Scientific Working Group

Report on

Insect vectors and human health

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Executive summary

Vector-borne diseases still represent a significant threat to human health despite considerable national and international control efforts. Population growth, urbanization and migration, and poor environmental sanitation are some of the major causes of the emergence and re-emergence of vector-borne diseases in developing countries. In the past, and today, control of vectors has been a major component of disease management, but the effectiveness of the available vector control methods has been limited by logistic problems, high cost, insecticide resistance and by environmental pollution concerns. Therefore, novel and sustainable approaches to disease-vector control are urgently needed.

The Scientific Working Group (SWG), a multidisciplinary group of scientists representing academic and government institutions, was assembled to provide guidance to the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and partners regarding their vector research agenda and capacity building necessary for vector-borne disease control for the next five years. The recommendations of the SWG will provide a basis for TDR to define its own vector research programmes, taking into account its comparative advantages in both research and capacity-building activities.

Recommendations

The goal of the meeting was to provide technical guidance for the improvement and diversification of vector research in order to meet the needs of expanding and emerging disease situations. TDR-mandated activities on African trypanosomiasis, Chagas disease, dengue fever, leishmaniasis, lymphatic filariasis, malaria and onchocerciasis were critically reviewed and accomplishments were noted.

The meeting provided an opportunity to identify areas of knowledge that could be exploited for the improvement of existing tools for the management of disease vectors and the development of new tools. The SWG noted that there were major gaps in knowledge of the biology, ecology and behaviour of many disease vectors, and of vector/pathogen interactions. Research into the discovery and development of new control strategies and the implementation of research in the field is inadequate to meet the needs of many disease-vector control programmes around the world. The group emphasized that expanded research activity in these areas might identify vector weaknesses that could be exploited. It was also noted that TDR had had a successful track record in finding and developing biological vector control measures and that this area of research would provide strategies for the implementation in integrated vector control programmes.

The SWG recommended that the following areas of research should receive special attention:

- The biology and ecology of vectors in relation to the parasite/pathogen transmission
- Improvement of existing vector control tools
- The use of innovative approaches to the interruption of the pathogen life cycle
- Research capacity strengthening in disease endemic countries, especially in the basic sciences, including bioinformatics and genomics
- Co-funding of tropical disease research activities in cooperation with interested partners.

Specific recommendations on research, training and implementation priorities for each of the vector-borne diseases on which TDR focuses are included later in this report.

Introduction

Since its establishment, the Special Programme for Research and Training in Tropical Diseases (TDR) has participated in the search to identify and develop integrated vector control strategies which combine all appropriate techniques (chemical, biological, physical, environmental) in a systematic and coordinated manner. Among the ten diseases on which TDR focuses, seven (African trypanosomiasis, Chagas disease, dengue fever, leishmaniasis, lymphatic filariasis, malaria, onchocerciasis) are transmitted by insects.

In the past 20 years, TDR has supported research into and development of new tools for vector control, including fumigant canisters and insecticidal paints for the control of the triatomine bugs that transmit Chagas disease, pyramidal and monoconical tsetse fly traps and insecticide-impregnated bednets for malaria vector control. The research activities that the TDR Biological Control of Vectors (BCV) Steering Committee (SC) supported led to the isolation, characterization and evaluation of a vast array of entomopathogenic organisms, of which *Bacillus thuringiensis* subsp. *israelensis (Bti)* and *Bacillus sphaericus (Bs)* were quickly developed and accepted into vector control programmes for the prevention of onchocerciasis, lymphatic filariasis and malaria around the world.

In 1994, TDR set up the strategic research programme on Molecular Entomology, with the overall goal of developing tools for the genetic engineering of malaria vectors in which the parasite was unable to develop, and to use these transgenic mosquitoes to replace wild populations, thus interrupting parasite transmission. Research activities in the molecular entomology programme are now limited to malaria and dengue vectors (*Anopheles* and *Aedes*). This field of research is progressing well: the genetic transformation of the vectors, and the blocking of pathogen development in transgenic vectors and partial interruption of transmission have been achieved under laboratory conditions.

Genomics is a crucial component of 21st century biomedical research. Complete genetic information on the pathogen, its natural host and vector provides opportunities for increased insight into the biology of host/pathogen and vector/pathogen interactions, as well as for genetic modifications of natural enemies of vectors for increased effectiveness and sustainability.

Opportunities are being made available to tackle other innovative areas of research that will yield novel tools in the near future (e.g. the search for new insecticide resistance mechanisms and targets against which new generations of insecticides can be designed) for use in established vector control programmes. It is equally important to explore new biological control agents or improve the regulatory force of the existing biological control agents. The application of molecular genetics to the alteration of pathogens is a promising area of research.

Developing tomorrow's tools from today's research advances is a long process and the targets of strategic research are long term – some could take more than 15 years to realize, but powerful tools developed in the process will facilitate the achievement of long-lasting interventions. Although long-term oriented research is needed in order to find long-term sustainable solutions, at the same time it is highly desirable that research focusing on finding solutions for the near future is also carried out. In this context, research on the development of novel control tools, and the improvement of existing tools, needs to be conducted.

The TDR Intervention Development and Implementation Research (IDE) team is responsible for increasing interaction with disease control programmes with a new focus on implementation research. It deals with the research needed for the introduction of a new disease control tool (including vector control) by the health systems of disease-endemic countries.

1. Objectives and expected outcomes

The SWG was to recommend an overall strategy and scientific direction for a global research agenda, capacity and partnership building for disease vector control for the next five years and beyond. This would provide a basis for TDR to define its own vector research programme, taking into account its comparative advantages. The objectives of the meeting were to discuss:

- A summary of the current status of insect disease-vector research, control and capacity building activities;
- A definition of current research needs in relation to gaps in knowledge, effectiveness of available control tools and strategies for their use in the next five years and beyond;
- A review of ongoing disease-vector research and control activities globally, and identification of the most promising of present scientific opportunities for future directions;
- A definition of the research capacity strengthening and partnership needs for disease vector research and control and an outline of the response required;
- Recommendations to all stakeholders, TDR included, as to how to address the needs and gaps for a global research agenda, capacity and partnership building for disease vector control.

2. Malaria and *Anopheles*-transmitted lymphatic filariasis

Despite more than a century of control efforts, malaria remains one of the leading causes of human mortality in the world. Most malaria control interventions are based on antimalarial drugs or insecticides, and the effectiveness of both of these tools is now being compromised. Insecticide-treated materials are an important component of the Roll Back Malaria global strategy for vector control, but the number of effective insecticides available is limited. In contrast, in the control of lymphatic filariasis, emphasis has recently shifted towards human drug treatment. However, it is unlikely that drug treatment alone will eliminate the disease, and vector control efforts need to be maintained.

CURRENT CONTROL METHODS

- Residual house spraying
- Insecticide-impregnated materials and other personal protection measures
- Larval control in the broadest sense (including environmental management).

Potential methodologies involve:

- Genetically-modified vectors
- Other approaches based on new biotechnology advances.

CHALLENGES AND OPPORTUNITIES

Integration of control strategies

Although vector control is often recognized as the major means to fight malaria, it cannot by itself achieve the desired goals. Thus, the aim should be to integrate all possible methods of control, including drugs and vaccine development.

Global mosquito control in the urban environment

Mosquitoes are both a nuisance and the host/vectors of disease-causing parasitic organisms. They colonize rural and urban environments. Mosquito management is an important challenge in countries in which malaria is endemic and which often suffer from insufficient resources and limited sanitation infrastructures.

Integrated multi-disease prevention

Some haematophagous insects act as vectors for two or more pathogens. There is a need to understand how the development of one parasitic microorganism potentially interferes with the development and transmission of the other.

Development and acceptability of new methodologies and tools

The development of new methodologies is required; appropriate implementation relies on informed choice and acceptance by the community.

Understanding of vector bionomics in relation to transmission dynamics

Existing and novel disease control methodologies rely on knowledge of the biology, ecology, behaviour, and genetics of the vectors. Most anopheline mosquitoes are members of species complexes, thus the challenge is to focus research on biological species in order to understand parasite transmission dynamics.

Insecticide resistance management and the development of new insecticide products

Resistance to insecticides is increasing in many areas, yet few insecticides and tools are available for resistance management.

Vector response to environmental and parasite changes

It is clear that mosquitoes are colonizing new ecological niches in response to inappropriate urbanization and increased man-made environmental changes. Drug pressure on parasite populations, resulting in the selection of drug-resistant phenotypes, may also alter the balance of the vector–parasite interaction. Research needs to keep pace with the rapid evolution of the vector in both cases.

Vector control impact assessment

Too often, control operations are implemented without concurrent assessment of the impact of these interventions on parasite transmission, and on the dynamics of the vectorial system. There is a need for the development of appropriate indicators.

Community motivation and political will for sustainable vector control

National and local legislation regarding consent must be respected before the introduction of any experimental interventions. However, the increased recognition of the individual's right to be informed and to make choices about any new procedures which may impact on health, suggests that locally appropriate methods for seeking individual and/or community informed consent should be developed. The knowledge of local people, the users of the control strategies, has been neglected for too long; this knowledge needs to be properly exploited if vector control is to be successful. This itself will aid the motivation of communities to sustain control strategies. Political commitment through appropriate advocacy is also required.

RECOMMENDATIONS FOR FUTURE RESEARCH

Research on vector biology and the processes of parasite transmission

- Understanding the ecology and population structure of vector populations in relation to vector control.

Many vector control activities are undertaken without regard for the presence of highly heterogeneous vectorial systems, e.g. species complexes with differential roles in transmission. Given the need for locally adapted, integrated vector control strategies, a better understanding of the ecology, biology, and population structure of the vectorial units is needed. This knowledge can be used for the evaluation of existing and novel control methodologies. Although good molecular tools to address these issues are now available, the development of additional tools is needed, e.g. computer modelling, geographic information systems (GIS), age-grading.

Key research areas:

– Population structure, speciation and gene flow (e.g. spread of insecticide resistance)
– Population dynamics and regulation mechanisms (e.g. niche partitioning/competitive exclusion) in relation to environmental change and vector control
– Evaluation of the impact of control interventions on the ecology, behaviour and epidemiology of species complexes.

- Understanding the biology of vector behaviour.

The heterogeneities mentioned above are directly related to behavioural differences.

Key research areas:

- Mating, feeding, resting, and oviposition behaviour of vectors
- Development, standardization, and optimization of sampling tools and sampling protocols in relation to behavioural differences.

- Understanding of vector–parasite interactions.

Understanding the interactions taking place in the midgut, the haemocoel, the salivary glands and, potentially, the salivary duct of the *Anopheles* mosquito may lead to the discovery of new targets for interventions aimed at stopping the parasite transmission cycle within the vector. For example, understanding of the sequential cross-communication between the parasite and the vector, the processes of invasion of the epithelia, and the mechanism used by the parasite to elude recognition and attack by the host immune system is still rudimentary. Moreover, the means by which the parasites take advantage of remodelling processes occurring at the tissue level as a result of haematophagy remain to be described in detail. The experimental strategies that can be used today include not only the classical biochemical and molecular ones, but also modern approaches such as RNA interference (RNAi) and post-genomic methodologies (e.g. RNA profiling). Furthermore, cell-biological and tissue-specific techniques, often using up-to-date microscopy, can help to understand these open questions.

Key research areas:

- Molecular and cell biology of vector–parasite interactions
- Genomic and postgenomic studies of vector–parasite interactions.

- Understanding of vector physiology and identification of novel targets for control.

Of the insecticide molecules identified by traditional high-throughput screening approaches, the majority attack a very limited number of targets within the vector organism, e.g. the nervous system. The availability of genomic and proteomic information, in association with a better understanding of insect physiology, will yield a larger number of potential targets for customized design of novel molecules for vector control.

Key research questions:

- What are the key insect-specific functions that can be targeted?
- What are the key stages within the insect life cycle during which these functions can be targeted?
- What are the optimum methods for targeting these functions?

- Vector genetics and genome structure, including transposable elements, mitochondrial DNA and *Wolbachia*.

Detailed study of vector genomes will provide valuable information regarding virtually all aspects of vector biology**.** Genome features such as highly polymorphic regions, chromosome inversions, the paternally-transmitted Y chromosome, and the maternally-transmitted mitochondrial DNA (mtDNA) can be used to understand the genetic and ecological structure of natural vector populations. Knowledge of the genome sequence will facilitate rapid progress in understanding those aspects of vector physiology and biochemistry that may prove suitable as targets for new strategies for the control of pathogen transmission. Finally, understanding evolutionary processes such as the propagation of transposable elements (TEs), the acquisition of chromosomal inversions, or the propagation of prokaryotic organisms like *Wolbachia* can be harnessed in genetic control strategies.

Research on existing control strategies

- Optimization of existing control strategies.

In operational control programmes, the impact of different vector control interventions on the reduction or interruption of parasite transmission is related to their respective coverage. However, the notion of coverage is not always clearly defined. A minimum coverage rate of 80% of houses in a community was established in WHO guidelines[1] for indoor residual spraying programmes. Many current programmes integrate several methodologies which have different effects. For example, indoor residual spraying controls parasite transmission by reducing vector density and longevity. Insecticide-treated nets can act either as a personal protection tool or as a vector control intervention that is partially dependent on the level of coverage. An important question is what level of coverage with insecticide-treated materials (ITMs) will have a mass effect on vector populations (in terms of reduction of population density and individual longevity) and be effective as a malaria control intervention. The same questions apply to interventions such as larval control (e.g. larviciding, environmental management) and the use of household products, including repellents.

- Impact of insecticide resistance and development of management strategies.

There are a limited number of insecticides in use for vector control, with a heavy reliance on pyrethroids. Resistance to pyrethroids and most other available insecticides is spreading rapidly in most of the major vectors. Resistance management has been the subject of extensive modelling, but limited implementation.

Key research questions:

- What are the major genes involved in insecticide resistance?
- What impact do these resistance genes have on vector behaviour and vectorial capacity?
- What influences the spread of these resistance genes within and between “species”?
- Will proposed management strategies work in the field?
- Can we use knowledge of resistance characteristics to develop novel management strategies?

- Social aspects of vector control implementation.

Description of socioeconomic factors and how they influence the adoption and sustainability of vector control strategies needs to be considered. Key groups of people in different communities need to be identified as communicators and as the targets of information, education and communication campaigns. Research into societal mechanisms for optimal cooperation of local and national government authorities to enable the participation of citizens in vector control programmes is required.

Development of novel approaches for the control of parasite transmission

- Exploration of transgenesis-based approaches to interrupt parasite transmission.

The development of genetic strategies for the control of pathogen transmission in wild vector populations has generated research in three important areas: the development of tools for genetic analysis and manipulation, the identification of genes that can be used to interfere with parasite differentiation, and the development of methods for driving those genes into vector populations. Several laboratories have already demonstrated that these tools can be used to create transgenic mosquito strains in which pathogen development

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[1] *Manual for indoor residual spraying. Application of residual sprays for vector control.* Geneva, World Health Organization (document no. WHO/CDS/WHOPES/GCDPP/2000.3 Rev. 1).

is almost completely blocked. While induction of refractoriness has been achieved in the laboratory, many problems remain to be solved before such a strategy can be introduced in the field. More effector genes need to be identified, the fitness cost of such genetic constructs needs to be understood, and most importantly, strategies for driving such genes into wild populations need to be developed. It is important to recognize, however, that almost all these aspects of research also have the wider value of providing a broad knowledge of basic aspects of insect physiology, insect–parasite interactions, and insect population biology.

- Identification of novel active compounds (e.g. insecticides, repellents, semiochemicals).

The development of novel active compounds is needed to broaden the present limited array of insecticides. Rapid identification and validation of novel targets should provide a pipeline for the development of new classes of active ingredients.

Key research questions:

– Can viable insect-specific active compounds be developed which can be introduced into field control programmes?

– What balance of insecticidal-activity spectrum, environmental impact and development cost is required to ensure development of new compounds for vector control through public–private partnerships?

- Immunological approaches.

Human antibodies against either parasite or vector antigens may be used to block the development of pathogens. Alternatively, human anti-vector antibodies could be used to kill the mosquito. The innate insect immune response may be enhanced to limit parasite development.

Key research areas:

– This work entails identifying potential targets, evaluating the human antibodies raised against them, and understanding the role of the mosquito's immune response (e.g. peptides, reactive oxygen species) in blocking parasite development.

RECOMMENDATIONS FOR CAPACITY AND PARTNERSHIP BUILDING

The recommendations presented below are relevant to all vector-borne diseases discussed in this document.

Expertise in tropical disease vector research has diminished drastically over recent years. It is essential that appropriate decisions be taken to strengthen research capabilities in this area, especially in countries in which such vector-borne diseases are endemic. This will require institutional strengthening of both infrastructure and trained scientists. TDR has a comparative advantage in this area and should take the initiative for developing a collaborative network for the management of vector research. The areas below have been discussed and recommended for promotion.

Training of scientists from disease-endemic countries is required in:

- Molecular genetic methods for taxonomy, population genetics, and functional genomics. Such training should occur within the context of the application of these methods to problems faced by disease-endemic countries.
- Disease transmission modelling (GIS, planning and monitoring of vector control programmes). The application of vector control tools is most effective when adapted closely to the local situation. Sophisticated modelling promises to improve the efficiency of the data collection and planning process by identifying the key parameters to be evaluated.

- Socioeconomic sciences and the formation of local networks structured in holistic approaches to vector control. The ethical issues related to consent at individual and community level will require persons with an adequate level of long-term commitment to a community, who are locally respected, and who have received training to provide a solid background in ethics, social sciences, and understanding of the legal issues related to conducting trials of new intervention strategies.

Improvement of partnerships between research and control

Better understanding by scientific institutions of field realities, the constraints, and the needs of control programmes would help in rapidly filling some important gaps in understanding. Regional workshops on vectors (identification, ecology, transmission), control tools and strategies, monitoring and evaluation should be organized, involving key scientists, representatives of major funding partners and institutions and staff from national control programmes. Countries in which the diseases are endemic should be encouraged, through WHO regional offices and partners, to include an operational research component in their national control programmes, with appropriate human, logistic and financial resource allocations.

Links between entomologists and epidemiologists

As testing of new vector control tools and strategies moves into the field, the evaluation of efficacy must include assessments of the impact on human health. Effectiveness should be measured in terms of broad epidemiological parameters that indicate public health burden (e.g. prevalence of infection, mortality or morbidity rates). Realization of these studies requires input from both entomologists and epidemiologists and incorporation of appropriate end-points in each protocol of the development plan.

Insecticide-resistance monitoring networks

Relationships between WHO and other partners should be strengthened in order to benefit from experience from agriculture in managing insecticide resistance and to share experiences. There is a need to create more regional monitoring networks involving scientific institutions and control programmes. There is a need for an efficient system by which information on vector resistance and resistance-management principles can be centralized, stored, and disseminated.

Institutional strengthening with a view to the evaluation of genetically-modified mosquitoes

Special care should be given to the adequate preparation of research institutions to conduct trials involving genetic modification of vectors for the purpose of vector control. Partnerships should be developed with the Food and Agricultural Organization (FAO), the United Nations Environment Programme (UNEP) and other organizations with experience in biosafety training. Special attention should be paid to the training of local persons to cope with the social and economic issues, and to develop two-way education strategies combined with the process of community consent and participation.

Involvement of industry (new insecticides, semiochemicals, etc.)

More productive collaboration and innovative partnerships between industry, scientific institutions, WHO, and control programmes should be encouraged. This relates to the development, testing, technology transfer and local production in disease-endemic countries of new vector control tools and methods. These partnerships should involve mutual investment of intellectual, infrastructural and financial resources.

Collaboration between the South and North

In all of the above items, a two-way process of exchange of information and expertise between South (developing countries) and North (developed countries) is essential. The process must be designed to establish sustainable networks that will optimize the use of human resources in a flexible and constructive manner, with a view to the empowering of local communities such that vector control strategies can be implemented with minimal need for ongoing external technical advice.

Table 1 – **SWG prioritization of needed research on malaria prevention and control**

	Research for which TDR has a comparative advantage	Activities most appropriate for other partners
High priority	**Research to better understand the biology of vectors and the processes of parasite transmission** ***Understanding the ecology and population structure of vector populations in relation to vector control*** - Population structure, speciation and gene flow - Population dynamics and regulation mechanisms in relation to environmental change - Evaluation of the impact of control interventions on the ecology and behaviour of vectors, and malaria epidemiology ***Understanding the biology of vector behaviour*** - Mating, feeding, resting, and oviposition behaviour of vectors - Development, standardization, and optimization of sampling tools and protocols in relation to behavioural differences ***Vector genetics and genomics, including transposable elements, genetic markers and* Wolbachia *symbionts, as well as bioinformatics*** **Research on existing control strategies (e.g. insecticide-treated materials, house-spraying, larval control, integrated control)** ***Optimization of existing control strategies*** - Scaling up the use of insecticide-treated materials - Better understanding of vector control strategies ***Social aspects of vector control implementation*** - Role of social factors on the adoption and sustainability of vector control - Optimal cooperation with local and government authorities **Development of novel approaches for the control of parasite transmission** ***Exploration of transgenesis-based approaches to interrupt parasite transmission*** - Development of a robust technique for mosquito transformation - Identification of genes for interruption of the malaria parasite cycle - Elaboration of the gene-driving mechanisms for natural population replacement	**Research to better understand the biology of vectors and the processes of parasite transmission** ***Understanding of vector–parasite interactions*** - Molecular and cell biology of vector–parasite interactions - Genomic and postgenomic studies of these interactions ***Understanding of vector physiology and identification of novel targets for control*** - What are the key insect-specific functions that can be targeted? - What are the key developmental stages within the insect for targeting these functions? - What are the optimum methods for targeting these functions? **Research on existing control strategies (e.g. insecticide-treated materials, house-spraying, larval control, integrated control)** ***Impact of insecticide resistance and development of management strategies*** - What are the major genes involved in resistance and their impact on behaviour and vectorial capacity? - What influences the spread of these resistance genes within and between "species"? - Will proposed management strategies work in practice? - Can we use knowledge of resistance characteristics to develop novel management strategies?
Medium priority		**Development of novel approaches for the control of parasite transmission** ***Identification of novel active compounds (e.g. insecticides, repellents, semiochemicals)*** - Development of viable insect-specific active compounds suitable for use in the field - Public–private partnerships for the development of new compounds for vector control ***Immunological approaches*** - Identifying potential targets, evaluation of human antibodies raised against them - Understanding the role of the mosquito's immune response in blocking parasite development

3. Dengue fever and *Aedes*-transmitted filariasis

A wide variety of measures are available for the control of the vectors of dengue fever/dengue hemorrhagic fever, but the continuing geographic spread and rising incidence of the disease suggests that these measures are either ineffective, inappropriate, or are not being correctly applied. Nevertheless, in the absence of an effective vaccine, vector control remains the only available approach to the prevention of dengue.

CHALLENGES AND OPPORTUNITIES

Defining the minimum vector population required for epidemic transmission

Many countries support dengue prevention and control programmes, but continue to experience high rates of transmission. Such programmes aim to reduce the vector population to levels at which transmission cannot occur, yet such levels have never been defined. The problem is complex, being dependent on factors involving the vector (e.g. local strains, survival rate, population, biting frequency), the pathogen (e.g. infectivity, virulence), and the host (e.g. exposure to bites). The contribution of such parameters to the reproductive rate of transmission will vary according to local conditions and herd immunity. An in-depth understanding of all these factors and their role in transmission dynamics at the local level would enable vector control objectives to be rationalized.

Estimating the adult vector population density

Currently, many authorities assess dengue vector populations by counting larvae-infested containers. Such counts do not address productivity, the number of adult mosquitoes produced over time (for this varies with container size), availability of nutrients, larval crowding and many other factors. Direct methods for estimating adult *Aedes aegypti* population densities are slow, labour-intensive and unsuitable for routine use. Practicable methods for making such estimates are required for studies of transmission dynamics, for routine assessment of the risk of transmission, and for evaluating the impact of control operations.

Assessing the potential for effective, sustainable vector control

Many factors are blamed for the failure of dengue vector control: explosive growth of urban areas, limited government resources, poor management, inadequate training of field personnel, over-reliance on insecticides, incorrect application of insecticides, insecticide resistance, overemphasis on government intervention and insufficient participation by the community. Assuming that reduction of the spread of dengue fever by control of mosquito populations is feasible, a rational procedure for assessing the potential for effective, sustainable control at the local level would be of great value.

RECOMMENDATIONS FOR FUTURE RESEARCH

Development and validation of robust dengue transmission models to predict transmission and rationalize control objectives

Current dengue transmission models are of limited value because critical parameters have not been adequately quantified under field conditions. Improved models are crucial for understanding dengue transmission dynamics, and forecasting epidemics and the impact of vector control measures. A major effort to measure these parameters – particularly longevity and dispersal – is therefore justified.

- Longevity of adult mosquitoes and the duration of the gonotrophic cycle.

Estimates of adult longevity are derived from the parous rate and the length of the gonotrophic cycle. Parous rate is determined by dissection of adults collected in the field. The gonotrophic cycle can be monitored by atomic emission spectroscopy of eggs laid by females fed with rubidium, a non-radioactive chemical marker. Field studies performed using both techniques in several locations over an entire season are required to determine the influence of environmental factors, including weather.

- Dispersal of *Aedes aegypti*.

Dispersal of infected mosquitoes contributes to the local movement of dengue virus and would be a key factor in successful dissemination of transgenic mosquitoes in local populations. Dispersal of *Aedes aegypti* is driven by the search for oviposition sites, and can be monitored with rubidium markers. Studies of factors that influence dispersal distance, e.g. physical barriers, mosquito population density, number of eggs laid per oviposition site, availability and characteristics of such sites, and the impact of source-reduction campaigns, are required.

Development of novel control approaches

- Functional genomics and bioinformatics.

The sequencing of the entire *Aedes aegypti* genome, anticipated to be completed within the next two years, will open up an unparalleled opportunity to explore the interaction of dengue viruses with the vector. Application of novel genomic tools, e.g. microarrays and bioinformatics, will provide research opportunities to explore virus–vector interactions at a whole-genome level.

- Transgenic and genetic approaches to interrupt virus transmission.
  - Improving transformation tools: Recent progress in genetic transformation of mosquitoes has permitted models of parasite transmission blocking to be tested. Further improvements in transformation techniques are required for future routine application, e.g. effective transposable element vectors, powerful tissue- and stage-specific promoters.
  - Identification of effector molecules: Little is known about dengue virus–mosquito interaction at the molecular level. Basic studies are required to identify the key events that determine successful virus development in the mosquito and that lead to effective transmission of the virus. Understanding (spatially and temporally) of the patterns of dengue virus infection in vector species such as *Aedes aegypti* and *Aedes albopictus* is required to establish whether tissue-specific promoters can effectively interfere with virus replication in targeted tissues such as the midgut or salivary glands. These studies will serve as a foundation for designing and characterizing new protein-mediated or RNA-silencing effectors for interfering with virus replication in the vector species. An important research question is: how can the replication of all four serotypes of dengue viruses (DEN-1, DEN-2, DEN-3, DEN-4) be interfered with?
  - Genetic drive mechanisms: The utility of genetically-modified mosquitoes as a disease control strategy depends on the successful identification of molecular tools for promoting the proliferation and stable maintenance of transgene constructs in mosquito populations. Basic studies are needed to determine the feasibility of genetic drive mechanisms of population replacement, e.g., loaded transposons, endogenous meiotic drivers and symbiont-driven cytoplasmic incompatibility.

  - Development of transmission-blocking vaccines against midgut component proteins. The dengue virus is a flavivirus, which like many viruses that encounter the gut of its host, may be proteolytically processed to facilitate infection of gut epithelial cells. Can vaccines be developed that produce antibodies against gut enzymes and thus inhibit infectivity of the virus? Another target that might be sensitive to the immunity effectors are the midgut receptors important in initial virus infection.

- Molecular and genetic basis of vector competence.

Research is needed into the physiological and biochemical mechanisms and underlying genetic causes of vector competence. This effort should include functional genomic approaches for: characterization and testing of candidate genes; development of techniques for mapping field populations for genotype association studies; development of regional, and eventually global geographic maps of vector competence phenotypes in *Aedes aegypti* populations for each DEN serotype. This should also involve protocols for identification of barriers to midgut infection and escape, and transmission barriers. Similar studies are needed into the vector competence of other potential vectors of dengue virus (e.g. *Aedes albopictus*, *Aedes polynesiensis*).

Improvement of existing control materials

- Lethal ovitraps using synthetic attractants.

Recent studies using polymerase chain reaction (PCR)-based experiments and rubidium-marked eggs have revealed that individual female *Aedes aegypti* distribute their eggs among many sites. This may explain why the control method used in the highly successful *Aedes aegypti* eradication campaign of the 1950s (in which larvae-infested containers and their surrounding surfaces were treated with DDT) was so effective: females searching for oviposition sites were highly likely to encounter the treated sites. This behaviour could be exploited by using an oviposition-attractant to lure gravid mosquitoes to an insecticide-treated surface. Hay infusions are an effective attractant, but a more convenient approach would be to identify the active chemicals that they contain. If an effective trap can be developed, it should be field-tested in areas where *Aedes aegypti* populations are already low.

An alternative to the use of insecticides would be a simple baffle device that would trap the live gravid mosquitoes. Attempts should be made to develop such a trap as a device for estimating adult *Aedes aegypti* populations.

- Biological control.

The efficacy of existing microbial insecticides could be improved by formulation development, e.g. the use of products designed to exploit larval feeding behaviour. Genetically-modified microbials with higher toxin production could reduce manufacturing costs and increase efficacy. A continued search for novel toxins and pathogens (perhaps including viruses) is also recommended.

- Chemical control.

Chemical control agents can provide effective management of dengue vectors in some situations. There remains a need for new compounds with novel modes of action, an acceptable margin of safety and minimal environmental impact.

RECOMMENDATIONS FOR CAPACITY AND PARTNERSHIP BUILDING

(see pages 11–13)

Table 2 – **SWG prioritization of needed research on dengue prevention and control**

	Research for which TDR has a comparative advantage	Activities most appropriate for other partners
High Priority	**Development and validation of dengue transmission models for prediction and rationalization of control objectives** ***Longevity of adult mosquitoes and duration of the gonotrophic cycle*** • Season-long field studies of adult longevity and the gonotrophic cycle in several locations are required to determine the influence of environmental factors, including weather. ***Dispersal of* Aedes aegypti** • Dispersal of infected mosquitoes would be a key factor in successful dissemination of transgenic mosquitoes in local populations. Studies on factors that influence dispersal distance and the impact of source-reduction campaigns are required. **Development of novel approaches for the control of virus transmission** ***Functional genomics and bioinformatics*** • Completion of the *Aedes aegypti* genome sequence and application of novel genomic tools for exploring the interaction of dengue viruses with the vector at a whole-genome level. **Improvement of the existing control methods** • Updating of sampling methods and indicators for entomological surveillance, monitoring and evaluation • Social, economic and biological factors related to promotion and support of community-based interventions	**Development of novel approaches for the control of virus transmission** ***Transgenic and genetic approaches to interrupt transmission*** • Improving transformation tools • Identification of effector molecules • Genetic drive mechanisms • Transmission-blocking vaccines directed against midgut component proteins ***Molecular and genetic basis of vector competence*** • Physiological, biochemical and genetic mechanisms • Functional genomic approaches for characterization of candidate genes • Mapping of field populations for genotype association studies • Development of regional and global maps of vector competence in *Aedes aegypti* and other potential vectors for each DEN serotype **Improvement of the existing control methods** ***Biological control*** • The efficacy of existing microbial insecticides could be improved by formulation development • Genetically modified microbials with higher production of toxins could reduce manufacture costs and increase efficacy • Continued search for novel toxins and pathogens.
Medium Priority	**Improvement of the existing control methods** ***Lethal ovitraps using synthetic attractants*** • Exploitation of oviposition attractants to lure gravid mosquitoes to an insecticide-treated surface • Identification of the active chemicals that they contain	**Improvement of the existing control methods** ***Chemical control*** • Search for new compounds with novel mode of action and an acceptable margin of safety as well as minimal environmental impact

4. African trypanosomiasis

Management of trypanosomiasis depends on active surveillance and treatment of infected hosts and, to a limited extent, on vector control. Disease control efforts are restricted by the lack of effective drugs, their high cost, adverse side-effects, and the emergence of drug resistance. However, long-term dependence on drugs will inevitably result in resistance and, more importantly, will require a permanent financial commitment that will be difficult to achieve. Furthermore, the realization that domestic animals can also serve as reservoirs for the parasite further complicates disease management strategies.

The insect remains at the centre of hope for developments for the long-term control of trypanosomiasis. Several methods that are effective in the local and area-wide control of tsetse flies have been developed over the years. These approaches aim to reduce numbers of, or eliminate adult, insects via the application of insecticides to various types of traps and screens and directly to animals as live bait, and have proven successful in the control of human sleeping sickness. In addition, area-wide tsetse control involving a variety of methods, and including the sequential aerial application of insecticides (SAT) and the sterile insect technique (SIT), has recently gained momentum.

Recent advances in molecular technologies and their application to insects have revolutionized the field of vector biology, although progress in the tsetse field has been slow. While the genomes of the African trypanosome and the human host have been entirely sequenced, genomic data for tsetse flies are sparse. This knowledge could potentially lead to development of new approaches that are environmentally acceptable, effective and affordable.

CHALLENGES AND OPPORTUNITIES

Challenges

- The adoption and application of community-based intervention strategies have not been sustainable.
- Disease outbreaks involving displaced communities in areas of civil strife have been difficult to resolve by conventional methods.
- Despite dependence on the use of insecticides, insecticide-resistance monitoring tools are not available.
- Tsetse fly distribution across national boundaries has made it difficult to develop cohesive control programmes.
- Tsetse fly research undertaken for human sleeping sickness has not been integrated with equivalent activities dealing with the animal disease.
- Adequate genetic and molecular tools for effective disease management have not yet been developed.
- There are very few active research groups working on tsetse flies.

Opportunities

- The low reproductive rate of tsetse flies and restricted gene flow between populations provide an opportunity to reduce populations effectively.
- The recent endorsement by the African Union for a campaign against African trypanosomiasis provides political support for tsetse research and control activities.
- The recent completion of the trypanosome genome sequencing project provides impetus for the development of tsetse genomics.
- There are extensive GIS-based tsetse fly distribution maps available for risk mapping.

- Genetic modification of the tsetse fly has been achieved by expressing foreign genes in its symbionts.
- The use of live-bait technique can reduce populations of other disease vectors, i.e. ticks and mosquitoes.

RECOMMENDATIONS FOR FUTURE RESEARCH

Development of basic information as a basis for decision-making for tsetse control

In order to choose the appropriate control strategy, it is necessary to understand in detail the genetic relationships between the target population and neighbouring populations. This genetic information can be used to identify the origin of populations that are likely to re-invade areas where an intervention has already been effected.

- Studies on population genetics and dynamics.

Molecular markers provide a powerful means to determine gene flow between populations. This information will enable decisions to be made on the basis of the degree of isolation of the target population and the potential for re-invasion. Research into the development of an effective sampling regime is needed to fully understand genetic variability of natural populations.

- Analysis of field population parameters to provide information on vector bionomics, and on the presence of, and variation in, symbionts associated with tsetse flies.

Hybrid sterility in tsetse has been demonstrated in numerous laboratory studies and the analysis of field populations provides the opportunity to revisit the use of this approach for control of certain species. The role of symbionts in such mating incompatibilities is currently unknown.

- Analysis of parasite prevalence in field populations will provide necessary information on the vector competence of genetically isolated subpopulations, and on transmission dynamics.

Development of genomics and post-genomics

The availability of genomic tools and their comparative functional analysis will provide information on the important components of tsetse–trypanosome interactions and the genetic basis of vector competence. In addition, these tools will greatly enhance our understanding of tsetse olfactory and visual responses.

- Information should be obtained on the structure and size of the tsetse genome, and selected expressed sequence tags (ESTs) and large DNA (bacterial artificial chromosome, BAC) libraries should be constructed and analysed.
- These tools can then be used to initiate initial functional genomic analysis to better understand tsetse–trypanosome interactions and important factors involved in vector competence.
- A tsetse genome sequencing project can be initiated to obtain new targets that can be used for developing new control strategies.

Development of novel control approaches

The nutritional and reproductive biology of tsetse flies is strongly influenced by their gut microbial flora. It has been possible to genetically transform one of the symbionts and reintroduce the engineered microorganism into the fly so that foreign gene products can be expressed in the midgut.[2] The expression of gene products which have antitrypanosomal activity is expected to render tsetse refractory for parasite transmission. The

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[2] see *http://info.med.yale.edu/eph/html/faculty/aksoy/*

use of refractory strains will enhance the release of conventional sterile insects, without the risk of disease transmission. Furthermore, the replacement of susceptible natural populations with their parasite-refractory counterparts may provide an alternative approach for disease control.

- Effector molecules which, when expressed in midgut symbionts, can block the development and/or differentiation of trypanosomes need to be identified.
- To have an impact on disease transmission, these refractory phenotypes need to be driven into natural populations. Gene-spreading mechanisms such as symbiont-mediated cytoplasmic incompatibility may be used for such population replacement. The presence of organisms such as *Wolbachia* and incompatibility phenotypes in field populations should be explored.

Baseline monitoring of insecticide susceptibility in order to detect possible occurrence of insecticide resistance.

Currently there are no documented cases of insecticide resistance in tsetse flies. However, the recent adoption of aerial spraying and the continued use of insecticides on traps and targets pose potential threats.

Investigation of conditions for sustainable implementation of the available community-based vector control approaches.

The available community-based approaches to tsetse control have been shown to be effective. However, their sustainable implementation has been difficult. Furthermore, periods of civil strife and natural disasters have made the implementation of local vector control approaches difficult. Research into ways of increasing the sustainability of these approaches could have immediate impact for disease control.

RECOMMENDATIONS FOR CAPACITY AND PARTNERSHIP BUILDING

(see pages 11–13)

Table 3 – **SWG Prioritization of needed research on African trypanosomiasis prevention and control**

	Research for which TDR has a comparative advantage	Activities most appropriate for other partners
High priority	**Gather information as a basis for decision-making for tsetse fly control** ***Studies on population genetics and dynamics*** • Molecular markers to study gene flow between populations • Research on genetic variability in natural populations ***Analysis of effects of field population parameters on vector bionomics*** • Presence and variation of the tsetse fly symbionts and their role in mating incompatibilities ***Analysis of parasite prevalence in tsetse fly populations*** • Vector competence and transmission dynamics **Development of genomic and post-genomic studies of tsetse flies** • Genome size, structure, EST and large BAC libraries • Functional genomic analysis for understanding tsetse–trypanosome interactions and vector competence **Development of novel approaches for the control of transmission** • To impact upon parasite transmission, the refractory tsetse phenotypes need to be driven into natural populations • Gene spreading mechanisms such as symbiont-mediated cytoplasmic incompatibility may be used for such population replacement. The presence of organisms such as *Wolbachia* and incompatibility phenotypes in field populations should be explored. **Community-based vector-control activities** • Social, economic and biological factors related to promotion and support of community-based interventions	**Development of novel approaches for the control of parasite transmission** • Effector molecules which, when expressed in midgut symbionts, can block the development and/or differentiation of trypanosomes need to be identified **Community-based vector-control activities** • Social, economic and biological factors related to promotion and support of community-based interventions
Medium priority	**Development of genomic and post-genomic studies of tsetse flies** • A genome sequencing project should be initiated to obtain new targets that can be used for developing new control strategies	**Monitoring of possible occurrence of insecticide resistance in tsetse flies** • Currently there is no documented case of insecticide resistance in tsetse flies; however, the recent adoption of aerial spraying and the continued use of insecticides on traps and targets pose potential threats.

5. Chagas disease

Chagas disease affects an estimated 16-18 million people throughout the Americas. Currently there are three multinational, intergovernmental control programmes: the Southern Cone, Andean Pact, and Central American control initiatives. Vector control efforts in all of these programmes focus on the application of insecticides (primarily synthetic pyrethroids) for the control of domestic triatomine populations. Due to high cost, house improvements designed to reduce bug infestations are utilized in a very limited manner. Screening of local and regional blood supplies has been initiated in many countries in the disease-endemic region.

CHALLENGES AND OPPORTUNITIES

Challenges

- Limited knowledge of aspects of general molecular genetics, biochemistry, immunology, and physiology that are relevant to transmission, new control approaches and insecticide resistance.
- Lack of standardized biological and/or molecular assays for assessing insecticide susceptibility/resistance.
- Limited knowledge of population structure (gene flow) and dynamics of local vector populations, relevant to peridomiciliary and sylvatic populations and domiciliary colonization.
- The need for reassessment of taxonomic concepts based on a multidisciplinary approach, specifically relating to species complexes.
- Lack of understanding of genetic factors governing vector competence and vector–parasite interaction in different triatomine species and/or populations.
- Lack of tools for determining the vector dispersion and the source/nature of insects in homes after insecticide intervention.
- Development of alternative control approaches (e.g. biological control agents, genetic modification methods) for use against low-density infestations in domestic and peridomestic situations.
- Lack of eco-epidemiologic modelling tools for predicting disease trends.
- Lack of public and private sector incentive and involvement for developing and/or improving control approaches.
- Perception in disease-endemic countries that the disease is not of great importance.

Opportunities

- Existence of applied field research that is coordinated with ongoing control activities.
- Partial availability of novel tools and technologies (e.g. molecular tools, GIS, remote sensing).
- Existence of networks for collaborations to create synergies for research, capacity building, and transfer of technology relating to vector-borne transmission.

RECOMMENDATIONS FOR FUTURE RESEARCH

Development of molecular tools for studying population structure and dynamics

These tools are crucial to our understanding of species complexes, gene flow, taxonomy, and house re-infestation after insecticide treatment.

Development of eco-epidemiologic models for predicting vector-borne disease transmission and trends

Chagas is a complex zoonotic disease, which involves multiple vectors and reservoir hosts, and diverse transmission cycles in different geographical regions. The use of eco-epidemiologic modelling tools will facilitate a better understanding of the risk factors associated with transmission and subsequent prediction of disease trends.

Research into the genetics and genomics of the primary domestic vectors of Chagas disease

Very little genetic and biochemical information is available regarding parasite–vector interaction, vector competence, insecticide susceptibility/resistance, and the development of new intervention strategies.

- Development of the tools, reagents, and resources that will provide an understanding of the genetic composition, structure and complexity of the key vector species (*Rhodnius prolixus* and/or *Triatoma infestans*), including genomic and cDNA libraries, expressed sequenced tags (ESTs), and the determination of basic genome size and complexity.
- Establishment of a triatomine genome project (*Rhodnius prolixus* and/or *Triatoma infestans*) that will lead to a fundamental knowledge of genome structure and function.

Development of novel tools for control of Chagas disease vectors, to be utilized as part of an integrated vector management programme

While great regional success has been achieved by the domiciliary use of insecticides, this approach is largely ineffective against peridomiciliary and sylvatic triatomine populations. Consequently, the development of alternative innovative control approaches, such as biological control agents, genetically-engineered bacterial symbionts or other novel strategies, for use as part of an integrated vector management programme, would be highly beneficial.

Development of standardized methods and procedures for insecticide susceptibility and resistance monitoring

The current vector control programmes rely heavily on insecticide use; consequently, it is imperative that standardized tools and protocols for susceptibility monitoring be developed.

Development of integrated community-based approaches for vector surveillance and control

The long-term success of any control strategy for Chagas disease is dependent upon community support and involvement.

RECOMMENDATIONS FOR CAPACITY AND PARTNERSHIP BUILDING

(see pages 11–13)

Table 4 – **SWG prioritization of needed research on Chagas disease prevention and control**

	Research for which TDR has a comparative advantage	Activities most appropriate for other partners
High priority	**Development of molecular tools for studying population structure and dynamics** These tools are crucial to our understanding of species complexes, gene flow, taxonomy, and house re-infestation following insecticide treatment. **Development of novel tools for control of Chagas disease vectors** While great regional success has been achieved by domiciliary insecticide use, this approach is largely ineffective against peridomiciliary and sylvatic triatomine populations. Consequently, the development of alternative innovative control approaches, e.g. biological control agents, genetically-engineered bacterial symbionts, for use as a part of an integrated vector management programme would be highly beneficial.	**Development of standardized methods and procedures for monitoring insecticide susceptibility/resistance** The current vector control programmes rely heavily on insecticide use; consequently, it is imperative that standardized tools and protocols for susceptibility monitoring be developed. **Development of integrated community-based approaches for vector surveillance and control** The long-term success of any control strategy for Chagas disease is dependent upon community support and involvement.
Medium priority	**Research into the genetics and genomics of the primary domestic vectors** Development of the tools, reagents and resources that will provide an understanding of the genetic composition, structure and complexity of the key vector species (*Rhodnius prolixus* and/or *Triatoma infestans*), including genomic and cDNA libraries, ESTs and the determination of basic genome size and complexity.	**Research into the genetics and genomics of the primary domestic vectors** Establishment of a triatomine genome project (*Rhodnius prolixus* and/or *Triatoma infestans*) that will lead to a fundamental knowledge of genome structure and function. **Development of eco-epidemiologic models for predicting vector-borne disease transmission and trends** Chagas is a complex zoonotic disease, which involves multiple vectors, reservoir hosts, and diverse transmission cycles in different geographical regions. Eco-epidemiologic modelling tools will allow a better understanding of the risk factors associated with transmission and subsequent prediction of disease trends.

6. Leishmaniasis

Indoor spraying with insecticides has been used in some regions, primarily in Europe, Asia and Africa, to control endophilic transmission of visceral and cutaneous leishmaniasis. Preliminary insecticide-treated bednet trials have also been carried out. In some locations, where dogs are acting as important reservoir hosts, insecticide-treated dog collars have been utilized for control of canine leishmaniasis and the prevention of visceral leishmaniasis in humans.

CHALLENGES AND OPPORTUNITIES

Challenges

- Limited knowledge of general molecular genetics, biochemistry, immunology, and physiology that are relevant to parasite–vector interaction, transmission, new control approaches, and insecticide resistance.
- Lack of molecular tools for assessing infection rates in natural vector populations and insecticide susceptibility/resistance.
- Limited knowledge of population structure (gene flow) and dynamics of local vector species/populations relevant to endophilic and exophilic transmission.
- The lack of molecular markers for phylogenetics, taxonomy, population genetics, and vector dispersion.
- Limited understanding of the role of vector salivary components in natural transmission, disease establishment and pathogenesis.
- Development of alternative control approaches (e.g. biological control agents, and environmental, home improvement, and genetic modification methods).
- Limited eco-epidemiologic modelling tools for predicting disease trends.
- Limited understanding of the focal and temporal distribution of disease transmission and local vector species/populations and densities.
- Limited existence of networks for collaborations to create synergies for research, capacity building, and transfer of technology relating to vector-borne transmission.

Opportunities

- Partial availability of novel tools and technologies (molecular, GIS, remote sensing, etc.).
- Existence of strong research programmes focused particularly on the parasite and host, and to a lesser degree on the vector.
- Recognition of the disease as a global priority.

RECOMMENDATIONS FOR FUTURE RESEARCH

Development of molecular tools for studying taxonomy, population structure and dynamics

These tools are crucial to our understanding of species complexes, gene flow, taxonomy, and the focal nature of disease transmission.

Development of eco-epidemiologic models for predicting vector-borne disease transmission and trends

The leishmaniases comprise a diverse group of zoonotic and anthroponotic diseases with a broad spectrum of clinical manifestations. These diseases are epidemiologically complex, involving multiple vector species and reservoir hosts, and diverse transmission cycles. Eco-epidemiologic modelling tools will allow a better understanding of the risk factors associated with transmission and subsequent prediction of disease trends.

Research into the genetics and genomics of the primary insect vectors of leishmaniasis

Very little genetic and biochemical information is available for phlebotomines, relevant to parasite–vector interaction, vector competence, insecticide susceptibility/resistance, and the development of new intervention strategies.

- Development of the tools, reagents, and resources that will provide an understanding of the genetic composition, structure and complexity of the key vector species *Lutzomyia longipalpis* and/or *Phlebotomus papatasi*, including genomic and cDNA libraries, ESTs, and the determination of genome size and complexity. Whilst these species are not the only important vectors, they are however the species for which the most genetic, biochemical, and physiologic information is available.
- Establishment of a phlebotomine genome project (*Lutzomyia longipalpis* and/or *Phlebotomus papatasi*) that will lead to fundamental knowledge of genome structure and function.

Identification and development of novel tools for control of leishmaniasis vectors, to be utilized as part of an integrated vector management programme

While domiciliary insecticides are highly effective for control of endophilic species, methods used to control exophilic species are largely ineffective. Consequently, the development of alternative innovative control approaches is important for the development of long-term vector control strategies.

Development of integrated community-based approaches for vector surveillance and control, as well as research on knowledge, attitudes, and perceptions related to vector-borne transmission

The long-term success of any control strategy for leishmaniasis is dependent upon community support and involvement.

Development of vaccines against vector salivary components

More is known about the role of vector saliva in the experimental transmission of cutaneous leishmaniasis in animal models than for any other vector-borne disease. This knowledge provides a basis for proposing the development of saliva-targeted vaccines.

RECOMMENDATIONS FOR CAPACITY AND PARTNERSHIP BUILDING

(see pages 11–13)

Table 5 – **SWG prioritization of needed research on leishmaniasis prevention and control**

	Research for which TDR has a comparative advantage	Activities most appropriate for other partners
High priority	**Development of molecular tools for studying taxonomy, population structure and dynamics** • These tools are crucial to our understanding of species complexes, gene flow, taxonomy, and the focal nature of disease transmission **Development of eco-epidemiologic models for predicting vector-borne disease transmission and trends** • Eco-epidemiologic modelling will allow a better understanding of the risk factors associated with transmission and subsequent prediction of disease trends	**Development of molecular tools for studying taxonomy, population structure and dynamics** • These tools are crucial to our understanding of species complexes, gene flow, taxonomy, and the focal nature of disease transmission **Identification and development of novel tools for control of vectors** • To be utilized as part of an integrated vector management programme • Alternative innovative control approaches are important for the development of long-term vector control strategies
Medium priority	**Research into the genetics and genomics of the primary human vectors of leishmaniasis** ***Development of tools, reagents, and resources*** • Understanding of the genetic composition, structure and complexity of the key vector species *Lutzomyia longipalpis* and/or *Phlebotomus papatasi* • Genomic and cDNA libraries, ESTs and determination of genome size and complexity **Development of novel tools for control of vectors** • New methods for control of exophilic sandfly species **Development of integrated approaches for vector surveillance and control** • Research on community-based support and involvement in vector control • Development of new strategies for the use of insecticide-treated materials	**Research into the genetics and genomics of the primary human vectors of leishmaniasis** Establishment of a phlebotomine genome project will lead to a fundamental knowledge of genome structure and function **Development of integrated community-based approaches for vector control** • The long-term success of any control strategy for leishmaniasis is dependent upon community support and involvement **Development of vaccines against vector salivary components** • The knowledge of the role of vector saliva in experimental transmission of cutaneous leishmaniasis in animal models provides a basis for the development of saliva-targeted vaccines

7. Lymphatic filariasis, *Culex*-transmitted

Lymphatic filariasis is a chronic disease caused by infection with filaria parasites (mainly *Wuchereria bancrofti*) which are transmitted by *Anopheles, Aedes, Culex,* and *Mansonia* mosquitoes. Traditional methods for controlling these species include sanitation, drainage and the application of larvicides. The recent development of new combinations of drug regimens for filariasis control have transformed the outlook for this disease. The Global Programme to Eliminate Lymphatic Filariasis (GPELF), coordinated by the WHO Secretariat, seeks to achieve its goal by 2020. In all countries, annual mass drug administration will be used to suppress or cure infection and interrupt transmission. Other available strategic interventions include morbidity control for disability alleviation and vector control to prevent transmission.

The control of the anopheline vectors of lymphatic filariasis is discussed in the malaria section, and control of *Aedes* mosquitoes in the dengue fever section.

Another very important vector is *Culex quinquefasciatus,* which breeds prolifically in polluted water. Control of this vector is thus often limited to routine larviciding and sanitation. Source reduction for the control of dengue transmitted by *Aedes aegypti* and *Aedes albopictus,* which breed in domestic water containers, contributes to *Culex* control when the larvae share the same habitats. A limited range of synthetic and biological larvicides is available for *Culex* control, and organophosphate resistance is relatively common. Physical methods such as drainage of water bodies, covering latrine pits, control of vegetation and use of expanded polystyrene beads can be highly effective, but are difficult to undertake in areas with high rainfall and extensive breeding sites in exposed ditches and flooded pits. Implementation research in various urban situations is urgently required to improve these methods.

CHALLENGES AND OPPORTUNITIES

Surveillance

Little is known about the relationship between vector densities, parasite infection and transmission risk. A primary requirement is that sampling methods for *Culex* adult mosquitoes be standardized to enable:

- Monitoring of vector density and seasonality
- Estimation of lymphatic filariasis transmission potential
- Evaluation of control operations.

Vector control

A wide range of mosquito-control methods is available, but in many cases impact is limited by technical and operational factors, thus:

- Programme planning and implementation should be based on results of operational research to determine the effectiveness of locally appropriate methods for *Culex* control.
- Evaluation should compare the relative roles of sanitation, physical, chemical and biological interventions for reduction of *Culex* productivity.
- Integrated vector management (IVM), implemented through the decentralized health programme, should be optimized.

RECOMMENDATIONS FOR FUTURE RESEARCH

Development and validation of transmission model

Several models of lymphatic filariasis transmission dynamics have been developed, e.g. LYMPHASIM, which was developed with TDR support. These models are intended to facilitate planning and assessment of the GPELF programme. Suitable models should be validated in various epidemiological settings.

Development of novel control approaches

- Functional genomics and bioinformatics.

Little information regarding the *Culex quinquefasciatus* genome is currently available. Basic genome studies should be initiated and programmes on identification of ESTs and development of large-fragment genome libraries should be expanded. These initiatives will facilitate the application of novel genomic tools, e.g. microarrays, bioinformatics, for the exploration of filarial–vector interactions at a whole-genome level.

- Transgenic and genetic approaches to vector control.
  - Improving transformation tools: Improvements in transformation techniques for *Culex* mosquitoes are required if this technology is to be applied for lymphatic filariasis, e.g. the development of effective transposable element vectors and powerful tissue- and stage-specific promoters.
  - Identification of effector molecules: Little is known about *Wuchereria bancrofti*–mosquito interactions at the molecular level. Basic studies are required to identify the key events that determine successful filaria development in the mosquito and that lead to effective transmission. A spatial and temporal understanding of *Wuchereria bancrofti* infection patterns in vector species such as *Culex quinquefasciatus*, *Aedes polynesiensis* and *Ochleratatus* (*Finlaya*) spp. is required in order to establish whether tissue-specific promoters can effectively interfere with parasite development in the tissues targeted, such as the midgut or in-flight muscles. These studies will provide a basis for interfering with pathogen development in the vector.
  - Genetic drive mechanisms: The utility of genetically-modified mosquitoes as a disease control strategy depends on the successful identification of molecular tools for promoting the proliferation and stable maintenance of transgene constructs in insect populations. Basic studies are needed to determine the feasibility of genetic drive mechanisms for population replacement, e.g. loaded transposons, endogenous meiotic drivers and symbiont-driven cytoplasmic incompatibility.

- Molecular and genetic basis of vector competence.

Research is needed into the physiological and biochemical mechanisms and underlying genetic causes of vector competence for filaria. This effort should include functional genomic approaches for the characterization and testing of candidate genes; development of techniques for mapping field populations for genotype-association studies; and development of regional, and eventually global geographic maps of vector competence phenotypes in *Culex quinquefasciatus*, *Aedes polynesiensis*, *Ochleratatus* (*Finlaya*) spp. populations for *Wuchereria bancrofti*. This should also involve protocols for identification of impediments to midgut infection and development, and escape of the parasite from flight muscles.

- Entomopathogenic viruses.

Viruses are now established control agents for lepidopteran pests of agriculture, but their potential value for the control of vectors of public health importance has not been exploited. Now that potent viruses which are pathogenic for *Culex* mosquitoes have been isolated, characterized and cultured, it is conceivable that they could be developed for operational control of vectors of lymphatic filariasis. Priority candidates for investigation are certain baculoviruses which infect *Culex,* and densoviruses which infect *Aedes*.

Improvement of existing control materials

- Insecticides and microbials.
  - The efficacy of existing microbials (biological agents with insecticidal properties) might be improved by the development of new formulations. Genetically-modified microbials (e.g. *B. sphaericus* into which *Bti* toxic genes have been inserted) with enhanced production of toxins might reduce manufacture costs and increase efficacy, and may be effective in reducing the risk of development of resistance.
  - Delivery of larvicides can be tailored in special presentations specifically for *Culex* control, e.g. slow-release or floating briquettes. Residual adulticides can be used to impregnate curtains and bednets, which might be made attractive to *Culex*.

- Personal protection.

In partnership with the Roll Back Malaria programme, the effectiveness of insecticide-treated materials should be evaluated for impact on lymphatic filariasis transmission. This urgently requires development of suitable protocols. The use of insecticide-treated materials for malaria control could possibly be encouraged by their reduction of the *Culex* nuisance levels and this should be investigated.

- Lethal ovitraps and resting sites.

Attractants for oviposition, feeding or resting can be used to lure mosquitoes to insecticide-treated surfaces or traps. With such baits, some experimental devices use mechanical arrangements to retain and kill the mosquitoes. These methods should be evaluated and further developed for lymphatic filariasis vector control, especially for *Aedes polynesiensis* as well as *Cx. quinquefasciatus.* Natural or synthetic attractants (semiochemicals) could enhance the performance of such devices.

- Integrated lymphatic filariasis vector management involving community participation and mobilization.

Considering the biting nuisance caused by *Cx. quinquefasciatus*, socio-behavioural and technical research is needed to explain control failures. Results should lead to new strategic combinations of interventions (integrated vector management, IVM) and their effective adaptation to achieve more successful control of lymphatic filariasis transmission potential in various socio-cultural situations.

RECOMMENDATIONS FOR CAPACITY AND PARTNERSHIP BUILDING

(see pages 11–13)

Table 6 – **SWG prioritization of needed research on lymphatic filariasis prevention and control***

	Research for which TDR has a comparative advantage	Activities most appropriate for other partners
High priority	**Develop and validate transmission models** • Several models of transmission dynamics have been developed with the intention of facilitating improved planning and assessment of the disease elimination programme. The most suitable models should be validated in various epidemiological settings. **Development of novel approaches for the control of parasite transmission** ***Molecular and genetic basis of vector competence*** • Physiological and biochemical mechanisms and underlying genetic causes of vector competence for filaria • Functional genomic approaches for characterization of candidate genes • Mapping of vector populations for genotype association studies • Regional, and eventually global, geographic maps of vector competence phenotypes in populations of *C. quinquefasciatus, Aedes polynesiensis, Ochlerotatus and Finlaya* spp.	**Develop and validate transmission models** • Several models of transmission dynamics have been developed with the intention of facilitating improved planning and assessment of the disease elimination programme. The most suitable models should be validated in various epidemiological settings. **Development of novel approaches for the control of parasite transmission** ***Functional genomics and bioinformatics*** • Basic genome studies for *Cx. quinquefasciatus* should be initiated • Identification of ESTs and development of genome libraries • Application of novel genomic and bioinformatics tools for exploration of filarial-vector interactions at a whole-genome level. ***Transgenic and genetic approaches to vector control*** • Improvements in transformation techniques for *Culex* mosquitoes • Identification of effector molecules • Basic studies to determine processes of filaria development in the vectors • Understanding *Wuchereria bancrofti* infection patterns in the vector • Basic studies of genetic drive mechanisms in mosquito populations. ***Entomopathogenic viruses*** • Potent baculoviruses pathogenic against *Culex* and densoviruses against *Aedes* mosquitoes have been isolated and might be developed for vector control. **Research and improvement of existing control strategies** ***Insecticides and microbials*** • Should be improved by development of new formulations • Genetically-modified microbials with increased efficacy • Residual adulticides can be used to impregnate curtains and bednets. ***Lethal ovitraps and resting sites*** • Attractants for oviposition, feeding or resting can be used to lure mosquitoes to insecticide-treated surfaces or traps.
Medium priority		**Research and improvement of existing control strategies** ***Personal protection*** • Effectiveness of insecticide-treated materials should be evaluated for impact on lymphatic filariasis transmission. ***Integrated vector management with community participation*** • Social, behavioural and technical research is needed to explain control problems • Adaptation of integrated vector-control management should be evaluated in various sociocultural situations.

*mainly for *Culex* mosquitoes; relevant topics for *Anopheles* and *Aedes* are placed under recommendations for malaria and dengue respectively

8. Onchocerciasis

Blackfly species that carry *Onchocerca* parasites are both numerous and diverse, and differ greatly in characteristics that are directly related to vectorial capacity. Thus, only if these species are identified precisely, and their behaviour patterns are carefully characterized, can any degree of vector control – as well as epidemiological understanding – be achieved. Larviciding is the only method of vector control that is currently in use.

The following techniques have been tested, and showed varying degrees of success:

- Adulticides – do not show much promise for the future
- Vegetation clearance – may have local value but is not of widespread applicability
- Control of outflow from dams – can have local value
- Personal protection (repellents, clothing, trapping, etc.) – could be of value if an effective repellent for personal use and/or an attractant for traps were to be developed
- *Bacillus thuringiensis israelensis* – is highly effective and has been used successfully in the Onchocerciasis Control Programme (OCP)
- Some other biological control agents (e.g. mermithid nematodes, chironomid larvae, viruses, fungi) – are not effective measures in isolation.

CHALLENGES AND OPPORTUNITIES

Challenges

- Cost is a major barrier to the more widespread use of larvicides.
- Larvicide resistance (e.g. as a result of agriculture-related runoffs) can be expected to develop in all vector control operations, except those of short duration.
- Re-invasion of controlled areas can prevent vector elimination. Changes in vector distribution may alter operational requirements during control programmes.
- The environmental impact of larvicides may not be trivial, especially when more than one larvicide, each with differing toxicities and effects, must be used.
- Chemotherapy will remain the method of choice for the control of onchocerciasis in the immediate future. However, such therapy currently relies on a single drug (ivermectin). The conditions under which this drug can be used to interrupt transmission are not understood, and the long-term efficacy of treatment of the community with ivermectin is unknown. Hence it is of immense importance that novel therapeutic interventions for onchocerciasis control be developed.
- The tools with which ivermectin distribution strategies are modelled do not currently take into account variation in vector parameters, thus making the rational choice of strategy incomplete.

Opportunities

- Larvicides could be used for vector elimination in isolated geographical foci where currently only ivermectin is used, e.g. Yemen and some areas in East Africa and Central/South America.
- Integration of larviciding with other disease control methods (e.g. ivermectin, nodulectomy) to improve effectiveness.
- The existing ivermectin distribution programmes in Africa and in Latin America provide numerous opportunities for research into interactions between chemotherapy and entomological aspects of parasite transmission.
- Onchocerciasis control will be more effective if ivermectin distribution is supported by vector control strategies, especially in the event of the appearance of ivermectin-resistant parasites.

RECOMMENDATIONS FOR FUTURE RESEARCH

Larviciding

The required logistic support, safety and cost of current insecticides make vector control methods, as used by OCP, very expensive and beyond the means of most disease-endemic countries. Therefore localized control methods will need to be implemented as cheaply as possible. Operational costs would be reduced if effective larvicides could be improved in terms of their carry distance and other characteristics. In addition, if the conditions (e.g. turbidity and conductivity) of rivers to which larvicides are applied are taken into account, it may be possible to reduce larvicide dosages.

- Improvements in larviciding agents:
  - Development of new larvicides and improvement of the carry distance of available larvicidal formulations
  - Identification of insecticide-resistant forms and populations of blackflies
  - Tailoring of larvicide formulation and delivery according to river conditions
  - Application strategies to reduce the environmental impact of larvicides.

- Study the feasibility of larviciding in isolated foci in addition to those already chosen by the African Programme for Onchocerciasis Control (APOC) (e.g. Yemen).

- Conduct a cost analysis of nuisance biting, allergenic manifestations and the veterinary significance of blackflies:

Blackflies can induce allergic reactions in humans due to their toxin-containing bites, and their harrying behaviour causes great annoyance and decreased labour efficiency. Blackfly bites also cause reactions and occasional deaths in domestic animals.

Research to improve ivermectin-use strategies

- Field studies of the effects of ivermectin on transmission, including vector parameters
- Taxonomic (including molecular) tools for entomological monitoring of ivermectin programmes
- Modelling to incorporate aspects of the biology of different vectors into existing and new models of onchocerciasis epidemiology.

Research into new control strategies

- Expressed sequence tags (ESTs).

The ability to manipulate the blackfly genome offers potential for the development of new genetic methods of vector control, in the same way as described for the mosquito genome and malaria. Almost no data concerning the blackfly genome exist, and hence it is not possible at present to identify potentially useful genes and transformation vectors. The first stage towards addressing this problem would be an EST project. Such a project would have important spin-offs in identifying a large number of genes for field biology (in terms of population genomics and phylogenomics) to study population structure, behavioural ecology, epidemiology, etc. The inability to establish colonies of onchocerciasis vectors in the laboratory will not hinder EST studies because experimental replication can be achieved by archiving large subsamples of material collected in the field.

- Investigate the development of new methods of trapping and of personal protection.

Greater understanding of the way in which blackflies respond to host stimuli, and the identification of semiochemicals, could lead to the development of new tools for sampling and control of blackfly populations.

- Blackfly population studies.

All methods of onchocerciasis control require a thorough understanding of blackfly population biology, including population subdivision, reinvasion and changes in vector distribution. In particular:

  - Taxonomic (including molecular) markers for the identification of migrants and studies of the vector competence of migrants
  - Species replacement to prevent re-invasion (“competitive exclusion”)
  - Factors determining species geographic limits and their vectorial significance.

RECOMMENDATIONS FOR CAPACITY AND PARTNERSHIP BUILDING

(see pages 11–13)

Table 7 – **SWG prioritization of needed research on onchocerciasis prevention and control**

	Research for which TDR has a comparative advantage	Activities most appropriate for other partners
High priority	**Vector studies relevant to improvement of ivermectin strategies** • Field studies on the effects of ivermectin on parasite transmission, including vector parameters • Modelling to incorporate aspects of the biology of different vectors into existing and novel models of onchocerciasis epidemiology **Research for new control strategies** • Study of the black fly genome by EST mapping • Discovery of genes relevant to field biology studies: population structure, behavioural ecology, epidemiology, etc. • Identification of semiochemicals suitable for development of new tools for the sampling and control of blackflies • Development of new trapping and personal protection methods **Blackfly population studies** • Taxonomic (including molecular) markers for identification of migrants and studies of their vector competence • Factors determining species distribution geographic limits and their vectorial significance	**Larvicidal control of blackflies** ***Improvements of larviciding agents*** • Development of new and improvement of carry distance of available formulations of larvicides • Formulation and delivery of insecticides according to river condition • Larviciding application strategies to reduce environmental impact • Identification of insecticide resistant forms and populations of blackflies ***Optimization of larviciding in isolated foci in the area of the African Programme for Onchocerciasis Control***
Medium priority	**Research to improve ivermectin strategies** Taxonomic (including molecular) tools for entomological monitoring of ivermectin programmes	**Larvicidal control of blackflies** ***Cost analysis of nuisance biting, allergenic manifestations and veterinary significance of blackflies*** Blackflies can induce allergic manifestations in humans due to their bites and toxins and their harrying behaviour causes great annoyance and decreased labour efficiency. They also cause reactions and occasional deaths in domestic animals.

Annex 1

AGENDA: Scientific Working Group on Insect Disease Vectors and Human Health

WHO/HQ, Geneva, Switzerland, 12–16 August 2002

Day 1 (12 August)		
09.00 – 09.15 09.15 – 09.30	Welcome address TDR research strategy	Dr C. Morel, Director, TDR Dr C. Morel, Director, TDR
09.30 – 09.40	Overview of planned activities of the SWG meeting	Dr Y. Touré
09.40 – 10.30	Overview of TDR disease vector research	Dr B. Dobrokhotov (30 min + 20 min discussions)
10.30 – 10.55	Current strategy for vector research in NIAID/NIH:	Dr K. Aultman (15 min + 10 min)
10.55 – 11.15	Coffee	
Issues and challenges for vector research		
11.15–12.05	Genetic control of disease vectors	Dr F. Collins (30 min + 20 min discussions)
12.05- 12.55	Chemical control and insecticide resistance	Dr J. Hemingway (30 min + 20 min discussions)
12.55 – 14.00	*Lunch*	
14.00 – 14.50	Environmental management for vector control	Dr R. Bos (30 min + 20 min discussions)
14.50 – 15.40	Biological control of vectors	Dr N. Becker (30 min + 20 min discussions)
15.40 – 16.00	*Coffee*	
16.00 – 16.50	Social and economical implications for vector control	Dr K.D. Ramaiah (30 min + 20 min)
16.50 – 17.40	Ethical, legal and social issues (ELSI) for the use of GM vectors for disease control	Dr D. Macer (30 min + 20 min)

Day 2 (13 August)		
Disease vector control specific issues		
09.00 – 09.30	Malaria vector control	Dr M. Coetzee (20 min + 10 min)
09.30 – 10.00	Dengue vector control	Dr U. Thavara (20 min + 10 min)
10.00 – 10.30	African trypanosomiasis vector control	Dr J. Ndung'u (20 min + 10 min)
10.30 – 11.00	*Coffee*	
11.00 – 11.30	Chagas disease vector control	Dr E. Garcia (20 min + 10 min)
11.30 – 12.00	Leishmaniasis vector control	Dr A.Warburg (20 min + 10 min)
12.00 – 12.30	Lymphatic filariasis vector control	Dr P.K. Das (20 min + 10 min)
12.30 – 14.00	*Lunch*	

14.00 – 14.30	Onchocerciasis vector control	Dr M. Wilson (20 min + 10 min)
14.30 – 14.40	Introduction of Working Group Strategy	Dr Y. Touré (10 min)
14.40 – 17.30	Working groups I, II, III, IV	**Salle B, M205, M505,M605**

Day 3 (14 August)		
09.00 – 10.30	Working groups: continued	
10.30 – 11.00	*Coffee*	
11.00 – 12.30	Working groups: continued	
12.30 – 14.00	*Lunch*	
14.00 – 14.40	Plenary report Working Group I	WG rapporteur (20min + 20min)
14.40 – 15.20	Plenary report Working Group II	WG rapporteur(20min + 20 min)
15.20 – 16.00	*Coffee*	
16.00 – 16.40	Plenary report Working Group III	WG rapporteur (20min + 20min)
16.40 – 17.20	Plenary report Working Group IV	WG rapporteur (20min + 20min)

Day 4 (14 August)		
09.00 – 10.30	Finalization of working group reports and setting of priorities for TDR research agenda	All WGs
	Drafting of SWG meeting report by plenary rapporteurs	SWG chairperson/rapporteurs
10.30 – 11.00	*Coffee*	
11.00 – 12.30	Drafting of SWG meeting report by plenary rapporteurs Distribution of draft report to meeting participants	SWG chairperson/rapporteurs
12.30 – 14.00	*Lunch*	
14.00 – 15.30	Plenary presentation, discussion and amendment of the SWG draft report	Chair, rapporteurs
15.30 – 16.00	*Coffee*	
16.00 – 16.30	Continuation of discussion and amendment of the SWG draft report Any other business	
16.30 – 17.00	Concluding remarks	-Dr. M. Mulla, Chairperson -Dr. A. Oduola TDR/STR

Day 5 (16 August)		
09.00 – 10.30	Finalization of SWG report	Chair, rapporteurs/consultant
10.30 – 11.00	*Coffee*	
11.00 – 12.30	Finalization of SWG report	Chair, rapporteurs/consultant
12.30 – 14.00	*Lunch*	
14.00 – 15.30	Finalization of SWG report	Chair, rapporteurs/consultant
15.30 – 16.00	*Coffee*	
16.00 – 17.30	Finalization of SWG report	Chair, rapporteurs/consultant

Annex 2

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Annex 3

Disease vector control: development and implementation of control methods

Paper for the WHO/TDR Scientific Working Group on Insect Vectors and Human Health, Geneva, Switzerland, 12–16 August 2002

DISEASE VECTOR CONTROL: DEVELOPMENT AND IMPLEMENTATION OF CONTROL METHODS

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1. THE BURDEN OF DISEASE ASSOCIATED WITH ARTHROPODS

It is appropriate to direct resources for disease control after consideration of the overall burden due to each disease. Disability Adjusted Life Years (DALYs) are used in an attempt to express (albeit imperfectly) the burden of deaths and of disability associated with a disease (World Bank, 1993). The cost of reducing the burden of each disease should also be considered, and careful note taken of regional variations in the relative burden of each disease, especially where the resources to be used for control are regional, national or local.

Curtis & Davies (2001) produced a table based on data on the WHO website on worldwide loss of DALYs for each of the major arthropod-related human diseases. Their table included the diseases whose pathogens have a biological process of development inside insect vectors and which constitute most of the WHO/TDR list of diseases.

The table of Curtis & Davies (2001) also included diarrhoeal diseases, which are the largest killer of children, trachoma, which is the largest cause of preventable blindness, and asthma, which is a growing problem among children in developed countries. It has long been thought that synanthropic flies (*Musca domestica* and *M. sorbens*) had a role in the mechanical transmission of the pathogens responsible for diarrhoeal diseases and trachoma. This has recently been proved in the most practical way by two trials investigating the effects of fly control using deltamethrin space spraying. Both trials showed a reduction of about 24% in incidence of diarrhoea in children; one of the trials also showed a 60-70% reduction in the incidence and prevalence of trachoma (Chavasse et al., 2000; Emerson et al., 2000). The etiology of asthma is complex, but allergy to the faeces of house dust mites (*Dermatophagoides* spp.) is certainly involved (Løvik et al., 1998) and a 30% reduction in the burden of asthma is estimated to be possible, if complete control of house dust mite allergy could be achieved, e.g. using the presently very encouraging method of permethrin-treated mattress covers (Cameron & Hill, 2002).

Of all the vector-borne diseases, malaria causes by far the largest worldwide disease burden, largely because of its huge impact in rural Africa. It may be noted that the worldwide burden in terms of DALYs due to malaria is about 80 times that due to dengue. This should be taken into account when allotting international donor funding for research or control. However, in some relatively affluent parts of the tropics, such as Singapore and the coastal cities of Brazil, dengue is perceived as much more locally important than malaria. Local funding would understandably be allotted accordingly. It is also noteworthy that, according to WHO figures in the 1990s, visceral leishmaniasis (*kala azar*) caused more loss of DALYs in India than did malaria. After malaria, diarrhoeal diseases and asthma have prominent positions in the worldwide rating of the potential achievements of control of the relevant arthropods (Curtis & Davies, 2001). This emphasizes the urgency of developing, fully evaluating and applying more sustainable fly-control methods than the space spraying used in the above mentioned trials, e.g. fly traps (Cohen, 1991), ventilated improved pit latrines (Morgan, 1977) and improved solid waste disposal. Pursuing effective control of house dust mites should also be encouraged, using methods such as the treated mattress covers mentioned above.

2. INDOOR RESIDUAL SPRAYING OF INSECTICIDES

2.1. DDT

2.1.1. Anophelines and malaria

Malaria incidence in India was reduced in the 1950s and 1960s by more than 99.8% (from about 75 million cases per year to about 100 000) primarily by indoor residual spraying with dichloro-diphenyl-trichloro-ethane (DDT). At about the same time in Zanzibar, Tanzania, this method achieved a far greater reduction in malaria prevalence than in any of the recent trials with insecticide-treated bednets (see review by Curtis and Mnzava, 2000). In both India and Zanzibar in more recent times, results obtained with DDT have been much worse than in the past. This is presumably at least partly because of the development of resistance to DDT in the insect vectors in parts of India and in Zanzibar, but DDT still has an impact by driving resistant mosquitoes out of houses because it acts as an irritant (Sharma et al., 1982; Roberts et al., 1997). Apart from the effects of resistance, it also appears that operational spray teams are less highly motivated to achieve adequate rates of household coverage than in the past. In India, a DDT dose of only 1 g/m2 is used instead of the WHO recommended dose of 2 g/m2, but no improvement in results was seen in an area in India where vectors are resistant to DDT when the dose was increased from 1 to 2 g/m2 (Sharma et al., 1982).

In the 1990s, DDT spraying was associated with the termination of the disastrous malaria epidemic which is estimated to have caused about 40 000 deaths in the highlands of Madagascar (Romi et al., 2002). Switching back to DDT spraying was also associated with a turn-around of the malaria situation in South Africa. It is remarkable that after 50 years of successful use of this insecticide in South Africa, no resistance had evolved in the vectors but, under pressure from environmentalists, a switch was made to pyrethroid spraying in 1996. Within four years, recorded malaria cases had increased four-fold and *Anopheles funestus* with resistance to pyrethroids, but not DDT, had appeared in the country (Hargreaves et al., 2000). A switch back to DDT spraying in 2001 has been associated with complete elimination of mosquitoes exiting from sprayed houses and with a 91% decline in malaria cases (South African Dept of Health. 2003). High levels of resistance of *P. falciparum* to sulfadoxine-pyrimethamine appeared, and a switch to artesunate as a first-line antimalarial drug was made at about the same time as the switch back to DDT; it would be of great interest to distinguish the relative impacts of these two changes. DDT is only sprayed where there are mud houses and not those with plastered walls, where the visible deposits of 2 g DDT/m2 are considered to be intolerable by householders. It would be of interest to examine the details of available data to determine whether malaria is now better controlled among people living in mud houses where DDT is sprayed, compared with communities predominantly with houses with plastered walls and without DDT spraying.

Many otherwise well-informed people believe that Rachel Carson, in her book *Silent spring*, proved that DDT, as used against malaria vectors, was harmful to non-target organisms in the environment or to humans. However, all reports about environmental harm due to DDT date from the days of massive outdoor use in agriculture, in contrast to indoor use against *Anopheles* and, in India, against endophilic *Phlebotomus*. In India, DDE (an impurity in DDT as well as a biodegradation product of DDT) residues, have been detected in soil in areas where indoor spraying takes place (Dua et al., 1996), but there is no evidence as to whether the measured levels are harmful. With regard to possible effects on humans, it has not proved possible to duplicate claims about carcinogenicity (Key & Reeves, 1994; Smith, A.G., 2000; 2001). However, a recent report of a highly significant association of the concentration of DDE in sera of mothers giving birth to premature and underweight babies seems more substantial (Longnecker et al., 2001). The serum samples from the USA examined in this study had been stored since the 1950s-60s when DDT was extensively used there in agriculture and therefore had contaminated food. However, it is disquieting that DDT derivatives in breast milk have been associated with antimalaria spraying in South Africa (Bouwman et al., 1990). There is no direct evidence that such residues are harmful, but it is desirable that a study should be made of any potential harmful effects, e.g. on premature and underweight births. If there are any harmful effects of DDT on infant survival they are certainly outweighed by the major benefits to maternal health and infant survival due to suppression/eradication of hyperendemic malaria, as demonstrated by the DDT spraying programmes in Guyana (Giglioli, 1972) and Sri Lanka (Abeykoon, 1995). It has been suggested that the use of DDT is being phased out and therefore studies of possible toxic side effects are redundant. However, at the final round of negotiations of the International Convention on Persistent Organic Pollutants in December 2000, approximately 150 national delegations agreed *nem. con.* to an amendment which allows the continued use of DDT for vector control, provided that WHO guidelines are followed. Thus further research of any possible harmful effects is to be recommended. This is especially so if indoor residual spraying is to be revived in African countries, which have by far the world's largest malaria burden but which, in recent decades, have carried out little or no organized vector control. The same applies to Latin America where, since the 1970s, there has been a resurgence of malaria as DDT spraying has declined (Roberts, 1997).

2.1.2 Phlebotomines and leishmaniasis

When DDT was very extensively used against malaria in India, it had the unforeseen effect of eliminating visceral leishmaniasis which in India is carried by the endophilic species *Phlebotomus argentipes* (Mukhopadhyay et al., 1996). A considerable fraction of India's production of DDT is used to control these sandflies, which is appropriate considering the above mentioned importance of leishmaniasis versus malaria in India.

2.2 Other insecticides for indoor residual spraying

2.2.1 *Anophelines and malaria*

Because many authorities believe that DDT is too irritant to be optimally effective, or it is ineffective due to resistance, or it is environmentally harmful or even is illegal, there has been a trend to opt for spraying with organophosphates, carbamates or pyrethroids. Use of these agents is more expensive per house protected per year than DDT. However, there are surprisingly few comparative data on the cost–effectiveness of alternatives to DDT (Sharp et al., 1994; Doke et al., 2001). This question ought to be thoroughly investigated for all important vectors. Insecticides which were discovered by the pesticide industry, but have not been developed for the agriculture market, seem to be a source of candidates for vector control which would not require the huge expense of developing new compounds from scratch (Zaim and Guillet, 2002). The organophosphate chlorpyrifos methyl, which has low mammalian toxicity, has been approved by WHO Pesticide Evaluation Scheme for house spraying, and more compounds with novel modes of action are currently under investigation. Tests against Indian leishmaniasis vectors (Sanyal et al., 1979) should be included especially if, as seems likely, India is to reduce reliance on DDT.

2.2.2 *Triatomines and American trypanosomiasis*

Deltamethrin indoor residual spraying has been the mainstay of the Southern Cone Initiative against Chagas disease transmitted by domestic *Triatoma infestans*. This well-organized programme has been spectacularly effective, apparently because deltamethrin is sufficiently irritant to drive the triatomines out of crevices in mud walls and thatch roofs and kill them (Schofield, 2001; WHO, 2002a). In many areas, a single round of spraying has eradicated the vector population. However, in Venezuela, Colombia and Ecuador there are thought to be sylvatic, as well as domestic, populations of *Rhodnius* spp. In such cases, careful monitoring for possible re-infestation from sylvatic populations is required and a re-spraying capacity needs to be available in such cases.

2.2.3 *Evidence-based switching or pre-planned rotation of insecticides*

Where there is clear evidence that resistance to one compound is interfering with effectiveness of spraying, any reasonably well-organized vector control organization should be in a position to make a switch to an unrelated chemical which has been proved to be safe and to work better against the resistant population. The possibility of switching back to the previously-used chemical, if natural selection eventually lowers the frequency of the resistance gene(s) in the population, should be retained. There has been no field test of whether such a policy of evidence-based switching between insecticides would reduce the time before double resistance arises, compared with a pre-planned short-term (e.g. annual) rotation between the compounds. Simple population genetic models suggest that there would be no long-term difference in the "lifetime" of these two strategies (Curtis et al., 1993). However, one can make speculative models, with genes which can be selected to modify and normalize the fitness of resistance genes, which do suggest differences between these two approaches; it seems important to clarify this question.

3. SPACE SPRAYING OF INSECTICIDES

3.1 Anophelines and malaria

Space spraying against flies using a modern, water-based deltamethrin formulation was mentioned above as a highly effective means adopted to suppress fly populations in trials proving that diarrhoea and trachoma incidence could be reduced by fly control. Space spraying is used in a few places against malaria vectors, but this seems very unlikely to be cost-effective. In some other areas, motorized space-spraying equipment emitting droplets of relatively large size is used to apply residues to walls, and this appears to have advantages of increased speed per house covered, compared with conventional compression sprayers.

3.2 *Aedes* and dengue fever

Trials in South-East Asia (e.g. Pant et al., 1971) suggested that, in the event of a dengue fever epidemic, outdoor space spraying would be an appropriate response, with the aim of rapidly killing *Aedes* which had already emerged from the aquatic stages and some of which would already be virus-infected. However, there are doubts about the effectiveness of outdoor or aerial space spraying in Latin American or Caribbean conditions, where houses are relatively enclosed and aerosols would be unlikely to drift indoors and encounter endophilic *Aedes* (e.g. Castle et al., 1999). Reiter and Nathan (2001) emphasized the need for locally-collected evidence on effectiveness before space spraying is adopted.

3.3 Tsetse and African trypanosomiasis

Aerial spraying has long been used against the tsetse fly vectors of African trypanosomiasis, but seemed to be undergoing replacement by use of tsetse traps (section 5.1). However, Allsopp (2002) has recently reported from the Okavango delta in Botswana that in high rainfall conditions it became impossible to gain access to many traps to repair and re-treat them. Under these conditions, use of aerial spraying was renewed. With modern navigational techniques and with due attention to a cycle of spraying designed to kill emergent female flies before they reach an age at which they could deposit their first larvae, elimination of the vector population could be achieved at an affordable cost.

4. INSECTICIDE-TREATED NETS AND OTHER TEXTILES

4.1 Mosquitoes, malaria and lymphatic filariasis

Insecticide treatment of bednets (ITNs) or eaves curtains, rather than spraying of walls and ceilings, seems a more targeted way of ensuring contact with night-feeding human-biting vectors (*Anopheles*, some triatomines, *Culex* and some phlebotomines) than does house spraying. It is expected that such treatment will make nets, which are an imperfect physical barrier, into a better form of personal protection. Where a whole community is using treated nets they may divert vectors which are not exclusively human biters to bite animals and hence to reduce their chances of acquiring human pathogens. Diversion to dogs has been observed with *Anopheles farauti* (Charlwood and Graves, 1987), one of the malaria vectors in Papua New Guinea, and to birds with *Culex quinquefasciatus*, one of the vectors of lymphatic filariasis in East Africa (Bøgh et al., 1998). This diversion was associated with a reduction in the annual transmission potential of *Wuchereria bancrofti* (Mukoko et al. cited in Bøgh et al., 1998). It remains to be seen, by prolonged widespread use of treated nets, whether this form of vector control can supplement the effect of antifilarial drugs in elimination of lymphatic filariasis as a public health problem, as has been shown for *Culex* larval control (see section 7.3).

Community-wide use of pyrethroid-treated nets is also expected to kill large numbers of susceptible vectors which have been attracted to the "baited trap" constituted by the insecticidal net with human odour emitted by its occupant. The mass killing of vectors, where a whole community is using treated nets, is expected to lower vector population density and survival. Hence the proportion of vectors surviving long enough for parasites to mature to the infective stage is decreased. Such effects on *Anopheles gambiae s.s.* and *Anopheles funestus* have been unequivocally and repeatedly seen with regard to malaria sporozoites, for example in Tanzania (Magesa et al., 1991; Curtis et al., 1998a; Maxwell et al., 1999a), and with regard to *W. bancrofti* L3 larvae in Kenya (Mukoko et al., cited in Bøgh et al., 1998). However, *Cx. quinquefasciatus* is less susceptible to the insecticide and only personal protection of net users and diversion to bird biting, but no mass killing, have been observed with this species. With regard to malaria vectors in, for example, The Gambia, treatment of nets in some villages, but not in other nearby ones, did not result in a clear-cut difference in vector density and survival between villages. This was presumably because of dispersal between treated and untreated villages from the riverine breeding sites used by the anophelines from all these villages (Quiñones et al., 1998).

In The Gambia, the impact of treated nets on malaria morbidity and mortality has been clearly demonstrated (e.g. Alonso et al., 1991) and, in view of the results mentioned above, the benefits apparently resulted from the improved personal protection provided by treatment of nets. Near Ifakara, Tanzania, reductions in morbidity and mortality have been reported in households who have paid for nets and insecticide, compared with those in the same villages who have not (Abdulla et al., 2001; Armstrong-Schellenberg et al., 2002). However, in villages near Muheza, Tanzania, where treated nets have been provided free of charge and widely used and re-treated annually for three to four years, impacts on malaria fever, anaemia and splenomegaly were significant even in individual children who no longer had nets in these villages, compared with nearby villages with no nets (Maxwell et al., 2002). This indicates an important contribution from the mass effect of these nets. Data were collected in a large trial near Kisumu, Kenya (Hawley et al., 2003) on morbidity and mortality along transects across the dividing line between zones with and without treated nets. This gave further evidence for the importance to people without nets of the mass effect of near neighbours with nets, who are killing some vectors for them.

Clarification of the relative importance of the personal protection and the mass effects of treated nets is relevant to the extent to which high community coverage is required to achieve the full potential of treated nets. Some authorities believe that it will only be possible to afford to protect children from malaria with nets and insecticidal treatment if their parents can be persuaded to pay (WHO, 2002b). Even the best of such social marketing projects have achieved disappointing rates of net treatment, whereas organized free provision in relatively few villages in Africa (Curtis & Maxwell, 2002) easily and quickly achieves high coverage rates. On a national scale in Viet Nam, the introduction during the 1990s of organized free net-treatment (Ettling, 2002), together with widespread use of artesunate, has been associated with a major national decline in malaria incidence. This is held up by WHO as one of the few recent national-scale success stories against malaria. The arguments in favour of organized free provision of insecticide-treated nets in rural areas, rather than attempting to market them, are presented by Curtis et al. (2003). Particular emphasis was given to the higher coverage with effectively treated nets and the higher productivity, and hence lower distribution costs, of free distribution compared with data on Social Marketing (Hanson et al, 2003).

To clarify the question of personal versus mass effects, it is proposed that incidence of infection should be measured firstly in cohorts of young children in several villages and who are provided with treated nets. The measurement would be repeated in the same cohort after everyone has been provided with nets. If protection against infection is much greater in the latter case, it would indicate that it would be in the interests of those who are able to or choose to pay for nets and insecticide, to ensure that everyone is provided for on a communal basis. This would prevent those who are not able and willing to buy treated nets from providing infective blood meals to mosquitoes, which could then find opportunities to bite and infect even those who have treated nets.

Companies have developed and patented methods which are claimed to make nets permanently insecticidal despite repeated washing. The “Olyset” net has remained effective for several years in domestic use (Curtis et al., 1996; N’Guessan et al., 2001) but is reported to be much more expensive than a conventional net. Another product (“Permanet”) is at present quite cheap but results have been mixed in tests of wash resistance after up to 20 washes, and prolonged domestic use of the versions which are now on the market, compared with conventionally-treated nets (e.g. Curtis, 2001; Gonzalez et al., 2002; Müller et al., 2002). It appears that there have been problems of quality control and it is important that these are resolved. Widespread availability of long-lasting insecticidal nets at little extra cost compared to existing nets would be quite helpful to programmes based on free provision through the public health system. They would be even more important to those who feel that they must charge for treated nets but have found it impossible to get large numbers of people to pay for insecticide for re-treatment.

Apart from some small-scale trials, all net treatments have so far been made with pyrethroids and there has been much concern about the threat of pyrethroid resistance to continued success of the method (e.g. Curtis et al., 1998b). In Côte d’Ivoire, where there is a high frequency of pyrethroid resistance due to the *kdr* resistance gene in *Anopheles gambiae* Savannah form, lambdacyhalothrin-treated nets still had an impact on malaria and a mass effect on the density and sporozoite rate in the vector population (Carnevale, 2001). In experimental huts, the impact on mosquitoes was at least as high where the *kdr* frequency was very high as when it was more moderate (Darriet et al., 2000). It is known that pyrethroid resistance causes reduced irritability as well as reduced susceptibility to being killed, and it is thought that where the mosquitoes are resistant they rest for a long period on the treated nets and eventually pick up a lethal dose. It is important to study the applicability of this idea to metabolic forms of pyrethroid resistance (such as that mentioned in section 2.1.1 regarding South African *Anopheles funestus*) and of the super-*kdr* type of resistance which is known in house flies (Sawicki, 1978) and which could presumably arise in mosquitoes. Meanwhile studies have been or are now going on with non-pyrethroid alternatives on nets, such as pirimphos methyl (Miller et al., 1991), carbosulfan (Kolaczinski et al., 2000; Guillet et al., 2001) and chlorpyrifos methyl. Some of the results already obtained suggest that, quite apart from responding to or forestalling a resistance crisis, appropriate non-pyrethroids may kill more mosquitoes and therefore have a better mass effect with widespread use than has been experienced with pyrethroid treated nets. This is important, as the WHO-sponsored trials in the 1990s of treated nets and their impact on mortality and malaria morbidity (Lengeler, 1998), in comparison with carefully-monitored trials of indoor residual spraying in Africa in the 1950s, 60s and 70s, show that results with the treated nets were distinctly inferior (Curtis & Mnzava, 2000). It may require net treatments which are less irritant, but which kill vectors, rather than merely driving them from one person to another, to bring community-wide malaria vector control up to the level of effectiveness that was achieved 30-45 years ago.

It may be that there is less of an immediate threat to successful use of ITNs from pyrethroid resistance in *Anopheles* than in *Cimex* bedbugs. Such resistance has been detected in each of five Tanzanian villages in which ITNs have been used for seven years, in contrast to each of five nearby villages without ITNs (Myamba et al., 2002). People in the netted villages are complaining that, whereas at the outset the ITNs eradicated the bedbug population, the bedbugs are back in force. This may reduce the very high rate of re-impregnation of nets which have regularly been achieved in these villages. The addition of chlorpyrifos methyl to nets and its impact on the pyrethroid-resistant bedbugs is being studied.

4.2 Phlebotomines and leishmaniasis, triatomines and American trypanosomiasis

Trials with pyrethroid-treated nets have been carried out against endophagic phlebotomine vectors of leishmaniasis in Colombia (Alexander et al., 1995) and Sudan (El Naiem et al., 1995). In Kabul, Afghanistan, treated nets were shown to have an impact on leishmaniasis comparable to that of house spraying and other ways of deploying insecticides – see section 5.2 (Reyburn et al., 2000).

Treated nets repel or kill domestic night-biting triatomines under experimental conditions. A proposal was made for a trial of treated nets against American trypanosomiasis; unfortunately it was refused on the grounds that it would divert attention from establishing indoor residual spraying throughout the area where this disease is a problem. The wisdom of this decision could be questioned since, especially in remote areas, the relative lack of training and resources required for a community to run a treated net programme against any domestic night-biting vector, compared with an indoor residual spraying programme against the same vector, could make the difference between feasibility and unfeasibility.

4.3 Treated curtains and tents

In Burkina Faso, where houses are very small, it has been decided that it is necessary to use eaves and door curtains as the surface for treatment rather than bednets against vectors of malaria (Habluetzel et al., 1997) and leishmaniasis (Majori et al., 1987). These curtains are rather difficult to take down for re-treatment. A direct comparison of treated nets with curtains in experimental huts showed that the former were much more effective (Curtis et al., 1996). In a trial in Burkina Faso, permethrin-treated curtains did not significantly reduce the numbers of *Anopheles gambiae* entering houses, compared with untreated curtains, but carbosulfan-treated curtains did produce a significant reduction (Fanello et al., 2001). For this study, nets were provided to use in conjunction with mosquito-monitoring light traps. Villagers were very pleased to receive the nets and had no problems in fitting them into their houses. It may be that the decision to favour curtains over nets should be re-visited.

In refugee camps, epidemics of vector-borne disease often occur because of high population densities, lack of immunity in people arriving in areas of high endemicity from areas of low endemicity, and living in tents which give easier access to insects than do houses. Studies are in progress of means of treating plastic tent material with insecticide deposits so that they are as persistent as they are on polyester netting (Hewitt et al., 1995).

5. TRAPS AND TREATMENT OF LIVESTOCK, ANIMAL COLLARS AND HUMAN CLOTHING

5.1 Tsetse flies and African trypanosomiasis

Apart from house flies (see section 1), tsetse flies are the only vectors for which trapping has been extensively practised as a control (not just a monitoring) technique (Lavassière et al., 1990). The traps may aim to catch and confine the flies until they die in the midday sun or they may be insecticidally treated and as structurally simple as an appropriately coloured screen. The problems sometimes encountered in sustaining insecticidal re-treatment of these traps in remote areas have been mentioned in section 3.3. For the *Glossina palpalis* group, which are the principal vectors of the *gambiense* form of human sleeping sickness, visual stimuli of appropriate colour and shape are used to attract the flies into the traps. For the *G. morsitans* group, which are the principal vectors of the *rhodesiense* form of sleeping sickness as well as of animal trypanosomiasis, odour baits have been developed.

In the belt of land extending from Angola through the Democratic Republic of Congo to Sudan, where the population has been disastrously affected by prolonged civil wars, human sleeping sickness is infecting huge numbers of people and killing most of those infected because drug treatments are difficult to obtain, expensive, and have dangerous side-effects. Some charities are making available kits which communities could use to make their own tsetse traps, especially in such disaster areas. These efforts ought to be given every encouragement and assistance by medical entomologists.

For zoophilic tsetse flies, pyrethroid treatment may be effectively applied to livestock (e.g. Fox et al. 1993). Where the livestock have to be rounded up at frequent intervals to dip, to protect them from tick-borne diseases, this can be a cost-effective method.

5.2 Anophelines and malaria, and phlebotomines and leishmaniasis

In Pakistan, where *Anopheles culicifacies* malaria vectors are predominantly zoophilic, it has been found possible to have a major impact on malaria transmission by pyrethroid sponging of cattle owned by small-scale farmers (Rowland et al., 2001). The side-effect of this in killing ticks and improving the yield from the cattle made the procedure very popular with the farmers.

It is now planned to test this method against *Anopheles arabiensis*, the malaria vector in Tanzania and which is opportunistic in its feeding. In Ethiopia, the joint use of livestock dipping against tsetse and *Anopheles arabiensis* is being studied (Habtewold et al., 2002).

Dogs are important reservoir hosts of several species of *Leishmania*. Attempts have been made, with uncertain success, to carry out dog culling programmes (Tesh, 1995). A more promising idea seems to be to provide pyrethroid-impregnated dog collars, from which the insecticide diffuses across the dog's skin and kills or prevents feeding by sandfly vectors, thus greatly reducing the likelihood of their transmitting the parasites from the dogs to humans (Killick-Kendrick, 1997; Gavgani et al., 2002).

Pyrethroids of low toxicity and relatively low irritancy to humans can be applied to human clothing or bedclothes (Rowland et al., 1999; Reyburn et al., 2000). In Pakistan and Afghanistan, where *chaddars* (Islamic scarves) are universally worn by women in daytime and used as bedclothes by both sexes at night, results with pyrethroid treatment against malaria and leishmaniaisis were as good as with treated bednets. Moreover, less insecticide is required because the area to be treated is smaller.

Treatment of army uniforms with permethrin had a significant impact on both malaria and leishmaniasis in Colombian soldiers patrolling in the jungle (Soto et al., 1995).

These two examples refer to clothing of types which, for different reasons, are universally used by particular communities. It remains to be seen whether this method would be effective where choice of clothing is not so circumscribed and where clothing is washed frequently.

6. REPELLENTS

Synthetic repellents, such as DEET (N,N-diethyl-meta-toluamide) and hydroxyethyl isobutyl piperidine carboxylate, are more or less effective against all haematophagous insects for someone who has applied the substance to all exposed surfaces, including socks through which insects can bite. Much emphasis is placed on duration of protection, but this tends to be exaggerated in the labelling of some of the proprietary products. For people who will only be exposed to biting for a short period in the evening before getting under a net, one application would be enough but, where someone is to be exposed to biting for many hours, re-applications would be necessary. There has been much discussion about the toxicity of DEET. However, when the number of reported cases is compared with the very large number of containers of DEET sold, it is found that the frequency of adverse effects is comparable to that of household products such as bleach (Veltri et al., 1994). Apart from possible human toxicity, DEET can damage hard plastics such as watch faces, if incautiously used. A proprietary repellent based on p-methane diol from the plant *Eucalyptus maculata citriodon* has been shown to be as effective and persistent as DEET (Trigg, 1996) and has been successfully commercialized.

Repellents are mainly used to counteract a biting nuisance, but tourists are frequently advised to use them to reduce the risk of insect-borne disease. Presumably they do reduce these risks, but almost no quantitative evidence for this has been collected. Recently, however, a just significant impact of soap containing DEET and permethrin was reported from refugee camps in Pakistan (Rowland et al., in press).

There are many reports by ethnobotanists of plants which are used by villagers as repellents on the skin or to prevent biting insects from entering rooms. It is often suggested that these are a virtually cost-free means of controlling insect-borne disease. However, in order to investigate this idea, three questions need to be asked:

(i) Do the plants actually repel insects and are their effects persistent for long enough to be feasible for routine use and competitive with commercial products?
(ii) Do they work well enough to reduce disease risks to the user?
(iii) Do the plants simply divert the biting from a user to a non-user, and would an insecticide therefore be better for the community as a whole?

Question (i) can be investigated for skin repellents by biting catches with non-infective insects, or insect traps for testing plants which are claimed to repel insects from rooms. Question (ii) requires careful studies of disease incidence in users and non-users, of a kind that few have been willing to attempt so far. It is suggested that question (iii) could best be approached using the bednet traps developed by Mathenge et al. (2002) used in neighbouring houses with a rota of use of the plant in one or other house, or neither, on different nights. It would be hoped that the repellent would divert opportunistically-feeding vector species not to other humans but to nearby animals. This possibility could be investigated by net traps over the tethered animals.

7. LARVAL CONTROL

7.1 Source reduction

Larval control has always been the principal method of controlling culicine mosquitoes and has application in certain circumstances against anophelines. Ideally, source reduction can make a major contribution by:

(a) clearing garbage which retains rainwater or placing lids on pots in which *Aedes aegypti* breeds;
(b) clearing blockages in open drains which provide stagnant polluted water used by *Cx. quinquefasciatus* (Rajagopalan et al., 1990);
(c) modifying irrigation practices, as in Sichuan Province, China, where this has recently been associated with a major reduction in *Anopheles* adult populations and malaria (Gao et al, 2000; Liu et al, in press).

Innumerable demonstration trials have shown the efficacy of source reduction against culicine mosquitoes. However, making such a system work on a routine basis requires combinations of impartial enforcement of national or local laws (e.g. Chan Kai Lok, 1990) and patient health education. This could be provided especially via schools, with informal transmission of information back to parents, and by the use of advertisements whose biological accuracy has been carefully vetted by entomologists, in order to avoid the kinds of erroneous statements which are regrettably frequent in such advertisements.

7.2 Larvicides for mosquitoes

Organophosphate larvicides such as temephos in clean water, including drinking water, and chlorpyifos in polluted water have been the standard method of controlling culicine larvae. There is widespread resistance due to elevated levels of esterases in *Cx. quinquefasciatus,* including filaria vector populations (Curtis & Pasteur, 1981). This mechanism does not prevent a spraying of chlorpyrifos at the recommended dosage from killing larvae when it has been freshly applied but, as the insecticide is diluted and decays, a survivable dose is far more quickly reached with the resistant strains than with susceptible ones (Curtis et al., 1984). Resistance to temephos in *Aedes aegypti* is now being frequently detected, especially in the Caribbean area (Rawlins, 1998). It is not yet clear, however, to what extent this would interfere with effective use in the field.

7.3 Expanded polystyrene beads

Floating layers of expanded polystyrene beads in breeding sites completely surrounded by walls, such as pit latrines and soakage pits, suffocate larvae of *Cx. quinquefasciatus,* remaining effective for years unless the pit floods. It has been shown in Zanzibar, Tanzania, and in south India, that adult populations of *Cx. quinquefasciatus* can be massively reduced by a campaign of searching out and treating of all wet pits with polystyrene beads. This needs to be sustained by keeping a careful check for new or newly-wet pits, or pits from which the beads were lost by flooding (Maxwell et al., 1990; Reuben et al., 2001). In both areas, these mosquitoes were the vectors of *W. bancrofti* and, in both cases, mosquito larval control was integrated with time-limited campaigns of mass drug treatment with antifilarial drugs. Villages with the integrated control were compared with those with time-limited drug treatment alone. Whilst the drug treatment was effective so long as it continued, when it ceased in the absence of vector control, there was resurgence of infection. However, combination with vector control using polystyrene beads prevented this resurgence (Maxwell et al., 1999b; Reuben et al., 2001; Sunish et al., 2002; Curtis et al., 2002). This provides an argument that sustainable vector control to prevent re-infection of cured patients should be added to the current worldwide campaign to eliminate lymphatic filariasis as a public health problem, based on mass drug treatment. It must be admitted that there are no data yet on the outcome of keeping up the recommended annual drug treatment regimes for the time which is expected to be required for the adult worms to die out. It may be that this alone will be found to be enough to eliminate this disease. However, with modern mobile populations, sufficiently complete coverage of the human population is difficult to achieve. Also, elimination of the nuisance burden of *Cx. quinquefasciatus* greatly improves the popularity of an integrated programme.

7.4 Insect growth regulators

Insect growth regulators are synthetic compounds which operate in a more subtle way than insecticides to prevent development of the insect to the adult stage, e.g. methoprene and pyriproxyfen, which act like juvenile hormone and prevent metamorphosis. A recently reported trial in gem pits in Sri Lanka showed that malaria vector species which breed intensively in the pits could be prevented from metamorphosis. This required treatment of the pits with a low dose of a sand granule formulation only twice a year, far less frequently than was required with temephos or oiling (Yapabandara et al., 2001; 2002). Treatment of all the hundreds of pits in four gem-mining villages, leaving four neighbouring villages as untreated controls, showed a highly significant impact on the adult vector densities in the treated villages. There were also significant reductions in the prevalence of *P. falciparum* and *P. vivax* infection, as detected by mass surveys of blood, and the incidence of patients reporting to health facilities with fever plus parasitaemia. This is an exceptional case where larval control had a major impact on malaria. The key factor, apart from the ease of application of an effective dose of the chemical, appears to have been that the location of all the gem pits was well known to the villagers. Thus directing the treatments to virtually all the relevant breeding sites within flight range of the villages was relatively easily organized. Further trials have been carried out in areas where the main breeding sites are in river bed pools (Yapabandara et al, in preparation).

7.5 Biopesticides

The bacteria *Bacillus thuringiensis israelensis* (Bti) and *B. sphaericus* produce highly specific toxins which are lethal when eaten by mosquito larvae and a few other organisms. They are available commercially and are widely used, in those developed countries which forbid or limit the use of synthetic insecticides, against larvae of nuisance mosquitoes (Becker, 1997). In India, *B. sphaericus* has been used against vectors of malaria (Kumar et al., 1994). One of the advantages of Bti and *B. sphaericus* is that, because they are ineffective against agricultural pests, there is no incentive for antimalarial-spray people to try illegally to sell their supply of the toxin to farmers, unlike the case with broader spectrum insecticides.

B. sphaericus is more effective than Bti in polluted water. In a series of WHO-sponsored trials, *B. sphaericus* had an impact on *Cx. quinquefasciatus* populations (Hougard et al., 1993b), but unlike expanded polystyrene beads, it has not yet been shown to contribute usefully to control of filariasis transmission (Maxwell et al., 1999b).

There is no recorded resistance to Bti despite more than a decade of field use (Becker & Ludwig, 1993). On the other hand, *B. sphaericus* resistance has been reported in several areas (Rao et al., 1995; Silva-Filha et al., 1995; Wirth et al., 2000). The difference seems to arise from the fact that Bti contains several diverse toxins (Georghiou & Wirth, 1997) but *B. sphaericus* has only one. Thus Bti appears to have evolved a resistance-management system comparable to combination therapy used against TB, leprosy and HIV. This gives support for the idea of testing insecticide mixtures for resistance management in vectors.

7.6 Biological control

Remarkable results have been reported from Viet Nam on use of the micro-crustacean predator *Mesocyclops* to eradicate *Aedes aegypti* from certain villages (Vu Sinh Nam, 1998). This method did not require extensive rearing and introduction of the control agent from outside the community, but did require constant checking that all water-tank breeding sites had adequate populations of the predator and, where they did not, “seeding” from an infested tank. The success of this method is being extended to other parts of Viet Nam and determination of other potential locations worldwide where it could play a role is urgently necessary.

In western India, extensive areas are reported to successfully employ bio-environmental control, based partly on use of fish which consume the larvae of malaria vectors (Sharma et al., 1987). More details are awaited concerning the quantitative impact of this method on malaria incidence, in comparison with what can be achieved by indoor residual spraying.

7.7 Larval control of *Simulium*

The only feasible method of controlling the *Simulium* vectors of onchocerciasis is larval control, as the wholly exophilic adults are completely inaccessible to control. The Onchocerciasis Control Programme (OCP) in Africa, west of Nigeria, has been one of the most successful operations in the history of medical entomology (Molyneux, 1995). Unfortunately this programme has now been terminated with the hope that prevention of resurgence and elimination of the disease elsewhere, such as Nigeria, can be achieved by mass administration of the drug ivermectin alone. In Latin America, also, the emphasis for onchocerciasis control appears to be on ivermectin with little consideration to the possibility of vector control.

The OCP was based on aerial spraying of appropriate larvicides upstream of the rapids in which *S. damnosum s. l.* larvae live. It was planned to continue suppression of the vector populations for about

15 years until the *Onchocerca* infestations in humans in the area died out. In this the OCP was remarkably successful.

Initially only temephos was used for spraying, after ascertaining its effectiveness against *S. damnosum* and its relative harmlessness to non-target organisms. Before the completion of the planned period of spraying, resistance to temephos was detected in some of the vector populations. Fortunately there had been advanced planning for this possibility, and the safety and effectiveness had been ascertained of a range of alternative synthetic insecticides, as well as Bti. These were used successfully to manage the resistance problem (Hougard et al., 1993a). The system by which this was done is commonly referred to as a rotation, but it seems that it had more the character of the process of evidence-based switching between insecticides, referred to in section 2.2.3, than a pre-planned rotation. Other long-term programmes should emulate the OCP in making sure that chemically-unrelated alternatives are available and tested for safety and efficacy in advance of any resistance crisis. However, in other respects, important differences should be noted between the OCP and programmes of indoor residual spraying or net treatment, where insecticide residues are intended to remain for months, and in which incomplete kills of the target populations are inevitable. In contrast, in the OCP, insecticide residues were rapidly dispersed in the fast-flowing rivers and practically 100% insecticidal kills were achievable. Thus whilst rapid switches between different compounds were possible in the OCP, switches of insecticide would take much longer to have their full effect in other programmes. When the OCP made a switch to a new compound, the resistance genes to the previous one were completely eliminated with the killed larvae, and the new larval population was regenerated by long-distance migrant *Simulium* from fully-susceptible populations originating outside the area of resistance. In contrast in other programmes, old resistance genes are propagated from local insects which avoid the treatments and the resistance gene frequencies only decline slowly due to natural selection or, in some cases, do not decline at all for many years.

8. GENETIC CONTROL

8.1 Sterile insect technique

Until recently, the only form of genetic control of vectors which had been attempted was the sterile insect technique (SIT). This has achieved successful eradication of *Glossina austeni* from Zanzibar, Tanzania, (Msangi et al., 2000) and of the New World screw worm fly (*Cochliomyia hominovorax)* from the southern USA to Panama (Wyss, 2000). In the 1970s, there were programmes to test various forms of SIT against culicine mosquitoes in India (Curtis, 1977) and *Anopheles albimanus* in El Salvador (Dame et al., 1981). Unfortunately both of these programmes were destroyed by political troubles – their closure was not because the methods studied were failures.

There now appears to be a revival of interest based on: (a) an attempt to extend the successful eradication in Zanzibar by radiation-sterilized tsetse males to adequately isolated tsetse belts on the African mainland, (b) the new availability of transgenic *Aedes* (Jansinskiene et al., 1998) and *Anopheles* (Catteruccia et al., 2000). This might be exploited to improve on existing SIT technology via the RIDL (Release of Insects carrying a Dominant Lethal) technique (Thomas et al., 2000). This provides both improved reliability of elimination of biting females from batches of males being prepared for release, as well as non-production of female progeny from matings between released males and wild females. This sterility is produced without the need for irradiation, which may damage competitiveness, or use of mutagenic chemicals, which may not be permitted in the present time because of minute but detectable residues. If SIT is to be used, the aim should be eradication of isolated populations. The best targets for this, which have considerable human importance, would seem to be “urban islands” such as:

- *Anopheles arabiensis* in southern Nigerian cities surrounded by *Anopheles gambiae s. s.* in the surrounding rural areas (Coluzzi et al.,1979);
- *Anopheles stephensi stephensi* in Indian cities surrounded by *Anopheles culicifacies* and *Anopheles stephenis mysoreniss* in surrounding rural areas;
- *Aedes aegypti* and *Aedes albopictus* which continue to transmit dengue in Singapore despite intensive efforts to control them by source reduction and larviciding (Ooi, 2001).

8.2 Driving refractoriness into wild populations

Another possible use of transgenic mosquitoes would be the production of improved forms of refractoriness to *Plasmodium* or dengue virus, compared to those already selected by more conventional methods. Ito et al. (2002) have reported a transgenic strain of *Anopheles stephensi* which is 70-80% refractory to rodent malaria. No doubt it will be possible to produce strains refractory to *P. falciparum*. Boëtte and Koela (2002) have emphasized that, to be effective in areas of intense malaria transmission, strains with refractoriness close to 100% will be needed. Before any releases are made it will be necessary to check for any unexpected rise in susceptibility to viral or filarial pathogens. There would be little point in trying to introduce the refractoriness by mass release, as sterile males would do a more efficient job because, with SIT, the wild target population would decline in successive generations and become more readily dominated from a given production facility. A refractoriness gene would only be useful if it could be driven into a wild population from a relatively small "seeding" and could "resist" the effects of immigration, which defeats attempts to eradicate with SIT. There has so far been insufficient attention to the problem of producing a reliable driving system. The following are among the systems considered:

– Mobile autonomous transposons somewhat comparable to the P elements which spread spontaneously through the world's *Drosophila melanogaster* population in the last few decades (Kidwell and Ribeiro, 1992); it is generally considered that at present such mobile elements are too unpredictable and ill-understood to be usable for a release.
– *Wolbachia* symbionts, which are maternally transmitted and cause sterility in matings of *Wolbachia*-infected males to uninfected females, and are therefore selected in a mixed population (Turelli and Hoffmann, 1991). It has not yet been possible to introduce *Wolbachia* into *Anopheles*.
– The system of lethals and suppressors proposed by Davis et al. (2001) as a means of generating negative heterosis; it seems that this system could be produced by transgenesis in *Anopheles* or *Aedes* and seems at present the most hopeful prospect for a safe and effective driving system.

Even if a driving system can be produced, the problems are far from over – absolutely reliable linkage of the desired pathogen refractoriness gene(s) must be achieved, and there must be no back mutation to the susceptible type, otherwise the driving system detached from the refractoriness gene would probably be selected in preference to the complete system (Curtis, 1968, 2002). The first published measurements of the viability of transgenic mosquitoes showed that they had severely reduced fitness, but at least part of this was apparently due to inbreeding depression which could probably be avoided by appropriate crossing schemes (Catteruccia et al, 2003).

As a precaution against evolution of the pathogen to evade a single refractoriness gene, the inclusion of two or more independently-acting refractoriness genes should be aimed for. This increase in number of genes to be rigidly linked to the driving system further increases the difficulty (and perhaps makes impossible) the linked system that would be required. Very rare recombination repeatedly showed up as a problem when genetic sexing systems for the Mediterranean fruit fly (*Ceratitis capitata*) were expanded to the large scale in fly "factories". Eventually, a carefully planned system of purging colonies of recombinants has been introduced (Caceres, 2002), but this would be completely inapplicable to wild populations and attempts to use linked driving and refractoriness systems in them.

The present author therefore considers that the only genuinely foreseeable use of transgenic mosquitoes to improve disease control is to improve SIT by the RIDL technique applied to urban vector populations.

9. POSSIBLE "REBOUND" IN RESPONSE TO VECTOR CONTROL

For vector-borne diseases, such as malaria and dengue, where human immunity is important, it has been repeatedly suggested that the benefits of vector control, short of eradication, might be nullified because immunity levels would not build up in children born after the introduction of the vector control programme. Thus infection might be postponed until a later age, and the lifetime disease burden might be much the same, even though transmission had been made much less intense. If the effects of infection were worse at an older age, as has been suggested for cerebral malaria (e.g. Snow and Marsh, 1998) and for dengue (P. Coleman et al., in preparation), vector control could, paradoxically, make the situation worse. Some therefore consider that malaria vector control should not be attempted in the African rural lowlands (Trape et al., 2002) and that vector control should only be attempted in towns or highlands (Touré and Coluzzi, 2000). In Singapore, dengue vector control is now so effective that it is suggested that it may have contributed, paradoxically, to making the dengue disease situation worse. Only if the dengue vector populations could be sustainably eradicated (e.g. with SIT – section 8.1) could more intense vector control be justified.

Ellman et al. (1998) showed that, in nearby highland and lowland areas in Tanzania, transmission and several aspects of morbidity was lower in the former. Furthermore there was no sign of morbidity being postponed to later in childhood. This has been confirmed more recently in the same areas (Maxwell et al., in press) and introduction of insecticide-treated bednets in both areas was beneficial against morbidity by about the same percentage in each area. Continued use of the nets for three to four years (with annual re-impregnation) continued to be beneficial (Maxwell et al., 2002), despite the fact that reduction in levels of antibodies to variant surface antigens could be detected (Askjaer et al., 2001).

Conflicting conclusions have been presented about the relationship of transmission intensity and malaria mortality in different areas. However, the most recent and thorough review concludes that mortality is positively correlated with transmission so that, if transmission was artificially lowered, a reduction in malaria mortality could be expected (Smith TA et al., 2001).

Thus, for malaria, the reassuring view seems to be gaining support that one need have no reservations about trying to apply vector control anywhere and everywhere. However, vigilance on this issue is still to be recommended, since the possibility cannot be completely excluded that, by doing our best to control vectors, we might in some circumstances actually be worsening human health.

It is to be hoped that vaccination will replace the immunity which may be lost as a result of reduction of transmission due to vector control. Conversely, the control of intense transmission should reduce the likelihood of "swamping" of the effect of a vaccine. Thus there is reason to hope that vaccination and affordable vector control, e.g. with ITNs, will synergize each other. Therefore trials of the integrated use of the two methods should have high priority.

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Annex 4

Genetic modification of insects of medical importance: past, present and future

Paper for the WHO/TDR Scientific Working Group on Insect Vectors and Human Health, Geneva, Switzerland, 12–16 August 2002

GENETIC MODIFICATION OF INSECTS OF MEDICAL IMPORTANCE: PAST, PRESENT AND FUTURE

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1. INTRODUCTION

Insect-transmitted diseases impose an enormous burden on the world's population in terms of loss of life (millions of deaths per year) and morbidity. These diseases are also responsible for huge economic losses, both in terms of health-care costs and lost productivity, mostly in countries that can least afford them. Three basic approaches have been used in attempts to control these diseases:

- development of vaccines that prevent infection;
- treatment of infected people with drugs that kill the pathogen;
- control of insect vector populations.

1.1 Vaccines

The only existing vaccine that is effective against an arthropod-transmitted pathogen is the yellow fever vaccine. Even in this case, it has not yet been possible to eradicate the disease. Decades of intense research aimed at the development of other vaccines, notably for malaria and dengue fever, have yet to yield a viable product. At the heart of the problem is the fact that during thousands of years of association with humans, pathogens have been evolutionarily selected for their ability to efficiently evade the host immune system. High genetic diversity, variability of potential immune target molecules (e.g. *Plasmodium var* genes), and intracellular sequestration are strategies frequently used by pathogens to elude immune attack. There continues to be hope that an antimalarial vaccine will be developed; however the current fight against the ability of the malarial parasite to evade the human immune system has not yet been won.

1.2 Drugs

Drugs have been at the forefront of the control of many diseases that are transmitted by arthropods. Chloroquine, for example, was used successfully for many decades to treat malaria. However, with time, pathogens undergo natural selection and become resistant to drugs. The drugs used for treatment thus become ineffective and the development of new drugs becomes necessary. Unfortunately, this cycle of drug discovery followed by development of drug resistance becomes more and more difficult to perpetuate with the passage of time. In principle, combination drug therapy would greatly alleviate this problem. In practice, other factors (mostly economic) prevent the implementation of this strategy. While drugs are extremely useful in containing and treating diseases, they are not sufficient for disease eradication. Clearly, a combination of control strategies is needed.

1.3 Control of insect populations

Reduction of vector insect populations will decrease disease transmission. Reduction can be accomplished in various ways, for instance, with insecticides, by managing the environment (elimination of breeding sites) or by interfering with reproduction (e.g. sterile insect release). Recent technological advances suggest that an alternative approach may be feasible, namely, the genetic modification of the capacity of the arthropod to transmit pathogens ("vectorial capacity").

This article reviews the various strategies available for the control of insect populations, emphasizing recent advances in genetic manipulation. As the intent of this article is to stimulate discussion, controversial statements were included intentionally.

2. INSECTICIDES

In appropriate circumstances, insecticides are powerful weapons in the fight against vector-borne diseases. For instance, they have been crucial in the eradication of malaria in Europe and Brazil and they are also important in the control of sudden disease outbursts (e.g. dengue, West Nile fever) in urban areas. The introduction of the insecticide dichloro-diphenyl-trichloro-ethane (DDT) in the late 1940s heightened hopes for the eradication of insect-transmitted diseases. A case in point is the WHO campaign to eliminate malaria, using DDT spraying which was successful at its inception, resulting in the almost complete disappearance of malaria from the Indian subcontinent. However, problems such as the development of insecticide resistance by mosquitoes and discovery of the harmful effects of DDT on the environment and non-target organisms led to the reversal of most initial successes.

One aspect of the use of insecticides is frequently overlooked: insecticides usually leave intact the biological niche where the target insects reproduce. Therefore, insect populations rapidly return to pre-treatment levels as soon as spraying is halted, which is a very serious problem. For instance, it is unlikely that large-scale mosquito population reduction can be achieved in Africa, if one considers that management of all breeding sites (many widely-distributed small pools of water) would be required. Even if residual spraying of house interiors or use of bednets lowered transmission rates and perhaps reduced prevalence and incidence of infections, these mosquito breeding sites would continue to produce mosquitoes, and the development of insecticide resistance is likely to be hastened. Thus, insecticides are useful to bring temporary relief but cannot be considered as solutions in themselves. In the future, insecticides are likely to become key weapons when used in combination with other approaches, such as the use of vaccines or drugs, or to facilitate population replacement.

3. STERILE INSECT TECHNIQUE

The control of disease-vector insect populations by the release of sterile males (sterile insect technique, SIT) is based on the observation that females of many insect species usually mate only once during their reproductive life. Thus, a female that mates with a male that has no sperm or whose sperm has been rendered unviable, will never have any progeny. When many sterile males are released, the local insect population declines. There are a number of cases of the successful local application of this technique, for example, in the control of the Mediterranean fruit fly in Latin America, the New World screw-worm in the Americas and Libya, and the tsetse fly in Africa. SIT also has been applied to *Culex* mosquitoes in India and *Anopheles albimanus* mosquitoes in El Salvador.

The crucial parameter is the ratio of the numbers of released sterile males to females in the local population: ideally this should be around 10. Therefore, control using SIT is only effective when the resident population is small relative to the number of sterile males that can be mass-produced for release. It is highly desirable that only males be released for two reasons: (1) frequently, only female insects bite and transmit disease and sterile females remain able to transmit; (2) resident males could mate with the sterile released females instead of local females, thus reducing the efficacy of the programme (Alphey & Andreasen, 2002). Large-scale production by non-genetic means in the laboratory of a population consisting entirely of males may be problematic. Methods by which the sexes can be distinguished may rely on sex-specific differences of pupal size (culicine mosquitoes) or adult eclosion times (tsetse fly), but these protocols rarely yield a 100% male population. Clearly, genetic sexing methods (see below) are far superior.

The most commonly-used technique for male sterilization is exposure to high levels of radiation, a procedure that damages chromosomes and results in the production of unviable sperm. Sterilization by chemical means has also been employed. Because of the large numbers of insects required, it is crucial that the effectiveness of the sterilization procedure approaches 100%. However, the large doses of radiation and chemicals needed to achieve this level of efficacy may reduce the fitness, survival and mating competitiveness of the insects produced. Population control strategies can fail if the laboratory-reared males do not mate as effectively as their field counterparts.

The advent of germline transformation technology for a number of different insects has led to the development of genetic alternatives to production of sterile insects (Heinrich & Scott, 2000; Thomas et al., 2000). In one approach (Release of Insects carrying a Dominant Lethal, or RIDL; Thomas et al., 2000), a conditional dominant lethal gene is introduced into the target insect genome. This gene has two important properties: (1) it is expressed only in females (or it kills only females); and (2) it is effectively repressed by a compound that does not occur normally in nature (e.g. tetracycline). Large insect populations are maintained in the laboratory by rearing them in the presence of tetracycline, which represses the dominant lethal gene and allows the survival of equal numbers of males and females. Prior to release, tetracycline is withdrawn, allowing the dominant lethal gene to be expressed and thus causing the death of all females. The resulting males can be released without further manipulation or treatment. Males are homozygous for (carry two copies of) the dominant lethal gene. When these males mate with resident females in the wild, all female progeny will die and only males will be produced. Since these surviving males are heterozygous for (carry a single copy of) the dominant lethal gene, the population-reducing effect is still manifest in the second generation.

It should be emphasized that the effectiveness of SIT is dependent on population structure and dynamics. Furthermore, this technique leaves intact the biological niche in which the target insect is found. SIT is most likely to succeed in situations where target populations are sporadic, the number of target insects is low, and the target area is surrounded by a "buffer zone" where these insects are absent. The presence of a buffer zone reduces the speed at which re-invasion occurs. SIT is unlikely to be effective in areas of Africa where these diseases are endemic, and where the mosquito population is large and where poorly-interbreeding mosquito populations coexist.

4. GENETIC MANIPULATION OF VECTORIAL CAPACITY

4.1. Germline transformation

Drosophila melanogaster was the first multicellular organism to be stably transformed through the transposition of cloned P elements into germline chromosomes. The surviving embryos showed evidence of P element-induced mutations in a fraction of their transgenic progeny (Spradling & Rubin, 1982). The same general principles used in this pioneering work are still employed today for all insect germline transformations (Atkinson & James, 2002). The insect embryos are injected with two DNA constructs. The first construct contains two genes, the gene of interest and a gene encoding a dominant selectable marker (e.g. eye colour or expression of green fluorescent protein, GFP), which allows the transformed individuals to be identified. Each gene is driven by a separate promoter, and the sequence of DNA containing these components is flanked by the inverted repeats of a transposable element. The second construct encodes a transposase, an enzyme that recognizes the inverted repeats of the first construct and catalyses the insertion of the intervening sequence into the genome of the host insect. The net result of germline transformation is thus the integration into the host genome of a relatively large DNA sequence (12 kb or more) containing the genes of interest, flanked by the inverted repeats of the transposable element. The integrated DNA is usually stable and transmitted in a Mendelian manner from one generation to the next. The insects produced in this way are referred to as "transgenic".

It was more than a decade after the landmark work on *Drosophila* before another insect species was successfully transformed. It took from the early 1980s until the mid 1990s to develop two crucial technologies: an appropriate transposable element system (after it was realized that the P transposable element is not active in non-*Drosophila* organisms) and a suitable transformation marker. Since then, germline transformation of many insects has been accomplished, but mosquitoes (*Aedes*, *Anopheles*, *Culex*) are the only insects of medical importance among these. Importantly, both *Anopheles stephensi* and *Anopheles gambiae* can be transformed, though the success rate in the latter case still seems to be low. It would be desirable to develop germline transformation procedures for other medically-important insects, such as sandflies. Current technology cannot be applied to germline transformation of tsetse because these flies do not lay eggs, but produce fully-formed larvae.

In the following text we will consider transformation of insects to induce expression of genes that affect the insect's ability to transmit pathogens. We will start by considering promoters that can be used to drive expression of these effector genes and secondly, effector genes that are capable of interfering with parasite development.

4.2. Promoters

Strong promoters (that cause the effector gene sequence to be transcribed in large quantities) are required to drive the expression of effector genes in transgenic insects. This is because the effectiveness of the gene products is expected to improve with increased abundance. Two general types of promoters can be considered: ubiquitous and tissue-specific. In principle, ubiquitous promoters are less desirable because general expression of a foreign gene product in all tissues of the insect and at all times is likely to impose a fitness load on the host. The advantages of tissue-specific promoters are that the gene product is restricted to a particular tissue by means of a signal sequence, and is often regulated developmentally. Strong tissue-specific promoters that have been characterized in mosquitoes include gut carboxypeptidase (Moreira et al., 2000), fat body vitellogenin (Kokoza et al., 2000) and gut peritrophic matrix (Jacobs-Lorena laboratory, unpublished).

The carboxypeptidase promoter is induced by blood intake, which coincides with the arrival of the parasite in the gut. The carboxypeptidase signal sequence promotes secretion into the midgut lumen, the compartment in which the parasite initially resides. The peritrophic matrix promoter and signal sequence direct synthesis and storage of the effector protein in midgut cells prior to a blood meal, and immediate release into the midgut lumen following blood ingestion. The vitellogenin promoter is also induced by the ingestion of a blood meal with a delay of about 24 hours and its signal sequence promotes secretion of the effector protein into the mosquito body cavity, where the parasite later develops. Since the malaria sporozoite is released into the hemocoel about one to two weeks after a blood meal, it would be desirable to find promoters that activate synthesis and secretion of proteins into this compartment at about that time. While promoters that direct secretion into the salivary gland lumen have been identified, their expression levels are low (Coates et al., 1999). While the identification of a strong salivary gland-specific promoter which induces effector genes targeting pathogens stored in the salivary glands would be desirable, the fact that mosquito saliva is transferred to its vertebrate (human) host raises safety and ethical questions.

4.3. Effector genes

The term "effector gene" is used here to describe genes whose products interfere with the development of a pathogen. At least three classes of effector genes can be identified:

— Genes whose products interact with insect host tissues that are crucial for parasite development. Examples of this class of gene products are SM1, a peptide that occupies putative salivary gland and midgut receptors for the malaria parasite (Ito et al., 2002) and phospholipase A2 (PLA2), a protein that interferes with the malaria ookinete invasion of the midgut (Ziegler et al., 2001; Moreira et al., 2002).

— Genes whose products interact with the pathogen. Examples of this class are genes encoding single-chain monoclonal antibodies that bind to the parasite's outer surface thus blocking its development (Capurro et al., 2000).

— Genes whose products kill the pathogen. Examples are peptides from the insect innate immune system, such as defensins and cecropins, and peptides from other sources that act as selective toxins to parasites but do not affect the host insect, such as magainins, Shiva-1, Shiva-3 and gomesin.

Most published work on effector genes concerns the malaria parasite and little is known about such genes for other protozoan or metazoan pathogens. In particular, it is not clear which class of effector genes would be useful for nematodes (filaria). Since nematodes may be encapsulated in certain mosquito strains, genes that activate encapsulation could be considered as possible effector genes. For viruses, genes of the first class (interference of host tissue invasion) or genes that interfere with virus replication (Olson, 1996) are possible candidates.

4.4. Genetically-modified mosquitoes

Successful development of the technology described above namely, transgenesis, promoter characterization and effector gene identification, has permitted the creation of genetically-modified mosquitoes that are impaired in their ability to transmit the malaria parasite. An early example was the creation of an *Aedes aegypti* expressing defensin in the haemolymph (Kokoza et al., 2000). However, the effect of defensin on malaria parasite development was not reported. At about the same time, it was reported that a single-chain monoclonal antibody to a sporozoite surface protein was able to inhibit invasion of the salivary gland (Capurro et al., 2000). In this instance, the effector gene was transiently expressed from a viral vector that was not inherited by the mosquito progeny. Recently, the Jacobs-Lorena laboratory showed that a stably integrated gene encoding SM1 strongly inhibits parasite development in transgenic mosquitoes (Ito et al., 2002). In another example, transgenic mosquitoes expressing PLA2 were shown to have much reduced vectorial capacity (Moreira et al., 2002). Thus, it is clear that mosquitoes can be genetically modified to reduce their vectorial capacity. However, to date, all reported experiments have been carried out with non-human malaria parasites. An important next step is the transfer of this technology to the main vectors of human disease.

4.5. From now until field release

While genetic modification of mosquitoes to confer resistance to the malaria parasite is clearly feasible in a laboratory setting, many issues remain to be addressed before implementation of this approach in the field can be envisaged. Examples of unresolved issues include:

(i) Insect fitness

For the successful introduction of an effector gene into a mosquito population, it is important that the gene confers the minimal possible detrimental effect on mosquito survival or reproduction ("fitness load"). This parameter can be tested initially in the laboratory. For instance, SM1-transgenic mosquitoes do not seem to suffer any fitness load, while PLA2-transgenic mosquitoes lay considerably fewer eggs and therefore carry a significant fitness load (Jacobs-Lorena, manuscript in preparation). Recently, Cateruccia et al. (2003) suggested that transgenic mosquitoes may have a fitness disadvantage, but in their experiments, loss of fitness was mainly due to inbreeding, and perhaps to generalized foreign gene expression from an ubiquitous promoter (see above). Eventually tests will have to be devised to allow the measurement of insect fitness in the field where the parameters may be different. It is also likely that laboratory mosquitoes will not compete well with their field counterparts, a consideration that will be important for release studies. One possible solution to this issue is to introduce the effector genes *in loco*, into the offspring of mosquitoes caught in the wild, or to outcross transgenic populations with wild populations.

(ii) Parasite resistance and multiple effector genes

Parasites tend to have a heterogeneous genome that favours the survival of individuals that are able to overcome challenges such as drugs or possibly effector gene products. It will therefore be essential that transgenic mosquitoes incorporate more than one (ideally several) effector genes, each of which uses a different mechanism to block parasite development.

(iii) Safety concerns

While there is no reason to believe that any of the effector genes identified to date have any effects on non-target organisms, concerns are being raised by the scientific and lay communities regarding the safety of the transgenic mosquitoes. For instance, it has been suggested that these genetically-modified mosquitoes might be better vectors for other (non-malaria) pathogens. While there is no evidence to suggest that this is the case, caution should be used and these possibilities should be tested. Another concern is that of horizontal gene transfer from the transgenic mosquitoes to other organisms, including humans. This possibility, and in particular the possibility of horizontal transfer of the effector gene to the germ cells of another organism, is remote. Finally, most (if not all) effector genes being considered are expected to be innocuous to higher organisms.

(iv) Introduction of effector genes into field populations

A major issue that remains to be resolved is how to distribute the effector genes into field populations. Several approaches can be envisioned.

— **Population replacement by inundatory release**. A possible scenario would be to start with an isolated area (e.g. an island) where malaria is prevalent and reduce to the maximum extent possible the mosquito population by use of insecticides. As discussed above, this would leave an empty biological niche. The next step would be the release of transgenic mosquitoes to occupy this niche. Transgenic releases could be repeated periodically. The original mosquito population would be expected to be replaced by the transgenic one. Malaria transmission before and after population replacement could then be compared. While this approach could conceivably be used for small areas, it would be difficult to implement on a country- or continent-wide scale. Thus, this would be a good way to test the transgenic mosquito concept in a field situation.

— **Use of transposable elements.** There is an excellent example in *Drosophila melanogaster* of how transposable elements can spread through wild populations. In a matter of a few decades, the P element was able to spread through virtually all *D. melanogaster* populations in the world. Presumably, this happened because the transposase causes the element to multiply in the genome, resulting in non-Mendelian transmission. Unfortunately, P elements are only active in *Drosophila* and more importantly, no transposable element with similar properties has been identified in mosquitoes. Even if such an element is identified, it will be important to determine whether this approach is feasible for the spread of transgenes. This is because the elements tend to become truncated as they replicate, with the possible inactivation of the gene carried by the element. Another consideration is that in some instances, insects carrying a transposable element accumulate a repressor of the transposase, precluding introduction of a different gene with the same transposable element into the same population. In other words, this is a “one-shot” proposition: should one discover that the wrong transgene was used, or if resistance developed, there would not be a second chance with the same element.

— **Symbionts.** Symbionts are intracellular organisms that are present in many insects. Some symbionts may be essential for the survival of the host insect, as is the case for the tsetse fly symbiont the *Wolbachia* bacterium**.** Conceivably, an effector gene could be introduced into the symbionts and expressed from them (paratransgenesis). Feasibility of this approach has been demonstrated by expressing a cecropin in *Rhodnius prolixus* to prevent transmission of Chagas disease (Durvasula et al., 1997). Certain symbionts, such as *Wolbachia*, also can drive themselves into populations. One problem is, however, that these organisms are difficult to culture and therefore difficult to manipulate genetically (i.e. the introduction of an effector gene may be problematical).

— **Meiotic drive.** Population replacement can be driven by certain genes, such as the *Drosophila segregation distorter* gene, that themselves favour the survival of individuals inheriting the gene compared to individuals without the gene. Unfortunately, very little is known about such genes in insects of medical importance. Moreover, if such genes were to be employed to drive effector genes into populations, the meiotic drive genes and the effector genes would have to be tightly linked to avoid loss of effectiveness due to recombination.

(v) Population structure

It is quite clear that at least for mosquitoes, population structure is complex. This is because a number of morphologically identical but chromosomally different (cytotypes) mosquito populations can coexist in any given area. Importantly, these populations may not freely interbreed and this may seriously affect efforts to spread effector genes through populations. A better understanding of population structure and mosquito ecology should be given a high priority.

4.6. Political, social and ethical considerations

In addition to scientific concerns, it is important to address concerns of public perception. The issues and debates over genetically-modified crops provide a useful precedent from which we should try to learn. It will be important to educate the public at large about the benefits and risks of transgenic technology. The time to start is now, because public education regarding these issues may take a full generation to accomplish. The targets of such a campaign must include the communities concerned and the decision-makers. The results of safety tests should be honestly and broadly divulged. It is important to emphasize that no approach will be entirely risk-free and that the balance between potential benefit and risk should be considered. It is also crucial to make it clear that a single approach cannot be completely effective on its own, and that a final solution will have to incorporate a number of weapons, such as drugs, insecticides, bednets, and hopefully vaccines and genetically-modified insects.

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