sion of the infection. There must be some block to the development of the parasite in *Culicine* mosquitoes that the human parasite is unable to overcome.

Introduction of genes encoding refractoriness into field populations of mosquitoes remains a major obstacle to implementation of such control strategies. A transposable element, the P element, first appeared in *Drosophila melanogaster* around 1950 and spread in a non-Mendelian fashion to flies of this species around the world (*9*). We might use an analogous transposable element of mosquitoes in a cassette with genes for refractoriness to malaria to introduce these genes into vector populations.

The third leg of basic research aimed at developing new tools is the identification of biochemical pathways unique to the malaria parasite and subsequent development of poisons specific to the parasite. One such area is hemoglobin digestion. The parasite, after digestion of hemoglobin, reorganizes heme into a nontoxic compound, hemozoin pigment. The polymeric structure of hemozoin pigment has recently been identified, and the polymerizing enzyme activity is blocked by chloroquine and other related antimalarial compounds (*10*). Furthermore, the locus for the chloroquine resistance gene (*11*), which encodes a drug-efflux mechanism (*12*) unrelated to the polymerase, has been identified. Understanding the structure and function of the polymerase and efflux mechanism should open the way for drug design to reverse chloroquine resistance.

Malaria, unlike AIDS, is not a major health problem for citizens of the United States; it is a problem of the unseen sick and dying in the villages of the tropical world. Our goal must be to develop tools and ways of delivering these tools to limit disease and to prevent malaria at a cost that is affordable and sustainable in these populations with limited resources. Success will demand continual support of basic scientists and involvement of industry. Can we ignore this challenge?

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Mosquito Molecular Genetics: The Hands That Feed Bite Back

Anthony A. James

Mosquitoes are the most important vector of human parasitic diseases. Every year they transmit over 250 million new cases of malaria, filariasis, and viral disease. It is therefore surprising that only recently has mosquito molecular genetics been pursued with the same rigor and zeal characteristic of other aspects of parasitic disease control and treatment. In the last few years, research efforts funded by the John D. and Catherine T. MacArthur Foundation and the National Institutes of Health here in the United States, and by the Wellcome Trust in Great Britain, have catalyzed a renaissance of interest in the vectors of parasitic diseases. New knowledge of the molecular bases of vector-parasite interactions and population structure in mosquitoes should ultimately lead to novel ways to control disease transmission.

A workshop (*1*) sponsored by the MacArthur Foundation brought together vector molecular biologists with scientists who have made significant progress in other, well-studied organisms, most notably *Drosophila melanogaster*. Together, these two groups assessed the state of mosquito molecular biology and molecular genetics.

Many of the investigators are working toward developing strains of mosquitoes that are refractory or resistant to parasites. These strains will be released to control the transmission of disease by replacing the existing populations. Better diagnostic methods will help these efforts by defining those species or strains that should be the targets of genetic control.

In Africa there are six described members of the *Anopheles gambiae* complex, three of which overlap in their geographical range. Different members of a complex can coexist in a single locale, but only one may be actually transmitting the parasites. Members of the *An. gambiae* species complex have been differentiated by their polytene chromosome banding patterns (*2*), and the derived maps are as detailed as those available for *Drosophila*. However, these maps have yet to lead to techniques that would allow quick and accurate identification of large numbers of animals in the field. At the workshop, various molecular approaches for species identification were presented. Ribosomal (F. H. Collins, Centers for Disease Control–Atlanta) and mitochondrial (A. Cockburn, U.S. Department of Agriculture–Gainesville) DNA variation potentially can provide sensitive measures of species differentiation. The ribosomal RNA genes of mosquitoes have the typical structural features of the eukaryotic rDNA cistrons. Included in the intergenic spacer regions (IGS) are sequences of DNA that vary among populations (*3*). Regions of the IGS were scanned for two base-pair sequence polymorphisms among the members of the species complexes. Polymerase chain reaction (PCR) primers containing two 3′-terminal nucleotides that overlap the polymorphisms can distinguish among members of the complex. Only those animals with an exact match to the primers amplify a product. Mitochondrial DNA variability is also being evaluated in the *An. quadrimaculatus* species complex of the Americas, but probes have yet to be developed. PCR techniques with random amplified polymorphic DNA (RAPD) sequences (C. Louis, Institute of Molecular Biology and Biotechnology–Crete) are being investigated, but it remains to be seen whether they are generally applicable.

Another necessary step in developing strains of mosquitoes that are resistant to parasites is the identification of the gene or genes required for disease transmission. For example, certain genetic variants of *An. gambiae* and *Aedes aegypti*, the yellow fever mosquito (see figure), have reduced capacity to transmit the pathogens responsible for parasitic diseases, including malaria and filariasis. In order to identify the genes responsible for this reduced transmission, certain technological achievements must be made. The three central workshop topics—genome mapping, transposable elements, and transformation strategies—point to the challenges facing molecular biologists working with mosquitoes, notably, a lack of ability to transform mosquitoes at useful frequencies and an inability to isolate genes

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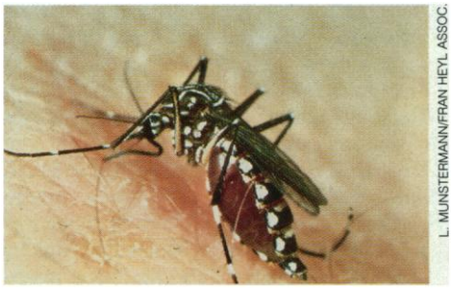
involved in refractoriness or susceptibility to specific parasites. However, encouraging reports from several laboratories suggest that solutions to these problems will ultimately be available.

The major vector species, *An. gambiae* and *Ae. aegypti*, are the targets of genome mapping efforts spearheaded, respectively, by F. Kafatos (Harvard University, Institute of Molecular Biology and Biotechnology–Crete) and B. Beaty (Colorado State University). In an effort that parallels their work in *Drosophila*, Kafatos and his colleagues have constructed division-specific probes and libraries from microdissected polytene chromosomes of *An. gambiae* (*4*). Each chromosomal division represents a cytologically distinguishable and contiguous portion of the mosquito genome. Polytene chromosomes are spread on microscope slides, and divisions delimited by identifiable chromosome bands are dissected. The microdissected DNA is extracted, and linker DNA is ligated to the fragments. Then, by using PCR primers homologous to the linker DNA sequences, each division-specific DNA preparation is amplified. At present, 46 of 54 recognized divisions have generated specific probes, as measured by hybridization of the probes to polytene chromosomes. Cloned DNA fragments can be localized by using hybridization filters



L. MUNSTERMANN/FRAN HEYL ASSOC.

A feeding female *Aedes aegypti*.

spotted with each of the division-specific DNA preparations. In conjunction with the *An. gambiae* genome project, a restriction fragment length polymorphism (RFLP) map with random and characterized complementary DNAs (cDNAs) as probes is being constructed (P. Romans, University of Toronto), as is a microsatellite map based on GT nucleotide repeat arrays that are polymorphic in length (L. Zheng and F. Kafatos, Harvard University). It is estimated that these molecular genetic maps should have a resolution of one centimorgan in order to be useful for cloning genes involved in vector competence.

The genome mapping project for *Ae. aegypti* is complicated by the relatively large size ($\sim 8 \times 10^8$ base pairs) of its genome. Polytene chromosomes are not readily visualized, but a large number of visible mutations have been described and meiotically mapped. An RFLP map based on polymorphisms revealed by hybridization of known and random complementary DNAs to Southern blots of genomic DNA is in progress (D. Severson, University of Wisconsin). An advantage of this species is that the RFLPs can be mapped meiotically with reference to a number of visible mutations such as *red-eye* and with reference to the sex-determining locus. A number of genes involved with vector competence have been mapped meiotically, and the efforts in this vector species may yield the first examples of cloned genes of this type.

The ability to transform mosquitoes with DNA is a necessary prerequisite to molecular genetic manipulation. Unfortunately, the P-element of *D. melanogaster* does not mobilize in mosquito embryos in experiments requiring the microinjected host to transcribe and translate the P-transposase. However, exogenous DNA will integrate into mosquito chromosomes at a low frequency, presumably as a result of nonhomologous recombination. There are several efforts underway to manipulate heterologous transposable elements and identify mosquito homologous elements, construct virus-based transformation vectors, and insert high-frequency, site-specific recombination hot spots into the mosquito genome. Although no transposable element equivalent to the P element exists in mosquitoes, a number of dispersed DNA sequences have been identified that share structural features with known elements [pX16 in *Ae. aegypti* (J. M. Crampton, Liverpool School of Tropical Medicine)] (*5*), and in the future these may serve as transformation vectors. The *D. melanogaster hobo* element appears more wide-spread than the P element and efforts to identify homologous elements in mosquitoes could prove fruitful. Alternately, the purification of active recombinant P-transposase from *Drosophila* cells (D. Rio, University of California–Berkeley) (*6*) opens up the possibility of using the *Drosophila* element in heterologous mosquito systems.

The low-frequency, nonhomologous recombination events that occur in mosquitoes could be exploited if they are used to incorporate into the genome a DNA sequence that serves as a docking site for high-frequency integration. The FLP-FRT site-specific recombination system of the yeast 2-μm plasmid consists of a recombinase, FLP ("flip"), and a minimal 34–base pair DNA sequence, FRT, that serves as a substrate for the reaction. This system functions in *Drosophila* (K. Golic, University of Utah; F. C. Kafatos and collaborators) (*7*) and can work in *Ae. aegypti* (A. A. Morris and A. A. James, University of California–Irvine) (*8*), where it catalyzes interplasmid recombination at endogenously derived or synthetic FRT sites in events that are functionally equivalent to integration. Efforts are underway to produce a strain of *Ae. aegypti* with an FRT site introduced into the genome that will serve as a docking site for high-frequency integration. The availability of purified FLP recombinase (P. D. Sadowski, University of Toronto) will facilitate the evaluation of this system as a means of transforming mosquitoes.

Several practical issues critical to an emerging field were addressed in the workshop: the need for centers where reference mosquito stocks could be kept, the need for high-density genetic maps, perhaps with microsatellite sequence DNAs, and the pressing need for an open and rapid method of communication of results. On advice from M. Coluzzi, the Suakoko strain was selected as a standard for *An. gambiae*. The Centers for Disease Control–Atlanta and University of Rome will be two of the sites at which these and other reference strains will be kept. Extensive communication between researchers has facilitated progress in understanding key basic questions in *D. melanogaster* and the yeast, *Saccharomyces cerevisiae*. It is hoped that an electronic mail bulletin board will be established in the United States and be accessible worldwide through the World Health Organization, serving the same vital role for the mosquito researchers.

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