Peer Community In
Evolutionary Biology

Nutritional symbioses in triatomines: who is playing?

Natacha Kremer *based on peer reviews by* ***Olivier Duron*** *and* ***Alejandro Manzano Marín***

Jonathan Filée, Kenny Agésilas-Lequeux, Laurie Lacquehay, Jean Michel Bérenger, Lise Dupont, Vagner Mendonça, João Aristeu da Rosa, Myriam Harry (2023) Wolbachia genomics reveals a potential for a nutrition-based symbiosis in blood-sucking Triatomine bugs. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/2022.09.06.506778

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Nearly 8 million people are suffering from Chagas disease in the Americas. The etiological agent, *Trypanosoma cruzi*, is mainly transmitted by triatomine bugs, also known as kissing or vampire bugs, which suck blood and transmit the parasite through their feces. Among these triatomine species, *Rhodnius prolixus* is considered the main vector, and many studies have focused on characterizing its biology, physiology, ecology and evolution.

Interestingly, given that *Rhodnius* species feed almost exclusively on blood, their diet is unbalanced, and the insects can lack nutrients and vitamins that they cannot synthetize themself, such as B-vitamins. In all insects feeding exclusively on blood, symbioses with microbes producing B-vitamins (mainly biotin, riboflavin and folate) have been widely described (see review in Duron and Gottlieb 2020) and are critical for insect development and reproduction. These co-evolved relationships between blood feeders and nutritional symbionts could now be considered to develop new control methods, by targeting the 'Achille's heel' of the symbiotic association (i.e., transfer of nutrient and / or control of nutritional symbiont density). But for this, it is necessary to better characterize the relationships between triatomines and their symbionts.

R. prolixus is known to be associated with several symbionts. The extracellular gut symbiont *Rhodococcus rhodnii*, which reaches high bacterial densities and is almost fixed in *R. prolixus* populations, appears to be a nutritional symbiont under many blood sources. This symbiont can provide B-vitamins such as biotin (B7), niacin (B3), thiamin (B1), pyridoxin (B6) or riboflavin (B2) and can play an important role in the development and the reproduction of *R. prolixus* (Pachebat et al. (2013) and see review in Salcedo-Porras et al. (2020)). This symbiont is orally acquired through egg smearing, ensuring the fidelity of transmission of the symbiont from mother to offspring. However, as recently highlighted by Tobias et al. (2020) and Gilliland et al. (2022), other gut microbes could also participate to the provision of B-vitamins, and *R. rhodnii* could additionally provide metabolites (other than B-vitamins) increasing bug fitness. In the study from Filée et al., the authors focused on *Wolbachia,* an intracellular, maternally inherited bacterium, known to be a nutritional symbiont in other blood-sucking insects such as bedbugs (Nikoh et al. 2014), and its potential role in vitamin provision in triatomine bugs.

After screening 17 different triatomine species from the 3 phylogenetic groups *prolixus*, *pallescens* and *pictipes,* they first show that *Wolbachia* symbionts are widely distributed in the different *Rhodnius* species. Contrary to *R. rhodnii* that were detected in all samples, *Wolbachia* prevalence was patchy and rarely fixed. The authors then sequenced, assembled, and compared 13 *Wolbachia* genomes from the infected *Rhodnius* species. They showed that all *Wolbachia* are phylogenetically positioned in the supergroup F that contains *w*Cle (the *Wolbachia* from bedbugs). In addition, 8 *Wolbachia* strains (out of 12) encode a biotin operon under strong purifying selection, suggesting the preservation of the biological function and the metabolic potential of *Wolbachia* to supplement biotin in their *Rhodnius* host. From the study of insect genomes, the authors also evidenced several horizontal transfers of genes from *Wolbachia* to *Rhodnius* genomes, which suggests a complex evolutionary interplay between vampire bugs and their intracellular symbiont.

This nice piece of work thus provides valuable information to the fields of multiple partners / nutritional symbioses and *Wolbachia* research. Dual symbioses described in insects feeding on unbalanced diets generally highlight a certain complementarity between symbionts that ensure the whole nutritional complementation. The study presented by Filée et al. leads rather to consider the impact of multiple symbionts with different lifestyles and transmission modes in the provision of a specific nutritional benefit (here, biotin). Because of the low prevalence of *Wolbachia* in certain species, a "ménage à trois" scenario would rather be replaced by an "open couple", where the host relationship with new symbiotic partners (more or less stable at the evolutionary timescale) could provide benefits in certain ecological situations. The results also support the potential for *Wolbachia* to evolve rapidly along a continuum between parasitism and mutualism, by acquiring operons encoding critical pathways of vitamin biosynthesis.

References:

Duron O. and Gottlieb Y. (2020) Convergence of Nutritional Symbioses in Obligate Blood Feeders. Trends in Parasitology 36(10):816-825. https://doi.org/10.1016/j.pt.2020.07.007

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Computational and Structural Biotechnology Journal. 3395-3401. https://doi.org/10.1016/j.csbj.2020.10.031

Reviews

Evaluation round #3

Reviewed by Alejandro Manzano Marín, 15 February 2023

Dear authors,

After reading the last round of rebutals, I can now say I am confident I can recommend the paper for publication as is. I believe the paper reports clearly its findings and the conclusions, as presented, are well supported by the author's results. I would like to particularly congratulate the authors for a nice piece of work which is a great addition to the *Wolbachia* literature and that of symbiotic interactions involving multiple organisms.

Sincerely,

Alejandro Manzano Marín

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2022.09.06.506778

Version of the preprint: 2

Authors' reply, 09 February 2023

Download author's reply

Decision by Natacha Kremer, posted 01 February 2023, validated 01 February 2023

PCI Evol Biol: Decision concerning your submission of the preprint https://doi.org/10.1101/2022.09.06.506778

Dear Dr. Filée and collaborators,

Thank you for submitting a revised version of the manuscript. Your preprint has now been reviewed again by the two initial reviewers. One is satisfied with the changes made, but the other still has a major concern about the interpretation and conclusions drawn from the results (and a few other more minor points). Before making a final recommendation of the paper, I encourage you to revise your preprint, and especially to rephrase/nuance the conclusions, indicating instead of "an ancient and dual association" a potential for complementation and complex coevolutionary interplay.

I therefore encourage you to resubmit it to PCI Evolutionary Biology together with a point-by-point response to the reviewers' comments. Please be sure to indicate in each response what changes you have made to the manuscript.

Yours sincerely,

Natacha Kremer **Download recommender's annotations**

Reviewed by Alejandro Manzano Marín, 30 January 2023

You can find my response to the authors in the attached PDF file.

Download the review

Reviewed by Olivier Duron, 04 January 2023

The authors have modified their manuscript in accordance with the reviewers' comments and suggestions - thanks for this. The new version of the manuscript is an interesting addition to the field of Wolbachia/symbiosis/hematophagy. I have no additional comment.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2022.09.06.506778

Authors' reply, 13 December 2022

Download author's reply

Decision by Natacha Kremer, posted 17 October 2022, validated 18 October 2022

PCI Evol Biol: Decision concerning your submission of the preprint https://doi.org/10.1101/2022.09.06.506778

Dear Dr. Filée and collaborators,

Your preprint has now been reviewed by two scientists in the field, and you will find their comments associated with this letter. While both reviewers acknowledge the quality of the work and the interest of the scientific question you tackle, they both have major reservations about the interpretation of the results and the presentation of the conclusions. Notably, both support that *Wolbachia* could provide a nutritional benefit to their host, but they separately question the ecology/stability of the association (diet, vertical transmission route, prevalence, co-speciation) and the role of B-vitamin operons (in *Wolbachia* genomes, in *Rhodnius* genomes or in the genomes of organisms potentially colonizing the triatomine bug) in the beneficial relationship with the host. Their arguments suggest that you will need to rethink some of your interpretations and to revise them accordingly, with the possible clarifications and genomic analyses suggested.

I therefore encourage you to revise your preprint, and to resubmit it to PCI Evolutionary Biology together with a point-by-point response to each of the reviewers' comments. Please be sure to indicate in each response what changes you have made to the manuscript. We will then contact both reviewers again to ensure that their concerns have been properly addressed before we can make any recommendation.

Yours sincerely,

Natacha Kremer

Reviewed by Olivier Duron, 27 September 2022

Filée et al. have produced a commendable piece of work which clearly articulates the evidence that a maternally inherited bacterium, Wolbachia, could be a nutritional endosymbiont of the Rhodnius spp. vampire bugs. This is a specific topic that has been the subject of little recent research, although these bugs are vectors of diseases of medical interest (eg Chagas disease): Significant research efforts have been undertaken to understand their biology and better control them, but not recently on their nutritional endosymbionts. Symbiosis with maternally inherited bacterium is essential for the nutrition of arthropods with an obligate blood-feeding habit. In these arthropods, divergent lineages of intracellular bacteria have independently evolved functional interactions with obligate blood feeders, but all converge to an analogous biochemical feature: The provisioning of B vitamins. Similar features have been characterized in bed bugs, ticks, tsetse flies, bat flies, head lice, etc, but surprisingly not in Rhodnius bugs: Previous studies suggested that the provisioning of B vitamins in Rhodnius spp. does not depend on maternally inherited/intracellular bacteria but a rather on a extracellular gut symbiont, Rhodnius rhodnii. However, as pointed by the authors, many contradictory results tend to demonstrate that the nutritional mutualism between R. rhodnii and Rhodnius is not strictly obligatory but depends mostly on rearing condition, host bloods or symbiont strains. In the present study, the authors have done an excellent job synthesising these different lines of evidence, and together with their own data present a cohesive argument showing that nutritional symbiosis is more complex in Rhodnius bugs than previously expected. Indeed, the authors sequenced and assembled 13 novel Wolbachia genomes (all belonging to supergroup F) and present genomic evidences suggesting that Wolbachia is a B vitamins provisioning endosymbiont for some Rhodnius spp. Analyses of bug genomes further evidences of Wolbachia-to-bug gene transfers, suggesting a complex evolutionary interplay between these organisms. More specific comments are below. Overall, a great piece of work that I can recommend for publication pending some revisions.

Major comments:

- About horizontal transmission of R. rhodnii: The authors mention in their manuscript (eg at lines 71, and further) that R. rhodnii is horizontally transmitted. This is not entirely true and it should be corrected. To be exact, it has been shown that egg surfaces (and adult feces) transmit R. rhodnii to the gut epithelium of the newborn insect. It is an orally acquiring symbiont: during oviposition, females smear egg masses with symbiont-containing feces, which are ingested by newly hatched nymphs, allowing the symbiont to pass through their digestive tract and establish in the midgut. This transmission route of nutritional gut symbiont through egg smearing is a distinctive trait in many hemipteran species as stinkbugs and others. In this context, the transmission route is vertical/maternal (although not transovarial), and not horizontal. It implies that there is a fidelity in the transmission of R. rhodnii, and thus a relative stability of the association.
- Line 152: The detection of mtDNA introgression is interesting in the context of maternally inherited endosymbionts, but the importance of this process is not really discussed further in the text. What consequences does this process have on the interpretation of the results? In particular on the distribution and prevalence variations of Wolbachia between the different triatomine species?
- One of the most disturbing results is that Wolbachia is not fixed for all bug species. Obligate mutualistic symbionts generally have a 100% prevalence, at least in females. This is not the case in this study and this is a difficult result to explain. As I suggest just above, could this be the result of cytoplasmic introgression with a Wolbachia introduced through this way into a bug species without Wolbachia? Moreover, the Wolbachia detected here belong to supergroup F, a clade that is also often found in nematodes including filaria. In this context, how to distinguish between Wolbachia specific to bugs, and those from filaria that could infect bugs (in which case the presence of Wolbachia should be interpreted as a false positive due to cross contamination). Indeed, this could be the case for Wolbachia from Rhodnius amazonicus which presents an extremely degraded and pseudogenized biotin operon.
- Apart from phylogenomic data, the authors do not really detail the levels of divergence between bug Wolbachia: Variations in genetic composition (besides B vitamin genes), pseudogenization rate, GC%, abundance of IS, ect, between strains should be presented and discussed. This is important for understanding how similar - or divergent - these Wolbachia strains are between bug species.
- About Wolbachia nomemclature: The authors nammed Wolbachia wRho all Wolbachia that they have sequenced from several bug species. However these Wolbachia strains have genomic differences and should be named differently. This is quite confusing, even though these strains are phylogenetically close. The basic rule for Wolbachia is to name each strain differently: for example for the Wolbachia of bed bugs, wClec, the first letter, w, stands for Wolbachia, the second, C, is for Cimex, and the next, le, are for lecturalis (only Drosophila Wolbachia has different rules for historical reasons). The same logic should be applied here for Wolbachia of bugs.
- About genomic insertions in bug genomes: There are several Wolbachia genes inserted in bug genomes, but does this have any impact on PCR survey for estimaning Wolbachia prevalence? For example, if the target genes of the screen (coxA and FtsZ) are present in bug genomes, it will completely bias the prevalence results. Another related question: What percentage of these inserted genes are pseudogenized

and therefore non-functional? Conversely, do any of the inserted Wolbachia genes seems functional (based on their sequences and orf prediction) and could they have a (nutritional) function for the bugs?

- About the biotin operon: I fully agree that this operon is rare in Wolbachia and moves through lateral gene transfers from Wolbachia-to-Wolbachia, but the transfer capabilities of this biotin operon are not limited to Wolbachia. Accumulating genomic sequences confirm that lateral transfer of this compact, streamlined biotin operon is rampant in nutritional symbioses of obligate blood feeders: related biotin operons (i.e., that diverged recently from the same operon ancestor) have been detected in diverse B vitamin-provisioning symbionts, including Midichloria and Rickettsia in ticks (https://doi.org/10.7554/eLife.72747) and Legionella in rat lice. Its extensive spread across bacterial lineages is definitely a key driver of the emergence of novel nutritional symbioses with obligate blood feeders (reviewed here: https://doi.org/10.1016/j.pt.2020.07.007).
- Line 339: The authors estimated the divergence between wCle and wRho around 5My. I would be careful about this estimate: the evolutionary rate they used was based on a different biological interaction (facultative Wolbachia in bees), so my feeling is that the latter did not evolve at the same rate as expected for a nutritional endosymbiosis.

Minor comments:

- Lines 52-54: Perhaps this sentence is a little bit too speculative: The observed results do not really allow to be conclusive on this point. I would recommend removing it from the abstract.
- I am surprised that there is no mention of the use of R. rhodnii as a potential method of control for triatomine bugs. Over the last 20 years, several studies have focused on R. rhodnii genetic transformation (paratransgenesis) to eliminate pathogens from vector populations. The strategy was to engineer R. rhodnii to express proteins such as Cecropin A that are toxic to Trypanosoma cruzi or that block the transmission of T. cruzi. The success of this strategy mainly depends on the positive fitness effect of R. rhodnii. This should be at least discussed in the discussion.
- Worth to mention somewhere that these genomic results will have to be proven experimentally. All previous studies on bugs have been done by cleaning the eggs (to remove the R. rhodnii deposited from egg smearing) but never with antibiotic treatments which are needed to remove Wolbachia.

Reviewed by Alejandro Manzano Marín, 16 October 2022

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