

Revision round #2

Author's Reply:

Dear Dr Haag,

Thanks for handling the review of our manuscript. Please find enclosed new a revised version. We agree that the comments of Jon Agren have further improved the quality of this manuscript and we tried to answer to all of them (see the point-by-point reply below). We provide a track-changes version where the changes in the main text and supplementary files are highlighted in bold. We hope that you will find this updated version of our manuscript suitable for recommendation by PCIEvolBiol and would be happy to take any further comments if you judge it would improve the manuscript.

Laurène Gay, on behalf of all the coauthors

Decision for round #2 : *Revision needed*

Dear Dr Gay,

Your revised preprint "Evolution of flowering time in a selfing annual plant: Roles of adaptation and genetic drift" has now been reconsidered by two of the original reviewers. As you will see, while one of them is satisfied with the new version, the other is positive but recommends an additional round of minor revision. From my own reading, I agree that the suggestions by the reviewer will likely further strengthen the manuscript. Therefore, before reaching a final decision, I would like to ask you to consider these suggestions, and to revise the manuscript accordingly. When you submit the revised version, please include a letter in which you describe how you have responded to each of the referees comments.

Best wishes, and many thanks for submitting to PCI Evol Biol,

Christoph Haag

by [Christoph Haag](#), 2021-04-19 11:50

Manuscript: <https://www.biorxiv.org/content/10.1101/2020.08.21.261230v2>

version 2

Review by [Jon Agren](#), 2021-04-16 11:54

I think the presentation has benefitted from the revisions made by the authors. Below is a list of comments on details regarding terminology and presentation that the authors may want to consider.

p. 1, Abstract first sentence. Resurrection experiments can detect correlations between trait modifications and changes in the environment, but this is not really a test of a causation, is it? Or is the argument here that simultaneous parallel changes in many populations indicate a

change in the environment acting over a large area? This could be indicated with a slight rewording.

This is true, using resurrection experiments is an intrinsically correlative approach. Experimental evolution would be a more accurate way to test a causation. We changed this sentence to avoid the confusion.

p. 1, Abstract first sentence. Change “traits modifications” to “trait modifications”.

Changed accordingly

p. 2, first paragraph. Not fully clear what the important difference is between experimental and natural populations. In both cases, an estimate of effective population size is required.

The major difference between experimental and natural populations that we had in mind here is that experimental settings generally include independent replicates for experimental populations. Following a comment from C. Haag in the previous round of review, we added the possibility to consider a set of natural populations as replicates, but this requires a few assumptions. Provided we have replicates, pattern tests can be applied and do not require an estimate of effective population size. We tried to clarify this at the end of page 1 and hope it is clearer now.

p. 2, right column, line 3. What does “>0.5” refer to? A broad-sense heritability estimate?

Yes it is a broad-sense heritability. We added this in the text.

p. 2, right column, line 27. Insert “selfing” after “predominantly”.

Changed accordingly

p. 2, right column, line 36, “across 22 generations”. Does this species have any seed bank that may affect “effective generation time”?

Yes, there is physical dormancy in *Medicago truncatula* and it seems to be highly variable depending on the accession and environmental conditions (Renzi et al. 2020. Release of *Medicago truncatula* Gaertn. and *Pisum sativum* subsp. *elatius* (M. Bieb.) Asch. et Graebn. Seed Dormancy Tested in Soil Conditions. *Agronomy-Basel* **10**; Renzi et al. 2020. Physical dormancy release in *Medicago truncatula* seeds is related to environmental variations. *Plants* **9**). Yet, little is known about seed dormancy in the natural environment and its consequences for seed bank. If the seed bank is large, generations will overlap and generation time will be reduced. Not taking this effect into account would mainly lead to an overestimation of the effect of drift. We added this point in the presentation of the method, page 5 lines 481-485.

p. 2, right column, line 47, “taking into account the multilocus genotypic composition...”. Unclear how this should be understood. Reword?

We were referring to our estimation of the realized fitness based on the changes in frequency of the multilocus genotypes through time. We changed this sentence, lines 194-197.

p. 2, right column, line 50, “for neutrality”. I suggest the authors indicate how this is achieved. – By using estimates of genotypic values for flowering time and assuming flowering time is a neutral trait?

Not exactly. We test for neutrality using a rate test, as described above in the Introduction: we estimate the effective population size and then simulate how the frequency of the multilocus genotypes would change under genetic drift alone. We added further details lines 199-201.

p. 3, first paragraph. I still find the procedure for building “families of full sibs” unclear: I suggest the authors state explicitly whether the families multiplied in 2011 each originated from a different pod collected in the field, or whether the families originated from seeds that had been randomly selected from pooled samples of seeds from 1987 and 2009, respectively.

Seeds for the generation of multiplication were randomly selected from pooled samples of seeds from 1987 and 2009. We added this sentence in the text lines 223-226.

p. 3, right column, paragraph “Temporal changes in sensitivity to vernalization”, “measured as the slope...” This needs some more explanation. Are differences calculated between all possible pairs of plants in the two treatments?

No, for each family, we paired each plant from block 1 in treatment 1 with the plant from block 1 in treatment 2. We detail this line 294-296.

p. 4, third paragraph, “good approximation of the additive genetic covariance”. What about maternal environmental and genetic effects?

Maternal environmental effects are controlled for by the generation of multiplication in the greenhouse, as explained line 222. We added in the text that maternal genetic effects are neglected, line 335.

p. 4, right column, first paragraph. State explicitly that the individuals analysed represented 145 different families?

We added that the 145 individuals represent 145 families, line 371.

p. 5, second paragraph, “As a preliminary step,...”. To me the argument would make more sense in the reverse order, as the changes in flowering time and MLG frequency between 1987 and 2009 are the most direct estimates of evolutionary change. In other words, starting from the observation of the changes in flowering time and MLG frequency, one can examine the strength of the association between flowering time and MLG in the greenhouse, and whether the change is consistent with selection observed in the greenhouse. I see no a priori reason why selection on flowering time in the greenhouse should mirror that at the site of the focal population. To make this order of logic clear, the authors may want to move the description of the selection gradient analyses to after this argument has been formulated.

The logic we had in mind was that if the fitness measured in the greenhouse correlates with the changes in frequency, it means that it reflects the “realized” fitness in the natural environment. We used the Robertson-Price identity with the genetic covariance to estimate the selection gradient so that it is not influenced by the environment (i.e. genes that make the plant flower early also increase the seed production). Yet, it is true that GxE interactions can

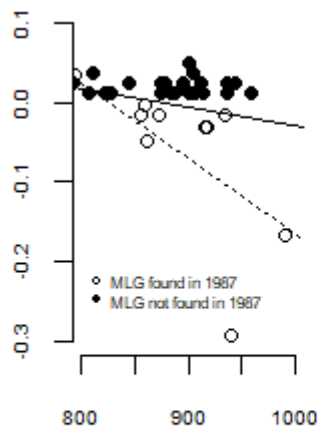
change the ranking of genotypes, and alter the conclusions. Therefore, we would rather keep the analyses in this order, but we fully agree that GxE are a major limit to this kind of studies, and we acknowledge it in the text, as recommended in your final comment.

p. 5, second paragraph, “whether selection is quantified in the greenhouse is likely to mirror selection in the field at present and 22 years ago”. To be strict, it would only need to mirror the predominant selection between 1987 to 2009 to be correlated with the change observed, right? Current selection in the field should matter little?

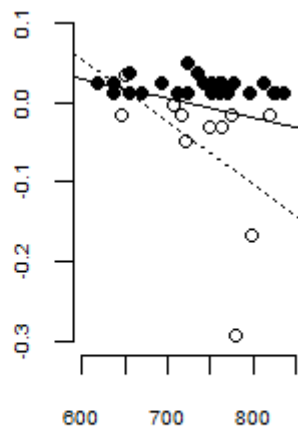
This is true, we changed the text accordingly

p. 5, second paragraph, “We then measured...”. I like this approach! The authors should indicate which measure of flowering time was used in this analysis. The legend of Fig. 3 speaks about “average flowering time”. The sensitivity to vernalization treatment varied among genotypes. Are the results of this analysis essentially the same if the analysis is conducted separately for treatment 1 or 2, or separately for estimates of flowering time obtained based on the seed sample from 1987 and from 2009, respectively?

We used the genetic value of flowering time, estimated as the average flowering time for a given MLG measured in the short vernalization treatment. But the results and conclusions were unchanged when we used the flowering times measured in the long vernalization treatment (the slopes are very close, as you can see on the figure below). Yet the regression that was significant with the sample restricted to the MLGs present in 1987 is non-significant using the long vernalization treatment (due to the very low sample size and lower variance in this treatment). We added this in the text lines 661-662.



MLG average flowering time in T1



MLG average flowering time in T2

The analysis conducted on the seed sample from 1987 was already in the text (white dots in Fig 3.) and the effects were more significant on this data, but the sample size is very small.

p. 6, first paragraph; Table 3. Since a single line was sampled in each population, it is a bit misleading to call the examined effect a “population effect”. Change to “line effect”?

This is true, even if for a predominantly selfing population, a single line can represent a very large proportion of the population (sometimes 90 or even 100%, see Jullien et al. 2019). We nevertheless changed to “line effect” in Table 3.

p. 7, first paragraph, “predict an evolution of towards earlier flowering”. Since estimates of selection and heritabilities are specific to a given environment, this prediction is valid for the greenhouse and not necessarily for other environments.

This is true, we added this precision in the text line 599-600.

p. 7. Was there an effect of year of sampling on estimates of flowering time for MLGs sampled in both 1987 and 2009?

We tested the year effect on the subset of families that belonged to the five MLGs that were present in 1987 and 2009, using a mixed model similar to the one we used in the manuscript (random bloc and effects, fixed effect year and treatment. We found no year effect (LRT: $\chi^2 = 2.81$; $df = 1$; $p = 0.094$).

p. 7, right column, second paragraph, “were persistent through time”. Change to “were observed in both years” to make the fact that altogether 5 lines were observed in both the 1987 and 2009 sampling more obvious?

Changed accordingly

p. 7, right column, second paragraph, “Fig. 3A, regression only significant...”. Add sample size (i.e., number of family means included in this regression).

We added the sample size here: 12 MLGs (the unit is the MLG, and there can be several families representing some MLGs)

p. 11, second paragraph, “Munguia-Rosas et al.”. Note that selection estimates considered in this meta-analysis largely ignores the effect of variation in number of flowers and plant size, suggesting that many of them rather reflect a correlation between plant condition and fitness.

We added a sentence in the Discussion to note this, lines 918-922. But we kept the paragraph because it still seems interesting to discuss what is expected if flowering was under directional selection.

Finally, I suggest the authors somewhere add a caveat regarding possible G x E interactions for flowering time (greenhouse vs. field), when discussing the possible association between flowering time as expressed in the greenhouse and fitness and evolutionary change in the field.

We now mention the possible GxE interactions at the beginning of the Discussion, line 732-736.

Review by [Stefan Laurent](#), 2021-03-20 17:00

I am satisfied with the answers to my comments and with the modifications to the main text. The qqplots should be added to the supplementary figures linked to main figure 3.

We added the qqplots as supplementary figure S3.