Unravelling the phenology-phylogeny tangle.

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Results & Discussion

Most species are sensitive to all three primary cues—forcing, chilling, and photoperiod (Figs. 1, Supporting Table **??**)(see also Laube et al., 2014; Ettinger et al., 2020)—with sensitivity to chilling approximately five-fold greater than sensitivity to photoperiod, on average (phenological advances of 7.2 days per standardized unit vs 1.4 days, for chilling and photoperiod, respectively; see Table **??**). However, these average sensitivities estimated across species, fail to capture the large differences in responses to chilling and forcing among species (Figs. 1, Supporting Table **??**).

By allowing species responses to vary, using a model that explicitly accounted for species' shared evolutionary history, we found species differences dwarfed the magnitude of differences between cues (Fig. 1): chilling varied 25-fold between species, while variation to forcing varied 6-fold. Our results suggest the strong focus on comparing cues misses the largest source of variation in phenological responses. Robust phenological forecasts must account for both the complexity of multiple cues and species-level variation in responses to them.

Differences across clades & cues

The large differences across species, however, were not random, but showed strong phylogenetic patterning, producing striking differences between clades. For example, oaks and beeches (Fagaceae), elms (Ulmaceae) and buckthorns (Rhamnaceae) are highly sensitive to chilling while rhododendrons (Ericaceae), butterfly bushes (Scrophulariaceae) or spindles (Celastraceae) show little to no response to chilling (Fig. 1 a). Similar clade-level variation was observed for forcing, where some clades—e.g., Ericaceae, Rhamnaceae, Ulmaceae, and Fagaceae—were particularly sensitive (advancing their budburst more than 10 days per standardized unit of forcing), while others such as the Sapindaceae, Cornaceae or Juglandaceae families showed only weak response (Fig. 1 b).

In the data analysed, we found that some species responded strongly to both temperature cues (chilling and forcing), which could suggest the existence of syndromes where the genetic basis for responses to one cue (e.g., forcing) has been been selected for alongside responses to another cue (e.g. chilling). We might expect, for example, early-season species to be sensitive to multiple cues as mistiming phenology could result in exposure to harsh conditions, leading to tissue loss or death (Sakai and Larcher, 1987), and sensitivity to multiple cues likely provides greater insurance against mistiming (Wolkovich and Donahue, 2021). Alternatively or additionally, linkage or pleiotropism among loci associated with sensitivity to different cues (Nakagawa et al., 2005) could induce correlated cues. However, the correlation in species sensitivities across cues was weak (r = 0.31; between forcing an chilling) and some genera, such as *Tilia* and Ericaceae[genus?], displayed strong responses to forcing but weak responses to chilling, while others, such as XXXXExamplesXXXX, show strong

responses to chilling but weak responses to forcing (Fig. 1). Species sensitivity to one cue, thus, does not constrain sensitivity to another cue, and selection might operate independently on responses to different cues (Bonamour et al., 2019).

In contrast to both chilling and forcing, species-level responses to photoperiod were of smaller effect and almost uniform across species. This observation provides novel insight on a large debate over the prevalence of photoperiod cues in temperate trees, where previous experiments (Basler and Körner, 2012; Zohner et al., 2016) and modeling exercises (e.g., Hunter and Lechowicz, 1992; Schaber and Badeck, 2003) indicated important variability across species that could constrain the responses of certain species to warming (Way and Montgomery, 2015). Our results suggest variability is broadly limited to a handful of species in Fagaceae, which have been heavily studied, such as *Fagus sylvatica* (e.g., Basler and Körner, 2012; Zohner et al., 2016; Kramer et al., 2017). Perhaps not coincidentally, these same few species are also where we find most evidence of local adaptation in photoperiod cues (e.g., Kramer et al., 2017). Given that studies included in our analysis spanned a range of latitudes (with many species studied in only a narrow latitudinal range), if local adaptation was high in photoperiod cues we would have expected more variability across species. The uniformity of response to photoperiod that we found thus supports an alternative view of generally low local adaptation in photoperiod cues for spring phenology, consistent with common garden studies of other species, which have reported little evidence of local adaptation in spring (but not fall) phenology (Aitken and Bemmels, 2016).

Our results suggest that while chilling and forcing are the dominant cues in terms of the magnitude of their effect sizes, most species are also sensitive to photoperiod, highlighting the potential importance of this cue to both climate change responses in the future, and through evolutionary history. In plants, photoperiod regulates a number of fundamental processes including growth, flowering, stress tolerance, and circadian rhythm (Serrano-Bueno et al., 2017; Singh et al., 2017), with photoperiodic sensing and adaptation to shifting daylength likely occurring early in the evolution of plants, perhaps with origins in the green algae (Serrano-Bueno et al., 2017). It is possible, therefore, that phenological responses to photoperiod reflect ancestral sensitivities with origins in deep time. Large plasticity in responses to additional environmental cues (e.g., temperature) for a given photoperiod cue (e.g., Kramer, 1995) may allow species to track interannual variation in climate with little directional selection on photoperiod sensitivities. Alternatively, photoperiod might provide a reliable calibration of the underlying biological clock (Jackson, 2009) upon which seasonal variation in climate modifies the tempo.

Phylogenetic structure of phenological cues

Variation—or lack thereof—in cues across species and clades, alongside related parameter estimates from our phylogenetic modeling approach, provides unique insights into the evolution of cues across the plant tree of

life. While responses to each cue were phylogenetically structured, the strength of phylogenetic conservatism in responses differ between cues (Fig. 3). Responses to temperature (forcing and chilling) were moderately structured ($\lambda = 0.65$ and $\lambda = 0.54$, for forcing and chilling, respectively), such that closely related species exhibiting more similar sensitivities than distantly related species. However, phylogenetic structure in species responses to photoperiod was comparatively weaker ($\lambda = 0.39$) (see Fig. **??**, Table **??**), emphasizing again that photoperiod diverges from other climate cues. In addition to being weaker and more uniform across species, sensitivity to photoperiod appears to also be less evolutionarily constrained than sensitivities to temperature cues.

What might drive phylogenetic structure in species temperature responses? Differences between species in their responses represent shifts in the slope of the relationship between the observed phenology and the cue. Thus, the observed phylogenetic structure in temperature responses (forcing and chilling) would be consistent with an interaction with a non-measured trait that moderates responses, and which also covaries with phylogeny (Davies et al., 2019). This fits fundamentally with the idea that early-season phenology plays a critical role in shaping species temporal niches (Gotelli and Graves, 1996) and thus should covary with a suite of life-history traits, including whether species are early-active with rapid return on investment trait, or start later in the season and have traits associated with higher competitive abilities (e.g., Grime, 1977; Wolkovich and Cleland, 2014). Geography may additionally drive structured phylogenetic variation, especially in regions where clades have radiated such that close relatives have experienced the same suite of environmental cues and thus been subject to similar selection regimes over their evolutionary history. Conversely, if species are not generally geographically constrained, as may be more likely the case, especially across deep time (Fitzpatrick and Turelli, 2006), then we might expect phylogenetic structure in phenology to also weaken, especially when aggregating across locations with different cues (Davies et al., 2013).

Weak phylogenetic signal in photoperiod sensitivity (Fig. 3) might seem at odds with observations that distantly related species respond more similarly (and less variably) to photoperiod than they do to forcing or chilling. However, somewhat counterintuitively, both uniform and random responses can manifest as low phylogenetic signal when estimated relative to Brownian motion expectations (see Wiens et al.). For example, rapid local adaptation within species might erase the phylogenetic structure in photoperiod responses, but this scenario is inconsistent with the uniformity we find in species' responses. Alternatively, if responses to photoperiod evolved early in plants, as we suggest above, and subsequent selection on photoperiod sensitivity was constrained by stabilizing selection operating on other life-history attributes sensitive to photoperiod (e.g., Rinne et al., 1994; Wilczek et al., 2014; Azeez and Sane, 2015), we would predict both low interspecific variation and weak phylogenetic signal in responses, better matching our observations. This latter interpretation is also consistent with our estimates of lower σ for photoperiod responses (CITE FIGURE). Here, as in more traditional phylogenetic comparative methods, σ represents the rate of evolution, and thus our results

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suggest photoperiod responses are also evolving slower than temperature responses. As we also show (see Appendix XXX), lower σ may capture the evolutionary dynamics of an 'early burst' model, in which trait variation accumulates rapidly early in the history of a clade and then slows through time (XXXcite Harmon: https://doi.org/10.1111/j.1558-5646.2010.01025.x), concordant with our interpretation of photoperiod evolution.

Phylogenetic conservatism (high λ) and slow evolutionary rates (low σ) in traits has sometimes been interpreted as indicative of evolutionary constraints to adaptive change (cite Wiens et al. and others cites XXX). If this were the case, we might then suggest that species with strong forcing response might be more vulnerable to future warming because phylogenetic conservatism (λ) in responses to forcing is higher compared to other cues, and its evolutionary rate (σ) is lower. This is misleading, however, as estimates of λ are independent from the rate of evolution, and macroevolutionary rates estimated on phylogenetic trees that integrate across millions of years of evolutionary history, and thus do not necessarily inform us of maximum possible rates of evolution over much shorter timescales. Indeed, there is accumulating evidence for rapid evolution to shifting climates (Bradshaw and Holzapfel, 2006; Franks et al., 2014). Our estimates of phylogenetic structure are thus more useful in improving our understanding of the evolutionary history of species phenological cues and how they should be modelled, and emphasise the importance of robustly estimating species differences in ecological forecasts.

Forecasting species-level responses

Our results highlight that species-level variability can be extremely high—when properly estimated. Our approach, which partially pools species responses based on their shared evolutionary history, did not have large influence on the average coefficients across species (model slopes for forcing and chilling shifted by 7.2% and 3.7%, respectively; Fig. 4), but estimated substantially higher variation among species compared with widely used hierarchical models. This was especially noticeable in temperature responses (for chilling variance across species was $\beta_{phylo} = 23.45$ compared to $\beta_{non-phylo} = 17.47$, for forcing it was $\beta_{phylo} = 8.74$ compared to $\beta_{non-phylo} = 5.01$) while photoperiod, which had low phylogenetic structure was more similar across approaches (variance of $\beta_{phylo} = 0.82$ compared to $\beta_{non-phylo} = 0.93$). Counterintuitively, a reduction in cross-species variance, far from increasing model accuracy could lead to increased type-II error by failing to detect actual relationships among cues and responses that would only emerge when phylogeny is correctly accounted for (see Supporting Information XX).

Importantly, the increase in variability across species allowed for in our model with phylogenetic structure decreased the uncertainty in estimates for individual species' temperature responses. Thus, traditional approaches that partially pool across species ignoring ther evolutionary affiliations (i.e. most hierarchical models in ecology) may lead to less precise predictions for individual species, although overall model accuracy might

still appear reasonable (see Appendix XX in Supporting Information).

Our model help inform which clades will be more sensitive to different axes of climate change—e.g., changes temperatures over winter (associated with chilling) versus spring (associated with forcing). For example, using a hierarchical model would not have identified oaks (genus *Quercus*) as being among the most sensitive to forcing and chilling (see e.g., (Ettinger et al., 2020)), but we show that species within this genus advance phenology by 2 days per standard unit of forcing and 4 days per standard unit of chilling. Our analyses highlight the need to move beyond current very general classifications currently used in models (e.g., functional groups ... Cat/Dan, can you offer better ref? Moorcroft, 2003) or groupings at coarse taxonomic levels (e.g., Ettinger et al., 2020) to make improved ecological forecasts for individual species.

The contrasts between temperature sensitivities and photoperiod sensitivities—in both their variability across species and phylogenetic structure—also allow for improved multi-species forecasts. For temperature responses, the large variability among species makes predicting species' individual responses challenging, but the phylogenetic structure in responses lets us borrow information from close relatives to improve our estimates. While this must be done with care (Molina-Venegas et al., 2018), especially given our very limited sampling of species, our results could provide reasonable predictions for many temperate woody plant species from clades for which we have multiple species and experimental observations at varying treatment levels. For photoperiod, the weak phylogenetic structure in responses indicate that phylogeny retains little information, but because species responses are generally more uniform, we can be more confident in assuming the mean species response across species, comforted in the knowledge that small errors will likely not have large impact given the relatively weak overall contribution of this cue, except for *Fagus sylvatica*. As *Fagus sylvatica* is nearly five times more sensitive to photoperiod than most other measured tree species, our results caution against using it to draw inferences of photoperiod responses more widely.

While we focused on spring phenology here, our modeling approach suggests a path towards more robust forecasting via integrating species-level estimates of climate change responses. Our results show how including the phylogenetic relationships among species can overcome major limitations of most current hierarchical models (producing biased model estimates, underestimating the full variability across species, and increasing uncertainty around individual species' estimates) while at once providing insight into the evolutionary history of biological responses. [And, bam, something a little more here ...]

References

Aitken, S. N., and J. B. Bemmels. 2016. Time to get moving: assisted gene flow of forest trees. Evol Appl 9:271–90.

Azeez, A., and A. P. Sane. 2015. Photoperiodic growth control in perennial trees. Plant signaling & behavior 10:e1087631.

Basler, D., and C. Körner. 2012. Photoperiod sensitivity of bud burst in 14 temperate forest tree species. Agricultural and Forest Meteorology 165:73–81.

Bonamour, S., L. M. Chevin, A. Charmantier, and C. Teplitsky. 2019. Phenotypic plasticity in response to climate change: the importance of cue variation. Philosophical Transactions of the Royal Society B-Biological Sciences 374.

Bradshaw, W. E., and C. M. Holzapfel. 2006. Evolutionary response to rapid climate change. Science 312:1477–1478.

Davies, T., E. Wolkovich, N. Kraft, N. Salamin, and S. E. Travers. 2013. Phylogenetic conservatism in plant phenology. Journal of Ecology 101:1520–1530.

Davies, T. J., J. Regetz, E. M. Wolkovich, and B. J. McGill. 2019. Phylogenetically weighted regression: A method for modelling non-stationarity on evolutionary trees. Global ecology and biogeography 28:275–285.

Ettinger, A., C. Chamberlain, I. Morales-Castilla, D. Buonaiuto, D. Flynn, T. Savas, J. Samaha, and E. Wolkovich. 2020. Winter temperatures predominate in spring phenological responses to warming. Nature Climate Change pages 1–6.

Fitzpatrick, B., and M. Turelli. 2006. The geography of mammalian speciation: Mixed signals from phylogenies and range maps. Evolution 60:601–615.

Franks, S. J., J. J. Weber, and S. N. Aitken. 2014. Evolutionary and plastic responses to climate change in terrestrial plant populations. Evolutionary Applications 7:123–139.

Gotelli, N. J., and G. R. Graves. 1996. The temporal niche. Pages 95–111 *in* N. J. Gotelli and G. R. Graves, eds. Null Models in Ecology. Smithsonian Institution, Washington, DC.

Grime, J. P. 1977. Evidence for existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111:1169–1194.

Hunter, A. F., and M. J. Lechowicz. 1992. Predicting the timing of budburst in temperate trees. Journal of Applied Ecology 29:597–604.

Jackson, S. D. 2009. Plant responses to photoperiod. New Phytologist 181:517–531.

Kramer, K. 1995. Phenotypic plasticity of the phenology of 7 european tree species in relation to climatic warming. Plant Cell and Environment 18:93–104.

Kramer, K., A. Ducousso, D. Gomory, J. K. Hansen, L. Ionita, M. Liesebach, A. Lorent, S. Schueler, M. Sulkowska, S. de Vries, and G. von Wuehlisch. 2017. Chilling and forcing requirements for foliage bud burst of European beech (*Fagus sylvatica* L.) differ between provenances and are phenotypically plastic. Agricultural and Forest Meteorology 234:172–181.

Laube, J., T. H. Sparks, N. Estrella, J. Höfler, D. P. Ankerst, and A. Menzel. 2014. Chilling outweighs photoperiod in preventing precocious spring development. Global Change Biology 20:170–182.

Molina-Venegas, R., J. C. Moreno-Saiz, I. Castro Parga, T. J. Davies, P. R. Peres-Neto, and M. Á. Rodríguez. 2018. Assessing among-lineage variability in phylogenetic imputation of functional trait datasets. Ecography 41:1740–1749.

Moorcroft, P. R. 2003. Recent advances in ecosystem-atmosphere interactions: an ecological perspective. PROCEEDINGS OF THE ROYAL SOCIETY OF LONDON SERIES B-BIOLOGICAL SCIENCES 270:1215–1227.

Nakagawa, H., J. Yamagishi, N. Miyamoto, M. Motoyama, M. Yano, and K. Nemoto. 2005. Flowering response of rice to photoperiod and temperature: a qtl analysis using a phenological model. Theoretical and Applied Genetics 110:778–786.

Rinne, P., A. Saarelainen, and O. Junttila. 1994. Growth cessation and bud dormancy in relation to ABA level in seedlings and coppice shoots of *Betula pubescens* as affected by a short photoperiod, water stress and chilling. Physiologia Plantarum 90:451–458.

Sakai, A. K., and W. Larcher. 1987. Frost Survival of Plants. Ecological Studies. Springer-Verlag.

Schaber, J., and F. Badeck. 2003. Physiology-based phenology models for forest tree species in germany. International Journal of Biometeorology 47:193–201. International Conference on the Times they are a Changin, WAGENINGEN, NETHERLANDS, DEC 05-07, 2001.

Serrano-Bueno, G., F. J. Romero-Campero, E. Lucas-Reina, J. M. Romero, and F. Valverde. 2017. Evolution of photoperiod sensing in plants and algae. Current Opinion in Plant Biology 37:10–17.

Singh, R. K., T. Svystun, B. AlDahmash, A. M. Jönsson, and R. P. Bhalerao. 2017. Photoperiod-and temperature-mediated control of phenology in trees–a molecular perspective. New Phytologist 213:511–524.

Way, D. A., and R. A. Montgomery. 2015. Photoperiod constraints on tree phenology, performance and migration in a warming world. Plant, Cell & Environment 38:1725–1736.

Wilczek, A. M., M. D. Cooper, T. M. Korves, and J. Schmitt. 2014. Lagging adaptation to warming climate in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences 111:7906–7913.

Wolkovich, E. M., and E. E. Cleland. 2014. Phenological niches and the future of invaded ecosystems with climate change. AoB Plants 6:plu013.

Wolkovich, E. M., and M. J. Donahue. 2021. How phenological tracking shapes species and communities in non-stationary environments. Biological Reviews .

Zohner, C. M., B. M. Benito, J. C. Svenning, and S. S. Renner. 2016. Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. Nature Climate Change 6:1120–1123.
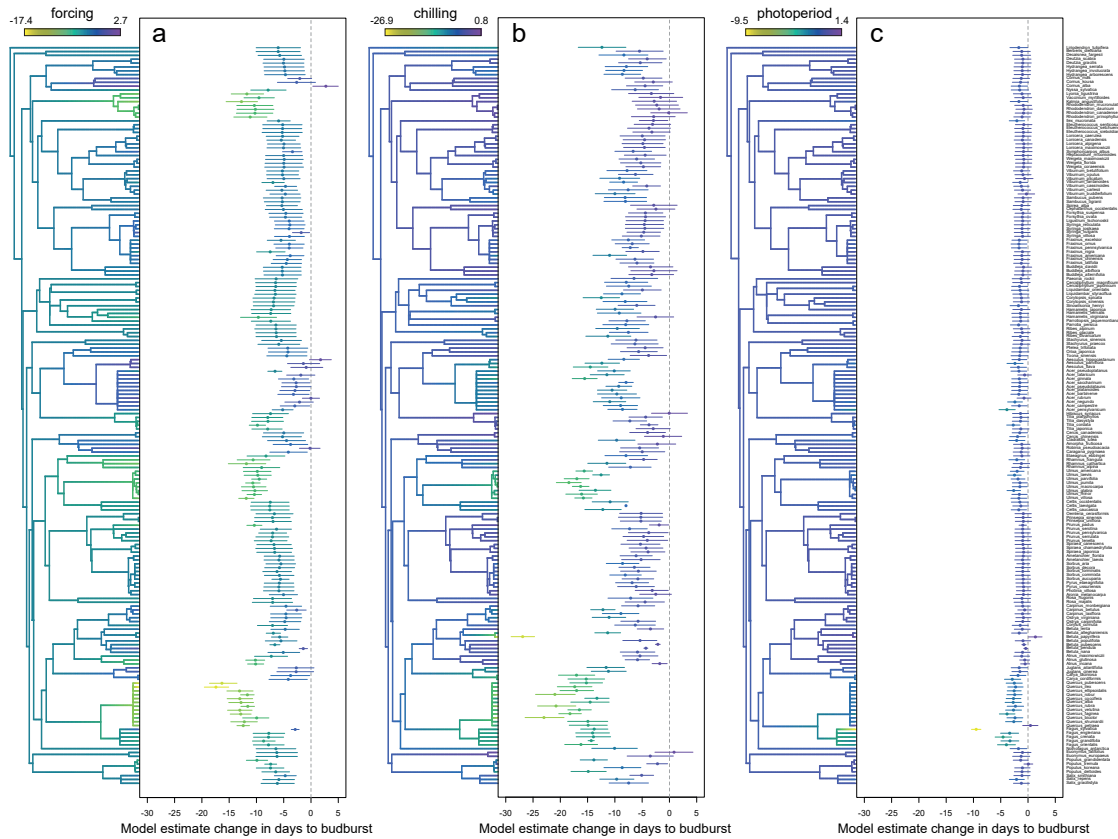
Tables and Figures

Figure 1: Phenological sensitivity to thee environmental cues, forcing (a), chilling (b) and photoperiod (c) measured in change in days to budburst per standardized unit (z-transformation) of the cues across 192 angiosperm species. The same phylogenetic tree is shown in each panel, colored acording to an estimation of ancestral character states, being the states at the tips the model slopes of our hierarchical phylogenetic model. Note that the color scale varies in each panel. Total tree depth is 81. My.
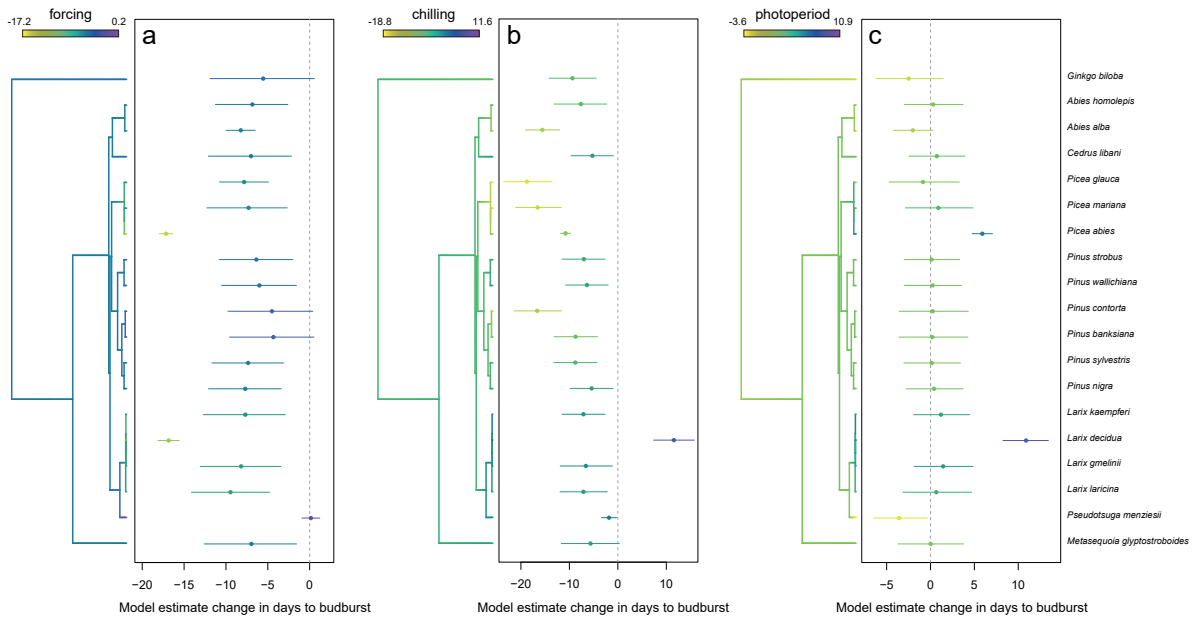
Figure 2: Phenological sensitivity to thee environmental cues, forcing (a), chilling (b) and photoperiod (c) measured in change in days to budburst per standardized unit (z-transformation) of the cues across 19 gymnosperm species. The same phylogenetic tree is shown in each panel, colored acording to an estimation of ancestral character states, being the states at the tips the model slopes of our hierarchical phylogenetic model. Note that the color scale varies in each panel. Total tree depth is 81. My.
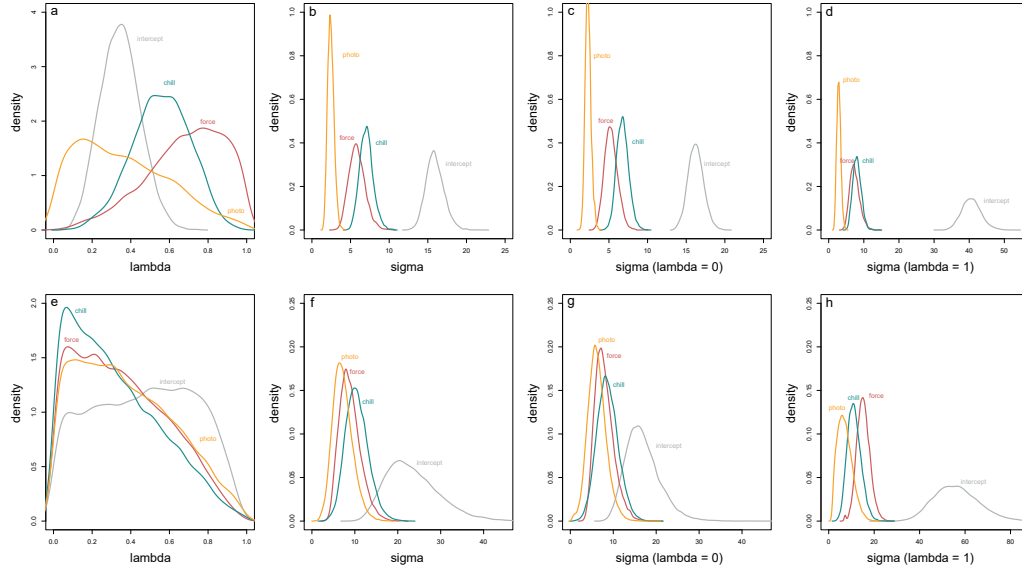
Figure 3: Density plots for the posterior distribution of phylogenetic signal measured by lambda for each cue included as a predictor in the model for angiosperms: forcing (red), chilling (blue), photoperiod (orange) and for the model intercept (grey). Panels correspond to angiosperms (a-d) and gymnosperms (e-h). Note that lambda estimations corresponding to panels c-d and g-h as they are constrained to be either equal zero or equal 1.
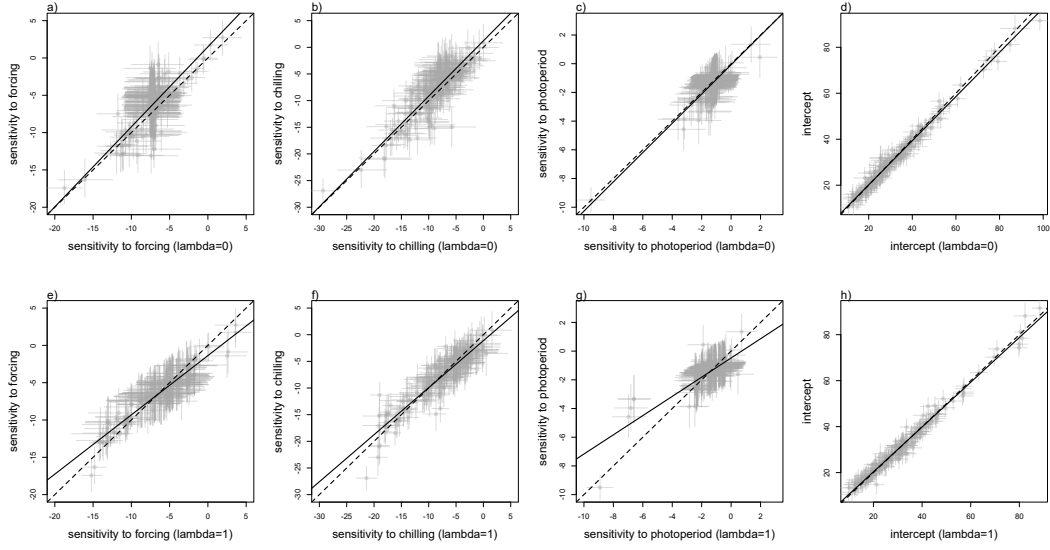
Figure 4: Correlations between model parameters as estimated by the full model and the models where lambda is constrained to be equal zero (upper row) or one (bottom row), for angiosperms. Panels correspond to sensitivity to forcing (a,e), to chilling (b,f), to photoperiod (c,g) and to model intercepts (d,h).
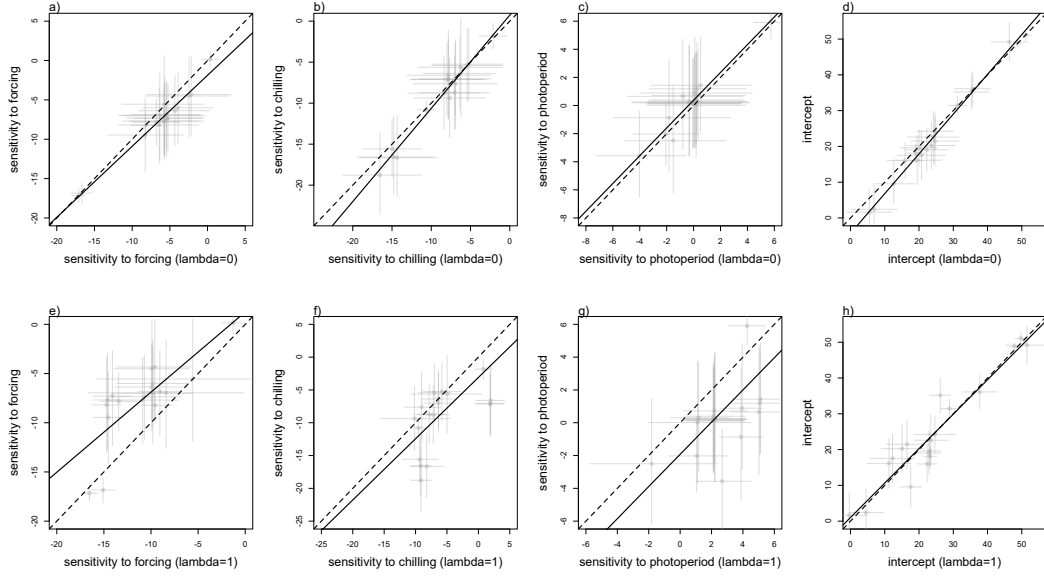
Figure 5: Correlations between model parameters as estimated by the full model and the models where lambda is constrained to be equal zero (upper row) or one (bottom row), for gymnosperms. Panels correspond to sensitivity to forcing (a,e), to chilling (b,f), to photoperiod (c,g) and to model intercepts (d,h).

Table 1: Full model parameters estimated for 192 angiosperm species.

parameter	mean	sd	2.50%	50%	97.50%	n_eff
μ_α	30.57	3.41	23.68	30.59	37.14	5031.19
$\mu_{\beta forcing}$	-5.84	2.01	-9.72	-5.89	-1.79	2374.73
$\mu_{\beta chilling}$	-7.19	2.03	-11.15	-7.18	-3.18	3694.93
$\mu_{\beta photoperiod}$	-1.37	0.76	-2.92	-1.35	0.14	1565.41
λ_α	0.35	0.10	0.16	0.34	0.56	3416.51
$\lambda_{\beta forcing}$	0.68	0.20	0.23	0.71	0.98	185.35
$\lambda_{\beta chilling}$	0.56	0.15	0.25	0.56	0.83	738.57
$\lambda_{\beta photoperiod}$	0.36	0.24	0.02	0.33	0.88	296.51
σ^2_α	15.93	1.17	13.84	15.85	18.41	2988.37
$\sigma^2_{\beta forcing}$	5.84	1.04	4.03	5.78	8.15	502.74
$\sigma^2_{\beta chilling}$	7.05	0.87	5.48	7.02	8.92	1026.77
$\sigma^2_{\beta photoperiod}$	2.45	0.41	1.74	2.42	3.32	469.46
σ^2_y	12.81	0.18	12.47	12.80	13.17	4017.16

Table 2: Full model parameters estimated for 19 gymnosperm species.

parameter	mean	sd	2.50%	50%	97.50%	n_eff
μ_α	25.75	4.50	16.88	25.73	34.73	33151.86
$\mu_{\beta forcing}$	-5.92	3.80	-12.97	-6.05	1.90	16443.03
$\mu_{\beta chilling}$	-8.11	3.63	-15.31	-8.09	-0.94	21379.81
$\mu_{\beta photoperiod}$	-0.88	3.33	-8.01	-0.67	5.19	16301.93
λ_α	0.47	0.26	0.02	0.48	0.90	15934.03
$\lambda_{\beta forcing}$	0.36	0.23	0.02	0.33	0.84	14336.60
$\lambda_{\beta chilling}$	0.32	0.23	0.01	0.28	0.82	13230.88
$\lambda_{\beta photoperiod}$	0.37	0.24	0.02	0.34	0.88	11199.49
σ^2_α	23.47	6.20	13.87	22.59	37.81	18272.58
$\sigma^2_{\beta forcing}$	8.89	2.45	4.96	8.60	14.51	8126.51
$\sigma^2_{\beta chilling}$	10.47	2.66	5.78	10.30	16.17	8539.38
$\sigma^2_{\beta photoperiod}$	7.18	2.29	3.29	6.96	12.25	5625.69
σ^2_y	15.81	0.41	15.04	15.81	16.63	28640.16