Flower-leaf phenological sequences in the American Plums (*Prunus* sect. *Prunocerasus*) reflect adaption to aridity

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Introduction

Woody perennials have a unique ability among plants to seasonally begin reproduction prior to vegetative growth. This flowering-first phenological sequence known as hysteranthy, proteranthy 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 hysteranthy 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 hysteranthy 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 hysteranthy 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 hysteranthy 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 their 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 refered to as the 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 to evaluate whether hysteranthy 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. Hysteranthy may be linked to other reproductive traits associated with dry environments such as

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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 hysteranthy 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, un-measured 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 func-tional significance of hysteranthy 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 hysteranthy 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 be used widely to investigate variability of flower-leaf sequences variation among and within species.

In this study,we used herbaria records to to quantify flower-leaf sequence patterns in the American plums, (subspecies *prunus*, sect. *prunocerasus*. We then evaluated the association between hysteranthy and several ecological and morphological traits to test the predictions of the hydraulic demand hypothesis of hysteranthy. 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* subspp. *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 sequence variation within and across species we randomly sample 200 specimens for each species and scored the phenological development of flower and leaves 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 Grafen & Hamilton (1989) 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" ().

Need to write this part more professionally. To compute a phylogentic signal for flower-leaf sequence variation, we calculated the mean of the log(vegetative BBCH observed during flowering) for each species and calculated Bloomberg's K using the function phylosig ().

To quantify FLS variation, we fit an ordinal, hierarchical, Bayesian, phylogenetic mixed model (de Villemeruil P. Nakagawa, 2014) to assess the likelihood an individual would be at any given vegetative BBCH phase while flowering. Because we expect that hysteranthy 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 effect in the model and species and phylogeny as random effects. The model is written below:

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

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

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 of 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% quantiles 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 hysteranthous) to 4 (hysteranthous through late season (Q75)), where 1= hysteranthous at start of season, 2= hysteranthous through early season (Q25) and 3 = hysteranthous through mid season (Q50). We also used two alternative indexing schemes (>25% of the probability distribution occured at BBCH 0 and >40% of the probability distribution occurred at BBCH 0 and BBCH 09).

0.3   Evaluating the hydralic demand hypothesis

To test the predictions of the hydralic 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 than 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 a environmental trait rather than a biological one. *Question for Jonathan: Does this check out?*

The model structure is written below:

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

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

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. Though these alternative classification scheme did change the hysteranthy index score for some species (Fig. S1), they changes did not substantially impact the interence from our models (see Tab. S1 for comparisons).

Results

Quantifying flower leaf sequences in the American plums

We found substantial inter-specific differences in flower-leaf sequences within the American plums (Fig. 2, S1). The phylogenetic signal was relatively weak (Phylogenetic signal K : 0.28), and flower-leaf sequences patterns

were strongly dependent on the day of observations, with observations later in the the flowering season of each species decreasing the likely hood of finding flowers open during early vegetative BBCH phases (β_{doy} 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. 3). 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 3).

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. 4a.), 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 (-.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. 4b.,c.).

Discussion

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 indicate that even though water limitation less common during the flowering season in temperate trees, the temporal segregation of flowering and leaf phenology can impact whole plant-water status later in the season.

Studies that have compared the transpiration rates among flowers and leaves that occur simultaneously provide insights to the potential importance of this seasonal partitioning for maintaining water status. These studies report floral transpiration rates of flowers can range from 20%-60% of that of leaves under comparable conditions (Whiley *et al.*, 1988; Roddy & Dawson, 2012). This additional hydraulic demand can drive loss of stomatal conductance and decrease photosynthetic rates (Galen *et al.*, 1999). A recent study Liu *et al.* (2017) comparing hydraulic properties of flowers and leaves in two hysteranthous tree species (*Magnolia spp*), found that sap flow to flowers (a measure of water movement) was 22-55% that of leaves. When considering species in or 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). Taken together, magnitude of water loss through floral organs in these physiological measurements demonstrate an underlying mechanism for the macro-ecological patterns we observed in our data.

Our finding that smaller flowers were associated with extended hysteranthy in the Amerian plums may be surprising when viewed in the context of a classic ecological tradeoff. It is well established that larger flowers demand more resources to maintain turgor and reproductive function than smaller ones(Galen *et al.*, 1999; Lambrecht & Dawson, 2007), therefore one might expect that hysteranthous flowering serves to compensate for maintaining larger flowers and in dry environments. The fact that we observed a negative association between the degree of hysternathy and flower size suggests rather, that hysteranthy might be part of a suite of traits that operate to increase the aridity tolerance of a species.

The negative relationship between hysteranthy and flower size we observed is also expected when considered in the context of resource allocation. A negative trade-off for the benefits gained by hysteranthous flowering is that hysteranthous woody plants much begin their reproductive investment from stored carbon alone; at the time of their annual cycle when their stored reserved are likely at their lowest (). The association between smaller flower and hysteranthous flowering has been observed in other clades (), though to our knowledge has not been investigated on the context of hydraulic costs or aridity tolerance. It it, however, clear that hysteranthous species have evolved specialized mechanisms for mobilizing water and carbohydrates early in the season to accommodate this resource partitioning strategy (). Sum up this paragraph.

Of course, selection on both phenology and floral traits is driven by a number of other factors than just plant hydraulics and resource allocation. The support we found for the hydraic 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 hysteranthy with flowering and fruit size could also be evidence for alternative hypotheses for FLS.

Pollinator attraction is major selective force on both floral phenology and morphology () and it has been alternatively suggested that hysteranthous flowering is an adaptation to increase the visibility of flowers to visually-foraging pollinators (). To our knowledge this hypothesis has not bee widely tested though there is evidence that background contrast does impact pollinators ability to locate flowers (). This hypothesis and the hydraulic demand hypothesis that we tested may be related, with loss of pollinator visibility that is associated with accompany the reduction in flower size () due to aridity compensated for by hysteranthous flowering. While we cannot make this mechanistic link from our analysis, our findings that hysteranthy is associated with aridity and reduced flower size set up intriguing follow up work to elucidate the ecological and evolutionary links between floral morphology and function, aridity and hysteranthous flowering.

In this study we intentionally chose to analyze a small, and morphologically restricted taxonomic clade in order to reduce the impact of unmeasured biological variation on our traits of interest. Our findings compliment and clarify previous analyses performed at large taxonomic scales which suggested that aridity may be a more important driver in biotically pollinated taxa than wind pollinated ones (). It was interesting we found a relatively weak influence of phylogeny at our restricted scale, while it appears to be quite strong at larger ones (). *Jonathan is there something interesting we can say about this in a line or two?*

To compliment the work that has been done at both very fine and course taxonomic resolution, it would be useful to explore the drivers of hysteranthy at an intermediate scale to better understand if the associations we observed in the American plums emerged at the genus or family level, or in other clades with strong intra-generic flower-leaf sequence variation like Rhododendron, Magnolia, Acer, Cornus.

Flower-leaf sequences patterns can vary substantially on an interannual basis with populations and even individuals (). It has been suggested that this variation is a product of differential sensitivity to temperature and light cues between flowers and leaves (), but if water limitation drives the evolution of hysteranthous flowering at the species level, it is possible that water availability may influence the plasticity of flower-leaf sequence on a seasonal timescale. Executing experimental or observational studies about this (say better) is an important step of for understanding the significance to the water dynamics hypothesis and for predict how woody plant phenological sequences may shift with climate change as local patterns of both temperature and water availability continue to change in the coming decades.

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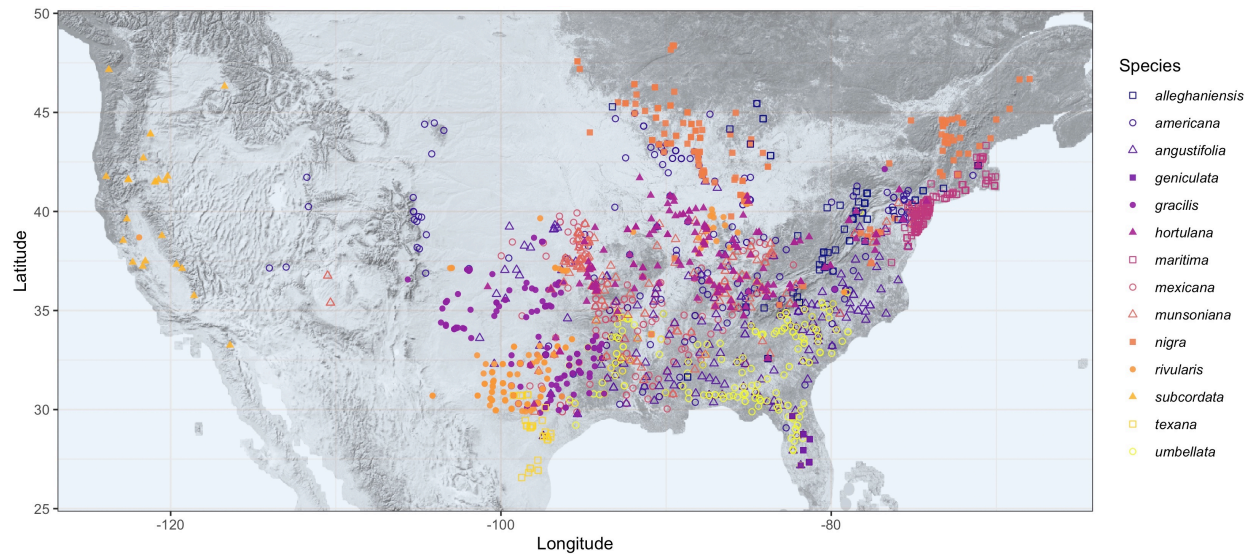
Figures

Figure 1: This is a map of all the herbaria records of our focal clade. Maybe better in the supplement
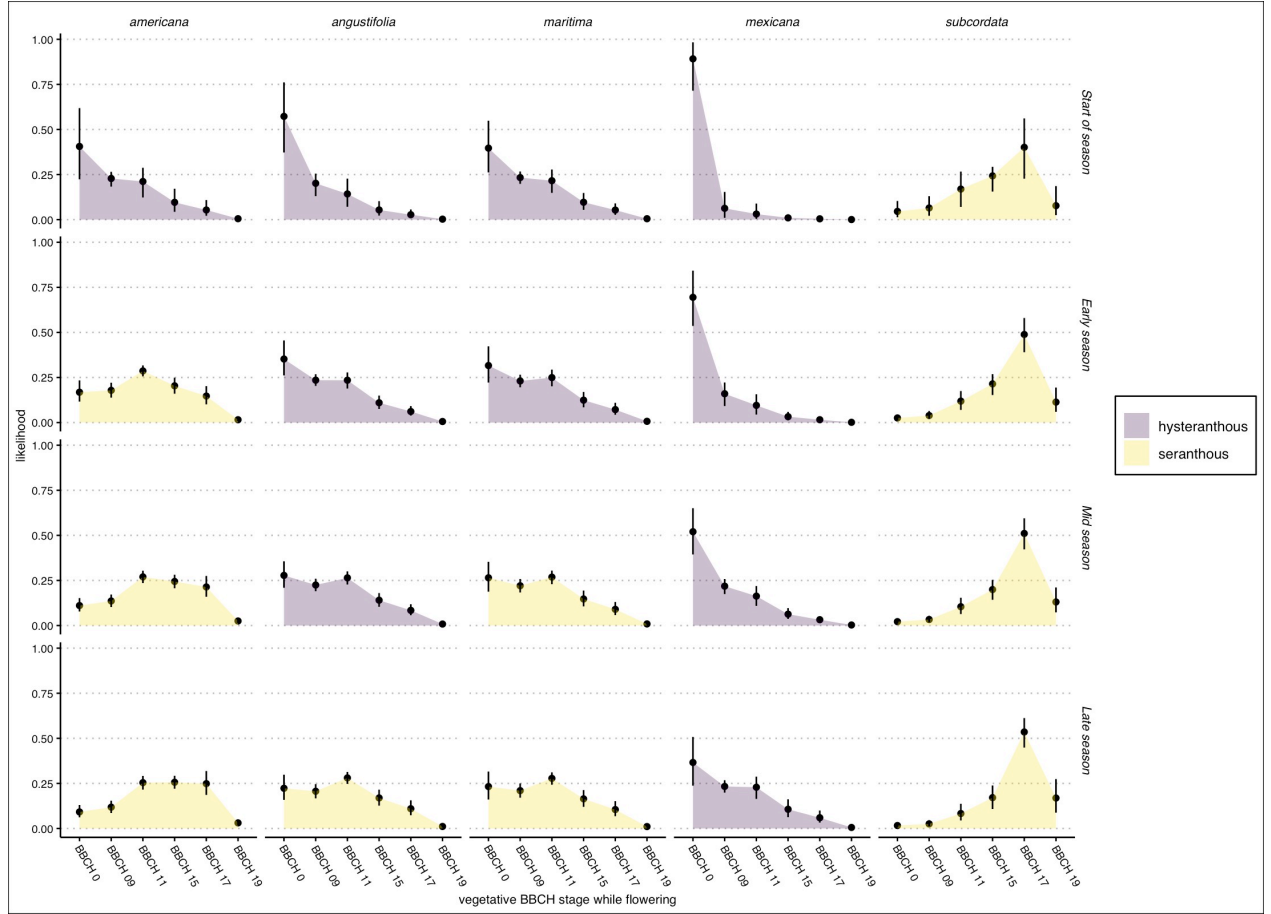
Figure 2: Predicted likelihood that a species would be in flower during each vegetative BBCH phase for five example species in the American plums. Points are the mean likelihood and bar the 95% uncertainty intervals. Species were classified as hysteranthous if greater than 50% probability flowering occured in BBCH 0 and BBCH 09 (colors) for each part of the flowering seaion. See Fig. S1 for all species and alternative hysteranthy classification schemes.
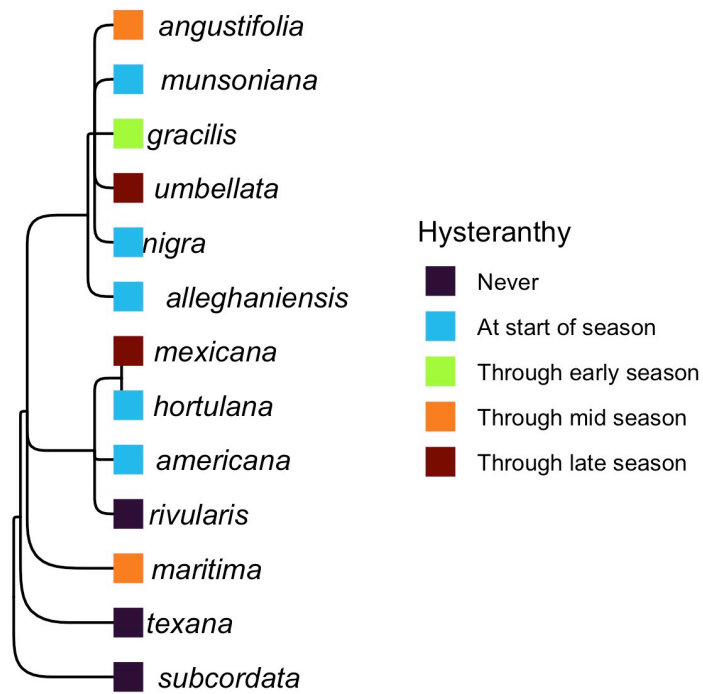
Figure 3: Phylogenetic relationships amoung the American plums and the duration of their flowering perion they are hysterathous. These categorizations are based on ordinal phylogenetics mixed models. Tree topology is from Shaw.
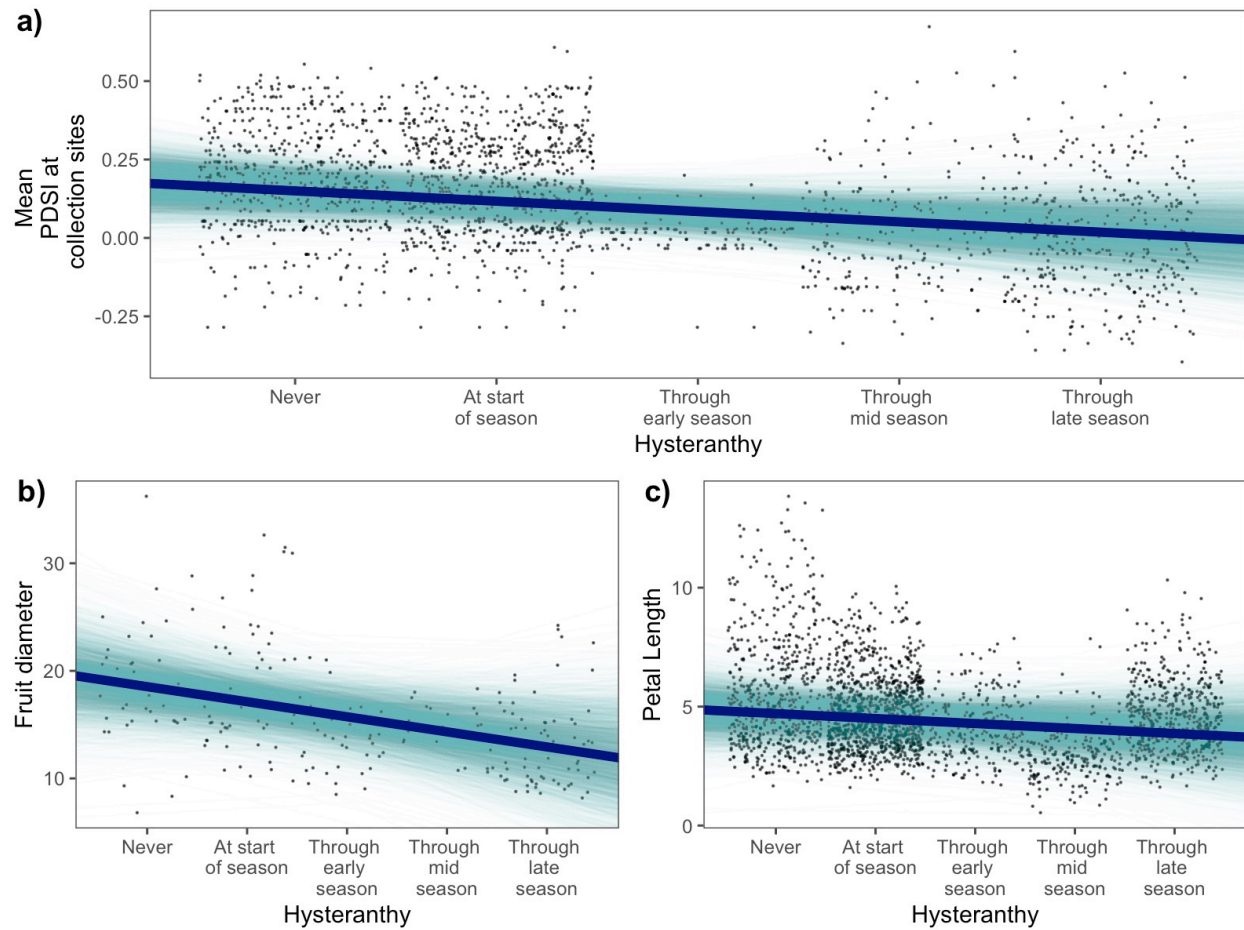
Figure 4: Relationships between the duration of hysteranthy across the flowering period and environmental and biological traits based on Bayesian phylogenetic mixed models.