

Aridity and floral architecture drive hysteranthous flowering in the North American cherries (*Prunus* spp.)

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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 hysteranthly, proteranthly or precocious flowering is particularly common in temperate forests around the globe (). A number of studies suggest that this flower-leaf sequences (FLSs) are under selection, and that flowering first has functional significance ().

The most common, and well-tested explanation for the evolution of hysteranthly 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 (). However, this hypothesis fails to address the prevalence of hysteranthous taxa that are biotically-pollinated. Approximately 30% of species of Eastern temperate forests of North America flower before leafing out, and of those, approximately 20% are biotically pollinated (). Despite the pervasiveness of this phenological syndrome, direct tests of the function of hysteranthly in biotically pollinated taxa are exceedingly rare.

Yet, based on decades of natural history accounts of hysteranthous species around the globe, we present two hypotheses regarding the function of hysteranthly in biotically-pollinated taxa. Each hypothesis makes logical predictions about how hysteranthous flowering should other plant traits should co-vary, and these hypotheses and their predictions can be used to guide further inquiry into the adaptive significance of hysteranthly.

The **water dynamics hypothesis** suggests that hysteranthly is an adaptation to arid environments, allowing for plant to partition the hydraulic demand of hydrated flowers and transpiring leaves across the growing season (). If this is the case, this hypothesis predicts that hysteranthous species should be more commonly found in dry environments.

The **pollinator visibility hypothesis** suggests that hysteranthly is an adaptation to attract visually-foraging pollinators (). If this is the case, this hypothesis predicts that hysteranthous species may invest less in other floral traits for pollinator attraction such as size of floral display or chemical attraction.

Still others have suggested that hysteranthly is simply the by-product of selection for early flowering (), and that variation in flower-leaf sequences among species is driven by developmental, physical or phylogenetic constraints than adaptive selection (). However, even this null hypothesis make testable predictions. If this is the case, hysteranthly should co-vary with other early-flowering associated traits like long fruit development periods or large fruit sizes () and the phylogenetic signal for hysteranthly should be strong.

With mounting evidence that climate driven shifts in phenological phases are altering the duration and order of flower-leaf sequences () understanding the functional significance of hysteranthly 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 these hypotheses:

First, characteristics like aridity tolerance, pollinator attraction, and reproductive investment are the emergent product of a suite of biological traits (). 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 hysteranthly in woody plants is through character deconstruction (); comparing flower-leaf sequences variation for only a subset of taxa of shared phylogenetic and morphological character.

A second challenge for robust testing of hysteranthly 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 sensitive to observer bias (see, ()).

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 (), but have not been used widely to investigate variability of flower-leaf sequences variation among and within species.

In this study, we begin by combining a large data set of occurrence records with published descriptions of flower-leaf sequences and plant traits for North American species in the genus *Prunus* to test the predicted trait associations of the major hysteranthly hypotheses at the genus level. We then shift our focus to one sub-clade within the genus, the American plums, (subsp *Prunus*, sect. *Prunocerasus*). Using herbaria records, we make detailed measurements to quantify flower-leaf sequences and trait variation both within and among species to test the predicted trait relationship with higher resolution data, and to evaluate these the hysteranthly hypotheses at contrasting taxonomic scales.

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Methods

0.1 Study system

The genus *Prunus* comprises approximately 200 species distributed across the globe (). Of 40-50 species native to or naturalized in North America, *Prunus* species display the full spectrum of variation in flower-leaf sequences () and show marked inter-specific variation in habitat requirements and functional traits making them an ideal system in which to investigate the inter-relationship between hysteranthly and traits predicted by the two major hysteranthly hypotheses.

Within the genus, The American plums (*Prunus* subsp. *Prunus* sect. *Prunocerasus*) offer potential for an additional, higher resolution investigation of drivers of hysteranthous flowering. Like the genus at large, the 16 species that make up the section are distributed across North America and 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 have solitary inflorescences () allowing for even more refined character deconstruction. Species in this section are well represented in herbaria records, making them a tractable group to measure and assess

intra-specific variation in flower-leaf sequences as well as other ecological and morphological characteristics related to the hysternanthy hypotheses described above.

0.2 Genus level analyses

To assess the relationship between hysternanthous flowering and morphological and ecological traits related to the hysternanthous hypothesis we obtained flower-leaf sequence descriptions and mean estimates of flower petal length, flowers per inflorescence, and fruit diameter for 44 *Prunus* species from the Flora of North America. As a measure of aridity tolerance, we obtained the coordinates of herbaria occurrence records of all 44 species from the Consortium of Midwest Herbaria digital Archeives (n=23,272). For records that we not geo-referenced was assigned coordinates to the county centroid in which the specimen was observed. We than extracted the average value of Palmer Modified Drought Index from at each local from the North America Drought Atlas () averaging these values across all occurrence per species for a coarse estimate of species level aridity tolerance.

To assess the relationship between inter-specific flower-leaf sequences variation and the traits predicted by the two hysternanthy hypotheses we fit a Bayesian ordinal regression model with each measured trait (mean pdsi, fruit diameter, flower petal length and flowers per inflorescence) as main effects. In this model, we included an interaction term between flower petal length and number of flowers per inflorescence to account for a well established trade off between these two elements of floral displays (). To be able to directly compare the effects of the of our multi-scale trait and environmental variables, we standardized all predictors in the model through z-scoring (). We fit all models using the R package “brms” (?) on four chains with 4000 iterations and a 3000 iteration warm up for a total of 4000 posterior draws for each parameter. We used weakly informative priors, and assessed model performance through ensuring \hat{R} s were between 1 and 1.01 and bulk and tail effective sample sizes were high (1800-2800 for most parameters, but as low as 800-900 for some).

0.3 Section level analyses

For our analysis of the section *Prunocerasus* we obtained digital herbarium species for all member of the section from the Consortium of Midwest Herbaria Database. For this analysis, we measured FLS sequence variation, petal length, fruit diameter and mean pdsi. We did not include flowers/inflorescence in this analysis because all members of the section *Prunocerasus* have solitary flowers ().

To evaluate the FLS 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 (?). In total, we evaluated the phenology of 2521 specimens, but only specimens with visible flower were included in this analysis (n=1009). 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). As in our previous genus level analyses, we computed the average Palmer Modified Drought Index for every *Prunocerasus* specimen in the database (n=2305) from the North America Drought Atlas ().

Because our data dependent and independent were collect we employed a sequential modeling approach to first estimate the mean and standard error of the posterior distribution of trait values for each species then model the relationship between these estimate and the likelihood of hysternanthy using Bayesian measurement error models. This approach propagates the error in the initial estimates of trait values into the our final

model, yielding a more accurate evaluation than using mean trait values alone (). For each parameter of interest, we ran Bayesian phylogenetic mixed-effects models with our measured traits as the response variable and species as the random effect. For traits like flower petal length and fruit diameters that included multiple measurements per specimen, we included specimen ID as an additional random effect. The model structure is written below:

Then using each of these trait mean estimates as predictors, we modeled their associations with flower leaf sequences OF using a repeat measure phylogenetic mixed ordinal regression in brms (). Because we found the three predictors of interest to be highly colinear (pairwise correlations >0.5), we ran one regression model per predictor trait to avoid skewing our model inference due to multi-collinearity (??).

For all models in the sequences, we use weakly informative priors and ran the models of four chains with a warmup of 2,500 iterations and 4000 sampling iterations for a total of 6,000 sampling iterations.

Results

Drivers of hysteranthly in *Prunus* spp. of North America

Increased likelihood of hysteranthly was associated increasing aridity, flowering and inflorescence size and their interaction. Fruit size had little effect of the likelihood of hysteranthly and at the genus level, the phylogenetic signal in hysteranthly was X suggesting weak to moderate phylogeny structure.

Drivers of hysteranthly in sect. *Prunocerasus*

Within the section *Prunocerasus*, hysteranthly was associated with smaller fruit (). There was no relationship between flower size, fruit phenology, or mean pdsi and the phylogenetic signal of hysteranthly was virtually non-existent within this sub-clade. When we investigated

Discussion

The associations between the likelihood of hysteranthly flower/inflorescence size and aridity between we detected at the genus suggests tacit support for both the insect visibility hypothesis and the water dynamics hypothesis. However we must emphasize that our analysis detected an association between hysteranthous phenology and reduced floral displays, but we cannot empirically identify the evolutionary mechanisms driving this relationship. While the relationship certainly may indicate a trade-off between the apparent visibility of flowers and investment in the floral structures that is predicted by the insect visibility hypotheses, there are numerous other evolutionary or developmental forces that could drive this relationship. For example, species with large inflorescence may not be able to store enough carbohydrates to produce and maintain these structure before leaf emergence, and the relationship we detected could a readily be evidence of a developmental constraint on hysteranthous flowering as evidence of adaptive selection. Sespite the strong relationship between flower display size and hysteranthly we detected in our study that follows the predicted pattern of insect-visibility hypothesis, we cannot unequivocally assert that hysteranthly is an adaptation for pollinator attraction. What can we say? I think that the relationship suggests this is an avenue to pursue and here is a place we could talk about mechanisnistic experiments.

In contrast to the strong associations we detected at the genus level, within the section of *Prunocerasus*,

fruit size was the only trait significantly associated with increased likelihood of hysteranthly. However, the estimated effect was relatively weak, and the positive relationship between fruit size and FLS category (suggestion that hysteranthly is associated with smaller fruit), is counter to the expectation about the relationship between fruit size and flowering time ().

The dissimilarity in the relationships between the likelihood of hysteranthly and predicted traits we observed at the genus and section level could describe a biological phenomenon indicating at what phylogenetic scale the evolution of hysteranthous flowering may be adaptive. Alternatively, because our analyses at the genus and section level leveraged different kinds of phenological and trait data, and differed in their ability to account for intra-specific variation and measurement error, the differences we observed among taxonomic scales may be a statistical issues rather than a biological one. While we cannot definitively separate the effects of biology and methodology on the outcomes of this study (), following each line of reason provides important paths forward for future studies of hysteranthly flowering and trait-based evolutionary ecology in general.

Why a strong

Unpacking the Biology

It is not terribly surprising that the association between hysteranthly and flower/inflorescence size was considerably stronger at the genus level than at the section level.

Unpacking the Methodology

Floral display in hysteranthly

Figures

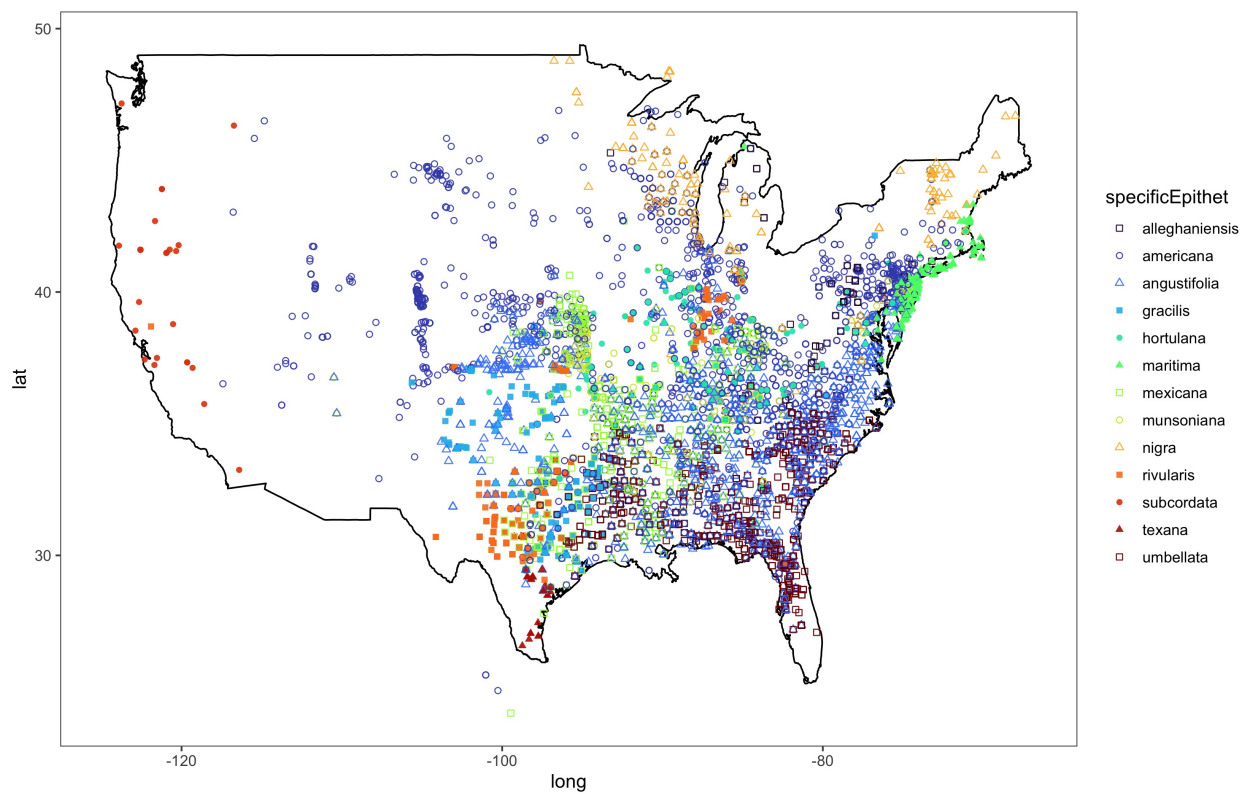


Figure 1: Map to show where data come from and to point out the two never hysteranthly species are highly endemic

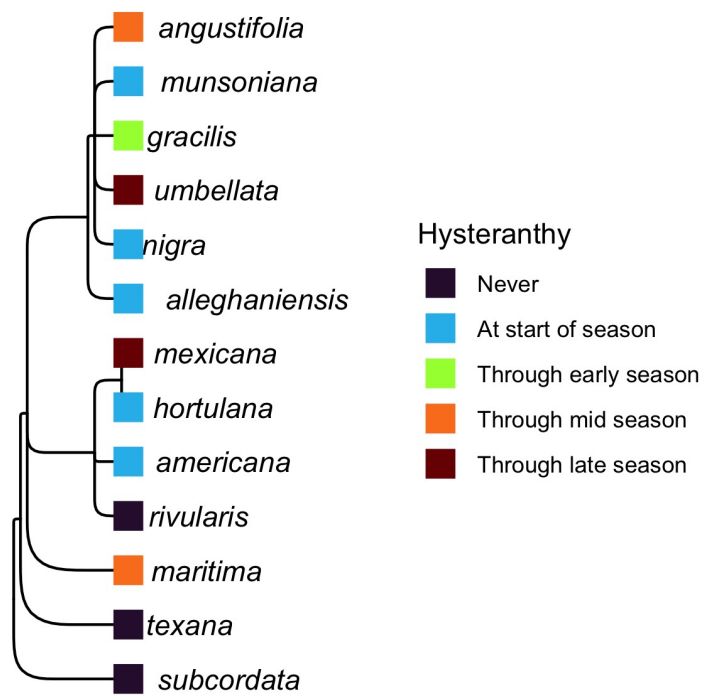


Figure 2: place holder for the phylogenies: Ideally will have all N.A. *Prunus* and *Prunocerasus*

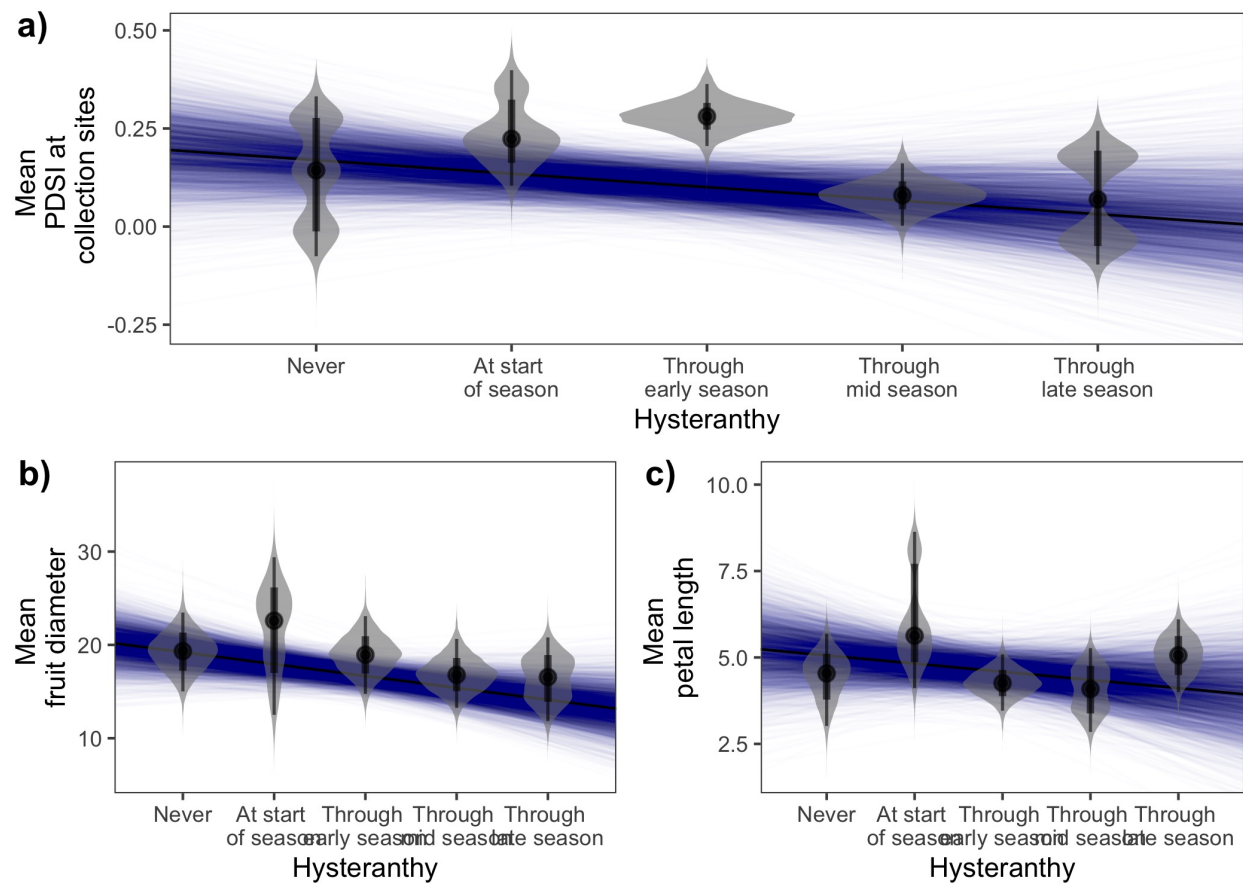


Figure 3: Relationships between the duration of hysteresis across the flowering period and environmental and biological traits