New Basic Knowledge

New basic knowledge about the biological, social, economic, health systems, and behavioural determinants of ill health is essential for improving and making more effective tools for the control of infectious diseases. In stimulating the generation of, and making accessible, new basic knowledge, TDR works at national and international levels. Success indicators include the number of new, significant and relevant scientific advances that are applied to neglected tropical diseases.

Fluorescing mosquito larvae: genetic manipulation opens up a wide variety of new possibilities for disease control
Innovative research in the biomedical and social sciences, leading to new interventions for tropical diseases, is encouraged through TDR’s Basic and Strategic Research (STR) component. The idea that powerful tools will facilitate long-lasting interventions is behind this area of work, in which advances and leads that will move science in new and productive directions are identified. However, the targets and objectives of STR are long-term and the research is often a lengthy process that can take decades rather than years. It includes research on pathologic mechanisms and on social and economic factors of disease, development of research models, and identification of drug, diagnostic and vaccine targets.

Significant changes in TDR’s way of working in its STR area were introduced during the biennium. Firstly, the separate steering committees on pathogenesis and genome were combined into one – the Steering Committee on Pathogenesis and Applied Genomics. This is a reflection of the advances in science – great leaps in knowledge in recent years mean that the post-genomics revolution is now well under way. The focus is no longer only on ‘sequencing’ genomes, i.e. on deciphering the order of bases along the length of deoxyribonucleic acid (DNA), but, based on this information, on identifying and understanding the roles of genes and proteins in each type of organism and on putting this information to use in developing new treatments and other means of disease control (see Box 1 for list of genome websites). For this reason, work on genome and pathogenesis is now seen as a continuum, allowing the exploration of new technologies, e.g. genomics, proteomics, bioinformatics and high-throughput screening, to open new ways for accelerated discovery of new drugs, vaccines and diagnostics.

The second major change introduced in STR during the biennium was to include social, economic and behavioural research, under a new Steering Committee on Strategic Social, Economic and Behavioural Research (SEB). SEB is located in STR to emphasize the importance of the basic social sciences for identifying needs and opportunities for improved prevention and control of TDR diseases.

The above steering committees represent two of three major thrusts of TDR/STR. The third area is molecular entomology. Important progress has been made in this area during the biennium.

Research on immunology of leprosy was a separate area managed in collaboration with the World Health Organization’s (WHO’s) Department of Vaccines and Biologicals through the Immunology of Mycobacteria Steering Committee (IMMYC) since 1994. IMMYC was disbanded in late 2000 in order to promote a broader approach to leprosy research across the spectrum of TDR activities, and all the relevant TDR committees are now open to leprosy research proposals.

NEW, SIGNIFICANT AND RELEVANT SCIENTIFIC ADVANCES

Pathogenesis and applied genomics

When a disease is acute, it is rapidly overcome and very few pathological changes develop in its wake. However, when an infectious disease is chronic, the pathology is much more serious; it arises when the immune system is caught between the need to prevent further invasion by an organism and the need to effect a response to any similar organisms already present in the body. Parasites, in contrast to the great majority of other infectious agents, commonly strike a balance with their hosts and continue to re-infect them. This situation forces the host’s immune system to display a bewildering variety of responses in terms of effector cells, antibodies and messenger/signalling molecules. Our understanding of the development of pathology and resistance in most of the parasitic diseases is incomplete, although a consolidated picture of interplay between opposing immunological mechanisms is beginning to emerge.

**Box 1. Genomes on the Web**

- **Malaria (Plasmodium) Genome Project**
- **The WHO/UNDP/World Bank Schistosoma Genome Network**
  [http://www.nhm.ac.uk/hosted_sites/schisto/](http://www.nhm.ac.uk/hosted_sites/schisto/)
- **The Filarial Genome Network**
  [http://helios.bto.ed.ac.uk/mbx/fgn/filgen.html](http://helios.bto.ed.ac.uk/mbx/fgn/filgen.html)
- **The Leishmania Genome Network**
  [http://www.ebi.ac.uk/parasites/leish.html](http://www.ebi.ac.uk/parasites/leish.html)
- **The Trypanosoma cruzi Genome Project**
  [http://www.ebi.ac.uk/parasites/leish.html](http://www.ebi.ac.uk/parasites/leish.html)
- **The Trypansoma brucei Genome Project**
  [http://parsun1.path.cam.ac.uk](http://parsun1.path.cam.ac.uk)
- **MycDB: Mycobacterium**
  [http://www.biochem.kth.se/MycDB.html](http://www.biochem.kth.se/MycDB.html)
- **Database AnoDB**

TDR research is helping to clarify some of the pathologic mechanisms involved in the etiology and progression of different diseases, including the genetic background, and is contributing to the identification of potential drug and vaccine targets.
Each of the TDR target diseases displays its own unique profile of acute and chronic inflammatory responses based on the influence of various effector cells and isotypical antibody stimulated by the invading organism. Typically, malaria is associated with the destruction of red blood cells; lymphatic filariasis with the deformities of elephantiasis; leishmaniasis with an array of different pathologies depending on whether the skin, mucous membranes or internal organs are involved; schistosomiasis with pathology of the liver or urogenital organs; African trypanosomiasis with lesions of the central nervous system; onchocerciasis with blindness and skin lesions; Chagas disease with the heart and/or intestines; and leprosy with lesions of the peripheral nerve trunks, resulting in damage to the skin and distressing deformities. Research is supported with the aim of finding ways and means to modulate these responses in a way that reduces the pathological response without jeopardizing the body’s immunological defence system.

**MALARIA:** The pathology associated with *Plasmodium falciparum* malaria is, in particular, due to adherence of infected red blood cells in the brain, metabolic disturbances, and organ dysfunction.

Research into the mechanism underlying adherence of red blood cells to the lining of the blood vessels (endothelium) in the brain indicates that the parasite ligand PFEMP1 may be key. The multi-adhesive nature of this molecule enables binding to at least six different receptors in the host. The role of another adhesive molecule, the thrombospondin-related adhesive protein (TRAP), in parasite invasion of epithelial cells of the host’s liver (hepatocytes), has been studied in *P. berghei* mutants carrying different variants of the gene encoding TRAP. Production of mutations of the A-domain of TRAP has made it possible to study the mechanism and results indicate that it might be possible to reduce parasite invasion through interfering with the interaction between TRAP and its receptor on the host’s hepatocytes.

A different line of research in pathogenesis of cerebral malaria has shown that immunoglobulin E (IgE) levels are elevated in these patients, suggesting that this antibody may be playing a role. This claim is supported by the finding that IgE forms part of immune complexes which induce CD23-mediated production of tumour necrosis factor (TNF), a chemical messenger in the body’s immune system (cytokine), in monocytes in vitro.

In the area of drug development, efforts to express and recombine *P. falciparum* dihydrofolate reductase (DHFR) and thymidylate synthase (TS) for rapid drug screening assays have been successful, but stabilization of the bifunctional DHFR-TS is needed to avoid spontaneous aggregation. In addition, a pathway involved in the DNA repair process has been shown to be exclusive for *P. falciparum* and different from in the mammalian host. This is a long-patch base excision pathway involved in repair of apurinic/apyrimidinic sites in DNA.

The role of individual PxA25 malaria genes in colonization of the *Anopheles* mosquito has been evaluated. These genes have been shown to drastically reduce the capacity of the parasite to complete its life cycle in the mosquito host. However, complete elimination of transmission in this experimental system has not yet been achieved.

In the area of vaccine development, several potent inhibitors of the surface protease responsible for secondary processing of *P. falciparum* merozoite surface protein (MSP-1) have been synthesized. Efforts continue to find *P. falciparum* choline kinase and choline phosphotransferase variants which are sufficiently different from those of human cells to permit exploitation as vaccine targets.

**TUBERCULOSIS:** Tuberculosis (TB) researchers now have access to the Steering Committee on Pathogenesis and Applied Genomics which meets annually. The first grant in TB was awarded in September 2000 for the identification of genomic sequences potentially useful in the development
of new diagnostic tests. In the future, it is expected that grants will be awarded for use of genomic information to identify and describe targets for the development of new drugs and vaccines.

**LYMPHATIC FILARIASIS:** In filarial nematodes, a new approach to validating potential drug targets is being developed using the model nematode *Caenorhabditis elegans*. Initially, expression of genes encoding putative drug targets is suppressed using the newly-developed technique of ribonucleic acid interference (RNAi), in which nematodes are exposed to double-stranded RNA encoding a fragment of the gene whose expression is to be silenced. The resulting worms are then examined to determine whether viability, fertility or growth are adversely affected. If they are, and the phenotype is considered suitable, mutants with permanent loss of function are obtained for further analysis and development of drug screens.

The RNAi approach was used to evaluate the suitability of enzymes catalysing the metabolism of the sugar trehalose, which is thought to be important in the formation of the cuticle or outer surface of the worms. So far however, no simple changes in characteristics have been noted for any of the genes silenced individually, i.e. none of the genes has been found to be essential and therefore suitable as a drug target.

Scientists working to unravel the genome of *Brugia malayi* have succeeded in sequencing 2000 new expressed sequence tags (ESTs) from subtracted libraries and the genomic materials have been conserved. In addition, laboratory staff in endemic countries have been trained in bioinformatics.

**LEISHMANIASIS:** In a move of major importance for our understanding of the biology of cell/parasite interaction in leishmaniasis, investigators were able to block macrophage infection by the *Leishmania* amastigote stage through use of monoclonal antibodies to proteophosphoglycan, a constituent of the parasite’s surface. It was further demonstrated that amastigotes can readily infect dendritic cells which are involved in the initiation of immune responses and are known to be crucial to the development of an effective immune response against *Leishmania*.

**SCHISTOSOMIASIS:** The role of antigen-specific (idiotype) sites of antibodies in the development and regulation of pathology due to schistosome infection can now be addressed by means of a newly-developed experimental schistosomiasis model. Using this model, it was observed that cross-reactive idiotype present in the sera of experimentally infected mice is of the IgG2a subclass and responsible for the proliferation of CD4 cells (white blood cells bearing the glycoprotein CD4 on their surface). It appears that this antibody subclass in mice is important in inducing Th1 type responses, i.e. cell-mediated immunity, which may be crucial for resistance, at least to certain antigens.

Further studies of the role of the schistosome TNF-α receptor in the reproductive biology of schistosomes indicates that levels of TNF-α in the serum increase in relation to egg production and granuloma formation (growths which form around the eggs as a result of immune reaction). The roles of cytokines in host pathology are being evaluated by studying the responses in ‘knock-out’ animals, in which the genes for key signalling molecules have been removed. So far these have given some divergent results suggesting that TNF can even be protective against cachexia (general wasting away). Granuloma formation also seems to be influenced by STAT-6 (a signal transducer and activator of transcription), ICAM-1 (an intercellular adhesion molecule) and LFA-1 (a leucocyte functional antigen).

Research on the relation between chemokine receptors and clinical forms of schistosomiasis has shown that distinct cell populations in the blood produce specific cytokines in different clinical situations, e.g. granulocytes produce the Th1 interleukin (IL-) cytokines IL-4 and IL-12, while monocytes are an important source of IL-10 in acute schistosomiasis. Monocytes also produce TNF-α in the chronic form of the disease. CD4+ lymphocytes produce IL-4 and IL-10 while the latter cytokine is also produced by CD8+ lymphocytes in intestinal schistosomiasis.
In the area of genome studies, the sequence and gene order of the mitochondrial genomes of all the schistosome species infective for humans have been completed. This information makes it possible to compare gene composition, gene arrangement, codon usage, structure of ribosomal ribonucleic acids (rRNAs) and transfer (t)RNAs, and to provide information on non-coding sequences including intra- and inter-specific variation in schistosomes, opening the way for entirely new research directions. In addition, EST clones from the various stages of *Schistosoma mansoni* and *S. japonicum* have been generated and supplied by the Genome Network laboratories. Although the ultimate goal of generating the whole genome map is still far away, there has been good progress in ongoing work on the production of a low-resolution map for chromosome 3 of *S. mansoni* and the generation of an EST cluster map.

**AFRICAN TRYPANOSOMIASIS:** An experimental model of African sleeping sickness has been developed using intestinal loops. The infection has been shown to cause marked intestinal damage with increased leakage and elevation of endotoxins closely correlated with nitric oxide and alterations in the levels of the cytokines TNF-α, IL-1β, IL-6, interferon-gamma (INF-γ) in the circulation. Further, the study of metabolic pathways in the parasites has indicated that inorganic polyphosphates play a role in completion of the life cycle and growth of trypanosome populations. For example, there is a build-up of these compounds in the lag phase of *Trypanosoma brucei*. Progress has also been made in proteomic analysis of the proteins involved in the differentiation of *T. brucei* from blood stream procyclic forms.

In the area of genome research, approximately 2000 plasmids with inserts larger than 500 base pairs had been sequenced before the beginning of 2001, and more than half of these were shown to have no homology to ESTs present in public databases (i.e. they show no similarity to genes from other organisms). In addition, great strides have been made towards completing a physical map of the *T. brucei* genome. A physical library is ready, and several high-density filters have been prepared and distributed to the scientific community.

**ONCHOCERCIASIS:** Recent results indicate that the first step towards the development of blindness is due to certain *Onchocerca volvulus* proteins which are implicated in the growth of new blood vessels in the eye. Identifying these proteins was a breakthrough, but much research remains to be done, e.g. it appears that the proteins are neither immunogens nor mitogens, thus ruling out an immunological explanation. In addition, the role of eosinophil eotaxin (a type of cytokine) in diethylcarbamazine (DEC)-induced skin lesions has been confirmed.

Genetic research on *O. volvulus* has shown there to be intra- and inter-strain variation in polymorphic microsatellite loci between savannah and forest strains of the parasite, suggesting that different genetic constitutions are associated with the different clinical manifestations.

**CHAGAS DISEASE:** In research on Chagas disease, a common pattern of cardiomiocyte G protein-coupled receptors and *Trypanosoma cruzi* ribosomal P proteins has been found. This antigenic determinant (P proteins), identified and mapped using synthetic peptides, strongly suggests that immunological cross-reaction is the culprit in the commonly seen heart-related pathogenesis in Chagas disease. Further, glycosylphosphatidylinositols (GPIs) that function as anchors for mucins appear to be hundreds of times more active in trypomastigotes (stages in the life cycle that occur in the mammalian host) than GPIs isolated from epimastigates (forms that occur in the insect host). These anchors appear to stimulate phosphorylation of three distinct mitogen activated protein (MAP)-kinase macrophase cascades, two of which are responsible for TNF-α induction and the third responsible for nitric oxide production. In a different line of research, the 3-dimensional structure of *T. rangeli* sialidase has been obtained and there has been progress on the *T. cruzi* counterpart. Mutagenic studies, based on crystallographic data, have revealed that only very few amino acids confer trans-sialidase activity to a sialidase scaffold. Single or double point mutations in or near the active site were able to abolish or maintain trans-sialidase and simultaneously increase the sialidase effect. As it has been shown that *T. cruzi* trans-sialidase is implicated in the invasion of mammalian cells, elucidating its mechanism of action is relevant for rational drug design against this parasite.

Calreticulin, an important chaperone for glycoprotein folding, has been shown to be important, possibly essential, for *T. cruzi* viability. Research using experimental infection in mice has confirmed that the genetic make-up of the host and the parasite clone used for infection play an important role in tissue tropism (i.e. which tissues the parasites invade).
**LEPROSY:** A high point of recent leprosy research has been to identify the molecular basis of the Mycobacterium leprae attraction to nerve tissue (neurotropism). M. leprae needs to invade peripheral nerve cells in order to survive, replicate and establish infection; during this process it causes significant damage to peripheral nerves, leaving patients with disabilities and deformities. Receptors on particular host cells act as initial targets with which the leprosy bacillus interacts. Attachment of the bacillus is either direct, between bacterial ‘adhesin’ and host cell receptor, or indirect, through absorption onto the bacterial surface of a bridging molecule of host cell origin. The initial and crucial step in the disease process is the attachment of M. leprae to the basal lamina that surrounds the Schwann cell-nerve axon unit. A TDR-sponsored study has shown that the G domain of the alpha2 chain of endoneurial laminin (filling material between cells) is crucial to the invasion of peripheral nerves by M. leprae. Furthermore, the receptor on the Schwann cell that binds to the laminin alpha2-G receptor has been identified as alpha-dystroglycan, and a candidate protein receptor on the surface of M. leprae that binds to this same laminin receptor has also been identified. This work makes a profound contribution to our understanding of the pathogenesis of leprosy and may have important implications for the design of interventions to control leprosy-induced nerve damage.1-5

Sequencing of the genome of M. leprae was initiated and catalysed by TDR, even though the majority of funds for the activity came from outside. TDR’s initial investment in 1989 proved a stimulus to in-depth analysis and genome sequencing by an international network of laboratories such that, by the end of 2000, sequencing was completed. The complete sequence, generated from a combination of cosmid and whole-genome shotgun sequencing, is 3,268,203 base-pairs in length. Less than half the genome contains functional genes (1,604 protein-coding genes identified), but pseudogenes (with intact counterparts in M. tuberculosis) abound. Gene deletion and decay have eliminated many important metabolic activities, and may be the explanation for the extremely slow growth of M. leprae and its refractoriness to cultivation in vitro. Both the sequence and annotation have been deposited in the public databases with the accession number AL450380.4

Sequence data are being used in TDR-sponsored research to develop a diagnostic skin test capable of determining whether or not an individual has been exposed to M. leprae. The test is expected to help in understanding the transmission of leprosy and its epidemiology. M. leprae-specific peptides should have unique amino acid sequences, or significant sequence dissimilarity from other mycobacteria. Peptides 15 amino acids in length were synthesized from 33 genes or open reading frames. They were tested against cell preparations from tuberculoid leprosy patients from Brazil, Ethiopia, Pakistan and Nepal, with UK blood donors serving as non-exposed/non-infected controls. Peptides which induced potentially specific responses in leprosy patients but not in UK controls, as well as peptides that were cross-reactive (present both in patients and controls), were identified. A difference of five amino acids from the equivalent M. tuberculosis sequence did not, by itself, identify peptides that were M. leprae specific, a finding which suggests that many of these peptides may have homologues in environmental mycobacteria. Nevertheless, a number of peptides have been identified that provide greater than 90% specificity and 19-47% sensitivity for diagnosis. These peptides, as well as new candidate peptides identified in the course of the leprosy sequencing project, will undergo further testing. Such peptides would have great potential as T-cell reagents, formulated either as skin test reagents or as antigens for testing in vitro, with which to monitor exposure to M. leprae within communities.

**MALARIA:** Molecular entomology

The vision of the malaria-transmitting mosquito as a harmless insect that doesn’t transmit the disease is becoming more tangible year by year. In the last two years, significant progress has been made in this area of TDR’s work. The ultimate aim is, through manipulation of mosquito genes, to replace the natural vectors of malaria in the wild with populations of anopheline mosquitos that are unable to support development of malaria parasites. The mosquitos would live in their normal environment but be unable to transmit malaria. This work began in 1991, when 36 specialists were brought together by TDR, the Wellcome Trust and the MacArthur Foundation to discuss the most promising lines of research in this new approach to mosquito control. It was estimated that the task would take 10-15 years to complete. Three main areas of research were recognized. The second line of research is to develop methods for inserting genes into the mosquito genome. At first, model genes are

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3 to Stewart Cole, Pasteur Institute, Paris.
inserted – such as green fluorescent protein, which makes a transformed mosquito easy to distinguish. But the aim is to insert genes selected from the ongoing projects on gene identification, and this has begun. The third line of research, and potentially the most problematical, not only because of the technology required, but also because of ethical, safety and regulatory issues, is to drive the selected genes into wild mosquito populations. Work in this area covers the genetics and dynamics of malaria-transmitting mosquito populations, and mosquito behaviour. For example, we need to know why a mosquito might prefer to feed on humans rather than animals, what natural chemicals it responds to in its life, and ultimately which genes are involved and might be used to change the mosquito’s behaviour by inserting into its genome – changing it into an insect that doesn’t like to feed on humans, for example. Developments along these three lines of research in 1999-2000 are outlined below. They mostly concern *Anopheles gambiae*, the main malaria vector in Africa.

Issues of ethics, risk perception, assessment, communication, choice of sites and plans for deploying a genetically engineered mosquito, and the socioeconomic issues associated with such an undertaking, are being addressed through a joint initiative under formulation by the TDR committees of Molecular Entomology, and Social, Economic and Behavioural Research.

**Analysing the mosquito genome and identifying genes**

A variety of activities contribute to the analysis of a genome. These include sequencing the mosquito genome and mapping together the fragments of genome information as they arise.

In 1999-2000, TDR supported a pilot gene discovery project based on EST analysis of high quality complementary DNA (cDNA) libraries from tissues critical to the interaction of the malaria parasite with the mosquito vector. More than 6000 ESTs have been cloned and analysed, leading to the discovery of a large number of genes. Integrated genetic and physical maps compiled through *in situ* hybridization of several hundred bacterial artificial chromosomes (BACs) to polytene chromosomes and a high resolution microsatellite map of *A. gambiae* have been completed. This will permit intensive analysis of regions of the genome associated with malaria related genotypes and coordination of complete genome sequencing efforts.

Building on the above, an international network of partners was formed (see list) and a global effort in sequencing the *A. gambiae* genome matured through the biennium. It was formally launched in March 2001. The project will build on the initial genomics research carried out at some of the participating institutions, and draw on the strengths of the different partners. TDR will play a coordinating role. Research capacity strengthening of malaria endemic country laboratories in contemporary genomics techniques is a priority of the network. It is expected that complete sequencing of a laboratory strain of *A. gambiae* will be available by the end of 2001. The approach being taken is to search for genes directly using the ‘shotgun’ approach, then to map the physical location of the genes on the chromosomes, from which their DNA sequences can be ascertained.5, 6

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**Sequencing the Anopheles gambiae genome**

**Current funding partners**
- TDR
- National Institutes of Health, USA
- French Government
- Multilateral Initiative on Malaria
- European Commission

**Current research partners**
- Institut Pasteur (France)
- European Molecular Biology Laboratory (EMBL)
- University of Notre Dame (USA)
- French National Sequencing Centre (Genoscope, France)
- Celera Genomics (USA)
- European Bioinformatics Institute (Ensembl/EBI, UK)
- Institute for Genomic Research (TIGR, USA)
- Institute of Molecular Biology and Biotechnology (IMBB, Greece)
- Organization for Nucleotide Sequencing and Analysis (ONSA network, Sao Paulo, Brazil)
- leading mosquito researchers around the world

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To accommodate the data emerging from the network, a new public database devoted to the genome of *A. gambiae* was established – AnoBase. As well as sequence information, this database will include full information about the role and function of genes as it becomes available. Another database, the *Anopheles* database AnoDB (see box 1), was started by TDR in 1996. This database contains data on the biology and genetics of anopheline mosquitoes. During 1999-2000, it was enhanced with new software (AceDB) and underwent some restructuring.

Knowledge of the total genomic information of the three organisms that participate in the malaria transmission cycle (the parasite, the human, the mosquito) could open up unprecedented opportunities for understanding and blocking disease transmission, and will lead to the discovery of targets against which new drugs and vaccines can be produced.

**Discovering mosquito genes which stop the parasite developing**

As a malaria parasite moves through the mosquito, it has to cross several barriers. These include the epithelia of the midgut and the salivary glands, where mosquito defences can stop the parasite’s development. Another barrier is provided by the mosquito’s immune system. TDR is supporting studies to gain insight into molecules which prevent the parasite from invading the midgut and salivary gland epithelia, and into molecules which allow the parasite to survive these barriers and complete its life cycle.

Activation of parasite gametes begins in the midgut, soon after a mosquito has taken a blood meal from a malaria infected person. More details of gametocyte activation factors have been obtained, and cell surface molecules and their roles in mosquito-parasite interactions have been identified. The localization of peptides that act as signals for the malaria parasite in the midgut is being investigated, and a new high-quality cDNA library has been generated, containing genes expected to be expressed in the adult female midgut. A new cell type – Ross cells – was discovered in the midgut epithelium of mosquitoes. These cells are preferentially invaded by the parasite’s ookinete stages when they cross the midgut epithelium.7,8,9

Attention is also being paid to the mosquito’s immune system. In some strains of mosquito, the immune response is so strong that the malaria parasites are killed, but normally the parasites manage to survive in small numbers. Immune reactions have been demonstrated at the molecular level in the midgut and salivary gland epithelia, in haemocytes, and in the fat body (the insect equivalent of the liver). A pilot study using cultured mosquito immune cells identified 30 putative genes, of which 19 are thought likely to be involved in the mosquito immune system and possibly in blocking development of the malaria parasite. Another study focused on identifying genes which determine the susceptibility of *A. gambiae* to pathogens. Molecular variation in the defensin gene was analysed, defensin being a protein that is active against pathogens. Very high genetic variation was found within a number of mosquito populations – 76 distinct sequences of the defensin gene were found altogether. Extensive analysis is necessary to understand the role and control of this gene.

Knowledge of how the malaria parasite interacts with the various organs and the immune system in the mosquito may lead to development of novel strategies for blocking disease transmission. But because it is difficult to obtain material from mosquitoes, TDR supports the development of mosquito cell lines, which are safe to work with and much easier to use than actual mosquitoes. Cell lines of mosquito midgut and immune cells have been generated – midguts of early 4th, and perhaps 3rd, larval stages (where most multiplication occurs) were found to be the most successful for this. Cultures of mosquito cells active in immune response have also been developed which, [7 Malaria. TDR Final Report series, no. 15, October 1999.](#) [8 Shahabuddin M, Pimenta PF. *Plasmodium gallinaceum* preferentially invades vesicular ATPase-expressing cells in *Aedes aegypti* midgut. *Proceedings of the National Academy of Sciences*, USA, 1998, 95(7):3385-3389.](#) [9 Zeiler H, Nawrocki JP, Shahabuddin M. *Plasmodium gallinaceum* ookinetes adhere specifically to the *Aedes aegypti* midgut epithelium by interaction with a carbohydrate ligand. *Journal of experimental biology*, 1999, 202(pt5):485-495.](#)
when exposed to an infectious agent e.g. a bacterium, respond by switching on specific genes, ultimately resulting in the synthesis of specific proteins to fight the infection. It was from these cells that the 30 putative genes mentioned above were isolated.

Novel immune response genes, and several specific transcriptionally co-regulated gene clusters elucidating new aspects of vector mosquito antimicrobial and antimalarial responses, have been discovered through cDNA microarray analysis. Further definition and dissection of the regulatory pathways and effectors involved in restricting malaria parasite development in the mosquito will be facilitated by global expression profiling of *A. gambiae* immune responses.

The malaria parasite can only be transmitted to a person after it has invaded the mosquito salivary glands. One study supported by TDR is looking at the control of genes in the salivary glands, at identifying sections of DNA which act as control or ‘promoter’ regions for genes, switching them on and off as directed. The first salivary gland specific gene to be isolated from *A. gambiae* was a putative apyrase gene (apyrase helps prevent coagulation of blood). In another study, a peptide was isolated which strongly inhibits the sporozoite stage of the malaria parasite from entering the salivary glands and midgut. This provides a potential tool – it represents a potential gene that could be inserted into the mosquito genome.

**Development of genetic and molecular tools for inserting selected genes into the mosquito genome**

In this area of work, a landmark event announced in June 2000 was the insertion of a gene into *Anopheles stephensi*, a major malaria vector on the Indian subcontinent. This was the first stable germline transformation of an *Anopheles* species. In this instance, the model gene for green fluorescent protein (GFP) was inserted, making the transformed mosquitoes glow green when excited by ultraviolet light. At a later stage, the GFP gene might be replaced by genes which inhibit parasite development. Work has already begun on one particular gene family – the *Anopheles* trypsin gene family, which consists of at least seven genes tightly clustered in a single locus. Trypsin is involved in blood feeding, and all the genes are expressed in the gut of female mosquitoes (male mosquitoes don’t feed on blood). Researchers have been able to insert a promoter for the trypsin gene into the germline of *A. stephensi*.

In this work, a transposon – a small mobile piece of DNA which inserts randomly into chromosomes – known as Minos was used to help transfer the gene into the *A. stephensi* genome. Preliminary results from work on *A. gambiae* indicate that another transposable element, known as piggyBac, can be used to transform this species of *Anopheles*. In the first instance of insertion into the *A. gambiae* genome, the gene for GFP was again used as the model gene.

These studies have now demonstrated the feasibility of transposable element-based systems for transformation of mosquitoes. It is likely that more and more laboratories will be able to apply the power of transgenesis in the investigation of the molecular biology of vectors.

**Development of methods to spread selected genes in wild mosquito populations**

Steady progress has also been made in this area of work during 1999-2000. In order to develop methods and strategies to spread selected genes through wild mosquito populations, we need to understand how genetic information flows between mosquito populations i.e. how related they are to each other, and what influences mosquito behaviour.
An ongoing study is looking at gene propagation between different populations of mosquitos. Results indicate that there is little genetic variation among populations of *A. gambiae* and *A. arabiensis* – in other words, few subpopulations are found across the continent of Africa. However, the Rift Valley of eastern Africa was seen to act as an ecological barrier to gene flow, separating genetically distinct eastern coastal populations of *A. gambiae* from populations west of the Rift Valley which stretch as far as West Africa.

Data collected in another study suggest that this lack of genetic diversity may be accounted for by the amount of gene dissemination among wild mosquitos. Significant levels of gene flow were found to occur between different molecular forms of *A. gambiae*, as revealed by DNA analysis of sperm from the reproductive systems of female mosquitos. A diagnostic tool, under development to differentiate between chromosomal forms of *A. gambiae* and determine whether there is gene movement between them, showed the amount of genetic diversity to be drastically reduced in laboratory colonies as compared to a field colony. These results were based on microsatellite (highly variable pieces of DNA) polymorphism at nine loci on chromosome 3 in the different colonies of mosquitos.

Populations of mosquitos build up very rapidly after onset of the rainy season – more rapidly than can be explained by the mosquito reproduction rate. Preliminary results in Western Kenya indicated that anopheline eggs are capable of surviving long periods of dry season before hatching into larvae after onset of the rainy season (in contrast to conventional wisdom that anopheline eggs hatch shortly after they are laid). This study on effective population size and gene flow in *A. gambiae* is looking to predict the occurrence and abundance of major malaria mosquitos.

The data produced in all these and similar studies will be useful, in due course, for identifying sites where transformed mosquitos might be released under the TDR molecular entomology programme. But before appropriate strategies to spread new genes among field populations can be designed, baseline information on mosquito behaviour and life history in the field is also needed. Research in this area includes a study of mosquito egg laying behaviour. This work has validated the hypothesis that sites selected by *A. gambiae* for egg laying are associated with certain species of bacteria and with microbial metabolites, which gravid female mosquitos respond to. Another line of ongoing TDR research is to characterize odorant binding proteins (OBPs) involved in mosquito recognition of human odours.

Thus this work helps us to understand gene flow in mosquito populations and to predict how malaria resistance genes will spread. Selected genes will have to be driven into wild populations of mosquitos. Possible driving forces have been described, e.g. transposable elements, which have the ability to spread through natural populations. Another potential gene driving mechanism is the 'cytoplasmic incompatibility' induced in mosquitos by infection with the symbiont *Wolbachia*; identification of genes and genome sequencing of two *Wolbachia* strains has begun.

The hypothesis that genetic manipulation of vectorial competence could be used to control the transmission of many vector-borne pathogens has stimulated much research in insect immunity, control of gene expression, transgenic technology, ecology, and prophylaxis of tropical diseases. Gene transfer technology offers a powerful tool to investigate the role of insect molecules involved in parasite-host cell interactions and opens up a wide range of applications with which to explore genomic data for disease control.

**Strategic social, economic and behavioural research**

The first meeting of the newly established Steering Committee on Strategic Social, Economic and Behavioural (SEB) Research was held in Geneva in September 2000, when a vision was developed for the next five years and a detailed workplan constructed for the coming two years. This committee was created following recommendations from the Joint Coordinating Board (JCB) in June 1999, and was preceded by a Scientific Working Group (SWG) of experts from a range of social, economic and policy sciences who met in Geneva, June 2000, to set the overall direction for SEB. The first call for grant applications was issued in October 2000.

Over the next few years, SEB will focus on gaining better understanding of how globalization and changing social, political and civil structures affect people’s access to health care and occurrence of TDR diseases – whether the diseases persist, resurge, or re-emerge. An important aspect of the Committee’s work will be to support capacity building.

**Amongst other things, studies have shown Anopheles gambiae populations in Africa to be closely related to each other, with little genetic variation across the whole continent.**

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From the beginning, TDR has placed considerable emphasis on the social and economic aspects of tropical infectious diseases and their control. From 1979-1994, social science research was supported through the Steering Committee on Social and Economic Research (SER), and, after 1994, through the Applied Field Research (now Intervention Development and Implementation Research) team. SEB is located within the STR area to reflect a focus on *basic* rather than *applied*, social, economic and behavioural research issues of trans-disease and global importance.

The focus of SEB reflects the growing interest in the complex relationship between poverty and health. On a worldwide scale, infectious and parasitic diseases disproportionately affect populations living in poverty. Social, political and economic inequalities are central to the persistence and spread of these diseases, and the performance of health systems in protecting vulnerable populations from the impact of these diseases often falls far short of what is needed. Over the next several years, the SEB Steering Committee will examine these issues within the context of globalization, the changing role of the state, and the emerging role of non-state actors (the private sector, non-governmental organizations and civil society).

**Gender issues**

The Intervention Development and Implementation Research (IDE) Task Force on Gender Sensitive Interventions (GSI) was created in 1998, with the overall aim to develop a conceptual framework and practical guidelines for incorporating gender considerations into tropical disease control programmes and services. To accomplish this, GSI sought to support studies that:

- develop and test methods for identifying potential gender inequities in the way tropical disease control programmes and services are designed, delivered, received and evaluated.
- develop and evaluate interventions that incorporate a gender perspective in tropical disease control.

However, with the creation of the new SEB Steering Committee, it was decided that gender research would benefit from the broader perspective on equity in health care taken by SEB. Therefore, the gender portfolio will be continued under the auspices of SEB, which will seek to develop a more comprehensive research agenda on gender equity in the prevention and control of neglected infectious diseases.

Ongoing work includes a study in Ghana to develop and pilot a district-level participatory planning process to identify and address gender issues in malaria control; a study in Myanmar to develop gender-sensitive approaches to reducing ocular disabilities in leprosy patients; a study in Brazil to identify gender specific barriers to leprosy detection and treatment; a study of the
feasibility of women’s support groups for lymphatic filariasis; two studies on schistosomiasis of the reproductive tract; and a four-country study of gender specific barriers to TB control. This latter work was initiated by the WHO Global Tuberculosis (TB) programme and continues in TDR. It is an investigation into the effects of sex and gender on the performance of TB control services in Malawi, Colombia, Bangladesh, and India. Country level work began following a protocol development workshop in Geneva in December 2000.

Results and experiences from these studies will contribute to the development of general guidelines for identifying and addressing gender specific needs, barriers and opportunities in infectious disease control.

Health sector reform

Following the report of the Ad Hoc Committee on Health Research Relating to Future Intervention Options, TDR managed, outside its normal activities, a research portfolio entitled Comparative Studies on Health Sector Reform. Some of the stakeholders in the then accelerating number of health sector reforms in countries were concerned about the effects of these reforms. With support from the Norwegian Government and in collaboration with the International Clearinghouse for Health System Reform Initiatives (ICHSRI) in Mexico, 54 individual studies were initiated in three rounds (1996, 1997, 1998). On establishment of the Steering Committee on SEB in 2000, finalization of the work was integrated into this committee.

During 1999 and 2000, the studies from the first two rounds were completed and results are being prepared for publication – eight are to be published in an international peer reviewed journal. Most of the 1998 studies, which all address equity, have also been completed. These studies will be supported technically during 2001 and the results made internationally available.

Results from the studies show that health sector reforms do indeed have many unintended and undesirable side-effects, e.g. shifting provider behaviours towards less cost-effective procedures and prescription practices, and increased inequity in access to services. While the intentions of the reforms were good, the problems lay in implementation, lack of monitoring, and lack of timely and adequate corrective measures when things start to go wrong.

The first three rounds of studies did not specifically focus on issues related to tropical diseases, but during 2000, a call for proposals was announced under the heading Health Sector Reform and Tropical Diseases 2000. The overarching aim is to study the opportunities and threats to the control of tropical diseases posed by ongoing health sector reforms. All the TDR diseases disproportionately affect poor and marginalized people, those who are often adversely affected by the approaches to reform as shown in the first rounds of reform studies. Two of the diseases, TB and malaria, are high on the international agenda, while the other eight diseases tend to be more neglected, although four (lymphatic filariasis, onchocerciasis, Chagas disease, leprosy) are targeted for elimination. Sixteen proposals were selected for funding and will be provided with technical support during 2001/2002 to develop appropriate methods and conduct the studies.