

## Assessing the role of host-symbiont interactions in maternal care behaviour

**Trine Bilde**  based on peer reviews by **Nadia Aubin-Horth**, **Gabrielle Davidson** and 1 anonymous reviewer

Sophie Van Meyel, Séverine Devers, Simon Dupont, Franck Dedeine and Joël Meunier (2021) Alteration of gut microbiota with a broad-spectrum antibiotic does not impair maternal care in the European earwig. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Evolutionary Biology. <https://doi.org/10.1101/2020.10.08.331363>

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The role of microbial symbionts in governing social traits of their hosts is an exciting and developing research area. Just like symbionts influence host reproductive behaviour and can cause mating incompatibilities to promote symbiont transmission through host populations (Engelstadter and Hurst 2009; Correa and Ballard 2016; Johnson and Foster 2018) (see also discussion on conflict resolution in Johnsen and Foster 2018), microbial symbionts could enhance transmission by promoting the social behaviour of their hosts (Ezenwa et al. 2012; Lewin-Epstein et al. 2017; Gurevich et al. 2020). Here I apply the term ‘symbiosis’ in the broad sense, following De Bary 1879 as “the living together of two differently named organisms” independent of effects on the organisms involved (De Bary 1879), i.e. the biological interaction between the host and its symbionts may include mutualism, parasitism and commensalism. So far, we have relative few studies that explore the role of symbionts in promoting social behaviours such as parental care. Clearly, disentangling cause and effect when assessing the functional significance of symbiotic relationships in general is extremely challenging, and perhaps even more caution is needed when assessing the role of symbionts in the evolution of parental care, given the high fitness benefits to the offspring of receiving care. An interesting study on the symbiotic relationship between termites and their eukaryotic gut symbionts proposes a role of gut flagellates in the origin of subsocial behaviour (extended offspring care) in the termites through proctodeal trophallaxis (i.e. anus-to-mouth feeding), driven by mutualistic beneficial interactions (Nalepa 2020). Van Meyel et al. (2021) hypothesized a role of gut symbionts in promoting maternal care behaviour in the European earwig, and set out to test this idea in a carefully executed experimental study. They used a broad-spectrum antibiotic treatment to alter gut microbiota in mothers and examined its effect on maternal care provisioning. While the antibiotic treatment altered the gut microbiome, no effect on pre- or post-hatching maternal care was

detected. The authors also investigated a broad range of physiological and reproductive traits measured over a major part of a female's lifetime, and detected no effect of microbiome alteration on these traits. The study therefore does not provide evidence for a direct role of the gut microbiome in shaping offspring care in this population of European earwigs. Within populations, earwigs show inter-individual variation in the expression of maternal care (Meunier et al. 2012; Ratz et al. 2016), and there is evidence that genetic and environmental factors contribute to this variation (Meunier and Kolliker 2012; Kramer et al. 2017). The study by Van Meyel et al. (2021) is the first to analyse microbiome composition of the European earwig, and they study host-symbiont associations in a single population. A next step could be to explore among population variation in the gut microbiome, to achieve a better understanding on host-microbiome variation and dynamics in wild populations. Depending on the nature of host-symbiont associations across populations, new perspectives on their functional significance may arise (Hird 2017; Johnson and Foster 2018). It is therefore too early to conclusively confirm or reject the role of microbial symbionts in the expression of parental care in this system.

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## Reviews

### Evaluation round #2

DOI or URL of the preprint: <https://doi.org/10.1101/2020.10.08.331363>

Version of the preprint: version 4

### Authors' reply, 24 February 2021

Dear Prof. Bilde,

Thank you again for the time you have spend editing our manuscript and for your last comments. We have corrected the text according to the minor modifications and we have edited our manuscript to make it clear that our study is the first to describe the microbiome of the european earwig.

Sincerely,

S. Van Meyel, S. Devers, S. Dupont, F. Dedeine and J. Meunier

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### Decision by **Trine Bilde** , posted 14 February 2021

#### minor revision

Dear Author

Thank you very much for the thorough revision in response to the reviewers' and my comments. When I read the revised version, I came to think about one more point that I would ask you to consider: If the gut microbiome is expected to shape maternal care behaviour, this assumes that there is a tight association between the gut microbiome (or specific taxonomic units OTUs) and the host. You allude to this when you mention transmission from mothers to offspring. Do you have any prior information on the microbiome composition or on inter-individual variation in the gut microbiome of your species, to support this assumption? Or is your study the first of the microbiome composition of the species? In either case, you could mention that this is an expected relationship. Your data allows you to assess this assumption directly. Would you consider

including a few sentences in relation to this point in the introduction/discussion?

Minor editorial changes:

Line 24 (in marked documents) – remove ‘a’ so it says ‘in host biology’.

Line 88: change ‘antibiotherapy’ to ‘antibiotic treatment’

Line 352: ‘strains’ instead of ‘strain’.

Otherwise, I am pleased to recommend your preprint.

Best regards

Trine Bilde

## Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2020.10.08.331363>

### Authors' reply, 12 February 2021

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### Decision by [Trine Bilde](#) , posted 08 January 2021

#### Decision on article: revision

Dear Authors,

Thank you for submitting the preprint of your article "Alteration of gut microbiota with rifampicin does not impair maternal care in the European earwig" to PCI (<https://doi.org/10.1101/2020.10.08.331363>).

I apologize for the handling time, the combination of the pandemic and the festive season made it difficult to find and secure reviews. I am happy to inform that I have received three reviews, and all are positive about the study, while providing constructive comments and recommendations for revision. I enjoyed reading your article, and include a few additional comments (attached in the PDF), for your consideration.

I would be happy to recommend the article pending revision according to the comments provided. Please consider the comments from the reviewers carefully, you will them pasted in below.

With best wishes for a Happy New Year!

Trine Bilde

Aarhus University [Download recommender's annotations](#)

### Reviewed by [Nadia Aubin-Horth](#), 02 December 2020

Van Meyel and colleagues aim to quantify if modifying the gut microbiota of females in a species exhibiting maternal care will affect directly or indirectly their level of care to their offspring. To assess if this effect (if any) is direct or through effects on other parts of the phenotype, they also quantified physiological and reproductive traits in these females. To obtain females with altered gut microbiota, they used a treatment with a broad-spectrum antibiotic (rifampicin) known to alter gut microbiota in other insect species at this dose. They first confirmed that indeed this treatment affects the structure, composition, and diversity of the microbiota in earwig females. They then showed that none of the 6 maternal care behaviours were significantly changed in females treated with the antibiotic rifampicin, but affected 3 life-history traits.

This ms presents a clear lack in our knowledge of the association between gut microbiota and maternal care behaviour and a clear objective. The method is straightforward for a non-specialist of the microbiota like myself and the quantification of the different parental behaviours is adequate. The measurements of life-history traits in females is also based on standard protocols. The authors had planned a paired design to analyse effects of treatment on behaviour and life history traits, with siblings in the control and treated groups to be compared directly, but mortality prevented them to use this approach, and they acknowledge clearly that they replaced it by general average comparisons (and they provide the paired analysis on a smaller sample in supplementary material). They also used the appropriate adjustment of p-values to account for multiple testing. The main text contains enough explanations and the supplementary material is exhaustive and well-made.

The results are clear and well presented. The figures are clear and I think presenting individual samples on the box-plot is an excellent idea, as it helps the reader appreciate biological variation within a treatment. The treatment had very little effects on the phenotypes studied, but it does not seem to be the result of a lack of statistical power. The authors also discuss the possibility that while the antibiotic treatment did affect the gut microbiota, it may have not targeted the microbes (fungi, protist...) that may be the driver of behaviour (if any). The data is available on public repositories.

This manuscript is well-written, clear and presents significant new knowledge about this question, using a well-designed experimental study.

Minor comments

I would like to see the variables presented in figure 4 included in table 1 instead of table S1

**Reviewed by [Gabrielle Davidson](#), 06 January 2021**

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**Reviewed by anonymous reviewer 1, 22 December 2020**

A highly influential study by Buffington in 2016 (cit 17) showed that in mice, maternal diet can affect the gut microbial composition in offspring, and altered compositions result in different social behaviors of offspring. The Buffington study has motivated research in other species, and this research tends to show correlations between social behaviors and gut microbiota. The present study uses antibiotic (and control) treatments to test if the expression of female parental care (a key form of social behavior) is modulated by gut microbiota in earwigs. A large number of care behaviors are quantified, but the authors do not detect an effect of different microbiota on care behaviors.

Studies like these, because they show absence of certain patterns, can be very important to prevent the scientific community from extrapolating isolated findings in one animal to animals in general. However, because it is inherently more challenging to show the absence than the presence of an effect, I suggest the authors give more weight towards assessing the robustness of their finding.

Specifically, I suggest evaluating two points: 1) what is the power to detect behavioral differences between treatment groups if there were any differences? To conduct power estimates, one would of course require using expected effect sizes (which are unknown), but the authors could use previous behavioral studies to formulate an educated guess for a reasonable effect size range. 2) is the detected shift in microbial communities upon treatment significant enough to expect consequences? (or could it be that, for some reason, the microbial gut communities were, in biological/functional terms, only mildly altered by the treatment?). Again, this would require evaluation of the biological meaning ("effect size") of the statistically significant community differences. In this respect, I was wondering whether the summary of the 161 unique OTUs into 6 phyla was pertinent, or whether we might lose too much information here. For example, are there any OTUs among the 161 that are completely lost upon antibiotic treatment, as was the case in the mouse study I believe? Or did the treatments mostly affect the relative frequencies of different phyla as appears to be the case from Figure 6 – from this

figure it appears that the transient stop in food intake in the control group has in fact a similarly strong effect on microbiota as the antibiotic treatment, which may suggest that perhaps antibiotic treatment did not cause the warranted shift in microbiota communities? (I was also wondering whether the diversity analyses were in fact based on the 161 OTUs and not the 6 phyla? If so, then please clarify in the text).

Minor comments.

I was surprised that the authors continued antibiotic treatment after egg hatching as this prevents distinguishing between direct effects of diet on juveniles from indirect effects mediated by maternal care (the focus of the study). A short explanation in the methods of the rationale for this decision would be helpful.

L7 I would say increase instead of maximise

L150 replace “appeared” by “are indicated in”

L187 The number of replicates per treatment or in total?

L205 I would reformulate to “whether females produced a second clutch (yes or no)” (you are looking if the event happened or not, not at the probability of it happening)

L236 corrected for multiple testing?

L320 “Our experiment first demonstrates that the ingestion of rifampicin by earwig females induced stage-specific modifications in the species composition ( $\beta$ -diversity) of the gut microbiota but did not shape its species richness ( $\alpha$ -diversity).” I am not convinced by this statement as stage-specificity may be caused solely by females not feeding for some time (i.e., a difference between treatment and transient absence of treatment, and not caused by stage-specific effects of the treatment)

intake

L381. For me, the following statement is a strong overinterpretation: “In the present study, however, heavier eggs and newly hatched juveniles did not translate into higher offspring survival and improved development compared to lighter counterparts. On a proximate level, these findings suggest that rifampicin breaks the association between offspring weight and quality, either due to alteration in gut microbial communities and/or antibiotic toxicity.” I suggest a simple alternative explanation: the ad libitum food available under lab conditions simply masks the effect small eggs would have on juvenile survival/development under more natural conditions.

Table S3 (with Affiliation Table with BLAST scores) needs explanations for the different column headers.