

Evolutionary history—more than phenological cues—explain temporal assembly of woody plant communities

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Introduction

1. Plant phenology is changing with climate change:
 - (a) Timing of spring bb is changing with anthropogenic climate change
 - (b) But changes are not uniform with some regions experiencing greater warming than others.
 - (c) Responses are also species specific and highly variable
 - (d) Budburst in forest communities is the product of species differences as well as geographic variability in environmental cues.
 - (e) Understanding the relative importance of these different drivers of budburst is needed to understand and predict future changes in spring phenology—and their ultimate impacts on growing season length, carbon cycle, species interactions
2. Cues that shape bb
 - (a) For woody plants, we do know there are three important environmental cues for bb:
 - i. Forcing: spring temperatures
 - ii. Photoperiod/daylength
 - iii. Chilling: winter length and temperatures
 - (b) But these cues interact—forcing can offset low chilling—photoperiod offsets weak forcing (Heide, 1993; Chuine, 2000; Caffarra and Donnelly, 2011; Flynn and Wolkovich, 2018)
 - (c) The consistency and strength of these interactions across species and populations remains unclear.
3. Cues can vary geographically and temporally—under future climates—likely to create differences in community dynamics and species composition.
 - (a) Increasing winter and spring temperatures = faster accumulation of chilling and forcing (Guy, 2014)—associated with earlier budburst in many temperate plant species.
 - (b) Could create novel niche space early in growing season—facilitating spp invasions or novel community assemblages

- 35 (c) Will also reduce fitness in spp with strong photoperiod cues = limited in their ability to
 36 advance (Körner and Basler, 2010) = different competitive landscape later in the growing
 37 season
- 38 (d) Differences in species cue responses are likely to shape the diversity and persistence of species
 39 across forest communities.
- 40 4. Variation in bb phenology across species in a community
- 41 (a) Timing of bb in a forest community can span several weeks—species fill different temporal
 42 niche
- 43 (b) e.g. understory shrub spp tend to bb earlier than canopy species, likely reflecting overarching
 44 differences in growth strategies.
- 45 (c) But differences in budburst responses are likely to also exist across a species ranges—need
 46 to know the relative effects of pop vs sp
- 47 5. While climate change may cause climates to shift rapidly, forest communities have assembled
 48 over longer timescales, with traits like phenology responding to local conditions.
- 49 (a) Across a species' spatial distribution—we can expect to see differences in both cues and
 50 therefore in spp responses in bb that have evolved over time
- 51 (b) Species with large latitudinal distributions experience differences in cues—e.g. photoperiod
 52 cues
- 53 (c) But few studies have explored how cue use may differ across spatial gradient for the same
 54 forest communities and the role of local environments and biotic communities in shaping
 55 budburst across North America.
- 56 (d) To better predict how forest communities will respond and assemble under continued climate
 57 change we need to identify the cues that drive budburst in our dominant forest species and
 58 functional groups, and how these responses can change across populations.
- 59 6. In this study we:
- 60 (a) Combined results from two growth chamber studies of woody plant phenological cues
- 61 (b) Data from four populations, from eastern to western North America and a range of 4-6°
 62 latitude
- 63 (c) Allows us to detect general trends in how bb of N Am. deciduous forest communities respond
 64 to forcing, chilling, photoperiod
- 65 (d) But also community specific responses—detect differences between Western and Eastern
 66 forest communities, and at different latitudes
- 67 (e) And trends across different functional groups, exploring differences between the shrubs that
 68 dominate the forest understory and tree species.

Materials and Methods

Field sampling

We combined data from two growth chamber studies using branch clippings of North American deciduous woody plants. Our first study used samples taken from two eastern populations—Harvard Forest, Massachusetts, USA (42.55°N, 72.20°W) and St. Hippolyte, Quebec, Canada (45.98°N, 74.01°W), which we sampled from the 20-28 January, 2015. The second growth chamber study spanned two western populations—Smithers (54.78°N, 127.17°W) and E.C. Manning Park (49.06°N, 120.78°W), British Columbia, Canada, respectively, sampled from the 19-28 of October, 2019.

We observed budburst for 47 species spanning our two transects, with 28 species at our eastern transect and 22 species at our western transect, 3 of which occur at both transects. We selected these species as they were dominant in the forest communities at each population and to maximize the number of species occurring across sites. Of the species we sampled in our eastern transect, 13 were shrubs and 15 were trees, while in our western forest community we sampled 18 shrub and 4 tree species. At all sites, we tagged between 15 and 20 healthy, mature, individuals for each species in the summer prior to the growth chamber study. Depending on the size of the individual, we collected between 1-16 cuttings from six to 20 tagged plants, with samples being taken using a pole pruner from the ground. We kept samples cold during sampling and immediately placed them in water upon returning from the field. Our eastern study was conducted at the Arnold Arboretum, Boston, MA, USA, and the western study at the University of British Columbia, BC, Canada. We used daily weather data from local weather stations to calculate the field chilling prior to collection (see Table S2).

Growth chamber study

To estimate the effects of chilling, forcing and photoperiod, we used growth chambers with two levels of chilling—with either no additional chilling or 30 days at 4°C for our eastern study, and 30 days or 70 days of chilling at 4°C for our western study (all dark)—after which plants were moved to one of two levels of forcing—a cool regime of 15°C:5°C and a warm regime of 20°C:10°C—crossed with two photoperiods of either 8 or 12 hours—for a total of 8 distinct treatments in each study. Our design was similar for both eastern and western species, except for the thermoperiodicity in forcing treatments. In our eastern study, we set the duration of forcing temperatures to correspond with the photoperiod treatments. While this is a widespread and common approach in phenology chamber studies, it leads to a confounding effect in the thermoperiodicity (Buonaiuto et al., 2023). In contrast, we applied forcing temperatures for 12 hours independent of the duration of the photoperiod treatments in our western study/ We had seven to ten replicates per treatment in our eastern study and eight replicates in our western study, with the exception of *Sorbus scopulina*, for which we only had six replicates per treatment. Further details regarding the re-cutting of samples, changing water in containers, and rotation through chambers are presented in Flynn and Wolkovich (2018).

Phenological observations of budburst were assessed using the BBCH scale, adapted for our specific species (Finn et al., 2007). We observed each sample every 1-3 days and recorded all phenological stages up to full leaf expansion (defined as code 17 by Finn et al., 2007). Here, we present the response in budburst date, defined as the day of budbreak or shoot elongation (defined as code 07 by Finn et al. (2007)). In total, our eastern study spanned 82 days, with over 19320 phenological observations. The western study in turn spanned 113 and we made 47844 phenological observations across all samples.

Statistical Analysis

To test for differences in cue responses across species and populations, we used a phylogenetic mixed effect model with partial pooling across species. This approach allowed us to account for both the

evolutionary relatedness of our species, and estimate the species level cue responses and differences across sites. Species phylogenetic relatedness was obtained by pruning the Smith and Brown (2018) megatree of angiosperms.

In our model, we included each of our three cues as continuous variables. To account for the greater amounts of field chilling experienced in our eastern study, we converted our chilling treatments to chill portions for each individual population using local weather station data and the chillR package (Luedeling, 2020). Chill portions were calculated for both the chilling experienced in the field prior to sampling and during the experiment in our 4°C chilling chambers. We also converted forcing temperatures to mean daily temperatures in each treatment to account for differences in thermoperiodicity between the two studies (Buonaiuto et al., 2023). Finally, we z-scored each cue and site using two standard deviations to allow results across parameters to be directly compared (Gelman, 2008). In addition to the overall predictors, we included all two-way interactions between cues and between cues and sites:

$$\begin{aligned} \hat{y}_i \sim & \text{normal}(\alpha_{sp_i} + \beta_{site2} + \beta_{site3} + \beta_{site4} + \\ & \beta_{chill_{sp_i}} + \beta_{force_{sp_i}} + \beta_{photo_{sp_i}} + \\ & \beta_{force \times chilling_{sp_i}} + \beta_{chilling \times photo_{sp_i}} + \beta_{photoperiod \times chilling_{sp_i}} + \\ & \beta_{force \times site2_{sp_i}} + \beta_{force \times site3_{sp_i}} + \beta_{force \times site4_{sp_i}} + \\ & \beta_{chilling \times site2_{sp_i}} + \beta_{chilling \times site3_{sp_i}} + \beta_{chilling \times site4_{sp_i}} + \\ & \beta_{photoperiod \times site2_{sp_i}} + \beta_{photoperiod \times site3_{sp_i}} + \beta_{photoperiod \times site4_{sp_i}}) \end{aligned}$$

$$\begin{aligned} \beta_{force_{sp}} & \sim \text{normal}(\mu_{force_{sp}}, \sigma_{force}^2) \\ \dots & \\ \beta_{photoperiod \times site4} & \sim \text{normal}(\mu_{photoperiod \times site4}, \sigma_{photoperiod \times site4}^2) \end{aligned}$$

We included the phylogenetic effect as a variance covariance matrix in the parameterization of the normal random vector:

$$\begin{aligned} \boldsymbol{\alpha} = [\alpha_1, \dots, \alpha_n]^T \text{ such that } \boldsymbol{\alpha} & \sim \text{multi-normal}(\mu_{\boldsymbol{\alpha}}, \mathbf{V}), \\ & (2) \end{aligned}$$

The timing of budburst prior to evolution is represented as root values of the means of the multivariate normal distributions. For more detail on this method of phylogenetic modeling, see Morales-Castilla et al (XXXX).

We used semi-informative priors for each of our model parameters, validating the model code using test data prior to analysis. Our model produced \hat{R} values close to one, well mixed chains, and n_{eff} values that exceeded 10% of the model iterations. Our model was fitted using the Stan language (Stan Development Team, 2018) using the rstan package in R (R Development Core Team, 2017) (version).

We present model estimates, relative to baseline conditions of low chilling, low forcing, and short photoperiod treatments, and—when relevant—for the Smithers population.

Results

On average, we observed species budburst 28.1 days (uncertainty interval: 45, 15.2) after the start of our forcing and photoperiod treatments (all estimates are given as mean \pm 90 uncertainty intervals, henceforth ‘UI,’ and budburst dates given as relative to baseline conditions, see methods for more details). But some species, such as *Aronia melanocarpa*, budburst as early as day 13.6, and as late as day 52.1 for *Quercus velutina*. The difference in timing of our earliest and latest species spanned a period of 38.5 and 30.7 days for our eastern and western species respectively. Overall we found species budburst to be strongly phylogenetically structured (λ of 0.8, UI: 0.9, 0.6) and that all cues led to an advance in budburst date. Of the three cues, chilling had the strongest effect (-15.2, UI: -17.3, -13.2) and photoperiod the weakest (-3.6, UI: -4.3, -3.0, Fig. 1). But we found a strong interaction between forcing and chilling (9.1, UI: 7.6, 10.5), which produced a delaying effect. The effects of low chilling to delay budburst can therefore be offset by high forcing conditions (Fig. 2 and see Table 1 for model output).

Overall we found population effects were small compared to differences between cues. Across all species, there was considerable overlap in the responses of our four populations to each cue (Fig. 1). As such, low forcing, chilling, and shorter photoperiods all produced later estimated budburst dates compared to higher temperature and longer photoperiod treatments across all populations (Fig. 3 a-c, shown for baseline conditions) and across transects (Fig. 3, see Table 1 for model output). Populations were similar in their overall budburst dates, but eastern populations were marginally earlier (34.1, UI: 40.7, 27.1) compared to our western populations (46.5, UI: 52.7, 39.4, see also Table 1 for model output).

Budburst of individual species showed distinct differences in their timing and the relative importance of cues. Both chilling and forcing responses varied with budburst, with later budbursting species having slightly stronger responses to each cue respectively (Fig. 4). Despite our expectations that species responses should differ across the canopy layer, with shrubs being less sensitive to cues than tree species, we did not find strong differences across these two functional groups (Fig. S3). Many of our earliest species were shrubs with weaker responses to chilling and forcing, like *Cornus stolonifera*. But 36.7 percent of our shrubs species, including *Menziesia ferruginea* and *Symphoricarpos alba*, exhibited the opposite response and budburst relatively late (Fig. S3). Similarly for tree species, some matched our predictions for their cue responses, but 23.5 percent budburst earlier than expected. Specific tree species, like *Quercus velutina*, did have stronger chilling and photoperiod responses as predicted, and of all our focal species *Fagus grandifolia* produced the strongest photoperiod response. But overall there were no clear differences between the cue responses of trees and shrubs across our four forest communities.

Our results depict the relative importance of phenological cues to variation across species, but we found the contribution of cues to budburst order and timing to be dependent of the magnitude of the cue, but small compared to overall species differences. Under high cue conditions, the rank order of species estimated budburst changed more than under low cues (Fig S4). Changes in species rank order as cue strength increased were greater for our western species, with a mean change in rank order of 6, compared to our eastern species for which the mean change in rank was 4. Comparing the change in budburst ranking for the three species that occurred in both transects showed no clear trends with cues or transects (Fig. S5). In comparing our earliest and latest budbursting species, we found the differences in the cue responses were relatively small (Fig. 4). This is contrary to our prediction of stronger cue requirements—especially chilling cues—for later budbursting species. For example, an early (shrub) species *Lyonia ligustrina* had chilling and forcing cues of -15.4 and -10.4 respectively, which are comparable to the estimates of -16.6 and -10.4 for the much later tree species, *Quercus alba*. Yet our model predicts these species to have a 15.9 day difference in day of budburst. This highlights how the timing of budburst across species could mostly be attributed to species level differences outside of cue responses (Fig. 5). In fact, across the range of cue values, they explained only between 38.6 and

67.0 percent of variation for eastern species and between 48.3 and 60.6 percent for western species, with the intercept explaining the rest (Fig. 5).

Discussion

Quick overview

1. In our study of woody plant budburst phenology across North America, we found the assembly of species' temporal niche to be poorly explained by environmental cues and population level variation.
2. But within a community, there was considerable species level variation.

Temporal assembly across North America

1. While our western and eastern forest communities differ in both their species composition and local environments—only small differences in budburst phenology or cue responses across populations
 - (a) Expected local habitat selection = differing selective pressures across populations = leading to differences in cues responses across populations
 - (b) But we only observed slightly earlier budburst in our eastern populations when compared to western populations (Fig 1).
 - (c) Also found no latitudinal gradients across populations despite differences in local photoperiods (Fig 1)—contrasts previous work in which poleward populations were later (Lieth1974, Zettlemoyer2021)
 - (d) Lack of population-level trends suggests the drivers that determine the timing of budburst are not shaped by geography, at least at our continental scale

Community composition and interspecific variation in phenology

1. We found high variation in species cue responses
 - (a) Species varied in the timing of budburst—early to late budbursting spp span a similar period as natural communities (Maycock1961)—suggesting that our experiment captures a realistic breadth in phenology within our forest communities
 - (b) Generally—all species showed some cue response
 - (c) Cues consistent with previous studies—ie.chilling being strongest, photoperiod weakest, but complex interactions between cues—eg (Heide, 1993; Caffarra and Donnelly, 2011; Flynn and Wolkovich, 2018)
 - (d) May be an important adaptation under future warmer winters when chilling might be insufficient and higher forcing needed to offset it
 - (e) While spp do differ in cue responses, identifying general trends cue responses across assemblages of species remains challenging.
2. Shrub and tree species differ greatly in their physiology—filling unique ecological niche—but we found little variation in their cue responses on a whole.
 - (a) Most tree species budburst later than shrubs species—in line with previous work—earlier in shrubs compared to trees (Panchen2014, Yu2015)

- (b) But about a quarter of tree species did show earlier budburst dates and a third of shrubs budburst later than expected—suggesting more nuance than previously found—advantage of us having such a large assemblages of species
- (c) As trees advance phenologically—with earlier canopy closure and reduced light predicted (Donnelly2019)—some less responsive shrubs will experience reduced fitness—but our finding suggests many have similar cue responses and the capacity to maintain their relative temporal niche space.
- (d) A more detailed examination of the the underlying mechanism, whether they be constraints by species interactions, or other functional traits that shape species growth, is needed to understand the considerable variation within each group.

Community assembly in responses to cues versus evolutionary history

1. In accounting for the effects of species' phylogeny, we found a high level of phylogenetic relatedness among clades of species in the timing of budburst.
 - (a) This is reflected in the high proportion of variation in budburst explained by species' level effects and relatively weak contribution by our three cues (Fig 4)
 - (b) Suggests species' budburst is constrained by their evolutionary history and not as strongly shaped by current environmental conditions as we would expect.
 - (c) This phylogenetic niche conservatism is surprising—given phenology is thought of as a highly plastic trait, with local conditions expected to drive variation in the type and magnitude of cue responses
 - (d) May be evidence of phenotypic attraction or environmental filtering of similar, more closely related species in temperate forests.
 - (e) But could also be an indication of limited evolutionary time—some temperate species, like *Nyssa* and *Fagus* have had a shorter geologic time in which to adapt to temperatures
 - (f) Results improve our ecological understanding of budburst phenology at the community level—illustrating the critical role of species' evolutionary history in shaping this trait.
 - (g) But also concerning—as it could limit species' ability to respond to changing climates and future community dynamics
2. Ecological communities are thought to be shaped by habitat selection—species that withstand local abiotic conditions persist—and phenotypic repulsion—limiting niche overlap and competition—but seems not to be the case for species temporal niche (or spring temporal niche?)
 - (a) While species in our forest communities varied in their timing of bb by several weeks—the similarity across populations suggests strong phylogenetic niche conservatism and stabilizing selection with little change in response to local habitats
 - (b) This is despite communities having very unique regional spp pools—western community dominated by shrub spp vs eastern communities with more canopy tree spp
 - (c) Indicates that cues we think are important for bb may also be selecting for other functional traits—such as traits related to light capture and photosynthesis or nutrient uptake
 - (d) Exploring how suites of other traits contribute to species cue response could = greater insights into the mechanisms driving species temporal niche

Predicting budburst phenology under future climates

1. Integrating the relative effects of climate, evolutionary history, and ecological process is critical to forecasting future phenologies under climate change.
2. Requires community wide approaches such as ours—one of the first to include these three types of drivers at a large geographic scale and across forest communities.
3. Critical bc provides greater diversity of spp and insights into how communities as a whole will respond
4. Our approach address many of the challenges to predicting species responses to further climate change—complexity of regional and increasingly variable seasonality
5. And yet, our findings highlight our limited understanding of the factors underlying phenological variation within forest communities and groups of species.
6. to forecast future changes = we need to identify the key underlying ancestral traits that link species' evolutionary histories and are driving the high spp variability observed within temperate forest communities.

1 Tables and figures

Table 1: Summary output from a phylogenetic Bayesian model in which species are partially pooled and phylogeny is included on the intercept. The model includes photoperiod and site as dummy variables, while the forcing and chilling effect is included as continuous. Parameter estimates were used to estimate day of budburst for sites under low cue conditions and for Smithers only when relevant.

	mean	sd	5%	95%
Intercept	12.51	3.14	7.40	17.60
Phylogenetic effect	0.79	0.12	0.60	0.90
Forcing	-9.55	0.74	-10.70	-8.30
Photoperiod	-3.62	0.41	-4.30	-3.00
Chilling	-15.21	1.25	-17.30	-13.20
Manning Park	2.09	0.36	1.50	2.70
Harvard Forest	-6.04	1.03	-7.80	-4.40
St. Hippolyte	-8.71	0.97	-10.30	-7.10
Forcing x photoperiod	0.23	0.71	-1.00	1.40
Forcing x chilling	9.06	0.90	7.60	10.50
Photoperiod x chilling	-0.67	0.90	-2.20	0.80
Forcing x Manning Park	-1.76	0.77	-3.00	-0.50
Photoperiod x Manning Park	0.58	0.79	-0.70	1.90
Chilling x Manning Park	-0.36	1.60	-3.00	2.20
Forcing x Harvard Forest	3.81	1.22	1.80	5.80
Photoperiod x Harvard Forest	-1.96	0.86	-3.30	-0.60
Chilling x Harvard Forest	9.97	2.03	6.60	13.40
Forcing x St. Hippolyte	5.25	1.19	3.20	7.20
Photoperiod x St. Hippolyte	-2.13	0.84	-3.50	-0.70
Chilling x St. Hippolyte	8.65	1.70	5.90	11.50

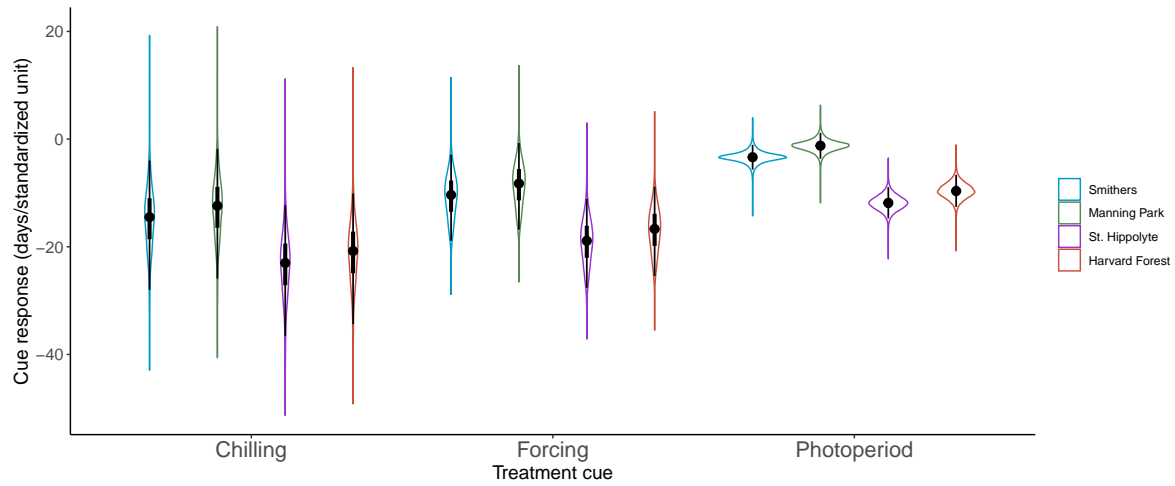


Figure 1: Posterior distributions of estimated chilling, forcing, and photoperiod responses with site level effects for individual populations. Black circles represent the median cue response, thicker black lines the 50% uncertainty interval, while the thinner black line the 90% uncertainty interval. The y-axis spans the entire range of the data. Cues were z-scored using two standard deviations.

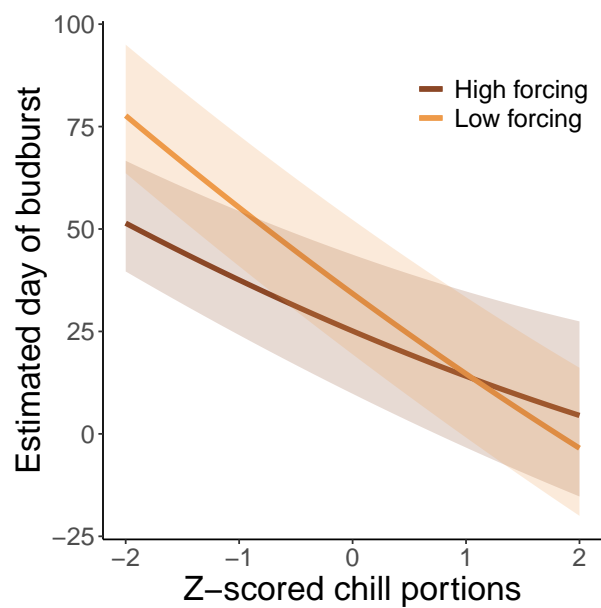


Figure 2: Estimated day of budburst of first bud in response to chill portions and forcing, estimated for our defined baseline conditions.

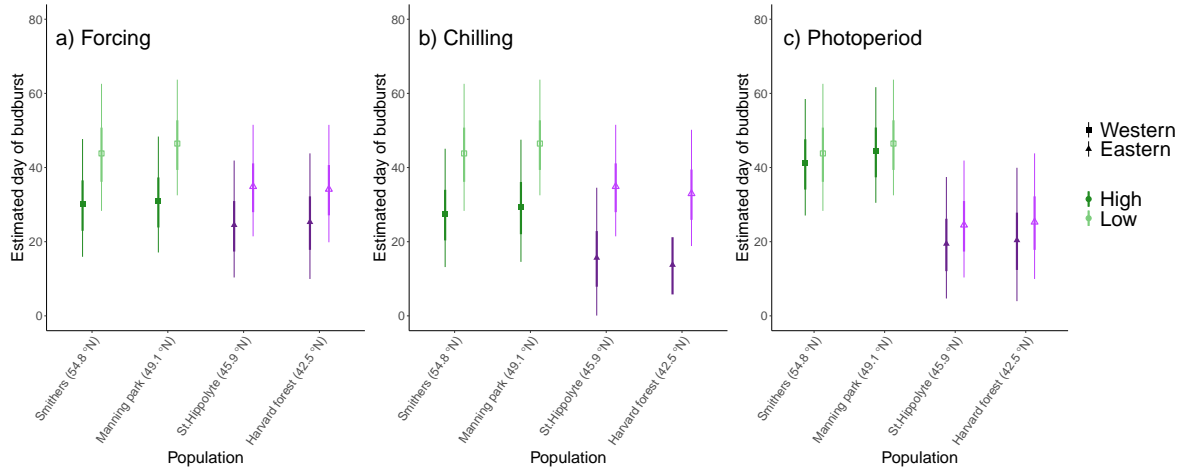


Figure 3: Estimated day of budburst of first bud in response to (a) forcing across populations under low chilling conditions and short photoperiods, (b) chilling across populations under low forcing and short photoperiods, and (c) across photoperiods under our baseline forcing and chilling conditions for species sampled from our four populations. The thin error bars represent the 90% uncertainty interval, while the thicker error bars represent the 50% uncertainty interval.

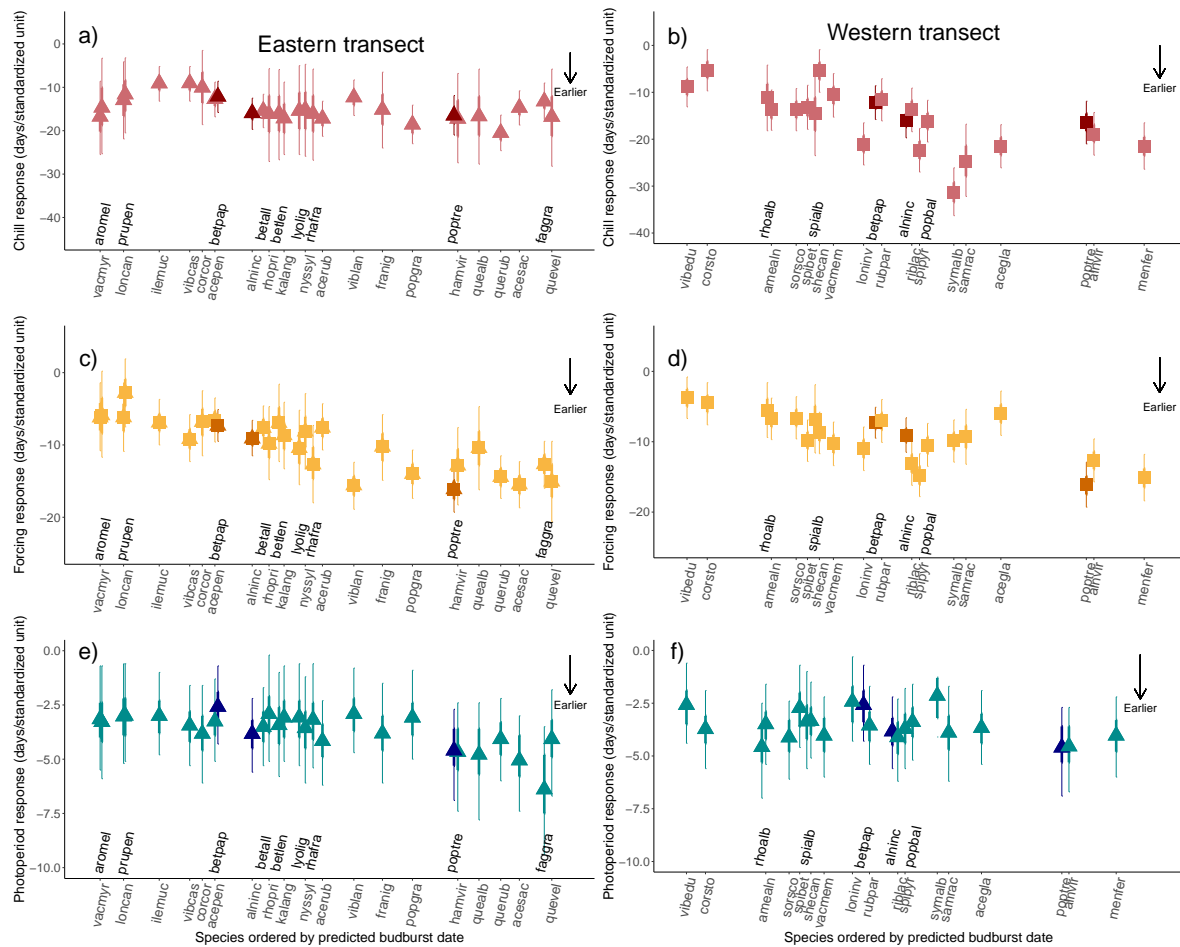


Figure 4: Estimated species' chilling (a,b), forcing (c,d) and photoperiod (e,f) responses ranked by increasing estimated budburst dates for both the eastern (a, c, e) and western (b, d, f) populations. Cues are plotted on differing y-axis to better depict species differences. For each species, the thinner error bars represent the 90% uncertainty interval, and thicker bars represent the 50% uncertainty interval. Species that occur in both the eastern and western transects are depicted using darker colours. Each cue was z-scored, using two standard deviations, to make the results across cues directly comparable.

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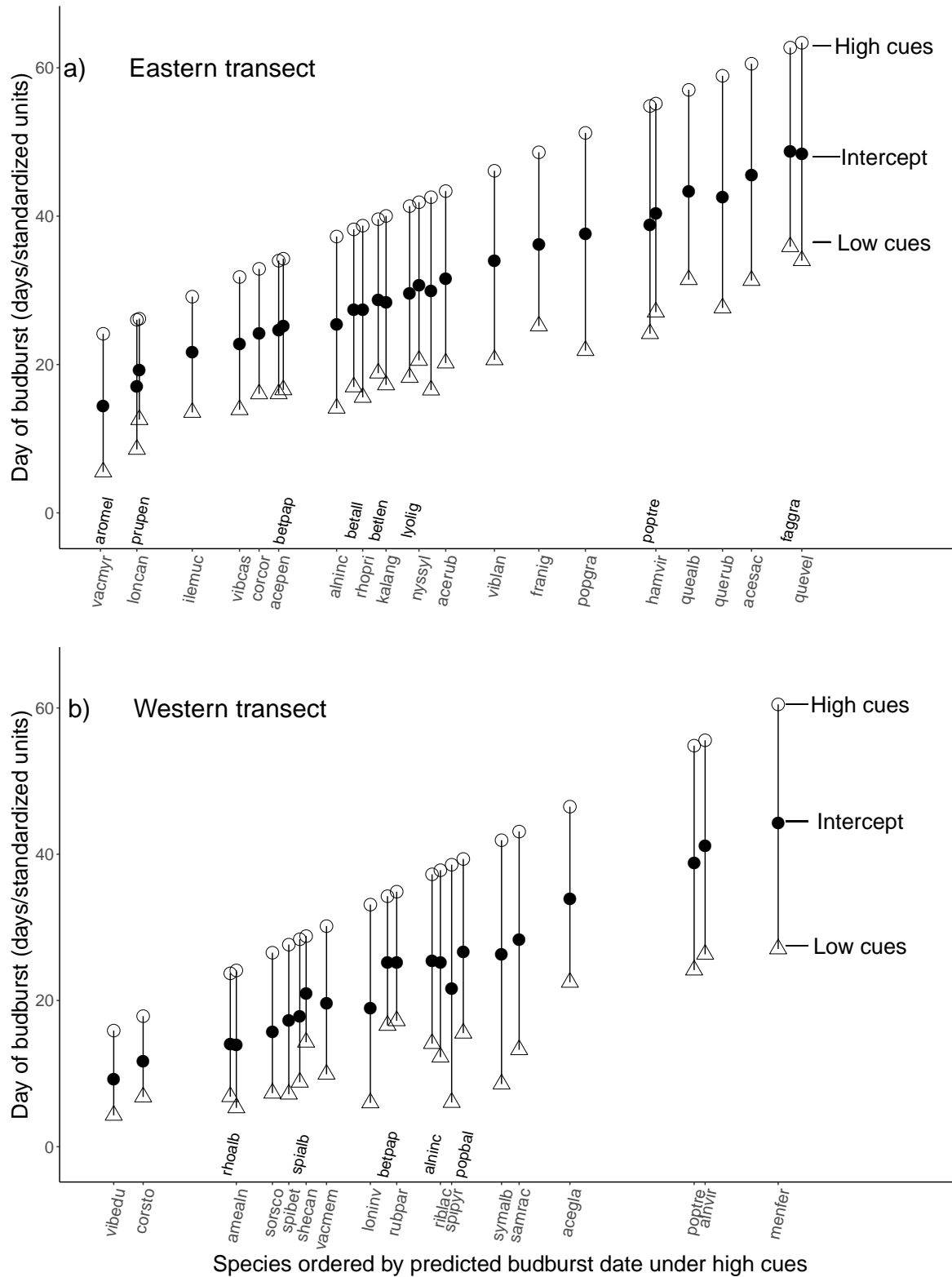


Figure 5: Comparisons of our estimated budburst based on the full model (intercept plus all cues), shown in white, versus the intercepts only (without cues), shown in black, ordered according to differences in species estimated budburst dates for both the eastern (a) and western (b) populations under high cue conditions, depicted as circles, and low cue conditions, depicted as triangles.