PCI Evol Bio submission #2

Response to referees

Decision

by Wolf Blanckenhorn, 2019-08-29 10:41

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Re-review: The plastic effects of gut bacteria and nutritional environment on developmental life history traits in Drosophila melanogaster

It is well known that the rearing environment has strong effects on life history and fitness traits of organisms. Microbes are part of every environment and as such likely contribute to such environmental effects. Gut bacteria are a special type of microbe that most animals harbor, and as such they are part of most animals' environment. How much do selected gut bacteria affect the organismal phenotype, in terms of life history and larval foraging traits, of the fruit fly Drosophila melanogaster, a common laboratory model species in biology? This was the main question underlying the laboratory study by Guilhot et al., which was previously assessed by two reviewers and myself for PCI. To investigate the above question, the authors isolated 4 types/species of bacteria from the gut of their lab strain of Drosophila, and subsequently let Drosophila eggs and larvae develop in both the usual artificial laboratory medium as well as grapes (a natural habitat for Drosophila larvae) inoculated with theses bacteria, singly and in combination, plus a bacteria-free control. By investigating various relevant developmental traits, the authors found that adding particularly Enterobacteria had some visible improving effects on several traits (with three other types of bacteria showing only minor or even no effects), both upward (indicting improvement) and downward (being detrimental). In general, the grape medium reduced performance relative to the standard lab medium. Strongest interactive effects occurred for development time and body size, with lesser such effects on some related feeding behavioral traits (Figs. 2,3).

Thank you for this new assessment.

There are several points we would like to clarify before revising the manuscript. Please find below our questions and clarifications.

The study overall was conducted correctly. In response to the previous reviews the Introduction was improved in the revised version to make clearer the general objectives of the study. The study remains largely descriptive in that no particular a priori hypotheses or predictions in relation to the particular bacteria isolated were tested, not least because the bacteria were somewhat arbitrarily chosen and there were apparently no particular prior studies from which to derive concrete predictions. Overall, the results of this study should be of interest to the community of evolutionary ecologists, at least those working on nutritional and microbiome effects on animal life histories. As the strictly evolutionary content is limited (to non-existent), this paper would be best suited for a (microbial or behavioral or physiological) ecology outlet.

The inclusion of a natural medium (grapes), in addition to the artificial lab medium, must be commended, because this should permit inferences and conclusions for at least one natural environment, as inferences drawn from laboratory studies for the natural situation are typically limited. The choice of bacteria isolated and used remains arbitrary, though there are many. Nevertheless a good start. It could be asked why not natural but artificial lab populations of Drosophila were used for this experiment, if the aim is to draw inferences for the wild situation. But again: a good start.

While these specific data are novel, they are not very surprising. If we grow animals in different environments we can expect some detectable effects of these environments, including the bacterial (microbiome) environment, on the hosts life history.

We agree that the environment almost always affects animal life history, it is therefore unsurprising to observe phenotypic differences among insects reared in different environments.

Our study aims to go beyond the trivial description of environmental effects. Indeed, such effects raise the following questions (i) why do such environmental effects occur; (ii) what are the evolutionary consequences of these effects?

Here, we answer these two questions through i) the description of some notable ecology of the Enterobacteriaceae, ii) the use of an new approach that enables separating the effects of bacteria on host developmental plasticity and resource acquisition, iii) discussion of ideas on the evolutionary consequences of such effects.

The standard and predicted (Stearns & Koella 1986 Evolution) life history response of Drosophila melanogaster (but not all insects: Blanckenhorn 1999: Evol Ecol) facing stressful nutritional environments is to extend development but come out smaller in the end. This is what happened here (Figs. 3,4): size was smaller and development time longer in the grape medium, and some of the bacteria also weakly induced this (or the opposite) response. The biological interpretation is that individuals have more trouble ingesting and/or digesting the nutrients (thus prolonging their foraging period and development) and still cannot convert the nutrients effectively into body size increments (hence emerging smaller). This is what the authors here refer to as developmental plasticity, which is, still, ultimately nutritionally mediated. That is, the conclusion that this is not mediated by resource acquisition is misguided.

Through this paragraph, we understand:

1) Literature predicts that *D. melanogaster* larvae that develop in nutritionally poor environments grow more slowly and give smaller adults than larvae that develop in nutritionally richer environments.

2) Our observations are congruent with these predictions: control larvae (i.e. bacteria-free larvae) grow faster and give larger adults in the laboratory environment, created to optimize the fitness of *Drosophila*, than in grapes, where nutrients are diffuse and/or difficult to ingest and exploit.

3) These changes between environments are mediated by resource availability.

We have no objections on the points 1), 2) and 3).

4) We refer these changes between environments as developmental plasticity.

We disagree on this point. Indeed, what we refer to developmental plasticity is when larvae grow fast and give small adults, or grow slow and give large adults. In other words, existing resources are allocated to one trait at the detriment of the other. This is what we observe when we remove the 'medium main effect': we are certain that the

responses of phenotypes are only driven by bacterial identity or interaction between bacterial identity and environment (laboratory medium and grape berry).

That microbe affect host plasticity along life history trade-offs is not trivial. Recent studies, which we only discovered since submission of the manuscript, investigated this question using similar analyzes based on another trade-off, between fecundity and adult life (Gould et al., 2018 PNAS 'Microbiome Interactions shape host fitness', Walters et al., 2018 bioRxiv 'The microbiota influences the Drosophila melanogaster life history strategy'). They show that bacteria move host phenotypes along this trade-off (see for example Gould et al. 2018 Figure 1.C. p.2).

We found bacteria affect the phenotypes along the developmental trade-off. However, this effect is not necessarily universal. For example, current experiments in our group suggest that environmental yeasts improve both larval development rate and adult size of *Drosophila melanogaster* (Figure 1).
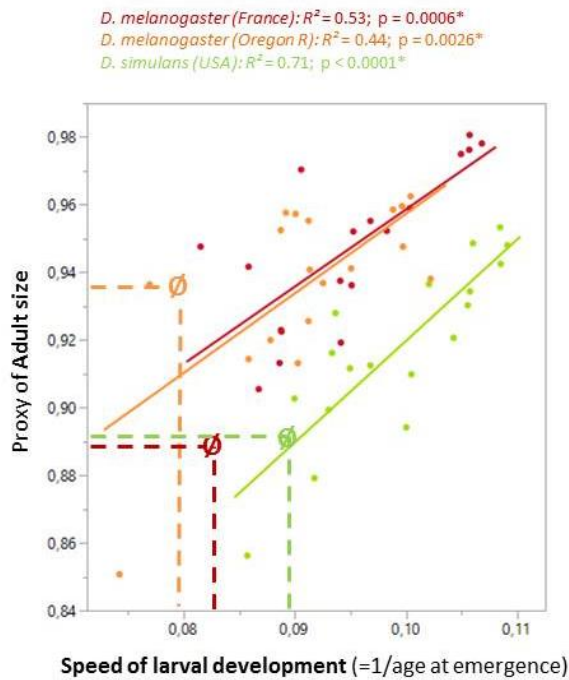

D. melanogaster (France): $R^2 = 0.53$; $p = 0.0006*$
D. melanogaster (Oregon R): $R^2 = 0.44$; $p = 0.0026*$
D. simulans (USA): $R^2 = 0.71$; $p < 0.0001*$

Figure 1: Relationship between yeast effects on age of emerging adults and on adult size for three *Drosophila* genotypes. Each point indicates the mean trait values for a particular strain of yeast. Colors indicate the *Drosophila* genotype. Ø symbols indicate control values.

I point out that the main and strongest interactive effects between medium and bacterial type are really only apparent for the enterobacteria, and they probably also mediate the overall effect of the mixture. For simplicity, I therefore suggest to initially present all data, but, in a second step, to only analyse control, enterobacteria & mixture, which would make plots and analyses much simpler and clearer. I say this because the new analyses presented in response to our previous statistical comments are actually no improvement whatsoever, but worse than before.

In general, more encompassing and increased questions in this context to be researched in the future could be: 1) are these effects predictable (not (yet) at this point, or so it seems), and 2) how strong are these environmental bacterial effects relative to other, more standard effects (e.g. relative to genetic variation, population variation, etc., or relative to other types of environmental effects like, say, temperature)? Although Genotype x Environment effects are invoked, they were not tested here due to lacking genetically different Drosophila families or populations tested. I consider this a major weakness of the study because it does not allow comparisons with other environments or situations. So, while interesting in principle, the study appears a bit basic in the end.

As mentioned before, and outlined above, the negative correlation between development time and body size is expected in stressful nutritional environments, which the enterobacteria apparently create. The multivariate analysis presented (which in the end analyses the effects of fruit salad instead of apples and oranges separately) is not what should be done; authors need to test for a correlation (between size and development time) within treatments properly with ANCOVA, entering ALL main factors (medium, bacteria, sex, plus interactions; cf. Table 1), such that the remaining correlation is indeed tested WITHIN treatment combinations. The negative regression ACROSS treatments is not really relevant because the tested environments are arbitrary. Suffice it to show that body size declines relative to control, while development time prolongs much more in relation (Fig. 4).

> Our interpretation of the previous paragraph is that developmental plasticity (the negative correlation between speed of development and adult size) should be tested within treatments. However, the relationship between the two traits within treatments would be mediated by uncontrollable sources of variation like micro-environmental and genetic differences. As we are only interested by the effect of bacterial *

environment interaction, we have to test the relationship between the two traits among treatments using MANOVA.

That this somewhat differs between the two nutritional treatments is interesting, of course. I don't see much "accelerated" but mostly decelerated development, in standard medium females in particular. The flies grow better in standard medium than on grapes (Fig. 4), which is perhaps a bit surprising. The reversal of sexual size dimorphism across bacterial treatments in the grape environment visible in Fig. 3 is interesting too, though I don't understand why this happens. If I understand correctly your sample size should be 15 (replicates) x 2 nutritional media x 6 bacteria treatments = 180. When analysing sexes separately this number doubles. The latter should be the magnitude of the error term in your full analyses. I don't see a need to analyse treatment means (you can plot them all right!); just present the overall ANOVAs of Table 1 (plus a corresponding ANCOVA; see above). (And reduce the data set to 3 bacteria treatments to do the same analysis, as suggested above.) I found it difficult to edit the tracked-changes version, which did not save my comments after all. In summary, some further revisions are in order and then the paper could be recommended by PCI EvolBiol.

We do not intend to initiate an endless argument and understand some scientific interpretations may be difficult to conciliate. Please be direct if you deeply disagree with our approach.

Thank you for your time.

The authors.