

Review “**Experimental evidence for short term directional selection of epigenetic trait variation**”

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- **Title and abstract**

- Does the title clearly reflect the content of the article? [☒] Yes, [☐] No (please explain), [☐] I don't know
- Does the abstract present the main findings of the study? [☐] Yes, [☐] No (please explain), [☐] I don't know

In the abstract expression “in real time” is not intuitive here. The authors should clarify what is the idea behind. Also, unless I missed something, the selection was tested for 4 growth and flowering related traits and not only rosette size and plant height as it is mentioned in the abstract.

- **Introduction**

- Are the research questions/hypotheses/predictions clearly presented? [☒] Yes, [☐] No (please explain), [☐] I don't know
- Does the introduction build on relevant research in the field? [☒] Yes, [☐] No (please explain), [☐] I don't know

General comments

The introduction provides many references that are useful to justify the context of the study and the associated questions. Nevertheless, I believe that is that several of them do not exactly illustrate the meaning of the sentence. For instance, p.1 lines 43-44, the main highlights in Stajic et al. (2019) are that “short term epigenetic inheritance may interact with genetic changes by modifying the rate and type of adaptive mutations”. This could be used further in the discussion. Unfortunately, we cannot see if the reference was used later in the text because from the Material and Methods, the references are numbered. We can assume that they follow the order in the References List but this has to be fixed.

My other concern is about the use of “transgenerational”. I guess that the authors should give one or two references that define it. It seems that throughout the text, the term transgenerational is used irrespectively to describe inheritance in experimental designs that imply a two or more generations. To my knowledge, Lamke and Baurle (2017) provided a straightforward distinction between trans- and intergenerational inheritance and the present design seems to fall into the later.

Other minor comments:

-p2, l.58-62: split the sentence

-p2, l.66-68: specify that it has never been done before (e.g. Schmid et al. 2018)

-p2, l.78: add These “EpiRILS are a set...” to make the link with the previous sentence.

-p2, l.83: “We therefore expect that a selection differential imposed to...” Edit the sentence

- **Materials and methods**

- Are the methods and analyses sufficiently detailed to allow replication by other researchers? ☐ Yes, ☐ No (please explain), ☒ I don't know
- Are the methods and statistical analyses appropriate and well described? ☐ Yes, ☐ No (please explain), ☒ I don't know

General comments

As being non-familiar with the procedure of such analyses, I can not edit this part.

Nevertheless, for non-specialist it looks detailed and the attempts to justify concerns about the samplings of the two populations and the consequence on the reliability of the statistics are convincing. I believe that the approach based on two selection pressures (“weak” and “strong”) is a solid added value to the experimental design.

Nevertheless, the fact that the two populations P1 and P2 were not tested at the same time raises the question of the condition of storage of the seeds used to produce G0. The authors should describe the storage conditions and justify that no effects of the seedlots from the two populations could have interfered or overruled the selection effects tested in each population. In relation with the results on selection for earlier and later flowering plants (p. 14-15) and the observed differential responses to selection between the two population, this could be discussed. Did the authors notice difference in germination rates between the two populations?

Otherwise, I guess that the Figures 1a and b could be cited here.

- **Results**

- In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? ☐ Yes, ☐ No (please explain), ☒ I don't know
- Are the results described and interpreted correctly? ☒ Yes, ☐ No (please explain), ☐ I don't know

General comments

The results section is nicely presented with dedicated sub sections notably on phenotypic response to selection and epigenetic signature of selection. These results are consistent and highlight evidences of epigenetic-driven features of selection in a similar genetic background. The four characteristics tested displayed different response to selection which could be further discussed.

Other minor comments:

-p12, l.31-32: “Heritability...to selection” should be moved further above l.22 when first presenting the results on heritability. For instance, Heritability which is the heritable part (...) was estimated...”.

-p14, l.80: Thus, the divergent selection for ~~rosette~~ wider and narrower rosette...”

- **Discussion**

- Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? ☒ Yes, ☐ No (please explain), ☐ I don't know

- Are the conclusions adequately supported by the results (without overstating the implications of the findings)? ☒ Yes, ☐ No (please explain), ☐ I don't know

General comments

The discussion is short but summarized the main results. The limitations of the study and the perspectives are explained in a dedicated sub section.

I believe that the experimentation was nicely designed to reach the objective of the study. Perspective of selection over 2 generations could have been mentioned, as truly transgenerational studies, thus highlighting the need of high throughput phenotyping and epigenotyping.