Evolutionary inference from Qst-Fst comparisons: disentangling local adaptation from altitudinal gradient selection in snapdragon plants

This manuscript provides new and relevant insights on local adaptation of snapdragons based on quantitative genetic data from a common garden and previously published nuSSR data. However, I found confusing the way the authors constructed the paper, contrasting what they called 'local adaptation' (i.e. populations standing alone) versus 'adaptation to altitudinal gradients'. From my perspective, adaptation to an environmental gradient involves also local adaptation (of each of the population of the gradient). In addition, other traits with Qst > Fst but not associated with altitudinal gradients (and thus part of the 'local adaptation' group) may still be associated to other environmental gradients (that have not been measured in this study, e.g. for soils). In addition, the strongest evidence of local adaptation along the altitudinal gradient does not come from the overall Qst-Fst comparison but from the pairwise analyses and the simple correlations between phenotypic means and altitude (Figures 3 and 4). Finally, please, have a careful read of the manuscript to correct errata, uncomplete sentences, unfinished citations, etc.

Some more specific comments follow:

Introduction

1. The Qst-Fst method, as applied here, has also important flaws compared to reciprocal transplantation approaches, which I think should be presented and briefly discussed in the Introduction. Perhaps the most important one is the bias produced by the common garden testing environment. Parameters like heritability or Qst may differ greatly when computed in different environments, and in particular field estimates are very different from those obtained in common gardens. This should be acknowledged.

2. Despite being common to use adaptation to altitude as a first approach to understand adaptation to climate change, there are some fundamental differences that make the two processes very different, in particular for traits related to photoperiod where, for example, day length is also relevant. These differences should be acknowledged and discussed in the Introduction and in the Discussion.

3. I like frogs, but still, why to provide examples of frog adaptation to altitude in the Introduction? There is a rich literature in plants that could be used instead, to illustrate patterns of genetic adaptation and plasticity along altitudinal (and latitudinal, which are perhaps more relevant to discuss climate change) gradients.

Material and Methods

4. Key information is missing about the quantitative genetics models used by the authors, in particular about sample sizes. Also, it would be good to write down the model itself, as the experimental design is not very clear. For example, did the experiment follow any block design? How many families? How many full-sibs per family? I have seen Table S1, but number of samples seem similar to number of families, which I don't understand…

5. In my opinion, the most original test presented in the manuscript is the pairwise analysis of Qst versus altitudinal differences (Figure 3). I am aware that the authors designed the study to include different altitudinal gradients so that dependence with distance is broken, but still some distance effects may remain and I think pairwise distance between populations should be included in these analyses (by means of a partial Mantel test or by using it as covariate).

6. "Potential effects on population genetic differentiation… for by using AMOVA". Not clear how the authors did this… and also, should not Qst estimates be also corrected in the same way? I find a bit weird, for example, that different subspecies are pooled to estimate heritability and Qst.

Results

7. The manuscript focuses on the power of the Qst-Fst method to detect adaptation to gradients, but still most of the traits with Qst > Fst did not correlate with altitudinal variation but with other, unknown factors. The story the paper tells is, of course, the author's own business, but still it makes me wonder whether these other traits with Qst > Fst do not deserve more attention (for example in the Discussion) and a substantial change of the main argumentation line.

8. Figure 3. Differences in slope are pretty clear indeed, but I still think that they should be tested formally, using a test for differences of regression slopes.

Discussion

9. Overall I found the discussion a bit repetitive and unbalanced towards supporting the Qst-Fst approach as a means to detect adaptation along gradients. In my opinion, the advantages of the approach are not so well supported by the results. I appreciate the pairwise approach (Figure 3), which is relatively original, but still, as commented before, it does not take advantage of sampling along the gradients to produce more robust estimates.

10. “…trait homogenisation caused during the experiment by phenotypic plasticity might be another plausible explanation.” I really don’t see this point… I would say that is the other way around, that the differences you see in the field are the ones that are caused by phenotypic plasticity (please, notice that substantial additive variance and differences among families are still present in the common garden, as shown by high heritability for time to flowering and germination rate).

11. I also found weird that germination rate does not have Qst > Fst overall but it is significant in the pairwise test, i.e. Qst > Fst along altitude for pairs of populations.

Tables

12. TABLE S3. Please add the standard error to the heritability estimates.