20-May-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

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 result in a very complex table, we present the unstandardized coefficents 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, L200-202).

*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 (P16, L375-378). Yet, the strength of selection on phenology (estimated by the selection gradient) was unrelated to mean population phenology (P10, L258-259, P16, L383-384). 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 the fact that this effect is not completely independent from the predation effect. In plants that flower late in the season, late-opening flowers might be able to escape predation due to a temporal mismatch with the butterfly oviposition period. On the contrary, in plants that flower early in the season, both early- and late-opening flowers could potentially be oviposited, due to a highest degree of overlap with the oviposition period of the predator. Although the coefficients in the path analyses are supposed to represent independent effects, we are here considering the effect of intensity of predation measured as number of eggs. The direct effect of phenology on fitness is therefore independent of the number of eggs oviposited on the plant. However, a plant flowering very early and another one flowering very late could have exactly the same number of eggs, but these eggs could be distributed differently among the different flowers in each plant. In this sense, the proportion of individual flowers that are attacked in a plant may be related to its flowering phenology, and there could be attacked late-flowering plants where some flowers escape seed predation because their bud development starts when the adult butterflies are already absent. This would explain the fitness increase in late-flowering plants, which would be driven by these late flowers which are able to escape predation. We unfortunately lack data on counts of eggs on individual buds in order to confirm this hypothesis, but other studies (Mahoro 2003, Ehrlén et al. 2015) have reported late-opening flowers escaping from seed predation, so we think that the same mechanism could be operating, at least in some populations, in our study system. We now include this in the discussion (PL).

*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 open. 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 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 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, L131-133).

*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).

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.*

think the editor’s suggestion to incorporate path models into the main document is a good idea and we newSee 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 maintain 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 above 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 how a given change in phenology influences the probability of predation and how this change in risk of predation translates into decreases in fitness (P15, L348-354).

We have also added new analyses examining how effect of caterpillar presence compare to the range of natural variation in fitness within and among populations.

On the other hand, to evaluate how much does the effect of caterpillar presence compare to the range of natural variation in fitness within and among populations, we have now included results from analyses of: 1) differences in number of intact fruits among attacked and non-attacked plants within each population, and 2) differences in mean number of intact fruits among populations with and without the predator.

2) 2010: One-way ANOVA, Estimate = -0.1413 (ns), reduction of 15%

2011: One-way ANOVA, Estimate= -0.1509 (ns), reduction of 20%

*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.

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.