Molecular Systematics of malaria parasites

The malaria parasites are included within the order Haemosporidia, phylum Apicomplexa. At least 15 genera of haemosporidians have been placed into the family Plasmodiidae. The best-known genera are *Plasmodium*, which is the cause of human malaria (more on those species below), and *Haemoproteus*, found primarily in birds.

The common term, "malaria parasites," is controversial in its use. Malaria is a disease, not a parasite. Thus, it is not appropriate to write or speak of "malaria infecting a bird." So, the parasites should be referred to as "malaria parasites" just as we might refer to "the cold virus." But, malaria is a human disease, with characteristic symptoms. Infection causes pathology in other vertebrate hosts, but is it the same disease? Also, should the term be restricted to *Plasmodium*, and not to other genera in the family? We prefer that common names be based on monophyly, but the choice of a node on a phylogenetic tree to define any higher-level taxon (above species level) is subjective. We therefore call all genera in the family, the "malaria parasites," and include *Leucocytozoon* in this group. *Leucocytozoon*, though, could well be placed into a separate family because it is an outgroup to all the others studied to date.

The genera are defined by life history traits. Picking the two best known, *Plasmodium* and *Haemoproteus*, illustrates this point. *Plasmodium* when it first enters a vertebrate host, undergoes a bout or several bouts of asexual replication in solid tissues, then emerges in the blood to infect blood cells. Asexual replication continues, and eventually non-replicating gametocytes are produced. The male and female gametocytes are the stages that are transmitted to the biting vector where sexual recombination occurs. *Haemoproteus* is similar except all the asexual replication in the vertebrate occurs in the solid tissues, and only gametocytes are seen in the blood. This must have been a major evolutionary step, and some authors suggest that *Haemoproteus* is the ancestral life cycle.

A more extreme difference is seen in *Hepatocystis*. Again, only gametocytes are seen in the blood, but they are huge, filling the entire red blood cell, and do not look at all like those of *Plasmodium* or *Haemoproteus*. The asexual stages are found in the liver (thus the generic name), and the dividing cell is huge, even visible to the naked eye.

How are these genera related? Molecular phylogenies should reveal their relationships. But, what gene should be used? A rapidly evolving gene would be useless because the relationships are deep, and the splits occurred

very long ago. *Plasmodium* is found in lizards in North and South America, Australia, Asia, and Africa, and thus the genus must antedate the split of the continents. A rapidly evolving gene would suffer repeated mutations at all sites that are able to tolerate changes, thus becoming "saturated" over such long periods of time, and any phylogenetic signal would be lost.

The gene first used in early studies of the molecular phylogeny of malaria parasites was the small subunit of the ribosomal RNA gene. This is a gene widely used to recover deep nodes on phylogenetic trees. However, in *Plasmodium* and related parasites, there are multiple copies of this gene, which become active during different stages of the life cycle (asexual stage, sexual stage, etc.). These copies of the rRNA gene in the parasites do not evolve together, and thus diverge over evolutionary time. Susan Perkins of the American Museum of Natural History has been studying this gene in detail for the entire phylum and concludes that it is a poor key to the phylogeny of the parasites.

Allison Creasey, from the malaria group at the University of Edinburgh, appears to have been the first researcher to use the cytochrome b gene for systematic studies of *Plasmodium*. Cytochrome b is normally a rapidly evolving gene, but for the malaria parasites is unusually slow. Perkins and Schall have used the gene in a large study of the phylogeny of malaria parasites (2002, *Journal of Parasitology*), and found no evidence of saturation. This study incorporated 52 taxa from throughout the world, and from bird, reptile, and mammal hosts.

The range of taxa studied is important. An early found that *P. falciparum* was closely related to bird malaria parasites suggesting its virulence is a result of a recent transfer from birds to humans. (Recent is relative! Thousands of years is a very long time for these parasites, so the high virulence cannot be a result of a "recent" transfer.) However, *P. falciparum* is quite different from other malaria parasites of mammals, and including a single bird malaria parasite resulted in "long branch attraction" of the most different taxa, a spurious clustering.

The Perkins and Schall study found that the malaria parasites of mammals form a well-supported clade. Rodent parasites are one clade, and the sister clade is the primate parasites. *P. falciparum* and *P. reichenowi* form a distant sister clade to the rest of the mammal parasites. Thus, *P. falciparum* really is a different parasite, but falls within the mammal malaria parasite clade.

The "genus" *Hepatocystis* falls within the *Plasmodium* clade of parasites. Thus, these parasites should be considered *Plasmodium*. Likewise, the *Haemoproteus* are polyphyletic (bird vs. reptile parasites), but both forms are contained within the *Plasmodium*. Again, *Haemoproteus* is not a valid genus, but actually are at least two groups within *Plasmodium*.

The relationships of species within *Plasmodium* have long been defined by number of merozoites produced by a schizont (daughter cells per mother cell) or host class. These are also not phylogenetically informative. Species of *Plasmodium* producing few merozoites can be closest relatives of those producing many (tiny *P. agamae* and massive *P. giganteum* of lizards, for example).

Thus, the standard characters used to define genera and the relationships within genera are not phylogenetically informative. Life history traits have evolved convergently within the *Plasmodium* clade.

Should we abandon the term "*Haemoproteus*"? These parasites really are distinct in life cycle, and often morphology, and are primarily parasites of birds. We continue to use this term and discuss the "genus" *Haemoproteus*; it has a long history, and the term is still useful.

Ah, but what will an analysis using another gene reveal? Is it possible that mitochondria (the home of the cytochrome b) move between quite different parasites (lateral transfer)? This has been found between closely related species of rodents, for example, but could it have happened between *Plasmodium*, *Hepatocystis*, and *Haemoproteus*? If so, all bets are off. We need data on a nuclear gene!

Another important issue: The *Plasmodium* and *Haemoproteus* of birds are quite diverse based on morpho-species, and also based on cytochrome b sequences. Some researchers argue that a single base difference in this gene indicates a species. If so, there appears to be a vast cryptic diversity of these parasites. For example, the studies in Vermont and Europe both show about as many cytochrome b lineages as species of birds studied (this is not a result of host specificity, though). Could there be thousands of cryptic species of avian malaria parasite? Unfortunately, we have very little data on the diversity of cytochrome b within species of the parasites. That is, we must differentiate within-species vs. among-species variation in the gene.

Cryptic species have been discovered using gene sequences. Perkins has found that *P. azurophilum* of *Anolis* lizard in the Caribbean islands is actually two species with identical morphology. This story is presented in the *P. azurophilum* section in Lizard Malaria Parasites on this website. For many years, we have thought there are four species of *Plasmodium* infecting humans, but a recent study found another, cryptic with *P. ovale*. Thus, we have five *Plasmodium* infecting people. There must be many more of these cryptic species to be discovered in the many hosts exploited by malaria parasites.

Perkins, S. L. and J. J. Schall. 2002. A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. *Journal of Parasitology* 88:972-978.