Vector Biology and the Control of Malaria in Africa

Frank H. Collins and Nora J. Besansky

Malaria, the most important parasitic disease in the world today, is disproportionately prevalent in tropical Africa, where approximately 10 percent of the world's population suffers more than 90 percent of the world's malaria infections (1). This can be attributed almost entirely to the mosquitoes *Anopheles gambiae*, *A. arabiensis*, and *A. funestus*, three of the most efficient malaria vectors in the world (Fig. 1). All live almost exclusively in close association with humans and feed on blood, primarily from humans. The power of *A. gambiae* as a malaria vector is well illustrated by its accidental introduction into Brazil, where in 1938, after a series of small but intense local outbreaks, it caused the worst epidemic of malaria ever recorded there, with over 14,000 deaths in less than 8 months. Only the subsequent eradication of the mosquito saved Brazil, and possibly much of the Americas, from levels of endemic malaria comparable to those now found in tropical Africa (2).

These three African mosquitoes are so efficient as malaria vectors because of their marked preference for human environments and humans as hosts and because they adapt so rapidly to changes in the environment induced by human habitation and agriculture. This genetic plasticity is evident in the often observed ability of the sibling species *A. gambiae* and *A. arabiensis* to rapidly evolve new behavior patterns, such as the shift from indoor to outdoor blood feeding and resting in response to indoor insecticide control programs. The intensity of malaria transmission by these mosquitoes is determined largely by environmental conditions, and where conditions support large mosquito populations and year-round abundance, parasite inoculation rates can exceed 300 infective mosquito bites per person per year (3). All past efforts to control malaria in those parts of Africa with moderate to high levels of transmission have failed.

A recent response to this problem has been the initiation of research to replace natural vector populations with populations of mosquitoes unable to support normal parasite development. An important advantage of such a strategy is that, unlike insecticide-based population reduction schemes, it is not subject to the rapid population rebound from immigration or reproduction of which mosquitoes are capable. *Anopheles gambiae* is the focus of this effort.

This strategy depends on progress in three areas: the identification of parasite-inhibiting genes, the development of technology for introducing such genes into the mosquito genome, and the development of mechanisms for moving these genes into the natural population. Interference with

Fig. 1. Blood-feeding *Anopheles gambiae*. [Photograph by Jim Gathany, CDC Photoservices]

any of several stages of malaria parasite development in the mosquito holds promising opportunities for parasite inhibition. For example, penetration of the peritrophic matrix, a chitin-containing barrier that physically separates the midgut contents from the surrounding midgut tissue, requires an ookinete-produced chitinase, the activity of which can be blocked by chitinase inhibitors or trypsin-specific antibodies (4). The thousand or more sporozoites released by the oocyst invade certain salivary gland cells by a mosquito receptor-parasite ligand interaction that could be inhibited by inappropriately expressed receptors (5).

The mosquito's own natural defense system is also under investigation. Inbred strains of *A. gambiae* have been selected that are refractory to parasite infection by several different mechanisms. One strain kills parasites by encapsulating ookinetes in a melanin capsule, a process that involves elevated phenoloxidase and hemolymph serine protease activity. Another strain lyses the ookinetes shortly after they penetrate the midgut epithelial cells (6). The recent development of a high-resolution genetic map for *A. gambiae*, combined with efficient techniques for localizing cloned genes by in situ hybridization to mosquito polytene chromosomes, will enable the cloning of these and other important mosquito genes by conventional genetic techniques (7). Whether foreign or endogenous, such parasite-inhibiting genes can then be efficiently introduced into the mosquito germline once genetic transformation tools have been developed.

Most problematic is the clear definition of a strategy to introduce and spread parasite-inhibiting genes in natural vector populations. Theoretical studies and preliminary experimental evidence support the feasibility of population replacement mechanisms involving "loaded" mobile elements or transformed endosymbionts (8), but the success of such mechanisms will require a detailed knowledge of the population structure of the target species. Genetic evidence, based on frequencies of paracentric chromosomal inversions, indicates that West African populations of *A. gambiae* and *A. arabiensis* have highly structured populations within which gene flow may be quite restricted. For example, five chromosomally distinct forms of *A. gambiae* have been recognized from West Africa (9). Most forms have relatively discrete distributions and genetically intergrade only where their distributions overlap. Two of the savanna forms, however, Bamako and Mopti, have coincident distributions in Mali and yet show no evidence of chromosomal intergradation. Reproductive isolation is not complete since both genetically intergrade with a third Savanna form, but nothing is known about the rates of gene flow among or even within these types.

In many West African populations of *A. gambiae* and *A. arabiensis*, a number of different inversions change in local frequency in a manner suggesting adaptation to climatic gradients from forest to dry zones; East and southern African populations, on the other hand, have fewer polymorphic inversions, and inversion frequencies are stable over much broader geographic areas. In addition, certain inversion karyotypes in local East and West African populations of these species show significant associations with *Plasmodium falciparum* infections and

The authors are in the Division of Parasitic Diseases, Centers for Disease Control and Prevention, Mailstop F22, 4770 Buford Highway, Chamblee, GA 30341, USA.

the propensity to feed on the blood of humans rather than animals (10).

Populations of A. gambiae and A. arabiensis in parts of Africa may experience large reductions and possibly local extinctions as the weather cycles from rainy to dry seasons. However, even in areas with extremely long dry seasons during which adults are impossible to find, chromosomal inversion frequencies are remarkably stable. This suggests that either relatively large local populations manage to survive undetected during such dry seasons (11) or local inversion frequencies are determined more by natural selection in response to ecological conditions than by drift during population bottlenecks.

Clearly, evidence of restricted gene flow will change the nature of a control strategy based on genetic transformation of an entire species. To date, almost all population genetic studies have been based on karyotyping of polytene chromosomes, which can only be scored from fourth instar larvae or females with developing ovaries (Fig. 2). The use of genetic markers that can be detected by the polymerase chain reaction (PCR), in conjunction with cytogenetic data (when available), will allow a more comprehensive examination of the relations among inversion karyotypes, ecological conditions, and mosquito behavior. It will be important to extend population studies to other locations in Africa, measure population size and its spatial and temporal stability, and define rates of gene flow over a range of geographic scales.

Although the genetic structure of populations of both A. gambiae and A. arabiensis would be expected to restrict the spread of an introduced genetic construct, other observations suggest opportunities for gene flow even between these species. For example, analysis of mitochondrial and nuclear genes in five members of the A. gambiae species complex shows that A. gambiae is most closely related to A. arabiensis and suggests that the two species may still be experiencing some interspecies gene flow, at least of mitochondrial DNA (12). In fact, chromosomally identified hybrids of all sympatric members of the A. gambiae complex have been encountered in nature at frequencies of 0.05 to 0.1 percent, and all hybrid females are fertile, some even showing higher fertility than the female parent. Also, in nature A. gambiae and A. arabiensis share some chromosome

inversions that have been moved from one species to another in laboratory experiments. This suggests that, if a transposable element or endosymbiont were used to alter A. gambiae, A. arabiensis would be affected as well at some low but potentially significant rate.
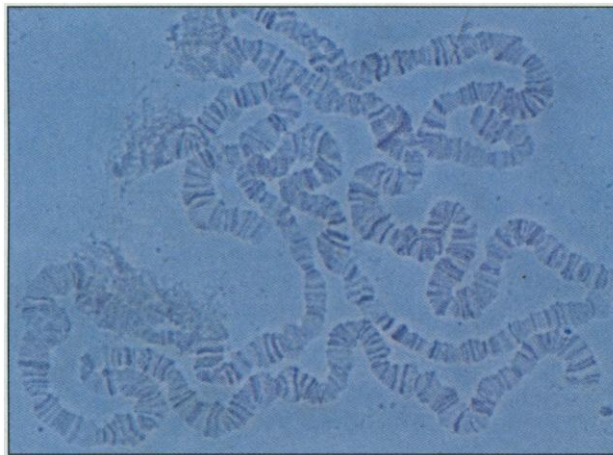

Fig. 2. Polytene chromosomes from ovarian nurse cells of Anopheles gambiae. [Photograph by Odette Mukabayire, CDC Division of Parasitic Diseases]

Control of malaria in Africa will require that all major vectors be targeted. Because one of these, A. funestus, is not closely related to A. gambiae, methods to genetically alter the vectorial capacity of A. gambiae might not be suitable for A. funestus. A single approach should be developed that would target all three, although the population biology of A. funestus and its relatives is not well understood. A. funestus contains several polymorphic inversions and it may also be genetically differentiated into locally adapted populations throughout its range in tropical Africa (13). Because this mosquito breeds in semipermanent bodies of water, there are greater discontinuities in its distribution than that of A. gambiae and A. arabiensis, which breed in temporary pools. Is reduced gene flow associated with these discontinuities?

Another approach is possible. Unlike A. gambiae and A. arabiensis, A. funestus is very susceptible to residual insecticides; previous residual spray programs have virtually eradicated this mosquito from large parts of southern Africa. In other areas of Africa where residual spray programs were undertaken, numbers of A. funestus quickly declined and recolonization was slow (13). If malaria transmission by A. gambiae and A. arabiensis can be eliminated by genetic means, transmission by A. funestus could perhaps be dealt with by more conventional technology.

Within the next two decades we will surely see laboratory colonies of A. gambiae that have been genetically engineered to be refractory to P. falciparum, given the pace

at which recombinant DNA technology and genome mapping techniques have advanced. To move the vector replacement strategy beyond the laboratory in the same time frame will take a well-executed effort, since success will depend on a continent-wide understanding of vector population biology. A transposable element newly introduced into a species could easily spread species-wide within decades, even with restricted gene flow between populations. However, for malaria control, replacement must be accomplished in years rather than decades by numerous releases. The strategy for transforming the wild population urgently needs theoretical definition with modeling and with laboratory experiments, as well as by extensive study of vector populations in the malaria-endemic countries. Rarely voiced is the need for an international consensus concerning the release of transgenic mosquitoes. What better way to foster a climate of cooperation and trust in the field than by making this effort a truly international collaboration.

References

1. J. Cattani, D. Davidson, H. Engers, Tropical Disease Research: Progress 1991–1992 (WHO/TDR Program Report No. 11, 1993).
2. F. L. Soper and D. B. Wilson, Anopheles gambiae in Brazil: 1930-1940 (Rockefeller Foundation, New York, 1949).
3. J. C. Beier et al., J. Med. Entomol. 27, 570 (1990).
4. M. Huber, E. Cabib, L. H. Miller, Proc. Natl. Acad. Sci. U.S.A. 88, 2807 (1991); M. Shahabuddin, T. Toyoshima, M. Aikawa, D. C. Kaslow, ibid. 90, 4266 (1993).
5. R. Rosenberg, Am. J. Trop. Med. Hyg. 34, 687 (1985); M. G. Touray, A. Warburg, A. Laughinghouse, A. U. Krettli, L. H. Miller, J. Exp. Med. 175, 1607 (1992).
6. F. H. Collins et al., Science 234, 607 (1986); S. M. Paskewitz, M. R. Brown, F. H. Collins, A. O. Lea, J. Parasitol. 75, 594 (1989); S. M. Paskewitz and F. H. Collins, unpublished data; K.D. Vernick and L. H. Miller, unpublished data.
7. L. Zheng, F. H. Collins, V. Kumar, F. C. Kafatos, Science 261, 605 (1993); V. Kumar and F. H. Collins, Insect Mol. Biol. 3, 41 (1994).
8. M. G. Kidwell and J. M. C. Ribeiro, Parasitol. Today 8, 325 (1992); J. M. C. Ribeiro and M. G. Kidwell, J. Med. Entomol. 31, 10 (1994); G. A. Meister and T. A. Grigliatti, Genome 36, 1169 (1993); H. R. Braig, H. Guzman, R. B. Tesh, S. L. O'Neill, Nature 367, 453 (1994).
9. M. Coluzzi, V. Petrarca, M. A. Di Deco, Bol. Zool. 52, 45 (1985); Y. T. Toure et al., unpublished data.
10. M. Coluzzi, A. Sabatini, V. Petrarca, M. A. Di Deco, Nature 266, 832 (1977); V. Petrarca and J. C. Beier, Am. J. Trop. Med. Hyg. 46, 229 (1992).
11. C. E. Taylor, Y. T. Toure, M. Coluzzi, V. Petrarca, Med. Vet. Entomol. 7, 351 (1993).
12. N. J. Besansky et al., Proc. Natl. Acad. Sci. U.S.A., in press.
13. M. T. Gillies and B. De Meillon, The Anophelinae of Africa South of the Sahara (The South African Institute for Medical Research, Johannesburg, 1968), pp. 127–152; M. T. Gillies and M. Coetzee, A Supplement to the Anophelinae of Africa South of the Sahara (The South African Institute for Medical Research, Johannesburg, 1987), pp. 78–87.
14. We thank L. H. Miller, M. Coluzzi, J. M. C. Ribeiro, and our colleagues at the Centers for Disease Control for helpful suggestions.