Bridging the gaps in vector biology

Workshop on the Molecular and Population Biology of Mosquitoes and other Disease Vectors

David S. Schneider¹ & Anthony A. James²⁺

¹Department of Microbiology & Immunology, Stanford Medical School, Stanford, CA, USA, and ²Departments of Microbiology & Molecular Genetics and Molecular Biology & Biochemistry, University of California, Irvine, CA, USA

This EMBO Workshop on the Molecular and Population Biology of Mosquitoes and other Disease Vectors took place between 22 and 30 July 2005 in Kolymbari, Crete, Greece. The workshop was organized by C. Louis (Chair), B. Beaty, N. Besansky, P. Brey, M. Coetzee, F. Collins, D. Fontenille, J. Hemingway, J. Hoffmann, A.A. James, F.C. Kafatos, J. Law, J. Ribeiro, M. Rodriguez, T. Scott, D. Severson, R. Sinden, A. della Torre and Y. Toure. The photo is of the sunset on the Monastery of Gonia, viewed from the Orthodox Academy of Crete where the Workshop took place (photo courtesy of C. Louis).

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Introduction

More than 100 scientists gathered in the peaceful setting of the Greek Orthodox Academy in Crete to share the latest research on the arthropod vectors of human diseases (abstracts available at http://skonops.imbb.forth.gr/AnoBase/Conferences/home_page.html).

¹Department of Microbiology & Immunology, Stanford Medical School, Stanford, CA 94303, USA

²Departments of Microbiology & Molecular Genetics and Molecular Biology & Biochemistry, 3205 McGaugh Hall, University of California, Irvine, CA 92697-3900, USA

+Corresponding author. Tel: +1 949 824 5930; Fax: +1 949 824 2814; E-mail: aajames@uci.edu

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The meeting participants were a mixture of basic scientists addressing fundamental biological questions and applied researchers whose intent is to reduce the frightful toll of diseases such as malaria, dengue fever and filariasis by targeting the vectors. This emphasis on vectors is justified by the success of control programmes that have used insecticides, environmental management and personal protection such as repellents and nets. New insights and technologies are needed to counteract the erosion of the efficacy of these approaches—for example, the emergence of insecticide resistance—and to address regions of the world where these approaches have either not been applied or have failed. This series of workshops promotes interactions among scientists to bridge the gaps between the laboratory development of anti-vector tools and the knowledge of field properties of mosquitoes, and to stimulate links between scientists in the developed world with disease endemic country (DEC) scientists who would be responsible for implementing new technologies. The research and applications that were discussed have a strong base in advanced technology and much of it involves potential control strategies. Thus, the widely recognized synergy between basic science and technology, through which advances in either propel the other, is now being played out in full force in vector biology. However, the big challenge remains the same—to develop practical ways to use the new knowledge and tools to alleviate suffering.

Research on the biology of vector–pathogen and vector–host interactions, population structure and genetics, insecticide resistance and genetic control methods has benefited from the development of genomics, molecular tools for species and gene diagnostics, transgenesis (Fig 1), reverse genetics and comparative species analyses. The majority of recent work has focused on mosquitoes. Although pathogens transmitted by sand flies, reduviid bugs and ticks are significant, more than half of the world's population is at risk from those that are carried by mosquitoes. This emphasis was evident in the number of presentations that reported on mosquito research.

Genomics

The rationale for genomic efforts is an anticipation that a full knowledge of the genome will lead to the identification of new and unexpected ways to manage or manipulate vector populations.

Fig 1 | Technological advances are driving basic discoveries in vector biology. Transgenic mosquitoes (*Anopheles stephensi*) expressing the cyan fluorescent protein in its fat-body tissues.

Genomes also serve as platforms to develop bioinformatic and micro-array technologies that can be used to study complex genetic phenotypes associated with pathogen development and transmission, and interaction with vertebrate hosts. The genomes of two of the main mosquito species, *Anopheles gambiae*, the principal vector of human malaria parasites in sub-Saharan Africa, and *Aedes aegypti*, the cosmopolitan vector of dengue and yellow fever viruses, have been sequenced (Holt *et al*, 2002; http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=a_aegypti). There is considerable interest in sequencing the genomes of additional mosquito species (*An. funestus* and *Culex quinquefasciatus*), a hard tick (*Ixodes scapularis*) and the tsetse fly—the vectors of African trypanosomiasis. Rigorous bioinformatics tools are being applied to the comprehensive annotation of the *An. gambiae* and *Ae. aegypti* genomes, and expression data for a large number of *An. gambiae* genes is now available (<http://www.angagepuci.bio.uci.edu/>). Enthusiasm is increasing for comparative genomics, and Vectorbase (<http://www.vectorbase.org/>) is being developed as a central resource to integrate information from a wide diversity of vector and non-vector insects.

Vector–pathogen and vector–host interactions

The study of vector–pathogen interactions in mosquitoes and other vectors covers a wide field of overlapping interests. Identification and characterization of crucial interactions should lead to new strategies for disrupting pathogen transmission. Several key observations are fuelling these studies. The first is that not all vector species are capable of transmitting all pathogens. For example, in mosquitoes, only members of the subfamily Anopelinae transmit malaria to humans, whereas those in the Culicinae are the principal vectors of many viruses. The genetic basis for this specificity has stimulated studies to identify physiologies that affect pathogen propagation. Identifying the relevant genes has drawn mosquito biologists into the larger landscape of innate and acquired immunity. The wealth of tools available for studying basic biology in the fruit fly, *Drosophila melanogaster*, makes it a trusted model for innate immunity and a source of both envy and inspiration for vector biologists. Whereas fly workers can

develop convenient genetic systems for analysing immunity (Dionne & Schneider, 2003), the mosquito researcher must come up with creative ways to study their host–pathogen pair and several methods were presented at the meeting. For example, researchers are looking at the development of the causal agents of malaria, *Plasmodium* parasites, in the mosquito. Reverse genetics and RNA interference (RNAi)-mediated gene-knockout approaches have made possible the functional dissection of immune responses (Blandin *et al*, 2002). A multi-gene pathway similar to that observed in the worm *Caenorhabditis elegans* (Ellis *et al*, 1991) regulates the melanization process—the encapsulation of pathogens by a layer of cross-linked melanin—and implicates two distinct pathways for phagocytosis (F. Kafatos, London, UK). The mechanisms by which other genes alter physiological states to interfere with parasite development are also being examined. For instance, nitric oxide is important as an effector in anti-plasmodium defence (Luckhart *et al*, 1998). Recent evidence shows that the pathways that are stimulated to produce nitric oxide are conserved and are related to the insulin-signalling pathway (S. Luckhart, Davis, CA, USA). Peroxidases might also have a significant role in defence reactions in the mosquito midgut (C. Barrilas-Mury, Rockville, MD, USA). RNAi-mediated knockout of a peroxidase gene prevents parasite killing and melanization in mosquitoes otherwise resistant to *Plasmodium*. Similar knockout experiments have established melanization as the key effector of mosquito resistance to filarial worms (C. Chen, Taipei, Taiwan). The terms ‘refractory’ and ‘resistant’ are used interchangeably by vector biologists to describe insects that do not support the development of specific pathogens. However, some authors have made a distinction and use ‘resistant’ to refer to active processes that affect pathogens and ‘refractory’ to describe what seem to be passive mechanisms—such as a lack of appropriate receptors (Beerntsen *et al*, 2000).

Insects have been described as having innate immunity but no acquired immune system. Recent work in a variety of insects has shown otherwise, and work on the *An. gambiae* homologue of the *D. melanogaster* gene *Dscam* (Watson *et al*, 2005) might provide a molecular explanation for some of these effects (G. Dimopoulos, Baltimore, MD, USA). The *An. gambiae* *Dscam* encodes an immunoglobulin-domain-containing protein with an enormous capacity for splice variation; more than 30,000 splice variants are possible. This complexity could provide a mechanism for increasing the diversity and specificity of the immune system. An experimental infection of mosquitoes with different bacterial species produces different splice variants, and RNAi-mediated knockouts of these variants decreases survival rates.

A second observation fuelling research in vector–parasite interactions is that malaria parasites tend to invade specific tissues of the mosquito, notably the midgut and salivary glands. Indeed, some of the phenotypes of parasite-resistant mosquitoes can be interpreted as the inability of a parasite to recognize tissues. These observations provide support for the hypothesis that parasites must have ligands that interact with specific receptors on the target mosquito tissues. Blocking these interactions could produce refractory mosquitoes. Whereas potential ligands on both protozoan parasites and viruses have been discovered, the search for mosquito receptors has proven more difficult. Affinity-based techniques have been used to show that laminins expressed in the midgut epithelial cells bind malaria parasites (C. Louis, Crete, Greece). More recent data suggest that annexins and cadherins also have a role. Calreticulin is proposed to be the receptor for *Plasmodium*

vivax in *Anopheles albimanus* (M. Rodriguez, Cuernavaca, Mexico). The protein saglin is thought to be a salivary-gland receptor for parasites on the basis of its ability to bind the sporozoite surface protein TRAP (M. Jacobs-Lorena, Baltimore, MD, USA). These studies are supported by observations that blocking interactions with putative receptors reduces the average number of parasites in infected mosquitoes and can have modest effects on prevalence.

The epidemiology of malaria transmission is such that if the blocking strategies described above are to be effective, the end target must be no parasites (zero prevalence) in salivary glands (R. Sinden, London, UK). Furthermore, it is highly unlikely that any programme based only on transmission blocking would be sufficient to control the disease. These strategies must be integrated into broader programmes that use drugs, vaccines and other vector control methods. Only by achieving this is there a realistic expectation of seeing a reduction in human disease.

Much of the discussion so far has focused on anopheline vectors and malaria parasites, however some aspects of mosquito–pathogen interactions are not represented in anophelines. Many culicines live in temperate climatic zones and can survive dry and/or cold seasons by altering their physiology through aestivation or diapause. The viruses carried by these vectors require the host transcriptional machinery to replicate themselves. Diapause, with its attendant reduction in overall host metabolism, presents a challenge to these viruses. La Crosse virus is a member of the Bunyaviridae transmitted by *Ochlerotatus* (formerly *Aedes*) *triseriatus*, and is a main cause of encephalitis and aseptic meningitis in children in the United States. It has 5'-end untranslated region sequences derived from host mRNAs, principally from a gene that encodes an inhibitor of apoptosis protein. This 5'-end scavenging presumably allows the virus to maintain itself in the vector during diapause (B. Beaty, Fort Collins, CO, USA).

One of the more intriguing developments in vector–host interactions is the dissection of the mosquito olfactory system. This knowledge may assist the development of compounds that repel mosquitoes, act as competitive attractants to lure questing female mosquitoes away from humans, or to mask human odour cues making it impossible for the mosquitoes to detect hosts. Pattern recognition algorithms were used in a bioinformatics approach to screen the *An. gambiae* genome to identify odorant-binding proteins and receptors, and physiological and behavioural experiments are being used to associate specific odorant ligands with individual proteins (L. Zwiebel, Nashville, TN, USA).

Vector biologists are asked frequently whether pathogens have any impact on the fitness of the infected insect. Early work showed effects of infection on feeding success (Rossignol *et al.*, 1985), but more recently, data are accumulating that parasites might affect the reproductive fitness of the insects (Hurd, 2003). Changes in resource utilization in mosquitoes infected with the rodent malaria parasite, *Plasmodium yoelii*, reduce both fecundity and fertility as follicles are reabsorbed (H. Hurd, Keele, UK). A systemic signal originating from the parasitized midgut is proposed to affect the reproductive physiology of the mosquito by inducing apoptosis in cells in the follicular epithelium. However, selection of the whole mosquito population for genes conferring resistance is unlikely in this scenario. Only a small fraction of the total population is parasitized at any one time, and this coupled with the observed decrease in offspring in parasitized animals is probably insufficient to select for resistance.

Population biology and genetics

Knowing the population biology of vectors is important for understanding the transmission dynamics of specific pathogens. Knowing which species transmit which pathogen is crucial when designing approaches to control epidemics. There is an amazing diversity of mosquito population structure and genetics. It seems that the majority of anophelines belong to species complexes comprising taxonomically similar, geographically overlapping (sympatric) populations that display a wide variety of adaptations for breeding sites, host preference, wet–dry season abundance and susceptibility to pathogens. Evidence is accumulating of reproductive isolation *in situ* for the members of species complexes, most probably owing to selective conditions originating from human intrusion on the landscape. The replacement of animals by humans as the principal source of blood meals, and the changes in environments brought about by farming practices and community development, might be driving incipient speciation in many anopheline vectors. As insects in the process of speciation are a 'moving target', their study presents several challenges to population biologists. Molecular tools are crucial for distinguishing reproductively isolated populations among superficially identical mosquitoes. This has led to revisions in the ways the insects are being analysed—for example, by single-strand conformational polymorphisms or microsatellite markers—and should have a significant impact on how they are controlled. Scientists are studying past mosquito invasions as one approach to understanding the conditions that lead to rapid changes in population structure (J. Powell, New Haven, CT, USA).

Insecticide resistance

Lurking in the background of every effort designed to take advantage of the new tools is the question of what can be done now to control vector-borne diseases. The answer is clear—use insecticides. Specific applications of chemicals are the most effective means of quelling epidemic outbreaks of malaria and dengue fever. The recent control of an outbreak of malaria in Madagascar was achieved by classic indoor residual spraying (Curtis, 2002). The challenges of the continued use of insecticides are well-documented: unacceptable effects on non-target organisms, the development of insecticide-resistance among targets, and a lack of industry involvement in discovering new and targeted products to replace old insecticides. Although concerted efforts are being made to improve biological agents such as *Bacillus thuringiensis israelensis* (BTI; B. Federici, Riverside, CA, USA), the general approach is not to discover new insecticides, but to learn how to manage the ones we have in place to prevent resistance (A. Rodriguez, Tapachula, Mexico). This has spurred a lot of good biochemistry and molecular biology to identify genes that mediate resistance and to develop inexpensive, fast, and field-ready diagnostic procedures. The latter are needed to monitor insecticide resistance and to provide a basis for proactive management of this phenomenon. Towards this end, a gene chip has been developed that has a large array of sequences representing cytochrome P450s, glutathione synthetases and other genes involved in resistance (J.-P. David, Liverpool, UK). This chip is an excellent laboratory tool for looking at complex resistance phenotypes (those that involve the activity of more than one gene) and for identifying hierarchical aspects of the genetic control of resistance.

Genetic control technologies

Genetic control technologies focus on methods to limit the size of vector populations (population reduction) or to alter the populations so that they are resistant to pathogens. Crucial to many of the proposed strategies is the ability to genetically manipulate the target species. New developments in transgenesis technologies are needed to achieve higher efficiencies of integration (especially for *An. gambiae*), site-specific integration for gene promoter analyses, remobilization of transposons for gene tagging and enhancer trapping, and to determine whether transposons can be used as gene drivers for introgressing new genes at high rates into target populations (James, 2005). Exciting new evidence about a class II transposable element in *An. gambiae* was presented at the meeting (D. O'Brochta, Baltimore, MD, USA). This element could be particularly important if it increases the efficiency with which this mosquito can be transformed. Transposon-mediated insertion of exogenous DNA is essentially a random event. As such, there are insertion-site effects that modulate differentially the expression of the genes carried by the transposon. A site-specific integration system based on phage $\phi 31$ (Groth *et al.*, 2004) has been adapted to *Ae. aegypti* (P. Eggleston, Keele, UK). The specific targeting of a site in the genome and the high frequency of integration of this system are valuable for the analysis of gene constructs (Nimmo *et al.*, 2005).

Cytoplasmic incompatibility is caused by endosymbionts belonging to the genus *Wolbachia* and results in asymmetric crossing sterility between infected and uninfected mosquitoes. A properly formulated release of mosquitoes carrying these endosymbionts might result in reductions in insect populations that will presumably reduce the transmission of pathogens. New data were presented indicating that *Wolbachia* may also be used in gene-drive technology (S. Dobson, Lexington, KY, USA). For the first time, investigators have been able to introduce exogenously derived endosymbionts into *Ae. aegypti*, a species that does not have them naturally (Xi *et al.*, 2005). With appropriate release ratios, the endosymbionts appeared in all animals within seven generations in cage studies of mosquito populations.

Concluding remarks

The Kolymbari workshop is scheduled to take place every two years. As such, this congregation holds the promise of being one of the most important meetings in vector biology. The format in which each attendee speaks for 20 minutes limits the number of participants to approximately 100, but at the same time ensures that everyone has a chance to tell their story. The organizers are looking to see greater representation in future meetings from qualified DEC scientists (only eight of the 23 countries represented could be considered DEC). If the current trend continues, the 2007 meeting promises astonishing new discoveries.

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