

Flower-leaf phenological sequence variation in the American Plums (*Prunus* sect. *Prunocerasus*) reflects adaption to aridity

August 16, 2022

Introduction

Woody perennials have a unique ability among plants to seasonally begin reproduction prior to vegetative growth. This flowering-first phenological sequence known as hysteranthly, proteranthly or precocious flowering is particularly common in temperate forests around the globe (Rathcke & Lacey, 1985). A number of studies suggest that this flower-leaf sequences (FLSs) are under selection, and that hysteranthly has functional significance (Gougherty & Gougherty, 2018; Buonaiuto *et al.*, 2021; Guo *et al.*, 2014).

The most common, and well-tested explanation for the evolution of hysteranthly in temperate forests is that it is adaptive for wind-pollination, as leafless canopies increase wind speeds for pollen transport and reduce the likelihood of pollen interception on vegetation (Whitehead, 1969; Niklas, 1985). However, this hypothesis fails to address the prevalence of hysteranthous taxa that are biotically-pollinated. Approximately 30% of woody plant species of Eastern temperate forests of North America flower before leafing out, and of these, approximately 20% are biotically pollinated (Buonaiuto *et al.*, 2021). Despite the pervasiveness of this phenological syndrome, direct tests of the function of hysteranthly in biotically pollinated taxa are rare for temperate forest species.

Yet looking to other biomes in which hysteranthous flowering is also common offers important insights regarding the function of hysteranthly in temperate, biotically-pollinated taxa. In the dry-deciduous tropics of South and Central America, flowering during the leafless period is also common (Rathcke & Lacey, 1985; Franklin, 2016). In these ecosystems, flowering is associated with a recovery in plant water status due to leaf drop (Borchert, 1983; Reich & Borchert, 1984). By temporally separating leaf and flower activity, woody plants can partition the hydraulic demand across the season, alleviating water stress (Gougherty & Gougherty, 2018; Franklin, 2016). These physiological observations suggest that hysteranthous flowering may be an adaptation to arid environments.

It is unclear whether this hydraulic demand hypothesis (also known as water dynamic hypothesis (Gougherty & Gougherty, 2018) or water limitation hypothesis (Buonaiuto *et al.*, 2021)) is relevant in the temperate zone where forests are rarely water-limited in the early season during which flowering and leafing occur (Polgar & Primack, 2011). Yet the hypothesis yields several predictions that can be tested to evaluate whether hysteranthly serves to increase aridity tolerance in temperate flora:

1. Hysteranthous taxa should be found in dryer habitats compared to closely related, non-hysteranthous species.
2. Hysteranthly may be linked to other reproductive traits associated with dry environments such as

reduced flower and fruit size (Herrera, 2009; Liu *et al.*, 2013).

With mounting evidence anthropogenic climate change is both driving shifts in flower-leaf sequences (Ma *et al.*, 2020) and changing geographic patterns of water availability (Overpeck & Udall, 2020), understanding the functional significance of hysteresis is vital to forecasting the demography and performance of forest communities in an era of global climate change. However, there are two major methodological challenges to testing the hydraulic demand hypothesis:

First, characteristics like aridity tolerance, are the emergent product of a suite of biological traits (Šímová *et al.*, 2017). Thus, when analyzing selective drivers of any particular trait at large taxonomic scales, unmeasured trait differences may obscure the estimated effects of the trait of interest, biasing results. This is a common problem in trait-based ecology, and one of the most promising solutions for understanding the functional significance of hysteresis in woody plants is through character deconstruction (Terribile *et al.*, 2009); comparing flower-leaf sequences variation for only a subset of taxa of shared phylogenetic and morphological character.

A second challenge for robust testing of hysteresis hypotheses is that most characterizations of flower-leaf phenological sequences are based on expert-opinion verbal descriptions (e.g. “flowers before leaves” or “flower before/with leaves”), which make comparisons across taxa, time and space difficult and sensitive to observer bias (see; Buonaiuto *et al.*, 2021).

This problem can be overcome by adopting standardized quantitative measures of plant phenology for observational studies and applying them to historic data records. Herbarium records are an excellent source of data that can be leveraged for quantitative phenological measurements (Willis *et al.*, 2017), but have not been used widely to investigate variability of flower-leaf sequences variation among and within species.

In this study, we used herbaria records to quantify flower-leaf sequences both within and among species in the American plums, (subspecies *prunus*, sect. *prunocerasus*). We then evaluated the association between hysteresis and several ecological and morphological traits to test the predictions of the hydraulic demand hypothesis of hysteresis. Our findings both clarify the hypothesized function of flower-leaf sequence variation in biotically-pollinated taxa, and offer insights into how flower-leaf sequences may impact species distributions as climate continues to change.

Methods

0.1 Study system

The genus *Prunus* comprises approximately 200 species distributed across the globe (Chin *et al.*, 2014). Within the genus, The American plums (*Prunus* subsp. *prunus* sect. *prunocerasus*) offer potential for a higher resolution investigation of drivers of hysteranthous flowering. The 16 species that make up the section are distributed across North America and, like the genus *Prunus* at large, show pronounced inter-specific variation in flower-leaf sequences. While within the larger genus species can be separated into three distinct morphological clades by inflorescence architecture (solitary, corymbose or racemose) all members of the section share solitary inflorescences (Shaw & Small, 2004) allowing for refined character deconstruction. Species in this section are well represented in herbaria records (Fig. 1), making them a tractable group to measure and assess variation in flower-leaf sequences as well as other ecological and morphological characteristics related to the hydraulic demand hypothesis.

0.2 Quantifying flower-leaf sequence variation

We obtained digital herbarium specimens for all member of the section *Prunocerasus* from the Consortium of Midwest Herbaria Database. To quantify the flower-leaf sequence variation within and across species we randomly sample 200 specimens for each species and scored the phenological development of flower and leaves in accordance with using a modified BBCH scale for woody plants (Finn *et al.*, 2007). In total, we evaluated the phenology of 2521 specimens, but only specimens with visible flower were included in this analysis (n=1009). We reconstructed the phylogenetic relationships among species in this group based on the tree topology in Shaw & Small (2004). Following the methods of ? we computed branch lengths for this phylogeny by assigning each node a height and computing the distance between upper and lower nodes using the R package “ape” ().

To quantify FLS variation, we fit an ordinal, hierarchical, Bayesian, phylogenetic mixed model (de Villemereuil P. Nakagawa, 2014) to assess the likelihood an individual would be at any given vegetative BBCH phase while flowering. Because we expect that hysternathy is more likely to occur earlier in the flowering period and species differ in their flowering periods, we included the day of the observation as a varying slope, main covariate effect in the model and species and phylogeny as random effects. The model is written below:

$$\text{logit}(P(Y \leq j)) = \beta_{[j]sp[i]} + \beta_{[j]sp[i]} + \beta_{dayofyear[sp[i]]} * X_1 + \epsilon_i$$

$$\epsilon_i \sim N(0, \sigma_y^2)$$

where Y is the ordinal outcome (leaf stage) and j is the number of categories (1,2,...6). $P(Y \leq j)$ is the probability of Y less than or equal to a category j=1,...j-1. In this varying slope and intercept model, $\beta_{[j]}$ describes an intercept for each category [1,2,...6], while slope $\beta_{dayofyear[sp[i]]}$ is constant across categories.

The influence of the phylogeny α_{phylo} was modeled as follows:

$$\alpha_{sp} \sim N(\mu_\alpha, COR[\sigma_{phylo}^2])$$

The α for species effects independent of the phylogeny was modeled as follows:

$$\alpha_{sp} \sim N(\mu_\alpha, \sigma_{species}^2)$$

We fit the model in the R package “brms” (Bürkner, 2018) using weakly informative priors, and ran the model on four chains with a warmup of 3,000 iterations and 4,000 sampling iterations for a total of 4,000 sampling iterations. Model fit was assessed with Rhats <1.01 and high effective sample sizes and no divergent transitions.

Because the day of observation strongly influenced the BBCH stage likelihood, quantifying flower-leaf sequences among species was intractable without accounting for this temporal trend. To address this issues, we used our model to predict the likelihood each species would be observed at a given vegetative BBCH stage during flowering at the 0%, 25% 50% and 75% quartiles of their flowering period. We then developed a flower-leaf sequence index, by assigning a numerical score to each species per seasonal quantile, and summing over the full flowering season. In each seasonal quantile, species received a 1 if more that 50% of their probability distribution occurred at BBCH 0 and BBCH 09 and a 0 if not. These values were summed across the season generating an index from 0 (never hysternanthous) to 4 (hysternanthous through late season (Q75)), where 1= hysternanthous at start of season, 2= hysternanthous through early season (Q25) and 3 = hysternan-

thous through mid season (Q50). We also used two alternative indexing schemes (>25% of the probability distribution occurred at BBCH 0 and >40% of the probability distribution occurred at BBCH 0 and BBCH 09).

0.3 Evaluating the hydraulic demand hypothesis

To test the predictions of the hydraulic demand hypothesis of hysteranthy we obtained data on petal length, fruit diameter and directly from herbarium specimens and characterized the aridity of the sites specimens were collected from using the Palmer Modified Drought Index (PDSI).

For our morphological measurements, we sampled an additional 321 specimens measured the petal length of up to 10 randomly selected petals per specimen (n=2757) using ImageJ image processing software. We also used ImageJ to measure the diameter of fruits on an additional 316 specimens, measuring up to 5 fruit per specimen (n=224). We computed the average Palmer Modified Drought Index score from 1900-2017 for every *Prunocerasus* specimen in the database (n=2305) from the North America Drought Atlas (Cook & Krusic, 2004).

We then used Bayesian phylogenetic mixed models to test the relationship between flower-leaf sequence index scores and each of the variables. In these models, we included species and phylogeny as the random effect. For our PDSI model, we did not include phylogeny as a random effect as PDSI is an environmental trait rather than a biological one. *Question for Jonathan: Does this check out?*

The model structure is written below:

$$y_i = \alpha_{ind/sp[i]} + \alpha_{phylo[i]} + \beta_{hyst.index} * X_{hyst.index} + \epsilon_i$$

$$\epsilon_i \sim N(0, \sigma_y^2)$$

The effect of the phylogeny was model as above.

Like above, we fit these models in the R package “brms” (Bürkner, 2018) using weakly informative priors, and ran the model on four chains with a warmup of 3,500 iterations and 4,500 sampling iterations for a total of 4,000 sampling iterations. Model fit was assessed with Rhats <1.01 and high effective sample sizes and no divergent transitions. We also ran each model using our two alternative FLS indexing approaches to make sure our particular indexing approach was not influencing our results (see Supplement for details).

Results

Quantifying flower leaf sequences in the American plums

We found substantial inter-specific differences in flower-leaf sequences within the American plums, and that patterns were strongly dependent on the day of observations, with observations later in the flowering season of each species decreasing the likely hood of finding flowers open during early vegetative BBCH phases (β_{day} 0.03, CI_{50} [0.02,0.03]). Based on our flower leaf sequence index, two species (*P. umbellata*, *P. mexicana*) were likely to be hysteranthous regardless of the time of observation and three species (*P. rivularis*, *P. subcordata*, and *P. texana*) were always most likely to flower after level expansion began (Fig. 2). All other species displayed intermediate phenotypes with five species mostly likely to hysteranthous at the start of the

season (*P. alleghaniensis*, *P. americana*, *P. hortulana*, *P. munsoniana* and *P. nigra*), one species through early season (*P. gracilis*) and two species through mid season (*P. angustifolia*, *P. maritima*) (Fig 2).

Evaluating the Hydraulic demand hypothesis

We found a negative association between flower-leaf sequence index and mean pdsi (β : -0.03, CI_{50} [-0.05, 0.02], Fig. 3a.), suggesting that species that displayed hysteranthous flowering later into their flowering season were found in dryer locations.

We found a negative association between flower-leaf sequence index and both petal length and fruit diameter (-0.21, CI_{50} [-0.38 -0.04], -1.40, CI_{50} [-1.97 -0.82] respectively), though the relationship between FLS index and fruit size was much stronger (Fig. 3b.,c.).

Discussion

Aridity tolerance and flower-leaf sequence variation

Our analyses suggest that within the American plums, hysteranthous taxa occur in more arid environments and are associated with drought-tolerant reproductive traits like reduced flower and fruit size. These associations support the hydraulic demand hypothesis of hysteranthous flowering. These results suggest that even though water limitation less common during the flowering season in temperate trees, the temporal segregation of flowering and leaf phenology can still impact whole plant water status later in the season.

Studies that have compared the transpiration rates among flowers and leaves that occur together provide insights to the potential importance of this seasonal partitioning for maintaining water status. These studies report floral transpiration rates can range from 20%-60% of leaves under comparable conditions (Whiley *et al.*, 1988; Roddy & Dawson, 2012) which can drive loss of stomatal conductance and low photosynthetic rates (Galen *et al.*, 1999). A recent study Liu *et al.* (2017) comparing hydraulic properties of flowers and leaves in two hysteranthous Magnolias, found that sap flow to flowers was 22-55% that of leaves. When considering species in our study specifically, the xylem conductivity of spring floral branches of *Prunus americana* is reported to be 20% of summer foliage branches (McMann *et al.*, 2022). Together these magnitudes of water loss through flowers suggested support our hypothesis (say better).

The relationship between flower and fruit size and hysteranthous needs some thinking about. It is well established that larger flowers demand more resources to maintain turgor and reproductive function (Galen *et al.*, 1999; Lambrecht & Dawson, 2007). Therefore a classic ecological tradeoff might predict hysteranthous compensates for larger flowers and fruits in dry environments. The fact that we found the opposite suggest rather, that these drought adaptations might be part of a suite of traits that operate to increase the aridity tolerance of a species.

Of course, selection on both phenology and floral traits is driven by a number of other factors than just plant hydraulics. The support we found for the hydraulic demand hypothesis does not rule out other eco-evo drivers shaping the flower-leaf sequences of insect-pollinated. In fact, the relationship we observed between hysteranthous with flowering and fruit size could also be evidence to alternative hypotheses for FLS.

Alternative hypotheses of flower-leaf sequence variation in biotically-pollinated woody plants

The negative relationship between hysternathy and flower size we observed in our analyses reflects a tradeoff predicted by the insect visibility hypthothesis of hysternanthous flower. This hypothesis suggests that flow

References

- Borchert, R. (1983) Phenology and control of flowering in tropical trees. *Biotropica* **15**, 81–89.
- Buonaiuto, D.M., Morales-Castilla, I. & Wolkovich, E.M. (2021) Reconciling competing hypotheses regarding flower–leaf sequences in temperate forests for fundamental and global change biology. *New Phytologist* **229**, 1206–1214.
- Bürkner, P.C. (2018) Advanced bayesian multilevel modeling with the r package brms. *R Journal* **10**, 395–411.
- Chin, S.W., Shaw, J., Haberle, R., Wen, J. & Potter, D. (2014) Diversification of almonds, peaches, plums and cherries –molecular systematics and biogeographic history of prunus (rosaceae). *Molecular Phylogenetics and Evolution* **76**, 34–48.
- Cook, E. & Krusic, P. (2004) *The North American Drought Atlas*.
- de Villemeruil P. Nakagawa, S. (2014) *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*, chap. General quantitative genetic methods for comparative biology, pp. pp. 287–303. Springer, New York.
- Finn, G.A., Straszewski, A.E. & Peterson, V. (2007) A general growth stage key for describing trees and woody plants. *Annals of Applied Biology* **151**, 127–131.
- Franklin, D.C. (2016) Flowering while leafess in the seasonal tropics need not be cued by leaf drop: evidence from the woody genus brachychiton (malvaceae). *Plant Ecology and Evolution* **149**, 272–279.
- Galen, C., Sherry, R. & Carroll, A. (1999) Are flowers physiological sinks or faucets? costs and correlates of water use by flowers of polemonium viscosum. *OECOLOGIA* **118**, 461–470.
- Gougherty, A.V. & Gougherty, S.W. (2018) Sequence of flower and leaf emergence in deciduous trees is linked to ecological traits, phylogenetics, and climate. *New Phytologist* **220**, 121–131.
- Guo, L., Luedeling, E., Dai, J. & Xu, J. (2014) Differences in heat requirements of flower and leaf buds make hysternanthous trees bloom before leaf unfolding. *Plant Diversity and Resources* **36**, 245–253.
- Herrera, J. (2009) Visibility vs. biomass in flowers: exploring corolla allocation in mediterranean entomophilous plants. *Annals of Botany* **103**, 1119–1127.
- Lambrecht, S.C. & Dawson, T.E. (2007) Correlated variation of floral and leaf traits along a moisture availability gradient. *Oecologia* **151**, 574–583.
- Liu, H., Xu, Q.Y., Lundgren, M.R. & Ye, Q. (2017) Different water relations between flowering and leaf periods: a case study in flower-before-leaf-emergence *magnolia* species. *Functional Plant Biology* **44**, 1098–1110.
- Liu, J., Bai, Y., Lamb, E.G., Simpson, D., Liu, G., Wei, Y., Wang, D., McKenney, D.W. & Papadopol, P. (2013) Patterns of cross-continental variation in tree seed mass in the canadian boreal forest. *PLOS ONE* **8**, e61060–.

- Ma, Q., Huang, J.G., Hänninen, H., Li, X. & Berninger, F. (2020) Climate warming prolongs the time interval between leaf-out and flowering in temperate trees: Effects of chilling, forcing and photoperiod. *Journal of Ecology* **n/a**.
- McMann, N., Peichel, A. & Savage, J.A. (2022) Early spring flowers rely on xylem hydration but are not limited by stem xylem conductivity. *New Phytologist* **233**, 838–850.
- Niklas, K.J. (1985) The aerodynamics of wind pollination. *The Botanical Review* **51**, 328–386.
- Overpeck, J.T. & Udall, B. (2020) Climate change and the aridification of north america. *Proceedings of the National Academy of Sciences* **117**, 11856–11858.
- Polgar, C. & Primack, R. (2011) Leaf-out phenology of temperate woody plants: From trees to ecosystems. *New Phytologist* **191**, 926–41.
- Rathcke, B. & Lacey, E.P. (1985) Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics* **16**, 179–214.
- Reich, P. & Borchert, R. (1984) Water-stress and tree phenology in a tropical dry forest in the lowlands of costa-rica. *Journal of Ecology* **72**, 61–74.
- Roddy, A.B. & Dawson, T.E. (2012) Determining the water dynamics of flowering using miniature sap flow sensors. 951, pp. 47–53, International Society for Horticultural Science (ISHS), Leuven, Belgium.
- Shaw, J. & Small, R.L. (2004) Addressing the "hardest puzzle in american pomology:" phylogeny of prunus sect. prunocerasus (rosaceae) based on seven noncoding chloroplast dna regions. *Am J Bot* **91**, 985–996.
- Šímová, I., Rueda, M. & Hawkins, B.A. (2017) Stress from cold and drought as drivers of functional trait spectra in north american angiosperm tree assemblages. *Ecology and evolution* **7**, 7548–7559.
- Terribile, L.C., Diniz-Filho, J.F., Rodríguez, M.Á. & Rangel, T.F.L.V.B. (2009) Richness patterns, species distributions and the principle of extreme deconstruction. *Global Ecology and Biogeography* **18**, 123–136.
- Whiley, A., Chapman, K. & Saranah, J. (1988) Water loss by floral structures of avocado (*persea americana* cv. fuerte) during flowering. *Australian Journal of Agricultural Research* **39**, 457–467.
- Whitehead, D.R. (1969) Wind pollination in the angiosperms: Evolutionary and environmental considerations. *Evolution* **23**, 28–35.
- Willis, C.G., Ellwood, E.R., Primack, R.B., Davis, C.C., Pearson, K.D., Gallinat, A.S., Yost, J.M., Nelson, G., Mazer, S.J., Rossington, N.L., Sparks, T.H. & Soltis, P.S. (2017) Old plants, new tricks: Phenological research using herbarium specimens. *Trends in Ecology & Evolution* **32**, 531–546.

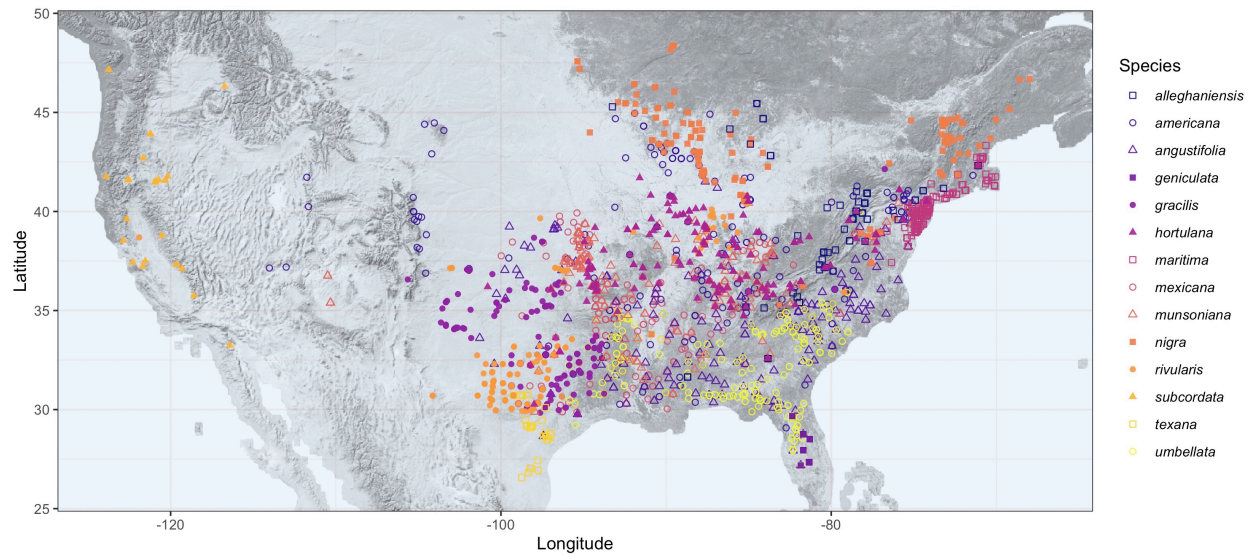


Figure 1:

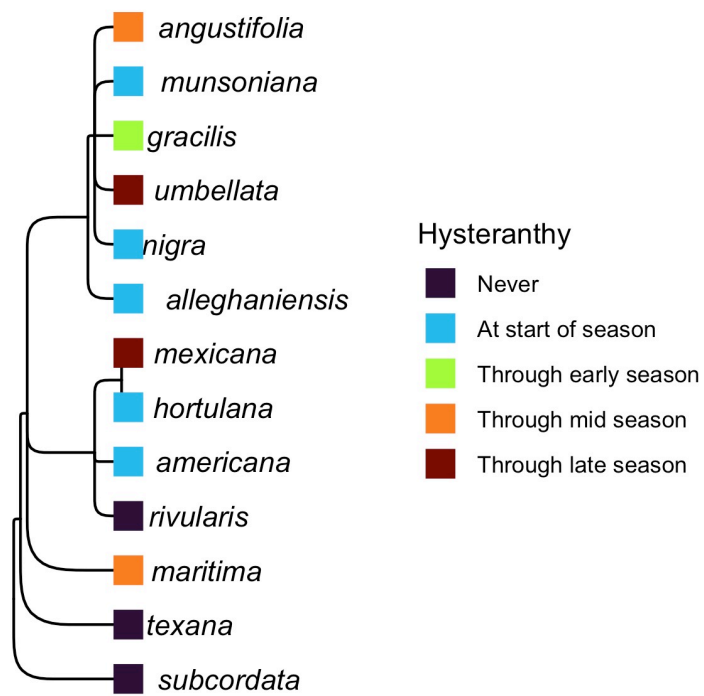


Figure 2:

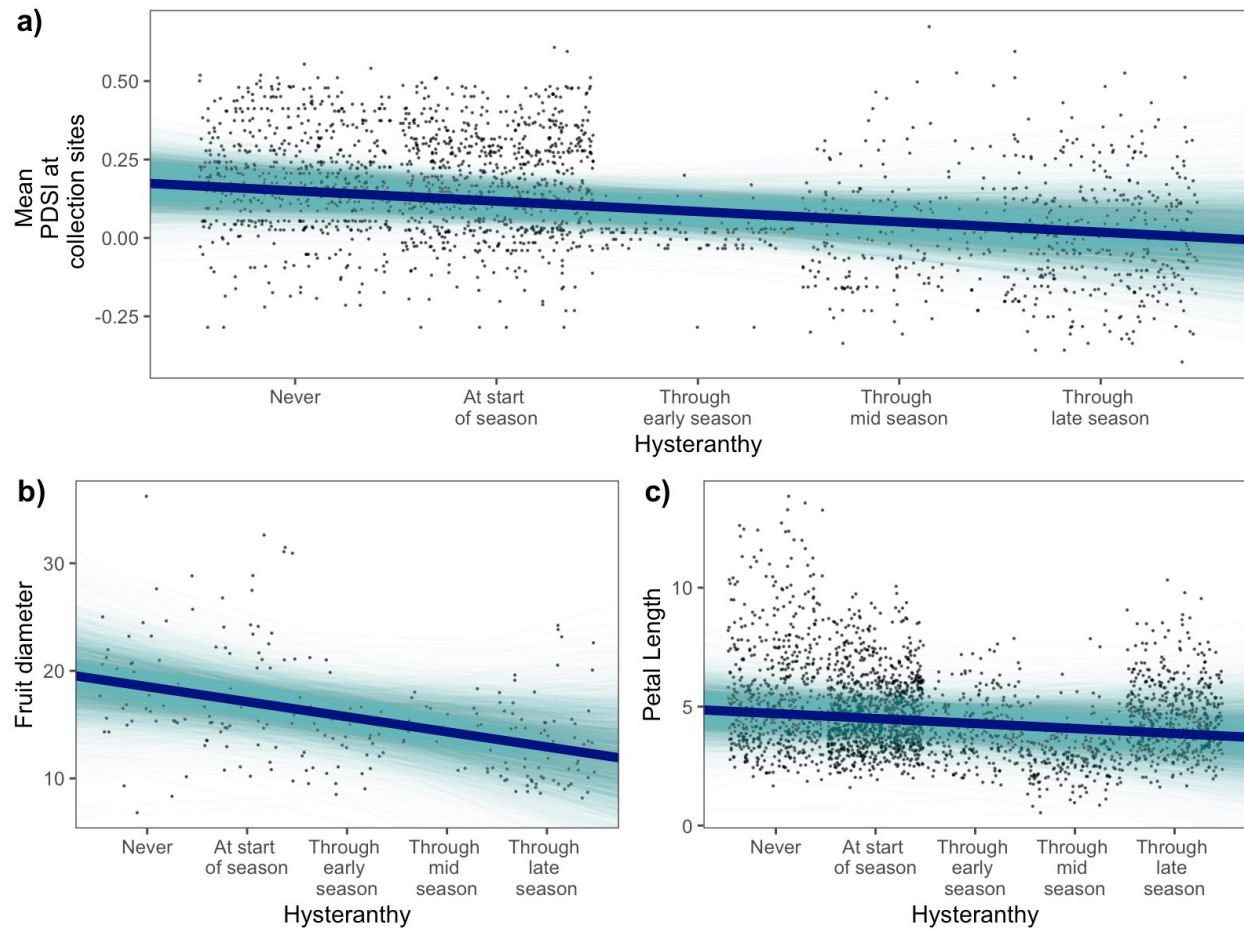


Figure 3: Relationships between the duration of hysteresis across the flowering period and environmental and biological traits