Evolutionary history as important as phenological cues in the temporal assembly of woody plant communities

Deirdre Loughnan[1] and E M Wolkovich[1]

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[1] Department of Forest and Conservation, Faculty of Forestry, University of British Columbia, 2424 Main Mall Vancouver, BC, Canada, V6T 1Z4.

Corresponding Author: Deirdre Loughnan, deirdre.loughnan@ubc.ca

Introduction

Climate changes is altering phenology, or the timing of life history events, for species across the tree of life. These effects of climate change, however, have not been uniform, given that some regions are experiencing greater warming than others (IPCC, Schwartz2006). This has produced high variability in phenological shifts, with unique responses observed for different species and across habitats (Fitter2002,Menzel2006, Yu2010,Fridley2012).

Species differences in phenological responses can be attributed to species-level differences and geographic variability in environmental cues (Vitasse2009, Wolkovich2014,Zohner2014, Gerst2017, Vitasse2018, CITE non-plant examples). Identifying the relative importance of different drivers of key life event is necessary to understand and predict future changes in phenology, and their ultimate impacts on community dynamics and ecosystem services, including growing season length, carbon cycles, and species interactions (Gotelli1996,Cleland2007,Richardson2009,Richardson2013,Keenan2014).

In a community, the timing of individual phenological events can vary over weeks or months (Lechowicz1984). This breadth in the period of when individual species' events occur allows species' to fill different temporal niche (Gotelli1996). In plant communities, understory species often budburst earlier than taller canopy species, a niche difference predicted to infer overarching differences in species growth strategies. Similarly, in Mediterranean marsh communities, native bird species have a distinct temporal niche from exotic species, reproducing earlier in the season (SanzAguilar2015). In addition to these within community differences, variation in phenological responses is likely to also exist between different populations across species' ranges (cite). At present, however, we lack a strong understanding of the relative effects of population versus species-level variation in shaping budburst of individual species and forest communities overall.

Decades of research has been done to understand the mechanisms shaping budburst phenology in woody plants, making them a good system to study spatial patterns in phenological cues. Woody plants vary in their growth strategies, but under controlled environments have consistent cue responses. Temperate woody plants show strong responses to temperature, particularly winter and temperatures, and daylength (Chuine2010,Polgar2011,Cook2012,Basler2014,Laube2014). While each of these three cues vary in their relative importance for a given species, they can also interact (????). Previous work has shown that spring temperatures, also referred to as forcing cues, can offset the effects of warm winter

temperatures, or chilling (**????**). Photoperiod responses also offsets weak forcing cues in cool springs conditions (**????**). The highly variable nature of both species cue requirements and the magnitude of their responses, however, has made it challenging to draw generalizable trends across species with large distributions.

The environmental cues that shape species budburst can vary geographically and temporally. Under future climates this could create differences in community dynamics and species composition. Increasing winter and spring temperatures will lead to the faster accumulation of chilling and forcing cues (**?**), which is associated with earlier budburst in many temperate plant species. Changes in temperature cues with climate change could therefore create novel niche space early in growing season and facilitate species invasions or alter community assemblages (Willis2010,Wolkovich2013). Species with strong photoperiod cues may experience a reduction in their fitness, as their ability to adapt will be constrained by constant daylengths (Way2015). Differences in species cue requirements will therefore shape species adaptive potential to future change, altering the competitive landscape experienced across the growing season, and reshaping the diversity and persistence of species across forest communities.

While climate change has caused temperatures to change over recent decades, it is also important to account for the longer timescales over which forest communities have assembled (Davies2013). It is local, historic conditions that have shaped traits like phenology, the results of which we observe today. Across a species' spatial distribution we expect local cues to have differed, driving differences in their budburst phenotype and cue responses. Species with large latitudinal distributions, for example, experience differences in their photoperiod cues across their spatial range and may differ in their responsiveness to photoperiods across populations. But to date, few studies have explored how cue use differs across spatial gradients using the same forest communities, testing the relative role of local environments and biotic communities in shaping budburst. To better predict how forest communities will respond and assemble under continued climate change we need to identify the cues that drive budburst in our dominant forest species and functional groups, and how these responses can change across populations.

In this study we combined results from two growth chamber studies of woody species budburst cues. We used data from four populations, from eastern to western North America, with pairs of populations on each coast spanning 4-6° latitude. Our approach allowed us to detect general trends in how budburst of North American deciduous forest communities respond to forcing, chilling, and photoperiod cues. We were also able to explore community specific responses and detect differences between western and eastern forest communities, and across different latitudes. By including diverse assemblages of species, we also tested for trends across different functional groups, exploring differences between the dominant shrubs and tree species that characterize the understory and canopy of our forest communities.

Materials and Methods

Field sampling

We combined data from two growth chamber studies using branch clippings of North American deciduous woody plants. Such cutting experiments are a commonly used and powerful approach to understanding the responses of adult trees to known environmental conditions (Cite Vitasse). 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 the dominant deciduous species 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 15-20 healthy, mature, individuals for each species in the summer prior to the growth chamber study. Depending on the size of the individual, we collected 1-16 cuttings from 6-20 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 **??**).

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 15:5°C and a warm regime of 20:10°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 (**?**). Thus 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 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 **?**.

We assessed phenological observations of budburst were assessed using the BBCH scale, adapted for our specific species (**?**). Every 1-3 days, we observed each sample and recorded all phenological stages up to full leaf expansion (defined as code 17 by **?**). Here, we present the response in budburst date, defined as the day of budbreak or shoot elongation (defined as code 07 by **?**). 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 **?** 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 (**?**). 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

138 studies (**?**). Finally, we z-scored each cue and site using two standard deviations to allow results across
139 parameters to be directly compared (**?**). In addition to the overall predictors, we included all two-way
140 interactions between cues and between cues and sites:
141

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

142
143

$$\beta_{force_{sp}} \sim \text{normal}(\mu_{force_{sp}}, \sigma^2_{force})$$
$$\dots$$
$$\beta_{photoperiod \times site4} \sim \text{normal}(\mu_{photoperiod \times site4}, \sigma^2_{photoperiod \times site4})$$

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

$$\boldsymbol{\alpha} = [\alpha_1, \dots, \alpha_n]^T \text{ such that } \boldsymbol{\alpha} \sim \text{multi-normal}(\mu_\alpha, \boldsymbol{V}), \tag{1}$$
$$\tag{2}$$

146 The timing of budburst prior to evolution is represented as root values of the means of the multivariate
147 normal distributions. For more detail on this method of phylogenetic modeling, see Morales-Castilla
148 et al (XXXX).
149
150 We used semi-informative priors for each of our model parameters, validating the model code using
151 test data prior to analysis. Our model produced $\hat{R}$ values close to 1, well mixed chains, and n_{eff} values
152 that exceeded 10% of the model iterations. Our model was fitted using the Stan language (**?**) using
153 the rstan package in R (**?**) (version).
154
155 We present model estimates, relative to baseline conditions of low chilling, low forcing, and short pho-
156 toperiod treatments, and—when relevant—for the Smithers population. We present results as means
157 and 90% posterior uncertainty intervals from our Bayesian phylogenetic models.
158

159 Results

160 On average, we observed species budburst 28.1 days (uncertainty interval: 45, 15.2) after the start of
161 our forcing and photoperiod treatments (all estimates are given as mean ± 90 uncertainty intervals,
162 henceforth 'UI,' and budburst dates given as relative to baseline conditions, see methods for more
163 details). 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, positive interaction between forcing and chilling (9.1, UI: 7.6, 10.5). This results in subadditive effects between chilling and forcing cues, with low chilling being 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. **??**). Many of our earliest species were shrubs with weaker responses to chilling and forcing, like *Cornus stolonifera*. But 36.7% of our shrubs species, including *Menziesia ferruginea* and *Symphoricarpos alba*, exhibited the opposite response and budburst relatively late (Fig. **??**). Similarly for tree species, some matched our predictions for their cue responses, but 23.5% budburst earlier than expected. Specific tree species, such as *Quercus velutina*, did have stronger chilling and photoperiod responses as predicted, and of all our focal species, such as *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, and small compared to overall species differences. The rank order of species estimated budburst changed more under high cue conditions than under low cues (Fig **??**). The budburst ranking for the three species that occurred in both transects remined relatively consistent across the two communities (Fig. 4), with only *Alnus incana* in our western community experiencing a large change in rank with cues (Fig. **??**).

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 was mostly attributed to species level differences outside of cue responses (Fig. 5). Across the range of cue values, cues explained only between 38.6 and 67.0% of variation for eastern species and between 48.3 and 60.6% 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 only be partially explained by environmental cues and population level variation.

2. Within a community, there was considerable species level variation.

3. Evidence of a strong phylogenetic structure to the timing of species bb

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)—such negligible differences could be due to differences in the methodologies used—eg differences in timing of sample collection or the slightly greater levels of chilling eastern spp experienced as a result

   (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 current 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 responses were consistent with previous studies—ie.chilling being strongest, photoperiod weakest, but complex interactions between cues that are advantageous under warming climates (**???**)

   (d) While spp do differ in cue responses, we did not find the clear, generalizable trends we expected across species with similar growth strategies.

2. Shrub and tree species differ greatly in their physiology—filling different 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 bb in shrubs compared to trees (Panchen2014, Yu2015)

   (b) But about a quarter of tree species = earlier budburst dates, more similar to the timing of shrub species, and a third of shrubs budburst at similar times as trees —suggesting more nuance to this 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 are likely to also advance with warming with the potential to maintain their relative temporal niche space.

3. Given the number of shrub and tree species exhibiting uncharacteristic budburst phenologies— suggests cues we think are important for bb may also be selecting for other functional traits

    (a) Selection for earlier budburst may correlate with traits related to light capture and photosynthesis in understory shrub species or nutrient uptake in trees associated with early successional stages or disturbance.

    (b) The fitness of species across different temporal niche = determined by species overall suite of traits— but currently we lack a sufficient understanding of the the underlying mechanisms

    (c) By incorporating phenology into a broader trait framework = greater insight into the considerable variation observed within species functional groups.

Community assembly in responses to cues versus evolutionary history

1. With continued warming, our ability to predict the timing of spring growth will depend on our understanding of cue responses at the species and community level.

    (a) Within a community, species varied in their timing of bb by several weeks— but the similarity across populations suggests strong niche conservatism and stabilizing selection with little change in response to local habitats

    (b) While the effects of population level variation on cue responses appears be negligible, individual species within community assemblages do differ in their responses to cue (Fig. 5).

    (c)

    (d) But can't simply focus on current cues—species current phenotypes are the result of multiple interacting and complex environmental cues that have shaped species over evolutionary time scales (Ackerly2009).

2. The high degree of phylogenetic relatedness among species suggests that species with shared ancestry, and presumably more similar phenotypes and growth strategies, exhibit similar timing in budburst.

    (a) A high proportion of variation in budburst is explained by species' level effects, with cues only explaining about two-thirds of variation in budburst (Fig 4)

    (b) Our study applied extreme differences in cues to test for responses—useful for longer-term predictions/parameterizing process based models—but variation due to phylogenetic structure likely underestimated

    (c) Surprising evolutionary history has such a large effect—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) But it indicates we are not accounting for other key drivers of spring phenology—additional cues or functional traits—limiting our predictive abilities.

3. The evolutionary history and ancestral phenotype of a species has the potential to effect their ability to adapt to future climates.

    (a) Could prevent adaptation to new environmental conditions, if spp are physiologically constrained by their ancestral, optimum phenotype

(b) Potential for processes occurring across different time scales to be strongly interdependent—the evolution of traits shaped over 'deep' timescales is influencing species' abilities to respond to several decades of climate change.

(c) Constraints could skew species assemblages, favouring spp with traits adapted for warmer climates—lower chilling and photoperiod requirements—better able to track and benefit from future climate conditions

(d) But could also indicate limited evolutionary time—some temperate species, like *Nyssa* and *Fagus* have had a shorter geologic time in which to adapt to temperatures—especially in populations at northern range limits.

(e) Identifying how and to what extent latent traits contribute to the phylogenetic structuring of temperate forest communities = unknown, but important if we are to predict how communities will respond to continued changes in climate.

Predicting budburst phenology under future climates

1. Our results provide new insights into the key factors and ecological processes that are critical to forcasting future phenoloiges under climate change.

   (a) Provides evidence that changes in budburst cues will produce consistent trends across populations—allowing us to forecast across populations for which we have limited local phenological data but similar species assemblages.

   (b) The high phylogenetic structure in budburst timing also allows us to use our existing knowledge of species' phenological cue responses to forecasts trends in phylogenetically related species.

   (c) But must also be cautious in our assumptions—as we did not find strong general trends at coarser taxonomic groupings (shrubs vs trees)—the number of exceptions within tree and shrub functional group suggests still selective pressures shaping bb we do not understand.

2. Our ability to predict changes in spring phenology and mitigate the cascading effects on forest communities is increasingly urgent in light of anthropogenic climate change.

   (a) 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.

   (b) Critical bc provides greater diversity of spp and insights into how communities as a whole will respond

   (c) 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
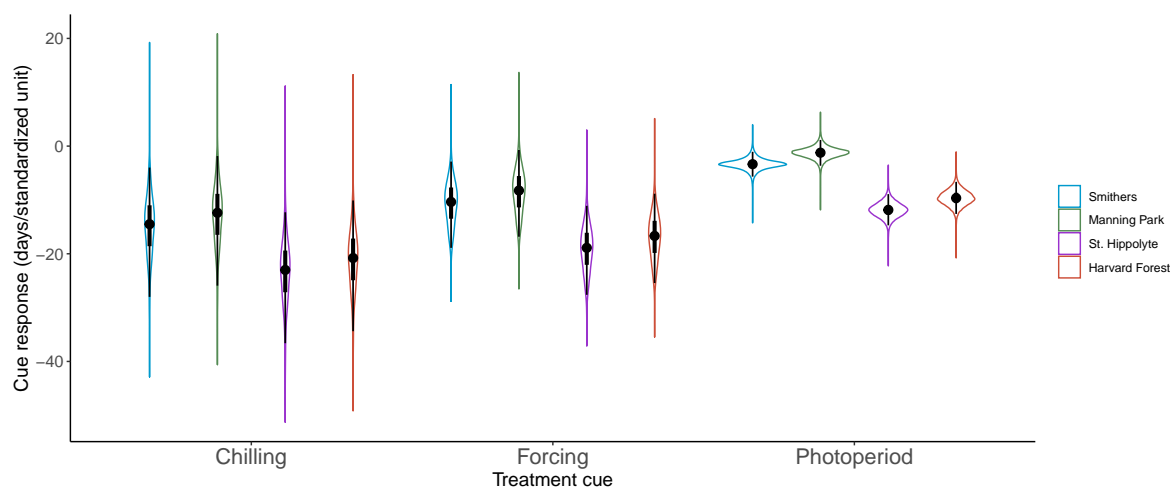
Figure 1: Posterior distributions of estimated chilling, forcing, and photoperiod responses with site level effects for individual populations. Black circles represent the median cue response, thicker black lines the 50% uncertainty interval, while the thinner black line the 90% uncertainty interval. The y-axis spans the entire range of the data. Cues were z-scored using two standard deviations, see methods.
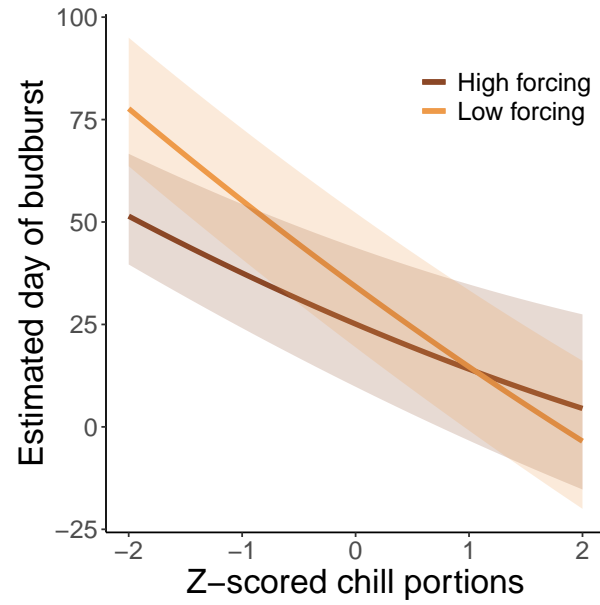
Figure 2: Estimated day of budburst of first bud in response to chill portions and forcing, estimated for our defined baseline conditions. We found a positive interaction between chilling and forcing cues, with high forcing compensating for low chilling to produce earlier budburst, while budburst occurred earlier under low forcing following high chilling.
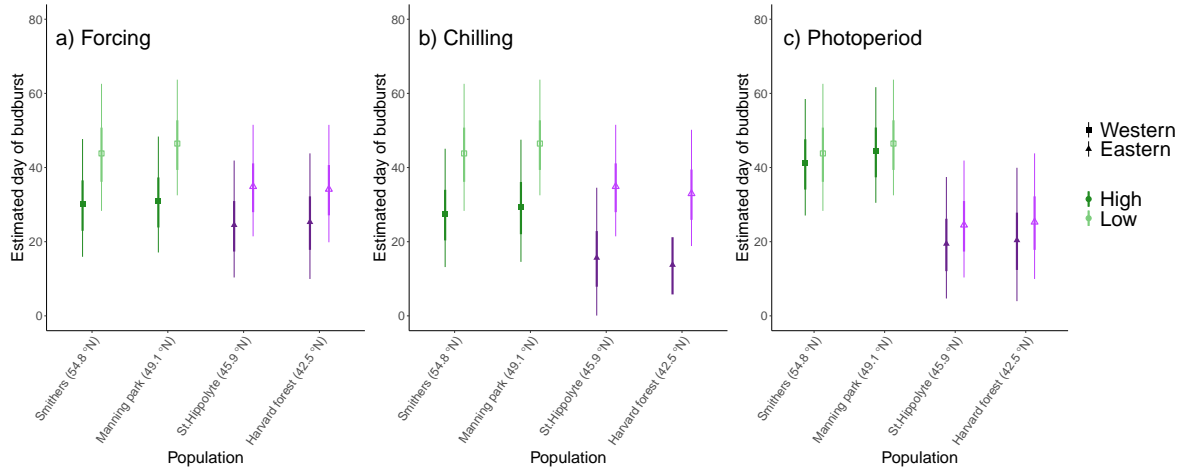
Figure 3: Estimated day of budburst of first bud in response to (a) forcing across populations under low chilling conditions and short photoperiods, (b) chilling across populations under low forcing and short photoperiods, and (c) across photoperiods under our baseline forcing and chilling conditions 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.
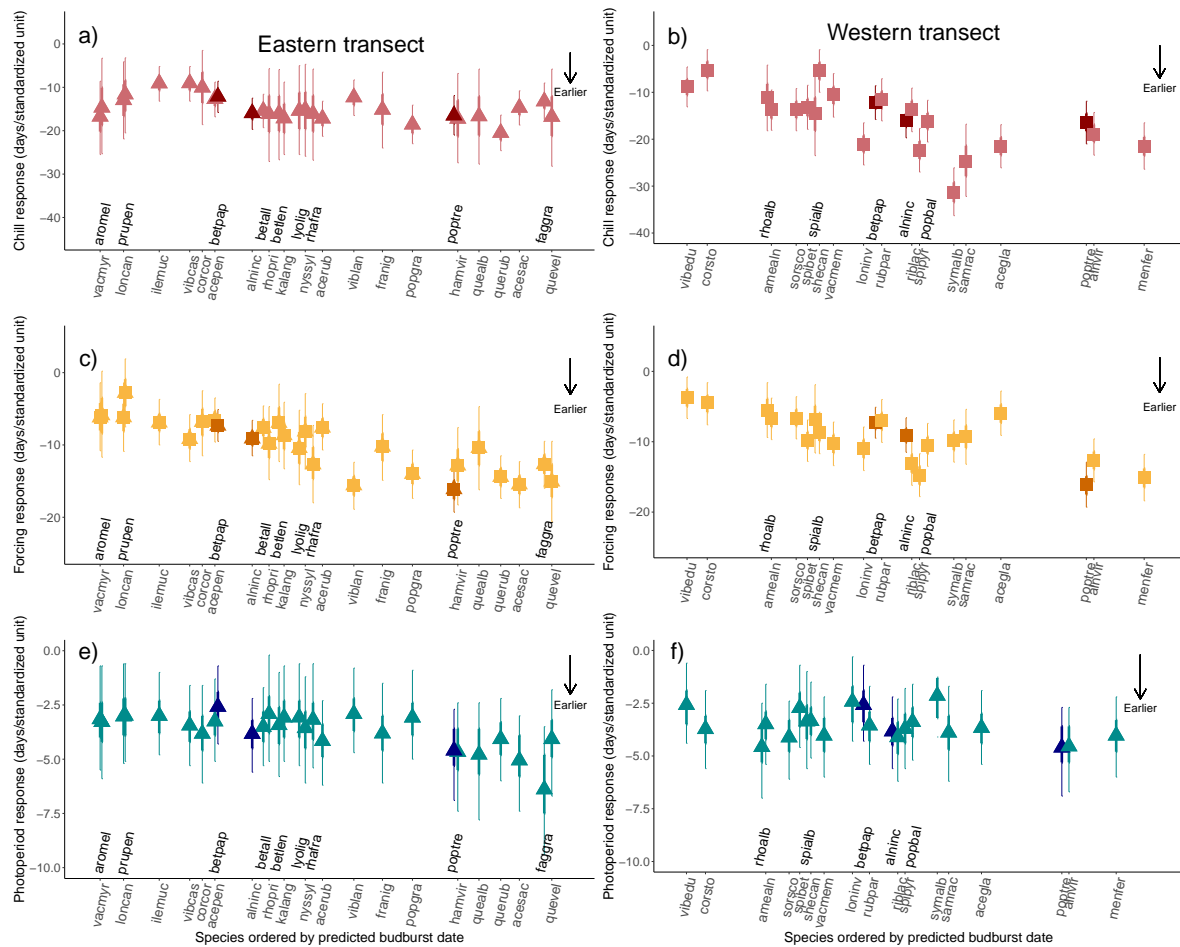
Figure 4: Estimated species' chilling (a,b), forcing (c,d) and photoperiod (e,f) responses ranked by increasing estimated budburst dates for both the eastern (a, c, e) and western (b, d, f) populations. Cues are plotted on differing y-axis to better depict species differences. For each species, the thiner 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, see methods.
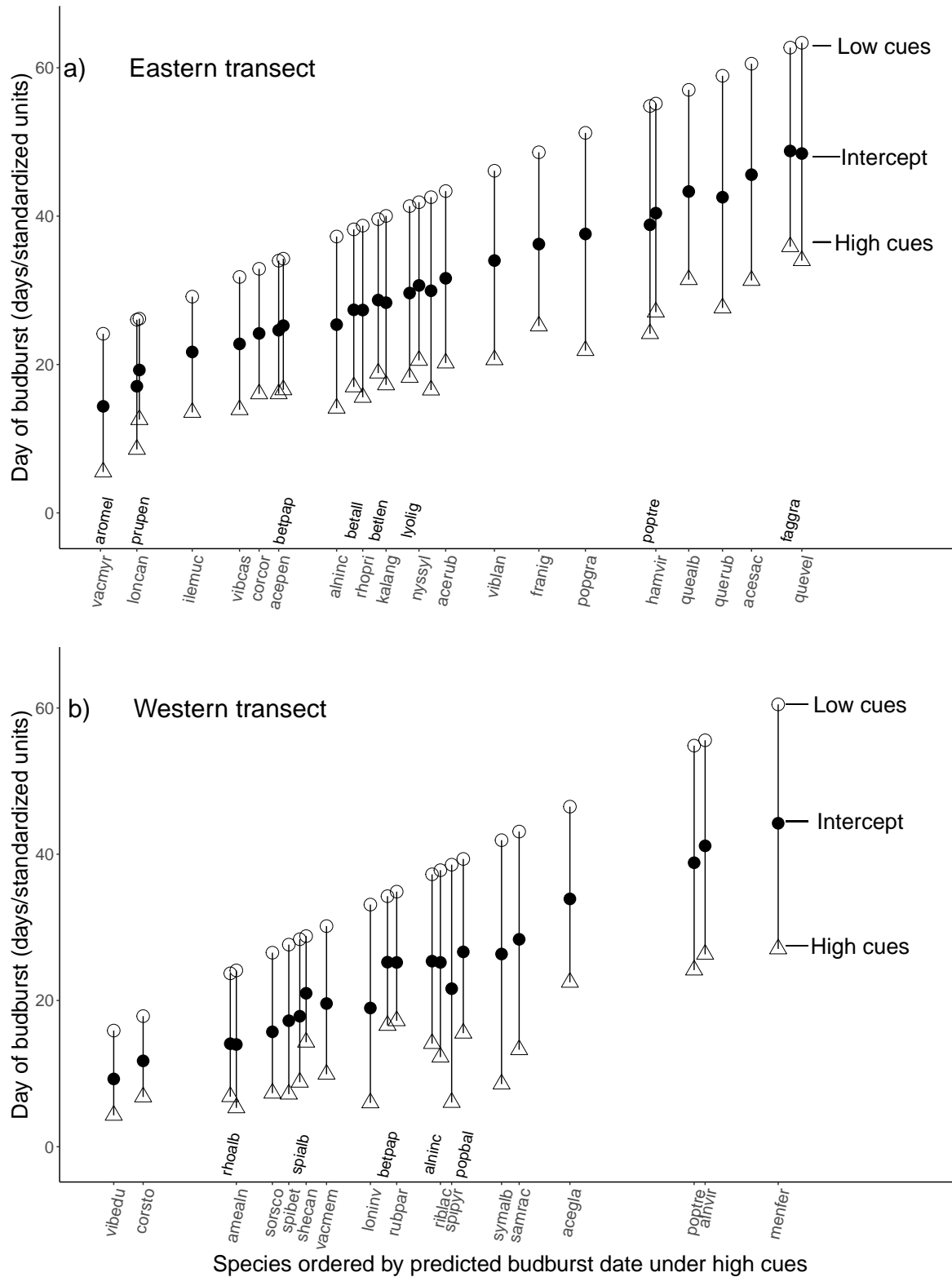
Figure 5: Comparisons of our estimated budburst based on the full model (intercept plus all cues), shown in white, versus the intercepts only (without cues), shown in black, ordered according to differences in species estimated budburst dates for both the eastern (a) and western (b) populations under high cue conditions, depicted as circles, and low cue conditions, depicted as triangles.