02-Jun-2016

Dear Editor,

Thank you for the invitation to submit a revised version of our manuscript ECY16-0167, now entitled ‘Caterpillar seed predators mediate shifts in selection on flowering phenology in their host plant’. We found the comments by you and the reviewer very helpful and think that thoroughly revising the manuscript according to these suggestions has substantially improved it. We have now moved part of the analyses in the supplementary material to the main text, and include a discussion of the effect sizes observed in the path analyses, focusing on the effects of phenology. We also explain why we consider our measure of phenology appropriate, and in some respects more adequate than records of calendar dates, for assessment of natural selection. Lastly, we have added new analyses with populations as the level of replication, as suggested by the reviewer.

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 reviewer and the editor.

Thanks again for your consideration of our manuscript.

Yours sincerely,

Alicia Valdés, on behalf of all authors

Response to review

Editor’s comments

*As mentioned, I have little to add to the thorough comments of the reviewer.  I second the reviewer's opinion that too much is buried in the supplementary material.  As the arrangement now stands, the two main figures are not terribly interesting (even if they are important).  My suggestion is to combine those figures into one two-panel figure, and then to bring some version of a path analysis into the main document, as it is the complexity of the path analyses that is (in my opinion) the real interest of this paper.*

We think the editor’s suggestion to incorporate path models into the main document is a good idea and we have now added a new Fig. 2 showing two general path analyses, one for each year. See response to the reviewer for details.

We have thoroughly considered the possibility of merging previous figures 1 and 2. Yet, we feel that each of these figures provides a clear illustration of the answers to the two main questions. They thus, in our opinion, constitute the core of the paper. At the same time, we feel that they refer to qualitatively different interactions (Fig. 1 shows differences in selection gradients among populations with and without the butterfly, while Fig. 2 – now Fig. 3 - shows the relationship between ant abundance and presence/absence of butterflies) and that it is thus conceptually difficult to merge them into one two-panel figure. We would prefer to keep them as two separate figures, but we are willing to combine them into a two-panel figure if the editor maintains that this is a good idea.  *With respect to what is now Fig 2, I would consider using either semi-transparent dots or hollow circles, so that overlapping points could be more readily deciphered (a slight jitter in the vertical direction could also be useful).*

We have now modified this figure (now Fig. 3) by using larger grey dots with a slightly darker border, and by making the dots semi-transparent. We hope that the result is a much clearer figure.

*With respect to a new path analysis figure, one possibility would be to show 6 or even 8 individual path diagrams as representatives in one large, multi-panel figure.  That could work in particular if coefficients were moved to a table and line thickness was made proportional to the strength and significance of the coefficients.  The reviewer suggests a general path model, and I agree that might be better, if possible.  Looking through the path models, it seems to me like an estimation of coefficients across populations could be useful (despite the significant heterogeneity indicated by the multi-group analysis).*

We agree that showing general path models is a better option than showing several path diagrams for different populations. As explained below in the response to the reviewer’s comments, we have now fitted one general model for each of the two years. These two general models are now shown in a new figure (Fig. 2). According to the reviewer’s suggestion, we now also include the coefficients for models fit to each population and year in several tables.

*The only other general comment I have is that I would find more discussion of effect sizes useful.  I appreciate the fact that the standardized coefficients are appropriate and effective for comparing different types of predictors and across populations, but it leaves the reader wondering about magnitude of ecological effects.  In other words, I believe it is appropriate subsequent to these path analyses to work backwards, out of the standardized variables and interpret slopes of relationships.  For example, in terms of seeds produced, how much does the effect of caterpillar presence compare to the range of natural variation in fitness within and among populations.  This would also provide a context for the discussion of other (as-of-yet-unmeasured) factors that likely influence flowering phenology in this system.*

We now present both the standardized and the unstandardized coefficients (in Appendices) for the path analyses fitted for each population and year. Effect sizes can be interpreted from the unstandardized coefficients, as they represent the change in absolute units of the response caused by a change of one absolute unit of the predictor, while holding all other predictors constant.

We now also provide information of effect sizes in terms of how a given change in phenology influences the probability of predation and how this change in risk of predation translates into decreases in fitness (P13-14, L274-282).

We have also added new analyses examining how the effect of caterpillar presence compares to the range of natural variation in fitness within and among populations. We did not find significant differences in fitness among populations with and without the predator, although mean number of intact fruits was higher in populations without the predator in both study years. The presence of the predator reduced mean population fitness by 15% in 2010 (mean fitness was 0.97 in populations without the predator and 0.83 in populations with the predator) and by 20% in 2011 (mean fitness was 0.76 in populations without the predator and 0.61 in populations with the predator). We have also explored relationships among fitness (i.e. number of intact fruits) and the proportion of predated fruits, flowers and buds in populations where the predator was present, in order to assess the percentage of the variation in fitness within and among populations that was explained by the effect of the predator. We found that predation explained between 0.22 and 49.54 % of the variation in fitness within populations and years (27.72 % on average, R2 values from Poisson GLMs relating the number of intact fruits per plant to the proportion of predated fruits, flowers and buds). Predation explained also 26.39 % of the variation in fitness among populations and years (R2 value from a linear model relating the mean number of intact fruits per plant to the mean proportion of predated fruits, flowers and buds). These results are now mentioned in the text (P14, L282-290).

*Finally, I have a pretty trivial suggestion, which you can decide to ignore if you like.  Personally, I would change the title to "Caterpillar seed predators...".  After all, it is the caterpillar that's doing the predating.*

We have now changed the title as well as the wording in this respect throughout the text, according to the editor’s suggestion.

Reviewer(s)' Comments to Author:  
  
Reviewer: 1  
  
*Comments to the Author  
This fine study by Valdés and Ehrlén makes some needed strides in explaining the sources of variation in selection on ecologically-important traits.  I have signed this review.  
  
The key trait under study is flowering phenology, and the system includes a plant, and a butterfly/caterpillar that is a predispersal seed predator in its early instar (and the host for the late-instar caterpillars). Selection favors later flowering in populations where the butterfly is present, while early flowering is favored in its absence.  Within populations with butterflies, earlier flowering individuals tend to suffer greater attack and lower fitness.  
  
One of the great strengths of this study is the number of populations examined.  The large number of populations also leads to an unfortunate but completely forgivable, that is, the imprecise measures made of flowering phenology and attack intensity that is no doubt due to the effort required to reach all sites in a timely manner.  The uncertainty involved in the analysis of each single population is very likely overcome by looking at so many populations.  
  
A very large amount of information has been put into appendices:  the paper will be greatly improved if some of it is moved up into the main body.   (I hesitate to make this suggestion, as I wonder if these analyses were moved to the back in response to reviewer/editor comments on a previous submission.  If so, I disagree with the move).  At the very least, a general path diagram and table S9 should be moved up into the body of the text.  Table S9 should alo be expanded to display all path coefficients.  (The individual path diagrams in the appendix would thus no be needed.)*

We now include a new figure (now Fig. 2) showing two general path analyses, one for each study year. According to the reviewer’s suggestion, we also now include all the coefficients for models fit to each population and year in Table 4 and Appendices S3-5. For the sake of brevity, we have chosen to present the results from the model where indirect effects are mediated by the intensity of predation (i.e. number of eggs) in the main text, and the results from the model where they are mediated by the probability of predator attack in the supplementary material (Appendices S3-5). In Table 4, we show the standardized coefficients for the model with number of eggs. Because showing both the standardized and the unstandardized (which are useful for interpretation of effect sizes) coefficients for all models in Table 4 would result in a very complex table, we present the unstandardized coefficients in Appendix S9. As all the coefficients are now shown in tables, we have removed the two appendices with the separate path diagrams for each population and year.   
  
*With regard to the path analysis, it is not clear if the data were standardized within populations of across populations.*

The data were standardized within populations. This is now mentioned in the text (P10-11, L209-210).

*There is not much said about the fact that in the path analyses, the direct effect of phenology on fitness is zero or negative in the populations with butterflies.  In populations without butterflies (and so all of the effect is direct) it is zero or positive.  Thus, I would expect that the direct effect of phenology on fitness also be zero or positive after taking account of the indirect effects of butterfly attack.  Why the reversal in the direct effect between the 0 and 1 populations?  Do the populations differ for mean phenology?*

*This leads to a suggestion for an additional analysis using population as the level of replication -- an analysis that addresses questions such as:  1) do populations with early mean phenology tend to have greater attack rates?, and, 2) is selection on phenology stronger/weaker in populations with early mean phenology?.  It is plausible that a very-early flowering population could escape butterfly attack, but still experience selection against early flowering due to temporal mismatch with pollinators, poor seasonal climate, etc.   Floral abundance and timing could have a density/frequency-dependent effect on butterfly oviposition that would leave a signal in the response of the enemy to the mean host phenotype.  The authors can consult a paper I wrote a number of years ago on variation in selection across populations and generations as an example (Weis, A.E., and A.D. Kapelinski.  1994. Variable selection on Eurosta's gall size, II: A path analysis of the ecological factors behind selection. Evolution 48:734-745; the analysis in this paper may not meet today’s standards, but is at least indicative of the issues).*

We have now performed the additional analyses suggested but the reviewer. The results of these population-level analyses show that populations with and without butterflies did not differ in mean phenology in any of the two years studied. They do, however, show that combinations of populations and years with earlier mean phenology have higher proportions of plants attacked by the butterfly (binomial GLM estimate = 0.528, p < 0.001). This implies that butterfly seed predators not only prefer earlier-flowering plants within populations, but also that attack rates are higher in earlier-flowering populations. These results are now provided in the text (P17, L362-365). Yet, the strength of selection on phenology (estimated by the selection gradient) was unrelated to mean population phenology (P17, L365-367). We also do not think that populations flowering very early suffer from a temporal mismatch with pollinators, as flowers would open at the earliest in the beginning of July in these populations, and at this time of the year availability of pollinators should not be a problem.

We think that the direct negative effect of phenology on fitness in some of the populations with the predator might be due to phenology affecting egg concentration among the different flowers in an individual plant. We show that an early phenology increases the number of eggs per plant, but we have not assessed the distribution of eggs among the different flowers within an individual. For a given number of eggs per plant, late-flowering plants might show higher among-flower variation in egg numbers than early-flowering plants. Late-opening flowers in late-flowering plants might escape predation due to a temporal mismatch with butterfly oviposition (i.e. these flowers might develop when the oviposition period is over). On the contrary, in early-flowering plants, all flowers would develop during the oviposition period and therefore would be potentially available for oviposition, leading to lower among-flower variation in egg number. We hypothesize that the direct increase in fitness in late-flowering plants observed in some populations and years could be driven by these late-opening flowers that are able to escape predation, as reported in other studies (Mahoro 2003, Ehrlén et al. 2015). We now include this argument in the discussion (P16, L329-334).

*SPECIFIC COMMENTS:  
  
Line 34:  there is a paper by Emily Austen in Evolution, 2015, that looked for selection on age at flowering versus flowering calendar date—seed predators select for early flowering date.*

We have now included this reference, and we thank the reviewer for the suggestion. *Line 95:  change “Contrary to..” to “Distinct from…”*

This is now changed. *Lines 117 and 128:  These are low precision measures of flowering phenology and attack rate.  In addition, they will be biased if visits the first and last sites were made even just a few apart.   Can the date of the visit be used as a covariate to correct for any such bias?*

There are two principal ways to assess differences in timing of development and phenology among individuals. The traditional way is to record the calendar date at which an individual reach a pre-defined stage of development, e.g. the arrival of birds in spring or the opening of the first flower. An alternative is to assess differences in the stage of development at a single occasion. Given that recordings are carried synchronously and at a relevant stage, this second option has two main advantages. First, it allows assessment of differences in development among a large number of individuals during a single recording. Second, it provides a measure of differences in the rate of development that is much less dependent on seasonal variation in temperature, i.e. similar differences in development stage may correspond to two days or two weeks depending on spring temperatures. We would thus argue that for the main objective of this study, to assess how selection gradients – estimated using parameter values that are standardized within populations – depend on the presence of the butterfly, our estimate of differences in floral development/phenology is not just necessary to achieve reasonable sample sizes in 20 populations, but is also a more adequate trait to measure than Julian dates of opening of flowers. We have now added such a motivation to why we used this measure of differences in floral development among individuals to the methods section.

At the same time, it is clear that one possible disadvantage of measuring developmental rate at one occasion is that unstandardized estimates might be difficult to compare among populations if recordings are not carried out simultaneously. However, in the current study we do not think that this was a problem. This is because, although visits to the different populations were not made on a single day, they were still made within a short period of time, and our measure of phenology was not correlated with the date of recording in any of the two years (linear regressions of population mean phenology vs. recording date, p = 0.326 for 2010 and p = 0.388 for 2011). This is now specified in the Methods section (P7-8, L136-142).

*Line 161:  Were quadratic selection gradient doubled, as per Stinchcombe, J. R., Agrawal, A. F., Hohenlohe, P. A., Arnold, S. J., & Blows, M. W. (2008). Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing?. Evolution, 62(9), 2435-2440.?*

Yes, we did this and it is now stated in the tables where we include quadratic coefficients (i.e. in the description of Table 1 and in Appendix S2).

REFERENCES

Ehrlén, J., J. Raabova, and J. P. Dahlgren. 2015. Flowering schedule in a perennial plant; life-history trade-offs, seed predation, and total offspring fitness. Ecology 96:2280–2288.

Mahoro, S. 2003. Effects of flower and seed predators and pollinators on fruit production in two sequentially flowering congeners. Plant Ecology 166:37–48.