Effects of malaria infection on mosquito olfaction and behavior: extrapolating data to the field

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Abstract

Vector-borne pathogens have been shown to influence behavioral and other traits of their hosts and vectors across multiple systems, frequently in ways that enhance transmission. In malaria pathosystems, *Plasmodium* parasites have been reported to alter mosquito physiology, fitness and host-seeking behavior. Such effects on vector behavior have obvious medical relevance given their potential to influence disease transmission. However, most studies detailing these effects have faced methodological limitations, including experiments limited to laboratory settings with model vector/pathogen systems. Some recent studies indicate that similar effects may not be observed under natural field conditions; furthermore, it has been suggested that previously reported effects on vectors might be explained by immune responses elicited due to the use of pathogen-vector systems that are not co-evolved. In light of these developments, further work is clearly needed to determine the validity of extrapolation from laboratory studies to field conditions and to understand how parasite effects on vectors affect transmission dynamics in real-world settings.

Introduction

Evidence from a wide range of studies suggests that vector-borne pathogens frequently manipulate host-vector interactions in ways that influence transmission [1-11]. In human disease systems, extensive research has focused on interactions between malaria parasites (*Plasmodium* spp.) and their mosquito vectors because of their profound impacts on global health [12-26]. Malaria transmission may potentially be affected by parasite effects on several different aspects of the host-vector interaction, including the host-seeking and feeding behavior of infected mosquitoes, as well as the relative attractiveness of infected and uninfected hosts to vectors [2](Fig 1). Furthermore, there is considerable evidence, emerging primarily from laboratory-based studies, that malaria parasites do indeed influence such interactions [12,14,17-24,26-28], and this work has potentially important implications for understanding malaria transmission and consequently for disease control efforts [29,30]. Here we discuss key issues regarding the extrapolation of past findings to predict transmission under real-world conditions [31-33] and highlight the pressing need for additional research on this topic.

Evidence for *Plasmodium* manipulation of mosquitoes

Several laboratory studies have explored the possibility that *Plasmodium* parasites might influence mosquito feeding behavior, and a majority have reported such effects, including increased likelihood, frequency, persistence and duration of probing by infected mosquitoes, (Table 1) [12,14,18,22]. Meanwhile, only two studies reported no effects of infection status on relevant traits, including the duration of probing and blood meal size [17,34]. Interestingly, the reported effects on mosquito behavior were observed exclusively during the transmissible sporozoite stage of the parasite's life cycle but not during the earlier, non-transmissible oocyst stage, consistent with the hypothesis that such effects reflect active manipulation by the parasite to enhance transmission. It has also been suggested that a decrease in biting behavior during non-transmissible stages may benefit the parasite, as feeding entails a risk of vector mortality [3,29]. Studies examining short- and mid-range host-seeking by *Plasmodium*-infected mosquitoes have reported a similar pattern of effects, with mosquitoes showing increased flight activity and attraction to hosts when the transmissible stage of the parasite is present but a decrease in these behaviors when only the non-transmissible stage is present [19,20,24,26]. Such findings have important implications for disease control, as they suggest that current models may significantly underestimate the intensity of transmission in the field and that incorporating the observed effects could conceivably lead to a 3- to 5-fold increase in predicted transmission rates [29,30].

Can mosquito immune responses explain behavioral alterations?

As noted, the findings discussed above are broadly consistent with parasite manipulation; however, recent findings raise questions about this interpretation [19,35]. *Plasmodium* infection has many effects on mosquito physiology (e.g., increased reproductive costs, altered metabolism, and changes in lifespan), many of which are mediated by the mosquito's immune response to infection [2,36-40]. The demonstration by Cator *et al.* that inoculation with heat-killed *Escherichia coli* caused behavioral and electrophysiological changes in *Anopheles stephensi* mosquitoes similar to those reported following infection by *Plasmodium yoelii* suggested that immune responses might play an important role in mediating effects of infection on mosquito behavior [19], and these authors speculated that the effects of a general immune response, rather than active manipulation by the parasite, could explain many of the behavioral effects previously reported from *Plasmodium* systems, possibly due to adaptive behaviour on the part of the mosquito to overcome the immune challenge. Consistent with this possibility, a follow-up study from the same group linked *E. coli* immune challenge to changes in insulin signalling that might potentially affect the sensitivity of olfactory receptor neurons [27]. While these findings raise questions about the interpretation of previous results, they are not necessarily incompatible with the hypothesis that *Plasmodium* infection alters mosquito behavior in ways that influence transmission, as it is plausible that the parasite might exploit the natural immune responses of the vector in order to manipulate behavior, and there may also be *Plasmodium*-specific effects that remained unexplored. However, the questions raised by these recent findings do highlight the need for more information about the exact nature of the behavioral effects that occur in natural populations.

Box 1**Potential confounding factors between laboratory and field systems**

If immune responses mediate *Plasmodium* effects on mosquito behavior [18], other factors affecting the immune system may also influence behavioral responses. Under field conditions, mosquitoes are exposed to natural environmental variation [46], have local gut microbiota that can impact the immune response to malaria [40], and in some populations may be insecticide resistant, which can also affect vector competence and the immune system [47]. *Plasmodium* infections in the field also typically exhibit parasite burdens much lower than those observed under laboratory conditions [44-45].

Co-evolution of vectors and parasites in sympatric populations may lead to an adaptive immune response that is difficult to mimic in artificial systems [25,41-43].

Laboratory colonies of mosquitoes may be genetically dissimilar to current field populations and may exhibit extremely limited genetic variation [48]. Likewise, laboratory *Plasmodium* cultures are usually derived from identical isolates. Genetic differences between laboratory and field populations may make it difficult to reliably predict field response based on laboratory assays.

A wide range of mosquito/*Plasmodium* species have been examined, but in relatively few studies each, making it difficult to make comparisons across systems or to field data [29].

Analysis of rodent or avian model systems can yield interesting results, but human, avian and rodent *Plasmodium* have been shown to develop differently within the vector [49], raising questions about how reliably this data can be extrapolated to human systems.

The potential significance of parasite-vector co-evolution in natural systems

Until recently, only a few studies from the 1990s had examined the interaction between *Plasmodium* infection and mosquito biting behavior in field systems [13,15,16]. For example, Koella *et al.* collected mosquitoes from huts and found that those infected with *P. falciparum* displayed higher levels of engorgement and were more likely to contain blood taken from more than one host compared to uninfected individuals [13,15]. Meanwhile, a similar study by Wekesa *et al.* reported a positive effect of infection on the percentage of field-collected mosquitoes probing hamsters in the laboratory [16]. As each of these studies used mosquitoes collected directly from the field, the stage of *Plasmodium* infection was unknown, and host-location behavior was not examined.

In contrast to these early field studies—and the majority of previous laboratory studies discussed above [12-16,18-20,22,24,26]—a recent study by Vantaux *et al.* examining the effects of *P. falciparum* on *An. coluzzii* behavior in naturally co-occurring populations reported no evidence of parasite effects on mosquito feeding behavior or on short- or long-range host-seeking in laboratory choice tests [25]. The authors of this study hypothesized that the divergence of their findings from previous studies might be related to co-adaptation between the parasite and its vector in natural populations, or rather to the lack thereof in most laboratory-based study systems, which frequently employ non-sympatric and co-evolved pathogen and vector models. Given the previously discussed observation that behavioral effects are often mediated by mosquito immune responses, such effects might well be heightened if responses are stronger when mosquitoes are infected by novel parasite strains; however, this would not explain differences previously reported for different species of field-collected mosquitoes [13,15,16], raising the possibility of variation across different species of *Plasmodium*/mosquitoes.

There is clear evidence of local adaptation in *Plasmodium*-vector systems [41,42]. For example, sympatric infections of *P. falciparum* in *An. gambiae* resulted in lower infection prevalence compared to foreign ones [43]. Furthermore, infections in laboratory settings have been shown to lead to higher parasite burden in the vector than that observed under field conditions [44], and there is evidence that the intensity of infection by *P. falciparum* can have significant effects on mosquito gene transcription [45]. Thus, it is plausible that the higher parasite counts observed in artificial infections in the laboratory may trigger correspondingly stronger immune responses that, in turn, affect mosquito behaviour, causing differences between laboratory and locally-adapted systems [2]. In addition to co-evolutionary effects, other expected differences between laboratory and field infections, such as the level and type of vector gut microbiota or the presence of insecticide resistance alleles, have also been suggested to decrease or increase, respectively, the level of *Plasmodium* infection [40,46-48] [Box 1].

Challenges in extrapolating findings across diverse systems

The use of disparate model systems in different studies poses additional complications in reconciling the results of laboratory findings and field observations. For example, the field study by Vantaux *et al.* [25] discussed above employed a pathosystem—comprising *P. falciparum* and *An. Coluzzii*—in which behavioral effects have not previously been examined, making it difficult to determine whether the absence of parasite effects observed in this study is indeed explained by parasite-vector co-evolution in natural populations or simply by the absence of effects in this particular interaction. More generally, the diverse studies in which *Plasmodium* has been reported to have an effect on vector behavior have rarely focused on the same systems (Table 1). Even the most studied parasite-vector association, *An. gambiae* infected with *P. falciparum*, has been examined by only a limited number of studies focused on vector behavior [15,16,23,24], while many other combinations have been examined only by single study. Meanwhile, work exploring how behavioral changes are mediated by immune responses has been carried out only with *An. stephensi*, with no confirmation that similar effects occur in other species [19,27]. Likewise, different studies frequently examine different aspects of behavior or use different methods, making it difficult to directly compare the findings of one study with another. Thus, while it is tempting to make

assumptions about behavior across studies it is important to carefully consider where direct comparisons can be made (as reviewed by Cator *et al.* [29]).

Still another factor that complicates efforts to extrapolate findings from laboratory studies to the field is that these studies frequently employ rodent and avian model systems [12,14,17-20,22,26]. The use of these models has obvious advantages, allowing for carefully controlled manipulative experiments that would often be impossible to perform with human subjects given logistical constraints and ethical concerns. However, it also raises additional questions about the confidence with which inferences drawn from the results can be extended to real-world human populations. For example, it has been shown that *Plasmodium* oocysts develop differently in human, rodent and avian hosts [49], which may also impact immune responses. Only two host-seeking studies have been carried out with the human malaria parasite, *P. falciparum*, with one reporting increased short-range host-seeking by *An. gambiae* when transmissible stage parasites were present [24] and the other reporting no effect on short- or long-range host seeking by *An. coluzzii* [25]. Given contradictory findings in human *Plasmodium* systems, it is important to clarify the extent to which behavioral alteration might be happening in order to judge the reliability of extrapolating from model systems.

Conclusions

While *Plasmodium* has been shown to alter host-seeking and biting behavior in laboratory and early field studies [12-16,18,19,22,24,26], increased recognition of the effects of parasite-vector co-evolution and differences between natural and laboratory systems suggest that such effects may not be consistently observed across different vector/*Plasmodium* systems in the field [25,43-46]. Furthermore, questions have been raised about the extent to which the “manipulative” effects of malaria parasites on vector behavior may be explained as by-products of a generalized immune response [3,19,29]. Consequently, our current knowledge does not allow us to assess the likely effects of vector behavioral alteration on field transmission rates with any degree of confidence.

In order to formulate more robust conclusions regarding the effect of *Plasmodium* on mosquito behavior, additional research is needed. In particular, studies that directly assess the extent to which behavioral modification occurs in human *Plasmodium*/vector systems under field conditions are urgently needed to facilitate accurate modelling of transmission rates, notwithstanding the considerable practical challenges entailed in such work. In addition, findings from field studies should be complemented by laboratory work focused on the same species in order to gain a robust understanding of the possible effect of co-evolution within particular pathosystems, as well as the influence of environmental variation and the variability of effects among *Plasmodium*/mosquito systems.

Table 1. A summary of laboratory/model and field (or natural, coevolved systems) studies examining the effect of malaria infection on mosquito behavior.

Laboratory/Natural	Mosquito <i>spp.</i>	Malaria <i>spp.</i>	Study	Finding
Laboratory	<i>An. gambiae</i>	<i>P. falciparum</i>	Nyasembe VO <i>et al.</i> 2014 [23]	Oocyst and Sporozoite Infected mosquitoes show increased attraction and probing to nectar sources
			Smallegange RC <i>et al.</i> 2013 [24]	Infected mosquitoes show increased short-range host-attraction at the transmissible sporozoite stage
	<i>An. stephensi</i>	<i>P. yoelii</i>	Anderson RA <i>et al.</i> 1999 [12]	Feeding persistence is altered at different stages of malaria infection
			Cator LJ <i>et al.</i> 2013 [19]	Host attraction in choice tests and electrophysiological response is altered due to <i>Plasmodium</i> infection and immune response
		<i>P. berghei</i>	Li X <i>et al.</i> 1995 [17]	No difference in time to locate host between infected and uninfected
		<i>P. chabaudi</i>	Ferguson HM <i>et al.</i> 2004 [22]	Increased likelihood of infected mosquitoes taking a bloodmeal
		Heat killed <i>E. coli</i>	Cator LJ <i>et al.</i> 2015 [27]	Immune challenge can mimic altered feeding behavior seen in malaria infection
	<i>Cx. pipiens</i>	<i>P. relictum</i>	Cornet S <i>et al.</i> 2013 [20]	Increased attraction/feeding in malaria infected mosquitoes
	<i>Ae. aegypti</i>	<i>P. gallinaceum</i>	Koella JC <i>et al.</i> 2002 [14]	Malaria infection stage dependent changes in threshold for continuation in bloodfeeding
			Rossignol PA <i>et al.</i> 1986 [26]	Sporozoite infected mosquitoes showed increased olfactometer response to host
			Rossignol PA <i>et al.</i> 1984 [18]	Sporozoite infected mosquitoes probed for longer
Field/Natural	<i>An. gambiae</i>	<i>P. falciparum</i>	Koella JC <i>et al.</i> 1998 [15]	Infected mosquitoes (all stages) collected from the field were more likely to contain blood from multiple sources
	<i>An. punctulatus</i>	<i>P. falciparum</i> and <i>P. vivax</i>	Koella JC <i>et al.</i> 1996 [13]	Infected mosquitoes collected from the field were more likely to have taken a full bloodmeal in a short time period
	<i>An. coluzzii</i> *(refreshed from field)	<i>P. falciparum</i>	Vantaux A <i>et al.</i> 2015 [25]	No difference in infected and uninfected mosquito response to behavioral choice tests
	<i>An. gambiae</i> and <i>An. funestus</i>	<i>P. falciparum</i>	Wekesa JW <i>et al.</i> 1992 [16]	Increased probing % and length of time in infected mosquitoes *(hamster attractant)

Fig 1. Examples of behavioral alterations observed in malaria-infected mosquitoes and humans which may affect the transmission cycle. At oocyst stage, malaria parasites are non-transmissible; at sporozoite stage they can be transmitted by biting.

Table 1. A summary of laboratory/model and field (or natural, coevolved systems) studies examining the effect of malaria infection on mosquito biting and feeding behavior. Studies are grouped by laboratory/field, mosquito species and malaria species.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

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References

1. Hughes DP, Brofeur, J., and Thomas, F.: *Host Manipulation by Parasites*: Oxford University Press; 2012.
2. Hurd H: **Manipulation of medically important insect vectors by their parasites.** *Annu Rev Entomol* 2003, **48**:141-161.
3. Lefevre T, Thomas F: **Behind the scene, something else is pulling the strings: emphasizing parasitic manipulation in vector-borne diseases.** *Infect Genet Evol* 2008, **8**:504-519.
4. Lima-Camara TN, Bruno RV, Luz PM, Castro MG, Lourenco-de-Oliveira R, Sorgine MH, Peixoto AA: **Dengue infection increases the locomotor activity of Aedes aegypti females.** *PLoS One* 2011, **6**:e17690.
5. Sylvestre G, Gandini M, Maciel-de-Freitas R: **Age-dependent effects of oral infection with dengue virus on Aedes aegypti (Diptera: Culicidae) feeding behavior, survival, oviposition success and fecundity.** *PLoS One* 2013, **8**:e59933.
6. Maciel-de-Freitas R, Sylvestre G, Gandini M, Koella JC: **The influence of dengue virus serotype-2 infection on Aedes aegypti (Diptera: Culicidae) motivation and avidity to blood feed.** *PLoS One* 2013, **8**:e65252.
7. Keating JA, Bhattacharya D, Rund SS, Hoover S, Dasgupta R, Lee SJ, Duffield GE, Striker R: **Mosquito protein kinase G phosphorylates flavivirus NS5 and alters flight behavior in Aedes aegypti and Anopheles gambiae.** *Vector Borne Zoonotic Dis* 2013, **13**:590-600.

8. Newman CM, Anderson TK, Goldberg TL: **Decreased Flight Activity in *Culex pipiens* (Diptera: Culicidae) Naturally Infected With *Culex flavivirus*.** *Journal of Medical Entomology* 2016, **53**:233-236.
9. Rogers ME, Bates PA: ***Leishmania* Manipulation of Sand Fly Feeding Behavior Results in Enhanced Transmission.** *PLoS Pathog* 2007, **3**:e91.
10. Ready PD: ***Leishmania* manipulates sandfly feeding to enhance its transmission.** *Trends Parasitol* 2008, **24**:151-153.
11. Platt KB, Linthicum KJ, Myint KS, Innis BL, Lerdthusnee K, Vaughn DW: **Impact of dengue virus infection on feeding behavior of *Aedes aegypti*.** *Am J Trop Med Hyg* 1997, **57**:119-125.
12. Anderson RA, Koella JC, Hurd H: **The effect of *Plasmodium yoelii nigeriensis* infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle.** *Proceedings of the Royal Society B-Biological Sciences* 1999, **266**:1729-1733.
13. Koella JC, Packer MJ: **Malaria parasites enhance blood-feeding of their naturally infected vector *Anopheles punctulatus*.** *Parasitology* 1996, **113 (Pt 2)**:105-109.
14. Koella JC, Rieu L, Paul REL: **Stage-specific manipulation of a mosquito's host-seeking behavior by the malaria parasite *Plasmodium gallinaceum*.** *Behavioral Ecology* 2002, **13**:816-820.
15. Koella JC, Sorensen FL, Anderson RA: **The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*.** *Proceedings of the Royal Society B-Biological Sciences* 1998, **265**:763-768.
16. Wekesa JW, Copeland RS, Mwangi RW: **Effect of *Plasmodium Falciparum* on Blood Feeding Behavior of Naturally Infected *Anopheles* Mosquitoes in Western Kenya.** *The American Journal of Tropical Medicine and Hygiene* 1992, **47**:484-488.
17. Li X, Sina B, Rossignol PA: **Probing behaviour and sporozoite delivery by *Anopheles stephensi* infected with *Plasmodium berghei*.** *Med Vet Entomol* 1992, **6**:57-61.
18. Rossignol PA, Ribeiro JM, Spielman A: **Increased intradermal probing time in sporozoite-infected mosquitoes.** *Am J Trop Med Hyg* 1984, **33**:17-20.
19. **Cator LJ, George J, Blanford S, Murdock CC, Baker TC, Read AF, Thomas MB: **'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* 2013, **280**:7.
This study examined the behavioral and electrophysiological impact of *P. yoelii* infection in *An. Stephensi*, demonstrating that mosquitoes were affected differentially at different points in the infection cycle. This paper was the first to examine whether an immune challenge with heat-killed *E. coli* could produce a similar effect, prompting the need for further investigation into the basis for *Plasmodium*-based behavioral alterations.
20. Cornet S, Nicot A, Rivero A, Gandon S: **Both infected and uninfected mosquitoes are attracted toward malaria infected birds.** *Malaria Journal* 2013, **12**:8.
21. Cornet S, Nicot A, Rivero A, Gandon S: **Malaria infection increases bird attractiveness to uninfected mosquitoes.** *Ecol Lett* 2013, **16**:323-329.
22. Ferguson HM, Read AF: **Mosquito appetite for blood is stimulated by *Plasmodium chabaudi* infections in themselves and their vertebrate hosts.** *Malaria Journal* 2004, **3**.

23. *Nyasembe VO, Teal PE, Sawa P, Tumlinson JH, Borgemeister C, Torto B: **Plasmodium falciparum infection increases Anopheles gambiae attraction to nectar sources and sugar uptake.** *Curr Biol* 2014, **24**:217-221.
This study demonstrated that *An. gambiae* infected by *P. falciparum* displayed increased attraction to plant odors and probing activity, as well as altered sugar uptake. This is not only an exploration of the physiological changes caused by infection, but also suggests that there may be widespread changes in the olfactory system which have previously only been examined in the context of host-attraction.
24. **Smallegange RC, van Gemert GJ, van de Vegte-Bolmer M, Gezan S, Takken W, Sauerwein RW, Logan JG: **Malaria Infected Mosquitoes Express Enhanced Attraction to Human Odor.** *Plos One* 2013, **8**:3.
Using *An. gambiae* infected by *P. falciparum*, this study was the first to show evidence of short-range host seeking manipulation caused by infection in a human malaria system. Attraction to host odors was suppressed at the oocyst stage and increased at the transmissible sporozoite stage, supporting the evidence of previous mouse and avian studies.
25. **Vantaux A, de Sales Hien DF, Yaméogo B, Dabiré KR, Thomas F, Cohuet A, LEFEVRE T: **Host-seeking behaviors of mosquitoes experimentally infected with sympatric field isolates of the human malaria parasite Plasmodium falciparum: no evidence for host manipulation.** *Frontiers in Ecology and Evolution* 2015, **3**.
Having examined sympatric *Plasmodium* and *An. coluzzii* populations, this study proposed that co-evolution may lead to a lack of behavioural impact of infection. There was no alteration in host-seeking or probing responses of infected mosquitoes, emphasizing the need for future studies to focus on field populations.
26. Rossignol PA, Ribeiro JM, Spielman A: **Increased biting rate and reduced fertility in sporozoite-infected mosquitoes.** *Am J Trop Med Hyg* 1986, **35**:277-279.
27. **Cator LJ, Pietri JE, Murdock CC, Ohm JR, Lewis EE, Read AF, Luckhart S, Thomas MB: **Immune response and insulin signalling alter mosquito feeding behaviour to enhance malaria transmission potential.** *Scientific Reports* 2015, **5**:11947.
This study further examined the degree to which general immune challenge could alter mosquito behaviour, showing that behavioural effects were only produced when the challenge of heat-killed *E. coli* was synchronous with a bloodmeal, and increased at higher doses. The resulting behavioural changes were linked to changes in expression in insulin-like peptides, manipulation of which altered behavioral phenotype, and suggesting a possible mechanism.
28. *De Moraes CM, Stanczyk NM, Betz HS, Pulido H, Sim DG, Read AF, Mescher MC: **Malaria-induced changes in host odors enhance mosquito attraction.** *Proceedings of the National Academy of Sciences of the United States of America* 2014, **111**:11079-11084.
The complexities of host/vector manipulation were investigated in this study, in which *Plasmodium* infected mice were shown to have an altered volatile profile which differentially attracted mosquito vectors. Mosquitoes were preferentially attracted to infected hosts at the parasite's transmissible stage. When considering the effect of *Plasmodium* infection on transmission studies it is important to consider the full dynamic of host, vector and parasite manipulation.
29. Cator LJ, Lynch PA, Read AF, Thomas MB: **Do malaria parasites manipulate mosquitoes?** *Trends in Parasitology* 2012, **28**:466-470.

30. *Cator LJ, Lynch PA, Thomas MB, Read AF: **Alterations in mosquito behaviour by malaria parasites: potential impact on force of infection.** *Malaria Journal* 2014, **13**:11.
Using a mathematical model, this paper investigated the potential changes to transmission models from alterations in mosquito behavior due to *Plasmodium* infection. Incorporating different factors could lead to a 2-5 fold increase in the force of infection, based on the the degree to which *Plasmodium* causes changes in mosquito behavior which benefit parasite transmission.
31. Tripet F: **Ecological Immunology of mosquito-malaria interactions: Of non-natural versus natural model systems and their inferences.** *Parasitology* 2009, **136**:1935-1942.
32. Aguilar R, Dong Y, Warr E, Dimopoulos G: **Anopheles infection responses; laboratory models versus field malaria transmission systems.** *Acta Tropica* 2005, **95**:285-291.
33. Boëte C: **Malaria parasites in mosquitoes: laboratory models, evolutionary temptation and the real world.** *Trends in Parasitology* **21**:445-447.
34. Hogg JC, Hurd H: **Plasmodium yoelii nigeriensis: the effect of high and low intensity of infection upon the egg production and bloodmeal size of Anopheles stephensi during three gonotrophic cycles.** *Parasitology* 1995, **111**:555-562.
35. Lefevre T, Thomas F, Schwartz A, Levashina E, Blandin S, Brizard JP, Le Bourligu L, Demettre E, Renaud F, Biron DG: **Malaria Plasmodium agent induces alteration in the head proteome of their Anopheles mosquito host.** *Proteomics* 2007, **7**:1908-1915.
36. Ahmed AM, Hurd H: **Immune stimulation and malaria infection impose reproductive costs in Anopheles gambiae via follicular apoptosis.** *Microbes Infect* 2006, **8**:308-315.
37. Chege GMM, Beier JC: **Effect of Plasmodium falciparum on the Survival of Naturally Infected Afrotropical Anopheles (Diptera: Culicidae).** *Journal of Medical Entomology* 1990, **27**:454-458.
38. Dong Y, Aguilar R, Xi Z, Warr E, Mongin E, Dimopoulos G: **Anopheles gambiae Immune Responses to Human and Rodent Plasmodium Parasite Species.** *PLoS Pathog* 2006, **2**:e52.
39. Zhao YO, Kurscheid S, Zhang Y, Liu L, Zhang L, Loeliger K, Fikrig E: **Enhanced Survival of Plasmodium Infected Mosquitoes during Starvation.** *PLoS ONE* 2012, **7**:e40556.
40. Dong Y, Manfredini F, Dimopoulos G: **Implication of the Mosquito Midgut Microbiota in the Defense against Malaria Parasites.** *PLoS Pathog* 2009, **5**:e1000423.
41. Joy DA, Gonzalez-Ceron L, Carlton JM, Gueye A, Fay M, McCutchan TF, Su XZ: **Local adaptation and vector-mediated population structure in Plasmodium vivax malaria.** *Mol Biol Evol* 2008, **25**:1245-1252.
42. Molina-Cruz A, DeJong RJ, Ortega C, Haile A, Abban E, Rodrigues J, Jaramillo-Gutierrez G, Barillas-Mury C: **Some strains of Plasmodium falciparum, a human malaria parasite, evade the complement-like system of Anopheles gambiae mosquitoes.** *Proc Natl Acad Sci U S A* 2012, **109**:E1957-1962.
43. Harris C, Morlais I, Churcher TS, Awono-Ambene P, Gouagna LC, Dabire RK, Fontenille D, Cohuet A: **Plasmodium falciparum Produce Lower Infection Intensities in Local versus Foreign Anopheles gambiae Populations.** *PLoS ONE* 2012, **7**:e30849.

44. Cohuet A, Osta MA, Morlais I, Awono-Ambene PH, Michel K, Simard F, Christophides GK, Fontenille D, Kafatos FC: **Anopheles and Plasmodium: from laboratory models to natural systems in the field.** *EMBO reports* 2006, **7**:1285-1289.
45. Mendes AM, Awono-Ambene PH, Nsango SE, Cohuet A, Fontenille D, Kafatos FC, Christophides GK, Morlais I, Vlachou D: **Infection Intensity-Dependent Responses of Anopheles gambiae to the African Malaria Parasite Plasmodium falciparum.** *Infection and Immunity* 2011, **79**:4708-4715.
46. Lefevre T, Vantaux A, Dabire KR, Mouline K, Cohuet A: **Non-genetic determinants of mosquito competence for malaria parasites.** *PLoS Pathog* 2013, **9**:e1003365.
47. Alout H, Ndam NT, Sandeu MM, Djégbe I, Chandre F, Dabiré RK, Djogbénou LS, Corbel V, Cohuet A: **Insecticide Resistance Alleles Affect Vector Competence of Anopheles gambiae s.s. for Plasmodium falciparum Field Isolates.** *PLoS ONE* 2013, **8**:e63849.
48. Norris DE, Shurtleff AC, Toure YT, Lanzaro GC: **Microsatellite DNA polymorphism and heterozygosity among field and laboratory populations of Anopheles gambiae ss (Diptera: Culicidae).** *J Med Entomol* 2001, **38**:336-340.
49. Orfano AS, Nacif-Pimenta R, Duarte APM, Villegas LM, Rodrigues NB, Pinto LC, Campos KMM, Pinilla YT, Chaves B, Barbosa Guerra MGV, et al.: **Species-specific escape of Plasmodium sporozoites from oocysts of avian, rodent, and human malarial parasites.** *Malaria Journal* 2016, **15**:394.

Infected humans
More attractive to mosquitoes

Increased
Transmission

Increased
Transmission

Increased
Survival

Plasmodium sporozoite stage
Mosquitoes more likely to
host-seek and bite

Plasmodium oocyst stage
Mosquitoes less likely to
host-seek

Laboratory

Field/Natural

Non-sympatric, colony lines
No co-evolution

Co-evolution between
sympatric systems

Differential effects of *Plasmodium* on mosquito immune system and physiology

Possible impact upon behavioral changes caused by *Plasmodium* infection?