

Reconciling competing hypotheses regarding flower-leaf sequences in temperate forests for fundamental and global change biology

D.M. Buonaiuto^{1,2,a}, I. Morales-Castilla³, E.M. Wolkovich⁴

Author affiliations:

¹Arnold Arboretum of Harvard University, Boston, Massachusetts, USA

²Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA

³Global Change Ecology and Evolution (GloCEE), Department of Life Sciences, University of Alcalá 28805, Alcalá de Henares, Spain

⁴Forest & Conservation Sciences, Faculty of Forestry, University of British Columbia, Vancouver, British Columbia, Canada

^aCorresponding author: 617.823.0687; dbuonaiuto@g.harvard.edu

Keywords: deciduous forests, flower-leaf sequences, global change, hysteresis, phenology, phylogeny

Paper type: Viewpoint

Counts: Words: Summary: 192; Main text: 3180; References: 39; Figures: 4 (all color). Supporting Information: 4 supplemental figures and Methods.

Summary

Phenology is a major component of an organism’s fitness. While individual phenological events affect fitness, growing evidence suggests that the relationship between events may be equally or more important. This may explain why temperate deciduous woody plants exhibit considerable variation in the order of reproductive and vegetative events, or flower-leaf sequences (FLSs). There is evidence to suggest that FLS may be adaptive, with several competing hypotheses to explain their function. Here, we advance existing hypotheses with a new framework that accounts for quantitative FLS variation at multiple taxonomic scales using case studies from temperate forests. Our inquiry provides several major insights towards a better understanding of FLS variation. First, we show that support for FLS hypotheses is sensitive to how FLSs are defined, with quantitative definitions being the most useful for robust hypothesis testing. Second, we demonstrate that concurrent support for multiple hypotheses should be starting point for future FLS analyses. Finally, we highlight how adopting a quantitative, intra-specific approach generates new avenues for evaluating fitness consequences of FLS variation and provides cascading benefits to improving predictions of how climate change will alter FLSs and thereby re-shape plant communities and ecosystems.

Introduction

Phenology, the timing of seasonal life cycle events, allows organisms to synchronize their activity with optimum environmental conditions (Forrest & Miller-Rushing, 2010). It is not only individual phenological stages that affect an organism’s performance, but also their chronology (Ettinger *et al.*, 2018; Firmat *et al.*, 2017; Vitasse *et al.*, 2010).

One phenological relationship that has long received scientific interest (see Robertson, 1895) and, recently, increased attention (e.g. Gougherty & Gougherty, 2018; Savage, 2019) is the flower-leaf phenological sequence (FLS) of temperate deciduous woody plants. In a typical model of plant life-history, vegetative growth precedes reproduction. However, for many species in the forests of

Eastern North America (and other temperate regions of the Northern Hemisphere), it is not the green tips of new shoots that mark the commencement of the growing season, but the subtle colors of their flowers. Previous work by Gougherty & Gougherty (2018) found that as many as 30% of tree species of the Midwestern United States flower prior to leafout. The prevalence of this FLS may be surprising as it requires plants to invest in reproduction from stored carbohydrates at a time when their reserves are most depleted (Primack, 1987), but this trade-off suggests that flowering-first has some adaptive significance (Rathcke & Lacey, 1985).

Understanding this phenological pattern is timely because anthropogenic climate change is altering FLSs. Long-term data shows that the number of days between flowering and leafout is increasing as a result of climate change, but the rate of change differs up to five-fold among species, with flowering-first species seemingly more sensitive to climate change (Fig. 1). If FLSs are indeed an important component of woody plant fitness, this inter-specific variation will exacerbate fitness differences between species, influencing which species will persist under altered climate conditions.

Long-term datasets also demonstrate high within-species variability in FLSs. Despite recent advances in understanding the physiology and evolution of FLSs (Gougherty & Gougherty, 2018; Savage, 2019), most analyses have not directly addressed this variability—potentially slowing progress in predicting how FLSs will respond to climate change. While the literature provides some general correlations between flower and leaf phenology (e.g. Ettinger *et al.*, 2018; Lechowicz, 1995), there have been few, if any, analyses of higher-resolution patterns (Gougherty & Gougherty, 2018).

We propose a new framework for the study of FLSs built on quantitative measures of both inter- and intra-specific FLS variation. This shift will improve predictions of how FLS patterns will change in the future, and may reveal novel avenues to better understand the fundamental biology of this phenological sequence. Here we 1) review hypotheses of the function of FLS variation 2) evaluate the biological basis of the current categorical FLS framework and 3) present our proposed

quantitative framework using a detailed case study of long-term phenology records from Harvard Forest in Petersham, MA.

Hypotheses for flower-leaf sequence variation

Wind pollination

The most prevalent FLS hypothesis suggests that flowering-first is an adaptation for wind-pollination, with leafless flowering allowing for more efficient pollen transfer (Whitehead, 1969)(Fig. 2a). The primary evidence for this hypothesis comes from pollen diffusion studies (e.g., particle movement through closed and open canopies, Milleron *et al.*, 2012; Niklas, 1985) and suggests canopy structure encumbers pollen movement.

Water limitation

Another hypothesis suggests that flowering before leaf development is an adaptation to reduce water stress caused by concurrently maintaining floral hydration and leaf transpiration (Franklin, 2016) (Fig. 2b). Observations from the dry tropics, where this FLS is also common, confirm that the timing of flowering in many species is associated with a water status recovery due to leaf drop (Borchert, 1983; Reich & Borchert, 1984), and that flowers tissue is more resistance to drought damage than leaf tissue (Zhang & Brodribb, 2017). Despite the fact that temperate forests are rarely water-limited during the spring when flowering and leafing occur (Polgar & Primack, 2011), a recent analysis by Gougherty & Gougherty (2018) found strong associations between flowering-first and water use traits for temperate species. This suggests that this hypothesis merits broader consideration and further development for the temperate zone as well (see Supporting Information , “The water limitation hypothesis in wet temperate forests”).

Early flowering

A third possibility is that the flowering-first FLS is a byproduct of selection for early flowering (Fig. 2c). Flowering-first species are among the earliest in a community to flower seasonally, which may be an adaptation to accommodate later phenological events such as the maturation of large fruits or seeds (Ettinger *et al.*, 2018; Li *et al.*, 2016; Primack, 1987) or avoiding seed predation (Schermer *et al.*, 2020). This may be particularly important at the high latitudes where selection on flowering time is strong due to a shorter growing season (Munguia-Rosas *et al.*, 2011). Recent work from Savage (2019) demonstrated that spring flower phenology is less constrained by prior phenological events than leaf phenology, which would allow selection to drive flowering into the early season, producing the flowering-first FLS. With this hypothesis there is no specific advantage to a species flowering before or after leafing; all that matters is its absolute flowering time.

Constraint

The previous hypotheses suggest that a flowering-first FLS may be adaptive, but the greater diversity of FLS patterns observed in temperate forests may be the product of phylogenetic (Gougherty & Gougherty, 2018) or physical (Diggle, 1995, 1999; van Schaik *et al.*, 1993) constraints among species (Fig. 2d). It is possible that FLSs are highly conserved traits for which FLS variation reflects macro-evolutionary relationships among taxa. If this is the case, we would expect to see a strong phylogenetic signal for FLS variation as was reported in a recent analysis by Gougherty & Gougherty (2018). A strong phylogenetic pattern in FLS would not preclude any of the adaptive hypotheses presented above, as many different evolutionary processes can yield comparable phylogenetic signals (Revell *et al.*, 2008).

Phylogenetic patterning for FLSs may be driven by developmental or architectural differences among species. For example, the reproductive phenology of species that produce flower from axillary buds set in previous season may be more independent of leaf phenology than species with

determinate growth (Borchert, 1983; Rathcke & Lacey, 1985; van Schaik *et al.*, 1993). Previous work also has suggested that differences in xylem anatomy may constrain spring phenology (Lechowicz, 1995), though Savage (2019) determined that for 20 spring-flowering species, reproductive buds were hydrated primarily by the phloem, suggesting the flowering-first FLS may be independent of xylem activity.

Evidence to date

While decades of inquiry have advanced each of these hypotheses independently, there is no clear consensus regarding their comparative merits. Most previous studies on FLSs have not compared hypotheses, and those that did have generally found support for multiple hypotheses (see Bolmgren *et al.*, 2003; Gougherty & Gougherty, 2018). There is no expectation that FLS hypotheses must be mutually exclusive. Indeed, understanding the relative importance of each one and the relationships between them may provide the most useful path forward, if they can be robustly compared.

We argue that a sensible reconciliation of these hypotheses is possible with a shift to a new conceptual framework for the study of FLSs. Under the current framework, FLSs are described qualitatively, and prescribed at the species level. We suggest that quantitative measures of FLS which include observations below the species level are more compatible with the biological processes underlying FLS variation. Below we present an overview of the current approach to describing FLSs and highlight some of the challenges that can arise when using it.

The current flower-leaf sequence framework

Describing FLSs

The current framework describes three main FLS categories: flowers before leaves (hysteranthy, proteranthy, precocious flowering); flowers with leaves (synanthy); and flowers after leaves (seranthy) (Heinig, 1899; Lamont & Downes, 2011). Some data sources (e.g. Barnes & Wagner, 1981,2004; Burns & Honkala, 1990) include additional categories: “flowers before/with leaves” and “flowers with/after leaves”, but it is unclear whether these categories describe intermediate FLS patterns or FLS variability in these species. While these categories are conceptually reasonable, applying them to real phenological sequences is not always straightforward.

Both reproductive and vegetative phenological sequences consist of multiple sub-stages, and this introduces significant ambiguity into how we interpret qualitative FLS descriptions. Consider a species with the following FLS:

**flower budburst → leaf budburst → first flowers open → leafout → peak flowering →
end of leaf expansion**

Observers could justifiably classify this species as: 1) Hysteranthous because flower budburst precedes leaf budburst, 2) Synanthous because flowers open during the budburst-leafout inter-phase, 3) Seranthous because peak flowering occurs after leafout. This problem extends beyond this simple example to real datasets, (e.g. O’Keefe, 2015) where the same ambiguities exist (Supporting Information Fig. 1). Not surprisingly then, different sources may classify the same species differently. We compared species-level FLS descriptions in two of the most comprehensive records of FLS, Michigan Trees and its companion volume Michigan Shrubs and Vines (MTSV) (Barnes *et al.*, 2016; Barnes & Wagner, 1981,2004) with The USFS Silvics Manual Volume II (Burns & Honkala, 1990). Of the 49 overlapping species, 30% were classified differently. Such different clas-

sifications could reflect interesting temporal or geographic variability in FLSs, but—given current definitions—they could equally be the product of observer classification decisions.

Categorization can often introduce biases in analyses (Edwards *et al.*, 2015) and highlight ambiguity in hypotheses; this may be particular prevalent for the study of FLSs. The wind pollination hypothesis hinges on the fact that leaves create a substantial barrier to pollen transfer, which may not be true during the early stages of leaf expansion. Rather, trees that flower during the early stages of leaf expansion should gain similar advantage to those who complete their flowering before any leaf activity and therefore it would be most biologically appropriate to bound the category of hysternanthy to include species that for which early leaf development overlap with flowering. (Fig. 2a). Alternatively, because transpiration intensifies as soon as leaves begin to expand (Wang *et al.*, 2018), the water limitation hypothesis asserts there should be a cost to maintaining floral structures during any stage of leaf activity. Here, only species where flowering occurs before any leaf expansion should gain a hydraulic advantage, and to most accurately address this hypothesis, the category of hysternanthy should only include species that flower before any leaf development. (Fig. 2b).

Given the differences in biological processes underlying these hypotheses, statistical relationships between FLS and traits will fluctuate depending on where categorical boundaries are drawn. If these hypotheses require different categorization schemes to accurately capture the underlying biology, it becomes difficult to compare them in the same modeling framework.

We found that associations between FLSs and functional traits related to each hypothesis were highly sensitive to how FLSs were defined (Supporting Information Fig. 2, e.g. pollination syndrome, Supporting Information Fig: 3). We applied two alternative FLS categorizations in two major datasets (MTSV and USFS, see Supporting Information Methods S1); physiological hysternanthy, which allowed for no overlap between floral and leaf phenophases, and functional hysternanthy,

which allowed for a degree of overlap (see Supporting Information Methods S1). These alternate categorization boundaries re-shuffled the species included in each classification, affecting both the trait distributions within each category and the phylogenetic patterning across the tree (Supporting Information Fig. 4).

This suggests that a new approach that relaxes the assumptions of categorization could help to fairly evaluate FLS hypotheses. Below we present a new framework for the study of FLSs built on 1) quantitative measures and 2) intra-specific investigations of FLS variation. This simple shift can capture biological variation missed by current approaches, and offer novel avenues for understanding the scope and consequences of FLS variation in an era of global change.

A new framework for flower-leaf sequences

Quantitative measures of FLS

In the current FLS framework species are classified based on sequence alone. The duration of and time between phases, however, also matters (Inouye *et al.*, 2019). When considering measures of time, FLSs of species within each category can be quite different (Fig. 3a). Measure of FLS based on continuous data—i.e. reporting the number of days between specific phenophases, suggest there is much greater diversity in FLS patterns in a given forest community than provided by the three categories of the current framework.

Treating FLSs like other quantitative measures of phenology (e.g. the BBCH scale, Finn *et al.*, 2007) would: 1) improve FLS-trait association models by reducing the noise from unmeasured variation and 2) standardize data across time and space, observer, and analyst. Adopting quantitative measurements would facilitate comparing FLS patterns across larger temporal, geographic, and taxonomic scales, giving researchers more power to accurately address questions about FLS variation.

211

212 An additional benefit of a quantitative approach to FLSs is that it allows for variation to be
213 evaluated below the species level. We argue that intra-specific inquiries into FLS variation are vital
214 to thoroughly answer both questions about the basic mechanisms that generate FLS variation, and
215 applied questions regarding the magnitude and impact of FLS shifts with climate change.

216 **Intra-specific data on FLSs**

217 Quantitative measurements of FLSs reveal significant variation among individuals and years (Fig.
218 3 b). This variation can be leveraged to further improved FLS-trait models at the species level,
219 and to generate and test novel questions about the fitness value of this trait.

220

221 Observations at multiple taxonomic scales should improve FLS-trait association models by allowing
222 researchers to explicitly incorporate multiple levels of variation, for example, by nesting individual
223 or population level FLS observations within a species grouping in a hierarchical model. When
224 intra-specific variation for a given trait is high, simply using species' mean trait values could
225 mis-represent inter-specific differences. Interestingly, this implies that incorporating intra-specific
226 variation to these models may be one of the most robust ways to accurately assess inter-specific
227 variation

228 Intra-specific inquiry is also a critical step to better understand the consequences of FLS shifts.
229 At the core of each FLS hypothesis is a fitness prediction that is best interrogated below the
230 species level. If FLSs are functionally important, individual variability in FLSs should correlated
231 with changes in performance as has been shown for other phenophases (e.g. Schermer *et al.*, 2020).
232 Evaluating the relationship between FLS variation and performance is critical to determine whether
233 at its biological core, FLS variation is merely an interesting natural history note of temperate forests
234 or an important functional trait that will impact the structure and function of these communities
235 in the future.

236

Testing the new framework

Quantitative measures

To compare categorical and quantitative approaches to FLSs, we used long-term phenological records for woody species at Harvard Forest (O’Keefe, 2015) to model the associations between FLSs and functional traits using both a categorical FLS framework and a simple quantitative metric; the mean number of days between flower and leaf budburst for each species (see Supporting Information Methods S1). We investigated functional traits related to each of the FLS hypotheses. We used pollination syndrome as a predictor for the wind pollination hypothesis. We used mean precipitation across a species’ range and two alternative predictors; species’ moisture use and minimum temperature across a species’ range as predictors for the water limitation hypothesis. We used flowering time and two alternative predictors; mean fruit dispersal time and seed mass to represent the early flowering hypothesis. We accounted for the influences of phylogenetic constraints by running these models in a phylogenetic modeling framework (Ives & Garland, 2010). In all cases, model results with alternative predictors were comparable to the sign and rank of the main results and can be viewed in the Supporting Information (Fig. ??).

Using the categorical approach, we detected only a weak relationship between hysteranthly and wind-pollination. However, with the improved predictive power of the quantitative approach, we found that increasing time between flower and leaf budburst was strongly associated with wind-pollination and early flowering, and that the longest FLS interphases were found in species with both of these traits (Fig. 4a,b).

Intra-specific variation

To test how model inference changed when accounting for intra-specific variation, we re-analyzed the same FLS data from Harvard Forest presented above using a Bayesian hierarchical model

that incorporated within-species variation in FLSs and flowering time (see Supporting Information Methods S1).

As in the model based on species' mean trait values, we found strong effects of flowering time, pollination syndrome and phylogeny on FLS variation, with only a weak signal for the water limitation hypothesis (Fig. 4c, Fig. Supporting Information 3). However, the hierarchical approach leveraged all the available data (1636 rows instead of 23 rows for the mean-based quantitative approach) at the most relevant biological scales, and with this improved power, we identified strong interactions between predictors. Of note, the effect of early flowering on FLS variation was more pronounced in biotic-pollinated taxa despite the fact that wind-pollinated species always had a longer FLS inter-phase. Additionally hydraulic demand was associated with increased time between flowering and leafing in biotically-pollinated taxa but not wind-pollinated taxa (Supporting Information Fig. 6). Together, these systematic differences suggest that flowering-first FLSs in these functional groups may have evolved under radically different environments and converged in temperate forests.

Even with a quantitative framework, analyses are inherently sensitive to how FLS's are defined. It is not surprising that traits association vary in strength when FLSs are defined based on different sub-phases of flowering and leafing; for example, days between flower budburst and leaf budburst vs. days between peak flowering and leaf expansion. We found that incorporating intra-specific variation into the modeling appeared to reduce this bias (see Fig. 7), which may allow researchers to robustly compare existing FLS data that are not perfectly standardized with each other (Supporting Information Tab. 1).

Future directions:

Our findings suggest that the tendency for previous studies to find support for multiple hypotheses (Bolmgren *et al.*, 2003; Gougherty & Gougherty, 2018; Savage, 2019) is consistent with the biological processes that shape FLSs. Multiple hypotheses should be the starting point for future FLS research. While large scale analyses may continue to be beneficial, a more nuanced understanding about function of FLS variation may result from pattern deconstruction (i.e. grouping of species according to sub-clades or trait commonalities, Terribile *et al.*, 2009). For example, it is clear that wind-pollination efficiency is not driving hysteranthous flowering in insect-pollinated taxa, so considering this group of species alone rules out one major FLS hypothesis, allowing for a better evaluation of alternatives.

While trait associations point to past selection, much of the current interest in FLSs relates to how shifting FLS patterns will impact woody plants in the future. Shifting research to focus on intra-specific FLS data may importantly provide insight into the biological levels of organization that determine how species can respond to climate change from the individual to population to species level. Variation among and within individuals provides insights regarding micro-climate effects, heritability, selection and plasticity for FLSs (Denéchère *et al.*, 2019). While not addressed specifically in our data, population level variation in FLSs is also high (Supporting Information Fig. 8), and critical to better understand the specifics of how environmental conditions shape FLSs (Vitasse *et al.*, 2009), and how FLS variation interacts with landscape scale processes like gene flow and dispersal (Manel *et al.*, 2003). Taken together, investigations at these lower taxonomic levels could provide a more robust assessment of the potential magnitude of FLS shifts with climate change.

As mentioned above, future FLS research should aim to test the performance consequences of FLSs by leveraging intra-specific variation. However, this may require more general focus on trait data at the same scale as FLS variation. For example, the wind-pollination hypothesis suggests that

decreasing the time between flowering and leafing should result in reduced pollination success. To test this prediction, studies tracking individual FLS variation in the field or controlled environments must also track performance metrics at this scale, for example, reproductive outcomes such as pollination success or fruit set. These kind of studies are critical for evaluating the implications of FLS shifts in the future.

Conclusion

In demonstrating our proposed framework for the study of FLSs we found that, in accordance with previous work, flowering time, pollination syndrome and phylogeny are important drivers of hysteranthly (Gougherty & Gougherty, 2018). Our work adds to the growing literature that infers the adaptive significance of FLSs from macro-evolutionary patterns and opens new avenues for testing the effects of FLS variation on woody plant performance below the species level. While it is clear the FLSs are highly variable and shifting with global climate change, research must directly examine the effects of FLS variation to better assess the consequences of future FLS shifts.

While much of research on the evolution of plant phenology focuses on specific phenophases (e.g. Ollerton & Lack, 1992; Savage & Cavender-Bares, 2013), selection likely acts on phenological sequences. With growing evidence that adaptation drives both the absolute timing of individual phenophases and the relative timing between them we must continue to develop analytical tools that improve our understanding of the drivers of phenological events as part of a phenological syndrome, rather than as discrete, separate events. Our treatment of FLSs here is a small part of this work, but understanding how selection shapes phenology both throughout the whole growing season and across years remains a major frontier for the study of phenology (Wolkovich & Ettinger, 2014). This is an essential step towards a more complete understanding of the fundamental biology of temperate woody plants, and for predicting the fate of these species as global climate continues to change.

Acknowledgements

We thank T.J. Davies and J.J. Grossman and three anonymous reviewers for their comments on this manuscript.

Author contributions

DMB developed the concept for the paper; DMB and IMC performed the analysis, DMB and EMW wrote the manuscript.

Data and code availability

Data for the FLS and climate change analysis is publicly available from PEP725 at <http://www.pep725.eu/>. The Harvard Forest phenology data is also publicly available in the Harvard Forest Data Archive <https://harvardforest.fas.harvard.edu/harvard-forest-data-archive> (dataset: HF003-05). The compiled data from the MTSV and USFS guidebooks will be available on KNB upon publication. All modeling code will be made available upon request.

References

- Barnes BV, Dick CW , Gunn ME.** 2016. *Michigan Shrubs Vines: A guide to species of the Great Lakes Region*. University of Michigan Press.
- Barnes BV , Wagner WHJ.** 1981,2004. *Michigan Trees: A guide to the Trees of the Great Lakes Region*. University of Michigan Press.
- Bolmgren K, Eriksson O , Linder HP.** 2003. Contrasting flowering phenology and species richness in abiotically and biotically pollinated angiosperms. *Evolution*, **57**: 2001–2011.
- Borchert R.** 1983. Phenology and control of flowering in tropical trees. *Biotropica*, **15**: 81–89.

- Burns RM , Honkala BH. 1990.** Silvics of north america: Volume 2. hardwoods. Tech. rep.,
United States Department of Agriculture (USDA), Forest Service.
- Denéchère R, Delpierre N, Apostol EN, Berveiller D, Bonne F, Cole E, Delzon S, Dufrêne E, Gressler E, Jean F *et al.* 2019.** The within-population variability of leaf spring and autumn phenology is influenced by temperature in temperate deciduous trees. *International Journal of Biometeorology*.
- Diggle PK. 1995.** Architectural effects and the interpretation of patterns of fruit and seed development. **26:** 531–552.
- Diggle PK. 1999.** Heteroblasty and the evolution of flowering phenologies. *International Journal of Plant Sciences*, **160:** S123–S134.
- Edwards EJ, de Vos JM , Donoghue MJ. 2015.** Doubtful pathways to cold tolerance in plants. *Nature*, **521:** E5–E6.
- Ettinger A, Gee S , Wolkovich E. 2018.** Phenological sequences: how early season events define those that follow. *American Journal of Botany*, **105**.
- Finn GA, Straszewski AE , Peterson V. 2007.** A general growth stage key for describing trees and woody plants. *Annals of Applied Biology*, **151:** 127–131.
- Firmat C, Delzon S, Louvet JM, Parmentier J , Kremer A. 2017.** Evolutionary dynamics of the leaf phenological cycle in an oak metapopulation along an elevation gradient. *Journal of Evolutionary Biology*, **30:** 2116–2131.
- Forrest J , Miller-Rushing AJ. 2010.** Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365:** 3101–3112.

- Franklin DC. 2016.** Flowering while leafless 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.
- Gougherty AV , Gougherty SW. 2018.** Sequence of flower and leaf emergence in deciduous trees is linked to ecological traits, phylogenetics, and climate. *New Phytologist*, **220**: 121–131.
- Heinig R. 1899.** Glossary of the botanic terms used in describing flowering plants. Calcutta, India.
- Inouye BD, Ehrlén J , Underwood N. 2019.** Phenology as a process rather than an event: from individual reaction norms to community metrics. *Ecological Monographs*, **89**: e01352.
- Ives AR , Garland Jr. T. 2010.** Phylogenetic logistic regression for binary dependent variables. *Systematic Biology*, **59**: 9–26.
- Kharouba HM, Ehrlén J, Gelman A, Bolmgren K, Allen JM, Travers SE , Wolkovich EM. 2018.** Global shifts in the phenological synchrony of species interactions over recent decades. *Proceedings of the National Academy of Sciences*, **115**: 5211.
- Lamont BB , Downes KS. 2011.** Fire-stimulated flowering among resprouters and geophytes in Australia and South Africa. *Plant Ecology*, **212**: 2111–2125.
- Lechowicz MJ. 1995.** Seasonality of flowering and fruiting in temperate forest trees. *Canadian Journal of Botany*, **73**: 175–182.
- Li X, Jiang L, Meng F, Wang S, Niu H, Iler AM, Duan J, Zhang Z, Luo C, Cui S et al. 2016.** Responses of sequential and hierarchical phenological events to warming and cooling in alpine meadows. *Nature Communications*, **7**: 12489.
- Manel S, Schwartz MK, Luikart G , Taberlet P. 2003.** Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, **18**: 189–197.

- 401 **Milleron M, Lopez de Heredia U, Lorenzo Z, Perea R, Dounavi A, Alonso J, Gil L ,**
 402 **Nanos N. 2012.** Effect of canopy closure on pollen dispersal in a wind-pollinated species (*Fagus*
 403 *sylvatica* L.). *Plant Ecology*, **213**: 1715–1728.
- 404 **Munguia-Rosas MA, Ollerton J, Parra-Tabla V , Arturo De-Nova J. 2011.** Meta-analysis
 405 of phenotypic selection on flowering phenology suggests that early flowering plants are favoured.
 406 *Ecology Letters*, **14**: 511–521.
- 407 **Niklas KJ. 1985.** The aerodynamics of wind pollination. *The Botanical Review*, **51**: 328–386.
- 408 **O’Keefe J. 2015.** Phenology of woody species at harvard forest since 1990. *Harvard Forest Data*
 409 *Archive: HF003*.
- 410 **Ollerton J , Lack A. 1992.** Flowering phenology: An example of relaxation of natural selection?
 411 *Trends in Ecology & Evolution*, **7**: 274 – 276.
- 412 **Polgar C , Primack R. 2011.** Leaf-out phenology of temperate woody plants: From trees to
 413 ecosystems. *New Phytologist*, **191**: 926–41.
- 414 **Primack RB. 1987.** Relationships among flowers, fruits, and seeds. *Annual Review of Ecology*
 415 *and Systematics*, **18**: 409–430.
- 416 **Rathcke B , Lacey EP. 1985.** Phenological patterns of terrestrial plants. *Annual Review of*
 417 *Ecology and Systematics*, **16**: 179–214.
- 418 **Reich P , Borchert R. 1984.** Water-stress and tree phenology in a tropical dry forest in the
 419 lowlands of costa-rica. *Journal of Ecology*, **72**: 61–74.
- 420 **Revell LJ, Harmon LJ , Collar DC. 2008.** Phylogenetic signal, evolutionary process, and rate.
 421 *Systematic Biology*, **57**: 591–601.
- 422 **Robertson C. 1895.** The philosophy of flower seasons, and the phaenological relations of the
 423 entomophilous flora and the anthophilous insect fauna. *The American Naturalist*, **29**: 97–117.

- 424 **Royston P, Altman DG , Sauerbrei W. 2006.** Dichotomizing continuous predictors in multiple
425 regression: a bad idea. *Statistics in Medicine*, **25**: 127–141.
- 426 **Savage JA. 2019.** A temporal shift in resource allocation facilitates flowering before leaf out and
427 spring vessel maturation in precocious species. *American Journal of Botany*, **106**: 113–122.
- 428 **Savage JA , Cavender-Bares J. 2013.** Phenological cues drive an apparent trade-off between
429 freezing tolerance and growth in the family salicaceae. **94**: 1708–1717.
- 430 **van Schaik CP, Terborgh JW , Wright SJ. 1993.** The phenology of tropical forests: Adaptive
431 significance and consequences for primary consumers. *Annual Review of Ecology and Systematics*,
432 **24**: 353–377.
- 433 **Schermer É, Bel-Venner MC, Gaillard JM, Dray S, Boulanger V, Le Roncé I, Oliver
434 G, Chuine I, Delzon S , Venner S. 2020.** Flower phenology as a disruptor of the fruiting
435 dynamics in temperate oak species. *New Phytologist*, **225**: 1181–1192.
- 436 **Templ B, Koch E, K.Bolmgren, Ungersböck M, Paul A, Scheifinger H , et al.. 2018.**
437 Pan european phenological database (pep725): a single point of access for european data. *Int.*
438 *J. Biometeorology*.
- 439 **Terribile LC, Diniz-Filho JF, Rodríguez MÁ , Rangel TFLVB. 2009.** Richness patterns,
440 species distributions and the principle of extreme deconstruction. *Global Ecology and Biogeogra-*
441 *phy*, **18**: 123–136.
- 442 **de Villemeruil P. Nakagawa S. 2014.** *Modern Phylogenetic Comparative Methods and Their*
443 *Application in Evolutionary Biology*, Springer, New York, chap. General quantitative genetic
444 methods for comparative biology, pp. pp. 287–303.
- 445 **Vitasse Y, Bresson CC, Kremer A, Michalet R , Delzon S. 2010.** Quantifying phenological
446 plasticity to temperature in two temperate tree species. *Functional Ecology*, **24**: 1211–1218.

- Vitasse Y, Delzon S, Bresson CC, Michalet R , Kremer A. 2009.** Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. *Canadian Journal of Forest Research*, **39**: 1259–1269.
- Wang Y, Li G, Di N, Clothier B, Duan J, Li D, Jia L, Xi B , Ma F. 2018.** Leaf Phenology Variation within the Canopy and Its Relationship with the Transpiration of *Populus tomentosa* under Plantation Conditions. *Forests*, **9**.
- Whitehead DR. 1969.** Wind pollination in the angiosperms: Evolutionary and environmental considerations. *Evolution*, **23**: 28–35.
- Wolkovich EM , Ettinger AK. 2014.** Back to the future for plant phenology research. *New Phytologist*, **203**: 1021–1024.
- Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG, McGlenn DJ, O’Meara BC, Moles AT, Reich PB *et al.* 2013.** Three keys to the radiation of angiosperms into freezing environments. *Nature*, **506**: 89–92.
- Zhang FP , Brodribb TJ. 2017.** Are flowers vulnerable to xylem cavitation during drought? *Proc Biol Sci*, **284**.

Supplemental Information

Fig. S1: Flower-leaf sequences of species at Harvard Forest 1990-2005.

Fig. S2: Effect-size summary plots of FLS predictors for the MTSV and USFS case studies.

Fig. S3: Phylogenetic signals for FLS variation.

Fig. S4: Visualization of FLS patterning across the phylogeny for the MTSV and USFS case studies.

Fig. S5: Effect-size summary plots of models with alternative functional traits as FLS predictors.

Fig. S6: Marginal effect plots graphically interpreting interactions among predictors for a hierarchical FLS model.

Fig. S7: Effect-size summary plots of models using alternative flower and leaf sub-phases to define FLSs.

Fig. S8: Population level variation in FLSs for *Fraxinus excelsior* mapped across Germany.

Table S1: Approximate conversions of phenophases described in the Harvard Forest dataset to the BBCH scale.

Methods S1: Methods for: FLS and climate change modeling, modeling FLS variation in MTSV and USFS data, modeling FLS variation in the HF data, and calculating the phylogenetic signals in FLS variation.

Figures

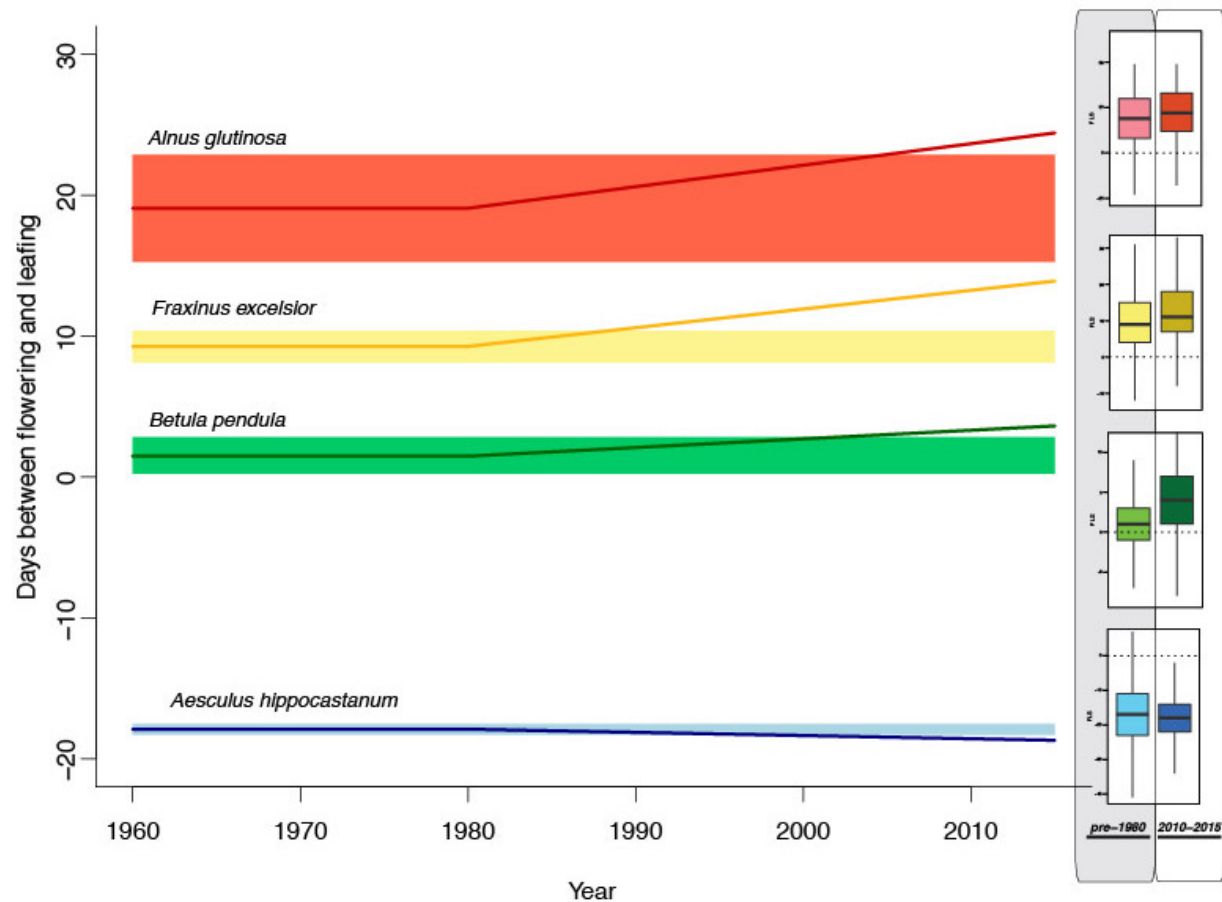


Figure 1: **Flower-leaf sequences (FLSs) across Europe for four tree species from 1960 to 2015 suggests climate change has generally increased the time between flowering and leafing**, but the direction and rate of change differs across species, which may exacerbate fitness differences within forest communities. To detect the effect of climate change on average FLS, we used models that allow for shifts in FLS after 1980 (Kharouba *et al.*, 2018). Lines represent the mean trend in FLS per species among populations, and the shaded regions indicate historic range of FLS variability (95% credible intervals of the pre-1980 average) from the PEP725 database (Templ *et al.*, 2018). The boxplots compare the FLS measurements prior to 1980 to the recent period (2010-2015), confirming shifts in FLSs over time for most species, but indicate high variability in the FLSs below the species level.

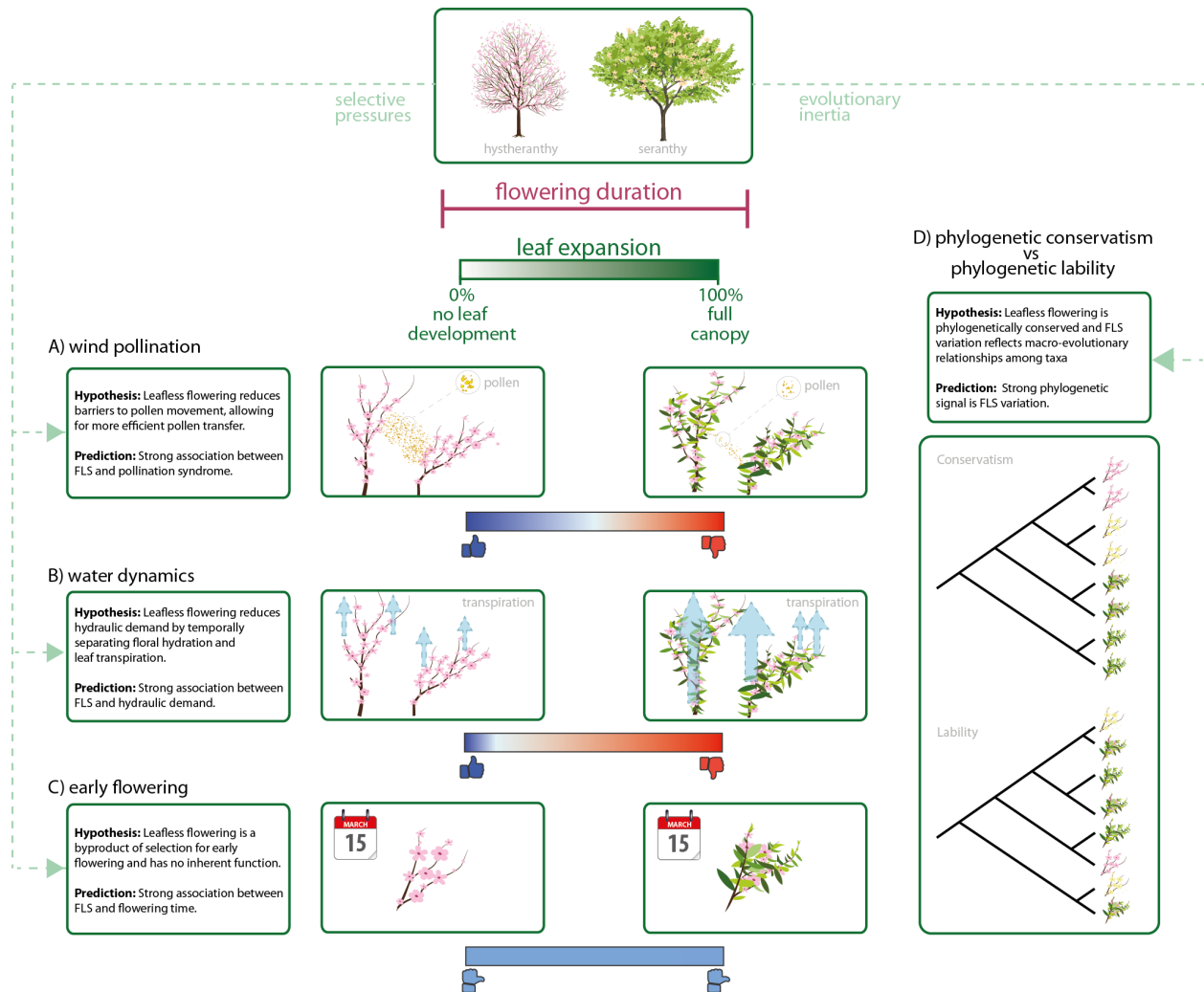


Figure 2: Several hypotheses have been proposed to explain flower-leaf sequence (FLS) variation in temperate, deciduous woody plants. The wind pollination hypothesis (a) suggests that leafless flowering reduces barriers to pollen movement. The water limitation hypothesis (b) suggests the temporal separation between flowering and leafing reduces hydraulic demand. The early flowering hypothesis (c) suggests FLS variation is a byproduct of selection for early flowering, possibly driven by later phenological event such as seed dispersal, the relative timing of flowers and leaves is inconsequential compared to the absolute time of flowering. As depicted by the scale bars in the center of the figure, the biology behind each hypothesis predicts different degrees of overlap between flowering and leaf development. Transpiration intensifies as small leaf primordia expand, but leaf development only affects environmental structure once leaves are sufficiently large, therefore the water limitation hypothesis accommodates little overlap between flower and leaves, while the wind pollination hypothesis encompasses some overlap. The early flowering hypothesis predicts no fitness differences whether or not flowers and leaves overlap. Additionally, inter-specific patterns of FLS variation may also be a product of phylogenetic conservatism or lability driven by physical constraints. (d).

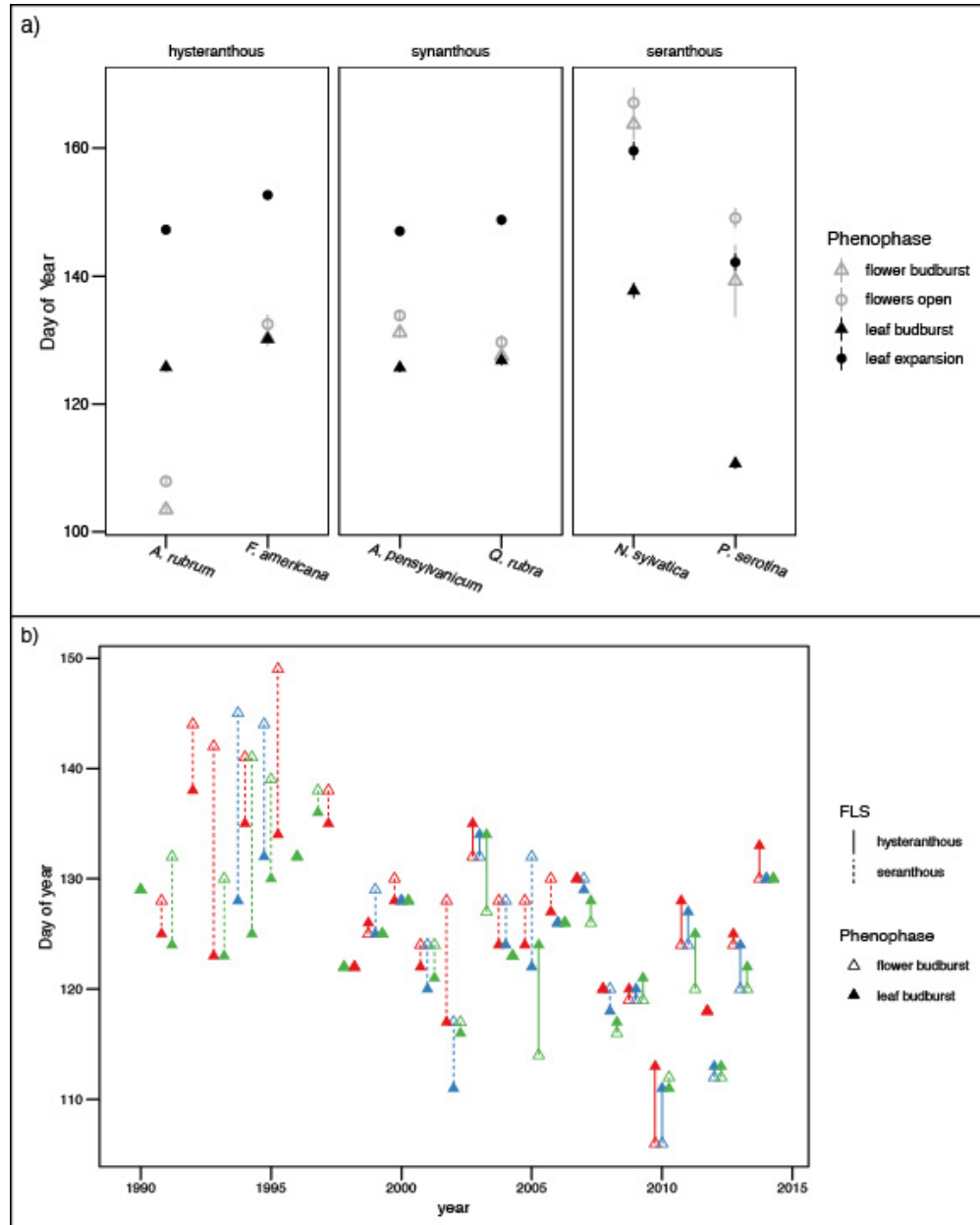


Figure 3: **The shift from categorical/inter-specific descriptions to quantitative/intra-specific measures of flower-leaf sequences (FLSs) reveals substantial variation.** Under the current framework, species are assigned to FLS categories by the order of phenophases alone. However, observations from Harvard Forest in Petersham, MA demonstrate that measuring the time between phenophases reveals substantial differences among species within each category (a). These records show that at the individual (b), the time between flowering and leaf activity can vary significant among individuals and for an individuals across years. In some species like the *Quercus rubra* depicted in here, an individual's sequence itself regularly switches across time. This inter- and intra- specific variation is key understanding the function of FLS variation in deciduous, woody plants. Quantitative measures also reveal variation in FLSs below the species level. This inter- and intra- specific variation is key understanding the function of FLS variation in temperate deciduous, woody plants. Data comes from O'Keefe (2015)

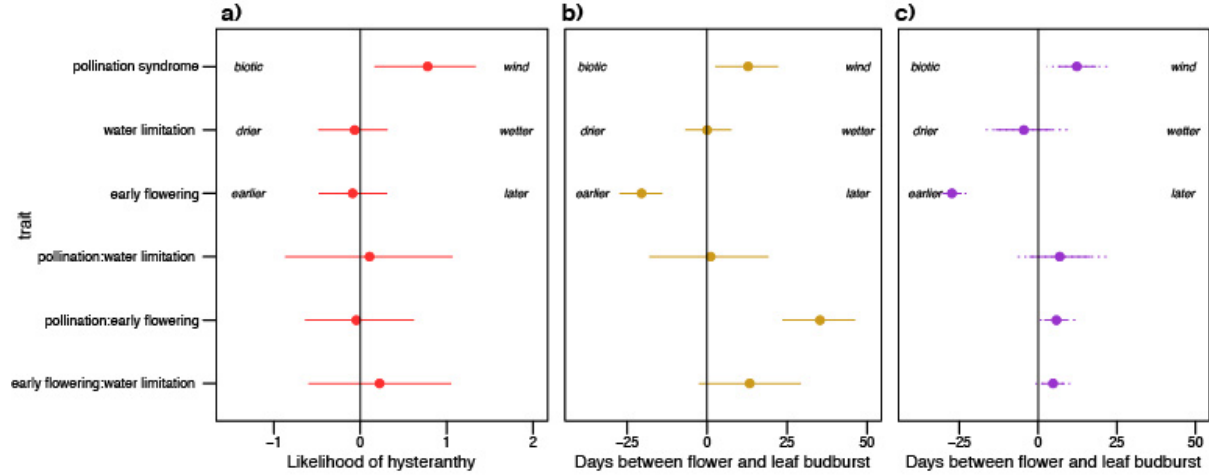


Figure 4: Mean estimates of the effects of flower-leaf sequence (FLS) predictors on the timing between flower and leaf budburst for woody plants at Harvard Forest between 1990-2015 reveal important differences between categorical and quantitative frameworks of FLSs. With the categorical approach in (a), there is a strong effect of pollination syndrome on FLS variability, with no detectable effect of other predictors. With quantitative measures based on the species level means of days between flower and leaf budburst in (b), there are strong effects and interactions of both flowering time and pollination syndrome. Finally, incorporating variation below the species level through hierarchical modeling in (c), reveals strong interactions between the predictors. These interactions suggest multiple drivers of FLS variability in the temperate zone. All models use standardized predictors to allow for comparisons between them. Symbols represent mean estimated effect of each predictor, with solid lines in (a) and (b) representing the 95% bootstrap intervals of the phylogenetic linear regression models (Ives & Garland, 2010) and dotted lines in (c) representing 50 and 95% credible intervals respectively for a phylogenetic mixed model (de Villemereuil P. Nakagawa, 2014). Graphical interpretation of the model interactions of the hierarchical model can be found in the Supporting Information (Fig. 6)