

# Unravelling the phenology-phylogeny tangle.

December 10, 2021

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# Abstract

Plants have evolved responses to environmental cues able to inform them about the temporal distribution of key resources—i.e. energy and light. The responses to individual cues such as forcing (or spring warming) have shown to be subjected to some degree of evolutionary conservatism. Yet, plants do not respond to isolated cues but to a combination of interacting cues, which difficults accurate predictions of phenology in the face of environmental change. Whether and how evolution has constrained phenological responses to combinations of interacting cues is not yet understood even when this knowledge could enhance model predictions and inform how different plant lineages have adapted to environmental change along their evolutionary histories. Here we use Bayesian hierarchical models and the most complete dataset on tree species phenological responses measured in experimental conditions to: (a) test if phenological responses to three major interacting cues are conserved phylogenetically when considered jointly, (b) compare the phylogenetic signal in the responses to different cues and, (c) test whether coefficient estimates differ between models assuming phylogenetic independence among species and models that explicitly incorporate phylogeny. Results show non-random phylogenetic structuring of phenological responses, highly variable across species and cues. More interestingly, regression coefficients shift when models control for phylogenetic effects, particularly so for forcing, which becomes the most important cue. Taken together, our results suggest that phylogeny should be incorporated into studies modelling multi-species phenological responses, as such responses have been jointly constrained through evolution and thus are not independent.

# Introduction

1. Understanding how different plant lineages have evolved their phenotypic responses to the joint effects of interacting environmental cues remains a challenge.
  - (a) Environmental cues matter as they inform organisms about the temporal distribution of key resources.
  - (b) Responses (and their evolution) to cues are usually studied individually assuming that a given phenotypic response (e.g. time of leafout) is linked to a single cue, when likely multiple ones operate interactively (and have done so across evolutionary history) to shape that response.
  - (c) Out of the three major cues that affect plants (forcing, chilling, photoperiod), few multi-species analyses have considered all three simultaneously, with repeated consensus that chilling and forcing would prevail, but would this pattern hold if evolution/phylogeny was accounted for?
2. Phenology makes for an ideal study case of species' responses to interacting environmental cues.

- (a) It is amongst the few phenotypic characters (if any other exists) for which there are multi-species experimental data on its responses to the three major environmental cues.
  - (b) It is evolutionary conserved (to some extent, review antecedents).
  - (c) Research in this area has focused on the phenotype (phenological event or its shifts) rather than on the cues—i.e. how shifts in the environment trigger species responses. For example, first flowering is significantly conserved (Davies et al., 2013).
  - (d) When using OU models so are shifts in first flowering and the slopes of the relationship between flowering and year (Rafferty and Nability, 2017).
  - (e) But many questions remain open: in a multi-species context, have specific lineages adapted more strongly to some of the cues? or to any combination of cues? Is there any cue that is particularly labile?
  - (f) Answering these questions may: (i) inform about the need to account for phylogeny in phenological models and predictions, and (ii) expand our knowledge on how phenological responses have been constrained so far, which would be relevant in a context where species’ sensitivities to warming temperatures seem to decline.
3. Current methods have prevented further advances in understanding how specific lineages have adapted phenotypic responses to interacting environmental cues.
- (a) Beyond data unavailability, common methodological approaches to studying phylogenetic constraints to phenological responses are not designed to explicitly test constraints in cue responses evolved jointly (nothing wrong with current methods, but they answer a different question). For example most common approaches have:
    - i. Used phylogenetic regression either hierarchical (PMM) or not (PGLS), where only phylogenetic signal or effects are modelled for the residuals (or included as a grouping or random factor). Doing so ‘hides’ information on how each cue’s associations with the response have been constrained through evolution while operating in concert with other cues. Research interested in how a given response to a cue would have been constrained through evolution would have, for example, looked at the phylogenetic signal of that particular response.
    - ii. Disregarded phylogenetic non-stationarity (but see (?))
    - iii. Focused on flowering (and leafout some) times and shifts in them (but see Joly et al. (2019), and add REFs!! on other phenological stages: budburst, ripening)
    - iv. Studied trait correlation (Bolmgren and D. Cowan, 2008) (not a limitation, but a different focus)

- v. Studied different evolutionary models best fitting the data (Rafferty and Nabity, 2017)
  - vi. measured shifts based on field observation data for both climate and phenology (when slopes are available, they represent shifts with time, not shifts with the environment).
  - vii. most efforts are on the phenotype rather than on the magnitude of species phenological responsiveness to different environmental cues.
4. Here, we expand previous phylogenetic regression settings to explicitly estimate phylogenetic constraints on the interactions among predictors (cues).
- (a) Common phylogenetic regression accounts for phylogenetic relationships as a grouping factor either explicitly (PMM) or implicitly (PGLS). Regardless, it fails to account for more complex interactions going on among predictors, which would be reflected in the species-level slopes being allowed to vary as a function of the phylogeny, rather than keeping slopes constant and only allowing the intercepts (or residuals) to vary.
  - (b) We ignore whether this is important and maybe current models are fine.
  - (c) In a first attempt at establishing whether or not it is important, we compare results from a common hierarchical model with partial pooling on the slopes that does not allow for phylogenetic constraints to affect slope estimates against results from a phylogenetic hierarchical model allowing phylogeny to constrain partially pooled slopes.
  - (d) We do so for an unprecedented dataset on phenological responses to environmental cues determined experimentally.
  - (e) This is one possible approach but there may be alternative ones.
5. Questions rather than specific hypotheses
- (a) Based on previous research on phylogenetic signal of phenological responses, we expect non-random phylogenetic structuring of the responses to environmental cues (Davies et al., 2013; Rafferty and Nabity, 2017; Joly et al., 2019) and expect that temperature-related cues display higher phylogenetic signal than photoperiod because the latter has remained more constant through evolutionary time. Yet, rather than specific hypotheses for different lineage-level responses, our work aims at exploring and discussing the following questions:
    - i. Do we need to account for phylogeny in multi-species, multi-cue modelling of the magnitude (strength) and variation of phenological responses to cues? This is, we worry about what are the biggest cues, and we think we may know which are those but if we have the wrong model, we may make the wrong inference or get estimates wrong.

- ii. If so, can accounting for phylogeny shed light on the ongoing debate on declining sensitivities? For example, if particular lineages have very different evolutionary constraints on their responses to the cues, they may also display very different declines in their sensitivities to the cues.
- iii. How can we interpret lambdas and sigmas for each cue, and for the intercept?
- iv. What are the implications for phenological predictions and forecasts?
- v. Is this approach transferable to different taxa or biological responses?

# Methods

## Phenological and Phylogenetic Data

1. Description of the OSPREE database (where it comes from, number of species, studies, etc.).
2. We analyze 5 different subsets of species in the OSPREE database to explore differences across taxa (effect of gymnosperms?) and to test to what extent data resolution affects the results:
  - (a) Species grouped in generic complexes, to ensure enough cross-treatment data, as in Ettinger et al. (under review) (including 52 complexes)[flags.for.mainmodel=T]
  - (b) All species in the main model (including 117 species resulting from ) [flags.for.mainmodel=T]
  - (c) All angiosperm species in the main model (including 110 species)[flags.for.mainmodel=T]
  - (d) All species in the latest version of OSPREE (including 231 species resulting from ) [flags.for.allspmodel=T]
  - (e) All angiosperm species in the latest version of OSPREE (including 215 species)[flags.for.allspmodel=T]
3. Two phylogenetic hypotheses have been considered to build a tree containing the species in OSPREE. First the vascular plant megatree by Zanne et al. (2014);Nature and, second the megatree by Smith & Brown (2019);AJB.
4. The backbone phylogenies were pruned to contain only the studied species in each subset.
5. Species not in the backbone phylogeny were added as polytomies at the generic level ( using the function *congeneric.merge*; (Pearse et al., 2015)).
6. To build a phylogeny for species complexes, the terminal branches of species belonging to the same complexes were collapsed.

Help here  
would be  
much appre-  
ciated!

## The Bayesian hierarchical phylogenetic model

For each of  $n$  species, we assumed that data were generated from the following sampling distribution:

$$y_j \sim \mathcal{N}(\mu_j, \sigma_e^2) \quad (1)$$

where

$$\mu_j = \alpha_j + \beta_{1,j}X_2 + \beta_{2,j}X_2 + \beta_{3,j}X_3 \quad (2)$$

Predictors  $X_1$ ,  $X_2$ ,  $X_3$  are standardized forcing, chilling, and photoperiod, and their effects on the phenology of species  $j$  are determined by parameters  $\beta_{1,j}$ ,  $\beta_{2,j}$ ,  $\beta_{3,j}$  representing traits. These traits, including the species-specific intercept  $\alpha_j$ , are elements of the following normal random vectors:

$$\begin{aligned}\boldsymbol{\alpha} &= \{\alpha_1, \dots, \alpha_n\}^T \text{ such that } \boldsymbol{\alpha} \sim \mathcal{N}(\mu_{\alpha}, \boldsymbol{\Sigma}_{\alpha}) \\ \boldsymbol{\beta}_1 &= \{\beta_{1,1}, \dots, \beta_{1,n}\}^T \text{ such that } \boldsymbol{\beta}_1 \sim \mathcal{N}(\mu_{\beta_1}, \boldsymbol{\Sigma}_{\beta_1}) \\ \boldsymbol{\beta}_2 &= \{\beta_{2,1}, \dots, \beta_{2,n}\}^T \text{ such that } \boldsymbol{\beta}_2 \sim \mathcal{N}(\mu_{\beta_2}, \boldsymbol{\Sigma}_{\beta_2}) \\ \boldsymbol{\beta}_3 &= \{\beta_{3,1}, \dots, \beta_{3,n}\}^T \text{ such that } \boldsymbol{\beta}_3 \sim \mathcal{N}(\mu_{\beta_3}, \boldsymbol{\Sigma}_{\beta_3})\end{aligned}\tag{3}$$

where the means of the multivariate normal distributions are root trait values (i.e., trait values prior to evolving across a phylogenetic tree) and  $\boldsymbol{\Sigma}_i$  are  $n \times n$  phylogenetic variance-covariance matrices of the form:

$$\begin{bmatrix} \sigma_i^2 & \lambda_i \times \sigma_i \times \rho_{12} & \dots & \lambda_i \times \sigma_i \times \rho_{1n} \\ \lambda_i \times \sigma_i \times \rho_{21} & \sigma_i^2 & \dots & \lambda_i \times \sigma_i \times \rho_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ \lambda_i \times \sigma_i \times \rho_{n1} & \lambda_i \times \sigma_i \times \rho_{n2} & \dots & \sigma_i^2 \end{bmatrix}\tag{4}$$

where  $\sigma_i^2$  is the rate of evolution across a tree for trait  $i$  (here assumed to be constant along all branches),  $\lambda_i$  scales branch lengths and therefore is a measure of the “phylogenetic signal” within a species trait, and  $\rho_{xy}$  is the phylogenetic correlation between species  $x$  and  $y$ , or the fraction of the tree shared by the two species.

The above specification is exactly equivalent to writing equation 2 in terms of root trait values and residuals, such that:

$$\mu_j = \mu_{\alpha} + \mu_{\beta_1} X_1 + \mu_{\beta_2} X_2 + \mu_{\beta_3} X_3 + e_{\alpha_j} + e_{\beta_{1,j}} + e_{\beta_{2,j}} + e_{\beta_{3,j}}\tag{5}$$

where the residual error terms (e.g.,  $e_{\alpha_j}$ ) are elements of normal random vectors from multivariate normal distributions centered on 0 with the same phylogenetic variance-covariance matrices as in equation 4.

## Interpretation of $\lambda_i$

In contrast to classic approaches to controlling for phylogenetic non-independence of analysis units (i.e. species), see (Freckleton et al., 2002), where Pagel’s Pagel (1999)  $\lambda$  is assumed constant across multiple predictors if those enter a PGLS model, our approach retrieves

The  $\lambda$  in our models is analogous to, but not fully equivalent to Pagel's Pagel (1999)  $\lambda$  parameter (Housworth et al., 2004), constrained to range from 0 to 1, with values of 0 indicating absence of phylogenetic relatedness, and values of 1 indicating *Brownian Motion* evolution (BM). This is because in our approach, a  $\lambda$  is estimated for each predictor in the model whilst in PGLS and similar approaches,  $\lambda$  is computed simultaneously across the predictor matrix.

## Phylogeny in forecasts of phenology

This sections needs to be fleshed out, but first we need to think and decide how to proceed (or if we want to proceed at all):

1. How we define the two scenarios (regular scenario; climate change scenario; see below)?
2. For which subset of species do we test it?
3. Are we predicting with and without phylogeny? I'm still not sure about how to do this.

## Results

### Cue sensitivities: are there major shifts when phylogeny is accounted for?

Perhaps we can compare the new results against those in Ailene's paper more closely ??, and ??.

A first glance comparing results here with those in the NCC paper suggest that after taking phylogeny into account, the associations with photoperiod may decrease and the variance around estimations of sensitivity to chilling gets larger.

### Phylogenetic signal in phenological responses

1. Phenological responses to the three studied cues are overall phylogenetically conserved but estimates of phylogenetic signal differ strongly across species subsets (angio vs. gymno).
2. When angiosperm species (from main model) are considered, responses to forcing are more conserved ( $\lambda = 0.64$ ) than responses to chilling ( $\lambda = 0.66$ ) or to photoperiod ( $\lambda = 0.35$ ) (see Figure 4).
3. When gymnosperm species are considered, all responses to cues are similarly low (yet different from zero): forcing ( $\lambda = 0.36$ ), chilling ( $\lambda = 0.32$ ) and photoperiod ( $\lambda = 0.37$ ) and show almost overlapping posterior distributions, which may be driven by a low number of species (19 ??).



## Budburst models, phylogenetic vs. non-phylogenetic

1. Here goes text comparing results with  $\lambda \bar{0}$  against results with estimated  $\lambda$ .

## Discussion

To be fleshed out.

## References

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## Tables and Figures

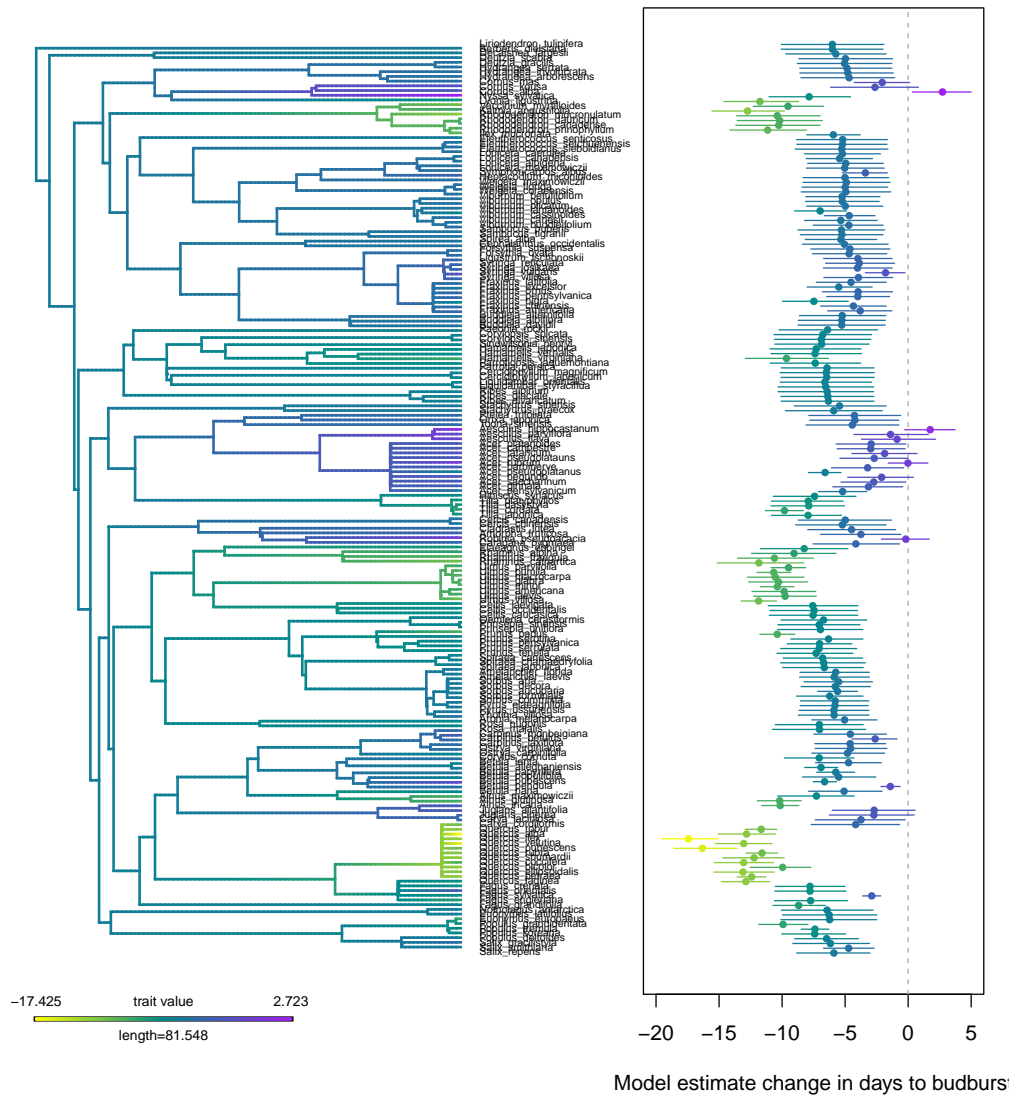


Figure 1: Cue sensitivity estimation by hierarchical phylogenetic model showing slopes for forcing, for 194 angiosperm species.

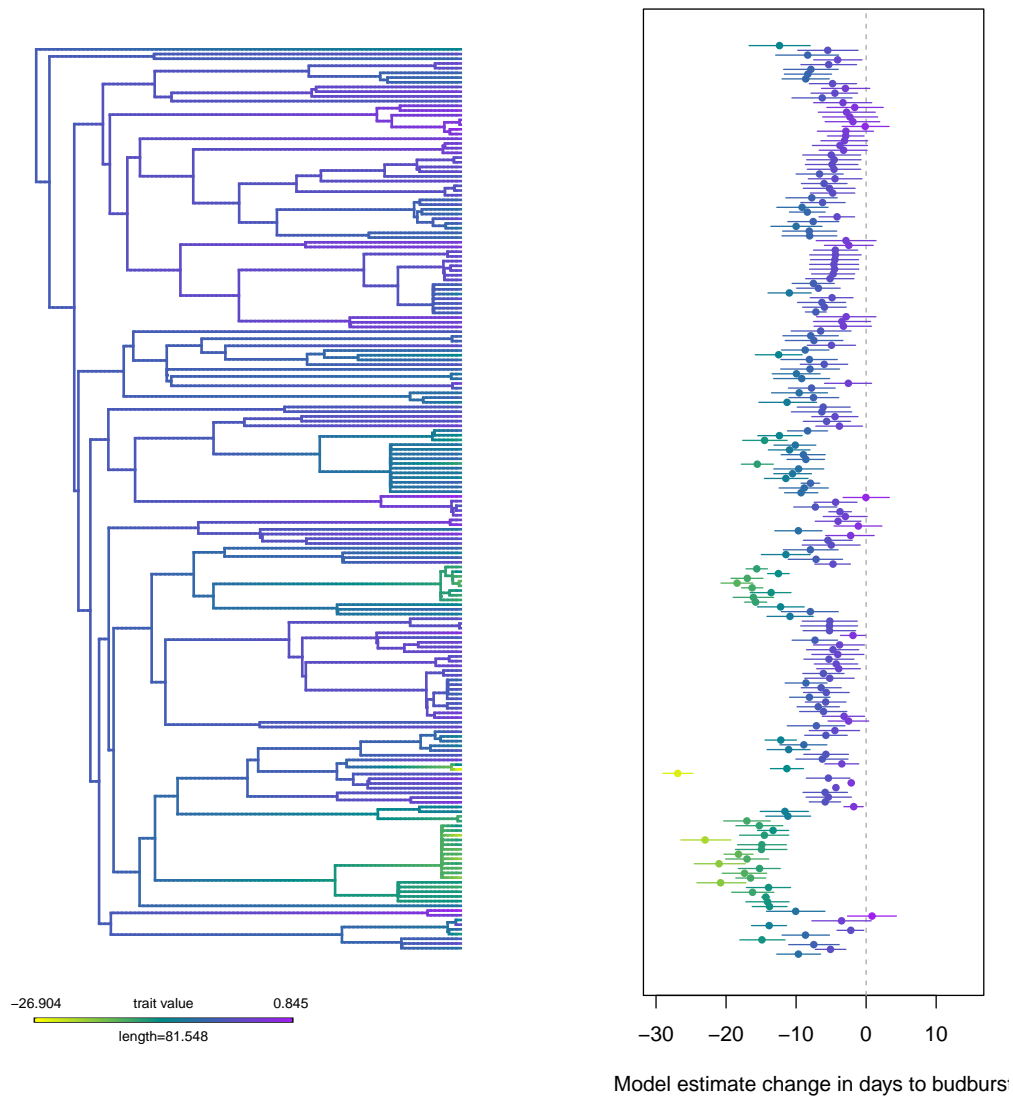


Figure 2: Cue sensitivity estimation by hierarchical phylogenetic model showing slopes for chilling, for 194 angiosperm species.



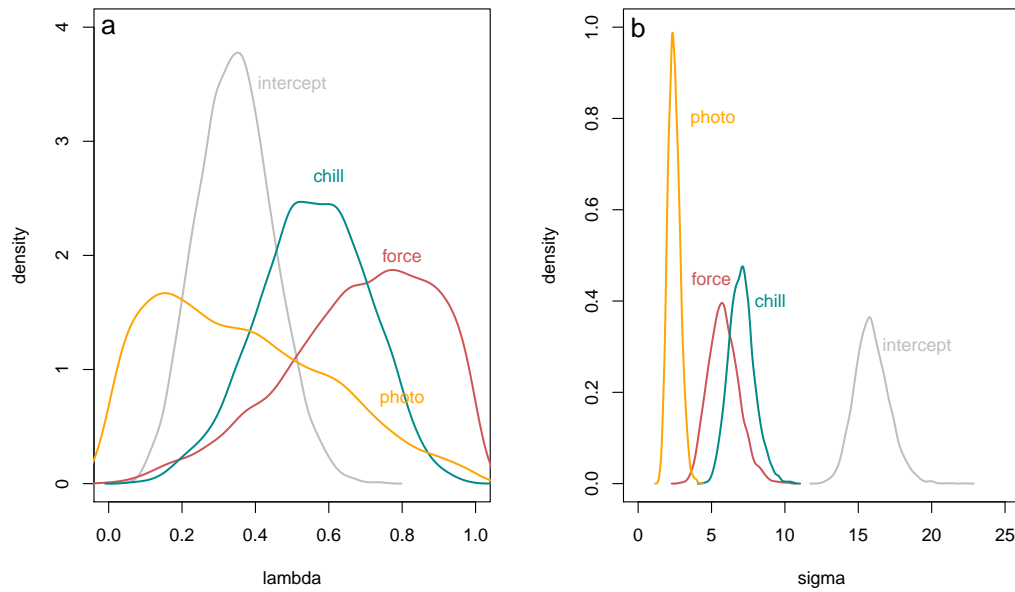


Figure 4: Posterior distribution of phylogenetic signal measured by  $\lambda$  for each cue included as a predictor in the model for angiosperms: forcing (red), chilling (blue), photoperiod (orange) and for the model intercept (grey).

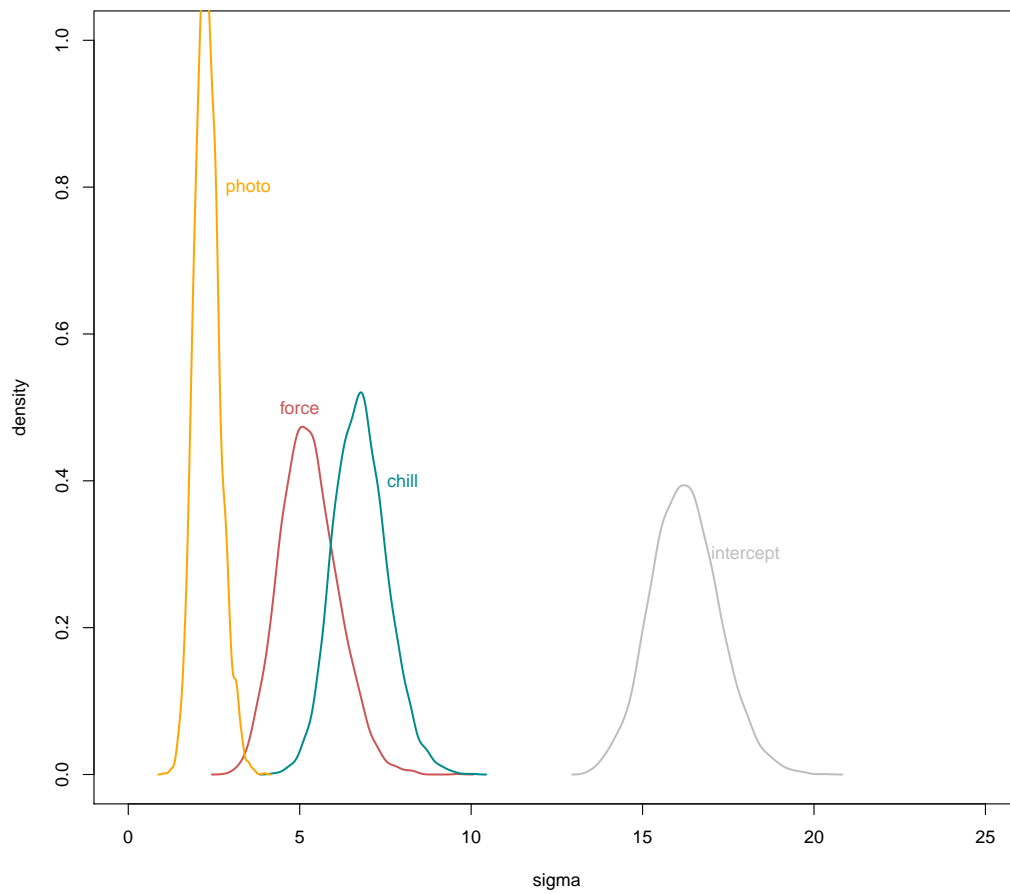


Figure 5: Posterior distribution of sigma for each cue included as a predictor in the model for angiosperms: forcing (red), chilling (blue), photoperiod (orange) and for the model intercept (grey).

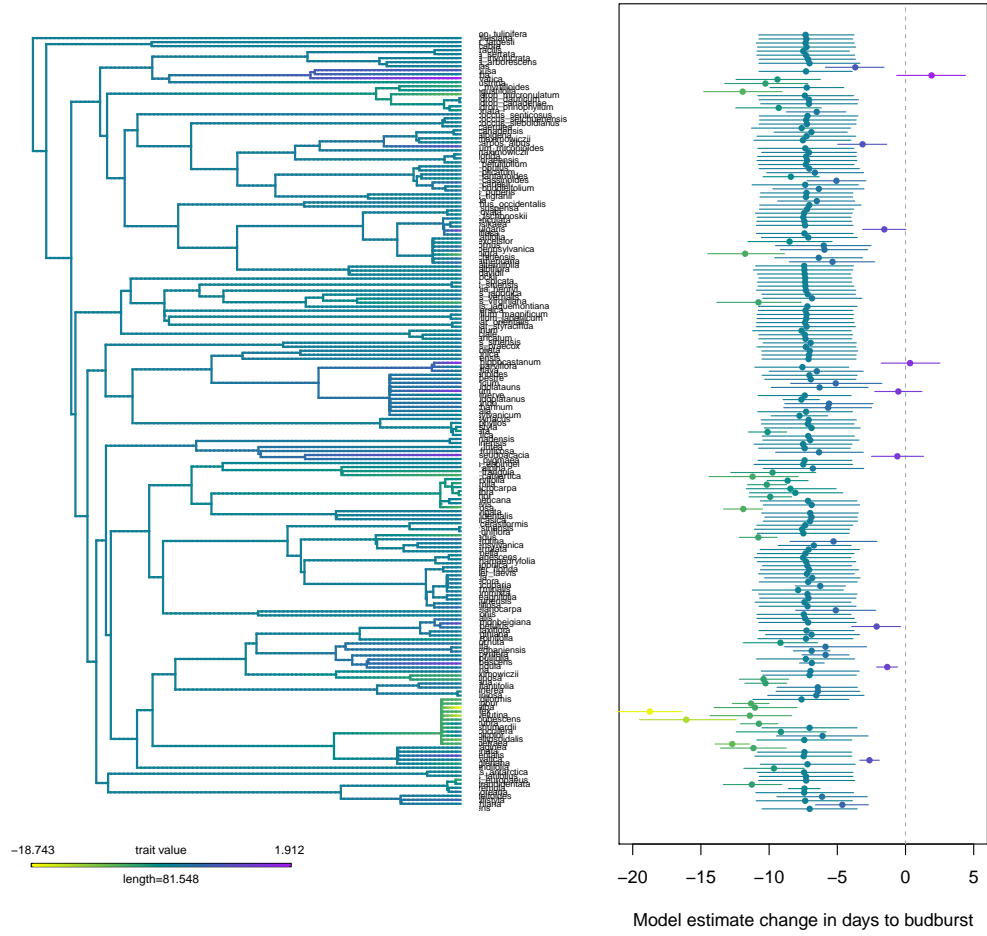


Figure 6: Cue sensitivity estimation by hierarchical phylogenetic model showing slopes for forcing making  $\lambda = 0$ , for 194 angiosperm species.

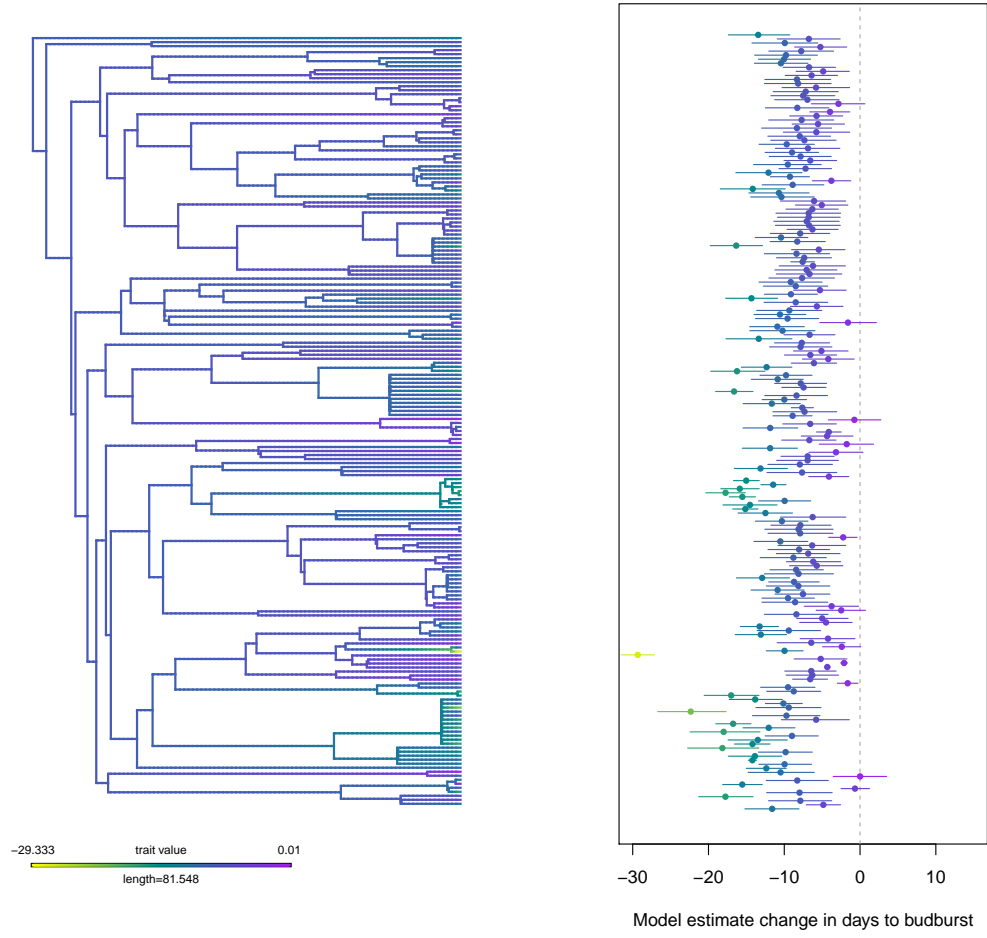


Figure 7: Cue sensitivity estimation by hierarchical phylogenetic model showing slopes for chilling making  $\lambda = 0$ , for 194 angiosperm species.



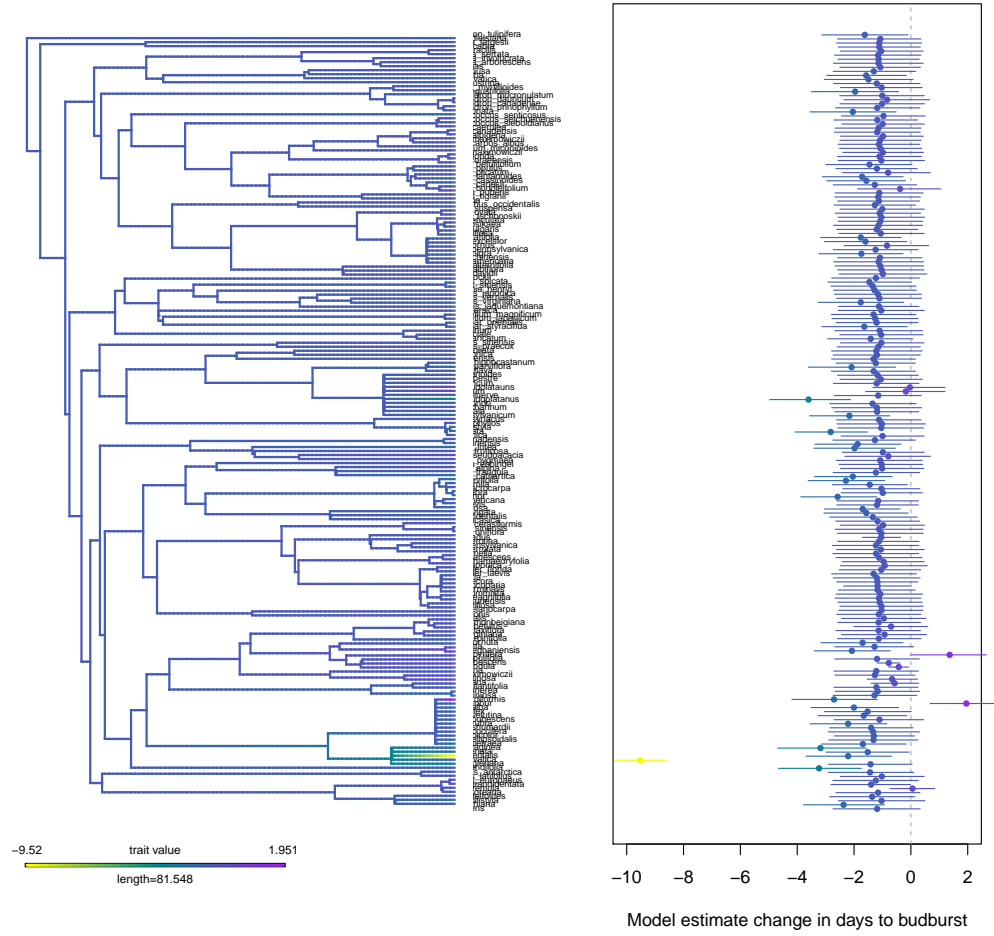


Figure 8: Cue sensitivity estimation by hierarchical phylogenetic model showing slopes for photoperiod making  $\lambda \bar{0}$ , for 194 angiosperm species.