Review in Advance first posted online on October 3, 2016. (Changes may still occur before final publication online and in print.)

Mosquito Vectors and the Globalization of *Plasmodium falciparum* Malaria

Alvaro Molina-Cruz,[1] Martine M. Zilversmit,[2] Daniel E. Neafsey,[3] Daniel L. Hartl,[4] and Carolina Barillas-Mury[1]

[1]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland 20852; email: cbarillas@niaid.nih.gov

[2]Richard Guilder Graduate School and Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, NY 10024

[3]Genome Sequencing and Analysis Program, Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142

[4]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138

Annu. Rev. Genet. 2016. 50:20.1–20.19

The *Annual Review of Genetics* is online at genet.annualreviews.org

This article's doi: 10.1146/annurev-genet-120215-035211

Keywords

malaria globalization, *Plasmodium falciparum*, *Anopheles* mosquito, antiplasmodial immunity, immune evasion

Abstract

Plasmodium falciparum malaria remains a devastating public health problem. Recent discoveries have shed light on the origin and evolution of *Plasmodium* parasites and their interactions with their vertebrate and mosquito hosts. *P. falciparum* malaria originated in Africa from a single horizontal transfer between an infected gorilla and a human, and became global as the result of human migration. Today, *P. falciparum* malaria is transmitted worldwide by more than 70 different anopheline mosquito species. Recent studies indicate that the mosquito immune system can be a barrier to malaria transmission and that the *P. falciparum Pfs47* gene allows the parasite to evade mosquito immune detection. Here, we review the origin and globalization of *P. falciparum* and integrate this history with analysis of the biology, evolution, and dispersal of the main mosquito vectors. This new perspective broadens our understanding of *P. falciparum* population structure and the dispersal of important parasite genetic traits.

INTRODUCTION

Malaria is transmitted by anopheline mosquitoes and is one of the most devastating parasitic human diseases (109). Although the current control strategies have reduced the number of malaria cases worldwide by 37% over the past 15 years, it is estimated that in 2015 there were 214 million new cases and 438,000 deaths, mostly of children from sub-Saharan Africa (109). *Plasmodium falciparum* is the most virulent of the five malaria parasites that infect humans and is responsible for 94% of cases and 99% of malaria-related deaths world-wide (109). *P. falciparum* malaria originated in Africa and became global as humans migrated to other continents. During this process, parasites encountered different mosquito species, many of which were evolutionarily distant from African vectors. Those parasites that were able to develop in local vectors continued to be transmitted and became established in new geographic regions. Today, malaria is transmitted to humans by more than 70 different anopheline vector species around the world (91).

Coevolving organisms influence each other's evolution and adaptation, and the specialized lifestyle of *Plasmodium* and its antagonistic relationship with its vertebrate host is revealed in both the host and pathogen's genomes. There is ample evidence that the vertebrate immune system drives *P. falciparum* selection for diversity of surface proteins, allowing the parasite to avoid recognition (reviewed in 18). For example, in the *var* gene family, which consists of different forms of the PfEMP1 protein (93, 94), great diversity was generated by gene duplication and reorganization of multiple domains. There are approximately 60 different *var* genes in the genome (41, 82), and they show such extreme levels of diversity that individual parasites often have unique repertoires of these gene types even within the same population (6, 41), with multiple novel forms evolving from parental types in a single recombination event (28). The parasite avoids immune detection by expressing only one variant of the gene at a time, and when the immune system adapts to recognize this variant, a small subset of parasites expressing a different gene variant on the erythrocyte surface survives and thrives (23, 27).

It is also clear that malaria has driven selection for some genetic traits, such as sickle cell anemia and other hemoglobinopathies, in human populations living in highly endemic areas (98), because these traits protect the host from severe malaria (12, 72). However, until recently, there has been no clear evidence that the mosquito immune system exerts a selective force on *Plasmodium* parasite populations or that the parasite exerts a selective pressure on mosquito vectors. When *Anopheles gambiae* mosquitoes from an endemic region were infected with parasites circulating in humans from the same area, the mosquito immune system was unable to mount an effective antiplasmodial response (17). This led many in the field to conclude that malaria transmission is a passive process. Although the observations are accurate, the interpretation is incorrect. In the past six years, several studies indicate that the mosquito complement-like system can limit *P. falciparum* infection to some extent; however, in general, the effects observed are modest (20, 52, 66, 108). The problem of analyzing the interactions between mosquitoes and parasites circulating in a given region is that one cannot observe all the parasite genotypes that were unsuccessful, because they have been eliminated. When one looks at the dynamics of malaria transmission at a global scale, a completely different picture emerges. One realizes, for example, that common parasite haplotypes in Africa are absent in other continents. In this review, we provide a synopsis of our current understanding of the global evolutionary history of mosquito vectors of *P. falciparum* malaria and integrate this history with the origin, dispersal, and population genetics of the parasite. We also discuss the molecular mechanisms by which the immune systems of various anopheline mosquitos can act as barriers for malaria transmission and how immune evasion mediated by the parasite protein *Pfs47* may affect malaria transmission worldwide.

ORIGIN OF *PLASMODIUM FALCIPARUM* MALARIA

Two questions are at the heart of efforts to understand the origin of falciparum malaria. First, where did this most lethal form of malaria come from? Second, how did it subsequently spread across the globe?

Malaria is not exclusive to humans, and parasites of the genus *Plasmodium* infect and cause malaria in multiple vertebrate groups, including apes, monkeys, and rodents. In addition, related vector-borne parasites infect the blood of bats, lizards, and snakes (75). One consistent characteristic of malaria parasites, particularly those of mammals, is that they tend to be specific to their vertebrate host, e.g., macaque parasites do not infect chimpanzees and vice versa (75). However, *Plasmodium* parasites tend to have broader host specificity in the mosquito and can often infect several different anopheline species (91). Based on the narrow vertebrate host range, it appears that malaria has been infecting humans and our ancestors for tens if not hundreds of millions of years, with occasional host switches that lead to new species of parasite. *Plasmodium falciparum* is a species that apparently arose from such a leap from one vertebrate host to another and subsequently spread throughout the world from its origin in Africa.

One of the early discoveries from phylogenetic analyses of the genus *Plasmodium* was that the five species of parasites that infect humans (*P. falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*) are not all closely related (24, 75). This finding indicates that different types of malaria came into the human lineage independently. Molecular evidence has shown that the great apes in parts of Africa are teeming with malaria parasites related to *P. falciparum*. The discovery of a new species, designated *Plasmodium gaboni*, in pet chimpanzees in Gabon (69) was quickly followed by studies reporting *P. falciparum* and other distinct malaria parasites in chimpanzees and gorillas (21, 77, 87). However, it is not known whether infected apes, especially juveniles, ever suffer mortality or symptoms of severe malaria. The most ambitious study of *Plasmodium* in great apes examined approximately 1,800 chimpanzees, 800 gorillas, and 100 bonobos living in remote forest areas (46). Infection with species related to *P. falciparum* was found to be endemic in chimpanzees from both western and eastern Africa as well as in western lowland gorillas, with minimum estimates of prevalence of 32–48%. This level of prevalence is comparable to that of *P. falciparum* in human populations in regions of hyperendemic transmission (83). Among the infected apes, approximately 70% were co-infected with multiple lineages, often with two or more distinct but related species. The sequences of the new parasites found in chimpanzees and western gorillas clustered into six distinct clades, three specific to chimpanzees and three to gorillas. Three of these clades had not been previously reported. No evidence was found for recombination between the chimpanzee and gorilla clades, suggesting that the clades are largely host-specific. The authors have suggested that each of these six clades constitutes a separate species (46), and a nomenclature for these species has been proposed (83).

In this sampling of great apes (46), no sequences closely related to *P. falciparum* were recovered among more than 200 distinct sequences from the chimpanzee samples. *P. falciparum* parasites in humans have low genetic diversity and are closely related to *P. praefalciparum* in gorillas. This led the authors to propose that all extant human lineages derive from a single horizontal transfer from gorillas to humans (46). Others have proposed that the parasite originated in humans and transferred to gorillas and then many parasite lineages in humans went extinct and this reduced the genetic diversity of *P. falciparum* in the human population. This more intricate hypothesis seems less likely (78). The mosquito vector responsible for the initial transmission between gorillas and humans has not been identified, but *Anopheles moucheti*, *A. vinckei*, and *A. marshallii*, have been proposed as a candidate because *P. praefalciparum* has been detected in mosquitoes of this species recovered in the wild (50, 54, 74).

ENTRY OF *PLASMODIUM FALCIPARUM* INTO THE HUMAN LINEAGE

kya: thousand years ago

SNPs: single-nucleotide polymorphisms

Inbreeding coefficient (F_{IS}): the level of inbreeding in a population by measuring the deficiency of heterozygous genotypes relative to random mating

If *P. falciparum* in humans arose from a single cross-species transmission event from gorillas, the next issue to be considered is the timing of this event and the subsequent spread of *P. falciparum* malaria. Ample evidence shows that the disease was already well established in human populations several thousand years ago (kya). For example, approximately 2.4 kya, Hippocrates so accurately described malaria's characteristic intermittent fevers, as well as its seasonal variation and association with marshes, that he is often considered the first malariologist. Other direct evidence of malaria in antiquity comes from *P. falciparum* DNA recovered from Egyptian mummies that are approximately 4,000 years old (64).

Both direct and indirect evidence suggest that epidemic malaria emerged in the human population approximately 10 kya. The climate in equatorial regions of Africa at that time was warm and humid (67), allowing for ideal breeding conditions for mosquito vectors. At about the same time, the human population density increased owing to the spread of slash-and-burn (swidden) agriculture (89), an important factor in increasing the ease of transmission and promoting the evolution of higher virulence phenotypes (84). In the human genome, the spread of epidemic malaria is attested to by the origin, approximately 10 kya, of the resistance mutations resulting in sickle-cell hemoglobin (47, 110) and deficiency in glucose-6-phosphate dehydrogenase (33, 99). In addition, the distribution of pairwise mismatches of single-nucleotide polymorphisms (SNPs) in *P. falciparum* mitochondrial DNA suggests an exponential increase in parasite population size in Africa approximately 10 kya (39).

However, additional data indicate that this population increase was not the first such expansion for the parasite. Extensive mitochondrial genome sequencing analysis supports the hypothesis of a rapid population expansion approximately 10 kya but also suggests that some lineages are much more ancient, with origins ranging from 50 kya to 100 kya (39). This evidence has been critical in resolving a controversy about the origin of *P. falciparum* malaria; multiple rigorous studies of nuclear loci had conflicting results, with some showing a very recent origin (86, 104) and others showing one much earlier (61, 95). In retrospect, the virtual absence of the initially observed synonymous polymorphism seems to be due in part to the sampling of a small number of genes from a limited number of isolates, but it might also be due in part to a demographic effect of epidemic transmission that intensifies both purifying selection and, paradoxically, random genetic drift (11). Although it is unclear when *P. falciparum* was transferred from gorillas to humans, the recent data point to a relatively recent (in evolutionary time) event that took place approximately 10–100 kya. Nevertheless, it is clear that speciation of anopheline mosquitoes preceded the appearance of *P. falciparum* malaria in humans.

GLOBAL POPULATION STRUCTURE OF *PLASMODIUM FALCIPARUM* MALARIA

With a new understanding of the origin of *P. falciparum* malaria in Africa, attention naturally turns to how it spread outside of the continent. A number of studies have addressed this question by analyzing genetic diversity and structure within and among populations worldwide (1, 39, 51, 60, 62, 105, 106, 112). These studies reveal several differences among the different African populations of *P. falciparum*. For example, on a continental scale, African *Plasmodium* parasites show fewer genetic differences among subpopulations and are less likely to undergo inbreeding than *Plasmodium* parasites in Southeast Asia or South America. West African populations have an inbreeding coefficient (F_{IS}) in the range 0.30–0.40 (4, 35, 51), compared with approximately 0.60 in populations in East Africa (51), approximately 0.70 in Southeast Asia (51), 0.60–0.90 in South America (1, 49), and approximately 0.90 in Papua New Guinea (51, 73).

FIXATION INDEX

F_{ST} (fixation index) is a parameter that measures the genetic distance between subpopulations. The theoretical value of FST ranges from 0 to 1. Higher values indicate greater genetic distance between subpopulations and more genetic structure in the population as a whole. An $F_{ST} = 0$ indicates freely interbreeding subpopulations, whereas FST = 1 indicates genetically distinct subpopulations.

Differences in levels of inbreeding also affect effective recombination rates, as revealed by the extent of linkage disequilibrium (LD) across the genome (1, 51, 60, 62). The level of LD approaches background levels at distances of 1 kb in African populations, 10 kb in those from Southeast Asia, and 100 kb in those from South America (62). Inbreeding levels also contribute to the greater genetic variation observed among subpopulations than within subpopulations. For example, the genetic variation among subpopulations in South America, as measured by the fixation index (F_{ST}) (see sidebar, Fixation Index), is estimated as 0.364, compared with 0.007 for subpopulations in Africa (1). Parasite populations from different continents are also genetically differentiated ($F_{ST} = 0.20$–0.45) and easily separated by principal components analysis (51, 60, 62, 103).

The patterns of overall genetic diversity indicate that African populations of *P. falciparum* are not only more homogeneous than those from other continents but also more genetically diverse (51, 60, 62, 103). For example, the average heterozygosities of microsatellite polymorphisms were 0.30–0.40 in populations in South America, 0.51–0.65 in those in Southeast Asia, and 0.76–0.80 in those in Africa (1). The high genetic diversity of the parasite in Africa is consistent with the idea that Africa is the place of origin of malaria, and the greater homogeneity may be the result of high disease transmission by efficient vectors.

Taken together, the available evidence supports a path out of the cradle of Africa, initially to Asia and later, in a separate migration out of Africa, to the New World. Notably, although the pattern of *P. falciparum* dispersal is the same as that of humans colonizing these regions, the timing is apparently quite different. The geographic isolation of some of the human mutations that confer resistance to severe malaria indicates that they have an independent origin; some, such as thalassemia and ovalocytosis (10), emerged outside of Africa, indicating that the disease was still spreading to other regions after the initial waves of human migration out of Africa had already occurred (25, 34). Because malaria transmission is completely dependent on the mosquito, one must consider the vector species that allowed the disease to become established in different regions.

ORIGIN, SPECIATION, AND DISTRIBUTION OF MOSQUITO VECTORS OF *PLASMODIUM FALCIPARUM* MALARIA

The five species of *Plasmodium* parasites known to infect humans are exclusively transmitted by approximately 70 species of mosquitoes belonging to the genus *Anopheles* (91). Therefore, an understanding of the global history of malaria must consider the evolutionary history and phylogeography of its requisite vectors. Unlike the distribution of the *Aedes* spp. mosquito vectors of yellow fever, dengue, and chikungunya, which exhibit cosmopolitan or rapidly expanding geographic ranges due to anthropogenic dispersal, the distribution of anopheline mosquito species has been impacted remarkably little by humans in the modern era. One notable exception was the accidental introduction of the African species *Anopheles arabiensis* into Brazil in the twentieth century, but this transplant was fortunately eliminated before it could spread more extensively through the neotropics (71).

Linkage disequilibrium (LD): nonrandom association of alleles at different loci

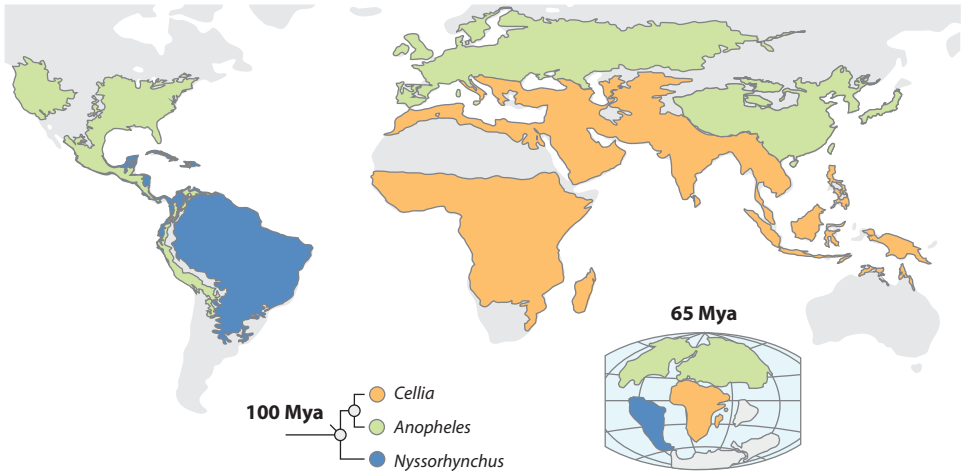

Figure 1

Origin and present geographic distribution of the three major anopheline mosquito subgenera that transmit malaria; subgenus *Nyssorhynchus* is in blue, *Cellia* is in yellow, and *Anopheles* is in green. The schematic phylogenetic tree indicates the evolutionary relationship among subgenera, with the divergence of *Nyssorhynchus* occurring approximately 100 million years ago (Mya). The inset shows the spatial relationship between the continents and the probable general location of these three subgenera approximately 65 Mya, when the continents drifted apart. It is clear that at that time, South America and Africa had separated but the northern regions were still in contact, allowing the migration of mosquitoes of the *Anopheles* subgenus from Europe and Asia to North America. The time and mechanism of speciation between the *Cellia* and *Anopheles* subgenera is not well established. The current map is based on the predicted distribution of malaria vectors reported by the Malaria Atlas Project (91).

Anopheles mosquito species are highly adapted to specific ecological niches and generally inhabit the geographic regions where they originally evolved. Their global distribution and speciation are therefore determined in part by geographic isolation resulting from the forces of continental drift (**Figure 1**). The *Anopheles* genus is very old, and these mosquitoes were originally residents of Pangaea. Molecular estimates suggest that the *Anopheles* lineage diverged from the culicine mosquito lineage approximately 217 million years ago (Mya) (85) and that extant species shared their most recent common ancestor at least 100 Mya (58). The three major subgenera of *Anopheles* (*Anopheles*, *Cellia*, and *Nyssorhynchus*) that transmit malaria to humans have different geographic ranges (**Figure 1**). Whereas subgenus *Anopheles* is cosmopolitan, the latter two subgenera have ranges that reflect the rift that developed 125–115 Mya (100) between the landmasses that would become South America and Africa; *Cellia* is restricted to the Old World, and *Nyssorhynchus* is limited to tropical regions of the New World (reviewed in 32) (**Figure 1**). The subgenus *Kerteszia*, present in Central and South America, also includes some malaria vectors (e.g., *Anopheles cruzzi*, *Anopheles bellator*, and *Anopheles neivai*). The *Kerteszia* is a small subgenus whose larvae have specific ecological requirements, as they can only develop within water that accumulates at the base of bromeliads, a flowering plant native to the neotropics (43).

Substantial evolutionary divergence is evident between the New World and Old World lineages of *Anopheles*, as was revealed by a recent effort to sequence and analyze the genomes of a geographically diverse collection of 16 *Anopheles* species exhibiting varying degrees of vectorial capacity (63). The genes and genomes documented by this effort were analyzed in order to understand the general evolutionary characteristics of this genus, as well as specific evolutionary patterns relevant to malaria transmission. *Anopheles* genomes have been evolving much more dynamically

Mya: million years ago

than their counterparts in *Drosophila*, another dipteran genus boasting comparable genomic resources. Compared to *Drosophila*, *Anopheles* genomes exhibit a fivefold higher rate of gene gain and loss, a higher rate of rearrangement on the X chromosome relative to autosomes, and a higher rate of intron loss (63).

In addition to facilitating the exploration of evolutionary signals spanning the entire *Anopheles* genus, comparative genome sequence analysis has also clarified the recent evolution of the *Anopheles gambiae* species complex, a clade of morphologically identical, closely related species that includes several major vectors of human malaria in sub-Saharan Africa. In particular, this work has refined our understanding of two shallowly diverged lineages only recently recognized as species (15), *A. gambiae* sensu stricto (formerly *A. gambiae* Molecular Form S) and *Anopheles coluzzii* (formerly *A. gambiae* Molecular Form M). Both species exhibit high anthropophily, i.e., a predilection for biting humans over other animals, making them important vectors in sub-Saharan Africa. *A. coluzzii* exhibits a tendency to select larger or more permanent bodies of water (e.g., rice paddies) as breeding sites compared to *A. gambiae* s.s., which prefers puddles, leading to speculation that human agricultural modification of the environment has driven the speciation process in these lineages and exacerbated malaria transmission in Africa. Phylogenomic analysis of the genome sequences from these two taxa, as well as from five other members of the *A. gambiae* species complex, indicates, however, that divergence of the *A. coluzzii* and *A. gambiae* s.s. lineages likely began 0.5 Mya, too early for agricultural modification of the environment to be a cause (26). The long coexistence of humans with these mosquito lineages in Africa has likely contributed to their shared ancestral predilection for human feeding. However, their recent ecological differentiation in response to human-mediated changes to the environment was preceded by their original divergence and by the switching of *P. falciparum* to human hosts. Some characteristics of the mosquito species present in a given region, such as their proclivity to feed on humans and their ability to support the complete life cycle of *Plasmodium*, are key determinants of their potential as disease vectors. Phylogenomic analysis of another member of this species complex (and another major vector of African malaria), *A. arabiensis*, also indicates that a large amount of introgressive gene flow occurred between the ancestor of that lineage and the ancestor of *A. coluzzii* and *A. gambiae* s.s., potentially disseminating anthropophilic feeding behaviors or other components of vectorial capacity into the *A. arabiensis* lineage (26). Although this event also likely preceded the origin of *P. falciparum* as a human parasite, it potentially helped to set the stage for the highly efficient transmission of *P. falciparum* in Africa and the emergence of that parasite as a major threat to global health.

Species complex: a group of closely related species that are difficult to differentiate from each other

Phylogenomic analysis: analysis of the evolutionary history of different species

Introgressive gene flow: movement of a gene from one species to another by repeated backcrossing of an interspecific hybrid with one of the parent species

Anthropophilic: the propensity of an arthropod vector to feed on a human over animals

PLASMODIUM BIOLOGY IN THE MOSQUITO AND VECTORIAL CAPACITY

Our understanding of the genetic and environmental factors that determine vectorial capacity for malaria transmission has increased substantially in the past two decades. *Plasmodium* parasites undergo a complex developmental program in the mosquito that involves several different stages, including sexual reproduction. The parasite life cycle is initiated when a female anopheline mosquito takes a blood meal containing male and female *Plasmodium* gametocytes. Within minutes, these sexually differentiated forms transform into gametes in the mosquito midgut lumen and fertilization occurs, creating a zygote. The zygote develops into a motile ookinete that must traverse the midgut epithelia. The ookinetes transform into oocysts as they reach the midgut basal lamina and start to divide constantly, eventually releasing thousands of sporozoites into the mosquito hemolymph. Some sporozoites are able to invade the salivary gland and are transmitted when an infected mosquito bites a human host. To complete their life cycle, parasites must cross

multiple barriers, including the epithelial layers of the midgut and salivary glands, and survive the epithelial defense responses and the systemic antiplasmodial responses mounted by the mosquito immune system (5, 13, 14, 19). This is a difficult and dangerous journey, and the parasite suffers major losses at each developmental step in the mosquito (90).

Several environmental, behavioral, cellular, and biochemical factors determine the ability of a mosquito to transmit malaria (i.e., the mosquito's vectorial capacity). These include physical conditions such as geographic colocalization with the parasite, temperature, and humidity as well as mosquito characteristics, including host preference, feeding rate and behavior, mosquito population density, longevity, and the mosquito's susceptibility to infection by *Plasmodium* (16, 111). Different mosquito species exhibit major differences in vectorial capacity. For example, some anopheline species, such as *A. gambiae*, are efficient vectors of malaria in part because they are anthropophilic; others, such as *Anopheles albimanus*, are more zoophilic and prefer to feed on animals over humans, making them less efficient vectors of malaria (92).

Comparative genomic analysis has also revealed insights into the molecular biology of vectorial capacity (63). Although anophelines exhibit high rates of gene gain and loss, little dynamism was observed in gene families associated with chemosensation, a biological process relevant to several aspects of mosquito biology, including host-seeking behavior. The number of olfactory receptor genes (approximately 60) has remained remarkably constant among the sequenced members of the *Anopheles*, *Cellia*, and *Nyssorhynchus* subgenera, despite a gain of 12 genes in this family by an ancestor of the *A. gambiae* species complex. Although the size of the chemosensation gene family has remained relatively static, many genes in this category exhibit high rates of nonsynonymous (amino acid–changing) sequence evolution, suggesting selection to diversify chemosensory capacity (63).

Several nongenetic factors, such as temperature, food, gut microbiota, co-infection with other microbes, and mosquito body size, can affect vectorial capacity (reviewed in 44). Some of these are being developed as possible methods of decreasing malaria transmission. For example, the gut microbiota has been shown to increase substantially during blood digestion and can block *Plasmodium* development by triggering mosquito immune responses or by inhibiting the parasite directly through the production of reactive oxygen species (13). It is also clear that the mosquito immune system can mount effective immune responses that limit *Plasmodium* infection.

MOSQUITO ANTIPLASMODIAL IMMUNITY

Ookinete midgut invasion causes irreversible damage to the epithelial cell, leading to apoptosis (31). The invaded cell activates a two-step nitration response involving the induction of nitric oxide synthase expression (30, 48), followed by the induction of a peroxidase-mediated reaction that is regulated by the JNK pathway and potentiates the nitration response (42, 68). When ookinetes emerge from the midgut epithelium, they come into contact with the mosquito hemolymph and must survive the complement-like system. Thioester-containing protein 1 (TEP1), which is similar to the C3 complement factor in vertebrates, is a key mediator of mosquito antiplasmodial immunity, which targets the ookinete stage. TEP1 binding to the ookinete surface triggers the formation of a complex that ultimately kills the parasite (8). It is clear that there is a functional link between epithelial nitration and complement activation, because disrupting epithelial nitration greatly reduces TEP1 binding and enhances parasite survival (68). Thus, epithelial nitration appears to tag parasites as they traverse the mosquito midgut, making them detectable by the mosquito complement-like system.

Pre-exposure of mosquitoes to *Plasmodium* infection when bacteria from the gut microbiota are present at the time ookinetes invade the midgut enhances their immune response to subsequent

infections (88). The priming response involves the constitutive release of hemocyte differentiation factor (HDF) into the mosquito hemolymph, which increases the proportion of circulating granulocytes and enhances antiplasmodial immunity (88). HDF consists of Lipoxin A4 bound to Evokin, a mosquito member of the lipocalin protein family (79). Although hemocytes are known to play an important role in early antiplasmodial responses (80), the precise mechanism by which they limit *Plasmodium* infection is not well established.

Genome-wide comparative analysis indicates that immunity-related genes exhibit a high rate of sequence evolution, and experimental work has implicated several immunity-related genes as crucial determinants of malaria vectorial capacity (8, 70, 76, 107). *Anopheles* immunity-related genes encoding classical recognition proteins and effector enzymes evolve at an unremarkable rate relative to other loci in the genome. However, genes encoding key components of the mosquito's complement-like immune system, such as leucine-rich repeat proteins, thioester-containing proteins, and short antimicrobial peptides, evolve with exceptional rapidity (63). Allelic variation in one thioester-containing protein in particular, TEP1, has been identified as an important determinant of malaria infection in *A. gambiae* (8, 108). Furthermore, the rapid evolution of this gene family as a whole indicates that the molecular underpinnings of vectorial capacity may vary widely, especially between New World members of the *Nyssorhynchus* subgenus and their Old World counterparts.

PLASMODIUM EVASION OF MOSQUITO IMMUNITY

The mosquito immune system limits *Plasmodium* infection to different extents depending on the mosquito-parasite combination. Several animal models of malaria have provided strong experimental evidence of differences in compatibility. For example, disruption of the complement-like system greatly increases the likelihood of *Plasmodium yoelii* (murine malaria) infection in *A. gambiae*, but has no effect when *Anopheles stephensi* is infected with the same parasite (38). Likewise, the *A. gambiae* complement-like system limits infection by *P. yoelii* and *Plasmodium berghei* (also a murine malaria parasite) (8, 38) but has little effect on infection by some African *P. falciparum* strains (17, 56, 66). For example, the African strains GB4 and NF54 of *P. falciparum* are able to evade the complement-like system, even in an *A. gambiae* strain that was genetically selected to be highly refractory to *Plasmodium cynomolgi* (monkey malaria) infection and to eliminate most other parasite species and strains (56, 57). In contrast, a *P. falciparum* strain from Brazil was efficiently eliminated by this refractory *A. gambiae* strain, indicating that the Brazilian *P. falciparum* strain was readily detected by the *A. gambiae* immune system (56).

Genetic studies using linkage mapping and functional genetics identified *Pfs47* as the *P. falciparum* gene that allows the parasite to evade the mosquito immune system (57). Parasites expressing *Pfs47* disrupt JNK signaling in invaded midgut cells (81) and fail to trigger an effective nitration response (57), allowing the ookinete to avoid TEP1-mediated killing. The fact that *P. falciparum* has evolved the capacity to evade the mosquito immune system suggests that mosquito defenses are an important barrier to malaria transmission.

EVIDENCE OF *PLASMODIUM* ADAPTATION TO MOSQUITO VECTORS

Several lines of evidence suggest that the global dispersion of *Plasmodium* required the parasite's adaptation to evolutionarily diverse mosquitoes and may have involved natural selection of the parasite by the vector. For example, both a laboratory *P. falciparum* line of putative African origin (NF54) and a clone from this line (3D7) have low infectivity in or fail to infect New World vectors

such as *A. albimanus* and *Anopheles darlingi* (7, 29). Furthermore, *A. gambiae* mosquitoes (African malaria vectors) are infected more efficiently with African *P. falciparum* isolates than with isolates from Thailand (37). These results are consistent with the hypothesis that natural selection of *P. falciparum* strains on different continents is influenced by the anopheline vectors present in those locations.

In laboratory infections with *P. vivax*, another human malaria parasite, *A. albimanus*, was readily infected by isolates originating from the New World, but the same mosquitoes were poorly infected, or not infected at all, by *P. vivax* isolates from Asia (45). Furthermore, in Southern Mexico, a different *P. vivax* population circulates in the lowlands, where *A. albimanus* is the main vector, than in the highlands, where *Anopheles pseudopunctipennis* is the main vector (40). Laboratory infections demonstrated that *A. albimanus* is more susceptible to infection with *P. vivax* from the lowlands and *A. pseudopunctipennis* is more susceptible to infection with parasites from the highlands (40), consistent with the hypothesis that these two vectors influence selection in the parasite populations circulating at different altitudes. A natural vector can simultaneously transmit multiple compatible parasite haplotypes and maintain the genetic diversity of the parasite population found in the human host (59). In addition, passage of *P. falciparum* through the mosquito can enhance the genetic diversity of the parasite by allowing genetic recombination during meiosis.

The plasticity of *Plasmodium* spp., which allows them to adapt to different vectors, is especially evident in *Plasmodium gallinaceum* (avian malaria) because extensive host shifts have been documented in avian *Plasmodium* spp. (65). It has even been possible to select for strains of *P. gallinaceum*, a parasite normally transmitted by *Culex* spp., that can be transmitted by an anopheline mosquito (36). Selection of arboviruses by the mosquito innate immune defense has also been demonstrated (9).

PLASMODIUM IMMUNE EVASION AND THE GLOBALIZATION OF *PLASMODIUM FALCIPARUM* MALARIA TRANSMISSION

Sequence analysis of *Pfs47* from many different isolates collected around the world indicates that although the predicted proteins have very high protein sequence identity (approximately 99%), multiple haplotypes are present (55). Furthermore, analysis of the geographic distribution of *Pfs47* has revealed striking differences between continents (55), in agreement with previous reports (2, 51). A total of 42 *Pfs47* haplotypes were identified based on the predicted full-length protein of 364 different isolates. The majority of African haplotypes (30/34) never migrated to another continent, whereas two different minor haplotypes in Africa have the widest circulation in Asia and the Americas, respectively (55). In general, the reduced genetic diversity of *P. falciparum* outside Africa is thought to be due to genetic drift and population bottlenecks. However, studies on the natural diversity of *P. falciparum* populations through genome-wide SNP analysis have revealed that some SNPs in *Pfs47* have exceptionally high fixation indices (F_{ST}) between Asian and African isolates relative to the rest of the genome (51). It has been suggested that this finding could be indicative of geographic drivers of evolutionary selection, possibly for gamete recognition and compatibility, because *Pfs47* is expressed in female gametes (102) and is required for fertilization in *P. berghei* (101). However, *Pfs47* does not seem to play an essential role in *P. falciparum* fertilization, as disruption of the gene had no effect on infection in *A. stephensi* (Nijmegen strain) mosquitoes (57). Given that the most striking geographic difference affecting malaria transmission is the species of mosquito vectors present on each continent, and that *Pfs47* enhances transmission by allowing *Plasmodium* to evade the mosquito immune system, an alternative explanation is that parasites

undergo selection as they adapt to mosquitoes that are different from those vectors present in Africa.

To test this hypothesis, colonized anopheline mosquitoes that are major malaria vectors in three different continents were infected with gametocyte cultures of *P. falciparum* lines from different regions (55). This made it possible to compare each line's infectivity for different mosquito vectors and to evaluate the effect of the mosquito immune system on compatibility in different parasite-mosquito combinations. This study showed that mosquito vectors from Africa (*A. gambiae*), Asia (*Anopheles dirus*), and the Americas (*A. albimanus*) are highly susceptible to infection by *P. falciparum* isolates from the same region, but they suffer limited infection by isolates from a different continent. The study also indicated that the differences in compatibility are determined by the mosquito immune system (55). These findings raise the question of why different mosquito species are susceptible to infection with different parasite lines.

The parasite lines chosen for the compatibility experiments (two lines from Africa, two from Asia, and one from South America) expressed different *Pfs47* haplotypes common in their continents of origin, but the lines also differed in their genetic background. The role of *Pfs47* in vector-parasite compatibility was explored by generating transgenic lines with the same genetic background (NF54) that differed only in the *Pfs47* haplotype they expressed. Replacement of the *Pfs47* haplotype in a *P. falciparum* isolate was sufficient to change its compatibility to different mosquito vectors (55). Moreover, when a parasite expressed a compatible *Pfs47* haplotype, it was no longer detected by the mosquito immune system (55).

This study indicates that the mosquito immune system is a major evolutionary force that continuously drives selection in the parasite populations circulating in a given region (55). On the basis of these observations, the lock and key model of malaria globalization was proposed. In this model, *Pfs47* acts as a key that allows the parasite to turn off detection by the mosquito immune system by interacting with some receptor protein(s) in the mosquito (the lock). The locks can be substantially different between evolutionarily distant mosquito species. There are also different variants of *Pfs47*, and the parasite must have the right key for a given mosquito vector to avoid detection by the mosquito immune system, survive, continue to be transmitted, and become established in a new geographic region (55).

PLASMODIUM FALCIPARUM GENETIC ADAPTATION DURING GLOBALIZATION

As *P. falciparum* became global, selection likely occurred for adaptation to human populations with diverse lifestyles and to geographic regions with different climates. As the parasite colonized the globe, it also had to adapt to dozens of highly diverse mosquito species (**Figure 2**). Genetic diversity studies reveal the likely route by which *P. falciparum* invaded Southeast Asia. The pairwise genetic differentiation of SNPs (as measured by F_{ST}) among 519 parasite isolates from locations around the world matches a pattern of isolation by distance, with a significant positive correlation ($R^2 = 0.56$) between F_{ST} and geographic distance from a point in sub-Saharan Africa. The within-population genetic diversity shows an even stronger negative correlation ($R^2 = 0.95$), suggesting sequential bottlenecks that tend to accompany migration because multiple founder events lead to a progressive loss of genetic variation (97). In terms of disease transmission, one can envision a sequential adaptation of parasites to different mosquito species, which could be in part responsible for the observed population bottlenecks. The vector immune system would eliminate some parasites, but those able to evade detection would survive and gain a selective advantage. This would explain why some minor *Pfs47* African haplotypes in Asia expanded greatly, whereas some that are common in Africa were not able to establish a presence. The extremely high geographic differentiation

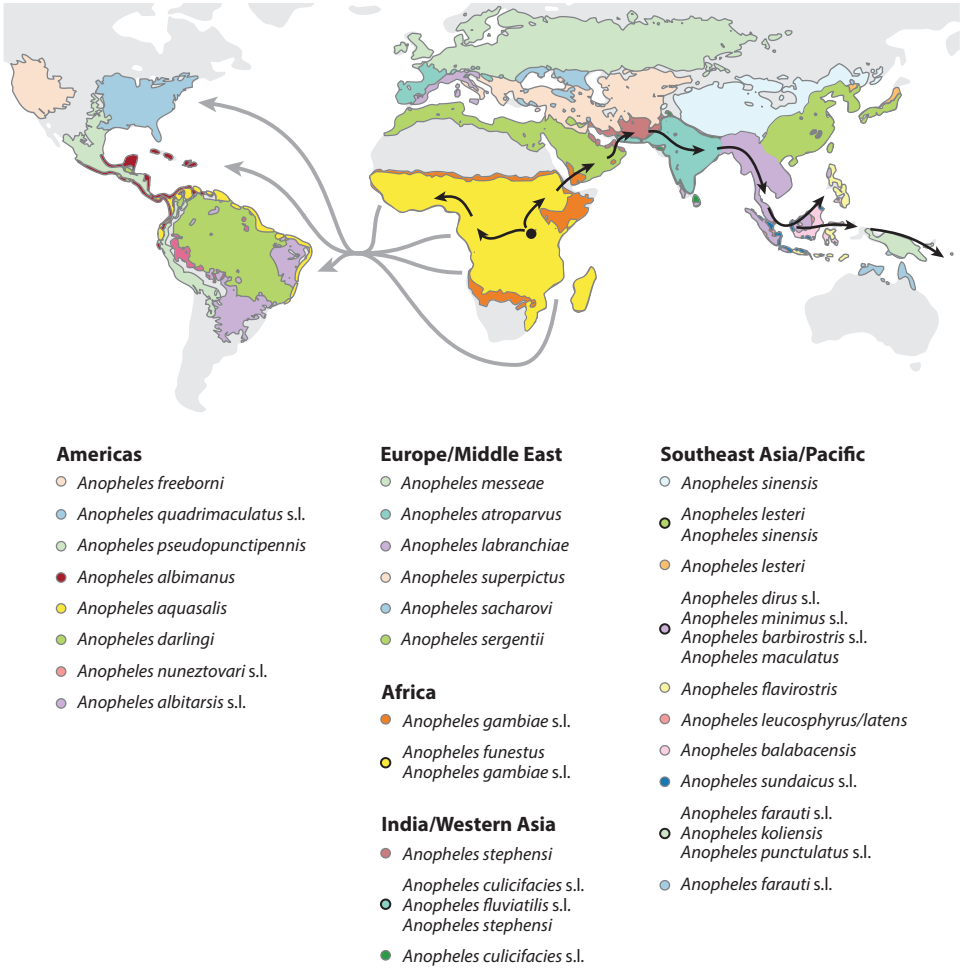

Figure 2

Global dispersal routes of *Plasmodium falciparum* malaria and current predicted geographic distribution of the major anopheline malaria vectors around the world. The route of dispersal of *P. falciparum* malaria as humans migrated from Africa to Southeast Asia is indicated by the black line (97), and the more recent dispersal to the Americas, mainly through the slave trade, is indicated by the gray arrows (112). The diversity and current geographic distribution of the major mosquito vector species are shown schematically via the colored areas. Regions in which there are multiple major vectors are shown with an additional layer. Malaria is transmitted by more than 70 different mosquito species, and vector diversity is much higher if one also considers secondary vectors. It is clear that *P. falciparum* encountered many different anopheline mosquitoes as humans migrated to new regions, and adaptation to these mosquito species was critical to maintain the transmission cycle. Sensu lato (s.l.) refers to a species complex comprising closely related mosquito species. The vector distribution in this figure is based on the map reported by the Malaria Atlas Project (91).

($F_{ST} = 0.795$) of *Pfs47* between Africa and Asia suggests that this gene could be under strong selective pressure.

The geographic route of *Plasmodium* expansion into southeast Asia that gives the strongest correlations with genetic distance begins near Lake Mweru on the border between Zambia and the Democratic Republic of the Congo, then traces a route northeast and crosses into Yemen

near Djibouti, follows the coast of the Arabian Sea into western India, crosses India to the Bay of Bengal, and then travels further along the coast to Thailand, Malaysia, Indonesia, Papua New Guinea, and the Pacific islands beyond (**Figure 2**). The estimated time of the initial migration of the parasite, based on computer simulations of serial colonization rates and subsequent migration between subpopulations is 33–96 kya (97). These results, based on 63 SNPs from the nuclear housekeeping genes that encode P-type Ca^{2+} ATPase and adenylosuccinate lyase, were later supported by analysis of 88 SNPs from mitochondrial loci from the same isolates (96). The hypothesized date range for the arrival of *P. falciparum* in Asia was based on a split between *Plasmodium reichenowi* and *P. falciparum* thought to have occurred approximately 6 Mya (97), but a subsequent study revealing the origin of *P. falciparum* as a gorilla parasite (46) suggests the split is more likely to have occurred approximately 8 Mya, which would increase the age of the initial migration to 44–128 kya. Both of these estimates are consistent with the migration of modern humans out of Africa approximately 75 kya. The dispersal of *P. falciparum* from Africa to Asia was a gradual process that took place over hundreds or thousands of years and, probably, in multiple waves as infected humans migrated out of Africa.

Population structure: genetic pattern of differentiation of organisms within a population

The last piece of the puzzle of *P. falciparum* malaria globalization is the colonization of the New World. It is unlikely that *P. falciparum* arrived with the first humans in the area because they, unlike the colonists of Asia, did not travel a route known for falciparum malaria endemicity. In contrast, they traveled a cold route through the northern reaches of first Asia and then the Americas. In addition, earlier work published 16 years ago showing lower genetic diversity and higher levels of population structure in *P. falciparum* present in the Americas (1) indicated a more recent arrival of the parasite, after human populations had become established in the New World.

More recent research analyzing genetic variation in microsatellite and SNP polymorphisms among 577 *P. falciparum* isolates from 24 populations in different parts of the world strongly suggests the introduction of *P. falciparum* malaria to South America via the African slave trade between the sixteenth and the mid-nineteenth centuries (**Figure 2**) (112). During these approximately 350 years, millions of Africans were transported to South America by Spanish and Portuguese slavers, the Spanish disembarking at ports in the West Indies, Mexico, and Colombia and the Portuguese at ports in Brazil (**Figure 2**) (112). *P. falciparum* consists of two clusters of subpopulations, one including those from Colombia and the other including those from Brazil, Bolivia, and French Guiana. Parasite mitochondrial DNA is also consistent with multiple independent introductions into South America (39). The parasite genetic diversity in South America is far less than would be expected in light of the more than 7 million African slaves imported (22, 112), which suggests that many of the parasite lineages that arrived in the Americas failed to persist. For example, selection of parasites with a compatible *Pfs47* haplotype by New World vectors could have decreased the genetic diversity of *P. falciparum* in the Americas (53). This hypothesis is supported by the strong geographic differentiation of *Pfs47* haplotypes between the Americas and the African ($F_{ST} = 0.754$) and Asian ($F_{ST} = 0.876$) continents.

The initial introduction of *P. falciparum* to the New World was abrupt, as many infected humans migrated in a relatively short period. The selective force of the vector was probably stronger in the New World than elsewhere, because anopheline vectors in the Americas are evolutionarily distant from those in Africa and Asia (**Figure 1**). It is also worth mentioning that the two clusters of subpopulations observed in South America (112) could be the result of selection by different mosquito vectors, for example by *A. pseudopunctipennis* (subgenus *Anopheles*), an important vector in the northern region of South America (Colombia and Peru), or by *A. darlingi* or other anophelines of the subgenus *Nyssorhynchus*, which are the main malaria vectors in the South American lowlands (**Figures 1** and **2**).

The action of the mosquito immune system as a barrier for the dispersal of *P. falciparum* has important implications for the epidemiology of malaria. For example, the *Pfs47* gene is located on chromosome 13, in close proximity to a major determinant of artemisinin resistance (the *K13-Propeller* gene) (3), and genetic linkage between these genes could affect the dispersal of resistance to this drug. Our findings highlight the extent to which the parasite depends on *Pfs47*-mediated immune evasion to become established and persist in an endemic area and suggest that *Pfs47* is a potential target to disrupt malaria transmission (55).

SUMMARY AND ROAD FORWARD

It is remarkable that a parasitic disease that originated from a single horizontal transfer from gorillas to humans has spread around the world and has become one of the most devastating human diseases. During the relatively recent dissemination of malaria in the human population, *P. falciparum* parasites have had to adapt to many different anopheline mosquito species, some of them evolutionarily distant from the mosquito vectors of Africa, where malaria originated. There is clear evidence that the mosquito immune system can greatly limit malaria infection, and the ability of the parasite to evade immune detection, through a *Pfs47*-mediated mechanism, appears to be critical to overcoming this transmission barrier. The selection of *Pfs47* and other *Plasmodium* genes by the different mosquito vectors may be an important force shaping the population structure of *P. falciparum* and may help researchers to understand and predict the dissemination of important traits such as parasite virulence and drug resistance. These are exciting times, as new genetics and genomics tools allow us, for the first time, to analyze many natural *Plasmodium* isolates and multiple anopheline mosquito species at a whole-genome level. We have also made great strides in understanding the basic biology of some of the important vector-parasite interactions that drive malaria transmission. An integrated understanding at a global level of the genetic interactions of *Plasmodium* parasites with their human hosts and their anopheline vectors is critical to ongoing malaria eradication efforts.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The work of A.M.-C. and C.B.-M. is supported by the Intramural Research Program of the Division of Intramural Research, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), grant number Z01AI000947. The research of D.L.H. is supported by NIH grants AI106734 and AI099105. M.M.Z.'s work is supported by a Gerstner Scholars Fellowship from the Gerstner Family Foundation and by the American Museum of Natural History. D.E.N.'s work is supported by funds from NIAID/NIH under grant number U19AI110818. We thank Austin J. Athman of NIAID/NIH for his help preparing the graphics.

LITERATURE CITED

1. Anderson TJ, Haubold B, Williams JT, Estrada-Franco JG, Richardson L, et al. 2000. Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*. *Mol. Biol. Evol.* 17:1467–82

2. Anthony TG, Polley SD, Vogler AP, Conway DJ. 2007. Evidence of non-neutral polymorphism in *Plasmodium falciparum* gamete surface protein genes *Pfs47* and *Pfs48/45*. *Mol. Biochem. Parasitol.* 156:117–23
3. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, et al. 2014. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* 505:50–55
4. Babiker HA, Ranford-Cartwright LC, Currie D, Charlwood JD, Billingsley P, et al. 1994. Random mating in a natural population of the malaria parasite *Plasmodium falciparum*. *Parasitology* 109(4):413–21
5. Barillas-Mury C, Kumar S. 2005. *Plasmodium*-mosquito interactions: a tale of dangerous liaisons. *Cell Microbiol.* 7:1539–45
6. Barry AE, Leliwa-Sytek A, Tavul L, Imrie H, Migot-Nabias F, et al. 2007. Population genomics of the immune evasion (*var*) genes of *Plasmodium falciparum*. *PLOS Pathog.* 3:e34
7. Baton LA, Ranford-Cartwright LC. 2012. Ookinete destruction within the mosquito midgut lumen explains *Anopheles albimanus* refractoriness to *Plasmodium falciparum* (3D7A) oocyst infection. *Int. J. Parasitol.* 42:249–58
8. Blandin S, Shiao SH, Moita LF, Janse CJ, Waters AP, et al. 2004. Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell* 116:661–70
9. Brackney DE, Schirtzinger EE, Harrison TD, Ebel GD, Hanley KA. 2015. Modulation of flavivirus population diversity by RNA interference. *J. Virol.* 89:4035–39
10. Carter R, Mendis KN. 2002. Evolutionary and historical aspects of the burden of malaria. *Clin. Microbiol. Rev.* 15:564–94
11. Chang HH, Moss EL, Park DJ, Ndiaye D, Mboup S, et al. 2013. Malaria life cycle intensifies both natural selection and random genetic drift. *PNAS* 110:20129–34
12. Chotivanich K, Udomsangpetch R, Pattanapanyasat K, Chierakul W, Simpson J, et al. 2002. Hemoglobin E: a balanced polymorphism protective against high parasitemias and thus severe *P. falciparum* malaria. *Blood* 100:1172–76
13. Cirimotich CM, Dong Y, Garver LS, Sim S, Dimopoulos G. 2010. Mosquito immune defenses against *Plasmodium* infection. *Dev. Comp. Immunol.* 34:387–95
14. Clayton AM, Dong Y, Dimopoulos G. 2014. The *Anopheles* innate immune system in the defense against malaria infection. *J. Innate Immun.* 6:169–81
15. Coetzee M, Hunt RH, Wilkerson R, Della Torre A, Coulibaly MB, Besansky NJ. 2013. *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa* 3619:246–74
16. Cohuet A, Harris C, Robert V, Fontenille D. 2010. Evolutionary forces on *Anopheles*: What makes a malaria vector? *Trends Parasitol.* 26:130–36
17. Cohuet A, Osta MA, Morlais I, Awono-Ambene PH, Michel K, et al. 2006. *Anopheles* and *Plasmodium*: from laboratory models to natural systems in the field. *EMBO Rep.* 7:1285–89
18. Conway DJ. 2015. Paths to a malaria vaccine illuminated by parasite genomics. *Trends Genet.* 31:97–107
19. Crompton PD, Moebius J, Portugal S, Waisberg M, Hart G, et al. 2014. Malaria immunity in man and mosquito: insights into unsolved mysteries of a deadly infectious disease. *Annu. Rev. Immunol.* 32:157–87
20. Dong Y, Dimopoulos G. 2009. *Anopheles fibrinogen*–related proteins provide expanded pattern recognition capacity against bacteria and malaria parasites. *J. Biol. Chem.* 284:9835–44
21. Duval L, Fourment M, Nerrienet E, Rousset D, Sadeuh SA, et al. 2010. African apes as reservoirs of *Plasmodium falciparum* and the origin and diversification of the *Laverania* subgenus. *PNAS* 107:10561–66
22. Emory Univ. 2016. *Voyages, The Trans-Atlantic Slave Trade Database.* Atlanta, GA: Emory Univ. **http://www.slavevoyages.org**
23. Enderes C, Kombila D, Dal-Bianco M, Dzikowski R, Kremsner P, Frank M. 2011. *Var* gene promoter activation in clonal *Plasmodium falciparum* isolates follows a hierarchy and suggests a conserved switching program that is independent of genetic background. *J. Infect. Dis.* 204:1620–31
24. Escalante AA, Freeland DE, Collins WE, Lal AA. 1998. The evolution of primate malaria parasites based on the gene encoding cytochrome b from the linear mitochondrial genome. *PNAS* 95:8124–29
25. Flint J, Harding RM, Boyce AJ, Clegg JB. 1998. The population genetics of the haemoglobinopathies. *Bailliere's Clin. Haematol.* 11:1–51
26. Fontaine MC, Pease JB, Steele A, Waterhouse RM, Neafsey DE, et al. 2015. Extensive introgression in a malaria vector species complex revealed by phylogenomics. *Science* 347:1258524

27. Frank M, Dzikowski R, Amulic B, Deitsch K. 2007. Variable switching rates of malaria virulence genes are associated with chromosomal position. *Mol. Microbiol.* 64:1486–98
28. Freitas-Junior LH, Bottius E, Pirrit LA, Deitsch KW, Scheidig C, et al. 2000. Frequent ectopic recombination of virulence factor genes in telomeric chromosome clusters of *P. falciparum*. *Nature* 407:1018–22
29. Grieco JP, Achee NL, Roberts DR, Andre RG. 2005. Comparative susceptibility of three species of *Anopheles* from Belize, Central America, to *Plasmodium falciparum* (NF-54). *J. Am. Mosq. Control Assoc.* 21:279–90
30. Han YS, Barillas-Mury C. 2002. Implications of Time Bomb model of ookinete invasion of midgut cells. *Insect Biochem. Mol. Biol.* 32:1311–16
31. Han YS, Thompson J, Kafatos FC, Barillas-Mury C. 2000. Molecular interactions between *Anopheles stephensi* midgut cells and *Plasmodium berghei*: the time bomb theory of ookinete invasion of mosquitoes. *EMBO J.* 19:6030–40
32. Harbach RE. 2013. The phylogeny and classification of *Anopheles*. In Anopheles *Mosquitoes—New Insights into Malaria Vectors*, ed. S Manguin. Rijeka, Croat.: InTech.
33. Hedrick PW. 2011. Population genetics of malaria resistance in humans. *Heredity* 107:283–304
34. Hill AV. 1992. Molecular epidemiology of the thalassaemias (including haemoglobin E). *Bailliere's Clin. Haematol.* 5:209–38
35. Hill WG, Babiker HA, Ranford-Cartwright LC, Walliker D. 1995. Estimation of inbreeding coefficients from genotypic data on multiple alleles, and application to estimation of clonality in malaria parasites. *Genet. Res.* 65:53–61
36. Hume JC, Hamilton H III, Lee KL, Lehmann T. 2011. Susceptibility of *Anopheles stephensi* to *Plasmodium gallinaceum*: a trait of the mosquito, the parasite, and the environment. *PLOS ONE* 6:e20156
37. Hume JC, Tunnicliff M, Ranford-Cartwright LC, Day KP. 2007. Susceptibility of *Anopheles gambiae* and *Anopheles stephensi* to tropical isolates of *Plasmodium falciparum*. *Malar. J.* 6:139
38. Jaramillo-Gutierrez G, Rodrigues J, Ndikuyeze G, Povelones M, Molina-Cruz A, Barillas-Mury C. 2009. Mosquito immune responses and compatibility between *Plasmodium* parasites and anopheline mosquitoes. *BMC Microbiol.* 9:154
39. Joy DA, Feng X, Mu J, Furuya T, Chotivanich K, et al. 2003. Early origin and recent expansion of *Plasmodium falciparum*. *Science* 300:318–21
40. Joy DA, Gonzalez-Ceron L, Carlton JM, Gueye A, Fay M, et al. 2008. Local adaptation and vector-mediated population structure in *Plasmodium vivax* malaria. *Mol. Biol. Evol.* 25:1245–52
41. Kraemer SM, Kyes SA, Aggarwal G, Springer AL, Nelson SO, et al. 2007. Patterns of gene recombination shape *var* gene repertoires in *Plasmodium falciparum*: comparisons of geographically diverse isolates. *BMC Genom.* 8:45
42. Kumar S, Gupta L, Han YS, Barillas-Mury C. 2004. Inducible peroxidases mediate nitration of *Anopheles* midgut cells undergoing apoptosis in response to *Plasmodium* invasion. *J. Biol. Chem.* 279:53475–82
43. Laporta GZ, Burattini MN, Levy D, Fukuya LA, de Oliveira TM, et al. 2015. *Plasmodium falciparum* in the southeastern Atlantic forest: a challenge to the bromeliad-malaria paradigm? *Malar. J.* 14:181
44. Lefèvre T, Vantaux A, Dabiré KR, Mouline K, Cohuet A. 2013. Non-genetic determinants of mosquito competence for malaria parasites. *PLOS Pathog.* 9:e1003365
45. Li J, Collins WE, Wirtz RA, Rathore D, Lal A, McCutchan TF. 2001. Geographic subdivision of the range of the malaria parasite *Plasmodium vivax*. *Emerg. Infect. Dis.* 7:35–42
46. Liu W, Li Y, Learn GH, Rudicell RS, Robertson JD, et al. 2010. Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. *Nature* 467:420–25
47. Livingstone FB. 1958. The distribution of the sickle cell gene in Liberia. *Am. J. Hum. Genet.* 10:33–41
48. Luckhart S, Vodovotz Y, Cui L, Rosenberg R. 1998. The mosquito *Anopheles stephensi* limits malaria parasite development with inducible synthesis of nitric oxide. *PNAS* 95:5700–5
49. Machado RL, Povoa MM, Calvosa VS, Ferreira MU, Rossit AR, et al. 2004. Genetic structure of *Plasmodium falciparum* populations in the Brazilian Amazon region. *J. Infect. Dis.* 190:1547–55
50. Makanga B, Yangari P, Rahola N, Rougeron V, Elguero E, Boundenga L, et al. 2016. Ape malaria transmission and potential for ape-to-human transfers in Africa. *PNAS* 113:5329–34
51. Manske M, Miotto O, Campino S, Auburn S, Almagro-Garcia J, et al. 2012. Analysis of *Plasmodium falciparum* diversity in natural infections by deep sequencing. *Nature* 487:375–79

52. Mitri C, Jacques J-C, Thiery I, Riehle MM, Xu J, et al. 2009. Fine pathogen discrimination within the *APL1* gene family protects *Anopheles gambiae* against human and rodent malaria species. *PLOS Pathog.* 5:e1000576
53. Molina-Cruz A, Barillas-Mury C. 2014. The remarkable journey of adaptation of the *Plasmodium falciparum* malaria parasite to New World anopheline mosquitoes. *Mem. Inst. Oswaldo Cruz* 109:662–67
54. Molina-Cruz A, Barillas-Mury C. 2016. Mosquito vectors of ape malarias: another piece of the puzzle. *PNAS* 113:5153–54
55. Molina-Cruz A, Canepa GE, Kamath N, Pavlovic NV, Mu J, et al. 2015. *Plasmodium* evasion of mosquito immunity and global malaria transmission: the lock-and-key theory. *PNAS* 112:15178–83
56. Molina-Cruz A, Dejong RJ, Ortega C, Haile A, Abban E, et al. 2012. Some strains of *Plasmodium falciparum*, a human malaria parasite, evade the complement-like system of *Anopheles gambiae* mosquitoes. *PNAS* 109:1957–62
57. Molina-Cruz A, Garver LS, Alabaster A, Bangiolo L, Haile A, et al. 2013. The human malaria parasite *Pfs47* gene mediates evasion of the mosquito immune system. *Science* 340:984–87
58. Moreno M, Marinotti O, Krzywinski J, Tadei WP, James AA, et al. 2010. Complete mtDNA genomes of *Anopheles darlingi* and an approach to anopheline divergence time. *Malar. J.* 9:127
59. Morlais I, Nsango SE, Toussile W, Abate L, Annan Z, et al. 2015. *Plasmodium falciparum* mating patterns and mosquito infectivity of natural isolates of gametocytes. *PLOS ONE* 10:e0123777
60. Mu J, Awadalla P, Duan J, McGee KM, Joy DA, et al. 2005. Recombination hotspots and population structure in *Plasmodium falciparum*. *PLOS Biol.* 3:e335
61. Mu J, Duan J, Makova KD, Joy DA, Huynh CQ, et al. 2002. Chromosome-wide SNPs reveal an ancient origin for *Plasmodium falciparum*. *Nature* 418:323–26
62. Neafsey DE, Schaffner SF, Volkman SK, Park D, Montgomery P, et al. 2008. Genome-wide SNP genotyping highlights the role of natural selection in *Plasmodium falciparum* population divergence. *Genome Biol.* 9:R171
63. Neafsey DE, Waterhouse RM, Abai MR, Aganezov SS, Alekseyev MA, et al. 2015. Highly evolvable malaria vectors: the genomes of 16 *Anopheles* mosquitoes. *Science* 347:1258522
64. Nerlich AG, Schraut B, Dittrich S, Jelinek T, Zink AR. 2008. *Plasmodium falciparum* in ancient Egypt. *Emerg. Infect. Dis.* 14:1317–19
65. Njabo KY, Cornel AJ, Bonneaud C, Toffelmier E, Sehgal RN, et al. 2011. Nonspecific patterns of vector, host and avian malaria parasite associations in a central African rainforest. *Mol. Ecol.* 20:1049–61
66. Nsango SE, Abate L, Thoma M, Pompon J, Fraiture M, et al. 2012. Genetic clonality of *Plasmodium falciparum* affects the outcome of infection in *Anopheles gambiae*. *Int. J. Parasitol.* 42:589–95
67. Olago DO. 2001. Vegetation changes over palaeo-time scales in Africa. *Clim. Res.* 17:105–21
68. Oliveira GdA, Lieberman J, Barillas-Mury C. 2012. Epithelial nitration by a peroxidase/NOX5 system mediates mosquito antiplasmodial immunity. *Science* 335:856–59
69. Ollomo B, Durand P, Prugnolle F, Douzery E, Arnathau C, et al. 2009. A new malaria agent in African hominids. *PLOS Pathog.* 5:e1000446
70. Osta MA, Christophides GK, Kafatos FC. 2004. Effects of mosquito genes on *Plasmodium* development. *Science* 303:2030–32
71. Parmakelis A, Russello MA, Caccone A, Marcondes CB, Costa J, et al. 2008. Historical analysis of a near disaster: *Anopheles gambiae* in Brazil. *Am. J. Trop. Med. Hyg.* 78:176–78
72. Pasvol G, Weatherall DJ, Wilson RJ. 1978. Cellular mechanism for the protective effect of haemoglobin S against *P. falciparum* malaria. *Nature* 274:701–3
73. Paul RE, Packer MJ, Walmsley M, Lagog M, Ranford-Cartwright LC, et al. 1995. Mating patterns in malaria parasite populations of Papua New Guinea. *Science* 269:1709–11
74. Paupy C, Makanga B, Ollomo B, Rahola N, Durand P, et al. 2013. *Anopheles moucheti* and *Anopheles vinckei* are candidate vectors of ape *Plasmodium* parasites, including *Plasmodium praefalciparum* in Gabon. *PLOS ONE* 8:e57294
75. Perkins SL, Schall JJ. 2002. A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. *J. Parasitol.* 88:972–78
76. Povelones M, Waterhouse RM, Kafatos FC, Christophides GK. 2009. Leucine-rich repeat protein complex activates mosquito complement in defense against *Plasmodium* parasites. *Science* 324:258–61

77. Prugnolle F, Durand P, Neel C, Ollomo B, Ayala FJ, et al. 2010. African great apes are natural hosts of multiple related malaria species, including *Plasmodium falciparum*. *PNAS* 107:1458–63
78. Prugnolle F, Ollomo B, Durand P, Yalcindag E, Arnathau C, et al. 2011. African monkeys are infected by *Plasmodium falciparum* nonhuman primate-specific strains. *PNAS* 108:11948–53
79. Ramirez JL, de Almeida Oliveira G, Calvo E, Dalli J, Colas RA, et al. 2015. A mosquito lipoxin/lipocalin complex mediates innate immune priming in *Anopheles gambiae*. *Nat. Commun.* 6:7403
80. Ramirez JL, Garver LS, Brayner FA, Alves LC, Rodrigues J, et al. 2014. The role of hemocytes in *Anopheles gambiae* antiplasmodial immunity. *J. Innate Immun.* 6:119–28
81. Ramphul UN, Garver LS, Molina-Cruz A, Canepa GE, Barillas-Mury C. 2015. *Plasmodium falciparum* evades mosquito immunity by disrupting JNK-mediated apoptosis of invaded midgut cells. *PNAS* 112:1273–80
82. Rask TS, Hansen DA, Theander TG, Gorm Pedersen A, Lavstsen T. 2010. *Plasmodium falciparum* erythrocyte membrane protein 1 diversity in seven genomes—divide and conquer. *PLOS Comput. Biol.* 6:e1000933
83. Rayner JC, Liu W, Peeters M, Sharp PM, Hahn BH. 2011. A plethora of *Plasmodium* species in wild apes: a source of human infection? *Trends Parasitol.* 27:222–29
84. Read AF. 1994. The evolution of virulence. *Trends Microbiol.* 2:73–76
85. Reidenbach KR, Cook S, Bertone MA, Harbach RE, Wiegmann BM, Besansky NJ. 2009. Phylogenetic analysis and temporal diversification of mosquitoes (Diptera: Culicidae) based on nuclear genes and morphology. *BMC Evol. Biol.* 9:298
86. Rich SM, Ayala FJ. 1998. The recent origin of allelic variation in antigenic determinants of *Plasmodium falciparum*. *Genetics* 150:515–17
87. Rich SM, Leendertz FH, Xu G, Lebreton M, Djoko CF, et al. 2009. The origin of malignant malaria. *PNAS* 106:14902–7
88. Rodrigues J, Brayner FA, Alves LC, Dixit R, Barillas-Mury C. 2010. Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science* 329:1353–55
89. Salamini F, Ozkan H, Brandolini A, Schafer-Pregl R, Martin W. 2002. Genetics and geography of wild cereal domestication in the near east. *Nat. Rev. Genet.* 3:429–41
90. Sinden RE, Dawes EJ, Alavi Y, Waldock J, Finney O, et al. 2007. Progression of *Plasmodium berghei* through *Anopheles stephensi* is density-dependent. *PLOS Pathog.* 3:e195
91. Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, et al. 2012. A global map of dominant malaria vectors. *Parasit. Vectors* 5:69
92. Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, et al. 2010. The dominant *Anopheles* vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic precis. *Parasit. Vectors* 3:72
93. Smith JD, Chitnis CE, Craig AG, Roberts DJ, Hudson-Taylor DE, et al. 1995. Switches in expression of *Plasmodium falciparum var* genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell* 82:101–10
94. Su XZ, Heatwole VM, Wertheimer SP, Guinet F, Herrfeldt JA, et al. 1995. The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*–infected erythrocytes. *Cell* 82:89–100
95. Su XZ, Mu J, Joy DA. 2003. The "Malaria's Eve" hypothesis and the debate concerning the origin of the human malaria parasite *Plasmodium falciparum*. *Microbes Infect.* 5:891–96
96. Tanabe K, Jombart T, Horibe S, Palacpac NM, Honma H, et al. 2013. *Plasmodium falciparum* mitochondrial genetic diversity exhibits isolation-by-distance patterns supporting a sub-Saharan African origin. *Mitochondrion* 13:630–36
97. Tanabe K, Mita T, Jombart T, Eriksson A, Horibe S, et al. 2010. *Plasmodium falciparum* accompanied the human expansion out of Africa. *Curr. Biol.* 20:1283–89
98. Taylor SM, Parobek CM, Fairhurst RM. 2012. Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. *Lancet Infect. Dis.* 12:457–68
99. Tishkoff SA, Varkonyi R, Cahinhinan N, Abbes S, Argyropoulos G, et al. 2001. Haplotype diversity and linkage disequilibrium at human G6PD: recent origin of alleles that confer malarial resistance. *Science* 293:455–62

100. Valencio DA, Vilas JF. 1969. Age of the separation of South America and Africa. *Nature* 223:1353–54
101. van Dijk MR, van Schaijk BC, Khan SM, van Dooren MW, Ramesar J, et al. 2010. Three members of the 6-cys protein family of *Plasmodium* play a role in gamete fertility. *PLOS Pathog.* 6:e1000853
102. van Schaijk BC, van Dijk MR, van de Vegte-Bolmer M, van Gemert GJ, van Dooren MW, et al. 2006. *Pfs47*, paralog of the male fertility factor Pfs48/45, is a female specific surface protein in *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 149:216–22
103. Van Tyne D, Park DJ, Schaffner SF, Neafsey DE, Angelino E, et al. 2011. Identification and functional validation of the novel antimalarial resistance locus *PF10_0355* in *Plasmodium falciparum*. *PLOS Genet.* 7:e1001383
104. Volkman SK, Barry AE, Lyons EJ, Nielsen KM, Thomas SM, et al. 2001. Recent origin of *Plasmodium falciparum* from a single progenitor. *Science* 293:482–84
105. Volkman SK, Hartl DL, Wirth DF, Nielsen KM, Choi M, et al. 2002. Excess polymorphisms in genes for membrane proteins in *Plasmodium falciparum*. *Science* 298:216–18
106. Volkman SK, Sabeti PC, DeCaprio D, Neafsey DE, Schaffner SF, et al. 2007. A genome-wide map of diversity in *Plasmodium falciparum*. *Nat. Genet.* 39:113–19
107. Waterhouse RM, Kriventseva EV, Meister S, Xi Z, Alvarez KS, et al. 2007. Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. *Science* 316:1738–43
108. White BJ, Lawniczak MKN, Cheng C, Coulibaly MB, Wilson MD, et al. 2011. Adaptive divergence between incipient species of *Anopheles gambiae* increases resistance to *Plasmodium*. *PNAS* 108:244–49
109. WHO (World Health Organ.). 2015. *World Malaria Report 2015*. Geneva, Switz.: World Health Organ.
110. Wiesenfeld SL. 1967. Sickle-cell trait in human biological and cultural evolution. Development of agriculture causing increased malaria is bound to gene-pool changes causing malaria reduction. *Science* 157:1134–40
111. Woodring JL, Higgs S, Beaty BJ. 1996. Natural cycles of vector-borne pathogens. In *The Biology of Disease Vectors*, ed. BJ Beaty, WC Marquardt, pp. 51–72. Niwot, CO: Univ. Press Colo.
112. Yalcindag E, Elguero E, Arnathau C, Durand P, Akiana J, et al. 2012. Multiple independent introductions of *Plasmodium falciparum* in South America. *PNAS* 109:511–16