Separating adaptation from drift: A cautionary tale from a self-fertilizing plant

Christoph Haag based on reviews by Jon Agren, Pierre Olivier Cheptou and Stefan Laurent

A recommendation of:

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Recommendation

In recent years many studies have documented shifts in phenology in response to climate change, be it in arrival times in migrating birds, budset in trees, adult emergence in butterflies, or flowering time in annual plants (Coen et al. 2018; Piao et al. 2019). While these changes are, in part, explained by phenotypic plasticity, more and more studies find that they involve also genetic changes, that is, they involve evolutionary change (e.g., Metz et al. 2020). Yet, evolutionary change may occur through genetic drift as well as selection. Therefore, in order to demonstrate adaptive evolutionary change in response to climate change, drift has to be excluded as an alternative explanation (Hansen et al. 2012). A new study by Gay et al. (2021) shows just how difficult this can be.

The authors investigated a recent evolutionary shift in flowering time by in a population an annual plant that reproduces predominantly by self-fertilization. The population has recently been subjected to increased temperatures and reduced rainfalls both of which are believed to select for earlier flowering times. They used a "resurrection" approach (Orsini et al. 2013; Weider et al. 2018): Genotypes from the past (resurrected from seeds) were compared alongside more recent genotypes (from more recently collected seeds) under identical conditions in the greenhouse. Using an experimental design that replicated genotypes, eliminated maternal effects, and controlled for microenvironmental variation, they found said genetic change in flowering times: Genotypes obtained from recently collected seeds flowered significantly (about 2 days) earlier than those obtained 22 generations before. However, neutral markers (microsatellites) also showed strong changes in allele frequencies across the 22 generations, suggesting that effective population size, Ne, was low (i.e., genetic drift was strong), which is typical for highly self-fertilizing populations. In addition, several

multilocus genotypes were present at high frequencies and persisted over the 22 generations, almost as in clonal populations (e.g., Schaffner et al. 2019). The challenge was thus to evaluate whether the observed evolutionary change was the result of an adaptive response to selection or may be explained by drift alone.

Here, Gay et al. (2021) took a particularly careful and thorough approach. First, they carried out a selection gradient analysis, finding that earlier-flowering plants produced more seeds than later-flowering plants. This suggests that, under greenhouse conditions, there was indeed selection for earlier flowering times. Second, investigating other populations from the same region (all populations are located on the Mediterranean island of Corsica, France), they found that a concurrent shift to earlier flowering times occurred also in these populations. Under the hypothesis that the populations can be regarded as independent replicates of the evolutionary process, the observation of concurrent shifts rules out genetic drift (under drift, the direction of change is expected to be random).

The study may well have stopped here, concluding that there is good evidence for an adaptive response to selection for earlier flowering times in these self-fertilizing plants, at least under the hypothesis that selection gradients estimated in the greenhouse are relevant to field conditions. However, the authors went one step further. They used the change in the frequencies of the multilocus genotypes across the 22 generations as an estimate of realized fitness in the field and compared them to the phenotypic assays from the greenhouse. The results showed a tendency for high-fitness genotypes (positive frequency changes) to flower earlier and to produce more seeds than low-fitness genotypes. However, a simulation model showed that the observed correlations could be explained by drift alone, as long as Ne is lower than ca. 150 individuals. The findings were thus consistent with an adaptive evolutionary change in response to selection, but drift could only be excluded as the sole explanation if the effective population size was large enough.

The study did provide two estimates of Ne (19 and 136 individuals, based on individual microsatellite loci or multilocus genotypes, respectively), but both are problematic. First, frequency changes over time may be influenced by the presence of a seed bank or by immigration from a genetically dissimilar population, which may lead to an underestimation of Ne (Wang and Whitlock 2003). Indeed, the low effective size inferred from the allele frequency changes at microsatellite loci appears to be inconsistent with levels of genetic diversity found in the population. Moreover, high self-fertilization reduces effective recombination and therefore leads to non-independence among loci. This lowers the precision of the Ne estimates (due to a higher sampling variance) and may also violate the assumption of neutrality due to the possibility of selection (e.g., due to inbreeding depression) at linked loci, which may be anywhere in the genome in case of high degrees of self-fertilization.

There is thus no definite answer to the question of whether or not the observed changes in flowering time in this population were driven by selection. The study sets high standards for other, similar ones, in terms of thoroughness of the analyses and care in interpreting the findings. It also serves as a very instructive reminder to carefully check the assumptions when estimating neutral expectations, especially when working on species with complicated demographies or non-standard life cycles. Indeed the issues encountered here, in particular the difficulty of establishing neutral expectations in species with low effective recombination, may apply to many other species, including partially or fully asexual ones (Hartfield 2016). Furthermore, they may not be limited to estimating Ne but may also apply, for instance, to the establishment of neutral baselines for outlier analyses in genome scans (see e.g, Orsini et al. 2012).

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Reviews

Toggle reviews

Revision round #2

2021-04-19

Author's Reply

Download author's reply (PDF file)Download tracked changes file

Dear Dr Haag,

Thanks for handling the review of our manuscript. 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. The new version is also available online on Biorxiv : https://www.biorxiv.org/content/10.1101/2020.08.21.261230v3

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 round #2

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

Reviewed 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.

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

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.

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

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

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

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

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?

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.

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?

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

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

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.

p. 5, second paragraph, "whether selection in 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?

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?

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"?

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.

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

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?

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

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.

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.

Reviewed 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.

Revision round #1

2020-10-26

Author's Reply

Dear Dr Haag, Please find enclosed a revised version of our manuscript. We are very grateful to you and the reviewers for the comments and suggestions that have improved the manuscript substantially. We tried to answer to all of them (see the point-by-point reply below). We provide a track-changes version with line numbers, where the changes in the main text and supplementary files are highlighted in bold. We also added a revised version that you can find after the track-changes (starting page 19). 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 round #1

Dear Dr Gay, Thank you for submitting your preprint "Evolution of flowering time in a selfing annual plant: Roles of adaptation and genetic drift" to PCI Evol Biol. Your work has now been considered by three reviewers, whose comments are enclosed. As you will see, the reviews are largely positive, and, based on these reviews as well as my own reading, I am happy to further consider your preprint for recommendation. However, before reaching a final decision, I would like you to revise your manuscript according to the recommendations by the reviewers. Besides the more minor points (which also should be considered carefully), I think there are two main issues that need particular attention: - First, the introduction (and perhaps also some other sections) would profit from some streamlining. In my opinion, this does not mean that you should entirely drop the discussion of the effects of selfing on the efficacy of selection. But this section should be reduced in length and care should be taken to clearly state the objective of the study early on without raising issues (e.g., comparison between selfers and outcrossers) that are not subsequently addressed. Incidentally, from my own reading, I also think that the last part of page 1 (where you give some more detail on the different possible approaches to investigate the influence of selection on phenotypic change) would profit from some reformulation: I found this part difficult to follow and its purpose is not entirely clear to me: Do you want to provide details on some of the approaches or do you want to explain why you used only some bot not others in your study? Moreover, the statement that natural populations cannot be replicated may also need to be nuanced (replication might in principle be possible across different populations or using independent samples from the same population). - Second, the analysis of the frequency changes of the multilocus genotypes needs some clarification, both in terms of potential effects of excluding rare genotypes and in terms of confidence intervals given (likely) non-normal distribution of residuals. If you submit a revised version, please include a letter in which you describe how you have responded to each of the referees' comments. Best withes, and apologies again for the delayed decision, Christoph Haag **Additional requirements of the managing board**: As indicated in the 'How does it work?' section and in the code of conduct, please make sure that: -Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data. -Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused. -Details on experimental procedures are available to readers in the text or as appendices. -Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: "XXX is one of the PCI XXX recommenders."

Reviewed by *Pierre Olivier Cheptou*, 2020-10-20 11:18

The study by Gay et al. reports empirical data on the evolution of flowering time in a highly selfing species: Medicago truncatula. The authors used several approach to investigate the question. In particular, they used a resurrection approach with seeds from 1987 and 2009. The aim of the study is to disentangle the role of drift and selection in the shift observed as well as estimating selection gradient of flowering time. The study is interesting and the different experiments (pop centered, regional) is consistent with a shift in flowering time. Below, my comments:

1-The introduction discuss the question of adaptation face to environmental change. While the text is rich and well referenced, I found that the introduction is a bit long. There is a long discussion on whether outcrossing/selfing traits influences adaptation. The logical consequence would be to compare outcrossing/selfing populations. Since the study does not compare outcrossing and selfing populations, I think this part should be greatly reduced. Also, the statement that bottlenecks are more frequent in selfers (if true !!) would be more striking if the references were reporting empirical data. To my knowledge, Schoen and Brown (1991) and Ingvargsson 2002 hypothesize that it is the case but did not demonstrated that selfers suffer from higher bottlenecks. In the following paragraph, I found confusing to assert that "self-fertilization mays have facilitated adaptations to agricultural practices" when discussing the role of mating system on adaptation. Is it because the traits were preadapted or because the genetic architecture of selfers facilitates adaptation? In short, the introduction should be more focused to introduce the question short term adaptation of flowering time in the face of warming.

2-Sum of temperature. The individual flowering time is converted in sum of temperature. The basal temperature is assumed to be 5°C, based on Moreau et al (2007). Would it be possible that Tb has evolved during the two decades? Would the conclusions be different if flowering time were measured as the number of days? At least, the possibility of a shift in Tb should be discussed as I found contradictory to evaluate adaptation to warming but keeping Tb constant.

3-Maternal effects. If I understood well, the results on the studied populations are corrected for maternal effects (one generation to refresh seeds stock) but the results of regional analysis are based on the F1 generation (without correcting for maternal effects). I was interested by the amplitude of the shift: two days in the cape Corsica populations but five days in the regional analysis. This may be a "true result" or an effect of correcting for maternal effects. Did the authors measure the flowering date in the F1 of the cape Corsica populations. I would suggest to mention this result in the discussion. Is it possible that the difference in flowering date reported have changed in Cape Corsica population because of the F1 generation in greenhouse? My feeling is that these results are, as such, interesting. We often see this pattern of a lower amplitude after one generation. If it was only noise, the first generation should exhibit either lower or higher difference than the F2. Epigenetic components of flowering could have played a role in adaptation to warming and these effect cannot be distinguished from true quantitative genetic effects if parts of these effects last more than one generation. Do the same MLG (from 1987 and 2009) have the same fitness? Because the authors have the chance to have the same MLG, it would be interesting to look at this relationship to investigate maternal effects.

4-Genetic analysis. If I understood well, the test for selection versus drift is based only on conserved multilocus genotypes, i.e. a fraction of the population. Why doing this choice? Why not using a Qst/Fst approach that would take into account all the individuals? (the design allows to estimate Qst, doesn't it?). In addition, I see a potential bias because it assumes that the population behaves as a fully selfing populations, which is not the case. While the authors point the potential differential selective response of outcrossers versus selfers, the results reported are based only on the full selfing fraction of the population, which I found contradictory.

Overall, I found the ms interesting and such long term dataset is rare. However, the ms would benefit from being more focused (particularly the introduction) in order to highlight the results and their biological interpretation.

Reviewed by Jon Agren, 2020-10-19 15:12

This study uses a resurrection experiment and simulations to explore the possible causes of changes in flowering time and genetic composition of a Medicago truncatula population across 22 generations. In the resurrection experiment, plants grown from seeds collected 22 years apart were raised in the greenhouse to produce selfed lines. These lines were then used to document possible changes in flowering time and to quantify selection on flowering time in the greenhouse. Changes in genetic composition were characterized by scoring 20 microsatellite loci (16 kept after filtering) and documenting changes in the frequencies of multilocus genotypes. The paper is well written and addresses interesting problems of wide general interest. However, I think the authors need to (a) motivate their approach to use estimates of selection obtained in the greenhouse to infer selection in the field, (b) provide more detail on the distribution of multi-locus genotypes and the power of their analysis of change in genetic composition, and (c) clarify a few details when it comes to sampling procedure (see below).

Main comments:

1. The authors appear to assume that selection quantified in the greenhouse is likely to mirror selection in the field at present and 22 years ago. This needs to be motivated.

2. I suggest the authors provide more detail on the distribution multilocus genotype (MLG) frequencies, and that this information is given already at the start of the third paragraph on p. 7. They report that 60 different MLGs were detected in their sample of 145 individuals. Two MLGs were common, and 12 MLGs were shared between the two sampling years. This suggests that most MLGs were rare and perhaps only represented by a single plant? The authors may want to discuss whether their sample sizes are sufficient to characterize changes in genetic composition of a population with such skewed distributions of MLGs.

3. I suggest the authors clarify a few details regarding sampling:

(a) For the resurrection experiment, "100 seeds per sampling were replicated" (p. 3, second paragraph). Were these seeds from 100 different pods and thus sampled from 100 plants, or were they a random sample of 100 seeds from a pooled seed sample from each year?

(b) For the genetic analysis, leaves were sampled from "the multiplication generation in the greenhouse" (p. 4, fifth paragraph), and after filtering 145 individuals remained in the data set to be analysed. Please, state explicitly that the "multiplication generation" refers to the plants derived from the 200 field-collected seeds (presumably representing seeds from 200 plants(?); see previous comment). Were seeds from the two sampling occasions equally represented among the 145 individuals included in the analysis?

Minor corrections:

Abstract, line 11 from bottom. Change "population" to "populations"

p. 7, first paragraph, second line from bottom, "in both years". From this wording, you easily get the impression that selection was quantified in two years. I suggest you add a few words to indicate that this rather refers to a similar negative relationship being observed among lines derived from each of the two years.

To make text in graphs readable, font size should be increased in Figures (in particular in Figs. 3-5).

Reviewed by Stefan Laurent, 2020-10-16 11:05

In this study, the authors test whether flowering time evolved in an experimental population of Medicago truncatula and whether this change could represent an adaptation to varying environmental conditions. For this, they measure changes in flowering time in a natural population over 22 generations (2 timepoints), they quantify the association between flowering time and fitness (as approximated by the number of seeds produced), they track changes in haplotype frequencies characterized by different approximated fitness values, and finally they also measure changes in flowering time in 17 populations from the same geographical region that have been sampled twice over a comparable time range.

The authors report a significant reduction in flowering in the main population and in the regional analysis that appears to be consistent with the specific effects of climate change in the Mediterranean region (i.e. limiting summer drought occurs earlier in the year). They also report a significant association between flowering time and seed production. However, the evidence for the effect of positive selection obtained by analyzing the changes in haplotypes is at best marginal; even if the authors do a good job in describing some of the uncertainty associated with this analysis, I think that one more aspect should be exposed.

Besides my major comment, I find the manuscript clearly written, the analyses carefully conducted and presented, and the intro and discussion very well written and informative, at least for the non-expert.

Major comment

My only major criticism refers to the results presented in Figure 3. The selection gradients measured here seem to be heavily influenced by two outlier points with low seed production and early flowering. As a result, the linear models (especially the one for MLG found in 1987) appear to be a poor fit to the data, as can probably be seen by inspecting the residuals, which are unlikely to be normally distributed. I think that the authors should report the uncertainty around the slopes and that this uncertainty should be further considered in the analyses presented in figure 4, which will likely cause the observed selection gradient to be non-significant under a larger range of Ne values. I am not sure about the best way to obtain confidence intervals for the selection gradients but I imagine that a bootstrap approach should be applicable.

Minor

I agree with the authors that the N_e value estimated from the temporal Fst is very likely underestimated. Comparing the expect heterozygosity under Ne=19 with the observed He would further support the idea that larger Ne values are indeed realistic. How does the observed heterozygosity in the population compares to the theoretical expectations given by Nordborg and Donnelly (1997)? Rescaling the census number (>2000) by 1/(1+F) would lead to a less conservative Ne value for the test for selection and may allow a putative selection signal to be detected even after considering the uncertainty around the observed selection gradient.