

University of Massachusetts Amherst

Dear Dr. Rausher,

Please consider this revised manuscript: “Ecological drivers of flower-leaf sequences: aridity and proxies for pollinator attraction select for flowering-first in the American Plums” as a “Full paper” in *New Phytologist*.

Our submission has now been reviewed multiple times by three reviewers, which has improved the study and how we present it. After our most recent set of revisions, we were pleased that the reviewer found our study to be creative and novel and that we had reasonably addressed their comments through our revisions. We felt remiss, however, that we did not make it clear we had tested some of the models requested by the reviewer—we had, but could have made this clearer, and now do.

In our current submission we now fully present an analysis assessing the influence of mean spring temperature on hysteranthly variation among species. We had previously tested this and found the model had lower explanatory power than the model with aridity, and we felt temperature was not as well connected to the hypotheses. However, we can now see that many readers may want to see this analysis—especially given its results. Mean temperature was not a strong predictor of flower-leaf sequences differences among species (contrary to the reviewer’s expectation). We believe that this result will help readers better understand the environmental drivers of hysteranthly. We hope it will spur ideas for future ways to investigate the flower-leaf sequence variation across multiple scales, and are grateful to you and the reviewer for pushing us to incorporate it into our manuscript. Our manuscript now includes a new table in the Supporting Information, and text in the Methods, Results and Discussion addressing the relationship between temperature and hysteranthly. We detail these changes, and the other adjustments we have made to our submission in response the reviewer’s feedback in the following pages.

The main text of this manuscript is now 5,022 words in length and it contains four figures. It is co-authored by T.J. Davies, S. Collins and E.M. Wolkovich. We hope that you will now find it suitable for publication in *New Phytologist*, and look forward to hearing from you.

Sincerely,

A handwritten signature in black ink, appearing to read 'D. Buonaiuto', with a stylized, cursive script.

Daniel Buonaiuto

Reviewer comments are in italics; author responses are in plain text.

Referee: 1

Comments to the Author

This is an innovative and creative paper that will be noticed and read. The authors have revised their paper in a reasonable way in response to the comments. Many of the suggestions have been addressed. Figure 3 is now greatly improved. The results are better explained, and the problems with the approach are more directly acknowledged. My view is that this paper is very creative and even novel, but also problematic. Among the phenology community, this article will stimulate conversation about whether or not the results are convincing, and if there was a better way to have done this study, which is a good fate for any paper.

We are grateful to the reviewer for their continued attention on our manuscript, and appreciate that they see the potential for substantial impact. We are pleased that they feel that our latest set of revisions improved this work.

The authors resist examining variation within species, arguing that their sample sizes are too small. However, there are 200 specimens per species, which is very large sample size for these types of analyses, and much greater than the number of species being compared. Readers will wonder why this analysis is not being done.

We appreciate the reviewer’s point that our inter-specific analyses naturally raise questions about intra-specific variation in flower-leaf sequences, and have expanded text in our Discussion (lines 363-366) regarding this. The text now reads:

Because our study was based on herbaria records collected on different individuals across space and time without repeat sampling, we could not robustly estimate how much flower-leaf sequences vary within vs. among species, and within individuals over time. Parsing in the influence of multiple climate drivers across multiple scales of ecological and evolutionary time would be an important next step for understanding how the environment and species interactions have shaped these phenological patterns.

We suspect that there are separate biological processes driving flower-leaf sequences on the different time scales of inter- and intra-specific adaptation, and that the physiological cues that control individual plasticity are not necessarily the same ones that influence intra-specific adaptation.

To understand these factors would require a model that is powerful enough to disentangle the effects of climate at each of the scales, and we do not feel we have the dataset or model(s) for that challenge. The sampling below the species-level in our current data is shown in Table S1 and ranges from 17 to 118 observations/species, with 50% of our species having fewer than 80 observations (thus, our within-species phenology sampling size is not 200/species). These diminishing sample sizes below the species-level are also spread unevenly across the geographic ranges of each species and across the 100+ year time series of our data—accounting for such biases would require a modeling approach that is beyond the scope of our current analyses. Nonetheless, we appreciate the reviewer’s interest in the drivers of variation below the species-level.

The authors resist examining the effects of temperature on hysteranthy. This would be such a simple analysis to do and carry out, as temperature data is readily available. To show the value of this approach, just compare the distributions of the 5 fully hysteranthus species with the 7 partially hysteranthus species. 4 of the 5 fully hysteranthus specie are southern USA species, whereas only 2 of the 7 partially hysteranthus species are southern species. It is a virtual certainty that the March temperatures of the hysteranthus species are warmer than the March temperatures of the partial hyseteranthus species, and these differences explain as much of the variation as aridity. Readers will wonder why such a simple geographic and temperature analysis was not carried out.

Reviewing this comment we realized that we did not make it sufficiently clear in our previous response letter that we did in fact follow the reviewer’s suggestion to examine the effects of temperature on hysteranthy. Because the results of this analysis indicated that mean spring temperature was not a good predictor of

species-level patterns of hysteresis, and the model explained substantially less variation than our model with aridity, we did not add these analyses into our previous submission. We now realize—based on the editor and reviewer’s points—that readers may be interested in seeing this relationship, and have added details regarding this analysis to our study (lines 194-198, 262-265, 291-293), and now include a new table in our Supporting Information (Table S4).

A detailed exploration of species’ distributions makes this lack of a relationship between temperature and hysteresis more understandable. Several of the species from the southern-most, warmest regions of the US (e.g., *P. texana* and *P. rivularis*) have only intermediate likelihoods of being hysteresis (index scores of .51, .44)—less than the likelihoods of the three northern-most species *P. nigra*, *P. alleghaniensis* and *P. americana* (index scores of 0.55, 0.58, and 0.62 respectively). Other more northern species (e.g., *P. maritima* and *P. angustifolia*) are among the most likely to be hysteresis (index scores of .68 and .76 respectively). Though we can see how the reviewer would feel this relationship between temperature and hysteresis should be expected based on broad biogeographical patterns of *northern vs. southern distributions*, the data highlights that this relationship is more complex. The results from our new analysis in Table S4 now quantitatively address this—so we are grateful to the reviewer and editor for pushing us to include this in our paper.

For the aridity index, the authors use aridity in June-August. However these plants flower Feb-April. It would be much better to use an index that corresponds to the flowering period.

The June-August period is a standard seasonal window developed by climatologists to capture variation in plant-relevant water stress. Further, as mentioned in our the previous letter, this is one of the only publicly available datasets that reconstructs historical aridity on a time-scale most relevant to the questions in our study.

We also believe, for our purposes, June-August is the more biologically appropriate window compared to the spring window. Selective pressures driving an adaptation to water stress would be strongest in the driest period of the season (June-August in our study system). As we discuss in lines 60 and 319, the water limitation hypothesis suggests hysteresis evolved to partition hydraulic demand across the season—such that leaves and flowers are not transpiring simultaneously and driving additive water loss during periods of water stress. This does not necessarily suggest that aridity measured during the flowering period only should affect this pattern. This is highlighted by the fact that the flowering of hysteresis species in the dry tropics (where this hypothesis was originally applied) is associated with the seasonal *recovery* of plant water-status (Franklin, 2016)—i.e., the expectation of the hypothesis is that flowering occurs outside of the driest seasonal window, but the dryness of those periods is the selective force on hysteresis. We hope this further clarifies our choice of metrics.

On line 323, it says that the values of aridity range from -0.5 to 0.2, but figure 3 shows values from -1.5 to 1.5. What is the explanation?

The values in Figure 3 are z-scored, allowing us to compare the estimates for PDSI to those for petal size. We have adjusted the figure caption to make this clear.

My intention in making these suggestions is to make the authors aware of these issues, even if they choose to keep the paper the way that it is.

We are grateful to the reviewer for their perspective on these issues.