




Peer Community In Microbiology

Stability in a microbe-insect interaction

Konstantinos (Kostas) Kormas  based on peer reviews by **Guillame Minard** and **Enric Frago**

Marie-Charlotte Cheutin, Manon Boucicot, Joel Meunier (2024) Microbiome turnover during offspring development varies with maternal care, but not moult, in a hemimetabolous insect. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Microbiology. <https://doi.org/10.1101/2024.03.26.586808>

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The degree of fidelity between microbes and their hosts varies considerably among different animal groups but also along the host's developmental stages and depends on the stability of their microbial communities. Cheutin et al. showcase experimentally the stability of whole body bacterial microbiome in a dermapteran insect species, the European earwig *Forficula auricularia*. The carefully designed experiments, which include a large number of investigated families and the related methodologies along with the data analysis, revealed that the bacterial communities of this insect are highly dynamic during the early developmental stages, but these changes are rather specific to each developmental stage and rather irrelevant to moulting. Some of these changes were reflected in the dominant predicted metabolic pathways. Another important finding of this study was that maternal care of the eggs has a detectable impact on the future shaping of the adult insect bacterial microbiome.

The findings of this paper clearly answer its working hypotheses, but they also generate a set of specific novel hypotheses for future studies. These hypotheses are of interest to the general field of animal-microbe interactions and, more specifically, to the driving forces of transmissibility of microbes from one generation to the next one. This study also depicts some of the most likely important metabolic pathways in this insect-microbe relationship that could be the focus of future studies with more specific methodologies.

References:

Cheutin M-C, Boucicot M, Meunier J. (2024). Microbiome turnover during offspring development varies with maternal care, but not moult, in a hemimetabolous insect. bioRxiv, ver.3, peer-reviewed and recommended by Peer Community In Microbiology. <https://www.biorxiv.org/content/10.1101/2024.03.26.586808v3>

Reviews

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2024.03.26.586808>

Version of the preprint: 2

Authors' reply, 08 July 2024

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Decision by **Konstantinos (Kostas) Kormas** , posted 17 June 2024, validated 18 June 2024

Moderate revision

Your manuscript has been reviewed by two experts. As I agree with their comments, the manuscript needs restructuring in presenting the experimental setup/treatments and the respective results, along with a few conceptual clarifications in order to avoid contradictory statements or misunderstandings. The reviewers have spotted specific parts of the manuscript that need this additional attention. Please provide a detailed point-by-point rebuttal letter to each of the reviewers' comments, along with your revised manuscript.

Reviewed by **Guillame Minard**, 01 May 2024

The manuscript synthesizes a study that refers to the effect of development, moult and maternal care on the bacterial microbiota associated with an Hemimetabolous species (*i.e.* the European earwigs).

All in all, I found the manuscript very well written.

The investigations are relevant in the light of the current literature in ecology. Indeed, the effect of development on the microbiota of hemimetabolous insects has poorly been regarded and the exact same remark goes for maternal care.

The experiments and data analyses have been seriously performed.

This leads me to recommend only minor edits that are listed in detail in the attached pdf document.

[Download the review](#)

Reviewed by **Enric Frago**, 31 May 2024

In this study Marie-Charlotte Cheutin, Manon Boucicot and Joël Meunier explore the dynamic changes in microbiota during development of an insect that has the peculiarity of having maternal care over young instars. The authors rear a group of earwigs from egg to adulthood and screen bacterial associates in different instars, and in two groups: one with and another without maternal care. Individuals not exposed to maternal care also reach adulthood as care by mothers is not necessary for the insect to reach adulthood. In addition to following the dynamics of the microbiota, the authors also apply an algorithm to detect microbes likely to be the core microbiota in this species, and they also associate the different bacteria found to putative functions. The manuscript is well written and timely, the topic of microbiota development in insects that do not perform a complex metamorphosis, and that show maternal care is important, and has been poorly studied. Despite these merits, I think there are few elements of this manuscript that could be changed to make it more appealing to readers.

My main criticism is on the way results are structured and presented. I think that this study presents two different experiments, but such separation is not clear to me. The first experiment explores how microbiota changes through insect development, the second (and more interesting to me) how maternal care influences

such development. These two parts should be clearly separated even if some insects are used in both parts. In the first experiment, microbiota development is assessed by screening bacteria present in all insect stages, whereas in the maternal care experiment only newborn insects and adults are screened. Newborn and adult insects in the first experiment can thus be used as controls in the second one. Parts where this distinction was particularly unclear to me is in the last paragraph of the introduction section and in figure 1 where the experimental design is presented. Maybe the most confusing part was when the authors mention that 20 groups of insects were used and that only 5 were exposed to maternal care. At that point I wondered about such an unbalanced design.

There are a few key results and analyses that are not clearly introduced. As a reader we discover these results and analyses once we reach the results section, but a clear statement of why they are important is missing in the introductory section. These include the analysis of core microbiome elements, and the study of the microbiota of young and old individuals within the same instar. Even if I see the value of these approaches, I believe they should be introduced earlier in the manuscript.

In the introduction section, I found the dichotomy between vertical and horizontal transmission unclear. The authors assume that environmental acquisition is always horizontal, but there are many instances in which parents deliver symbionts externally that are subsequently acquired by their offspring. For example, in L69 the authors state that "...juveniles can acquire these symbionts soon after hatching by ingesting their parents' feces". Wouldn't that be environmental and at the same time vertical transmission?

I think that the role of parental care in the species studied should be detailed more precisely. What do mothers do to eggs and young instars? They may protect them against predators, but also clean them from pathogens. I missed a basic assessment of fitness consequences once insects were prevented from maternal care. Also, I think that pathogens should be mentioned at some point in this manuscript. It is quite likely that what maternal care does is to remove pathogenic species.

Changes in alpha and beta diversity during offspring development. I think there is room for a better exploration of this quite exciting data. Pairwise comparisons are difficult to grasp in this example because there are many groups (particularly in Figure 3A). An alternative solution to analyse this data would be to transform the larval stage into a continuous numeric variable [from 0 (egg) to 5 (adult)] and include as a factor whether the insect was a young or an old individual (pre and post moult). This would allow for a formal test of diversity changes with time and status. Even if changes through development are not linear, at least plotting changes with a scatter chart with a smoothed line separating pre and post moult individuals would help visualising trends. To my taste this may help support several statements in the results section that suggest, for example, that microbial diversity increases with development.

I wonder if the information provided in Figure 3B and 4 are not redundant. In the first plot beta diversity differences are represented in a PCA and in the second with a clustered heatmap. I am aware that in the second figure only 62 genera indicators of developmental stages were selected, but it is not clear to me why samples were discriminated using a PCA technique in one section and with a heatmap in the other.

Some minor comments follow:

In L67 the authors consider that "mothers can deposit external secretions containing symbionts on the eggshell" as parental care, but I am not sure I agree.

L88. Please divide this sentence into 2.

L112. Please provide a scientific name.

L149. Was moist sand sterilised? It can be a source of microbes

Enric Frago

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(I sign all my reviews)