

7 *Primate malarias: evolution, adaptation, and species jumping*

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This female *Anopheles freeborni* is taking a blood meal from a human host. Photo by James Gathany and made available to the public domain and thus free of any copyright restrictions by the CDC <http://phil.cdc.gov/phil/home.asp>

Introduction

Malaria is one of the most widespread infectious diseases of modern vertebrates, with an endemic distribution that spans the globe's tropic, subtropic, and some temperate regions (Figure 7.1). The disease is caused by

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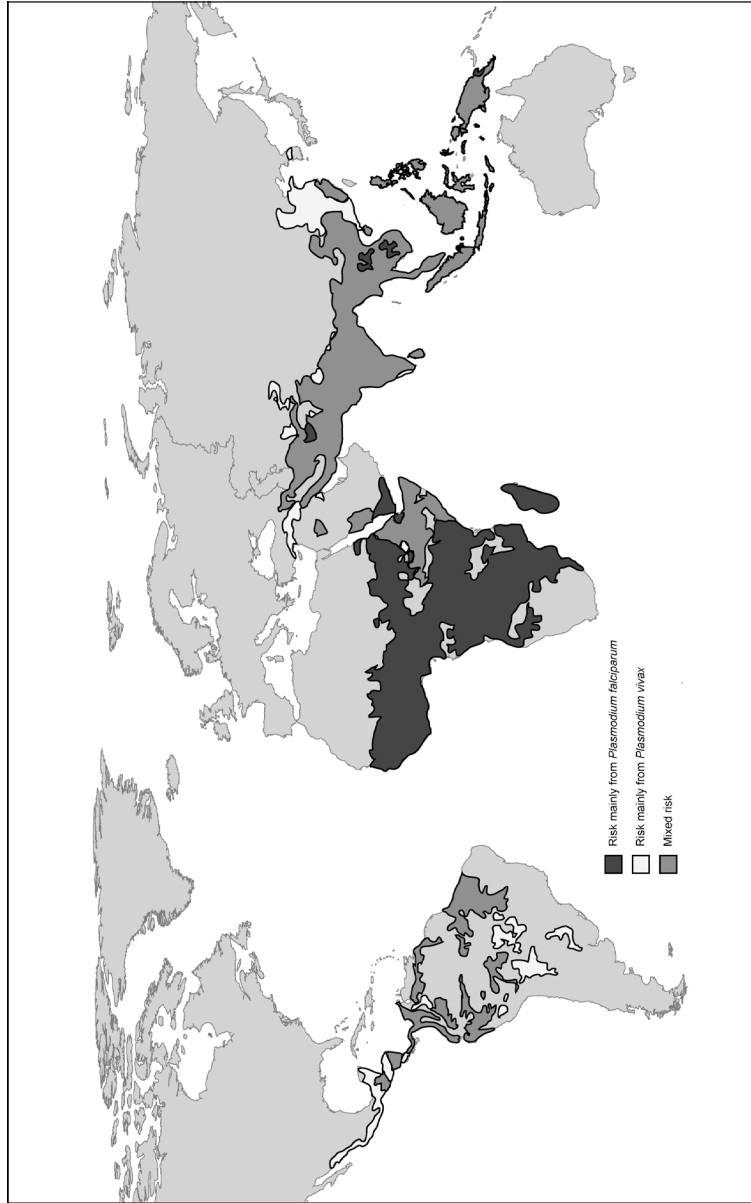


Figure 7.1. Distribution of human malarial risk in 2005. Redrawn from Guerra *et al.* (2006).

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protozoan parasites belonging to the Phylum Apicomplexa (Table 7.1). The most common agents of malaria are members of the genus *Plasmodium*, but species from several other genera can also cause disease in birds, squamates (lizards and snakes), and some mammals. Currently close to 200 species of the genus *Plasmodium* are recognized, based primarily on their life-history traits, morphology at different life cycle stages, and host species they infect. More than 50 species infect mammals (mainly primates and rodents) (Collins & Aikawa, 1993), over 30 infect birds (van Riper *et al.*, 1994), and close to 90 infect squamates (Telford, 1994). Genetic studies suggest that there also exist numerous “cryptic” species of *Plasmodium* and other genera of malarial parasites in lizards (Perkins, 2000) and birds (Bensch *et al.*, 2000, 2004; Hellgren, 2005). Thus, current estimates of the diversity of malaria-causing protists are likely to be low.

The global biological significance of malarial parasites is staggering. The World Health Organization estimated that 396 million cases of human malaria (and 1.1 million deaths) occurred in 2001 due to infection by *Plasmodium falciparum*, the most malignant of the four parasite species responsible for the disease in humans (WHO, 2003). For 2002, the estimated number of human cases of malaria due to *P. falciparum* infection was 515 million, with over 70% of those occurring in Africa (Snow *et al.*, 2005). Another 71 to 80 million cases are estimated to be caused annually by *P. vivax*, which is responsible for more than half of the cases of human malaria outside of Africa (Mendis *et al.*, 2001). More than 3.2 billion people – roughly half of the world’s human population – were estimated to be at risk for malarial infection in 2005 (Guerra *et al.*, 2006) (Figure 7.1), and projections suggest that over 80% of the global population, or more than 8.8 billion people, will be at risk for infection in 2080 (Arnell *et al.*, 2002).

In the future, the worldwide morbidity (i.e. rate of incidence) of human malaria is also expected to increase, in some areas, dramatically, because of population growth and urbanization in regions of high malarial risk, changing patterns of land use and land cover in the tropics, increased population mobility, and global climate change (Hay *et al.*, 2004; Sutherst, 2004). Climate changes – particularly altered rainfall patterns and increased global temperatures associated with higher levels of atmospheric CO₂ – are likely to impact the distribution of areas suitable for parasite persistence and influence the length of the potential transmission season (Kovats *et al.*, 2001; McMichael *et al.*, 2003, 2006; Patz *et al.*, 2005). Changes in temperature and rainfall can also influence the geographic distributions of the insect vectors most responsible for transmission of malarial parasites, not just among humans but among other animal species as well (Sutherst, 1998; Harvell *et al.*, 2002). Although not without controversy (Rogers & Randolph, 2000; Hay *et al.*, 2002a, 2002b; Thomas *et al.*, 2004), a number of recent modeling studies suggest that over the next

Table 7.1. Common genera and species of protozoal parasites from the *Phylum Apicomplexa*

Genus	Species	Common definitive host(s)	Common intermediate host(s)	Associated disease in host or zoonosis in humans	
Coccidiasina	<i>Cryptosporidium</i>	Rodents	None	Cryptosporidiosis	
	<i>Eimeria</i>	Waterfowl, marine birds	None	Coccidiosis	
	<i>Neospora</i>	Canids	None, Mammals	Neosporis	
	<i>Toxoplasma</i>	Felids	Mammals	Toxoplasmosis	
Piroplasmorida	<i>Sarcocystis</i>	Canids	Oviids, Rodents, Waterfowl	Sarcocystosis	
	<i>Theileria</i>	Ticks	Bovids	East Coast Fever, Theileriosis	
	<i>Babesia</i>	Ticks	Ungulates	Babesiosis	
	<i>Cytauxzoon</i>	Ticks	Felids	Cytauxzoonosis	
	<i>Haemoproteus</i>	Midges, louse-flies	Birds, Reptiles	Malaria	
	<i>Saurocytozoon</i>	Culex mosquitoes	Reptiles	Malaria	
	<i>Leucocytozoon</i>	Simuliid flies	Birds	Malaria	
	<i>Hepatocystis</i>	Midges	Squirrels, Old World monkeys, Bats	Malaria	
	Haemospororida	<i>Plasmodium falciparum</i>	Anopheles mosquitoes	Humans	Malaria
		<i>vivax</i>	Anopheles mosquitoes	Humans	Malaria
<i>malaria</i>		Anopheles mosquitoes	Humans	Malaria	
<i>ovale</i>		Anopheles mosquitoes	Humans	Malaria	
<i>reichenowi</i>		Anopheles mosquitoes	Chimpanzees	Malaria	
<i>gonderi</i>		Anopheles mosquitoes	Old World monkeys	Malaria	
<i>simiovale</i>		Anopheles mosquitoes	Old World monkeys	Malaria	
<i>knowlesi</i>		Anopheles mosquitoes	Old World monkeys	Malaria	
<i>cynomolgi</i>		Anopheles mosquitoes	Old World monkeys	Malaria	
<i>simium</i>		Anopheles mosquitoes	New World monkeys	Malaria	
<i>brasilianum</i>		Anopheles mosquitoes	New World monkeys	Malaria	
<i>berghei</i>		Anopheles mosquitoes	Rodents	Malaria	
<i>elongatum</i>		Culex mosquitoes	Birds	Malaria	
<i>gallinaceum</i>		Aedes and Culex mosquitoes	Birds	Malaria	
<i>wenyoni</i>		Culex mosquitoes	Snakes	Malaria	
<i>mexicanum</i>	Sand flies, mites	Lizards	Malaria		

50–75 years warming global climates could lead to dramatic increases in the number of people at risk for malaria and in the transmission potential of the disease, particularly in temperate latitudes, as well as result in modest increases in its likely latitudinal and altitudinal distribution (Martens *et al.* 1995, 1999; Tol & Dowlatabadi, 2001; Hartman *et al.*, 2002; Tanser *et al.*, 2003; van Lieshout *et al.*, 2004; Ebi *et al.*, 2005; Pascual *et al.*, 2006). As just one example, Martens *et al.* (1999) have projected that by 2080 more than 450 million *additional* people may be at risk for infection by *P. falciparum* and *P. vivax* as a result of global climate change than would be if current climate conditions remain stable.

Overview of the biology of malarial parasites

Plasmodium and other related genera of malaria-inducing protists are digenetic or “two-host” parasites – their life cycles involve both sexual reproduction (in the parasites’ “definitive,” invertebrate hosts) and asexual, clonal multiplication (in a vertebrate, “intermediate” host species) (Figure 7.2). Malarial parasites are transmitted between vertebrate hosts by hematophagous (“blood-eating”) insect and, less commonly, arachnid vectors. For mammals, these vectors are typically mosquitoes of the genus *Anopheles*, but other mosquito genera (e.g. *Aedes*, *Culex*) and other hematophagous arthropods (e.g. sand flies, midges, louse-flies, mites) may also serve as either common or occasional vectors for transmission of some primate, avian, and squamate malarial parasites.

A typical *Plasmodium* infection cycle in a human or non-human primate host is shown in Figure 7.2. The life cycles of *Plasmodium* in other hosts and of other genera of malarial parasites in their vertebrate hosts are fundamentally similar, although differences can be found in specific aspects of the cycle. For example, when reptiles and birds are infected with *Plasmodium*, the early rounds of asexual multiplication tend to take place in epithelial cells. The daughter merozoites produced are then released to the bloodstream both to invade circulating blood cells and to colonize solid tissues (e.g. liver, spleen) (Paul *et al.*, 2003). [And in](#) other genera of malarial parasites (e.g. *Haemoproteus*, *Hepatocystis*, and *Leucocytozoon*), asexual multiplication takes place solely within solid tissues in the body and not within circulating red blood cells in the peripheral blood system (Paul *et al.*, 2003).



Evolutionary history of malarial parasites

Origins and host-transfer in vertebrates

Over the last decade, as genetic data have accumulated on the malarial parasites and other Apicomplexa, a preliminary picture of the evolutionary history of the

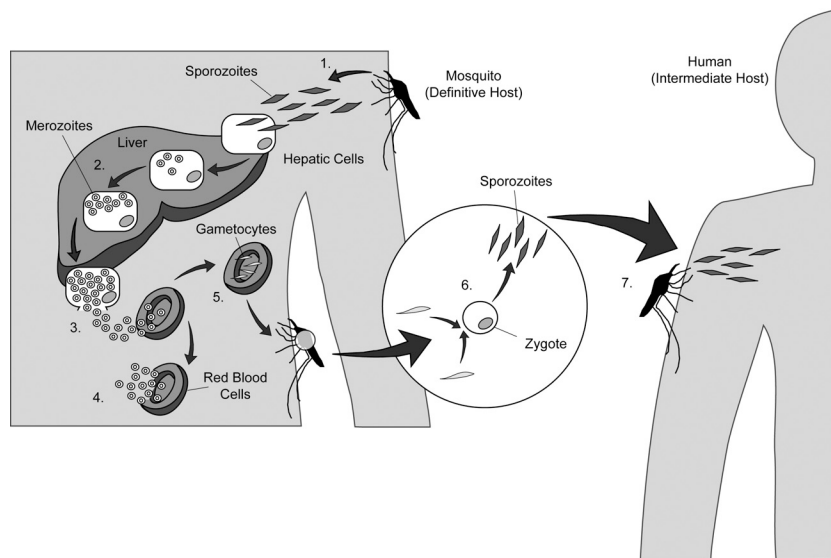


Figure 7.2. Basic life cycle of primate malarial parasites. Redrawn from www.encyarta.msn.com. (1) Sporozoites are inoculated into a primate host through the bite of an infected insect vector and move quickly via the bloodstream to the liver, where they penetrate hepatic parenchymal cells. (2) In the liver, the parasite multiplies asexually to form haploid merozoites. Some merozoites in the liver can remain dormant and become reactive years later. (3) After several replication cycles, these hepatic cells burst, releasing their merozoites into the blood stream where they invade red blood cells and continue reproducing asexually. The merozoites can infect either circulating immature or mature red blood cells, or, for some species of malarial parasites, white blood cells. (4) Once inside red blood cells, the merozoites multiply further, breaking down the constituent hemoglobin in those cells for nutrients and causing anemia in the host. On a ~24, ~48, or ~72 hour cycle, depending on the infecting species of *Plasmodium*, blood cells burst open synchronously, releasing large numbers of merozoites, which infect additional blood cells for further rounds of asexual multiplication. Successive rounds of parasite reproduction and bursting of infected red blood cells triggers an immune system response characterized by the periodic high fevers typically preceded by chills that are the classic clinical symptom of malaria in humans. (5) Within some infected blood cells, the merozoites develop further into haploid gametocytes, which can be ingested by another vector individual. (6) Within the vector's gut, the gametocytes are released from the blood cells, mature, and fuse to form diploid parasite zygotes. Zygotes develop into oocysts on the stomach wall of the vector, which then produce new sporozoites. (7) These sporozoites migrate to the salivary glands of the vector where they can be passed into a new host through subsequent blood feeding. See also Coatney *et al.* (2003) and Bannister & Mitchell (2003).

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malarial parasites and their relatives has emerged. Escalante & Ayala (1995) were the first to examine the evolutionary history of malarial parasites within the context of the Phylum Apicomplexa using molecular data. Based on a phylogenetic analysis of ~1550 base pairs of nuclear DNA sequence data from the slowly evolving small subunit ribosomal RNA (18S SSU rRNA) genes of *Plasmodium* – plus seven other genera of apicomplexans (including *Babesia*, *Toxoplasma*, and *Cryptosporidium*, all of which are potentially zoonotic for modern humans; Polley, 2005) and nine outgroup taxa – they concluded that the origins of the phylum Apicomplexa may date to as early as ~825 million years ago. This date precedes by several hundred million years the emergence of the land vertebrates that are the contemporary intermediate hosts for many apicomplexans. It similarly predates emergence of the Dipteran insects that are the definitive hosts for most malarial parasites (Benton & Donaghue, 2007). Apicomplexans most likely evolved originally as monogenetic parasites of marine invertebrates, with digenesis arising independently – and much more recently – in several of the major Apicomplexa lineages, as the parasites adapted to hematophagy on emerging terrestrial vertebrate hosts (Barta, 1989) (Figure 7.3).

Within the phylum Apicomplexa, the radiation of the genus *Plasmodium* likely dates to sometime during the Middle to Late Mesozoic. Based on applying a crude molecular clock to their 18S SSU rRNA sequence data, for example, Escalante & Ayala (1994, 1995), estimated the age of the last common ancestor of species of *Plasmodium* from birds, rodents, and humans as ~130 to 150 million years ago. Since this date is more recent than the divergence of mammals from birds and squamates (Benton & Donaghue, 2007) – the three groups of modern vertebrate intermediate hosts for malarial parasites – the present distribution of *Plasmodium* species among vertebrates requires, at minimum, several instances of lateral transfer across the vertebrate classes. Moreover, most analyses of 18S SSU rRNA data suggest that malarial parasites infecting mammals do not form a monophyletic group. Rather, avian and squamate malarías appear to nest within that group, again implying multiple cases of lateral transfer among vertebrates, although different analyses provide contradictory assessments of the position of rodent malarial parasites as either within (Escalante & Ayala, 1994, 1995; Qari *et al.*, 1996; Hagner *et al.*, 2007) or basal to (Escalante *et al.*, 1997; Leclerc *et al.*, 2004b) the remainder of the *Plasmodium* clade. Notably, in two early studies based on 18S SSU rRNA genes, the human parasite *P. falciparum* was found to be most closely related to certain avian species of *Plasmodium*, prompting the suggestion that the causative agent of the most virulent human malarial was acquired via recent lateral transfer from birds (Waters *et al.*, 1991, 1993), a position that several subsequent phylogenetic studies have disputed.

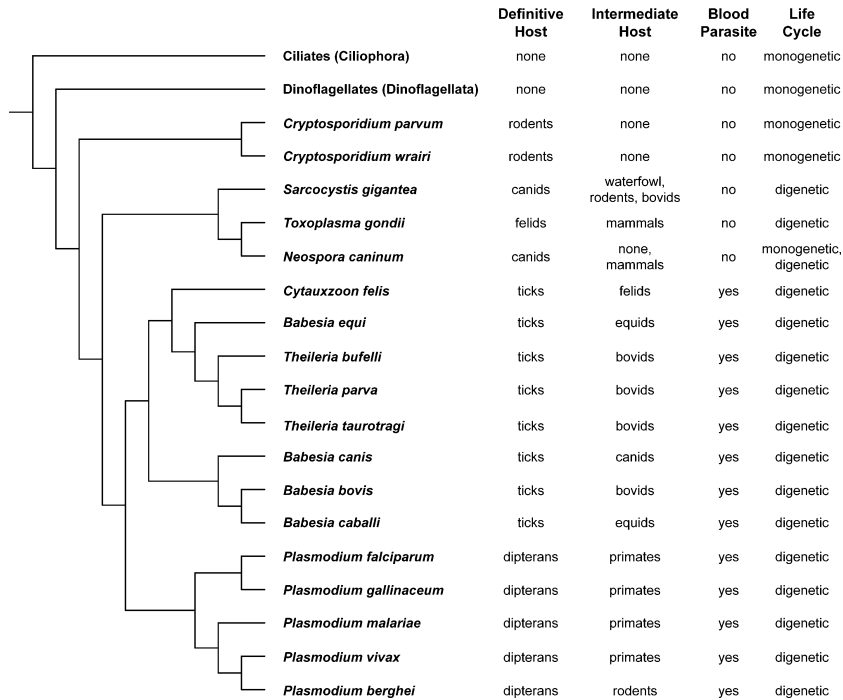


Figure 7.3. 18S SSU rRNA phylogeny of apicomplexan species and associated life-history characteristics based on Escalante & Ayala (1995) and Barta (1989).

A number of issues concerning the use of SSU rRNA sequence data require that caution be applied when interpreting some of these conclusions about the evolutionary history of *Plasmodium* and other apicomplexans. First, multiple copies of SSU rRNA genes are present in the apicomplexan genome, making it difficult to ensure that the sequence alignments used for phylogenetic analysis are based on comparing genes that are orthologous (identical by descent). In recognition of this issue, some analyses based on SSU rRNA data have used information on the secondary structure of the molecule to help guide alignments (Escalante *et al.*, 1997; Hagner *et al.*, 2007). Second, gene conversion (a poorly understood process of intra-chromosomal recombination) among different SSU rRNA genes can potentially confound inferences of phylogenetic relationships based on these loci. Finally, given the immense time depths under consideration and the lack of solid calibration points for determining the evolutionary rate of SSU rRNA loci, any divergence times assigned under a simple molecular clock model can only be regarded as tentative.

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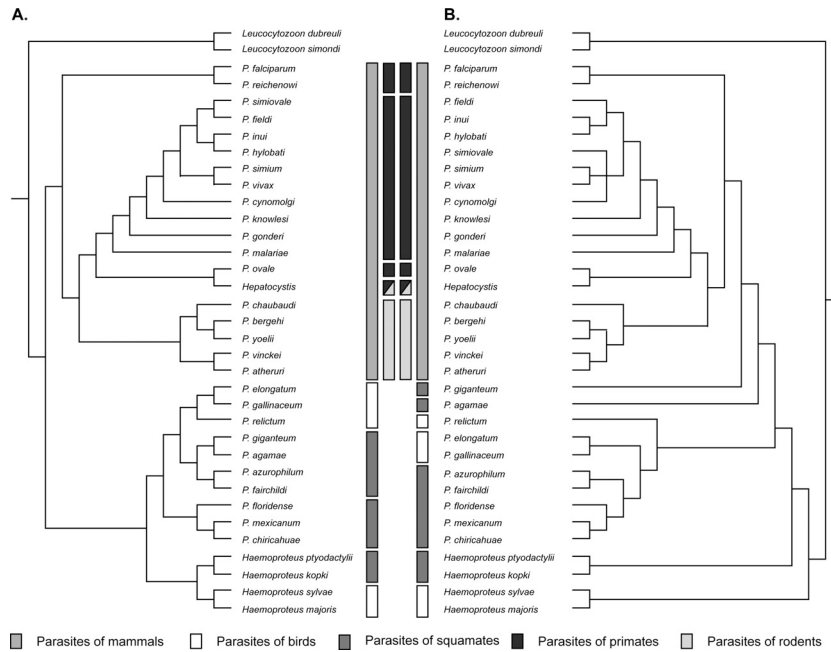


Figure 7.4. Simplified phylogenies of vertebrate malarial parasites based on cytochrome b mitochondrial DNA sequences from (A) Perkins & Schall (2002) and (B) Yotoko & Elisei (2006). The major vertebrate host of each parasite clade is indicated in the boxes adjacent to the clade at the center of the figure (i.e. bird vs. squamate vs. mammal and, within mammals, primate vs. rodent).

Other molecular studies of the evolutionary relationships among malarial parasites have focused only on a handful of additional loci. These include several, single-copy nuclear genes – circumsporozoite surface protein (*Csp*, ~1050 bases) (Escalante *et al.*, 1995; McCutchan *et al.*, 1996; Vargas-Serrato *et al.*, 2003), merozoite surface proteins 1 (*Msp-1*, ~6600 bases) (Polley *et al.*, 2005; Tanabe *et al.*, 2007) and 9 (*Msp-9*, ~2300 bases) (Vargas-Serrato *et al.*, 2003), and adenylosuccinate lyase (*ASL*, ~1400 bases) (Kedzierski *et al.*, 2002) – as well as one plastid gene, caseinolytic protease C (*Clp-C*, ~640 bases) (Rathore *et al.*, 2001; Hagner *et al.*, 2007), and the mitochondrial gene cytochrome b, (*cyt b*, ~1100 bases) (Escalante *et al.*, 1998a; Perkins & Schall, 2002; Ricklefs *et al.*, 2004; Yotoko & Elisei, 2006). With the exception of cytochrome b, none of these have been looked at in a broad range of parasite taxa.

Figure 7.4 shows two simplified evolutionary trees resulting from recent phylogenetic analyses of cytochrome b sequence data from more than 50 malarial parasites comprising a large number of species of *Plasmodium* that infect



mammals, birds, lizards, and snakes, plus representatives of additional genera of malaria-inducing protists (*Hepatocystis*, *Haemoproteus*, *Leucocytozoon*). While there are some inconsistencies between the trees, several important conclusions concerning the evolutionary history of vertebrate malarial parasites can be drawn (Escalante *et al.*, 1998a; Perkins & Schall, 2002; Yotoko & Elisei, 2006). First, all of the malarial parasites of mammals fall into a single clade that includes species attributed to the genera *Plasmodium* and *Hepatocystis*. Interestingly, this result stands in contrast to most of the phylogenies inferred from 18S SSU rRNA sequence data, and to the results of subsequent studies of *Csp*, *Clp-C*, *MSP-1*, and *ASL*, in which at least some avian species of *Plasmodium* fall inside the grouping of mammalian malarial parasites (Rathore *et al.*, 2001; Kedzierski *et al.*, 2002; Vargas-Serrato *et al.*, 2003; Polley *et al.*, 2005; Tanabe *et al.*, 2007).

Second, the primary causative agents of malaria in humans (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*) do not form a monophyletic group but rather have multiple, independent evolutionary origins that date to different times, a robust result found in all molecular phylogenies for *Plasmodium*. Nonetheless, according to the cytochrome b data, all human malarial agents do fall within a clade of mammalian parasites. With respect to *P. falciparum*, this observation runs counter to the hypothesis that the parasite entered the human population recently via lateral transmission from birds (Waters *et al.*, 1991, 1993; McCutchan *et al.*, 1996).

Third, the cytochrome b phylogeny corroborates a sister-taxon relationship of human *P. falciparum* and *P. reichenowi*, a malarial parasite of wild chimpanzees – an observation that was previously suggested by phylogenetic analysis of both 18S SSU rRNA and *Csp* sequence data (Escalante & Ayala, 1994; Escalante *et al.*, 1995, 1996; Qari *et al.*, 1996; Escalante *et al.*, 1997). Notably, in the cytochrome b phylogeny, as in most of the 18S SSU rRNA phylogenies, the *P. falciparum/reichenowi* clade appears basal with the mammalian malarial parasites, and these two species are quite divergent from other mammalian *Plasmodium*. Rodent *Plasmodium* then diverges subsequent to the origins of the *P. falciparum/reichenowi* clade, thus the group of malarial species that infect primates is paraphyletic. Given the estimated rate of nucleotide substitution at the 18S SSU rRNA and *Csp* loci, the human and chimpanzee malarial parasites are estimated to have diverged roughly 8–11 million years ago (Escalante & Ayala, 1994; Escalante *et al.*, 1995), which is around the time of divergence of their hosts. This suggests that the last common ancestor of those parasites transferred into a hominoid host prior to the human–chimpanzee split (Rich & Ayala, 2003).

Using a cytochrome b based phylogenetic tree and information on the class of vertebrate host infected by each extant parasite taxon, Yotoko & Elisei (2006)



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reconstructed the most-likely ancestral hosts at each internal node of the *Plasmodium* phylogeny, repeating the procedure for several different tree topologies. Their analyses yielded several key findings. First, under all topologies, a squamate host was reconstructed as most likely for the last common ancestor of all extant *Plasmodium*, *Haemoproteus*, and *Hepatocystis* species. Thus, a minimum of four host shifts across vertebrate classes would be required to arrive at the host distribution of modern malarial parasites – one from reptiles to mammals, one from reptiles to birds, plus various numbers of additional reptile–bird or bird–reptile switches, depending on the specific tree topology used. Second, for all topologies, only a single host-switch into mammals is required, although within the mammals, at least two additional ordinal host shifts (between primates and rodents and between primates and bats) are implied.

Despite the progress made thus far in understanding the deep evolutionary history of *Plasmodium*, further work is needed, particularly the accumulation of additional sequence data from multiple, independent loci in the parasite genomes. It is worth noting that all of the studies mentioned above were based on alignments of very small segments of DNA – only one was more than 2500 bases in length out of a genome more than 10 000 times that size – and it is not surprising that analyses of different, single loci yield incongruous, poorly resolved gene trees. In fact, in a recent reanalysis of the SSU rRNA, *Clp-C*, and cytochrome b datasets, among the largest and most comprehensive available, Hagner *et al.* (2007) concluded that the phylogenetic signal provided by the first two of these loci is insufficient for resolving the question of whether mammalian malarial parasites indeed form a monophyletic clade, or the related issue of whether *P. falciparum* might represent an avian zoonosis. Even for the cytochrome b dataset, the putative monophyly of mammalian parasites was only weakly supported. Fortunately, as genomic data become available for more species of *Plasmodium*, it should prove easier to accumulate the comparative data needed to develop a more complete picture of the evolutionary history of malarial parasites.



Host-shifts and parasite-host coevolution in primates

Primates are by far the most common mammalian intermediate hosts for malarial parasites, and the extent to which the relationships among primate malarial agents and their host species have been shaped by either cospeciation or by the lateral transfer of parasites among different primate lineages is of great interest, particularly given the global human burden of *Plasmodium* infection. To investigate the coevolution of malarial parasites and their primate hosts, well-resolved phylogenetic trees for each set of taxa must be available. To date,

Table 7.2. *Species of Plasmodium infecting primates and their natural primate hosts*

<i>Plasmodium</i> species	Genera of Natural Primate Host(s)
<i>P. brasilianum</i>	<i>Alouatta, Aotus, Brachyteles, Ateles, Lagothrix, Cacajao, Chiropotes, Callicebus, Cebus, Pithecia, Saguinus, Saimiri</i>
<i>P. bucki</i>	<i>Eulemur</i>
<i>P. coatneyi</i>	<i>Macaca</i>
<i>P. coulangesi</i>	<i>Eulemur</i>
<i>P. cynomolgi</i>	<i>Macaca, Presbytis</i>
<i>P. eylesi</i>	<i>Hylobates</i>
<i>P. falciparum</i>	<i>Homo</i>
<i>P. fieldi</i>	<i>Macaca</i>
<i>P. foleyi</i>	<i>Eulemur</i>
<i>P. fragile</i>	<i>Macaca</i>
<i>P. georgesi</i>	<i>Cercocebus</i>
<i>P. girardi</i>	<i>Eulemur</i>
<i>P. gonderi</i>	<i>Cercocebus, Mandrillus</i>
<i>P. hylobati</i>	<i>Hylobates</i>
<i>P. inui</i>	<i>Macaca, Presbytis</i>
<i>P. jefferyi</i>	<i>Hylobates</i>
<i>P. knowlesi</i>	<i>Macaca, Presbytis, occasionally Homo</i>
<i>P. lemuris</i>	<i>Eulemur</i>
<i>P. malariae</i>	<i>Homo, perhaps Pan</i>
<i>P. ovale</i>	<i>Homo</i>
<i>P. percygarnhami</i>	<i>Eulemur</i>
<i>P. petersi</i>	<i>Cercocebus</i>
<i>P. pitheci</i>	<i>Pongo</i>
<i>P. reichenowi</i>	<i>Pan, Gorilla</i>
<i>P. rodhaini</i>	<i>Pan, Gorilla</i>
<i>P. schwetzi</i>	<i>Pan, Gorilla</i>
<i>P. shortii</i>	<i>Macaca</i>
<i>P. silvaticum</i>	<i>Pongo</i>
<i>P. simiovale</i>	<i>Macaca</i>
<i>P. simium</i>	<i>Alouatta, Brachyteles, Ateles</i>
<i>P. uilenbergi</i>	<i>Eulemur</i>
<i>P. vivax</i>	<i>Homo</i>
<i>P. "vivax-like"</i>	<i>Homo</i>
<i>P. youngi</i>	<i>Hylobates</i>
<i>P. species (undescribed)</i>	<i>Eulemur</i>
<i>P. species (undescribed)</i>	<i>Mandrillus</i>

however, the most complete phylogenetic trees for primate malarial parasites only include around half of the more than 30 species of *Plasmodium* that infect various primates (Gysin, 1998; Coatney *et al.*, 2003) (Table 7.2). Nonetheless, it is very clear that the coevolutionary history of *Plasmodium* and its primate hosts is a complex one, involving multiple host switches within the primates.

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Recently, Mu *et al.* (2005) examined the co-evolution of 14 species of primate *Plasmodium* and their hosts, using a parasite phylogeny inferred from sequence data for the complete mitochondrial genome of eight species plus cytochrome b data from the remaining parasite taxa. Figure 7.5 summarizes the results of their study, incorporating one additional malarial parasite of New World monkeys, *P. brasilianum*, at the appropriate position in the parasite phylogeny (Ayala *et al.*, 1998). Two key points are clear from the figure. First, humans are host to several very divergent strains of malarial parasites, whose most recent common ancestor likely predated the split between primates and other orders of mammals, roughly 95 million years ago (Hedges *et al.*, 1996; Arnason *et al.*, 1998; Kumar & Hedges, 1998). *Macaca* is also host to a number of species of *Plasmodium*, but these most likely arose via cospeciation within the macaque radiation. Second, some distantly related primates are host to closely related parasite species (e.g. *Hylobates* with *P. hylobati* and *Macaca* with *P. inui*). To account for the current distribution of *Plasmodium* species among modern primates, a minimum of five cases of lateral transfer are required – one from macaques to gibbons (involving the common ancestor of *P. inui* and *P. hylobati*), one from macaques to humans (involving *P. knowlesi* as a zoonosis) (Jongwutiwes *et al.*, 2004; Singh *et al.*, 2004), one from macaques to either New World monkeys or humans (involving the common ancestor of *P. simium* and *P. vivax*) followed by more recent transfer into the other of those primate taxa, and a second case of transfer between humans and New World monkeys (involving *P. brasilianum* and *P. malariae*) where the direction of transfer is also controversial. As additional primate malarial parasites are sequenced and added to this phylogeny, it is possible that the number of identifiable cases of host-switching will increase. It is noteworthy that within birds, host-switching by *Plasmodium* and *Haemoproteus* malarial parasites also appears to be common (Bensch *et al.*, 2000; Ricklefs & Fallon, 2002; Ricklefs *et al.*, 2004).

Timing of *Plasmodium* transfers into humans

As alluded to above, there remains some controversy over the specifics of when and where various species of *Plasmodium* came to adopt humans as their intermediate hosts. At the source of the debate are the close phylogenetic relationships seen among several pairs of malarial parasites, one of which infects human and the other a non-human primate host. In one pair, the closest phylogenetic relative of the human parasite, *P. falciparum*, is *P. reichenowi*, a parasite of chimpanzees. Though sister taxa, these parasite species shared a last common ancestor ~8 million years ago, prompting the conclusion that an ancestral hominoid was likely to have been a host to the common ancestor

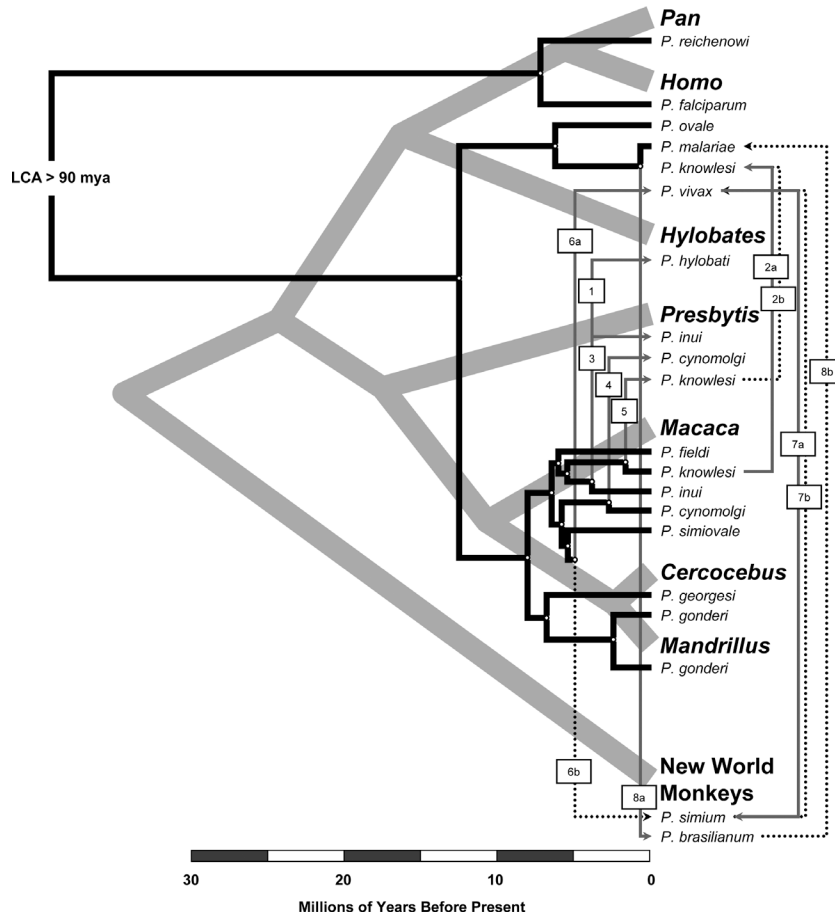


Figure 7.5. Comparison of consensus primate (thick grey) and primate malarial parasite (black) phylogenies showing eight putative host-switching events (thin numbered lines, with the direction of the switch indicated by the arrowhead) based on Ayala *et al.* (1998) and Mu *et al.* (2005). Alternative scenarios for several host switches are indicated by both solid and dotted numbered lines, identified, respectively, by the “a” or “b” following the host switch number. (1) *Macaca* (Old World monkey) to *Homo*, (2) *Macaca* or *Presbytis* (Old World monkey) to *Homo*, (3) to (5) *Macaca* to *Presbytis*, (6) and (7) *Macaca* (Cercopithecine) to *Homo* followed by *Homo* to New World monkeys or *Macaca* (Cercopithecine) to New World monkeys followed by New World monkeys to *Homo*, (8) *Homo* to New World monkeys or New World monkeys to *Homo*.



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of *P. falciparum* and *P. reichenowi*, and that the ancestral parasite cospeciated along with the divergence of its hominoid host into chimpanzees and humans (Escalante *et al.*, 1995; Rich & Ayala 2003).

Two other pairs of closely related *Plasmodium* species show a similar pattern, with one member being found in humans and the other in a non-human primate taxon. However, in these two cases the non-human primate host is only very distantly related to *Homo sapiens*. Thus, *P. vivax*, found in humans, is very closely related to (and perhaps genetically indistinguishable from) *P. simium*, a parasite of two genera of New World primates, and *P. malariae* in humans is a closely related sister taxon to *P. brasilianum*, a generalist parasite that infects 12 genera of New World primates (Escalante *et al.*, 1995; Ayala *et al.*, 1998). For these two cases, it seems most likely that host-switches between humans and non-human primate species have occurred in the very recent past, within the last several hundred years.



Emergence and expansion of Plasmodium falciparum

There is little doubt that the *P. falciparum* lineage has deep evolutionary roots. Phylogenetic analyses based on multiple regions of the genome all indicate, first, that *P. falciparum* from humans and *P. reichenowi* from chimpanzees are each other's closest relatives and, second, that these taxa are very distantly related to other human malarial parasites, having diverged from other lineages of *Plasmodium* prior to the origins of primates roughly 90 million years ago. Moreover, historical records strongly suggest that highly virulent *falciparum*-like malaria has been impacting human populations for several thousands of years, making *P. falciparum* the malarial agent with the longest known history of association with humans (Sherman, 1998, 2006).

At issue, then, is precisely how and when the modern population of *P. falciparum* came to assume its current global distribution and population size. Assuming that the parasite has been present in the *Homo* lineage since our divergence from chimpanzees, has it enjoyed a widespread distribution and large effective population size for hundreds of thousands to millions of years ago, pre-dating or coinciding with the initial spread of humans around the globe? Or, has the parasite population only recently expanded from a small effective population size – e.g. within the last several thousand years – a scenario referred to as the “Malaria’s Eve” hypothesis (Rich *et al.*, 1998)?

One way to address this issue is with data on the amount and structuring of genetic variation found within modern-day *P. falciparum*. If the parasite has enjoyed a large effective population size throughout a long history of association with humans, we would expect to see high levels of polymorphism

in the modern *P. falciparum* population, particularly at selectively neutral or nearly-neutral sites within the genome. Limited neutral polymorphism, by contrast, would reflect more recent expansion of the parasite from a small ancestral population. With data on the amount of genetic variation seen among modern-day *P. falciparum* and information on the rate of sequence evolution in the parasite, it should be possible to estimate the timing of the parasite's first significant emergence in humans – the so-called coalescence time for the last common ancestor of the modern parasite population.

Substantial genetic variation does exist in the global population of *P. falciparum* in some parasite genes. Rather than being neutral, however, much of the variation seen involves non-synonymous substitutions – i.e. single base pair mutations in the DNA that nonetheless do not result in a different protein product being made – in loci coding for a variety of cell membrane molecules that contribute to antigenic variation in the parasite, an adaptation for evading the host's immune system (Hughes, 1991, 1992; Hughes & Hughes, 1995; Escalante *et al.*, 1998b; Polley *et al.*, 2005). Coalescence times have been estimated at tens of millions of years for alleles at some of these loci. These non-neutral polymorphisms might have been maintained by positive selection over a time scale much greater than the parasite's association with humans, and indeed, much greater than the divergence between *P. falciparum* and *P. reichenowi* (Hughes, 1992; Hughes & Hughes, 1995; Hughes & Verra, 1998). However, since alleles subject to strong positive selection (such as those involved in the immune response or in resistance to antimalarial drugs), can greatly increase in frequency or become fixed in a population within a few generations conclusions drawn from this type of analysis are speculative and should be interpreted with caution (Rich & Ayala, 1998, 2003; Rich *et al.*, 1998).

Genetic studies that have focused on neutral or nearly-neutral variation – e.g. synonymous or “silent” substitutions in protein-coding regions and sequence polymorphisms in introns – may be more suitable for reconstructing the history of *P. falciparum*'s association with humans. These have provided widely divergent coalescence date estimates ranging from less than 10 000 years ago to more than 400 000 years (Conway & Baum, 2002; Hartl, 2004). Thus, at least some estimates based on coalescent analyses of neutral polymorphisms are compatible with alternative scenarios concerning the parasite's effective population size throughout its association with humans.

Rich *et al.* (1998) examined sequence data published in GenBank for 10 nuclear protein-coding genes in *P. falciparum* strains isolated from around the globe and found absolutely no synonymous (i.e. neutral) variation in more than 16 000 base pairs of compared sequence. Using estimates of the rate of silent mutation at these loci derived from comparing *P. falciparum* with other

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species of *Plasmodium*, Rich *et al.* (1998) calculated that the common ancestor (“Malaria’s Eve”) of the all modern-day populations of *P. falciparum* dates to 24 500 to 57 500 years ago at the earliest. Recent analysis of more than 22 000 base pairs of aligned data from 20 additional nuclear protein coding loci likewise revealed very little synonymous variation within the global population of *P. falciparum* and lend support to Rich *et al.*’s (1998) hypothesis of a recent expansion of the parasite in humans (Hartl, 2004).

Additional support for the “Malaria’s Eve” hypothesis comes from studies of neutral single nucleotide polymorphisms (SNPs) found in introns. On the basis of the very limited sequence diversity seen in 25 intronic regions from genes on two parasite chromosomes, Volkman *et al.* (2001) inferred the age of the most recent common ancestor of all modern *P. falciparum* to be at most 9500 to 23 000 years. Similarly, mitochondrial DNA sequence data also support the conclusion of a relatively recent though slightly older date for the origins and expansion of the parasite in humans. For example, Conway *et al.* (2000) found very little synonymous sequence diversity in the complete mitochondrial genomes of a worldwide sample of *P. falciparum* when compared to the divergence between *P. falciparum* and *P. reichenowi*, and they inferred an age for the last common ancestor of modern *P. falciparum* of less than 50 000 years. Similarly, based on the number of synonymous substitutions seen in protein-coding regions in a sample of 100 complete mitochondrial genomes from around the globe, Joy *et al.* (2003) concluded that the last common ancestor of worldwide populations of *P. falciparum* existed 70 000 to 98 000 years ago. Interestingly, though, Joy *et al.*’s (2003) analysis also suggests a dramatic increase in the parasite population in Africa within the last 10 000 years, coinciding roughly with the origins and spread of agriculture during the Neolithic and with the origin of the mosquito *Anopheles gambiae*, the main African vector of *P. falciparum* (Coluzzi, 1999).

Not all genetic data, however, support the conclusion of a recent expansion of *P. falciparum* in humans. For example, based on coalescent analysis of the sequence diversity found in a set of 23 nuclear protein-coding loci that show no evidence of having been under positive selection, Hughes & Verra (2001) have argued that the age of the last common ancestor of modern *P. falciparum* existed 290–390 thousand years ago. Similarly, in a large-scale survey of SNP variation on chromosome 3 of the parasite genome, Mu *et al.* (2002) used the number of neutral SNPs (i.e. synonymous substitutions and polymorphisms found non-coding regions) to estimate a date of 102 000 to 177 000 years ago for the common ancestor of all modern *P. falciparum*.

At present, based on the low level of genetic polymorphism seen in a broad range of neutral markers and loci, the genetic evidence seems to come down in favor of a recent and precipitous increase in the population size of *P. falciparum*

from a relatively small ancestral population. Some portions of the *P. falciparum* genome do have much older coalescence times, but that presumably reflects balancing selection to maintain diversity in the parasite's antigenic proteins, which help the parasite evade its hosts' immune defenses. Recent work has also indicated that some of the polymorphisms recognized in those studies estimating an older common ancestor for modern *P. falciparum* likely resulted from sequencing errors and undue reliance on unverified sequence data from GenBank and other databases (Barry *et al.*, 2003; Rich & Ayala, 2003; Hartl, 2004). Moreover, some putative polymorphisms are likely to result from gene conversion between paralogous copies of the gene in question that have arisen from a past gene duplication event (Nielsen *et al.*, 2003) and should not be used in estimating coalescence times under a model of neutral sequence evolution.

Origins and expansion of Plasmodium vivax

Plasmodium vivax is second only to *P. falciparum* in the number of cases of human malaria it is responsible for each year (Figure 7.1) (Mendis *et al.*, 2001), although the mortality rate is far lower. As noted above, it is genetically very similar to (and perhaps indistinguishable from) *P. simium*, a parasite of three genera of New World monkeys. As with *P. falciparum*, however, questions remain as to the timing of its significant expansion in humans and over the geographic region where the parasite originated, with various researchers suggesting Africa (Carter, 2003), South-East Asia (Escalante *et al.*, 2005; Jongwutiwes *et al.*, 2005; Cornejo & Escalante, 2006), and even the Americas (Ayala *et al.*, 1999; Rich & Ayala, 2003; Lim *et al.*, 2005).

As in the case of *P. falciparum*, there is a debate whether the association of *P. vivax* with the human lineage is ancient or recent. Part of the controversy results from the fact that different studies paint contrasting pictures of the level of genetic diversity present within modern-day populations of *P. vivax*, which then influences the conclusions drawn about the timing of emergence of the parasite as a significant agent of human disease. For example, two independent studies using sequence data from the complete mitochondrial genomes of a worldwide sample of *P. vivax* – one involving 106 isolates (Jongwutiwes *et al.*, 2005) and one 176 isolates (Mu *et al.*, 2005) – have yielded coalescence time estimates for the most recent common ancestor of the modern parasite population ranging from 53 000 to more than 300 000 years ago, depending on the combination of nucleotide substitution rate and population demographic history assumed. Cornejo & Escalante (2006) recently combined and reanalyzed the datasets used in these two studies. While they caution that any estimate of the date of the expansion of *P. vivax* is sensitive to assumptions made about the

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neutral mutation rate and the parasite population's demographic history and geographic structuring, all of the different permutations of these variables in their analyses nonetheless yield estimates in the range of 162 000 to 465 000 years, or well before the spread of modern humans around the globe (Cornejo & Escalante, 2006). Sequence data from *MSP-1* – a locus which shows evidence of having undergone positive and diversifying selection – also suggest a Middle Pleistocene coalescence date of ~594 000 years ago for modern *P. vivax* (Tanabe *et al.*, 2007). Finally, in a large-scale sequencing study covering roughly 100 kilobases from the genome of five *P. vivax* isolates, Feng *et al.* (2003) found a greater number of non-coding and synonymous SNPs than are present in the homologous region of the *P. falciparum* genome. They conclude that the *P. vivax* genome is highly diverse and, by implication, its radiation could not have been very recent. Thus, all of these studies support the idea that *P. vivax* underwent a relatively ancient population expansion prior to the emergence of modern *Homo sapiens*.

By contrast, Leclerc *et al.* (2004a) examined the variation seen at rapidly evolving microsatellite and other tandem-repeat loci – including the most polymorphic of those found in Feng *et al.*'s (2003) study – in a set of ~100 isolates of *P. vivax* from a worldwide sample and found far less diversity than is seen in *P. falciparum*. They conclude that the modern-day population of *P. vivax* is genetically depauperate, at least at neutral sites, which suggests that the population has either undergone an implausible series of recent selective sweeps or has rapidly expanded from a small effective population size in the very recent past, likely within the last 10 000 years (Leclerc *et al.*, 2004a). This position finds support from a more recent study of variation in *Csp* gene sequences, which found very few synonymous polymorphisms within a global sample of *P. vivax* isolates and likewise concludes that *P. vivax* became a significant human parasite with a global distribution only since the Holocene (Lim *et al.*, 2005). Interestingly, it appears that there may have been two independent host transfers of *P. vivax/simium* between humans and New World monkeys during this time, based on the fact that the same two strain types are found in both populations (Lim *et al.*, 2005).

Multiple lines of evidence implicate South-East Asia for the origin of *P. vivax*, particularly if the association of the parasite with humans is an ancient one. First, molecular phylogenies based on sequence data from multiple nuclear loci as well as complete mitochondrial genomes, place both *P. vivax* and *P. simium* squarely within a monophyletic clade whose other members comprise only malarias of Asian primates – macaques, leaf-monkeys, and gibbons – with the African primate malarias more basal (Escalante *et al.*, 1998a, 2005; Perkins & Schall, 2002; Mu *et al.*, 2005; Yotoko & Elisei, 2006). Within this clade, *P. vivax* and *P. simium* are also more similar to the malarial parasites of macaques than



to parasites of gibbons, suggesting that if the parasite indeed was introduced into humans in South-East Asia then the source was a cercopithecoïd rather than a hominoid primate. Results from cross-species screening of microsatellite and other simple sequence repeat loci echo these points: *P. vivax*-derived tandem-repeat loci amplify more reliably in several macaque parasites than in *P. hylobati*, a malarial agent in gibbons, or *P. gonderi*, a parasite of African cercopithecoïds (Leclerc *et al.*, 2004a). Finally, when different geographical subsamples of complete *P. vivax* mitochondrial genomes are analyzed separately, the Asian sample contains greater haplotype diversity and yields a much older estimate of the age of the most recent common ancestor than do the samples from either Africa or the Americas, lending additional support to the conclusion of an Asian origin for the parasite (Cornejo & Escalante, 2006).

The inclusion of *P. simium*, a parasite of New World monkeys, in a clade of principally South-East Asian parasites is something of a paradox. Under the Asian origins scenario, the close phylogenetic relationship between *P. vivax* and *P. simium* is interpreted as an anthroponosis – a case of host switching from humans to monkeys. This scenario would involve at least two host switches because two different strain types of *P. vivax/simium* infect both humans and platyrrhines (Lim *et al.*, 2005). The number of host transfers between humans and monkeys may have been greater than two because *P. simium* is a parasite of multiple platyrrhine genera. Alternatively, several transfers between platyrrhine species would have to have occurred after the original two transfers from humans. The original direction of transfer, however, could have been the reverse – i.e. *P. vivax* might be a zoonosis introduced very recently into humans via lateral transfer from New World monkeys, rather than vice versa (Escalante & Ayala, 1995; Ayala *et al.*, 1998; Rich & Ayala, 2003).

Several points, in fact, make a New World monkey-to-human host-switch more plausible than a human-to-monkey transfer. First, modern humans shared a common ancestor much more recently with Old World than New World monkeys and, likewise, have a much longer history of geographic contact with Old World primates – on the order of millions rather than thousands of years. Thus, if parasite transfer from humans to monkeys were to occur, it seems far more likely that it would have taken place in the Old World. Moreover, given that *P. simium* infects several different genera of New World monkeys that shared a common ancestor millions of years before the very recent emergence of the parasite, several host shifts between humans and monkeys (or at least two ~~between humans and monkeys~~, followed by additional transfers among monkey species) would be required to explain the parasite's current distribution. As others have noted, evolutionary parsimony thus favors a monkey-to-human host switch (Escalante & Ayala 1995; Ayala *et al.*, 1998; Rich & Ayala, 2003).



Origins of *Plasmodium malariae*

Another agent of human malaria, *Plasmodium malariae*, is also very closely related to a malarial parasite of New World monkeys, *P. brasilianum*. To date, intraspecific variation in *P. malariae* has not been well studied. Based on a very small number of samples ($n = 2$), Ayala *et al.* (1998) found the level of polymorphism at the *Csp* locus to be comparable to that seen in *P. vivax*, perhaps implying a similar time frame for the expansion of *P. malariae* in humans. Clearly, however, additional loci and a much larger set of samples need to be studied before any robust conclusions might be drawn.

As is the case for *P. vivax/simium*, the question of whether humans acquired *P. malariae/brasilianum* from or transferred the parasite to New World monkeys remains unresolved, but given the fact that *P. brasilianum* is known to infect at least 12 of the roughly 16 currently recognized genera of platyrrhines (Table 7.2), a strong, parsimonious case can be made for a single platyrrhine-to-human transfer. By contrast, any scenario involving host-switching from humans to monkeys would have to involve either numerous transfers from humans to different platyrrhines or multiple transfers among platyrrhine genera following an introduction from humans.

As others have noted (Ayala *et al.*, 1998; Rich & Ayala, 2003; Lim *et al.*, 2005), the critical data needed for resolving outstanding questions about the direction of transfer for both *P. malariae/brasilianum* and *P. vivax/simium* are estimates of the amount of neutral polymorphism found in natural populations of the New World parasites. If New World monkeys are in fact the source of the two human malarial agents, then the expected neutral genetic polymorphisms in *P. brasilianum* and *P. simium* should be much greater and should coalesce further back in time than those in *P. malariae* and *P. vivax*.

Parasite evolutionary dynamics and host adaptations

Virulence differences within and between species

A staple topic of study in host–parasite interactions is the evolutionary dynamics of parasite virulence. Conventional wisdom suggests that parasites that have a long relationship with a particular host taxon should, over time, evolve to become less virulent in their hosts, since overly virulent parasites can cause host death, thereby curtailing the parasite’s ability to transfer into a new host and its eventual reproductive success. However, other features of host demography (e.g. host population density), as well as features of parasite population ecology (e.g. the timing of transmission relative to host mortality, the parasite’s dependence upon or independence from one or more vectors, or the

prevalence of host coinfection by multiple parasite strains, which engenders within-host competition among parasites) are also expected to influence parasite virulence (Bull, 1994; Day, 2001, 2003). Some researchers have further suggested a tradeoff between host specialization and virulence, with lower virulence characterizing more “generalist” parasites (parasites that infect multiple host species) and higher virulence characterizing more “specialized” parasites (Woolhouse *et al.*, 2001; Gandon, 2004). All of these factors may contribute to the wide variation in virulence seen among and within parasite species.

Within primates, different species of *Plasmodium* are known to infect different numbers of host species, from one, in many cases (e.g. *P. falciparum*, which is specific to humans) to more than two dozen (*P. brasilianum* in New World monkeys) (Table 7.2). Recently, Garamszegi (2006) used the primate malaria host–parasite complex to test the hypothesis that parasite virulence covaries with host specialization. He found that average peak parasitemias – i.e. counts of infected cells per volume of blood in experimentally inoculated animals averaged across the set of host species – were negatively associated with the degree of host specialization of the parasite, lending support to the hypothesis that more generalist parasite species are less virulent.

With respect to intraspecific variation in virulence, it is known that in humans the severity of malaria caused by different strains of *P. falciparum* can differ markedly, from “mild” (associated with low host mortality) to “severe” (associated with host mortalities >10%, even with treatment; Gupta *et al.*, 1994). The most severe cases of *P. falciparum* malaria result when infected blood cells adhering to the walls of blood vessels – one of the parasite’s strategies for circumventing the host’s immune system by allowing it to remain sequestered in the peripheral circulatory system (Craig & Scherf, 2001; Beeson & Brown, 2002) – cause those vessels in the brain and other vital organs to become blocked and rupture. Malarial parasites accomplish this adhesion by causing several types of ligand proteins to be expressed on the surface of infected blood cells of their host – proteins which are coded for by several families of genes (e.g. *var*, *rif*, and *stevor* in *P. falciparum*; Crabb & Cowman, 2002). *Plasmodium falciparum* is unique among human malarias in that its merozoites are able to infect mature red blood cells.

Recent experimental studies have demonstrated that genetic variation among parasite strains within some species of *Plasmodium* is associated with differences in virulence, both in the intermediate host (Mackinnon & Read, 1999; Chotivanich *et al.*, 2000) and in their mosquito vectors (Ferguson & Read, 2002). In fact, in at least one model system involving the rodent parasite, *P. chaubaudi*, Ferguson & Read (2002) found a gene-by-environment effect on virulence in the parasite’s definitive host, the mosquito *Anopheles stephensi*. Thus, a clear mechanism exists whereby phenotypic variation in virulence may

be maintained in a population. The extent to which the combination of environmental and genetic variation account for variability in pathogenicity associated with *P. falciparum* infection in humans remains to be investigated.

Human and non-human primate adaptations to malaria

Over the course of modern human evolutionary history, malaria is possibly responsible for the deaths of more than half of all people who have ever lived (Sherman, 2006), and the disease is likely to be the single most important selective force to which modern humans have had to adapt (Kwiatkowski, 2005). In turn, the evolutionary dynamics of *Plasmodium* infecting humans and other non-human primates has also been shaped by selection pressures associated with the evolved defenses of their hosts. Not surprisingly, then, a wide range of genetic polymorphisms seen in modern humans have been linked to their role in resistance to infection by malarial parasites (Flint *et al.*, 1998; Evans & Wellems, 2002; Fortin *et al.*, 2002; Kwiatkowski, 2005; Williams, 2006). Many of these polymorphisms involve changes to the cell surface proteins and/or internal structure of red blood cells that either render them less susceptible to invasion by circulating *Plasmodium* merozoites, greatly reduce or destroy the ability of the parasites to grow in red blood cells, or enhance the process by which hosts develop natural immunity to *Plasmodium* infection (Friedman, 1978; Williams *et al.*, 2005a).

The classic example of one such polymorphism involves the β -hemoglobin (HBB) gene and is responsible for “sickle-cell anemia” in humans. The HBB gene codes for one of the peptides that makes up hemoglobin, the molecule in red blood cells responsible for binding and transporting oxygen. One form of the HBB gene, the hemoglobin A (HbA) allele, is by far the most common variant in human populations worldwide. However, in some populations where the prevalence of malaria is high – particularly in sub-Saharan Africa – an alternative allele known as HbS, achieves a high frequency. The HbS allele arises from a single base pair missense mutation, which changes one amino acid in the β subunits of the hemoglobin molecule. This change causes red blood cells to assume a reversible, sickled shape under hypoxic conditions; hence, HbS is often referred to as the “sickle-cell” allele. While individuals homozygous for the HbS allele suffer from debilitating anemia and painful vascular infarctions caused by sickled red blood cells blocking and sometime bursting vessels of the circulatory system, heterozygotes possessing one HbA allele and one HbS allele experience very few of the symptoms of the condition, except when oxygen deprived. Additionally, heterozygous individuals show resistance to *Plasmodium* infection, seemingly either because the normal metabolism of

the parasite in erythrocytes is disrupted (Friedman, 1978) or because infected blood cells are more effectively recognized and removed from the blood and destroyed in the spleen (Kwiatkowski, 2005). Thus, the HbA–HbS polymorphism is apparently maintained due to an increased fitness of heterozygotes in environments where malarial risk is high.

Two other single nucleotide polymorphisms in the HBB gene are responsible for yet other hemoglobin alleles that also convey substantial protection against *Plasmodium* infection (Williams, 2006): HbC, which like HbS is common in sub-Saharan and western Africa, and HbE, which is most common in South-East Asia (Hutagalung *et al.*, 1999; Agarwal *et al.*, 2000; Modiano *et al.*, 2001; Chotivanich *et al.*, 2002; Ohashi *et al.*, 2004). Linkage disequilibrium studies of the various HBB polymorphisms suggest that the HbS allele has arisen independently in several different geographical regions within Africa (Mears *et al.*, 1981; Antonarakis *et al.*, 1984; Pagnier *et al.*, 1984; Chebloune *et al.*, 1988; Flint *et al.*, 1998), and the same may be true for HbE. Moreover, these alleles seemingly arose relatively recently (<5 000 years ago) in human populations (Flint *et al.*, 1998; Currat *et al.*, 2002; Ohashi *et al.*, 2004), providing testament to the powerful selective role that malaria has played in recent human evolution and lending additional support to the idea that *P. falciparum* has only emerged as a significant pathogen of modern humans within the last several thousand years.

A large number of additional red blood cell polymorphisms have also been maintained in humans presumably as a result of the strong selective pressures imposed by malaria. For example, many different polymorphisms in the genes coding for either the α or β subunits of the hemoglobin molecule (and in the regulatory regions influencing transcription of these two genes) are responsible for thalassemias, a family of blood disorders in which red blood cells underproduce hemoglobin, which may result in mild to severe anemia. As in the case of heterozygous carriers of the HbS allele, individuals heterozygous for certain α - and β -thalassemias suffer from mild anemia but show markedly increased resistance to severe malaria (Flint *et al.*, 1986; Allen *et al.*, 1997; Williams *et al.*, 2005b).



Polymorphism at the X-linked glucose-6-phosphate dehydrogenase (G6PD) locus has long been known to be associated with the occurrence of malaria in humans (Allison & Clyde, 1961; Gilles *et al.*, 1967; Beutler, 1994). The G6PD enzyme is ubiquitous in animal cells where it plays a major role in glucose metabolism and in the production of nicotinamide adenine dinucleotide phosphate (NADPH), which is critical for cells – particularly red blood cells – to be able to cope with oxidative stress (Greene, 1993; Ruwende & Hill, 1998). A variety of mutations in the gene result in deficiencies in G6PD production, and the geographic distribution of G6PD-deficient variants corresponds well

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with areas of high malaria risk, suggestive of a selection-driven link between the condition and malaria resistance. Some *in vitro* studies have demonstrated that G6PD deficiency inhibits the growth of *P. falciparum*, at least in the early stages of infection (Roth *et al.*, 1983; Ruwende & Hill, 1998), and field studies have revealed that for both heterozygous female and hemizygous male children, one form of G6PD deficiency (G6PD A-) was associated with a 46–58% reduction in the risk of severe malaria in two African populations (Ruwende *et al.*, 1995). Different G6PD polymorphisms have arisen and been selected for in different parts of the world, and recent haplotype analysis of two of these resistance-conferring variants (G6PD A- and G6PD Med) suggest that they arose and spread rapidly within African and circum-Mediterranean populations within the last 1500 to 12 000 years (Tishkoff *et al.*, 2001; Saunders *et al.*, 2002). Another common G6PD deficiency allele variant in modern humans, G6PD A, also appears to be maintained through selection, but the age of that allele predates the recent emergence of severe malaria, suggesting a different adaptive function than malaria resistance (Verrelli *et al.*, 2002). Interestingly, a recent parallel study of variation at the G6PD locus in a large set of chimpanzees plus exemplar individuals from several other non-human primate taxa concluded that the evolution of the enzyme has been strongly constrained over the 30- to 40-million year history of anthropoid primates (Verrelli *et al.*, 2006). Thus, in contrast to the situation for humans, there is no evidence to support the idea of positive selection for malaria resistance at the G6PD locus in chimpanzees (Verrelli *et al.*, 2006).



Variation in the structure of several types of red blood cell membrane proteins also plays an important role in human susceptibility and resistance to *Plasmodium* infection. For example, to gain access to human erythrocytes, both *P. vivax* and *P. knowlesi* merozoites must recognize and bind to a specific chemokine receptor protein, the Duffy antigen, which is expressed on the surface of red blood cells (Miller *et al.*, 1975, 1976; Barnwell *et al.*, 1989). The Duffy antigen is coded for by a gene known as FY, which has three main allele types in humans, FY^A, FY^B and FY^{null}. Both *P. vivax* and *P. knowlesi* merozoites express a ligand protein that contains a Duffy-binding-like (DBL) domain, which allows the parasite to bind to and enter red blood cells that bear either FY^A or FY^B coded Duffy antigens. Most native sub-Saharan Africans are homozygous for the Duffy-negative allele (FY^{null}/FY^{null}) and thus fail to produce the Duffy antigen, rendering them effectively immune to infection by either *P. vivax* or *P. knowlesi*. Studies of both sequence polymorphism and microsatellite variation in the FY-gene region suggest that the locus has been under positive, directional selection in African populations, with the near fixation of the FY^{null} allele in Africa estimated to have arisen within the last 33 000 years, i.e. after the dispersal of anatomically modern humans out of

Africa (Hamblin & Rienzo 2000; Hamblin *et al.*, 2002), and perhaps much more recently (Seixas *et al.*, 2002).

In contrast to *P. vivax*, which can only infect human red blood cells that bear the Duffy antigen, *P. falciparum* merozoites express several different erythrocyte binding ligands that contain Duffy-binding-like domains and can use multiple, redundant invasion pathways to gain entry into mature red blood cells (Dolan *et al.*, 1994; Okoyeh *et al.*, 1999; Adams *et al.*, 2001; Chitnis, 2001; Gaur *et al.*, 2004). Not all erythrocyte surface proteins recognized by these ligands are known, but the glycophorins – membrane proteins that are highly glycosylated, bearing O-linked and N-linked glycans (oligosaccharide chains) rich in the terminal sugar sialic acid – are among the set of targets that are most commonly utilized by the parasite (Pasvol *et al.*, 1982a, 1982b, 1993; Friedman *et al.*, 1984; Dolan *et al.*, 1994; Lobo *et al.*, 2003; Mayer *et al.*, 2006). Glycophorin loci are among the fastest evolving genes in humans, especially at sites where glycans are attached (Baum *et al.*, 2002; Wang *et al.*, 2003), strongly suggesting that these glycoproteins have been the targets of positive selection at least in part due to the risk of *P. falciparum* infection. Indeed, variation in some human genes coding for the protein components of several glycophorins (e.g. GYPA, GYPB, GYPC) influences how readily those membrane proteins are bound by *P. falciparum* ligands (Gaur *et al.*, 2004; Mayer *et al.*, 2006), which, in turn, influences how susceptible the red blood cells bearing those proteins are to malarial infection.

The sialic acid component of red blood cell glycophorins is particularly important in the recognition of erythrocyte receptors by certain *P. falciparum* ligands. Thus, genes involved in sialic acid biochemistry and modification are also likely to be associated with human susceptibility/resistance to malaria and other pathogens that target sialic acids to invade animals cells (Varki, 2001). For example, the dominant invasion pathway for *P. falciparum* involves recognition of the sialic acid residue of glycophorin A (N-acetylneuraminic acid, or Neu5Ac) on human red blood cells by the erythrocyte-binding antigen (EBA) 175 of the parasite. Humans are almost unique among mammals in having Neu5Ac as the principal sialic acid associated with erythrocyte cell membrane proteins, while the red blood cells of chimpanzees (and most other mammals) instead carry a mixture of Neu5Ac and Neu5Gc, a related sialic acid synthesized from Neu5Ac (Varki, 2001). Since the human–chimpanzee divergence, the gene encoding the enzyme CMP-N-acetylneuraminic acid hydroxylase (CMAH), which is centrally involved in synthesis of Neu5Gc from Neu5Ac, has become deactivated in the human lineage (Chou *et al.*, 1998, 2002). Martin *et al.* (2005) have demonstrated that this difference in the form of sialic acid associated with glycophorin A is likely to be responsible for the remarkable host-specificity of *P. falciparum* and *P. reichenowi* for humans and chimpanzees, respectively,

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where inoculation of one of these hominoid species with the parasite of the other fails to produce sustained infection or significant parasitemia. Interestingly, the same study found that red blood cells of *Aotus*, a New World monkey genus commonly used in *P. falciparum* research, resembles human red blood cells by only carrying Neu5Ac.

With respect to non-human primates, a potential case of the impact of *Plasmodium* on the evolution of orangutans (*Pongo pygmaeus*) has recently come to light. A duplication of the α -globin gene has been discovered in orangutans that Steiper *et al.* (2006) suggest may be of adaptive significance. It is hypothesized that the activity of this locus can result in thalassemia-like conditions, which, as in humans, may provide some resistance to *Plasmodium* infection. Orangutans can be naturally infected by two malaria species, *P. pitheci* and *P. silvaticum* (Table 7.2), and evidence now indicates that some orangutans can be infected with the human parasite, *P. vivax*, and with the macaque malarial species, *P. cynomolgi* and *P. inui* (Wolfe *et al.*, 2002; Reid *et al.*, 2006). Given that orangutans once ranged all the way from China to the Celebes Islands, they have likely been under considerable pressure from malaria throughout much of their evolutionary history (Peters *et al.*, 1976).

Other α -globin duplications have been found in gorillas, chimpanzees, and crab-eating macaques (Takenaka *et al.*, 1993), raising the possibility that malaria has also been a selective force driving α -globin evolution in other tropical primates. It has even been hypothesized that the driving force behind the speciation of *Macaca mulatta* and *M. fascicularis* may have been malarial pressure (Wheatley, 1980). Whereas rhesus macaques (*M. mulatta*) show little to no variation in the constituent chains of the hemoglobin molecule, crab-eating macaques (*M. fascicularis*) show several variants (Barnicot *et al.*, 1966). This variation was hypothesized to correlate with the different selective pressures of malaria on these two species. *Macaca mulatta* is widely used as a model organism in malaria research because they show a strong, usually fatal, response to infection with *P. knowlesi* (a *falciparum*-like species). *Macaca fascicularis*, on the other hand, only exhibit minor, chronic infection with low level parasitemias when similarly infected (Schmidt *et al.*, 1977). However, peninsular Malaysian *M. fascicularis* gets as sick as rhesus macaques. This is interesting because molecular phylogenetic studies reveal that mainland *M. fascicularis* have hybridized with *M. mulatta* (Tosi *et al.*, 2002) and therefore may have similar genetic background in key anti-inflammatory and malaria resistance loci (Praba-Egge *et al.*, 2002; Ylostalo *et al.*, 2005).

From the parasite's point of view, it is clear that human and non-human primate adaptations to *Plasmodium* infection have also significantly influenced the course of the parasite's evolution, by pressuring the parasite to find ways to circumvent its hosts' defenses. One example is the development of multiple and

widespread drug-resistant strains of *P. falciparum* and *P. vivax* within the last half century, following concerted efforts by the World Health Organization and world governments to eradicate the disease. Another example is the rapid evolution of diversity in erythrocyte membrane molecules, which seems to have been matched by a corresponding evolution of diversity in parasite ligands. Recent studies have found a high level of non-synonymous polymorphism in the gene coding for EBA 175, the principal parasite ligand allowing *P. falciparum* merozoites to bind and invade mature erythrocytes (Baum *et al.*, 2003; Wang *et al.*, 2003), and additional variation has been seen in other ligands involved in the infection of immature red blood cells (reticulocytes) (Taylor *et al.*, 2002). Coupled with the high rate of evolution seen at the human glycoporphin A locus, this strongly suggests an ongoing evolutionary arms race between *P. falciparum* and its human hosts (Wang *et al.*, 2003). Indeed, the evolution and maintenance of diversity in the glycans associated with animal cell membrane glycoproteins and glycolipids may, in general, be driven by the co-evolutionary arms struggles between microbial pathogens and their hosts (Gagneux & Varki, 1999; Bishop & Gagneux, 2007). Consistent with this idea of rapid adaptive evolution between parasite and host, comparative genomic data for *P. falciparum* has shown that genes presumably coding for antigenic cell surface molecules – which influence the parasite’s ability to invade host cells and evade its host’s immune defenses – are characterized by much greater diversity than genes associated with more basic metabolic functions (Volkman *et al.*, 2007). Moreover, a recent genomic comparison between *P. falciparum* and the chimpanzee parasite, *P. reichenowi*, found that the key functional differences between these parasite genomes are also primarily found in those genes involved in mediating parasite–host interactions. For example, loci coding for membrane proteins have evolved at a much faster rate since the *P. falciparum/reichenowi* split than have genes coding for proteins active primarily within the cell (Jeffares *et al.*, 2007).

Given their much larger population sizes and much shorter generation times, the evolutionary dynamics of a parasite taxon theoretically should often outpace that of its host(s), thus the fact that malaria and other parasite-induced infectious diseases today remain such a challenge for long-lived species such as humans and other non-human primates is perhaps not surprising. Although the situation is obviously complex in a two-host system like malaria – where the life histories and biochemical milieu of the invertebrate “definitive” and vertebrate “intermediate” hosts differ dramatically from one another, as well as from that of the parasite – the relative speed of adaptation is still likely to be far more rapid for the parasite than for either host. Still, the complexity of a two-host system may make various vector control strategies (e.g. larviciding of vector hatching sites, use of insecticide-treated bed nets to reduce



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vector-human contact) a viable option for reducing the human toll of the parasite, rather than focusing public health efforts solely on the development of vaccines or more effective antimalarial drugs.

Future directions

Over the last decade, substantial progress has been made in understanding the evolutionary history of the malarial parasites, particularly as they relate to the various primate genera that are their most common mammalian intermediate hosts. Still, much work needs to be done. First, to date, fewer than half of the *Plasmodium* species known to infect non-human primates have been included in phylogenetic studies (Table 7.2), thus a complete picture of the evolutionary history of the malarial parasites and their primate hosts is lacking. Importantly, none of the parasite species infecting strepsirrhine primates (e.g. *P. lemuris*) have been included in phylogenetic analyses, nor have most of the hominoid parasites (e.g. *P. rhodani*, *P. schwetzi*, *P. pitheci*, *P. youngi*). Inclusion of these parasite species might reveal evidence of additional host transfers among non-human primates, influence the debate over the geographic origins of *P. vivax*, and provide insight into the evolutionary history of malaria among the Malagasy primates. For example, have the *Eulemur* malarias coevolved with their hosts over a long period of time or do they represent anthroponoses acquired in the last several thousand years since humans first colonized the island of Madagascar?

Second, further efforts are needed to characterize the natural variation found within populations of additional species of malarial parasites. To date, large-scale studies of intraspecific variation have only been carried out for two of the human parasites, *P. falciparum* and *P. vivax*, and not for any of the parasites targeting other genera of primates. Data on intraspecific variation in additional species of *Plasmodium* would be desirable not only for evaluating hypotheses about the source and timing of emergence of other human malarial agents (e.g. *P. ovale* and *P. malariae*), but also of widespread non-human primate malarial parasites like *P. knowlesi* and *P. brasilianum*. The debate over whether host transfer by *P. vivax/simium* and *P. malariae/brasilianum* occurred from humans to New World monkeys or vice versa could be answered by comparing the relative amount of neutral genetic variation found in the ~~primate versus human~~ parasites (Rich & Ayala, 2003; Lim *et al.*, 2005). Given that studies of intraspecific variation in *P. falciparum* have invigorated the search for ways to reduce the human toll of malaria (e.g. by suggesting novel vaccination strategies and therapies), it is likely that further appreciation of the variation within other species of *Plasmodium* may do the same.



Finally, apart from G6PD in chimpanzees (Verrelli *et al.*, 2006) and a few known hemoglobin variants in hominoids and macaques (Barnicot *et al.*, 1966; Takenaka *et al.*, 1993; Steiper *et al.*, 2006), the extent to which any non-human primate taxon exhibits variation at any of the loci that have been implicated in malaria resistance in humans is unknown, as are the functional reasons why some species of primates are resistant to infection by species of *Plasmodium* that infect their close phylogenetic relatives. Presumably, if malaria has asserted a significant evolutionary selective pressure on primates other than humans, then these species, too, should show genetic and functional signatures of their adaptations to infection by *Plasmodium*, but such signatures have not yet been widely looked for outside of humans.

Conclusions

The late 20th and early 21st centuries have been marked by substantial progress in genetic research on malaria. Phylogenetic data clearly demonstrate that the primate malarias have a complex evolutionary history vis-à-vis their hosts – a history characterized by both coevolution and cases of host-switching between sometimes very distantly related primate taxa. Among the human parasites, while some of the genetic variation present in *P. falciparum* and *P. vivax* appears to be ancient, most of the genetic data suggests that modern-day populations of these two species are descended relatively recently from a very small number of founders. It appears that malaria only became a significant health burden for humans recently in our evolutionary past, most likely within the last 6000 to 30 000 years, and certainly well after the origins of modern *Homo sapiens*. As others have suggested, the presumed timing of emergence of *P. falciparum*, in particular, seems to coincide well with the Neolithic transition to agriculture in Africa and the origins of anthropophilic species of mosquitoes such as *Anopheles gambiae*, the primary vector for *P. falciparum* transmission in sub-Saharan African populations (Coluzzi, 1999; Hume *et al.*, 2003; Rich & Ayala, 2003; Ayala & Coluzzi, 2005).

In addition to the results reviewed above, a wealth of comparative genomic data on *Plasmodium* is either currently available or forthcoming. For example, the complete genome of *P. falciparum* has recently been sequenced, assembled, and published (Gardner *et al.*, 2002), and for a number of additional *Plasmodium* species, whole genome data in various stages of assembly and annotation are also available (Carlton *et al.*, 2002; Hall *et al.*, 2005). These include three rodent malarial agents (*P. berghei*, *P. chaubaudi*, and *P. yoelii*), one Old World monkey parasite (*P. knowlesi*), one avian parasite (*P. gallinaceum*), and one additional human parasite (*P. vivax*) that have been sequenced by either the

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Wellcome Trust's Sanger Institute or by The Institute for Genome Research (TIGR). Additionally, the Wellcome Trust is part-way through the process of sequencing the *P. reichenowi* genome. Thus, complete genomes will soon be available for a suite of *Plasmodium* species that infect a wide range of vertebrate hosts, thereby facilitating comparative analyses of gene evolutionary history, structure, and function. PlasmoDB (www.plasmodb.org) is a comprehensive, searchable, web-based database for comparative *Plasmodium* genomics that makes much of this data publicly available (Kissinger *et al.*, 2002; Bahl *et al.*, 2003; Stoeckert *et al.*, 2006).

Compared to the progress made in understanding the evolutionary history of the malarial parasites, progress on reducing the human toll of malaria has been far less impressive. While concerted efforts to combat the disease succeeded in reducing the global burden of malaria for a portion of the mid-20th century, malaria is once again on the rise and is considered a re-emerging infectious disease that, in many places, has evolved resistance to some of the most effective treatments previously used (Carter & Mendis, 2002). The Roll Back Malaria Partnership – launched in 1998 by the World Health Organization in collaboration with the United National Development Program, UNICEF, and the World Bank – was aimed at reversing the disappointing increase in the global malarial burden that followed the interruption of eradication programmes in the 1970s. However, the Partnership's goal of reducing the annual number of worldwide deaths due to malaria by 50% by the year 2010 seems unreachable. Given that close to half the world's human population is at risk for malarial infection (Guerra *et al.*, 2006), that resistance to standard antimalarial drugs is increasing among parasite populations, and that global climate change is likely to dramatically increase the world regions facing malaria risk (Hay *et al.*, 2004; Sutherst, 2004), the contemporary significance of malaria for humans cannot be underestimated.

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