An Integrated Approach to Biology

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Zoology, being a basic bioscience, is essential for a better understanding of applied biological disciplines. A sound background in zoology can often be the key to success in modern day cutting-edge biological research. Students of zoology have many more interesting questions to ask now than ever before. Precisely when did humans take to clothing? How is losing body hair actually correlated to body louse speciation? What is so unique about a schistosome that enables it to parasitize two very different host species, to have a free living stage and show a broad range of host specificity in one life cycle? Genomics has already helped us to get the molecular expression profiles of many organisms. But to understand how all the information obtained at the molecular level fits into the scheme of things, we need a sound foundation in zoology. It is all the more fun studying zoology now with so many interesting questions to ask and ideas to explore.

1. Introduction

By the time I completed school and began college, zoology was somewhere at the bottom of the list of potential majors of students who wished to graduate in Life Sciences. Four years since, the condition has improved very little. Emerging fields like biotechnology continue to top the charts. Among the fistful few who take up zoology, a greater proportion view it as a temporary halt and subsequently move on to greener pastures which, no doubt, promise lucrative pay packages and excellent opportunities for career growth. The perennial question that I have faced from parents, peers and relatives alike was where zoology will ultimately lead me to. Not to blame them, I was somewhat amazed to discover that the society at large draws a unanimous conclusion that a student of zoology sticking to his/her subject steadfastly must either move on to teach, or at best bag a job in a zoo or

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conservation park. As most young people, I didn't have any antidote to this. Today of course I can face them better, thanks to my two-month summer training in a genomics laboratory. I will share two examples to make my point.

2. A Tale of Two Parasites

2.1 *Schistosome*

Schistosomiasis is a notorious parasitic disease, second only to malaria if the number of people affected globally is considered. It takes a heavy toll on the economy of countries having endemic areas within their political boundaries. Development of effective remedy against schistosomes, the causative agent, is an area where molecular biologists must join hands with zoologists.

Schistosomes are tiny blood flukes, adults measuring 10–20 mm in length. The complexity of their life cycle can be attributed to the fact that it involves parasitic stages in two hosts belonging to discrete unrelated genera (hence placed under Digenea: 'di' two, 'genea' related to genus) with a pulmonate snail as its intermediate host[1] and a mammalian definitive host[2]. They also have adaptations for surviving in freshwater as free-living forms (*Figure* 1). The details of the species causing the disease are listed in *Table* 1.

[1] Host in which the parasite reproduces asexually.
[2] Host in which the sexual phase of the parasite occurs.

What is it that makes schistosome so potent as a parasite, manipulating two very different host systems, besides having a free living stage? Whatever animal model one may wish to work with, having a good knowledge of its taxonomy (*Box* 1), evolutionary history, life cycle or so to say, its biology as a whole, becomes not only helpful but also essential. That is why we need zoologists to step in and collaborate with molecular biologists.

Schistosomiasis can be divided into three phases:

- The migratory phase lasting from penetration of the parasite up to its maturity.
- The acute phase which starts when the eggs are deposited.
- The chronic phase which is restricted to the endemic areas.

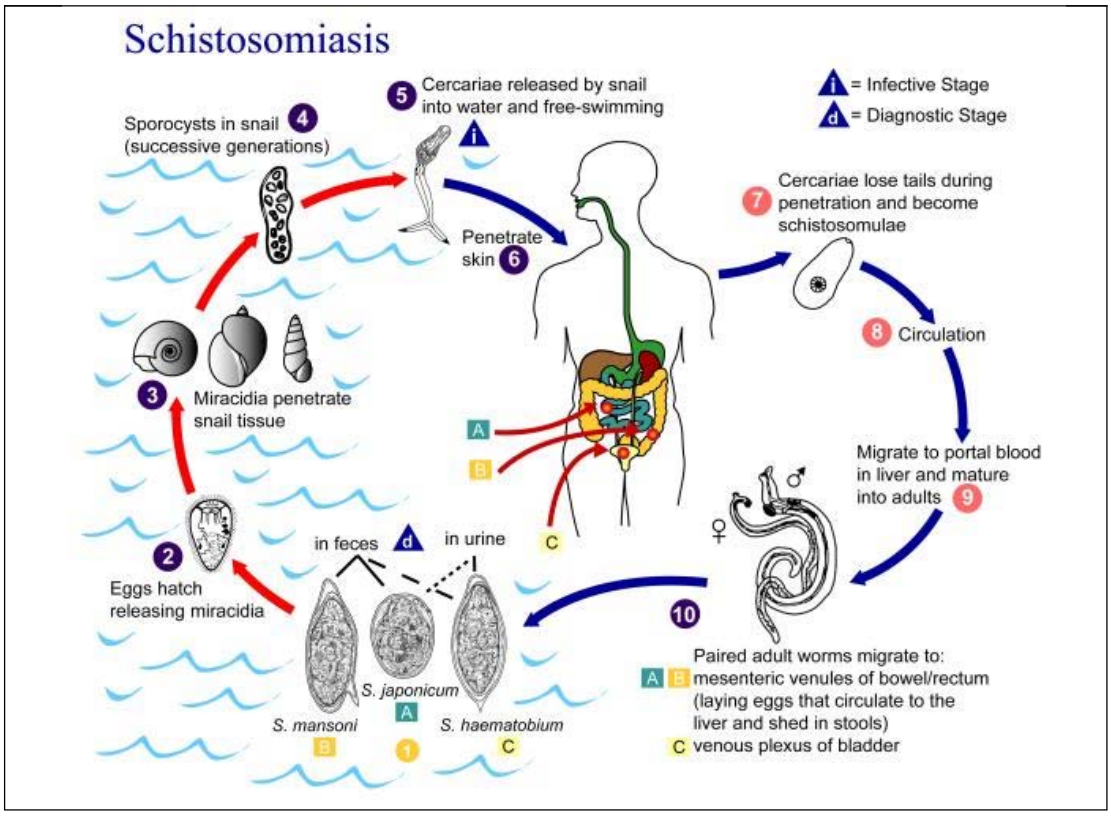

Figure 1. Schistosome life cycle and pathogenesis. (Courtesy: Wikipedia)

Table 1.

Species	Disease caused	Geographical area of occurrence	Alternative definitive hosts
S.japonicum	Intestinal and hepatosplenic schistosomiasis	China, Philippines, Indonesia	Cattle, dogs, pigs and rodents
S.mansoni	Intestinal and hepatic schistosomiasis	Africa and South America	Rodents, other primates
S.hematobium	Urinary schistosomiasis	Africa, Arabian peninsula, Middle East, Portugal and India	Baboons, monkeys (both very rare)

Box 1. Schistosome Zoology – A Glimpse

The name 'schistosoma' has been derived from two Greek words – '*skhistos*' which means split and '*soma*' which refers to body. This nomenclature was based on the male worm's morphology (split abdomen, wherein the male holds the female in its gynaecophoric canal). It is also called Bilharzia since the eggs of this parasite were first discovered by Theodor Maximilian Bilharz, a German pathologist.

Taxonomic Classification

Kingdom – Animalia
Phylum – Platyhelminthes
Class – Trematoda
Subclass – Digenea
Order – Strigeidida
Family – Schistosomatidae
Genus – *Schistosoma*

It is presumed to have originated as a parasite of hippopotamus (*S. hippopotami*). Rodents were probably the original hosts for the South East Asian species.

21 species are currently identified under genus *Schistosoma.* Their origin is from several ancestral branches (polyphyletic). Four broad groups are currently recognized :

- *indicum* group
- *japonicum* group
- *haematobium* group
- *mansoni* group

The *indicum* group, evolved probably during Pleistocene, includes *S. indicum, S. nasale* and *S. spindale*; *S. indicum* occurs mainly in India and Thailand. Its favourite intermediate host is a freshwater snail *Indoplanorbis exustus.*

Special features

Adult worms generally parasitize mesenteric blood vessels as intravascular parasites. They are dioecious (distinct male and female forms are recognized), a rarity among platyhelminths and cause infection directly by penetrating the host body.

Any anti-schistosome drug is known as a Schistosomicide.

The development of an effective remedy against schistosomes necessitates having a clear picture as to what it is that helps it to adapt to and exploit two different physiological systems with equal dexterity. The draft schistosome genome sequence has already been prepared by the Wellcome Trust Sanger Institute

and the Schistosome Genome Network set up in 1994 with active help from the World Health Organization. Functional genomics has successfully revealed gene expression profiles across gender and developmental stages of the parasite (*Box* 2). Thus a solid foundation has already been laid whereupon research can now be done to discover novel targets for chemotherapeutic drugs and candidates for vaccines to treat this dreadful disease.

Transcriptome[3] analysis of schistosomes has cast some light on the evolutionary pattern of metazoans and their phylogenetic position. Full length cDNAs with entire Open Reading Frames (ORFs) have already been isolated from the schistosomal Expressed Sequence Tag (EST) library. Proteomic data obtained using a plethora of techniques like mass spectrometry, 2D gel electrophoresis and liquid chromatography as well as experiments using RNAi[4] has provided us with some valuable insights into schistosome biology. Molecular mechanism of schistoso-

[3] Transcriptome is the total pool of transcripts from all active genes in a cell, tissue or organism.

[4] RNA interference

Box 2. Salient Features of the Schistosome Genome

- $2n = 16$ where n represents the haploid number of chromosomes. Out of these, 7 pairs are autosomes and the last pair is sex chromosome. Male is the homogametic sex and ZW–ZZ pattern of sex determination is observed.
- Chromosomes range in size from 18 to 73 MB and can be easily distinguished by size, shape and C banding pattern.
- The size of the haploid genome of *S. mansoni* is predicted to be 2.7×10^8 base pairs, composed of 60 % highly and moderately repeated DNA as well as 30 % single copy sequences.
- The genome size is ~300 Mbp as indicated by an assembly of 3.5 million reads, with ~13,339 protein coding genes (~4 % of the genome).
- The total GC content is ~34 %.
- 657 different repeat families are found which includes 29 types of retrotransposons constituting 40.1 % of the genome (long terminal repeats (LTR), non-LTR as well as *Penelope*-like elements). Non-LTR retrotransposons have significantly higher copy number (~12.6 % of the genome).
- Transposons are scarce.
- The mean coding sequence length is 1.2 kb, comparable to that of *C.elegans*.
- 7286 Single Nucleotide Polymorphism (SNP) sites are recently identified for *S.japonicum*. 60 % of SNPs only induce substitutions in coding regions and 40% may lead to protein variations.
- Of the 2806 indels (insertions/ deletions) discovered so far, 38.4 % are found in the coding regions. Length of indels varies from 1–3 nucleotides.

miasis has been tested using loss-of-function and gain-of-function genetic manipulation approaches.

The parasite tampers with the innate immunological defence and the neuroendocrine system of snails whereas immune evasion technique in the mammalian host involves the manipulative exploitation of the hormonal microenvironment.

Schistosomes lack the endogenous machinery to produce several steroid hormones as they cannot synthesize cholesterol. But they can make putative receptors with a high degree of similarity to mammalian steroid hormone receptors which allow them to exploit the host's progestin, progesterone, estrogen and adrenal hormones for their own development and maturation. Schistosomulum selectively expresses certain genes to make receptors so as to utilize host's ecdysteroids for renewal of its tegument during metamorphosis into the adult stage and also the host's allostatin, biochemically a polypeptide, to suppress the juvenile hormone secretion. The genes involved in stage-specific attributes are tabulated in *Table* 2. Cathepsin D is presumed to be an important enzyme in the mammalian stages of the parasite. Transforming Growth Factor beta (TGF-β) signalling plays a pivotal role in the embryogenesis of the parasite as well as in the development of vitelline cells in females by stimulus from male integument.

Schistosome genes may encode mammalian-like receptors and the parasite can accept certain hormones and cytokine signals from the mammalian host.

The mammalian immune system responds to eggs in the liver causing hypersensitivity, a necessary immune response to prevent damage to hepatocytes. The parasite's syncytial (multinucleated) tegument is shed every few hours, so that the host's antibodies binding to it can hardly bind for long. Schistosome can also take up host proteins and this causes molecular masking (*Figure* 2).

Praziquantel, a quinolone derivative, is the only effective drug in use today against schistosomes. It is thought to disrupt Ca^{2+} homeostasis in the parasite. However, resistant schistosome strains have already been reported from two endemic areas. This

Table 2. Stage-specific gene expression of Schistosomes.

Life cycle stage	Behaviour/ Feature	Genes involved
Cercariae	Non feeding, host seeking quest – energy demanding	Upregulation of mitochondrial genes
	Host skin invasion	Cercarial elastase
Schistosomula	Host immune evasion	Immunomodulators Sm16 and venom allergen homolog
	Protection against oxidative stress	Chaperones (HSP70), enzymes related to redox homeostasis
	Over expression of genes associated with immune response and stress response	Prostaglandins, paramyosin, glutathione-S-transferase
	Ingestion of RBC, breakdown of haemoglobin and lysosomal lipids	Cathepsins, asparaginyl endopeptidase, saposin
	Sugar transport	STGP4
	Genes associated with aerobic respiration	Cytochrome C_1, ferridoxin, aconitate hydratase
Adult (abundant and significantly diverse gene expression)		Cathepsin B, D, L, exopeptidase cathepsin C, genes involved in glucose metabolism
	Gonadal differentiation, egg production, reproductive functions	Antimullerian hormone receptor gene in males and retinoid X receptor, Smed family genes in females
Eggs	Viability and metabolism	Egg shell proteins, major egg antigen, nutrient transporters (SGTPs), Sm23, Sm14, redox balance enzymes, integral membrane protein 25, 26 & 28 glutathione-S-transferases
Mother Sporocyst	Successful infection of the snail host	Upregulation of Cathepsin C, preprocathepsin L, haemoglobinase (Sm32), elastase genes
Daughter Sporocysts	General protein synthesis machinery is active	Genes for 40S, 60S ribosomal subunits elongation factors

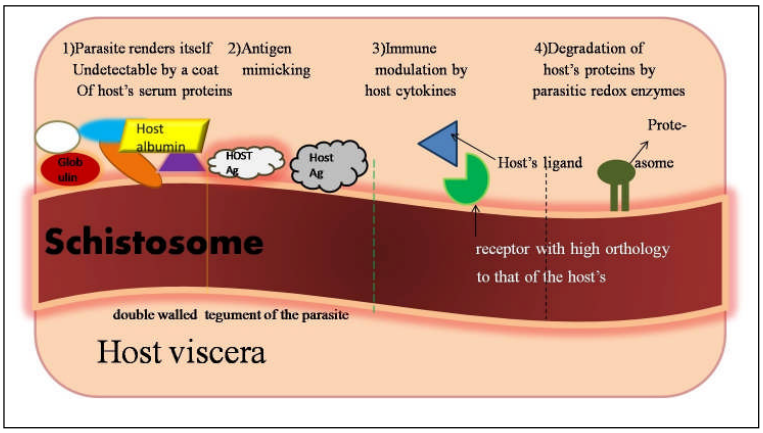

Figure 2. Molecular masking: A clever schistosomal strategy to avoid host immune response. Schistosome has devised various methods to evade host immune responses, which includes coating itself with host's serum proteins or antigens, exploiting host cytokine signals and degrading host proteins.

corroborates the urgency to identify potential drug targets for rational drug design of the next generation schistosomicidal medications. It has been seen that the parasite converts its trilaminate surface membrane tegument (found in the snail host) to a heptalaminate form to adapt to the mammalian environment. Hence examination of the parasite's integument proteome can reveal candidate proteins associated with host-specific infestation and these can be targeted by vaccines and drugs. Several drugs like cyclophilins, oxamniquine, hycanthone, tyrosylprotein sulfotransferases and oxadiazoles are under screening for their efficacy. Tetraspanins in the outer tegument is a promising vaccine antigen.

2.2 *Body Louse*

The advent of clothes has been a spectacular chapter in the evolutionary chronicle of *Homo sapiens* with both physiological as well as sociological overtones. Clothes have made it possible for humans to disperse and successfully establish themselves in extreme climates. Also the impact of clothes has been monumental in the progress of human civilization if viewed from an anthropological perspective. When exactly did humans start wearing clothes? It is an intriguing question worth serious consideration. The way it was answered is even more fascinating.

It is a well-known fact that the louse is a notorious obligate

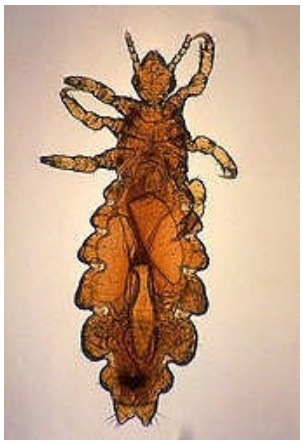

***Figure 3. Head Louse, Pediculus humanus capitis*. (*Magnification: 500*×).**

Mouth parts are highly adapted for piercing skin to suck blood and remain retracted to the insect's head, except during feeding. Short legs terminate with a single claw; opposing thumb grasps the host's hairs or clothes. Due to extreme adaptation for clinging, legs are unsuitable for jumping or walking over a flat surface.

(Courtesy: Wikipedia)

hematophagic[5] ectoparasite (*Figure* 3). There are precisely two kinds of them infesting humans – the head louse (*Pediculus humanus capitis*) and the body louse (*Pediculus humanus corporis*) with a clear-cut distinction in their ecological niches. Whereas the former spends all its life faithfully adhering to the scalp hair and sucking blood, the latter necessarily needs clothes to cling on to although its diet is no different. Hence if the molecular origin of body louse can be traced, answers to the time of origin of clothing would no longer remain an unsolved mystery.

[5] Feeding on blood exclusively.

It was experimentally determined that the body louse has diverged from head louse ~72,000 ± 42,000 years ago by molecular clock analysis using two mitochondrial genes ND4 (579bp) and CYTB (440bp) and two nuclear genes EF-1 (485bp) and RP-II (601bp). To ensure maximum accuracy of data analysis, a subunit of cytochrome oxidase1 (COX1) gene was also analyzed in a mixed global sample of 56 head and body lice. This would remove probable risks of data misinterpretation, as analysis of nuclear genes across generations is often jeopardized by recombination events. The chimpanzee louse (*Pediculus schaeffi*) was used as an outgroup assuming that the human and chimp louse have co-speciated with their hosts some 5.5 million years ago. The experiment revealed that samples of head louse collected from a single region of Africa (Ethiopia) exhibit greater diversity compared to a global sample of body louse (*Figure* 4). This would imply the

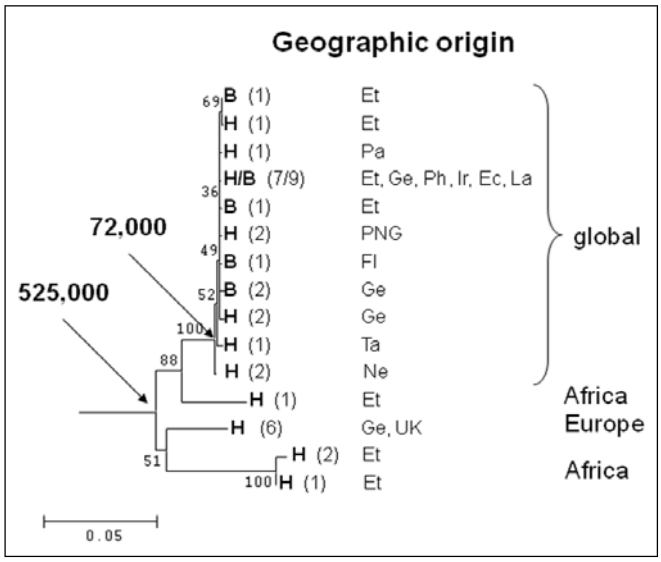

Figure 4. Phylogenetic tree to show the divergence and speciation of lice.
B: body louse, H: head louse. Geographical locations from where samples were collected: Et: Ethiopia, Pa: Panama, Ge: Germany, Ph: Philippines, Ir: Iran, Ec: Ecuador, La: Laos, PNG: Papua New Guinea, Fl: Florida (USA), Ta: Taiwan, Ne: Nepal and UK: United Kingdom. B appears only in a distinct clade which has separated from ancestral H about 72,000 years ago.
Reproduced with permission from author [2].

parasite's probable origin to be in Africa since a greater genetic variability is often a key feature associated with populations residing in a localized geographical area for a period of time which must be geologically significant. This, in turn, is a necessary corollary to the African origin of humans simply because the human louse is an obligate parasite which can parasitize no other host species. The divergence of the head and body lice is presumed to have occurred when modern humans resorted to the frequent use of clothing and lice speciated to occupy the new habitat so generated. Archaeological evidence proves that the tools required for cloth-making were a monopoly of *Homo sapiens* which no other pre-historic anthropoid species possessed. With the security of clothes to keep himself warm, man – and his parasite – subsequently moved to explore other cooler places on earth.

By comparison, rather contrast, one can say that the schistosome genome is tremendously flexible as inferred by its multiple host-seeking behaviour as well as its ability to speciate easily, infect novel host species and increase its virulence from time to time. The human louse, on the other hand, has been faithfully maintaining its parasitic relationship with the host, co-evolving and sometimes, even co-speciating (*Box* 3). This suggests that the louse

Box 3. Host–Parasite Interaction and the Concept of Co-Evolution.

If one must epitomize a classical example of a 'tug-of-war' continuing in nature from time immemorial, there is nothing better to cite than host-parasite interaction. It is an exceptional case wherein evolution is *rapid* and *reciprocal*. A host must continuously devise novel strategies to keep the parasite at bay and so must the parasite plan schemes to break the firewall of host's defence. Each time the host becomes increasingly immunized to the existing parasitic strain, it triggers the parasite to evolve changes at the molecular level to enhance its pathogenecity. However a judicious move on the part of the parasite would be never to incur lethal damage to the host since a host killed would mean a substantial loss to the parasite, both in terms of progeny as well as nutrients.

By definition, a parasite harms the host by draining the latter of resources and increasing its own fecundity (reproductive efficiency) in the process. But no host would tolerate such a situation indefinitely and so it evolves by selecting rare allelic variants or by promoting heterozygosity at many genetic loci to combat parasitic attack. The parasite too comes up with new genetic variants owing to the selection pressure acting upon it. They *co-evolve*.

Parasitism is essentially *heterospecific*. A foetus is never a parasite on its mother although it depletes her of vital life-resources.

genome is quite rigid wherein variability necessary to infect new host species is seldom welcome. Understanding their zoology would only improve our interpretation of their genomic data, and the duo working in synchrony would surely expedite the alleviation of the sufferings of people falling prey (or rather host!) to these parasites. They can be better managed, if not completely eradicated.

With the pre-defined rigid boundaries of individual disciplines fading into today's single arena of interdisciplinary research, it is imperative for one to move beyond the strict traditional confines of a single subject. As newer disciplines pose more and more meaningful questions in research, fundamental concepts of basic sciences – be it chemistry, physics, botany or zoology – become all the more necessary to answer them. Genomics is a tremendously useful tool no doubt, but the best use of it can only be made by the ones with a sound background in basic sciences. So to conclude, new disciplines of science are indeed very promising

but basic biosciences like zoology still remain far from being obsolete.

Acknowledgements

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Suggested Readings

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