



# Peer Community In Evolutionary Biology

## Malaria host manipulation increases probability of mosquitoes feeding on humans

**Alison Duncan** based on peer reviews by **Olivier Restif**, **Ricardo S. Ramiro** and 1 anonymous reviewer

Amelie Vantaux, Franck Yao, Domonbabele FdS Hien, Edwige Guissou, Bienvenue K Yameogo, Louis-Clement Gouagna, Didier Fontenille, Francois Renaud, Frederic Simard, Carlo Constantini, Frederic Thomas, Karine Mouline, Benjamin Roche, Anna Cohuet, Kounbobr R Dabire, Thierry Lefevre (2018) Field evidence for manipulation of mosquito host selection by the human malaria parasite, *Plasmodium falciparum*. bioRxiv, ver. 6, peer-reviewed and recommended by Peer Community in Evolutionary Biology.

<https://doi.org/10.1101/207183>

Submitted: 28 February 2018, Recommended: 09 November 2018

### Cite this recommendation as:

Duncan, A. (2018) Malaria host manipulation increases probability of mosquitoes feeding on humans. *Peer Community in Evolutionary Biology*, 100057. [10.24072/pci.evolbiol.100057](https://doi.org/10.24072/pci.evolbiol.100057)

Published: 09 November 2018

Copyright: This work is licensed under the Creative Commons Attribution 4.0 International License. To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

---

Parasites can manipulate their host's behaviour to ensure their own transmission. These manipulated behaviours may be outside the range of ordinary host activities [1], or alter the crucial timing and/or location of a host's regular activity. Vantaux *et al* show that the latter is true for the human malaria parasite, *Plasmodium falciparum* [2]. They demonstrate that three species of Anopheles mosquito were 24% more likely to choose human hosts, rather than other vertebrates, for their blood feed when they harboured transmissible stages (sporozoites) compared to when they were uninfected, or infected with non-transmissible malaria parasites [2]. Host choice is crucial for the malaria parasite *Plasmodium falciparum* to complete its life-cycle, as their host range is much narrower than the mosquito's for feeding; *P. falciparum* can only develop in hominids, or closely related apes [3]. The study only shows this stage-dependent parasite manipulation retrospectively (by identifying host type and parasite stage in mosquitoes after their blood feed [2]). There was no difference in the preferences of infectious (with sporozoites) or un-infectious (infected without sporozoites, or uninfected) mosquitoes between human versus cow hosts in a choice test [2]. This suggests that the final decision about whether to feed occurs when the mosquito is in close range of the host. This, coupled with previous findings, shows that vector manipulation is a fine-tuned business, that can act at multiple stages of the parasite life-cycle and on many behaviours [4]. Indeed, mosquitoes with non-transmissible *Plasmodium* stages (oocysts)

are more reluctant to feed than sporozoite-infected mosquitoes [5] as vectors can be killed by their host whilst feeding, doing so before they are ready to transmit is risky for the malaria parasite. Thus, it seems that *Plasmodium* is, to some extent, master of its vector; commanding it not to feed when it cannot be transmitted, to feed when it is ready to be transmitted and to feed on the right type of host. What does this mean for our understanding of malaria transmission and epidemics? Vantaux *et al* use a mathematical model, parameterised using data from this experiment, to highlight the consequences of this 24% increase in feeding on humans for *P. falciparum* transmission. They show that this increase raises the number of infectious bites humans receive from 4 (if sporozoite-infected mosquitoes had the same probability as uninfected mosquitoes) to 14 (an increase in 250%), for mosquitoes with a 15-day life-span, at ratios of 1:1 mosquitoes to humans. Longer mosquito life-spans and higher ratios of mosquitoes to humans further increases the number of infectious bites. These results [2] have important implications for epidemiological forecasting and disease management. Public health strategies could focus on possible ways to trap sporozoite-infected mosquitoes, mimicking cues they use to locate their human hosts, or identify the behaviour of mosquitoes harbouring non-yet infectious *Plasmodium*, and trap them before they bite. Moreover, the results of the model show that failing to take into account the preference for humans of sporozoite-infected mosquitoes could underestimate the size of pending epidemics. An important question previously raised is whether *Plasmodium*-induced alteration in host behaviour really is manipulation, or just a side-effect of being infected [4,5]. The fact that Vantaux *et al* show that these altered feeding behaviours increases the likelihood of transmission, in that a sporozoite-infected mosquito is more likely to feed on a human, strongly suggests that it is adaptive for the parasite [2]. Ultimately, to show that it is manipulation would require the identification of molecular factors released by *Plasmodium* that are responsible for physiological changes in the mosquito [6].

### **References:**

- [1] Thomas, F., Schmidt-Rhaesa, A., Martin, G., Manu, C., Durand, P., & Renaud, F. (2002). Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts? *Journal of Evolutionary Biology*, 15(3), 356–361. doi: [10.1046/j.1420-9101.2002.00410.x](<https://dx.doi.org/10.1046/j.1420-9101.2002.00410.x>)
- [2] Vantaux, A., Yao, F., Hien, D. F., Guissou, E., Yameogo, B. K., Gouagna, L.-C., ... Lefevre, T. (2018). Field evidence for manipulation of mosquito host selection by the human malaria parasite, *Plasmodium falciparum*. *BioRxiv*, 207183 ver 6. doi: [10.1101/207183](<https://dx.doi.org/10.1101/207183>)
- [3] Prugnolle, F., Durand, P., Ollomo, B., Duval, L., Arley, F., Arnathau, C., ... Renaud, F. (2011). A Fresh Look at the Origin of *Plasmodium falciparum*, the Most Malignant Malaria Agent. *PLOS Pathogens*, 7(2), e1001283. doi: [10.1371/journal.ppat.1001283](<https://dx.doi.org/10.1371/journal.ppat.1001283>)
- [4] Cator, L. J., Lynch, P. A., Read, A. F., & Thomas, M. B. (2012). Do malaria parasites manipulate mosquitoes? *Trends in Parasitology*, 28(11), 466–470. doi: [10.1016/j.pt.2012.08.004](<https://dx.doi.org/10.1016/j.pt.2012.08.004>)
- [5] Cator, L. J., George, J., Blanford, S., Murdock, C. C., Baker, T. C., Read, A. F., & Thomas, M. B. (2013). "Manipulation" without the parasite: altered feeding behaviour of mosquitoes is not dependent on infection with malaria parasites. *Proceedings. Biological Sciences*, 280(1763), 20130711. doi: [10.1098/rspb.2013.0711](<https://dx.doi.org/10.1098/rspb.2013.0711>)
- [6] Herbison, R., Lagrue, C., & Poulin, R. (2018). The missing link in parasite manipulation of host behaviour. *Parasites & Vectors*, 11. doi: [10.1186/s13071-018-2805-9](<https://dx.doi.org/10.1186/s13071-018-2805-9>)

## Reviews

### Evaluation round #3

DOI or URL of the preprint: [10.1101/207183](https://doi.org/10.1101/207183)

Version of the preprint: 4

#### Authors' reply, 19 October 2018

[Download author's reply](#)

[Download tracked changes file](#)

#### Decision by [Alison Duncan](#), posted 19 October 2018

##### Decision for preprint Vantaux et al

Before I recommend your Preprint please can you clarify the values of HBI used in your model for susceptible and sporozoite infected mosquitoes. See my comment below.

Lines 231 – 243: This paragraph is still not clear. Please see suggested corrections below.

Lines 231 – 243 and 341 - 366: Also, there is some inconsistency with regards to the values of HBI used, and for which category of mosquito. Lines 238 – 239 indicate that the HBI of susceptible mosquitoes was based on the range of values obtained from the CI of oocyst infected mosquitoes. But the text in lines 345 – 346 states that the HBI of susceptible mosquitoes was fixed at 0.62, the mean value for susceptible and uninfected mosquitoes. This is repeated in the legend for Figure 4, and here it also states that 'the ribbons represent the EIR values for different HBI of sporozoite-infected mosquitoes according to the CI of HBI in oocyst infected mosquitoes'. The response to Dr Ramiro in the first round of review states that the simulations were re-run using the minimum and maximum values of HBI CI of oocyst infected mosquitoes, for sporozoite infected mosquitoes. This is the clearest explanation of how the ribbon values were estimated.

Please state clearly the value(s) of HBI used for each category of mosquito. Also, why were the CI for oocyst infected mosquitoes used in the simulation and not sporozoite infected mosquitoes?

Lines 289 – 298: I think the abbreviation IC should be CI?

Lines 352 – 355: Maybe add to this sentence that it is the size of the increase in HBI for sporozoite infected mosquitoes that declines with increasing mosquito longevity?

Line 134: in a Petri Line 231: 'are born' Line 234: populations die at rate. Nm is the Line 235: the probability.... and the HBI Line 236: Nm is the Line 237: equals Lines 238 – 239: on the confidence intervals of oocyst-infected mosquitoes that were experimentally.... Line 240: the HBI Line 241: EIR) Line 247: remove 'similar'. Line 329: the highest Line 361: susceptible mosquitoes Line 395: preferences to Line 397: inform

### Evaluation round #2

DOI or URL of the preprint: [10.1101/207183](https://doi.org/10.1101/207183)

Version of the preprint: 3

#### Authors' reply, 07 September 2018

[Download author's reply](#)

[Download tracked changes file](#)

## Decision by [Alison Duncan](#), posted 07 September 2018

### Decision for preprint Vantaux et al

I agree with both reviewers that your manuscript is nearly suitable for Recommendation by PCI Evolutionary Biology following some minor corrections. The quality of the Figures needs to be improved, especially Figure 4 and the text for the equations is very blurred. Please also respond to my comments and those of Reviewer 1.

Lines 141 – 143: state here that you test for parity to control for age.

Line 199: did you check for an effect of village on AI?

Lines 234 – 239 and 253: the text for the equations is very blurred. Please check and replace.

Lines 245 -246: this sentence is unclear. Would 'The infectious period of infectious humans (I<sub>h</sub>) is equal to 1/ on average' work.

Lines 247 - 252: These sentences are also unclear. The explanation in the response to reviewer 1 and the figure legend is much clearer. Please can you re-word this section.

Figure 4 is blurred. Please can you increase its quality.

There are still references missing from the discussion (now lines 297, 312, 315, 333, 345 – 346). I disagree that these topics are specific to the discussion of the results obtained. Please provide suitable references. For example, line 389 the effect of temperature on malaria development, line 400 mosquitoes relying on CO<sub>2</sub> and other odours for long-range host detection, line 416 the circadian rhythms of mosquito activity, lines 428 – 429: models that assume infected and uninfected mosquitoes have a similar preference for humans, lines 435 – 437: the duration of parasite and vector lifespans.

The 'Epidemiological consequences' paragraph in the Supplementary Materials is very difficult to follow.

British and American English are used interchangeably throughout the manuscript, e.g. odour and odor, please choose one. Also, please can you correct the following typos.

Line 20: humans.

Line 22: preferences.

Line 40: responses.

Line 56: humans.

Line 57: chimpanzees, bonobos and gorillas).

Line 60: hosts.

Line 61: humans.

Line 62: humans.

Lines 70 and 80: in Burkina Faso, not of.

Line 88: chickens.

Line 110: the quantity.

Line 130: they were instead of it was.

Line 176: The head and thorax.... Line 188: was started by an initial.

Line 211: humans.

Line 216: two-ways.

Line 243: dies.

Line 246: parameters.

Line 248: the HBI.

Line 258: 'was' not 'has been'.

Line 284: humans.

Line 291: both their oocyst.

Line 307: hosts.

Line 311: hosts.

Line 323: humans.  
Line 324: intervals.  
Line 330: Figure 3.  
Line 340: humans or humans and animals.  
Line 341: intervals.  
Line 365: represents.  
Line 371: the mosquito to human.  
Line 372: measured.  
Line 386: humans.  
Line 394: humans.  
Line 402: moisture.  
Line 403: host specific, and informing of  
Line 404: engages.  
Line 415: of rest.  
Line 417: bed-nets.  
Line 419: an increased.  
Line 420: rhythms.  
Line 421: periods.  
Line 429: preferences.  
Line 431: increases in infectious period can have a dramatic.  
Line 439: taken.  
Line 444: humans.

Supplementary materials.

Line 54: humans or human-animal mixed blood meals. Line 55: villages.  
Line 56: intervals.  
Line 17 dwellings.

### **Reviewed by Ricardo S. Ramiro, 27 June 2018**

The authors carried a significant amount of work to improve the clarity of the manuscript and address my previous concerns. Just a few minor comments: - line 243: "each mosquito population die at rate" should be "Mosquitoes die at rate" ? - line 247-252: this section could be written more clearly - lines 294-301: It would just be good if the authors state here how often were there significant differences between sporozoite infected vs all other mosquitoes (e.g. in all randomisations or in a percentage of these?) - line 313: should read: "Soumouso only human dwellings were..."

### **Reviewed by Olivier Restif, 27 June 2018**

I am satisfied with the revisions made by the authors. Apart from a few typos (e.g. lines 438-439), I believe this manuscript is of sufficient quality to be published.

## **Evaluation round #1**

DOI or URL of the preprint: [10.1101/207183](https://doi.org/10.1101/207183)

Version of the preprint: 2

## Authors' reply, 26 June 2018

[Download author's reply](#)

[Download tracked changes file](#)

## Decision by Alison Duncan, posted 26 June 2018

### Revise

Vantaux et al investigate, whether Anopheles mosquitoes harbouring sporozoites, the stage of Plasmodium falciparum transmitted to the vertebrate host, are more attracted to, and likely to have fed on, human hosts. The study checked for the presence of Plasmodium in mosquitoes from 1) field, host-choice experiments for human or calf odours and 2) collections from occupied or unoccupied houses, or animal sheds. They show that both infected and uninfected mosquitoes are equally attracted to humans, but that mosquitoes harbouring sporozoites are more likely to have fed on humans. The experiment is coupled with an epidemiological model predicting that this preference could increase the number of infectious mosquito bites people receive by 250% (14 infectious bites compared to 4 if mosquitoes showed no host preference).

I enjoyed reading the manuscript and thought the experiments and epidemiological model provide a neat, novel body of work. Both Reviewers liked the manuscript, but request a number of revisions. I agree with both the Reviewers that the Methods would benefit from being fleshed out. More information is required about the model parameterisation. The methods would also be easier to follow if more information were provided in the last paragraph of the Introduction setting out the aims of the manuscript, and if the 'Mosquito host preference', 'Mosquito blood-feeding pattern' and 'Mathematical model' were more clearly highlighted as the major axes of the paper. The 'Mosquito blood-feeding pattern' section also needs a few sentences at the beginning describing the aims. Please state somewhere whether a live calf was placed in the tent for calf odours, and whether different volunteers and calves were used each night.

Please be reminded that the following information must be included in final version of the preprint.

-Data must be available to readers after recommendation, in the preprint or through deposition in an open data repository, such as Zenodo, Dryad or institutional repositories, for example.

-Details of the quantitative analyses (e.g. data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) in the recommended preprints must be available to readers in the text or as appendices or supplementary materials, for example.

Some very minor comments of my own: Lines 71 – 75: State more clearly that these are separate experiments.

Lines 140 – 142: I would put this information at the beginning of this section.

Lines 229 – 231: Please state in the text or Figure legends how HBI is impacted by shelter type and village.

Line 293: Doesn't this also allow you to distinguish from an intrinsic parasite characteristic?

There are some references missing from the discussion for example, lines 297, 312, 315, 333, 345 – 346,

Lines 323 – 328: Is anything known about mosquito phototactic behaviour?

## Reviewed by anonymous reviewer 1, 02 March 2018

The manuscript by Vantaux et al. provides evidence for mosquitoes, infected with Plasmodium falciparum, preferring to feed on humans. Interestingly, this preference is specific for mosquitoes infected with sporozoites, the mosquito-to-human transmissible stage. This is a well-written manuscript, using a combination of field work, behavioural experiments and theoretical modeling.

The authors measure mosquito preference for humans versus domestic animals, using three different assays: odour-baited entry traps; odour-baited double net traps and collection of indoor resting mosquitoes. In the first two assays, the mosquitoes are attracted to either calf or human odours. In the latter assay, indoor resting mosquitoes were collected in the morning from human dwellings, unoccupied houses and animal sheds. Mosquitoes for these assays were field mosquitoes from three different locations. All mosquitoes were

dissected and ELISA or PCR were used to determine their infection status and the host on which the mosquito fed.

My main criticisms are the following:

- The main result of the paper is that in the collected indoor resting mosquitoes there is an increased preference for humans in the mosquito population that is infected with sporozoites. However, it is unclear to me whether this result is or not fully dependent on the mosquitoes collected in the human dwellings. This is because the sample size varies a lot between the different shelter types. From fig. S4: 1731 mosquitoes from human dwellings; 198 from unoccupied houses; 399 from animal sheds. Thus the sample size in unoccupied houses and animal sheds is 11 and 23% of what the authors have for human dwellings. As the authors state in the main text that there is no interaction between shelter type and infection status, the effects of each variable can be tested separately. Thus, it is key to know whether the human blood index (HBI) is significantly affected by infection status within each shelter type.
- The authors model how the entomological inoculation rate depends on the HBI. However, the model appears to use fixed values of HBI whereas considerable variation is observed in the results from the field experiments. As the authors state that the ODE equation system is simulated throughout the season, would it be possible to allow HBI to vary (around some mean value) or at least discuss the implications of adding such variation
- The methods would benefit from more detail. Particularly in the section “laboratory processing of samples” where the genes/proteins used in PCR and ELISA assays should be given. Also, to help replication, product codes/brands should be added
- In the discussion the authors should be more clear in their discussion of the difference between the assays of the odour-baited traps and the indoor resting mosquitoes. This seems to be done from line 309 onwards, but the authors should make the comparison more explicit

Minor points:

- methods model section: not all parameters are described, please add them all. line 183: I think there must be a typo, it should be “lh”?
- results: when including means +/- error measure, please state what is the error measure
- Figures: could the authors please add the lower half of the error bars
- line 209: “with a significant attraction toward human odours” - This does not appear to be the case for sporozoite-infected mosquitoes - given the stats in front
- line 225: I find the statement of a 24% increase a bit strange. Given that the HBI is itself a percentage, isn't the percent increase just  $HBI(\text{sporozoite}) - HBI(\text{uninfected})$ , rather than one over the other, as I assume the authors have done?
- line 281: “The plain lines show how EIR changes according to HBI...” instead of “The plain lines show the evolution of EIR according to HBI...”
- line 324: if this was the case, one should expect a significant interaction between infection and shelter type?

**Reviewed by Olivier Restif, 02 March 2018**

Review of “Field evidence for manipulation of mosquito host selection by the human malaria parasite, *Plasmodium falciparum*” by Vantaux et al for *PLoS Evol Biol*.

Manuscript downloaded from BioRxiv (<https://www.biorxiv.org/content/early/2018/02/26/207183>) on 6 March 2018.

This is an original, innovative and interesting study of the effect of Plasmodium infection on mosquito blood feeding behaviour in a natural setting. The hypothesis and methods were clearly presented, and the results presented support the authors' conclusions. The use of a mathematical model to predict the epidemiological consequence of the observed behavioural differences is a useful addition.

I have found no major issues, but I would like to see a better justification of the model's assumptions and parameter values. Here I list suggested corrections which would help improve the clarity of the manuscript. Line numbers refer to the pdf version on BioRxiv.

- I.150: "reduced feeding attempts seem beneficial to the parasite": is that a theoretical prediction or an observation? and does it only apply to the oocyst stage?
- I.195: If possible, include Figure S2 (a and b) in the main text, as it's very helpful for readers not familiar with the traps.
- I.131: how large and representative was the subset? Is that sufficient to rule out other Plasmodium species in all the oocyst-infected mosquitoes in the study?
- I.133: are the ELISA and qPCR assays used specific to the sporozoite stage?
- I.175: replace "a SIR model" with "a compartmental model for Plasmodium transmission between humans and mosquitoes".
- I.176: please provide a reference for the validation of this particular model. In particular, is it valid to ignore latent infection in humans (*Eh*), and ignore human births and deaths? In addition, I'm surprised to see two different frequency-dependent terms for human-to-mosquito transmission and mosquito-to-human transmission: the former has  $N_m$  as the denominator and the latter has  $N_h$ . Both should be fractions of the same total number of mosquito bites. Although this would not affect the results as long as the two population sizes are constant, it seems to me that the equations are inconsistent.
- I.185: Table S1 is very succinct. Please provide justifications (and references) for the chosen parameter values and ranges.
- I.188: It's not clear from the formula how the 1-year duration was accounted for, as  $I_m$  represents the instantaneous number of infected mosquitoes. Did you allow the model to reach its steady state first?
- Figures 1 and 2: I'm not a big fan of bar plots (unless they show count data), and in this case they hide the bottom halves of the confidence intervals, which is not helpful.
- I.263: echoing my comment about Table S1, I'd like to see a justification for the choice of values for mosquito lifespans and densities.
- I.264: 0.62 is the mean of the HBI measured in three locations, which range from 0.4 to 0.8. It would be worth showing the effect of this variation too.
- Figure 3: please add a legend for the red, grey and black lines.