# The remarkable journey of adaptation of the *Plasmodium falciparum* malaria parasite to New World anopheline mosquitoes

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Plasmodium falciparum *originated in Africa, dispersed around the world as a result of human migration and had to adapt to several different indigenous anopheline mosquitoes. Anophelines from the New World are evolutionary distant form African ones and this probably resulted in a more stringent selection of* Plasmodium *as it adapted to these vectors. It is thought that* Plasmodium *has been genetically selected by some anopheline species through unknown mechanisms. The mosquito immune system can greatly limit infection and* P. falciparum *evolved a strategy to evade these responses, at least in part mediated by* Pfs47*, a highly polymorphic gene. We propose that adaptation of* P. falciparum *to new vectors may require evasion of their immune system. Parasites with a* Pfs47 *haplotype compatible with the indigenous mosquito vector would be able to survive and be transmitted. The mosquito antiplasmodial response could be an important determinant of* P. falciparum *population structure and could affect malaria transmission in the Americas.*

Key words: malaria - *Plasmodium falciparum* - Anopheles - adaptation - mosquito - Americas

In spite of the great advances in disease control achieved in the past decade, malaria remains one of the most devastating infectious diseases to humankind. The disease, caused by *Plasmodium* protozoan parasites, is transmitted by anopheline mosquitoes. It threatens 1.2 billion people worldwide, with an estimated 219 million infections and 660,000 deaths in 2010 (WHO 2012). Although 90% of the mortality from malaria occurs in Africa, the American continent and the Caribbean have 119 million people at risk, with one million infections and 1,100 deaths estimated for 2010 (WHO 2012). In the Americas, 76.7% of infections are caused by *Plasmodium vivax* and 23.3% caused by *Plasmodium falciparum*. Brazil has the largest incidence of malaria, with 41% of the cases (WHO 2012).

In general, anopheline mosquitoes from the New World are less efficient vectors of malaria than African ones. This became dramatically evident during the transitory introduction of *Anopheles arabiensis* (member of the *Anopheles gambiae* species complex) to Brazil in the 1930's, which caused a serious increase in the prevalence of malaria with mortality rates of 20-25% (Parmakelis et al. 2008). One of the factors that results in lower transmission of human malarias by New World anophelines is their feeding preference. For example, major malaria vectors from sub-Saharan Africa, such as *An. gambiae* and *Anopheles funestus*, have an anthropophilic index (the probability of feeding on humans over other animals) of 80-100%; while in the vectors from the Americas the index is usually less than 50% (Bruce-Chwatt et al. 1966, Carter & Mendis 2002, Hay et al. 2010, Sinka et al. 2010a, b). Nevertheless, other mosquito and parasite factors, including the mosquito immune system, may also be important determinants of the efficiency of malaria transmission.

In this review we discuss the origin and global dispersion of *P. falciparum* throughout the world and the evidence for adaptation of *Plasmodium* parasites to different mosquito vectors. The role of the mosquito immune system in determining susceptibility to *Plasmodium* infection and the evidence for a mechanism of *P. falciparum* evasion of mosquito immunity will be presented. This background information is used to explore the potential role of the mosquito immune system as a barrier to adaptation of *P. falciparum* to indigenous vectors of the Americas.

*Out of Africa: P. falciparum geographic origin and dispersal* - Several lines of evidence indicate that *P. falciparum* originated in sub-Saharan Africa. It has been recently found that great apes from Africa harbour high diversity of *Plasmodium* species closely related to *P. falciparum* and that west African gorillas (*Gorilla gorilla*) harbour infections with a parasite that is nearly genetically identical to *P. falciparum*, strongly suggesting that *P. falciparum* originated in African gorillas and transferred to humans relatively recently (Liu et al. 2010, Prugnolle et al. 2011). Previous molecular genetic analysis of *P. falciparum* isolates from around the world has consistently shown higher genetic diversity in African isolates (Hartl 2004). Furthermore, the genetic diversity of *P. falciparum* populations decreases as a function of geographic distance from sub-Saharan Africa, also consistent with an African origin (Tanabe et al. 2010, 2013).

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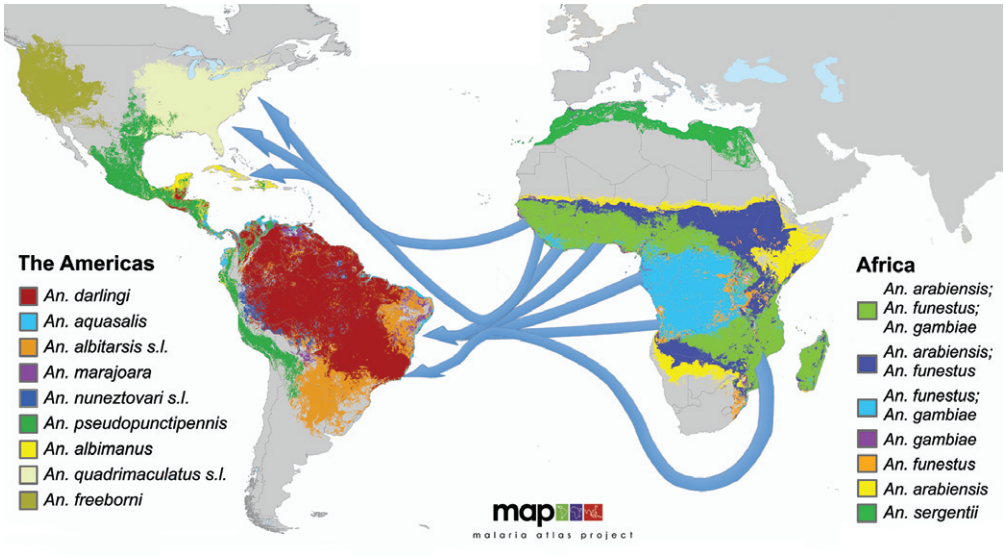
As malaria-infected humans migrated out of Africa, they carried *P. falciparum* with them, leaving their African mosquito vectors behind. This initiated a remarkable journey that led to the adaptation of the parasite to more than 34 different anopheline mosquito species worldwide (Sinka et al. 2012). Phylogenetic analysis of *P. falciparum* isolates indicates that the parasite population from the Americas has lower genetic diversity than those from the Old World (Anderson et al. 2000, Conway et al. 2000, Conway 2003, Joy et al. 2003, Neafsey et al. 2008, Yalcindag et al. 2012). Microsatellites, nuclear genes and mitochondrial DNA analysis indicate that American isolates are genetically closer to African haplotypes than to Asian ones, consistent with an introduction of African parasites into the American continent (Anderson et al. 2000, Joy et al. 2003, Yalcindag et al. 2012). Most historical, archaeological and genetic evidence suggest that malaria was introduced during or after the European conquest of the Americas (de Castro & Singer 2005, Webb Jr 2009, Yalcindag et al. 2012).

The *P. falciparum* populations that arrived to the Americas during the last 500 years probably had considerable genetic diversity. Historical evidence shows that more than seven million African slaves were brought to the New World between 1514-1866 (Voyages-Database, available from slavevoyages.org/tast/index.faces) (Figure). Today, the region in West Africa where most of these slaves originated presents a high prevalence of *P. falciparum* malaria, as well as large genetic diversity of the parasite (WHO 2012, Yalcindag et al. 2012). It is therefore very likely that a high percentage of African slaves carried *P. falciparum* infections and that the parasites they carried had a genetic diversity probably higher than what is found today in the Americas. The apparent decrease in genetic diversity could be the result of a random founder effect or genetic drift, due to the lower transmission in the New World. Alternatively, it may have been the result of adaptation by genetic selection or most likely, by a combination of these two factors.

There is historical evidence for significant malaria transmission that resulted in major outbreaks in Jamaica (1655-1656), Hispaniola (1794-1795, 1802) and Illinois (1850) (Webb Jr 2009). This suggests that a drastic founder effect due to low malaria transmission in the Americas probably did not take place. Adaptation through a genetic selection process could also have reduced the genetic diversity of *P. falciparum* in the Americas. Particular *P. falciparum* genotypes could have been selected by either or both of its obligatory hosts: humans and mosquitoes. In the case of humans, the large number of African slaves should have maintained high genetic diversity of *P. falciparum* in areas with significant transmission and there is no evidence of differential susceptibility to malaria in other ethnic groups that could have driven selection. More recently, however, the widespread use of the antimalarial drug chloroquine may have greatly reduced the genetic diversity of *P. falciparum* (Wootton et al. 2002).

Let us consider now the other host, the anopheline malaria vectors in the New World. Adaptation to local mosquitoes was undoubtedly an initial requirement for *P. falciparum* to be transmitted in the New World. According to the present geographic distribution of anophelines and the slave trade disembarking ports (Figure), some of the first indigenous vectors that *P. falciparum* encountered were *Anopheles albimanus*, *Anopheles aquasalis*, *Anopheles darlingi*, *Anopheles albitarsis* and *Anopheles quadrimaculatus*. Old and New World anophelines are evolutionary distant since they diverged some 95 million years ago, when the South American continent separated from what is now Africa (Moreno et al. 2010). It is, there-




Map of the major mosquito vectors of malaria in Africa and the Americas and slave-trade routes to the New World. *Plasmodium falciparum* is thought to have arrived to the Americas in malaria-infected Africans brought during the transatlantic slave trade between 1514-1866. The blue arrows indicate general transatlantic slave trade routes. The malaria vector map is from the Malaria Atlas Project (Sinka et al. 2012) and was modified to illustrate the slave trade routes.

fore, not surprising that there are marked genetic, ecological and behavioural differences between New World and African anopheline vectors (Sinka et al. 2010a, b). This probably resulted in a more stringent selection of *Plasmodium* as it adapted to these vectors.

*Evidence for adaptation of Plasmodium to different anophelines by genetic selection* - There are several lines of evidence for adaptation of *Plasmodium* to new vectors through genetic selection by different anopheline species. One case involves *P. vivax* transmission in different areas of Southern Mexico (Joy et al. 2008). In this region, the low altitude (< 100 m) mosquito *An. albimanus* transmits malaria in coastal areas, whereas in the foothills malaria is transmitted mainly by the higher altitude mosquito *Anopheles pseudopunctipennis* (Rodriguez et al. 2000). Interestingly, genetically distinct populations of *P. vivax* have been identified in these two geographic areas. Furthermore, laboratory infections showed that *An. albimanus* is more susceptible to infection by coastal *P. vivax* genotypes, whereas *An. pseudopunctipennis* is more susceptible to foothill parasite genotypes (Joy et al. 2008). This suggests that selection of *P. vivax* by local anophelines has led to adaptation of the parasite to different vectors.

In the case of *P. falciparum*, it has been found that a laboratory line of putative African origin (NF-54) and a clone from this line (3D7), infect poorly or not at all the New World *An. albimanus,* the main malaria vector in Central American and the Caribbean (Grieco et al. 2005, Garver 2006, Baton & Ranford-Cartwright 2012). Conversely, the African *An. gambiae* tends to be infected more efficiently with African *P. falciparum* isolates than with isolates from Thailand (Hume et al. 2007), consistent with genetic selection of different *P. falciparum* strains by anopheline vectors present in different continents. Taken together, these studies suggest that genetic selection of *Plasmodium* during its adaptation to different anopheline vectors does occur in nature. However, the mechanism of the adaptation has not been identified.

*The anopheline immune system as a barrier to Plasmodium adaptation* - *An. gambiae* mosquitoes can mount robust and effective antiplasmodial immune responses capable of greatly reducing or eliminating *Plasmodium* infection (Osta et al. 2004). The thioester containing protein-1 (TEP1), a key component of the *An. gambiae* complement-like system, is one of most potent antiplasmodial mosquito immune responses (Blandin et al. 2004, Fraiture et al. 2009, Povelones et al. 2009). TEP1 binds to the surface of *Plasmodium* ookinetes (the stage that invades the mosquito midgut epithelia) and triggers the formation of a complex that causes lysis and/or melanotic encapsulation of the invading parasite. Recent studies indicate that exposure of the parasite to nitration reactions during their transit through the invaded midgut epithelial cell is a prerequisite of TEP1 binding (Oliveira et al. 2012). Although the *An. gambiae* complement-like system has been shown to be very effective at eliminating some parasite species, such as *Plasmodium berghei* and *Plasmodium yoelii* (mouse malaria parasites) (Blandin et al. 2004, Jaramillo-Gutierrez et al. 2009), it is less active or not active against sympatric *P. falciparum* from Africa (Cohuet et al. 2006, Nsango et al. 2012). Furthermore, it was recently found that some African *P. falciparum* isolates are able to evade the complement-like system of an *An. gambiae* refractory strain that was selected to be refractory to *Plasmodium cynomolgi* and mounts a very strong antiplasmodial response. In contrast, isolates from non-African regions are efficiently killed and encapsulated by this refractory strain (Molina-Cruz et al. 2012). This suggests that sympatric *P. falciparum* strains are adapted to evade the *An. gambiae* immune system.

Using linkage mapping and functional genetics, *Pfs47* was identified as a gene that allows some African strains of *P. falciparum* to evade the *An. gambiae* immune system (Molina-Cruz et al. 2013). *Pfs47* is a member of the 6-cys protein family and is expressed on the surface of female gametocytes and ookinetes (van Schaijk et al. 2006, Molina-Cruz et al. 2013). The *Pfs47* homologue in *P. berghei* is required for female gamete fertility (van Schaijk et al. 2006), but *Pfs47* is not essential for *P. falciparum* fertilisation. Although the mechanism of action of *Pfs47* is not yet known, *Pfs47* appears to be actively inhibiting protein nitration in the mosquito midgut cell (Molina-Cruz et al. 2013), which is a prerequisite for TEP1-mediated parasite elimination (Oliveira et al. 2012). *Pfs47* inhibits the induction of two enzymes, NOX5 and HPX2, that mediate nitration in response to *Plasmodium* invasion (Molina-Cruz et al. 2013). Because the Jun N-terminal kinase (JNK) pathway mediates the induction of these two enzymes (Garver et al. 2013), *Pfs47* seems to disrupt JNK signalling by interacting with a critical mosquito target protein that remains to be identified.

The fact that *Plasmodium* evolved a mechanism to evade the mosquito complement-like system indicates that this defense mechanism is an important determinant of malaria transmission. Evidence that the mosquito immune system may be a barrier for adaptation of *Plasmodium* to a new vector comes from studies in *Anopheles quadriannulatus*, a non-malaria vector from Africa. In this anopheline, disruption of the mosquito complement-like system by silencing of TEP1 expression greatly increased susceptibility to *P. falciparum* infection (Habtewold et al. 2008).

The *Pfs47* gene is likely to be involved in adaptation to new vectors, due to its role in evasion of the mosquito immune system. Population genetic analysis has shown that *Pfs47* is a highly polymorphic protein with different haplotypes predominating in different continents, indicating strong geographic genetic structure (Anthony et al. 2007, Manske et al. 2012). In fact, analysis of whole genome sequences of 227 *P. falciparum* isolates from Africa, Asia and Papua New Guinea revealed that *Pfs47* has one of the highest levels of geographic genetic structure when compared to polymorphisms in the rest of the genome (Manske et al. 2012). *Pfs47* was found to have higher genetic diversity in African *P. falciparum* isolates compared to other continents (Anthony et al. 2007, Manske et al. 2012) and one of the polymorphisms in *Pfs47* has a fixed difference between Africa and other continents (Manske et al. 2012). We propose that the

geographic genetic structure of *Pfs47* may be largely determined by selection imposed by the immune system of anopheline mosquitoes from different regions of the world and that interaction of *Pfs47* haplotypes with particular haplotypes of its target in the mosquito has been critical for adaptation of the parasite to new vectors.

Based on this working hypothesis, one can draw several important predictions: (i) different anopheline vector species could harbour different haplotypes of the protein that interacts with *Pfs47* and malaria transmission would be enhanced when compatible parasite-vector combinations interact. As a result, the mosquito species present in a given geographic area selects for compatible *Pfs47* haplotypes; (ii) the population genetic structure observed for *Pfs47* (and for the parasite in general) may be better understood by correlating *Pfs47* haplotypes to mosquito vector species present in a given geographic region. This correlation should be even stronger with the haplotypes of the mosquito target of *Pfs47*, a gene that has not been identified; (iii) *Pfs47* may be a good target for a transmission blocking strategy in *An. gambiae*. Binding of antibodies from a vaccine against *Pfs47* or inactivation with a drug, could prevent the interaction of *Pfs47* with the mosquito target protein and disrupt the ability of the parasite to evade the mosquito immune system.

*Experimental models of malaria transmission in Brazil* - A wealth of information on mosquito-parasite interactions has been generated in the last 10 years, mostly in *An. gambiae* and *Anopheles stephensi*, the major malaria vectors in Africa and India, respectively. These two mosquito species can be readily colonised and are susceptible to infection with several parasite species, such as murine malarias (*P. berghei* and *P. yoelii*) (Blandin et al. 2004, Jaramillo-Gutierrez et al. 2009) or human malaria gametocyte cultures (*P. falciparum*) (Luckhart et al. 1998, Dong et al. 2006, Garver et al. 2009); making them robust laboratory models to study the biology of malaria transmission. Although malaria is an endemic disease in the Americas, especially in the Amazon Region, relatively little is known about the biology of parasite transmission by mosquito vectors in Brazil, such as *An. darling* or *An. aquasalis*. Research in this area would be greatly facilitated by establishing robust experimental systems. Until recently, *An. darlingi*, the major vector in Brazil, had not been colonised because this species does not mate readily in captivity. As a result, all experimental work has been done using field-collected larvae that are reared in the laboratory until they reach the adult stage. However, the recent report of adaptation of *An. darlingi* to laboratory conditions could be a major breakthrough in the field (Villareal et al. 2013).

In general, anophelines are highly adapted to a particular ecological niche and the immune systems of African and New World mosquitoes probably have evolved to deal with the microorganisms in their natural habitat. A broad genome-wide comparison of the immune effector genes between anopheline mosquitoes will soon be possible with the completion of the sequence of several genomes. This analysis may reveal important differences in organisation and/or expansions of specific immune effector genes in particular anopheline species. Given the great evolutionary distance between New World vectors and *An. gambiae*, it is likely that there are some important differences in their antiplasmodial responses. Recent studies on the immune response of *An. aquasalis* to *P. vivax* infection revealed that the participation of the signal transducer and activator of transcription pathway in antiplasmodial immunity, first described in *An. gambiae,* is also taking place in this system (Bahia et al. 2010). However, reducing detoxification of reactive oxygen species by silencing catalase enhanced *P. vivax* infection in *An. aquasalis* (Bahia et al. 2013), but had the opposite effect when *An. gambiae* mosquitoes are infected with the murine malaria parasite *P. berghei* (Molina-Cruz et al. 2008). These results highlighting the importance of comparative studies to validate models built based on different mosquito-parasite combinations, as some responses may not be universal.

Our working hypothesis also indicates that it is critical to consider the compatibility between the mosquito-parasite combinations being used when establishing experimental models of malaria transmission, as mosquitoes could mount strong immune responses to one parasite strain, but not respond at all when infected with a different strain. *P. falciparum* NF-54 is a laboratory strain of African origin that is commonly used in trials to assess the efficiency of transmission blocking drugs or vaccines. However, parasites with the NF-54 haplotype of *Pfs47* are rarely found in human isolates outside of Africa and infect *An. albimanus* very poorly; suggesting that parasites with this haplotype may not infect vectors outside of Africa very efficiently. The adaptation of *Plasmodium* isolates from the same geographic area as the mosquito vectors to in vitro culture may be the best way to ensure a compatible combination.

In conclusion, as humans migrated, they dispersed *P. falciparum* around the world and the parasite had to adapt to different indigenous anopheline mosquitoes. In the case of the Americas, *P. falciparum* encountered anophelines that are evolutionary distant from African vectors. Mosquitoes can mount effective immune responses that represent an important barrier to malaria transmission. In response, *P. falciparum* has evolved the capacity to evade the *An. gambiae* immune system through a mechanism that involves *Pfs47.* We propose that those parasites with *Pfs47* alleles that were compatible with the anopheline species present in a new geographic region could be transmitted because they were able to evade the mosquito immune system and that genetic selection by interaction with the vectors immune systems shaped the population of *P. falciparum* parasites present in the New World.

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