

HERBARIUM SPECIMENS MAY PROVIDE BIASED FLOWERING PHENOLOGY ESTIMATES FOR DIOECIOUS SPECIES

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Premise of research. Measuring phenology from herbarium specimens has become a widely used, powerful method for investigating the effects of climate change on plants across space, time, and phylogeny. However, it is not known whether and how biases in plant collecting might affect conclusions, particularly for dioecious species.

Methodology. We examined the effects of spring temperature and latitude on the flowering phenology of male and female blunt-lobed spicebush (*Lindera obtusiloba* Blume) plants from across East Asia using 88 herbarium specimens and digitized photographs collected from 1918 to 2018.

Pivotal results. Temperature and latitude did not affect the flowering phenology of either sex when analyzed separately. Surprisingly, however, female flowering plants were collected on average 25 d later than male plants. This difference is likely due to male plants being preferentially collected during the flowering season (with showy but more ephemeral flowers) and female plants being collected after the peak flowering season (with nonreceptive flowers persisting later into the season).

Conclusions. Researchers investigating phenology using herbarium specimens need to be aware of possible collecting bias in dioecious species and species with persistent flowers. Herbarium collectors and photographers should sample both male and female plants.

Keywords: blunt-lobed spicebush, climate change, collection bias, dioecy, *Lindera obtusiloba*, sex differences.

Introduction

The use of herbarium specimens in ecological and climate change research, especially to detect phenological patterns over time and across large spatial areas (Willis et al. 2017; Heberling 2022), has greatly expanded in recent decades. Herbarium specimens are increasingly accessible through widespread museum digitization efforts, with millions of herbarium specimens now openly available online (Soltis 2017). As a result, such herbarium-based studies of phenology and climate change are now easier and faster to carry out. There is even the potential to apply these herbarium specimen approaches to continental-scale geographical areas and to investigate ecological mismatches between plant species (e.g., Ellwood et al. 2019; Park et al. 2019; Gallinat et al. 2021; Park et al. 2021).

Despite this new wave of herbarium specimen use for phenological research, there is also a long-standing but growing recog-

ognition that herbarium specimens exhibit certain collection biases that may lead to skewed or erroneous results if left unaccounted for in analyses (Daru et al. 2018). Among such biases are spatial bias (e.g., areas near universities may be overcollected relative to remote areas), temporal bias (e.g., collecting effort may have been stronger in past than in recent years), taxonomic bias (e.g., collectors might more frequently sample certain species), and trait bias (e.g., plants with certain characteristics, such as short stature or conspicuous flowers, might be preferentially collected). Many herbarium-based phenological studies have considered collection bias through careful study design (i.e., species and specimen selection) or analyses (Pearse et al. 2017).

However, an important topic that has been largely overlooked in modern phenological and climate change research is the phenology of dioecious species (i.e., species with male and female flowers on separate individuals). Dioecy has evolved independently many times throughout the plant tree of life and is found in nearly half of all plant families and around 6% of angiosperm species studied (Renner 2014). A growing body of research has explored differences between growth performance and niche requirements between sexes of dioecious species and their differential responses to environmental change (Hultine

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et al. 2016), but very few studies have explored the extent to which dioecious species respond to climate change and the possibility that male (staminate) and female (pistillate) plants within the same species might respond differently. With many dioecious plants collected across the world now more accessible than ever through digitization, herbarium specimens provide an unprecedented opportunity to study the phenology of dioecious species and their responses to environmental changes.

Morphological, physiological, and life history traits of male and female individuals, including phenological traits, differ in complex ways among dioecious species (Barrett and Hough 2013). The timing of flowering onset and the duration of male and female flowers show variable responses across studies and species (Lloyd and Webb 1977), with expectations based on the theory of the individual cost of flower production and pollination success based on population sex ratio and pollinator activity (Forrest 2014). The null hypothesis is that male and female plants of the same species should respond similarly to climate change because both sexes are likely responding to the same environmental cues. Shared phenological cues between sexes within a species would be logical, as coflowering is required to ensure cross-pollination, fertilization, and fruit set. However, these expectations are complicated by a reported pattern found across many dioecious species of slightly advanced flowering onset in male plants (Forrest 2014) and longer flower longevity in female plants (Primack 1985). For example, the cumulative flowering of female flowers of blunt-lobed spicebush (*Lindera obtusiloba* Blume) was previously shown to lag behind the cumulative flowering of male flowers by approximately 1 wk when measured in situ (Dupont and Kato 1999).

What is missing from these studies is determining how the flowering phenology of males and females responds to a changing climate, particularly over large geographic areas. However, given the morphological and phenological differences between sexes, herbarium specimens of dioecious species may be subject to collector bias. Using digitized herbarium specimens of the widespread East Asian species blunt-lobed spicebush (*L. obtusiloba* Blume), we asked:

1. Do female and male *L. obtusiloba* have different flowering responses (i.e., different phenological sensitivities) to variations in spring temperature and latitude?
2. To what extent do male and female *L. obtusiloba* flowering periods overlap on average over the spatiotemporal extent of historical observations?
3. Is there any evidence of collection bias in this species that could suggest an impact on herbarium-based phenological and climate change studies of dioecious species?

Material and Methods

Study Species

Lindera obtusiloba, widely distributed in Bhutan, China, India, Japan, and Korea, is a small tree or shrub often sprouting new shoots from the base. The species is dioecious, with separate male and female individuals. In the early spring, plants produce small yellow flowers that appear on the twigs before the leaves. Male flowers are bright yellow in color, with prominent stamens, and are more conspicuous than the yellowish-green female flowers, with reduced nonfunctional stamens (fig. 1). In

studies of *L. obtusiloba* and other Asian species, males start to flower a few days before the females, but there is broad overlap in flowering between the sexes during the several-week flowering period (Dupont and Kato 1999). The fruit is a black drupe, and the leaves are ovate, often bluntly trilobed, and aromatic.

The sex ratios of *Lindera* species have been extensively investigated, especially in eastern North America and eastern Asia, with populations varying from having equal numbers of males and females to having female-biased sex ratios to having male-biased sex ratios (Primack 1985; Niesenbaum 1992; Cipollini and Whigham 1994; Dupont and Kato 1999; Cipollini et al. 2020). Sex ratios have also been investigated in other Lauraceae genera, such as *Ocotea* and *Laurus* (Wheelwright and Bruneau 1992; Forfang and Olesen 1998). Female plants of *Lindera benzoin* have been shown to have a greater cost of reproduction due to subsequent fruit production (Cipollini and Whigham 1994; Cipollini et al. 1994).

Herbarium-Based Phenology Scoring

We searched for herbarium specimens of *L. obtusiloba* in the Chinese Virtual Herbarium (<http://www.cvh.ac.cn>), which predominantly includes specimens from China, with a small number from Korea and Japan. We also included digitized photographic specimens from the Chinese Field Herbarium that were shot in the field (<http://www.cfh.ac.cn/>). These online searches resulted in a total of 1839 specimens.

We evaluated each specimen image to determine whether it showed plants in flower and identified sex on the basis of flower morphology (fig. 1). Evaluating sex in digitized specimens was challenging, as the flowers are small and often shrivel up after drying. Ninety-eight herbarium specimens and 60 field photographs included plants with flowers and the necessary metadata (i.e., date and precise location information). However, we could not reliably determine the sex of 21 specimens based on visual inspection of these images. After we removed duplicates, the final data set included 88 unique observations (23 photographic observations collected between 2000 and 2018 and 65 herbarium specimens collected between 1918 and 2015), all from China, whose sex could be identified and that had information on the date and place of collection. This modest number reflects both the difficulty of finding specimens that met our parameters and the smaller overall number of available specimens in East Asian herbaria in comparison with those in other regions of the world (Yang 2013). These samples were collected across China and are not dominated by any one plant collector.

Climate and Elevation Data

We georeferenced each specimen based on the locality descriptions on the labels. Historical spring temperature data (average of March and April monthly means) were acquired for the year of each specimen's collection date using the Climatic Research Unit temperature series data (Harris et al. 2020).

Data Analysis

Prior to analysis, we standardized explanatory factors to have a mean of 1 and standard deviation of 0.5, following Gelman (2008). This rescaling allows us to directly compare the relative

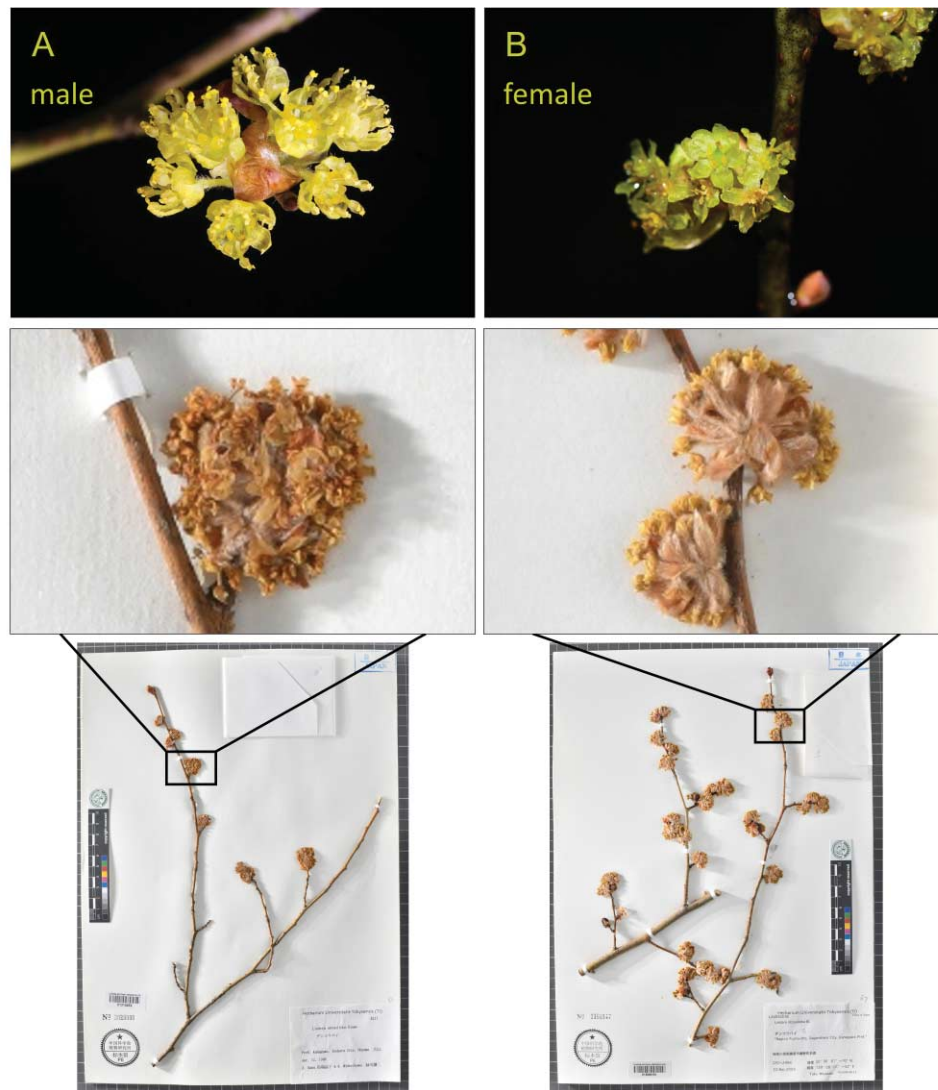


Fig. 1 Close-up images of field (*top*) and herbarium (*bottom*) specimens of blunt-lobed spicebush (*Lindera obtusiloba*), illustrating differences between male (*A*) and female (*B*) flowers. Tepals are approximately 2.5 mm long and 1 mm wide. Specimen credits: Herbarium (PE), Institute of Botany, the Chinese Academy of Sciences (*A*: Baba 8217, PE 2029000; *B*: Miyazaki 0903033, PE 2360147).

importance of different explanatory factors, as parameter estimates now represent standardized effect sizes.

We analyzed relationships between flowering times and environmental drivers (average spring temperature and latitude) using a hierarchical Bayesian mixed effects model:

$$\text{FloweringDay}_i \sim N(\mu_i, \tau)$$

$$\mu_i = \alpha + \beta_1 \times \text{SpringTemp}_i + \beta_2 \times \text{Latitude}_i.$$

Flowering day of observation i is modeled using a normal likelihood with mean μ and variance τ . The mean μ is then modeled following a linear relationship with the intercept α and β slope terms corresponding to fixed effects for spring temperature and latitude. We used minimally informative normal prior distributions for α and $\beta \sim N(0, 1000)$ and gamma distribution for the variance $\tau \sim \Gamma(0.01, 0.01)$. Models were run separately for male ($n = 31$) and female ($n = 57$) plants using OpenBUGS

software (Lunn et al. 2009), using three Markov chain Monte Carlo chains iterated 50,000 times with a burn-in period of 25,000 iterations. Parameter convergence was evaluated visually and using the Brooks-Gelman-Rubin statistic (Gelman and Rubin 1992).

We tested for differences in flowering time between male and female plants using Student's t -test (unpaired, two tailed). We tested for differences in the variance of flowering times using the var.test command in the stats package in R (R Core Team 2021).

Results

Flowering times (i.e., the period of flowering estimated from herbarium observations) were unaffected by spring temperatures and latitude for both male and female plants (95% Bayesian confidence intervals overlap; fig. 2A). Spring temperature

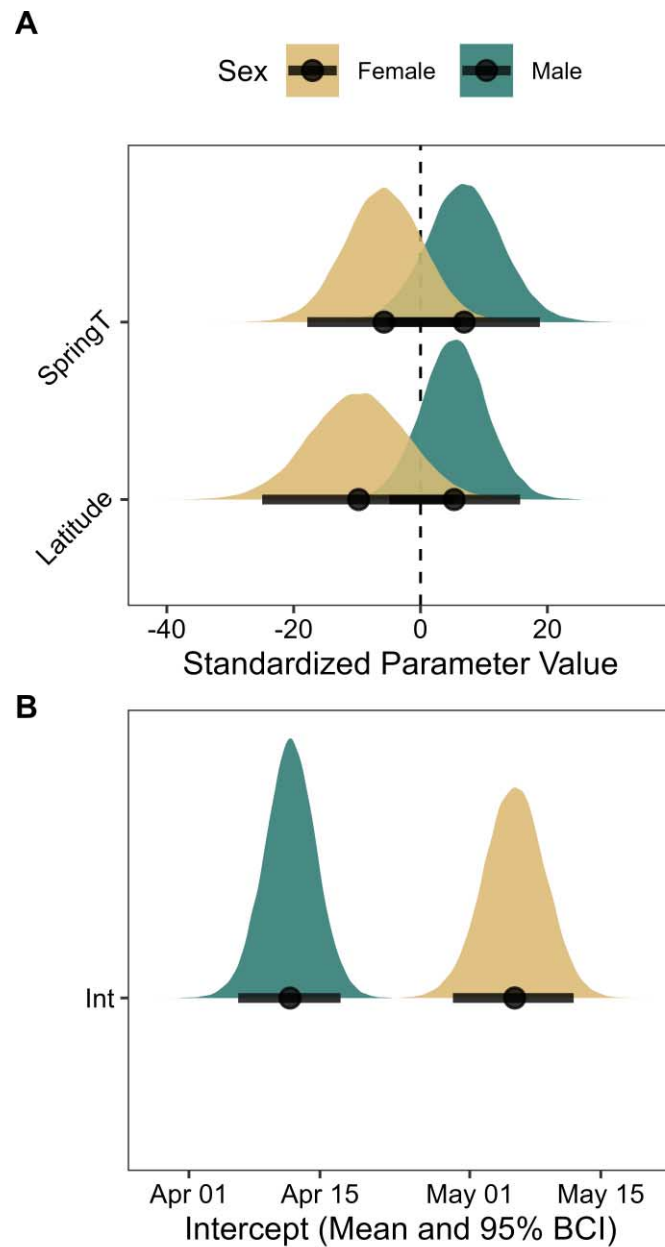


Fig. 2 Posterior distributions, estimated means (circles), and 95% Bayesian confidence intervals (BCIs; black bars) for the average spring temperature and latitudinal slope parameters (A) and model intercepts (B). Distributions show estimated parameter values for female and male plants. Parameter estimates are considered significantly different if 95% BCIs do not overlap. Int = intercept; SpringT = spring temperature.

was slightly more influential than latitude in cuing male flowering, as indicated by the absolute distance of the mean standardized effect size from 0, but the opposite was true for female flowering. Both drivers tended to have positive associations with male flowering (i.e., warmer temperatures and higher latitudes were associated with delayed male flowering), whereas female flowering tended to be negatively associated with both drivers (i.e., warmer temperatures and higher latitudes led to earlier female flowering).

While sensitivities of flowering time to environmental variables were varied and weak, flowering period significantly differed between male and female plants. This difference is evident in the posterior estimated intercept values, which were significantly earlier for male plants compared with female plants (fig. 2B). Furthermore, we found significant differences in flowering dates between sexes when we looked only at the observed (not estimated) flowering times. The mean flowering date for males (day 103) was 25 d earlier than the mean date for females

Table 1
Flowering Times (Day of Year) of Male and Female Specimens of *Lindera obtusiloba*

	Male	Female	Overall
Mean	103.1	128.0	119.2
SD	13.7	23.1	23.4
Range	76–126	80–217	76–217
N	31	57	88

(day 128; $t = 6.3$, $df = 85$, $P < 0.001$; table 1). Nearly all plants flowering before day 110 (April 20) were male, while those flowering after day 110 were primarily female (fig. 3). In addition to mean differences, female plants showed a greater degree of variability in flowering times in comparison with male plants (table 1; test of unequal variances between sexes: $F_{30,56} = 0.35$, $P = 0.002$). This difference between sexes is reflected in the greatly extended last date of flowering of female plants in comparison with male plants.

Discussion

Using herbarium specimens and historical photographic records, we asked whether the flowering phenology of male and female plants of a dioecious species (*Lindera obtusiloba*) re-

sponds differently to environmental differences. Many previous studies have demonstrated that spring-blooming plants flower earlier with warmer temperatures and at lower latitudes (Doi et al. 2017; Ellwood et al. 2019; Park et al. 2019; Piao et al. 2019), but this question has not been well explored for dioecious species. Contrary to our expectations, we found no evidence that *L. obtusiloba* flowers earlier with warmer conditions or at lower latitudes. Furthermore, these responses varied slightly between male and female flowers. While such effects of temperature and latitude are readily seen in many other species and studies, they are not apparent in this species (although we note that this may simply be a product of insufficient data availability). It is worth further investigation to determine why this species of spicebush does not show this pattern.

The most striking finding of this study is that female plants flowered substantially later than male plants, with an average difference of 25 d. The flowering of male plants before females (protandry) has been indicated as a somewhat common pattern in a meta-analysis of 32 dioecious species (Forrest 2014). Earlier flowering in males has even been reported for *L. obtusiloba* in field observations (Dupont and Kato 1999). However, this difference in flowering onset was only a few days earlier for males—far less than what we found in the current study using herbarium specimens. This difference in flowering times dominates the comparisons of phenological sensitivity to temperature and latitude (figs. 2, 3).

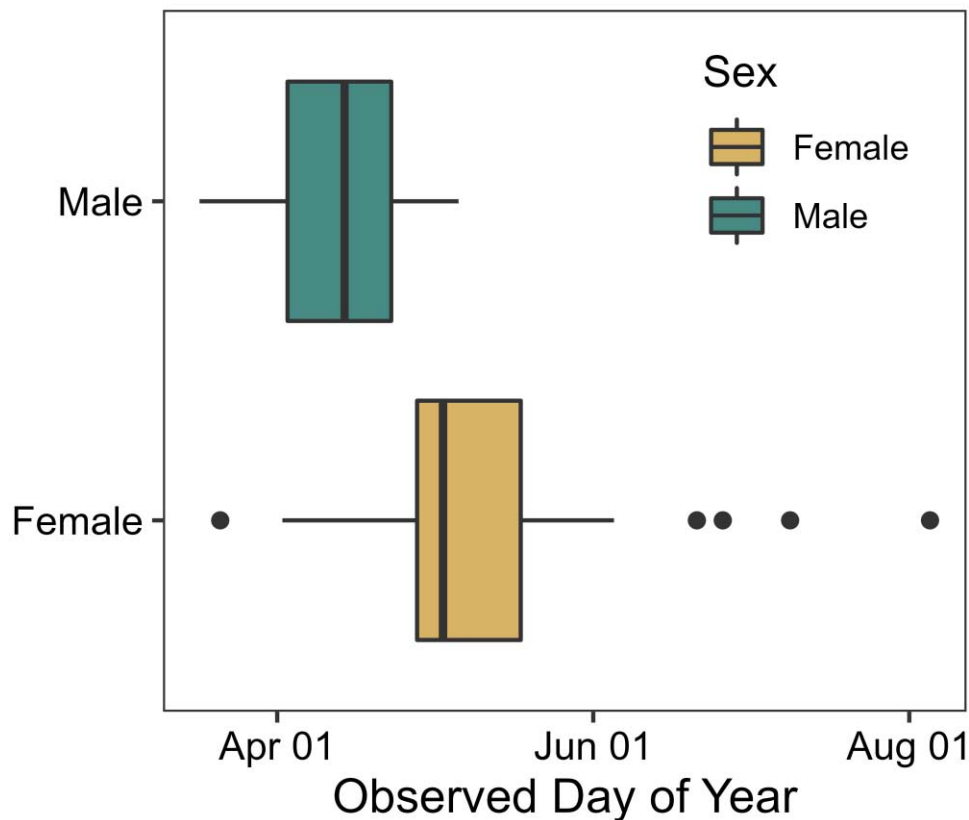


Fig. 3 Boxplots show flowering time differences between male and female specimens. Boxes show first and third quartiles with a median line; whiskers extend to 1.5 times the interquartile range, with outlier points.

If true, a 25-d separation in flowering time between males and females would lead to reproductive decline and failure. Rather, we suggest that the discrepancy between herbarium specimens and field observations might be mostly due to collection bias in herbarium specimens. If the true flowering time of *L. obtusiloba* across East Asia ranges roughly from early March through mid-May, then collectors during this flowering period would encounter both male and female plants in flower. However, it appears that the plant collectors mostly collected male plants during the main flowering period, probably because the bright yellow color and prominent stamens make them more conspicuous than the female flowers (fig. 1). This is consistent with recent findings from an Arctic collection suggesting that herbarium collections can be biased by plant aesthetic qualities (e.g., color and growth form) and by being concentrated during the time of peak flowering at the community level (Panchen et al. 2019). Similar bias has been suggested in a review of herbarium collection biases (Daru et al. 2018).

However, after May 5 (day 125), on average, the male plants quickly lose their flowers following anthesis, but the female plants still retain their flowers, which are developing into young fruits. As a consequence, after May 5, collectors will predominantly and then exclusively collect female plants with older, nonreceptive flowers, reversing the sex ratio of the plants represented in the herbarium collection across the season. A similar bias will affect the photographers who are making digital photographic collections. This suspected collection bias could be further complicated by male flowers blooming slightly earlier and in greater numbers in the first week of the flowering season (Dupont and Kato 1999), thereby also increasing the likelihood of the collection of male flowers earlier in the year, despite female flowers being present. This bias is not due to the biases of a particular collector or regions, as these *L. obtusiloba* specimens were collected across China by numerous collectors. It is unknown whether the pattern we observed for this species is widespread across other dioecious species. More studies are needed.

Researchers conducting phenology studies of dioecious species using herbarium specimens should record the sex of the specimens they are evaluating. Male and female plants may differ in their phenology and may exhibit differential phenological sensitivities to climate change and other environmental factors. For many species, male plants might be more effective for use in phenology studies because they are collected in greater abundance and because their flowers are more likely to be present only

during the active period of flowering and pollination. However, our results clearly show that plant sex cannot be ignored in herbarium-based or field-based studies, which is often done in community-scale phenological studies including dioecious and monocious species (e.g., Ellwood et al. 2013). They also highlight the need for other researchers to explore the extent to which this bias affects other dioecious species.

Conclusions

Our study highlights that phenology, although overlooked, can be an important area of trait bias among herbarium specimens of dioecious species. Further studies of *Lindera obtusiloba* and a broader range of dioecious species are needed to confirm this collection bias and understand differences between male and female phenological responses to climate change. Collectors of both herbarium specimens and photographs should be aware of this potential bias and make a deliberate effort to collect both male and female plants of dioecious species. Improving the quality of data curation in future collections will help us better elucidate the sex-specific responses of dioecious species to environmental variation.

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Data Availability

All data used in this study are openly available on Zenodo (<https://doi.org/10.5281/zenodo.6380698>).

Literature Cited

- Barrett SCH, J Hough 2013 Sexual dimorphism in flowering plants. *J Exp Bot* 64:67–82.
- Cipollini ML, NR Dingley, P Felch, NJ Bailey, JP Moss, MG Gaskin, S Williams 2020 Does sex ratio bias and sexual dimorphism occur in *Lindera benzoin* L. (Lauraceae) prior to fruit production? *J Torrey Bot Soc* 147:272–280.
- Cipollini ML, DA Wallace-Senft, DF Whigham 1994 A model of patch dynamics, seed dispersal and sex ratio in the dioecious shrub *Lindera benzoin* (Lauraceae). *J Ecol* 82:621.
- Cipollini ML, D Whigham 1994 Sexual dimorphism and cost of reproduction in the dioecious shrub *Lindera benzoin* (Lauraceae). *Am J Bot* 81:65–75.
- Daru BH, DS Park, RB Primack, CG Willis, DS Barrington, TJS Whitfeld, TG Seidler, et al 2018 Widespread sampling biases in herbaria revealed from large-scale digitization. *New Phytol* 217:939–955.
- Doi H, O Gordo, T Mori, MT Kubo 2017 A macroecological perspective for phenological research under climate change. *Ecol Res* 32:633–641.
- Dupont YL, M Kato 1999 Phenology and flower-visiting entomofauna of six species of *Lindera* (Lauraceae) in Japan. *Nord J Bot* 19:707–718.
- Ellwood ER, RB Primack, CG Willis, J HilleRisLambers 2019 Phenology models using herbarium specimens are only slightly improved by using finer-scale stages of reproduction. *Appl Plant Sci* 7:1–9.

- Ellwood ER, SA Temple, RB Primack, NL Bradley, CC Davis 2013 Record-breaking early flowering in the eastern United States. *PLoS ONE* 8:1–9.
- Forfang AS, JM Olesen 1998 Male-biased sex ratio and promiscuous pollination in the dioecious island tree *Laurus azorica* (Lauraceae). *Plant Syst Evol* 212:143–157.
- Forrest JRK 2014 Plant size, sexual selection, and the evolution of protandry in dioecious plants. *Am Nat* 184:338–351.
- Gallinat AS, ER Ellwood, JM Heberling, AJ Miller-Rushing, WD Pearse, RB Primack 2021 Macrophenology: insights into the broad-scale patterns, drivers, and consequences of phenology. *Am J Bot* 108:2112–2126.
- Gelman A 2008 Scaling regression inputs by dividing by two standard deviations. *Stat Med* 27:2865–2873.
- Gelman A, DB Rubin 1992 Inference from iterative simulation using multiple sequences. *Stat Sci* 7:457–511.
- Harris I, TJ Osborn, P Jones, D Lister 2020 Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. *Sci Data* 7:1–18.
- Heberling JM 2022 Herbaria as big data sources of plant traits. *Int J Plant Sci* 183:87–118.
- Hultine KR, KC Grady, TE Wood, SM Shuster, JC Stella, TG Whitham 2016 Climate change perils for dioecious plant species. *Nat Plants* 2:16109.
- Lloyd DG, CJ Webb 1977 Secondary sex characters in plants. *Bot Rev* 43:177–216.
- Lunn D, DJ Spiegelhalter, A Thomas, N Best 2009 The BUGS project: evolution, critique and future directions. *Stat Med* 28:3049–3067.
- Niesenbaum RA 1992 Sex ratio, components of reproduction, and pollen deposition in *Lindera benzoin* (Lauraceae). *Am J Bot* 79:495–500.
- Panchen ZA, J Doubt, HM Kharouba, MO Johnston 2019 Patterns and biases in an Arctic herbarium specimen collection: implications for phenological research. *Appl Plant Sci* 7:1–12.
- Park DS, I Breckheimer, AC Williams, E Law, AM Ellison, CC Davis 2019 Herbarium specimens reveal substantial and unexpected variation in phenological sensitivity across the eastern United States. *Philos Trans R Soc B* 374:20170394.
- Park IW, T Ramirez-Parada, SJ Mazer 2021 Advancing frost dates have reduced frost risk among most North American angiosperms since 1980. *Glob Change Biol* 27:165–176.
- Pearse WD, CC Davis, DW Inouye, RB Primack, TJ Davies 2017 A statistical estimator for determining the limits of contemporary and historic phenology. *Nat Ecol Evol* 1:1876–1882.
- Piao S, Q Liu, A Chen, IA Janssens, Y Fu, J Dai, L Liu, X Lian, M Shen, X Zhu 2019 Plant phenology and global climate change: current progresses and challenges. *Glob Change Biol* 25:1922–1940.
- Primack RB 1985 Longevity of individual flowers. *Annu Rev Ecol Syst* 16:15–37.
- R Core Team 2021 R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Renner SS 2014 The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. *Am J Bot* 101:1588–1596.
- Soltis PS 2017 Digitization of herbaria enables novel research. *Am J Bot* 104:1281–1284.
- Wheelwright NT, A Bruneau 1992 Population sex ratios and spatial distribution of *Ocotea tenera* (Lauraceae) trees in a tropical forest. *J Ecol* 80:425.
- Willis CG, ER Ellwood, RB Primack, CC Davis, KD Pearson, AS Gallinat, JM Yost, et al 2017 Old plants, new tricks: phenological research using herbarium specimens. *Trends Ecol Evol* 32:531–546.
- Yang Y 2013 Holdings of type specimens of plants in herbaria of China. *Biodivers Sci* 20:512–516.