Peer Community In Evolutionary Biology

Parasite-mediated selection promotes small body size in yellow dung flies

Rodrigo Medel *based on peer reviews by* ***Rodrigo Medel*** *and 1 anonymous reviewer*

Wolf U. Blanckenhorn (2017) Selection on morphological traits and fluctuating asymmetry by a fungal parasite in the yellow dung fly. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Evolutionary Biology. 10.1101/136325

Submitted: 10 May 2017, Recommended: 31 July 2017

Cite this recommendation as:

Medel, R. (2017) Parasite-mediated selection promotes small body size in yellow dung flies. *Peer Community in Evolutionary Biology*, 100027. 10.24072/pci.evolbiol.100027

Published: 31 July 2017

Body size has long been considered as one of the most important organismic traits influencing demographical processes, population size, and evolution of life history strategies [1, 2]. While many studies have reported a selective advantage of large body size, the forces that determine small-sized organisms are less known, and reports of negative selection coefficients on body size are almost absent at present. This lack of knowledge is unfortunate as climate change and energy demands in stressful environments, among other factors, may produce new selection scenarios and unexpected selection surfaces [3]. In this manuscript, Blanckenhorn [4] reports on a potential explanation for the surprising 10% body size decrease observed in a Swiss population of yellow dung flies during 1993 - 2009. The author took advantage of a fungus outbreak in 2002 to assess the putative role of the fungus *Entomopthora scatophagae*, a specific parasite of adult yellow dung flies, as selective force acting upon host body size. His findings indicate that, as expected by sexual selection theory, large males experience a mating advantage. However, this positive sexual selection is opposed by a strong negative selection on male and female body size through the viability fitness component. This study provides the first evidence of parasite-mediated disadvantage of large adult body size in the field. While further experimental work is needed to elucidate the exact causes of body size reduction in the population, the author proposes a variation of the trade-off hypothesis raised by Rantala & Roff [5] that large-sized individuals face an immunity cost due to their high absolute energy demands in stressful environments.

References:

[1] Peters RH. 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

[2] Schmidt-Nielsen K. 1984. Scaling: why is animal size so important? Cambridge University Press, Cambridge.

[3] Ohlberger J. 2013. Climate warming and ectotherm body size: from individual physiology to community ecology. Functional Ecology 27: 991-1001. doi: [10.1111/1365-2435.12098](https://doi.org/10.1111/1365-2435.12098)

[4] Blanckenhorn WU. 2017. Selection on morphological traits and fluctuating asymmetry by a fungal parasite in the yellow dung fly. bioRxiv 136325, ver. 2 of 29th June 2017. doi: [10.1101/136325](https://doi.org/10.1101/136325)

[5] Rantala MJ & Roff DA. 2005. An analysis of trade-offs in immune function, body size and development time in the Mediterranean field cricket, *Gryllus bimaculatus*. Functional Ecology 19: 323-330. doi: [10.1111/j.1365-2435.2005.00979.x](https://doi.org/10.1111/j.1365-2435.2005.00979.x)

Reviews

Evaluation round #2

Reviewed by Rodrigo Medel, 31 July 2017

I have reviewed carefully the revised version of this manuscript. The author did a good job in considering the suggestions made by reviewers and justified his point of view when he disagreed in some specific point. So, it is a pleasure to accept the current version of this interesting manuscript for publication in the PCI repository.
Best regards
Rodrigo Medel

Evaluation round #1

DOI or URL of the preprint: **10.1101/136325**
Version of the preprint: 1

Authors' reply, 04 July 2017

Thanks for the competent and swift review of my paper. This was kind of a curious test run for me using this new system. I now revised the paper according to the reviewer comments, to which I respond case-by-case below. In the revised manuscript, the key changes are indicated in BLUE. Sorry for the delay, but I had some students finishing their theses. Wolf Blanckenhorn **Reviewer #1 (Reviewed by Rodrigo Medel, 2017-06-13 18:23)**

This study is a nice case of phenotypic selection on body size. The surprising 10% body size decrease observed in a Swiss population of the yellow dung fly clearly calls for an explanation. The author took advantage of a fungus outbreak to assess the putative role of Entomopthora as selective force on body size through viability and reproductive fitness components. I have a list of major and minor suggestions that hopefully may help to improve the study.

Major comments 1. I would suggest the author to perform a Bonferroni adjustment on the selection coefficients shown in Table 1. I sincerely doubt the significance of coefficients change but it is necessary to present such analysis leaving the Bonferroni resistant gradients boldface.

Thanks for the competent and swift review of my paper.

Bonferroni corrections are usually not really of concern in the context of selection studies as far as I'm aware. Typically several traits and selection episodes are considered, as is the case here, and it is unclear to me what total number any correction is supposed to be based on: 5, 10 (subsuming size and FA traits), 40 (subsuming all numbers in Table 1)? In any case, even when sequentially adjusting for 40 estimates, the final threshold would be $P=0.05/40 \approx 0.001$. As all the bold-faced coefficients in Table 1 are below that P-level, nothing changes.

1. Figure 3 and related analyses. Have you considered to perform an interaction test between the factors mating (yes/no) and infection (yes/no) on tibia length? Visual inspection of Figure 3 suggests me such possibility. If so, a significant term would indicate that factors cannot be analyzed independently.

Yes, this is what I did. It is given on P10, L20, along with all other interaction tests in the Results (in BLUE).

2. In the discussion section, the author comments on an important information related to the specialization of the fungus on adult flies. It seems to me that such piece of evidence should be presented earlier in the manuscript, probably in the Introduction section, as it suggests from the beginning the idea of strong parasite-mediated selection rather than weak fitness impact.

That the parasite affects adult flies is first mentioned on P4, bottom, when first introduced. Not sure what else to do.

3. A potential line of discussion is to emphasize the mosaic nature of the host-parasite interaction with body size evolving in different directions depending on moisture and parasitism. You can include a paragraph introducing the topic after the first paragraph in the Intro section. Anyway, this is completely optional.

I suppose you mean that parasite prevalence is much greater in spring and fall than in summer? Yes, I realise that I could discuss this aspect more here, and how this might differentially affect seasonal size-dependent mortality. However, as the dependence of the fungus on humid climate was already known, I chose not to dwell on this point any further beyond the brief and somewhat extended discussion on P11.

Minor comments

1. Page 2. Line 12. I would suggest changing "females never laid..." by "All infected females died, so there...".

Changed.

2. Page 8 line 23. Please include the way relative fitness for clutch size was estimated. I assume individual values were divided by mean clutch size.

Changed, now on P9.

3. I would suggest changing "phenomenological" by "correlative" along the text.

I must say I prefer the former, which is more specific. Now defined on P12 top.

4. For clarity, I would move the final paragraph of the Results section to the beginning of Page 10. I'm not sure why you think this would be clearer. I left the order as is. Reviewer #2 (Reviewed by anonymous reviewer, 2017-06-13 18:23)

This study presents a nice case of an empirical demonstration of the negative effect of body size on viability through a fungus mediated effect. Despite several suggestions to improve presentation the study in generally clear, easy to follow and well written. Maybe the absence of a mechanistic explanation for the increased risk of infection of large hosts weaken the impact of the results. Overall the reduced host size over the last years can not be attributed to the fungus, but open the discussion about its potential role as a selective agent on body size. Please find below my specific comments to improve presentation.

Thanks. Clearly, any phenomenological selection study cannot really identify the mechanisms, but they are a first step. Previous studies of the yellow dung fly allowed discussing the immuncompetence hypothesis at least.

Abstract: I would mention more explicitly the putative causes for insect mortality and the way FA relates to the fungus.

I do, in the last sentence, rather than further up.

Introduction: After the first paragraph you jumped too fast to your study system before remarking the real problem that the study is going to address. There are plenty of evidence of selection on body size in several systems, and even in plants is feasible to extract a general pattern. What additional factors you think could run against body size advantages? Hypothesis are missing at this point.
I would move the third paragraph below after presenting the hypothesis. You haven't justified the way parasites come into action at this point.
Page 6, lines 6-9: This last idea should be presented above to connect body size with FA and the immune response. See my comment above.
Page 6: I would try to summarize the goals of the study.

I reckon from your comments that you do not like my style of introducing this study. It admittedly deviates from the standard hypothesis-testing approach, primarily because it is phenomenological. I now added a couple of general sentences about body size effects (P3). Nevertheless, the way the study and the paper are set up, I need to introduce specific info about the species early on to keep the flow of the argument. The main aim was to phenomenologically estimate parasite-mediated selection on body size and FA, which is introduced on P4, center paragraph. There is no specific hypothesis because the immunocompetence came as an interpretation after the fact. The last paragraph of the Introduction lists more specific expectations after having introduced the parasite in the previous paragraph. I hope you can accept this way of introducing the work. In the end, all necessary arguments are there, perhaps not in the order you are used to.

Page 7, Laboratory procedures: Do you have additional evidence that ensures dead flies were infected by the fungus? Is there others sources of mortality?

The fungus is pretty obvious (I now inserted a picture in Fig. 1!), but I now acknowledge some random background mortality. How many clutches of eggs a single female can oviposit? About one clutch per week, for as long as they live (up to 3 months has been documented in the field, up to 10 months in the lab. Is just one clutch representative of the true relative number of eggs among surviving females? I think so, as body size sets the number of ovarioles and hence eggs. I now made such a statement on P8, top. Of course, lifetime egg output would be better, but this cannot be reasonably estimated in the field, and in the lab it is not representative. So clutch size is all we have.

Page 8: Statistical analyses: So you finally used or not PC analysis to perform the selection analysis? Please be more clear on this point. What are your explanatory variables in the analyses? After reading Table 1 it is clear to me that you used both, but you never provide a reason.
If selection on PC1 is as intense as selection on individual traits why using PCA? It is clear to me that if selection acts on a PC but no selection is detected on individual traits then the combination of traits is the actual target of selection. In your case it doesn't seem that selection is acting on the combination of traits as each trait is probably a reasonable proxy of body size. You can check Fredric J. Janzen and Hal S. Stern. Logistic Regression for Empirical Studies of Multivariate Selection. Evolution, Vol. 52, No. 6 (Dec., 1998), pp. 1564-1571; that show how to perform selection analyses using binomial response fitness as in your case. Although following their approach is not mandatory, it would be useful to discuss this issue in more detail for clarity.

Yes, there is always a discussion about which traits best reflect body size. Some people prefer PCs (I don't), so I included it for completeness. Other people prefer body mass; I don't, because it includes confounding effects such as food, drink, eggs, etc. Fact is, all body size proxies are always highly correlated, and, as argued in the MS, selection on various body parts may potentially differ (not here), hence the multivariate approach. In the end multivariate regression approaches do not allow identifying the true target of selection in cases when traits are highly correlated (which for body size is always true). I now added a justification on P8, bottom. I am of course aware of Janzen & Stern's paper, but it turns out that selection studies since have largely followed the treaded standard Arnold & Wade method, for comparative reasons. So have I. The numbers would look similar after all.

I hope this response is satisfactory.

Best regards,
Wolf Blanckenhorn

Decision by Rodrigo Medel, posted 12 June 2017

Revision needed

Dear Dr. Blanckenhorn:

I am writing to inform you that you manuscript "Selection on morphological traits and fluctuating asymmetry by a fungal parasite in the yellow dung fly" has been reviewed. Both reviewers consider this is a nice study on a quite interesting system. They suggest a series of major and minor changes that need to be performed before acceptance by PCI Evolutionary Biology. Below you can find a list of suggestions that need to be considered in a second version. With best wishes

Dr. Rodrigo Medel Full Professor University of Chile

Reviewed by Rodrigo Medel, 09 June 2017

This is a nice study on a quite interesting system. The surprising 10% body size decrease observed in a Swiss population of the yellow dung fly clearly called for an explanation. The author took advantage of a fungus outbreak to assess the putative role of Entomopthora as selective force on body size through viability and reproductive fitness components. I have some suggestions that hopefully may help to improve the study.

1. I would suggest the author to perform a Bonferroni adjustment on the selection coefficients shown in Table 1. I sincerely doubt the significance of coefficients change but it is necessary to present such analysis leaving the Bonferroni resistant gradients boldface.

2. Page 8 line 23. Please include the way relative fitness for clutch size was estimated. I assume individual values were divided by mean clutch size.

3. Figure 3. Have you considered to perform an interaction test between the factors mating (yes/no) and infection (yes/no) on tibia length? Visual inspection of Figure 3 suggests me such possibility. If so, a significant term would indicate that factors cannot be analyzed independently.

4. In the discussion section, the author comments on an important information related to the specialization of the fungus on adult flies. It seems to me that such piece of evidence should be presented earlier in the manuscript, probably in the Introduction section, as it suggests from the beginning the idea of strong parasite-mediated selection rather than weak fitness impact.

5. A potential line of discussion is to emphasize the mosaic nature of the host-parasite interaction with body size evolving in different directions depending on moisture and parasitism. You can include a paragraph introducing the topic after the first paragraph in the Intro section. Anyway, this is completely optional.

   Minor comments

6. Page 2. Line 12. I would suggest changing "females never laid..." by "All infected females died, so there..."

7. I would suggest changing "phenomenological" by "correlational" along the text.

8. For clarity, I would move the final paragraph of the Results section to the beginning of Page 10.

Reviewed by anonymous reviewer 1, 11 June 2017

Dear Editors, This study presents a nice case of an empirical demonstration of the negative effect of body size on viability through a fungus mediated effect. Despite several suggestions to improve presentation the study in generally clear, easy to follow and well written. Maybe the absence of a mechanistic explanation for

the increased risk of infection of large hosts weaken the impact of the results. Overall the reduced host size over the last years can not be attributed to the fungus, but open the discussion about its potential role as a selective agent on body size. Please found below my specific comments to improve presentation.

1. Abstract: I would mention before the results what presumably causes the insect mortality by the fungus and what is the hypothesis that connect FA with the fungus.

2. Introduction: After the first paragraph you jumped to fast to your study system before remarking the real problem that the study is going to address. There are plenty of evidence of selection on body size in several systems, and even in plants to extract a general pattern. What factors could run against body size advantages??? Hypothesis are missing at this point.

3. I would move the third paragraph below after presenting the hypothesis. You haven't justify with the fungal parasite come into action at this point.

4. Page 6, lines 6-9: This last idea should be presented above to connect body size with FA and the immune response.

5. Page 6: I would try to summarize the goals of the study.

6. Page 7, Laboratory procedures: How can you be sure that dead flies were all infected by the fungus? there are others sources of mortality? How many clutches of eggs a single female can oviposit? is just one clutch representative of the true relative number of eggs among surviving females?

7. Page 8: Statistical analyses: So you finally used or not PC analysis to perform the selection analysis? Please be more clear on this point. What were your actual explanatory variables in the analyses? After reading Table 1 it is clear that you used both, but you never justify why? If selection on PC1 is as intense as selection on traits the account for PC1 what does using PC1 add beside reducing the number of variable in the analysis an increasing your power. It is clear to me that if selection act on a PC but no selection is detected on those traits accounting for the variation in that PC then the combination of traits is what is actual target of selection. In your case it doesn't seem that selection is acting on the combination of traits because probable most of them are reasonable proxies of body size. Additionally there is a paper by Fredric J. Janzen and Hal S. Stern. Logistic Regression for Empirical Studies of Multivariate Selection. Evolution, Vol. 52, No. 6 (Dec., 1998), pp. 1564-1571; that show how to perform selection analyses using binomial response fitness as in your case. Maybe you follow this approximation but it should be clear though.

8. Page 12: OK but if the fungus dispers through the wind by spores how can a selectivity of prey argument make sense in this case??