Perspective Piece: Needs for Monitoring Mosquito Transmission of Malaria in a Pre-Elimination World

Stephanie James,* Willem Takken, Frank H. Collins, and Michael Gottlieb

Science Division, Foundation for the National Institutes of Health, Bethesda, Maryland; Laboratory of Entomology, Wageningen University and Research Center, Wageningen, The Netherlands; University of Notre Dame, Notre Dame, Indiana

Abstract. As global efforts to eliminate malaria intensify, accurate information on vector populations and transmission dynamics is critical for directing control efforts, developing new control tools, and predicting the effects of these interventions under various conditions. Currently available sampling tools for mosquito population monitoring suffer from well-recognized limitations. As reported in this workshop summary, a recent gathering of medical entomologists, modelers, and malaria experts reviewed these issues and agreed that efforts are needed to improve methods to monitor key transmission parameters. Identified needs include standardized methods for sampling of both mosquito adults and larvae, improved tools for mosquito species identification and age-grading, and a better means for determining the entomological inoculation rate.

INTRODUCTION

Effective malaria control increasingly requires accurate spatio-temporal estimates of vector behavior and transmission intensity to understand both the species involved in transmission and the intervention tools that best could target them. Unfortunately, many existing entomological methods fail to provide realistic estimates of transmission and behavioral parameters. For example, currently available sampling tools for mosquito population monitoring, such as human landing catches, traps, and larval sampling, may collect only a small part of the mosquito population and thus provide limited and biased data. Moreover, these tools are difficult to standardize and do not work well under all ecological conditions. Methods for assessing outdoor biting are especially inefficient, yet this is fundamental to understanding the development of behavioral resistance to vector control measures and to measuring the fraction of parasite transmission that occurs primarily outdoors. The limitations of current methods for measuring malaria transmission by vector mosquitoes are expected to become even more pronounced as ongoing implementation of available control methods, including indoor residual spraying (IRS) and insecticide-treated nets (ITNs), drives down mosquito and malaria endemicity levels.¹ A recent workshop² brought together medical entomologists, modelers, and malaria experts to review the strengths and weaknesses of existing methods and to consider research needs for more effective tools and strategies to monitor key parameters involved in malaria transmission by the diverse group of anopheline vectors. This report provides a summary of the discussion and recommendations from that workshop.

PRIORITIES AND PROGRESS

Needs assessment. Malaria parasites are transmitted by several dozen different *Anopheles* species, many of which differ significantly in their ecologies.³ Mathematical models are being used to predict the efficacy of both existing and proposed new malaria interventions, allowing for evaluation of

assumptions and hypotheses under a range of conditions.^{4–6} Such models, however, are dependent upon accurate empirical data on important transmission-related parameters. Thus, models are calling attention to needs for better data on feeding location and timing (e.g., outdoor and crepuscular biting); host selection (overall biting rate on different species of host, number of feeds); sugar meal sources and feeding frequency; density dependence; mosquito migration and dispersal patterns (how far mosquitoes fly to find food and egg-laying sites, and what conditions influence these patterns); mix of local vector species (behavior and dynamics of minor vector species); effects of parasite infection on mosquito life history; and dry season dynamics (location of refugia).

Research to design new tools for vector and transmission control is highlighting needs for better data on the intrinsic rate of population increase and better methods for assessing population size of adult mosquitoes and their survival rates. Efforts to monitor and respond to the threat of insecticide resistance, and research to develop new interventions based on genetically modified mosquitoes, require an understanding of the rate at which genotypes of interest spread through the population, and what constitutes a barrier to gene flow.

Vector-targeted intervention tools are generally aimed at specific aspects of vector behavior. Insecticide-impregnated bed nets, for example, preferentially target late-night, indoor-feeding species; indoor residual sprays target indoor-resting species. Tools based on human attractants or sugar meal attractants could potentially reach a broader array of vector species than those targeted by indoor spraying and nets. Although the diversity of vector species is very broad, the diversity of behaviors that could constitute intervention targets may be far less complex because of shared behavioral traits across multiple species. To guide both the development and application of new behaviorally targeted interventions, it would be important to be able to behaviorally profile at least the major malaria vectors around the world.

***Anopheles* population monitoring.** Widely used standard mosquito sampling methods, including human landing catches, resting collections, pyrethrum spray catches, and Centers for Disease Control and Prevention (CDC) light traps are all subject to operator-related variability, such that results may not be reproducible or accurately reflective of the overall local population. Other issues with current methods include cost and inadequate sensitivity at low densities. Newer methods

*Address correspondence to Stephanie James, Science Division, Foundation for the National Institutes of Health, 9650 Rockville Pike, Bethesda, MD 20814. E-mail: sjames@fnihi.org

are using lures that mimic human odors to effectively attract female mosquitoes without requiring human “bait.” Blends of carboxylic acids have demonstrated good attractant characteristics in olfactometer studies.⁷ More recently, studies of human skin bacteria have identified 3-m-butanol and 3-m-butanoic acid as attractants, and combinations of a basic carboxylic acid odorant blend plus 3-m-butanol in MM-X traps were shown to be attractive for both *Anopheles gambiae* and *Anopheles funestus* in an African village setting.^{8,9} These odor-baited traps collect mosquito populations both indoors and outdoors, and therefore provide crucial information on two different locations where mosquitoes might blood feed. This instills optimism that odor-baited traps will improve our ability to estimate population densities of human-feeding vectors, to detect outdoor mosquito populations, and to rapidly assess remaining pockets of secondary vectors after control has substantially decreased the overall population level of primary vector species. Additional improvements will be required to make odor-baited traps a feasible tool for surveillance and vector control. Efforts are underway to test the use of odorant lures in an inexpensive trap design, including methods to ensure capture of blood-fed and gravid as well as non-blood fed females and to prolong the effectiveness of the lures. Identification of an appropriate CO₂ mimic could also improve the function of odor-baited traps.¹⁰ Efforts will be needed to assess ability to standardize such traps and to determine comparability in different settings and with different vector species.

Alternative methods will be required for monitoring male mosquitoes, and one possibility for *An. gambiae* is the use of swarms as a sampling tool. Males select landmarks to swarm, and these sites are very consistent over several years. The swarms are clustered, with “hot spots” that are likely related to a number of factors including visual cues.¹¹ Another possibility is the use of plant-based attractants for sampling.¹² These have the potential advantage of being attractive for both sexes, of functioning close to mosquito breeding sites and soon after emergence, and of catching females at all gonotrophic stages, all ages, and during diapause.¹³ More research will be needed to perfect the plant-based blends in the way that has been done for human odorants, to minimize the catch of non-target insects, and to develop functional yet inexpensive traps. For many *Anopheles* species, including both indoor and outdoor active species, barrier screens¹⁴ may prove useful to sampling as they move between blood feeding, resting, and oviposition sites. This relatively low-complexity method also allows exploration of flight patterns of male and female mosquitoes. The use of larval sampling for population monitoring is hampered by a lack of standardized methods, and the diversity and scale of breeding sites, which make it difficult to obtain representative samples.

***Anopheles* taxonomy.** Incomplete knowledge of the systematic taxonomy of the genus *Anopheles* compromises our understanding of many key parameters needed to determine the transmission dynamics of human malaria parasites. Such parameters include vector competence, mosquito behaviors, gene flow, susceptibility to and development of malaria parasites, spread of insecticide resistance, relationships among sibling species, and identification of cryptic species. The lack of comparability of results from both laboratory and especially field studies is in part a result of limited information in this area. The genus *Anopheles* is composed of 465 formally recognized species and more than 50 unnamed species¹⁵; of

these, ~70 have the capacity to transmit human malaria parasites; 41 are considered to be dominant vector species/species complexes that are capable of transmission at a level of major public health concern. Most vectors are morphologically indistinguishable members of recently evolved species complexes. Efforts are being made to develop a global map of the dominant vectors of malaria³ and to use this information to better map the distribution of shared behavioral characteristics across major vectors that could form the targets of existing or new intervention tools.⁶

The lack of adequate taxonomic information on the genus *Anopheles* is attributed to several factors. For one thing, the number of scientists with the relevant expertise in systematics is declining. Moreover, despite significant advances in DNA sequencing and comparative genomic approaches, GenBank records are incomplete and in some cases erroneous, and there are no agreed upon standardized molecular methods of identification. Efforts are underway to advance the development of an integrated, systematic approach to *Anopheles* identification through the Mosquito Barcoding Initiative (MBI) in conjunction with the Barcode of Life Database.^{16,17} The MBI initially uses a 685 bp sequence from the mitochondrially encoded cytochrome oxidase 1 (CO1) to uniquely identify *Anopheles* species. Anchored by museum specimens, the initiative links the barcode sequences with additional data, including geographic and morphological, on the type specimens. To date, MBI has completed analysis of 91% of known anophelines and the CO1 barcode successfully identifies 99% of the species in the genus. Although the MBI provides a useful tool some obstacles to this approach remain, such as the fact that some closely related species, including members of the *An. gambiae* complex, have not yet undergone complete lineage sorting. Additional work is needed to ensure that methods are standardized and that there is cooperation between the professional taxonomists and the broader user community of malaria vector biologists. In addition to the barcoding effort, work is needed to further the understanding of species population structure and behavioral ecology as intraspecific variation is essential to characterize the role of *Anopheles* species in transmission. For example, some epidemiologically important behavioral traits are labile and vary considerably within a species.^{18,19}

Cheaper and faster methods for whole genome DNA sequencing, made possible by next generation technologies, are being applied to *Anopheles* species; especially African vectors of the *An. gambiae* complex, to identify heritable traits that impact transmission²⁰; such traits include exo- and endophily, anthro- and zoophily, innate immunity, and insecticide target site and metabolic resistance mechanisms. Research needs to be expanded on standardized methods of collection, sequencing, and computational methods for genome-wide association studies of important phenotypic parameters and for determination of population structure. Results of such studies will have implications for determining vector competence and the evolution of sibling species, for estimating the spread of insecticide resistance variants, and for evaluating changes resulting from the introduction of new interventions including genetically modified organisms.²¹

***Anopheles* age grading.** Because of the length of time required for the malaria parasites to develop from ingested sexual stages to infectious sporozoites (sporogonic cycle) in the mosquito salivary glands, the survival time of infected

mosquitoes is perhaps the most important determinant of vectorial capacity. Small changes in mosquito longevity greatly amplify effects on malaria transmission. Therefore, validated and reproducible methods are needed to determine accurately mosquito survivability and to assess age structure changes in mosquito populations as a result of the introduction of vector control interventions. Although traditional methods such as parity dissection contribute to estimating mosquito age, there is a need for greater accuracy especially with respect to the timing at which mosquitoes become able to transmit infectious sporozoites to humans.

The application of near infrared (NIR) spectroscopy, currently being used in quality control efforts for crops by the agricultural industry, seeks to provide spectral data that correlate with mosquito age. Initial studies applied this method to laboratory-reared mosquitoes but subsequent studies have provided preliminary observations for semi-field and wild populations²²; with current methods, NIR spectroscopy has shown greater than 95% accuracy in distinguishing individuals of a newly colonized population of *Anopheles arabiensis* that are < 6 days old from those that are > 8 days old. Further validation using field-caught mosquitoes will need to be undertaken. It is envisioned that additional detailed analysis of the spectra will be helpful in further distinguishing mosquito species and potentially in determining infection with *Plasmodium* spp. and even other human pathogens. Although equipment costs currently are high and not readily field applicable, more research using this method may lead the way to less expensive and rapid methods to detect specific metabolites responsible for the differential spectral patterns.

Several -omics based approaches are in development in the effort to determine mosquito age. Building on work with *Ae. aegypti*, transcriptome profiling is being adapted to *An. gambiae*.^{23,24} Similarly, proteomics approaches are being extended from *Ae. aegypti* to *An. gambiae* to correlate specific proteins with age of the mosquito. Additional effort is required to assess the sensitivity and specificity of these methods for mosquitoes reared in the laboratory under different environmental conditions and for mosquitoes released into field cages in endemic areas. Not surprisingly, there is more variation in results obtained from mosquitoes reared in field cages compared with those reared in the laboratory. As in the case of NIR spectroscopy, more rapid and robust methods may be developed to identify specific age-dependent RNA transcripts and/or proteins and peptides. Research is needed to validate the findings with field-caught specimens. Given the potential labile nature of the age-specific determinants, whether a small molecule metabolite with an NIR signal, an RNA transcript, or a protein, research is also needed to establish conditions for using archived or preserved mosquito specimens or extracts from such specimens.

Primarily used for age grading of *Ae. aegypti* mosquitoes, gas chromatographic analysis of cuticular hydrocarbons (CHC), which are solvent-extracted from the legs of female mosquitoes, has been in development for some time.²⁵ These analyses have an advantage in that they may be used on preserved specimens. The relative abundance of C25, C29, and other hydrocarbons correlate with age using laboratory-reared mosquitoes, but there is more variability in studies using wild mosquitoes caught at different times of the year under different climatic conditions. The method has more recently been applied to *Anopheles* spp. in an attempt to identify and model

CHC ratios as a predictor of mosquito age. To date, such studies have produced data that can group mosquitoes into different age categories (young, intermediate, and old mosquitoes ± 3.5 days). Such determinations cannot, however, grade female mosquitoes older than 15 days—a critical time period for malaria transmission. More recently, methods such as matrix-assisted laser desorption/ionization–time of flight measurements using mass spectrometry and sophisticated statistical analysis are in development to determine age-related amounts of CHC and cuticular lipids.²⁶ These may offer improved sensitivity and specificity because of the capacity to assess an expanded number of age-grading factors. However, much more work is needed to field validate these methods.

Human-vector contact. Ultimately, all of the factors discussed previously influence vectorial capacity and must be related back to malaria transmission. There are few robust measures of vectorial capacity, and simpler measurements are needed to facilitate comparison among sites. Entomologic inoculation rate (EIR, an estimate of the number of bites by infectious mosquitoes per person per unit time) traditionally has been used as a measure of transmission intensity, but it is subject to variability and lack of standardization, and is influenced by a wide variety of intrinsic and extrinsic factors such as temperature, altitude, rainfall, and population density.²⁷

Moreover, there is doubt about the measurement of EIR when malaria case incidence is low. Calculation of EIR involves estimation of the fraction of vector mosquitoes that are infectious. This may be done by detecting sporozoite antigen in mosquitoes. Although enzyme-linked immunosorbent assay is a robust and inexpensive method for detecting parasite antigen, it may be insufficiently sensitive at low infection levels.²⁸

New serological methods may prove useful to complement or even supplant entomologic procedures under conditions of low mosquito density and/or low EIR from secondary vectors. One of these uses antibodies to mosquito salivary proteins as markers of human exposure to mosquito bites and risk of transmission. A salivary peptide has been identified that is specific to the *Anopheles* genus and highly conserved among different *Anopheles* species. Antibody reactivity to this peptide shows promising characteristics as a biomarker for human biting; increases in these specific antibody levels correlated with increased rainfall in a region of very low mosquito exposure and rapid decreases in these levels were observed in individuals after ITNs were introduced in areas of high malaria transmission.^{29–31} At this point, there is no ability to distinguish biting by different *Anopheles* species, although this would be important for estimating the relative contribution of each species to transmission. More information on the sensitivity of this assay also would be helpful, with regard to the number of bites required to elicit a measurable response. Antibodies against parasite antigens are being proposed as markers of exposure to infection,³² using response to sporozoite antigens as a surrogate for infectious bites, and response to asexual stage antigens as a surrogate for infection. Good correlation was observed between seroconversion to merozoite antigen and EIR in several African sites,³³ and seroprevalence to merozoite antigens in a low transmission setting reflected heterogeneous exposure.³⁴ There is a need to understand the duration of these responses more fully to determine their use as biomarkers for current transmission patterns. To improve the field use of these antibody-based assays, efforts

are underway to convert from use of blood specimens to detection of antibodies in human saliva.³⁵

An outstanding issue is the need to understand the extent to which human malaria vectors are feeding on non-human species, as this may influence the efficacy of certain control measures. In planning for eradication, another unknown is the potential impact on human infection of zoonotic introductions of malaria by vectors of non-human primates.

NEXT STEPS

The workshop raised a number of issues for further consideration. Participants recognized that monitoring approaches are complicated by enormous vector taxonomic and behavioral complexity, and that many widely used monitoring methods are insufficient at the very low abundance of vectors associated with widespread implementation of vector control tools such as IRS and ITNs. Presently, even measures of key transmission parameters like EIR are compromised by a lack of accuracy in estimates.

Work to improve the methods to monitor key parameters is warranted. This should include efforts to standardize measurement techniques to allow comparison of results from site to site, and development of new measurement methods for obtaining information critical for hypothesis testing. Modeling is useful for identifying these key parameters and for generating new hypotheses, but modeling requires an understanding of underlying vector dynamics. Local context is critical—for example, whether feeding and oviposition sites are close together or far apart will be an important influence on transmission—and improved precision in entomological sampling will be required to address many of the unknowns identified by modeling. Modeling has also highlighted a need for improved standardization of methods for data collection—an example is the lack of available studies that include data on both human biting rates and sporozoite rates that would contribute to more accurate estimates of parasite exposure.

The entomology community needs to converge on approaches that are as standardized as possible, despite the inherent difficulties involved. Needs expressed at this meeting include standardized methods for sampling of both mosquito adults and larvae; tools to make appropriate molecular taxonomic assignments; and better means for determining EIR.

New methods are under investigation that may help to solve problems of precision and standardization. Examples of additional measurement techniques needed include:

- New types of traps that allow recovery of samples that reflect the overall vector population and can facilitate understanding of mosquito population size and movement patterns. Such traps should preferably sample indoor and outdoor biting members of the vector population.
- New methods for mosquito species identification and age-grading.
- Better tools for assessing human-vector contact, to understand key parameters of human biting rate and proportion of infective mosquitoes.
- Methods to obtain information about poorly understood activities such as sugar feeding, swarming, and oviposition, which can provide insights for development of new control approaches.

With the commitment to a malaria elimination and eradication agenda, current interventions are being rolled out on increasingly larger scale and strategic decisions are being made about what additional interventions will be required.³⁶ Thus, participants felt some urgency about the necessity for improving methods to collect crucial transmission data to support research and surveillance. It will be important to be selective about what is being measured, based on the specific questions being asked and assumptions about the mechanism of action of the intervention being assessed. Efforts should focus on “need to know,” rather than “nice to know,” issues. Planning might include designation of sentinel sites for collecting and archiving specimens to allow tracking of long-term changes and application of advanced methods that are not yet available. Some of these needs could be addressed through better self-organization within the research community, even in the absence of additional directed funding. A community-wide effort is needed to prioritize and integrate ideas for improving monitoring of mosquito malaria transmission.

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Authors' addresses: Stephanie James and Michael Gottlieb, Science Division, Foundation for the National Institutes of Health, Bethesda, MD, E-mails: sjames@fnihi.org and mgottlieb@fnihi.org. Willem Takken, Laboratory of Entomology, Wageningen University and Research Center, Wageningen, The Netherlands, E-mail: willem.takken@wur.nl. Frank H. Collins, Notre Dame University, Department of Biological Sciences, Notre Dame, IN, E-mail: frank@nd.edu.

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