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Could culicine mosquitoes transmit human malaria?

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Abstract

Human malaria is known to be transmitted strictly by anopheline mosquitoes. Culicine mosquitoes such as *Aedes* spp. and *Culex* spp. are important vectors of other human pathogens including viruses and filarial worms, but have never been observed to transmit mammalian malarial parasites.

Culicines do transmit avian malarial parasites, and interestingly, allow partial development of mammalian-infectious *Plasmodium* parasites, implying that physiological barriers in the mosquitoes prevent parasite transmission. Although the mechanism(s) are not known, the mosquito immune system is likely involved in eliminating *Plasmodium*. However, *Plasmodium* has shown substantial capacity to adapt to new vectors, and current ecological changes caused by humans could promote adaptation of human-infectious *Plasmodium* parasites to culicines. Such an event could have widespread epidemiological implications and therefore merits attention.

Keywords

malaria; transmission; mosquito; *Culex* ; *Anopheles*

Mosquitoes that transmit human *Plasmodium* spp

Malaria is a devastating disease, which, despite all control efforts, still causes ~2 million clinical cases and 660 000 deaths annually [1]. The disease is caused by *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi*, and most importantly by *Plasmodium falciparum*, which is responsible for the greatest burden in terms of morbidity and mortality. Human-infectious *Plasmodium* spp., in fact all *Plasmodium* spp. that infect mammals, are known to be transmitted via the bite of an infected anopheline mosquito (see Glossary). However, culicine mosquitoes (e.g., *Aedes* spp., *Culex* spp., and *Mansonia* spp.), which are common worldwide, are also abundantly present in areas where malaria is endemic. They feed on humans and are, just like anophelines, important vectors for human pathogens, transmitting filarial worms and arboviruses (e.g., Yellow fever, Dengue, and West

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Nile Virus). Additionally, culicine mosquitoes are the vectors for avian *Plasmodium* spp. (Table 1). Nevertheless, they do not transmit human (mammalian) malaria parasites, and what prevents transmission remains a mystery. Besides having significant epidemiological implications, solving this question might shed light onto *Plasmodium* biology and its host specificity and help in uncovering powerful mechanisms that block *Plasmodium* development in the mosquito.

For the adaptation of a parasite to a new vector in nature, several conditions have to be met in terms of vector competence and capacity (Box 1). This would require at least one parasite genotype overcoming all of the physiological barriers encountered in the new vector, and ecological circumstances promoting adaptation to a new vector, which would necessitate a higher benefit than cost to the parasite.

Box 1

Determinants of vectorial capacity and competence

The ability of a mosquito to transmit a pathogen depends on environmental, behavioral, cellular, and biochemical factors that encompass the vectorial capacity of the mosquito. Among these factors are host preference, feeding rate and behavior, mosquito population density, longevity, and its susceptibility to transmit the pathogen, also known as vectorial competence. A number of environmental (reviewed in [68]) and physiological parameters can determine the vectorial competence to *Plasmodium*, including:

- Components of the mammalian host complement system are ingested during a blood meal by the mosquito vector and can be deleterious to *Plasmodium* in the midgut of the mosquito. In response to this, *P. falciparum* gametes have developed the ability to inactivate complement protein C3b by co-opting the complement regulating factor H from the blood [69].
- *Plasmodium* male gametogenesis is triggered by mosquito-released xanthurenic acid (XA) [13].
- The mosquito gut harbors microbiota that increase substantially during blood digestion and can block *Plasmodium* development by triggering a mosquito immune response or by inhibiting the parasite directly through the production of reactive oxygen species [70].
- The chitinous peritrophic matrix forms during digestion and lines the luminal side of the midgut epithelium, representing a physical barrier that the parasite must cross. Some *Plasmodium* parasites secrete chitinases to disrupt the peritrophic matrix [71].
- The mosquito innate immune system can develop an immune response that in some cases eliminates *Plasmodium*. The midgut epithelium is the first mosquito tissue that the parasite crosses. Inside the epithelial cell, production of reactive oxygen species [72] and protein nitration mediated by a peroxidase (HPX2) and a NADPH-oxidase (NOX5) system can lead to *Plasmodium* elimination [73]. Protein nitration of *Plasmodium* appears to be a prerequisite

for recognition and elimination of the parasites by the mosquito complement-like immune system. This involves the binding of thioester-containing protein 1 (TEP1) to the parasite to label it for destruction. Recently it has been found that *P. falciparum* parasites are able to evade the complement-like system and recognition by TEP1 in *An. gambiae* by a mechanism involving Pfs47, a parasite surface protein [74].

During the “normal” life cycle of *Plasmodium* within a susceptible mosquito (Figure 1), male and female gametocytes are taken up with the blood while the mosquito feeds on an infected vertebrate host (Figure 1, step 1). Inside the midgut lumen of the mosquito, the gametocytes are “activated” and transform into gametes (Figure 1, step 2), male gametes (microgametes) exflagellate and fertilize the females (macrogametes), and within an hour a diploid zygote is formed (Figure 1, step 3). The zygote develops into a motile ookinete (Figure 1, step 4) that crosses the mosquito midgut epithelium after 16 to 26 hours and transforms into an oocyst on the basal side of the midgut (Figure 1, step 5). Sporozoites mature in the oocyst and are released into the hemolymph after approximately 10–16 days (Figure 1, step 6). The sporozoites travel to the salivary glands (Figure 1, step 7), from where they are transmitted to another vertebrate host during a subsequent blood feeding of the mosquito. Culicine mosquitoes undoubtedly take up human-infectious *Plasmodium* gametocytes; however, parasites are not transmitted. Several questions arise concerning why this is the case: (i) what are the barriers that human malaria parasites encounter in culicines; (ii) can any of the human-infectious *Plasmodium* spp. overcome these barriers and be transmitted by a culicine mosquito; and (iii) what ecological conditions would promote the adaptation of human-infectious *Plasmodium* spp. to culicines?

Here, we discuss these questions in the context of the few studies that have investigated the development of mammalian *Plasmodium* spp. in culicine mosquitoes, including recent findings showing that the human malaria parasite *P. falciparum* can develop ookinetes in *Culex quinquefasciatus* and infects its midgut epithelium [2], indicating that a human-infectious malaria parasite is already able to develop partially in a culicine mosquito.

The development of mammalian *Plasmodium* spp. in culicine mosquitoes

Several studies have tested if different human, primate, and other mammalian malaria parasites can complete their development in culicine mosquitoes. Depending on the mosquito and *Plasmodium* species used, parasites were observed in more or less advanced stages of development, ranging from the absence of ookinetes in the midgut lumen [3, 4] to the presence of sporozoites in the salivary glands of the mosquito (Figure 1, steps 4–7) [5, 6]. Only a few studies have investigated development of human-infectious *Plasmodium* spp. in culicine mosquitoes. The earliest study of *P. falciparum* in a culicine mosquito of which we are aware was performed in 1937 by Williamson and Zain, who infected *Culex bitaeniorhynchus* with *P. falciparum* and other human-infectious *Plasmodium* spp. They observed oocysts on the midgut epithelium of the mosquitoes and sporozoites in the salivary glands after infections with *P. falciparum*, *P. vivax*, and *P. malariae* [6]. This observation is the closest to completion of the developmental cycle of human malaria parasites in a

culicine mosquito found thus far. However, in another study with *C. bitaeniorhynchus* and *P. falciparum*, no oocysts were observed on the midgut epithelium [7]. It remains unclear if the results were not replicated because of the use of different genotypes of parasite and vector [8–10].

In infections of *Mansonia uniformis* with *P. falciparum*, oocysts were observed on the midgut epithelium after feeding on an infected patient. Unfortunately, no mosquitoes were kept long enough to see if sporozoites would develop in the oocysts and invade the salivary glands [11]. A recent study of the development of *P. falciparum* in *C. quinquefasciatus* showed that the parasites develop into ookinetes in the blood meal of this mosquito in nearly the same numbers as in their natural vector, *Anopheles gambiae* [2]. But immediately after ookinetes cross the midgut epithelium and reach the basal side, they are lysed and no oocysts are seen.

Parasite development to near completion has not only been observed for human-infectious *Plasmodium* spp. [6], but also for the primate malaria parasite *Plasmodium cynomolgi*. In infections of *M. uniformis*, oocysts as well as sporozoites were found in the mosquitoes [5]. Yet, the sporozoites were seen in the hemolymph of the mosquitoes, but seemed to fail to invade the salivary glands. Only one sporozoite was reported to be inside a gland, and transmission experiments were unsuccessful, confirming that no infectious sporozoites were present in the salivary glands of the mosquitoes.

Conversely, the most extreme arrest of development was described for the rodent malaria parasite *Plasmodium berghei*. In studies with *Aedes aegypti* [3] and *Culex salinarius* [4], only limited ookinete formation could be observed in the blood meal of the mosquitoes, leading to the conclusion that the ookinetes were unable to cross the midgut epithelium [4]. This assumption was confirmed later; analyses of *P. berghei* in the blood meals of *Ae. aegypti* revealed that the transformation of male gametocytes into microgametes (measured as the number of exflagellations observed) was highly affected and rarely seen [3]. Feeding experiments with *in vitro* cultured *P. berghei* ookinetes, carried out in the same study, showed that significantly fewer ookinetes attach to the midgut epithelium in *Ae. aegypti* than in *Anopheles stephensi*. No oocysts were observed 48 h post-feeding, suggesting that *P. berghei* ookinetes are unable to cross the *Ae. aegypti* midgut epithelium [3]. Even when injected directly into the mosquito hemolymph, the ookinetes did not form oocysts, and sporozoites were never observed in the salivary glands of *Ae. aegypti* compared to *An. stephensi*, where 93% of the mosquitoes had high numbers of sporozoites in their glands 18–20 days after parasite injection. As would be expected, transmission experiments with *P. berghei* using *Ae. aegypti* were unsuccessful [3].

Taken together, the above described observations from *Plasmodium* spp. that can infect mammals point to several conclusions: first, there is no evidence for complete development and successful transmission of any of the studied parasites by a culicine mosquito. Second, the ability of mammalian-infectious *Plasmodium* parasites to develop in culicine mosquitoes is highly dependent on the combination of mosquito and parasite species used. It is apparent that each of the studied parasite species is affected at a different developmental stage in the mosquito (Figure 1) and therefore seems to encounter a unique physiological barrier (Box

1). This suggests that several mechanisms are responsible for the developmental arrest/death of the parasites and that species-specific adaptation is required for successful completion of parasite development in a vector.

A number of factors are known from studies of *Plasmodium* in *Anopheles* mosquitoes that are either required by the parasite or controlling infection. For example, it has been shown that *P. berghei* parasites need xanthurenic acid (XA), a metabolite found in the mosquito, to initiate exflagellation of male gametes in the midgut [12]. In *Ae. aegypti* and *C. salinarius*, *P. berghei* development is arrested at the early stage of microgamete activation, immediately after parasites reach the mosquito midgut (Figure 1, step 1) [3, 4]. XA concentrations are lower in culicine mosquitoes than in anophelines, and the sensitivity of the parasites to XA varies between *Plasmodium* spp. [12, 13]. Because *P. berghei* requires relatively high concentrations of XA, this could explain the lack of exflagellating microgametes in *Ae. aegypti*. This could be tested directly by increasing the concentration of XA in the blood meal by providing it to the mosquito or injecting it into a *P. berghei* infected mouse before mosquito infection. In addition to the components that are promoting parasite development, the mosquito blood meal contains factors such as vertebrate complement, antibodies, and mosquito gut microbiota, all of which have been shown to be harmful for the parasite (Box 1) and could be responsible for a developmental arrest. In the case of *P. falciparum*, the fact that parasites can be found on the basal side of the midgut epithelium [2], or in some cases even form sporozoites in culicine mosquitoes [5, 6], indicates that *P. falciparum*, in contrast to *P. berghei*, is able to cope with the conditions in the midgut lumen, cross the peritrophic matrix, and invade the midgut epithelial cells. In *C. quinquefasciatus*, the parasites are lysed immediately after they reach the basal side of the midgut epithelium and come into contact with the mosquito hemolymph. In insects, the hemolymph contains factors of the innate immune system, and members of several different immune pathways have been identified as major defenses against malaria parasites in *Anopheles* mosquitoes. The most powerful anti-plasmodial response known to date is probably achieved by the thioester-containing protein TEP1, a homologue of the human complement factor C3 [14–16]. TEP1 is a hemolymph protein released by hemocytes. It forms a complex with two leucine-rich repeat (LRR) proteins, LRIM1 (leucine-rich repeat immune protein 1) and APL1 (*Anopheles-Plasmodium*-responsive leucine-rich repeat 1), preventing it from non-specific binding [17–19]. Binding of TEP1 to *Plasmodium* ookinetes or early oocysts initiates their elimination and further lysis or melanization in *An. gambiae* [14] and has been shown to be the major determinant of refractoriness of *Anopheles quadriannulatus* [20]. Additionally, several other major immune pathways (IMD-, TOLL, JAK-STAT, JNK-pathways) have been shown to influence infection intensities in both *P. berghei* and *P. falciparum* infections [21–24]. Analyses of the *C. quinquefasciatus* and *Ae. aegypti* genomes (www.vectorbase.org) and the Immune gene database ImmunoDB (<http://cegg.unige.ch/Insecta/immunodb>) reveal the presence of several thioester-containing proteins, which could be part of a complement-like system in these culicine mosquitoes, as well as components of the IMD-, TOLL-, and JNK pathways. RNA-mediated silencing of these genes (which should lead to the survival of the parasites), could give insights into the role of the mosquito immune system in parasite killing in culicines. Silencing of some TEP genes in *C. quinquefasciatus* show an effect in

immunity against bacteria (Knöckel, J., unpublished), indicating that they do have a function in culicine immunity, but their effect on *Plasmodium* has to be determined.

Other explanations for the observed death of *P. falciparum* in *C. quinquefasciatus* could be a lack of specific nutrients or signals that are needed for the parasites to continue their development. However, in other culicine mosquitoes, sporozoites have been observed, some of which were located in the salivary glands of the mosquitoes. *Plasmodium* ookinetes can also transform into oocysts when injected into the *Drosophila* hemolymph [25], suggesting that lack of nutrients or chemical signals is an unlikely barrier. Mammalian-infectious *Plasmodium* spp. have been successful in adapting to anopheline mosquitoes throughout the world, but have not (yet) been shown to complete their development in culicine mosquitoes. Nonetheless, the question that comes to mind following the observations that mammalian *Plasmodium* can at least partially develop in culicine mosquitoes is: is there any *Plasmodium* sp. that has the capacity to be transmitted by both anopheline and culicine mosquitoes?

Transmission of *Plasmodium* spp. by anopheline and culicine mosquitoes

Avian malaria parasites are typically transmitted by culicine mosquitoes in nature (Table 1). Nevertheless, under laboratory conditions, *Plasmodium gallinaceum* has been successfully transmitted by *Anopheles quadrimaculatus* [26–28], *An. stephensi*, and *An. gambiae* [3, 29, 30]. Oocysts were observed on the midgut epithelium of *Anopheles freeborni*, but transmission of the parasites by the mosquitoes was not tested [26]. In the case of *Plasmodium relictum*, transmission between birds by anopheline mosquitoes has been demonstrated with *An. quadrimaculatus*, *Anopheles albimanus* [28, 31], *Anopheles crucians* [28], and *An. freeborni* [28, 32].

Even though many parasites seem to be eliminated in the midgut epithelium [33], a recent study showed that *An. stephensi* can transmit *P. gallinaceum* to chickens [30]. Feeding on an infected chicken led to infection in 2% of the mosquitoes. Furthermore, a highly susceptible *An. stephensi* line was genetically selected, which showed over 85% infection prevalence with *P. gallinaceum* within a few generations. A similar approach was used 25 years earlier by Collins and coworkers, who selected a *Plasmodium*-refractory and a *Plasmodium*-susceptible line of *An. gambiae* [29]. The selection was based on the observation that a small fraction of the colonized mosquitoes showed reduced susceptibility to *P. cynomolgi*, a primate malaria parasite. In those refractory mosquitoes, large numbers of melanized parasites could be observed on the midgut epithelium alongside a few or no live oocysts. Selective breeding using these mosquitoes, or on the other hand the mosquitoes that showed the highest level of live oocysts after infection, led to the establishment of two mosquito lines, one fully refractory and one highly susceptible to *P. cynomolgi*. Interestingly, the selection not only conferred refractoriness or susceptibility to *P. cynomolgi*, but also to the avian parasite *P. gallinaceum* and other *Plasmodium* spp., including some *P. falciparum* lines [29]. All parasite species tested are mostly encapsulated by melanization in the refractory *An. gambiae*. Since encapsulation is a major immune response to fight invading pathogens, this suggests that the mosquito immune system is responsible for the observed phenotypes. More recently, it has been confirmed that the complement-like immune system is mediating the selected refractoriness in the case of *P. falciparum* [34]. This example clearly shows

that the mosquito immune system is an important barrier for *Plasmodium* to adapt to a new vector.

Ecological aspects of pathogen adaptation to a new vector

In addition to the physiological barriers that the parasite encounters inside the mosquito, ecological factors must be in favor of promoting transmission. The benefit of adapting to a new vector has to be higher than any cost associated with a decrease in transmission efficiency in the original vector. Under certain ecological circumstances, pathogens are able to rapidly adapt to new vectors. One example is the Chikungunya virus (CHIKV), originally transmitted by *Ae. aegypti*. A single point mutation resulting in an amino acid replacement significantly increased the transmission efficiency of the virus in *Aedes albopictus* [35]. This same point mutation has occurred independently in at least three different geographical areas in just a few years, resulting in new disease epicenters: the islands of the Indian Ocean (e.g., La Reunion), Central Africa, and Asia [35–37]. During these adaptations *Ae. albopictus* was common in all three areas, whereas the original vector, *Ae. aegypti* was rare [35, 37]. Notably, the new mutant form of CHIKV spread in *Ae. albopictus* in La Reunion despite causing elevated mortality of the new vector [36]. Recently, it was shown that the mutation in CHIKV is also associated with slightly lower transmission efficiency by *Ae. aegypti* [38]. These studies suggest that pathogen adaptation to new vectors is governed to a large extent by the cost/benefit ratio, which can include reduced transmission efficiency in the original vector. Reducing access of the parasite to its original vector increases the prospects for an adaptation to a new, more abundant vector species. In the case of *Plasmodium*, similar ecological conditions may have occurred during the arrival of the parasites in the New World. It resulted in its adaptation to the local anopheline species, which had diverged from their African counterparts ~95 million years ago [39], when the continents separated. One example is *P. vivax* adaptation to different anophelines in Mexico [40]. In coastal areas, where *An. albimanus* is most common, the parasites are transmitted by *An. albimanus*, whereas at higher altitudes the main vector is *An. pseudopunctipennis*, a “foothill-mosquito” [41]. The populations of *P. vivax* in these geographic areas are genetically distinct, and consistent with a local adaptation of a parasite to a vector, the coastal *An. albimanus* is more susceptible to infection by the coastal *P. vivax* genotype, whereas *An. pseudopunctipennis* is more susceptible to foothill parasite genotypes [40].

The establishment of the parasite in new lands almost always depends on vector switches [42, 43]. Nevertheless, human-infecting *Plasmodium* spp. have only adapted to different anopheline species, but not to culicine mosquitoes. However, an adaptation to culicines changes in a way that promotes it. Although not specifically designed for this purpose (and maybe not reaching the tipping point to shift adaptive prospects), a significant change of ecological conditions has been occurring for several decades in Africa due to urbanization [44, 45] and large scale vector control in rural areas [45]. Because anophelines cannot tolerate pollution in urban centers whereas culicines can, vector composition in cities is dominated by culicines [44, 46–49]. Likewise, successful vector control campaigns in many rural areas, especially in East Africa, resulted in dramatic suppression of anopheline vectors with far less impact on *C. quinquefasciatus* [50]. The cost-benefit ratios for malaria parasites

in Africa may be changing as these lines are written. The prevailing trends increase the benefit of adaptation to a new vector such as *C. quinquefasciatus*.

An ideal experiment to test the ability of human-infectious malaria parasites to adapt to culicine mosquitoes could involve exposure of diverse parasite populations to large numbers of mosquitoes that represent “poor” vector species and testing parasite transmission to the vertebrate host over time in a series of replicated experiments. But which parasite-vector combinations would provide us with the most valuable information to predict the likelihood of a vector switch to happen in nature? Certainly, highly abundant mosquitoes with frequent human host contact that lasts long enough to allow sporozoite development and transmission would represent promising candidates for adaptation to a new vector. Nonetheless, since adaptation initially depends on rather low but successful transmission by the new vector, a mosquito that allows current parasites to develop into sporozoites (such as *M. uniformis* and *P. cynomolgi*) appears to be a better candidate than a mosquito that does not allow parasite development (*P. berghei* in *Ae. aegypti*). Accordingly, even if *P. falciparum* can complete development in *C. bitaeniorhynchus* (see above), it is not known to bite people in Africa, and therefore it represents a poor candidate for parasite adaptation in Africa. Nevertheless, it is common in Asia where it feeds on people frequently and thus warrants further attention. However, its wide distribution and variable phenotype suggest that it represents a species complex (http://www.wrbu.org/SpeciesPages_non-ANO/non-ANO_A-hab/CXbit_hab.html).

Concluding remarks

We discussed here why human (mammalian)-infectious *Plasmodium* spp. are not transmitted by culicine mosquitoes. Based on the fact that culicines such as *Aedes* spp. and *Culex* spp. are the main vectors for other human pathogens and are present in areas where malaria is endemic, their contact with human malaria parasites in nature is indisputable. It has been demonstrated that several mammalian-infectious *Plasmodium* spp. can reach advanced developmental stages in some culicine mosquitoes; nevertheless, there is no evidence that culicines transmit malaria to humans. This suggests that the physiological barriers in the mosquito are either too strong in culicines and/or the cost of adaptation to a new vector is still too high for the parasite. It has been repeatedly shown that the mosquito immune system plays a crucial role in defining vector competence of *Anopheles* mosquitoes for *Plasmodium* spp., and manipulating the mosquito immune response can influence parasite survival. Present evidence suggests that the culicine immune system is involved in preventing transmission of mammalian-infectious *Plasmodium* spp. On the other hand, *Plasmodium* has shown great capacity to adapt to new vectors on different continents, and recent results show that *P. falciparum* parasites are able to evade the mosquito immune system in *An. gambiae*. The observation that avian *Plasmodium* spp. can be transmitted by anopheline and culicine mosquitoes clearly indicates that the same could be possible for mammalian-infectious *Plasmodium* parasites. Based on our current knowledge it is difficult to say if this is ever going to happen and when, and more research would be needed to provide a more reliable prediction (Box 2). The current decrease in *Anopheles* population density in African urban areas concurrent with an increase in culicine mosquitoes could move the balance toward adaptation of the parasite to culicines. Such adaptation could have

significant consequences for public health and therefore attention for this possibility may be prudent.

Box 2

Outstanding questions

- What is the nature of the physiological barriers that prevent transmission of mammalian-infectious *Plasmodium* spp. by culicines?
- Which culicine species are more likely to transmit mammalian malaria?
- What are the precise ecological conditions that would favor adaptation of human-infectious *Plasmodium* spp. to culicine mosquitoes?
- How important is the parasite's fitness cost tradeoff when adapting to a different vector?
- Can we be sure that transmission of human malaria by culicine mosquitoes has not happened in nature?

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Glossary

Anopheline mosquito

mosquitoes are classified within the Culicidae family ("small flies"), which is, based on morphology, divided into two subfamilies, the Anophelinae and the Culicinae

Basal lamina (BL)

layer of extracellular matrix secreted by epithelial cells on the basal side of the epithelium, serving as support for the epithelial cells. Two major components of the BL are the proteins laminin and collagen

Complement factor C3b

plasma protein derived from Factor C3 that binds to antigens on pathogens to label them for destruction

Complement-like immune system

a part of the insect immune system, analogous to the vertebrate complement system. It includes the thioester-containing protein 1 (TEP1) and leucine-rich repeat proteins LRIM1 and APL1

Culicine mosquito

see anopheline mosquito

Exflagellation

process by which *Plasmodium* male gametes are released from the red blood cell. It is identified under the microscope as male gametes move their flagella vigorously

Factor H

serum protein component of the vertebrate complement system that inhibits complement activity by binding complement protein C3b

Gamete

sexually differentiated cell that fuses with a gamete of different sex to form a fertilized zygote during sexual reproduction. *Plasmodium* gametes are formed in the mosquito midgut lumen and are derived from gametocytes ingested by a mosquito in a blood meal

Gametocyte

sexual stages of *Plasmodium*, which are formed in the peripheral blood of an infected vertebrate host. They exist in two forms, male and female gametocytes

Hemolymph

circulatory fluid in insects similar to blood in vertebrates

IMD-pathway

immune signaling pathway that is primarily activated by gram- bacteria and has been shown to respond against *P. falciparum* in *An. gambiae*

JNK-pathway

the Jun-N-terminal kinase (JNK)-pathway appears to be involved in responding to oxidative stress, and regulates cell growth, differentiation, survival and apoptosis (cell death) and immunity. The pathway mediates an immune response against the rodent malaria parasite *P. berghei* in *An. gambiae* mosquitoes

Melanization

part of the innate immune response of insects and other arthropods in the defense against pathogens. Pathogens are coated by a layer of the black melanin pigment and killed. Melanization is also a mechanism of wound healing, covering injuries with melanin

Midgut epithelium (MGT-E)

single layer of cells lining the mosquito gut. The cells are lying on a basal lamina that separates them from the hemolymph and contain microvilli on the anterior side facing the midgut lumen. The main function of the midgut epithelium is secretion of digestive enzymes and absorption of nutrients

Midgut lumen

interior cavity of the mosquito midgut where the blood meal is contained and digested

Oocyst

Plasmodium developmental stage formed from ookinetes after they crossed the midgut epithelial cells. The oocysts are located on the basal side of the midgut epithelium between

the basal membrane of the epithelial cell and the basal lamina and give rise to thousands of sporozoites

Ookinete

motile stage of the *Plasmodium* parasite that develops in the midgut lumen of the mosquito from the fertilized zygote and crosses the midgut epithelium to come to rest on the basal side of the midgut cell, where it develops into an oocyst

Peritrophic matrix (PM)

chitin-containing membrane that is formed in the mosquito midgut after blood feeding. Matrix components are secreted by the midgut epithelial cells and surround the blood bolus, protecting the midgut epithelium from gut microbiota and pathogens

Salivary glands (SG)

saliva-producing glands of the mosquito. *Plasmodium* sporozoites invade the salivary glands in order to be transmitted to another vertebrate host in the mosquito saliva

Sporozoite

infectious stage of *Plasmodium*. Sporozoites are formed in the oocysts and are released approximately 10 days after the mosquito blood meal. They invade the salivary glands and are transmitted to a vertebrate host during a blood meal of the mosquito. In the vertebrate host, sporozoites infect the liver and give rise to merozoites that infect the red blood cells

Thioester-containing protein 1 (TEP1)

protein found in the hemolymph of mosquitoes, which has structural similarity to complement factor C3. Upon cleavage, it binds to the surface of *Plasmodium* and tags it for elimination

TOLL-pathway

Signaling pathway involved in insect embryonic development and immunity. It is mainly activated by gram+ bacteria, fungi, and viruses and has been shown to act against the rodent malaria parasite *P. berghei* in *An. gambiae*

Vectorial capacity

ability of a mosquito to transmit a pathogen (see Box 1)

Vectorial competence

susceptibility of a mosquito to be infected by a pathogen (see Box 1)

Vertebrate complement

part of the immune system of vertebrates, composed of serum proteins including “Factor C3” that upon activation lead to tagging of a pathogen for destruction

Xanthurenic acid (XA)

byproduct of eye-pigment synthesis in insects

Zygote

diploid cell, product of the fusion of two haploid gametes during sexual reproduction

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Highlights

- Culicine mosquitoes are exposed to human-infectious *Plasmodium* spp, but do not transmit it.
- Culicines allow partial development of several mammalian *Plasmodium* spp.
- Different *Plasmodium* spp. encounter diverse barriers that prevent transmission.
- The right ecological conditions might promote adaptation for transmission.

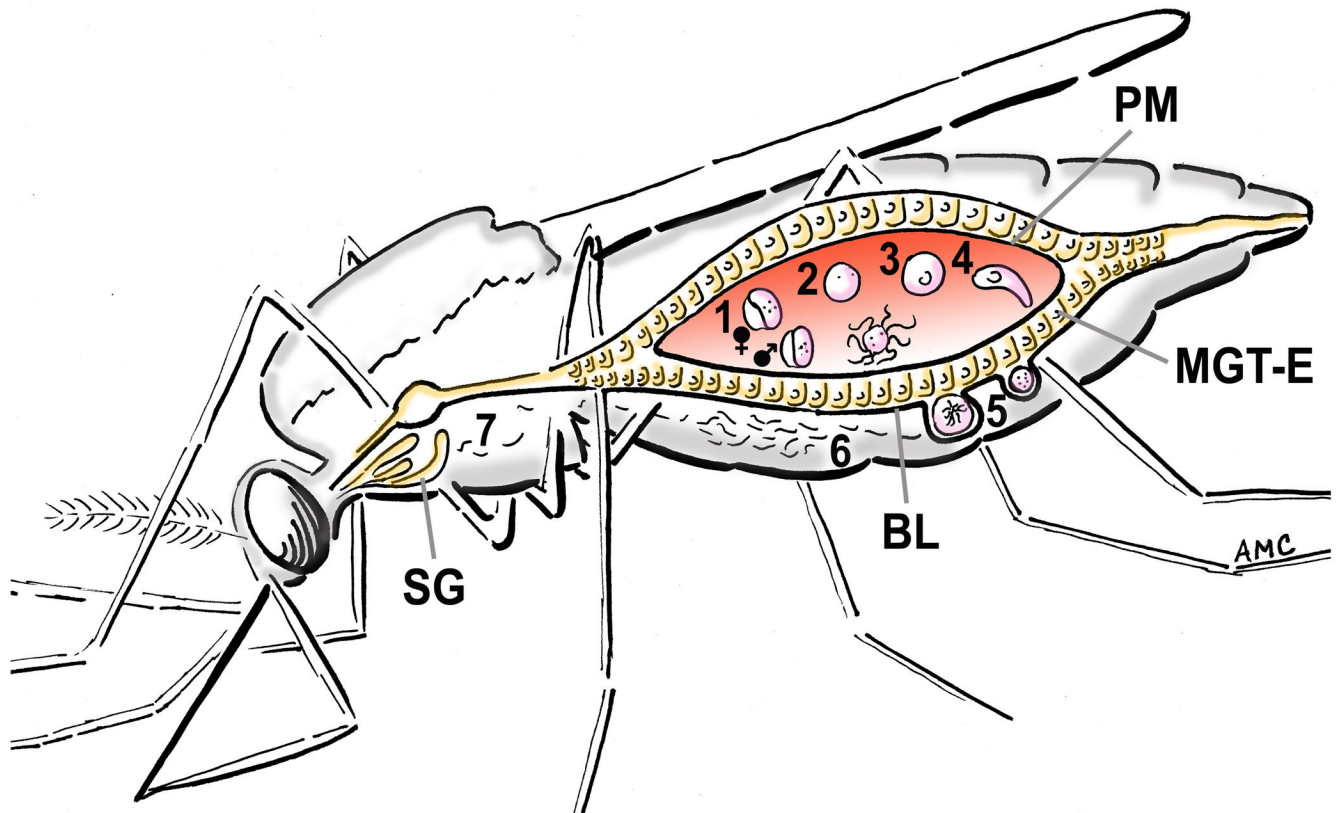


Figure 1. *Plasmodium* development in a mosquito vector

Successful transmission of *Plasmodium* spp. by a mosquito involves a complex developmental cycle within the vector. The mosquito must: (1) ingest a blood meal containing male and female *Plasmodium* gametocytes; (2) within minutes the gametocytes develop into gametes; (3) female macrogametes are fertilized by the male microgametes and a diploid zygote is formed; (4) the zygote develops into a motile ookinete; (4) the ookinete crosses the peritrophic matrix (PM) to invade the midgut epithelium (MGT-E) by 16–26 h post-feeding. (5) Successful ookinetes traverse the midgut epithelial cells and form oocysts, lying between the basal membrane of the epithelium and the basal lamina (BL). (6) The oocyst takes 7–21 days to develop thousands of sporozoites that are released into the mosquito hemolymph. (7) A fraction of the sporozoites invade the salivary glands (SG) and remain there to be injected into another vertebrate host when the mosquito takes another blood meal. Each step in the life cycle of *Plasmodium* in the mosquito can potentially represent a barrier for transmission (Box 1).

Table 1 Examples of pathogens transmitted by anopheline and culicine mosquitoes in nature and the laboratory

Pathogen	Mosquito					Refs
	Anophelinae	Culicinae			Mansonia spp.	
	<i>Anopheles</i> spp.	<i>Culex</i> spp.	<i>Aedes</i> spp.			
Human malaria	<i>P. falciparum</i>	✓ ^a	Ookinetes ^c [2], SGsporozoites ^d [6]	N/E	Oocysts ^e [11]	[51, 52]
	<i>P. vivax</i>	✓	SGsporozoites [6]	N/E	N/E	
	<i>P. malariae</i>	✓	SGsporozoites [6]	N/E	N/E	
	<i>P. ovale</i>	✓	N/E	N/E	N/E	
	<i>P. knowlesi</i>	✓	N/E	Sporozoites ^f (mentioned in [5], [53])	N/E	
Simian malaria	<i>P. cynomolgi</i>	✓	N/E	N/E	Sporozoites [5]	[54–56]
	<i>P. reichtenowi</i>	✓	N/E	N/E		
Rodent malaria	<i>P. berghei</i>	✓	No exflagellation ^e [4]	No exflagellation [3]	N/E	[57]
	<i>P. yoelii</i>	✓	N/E	N/E	N/E	
	<i>P. chabaudi</i>	✓	N/E	N/E	N/E	
Avian malaria	<i>P. gallinaceum</i>	✓	✓	✓	N/E	[3, 58]
	<i>P. relictum</i>	✓	✓	✓	N/E	
Flavi-viruses	Dengue	N/E ^b	N/E	✓	N/E	[59–62]
	Yellow fever	N/E	N/E	✓	N/E	
	West Nile	N/E	✓	✓	✓	
	Japanese encephalitis	✓	✓	✓	N/E	
α-viruses	St. Louis encephalitis	✓	✓	✓	N/E	[63–66]
	O'nyong'nyong	✓	N/E	N/E	N/E	
	Sindbis	N/E	✓	N/E	N/E	
Lymphatic filariasis	Chikungunya	✓	✓	✓	✓	[67]
	<i>Wuchereria bancrofti</i> <i>Brugia malayi</i>	✓	✓	✓	✓	

^a✓: successful transmission has been observed in nature and/or in the laboratory.

^bN/E: no evidence.

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^c Ookinetes: ookinetes have been found in the blood meal and on the midgut epithelium of the mosquito.

^d SGsporozoites: sporozoites can reach the salivary glands (SG) of the mosquito.

^e No exflagellation: activation of male gametocytes is impaired, and no exflagellation can be observed.

^f Sporozoites: sporozoites are present in the mosquito hemolymph but do not invade the salivary glands.

^g Oocysts: oocysts were observed on the midgut epithelium.