The unpredictable past of *Plasmodium vivax* revealed in its genome

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Until quite recently, very little information has been available about the genome content and structure of parasitic protozoa. This inadequacy has been rectified by the advent of high-throughput strategies that permit sequencing of whole genomes and enhanced computational capacities that render this information tractable. The current list of complete or near-complete genomes includes some of the greatest scourges of humans and their domesticated companions. Among these miscreants are several members of the genus *Plasmodium*, the agents of malaria. Scores of species of *Plasmodium* have been described and comprise pathogens of every major group of terrestrial vertebrates. At present, no less than seven of these species are subjects of major genome-sequencing projects, and it is anticipated that the comparison of these genomes will provide invaluable insights as to how genomic contents may mediate the interaction of the parasite with its host (1). Our understanding of genome structure and function has been facilitated by models of evolutionary biology, and these findings validate theoretical predictions about genome evolution that predate by several decades our ability to collate their contents (2). Coupled with these comparative approaches to understand how genomes have shaped different species are studies that use the new information about genome polymorphism to better under understand the population structure and evolutionary history of individual species. The article by Leclerc *et al.* (3) in a recent issue of PNAS exemplifies how "population genomics" provides a means of examining the evolutionary forces acting, in both the past and present, on a species. The authors assayed polymorphism at several genetic loci of the human malaria parasite, *Plasmodium vivax*, and compared the extent of this polymorphism with that of other closely related primate malaria species. Like previous reports of a paucity of genetic polymorphism in the malignant malaria parasite *Plasmodium falciparum* (4, 5), Leclerc *et al.* (3) found extremely low levels of polymorphism in *P. vivax* and concluded that a selective sweep and/or population bottleneck must have occurred in this species' recent evolutionary past.

Recent History of a Modern Scourge

At first, Leclerc *et al.*'s (3) finding that some profound reduction in population size has occurred in the recent evolutionary past of *P. vixax*, through either selective or stochastic processes, may not seem surprising. After all, selective sweeps and/or bottlenecks are not extraordinary among human pathogens, including the aforementioned *P. falciparum*, but also are known to have occurred in parasitic protists such as *Entamoeba* spp. and *Toxoplasma gondii* (6, 7). But *P. vivax* differs from these other parasites in several important respects. First, it is far more cosmopolitan in distribution and more tolerant of temperate climates than *P. falciparum*, which is restricted primarily to the world's tropics. Second, *P. vivax* is widely regarded to be a true anthroponosis rather than zoonosis, like many of the parasitic protozoa infecting the digestive system, and so it is thought to share a long-term evolutionary history with our species. But this latter assumption brings us to the second remarkable finding reinforced by Leclerc *et al.*'s (3) data. Most known species of primate malaria claim an Old World or Catarrhine monkey as their host species, and not surprisingly, these parasites have a phylogeny largely consistent with that of their respective hosts and reflect a diversification of the superfamily Cercopithecoidea, which includes various species of macaques. However, *P. vivax* appears to be genetically indistinguishable from *Plasmodium simium*, a parasite of the New World Cebidae monkeys. Indeed, *P. vivax* and *P. simium* share alleles at all 13 microsatellite (MSAT) loci and all but one of the larger tandem repeat (TR) loci examined by Leclerc *et al.* (3), whereas the other Old World primate malarias have alleles largely endemic to species.

Population genomics provides a means of examining the evolutionary forces acting on a species.

The virtual identity of *P. vivax* and *P. simium* raises the question whether these are monkey parasites recently introduced to humans or human parasites that have spilled over into wild monkey populations. On the basis of a stringent parsimonious interpretation of phylogeny and other biological factors, the monkey-to-human transfer has been put forth as more probable (8). Low levels of MSAT and TR polymorphism in *P. vivax* are consistent with the monkey-to-human transfer theory, wherein the global distribution of *P. vivax* would probably have originated from a small propragule transmitted from monkeys to humans in very recent historical times (3). However, the close phylogenetic relatedness of both these species to the Old World monkey malarias seems problematic for this hypothesis. To date, the most compelling argument against the recent introduction of *P. vivax* into human populations from a monkey donor has been the historical record of medical conditions evocative of *P. vivax* infection dating back to ancient times and thus preceding European colonization of the New World by millennia.

Parasite Flux Among Host Species

What can be stated with certainty based upon laboratory experimentation as well as field observations is that malaria parasites do appear to have a propensity for horizontal mobility among host species, and primate malarias are no exception. *P. simium* has been reported from at least three different genera of New World Cebidae (9). And so it may be that some of the confusion about the archetypal host of *P. vivax* may stem from the flawed assertion that it is necessary to impose a strict host relationship upon a parasite whose tastes run more catholic than was first suspected. In this light, host shifts need not be considered limiting, and thus we may be less restricted in a parsimonious interpretation of the distribution of parasites among host taxa.

See companion article on page 14455 in issue 40 of volume 101.

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Carter (10) has put forth a scenario in which the ancestor of *P. vivax* and *P. simium* diverged from the lineage leading to the diversification of Asian primate malaria ≈2–3 million years ago. He goes on to suggest that this ancestor was a human parasite that was relegated to glacial refugia during the last Pleistocene glacial period ≈100,000 years ago. According to Carter's model (10), the subSaharan African refugium would have been the ideal place for *P. vivax* proliferation, whereas elsewhere it became all but extinct, most notably in Southeast Asia. The evidence to support this hypothesis, albeit circumstantial, comes from several studies suggesting that human resistance to *P. vivax* infection among indigenous African populations arose as a direct result of selection from repeated long-term exposure of these populations to *P. vivax* infection. Specifically, the refractory phenotype, often referred to as Duffy group negative, is determined by mutations in a single genetic locus that trace back to a most recent common ancestor 97,000 to 6,000 years ago (11).

That *P. vivax* was originally a human parasite and that it left Africa with its hosts during the "out of Africa" diaspora seem to stand as hypotheses wholly inconsistent with the suggestion that *P. vivax* was introduced to humans only in recent times after European colonization of South America. But it may be possible to rectify the difference between these two story lines. Although Leclerc *et al.* (3) have reported extremely low levels of polymorphism among MSATs and TRs, there is an abundance of evidence for higher levels of polymorphisms at loci encoding proteins (12). Indeed, Li *et al.* (13) showed that *P. vivax* comprises two distinct lineages that can be distinguished by a chromosomal inversion and certain mutations in an ORF in the plastid genome.

Understanding the evolution of human malaria requires an account of the evolution of the mosquito.

Moreover, these two types showed a marked phenotypic difference with respect to their preferred mosquito vector. The two types were designated Old World and New World. *P. simium* appears to be like an Old World type along with several extant Eurasian human isolates of *P. vivax*, whereas New World types correspond to South and Central American human isolates. The investigators went on to demonstrate convincingly that the Old World types show markedly higher vector competence in *Anopheles freeborni* than did the New World types (13). This marks the importance of understanding that the evolutionary history of human malaria will require that we take account of the evolutionary history of the malaria's other host, i.e., the mosquito. Ultimately, the complete story of the origin of *P. vivax* will require further sampling of *P. simium* populations and an interpretation of these data in the context of the better-understood evolutionary histories of the primate and mosquito hosts. As pointed out by Leclerc *et al.* (3), it should be relatively straightforward to determine the direction of host transfer by identifying which group, *P. vivax* or *P. simium*, has the greater polymorphism and is hence the ancestor.

Genome information has provided evolutionary biologists with new tools in the form of genetic markers for better understanding the history of parasite species. This information has transformed the field by drawing investigators from diverse academic disciplines, epidemiology, immunology, and biochemistry, to name a few, to more fully appreciate Dobzhansky's adage (14) that the biological world is understandable only when we view not only the products but also the processes that have shaped the world around us. A deeper understanding of the origins of the agents of infectious disease provides not merely esoteric but also practical knowledge about how to best prevent their further proliferation. Leclerc *et al.* (3) have presented a population genomic analysis that suggests that *P. vivax* malaria, like *P. falciparum* malaria, may trace its ancestry to certain very recent events. Knowing how genes and genomes of these organisms have changed and understanding the mechanisms and rates of these changes will prove invaluable to the goal of developing strategies to intervene in parasite transmission.

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