# Field evidence for manipulation of mosquito host selection by the human malaria parasite, *Plasmodium falciparum*

Amélie Vantaux[a,b,], Franck Yao[b], Domonbabele FdS Hien[b], Edwige Guissou[b], Bienvenue K. Yameogo[b], Louis-Clément Gouagna[a], Didier Fontenille[a,d], François Renaud[a], Frédéric Simard[a], Carlo Costantini[a], Fréderic Thomas[a], Karine Mouline[a,b,c], Benjamin Roche [a,e], Anna Cohuet[a], Kounbobr R. Dabiré[b,c] , Thierry Lefèvre[a,b,c]

[a] Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle (MIVEGEC), UMR IRD 224-CNRS 5290-UM, Montpellier, France.

[b] Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso.

[c] Laboratoire mixte international sur les vecteurs (LAMIVECT), Bobo Dioulasso, Burkina Faso.

[d] Institut Pasteur in Cambodia, Phnom Penh, Cambodia.

[e] Unité de Modélisation Mathématique et Informatique des Systèmes complexes (UMMISCO), UMI IRD/UPMC209, Bondy, France

**Corresponding author**: AV: amelie.vantaux@gmail.com

AV current address: Malaria Molecular Epidemiology Unit, Institut Pasteur in Cambodia, 5 Bd Monivong, PO Box 983, Phnom Penh 12 201, Cambodia

## Abstract

Whether the malaria parasite *Plasmodium falciparum* can manipulate mosquito host choice in ways that enhance parasite transmission toward human is unknown. We assessed the influence of *P. falciparum* on the blood-feeding behaviour of three of its major vectors (*Anopheles coluzzii*, *An. gambiae* and *An. arabiensis*) in Burkina Faso. Host preferences assays using odor-baited traps revealed no effect of infection on mosquito long-range anthropophily. However, the identification of the blood meal origin of mosquitoes showed that females carrying sporozoites, the mature transmissible stage of the parasite, displayed a 24% increase in anthropophagy compared to both females harbouring oocysts, the parasite immature stage, and uninfected individuals. Using a mathematical model, we further showed that this increased anthropophagy in infectious females resulted in a > 250% increase in parasite transmission potential, everything else being equal. This important epidemiological consequence highlights the importance of vector control tools targeting infectious females.

## Introduction

There is mounting evidence that malaria parasites affect phenotypic traits of their vectors and hosts in ways that increase contacts between them, hence favouring parasite transmission (Hurd 2003, Lefèvre and Thomas 2008, Koella 2005). In addition to increased vertebrate attractiveness to mosquito vectors (De Moraes et al. 2014, Cornet et al. 2013, Lacroix et al. 2005, Batista, Costa, and Silva 2014, Busula et al. 2017, Emami et al. 2017), another frequently reported parasite-induced change is the alteration of vector motivation and avidity to feed (Cator et al. 2012, Stanczyk, Mescher, and De Moraes 2017). Mosquitoes infected with *Plasmodium*

sporozoites (the mosquito to human transmission stage) can indeed display increased (i) response to host odours (Rossignol, Ribeiro, and Spielman 1986, Cator et al. 2013), (ii) landing and biting activity (Rossignol, Ribeiro, and Spielman 1984, 1986, Wekesa, Copeland, and Mwangi 1992, Anderson, Koella, and Hurd 1999, Koella, Rieu, and Paul 2002, Smallegange et al. 2013), (iii) number of feeds (Koella, Sorensen, and Anderson 1998) and (iv) blood volume intake (Koella and Packer 1996, Koella, Sorensen, and Anderson 1998, Koella, Rieu, and Paul 2002). In contrast, mosquitoes infected with oocysts (the immature non-transmissible stage of the parasite), are less likely to attempt to feed (Anderson, Koella, and Hurd 1999, Koella, Rieu, and Paul 2002, Cator et al. 2013). Since biting is risky (e.g., host defensive behaviours can kill the vector and its parasite), reduced feeding attempts seems beneficial to the parasite (Schwartz and Koella 2001).

These "stage-dependent" behavioural alterations likely increase parasite transmission (Cator et al. 2014, Dobson 1988), provided that mosquito feeds are taken on a suitable vertebrate host species for the parasite. While malaria vectors can usually feed on a range of different vertebrate species (Takken and Verhulst 2013), the malaria parasites they transmit are often highly host-specific, infecting only one or a few vertebrate species (Perkins 2014). For example *P. falciparum*, which causes the most severe form of human malaria, displays an extreme form of specificity and can develop and reproduce in hominids only (predominantly in human and to a lesser extent in chimpanzee, bonobo, and gorilla) (Rayner et al. 2011, Prugnolle et al. 2011, Ngoubangoye et al. 2016), such that any mosquito bite on another vertebrate species would be a dead-end for the parasite. In contrast, the vectors of *P. falciparum* can feed on a wide range of vertebrate hosts species in the wild depending on the geographic area and the relative abundance of human and other vertebrates (Costantini et al. 1999, Takken and Verhulst 2013). Accordingly,

*P. falciparum* could modify its vector choice in ways that enhance transmission toward human and/or reduce mosquito attraction to other unsuitable host species (i.e. specific manipulation). A previous study testing this hypothesis found no effect of *P. falciparum* infection on host preference of three major vector species, *An. coluzzii*, *An. gambiae,* and *An. arabiensis* (Nguyen et al. 2017). However, this study examined the odour-mediated mosquito host preference in laboratory conditions using a dual-port olfactometer, not the final realised host choice which is of primary importance for parasite transmission.

Here, we assessed the influence of *P. falciparum* on *An. coluzzii, An. gambiae* and *An. arabiensis* blood-feeding behaviour in three villages of Burkina Faso. First, inherent mosquito host preferences were determined using odor-baited traps, set side by side in a choice arrangement, releasing either human or calf odors. Patterns of host selection were then assessed by the identification of the blood meal origin of indoor-resting samples.

## Material and methods

### *Collection sites*

The study was conducted in three villages of South-Western Burkina Faso: Soumousso (11°23’14”N, 4°24’42”W), Klesso (10°56’40.5”N, 3°59’09.9”W) and Samendeni (11°27’14.3”N, 4°27’37.6”W) (Figure supplement S1). The three villages are located in an area characterized by wooded savannah, where *Anopheles* females only have access to temporary, rain-filled puddles and quarries that permit larval development during the rainy season from June to November. The dry season extends from December to May. In these rural villages, domestic animals (including cattle, goats, sheep, pigs, chickens, donkeys, dogs) are usually kept in compounds in open conditions but a few households use separate roofed shelters for sheep,

goats, pigs and chicken. Most houses are mud-walled with roofs of iron sheets or thatch, but a few houses are made of bricks.

***Mosquito host preference***

Two odour-baited entry traps (OBETs as in Lefèvre et al. 2009, Costantini et al. 1996, Costantini et al. 1998) and two odour-baited double net traps (BNTs as in Tangena et al. 2015) baited with calf and human odours were used to assess the host preference of field populations of mosquitoes in Samandeni and Klesso villages (Figure supplement S2).The two OBETs were connected to a tent (Lxlxh: 250x150x150 cm) by air vent hoses (Scanpart®, DxL=10*300cm; Figure supplement S2a). The odours of the two hosts were drawn by a 12-V fan from the tents and into the OBETs by the air vent hoses, coming out of the traps at a speed of 15cm/s (±2cm/s), as measured with a Testo 425-Compact Thermal Anemometer (Testo, Forbach, France) equipped with a hot wire probe [range: 0 to + 20m/s, accuracy: ± (0.03 m/s + 5% of mv)]. Host-seeking mosquitoes responding to the host cues flew up the odour-laden streams and entered one of the two traps. The two odour-baited double net traps (BNTs) consisted of an untreated bed net (Lxlxh: 300x250x185 cm) from which each corner was raised 20 cm above ground and a smaller untreated bed net (Lxlxh: 190x120x150 cm) protecting the human volunteer in the human baited trap (Figure supplement S2b).

In both OBETs and BNTs, the human volunteers rested on a metal-framed bed (Lxl: 190x80 cm) and were protected from mosquito bites. OBETs and BNTs were operated from 19:00 to 05:30 hours, for 3 nights in June 2013, and 13 nights in September 2013 in Samendeni. The BDNTs only were set-up for 6 nights in September in Klesso. Trapped mosquitoes were retrieved in the morning using mouth aspirators. They were kept in a 20x20x20 cm cage with a humid towel on top and brought back to the laboratory for further processing (see below).

***Mosquito blood-feeding pattern***

Indoor resting mosquitoes were collected between 7 am and 9 am by insecticide pyrethroid spray catches as in Lefèvre et al. (2009). In Samandeni and Klesso, mosquito collections were carried out in the rainy season only (4 days in June 2013, and 13 days in September 2013 in Samendeni, and 6 days in September 2015 in Klesso), whereas in Soumousso it was conducted in both the rainy and the dry season (26 days between January and November 2009). . In Soumousso, human dwellings (from 10 neighbourhoods) only were sampled whereas animal sheds and unoccupied houses were also sampled in Samandeni and Klesso. A total of 27 human dwellings, 7 unoccupied houses and 20 animal sheds were sampled in Samendeni. A total of 7 human dwellings, 7 unoccupied houses and 9 animal sheds were sampled in Klesso. All mosquitoes were kept in petri dish with a humid paper towel to facilitate later dissection and brought back to the laboratory for further processing (see below).

***Laboratory processing of samples***

*Anopheles gambiae sl.* females were dissected in a drop of phosphate buffered saline (PBS) (pH 7.2). Blood-fed midguts were gently squeezed to get the blood out, which was mixed with PBS, absorbed on a filter paper, and then kept at -20°C until identification by an enzyme-linked-immunosorbent assay (ELISA) for Soumousso and Samendeni samples and by multiplex PCR as in Kent and Norris (2005) with minor modifications (see supplementary material) for Klesso samples. Each blood meal was discriminated between human, cattle, goat/sheep, chicken, dog, pig, and horse/donkey origins. The extracted midguts were then stained with 1% mercurochrome to detect with a microscope (20× magnification) the presence and number of *Plasmodium* spp. oocysts. PCR on a subset of oocyst-infected individuals confirmed that these oocysts belonged to *P. falciparum*. Head and thorax of individual mosquitoes were stored at −20°C in 1.5 mL

Eppendorf tubes. Sporozoite infection with *P. falciparum* was determined by ELISA for the Soumousso samples (Wirtz et al. 1987) and by qPCR for the samples from Samendeni and Klesso (Boissière et al. 2013).

This protocol allowed us to gather the following information for each collected individual mosquito: immature *Plasmodium* infection status (presence of oocysts in the midgut); mature *P. falciparum* infection status (presence of sporozoites in salivary glands); source of blood meal or trap (calf/human) chosen; shelter type (human dwellings, unoccupied houses, animal sheds). A total of 3447 blood-fed *Anopheles gambiae s.l.* collected indoors and 674 females collected in the choice traps were processed. In addition, a subset of 276 females collected indoors was used to determine parity (parous versus nulliparous) based on the condition of ovarian tracheoles. Similarly, a subset of 418 individuals was used to distinguish between the *Anopheles* species sensu stricto (*Anopheles arabiensis*, *Anopheles coluzzii* and *Anopheles gambiae*) using routine PCR-RFLP as described in Santolamazza *et al*. (2008).

***Statistical analyses***

*Mosquito host preference (OBETs and BNTs)*

The anthropophily index (AI) was expressed as the number of *Anopheles gambiae s.l.* caught in the human-baited trap over the total number of mosquitoes caught in both human- and calf- baited traps. We tested the effect of infection status (uninfected, infected with the oocyst immature stages and infected with the sporozoite transmissible stages), collection method (OBET vs. BNT), and their interaction on AI using a general linear model (GLM) with a binomial error structure.

*Mosquito blood-feeding pattern (blood origin of indoor resting samples)*

The human blood index (HBI) was expressed as the number of *Anopheles gambiae s.l.* fed on human including mixed human-animal bloodmeals over the total number of blood-fed *Anopheles gambiae s.l.*. We tested the effect of *Plasmodium* infection status (uninfected, oocyst-infected, sporozoite-infected individuals - 25 individuals with both oocysts and sporozoites were included in the sporozoite infected group and excluding these individuals from the analysis yielded similar results), village (Soumousso, Samendeni, Klesso), shelter type (human dwelling, unoccupied house, animal shed) and relevant two-ways interactions (infection status by shelter type and infection status by village) on HBI using a general linear model (GLM) with a binomial error structure. The effect of species (*Anopheles gambiae*, *An. coluzzii* and *An. arabiensis*), infection status, shelter type, and their interactions on HBI was assessed using the subset of females identified to the molecular level using a GLM with a binomial error structure. The effect of parity (nulliparous *vs.* parous) on HBI was assessed on a subset of females using a GLM with a binomial error structure.

We also verified for both AI and HBI whether choice significantly differed from a random distribution between humans and animals or whether mosquitoes displayed a statistically significant attraction to one type of blood meal or trap.

For model selection, we used the stepwise removal of terms, followed by likelihood ratio tests (LRT). Term removals that significantly reduced explanatory power ($P<0.05$) were retained in the minimal adequate model (Crawley 2007). All analyses were performed in R v.3.0.3.

***Mathematical model***

In order to explore the epidemiological consequences of variation in HBI, we built a SIR model (Keeling and Rohani 2008):

$$\frac{dS_m}{dt} = \mu N_m - ab\frac{S_m}{N_m}I_h\varepsilon_s - \mu S_m$$

$$\frac{dE_m}{dt} = ab\frac{S_m}{N_m}I_h\varepsilon_s - (\mu + \gamma)E_m$$

$$\frac{dI_m}{dt} = \gamma E_m - \mu I_m$$

$$\frac{dS_h}{dt} = -ac\frac{S_h}{N_h}I_m\varepsilon_i + \delta I_h$$

$$\frac{dI_h}{dt} = ac\frac{S_h}{N_h}I_m\varepsilon_i - \delta I_h$$

Susceptible mosquitoes ($S_m$) born at rate $\mu$ and become exposed ($E_m$) according to their biting rate ($a$), their probability to get infected ($b$) and the HBI of susceptible mosquitoes ($\varepsilon_s$). Then, exposed mosquitoes become infectious ($I_m$) according to their extrinsic incubation period ($\gamma$). Each mosquito population die at rate ($\mu$). Susceptible humans ($S_h$) get infected according to mosquito biting rate, probability to develop infection (c) and HBI of infectious mosquitoes ($\varepsilon_i$). Then, infectious humans remain infectious ($I_m$) during their infectious period equals to $1/\delta$ on average.

See parameters values in table supplement S1. We have fixed HBI of susceptible mosquitoes ($\varepsilon_s$) to the value that has been experimentally measured in this study and we explored the impact of HBI of infectious mosquitoes ($\varepsilon_i$, during the sporozoite stage) on the Entomological Inoculation Rate (EIR, representing the number of infectious bites received by a human during one year (Smith and Ellis McKenzie 2004), as defined by:

$$EIR = ma\frac{I_m}{N_m}$$

where $m$ is the ratio between mosquitoes and humans, and other parameters are similar as above. With an identical human population size of 100 individuals, we have assumed different ratio

values (m) between mosquitoes and humans (low: m=1, medium: m=10 and high: m=100) to explore the impact of different HBIs on the EIR in relation to mosquito densities. Then, the ODE system has been simulated for one season in order to estimate the proportion of infectious mosquitoes.

***Ethics***

Ethical approval was obtained from the Centre Muraz Institutional Ethics Committee under agreement no. 0003-2009/CE-CM and A0003-2012/CE-CM.

## Results

***Mosquito host preference*** – To assess the inherent mosquito host preference of field populations of mosquitoes, we used two odour-baited entry traps (OBETs) and two odour-baited double net traps (BNTs) releasing either calf or human odours. The anthropophily index (AI) was expressed as the number of *Anopheles gambiae s.l.* caught in the human-baited trap over the total number of mosquitoes caught in both human- and calf- baited traps. The infection status was successfully determined in 584 out of the 674 mosquitoes (86.6%) collected in the OBETs (383 individuals) and BNTs (201 individuals). Uninfected, oocyst-infected and sporozoite-infected females displayed similar host preferences ($X^2_2$ = 3.6, P = 0.17, Figure supplement S3), with a significant attraction toward human odours (uninfected females: 63.3 ± 4%, N=531, OR=0.58, 95% CI = 0.53-0.63, P <0.0001; oocyst-infected females: 55.2 ± 18 %, N=29, OR=0.81, 95% CI = 0.56-0.81, P=0.58; sporozoite-infected females: 45.8 ± 20 %; N=24, OR=1.18, 95% CI = 0.78-1.78, P=0.7). There was no effect of collection method on AI (OBETs: 64 ± 5%, BNTs: 59 ± 7%; $X^2_1$ = 1.5, P = 0.21), indicating that both methods are comparable to assess mosquito host

preference. There was no interaction between mosquito infection and collection method ($X^2_2$ = 0.26, P = 0.9; Figure supplement S3).

***Mosquito blood-feeding pattern*** – To assess the realized host selection of *Anopheles gambiae s.l.*, the blood meal origins of females collected indoors between 7 am and 9 am were identified. The human blood index (HBI) was expressed as the number of females fed on human (including mixed human-animal bloodmeals) over the total number of blood-fed females. Of the 3447 blood-fed *Anopheles gambiae s.l.* collected indoors, the blood meal origin was successfully identified in 2627 samples (76%). Among these 2627 samples, infection status was successfully determined in 2328 mosquitoes (88.6%). The following analyses are restricted to these 2328 females. HBI was significantly affected by mosquito infection status ($X^2_2$ = 13.007, P = 0.0015; Figure 1) with a 24% increase in HBI in sporozoite-infected females compared to both oocyst-infected and uninfected counterparts (sporozoite-infected: 77 ± 5.7%; N=209, deviation from random feeding: OR=0.3, 95% CI = 0.25-035, P <0.0001; oocyst-infected females: 63.6 ± 5.7%, N=118, OR=0.57, 95% CI = 0.47-0.69, P =0.004; uninfected females: 61.1 ± 2.1%; N=2001, OR=0.64, 95% CI = 0.61-0.66, P <0.0001). HBI was also significantly influenced by shelter type ($X^2_2$ = 145.92, P < 0.0001) and villages ($X^2_2$ = 139.5, P < 0.0001; see supplementary material for details). The HBI of sporozoite-infected mosquitoes was higher than that of oocyst-infected and uninfected females regardless of the village considered (infection status: village interaction: $X^2_4$ = 2.3, P = 0.68, Figure 1) or the shelter type in which mosquito females were collected (infection status: shelter type interaction: $X^2_4$ = 0.7, P = 0.95, Figure supplement S4).

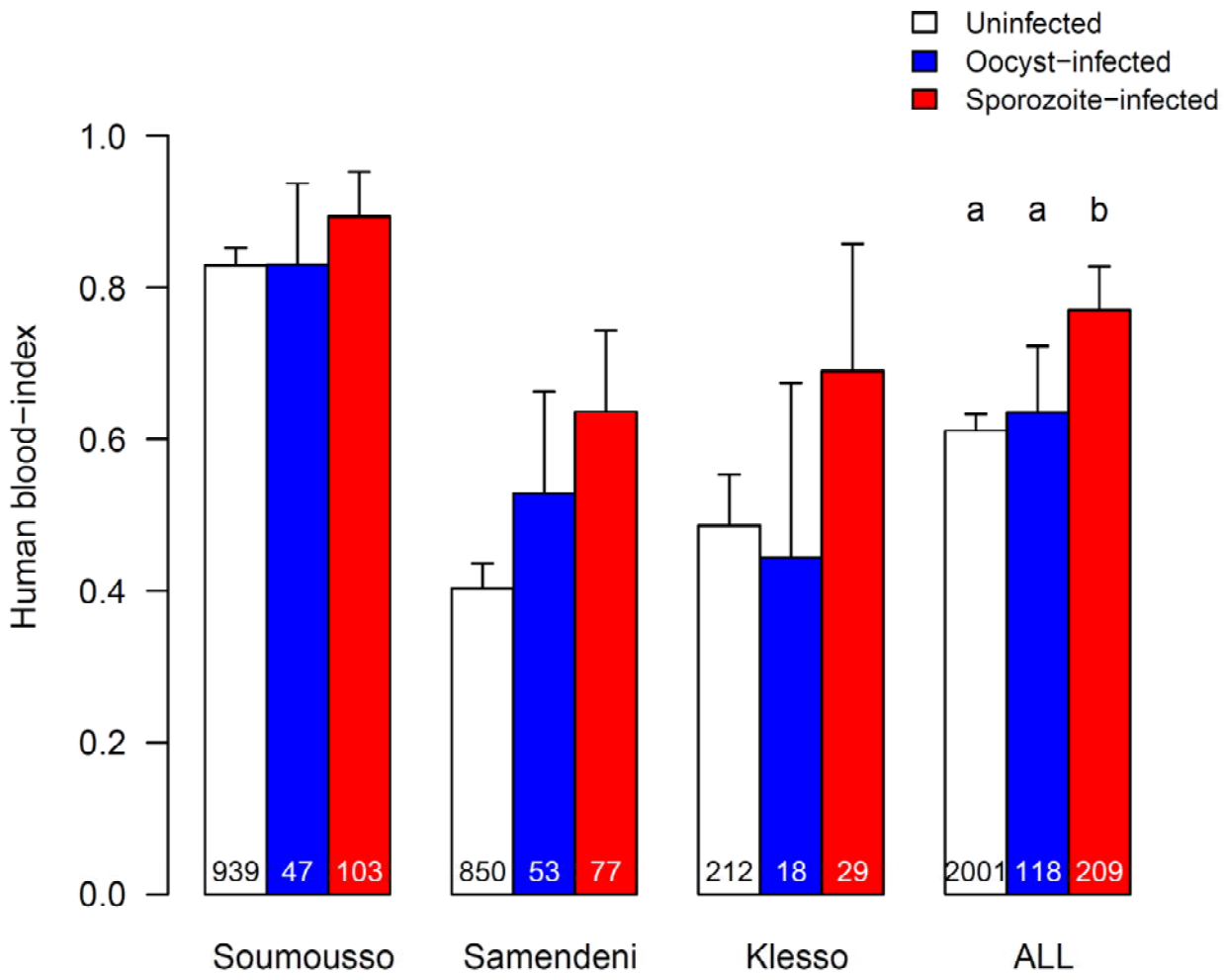


**Figure 1.** Effect of infection status on the human-blood index of *Anopheles gambiae s. l.* females expressed as the number of females fed on human out of the total number of blood-fed females for the three sampled villages. Data show proportion ± 95% confidence interval. Numbers in bars indicate the total numbers of mosquitoes. Different letters indicate differences between infection status (Chi-square *post-hoc* tests: sporozoite-infected *vs*. oocyst-infected females $X^2_1$=6.1, P=0.013; sporozoite-infected *vs*. uninfected females $X^2_1$=19.4, P<0.0001; oocyst-infected *vs*. uninfected females $X^2_1$=0.18, P= 0.67).

A significant species variation in HBI was observed ($X^2_2$ = 10.2, P = 0.006; Figure 2) with *Anopheles arabiensis* being significantly less anthropophagic (22.2 ± 15%, N=27, OR=3.5, 95% CI = 2.2-5.56, P = 0.007) than *An. gambiae* (54.8 ± 7.1%; N=186, OR=0.82, 95% CI = 0.71-0.95, P = 0.19 ) and *An. coluzzii* (55.1 ± 6.8%; N=205, OR=0.81, 95% CI = 0.71-0.94, P=0.14). Although HBI varied among mosquito species, sporozoite-infected individuals displayed highest anthropophagy regardless of the species considered (infection status: species interaction: $X^2_4$ = 4, P = 0.42; Figure 2 and supplementary material).

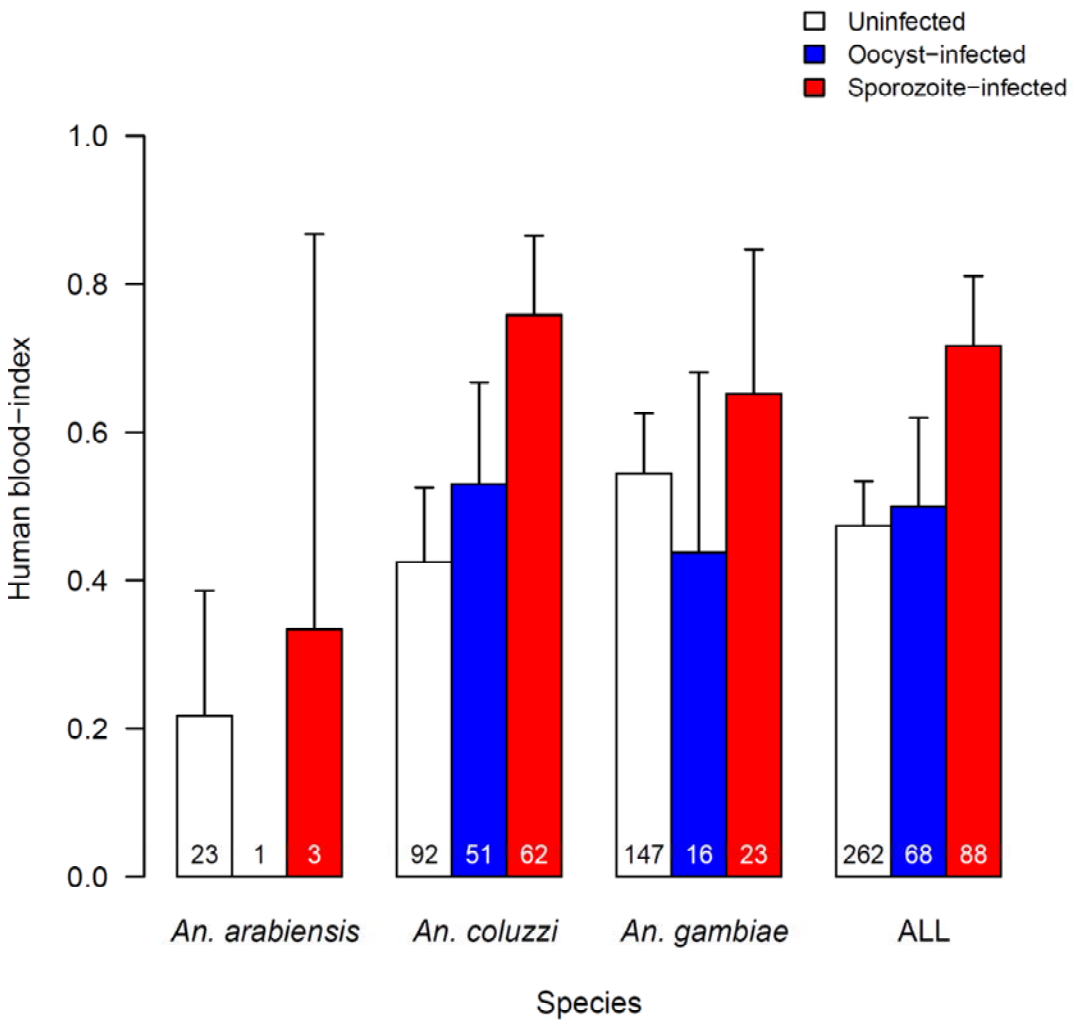

**Figure 2.** Effect of infection status and *Anopheles* species *sensu stricto* on the human-blood index expressed as the proportion of females fed on human or human and animal out of the total of blood-fed females. Data show proportion ± 95% confidence interval. Numbers in bars indicate the total numbers of mosquitoes.

Finally, HBI was not significantly affected by parity, a proxy used to estimate mosquito age (nulliparous females: 49.53 ± 9%, parous females: 45.6 ± 7.5%; $X^2_1 = 0.4$, P = 0.52)

***Epidemiological consequences*** – To investigate the epidemiological impact of a higher HBI in infectious females compared to oocyst-infected and uninfected females, we built a mathematical model based on the experimental values observed in this study. This model assessed the impact of different HBIs on the Entomological Inoculation Rate (EIR, number of infectious bites received by a person during one year) at different mosquito longevities and densities. The HBI of susceptible mosquitoes was fixed at 0.62 (as in uninfected and oocyst-infected mosquitoes) and the impact of HBI variation in infectious (sporozoite-infected) mosquitoes on parasite transmission potential was explored (Figure 3). For an average mosquito lifespan of 15 days (Figure 3a), an HBI of infectious mosquitoes of 0.62 (similar to that of susceptible mosquitoes) resulted in an EIR of 4 at a low ratio of 1 (1 mosquito per human), while an HBI of 0.77 (as observed here in infectious mosquitoes) resulted in an EIR of 14. In other words, a 24% increase in HBI resulted in a 250% increase in EIR, everything else being equal. Transmission consequences were even larger when the human-to-mosquito ratios were higher (5 *vs*. 19 with a ratio of 10 or 100, i.e. a 280% increase in EIR) but tended to decrease with increasing mosquito longevities (Figure 3c, 3d, and supplementary material).

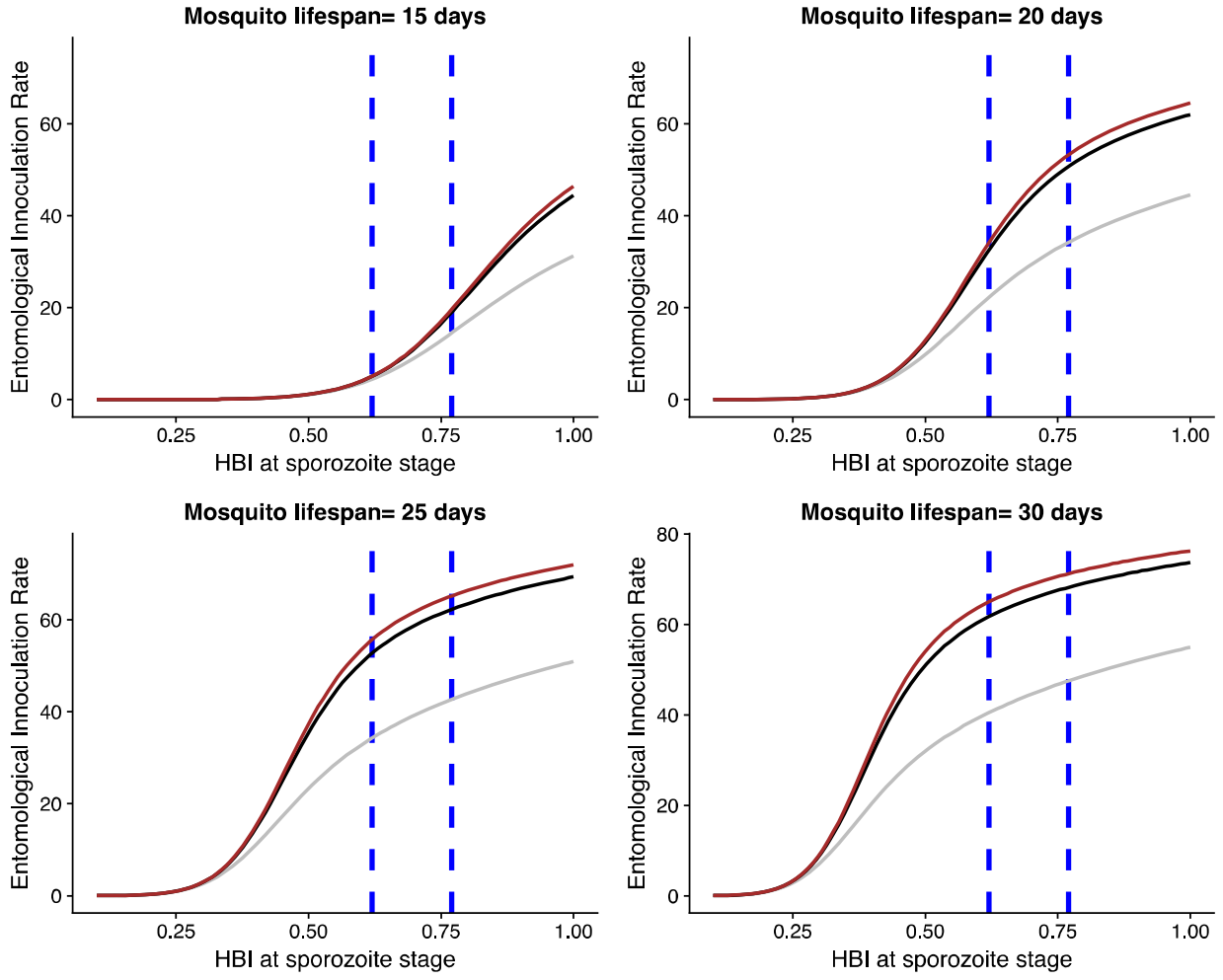


**Figure 3**. Expected epidemiological consequences of HBI variation for different values of mosquito lifespan and mosquito/human ratio. X axis represents the range of values considered for the HBI of infectious (sporozoite-infected) mosquitoes and the Y axis is the Entomological Inoculation Rate (EIR, number of infectious bites received by a person over one year) when the HBI of susceptible (uninfected and oocyst-infected) is 0.62. The plain lines show the evolution of EIR according to HBI of sporozoite infected mosquitoes for different values of mosquito-to-human ratio. The dashed lines represent the value considered for susceptible mosquitoes (0.62) and the value measure for sporozoite-infected mosquitoes (0.77). Ratio=adult mosquito/human

densities; grey lines = low ratio (m= 1); black lines = medium ratio (m=10); redlines = high ratio (m=100).

## Discussion

Consistent with the hypothesis of specific manipulation, the patterns of mosquito host selection showed that sporozoite-infected *An. coluzzi*, *An. gambiae* and *An. arabiensis* females were more likely to have fed on human than oocyst-infected and uninfected individuals. By distinguishing sporozoite and oocyst infection, we ruled out the potential confounding effect of a mere intrinsic mosquito characteristic. Infected mosquitoes may indeed exhibit increased anthropophagy not because of being infected but just because of an innate preference for human, thus making these mosquito individuals infected. Here, individuals infected with sporozoites displayed different HBI than individuals infected with oocysts, thus ruling out this possibility. Because *Plasmodium falciparum* takes about 10 to 18 days to complete its development (depending on temperature), there is an increased likelihood of sporozoite infection as mosquitoes become older. This means that mosquito age could be a confounding factor of infection, with infected mosquitoes displaying increased HBI not because they harbour sporozoites but because they are older. Such an age effect could be mediated by specific physiological requirements in old mosquitoes or by a positive reinforcement (learning / memory) of feeding on human. Our data does not support an age effect as we did not find a significant effect of parity (a proxy for age) on HBI (i.e. parous and nulliparous mosquito females displayed similar anthrophagy).

The preference assays (OBETs and BNTs) showed that infected mosquitoes displayed similar long-range attraction toward human odour as uninfected individuals regardless of parasite

developmental stages (oocyst vs sporozoite), confirming previous laboratory results (Nguyen et al. 2017).

The precise mechanisms responsible for increased anthropophagy in sporozoite-infected mosquitoes is not yet clear, but at least three hypotheses can be proposed. First, malaria parasites might manipulate mosquito short-range behaviours only, whereas at longer range when mosquitoes rely mainly on $CO_2$ and other volatile odours, sporozoite-infected mosquitoes display similar preference as uninfected and oocyst-infected individuals. At short range, mosquitoes rely on other cues including visual stimuli, moist, heat and skin emanations (Takken and Verhulst 2013). These stimuli can be host-specific and informing of host suitability for parasite development before the mosquito engage in selection and eventually in feeding. In addition to a possible preferential short-range attraction of sporozoite-infected mosquitoes toward host species suitable for parasite development, there could also be short-range repellence by unsuitable host species.

Second, the parasite may induce changes in the vector such as an alteration of microhabitat choice to spatially match the habitat of the suitable host. This could be achieved through parasite manipulation of mosquito endophagic/philic behaviours resulting in a higher degree of indoor -feeding and -resting of sporozoite-infected females. For example, infectious mosquitoes may exhibit an enhanced tendency to enter house interstices regardless of emitted odors. Such changes could also be mediated through a modification of mosquito phototactic response: while uninfected mosquitoes would respond normally causing the natural fraction of the population to leave the house following a blood-meal, infectious mosquitoes may display a negative phototactic response increasing their chances of staying inside human dwellings. Future

experiments exploring the phototactic response of infected and uninfected mosquitoes would help addressing this hypothesis.

Third, the parasite may induce changes in the vector such as an alteration of time activity in order to temporally match the time rest or activity of the suitable host. Mosquitoes exhibit circadian rhythms in many activities such as flight, host-seeking, swarming, egg-laying, etc. There is mounting evidence that, following bed-nets introduction, malaria vectors can display increased tendency to feed outdoors (Russell et al. 2011) or bite earlier in the evening or later in the morning (Moiroux et al. 2012). Accordingly, *P. falciparum* could manipulate mosquito host-seeking rhythm in a way that increases bites on unprotected people. Testing this hypothesis would require sampling mosquitoes at distinct period and comparing the proportion of uninfected, oocyst-infected and sporozoite-infected vectors among samples.

Sporozoite-induced change in mosquito host selection occurred in three major and related mosquito vectors, namely *An. coluzzii*, *An. gambiae* and *An. arabiensis*. This suggests that manipulation likely already occurred in the common ancestor of these three species and that the parasites might exploit a physiological pathway common to all three mosquito species to modify its vector host choice.

Transmission models generally assume that uninfected and infected vectors have similar preference for human. This study suggests that this assumption may not be valid and that these models possibly underestimate transmission intensity. Our modelling approach confirms that HBI increase in infectious mosquitoes can have dramatic impact on disease transmission. In particular, if we consider mosquito lifespans relevant to natural settings (i.e. 15 to 20 days; Gillies and Wilkes 1965, Saul, Graves, and Kay 1990, Killeen et al. 2000, Charlwood et al. 1997, Gillies 1961), the transmission potential was almost multiplied by 3 when the HBI increased

from 0.62 to 0.77 i.e. the value observed for the infectious mosquitoes in this study. For many mosquito–*Plasmodium* associations including *An. gambiae s.l.-P. falciparum*, the duration of the parasite's development within the mosquito is as long as the insect vector's average lifespan. This means that most mosquitoes do not live long enough to transmit the disease, and hence that feeds taken by infectious mosquitoes taken on unsuitable host species would have disastrous consequences for the parasite's fitness. The model suggests that the benefits of specific manipulation should be particularly high in vectorial systems in which transmission opportunities are rare (short vector lifespan, relatively long parasite development period, and diverse blood sources).

In conclusion, our results suggest that the human malaria parasite *P. falciparum* evolved the ability to enhance transmission toward human, the appropriate host species, by increasing mosquito anthropophagy (or decreasing zoophagy) with potentially profound public health consequences. Future laboratory and field studies will be essential to confirm these results and to better understand the epidemiological, ecological and evolutionary consequences of parasite manipulation of vector behaviours.

**Acknowledgements**

We would like to thank all volunteers for participating in this study as well as the local authorities for their support. We are very grateful to the IRSS staff in Burkina Faso for technical assistance.

**Competing interests**

We have no competing interests.

**Funding**

This study was supported by the ANR grant no. 11-PDOC-006-01 and the European Community's Seventh Framework Program (FP7/2007–2013) under grant agreements no. 242095 and no.223736. BR is supported by the ANR project PANIC.

## References

Anderson RA, Koella JC, and Hurd H. 1999. The effect of *Plasmodium yoelii nigeriensis* infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle. *Proceedings of the Royal Society of London Series B-Biological Sciences* **266**:1729-1733.

Batista E, Costa E, and Silva A. 2014. *Anopheles darlingi* (Diptera: Culicidae) displays increased attractiveness to infected individuals with *Plasmodium vivax* gametocytes. *Parasites & Vectors* **7**:251.

Boissière A, Gimonneau G, Tchioffo MT, Abate L, Bayibeki A, Awono-Ambéné PH, Nsango SE, and Morlais I. 2013. Application of a qPCR assay in the investigation of susceptibility to malaria infection of the M and S molecular forms of *An. gambiae* s.s. in Cameroon. *PLoS ONE* **8**:e54820.

Busula A, Bousema T, Mweresa C, Masiga D, Logan J, Sauerwein R, Verhulst N, Takken W, and de Boer J. 2017. Gametocytemia and Attractiveness of *Plasmodium falciparum*-Infected Kenyan Children to *Anopheles gambiae* Mosquitoes. *The Journal of Infectious Diseases* **216**:291-295. doi: 10.1093/infdis/jix214.

Cator L, Lynch P, Thomas M, and Read A. 2014. Alterations in mosquito behaviour by malaria parasites: potential impact on force of infection. *Malaria Journal* **13**:164.

Cator LJ, George J, Blanford S, Murdock CC, Baker TC, Read AF, and Thomas MB. 2013. 'Manipulation' without the parasite: altered feeding behaviour of mosquitoes is not dependent on infection with malaria parasites. *Proceedings of the Royal Society B: Biological Sciences* **280**:20130711. doi: 10.1098/rspb.2013.0711.

Cator LJ, Lynch PA, Read AF, and Thomas MB. 2012. Do malaria parasites manipulate mosquitoes? *Trends in Parasitology* **28**:466-470.

Charlwood JD, Smith T, Billingsley PF, Takken W, Lyimo EOK, and Meuwissen JHET. 1997. Survival and infection probabilities of anthropophagic anophelines from an area of high prevalence of *Plasmodium falciparum* in humans. *Bulletin of Entomological Research* **87**:445-453. doi: 10.1017/S0007485300041304.

Cornet S, Nicot A, Rivero A, and Gandon S. 2013. Malaria infection increases bird attractiveness to uninfected mosquitoes. *Ecology Letters* **16**:323-329. doi: 10.1111/ele.12041.

Costantini C, Gibson G, Sagnon N, Della Torre A, Brady J, and Coluzzi M. 1996. Mosquito responses to carbon dioxide in a west African Sudan savanna village. *Med Vet Entomol* **10**:220-7.

Costantini C, Sagnon N, Della Tori A, Diallo M, Brady J, Gibson G, and Coluzzi M. 1998. Odor-mediated host preferences of West African mosquitoes, with particular reference to malaria vectors. *American Journal of Tropical Medicine and Hygiene* **58**:56-63.

Costantini C, Sagnon N, della Torre A, and Coluzzi M. 1999. Mosquito behavioural aspects of vector-human interactions in the *Anopheles gambiae* complex. *Parassitologia* **41**:209-17.

Crawley MJ. 2007. *The R book*. The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England: John Wiley & Sons Ltd.

De Moraes CM, Stanczyk NM, Betz HS, Pulido H, Sim DG, Read AF, and Mescher MC. 2014. Malaria-induced changes in host odors enhance mosquito attraction. *Proceedings of the National Academy of Sciences* **111**:11079-11084. doi: 10.1073/pnas.1405617111.

Dobson AP. 1988. The population biology of parasite-induced changes in host behavior. *Q. Rev. Biol*. **63**:139-165.

Emami SN, Lindberg BG, Hua S, Hill S, Mozuraitis R, Lehmann P, Birgersson G, Borg-Karlson A-K, Ignell R, and Faye I. 2017. A key malaria metabolite modulates vector blood seeking, feeding, and susceptibility to infection. *Science* **355**:1076-1080. doi: 10.1126/science.aah4563.

Gillies MT. 1961. Studies on the dispersion and survival of *Anopheles gambiae* Giles in East Africa, by means of marking and release experiments. *Bulletin of Entomological Research* **52**:99-127. doi: 10.1017/S0007485300055309.

Gillies MT, and Wilkes TJ. 1965. A study of the age-composition of populations of *Anopheles gambiae* Giles and *A. funestus* Giles in North-Eastern Tanzania. *Bull Entomol Res* **56**:237-62.

Hurd H. 2003. Manipulation of medically important insect vectors by their parasites. *Annual Review of Entomology* **48**:141-161. doi: 10.1146/annurev.ento.48.091801.112722.

Keeling MJ, and Rohani P. 2008. *Modeling Infectious Diseases*. Princeton: Princeton University Press.

Kent RJ, and Norris DE. 2005. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome B. *Am J Trop Med Hyg* **73**:336-42.

Killeen GF, McKenzie FE, Foy BD, Schieffelin C, Billingsley PF, and Beier JC. 2000. A simplified model for predicting malaria entomologic inoculation rates based on entomologic and parasitologic parameters relevant to control. *The American Journal of Tropical Medicine and Hygiene* **62**:535-544. doi: doi:https://doi.org/10.4269/ajtmh.2000.62.535.

Koella JC. 2005. Malaria as a manipulator. *Behavioural Processes* **68**:271-273. doi: https://doi.org/10.1016/j.beproc.2004.10.004.

Koella JC, and Packer MJ. 1996. Malaria parasites enhance blood-feeding of their naturally infected vector *Anopheles punctulatus*. *Parasitology* **113**:105-109.

Koella JC, Rieu L, and Paul REL. 2002. Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*. *Behavioral Ecology* **13**:816-820. doi: 10.1093/beheco/13.6.816.

Koella JC, Sorensen FL, and Anderson RA. 1998. The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**:763-768.

Lacroix R, Mukabana WR, Gouagna LC, and Koella JC. 2005. Malaria infection increases attractiveness of humans to mosquitoes. *Plos Biology* **3**:1590-1593. doi: e298

10.1371/journal.pbio.0030298.

Lefèvre T, Gouagna LC, Dabire RK, Elguero E, Fontenille D, Renaud F, Costantini C, and Thomas F. 2009. Beyond Nature and Nurture: Phenotypic Plasticity in Blood-Feeding

Behavior of *Anopheles gambiae* s.s. When Humans Are Not Readily Accessible. *American Journal of Tropical Medicine and Hygiene* **81**:1023-1029. doi: 10.4269/ajtmh.2009.09-0124.

Lefèvre T, and Thomas F. 2008. Behind the scene, something else is pulling the strings: Emphasizing parasitic manipulation in vector-borne diseases. *Infection Genetics and Evolution* **8**:504-519. doi: 10.1016/j.meegid.2007.05.008.

Moiroux N, Gomez MB, Pennetier C, Elanga E, Djenontin A, Chandre F, Djegbe I, Guis H, and Corbel V. 2012. Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. *Journal of Infectious Diseases* **206**:1622-1629. doi: 10.1093/infdis/jis565.

Ngoubangoye B, Boundenga L, Arnathau C, Mombo IM, Durand P, Tsoumbou T-A, Otoro BV, Sana R, Okouga A-P, Moukodoum N, Willaume E, Herbert A, Fouchet D, Rougeron V, Bâ CT, Ollomo B, Paupy C, Leroy EM, Renaud F, Pontier D, and Prugnolle F. 2016. The host specificity of ape malaria parasites can be broken in confined environments. *International Journal for Parasitology* **46**:737-744. doi: http://dx.doi.org/10.1016/j.ijpara.2016.06.004.

Nguyen PL, Vantaux A, Hien DF, Dabiré KR, Yameogo BK, Gouagna L-C, Fontenille D, Renaud F, Simard F, Costantini C, Thomas F, Cohuet A, and Lefèvre T. 2017. No evidence for manipulation of *Anopheles gambiae*, *An. coluzzii* and *An. arabiensis* host preference by *Plasmodium falciparum*. *Scientific reports* **7**:9415. doi: 10.1038/s41598-017-09821-x.

Perkins SL. 2014. Malaria's many mates: past, present, and future of the systematics of the order Haemosporida. *J Parasitol* **100**:11-25. doi: 10.1645/13-362.1.

Prugnolle F, Durand P, Ollomo B, Duval L, Ariey F, Arnathau C, Gonzalez J-P, Leroy E, and Renaud F. 2011. A fresh look at the origin of *Plasmodium falciparum*, the most malignant malaria agent. *PLoS Pathog* **7**:e1001283. doi: 10.1371/journal.ppat.1001283.

Rayner JC, Liu W, Peeters M, Sharp PM, and Hahn BH. 2011. A plethora of *Plasmodium* species in wild apes: a source of human infection? *Trends Parasitol* **27**:222-9. doi: 10.1016/j.pt.2011.01.006.

Rossignol PA, Ribeiro JMC, and Spielman A. 1984. Increased intradermal probing time in sporozoite-infected mosquitoes. *American Journal of Tropical Medicine and Hygiene* **33**:17-20.

Rossignol PA, Ribeiro JMC, and Spielman A. 1986. Increased biting rate and reduced fertility in sporozoite-infected mosquitoes. *American Journal of Tropical Medicine and Hygiene* **35**:277-279.

Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, and Killeen GF. 2011. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malaria Journal* **10**:80. doi: 10.1186/1475-2875-10-80.

Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, and della Torre A. 2008. Insertion polymorphisms of SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. *Malaria Journal* **7**:163.

Saul AJ, Graves PM, and Kay BH. 1990. A Cyclical Feeding Model for Pathogen Transmission and Its Application to Determine Vectorial Capacity from Vector Infection Rates. *Journal of Applied Ecology* **27**:123-133. doi: 10.2307/2403572.

Schwartz A, and Koella JC. 2001. Trade-offs, conflicts of interest and manipulation in *Plasmodium*-mosquito interactions. *Trends in Parasitology* **17**:189-194. doi: 10.1016/s1471-4922(00)01945-0.

Smallegange RC, van Gemert G-J, van de Vegte-Bolmer M, Gezan S, Takken W, Sauerwein RW, and Logan JG. 2013. Malaria infected mosquitoes express enhanced attraction to human odor. *PLoS ONE* **8**:e63602.

Smith D, and Ellis McKenzie F. 2004. Statics and dynamics of malaria infection in *Anopheles* mosquitoes. *Malaria Journal* **3**:13.

Stanczyk NM, Mescher MC, and De Moraes CM. 2017. Effects of malaria infection on mosquito olfaction and behavior: extrapolating data to the field. *Current Opinion in Insect Science* **20**:7-12. doi: http://dx.doi.org/10.1016/j.cois.2017.02.002.

Takken W, and Verhulst NO. 2013. Host preferences of blood-feeding mosquitoes. *Annu Rev Entomol* **58**:433-453.

Tangena J-AA, Thammavong P, Hiscox A, Lindsay SW, and Brey PT. 2015. The Human-Baited Double Net Trap: An Alternative to Human Landing Catches for Collecting Outdoor Biting Mosquitoes in Lao PDR. *PLoS ONE* **10**:e0138735. doi: 10.1371/journal.pone.0138735.

Wekesa JW, Copeland RS, and Mwangi RW. 1992. Effect of *Plasmodium falciparum* on blood feeding-behavior of naturally infected *Anopheles* mosquitoes in Western Kenya. *American Journal of Tropical Medicine and Hygiene* **47**:484-488.

Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, and Andre RG. 1987. Comparative testing of monoclonal antibodies against

*Plasmodium falciparum* sporozoites for ELISA development. *Bulletin of the World Health Organization* **65**:39-45.

535