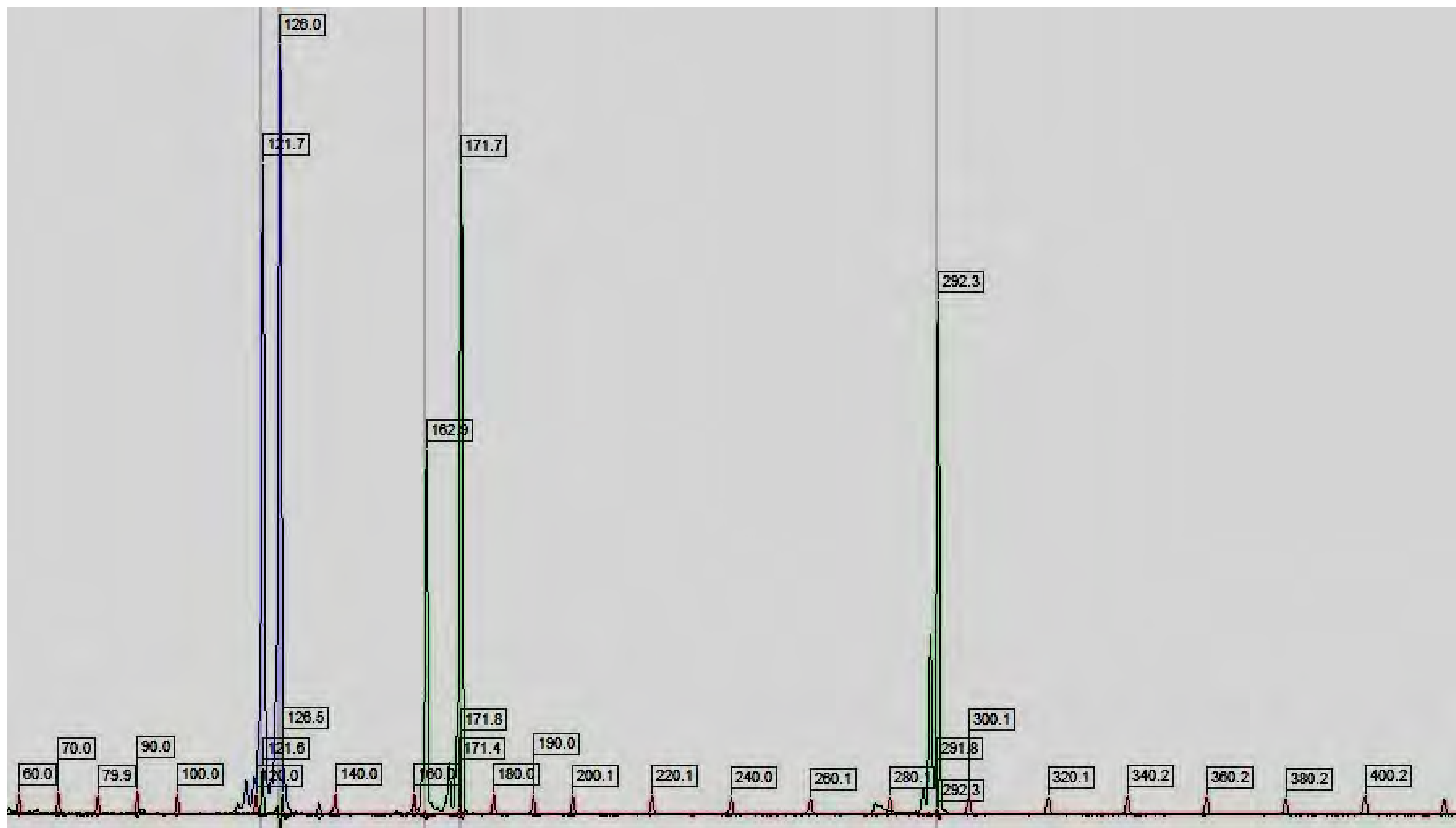

Figure 5. Chromatogram of Multiplex 3

3.2.1. Genetic diversity at population level

The mean number of alleles per locus (MNA) detected in each population and the observed heterozygosities were used as indicators of genetic polymorphism within the populations under study.

3.2.1.1. Allelic diversity

In this study a total of 164 alleles were scored in all of the four populations for all the eleven loci examined. There was no null allele detected. Individually a total of 127, 123, 102 and 94 alleles for all of the 11 loci were counted for Kemissie, Wondo Genet, Sille-Elgo and Ziway, respectively. The number of alleles (A) scored for each locus ranged from 4-22 (SMC1-SMDO11) with a mean value of 8.5 in Ziway; from 3-25 (SMD28-SMDO11) with a mean value of 11.5 in Kemissie; 4-22 (SMC1-SMDO11) with a mean value of 11.2 in Wondo Genet and, 3-23 (SMD28-SMDO11) with a mean value of 9.3 in Sille-Elgo for the eleven loci. The number of alleles in all of the four populations ranged from 8-34 (SMD28-SMDO11) with a mean value of 14.9. The mean number of alleles in Kemissie was 11.5, while in Wondo Genet, Sille-Elgo and Ziway it was 11.2, 9.3 and 8.5, respectively. Among the eleven microsatellite loci studied, SMDO11 had the highest number of allele count for all of the study sites (in the range of 22-25) while SMD28 has the lowest value in all of the study sites, ranging from 3-6 (Table5). In order to determine whether there was significant difference in number of alleles counted within the populations, a nonparametric Friedman test was calculated (χ^2= 10.941 at 3DF; P= 0.012).

Table 5. Number of allele count (A) for each locus in each population

Locus	Kemissie	Sille-Elgo	Wondo Genet	Ziway	All populations
R95529	11	4	11	5	14
SMC1	5	7	4	4	7
SMBR16	11	10	10	9	14
SMD57	17	15	19	11	21
SMDO11	25	23	22	22	34
SMDA28	14	7	12	11	15
SMS7	4	4	5	6	8
SMD28	3	3	6	4	6
SMBR10	12	11	11	9	15
L46951	13	9	12	9	16
SMD25	12	9	11	4	14
Mean	11.5	9.3	11.2	8.5	14.9

3.2.2.2 Allelic richness

The allelic richness (An) of the population at a particular locus is the total number of allele types present in the population at that locus. In this study the allelic richness was calculated based on minimum sample size of 29 diploid individuals (Table6). It ranged from 3.46 – 20.8 (SMD28-SMDO11) with a mean value of 9.9 in all of the four populations. Ziway had An value of 3.6 - 22.28 (SMD28-SMDO11) with a mean value of 9.06, Kemissie 2.99 - 18.93 (SMD28 -SMDO11) with a mean value of 9.44, Wondo Genet 3.79-16.81(SMD28-SMDO11) with a mean value of 9.18 and Sille-Elgo 3.0-18.16 (SMD28-SMDO11) with a mean value of 7.71. A nonparametric Friedman test was done to determine if there is significant difference in An among the four populations (χ^2= 3.327 at 3DF; P=0.344). Wilcoxon rank test indicated that Sille-Elgo had the lowest allelic richness (P=0.02).

Table 6. Allelic richness (An) for each locus in each population

Locus	Kemissie	Sille-Elgo	Wondo Genet	Ziway	All pop
R95529	9.20	4.00	8.31	4.53	8.01
SMC1	4.68	6.38	3.99	4.00	5.22
SMBR16	9.82	8.35	8.52	9.00	11
SMD57	14.20	12.95	15.19	10.53	15.7
SMDO11	18.93	18.16	16.81	18.86	20.8
SMDA28	10.40	5.90	10.71	10.87	10.75
SMS7	3.52	3.66	4.25	5.75	4.64
SMD28	2.99	3.00	3.79	3.60	3.46
SMBR10	10.04	7.42	9.90	8.42	9.92
L46951	9.77	7.35	10.09	8.08	10.1
SMD25	10.28	7.62	9.40	3.99	9.3
Mean	9.44	7.71	9.18	7.97	9.9

3.2.2.3 Heterozygosity

Genetic diversity of the study populations was measured using heterozygosity. The value of this measure ranged from zero (no heterozygosity) to nearly 1 (for a system with a large number of equally frequent alleles). Paired *t*-test was used to evaluate the statistical significance of the deviations between the observed and expected heterozygosity (t(10.367), 43DF, P=0.000). The expected heterozygosity was 73% in Sille-Elgo followed by Wondo Genet (71%), Kemissie (69%) and Ziway (66%). Similarly the observed heterozygosity was 52% for Sille-Elgo 49% for Wondo Genet, 44% for Kemissie and 37% for Ziway (Table7).

Table 7. Total number of sample size, loci typed and H_E and H_O for all populations

Population	Sample size	Loci typed	H_E	H_E Standard Deviation	H_O	H_O Standard Deviation
Kemissie	79	11	0.6975	0.0686	0.4427	0.0187
Sille-Elgo	79	11	0.7308	0.0352	0.5283	0.0180
Wondo Genet	82	11	0.7180	0.0657	0.4954	0.0181
Ziway	48	11	0.6668	0.0634	0.3771	0.0231

The highest and lowest observed heterozygosity in Kemissie was at the locus SMDO11 (0.75) and SMD28 (0.16). In Sille-Elgo the highest was at the locus SMDA28 (0.71) and the lowest at SMD25 (0.34). At Wondo Genet, the highest was for the locus SMD25 (0.69) and the lowest was at SMD28 (0.10). In Ziway the highest was at the locus SMDO11 (0.67) and the lowest at R95529 (0.16). The mean observed heterozygosity for Kemissie, Sille-Elgo, Wondo Genet and Ziway was 0.44, 0.53, 0.5 and 0.38, respectively, (χ^2=7.691 at 3DF; P=0.053) (Table8). It was also observed that the expected heterozygosity at all loci in each study populations is larger than that of the observed heterozygosity. The highest and lowest expected heterozygosity in Kemissie was at the locus SMDO11 (0.91) and SMD28 (0.24). In Sille-Elgo the highest was at the locus SMDO11 (0.92) and the lowest at R95529 (0.59). At Wondo Genet, the highest was for the locus SMD57 (0.90) and the lowest was at SMD28 (0.12). In Ziway the highest was at the locus SMDO11 (0.91) and the lowest at R95529 (0.29). The mean expected heterozygosity for Kemissie, Sille-Elgo, Wondo Genet and Ziway is 0.70, 0.73, 0.77 and 0.67, respectively. In all of the study populations F_{IS} has a high positive value different from zero for all of the 11 loci while only Sille-Elgo and Ziway has a respective negative value at the loci SMDA28 and SMBR10. Statistical test was done to determine the significant difference between the expected and observed heterozygosities (χ^2=32.818, 1DF, P=0.000).

Table 8. Expected (H_E) and Observed heterozygote (H_O) in each population for each locus with F_{IS} value

	Kemissie		Sille-Elgo		Wondo Genet		Ziway		Kemissie	Sille-Elgo	Wondo Genet	Ziway
Locus	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	F_{IS}	F_{IS}	F_{IS}	F_{IS}
R95529	0.81	0.34	0.59	0.40	0.72	0.50	0.29	0.16	0.58564	0.32500	0.30795	0.451
SMC1	0.69	0.42	0.72	0.67	0.64	0.48	0.57	0.34	0.39290	0.07477	0.24957	0.40111
SMBR16	0.78	0.43	0.81	0.58	0.79	0.48	0.84	0.41	0.44630	0.28459	0.38707	0.51092
SMD57	0.89	0.42	0.89	0.65	0.90	0.43	0.86	0.38	0.53463	0.27434	0.52226	0.53747
SMDO11	0.91	0.75	0.92	0.56	0.83	0.60	0.91	0.67	0.17017	0.38913	0.27443	0.27204
SMDA28	0.79	0.44	0.61	0.71	0.86	0.52	0.87	0.19	0.43963	-0.15352	0.39762	0.78089
SMS7	0.29	0.18	0.60	0.41	0.60	0.55	0.66	0.42	0.39753	0.30713	0.08779	0.36426
SMD28	0.24	0.16	0.66	0.40	0.12	0.10	0.35	0.22	0.35285	0.39274	0.19567	0.37233
SMBR10	0.75	0.64	0.66	0.62	0.81	0.66	0.56	0.57	0.15017	0.0698	0.18587	-0.02768
L46951	0.66	0.49	0.77	0.47	0.78	0.44	0.73	0.43	0.24916	0.39094	0.43511	0.40733
SMD25	0.86	0.60	0.80	0.34	0.86	0.69	0.70	0.35	0.30692	0.57500	0.19953	0.50472
Mean	0.70	0.44	0.73	0.53	0.72	0.5	0.67	0.38	0.36708	0.27853	0.31163	0.43473

3.2.3 Genetic diversity at infrapopulation level

3.2.3.1 Allelic diversity

Twenty four infrapopulations were analyzed for the number of alleles counted. The scored number of alleles in individual study subjects which were represented by five or more miracidia also had shown variation (Table 9). The number of allele count at a single locus for the twenty four infrapopulations range from 1 to 17. The largest mean number of allele was 7.54 and the lowest was 3.09. Most of the infrapopulations have a low allele count for the SMD28 locus. Among infrapopulations from Kemissie, K12 and K13 has each 10 allele count for the SMD57 locus and K3 and K12 has 12 and 14 allele count for SMDO11 locus, respectively. For infrapopulations from Sille-Elgo, SE12 and SE14 each has a count of 12 alleles for SMD57 and 16 and 14 for the locus SMDO11, respectively. For infrapopulations from Wondo Genet W89 has the maximum allele count of 10 for the locus SMDA28 followed by W38 and W47 which has 9 and 8 allele count for the locus SMDO11 respectively. Infrapopulations from Ziway had also a variable allele count. Z3 has 10 alleles at SMDA28; Z4 has 10 allele counts at each of SMDA28 and SMD57 loci and 17 at SMDO11. Generally, SMDO11 had a frequency of 34 in all of the subpopulations followed by SMD57 (18), L46951 (16) and the least being 4 for the locus SMD28. The total allele count for each infrapopulation at all of the 11 loci was in the range of 48-73 for Kemissie, 46-80 for Sille-Elgo, 42-54 for Wondo Genet and 40-83 for Ziway. The nonparametric Friedman test indicate that there is significant difference in the number of alleles scored in each infrapopulation (χ^2=93.485, 23DF, P=0.000). The mean number of alleles for each infrapopulation was determined, hence, the mean number of alleles for Kemissie was in the range of 4.36-6.64 with a standard deviation ranging from 1.80 to 3.47. The mean number of alleles for Sille-Elgo was in the range of 3.55-7.18 with a standard deviation of 0.93-3.95. For Wondo Genet the mean number of alleles was 3.27-4.91 with a SD of 1.33-2.50. In Ziway the mean number of alleles was 3.09-7.55 with a SD of 1.29-4.01 (Table11).

Table 9. Number of allele count at individual human host level

Locus	K1	K2	K3	K9	K12	K13	K14	K15	SE1	SE12	SE14	SE16	W21	W22	W38	W47	W51	W55	W62	W88	W89	Z3	Z4	Z5
R95529	6	4	5	8	4	4	4	7	3	4	4	3	2	4	4	5	3	3	5	3	4	2	4	2
SMC1	4	4	4	3	5	5	4	3	4	7	6	5	3	3	2	4	3	2	4	3	3	2	4	2
SMBR16	4	4	5	4	8	6	6	5	6	9	10	4	1	5	5	5	3	5	4	5	2	4	8	4
SMD57	7	6	6	8	10	10	8	6	5	12	12	5	7	7	6	5	6	6	4	7	6	3	10	3
SMDO11	7	12	7	9	14	9	7	4	7	16	14	5	5	5	9	8	3	3	7	5	5	5	17	8
SMDA28	6	9	5	5	7	8	7	4	4	6	5	3	2	5	7	5	5	5	6	3	10	5	10	4
SMS7	2	1	2	2	2	2	4	2	3	3	3	4	3	3	2	3	3	3	3	1	3	5	5	2
SMD28	2	2	1	3	2	2	2	2	3	3	3	3	1	1	3	1	1	2	1	1	1	2	4	1
SMBR10	7	7	6	5	7	5	7	5	3	7	8	5	4	6	6	5	5	5	5	5	4	5	9	2
L46951	6	7	6	3	7	5	4	3	6	5	9	4	3	4	4	5	5	4	4	3	5	4	8	3
SMD25	4	5	6	7	7	9	7	7	3	7	6	5	5	5	6	6	7	4	6	6	7	3	4	3
Total	55	61	53	57	73	65	60	48	47	79	80	46	36	48	54	52	44	42	49	42	50	40	83	34

3.2.3.2 Allelic richness

Allelic richness of *Schistosoma mansoni* isolates at individual host level ranged from 1 to 1.96 for each locus. At all over loci level allelic richness had a mean value of 1.59-1.73 (Table10). For all of the 24 infrapopulations, the allelic richness for each locus was in the range of 1.42-1.93 with a mean value of 1.76. The lowest and highest allelic richness observed was at SMD28 and SMDO11 loci, respectively. There is no statistically significant variation in allelic richness of *Schistosoma mansoni* among each infrapopulations (χ^2= 12.023 at 23DF; P=0.970).

As shown in Table10, the allelic richness based on one diploid individual for all 11 loci typed from Kemissie ranged from 1.11-1.89 (SMD28-SMDO11) for K1, 1-1.94 (SMS7-SMDO11) for K2, 1-1.89 (SMD28-R95529) for K3, 1.43-1.92 (L46951-SMDO11) for K9, 1.21-1.95 (SMD28-SMD57), 1.13-1.93 (SMS7-SMD57) for K13, 1.11-1.85 (SMD28-SMD57& SMBR16) for K14 and, 1.41-1.89 (SMS7-SMBR16) for K15. For infrapopulations from Sille-Elgo the An ranged from 1.28-1.88 (R95529-SMDO11) for SE1, 1.56-1.93 (R95529-SMDO11) for SE12, 1.57-1.91 (SMS7-SMDO11) for SE14, and 1.6-1.93 (SMD28-SMDO11) for SE16. Similarly in Wondo Genet allelic richness ranged from 1-1.93 (SMD28-SMD57) for W21, 1-1.96 (SMD28-SMD57) for W22, 1.17-1.94 (SMS7-SMDO11) for W38, 1-1.94 (SMD28-SMDO11) for W47, 1-1.93(SMD28-SMD25) for W51, 1.2-1.93(SMD28-SMBR16) for W55, 1-1.91(SMD28-SMD57) for W62, 1-1.91(SMD28-SMD57) for W88, and 1-1.93(SMD28-SMDA28) for W88. Also in Ziway allelic richness ranges from 1.2-1.89(SMD28-SMDA28) for Z3, 1.26-1.92(R95529-SMDO11) for Z4 and, 1-1.96(SMD28-SMDO11) for Z5.

Table 10. Allelic richness of infrapopulations based on minimum sample size of one diploid individual

	K1	K2	K3	K9	K12	K13	K14	K15	SE1	SE12	SE14	SE16	W21	W22	W38	W47	W51	W55	W62	W88	W89	Z3	Z4	Z5	All
R95529	1.88	1.58	1.89	1.88	1.73	1.73	1.64	1.80	1.28	1.56	1.64	1.61	1.43	1.71	1.65	1.80	1.71	1.46	1.82	1.73	1.82	1.20	1.26	1.53	1.70
SMC1	1.70	1.76	1.78	1.54	1.73	1.67	1.50	1.58	1.75	1.74	1.70	1.80	1.83	1.62	1.49	1.68	1.62	1.36	1.68	1.53	1.60	1.36	1.50	1.53	1.70
SMBR16	1.82	1.50	1.76	1.66	1.88	1.80	1.85	1.89	1.79	1.84	1.78	1.79	1.00	1.83	1.85	1.74	1.73	1.93	1.74	1.76	1.43	1.87	1.83	1.87	1.84
SMD57	1.89	1.84	1.86	1.87	1.87	1.93	1.85	1.78	1.82	1.89	1.89	1.84	1.93	1.96	1.91	1.83	1.89	1.91	1.71	1.91	1.88	1.71	1.84	1.73	1.92
SMDO11	1.89	1.94	1.81	1.92	1.95	1.91	1.90	1.80	1.88	1.93	1.91	1.93	1.79	1.89	1.94	1.94	1.71	1.71	1.93	1.73	1.82	1.80	1.92	1.96	1.93
SMDA28	1.82	1.89	1.81	1.55	1.52	1.70	1.81	1.76	1.50	1.66	1.59	1.64	1.54	1.83	1.91	1.83	1.85	1.82	1.80	1.68	1.93	1.89	1.86	1.87	1.84
SMS7	1.13	1.00	1.14	1.44	1.17	1.13	1.59	1.41	1.60	1.57	1.57	1.71	1.73	1.71	1.17	1.51	1.71	1.73	1.68	1.00	1.58	1.87	1.63	1.53	1.58
SMD28	1.11	1.11	1.00	1.49	1.21	1.47	1.11	1.47	1.57	1.64	1.67	1.60	1.00	1.00	1.44	1.00	1.00	1.20	1.00	1.00	1.00	1.20	1.41	1.00	1.42
SMBR10	1.82	1.78	1.79	1.66	1.58	1.82	1.83	1.79	1.51	1.67	1.67	1.76	1.75	1.78	1.87	1.87	1.67	1.76	1.87	1.80	1.64	1.80	1.53	1.43	1.72
L46951	1.68	1.74	1.77	1.43	1.76	1.62	1.55	1.62	1.82	1.77	1.78	1.82	1.69	1.65	1.79	1.67	1.83	1.71	1.80	1.60	1.80	1.78	1.73	1.71	1.83
SMD25	1.77	1.79	1.81	1.83	1.82	1.92	1.87	1.86	1.66	1.79	1.73	1.84	1.82	1.83	1.76	1.89	1.93	1.75	1.84	1.89	1.77	1.61	1.69	1.83	1.84
Mean	1.68	1.63	1.67	1.66	1.66	1.70	1.68	1.71	1.65	1.73	1.72	1.76	1.59	1.71	1.71	1.71	1.70	1.67	1.72	1.60	1.66	1.64	1.65	1.64	1.76

3.2.3.3 Heterozygosity

The number of samples analyzed and the results of the expected and observed heterozygosity of *Schistosoma mansoni* miracidia at each study populations for each of the eleven loci are indicated in Table11. From a total of 24 schistosomiasis positive individual a total of 247 miracidia were analyzed at 11 loci for their heterozygosity. It was observed that, the value is higher for the expected heterozygosity (59%-73%) than the observed heterozygosity (28%-59%). Paired *t*-test was used to evaluate the statistical significance of the deviations between the observed and expected heterozygosity (*t*(18.091), 23DF, *P*=0.000). F_{IS} value at infrapopulation level ranges from 0.23196-0.49892.

Table 11. Expected and observed heterozygosity with mean number of allele count and F_{IS} for each infrapopulation

Patient	Sample size	Loci typed	H_E	H_E SD	H_O	H_O SD	No. Alleles	No Alleles SD	F_{IS}	95%CI
K1	10	11	0.6824	0.0867	0.4601	0.0554	5.00	1.90	**0.34494**	(0.05579 -0.43622)
K2	10	11	0.6290	0.0938	0.3938	0.0515	5.55	3.14	**0.38889**	(0.20615 -0.43191)
K3	8	11	0.6753	0.0913	0.4074	0.0583	4.82	1.83	**0.41958**	(0.11086 -0.49138)
K9	10	11	0.6603	0.0560	0.4701	0.0489	5.18	2.44	**0.29971**	(0.15599 -0.33207)
K12	10	11	0.6559	0.0794	0.4735	0.0499	6.64	3.47	**0.29048**	(0.11189 -0.34722)
K13	10	11	0.6984	0.0714	0.5205	0.0518	5.91	2.77	**0.26863**	(0.05067 -0.30071)
K14	10	11	0.6808	0.0716	0.4436	0.0521	5.45	1.92	**0.36387**	(0.16972 -0.41061)
K15	10	11	0.7057	0.0487	0.3695	0.0594	4.36	1.80	**0.49892**	(0.23853 -0.53854)
SE1	9	11	0.6557	0.0526	0.4707	0.0541	4.45	1.75	**0.34882**	(0.09111 -0.42230)
SE12	29	11	0.7309	0.0372	0.5414	0.0295	7.18	3.95	**0.26324**	(0.17658 -0.32751)
SE14	29	11	0.7209	0.0335	0.5562	0.0293	7.27	3.66	**0.23196**	(0.13622 -0.30570)
SE16	5	11	0.7165	0.0365	0.5167	0.0762	3.55	0.93	0.30460	(-0.03072 -0.38731)
W21	5	11	0.5919	0.0977	0.4318	0.0755	3.27	1.85	0.28750	(-0.13514 -0.32817)
W22	6	11	0.7120	0.0777	0.4667	0.0649	4.36	1.63	0.37143	(-0.01250 -0.46882)
W38	6	11	0.7057	0.0743	0.5909	0.0630	4.91	2.17	0.17635	(-0.19171 -0.22309)
W47	6	11	0.7061	0.0795	0.5758	0.0623	4.73	1.74	0.20000	(-0.10236 -0.21839)
W51	6	11	0.6967	0.0756	0.4364	0.0657	4.00	1.73	**0.40496**	(0.09867 -0.40496)
W55	5	11	0.6683	0.0697	0.4455	0.0703	3.82	1.33	0.36087	(-0.05263 -0.38804)
W62	7	11	0.7161	0.0754	0.4680	0.0650	4.45	1.63	**0.36995**	(0.04770 -0.39774)
W88	6	11	0.6035	0.0960	0.4273	0.0639	3.82	1.94	0.31884	(-0.14187 -0.44037)
W89	8	11	0.6612	0.0799	0.4908	0.0593	4.55	2.50	0.27769	(-0.00187 -0.39571)
Z3	5	11	0.6437	0.0804	0.3485	0.0674	3.64	1.29	0.49727	(-0.21429 -0.56041)
Z4	32	11	0.6530	0.0638	0.4113	0.0283	7.55	4.01	**0.37517**	(0.26578 -0.46044)
Z5	5	11	0.6362	0.0821	0.2848	0.0752	3.09	1.87	0.59872	(-0.36842 -0.59872)

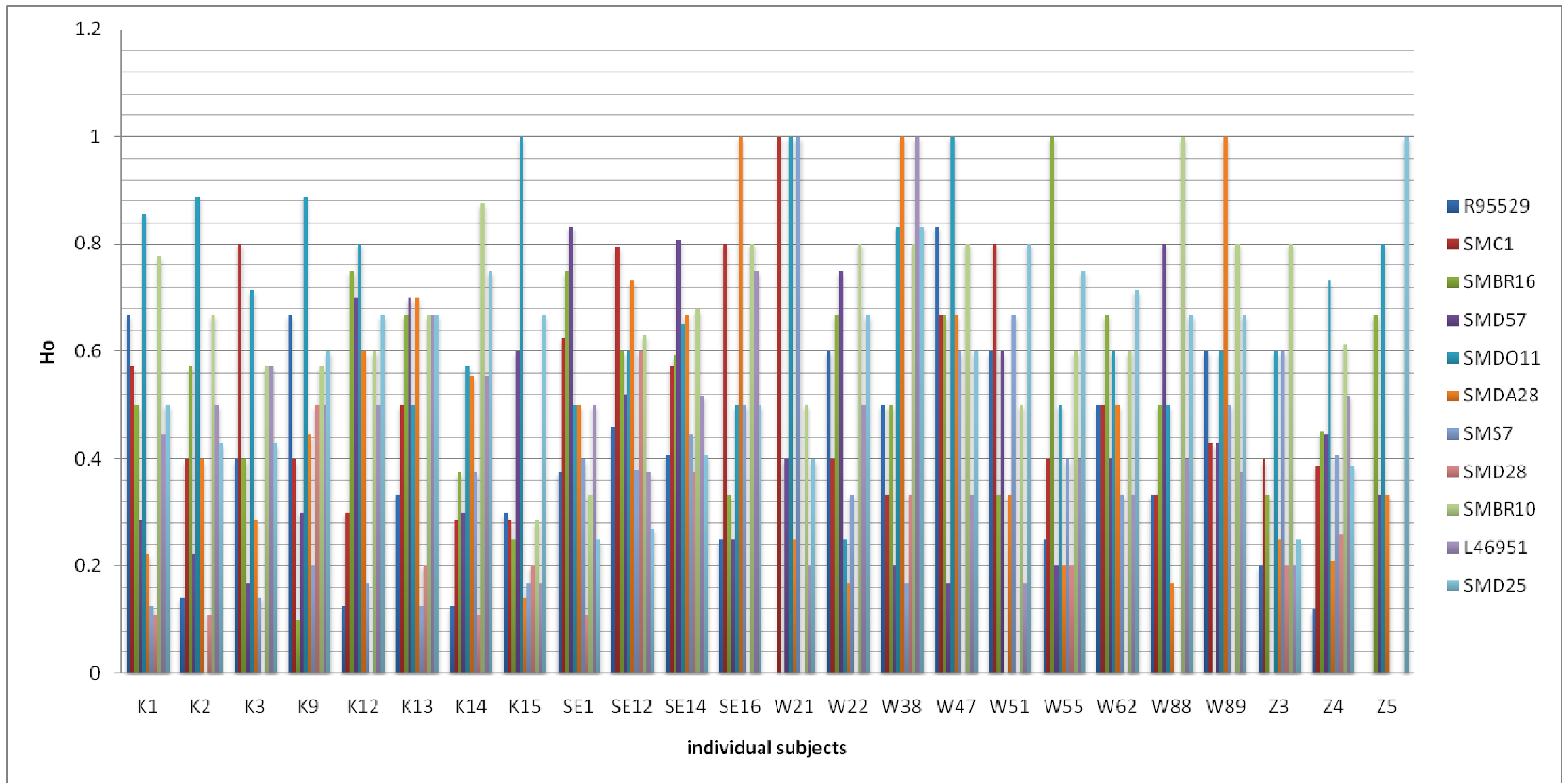

Figure 6. Observed Heterozygosity at infrapopulation level for each locus

3.2.3.4 Sibship determination

Microsatellite markers, due to their high polymorphism, are particularly well suited to studies of relatedness and kinship assessment (Queller *et al*., 1993; Blouin *et al*., 1996), and even with few loci, such molecular markers, without *a priori* pedigree information, have been successfully employed to the partitioning of individuals into families (Smith *et al*., 2001). The Colony software V1.1 (Wang, 2004), which takes into account the typing errors and mutations, has been developed and demonstrated to be able to accurately infer full- and half sibships from the molecular data of a high error rate. This program implements a maximum likelihood method to assign individuals from a population into full-sib families nested within half-sib families (colonies). It adopts an iterative procedure for updating allele frequencies with reconstructed sibships taken into account, and uses efficient algorithms for calculating the likelihood function and searching for the maximum-likelihood configuration (Wang, 2004).

In this study, Colony was applied without knowing parental information of *S. mansoni* larvae to the estimation of full-sib relationships between the sampled larval multi-locus genotypes. It was observed that, there were an estimated total of 37 genetically unique adult worm pairs, with a mean of 88% and 26 worm pairs shared among the eight individuals in Kemissie (Table12). Similarly, there were an estimated total of 36 genetically unique adult worm pairs, with a mean of 73%, and 19 worm pairs shared among the four individuals in Sille-Elgo. In Wondo Genet there were an estimated total of 32 genetically unique adult worm pairs, with a mean of 92%, and 15 worm pairs shared among the nine individuals. In Ziway, total of 24 genetically unique adult worm pairs, with a mean of 66% were observed and there were 4 worm pairs shared among the three individuals. Based on genetically unique adult worm estimation, the level of multiplicity of infection in an individual was analyzed. Thus, among the Kemissie infrapopulations, K2 had 100% genetically unique adult worm pairs, while K1, K12, K13 and K15 were infected with 90% genetically unique adult worm pairs each, and K9 and K14 with 80%. Similarly from Sille-Elgo, SE16 has 60%, SE14, 76%, SE1, 78% and SE12, 79% genetically unique adult worm pair infections. In Wondo Genet, W21, W22, W38, W55 and W62 had 100% while W47, W51 and W88 had 83% and W89 had 88% genetically unique adult worm pair infections. In Ziway Z3 had 80%, Z5 had 60% and Z4 had 59% genetically unique adult worm pair infection.

Table 12. Estimated sibship of *Schistosoma mansoni* at single human host level

Kemissie	Sample size	Estimated genetically unique adult worm pairs within hosts	Unique worm pairs	Shared pairs among patients
K1	10	9 (90%)	37	26
K2	10	10(100%)		
K3	8	7(88%)		
K9	10	8(80%)		
K12	10	9(90%)		
K13	10	9(90%)		
K14	10	8(80%)		
K15	10	9(90%)		
Mean		88%		
Sille-Elgo				
SE1	9	7(78%)	36	19
SE12	29	23(79%)		
SE14	29	22(76%)		
SE16	5	3(60%)		
Mean		73%		
Wondo Genet				
W21	5	5(100%)	32	15
W22	6	6(100%)		
W38	6	6(100%)		
W47	6	5(83%)		
W51	6	5(83%)		
W55	5	5(100%)		
W62	7	7(100%)		
W88	6	5(83%)		
W89	8	7(88%)		
Mean		92%		
Ziway				
Z3	5	4(80%)	24	4
Z4	32	19(59%)		
Z5	5	3(60%)		
Mean		66%		

3.3 Genetic structure of *Schistosoma mansoni* isolates of Ethiopia

In the present study the population structure of *Schistosoma mansoni* isolates of Ethiopia was determined using previously published 11 polymorphic loci. The structuration is presented both at population and infrapopulation level. Moreover, based on the Principal Component Analysis the distance between *Schistosoma mansoni* transmission sites within each endemic area are also determined.

3.3.1. Genetic structure at population level

In this study the mean F_{IS} value for all of the populations ranges from 0.27853-0.43473. The F_{ST} value between Ziway and Kemissie was 13.63%. It was also observed that it was 10.17% between Kemissie and Sille-Elgo, 9.15% between Wondo Genet and Sille-Elgo, 8.08% between Ziway and Sille-Elgo, 7.55% between Ziway and Wondo Genet and 3.57% between Kemissie and Wondo Genet where $P<0.05$ (Table13)

Table 13. Population pairwise F_{ST} values for the study populations at eleven loci

Location	Ziway	Kemissie	Wondo genet	Sille-Elgo
Ziway	_	13.63%	7.55%	8.08%
Kemissie	*	_	3.57%	10.17%
Wondo Genet	*	*	_	9.15%
Sille-Elgo	*	*	*	_

(F_{ST} above diagonal and their significance level below diagonal, * $p<0.05$)

The geographic distance between Kemissie and Sille-Elgo was 542.17Km while between Ziway and Kemissie it was 553.55Km. The respective geographic distance between Kemissie and Wondo Genet, Ziway and Wondo Genet, Ziway and Sille-Elgo and, Wondo genet and Sille-Elgo is 422.8Km, 132.5Km, 127.3Km and 178Km. Similarly, the genetic distance between Kemissie and Ziway is 16.21%, Kemissie and

Sille-Elgo is 10.7%, Sille-Elgo and Wondo Genet is 10.1%, Sille-Elgo and Ziway is 8.99%, Wondo Genet and Ziway is 8.21% and, Kemissie and Wondo Genet is 3.8%.

Table 14. Matrices of genetic distance (above diagonal) and geographic distance in Km (below diagonal) among study populations

Population	Kemissie	Sille-Elgo	Wondo Genet	Ziway
Kemissie		10.75%	3.8%	16.21%
Sille-Elgo	542.17		10.14%	8.99%
Wondo Genet	422.8	178		8.21%
Ziway	553.55	127.3	132.5	

Based on the geographic distance and percentage of differentiation, the map for the genetic distance for the populations was constructed as the following chart (Fig7).

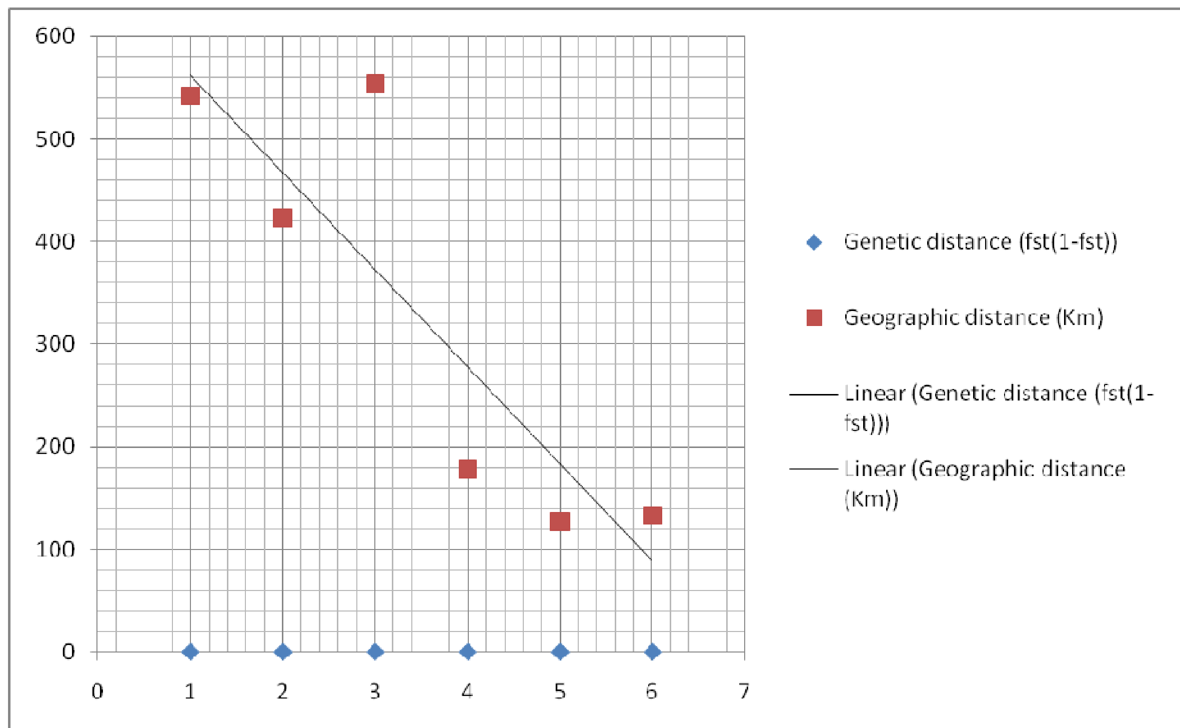

Figure 7. Geographic and genetic distance between the four study populations

Spatial genetic structuration or distribution of *Schistosoma mansoni* isolates in this study was determined by the Principal Component Analyses (PCA) using Genetix software. Each allele of each locus represents a variable that represents a total of 22

variables (11 loci x 2 alleles). Each individual was depicted by 22 variables and the PCA reduced the 22 variables into 2 (in 2D) or 3 (in 3D) variables. Each axe represented a part of the genetic variability (Fig8).

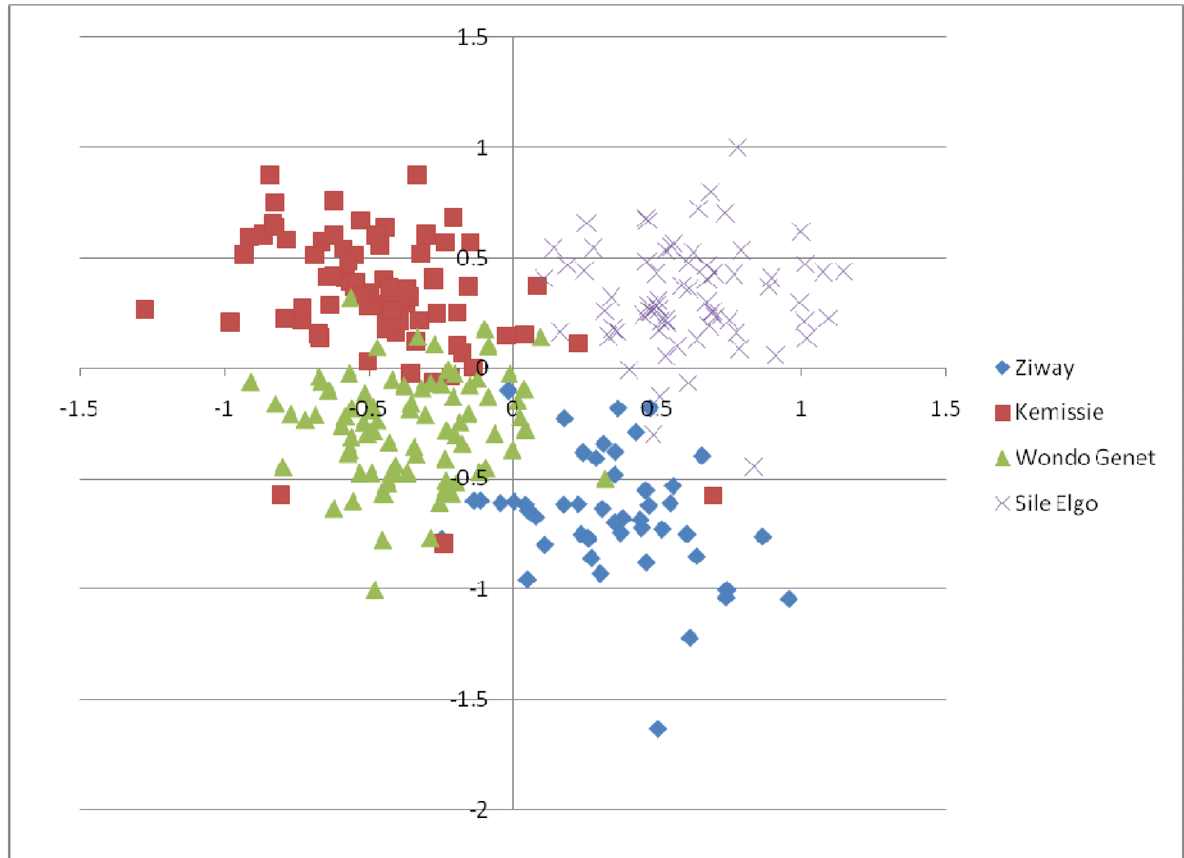

Figure 8. Assigned mean of population into clusters by Principal Component analysis

The Bayesian approach implemented in Structure software is a complementary than PCA approach because Structure assigns individuals in different clusters without consideration of the population of origin. Structure give the probability that the different individuals are assigned to K=1, 2 … to n clusters. This method does not rely on predefined populations and instead delineates clusters of individuals in Hardy-Weinberg equilibrium and assigns each individual a probability of belonging to a cluster (Pritchard *et al.*, 2000). In this study the maximum likelihood is obtained for

K=4 clusters; each cluster corresponding to one population. Based on the Bayesian approach STRUCTURE shows the mean assignation of the different populations in to four clusters (Fig. 9). Cluster 1 represents the structure of Ziway with 77% of its own and sharing about 23% from/with the rest. Cluster 2 represents Kemissie with 78% of its own and 22% sharing with the rest. Cluster 3 represents Sille-Elgo with 87% of its own and sharing 13% from the rest. Cluster 4 represents Wondo Genet having 68% of its own and sharing 32% with the others. It is observed that the percentage of each population sharing with the rest of the three populations is variable.

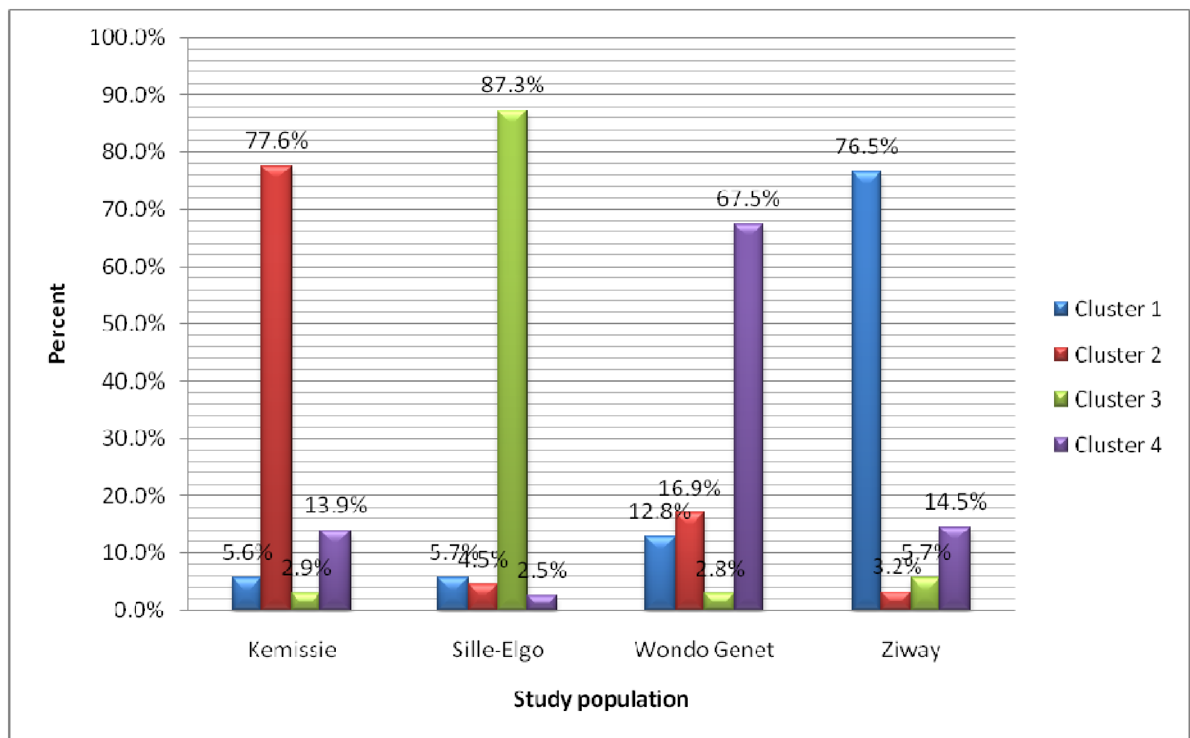

Figure 9. Assigned mean of the study populations into clusters

3.3.2. Genetic structure at infrapopulation level

At the infrapopulation level the F_{IS} also had a significantly higher value for all loci (0.23196-0.49892) indicating its departure from the Hardy-Weinberg equilibrium (Table11). Genetic structuration was determined at infrapopulation level based on PCA. Thus, considering a single individual patient the PCA had revealed four distinct infrapopulation structures (Fig10).

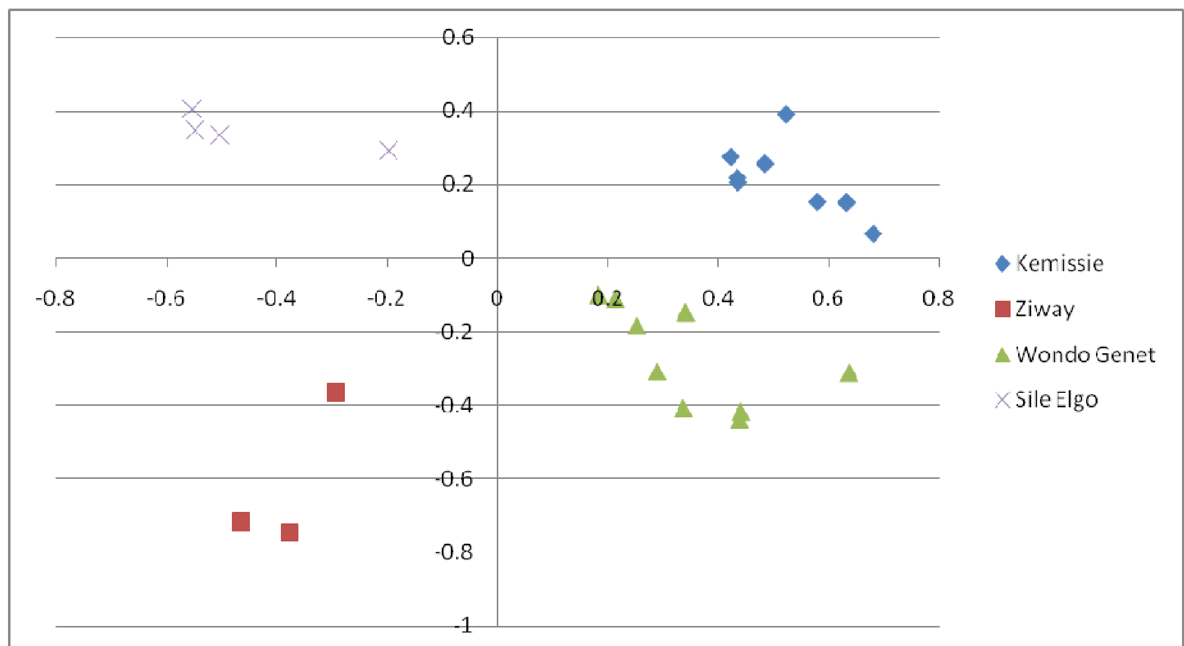

Figure 10. Assigned mean of infrapopulations in to clusters by Principal Component Analysis

Similarly, the Bayesian analysis using the STRUCTURE software had also assigned each of the 288 miracidia to their respective clusters. In the following graph one column corresponds to one individual with its assignation to the four different clusters (four colors) (Fig11). It was observed that there was variation from zero to hundred percent assignation of an individual to a different major cluster.

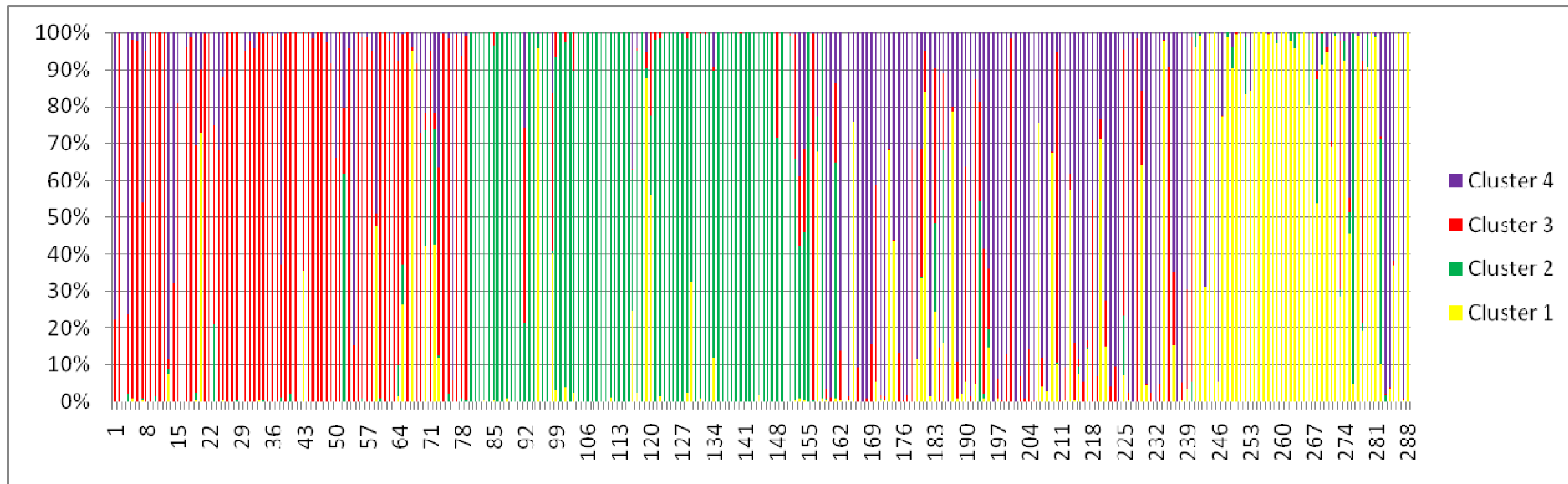

Figure 11 Assigned mean of individual miracidia to the four clusters

3.3.3. Determination of *Schistosoma mansoni* transmission site by PCA

One of the primary reasons for studying parasite population genetics is to understand demographic parameters, such as gene flow and population size, which are not readily observable using conventional ecological methods. These insights allow inferences regarding the patterns of transmission and recruitment within the environment (Thiele *et al*., 2008). Thus, based on this principle, in the current study, PCA had enabled us to determine the relative distance of *S. mansoni* transmission foci in each study sites (Fig. 12). The marks in the figure correspond to the infrapopulation of *S. mansoni* that infected individuals representing site of *S. mansoni* transmission. It was observed that the transmission sites for Kemissie were close to each other and aggregated. In Sille-Elgo most of the transmission sites were more aggregated in one area and very distant for some others. In Wondo Genet, the transmission sites were stretched and dispersed while in Ziway there were multiple sites of infection that are much far apart to each other.

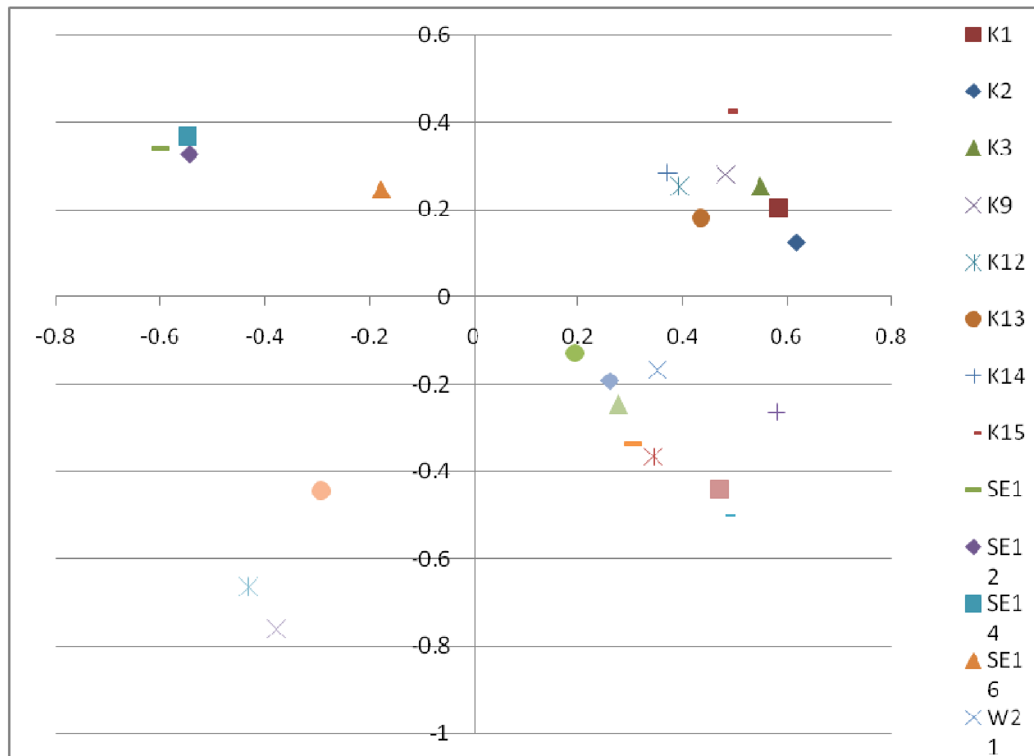

Figure 12. Transmission sites as determined by Principal Component Analysis

4. DISCUSSION

4.1. Prevalence and intensity of *Schistosoma mansoni* infection in different geographical areas

In the present study, it was observed that the prevalence of *Schistosoma mansoni* infection among the study participants in Kemissie, Wondo Genet and Sille-Elgo was 89.6%, 59.9% and 31.6%, respectively. Other helminth infections observed to be prevalent included *Ascaris lumbricoides* and *Trichuris trichiura* infections.

The complexity of schistosome infection is apparent at the phenotypic level in the marked variation between schistosome-infected individuals from the same endemic area, including variation in the intensity of infection, immune response to infection, and in the development of schistosome-related pathology among infected individuals. While these heterogeneities are consistent with host genetics, schistosomes do not multiply within the host and, therefore, such heterogeneity in infection may also result from the degree of exposure to infective stages (Bethony and Quinnel, 2008). Hence, observable variation in the prevalence and intensity of *Schistosoma mansoni* infection in the current study followed the same patterns.

In the current study the prevalence and intensity of *Schistosoma mansoni* infection among the three study sites was considerably variable in its distribution. In Kemissie 83.9% of males and 71.3% of females were found positive for *S. mansoni*. There was no significant variation observed in infection rate with regard to sex and age. However, there was variation in intensity of infection among the different age categories. It was observed that the age group 5-14years old were having similar proportions of light, moderate and heavy infection rate while those at the 15-19years of age group were having high proportion of heavy infection. Moreover, the 5-9years of age group were 1.517 times more likely than the 10-14years of age and 2.044 times more likely than 15-19years of age group to acquire high intensity of infection. However, there was no association observed with regard to intensity of infection and sex.

In previous study conducted around Kemissie (Cheretee), Berhe *et al*. (2007) reported *S. mansoni* infection prevalence of 50.2% of which 28.3% of them had an egg per gram count of greater than 100 with a geometric mean of 24.1. In contrast to this previous study, the current study showed higher prevalence (89.9%) and intensity (346 EPG) of *S. mansoni* infection. This indicates that there is a change in the dynamics of *S. mansoni* transmission.

The present study showed that *S. mansoni* infection was found to be more prevalent in males (65.5%) than in females (55%) in Wondo Genet. Males were 1.551 times at a higher risk than females. Furthermore, moderate and heavy intensity of infection was more prevalent in males than females, while the prevalence of light infection was relatively higher for females than males.

With regard to age, individuals in the 5-14 years age group had high intensity of infection than the rest. In a Brazilian study, among the demographic variables considered, a significant association was found between infection and gender, as well as age group. Males showed 3.39-times higher risk for infection than females (Enk *et al*., 2010). Their finding is consistent with the present study.

In Sille-Elgo, the proportions of males and females which were *S. mansoni* positive were 32.8% and 30.2%, respectively. There was no significant association between sex and infection rate. However, there was significant association between infection rate and age. Individuals in the 5-9 years of age group were 2.889 times at risk than those in the 15-19 years of age group. In Sille-Elgo, the intensity of *S. mansoni* infection was low (range, 24 to 3960 epg), compared to the other two sites in the present study. This shows the general trend that does exist between the intensity and prevalence of infection. A previous study to determine factors affecting prevalence and immune responses to *Schistosoma mansoni* in schoolchildren had shown that the age of the children significantly affected prevalence whereas sex had only marginal effects (Degu *et al*., 2002). This finding is also in agreement with the current study.

Generally the intensity of infection in Sille-Elgo was found to be associated with age. In the 5-9 years age group, the intensity of infection was 7.457 times that of 40-44 years age group. Children below 10 years of age are having the highest intensity of infection compared with the adults. In a Brazilian study the highest infection rates occurred in the age group from 10-30 years and the highest epg were found in the age group of children

up to 10 years (Enk *et al*., 2010). Similarly a pre and post-treatment comparative study in Egypt showed that the prevalence and intensity of infection were higher in the younger (5-19) than in the older (20-50) age group both before treatment and one year post-treatment. In terms of age in relation to high intensity of infection, these finding are in line with the current study.

Previous work by Berhe and colleagues (2007) demonstrated that in Sille-Elgo the prevalence of *S. mansoni* infection was 65.1%, the intensity of infection being over 100 epg (mean 24 epg) in 33.8% of the positive cases. However, in the present study the prevalence of *S. mansoni* infection was 31.6% with a maximum epg of 3960 (mean 91 epg). Although the prevalence of *S. mansoni* in Sille-Elgo decreased, the intensity of infection remains high. This could be due to overdispersion of *S. mansoni* infection in an endemic community. An important consequence of the overdispersed distribution in egg counts is that the majority of eggs excreted in a community come from a minority of residents. Results of a study conducted in Ethiopia indicated that 50% of the eggs excreted came from 5% of the community (Polderman, 1979), and in a Brazilian study the same proportion came from 6% of the community (de Lima e Costa *et al*., 1985). Moreover, day to day fluctuations in egg counts for a single individual had also been documented (Engels *et al*., 1996) as a contributing factor in the variation of intensity of infection.

The prevalence and intensity of *Schistosoma mansoni* infection is important in determining the morbidity of the disease. The distribution of *S. mansoni* infection among members of a population varies, among other factors, due to occupational and behavioral differences. Age and sex are important epidemiological factors in the determination of prevalence and intensity of infection of schistosomiasis (Degu *et al*., 2002). Previous investigation in Kemissie and Sille-Elgo showed that the morbidity level due to *Schistosoma mansoni* infection varied in different endemic villages and that it was dependent on sex and intensity of infection (Berhe *et al*., 2007). According to their finding based on ultrasonography, the proportion of schistosomal definite/periportal thickening/fibrosis in Cheretee, Chekorso and Worke Mado villages around Kemissie, and in Sille-Elgo was 2.6%, 26.6%, 5.9% and 0.6%, respectively. Therefore, the occurrence of variation in the intensity of infection in the current study

areas could result variation in the level of morbidity among the different *S. mansoni* positive subjects.

Results from the three study sites combined, the overall prevalence of *Schistosoma mansoni* infection was 60.48% (60.9% in males and 59.8%), indicating the absence of association between sex and rate of infection. However age has impact on infection rate. The risk in the 5-9 years age group was 2.262 times than that in the 15-19 years age group and 2.524 times than that in the 20-24 years age group. Other than being the risk for infection, age was also a factor in determining the intensity of infection.

Different confounding factors can be associated with intensity of infection in different age groups. There are speculations that the puberty-related hormone dehydroepiandrosterone sulphate (DHEAS) might influence the intensity of infection and immune responses during *Schistosoma* infections. A previous study conducted to evaluate the relationships between DHEAS, intensity of *Schistosoma mansoni* infection and humoral immune responses in 135 residents in northeastern parts of Ethiopia demonstrated that there was a significant negative correlation between serum levels of DHEAS and intensity of *S. mansoni* infection. A significant increase in serum levels of DHEAS in the age group 15-19 years was accompanied by a progressive decline in the intensity of infection. Peak level of DHEAS coincided with the lowest intensity of infection in the age group 20-29 years (Abebe *et al*., 2003). Thus, this factor can explain the occurrence of high level of *S. mansoni* infection in lower age groups than the elders in the current study.

4.2. Level of genetic diversity and multiplicity of *Schistosoma mansoni* infection

In the current study based on the number of alleles counted and allelic richness the level of genetic diversity at population and infrapopulation level as well as the occurrence of genetically different *Schistosoma mansoni* infections at intrahost level had been determined.

4.2.1. Genetic diversity at population level

4.2.1.1. Allelic diversity

The PCR products of DNA extracts of *Schistosoma mansoni* isolates from four geographically apart localities of Ethiopia provided a total of 164 unique alleles when genotyped for 11 microsatellite loci. Individually 127, 123,102 and 94 alleles were counted from Kemissie, Wondo Genet, Sille-Elgo and Ziway isolates, respectively. The frequency of alleles counted was used to measure heterozygosity. The number of alleles scored for each locus within the four study populations ranged from 3 to 25. Among the study localities, the largest mean number of alleles was recorded for Kemissie (11.5) while the lowest mean number of allele was counted for Ziway (8.5). This result indicated the occurrence of variation in the number of alleles counted among the study sites. The nonparametric Friedman test had shown that the difference in number of alleles counted among the study populations is statistically significant. Therefore, this difference in number of alleles among the study populations shows variation in degree of heterozygosity.
In a study from Kenya (Agola *et al*., 2006), a total of 114 alleles were recorded across all individuals at the five loci studied. In their study the total number of alleles per locus ranged from 11 for SMC1 to 43 for SMDO11 loci. The largest and lowest mean number of alleles counted for two populations was 13.67 and 8.22, respectively, a relatively similar finding with the current study.

Among the eleven microsatellite loci studied in the current study, SMDO11 had the highest number of allele count for all of the study sites (in the range of 22-25) while

SMD28 had the lowest value in all of the study sites, ranging from 3-6. The total number of alleles per locus for all four populations combined ranged from 6 for SMD28 to 34 for SMDO11.

When the number of total alleles scored for the loci SMC1 (7) and SMDO11 (34) in the current study was compared with the work of Agola et al. (2006) which was 11 and 43, respectively, there is difference between the Ethiopian and the Kenyan strains.

4.2.1.2. Allelic richness

Allelic richness (*An*), also referred to as allelic diversity or mean number of alleles per locus, is one of the most commonly reported measures of genetic variation (Leberg, 2002). In this study the allelic richness of *Schistosoma mansoni* was calculated based on minimum sample size of 29 diploid individuals. Accordingly, the allelic richness for each locus ranged from 3.46 – 20.8 (SMD28-SMDO11) with a mean value of 9.9 in all of the four populations. At individual population level, Ziway had allelic richness value of 3.6 - 22.28 (SMD28-SMDO11) with a mean value of 9.06, while Kemissie had allelic richness of 2.99 - 18.93 (SMD28 -SMDO11) with a mean value of 9.44; Wondo Genet had 3.79-16.81(SMD28-SMDO11) with a mean value of 9.18 and, Sille-Elgo 3.0-18.16 (SMD28-SMDO11) with a mean value of 7.71. Although the allelic richness scored was higher for Ziway followed by Kemissie, Wondo Genet and Sille-Elgo, the nonparametric Friedman test showed that there is no significant difference in allelic richness among the four populations. However, Wilcoxon rank test indicated that Sille-Elgo had the lowest allelic richness. Hence, there shows low genetic variation of *S. mansoni* isolates of Sille-Elgo compared to the others.

In a Kenyan study, the mean number of alleles reported among four school populations ranged from the minimum 8.22 to the maximum 9.48 (Agola *et al.*, 2009). Hence, the mean allelic richness value in the Kenyan study is equivalent with observations from the current study, indicating the presence of genetic variability among *S. mansoni* isolates.

4.2.1.3. Heterozygosity

One of the parameters used to measure the level of genetic diversity in a population is the degree of heterozygosity. In the current study, the observed heterozygosity at the population level ranged from 0.16-0.75 in Kemissie, 0.34-0.75 in Sille-Elgo, 0.10-0.69 in Wondo Genet and 0.16-0.67 in Ziway for each locus indicating the presence of high genetic diversity. However, there is a difference in the degree of heterozygosity when each locus is considered. The highest observed heterozygosity in Kemissie was at the

locus SMDO11 (0.75), in Sille-Elgo at the locus SMDA28 (0.71) in Wondo Genet at the locus SMD25 (0.69), and in Ziway at the locus SMDO11 (0.67). The mean observed heterozygosity for Sille-Elgo, Wondo Genet, Kemissie and Ziway was 0.53, 0.5, 0.44 and 0.38, respectively. Nevertheless variation in the level of heterozygosity among the populations was marginal.

The mean expected heterozygosity for Wondo Genet, Sille-Elgo, Kemissie, and Ziway was 0.77, 0.73, 0.70 and 0.67, respectively. The mean values of expected and observed heterozygosities for 11 loci studied in Brazil were 0.79 and 0.59, respectively (Rodrigues *et al*., 2007) which is a bit similar to our finding. In the current study there was statistically significant difference between the expected and observed heterozygosities showing significant departure from Hardy–Weinberg equilibrium. The F_{IS} value for all loci in the four populations is significantly greater than zero, indicating heterozygote deficiency, while Sille-Elgo and Ziway had less than zero value at the SMDA28 and SMBR10 loci, respectively indicating heterozygote excess.

A study by Agola *et al*. (2009) showed that the expected heterozygosity and observed heterozygosity has no statistically significant difference among four different school populations unlike the current finding. In a comparison of microsatellite polymorphism and heterozygosity among field and laboratory populations of *Schistosoma mansoni* isolates from Brazil, Stohler and colleagues (2004) had shown that there is low polymorphism and heterozygosity in the laboratory populations. They showed that a total of 127 alleles were detected in the field population across seven loci, with the worms of any one patient isolate having a range of 24-29 alleles. The average heterozygosity in the field population was 0.58. The field population also displayed a significant deficiency of heterozygotes across all loci, compared to the values expected from the allele frequencies, assuming Hardy-Weinberg equilibrium. Their finding on field populations is comparable to the current study. When comparable loci were analysed the expected and observed heterozygosity in their study for the the locus SMDA28, L46941, R95529, SMD28 and SMDO11 were (0.86, 0.73), (0.83, 0.54), (0.81, 0.58), (0.45, 0.36) (0.91, 0.65), respectively. While in the current study for the same loci the the maximum expected and observed heterozygosity within the four populations was (0.87, 0.71), (0.78, 0.49), (0.81, 0.50), (0.66, 0.40) and (0.92, 0.75), respectively.

4.2.2. Genetic diversity at infrapopulation level

4.2.2.1. Allelic diversity

Schistosoma mansoni infrapopulations (populations derived from a single host) in the current study had high level of genetic polymorphism as evidenced by a large number of alleles detected in each infrapopulation. The number of allele count at a single locus for the twenty four infrapopulations in the current study ranged from 1 to 17 with a mean value of 3.27-7.55. The largest mean number of allele was 7.54 and the lowest was 3.09. Most of the individuals had a low allele count for the SMD28 locus. It was observed that there is variation in the number of alleles counted from a single host for the eleven loci among the infrapopulations. Among individuals form Kemissie, the allele count for the loci SMD57 and SMDO11 was higher. Among the eight infrapopulations from Kemissie, K12 had the highest total allele count (73) compared to the rest. Among the four infrapopulations from Sille-Elgo, SE12 and SE14 had a total allele count of 79 and 80 which is higher than that of Kemissie. From Wondo Genet, among the nine infrapopulations, the highest allele count was for W47 (52) and W38 (54). Among the three infrapopulations from Ziway, Z4 had the highest allele count (83) compared to Z3 (40) and Z5 (34). Ziway showed the highest and lowest number of allele count at infrapopulation level when all four study sites are compared. The nonparametric Friedman test indicated that there is significant difference in the number of alleles scored in each individual. The mean number of alleles for Wondo Genet, Kemissie, Sille-Elgo and Ziway was 3.27-4.91, 4.36-6.64, 3.55-7.18 and 3.09-7.55, respectively. These results show that there is variation among and within infrapopulations in the number of alleles exhibited at the 11 loci level, suggesting that the degree of heterozygosity is highly variable among the study subjects at infrapopulation level.

In a Kenyan study conducted to determine genetic diversity and population structure of *Schistosoma mansoni* within human infrapopulations, it was observed that the number of alleles per locus ranged from 2 to 25 as assessed by microsatellite markers. The largest and lowest mean number of alleles for infrapopulations was 10.22 and 8.22, respectively (Agola *et al*., 2009), relatively a higher value compared to the current

study. In a comparison study of infrapopulations genetic diversity between two villages in Brazil, Thiele *et al*. (2008) reported that in one of the villages from 5 to 27 alleles were observed per locus in the 585 worms genotyped, with a mean of 14.1 alleles per locus. In the other village a total of 14 to 24 alleles were observed per locus, with a mean of 18.1 alleles per locus, which is much higher than the current study. In a previous study based on 21 microsatellite analysis of miracidia from Kenya, Steinauer and colleagues (2008b) had found that the number of alleles scored ranged from 2-15 which is much similar to the current finding.

Allelic diversity assessed in five *S. mansoni* isolates (four from human patients, one from field-collected snail intermediate hosts) from two villages in the state of Minas Gerais, Brazil, revealed extensive polymorphism (Curtis *et al*., 2001). In their finding it was observed that the maximum number of alleles scored for SMDA28, SMC1 and SMDO11 loci was 8, 7 and 6, respectively. In contrast to this the number of alleles for the same loci in the current study was 24, 9 and 43 indicating very high polymorphism.

The level of genetic diversity in schistosome populations is likely to be influenced by a variety of factors. On the one hand, repeated exposure of children in a selected number of transmission sites can result in the recirculation of related populations of schistosomes. If infection of humans and snails occurs at isolated transmission sites, then this would lead to development of distinct schistosome lineages as mutation and drift affects isolated populations within hosts and across geography (Agola *et al*., 2009). Rapid turnover of infected snails induces a temporal succession and renewal of different larval genotypes within a site and spatial aggregation of infected snails concentrate cercariae with different genotypes within a limited standing water area favoring multigenotype infection of definitive hosts (Theron *et al*., 2004). On the other hand, opportunities for genetic exchange resulting from snail and especially human movements may lead to coexistence of a large number of genotypes in an area (Brouwer *et al*., 2001).

4.2.2.2. Allelic richness

In this study, the allelic richness for each infrapopulation was determined based on one diploid individual for all 11 loci typed. The allelic richness for Sille-Elgo, Kemissie,

Wondo Genet, and Ziway ranged from 1.28-1.93, 1.11-1.95, 1-1.96 and 1.2-1.96, respectively. Though there is a genetic diversity in all infrapopulations, there was variation in allelic richness of each infrapopulation in relation to each locus. However, the Friedman nonparametric test showed that there is no significant difference in the allelic richness for all the infrapopulations typed for all of the 11 loci. Agola *et al.* (2009) reported that among twelve infrapopulation observed in their previous study the Friedman nonparametric test had shown no significant difference in the mean number of allele per locus which is in agreement with the current study.

4.2.2.3. Heterozygosity

In the present study, a total of 247 miracidia were analyzed from 24 *Schistosoma mansoni* positive human cases that represented 24 infrapopulations, at 11 loci for their heterozygosity. The expected heterozygosity had ranged from 59% to 73% while the observed heterozygosity had ranged from 28% to 59%. It has been shown that the expected heterozygosity value is higher than the observed heterozygosity. The nonparametric Friedman and Paired *t*-tests indicated a statistically significant difference between the expected and observed heterozygosity. The inbreeding coefficient (F_{IS}) value at infrapopulation level is significantly greater than zero indicating heterozygote deficiency. However, since the observed heterozygosity at individual level for all the eleven loci is higher there is genetic variation among infrapopulations of the study subjects. Agola *et al*. (2009) from Kenya had reported 68% to 70% expected heterozygosity among twelve infrapopulations which was not also different from the observed heterozygosity. The nonparametric Friedman test had also indicated the expected heterozygosity value difference was nonsignificant in their finding. Although the expected heterozygosity of their study is relatively similar, there is difference in the observed heterozygosity compared with the current study. This indicates that in the Kenyan case a Hardy Weinberg equilibrium is maintained while the Ethiopian sample shows departuere from Hardy Weinberg equilibrium due to some selective and migration forces.

4.2.2.4. Sibship determination

Based on the Colony software analysis Lu and colleagues inferred the number of genetically unique adult *S. japonicum* worm pairs within a host individual from the reconstructed families of the miracidia within that host. Thus, they were able to determine the full-sibship of *S. japonicum* from different hosts (Lu *et al*., 2010). Likewise in this study it was possible to infer the sibship of genetically unique adult worm pair estimates within the human hosts.

In this study, Colony was applied without knowing parental information of *S. mansoni* larvae, to the estimation of full-sib relationships between the sampled larval multi-locus genotypes. It was observed that in Kemissie there were an estimated total of 37 genetically unique adult worm pairs, with a mean of 88%, while there were 26 adult worm pairs shared among the eight individuals. Similarly, in Sille-Elgo it was observed that there were an estimated total of 36 genetically unique adult worm pairs, with a mean of 73% and 19 adult worm pairs shared among the four individuals. In Wondo Genet there were an estimated total of 32 genetically unique adult worm pairs, with a mean of 92% and 15 adult worm pairs shared among the nine individuals. In Ziway there were an estimated total of 24 genetically unique adult worm pairs, with a mean of 66% and 4 adult worm pairs shared among the three individuals. The estimation of genetically unique adult worm pairs within a single host proves the occurrence of genetically different *S. mansoni* infections within a single host.

Based on genetically unique adult worm estimation, the level of multiplicity of infection in an individual was also analyzed. Thus, among the Kemissie infrapopulations, K2 had 100% genetically unique adult worm pairs, while K1, K12, K13 and K15 were infected with 90% genetically unique adult worm pairs each and K9 and K14 with 80% genetically unique adult worms each.. Similarly from Sille-Elgo, SE16 had 60%, SE14, 76%, SE1, 78% and SE12, 79% genetically unique adult worm pair infections. In Wondo Genet, W21, W22, W38, W55 and W62 had each 100% while W47, W51 and W88 had each 83%, and W89 had 88% genetically unique adult worm pair infections. In Ziway Z3 had 80%, Z5 had 60% and Z4 had 59% genetically unique adult worm pair infection. In general Sille-Elgo and Ziway presented lower unique adult worm pairs (73% and 66%) than Kemissie or Wondo Genet (88% and 92%), respectively. These results are important indicators for the presence of multiple genotypes of *Schistosoma mansoni* infections in a single host.

An investigation on the genotypic composition of *Schistosoma mansoni* for its adult stages within the definitive host (the wild rat, *Rattus rattus*) and for the larval stages within the intermediate host (the snail, *Biomphalaria glabrata*) both collected at the same transmission site was conducted by Theron and colleagues (2004). Their analyses were based upon the recognition and distribution of 200 different multilocus genotypes generated by RAPD markers. The result showed that intramolluscan larval infrapopulations were characterized by a low infection rate (0·6% on average) and low

intra-host genetic diversity (1·1 genotype on average per infected snail), while adult infrapopulations within rats showed a high infection rate (94%) and a substantial intra-host genetic diversity (34 genotypes on average) linked to high intensities (160 worms per host on average). In a single definitive host bearing 105 different genotypes harbored 52% of the total genetic diversity detected within the whole parasite population. Analysis of the genetic data allowed them the identification of various ecological, behavioral and immunological factors which are likely to enhance transmission of multiple parasite genotypes towards the vertebrate hosts. This identification of infection of both the intermediate and definitive hosts with genetically different *S. mansoni* strains is in favor of the current finding where multiplicity of infection had occurred in human hosts based on sibship determination.

4.3. Genetic structure of *Schistosoma mansoni* isolates of Ethiopia

In the current study the genetic structure of *Schistosoma manso*ni isolates from Kemissie, Ziway, Wondo Genet and Sille-Elgo was determined based on FSTAT, the Principal Component Analysis and the Bayesian clustering STRUCTURE.

4.3.1. Genetic structure at population level

Schistosome population genetic structure plays an important role in schistosome epidemiology. If variation in *Schistosoma mansoni* is distributed as in a panmictic species, with sufficient gene flow within a region, or even across a country, to swamp localized selection pressures, then there is no population structure. The implication would be that the present variation is subject to frequent recombination as adult worms pair and mate within vertebrate hosts, continuously generating novel combinations of alleles while rapidly spreading alleles that are particularly favorable to the parasite (ie. praziquantel resistance) across all populations. If, on the other hand, different natural populations of schistosomes are adapting to their local environments and not frequently exchanging genes with populations in slightly different neighboring habitats, then there is the potential for population subdivision to occur. Each of these relatively isolated subpopulations could then evolve a disparate set of characters for traits such as drug susceptibility and pathology (Curtis and Minchella, 2000).

In the current study all loci were tested pairwise and based on 4400 permutations and adjusting P value to 0.000227, the result indicated that there was no linkage disequilibrium detected among them. Thus, the result obtained from the employment of these loci is amenable.

In order to document the extent of separation between the schistosomes from the different localities of Ethiopia F_{ST} was calculated for all possible pairs. F_{ST} indicated that there is highest percentage of differentiation among Ziway and Kemissie (13.63%) followed by Kemissie and Sille-Elgo (10.17%), Wondo Genet and Sille-Elgo (9.15%), Ziway and Sille-Elgo (8.08%), Ziway and Wondo Genet (7.55%) and, Kemissie and Wondo Genet (3.57%). Pairwise F_{ST} values between all populations were high and test

of significance showed that the difference in genetic differentiation among the study populations is very high. These results showed a clear and significant genetic differentiation, supporting distinctions among the four populations, thus implying that there is restricted gene flow among the schistosomes under study.

This high genetic diversity of Ethiopian *Schistosoma mansoni* isolates can also be explained as a result of coinfections by multiple genotypes from genetically different cercaria. Schistosome genetic diversity within molluscan host populations has been characterized in previous studies elsewhere. These previous studies showed that the biology of the schistosomes is such that dispersal of the parasite is dependent on the host dispersal and the dissemination of the free larval stages (miracidium and cercariae) (Agola *et al*., 2006). In the current study the large geographical distance separating the four study sites is likely to limit contacts between populations, thus promoting differentiation among them.

A previous study on the genetic diversity and population structure of seven populations of *Schistosoma mansoni* sampled in Kenya assessed using five microsatellite markers revealed values ranging from 5.2 to 10.7, 0.5–0.8 and 3.6–27.3% of mean number of alleles per locus, expected heterozygosity in Hardy–Weinberg equilibrium and pairwise F_{ST} test, respectively. These data showed that *S. mansoni* populations in Kenyan, have relatively high levels of genetic diversity and is significantly differentiated. These data combined with information on biogeography support the hypothesis that the strong genetic structure in Kenyan schistosomes is as a result of limited gene flow and large population sizes (Agola *et al*., 2006). This finding is in support of our finding where there is high genetic differentiation and there is no gene flow.

In this present study the Principal Component Analysis had shown the structuration of *Schistosoma mansoni* isolates into four clusters. The Bayesian approach implemented in STRUCTURE software is a complementary than PCA approach because STRUCTURE assigns individuals in different clusters without consideration of the population of origin. STRUCTURE gives the probability that the different individuals are assigned to K=1, 2 … to n clusters. This method does not rely on predefined populations and instead delineates clusters of individuals in Hardy-Weinberg equilibrium and assigns each individual a probability of belonging to a cluster (Pritchard *et al*., 2000).

STRUCTURE identifies groups of individuals corresponding to the uppermost hierarchical level, and performs well with both dominant and codominant markers (Evanno *et al.*, 2005). In this study based on the Bayesian approach the maximum likelihood is obtained for K=4 clusters; each cluster corresponding to one population.

A similar study in Brazil using Bayesian clustering of individuals indicated that *K* values of 4 and 5 were the most probable, with 4 having greater population differentiation and structuration (Thiele *et al.*, 2008) which is in agreement with this current study.

The Bayesian clustering analysis supports the conclusion of greater genetic differentiation in the study sites. The percentage of genetic differentiation for Ziway was 77% of its own and sharing about 23% from the rest. Kemissie had a genetic differentiation of 78% of its own sharing 22% from the other three. Sille-Elgo had a genetic differentiation of 87% of its own and sharing 13% from the rest. Wondo Genet had 68% of its own genetic differentiation and sharing 32% with the others. These four clusters indicate that there is high genetic differentiation among the study sites in which each population has its own major genetic makeup that differentiate it from the others. The result indicated that Sille-Elgo is more differentiated than Ziway, Kemissie and Wondo Genet.

Steinauer and colleagues (2009) examined the spatial structure of *Schistosoma mansoni* from natural infections at two levels: across the Lake Victoria basin of Kenya and among snail hosts. Using 20 microsatellite markers they examined geographic patterns of relatedness and population structure of cercariae and found weak, but significant structure detected by some, but not all analyses. The analyses with STRUCTURE, the Bayesian clustering analysis that makes no *a priori* assumptions of population structure, indicated that the most optimal partition of the data consisted of one cluster, with no subdivision, suggesting a single panmictic population. Also, when analyses were performed with more than one cluster, the estimated membership coefficients indicated that each individual was about equally likely to belong to all of the estimated clusters, which supports the presence of only one cluster. They hypothesized that structure created by aggregations of clonal individuals or adherence of hosts to local transmission sites is eroded by high amounts of gene flow in the region. Their finding is in contrast

to this study where four clusters of genetic structure are found and where there is no gene flow.

Isolation by distance, in the context of population genetics, is the process by which geographically restricted gene flow generates a genetic structure, because random genetic drift is occurring locally. It is an important phenomenon to consider whenever the genetic structure or the evolutionary trends of natural populations are to be analyzed spatially. Isolation by distance occurs in subdivided populations, when subpopulations exchange genes at a rate dependent upon the distance, or within a continuously distributed population, when dispersal of gametes and/or zygotes is spatially restricted (Hardy and Vekmans, 1999).

In this study, the geographic distance was higher between Ziway and Kemissie, followed by Kemissie and Sille-Elgo, Kemissie and Wondo Genet, Ziway and Wondo Genet, and, Ziway and Sille-Elgo. Similarly, the genetic distance was higher between Kemissie and Ziway followed by Kemissie and Sille-Elgo, Sille-Elgo and Wondo Genet, Sille-Elgo and Ziway, Wondo Genet and Ziway and Kemissie and Wondo Genet. Although the genetic distance between the study populations is comparably variable, it was not associated with difference in geographic distance.

A previous work in Zimbabwe demonstrated that schistosomes derived from snails along non-connected river systems showed a substantial genetic diversity with genetic distance increasing with geographic diversity (Davies *et al*., 1999). However, this is not in agreement with the findings of this current study where the genetic variation is not due to isolation by distance. Other previous studies at Guadeloupe island (Prugnolle *et al*., 2005), Brazil (Thiele *et al*., 2008) and Kenya (Agola *et al*., 2006; Agola *et al*., 2009) had shown that the genetic diversity of *S. mansoni* population was not associated with isolation by distance which is in agreement with our finding. As described by Agola *et al*. (2006), the probable reason for the absence of isolation by distance in this study could be due to a combination of factors that include restricted gene flow between populations, local adaptations and systematic variations in environmental conditions.

4.3.2. Genetic structure at infrapopulation level

Wright's F-statistics were employed to characterize the structures at the level of infra-populations. F_{IS} measures the within-host population magnitude of departures from Hardy-Weinberg equilibrium expectations (Wright, 1965). In this study at the infrapopulation level the F_{IS} also has a higher value (0.23196-0. 49892) indicating its departure from the Hardy-Weinberg equilibrium. This inbreeding coefficient shows a reduction of heterozygosity at individual level which implies high genetic inbreeding at infrapopulation level.

In the current study a single infrapopulation represented with at least five miracidia isolate indicated similar population structure like that of the whole population based on PCA. It was observed that there were four genetically differentiated clusters at infrapopulation level. This high genetic differentiation between infrapopulations could be associated with the nature of schistosome transmission.

Minchella *et al*. (1995) described that infection of snails with multiple genotypes will have two main epidemiological consequences. It increases the probability that humans will acquire a dual-sex infection and also facilitates the maintenance of genetic diversity among infrapopulations. Thus, the genetic differentiation observed within an individual in the current study could be associated with infection with different *S. mansoni* strains. In contrast to this finding a study in central Kenya showed that the PCA lacked clear geographical patterns suggesting the absence of strong substructure within the *S. mansoni* population. Assignment of individual to populations using GENECLASS software also indicated the same pattern of random assignment of individuals to population further indicating a lack of genetic structure (Agola *et al*., 2009).

A study conducted to evaluate epidemiological patterns of *Schistosoma mansoni* among human definitive hosts in the village Virgem das Gracas, the state of Minas Gerais, Brazil, where schistosomes are primarily transmitted in stream habitats, indicated that schistosomes were significantly substructured among individual patients and among households (separated by 1 m to 6 km). However, this subdivision explained only a small proportion of the total genetic variation across the region and subdivision was not correlated with distance among households (Thiele *et al*., 2008). Their finding suggests

substantial gene flow among schistosomes throughout the region. Contrasting results were found from schistosomes of another rural village, in the same state of Brazil, where schistosomes are also transmitted in stream habitats. In this region, schistosomes showed much higher differentiation over similar geographic scales, and differentiation was associated with distance (Curtis *et al*., 2002). These data suggest more localized transmission cycles. The current study showed that there is strong genetic differentiation within the infrapopulation in each of the four study sites.

In Kenya, boundaries of watersheds and water bodies restrict gene flow of *S. mansoni* and help define transmission foci. This species shows strong genetic structure across regions that encompass different watersheds in the east, west, and southwest portions of the country (Agola *et al*., 2006). Structure has also been detected among schistosome infrapopulations. In another study which is a rice farming irrigation region called Mwea, in the Kirinyaga District of central Kenya, samples of miracidia from school children showed significant, but low levels of pairwise subdivision among the infrapopulations of each child and among four schools that were 2–7 km apart (Agola *et al*.,2009). Thus, it appears that the geographic scale of differentiation identified can vary widely among different studies on *S. mansoni.*

Just as the schistosome life cycle affects genotype movement through biotic and abiotic environments, it also plays an important role in determining schistosome population structure. Infection foci for schistosomiasis must have three characteristics: (1) suitable snail habitat, (2) contamination of freshwater with host faeces and (3) areas where humans have contact with water containing infective parasite larvae. Areas that do not meet these criteria are not likely to become stable infection foci, and will serve as parasite population `sinks' in the host parasite landscape. Gene flow between parasite subpopulations will then depend on the rate at which schistosomes are transferred between infection foci, which in most cases, would depend on the movement of infected human hosts (Curtis *et al*., 2002). When transmission sites are not widely separated (e.g. on separate watercourses), the expectation of subdivision due to geographic factors diminishes. In this study, the Principal Component Analyses and Bayesian analyses confirm that the different sites are highly structured (i.e. no or few exchange between sites).

A study by Thiele *et al*. (2008) reported four infrapopulations with significantly less than zero F_{IS}, indicating heterozygote excess, and two infrapopulations significantly greater than zero F_{IS}, indicating heterozygote deficiency. While this finding is not in line with our data on another village their finding indicated the observance of overall slightly higher genetic diversity and highly inbred population which is in agreement with our findings.

4.3.3. Determination of *Schistosoma mansoni* transmission site based on PCA

One of the primary reasons for studying parasite population genetics is to understand demographic parameters, such as gene flow and population size, which are not readily observable using conventional ecological methods. These insights allow inferences regarding the patterns of transmission and recruitment within the environment (Thiele *et al*., 2008). Analysis of genetic structure data shows that transmission foci can be structured by watershed boundaries, habitat types, and host species (Steinauer *et al*., 2010). Thus, in the current study based on the Principal Component Analysis the spatial genetic structure of *Schistosoma mansoni* infrapopulations was determined. Moreover, the PCA enabled us to determine the relative distance of *Schistosoma mansoni* transmission foci in each study sites based on the genetic differentiation of the miracidia. It is observed that the transmission sites for Kemissie are near to each other and aggregated. In Sille-Elgo the transmission sites are more aggregated in one area and are very distant from the others. In Wondo Genet, the transmission sites are stretched and dispersed while in Ziway there are multiple sites of infection that are much far apart to each other.

5. CONCLUSION & RECOMMENDATIONS

5.1 Conclusion

This study provides insight into the current status of transmission, genetic diversity and structure of *Schistosoma mansoni* population in Ethiopia.

The study demonstrated that the prevalence of *Schistosoma mansoni* infection in the study areas was high and presents a public health problem. However, there was variation in the prevalence among the different localities, the highest in Kemissie followed by Wondo Genet and Sille-Elgo. Similarly intensity of infection was the highest for Kemissie followed by Wondo Genet and Sille-Elgo. It was observed that level of infection was generally affected by age and sex.

The current study had shown genetic variation of *Schistosoma mansoni* isolates within inter and intra-host as well as at geographical level. This genetic variation might also be associated with morbidity variation.

With regard to the genetic diversity of *Schistosoma mansoni* isolates of Ethiopia, a total of 164 unique alleles were counted which is a very high proportion compared to other studies conducted in East Africa. There were no null alleles. The allele count was higher for the isolates of Kemissie followed by Wondo Genet, Sille-Elgo and Ziway. Among the loci studied SMDO11 had the highest allele count in all of the study sites while SMD28 had the lowest allele count. In general there was a significant difference in the number of alleles counted among the four study populations. Moreover, in all of the four study populations the observed heterozygosity was lower than the expected heterozygosity when Hardy-Weinberg equilibrium is expected. However, the observed heterozygosity value was high and hence results high level of genetic differentiation. But significant heterozygote deficiency was observed in all of the four populations that could be due to restricted gene flow.

The molecular characterization results based on PCA and the Bayesian clustering STRUCTURE showed a clear and significant genetic differentiation that provided four

distinct population structures. There was high percentage of differentiation among Ziway and Kemissie followed by Kemissie and Sille-Elgo, Wondo Genet and Sille-Elgo, Ziway and Sille-Elgo, Ziway and Wondo Genet, and Kemissie and Wondo Genet populations. There was also high geographic and genetic differentiation among the study sites. However, this variation in geographic and genetic distance between the study sites was not associated with isolation by distance.

The result of this study had shown high genetic diversity and structuration of *Schistosoma mansoni* isolates of Ethiopia associated with limited or no gene flow among the different populations studied and thus there is limited opportunity for the spread of rare alleles that confer resistance to antihelminthics.

At the infrapopulation level, the number of allele count at a single locus for the twenty four infrapopulations in the current study showed variation. Like that of the population level SMDO11 had also a high frequency of allele counts at the infrapopulation level. Although there was a significant variation in the number of alleles counted among the infrapopulations, there was no variation observed in terms of allelic richness. At infrapopulation level the F_{IS} value was significantly greater than zero, indicating heterozygote deficiency, suggesting relatively inbred parasite infrapopulation.

The current study showed that there is strong genetic differentiation within the infrapopulation in each of the four study sites. Therefore, most of the infrapopulations were assigned to the four population clusters.

Based on the Colony software analysis, *Schistosoma mansoni* full-sib relationships were estimated and the result showed that there were multiplicities of infections within a single host with genetically different strains of *S. mansoni*. The degree of multiplicity of infection was higher among study subjects of Wondo Genet followed by Kemissie, Sille-Elgo and Ziway.

In general, high levels of genetic diversity and population differentiation characterizes the *Schistosoma mansoni* isolates studied in this investigation. This observation is consistent with a previous finding that showed a remarkable amount of variation in *Schistosoma mansoni*, strongly suggesting an East African origin of the parasite. Moreover, the presence of a rich snail fauna in East Africa (about half a dozen species

each of *Biomphalaria* and *Bulinus*), including four subspecies of *Bulinus truncatus* in Ethiopia suggests that schistosomiasis evolved in this region. Hence, based on the fact that there is rich snail fauna in Ethiopia and the high genetic differentiation and structuration of *Schistosoma mansoni* isolates demonstrated by this investigation it could be speculated that Ethiopia could be the probable country of origin for schistosomes. However, this conclusion requires further investigation using other genetic markers as well as comparisons with other countries isolates.

5.2 Recommendations

From this study, it is observed that there is a difference in the prevalence and intensity of *Schistosoma mansoni* infection as well as a strong genetic structure for each of the four clusters. It was also observed that there is multiplicity of infection in a single host. Thus, based on the findings of this work, the following are recommended for consideration of *S. mansoni* control.

1. Complex interplay of host genes and schistosomiasis that results in striking inter-individual variation in infection intensity and disease has been observed. The available evidence suggests that there is genetic control of infection intensity and pathology, though this has not been demonstrated in all populations. Heritability of infection intensity is variable, and the factors underlying this variation are unknown. Though major gene control of infection and pathology has been demonstrated in some populations, further segregation analyses are needed to confirm the generality of these results. Thus, investigation on the host genetics is important to explain the variation in intensity of infection and level of morbidity in Ethiopia.
2. As there are reports on variation in intensity of infection and pathogenicity of *S. mansoni* from different parts of the world, determining the morbidity variation in different endemic areas of Ethiopia could enable in determining intervention priority areas.

3. The selective pressures imposed by increasing levels of drug administration necessitate a clearer idea of population structure and genetics of the parasite to enable the prediction and monitoring of changes in, for example, drug susceptibility. Therefore, studies on Ethiopian isolates will have contribution in monitoring drug efficacy and the occurrence of drug tolerance/resistance at the molecular level.

4. Different African countries are involved in schistosomiasis control through national mass drug chemotherapy programmes in order to reduce morbidity and transmission. Thus, such programmes must be implemented in Ethiopia at least in school settings.

5. It is to be recalled that endod (*Phytolacca dodecandra*) was used as molluscicide and applied in the control of schistosomiasis in some parts of Ethiopia. Its biodegradability, being environmentally friendly and local availability is the good qualities for its application. Thus, continuation of endod based control in a large scale will curb the level of transmission as well as morbidity.

6. Long-term epidemiological studies are needed for accurate modeling of infection and transmission dynamics of *S. mansoni* at different infection foci. Basic population genetics data is lacking in *Biomphalaria pfeifferi* and *B. sudanica* the most important intermediate host of *S. mansoni* in Ethiopia. It is thus essential to study the population structure of these species so as to understand the parasite-snail coevolutionary dynamics. Thus, a work on determining the genetic structure of snail vectors will contribute to the control of schistosomiasis.

6 REFERENCES

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DECLARATION

I, the undersigned, declare that this PhD dissertation is my own original work and has not been presented for a degree in any other university, and all sources of materials used for the dissertation have been duly acknowledged.

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7 ANNEX

<u>በጥናቱ ለመሳተፍ ፈቃደኝነት መግለጫ ቅፅ</u>

ተራ ቁጥር: ________________

ስም: ____________________________________

እኔ ከላይ በስም ተጠቃሹ የአንጀት ጥገኛ በሽታ (*Schistosomiasis mansoni*) ሳይኖርብኝ እንደማይቀር ተነግሮኝ በሽታየን ለመመርመር የሠገራ ናሙና (*Stool specimen*) እንደሚያስፈልግ ተነገሮኛል:: ይህ ናሙና የበሽታየን ምንነት ለማወቅ የሚያስችል ምርመራ የሚካሄድበት ነዉ:: በተጨማሪም ከዚሁ ናሙና የተወሰነዉ ክፍል በሽታዉን አምጪ ለሆነዉ ጥገኛ ተዉሳክ ዝርያ (*Schistosomiasis mansoni genotyping*) ለመለየት ለሚደረገዉ ጥናት ሥራ አላማ እንደሚዉል ተገልፆልኛል:: አኔም ስለጥናቱ በሚገባ ከተረዳሁ በኋላ አስፈላጊ ናቸዉ ብዬ ያሰብኳቸዉን ጥያቄዎች ጠይቄ አስፈላጊዉን ማብራሪያ አግኝቻለሁ:: በጥናቱ መረጃ መሰብሰቢያ መጠይቅ ላይ የተዘረዘሩት መረጃዎች ሁሉ በሚስጥር የሚያዙ መሆናቸዉንና የስምምነት ቅጹም በሚስጥር ተይዞ ጥናቱ አንደሚካሄድ ተገንዝቤአለሁ::

ስለራሴ የምጠየቀዉን መረጃ ያለመስጠት፣ ለጥናቱ ያለመተባበርና በየትኛዉም ወቅት ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ መሆኑ የተገለጸልኝ ሲሆን ይህንንም በማድረጌ ምክንያት በአጠቃላይ በሽታዬን ከመታከም የሚያግደኝ ነገር የሌለ መሆኑን በሚገባ ተረድቻለሁ:: ስለሆነም ለተመራማሪዉ መስማማቴን በፊርማየ ያረጋገጥኩት ሁኔታዉን በመረዳቴ ነዉ:: በተጨማሪም በሽታዉ ከተገኘብኝ ከሚወሰደዉ ናሙና የሚገኘዉ የአንጀት ተዉሳክ ዝርያ ለጥናት ወደ ዉጭ አገር ቢላክ ተቃዉሞ የለኝም:: እንዲሁም ከጥናቱ የተረፈዉ ናሙና ወደ ፊት ለሌላ የዚሁ ተመሳሳይ የጥናት ተግባር ላይ ቢዉል ፈቃደኛ ነኝ::

ፊርማ: ____________________

Consent form (English)

S/No.: ________________

Full Name: ____________________________________

I the above mentioned________________________ have been told that I may have intestinal parasitic disease known by *Schistosomiasis mansoni* and would like get my stool specimen for identification of parasites. The stool specimen is important to know the disease causing parasite. Some portions of the stool specimen will be used to recover and extract *Schistosomiasis mansoni* larva for molecular genotyping and subgenotyping. I have asked questions relevant to the study and got satisfied answers with clarifications. Information and data in the survey questioner will be handled with strictly confidential and used only for the specified study.

I have the right not to give any information, not cooperate and resign from this study and this will not affect my right from diagnosis and getting treatment. So, I understand, agreed and signed this consent form. In addition to the above mentioned, if I am found positive for *Schistosomiasis mansoni*, I have no objection if the parasite obtained from the stool specimen be shipped to other country for further characterization. I have also agreed to the use of this sample for the same experiment in the future.

Signature: ___________________