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Current Biology *24*, 1–5, February 17, 2014 ©2014 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2013.12.022

Report

# *Plasmodium falciparum* Infection Increases *Anopheles gambiae* Attraction to Nectar Sources and Sugar Uptake

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## Summary

***Plasmodium*** **parasites are known to manipulate the behavior of their vectors so as to enhance transmission [1–4]. From an evolutionary standpoint, behavior manipulation by the parasite should expose the vector to limited risk of early mortality while ensuring sufficient energy supply for both it and the vector [5, 6]. However, it is unknown whether this vector manipulation also affects vector-plant interaction and sugar uptake. Here, we show that the attraction of** ***Anopheles gambiae s.s.*** **to plant odors increased by 30% and 24% after infection with the oocyst and sporozoite stages of** ***Plasmodium falciparum***, **respectively, while probing activity increased by 77% and 80%, respectively, when the vectors were infected with the two stages of the parasite. Our data also reveal an increased sugar uptake at the oocyst stage that decreased at the sporozoite stage of infection compared to uninfected** ***An. gambiae***, **with depletion of lipid reserves at the sporozoite stage. These results point to a possible physiological adjustment by** ***An. gambiae*** **to** ***P. falciparum*** **infection or behavior manipulation of** ***An. gambiae*** **by** ***P. falciparum*** **to enhance transmission. We conclude that the nectar-seeking behavior of** ***P. falciparum*****-infected** ***An. gambiae*** **appears to be modified in a manner governed by the vector's fight for survival and the parasite's need to advance its transmission.**

## Results

### Experimental Infection

Three- to five-day-old mosquitoes were fed on either nongametocytic blood (uninfected group) or *P. falciparum* gametocyte-positive blood (infected group) using membrane feeders. Three experimental infections were achieved with an average infection rate of 53.73% (geometric mean oocyst density ± SEM = 8.17 ± 1.97, n = 360). No oocyst was detected in the midgut of the uninfected group of *An. gambiae*.

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### *P. falciparum* Infection Increases *An. gambiae* Attraction to Nectar Sources

Olfactory cues play an important role in the location of nectar sources by *An. gambiae* [7]. We studied the olfactory responses of uninfected and *P. falciparum*-infected *An. gambiae* to three nectar sources, *Parthenium hysterophorus* (Asteraceae), *Ricinus communis* (Euphorbiacea), and *Bidens pilosa* (Asteraceae). A general linear model taking into account the infection rate and density was used to analyze the data. Our results revealed that parasite infection altered nectar-seeking behavior of *An. gambiae*. In the dual-choice olfactory responses, there was an overall preference for odors from the three nectar sources by both uninfected and *Plasmodium*-infected *An. gambiae.* Infection with *P. falciparum* increased nectar source attraction by 30% (0.42–0.86 confidence interval [CI], $p < 0.01$) at the oocyst stage and 24% (0.48–0.99 CI, $p < 0.01$) at the sporozoite stage compared to uninfected *An. gambiae* of corresponding ages. In terms of odor preference, significant differences were also detected among the three nectar sources at the oocyst ($F_{(2, 56)} = 17.94$, $p < 0.001$) and sporozoite ($F_{(2, 56)} = 6.35$, $p < 0.05$) stages of parasite development (Figure 1).

### *P. falciparum* Infection Increases *An. gambiae* Probing on Nectar Sources

Nectar feeding is preceded by landing and probing activity on floral and extrafloral parts of the plant. We conducted a no-choice probing assay to study the effect of *P. falciparum* infection on probing activity of *An. gambiae* on the three nectar sources. Similarly, a general linear model taking into account the infection rate and density was used to analyze the data. Overall, infection with both the oocyst and sporozoite stages of *P. falciparum* increased probing activity of *An. gambiae* by 77% (0.38–5.87 CI, $p < 0.001$) and 80% (0.44–6.87 CI, $p < 0.001$), respectively, on the three nectar sources. Significant differences in probing activity was also detected between the three nectar sources ($F_{(2, 80)} = 55.78$, $p < 0.01$), with *P. hysterophorus* having the highest number of *An. gambiae* probing (probing ratio [PR] = 1.66, 1.2023702–2.349070 CI, $p < 0.01$), followed by *R. communis* (PR = 1.27, 0.8815493–1.793855 CI), while *B. pilosa* was the least attractive (PR = 1). However, there was no significant interaction between nectar source and infection status (Figure 2).

### *P. falciparum* Infection Alters *An. gambiae* Sugar Uptake and Energy Reserves

As evidence of actual plant probing, we analyzed both uninfected and *Plasmodium*-infected *An. gambiae* for total sugar content using hot anthrone test after probing assays. Overall, infection with the oocyst stage of *P. falciparum* significantly increased the amount of sugar uptake by *An. gambiae* from the different nectar sources ($F_{(1, 24)} = 14.69$, $p < 0.001$), with *An. gambiae* obtaining the highest sugar amount from *P. hysterophorus* when infected ($p < 0.05$) (Figure 3). On the contrary, sugar uptake was significantly compromised at the sporozoite stage ($F_{(1, 24)} = 14.75$, $p < 0.001$). The uptake of sugar in uninfected *An. gambiae* was higher from each of the three nectar sources than that of their sporozoite-infected

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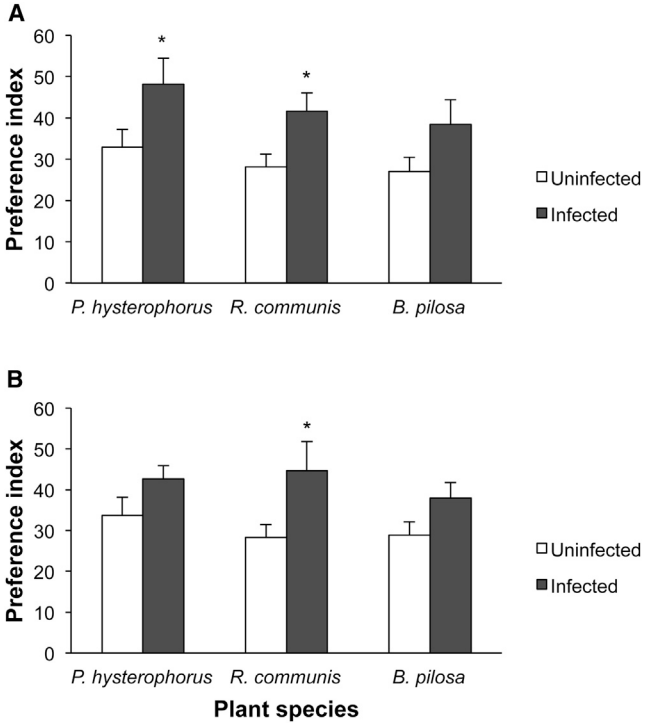


Figure 1. Olfactometer Responses of Different Stages of *Plasmodium*-Infected *Anopheles gambiae* to Intact Plant Odors

(A) Oocyst stage and (B) sporozoite stage are shown. Uninfected, comprising blood-fed *An. gambiae* of corresponding ages to oocyst- and sporozoite-stage infected mosquitoes were used as controls. Eight replicates of each experiment comprising ten mosquito per mosquito group/plant were conducted. Error bars indicate the SEM; bars capped with asterisks indicate significant difference between test and control for each plant species at *p < 0.05.

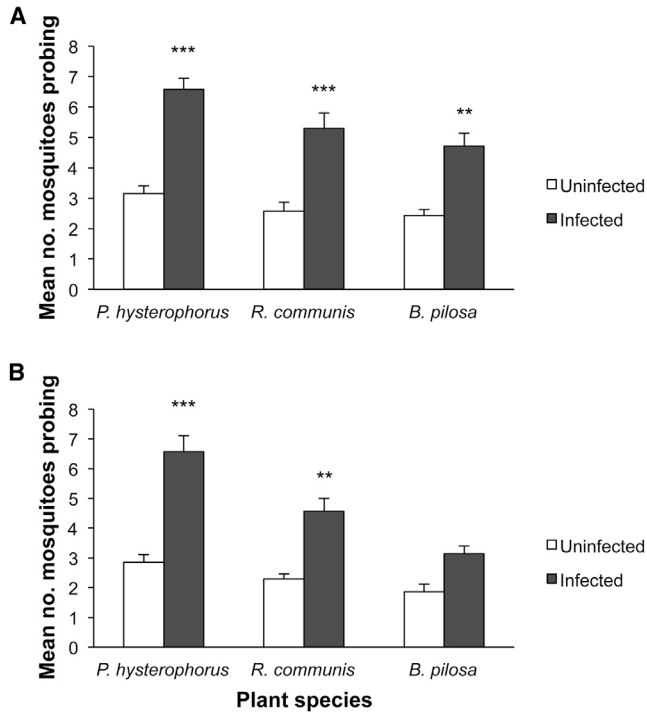


Figure 2. Probing Responses of Different Stages of *Plasmodium*-Infected *Anopheles gambiae* on Different Plant Species

(A) Oocyst stage and (B) sporozoite stage are shown. Uninfected, comprising blood-fed *An. gambiae* of corresponding ages to oocyst- and sporozoite-stage infected mosquitoes were used as controls. Eight replicates of each experiment comprising ten mosquito per mosquito group/plant were conducted. Error bars indicate the SEM; bars capped with asterisks indicate significant difference between test and control for each plant species at *p < 0.05, **p < 0.01, and ***p < 0.001.

counterparts, with a significant difference in the amount of sugar uptake detected among those probing on *R. communis* ($p < 0.01$).

In addition, we tested for the effect of *P. falciparum* infection on glycogen and lipid reserves after 7 days (oocyst stage) and 12 days (sporozoite) postinfection. Our results show that infection with both the oocyst and sporozoite stages of the parasite did not significantly affect the glycogen reserves, but the sporozoite stage severely depleted lipid reserves (uninfected = 0.61, infected = 0.39, $p < 0.001$) (Figure 4).

## Discussion

Our results clearly indicate that infection with *P. falciparum* alters the behavior of *An. gambiae* toward the three nectar sources. Both dual-choice olfactometer and probing assays showed a marked increase in plant attraction and acceptance at the oocyst and sporozoite stages of parasite development, suggesting either physiological adjustment in *An. gambiae* due to the infection resulting in change in behavior or behavior manipulation of the vector by the parasite. Behavior manipulation by malaria parasites on their host vectors has been reported for various *Plasmodium* species in vertebrate host-vector interactions, in which sporozoite-stage *Plasmodium*-infected mosquitoes were found to be highly attracted to their vertebrate host [1, 4, 8, 9]. Also, *Plasmodium*-infected vertebrate hosts have been reported to be more attractive to uninfected mosquitoes than uninfected hosts [3, 10]. Although nectar feeding is known to play a critical role in the survival of malaria vectors [11, 12], this is the first study to demonstrate possible physiological adjustment of *P. falciparum*-infected *An. gambiae* and/or behavior manipulation by *P. falciparum* of the vector toward nectar sources. Increased vertebrate host attraction of malaria vectors confers evolutionary advantage to the parasite as it increases host-vector contact and thus enhances chances of transmission [1, 6, 13]. On the other hand, increased vertebrate host attraction during nontransmissible stages of the parasite would be disadvantageous to the parasite since vertebrates are physically aggressive, hence the high risk of untimely vector mortality [5, 14]. This suggests that in the evolutionary arms race, the selective pressure on *An. gambiae* appears to favor their plant nectar feeding during the noninfective stages of the parasite development, thus reducing feeding-associated vector mortality.

Our results further point to increased sugar uptake by infected *An. gambiae* at the oocyst stage of the parasite, whereas at the sporozoite stage the sugar uptake was compromised. These results corroborate previous findings [15, 16], but they also underpin the important mechanisms involved in the possible vector manipulation by the parasite. While the increased sugar uptake at the oocyst stage of the parasite can be explained by either the adjustment by *An. gambiae* to compensate for the energy deficit created by parasite infection or parasite manipulation to increase sugar

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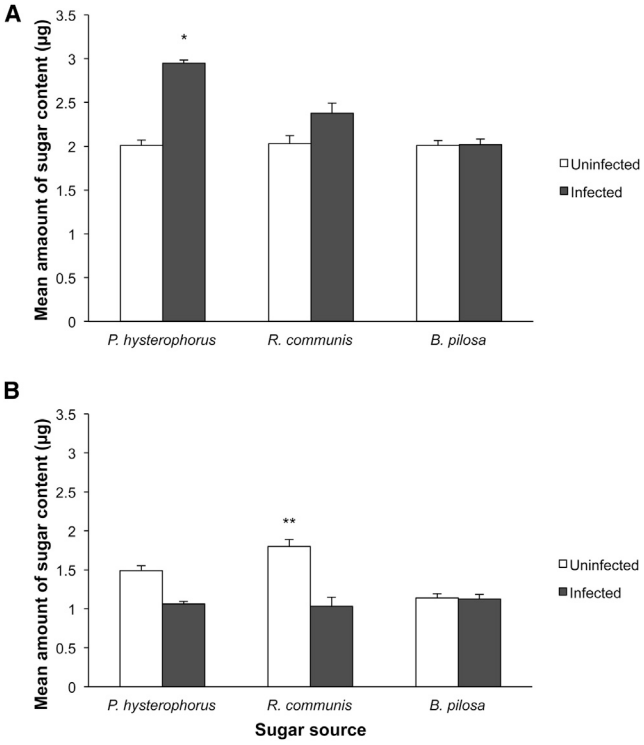


Figure 3. Mean Amount of Total Sugar Content in Oocyst- and Sporozoite-Stage *Plasmodium*-Infected *Anopheles gambiae*

(A) Oocyst stage and (B) sporozoite stage are shown. The total sugar content was measured on day 7 (during oocyst stage of parasite development) and day 12 (sporozoite stage) postinfection for each group of mosquitoes probing on each plant species. Uninfected, comprising blood-fed *An. gambiae* of corresponding ages to oocyst- and sporozoite-stage infected mosquitoes that probed on the three plant species were used as controls. Error bars indicate the SEM. The total number of each group of *An. gambiae* per plant species (n) = 40. Bars capped with asterisks are significantly different from the corresponding controls at *p < 0.05 and **p < 0.01.

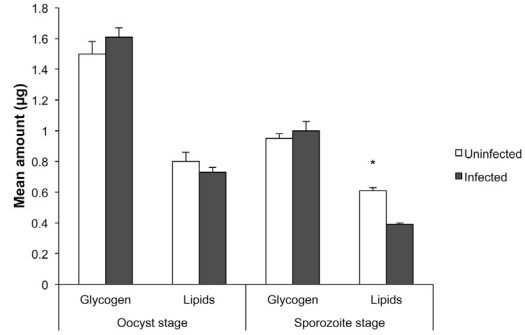


Figure 4. Mean Amounts of Glycogen and Lipid Content in Oocyst- and Sporozoite-Stage *Plasmodium*-Infected *Anopheles gambiae*

The total sugar content was measured on day 7 (during oocyst stage of parasite development) and day 12 (sporozoite stage) for each group of mosquitoes. Uninfected, comprising blood-fed *An. gambiae* of corresponding ages to oocyst- and sporozoite-stage infected mosquitoes were used as controls. Error bars indicate the SEM. The total number of each group of *An. gambiae* (n) = 120. The bar capped with an asterisk is significantly different from the corresponding uninfected mosquito counterparts at *p < 0.05.

intake for its own metabolism and for improved vector survival [15], the reduced sugar uptake at the sporozoite stage is not in tandem with the observed increase in probing activity. The invasion of the salivary glands of the vector by the sporozoite stage of the parasite has been linked to reduced apyrase activity with a resultant increase in probing time [8, 13, 17]. Sporozoite infection has also been associated with difficulties in taking complete blood meals, with resultant persistent attempts to initiate new blood uptake [2]. Further evidence also points to altered levels of a number of proteins in the head of *An. gambiae* after infection with the sporozoite stage of *Plasmodium berghei.* These include the synapse-associated proteins, which could potentially affect the olfactory system [18]. Whichever the case, this is expected to confer transmission advantage to the parasite as many sporozoites are transferred to new vertebrate hosts with every feeding attempt. Therefore, we suggest that the observed increase in plant probing activity accompanied by reduced sugar uptake could possibly be an extrapolated effect of reduced apyrase activity or an altered olfactory system or both, resulting in impaired ability to imbibe on plant nectars and/or increased plant attraction.

Given that most parasitic infections exert energetic costs to their host vectors [19, 20], with a resultant loss of reproductive potential and reduced lifespan [21–25], it is possible that the malaria vector's quest for increased probing is to meet its own metabolic demands and that of the growing oocyst. Studies on the effect of *Plasmodium* infection on vector longevity are conflicting, with the majority showing that vector survival is unaffected, but some showing reduced vector survival [24]. Selection for *Plasmodium*-vector interactions that favor vector survival over reproduction has been suggested [5, 26], but more studies are needed to fully understand the effect of parasite infection on the energetic budget of mosquito vectors [6]. Zhao et al. [27] recently demonstrated increased survival of *P. berghei*-infected *An. gambiae* and *An. stephensi* compared to uninfected mosquitoes when they are subjected to starvation. They attributed this to decreased carbohydrate catabolism accompanied by enhanced expression of insulin-like peptides that lead to higher glycogen accumulation. Our study further demonstrates no effect on glycogen reserves of *An. gambiae* after infection with *P. falciparum*, though the infected vectors had slightly higher glycogen reserves at the oocyst stage than did their uninfected counterparts. These results further point to possible vector manipulation by the parasite to ensure sufficient energy supply, and hence sustained vector survival that ensures completion of the sporogonic cycle, or physiological adjustment by the vector to parasite infection. However, further studies need to be carried out to fully understand the effect of *P. falciparum* infection on the vector energetic reserves.

The reduced lipid level, particularly at the sporozoite stage, is noteworthy. Lipids have been implicated in *Plasmodium*-mosquito interactions [28]. While our study serves to shed more light into possible involvement of lipids in these *Plasmodium*-vector interactions, more studies are needed to further elucidate their role in the outcome of such interactions. It is possible that lipid reserves are depleted by the parasites' invasion of the midgut epithelial cells either through destructive migratory activity or through formation of capsules around the oocyst stages [29]. Alternatively, the observed depletion of lipid reserves at the sporozoite stage of infection could be explained by the fact that developing oocysts normally sequester lipids for their structural development [28].

Rivero and Ferguson [15] alluded to a possible protective role played by high sugar intake, which increases the ability of *An. stephensi* to synthesize nitric oxide, a defense molecule in its immune response. The observed increase in sugar uptake at the oocyst stage further strengthens this argument, given that this is the most virulent stage of the parasite in the mosquito vector [30, 31]. However, substantive studies on the metabolic pathway involving sugar uptake in *P. falciparum*-infected *An. gambiae* are needed to verify this possibility. Overall, these studies highlight a possible coevolutionary relationship between the malaria parasite and its vector that results in minimal damage to both.

## Conclusions

In conclusion, our findings highlight the influence of *P. falciparum* on nectar-seeking behavior of *An. gambiae*, which is similar to the previous results found for the parasite-infected vectors seeking a vertebrate host for a blood meal. In both cases, it appears that the nectar-seeking behavior is governed by the physiological adjustment by the vector to a *P. falciparum* invasion or the parasite's need to advance its transmission while minimizing vector mortality. These results suggest evolutionary behavior modification that is advantageous for both survival of the vector and parasite transmission. This study exemplifies the need to understand the mechanisms underlying vector-parasite interactions in malaria systems, which is of paramount importance for disease control.

### Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.12.022.

## Author Contributions

V.O.N., P.E.A.T., J.H.T., C.B., and B.T. conceived and designed the experiments. V.O.N., P.S., and B.T. performed the experiments. V.O.N. and B.T. analyzed the data. V.O.N, P.E.A.T., P.S., J.H.T., C.B., and B.T. wrote the paper. All authors approved the final version for submission.

### Acknowledgments

We are grateful to all of the staff at the *icipe* Mbita and Duduville campuses, who provided support without which our research would not have been possible. Special thanks are extended to Woodbridge A. Foster for critical review of the manuscript and Daisy Salifu for assistance with the statistical analysis. We also appreciate the technical assistance from George Omweri of *icipe*'s St Jude clinic in Mbita; Tom Guda and his team of vector competence unit and the staff at the insectary unit at Mbita; and Onesmus Wanyama, Milka Gitau, and Richard Ochieng' at *icipe* Duduville. We thank Simon Mathenge (formerly of the Botany Department, University of Nairobi) for help in identification of plants. This study was funded in part by Centre for Medical, Agricultural, and Veterinary Entomology, US Department of Agriculture and by the US National Institutes of Health, National Institute of Allergy and Infectious Diseases grant R01A1077722 to Woodbridge A. Foster. V.O.N. was supported by *icipe* and the World Federation of Scientists. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Received: September 18, 2013
Revised: November 29, 2013
Accepted: December 10, 2013
Published: January 9, 2014

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