



Peer Community In Evolutionary Biology

Flexibility in Aphid Endosymbiosis: Dual Symbioses Have Evolved Anew at Least Six Times

Olivier Tenaillon based on peer reviews by **Alex C. C. Wilson** and 1 anonymous reviewer

Alejandro Manzano-Marín, Armelle Coeur d'acier, Anne-Laure Clamens, Corinne Cruaud, Valérie Barbe, Emmanuelle Jousselin (2023) Co-obligate symbioses have repeatedly evolved across aphids, but partner identity and nutritional contributions vary across lineages. bioRxiv, ver. 5, peer-reviewed and recommended by Peer Community in Evolutionary Biology. <https://doi.org/10.1101/2022.08.28.505559>

Submitted: 16 November 2022, Recommended: 11 May 2023

Cite this recommendation as:

Tenaillon, O. (2023) Flexibility in Aphid Endosymbiosis: Dual Symbioses Have Evolved Anew at Least Six Times. *Peer Community in Evolutionary Biology*, 100640. [10.24072/pci.evolbiol.100640](https://doi.org/10.24072/pci.evolbiol.100640)

Published: 11 May 2023

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/>

In this intriguing study (Manzano-Marín et al. 2022) by Alejandro Manzano-Marín and his colleagues, the association between aphids and their symbionts is investigated through meta-genomic analysis of new samples. These associations have been previously described as leading to fascinating genomic evolution in the symbiont (McCutcheon and Moran 2012). The bacterial genomes exhibit a significant reduction in size and the range of functions performed. They typically lose the ability to produce many metabolites or biobricks created by the host, and instead, streamline their metabolism by focusing on the amino acids that the host cannot produce. This level of co-evolution suggests a stable association between the two partners.

However, the new data suggests a much more complex pattern as multiple independent acquisitions of co-symbionts are observed. Co-symbiont acquisition leads to a partition of the functions carried out on the bacterial side, with the new co-symbiont taking over some of the functions previously performed by *Buchnera*. In most cases, the new co-symbiont also brings the ability to produce B1 vitamin. Various facultative symbiotic taxa are recruited to be co-symbionts, with the frequency of acquisition related to the bacterial niche and lifestyle.

Despite this diversity of associations, the evolution of co-obligate symbiosis in aphids commonly involves just a handful of nutritional pathways. These include tryptophan biosynthesis (twice), histidine biosynthesis, riboflavin biosynthesis (six times), and biotin biosynthesis (five times). Microscopy analyses suggest that some co-symbionts colonize different bacteriocytes. Yet, a few traces of horizontal gene transfers in *Buchnera* suggest

that some contact with other bacteria may occasionally occur.

The emergence of multiple co-symbioses highlights the success of a "ménage à trois". However, this success is achieved by adding a new co-symbiont to an already established pair. It is possible that the slow but irreversible decay of the bacterial genome under symbiosis may lead to a degradation of the partnership, creating a niche for the acquisition of new bacteria to maintain the symbiosis.

References:

Manzano-Marín, Alejandro, Armelle Coeur D'acier, Anne-Laure Clamens, Corinne Cruaud, Valérie Barbe, and Emmanuelle Jousset. 2023. "Co-Obligate Symbioses Have Repeatedly Evolved across Aphids, but Partner Identity and Nutritional Contributions Vary across Lineages." bioRxiv, ver. 5 peer-reviewed and recommended by Peer Community in Evolutionary Biology.

<https://doi.org/10.1101/2022.08.28.505559>.

McCutcheon, John P., and Nancy A. Moran. 2012. "Extreme Genome Reduction in Symbiotic Bacteria."

Nature Reviews Microbiology 10 (1): 13–26. <https://doi.org/10.1038/nrmicro2670>.

Reviews

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2022.08.28.505559>

Version of the preprint: 4

Authors' reply, 20 April 2023

Find attached a PDF containing the point-by-point answer to recommender's and reviewer's comments as well as a "tracked changes" document. This last has been compressed to fit the 5MB file limit, which has impacted the quality of some figures. The version at bioRxiv should be taken as the definite one (w/full quality figures).

[Download author's reply](#)

[Download tracked changes file](#)

Decision by Olivier Tenailon, posted 06 April 2023, validated 06 April 2023

Minor revisions

Decision:

I think the paper could easily be out with a few very minor changes proposed nicely by the reviewers.

My own comments:

In their study called "Co-obligate symbioses have repeatedly evolved across aphids, but partner identity and nutritional contributions vary across lineages" Alejandro Manzano-Marín and colleagues have sequenced the genomes of symbiotes of Aphids. While the association between aphids and their main symbiont Buchnera was supposed to be stable, they show multiple independent acquisition of co-symbiont. This large body of work reveals multiple interesting facets of bacterial/aphid symbiosis. Various species are recruited to be co-symbionts, some more than others relying on their lifestyle. Vitamin B2 synthesis seems to be a source of co-symbiosis as in

all cases the synthesis of this vitamin is achieved by the secondary symbiont. Some co-symbionts colonise different bacteriocytes.

The analysis are done according to the state of the art and the results are nicely and thoroughly presented. All reviewers were enthusiastic about the work and suggest mostly editing and rewording issues. I have a few additional suggestions of figures.

I think that one figure showing genome and gene content against the presence of a co-symbiont could be worth having. This is an important question connecting symbiosis to genome size. The presumed age of the cosymbiosis and the genome size could be plotted to see if there are some trends.

For perspectives: It could also be worth testing a model of genome size evolution with a rate of decay and to see if a two rate model is fitting the data better, the second rate occurring after cosymbiosis. (<https://academic.oup.com/sysbio/article/65/5/812/2223542?login=true>)

I was particularly interested in the *Buchnera* strains from Hormaphidinae, which hold rather large genomes (630 and 580 kbp) with a strikingly low number of CDSs (453 and 450). Having close to 30% of non coding DNA is a very interesting feature for bacterial genomes. Could a map of these genomes be given and compared to their closest relatives. What is the source of this non-coding DNA? A zoom on some regions **could be** valuable...

Here are some additional thought I had on the discussion:

Regarding the proximal scenario for cosymbiosis, most arguments were associated with gene loss. But it is highly probable that through drift or antagonistic pleiotropy, some of the bacterial function may be reduced, but not inactivated. This could offer a window during which a new symbiont providing an active complementation could be selected for until it completely takes over the least functional function of *Buchnera*.

Could there be a reason for riboflavin to be the source of switching? Is it particularly costly to produce?

Aş perspective, but beyond the paper: it could be interesting to test if the acquisition of new cosymbiont is associated with a benefit for the host that would be seen at the species level, through past demographic inferences for instance, in the case of recent acquisitions.

There are few sentences that were not clear:

-Our 16S rRNA amplicon survey revealed that *Fukatsuia*-related symbionts are found in populations of aphid species from different subfamilies, but much frequently than *Se. symbiotica*.

do you mean "much less frequently"

-Given the multiple benefits conferred by this endosymbiont, the potential for vertical transmission through the host plant of the aphid host

I am not sure here that transmission through the host could be said to be vertical transmission

Reviewed by anonymous reviewer 1, 13 February 2023

In the manuscript entitled "Co-obligate symbioses have repeatedly evolved across aphids, but partner identity and nutritional contributions vary across lineages", Manzano-Marín and collaborators have assembled and investigated a large metagenomic database generated from 25 aphid species, as well as an important metabarcoding database generated from 223 individual aphids from 147 species. In their study, the authors aimed at identifying and characterizing the insects' obligate symbionts and their evolution, particularly focusing on the occurrence of co-obligatory symbioses, as aphid symbioses are mostly known for the insect association with their main obligate symbiont *Buchnera*. With their approach, the authors were able to convincingly show that co-obligatory symbioses are not a rare event, as they independently and repeatedly appear within different lineages of aphids. The authors are also microscopically describing two co-obligatory symbioses that were poorly studied, which, despite feeling a little offbeat at first, validates the authors approach in showing that their inferences of co-obligatory symbioses can be validated and describes convergent pattern in the evolution of symbiotic associations in aphids.

Overall, the manuscript is nicely written and quite readable. The methods are sound and nicely detailed so it would be easy to reproduce what the authors did. I commend the authors for the nice job they have done in summarizing so relevantly the large amount of data that they have brought, processed and analyzed. The figures are particularly helping to understand this study's approach. The authors show a great knowledge over the literature surrounding their work, but maybe sometimes too specifically, to the point that there is fewer discussion out of the aphid and hemipteran world. It is not deleterious at all but the scope of their findings could have been broadened on some points (for instance on symbiont replacements, or on the discussion on the potential and interest to investigate co-obligatory symbionts in radically different insects or even other arthropods). This said, the discussion does not need to be expanded as it is already long enough, but surprisingly quick to read (it could be maybe shortened on some points, for instance on the metabolic paragraphs as this is the least "original" part of this study).

In conclusion, the present work is a relevant read to better understand the evolution of symbiotic associations in insects (especially aphids in this case). The conclusions of this study are quite convincing and combined with the easy-to-understand methods and approaches described here, could make this work a nice reference for further studies of the same nature.

Following are some rather picky comments and questions that could maybe be used to improve the manuscript.

Abstract

"Lastly, patterns ..." this sentence could be rephrased to be easier to understand. If I got it correctly, the authors want to highlight that it's not the number of genes lost over genome erosion that can promote the rise of co-obligate symbioses but rather the specificity of a few genes. The sentence could be streamlined.

"often thought of as exclusive, appears fragile" I disagree on such a strong statement to qualify the association between *Buchnera* and aphids. Granted that associations are not set in stones, but it does not mean they are "fragile" as most of them remain stable for millions of years. Maybe the authors could shift for something fitting their results but slightly less assertive. Maybe, something like "appears adjustable/movable/more flexible than initially thought".

Introduction

Spherical cell shape of endosymbiont: the statement is misleading as it suggests that it is a general feature of obligate endosymbionts with reduced genomes, while in total opposition, some endosymbionts with reduced genomes present rod-shape gigantism (e.g. *Wigglesworthia*) or intense elongation (e.g. *Nardonella*). The authors could rephrase the sentence in a less general context. For example, "is a characteristic shared among several aphid-associated endosymbionts."

Results

Microscopy on new co-obligate symbionts: How do the authors confidently assess the shape of the symbionts with their pictures? Especially for *Fukatsia*, the bacteriocytes are so packed with endosymbionts that it is very hard to conclude that the bacterium is spherical. Could the authors display a close-up picture? If relying solely on the finding from Michalik et al. 2014, maybe it could be more accurate to state that there is no observation of an obvious pattern that could be contradictory.

Discussion

If the authors want to broaden their point and extend generalization to holometabolous insects, they could cite that symbiont replacement has also been described in weevils (Lefèvre et al., 2004; Conord et al., 2008 in Mol. Biol. Evol).

“the latter being published just months before the preparation of this manuscript”. What is the relevance of this comment? Do the authors want to highlight a coincidental interest for the same subject? Do they want to support the importance of the finding by convergent studies? I don't see any point risen after this comment, which could be interpreted as a frustration of not being the first to describe the stated findings.

Figure 7: I find this figure very nice, but not as informative as it could be due to the format. For instance, the blue color chosen for *Se. symbiotica* is very hard to discriminate from the black as it is surrounded by grey. It could be improved as this is in my opinion one of the most relevant parts presented in this figure, as it shows clearly the repeated acquisitions of this symbiont as co-obligate. Also, maybe the co-obligate genera could be represented when they are facultative symbionts as well. It could emphasize that they regularly interact with the different aphids. As such, I was under the impression that it was rather a rare event to observe the rise of co-obligation and that these symbionts are not that present among the different aphids, while it's the opposite as I assume that the grey highlight represents that all these aphids' taxa are interacting with facultative symbionts.

Reviewed by **Alex C. C. Wilson**, 01 March 2023

Review of Manzano-Marín et al., “Co-obligate symbioses have repeatedly evolved across aphids, but partner identity and nutritional contributions vary across lineages”.

For a long time it has been understood that the endosymbiont of aphids is Buchnera. While there have been a few known exceptions to this rule, including two examples of Buchnera being replaced entirely, and others where Buchnera is supported by a second co-obligate symbiont, the extent to which aphid lineages are supported by co-obligate symbionts, and the nature and evolution of such systems across a diversity of aphids has been unknown. In this manuscript, using metagenomics on 25 aphid species representative of nine subfamilies, and 16S rRNA amplicon sequencing from 147 species representative of 12 subfamilies, the authors explore the prevalence of aphid species hosting co-obligate endosymbionts. The results of this work solidly shift the paradigm. No longer can we generalize that aphids have a primary symbiont Buchnera. The evolution of endosymbiosis in aphids is much more dynamic and variable than had previously been appreciated. This paper will become a well-cited piece of the story of endosymbiosis in aphids.

I really enjoyed reading this paper. It is well-written, mostly in an accessible, conversational style. The figures are excellent - they are clear and accessible, containing just the right amount of detail to communicate the data/results. The figure legends and keys within the figures are well-planned supporting the figures so that they stand alone from the text. Figures 2, 3 and 5 are real standouts (especially figure 3)! While the results are mostly unsurprising, they are interesting and make an important contribution to the field.

Things I will cite this paper for:

- *The evolution of co-symbiosis is common in aphids outside the Aphidinae.
- *Two distantly related Buchnera lineages have gained a gene by HGT!
- *The evolution of co-obligate symbiosis in aphids commonly involves just a handful of nutritional pathways, these include tryptophan biosynthesis (twice), histidine biosynthesis, riboflavin biosynthesis (six times), biotin biosynthesis (five times)
- *The evolution of co-obligate symbiosis in aphids appears to commonly expand the metabolic provisioning of the endosymbiosis to include thiamin.

Suggestions for improving the manuscript:

- *I suggest revising the abstract so that it reflects the fact that there were 25 newly sequenced genomes, AND 20 re-assembled and/or reannotated genomes – as this almost doubles the number of Buchnera genomes

that were included in the analysis.

*I suggest rewording “falling along this extremes”. The point you are trying to make is not as clear as it could be. I also suggest here rewording talking about GC content because 27.02% GC is still “low” GC content but the current wording of this section suggests that it is not. Maybe low GC and lower GC or lowest GC content?? With respect to Figure 1 – are there dots in between the 18.37 and 27.02% GC, or are they just the two sizes? Maybe reduce the size of the dots in the figure so that there is less overlap among the dots ... so that they can appear as discrete data points?

*Page 5, 2nd sentence, there is a word missing “As in previous work...”

*Page 6 is “deserted” the best choice of word? Also, did you look in these intergenic regions for the presence of repetitive elements or the small RNAs reported by Hansen, A. K. & Degnan, P. H. Widespread expression of conserved small RNAs in small symbiont genomes. *ISME Journal* 8, 1–13 (2014)??

*Page 6 “afore-mentioned” – check spelling/hyphenation

*Page 8 – “symptoms of long-term associations” I like this word choice, I also like your use of “auxotrophies” and “complementarities”.

*The discussion of transcriptional frameshifting only cites Tamas et al 2008, I am pretty sure that there is an earlier paper by Wernegreen’s group in ants that should also be cited here.

*Figure 4 – can you revise this figure to present the data in a way that corrects for sampling depth? If not, I suggest removing this figure because I think that it can lead to misinterpretation of what the situation is.

*Page 10 – discussion of a Bacteroidota symbiont in Fordini that was not found in this work – did you find Buchnera in those samples instead? If you did not find Bacteroidota, what did you find?

*Sodalis – I had missed the earlier papers! I find it exciting to learn that Sodalis is also found in aphids.

*Page 12 – “tissue trophism” can you define or reword. It wasn’t clear to me exactly what was meant here.

*The bacteriocytes occupied by Fukatsuia are very large!

*Page 14, first line, there is a space missing at the beginning of the sentence that starts “Along the same...”. Second line replace “relay” with “rely”?? Second last line of first paragraph – isn’t it three Eriosomatini?? Second paragraph, third last line “and Auchenorrhyncha...”

*Page 16, 5th line word missing “ but much more frequently...”. Later: replace “This mirrors” with “These patterns mirror...” Second paragraph “56% of samples were”.

*Page 16 last paragraph through page 17 – I find this part of the discussion weak, overly speculative, and not very helpful. I suggest either dropping this section or thinking more about it, and then revise the section strengthening the arguments.

*Page 19 Gil et al, Van Ham et al ... about pLeu. Shouldn’t Chong and Moran also be cited here?

*The HGTs – did you figure out their origins?