Coevolutionary Genetics of *Plasmodium* Malaria Parasites and Their Human Hosts

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**Synopsis.** Malaria has been invoked, perhaps more than any other infectious disease, as a force for the selection of human genetic polymorphisms. Evidence for genome-shaping interactions can be found in the geographic and ethnic distributions of the hemoglobins, blood group antigens, thalassemias, red cell membrane molecules, human lymphocyte antigen (HLA) classes, and cytokines. Human immune responses and genetic variations can correspondingly influence the structure and polymorphisms of *Plasmodium* populations, notably in genes that affect the success and virulence of infection. In Africa, where the burden from *Plasmodium falciparum* predominates, disease severity and manifestations vary in prevalence among human populations. The evolutionary history and spread of *Plasmodium* species inform our assessment of malaria as a selective force. Longstanding host-pathogen relationships, as well as recent changes in this dynamic, illustrate the selective pressures human and *Plasmodium* species place on one another. Investigations of malaria protection determinants and virulence factors that contribute to the complexity of the disease should advance our understanding of malaria pathogenesis.

**Introduction**

The global public health problem of malaria persists today, as drug resistance of *Plasmodium* malaria parasites and the limits of insecticides against mosquitoes undermine control measures that seemed so promising 50 yr ago. Bolstered by the initial successes of chloroquine as an antimalarial drug and DDT as an insecticide, the World Health Organization (WHO) had embarked on a campaign in 1955 to eradicate malaria (Jeffrey, 1976). Early successes in some areas of the globe were dramatic, and by the early 1960s malaria was reduced to very low levels in certain countries (Wernsdorfer, 1980). Unfortunately, maintenance of eradication measures became more difficult in the face of practical constraints and commitment limits, and the campaign began to lose force (Henderson, 1999; Aylward et al., 2000). Anopheline mosquito populations adapted to survive the height of DDT-spraying programs (Litsios, 1996), and chloroquine-resistant strains of *Plasmodium falciparum* were spreading in South America and Southeast Asia before 1960 (Payne, 1987). Malaria soon reestablished itself with devastating impact in India and other countries where eradication had seemed nearly within grasp (Sharma, 1996).

Natural selection in response to chemical control measures is a recent example of evolution in the relationships between malaria parasites, mosquitoes, and humans. Other examples of selection and genome-shaping events have arisen in the long history of interactions between these species. These include a variety of human genetic polymorphisms that have been shown to protect against the disease.

Human malarias are caused by four different *Plasmodium* species. *P. falciparum* is in a deep evolutionary clade of its own and is responsible for the most acute and deadly form of malaria. *P. vivax*, *P. malariae*, and *P. ovale* fall among other clades that include species with longstanding evolutionary relationships with numerous other primates, perhaps explaining the tendency of these parasites to produce less severe forms of disease. Here we review some central features of malaria and discuss genetic adaptations in the host-parasite relationship. Further reading may be found in three recent books that give comprehensive and up-to-date summaries of accumulated knowledge on malaria (Sherman, 1998; Coluzzi and Bradley, 1999; Wahlgren and Perlmann, 1999).

**Overview of *Plasmodium* Infections in Humans**

All human *Plasmodium* species have fundamental similarities in their transmission cycles but differ in the manifestations of the disease they cause. Bites from infected anopheline mosquitoes transfer parasites into the human bloodstream, where they quickly make their way to the liver and invade hepatocytes. The parasites emerge from the liver after 1–3 wk (*P. vivax* and *P. ovale* can also remain in the liver in latent form and produce relapses months or years later). After emerging from the hepatocytes, the parasites infect erythrocytes in the bloodstream and proliferate by cycling through rounds of erythrocyte invasion, multiplication, and host cell lysis. These cycles lead to exponential expansion of the parasite populations and the pathogenesis of malaria. Recurring fevers, paroxysms, rigors, and sweats occur as erythrocytes rupture and release new invasive parasites into the bloodstream. Different species can cause fevers at different intervals because the rounds of replication differ in cycle time. *P. falciparum*, *P. vivax*, and *P. ovale* reinvoke erythrocytes every 48 hr, whereas *P. malariae* does so every 72 hr, giving rise to the historical terms of tertian and quartan malaria, respectively (fevers on days 1, 3, . . . vs. fevers on days 1, 4, . . . ).
A dangerous feature of *P. falciparum*-infected erythrocytes is their ability to adhere to the endothelial cells that line the microvasculature and thereby sequester within critical tissues. This phenomenon is thought to enable the parasites to avoid passage through the spleen, where they could be destroyed. In addition, sequestration may favor parasite growth and reproduction through effects of the microcapillary gas environment. Unfortunately, sequestration in such organs as the brain, lungs, and placenta can produce serious complications. Severe anemia, coma, pulmonary edema, and placental compromise in pregnancy are among the most dangerous developments. Rosetting of infected erythrocytes with surrounding uninfected red cells may also contribute to sequestration within microvessels and aggravate the pathogenic effects of infection (for an overview of the clinical features of malaria, see Marsh, 1999).

While *P. falciparum* is responsible for nearly all deaths directly attributable to malaria, all *Plasmodium* species can cause exhausting and recurring illnesses that have profound effects on individual productivity and fitness. Of these, the greatest burdens are produced by *P. falciparum* and *P. vivax*. Economic assessments have shown that a single bout of malaria results in a loss of 5–20 working days, and one study has shown that an agricultural family afflicted by malaria may be as much as 60% less productive than a family without malaria (Oaks et al., 1991). Depending on the characteristics of disease transmission and pathogenesis, some economic estimates may underrepresent the impact of malaria in communities where children are primary victims. Recent analyses have weighed the hidden costs of pain and suffering, increased susceptibility to other infectious diseases, and adverse demographic effects of the disease (http://www.malaria.org/jdsachseconomic.html). When these standards are used to gauge the costs incurred by malaria, they show its enormous potential to act as a selective force.

While a single bite by an infected mosquito is sufficient to cause malaria, studies from Kenya have shown that only about half of such bites actually transmit the infection to children, and only small percentages of these children go on to develop severe complications from malaria (Marsh, 1992). Individuals who have had several episodes of malaria usually become less susceptible to the worst effects of the disease because of acquired clinical immunity (premunition), meaning that their immune systems have “learned” how to cope with the disease. Inherited traits may also contribute significant protection against severe complications of malaria. We now review what is known about many of these traits in the context of host-parasite evolution.

**Thalassemias and hemoglobinopathies protect against the severe effects of malaria**

Many human polymorphisms that protect against severe manifestations of malaria are erythrocyte related, reflecting the importance of this cell as the primary refuge of the parasite during infection and disease. Mutations responsible for hemoglobin underproduction (thalassemias) or hemoglobin variants (hemoglobinopathies) are well recognized in this regard.

Over 50 yr ago, J. B. S. Haldane hypothesized that thalassemia may offer a protective effect against malaria (Haldane, 1949). Since that time, thalassemias have been shown to result from a number of diverse mutations in either the α- or β-globin genes, causing a decrease or loss of globin production (Weatherall and Clegg, 1981). Several lines of evidence have supported the hypothesis that malaria is the selective force behind these mutations. First, the α- and β-thalassemias have a distribution that overlaps with malaria endemicity, even though thalassemias are associated with hundreds of alleles that arose in different times and places (Flint et al., 1998). Second, epidemiologic studies have demonstrated associations between protection against malaria and certain thalassemias (Flint et al., 1986; Allen et al., 1997; Sakai et al., 2000). Some reports have suggested that the protective effect imparted by α-thalassemia is due to modified antigen recognition on the surface of the parasitized erythrocyte (Luzzi et al., 1991). It has also been proposed that α-thalassemia may be associated with higher levels of *P. vivax* infection early in life and that this may heighten immunologic defenses and provide better protection from more dangerous, subsequent *P. falciparum* infections (Williams et al., 1996).

A few years after Haldane’s predictions, A. C. Allison advanced the hypothesis for malaria’s role in the selection of human polymorphisms when he proposed that individual heterozygotes for sickle-cell hemoglobin are protected against the disease (Allison, 1954). Abundant evidence subsequently reinforced the idea that individuals heterozygous for the sickle-cell allele (sickle-trait; HbAS) are protected against the severe effects of malaria (Hill et al., 1992; Aluoch, 1997). Although differences in the erythrocyte’s ability to support parasite growth *in vitro* have been advocated as a protective mechanism (Friedman, 1978, 1979; Pasvol et al., 1978), parasitemia data in normal and HbAS individuals have not been readily explained by these differences (Gendrel et al., 1991). Protection is more likely the result of an HbS effect that leads to an early and enhanced acquisition of protective immunity against malaria (Bayoumi, 1987; Bayoumi et al., 1990). The finding that the hemoglobin S (HbS) mutation has arisen on at least four different occasions in Africa and the Middle East (Wainscoat et al., 1983) is further support for the protective value of the HbS gene.

The homozygous sickle-cell condition (HbSS) is responsible for the deadly effects of sickle-cell anemia, whereas the heterozygous condition is usually asymptomatic. Selection for AS carriers by malaria and selection against SS individuals by sickle-cell anemia is thus an example of a balanced polymorphism whereby an allele that is detrimental in the homozygous state is
maintained due to a survival advantage among heterozygotes.

Other predominant hemoglobin variants also have geographic distributions that suggest selection by malaria. HbE is the second most prevalent hemoglobin variant in the world. It is distributed throughout much of Southeast Asia and has gene frequencies greater than 0.10 in some regions (Ingram, 1986). Protection from malaria has been the conclusion of several studies (Nagel et al., 1981; Vernes et al., 1986; Kitayaporn et al., 1992; Hutagalung et al., 1999).

HbC is another common variant in West Africa, where it occurs in some populations at gene frequencies of 0.12 or more (Ingram, 1986). An interesting feature of HbC is that it contains an amino acid substitution at exactly the same β-6 position as the HbS mutation. Reduced parasite growth in HbCC cells has been reported under in vitro conditions (Friedman et al., 1979; Olson and Nagel, 1986), but high parasitemias readily occur in vivo in P. falciparum-infected HbCC individuals (Agarwal et al., 2000). Recent epidemiologic data have provided evidence of a protective effect of homozygous and heterozygous HbC against malaria in certain populations of West Africa (Agarwal et al., 2000; Modiano et al., 2001). In these populations, the prevalence of HbS was found to be lower than that of HbC, in contrast to populations elsewhere in Africa where rates of HbC are lower and a protective effect of HbC trait could not be demonstrated (Molineaux and Gramiccia, 1980; Guinet et al., 1997). This evidence for different protective effects from HbC suggests the interesting possibility that population-specific factors, including differences in genetic background, may have a strong influence on the distributions of haemoglobin mutations in malaria regions.

**Other polymorphisms that protect against malaria**

A number of other polymorphic genes have also come to be seen as contributory to producing malaria resistance. Like the thalassemias and hemoglobinopathies, some of these mutations were first identified as medical conditions. As Geoffrey Pasvol points out, “To view many of these variants as ‘defects’... is naïve. Many of these polymorphic variants have served the human genome well and must be considered in the context of providing a selective advantage in the face of dangerous life-threatening pathogens” (Pasvol, 1996).

At the plasma membrane, the P. vivax merozoite requires the Duffy chemokine receptor for erythrocyte invasion. A large percentage of the African population, as well as many American blacks, is Duffy negative; these individuals lack the receptor on their red blood cells and as a result are not infected by P. vivax (Miller et al., 1975, 1976). In these individuals, expression of the gene is blocked only in developing erythrocytes by a mutation of the promoter region that disrupts binding of the GATA1 erythroid transcription factor (Tournamille et al., 1995).

Changes in the membrane of erythrocytes can also affect the outcome of infection. Some changes caused by mutations in the erythrocyte membrane anion exchanger (AE1 or Band 3) are examples. Changes in the coding region of Band 3 are known to produce a syndrome called Southeast Asian ovalocytosis (SAO). This syndrome is associated with malaria endemicity in parts of the western Pacific (Mgone et al., 1996). Ovalocytic erythrocytes are thought to have reduced susceptibility to parasite invasion (Castelino et al., 1981; Kidson et al., 1981; Hadley et al., 1983; Cattani et al., 1987). The mechanism of this inhibition is unclear, but it has been suggested that altered binding of SAO Band 3 to parts of the cytoskeleton could decrease cell deformability or limit the usual redistribution of the protein that is observed when the parasite tries to invade (Jarolim et al., 1991). Recent epidemiologic data also suggest a protective effect against cerebral malaria (Allen et al., 1999), which may point to an effect in another aspect of pathogenesis.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a syndrome that results in decreased activity of a metabolic pathway that protects the interior of the cell from oxidant stress. G6PD deficiency may be the most common human enzymopathy in the world, as it is present in nearly 400 million people. Like the patterns of other polymorphisms we have described, its distribution correlates with malaria endemicity in Africa, Asia, the Middle East, and the Mediterranean. Epidemiologic field data and in vitro studies support associations between malaria resistance and the enzyme deficiency (Ruwende et al., 1995; Ruwende and Hill, 1998). The mechanism by which it might protect against malaria is still unknown. Some evidence suggests that the accumulation of toxic oxidized substances in the cell can inhibit parasite multiplication (Golenser et al., 1988). Other studies suggest that infected cells may be more susceptible to phagocytosis or hemolysis as a result of increased cytolytic compounds and membrane damage (Janney et al., 1986; Cappadoro et al., 1998).

Immune system polymorphisms have been documented for malaria as for other infectious diseases. Epidemiologic data have suggested that the most common human leukocyte antigen type in West Africa (HLA-B53) is associated with resistance to severe malaria (Hill et al., 1991, 1992). This would be consistent with evolution of variation in the major histocompatibility complex (MHC) of the human immune system by infectious agents. Polymorphisms present within the promoter region of the tumor necrosis factor (TNF) cytokine have been implicated in cerebral malaria pathogenesis, but these are associated with the presence of, rather than protection from, severe cerebral manifestations (McGuire et al., 1994, 1999; Knight et al., 1999). Selection against these polymorphisms has been proposed to be counterbalanced by other biologic advantages, such as enhanced resistance against microbes, or the gene may be in linkage disequilibrium with another highly selected component of the MHC.
Evolution of Plasmodium species

Based on comparisons of genetic sequences from a number of parasite species, it is believed that the genus Plasmodium originated ~150 million years ago, possibly as early as the split between birds and reptiles (Escalante and Ayala, 1994). Today there is a variety of extant species that parasitize birds, reptiles, and rodents, as well as human and nonhuman primates. Species that parasitize humans are found in three deeply rooted branches; genetic data suggest the P. malariae and P. vivax branches diverged ~100 million years ago, and the branch that gave rise to P. falciparum split away even earlier (Escalante et al., 1995).

Comparisons of the available DNA sequences from several species were thought to suggest that P. falciparum may have come to humans by a recent lateral transfer from avian hosts (McCutchan et al., 1984; Waters et al., 1991, 1993). This hypothesis appeared to be consistent with the idea that relatively new pathogens are less well adapted to their host and correspondingly produce more malignant and dangerous disease. Additional data, however, have shown that P. falciparum is very closely related to a chimpanzee parasite, P. reichenowi, and that these two parasites share a common ancestor from 5–6 million years ago, about the time of the divergence of the human and the chimpanzee lineages (Escalante and Ayala, 1994; Escalante et al., 1995). Subsequent studies of sporozoite protein sequences have led to the conclusion that these two parasites share ancestral features with Plasmodium species that infect birds but, if transfer of a bird parasite to a primate occurred, such a transfer would have happened in an evolutionary distant progenitor to humans and chimpanzees (McCutchan et al., 1996).

Comparisons of genes from regional isolates have led to reports that P. falciparum emerged only recently through a population bottleneck in Africa (Rich et al., 1998; Rich and Ayala, 1998, 2000; Ayala et al., 1999; Conway et al., 2000). Some objections have been raised to these conclusions because of the need to interpret the relative paucity of synonymous mutations in P. falciparum genes (Hughes and Verra, 1998; Saul, 1999). Although low numbers of non-coding single nucleotide polymorphisms (SNPs) were reported to support an age of 3,200 to 7,700 years for the bottleneck from which all extant P. falciparum emerged (Volkman et al., 2001), an estimated age of 100,000 to 180,000 years was subsequently found for this bottleneck from an analysis of 62 synonymous and 31 non-coding SNPs (Mu et al., 2002).

Development of agriculture probably facilitated the spread of P. falciparum in Africa when human agricultural practices brought about changes in speciation and anthropophilic behavior of anopheline mosquito populations. This may have resulted in an increase in malaria transmission and perhaps selection of more aggressive strains of P. falciparum parasites (Coluzzi, 1999). Haplotype analysis indicates that the major G6PD enzyme deficiency, G6PD A−, arose as a malaria protective polymorphism between 3,000 and 11,000 years ago, in the timeframe of the spread of agriculture and animal domestication in the Middle East and Northeast Africa (Tishkoff et al., 2001). P. falciparum probably was not present in the Western Hemisphere until the colonization of the Americas by Europeans and the introduction of African slaves (Coatney et al., 1971).

Development of drug resistance in Plasmodium

The widespread use of synthetic antimalarials in the 20th century altered P. falciparum populations by selecting drug-resistant strains. The premiere example is the development and spread of chloroquine resistance into nearly all malarious regions—a dramatic example of selection pressure acting in our own time. Chloroquine resistance was first observed in the late 1950s at separate foci in South America and Southeast Asia (Payne, 1987). It has since been linked to mutations in the P. falciparum protein PfCRT, a molecule that likely functions as a transporter in the parasite’s digestive vacuole membrane (Fidock et al., 2000). Field studies and population surveys of PfCRT mutations have suggested that chloroquine resistance arose in four distinct geographic foci (Wellem and Plowe, 2001; Wootton et al., 2002). Chloroquine resistance in P. vivax was first reported in the late 1980s (Rieckmann et al., 1989). The three-decade interval between the appearance of resistance in P. falciparum and in P. vivax is thought to be consistent with genetic findings that there are different mechanisms of drug resistance in these two species (Nomura et al., 2001).

Plasmodium genome shaping by host-parasite interactions

In P. falciparum malaria, parasites evade immune destruction by altering the antigenic and adhesive properties of infected erythrocytes. This ability is attributed to a major variable erythrocyte membrane protein (PfEMP1) that parasites place on the erythrocyte surface (Baruch et al., 1995). In individual parasites, PfEMP1 is an exclusively expressed product from one of about 50 different var genes within the genome (Su et al., 1995; Chen et al., 1998; Scherf et al., 1998). Parasites occasionally and spontaneously switch expression from one var gene to another, producing different PfEMP1 molecules and altering the antigenic properties of the infected cells (Smith et al., 1995). During P. falciparum infections, such switching gives rise to antigenically diverse subpopulations that must be continually chased by the immune response (Newbold, 1999). In field populations, vast numbers of var gene repertoires are present among different parasites (Kyes et al., 1997). The capacity for generating new antigenic forms is comparable to that of another human parasite, the African trypanosome (Rudenko, 1999), and represents a critical survival strategy that has evolved under continuous pressure from host defenses (Freitas-Júnior et al., 2000).

There is evidence for coevolutionary cycles of se-
lecion and adaptation in gene-for-gene struggles between the parasite and the host. The protective effect imparted by the HLA-B53 type may involve an immune response to the *P. falciparum* liver-stage antigen LSA-1 (Hill et al., 1992). Analyses of immune responses to parasite LSA-1 polymorphisms have led to the suggestion that the prevalence of parasite polymorphisms may be affected by HLA types in human populations (Gilbert et al., 1998; Plebanski et al., 1999). It has also been proposed that the prevalence of sickle-cell trait can influence the genetic structure of parasite populations. Distributions of the *P. falciparum* genes encoding the merozoite surface proteins Mspl and Msp2 have been reported to be skewed with the occurrence of Hbs trait in some regions (Ntoumi et al., 1997; Konate et al., 1999).

**CONCLUSION**

The ability of *Plasmodium* spp. and their mosquito and human hosts to adapt in response to selective pressure emphasizes the need for continued research to understand and control malaria. The catalog of human polymorphisms that reduce disease severity and the continual generation of new diversity in parasite populations testify both to the impact of malaria and its persistence as a major public health burden. By exploring the protective determinants and virulence factors that arise from evolutionary pressures in malaria, we may come to better understand the disease and possibly identify new approaches to its control.

**REFERENCES**


COEVOLUTIONARY GENETICS IN MALARIA


