COMMENTARY

Malaria genotyping for epidemiologic surveillance

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Malaria control and elimination have, for more than a century, relied on traditional surveillance methods: catching mosquitoes or examining blood slides under a light microscope. Recent technological advances have started to change the game, including high-quality rapid diagnostic tests for malaria. At the same time, advances in the study of parasite genetics, especially for *Plasmodium falciparum*, have enabled important insights into specific questions regarding parasite biology, such as the mechanisms underlying immune evasion and the origins and evolution of drug resistance (1–3). Additional insights have come from genetic analysis of mosquito populations (4). Although genetic studies have improved our basic understanding of malaria, links between malaria genetics and transmission—and a path to generating operationally useful information—have been more elusive. Malaria epidemiology and population genetics have flirted with each other, but each side has tended to see the world from its own point of view. In PNAS, Daniels et al. (5) provide an example of what a successful marriage might look like.

Marrying Epidemiology and Genetics

A happy marriage requires good communication, but, until now, each academic discipline has tended to speak its own language. Most mathematical models designed to understand or predict malaria epidemiology relegate the complex dynamics of parasite clones within and between individuals to oversimplifications that do not provide much insight into what the parasite population might look like (6). On the other side, population genetic studies have demonstrated that areas with higher malaria transmission tend to have higher genetic diversity, that isolated areas (geographically or anthropologically) tend to have parasite populations that are more distinct from each other, and that decreases in transmission measured via traditional means can be seen in population genetic signals (7–10). Such studies are interesting and may affect malaria epidemiology in profound ways but do not provide advice that a malaria control program officer could respond do. What should a control program do in response to a doubling of the effective population size? What does it mean if the proportion of infected individuals harboring multiple parasite clones decreases by 10% after bednets are distributed?

Daniels et al. make some interesting observations regarding the population of *P. falciparum* in Thiès, Senegal.

Part of the problem is that there is no easy way to directly translate parasite genetic diversity into a measurement of parasite transmission. Even a simple genetic metric, e.g., the proportion of infected individuals harboring multiple parasite clones in a given geographic area, is affected by a complex network of processes including overall transmission intensity, spatial, temporal, and individual heterogeneity in transmission, host immunity, treatment seeking behavior, antimalarial drug efficacy, and connectivity of humans and mosquitoes to other areas where malaria is endemic.

Daniels et al. make some interesting observations regarding the population of *P. falciparum* in Thiès, Senegal. The authors genotyped 24 single-nucleotide polymorphisms (SNPs) in ∼1,000 samples collected from individuals diagnosed with symptomatic malaria over a period of 8 y. They noticed that specific combinations of SNP "barcodes" came up repeatedly in a given year and that some even persisted across years. Detecting identical barcodes would be unusual in most of Africa, because *P. falciparum* undergoes sexual replication in the mosquito during transmission from one person to the next, rapidly shuffling genomes via recombination when individuals are infected with multiple clones for the mosquito to ingest. The fact that the authors were able to detect more repeated barcodes over time, along with other genetic signals such as a decrease in effective population size, suggested that something untoward was happening to the parasite population. Indeed, such genetic signals have been previously associated with decreases in malaria transmission by the authors (11) and by others in different parts of the world (10).

However, how do we obtain quantitative information regarding transmission from these genetic signals? Ideally, simple mathematical models could perform this alchemy. In populations where malaria transmission has been constant and where prevalence and multiplicity of infection remain constant, the effective reproductive rate (R) is 1: each parasite infecting a human would be transmitted to exactly one other human. When two or more distinct parasite clones infect a single human, there is a chance that parasites will recombine during mosquito transmission to the next human host, generating genetic diversity balanced by loss via genetic drift. If the probability of recombining is D, branching processes predict the total number of offspring would be $1/(1-D)$. In a population where overall transmission is increasing or decreasing slightly, such that the number of offspring is $1 \pm E$, the total number of offspring would be slightly higher or lower at $(1 \pm E)/(1-D)$. When transmission is increasing, each clone will have slightly more offspring, increasing diversity, and vice versa. Although this example can qualitatively explain the authors' observations, it also highlights the limitations of such a simple model: how does one actually measure D, taking into account the complex interactions between hosts, parasites, and the environment?

Given the complexity of the relationship between genetic metrics and epidemiologic parameters, the authors took advantage of an

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agent-based model, in which individual mosquitoes, people, and the parasite genotypes they were infected with were simulated. The significant advance of this study is the synthesis of multiple observable genetic metrics, for instance, the number of sample barcodes in a year that were repeated, into quantitative estimates of parameters relevant for measuring transmission. In particular, the authors were able to detect changes in *R*, the effective reproductive rate of the parasite in endemic populations. By performing a large number of simulations under various potential scenarios (e.g., with different values for *R*), the model was calibrated to observed data to determine the scenarios that were most likely. The results from these simulations suggest that *R* for *P. falciparum* in Thiès very likely decreased from 2006 to 2010, and then rebounded sometime between 2010 and 2013. The validity of these conclusions is supported by traditional surveillance data, indicating that the incidence of malaria in Thiès decreased and rebounded over approximately the same time period.

Genetics for Surveillance?

The coupling of field-based genetic data with appropriate epidemiologic modeling, as demonstrated by Daniels et al., suggests that useful molecular epidemiology tools may soon be accessible to those responsible for malaria control. However, two challenges remain before decision makers on the ground can use these tools in an operational context. First, the genetic signals of falling transmission were clear in Thiès, where diversity was low and the parasite population appeared to be relatively isolated. Will the same signals be detectable in other settings, especially those in which transmission is affected by importation of parasites due to human population movement (12)? If not, can other signals be detected, and will they be amenable to straightforward sampling and genotyping methods? Fortunately, the flexible modeling approach proposed in this study should be able to incorporate a variety of different genetic inputs with appropriate modification. The bigger question is whether and in what situations such tools will be cost-effective and practical. In this paper, the authors present malaria incidence data to validate the accuracy of the conclusions obtained from molecular epidemiology, which is appropriate for proof of concept. Once the accuracy of such methods is validated in multiple settings, the higher burden will be to demonstrate that they add value to other surveillance methods, including entomological monitoring, passive and active case detection, and serology (13, 14). As suggested by the authors, it is possible that it may be easier in some areas, especially those with less developed malaria surveillance systems, to obtain some parasite samples than to ensure high-quality case data. More importantly, obtaining overall estimates of transmission may just be the tip of the iceberg. Parasite genetics has the potential to provide a rich set of data to more completely characterize parasite transmission, including distinguishing imported from locally transmitted cases (15), characterization of parasite flow between regions to identify the scales of transmission (8, 9), and identifying important drivers of transmission. If the work by Daniels et al. marks the beginning of an ongoing and productive conversation between malaria epidemiology and population genetics, such tools will hopefully become the expected offspring.

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