Resolving the phylogeny of malaria parasites

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Regardless of what aspect of malaria one might discuss, superlatives will always be abundant in the conversation. That is because human malaria parasites are among the deadliest scourges of our species, have been recorded farther back in our recorded history than any other pathogen, and are among the first pathogens to be identified and studied in a modern public health context. However, despite the extreme significance to the health of our species, malaria has been a most elusive subject of scientific inquiry, and hence a persistent challenge for those seeking to interrupt its transmission. In PNAS, Outlaw and Ricklefs (1) present a unique insight on the phylogenetic relationships among these parasites. To appreciate the relevance of these findings fully, it is necessary to contextualize them in light of the past 130 y of malaria research.

Malaria History

Before discovery of its protozoan agent by Laveran in 1880, malaria was believed to be caused by noxious fumes emanating from swamps. Nearly 2 decades after that, British and Italian scientists working separately would demonstrate that the parasite was transmitted by the bite of a mosquito.

By the time the mosquito-borne etiology of malaria was published, it was known that malaria in humans was a disease caused not by one but several different species of parasite. At least five of these species are now thought to be of public health significance, and one of these, *Plasmodium knowlesi*, was only very recently appreciated as a legitimate human threat (2). There are, in fact, hundreds of species of parasites that might broadly be described as malaria parasites infecting mammals, birds, and reptiles (3).

Coatney and Roudabush (4) pointed to another malaria superlative in 1949 when they noted that "the nomenclature of malaria parasites is one of the most confusing in all zoologic literature." This confusion stems, in part, from the fact that the group of organisms referred to as malaria parasites actually comprise several genera within the order Haemosporidia (Phylum Apicomplexa). Before the advent of molecular phylogenetics, the only means of determining the relationships among these genera were based on host and vector specificity, morphological characteristics, and/or life history traits. These phenotypic characters may derive from adaptive changes and not strictly adhere to phylogenetic descent, rendering them of limited use to resolve systematic relationships (5). Moreover, if these traits are used to generate putative species trees, it then becomes tautological to determine instances of adaptation, for example, of shifting host and/or vector specificity among the parasites themselves.

Malaria Phylogeny

Molecular phylogenetics brought promise for resolving this conundrum. If relationships of malaria species can be determined objectively by inferring the descent of individual gene sequences, the resulting species trees can serve as a framework for evaluating the likelihood of host shifts, morphological plasticity, and/or adaptation of life history traits. In particular, it is now plausible to determine how the characters long considered diagnostic of a "malaria parasite" were acquired and/or lost in their respective lineages. In particular, traits of "malarianess" could be examined objectively by means of independently derived phylogenies.

Outlaw and Ricklefs have offered an intriguing interpretation on the question of malarial origins.

Valuable insights came from the earliest molecular phylogenies of malaria parasites. For example, phylogenetic analyses allowed us to determine that the digenetic life style—that which requires two separate host species—has evolved multiple times within the phylum (6, 7). Also among the most interesting of these findings was the fact that the human malaria parasites are not a monophyletic group, indicating that strict descent with the vertebrate host is not the rule and that shift of host preference occurred repeatedly in the evolution of parasites of the order Haemosporidia (8). We also discovered the potential bias of taxon sampling when inferring origin of phenotypic traits (9). For decades, parasitologists had hypothesized that *Plasmodium falciparum* was so pathogenic because it had only been acquired recently as a human pathogen from a bird origin, and one the first 18S rDNA phylogenies seemed to confirm this hypothesis (10). Nonetheless, more thorough taxon sampling indicated that this was not the case and that, in fact, *P. falciparum* is part of a larger group of diverse hominid parasites (8, 11, 12).

Ultimately, rDNA sequences proved of limited use for resolving relationships within the order Haemosporidia. Thus, for greater resolution, systematists turned to mtDNA, particularly the cytochrome B locus (11, 13). Among the most comprehensive datasets was that of Perkins and Schall (11), who sampled three of the major genera, *Hepatocystis*, *Haemoproteus*, and *Plasmodium*, commonly regarded as malaria parasites *sensu latu* (14). They subsequently expanded this study to include DNA sequences from the three parasite genomes (nuclear, mitochondrial, and apicoplast) (5). With this comprehensive molecular phylogenetic evidence, Perkins and colleagues (5) provided a framework for testing the origin of key malarial characteristics. The two essential malarial traits they examined are central to the doctrinarian identity of malaria. The first is the existence of asexual proliferation in the intermediate host blood, referred to as erythrocytic schizogony or merogony. The second important trait is the presence of the "malarial pigment" hemozoin, a biocrystalized byproduct of hemoglobin digestion. Importantly, it was concluded that these two essential malarial traits did not adhere to a strictly parsimonious model of descent; that is, these characters have evolved multiple times in the evolution of the order (11).

Although comprehensive molecular phylogenetics of the haemosporidian genera have gone a long way toward resolving the origins of traits considered fundamental to the identity of malaria, efforts to date have been flawed by a single assumption that turns out to be incorrect. That flaw lies in the choice of outgroup comparison for generating the gene (and multigene) trees from which the species trees are inferred. In each instance, the authors chose related but undisputedly nonmalarial species for outgroup comparison. The choice of outgroup is fundamental in phylogenetic inference and

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establishes a priori the location of the root of the ingroup taxa. In the case of the two most comprehensive molecular phylogenetic studies of this group, the chosen outgroup was *Leucocytozoan*, a genus closely related to but not generally considered to be one of the malaria parasite groups because it lacked both pigmentation and merogony (5, 11).

Outlaw and Ricklefs (1) have used a means to determine the proper rooting for the group of Haemosporidia parasites. Rather than assuming *Leucocytozoan* is the outgroup, their approach infers the root from the data (15). The results are astounding and present a previously undescribed interpretation for the evolution of this group. Their analyses suggest that *Leucocytozoan* is a sister group to the avian parasites of the genera *Haemoproteus* and *Parahaemoproteus*. Moreover, the evidence supports monophyly of major clades corresponding to those parasites with mammalian intermediate hosts (*Plasmodium* and *Hepatocystis*) and to those parasites with avian/reptilian intermediate hosts (*Plasmodium*, *Haemoproteus*, *Parahaemoproteus*, and *Leucocytozoan*). A strict parsimony argument would suggest that the lesser known bat parasites of the genus *Polychromophilus* appear to have arisen by horizontal transfer from an avian host.

Malaria Taxonomy

Based on this interpretation of the molecular data, it now appears that the two fundamental phenotypic traits on which traditional taxonomies were based, in fact, evolved once within the group and then underwent secondary loss in subsequent lineages. In the case of merogony, the trait was lost in all lineages except those delineated as *Plasmodium*. In fact, the taxonomy of the genus *Plasmodium* is based on the presence of merogony; hence, these taxonomic designations no longer appear to be valid. That *Plasmodium* does not constitute a monophyly comes as no surprise, because every phylogeny of major avian/reptilian and mammalian parasites has demonstrated the paraphyly. However, this most recent study lays open the intriguing possibility that the major characteristic that has long been held up as making *Plasmodium* unique is, in fact, a primitive character in the order.

The nomenclatural ramifications of this latest systematics study are clear; there is grave need for taxonomic revision of these groups. In particular, we must consider reassignment of the parasites of birds/reptiles currently assigned to the genus *Plasmodium*. If the first half of the 20th century can be characterized by haemosporidian taxonomy in flux, the second half can be characterized as static. No major attempts have been made at taxonomic revision in the era of molecular systematics. Where debate has occurred, it has concerned the more esoteric question of what constitutes a malaria parasite (14, 16); however, because the genera within the opposing nomenclatural models do not themselves constitute monophyletic groupings, those interpretations are inherently flawed as well.

This recent phylogenetic interpretation of the Haemosporidia has implications beyond taxonomy. It is probably worth mentioning that the phylogeny of Outlaw and Ricklef (1) is only as good as the data on which it is based and that this situation may change as additional taxa are included and more genes are analyzed. Nonetheless, the lack of a priori assumptions about the position of the tree's root has great appeal. In the end, the greatest utility of these phylogenies is to be found in their biological relevance, particularly in their utility for establishing testable hypotheses that might not otherwise have been considered. Long-term research objectives should include probing the genomes of more haemosporidian parasites. If it is the case that merogony and hemozoin pigmentation are primitive traits lost in some lineages, there will most certainly be some signature of that loss of function in the genomes of the parasites that lack these traits. Verifying these losses will not only provide a test of the phylogeny of Outlaw and Ricklef (1) but will yield invaluable insights into the evolution of these parasites.

One of the promises that evolutionary studies of malaria (and other pathogens) hold for public health and epidemiological relevance is that by establishing accurate phylogenies, we will be better able to determine how parasite host and vector preference has changed (or remained the same) throughout the course of evolution. Robust phylogenies allow us to test hypotheses about how parasites have moved from one species to another, and knowing how this might have happened in the past might inform us about the likelihood that similar movements will occur in the future. This has proven to be of great interest in looking at the origins of the hominid malaria parasite in particular (12, 17, 18). Outlaw and Ricklefs (1) have offered an intriguing interpretation on the question of malarial origins that will likely stimulate much more work in the future.

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