26-Aug-2016

Dear Editor,

Thank you for the invitation to submit a revised version of our manuscript ECY16-0167.R1, entitled ‘Caterpillar seed predators mediate shifts in selection on flowering phenology in their host plant’.

ADD TEXT HERE…

Below, we provide a point-by-point list with answers to all questions and detailing the changes made in response to each of the comments by the reviewers and the editor.

Thanks again for your consideration of our manuscript.

Yours sincerely,

Alicia Valdés, on behalf of both authors

Response to review

Editor’s comments  
  
*Line 21.  The second reviewer addresses this issue, of the community context for selection.  I appreciate the reviewer's points, but I don't feel that the case is overstated.  Here, for example, the word "suggest" is clearly used.*

We agree that the reviewer makes an important point, and we have consistently tried to avoid overstating the importance of the community context, being aware that further data are needed to confirm that the community context drives among-population variation in selection.   
  
*30.  The word "mutualistic" is unnecessary at the start of that sentence.  Simply saying "Pollinators might select..." is sufficient.*

This is now changed.  
  
*43-44.  Re-order for clarity: "Variation in selection strength may potentially be driven by differences among populations in interaction intensities...".*

This is now changed.  
  
*66.  Change to "In this study, we asked if..." or "In this study, we examined the possibility that among-population variation...".*

This has now been changed, following the first suggestion.   
  
*127.  Change to "individual reaches."*

This is now changed.  
  
*129.  Change "carried" to "made".*

This is now changed.  
  
*130.  "second option" is confusing here.  Do you mean to say "our approach."*

Yes, that is what we meant. We have now changed this sentence.   
  
*130.  Break this sentence up, so that one sentence ends with "two main advantages." and the next sentence starts "First, it allows for an assessment of..."*

This is now changed.  
  
*131-133.  I have to say that I don't really understand this point, about being "less dependent on seasonal variation."  If one of your years had been much warmer or colder, wouldn't that have been reflected in the measurements you took in your window of observation?*

We agree that this was poorly worded and that the point is unclear. What we meant to say is that development strongly depends on ambient temperature and that two individuals with similar differences in development stage, e.g. large buds vs. just opened flowers, may differ in first date of flowering by just one or two days if temperatures are high but by up to ten days if temperatures are low. Hence, similar differences in developmental stage might translate into widely different differences in first flowering date if temperatures vary during the flowering season. We have now changed the text in this part to make our point clearer.

*172.  I second the reviewer's question here about the use of Type II SS.*

Please see answer to reviewer’s comment.

*186.  I'm not sure about your use of the term hierarchical here.  If you're using that term because the model includes nested elements, I believe it would be more straightforward to just say that and explain what was nested within what.*

Yes, trait × population interaction effects were nested within predation. We have now removed the word “hierarchical” and described the nesting in the text.

*261.  Is that a net decrease in fitness?  In other words, is there a number of flowers that actually reduces fitness beyond (for example) having just a few flowers and not attracting any oviposition?*

No, the net effect of flower number on fitness was positive, as is shown if we calculate the total effects using the path coefficients in Fig. 2:

2010: 0.46+0.26\*(-0.21)=0.41

2011: 0.48+(0.45\*(-0.29)=0.35

We have now clarified in the text that although predation increases with flower number and fitness decreases with predation, the net effect of flower number on fitness remains positive.

*268.  I appreciate what you have done here.  This is a nice discussion of effect sizes.*

*286.  Shouldn't this be "among"?  Because you're talking here about analyses where the unit of replication was populations not individuals within populations, right?*

Yes, these analyses had populations and not individuals as the unit of replication, so we now start this sentence with “Among populations where the butterfly was present…”.   
  
*304.  Change "during" to "in".*

This is now changed.  
  
*314.  Reword this to be "In apparent contradiction to the idea that selection...".*

This is now changed.  
  
*322.  Change to: "This leads to the idea that, for a given egg load per plant, a higher number..."*

This is now changed.  
  
*334 - 335.  This might be easier to read with parentheses starting before "that caterpillar" and then closing after "early-flowering individuals."*

We agree with the editor and have now added parentheses in this sentence.

*337.  Should be "have shown".*

This is now changed.  
  
*340.  Reword this sentence, it's not clear how this contrasts with the previous sentence, but a contrast is implied by "Our study instead."*

“Instead” has been now removed and this sentence has been modified.

*341 - 350.  See if you can't simplify the rest of this paragraph, as this text feels pretty repetitive with what has been said before.  In fact, for this next revision you should be aware of repetition throughout the Discussion and streamline wherever possible.*

We have now tried to simplify this paragraph and have carefully revised the discussion for repetition.  
  
*360.  When talking about among-population variance in butterfly presence, it might be more accurate to say "that this seed predator not only prefers earlier-flowering plants within populations but also tends to be associated with earlier-flowering populations."  After all, persistence might be more of an issue than preference per se at the population level.*

We agree and have changed the text accordingly.   
  
*393.  Remove "of" after "likelihood."*

This is now changed.

*395.  Re-order sentence to read "we also need to know."*

This is now changed.

Reviewers' comments

Reviewer: 1  
  
By re-arranging presentations of the data and performing additional analyses , Valdes and Ehlern clarify their findings strengthen their conclusions: the direction of selection for flowering phenology imposed by a pre-dispersal seed predator is reversed when that predator's a second prey species is absent.  
  
I am sympathetic to the argument in favour of the "stage-based" measure of phenology, and can certainly see how it was the only alternative given the scope of the study.   I would still argue that it has yet been demonstrated to be as good as repeated censuses.  But then this might just be me riding my hown hobby horse.  
  
Congratulations on a very fine study.  
  
  
Reviewer: 2  
  
Review of ECY16-0167.R1 “Caterpillar seed predators mediate shifts in selection on flowering phenology in their host plant”  
  
In this manuscript, the authors measure selection on flowering time of Gentiana pneumonanthe in 20 populations in each of two years.  They demonstrate that a butterfly pre-dispersal seed predator is an agent of selection on flowering time, and that the presence of butterflies depends on the present of their ant hosts.  Consequently, they conclude that variation in selection on plant traits depends in part on the community context (i.e., the presence of ants).  
  
*The authors have a very nice dataset; there are few studies of selection on plant traits that are both spatially and temporally replicated to this degree.  But I had three significant concerns about the manuscript as currently written.  First, I think that the authors overstate their case when it comes to the effect of the community context on selection.  They need to demonstrate that the ants affect selection on plant traits, not just the probability that a population will contain butterflies.  Second, the introduction does not do a compelling job of explaining how the current study differs from past studies of the causes of selection.  There are other studies of the effect of pre-dispersal seed predators on selection, so as a reader I need to know what new angle this study brings to the table.  Third, many of the interpretations in the discussion are tossed out without being well-developed (see numbered comments below for examples).*

In response to the first concern, we have, as stated above, consistently tried to avoid overstating the importance of the community context, being aware that further data are needed to confirm that the community context drives among-population variation in selection. We have now carefully gone over the text once more to make sure that we do not make any unwarranted claims in this respect. In response to the second concern, we have tried to further clarify how how our study differ from past studies in the introduction. In response to the third concern, we have now tried to develop the interpretations in the discussion folowing the suggestions by the reviewer (see below).

COMMENT 1-2-3  
  
*1.      Lines 41-56: The message of this paragraph is unclear.  I agree that there are many potential causes of variation in selection, but for the reader’s benefit I think that this paragraph should focus on the cause or causes that you are testing.*

We agree with this concern. We have now condensed this paragraph considerably and feel that it is now more focused.

*2.      Lines 57: The relationship between this paragraph and the one that came before it is unclear.  Why are we suddenly hearing about butterflies?*

We understand the reviewer’s concern, and now start this paragraph by talking about the potential of antagonistic interactors to mediate selection on plant traits, such as flowering phenology, introducing then the case of Large Blue butterflies as an example.

*3.      Lines 172-173: Why were Type II sums of squares used?*

…

*4.      Line 184: I don’t understand “to avoid basing contrasts on estimated selection coefficients”.  What is the perceived problem here?*

We agree that wording was a bit unclear. What we wanted to avoid was to base statistical analyses on values that were estimates with an error without accounting for the errors of estimates, i.e., avoid doing stats on stats. We have now changed the text to clarify this.

*5.      Lines 236-240: Is there a typo here?  I would think that a positive selection coefficient would mean selection for later flowering, and a negative selection coefficient would mean selection for earlier flowering.*

No, the text is correct, positive selection coefficients mean selection for earlier flowering, because higher values of the phenology measure indicate a more advanced floral development at the day of recording, i.e. an earlier flowering. This is indicated in the Methods section (P7L124-125).

*6.      Lines 245-249: It would be helpful if you took a little more time to describe these results.*

The main focus of our paper is on selection on flowering phenology and we wanted to give relatively less weight to the part describing selection of other traits. However, we have now modified this paragraph in order to better describe among-population variation in selection on flower number and shoot height and to state if this variation was associated with the incidence of the predator in plant populations.

*7.      Lines 254-256: How did the relationships vary?  In strength, direction, both?*

Most of these relationships were positive (see Table 4), so there was almost no variation in direction, although they largely varied in strength. This is now specified in the text.

*8.      Lines 257-282: This paragraph is very difficult to follow.  I am struggling to figure out how the different details presented here relate to each other.*

We agree that this paragraph contains a lot information that is not very easy accessible. However, we felt that it was important to try to describe the overall importance of seed predation on selection in terms of effect sizes and effects on fitness among and within populations. We have tried to simplify, clarify and partly restructure the text in this paragraph and hope that it is now clearer.

*9.      General comment: “The predator” and “the butterfly” are used interchangeably, which I found pretty confusing.  It would help me as a reader if you picked one set of terminology and stuck to it.*

We now use consistently “butterfly” or “butterfly seed predator” throughout the text.

*10.     Lines 295-298: I do not find this statement terribly persuasive.  For this to be the case, you’d need evidence that the strength of selection, not just the probability that butterflies are present, covaries with ant abundance or presence.  That link to selection seems to be missing here.*

We agree with the reviewer in the fact that more evidence is needed to claim that the community context influences selection. Our aim here is not to state that this is the case in our study, but to point out that the observed relationship among incidence of the predator and ant abundance suggests that the community context might contribute to among-population variation in selection. To make this even clearer we have now slightly modified the wording here (writing that “suggesting that the community context in terms of the second host of the butterfly *might* influence selection” instead of “influences selection”).

*11.     Lines 314-323: This is interesting, but it is a new idea that belongs in a new paragraph.*

We agree with the reviewer’s point and now start a new paragraph here.

*12.     Lines 300-304: Do you have any evidence supporting either of these hypotheses?*

We have now modified these sentences and added two references supporting the hypothesis.

*13.     Lines 354: What do you think that these unidentified factors could be, given what you know about your study system?*

As stated in the text, we think that abiotic factors could also play a role in selection on flowering phenology, and we have now specified temperature and humidity as two examples.

*14.     Lines 374-375: What do you think that the relevant environmental could be, given what you know about your study system?*

As specified in the text, we think that the height of the vegetation surrounding *G. pneumonanthe* plants might influence butterfly preferences for shoot height. We expect the preference for taller shoots to be stronger in populations with high surrounding vegetation, as these shoots protrude from the canopy, and would be more easily spotted by the butterflies. We have modified the text to make this clearer and added a reference.

*15.     Lines 392-404: The material in this paragraph seems peripheral/speculative and could be cut.*

This paragraph was added in response to the comments of a reviewer on the first version of this manuscript, asking us to acknowledge the limitations of our study and refer to unpublished data on population differentiation that could support our conclusions.

*16.     Lines 405-416: This seems to largely rehash what has already been said in the introduction and discussion.  Consequently, it is not the strongest way to end the paper.*

We have now rewritten the last paragraph to clarify what we feel is the most important contribution with the current study as well as the broader implications for the study of natural selection.

*17.     Your study is relatively unique in being both spatially and temporally replicated, but that strength is not effectively leveraged here.  How did the magnitude of spatial vs. temporal variation in selection differ?  Do you expect the butterflies and ants to have a larger effect on spatial or temporal variation in selection?*

Or, we could phrase it as:

We agree that examining the patterns of temporal vs. spatial variation is indeed an important topic. Yet, we feel that the design of this study with non-random set of populations with vs. without the predator present, and with only two study years makes it hard to make any strong conclusions in this respect. We have, however, now added information about variation in selection gradients for phenology among populations within years vs. differences between years within populations in the results section. We also provide detailed information about all selection gradients in the appendices.

We agree with the reviewer in that our study is well-suited to study spatial variation in selection. However, we only have two years of data and we do not think this is enough to make strong conclusions about temporal variation.

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Regarding the effects of butterflies and ants, we expect them to have a larger effect on spatial variation, as their abundances would probably vary more among populations than among years. However, although extreme variations in summer temperatures and/or precipitations could probably strongly modify the abundances of both butterflies and ants).