Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms

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4   **Herbarium records are reliable sources of phenological change driven by**

5   **climate and provide novel insights into species' phenological cueing**

6   **mechanisms**[1]

7

8   Charles C. Davis[2,7], Charles G. Willis[2,3], Bryan Connolly[4,5], Courtland Kelly[2], and

9   Aaron M. Ellison[6]

10

11   [2]Department of Organismic and Evolutionary Biology and Harvard University

12   Herbaria, Harvard University, Cambridge, Massachusetts, USA

13

14   [3]Harvard University Center for the Environment, Harvard University, Cambridge,

15   Massachusetts, USA

16

17   [4]Massachusetts Natural Heritage and Endangered Species Program, Division of

18   Fisheries & Wildlife, Massachusetts, USA

19

20   [5]Department of Biology, Framingham State University, Framingham, Massachusetts,

21   USA

22

23   [6]Harvard Forest, Harvard University, Petersham, Massachusetts, USA

24

25   [*]Author for correspondence (e-mail: cdavis@oeb.harvard.edu)

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27

40

41   [7] Author for correspondence (e-mail: cdavis@oeb.harvard.edu)

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43   ⬜ *Premise of the study*: Climate change has resulted in major changes in the

44   phenology of some species but not others. Long-term field observational

45   records provide the best assessment of these changes, but geographic and

46   taxonomic biases limit their utility. Plant specimens in herbaria have been

47   hypothesized to provide a wealth of additional data for studying phenological

48   responses to climatic change. However, no study to our knowledge has

49   comprehensively addressed whether herbarium data are accurate measures

50   of phenological response, and thus applicable to addressing such questions.

51

52   ⬜ *Methods*: We compared flowering phenology determined from field

53   observations (years 1852-1858; 1875; 1878-1908; 2003-2006; 2011-2013)

54   and herbarium records (1852-2013) of 20 species from New England, USA.

55

56   ⬜ *Key Results*: Earliest flowering date estimated from herbarium records

57   faithfully reflected field observations of first flowering date and substantially

58   increased the sampling range across climatic conditions. Additionally,

59   although most species demonstrated a response to inter-annual temperature

60   variation, long-term temporal changes in phenological response were not

61   detectable.

62

63   ⬜ *Conclusions*: Our findings support the use of herbarium records for

64   understanding plant phenological responses to changes in temperature, and

65   also importantly establish a new use of herbarium collections: inferring

66   primary phenological cueing mechanisms of individual species (e.g.,

67   temperature, winter chilling, photoperiod). These latter data are lacking from

68   most investigations of phenological change, but are vital for understanding

69   differential responses of individual species to ongoing climate change.

70

71   **Key words**: climate change, climate variability, phenology, herbarium specimens,

72   museum collections

73

74   The impacts of global climatic change on organisms have been well documented in

75   recent years (Walther et al., 2002; Root et al., 2003; Parmesan, 2006). Changes in

76   phenological events—i.e., the timing of specific life-history events—are used widely

77   to assess responses of different organisms to climate change. Typically, researchers

78   have focused on relatively common phenological events that are easily measured

79   and have a history of regular observation such as leaf-out, flowering, or fruiting in

80   plants, or eclosion and migration in animals (Sparks and Carey, 1995; Bradley et al.,

81   1999; Fitter and Fitter, 2002; Menzel, 2002; Parmesan and Yohe, 2003; Root et al.,

82   2003; Parmesan, 2007; Bertin, 2008; Miller-Rushing et al., 2008; Miller-Rushing and

83   Primack, 2008; Visser, 2008; Willis et al., 2008; Körner and Basler, 2010; Willis et

84   al., 2010; Panchen et al., 2014). However, the paucity of long-term data sets

85   necessary to identify the influence of climatic change on phenological events

86   remains problematic, even for regions where associated climatic data are available

87   (e.g., long-term temperature trends). Moreover, most data sets of this nature show a

88   strong geographical and taxonomic bias—they are largely from temperate regions,

89   mostly include a small subset of species or functional types within particular

90   assemblages (e.g., dominant woody species), and do not sample the variation in

91   phenological response across the range of a species (Wolkovich, Cook, and Davies,

92   2014).

93   The enormous collections of plants housed in herbaria around the world

94   provide a potential, largely untapped, alternative body of data for studying long-

95   term phenological responses to climatic change (Vellend et al., 2013; Kharouba and

96   Vellend, 2015). Herbarium specimens represent snapshots of phenological events

97   (e.g., flowering, fruiting) at a specific place and time. Observations from numerous

98   specimens collected at multiple locations and times may allow us to determine

99   whether a given species has changed its phenology in parallel with climate. Previous

100 efforts have used herbarium specimens in this manner (Primack et al., 2004; Miller-

101 Rushing et al., 2006; Robbirt et al., 2011; Panchen et al., 2012), but only recently has

102 this effort been scaled-up to investigate patterns of phenology across large numbers

103 of species and vast geographical areas. For example, Calinger *et al.* (2013) combined

104 data from herbarium specimens of 141 species with climatic records to determine

105   that peak flowering has advanced 2.4 days/°C of warming over the last century

106   across ~116,000 km^2 in north-central North America. They further identified

107   differences in phenological responses based on whether a species was native, its

108   pollination syndrome, growth form, functional group, and flowering season.

109   Similarly, Everill *et al.* (2014) examined ~1600 herbarium records from 1834-2008

110   of 27 common New England tree species. They reported that spring leaf-out dates

111   were strongly associated with spring temperatures and that tree species leafed out

112   ~2.0 days/°C earlier now than in the past.

113   We applaud these efforts to leverage herbarium data to investigate recent

114   effects of climate change. Despite the promise of these studies, however, the efficacy

115   and bias of herbarium records as accurate measures of phenological response have

116   seldom been assessed (Gardner et al., 2006; Moerman and Estabrook, 2006; Loiselle

117   et al., 2008; Sastre and Lobo, 2009). This is relevant because the purpose of

118   herbarium collections, at least historically, has not been to document phenological

119   phenomena *per se*, but rather to sample representative specimens of a species

120   throughout its geographic distribution. Thus, phenological data collected from

121   herbarium records are subject to numerous potential biases. For example, botanists

122   might collect samples at the same time every year out of habit or convenience, with

123   little regard to inter-annual climatic variation, occurrence of date of first flowering,

124   or time of spring leaf-out. Other sources of bias include: misidentification of closely

125   related species that vary in phenological response; temporal gaps in collecting effort

126   that impede efforts to assess long-term change [e.g., decline in collecting efforts

127   since the mid-20th century (Gardner et al., 2006)]; spatial gaps in collecting; and

128   spatial preferences when collecting samples (e.g., easily-accessible urban areas,

129   trails, roadsides).

130   At the same time, ecologists' emphasis on reconstructing phenology

131   overlooks other important uses of herbarium data. With rare exceptions (e.g., crops

132   and model species like *Arabidopsis thaliana* (L.) Heynh.), we know relatively little

133   about environmental cues that regulate onset and duration of phenological events

134   for most plant species. These cues govern physiological mechanisms that initiate

135   phenological events associated with fitness traits (e.g., initiation of buds or

136   flowering), and timing of these events ultimately may determine how species will

137   respond to future climatic change. For example, species for which flowering is most

138   sensitive to temperature likely will be strongly affected by changing temperatures,

139   especially when this could create temporal mismatches with key pollinators that are

140   more (or less) sensitive to temperature cues (Burkle, Marlin, and Knight, 2013).

141   Fortunately, recent advancements in process-based modeling and data-model fusion

142   have allowed researchers to distinguish among the relative importance of major

143   environmental cues (e.g., temperature, winter chilling, and photoperiod)

144   (Richardson et al., 2006; Morisette et al., 2008; Migliavacca et al., 2012; Archetti et

145   al., 2013; Siniscalco et al., 2014). These models, however, require extensive and

146   temporally dense (>50years) collections of standardized observational data. As a

147   result, the application of process-based models to studies of phenological change

148   has been limited to only a few dozen species, often from fairly restricted

149   phylogenetic and life history groups. To the extent that they prove to be reliable

150   measures of inter-annual phenological response, herbarium data offer the potential

151   for researchers to expand the temporal depth of phenological data for large

152   numbers of species, and simultaneously propose new hypotheses regarding

153   physiological controls on phenological events.

154   In this paper we address both the validity of herbarium records for

155   investigating phenological change and the use of herbarium records for identifying

156   potential cueing mechanisms of phenological events. Our focal region—New

157   England in the United States—is where researchers have suggested that many

158   plants today flower much earlier than in the past because they are responsive to

159   changes in temperature, which has been rising rapidly in this region (Miller-Rushing

160   and Primack, 2008). We first combine direct observational records and rich

161   herbarium records collected during the last 160 years from Concord, Massachusetts

162   and adjacent counties to assess whether herbarium flowering data correspond with

163   first flowering dates observed at irregular intervals in the field. We then leverage

164   these results to identify likely physiological cueing responses necessary for

165   regulating flowering phenology. We focused on 20 species previously shown to

166   exhibit dynamic responses to climatic change (Willis et al., 2008; Willis et al., 2010).

167 These species are abundant in New England herbaria because they are frequently

168 collected, conspicuous plants that produce large flowers, which are easy to score

169 from herbarium collections (e.g., lilies, orchids). Ultimately, results of our efforts will

170 help to build a refined regional picture of how climatic change has affected plant

171 phenology given the range of physiological mechanisms by which plants are cued to

172 flower and fruit, and how this is likely to shape plant diversity in the near future.

173

174 **MATERIALS AND METHODS**

175 *Study site*–Concord (42°27'38" N; 71°20'54" W) is a ~67 km^2 town in Massachusetts

176 (MA) with a wide range of habitats, including peatlands, deciduous hardwood

177 forests, and prairies. Although Concord has undergone extensive development since

178 the 1850s, ~60% of the town's land area remains undeveloped or has been

179 permanently protected from future development (Willis et al., 2008; Primack,

180 Miller-Rushing, and Dharaneeswaran, 2009). During the mid-19th and early 20th

181 centuries, Henry David Thoreau and Alfred Hosmer, respectively, documented plant

182 species occurrences and first-flowering dates there (Miller-Rushing and Primack,

183 2008; Willis et al., 2008; Primack, Miller-Rushing, and Dharaneeswaran, 2009).

184 These data, combined with contemporary observations, have suggested that,

185 depending on spring temperatures, some species flower as many as 8 days earlier

186 now than they did in the 1850s (Miller-Rushing and Primack, 2008; Willis et al.,

187 2008; Primack, Miller-Rushing, and Dharaneeswaran, 2009). Moreover, Willis *et al.*

188 (2008, 2009) identified phylogenetic effects in these data on species diversity:

189 clades whose first flowering time are less sensitive to temperature and have shown

190 little phenological change also have declined significantly in abundance.

191

192   *Study species*–We investigated 20 biennial or perennial species in nine families

193   (Table 1), each of which met four criteria. [1] The species were represented in

194   historical field observations by Hosmer (1878-1903) from Concord (Miller-Rushing

195   and Primack, 2008). These data are unique in providing a reliable, uninterrupted

196   15-year period of phenological monitoring. [2] The species had relatively large

197   flowers, which facilitated rapid and accurate assessment of flowering from

198   herbarium specimens. [3] The species were well represented in herbarium

199   collections from Massachusetts, including Middlesex County (which includes

200   Concord) and nearby counties. [4] Species with showy, ephemeral flowers and with

201   relatively short flowering time, such as orchids (Orchidaceae) and irises (Iridaceae),

202   were preferred because they were more likely to have been collected near to their

203   first flowering date (Robbirt et al., 2011). We also included non-native and invasive

204   species (e.g., *Barbarea vulgaris* [Brassicaceae], *Chelidonium majus* [Papaveraceae],

205   respectively) as well as species such as *Vaccinium angustifolium*, which previously

206   have been shown to be phenologically responsive to warming (Ellwood et al., 2013)

207   and thus more likely to exhibit long-term phenological shifts associated the secular

208   trend of rising mean temperatures. Additionally, taxon sampling is representative of

209   the breadth of seasonal flowering (e.g., spring ephemeral vs. summer flowering

210   species).

211

212   *Flowering time data*–Field observations of first flowering date for Concord were

213   recorded by Thoreau (1852–1858), Hosmer (1875, 1878–1903), Miller-Rushing &

214   Primack (2003–2006) (Miller-Rushing and Primack, 2008), and this paper's co-

215   authors Davis & Connolly (2011–2013: field data first reported and used herein).

216   Field observations by Davis & Connolly applied a similar method to the one outlined

217   by Primack et al. (2009): from April to September, multiple sites throughout the

218   Concord area were visited 1-3 times weekly by Davis & Connolly to systematically

219   record flowering dates. Similar to Primack et al., Davis & Connolly also consulted

220   local botanical experts about the location and flowering time of certain species.

221   Estimates of earliest flowering dates for herbarium records were based on

222   data collected during visits to the Harvard University Herbaria (HUH), New York

223 Botanical Garden's William and Lynda Steere Herbarium (NY), Yale University

224 Herbarium (YU), and University of Connecticut's George Safford Torrey Herbarium

225 (CONN) by co-author Kelly. These herbaria collectively represent the largest

226 holdings of plants of the Northeastern United States and include both very old

227 collections (HUH, YU) and more recent ones (CONN). We first identified flowering

228 specimens from MA for each of our target species. Following Primack et al. (2004),

229 we recorded locality, collection date, accession number (when provided), and

230 collector for specimens with fully open flowers. When multiple flowers were

231 present on a specimen, it was recorded as flowering if ≥ 75% of them were fully

232 opened. Specimens that had a majority of flower buds or fruit were ignored, as were

233 those with insufficient or illegible collection data. The majority of herbarium

234 specimens were collected between the late 1800s and mid-1900s. When there were

235 multiple specimens for the same species in a given year (which occurred only for

236 <3% of the data collected), we used the earliest record for a species × county

237 combination in a given year as our estimate of earliest flowering date.

238 Finally, we emphasize that the earliest flowering date estimated from

239 herbarium specimens is different from first flowering date recorded by field

240 observers. Observational records are the gold standard for phenological research,

241 and estimate first flowering date with a high degree of accuracy. In contrast, data

242 from herbarium specimens provides only an approximation of earliest flowering

243 date for those specimens, which may or may not be correlated with first flowering

244 date observed in the field. However, our goal was to assess changes in flowering as a

245 function of both inter-annual temperature variation and long-term changes in

246 climate. Although we expected differences between the observed first day of

247 flowering and estimated date of flowering estimated from herbarium specimens, the

248 aim of this study was to assess whether these two data types estimate similar

249 responses to both short-term and long-term climatic change. We hypothesized that

250 estimated changes in earliest flowering date determined from herbarium specimens

251 would be correlated with observed changes in first flowering date as the climate has

252 changed.

253

254   *Temperature records*–Mean monthly temperatures (1885–present) at Great Blue

255   Hill, ~33 km southeast of Concord were obtained from NOAA's Global Historical

256   Climatology Network (http://ncdc.noaa.gov/ghcnm/). We used the GHCNM v3

257   quality controlled unadjusted data. These data are highly correlated ($r \geq 0.995$) with

258   available, but sparser, climatological data from Concord (Miller-Rushing and

259   Primack, 2008). Monthly temperature data from 1831 – 1884 collected by the Blue

260   Hill Meteorological Observatory were provided to us by A. Miller-Rushing. Mean

261   annual temperatures (Fig. 1a) were calculated by averaging the mean monthly

262   temperatures for each year. Mean spring temperatures (Fig. 1b) were calculated as

263   the average of each year's February – May mean monthly temperatures. These

264   months were used because they had been found previously to represent the months

265   that are most predictive of flowering time (Miller-Rushing and Primack, 2008;

266   Primack, Miller-Rushing, and Dharaneeswaran, 2009).

267

268   *Statistical analysis*–We used linear mixed-effects models in the *nlme* library

269   (Pinheiro et al., 2015) of the R statistical software system (Team', 2014) version

270   3.1.0 to test for overall and species-specific relationships between spring

271   temperature, calendar year, and earliest flowering date. Earliest flowering date in a

272   given year—either from field or herbarium observations—was the response

273   variable in all models. In the "climate" model, mean spring temperature was treated

274   as a fixed predictor variable, whereas in the "year" model, calendar year was the

275   fixed predictor variable. In both models, data type—field observation or herbarium

276   record—was also treated as a fixed predictor variable. Species identity was included

277   as a random effect. The values of the random effects (i.e., equivalent to the y

278   intercept for each species) ordered the species from earliest to latest flowering, so

279   we also regressed (using a linear regression model) the rate of change in earliest

280   flowering date for each species (i.e., the slope of the line relating earliest flowering

281   date to climate) against its random effect term. This latter analysis provided

282   additional insights about potential species-specific sensitivity to spring temperature

283   as a phenological cue.

284   To test the hypothesis that estimated changes in earliest flowering date

285   determined from herbarium specimens were correlated with observed changes in

286   first flowering date as the climate has changed, we plotted the slopes of the lines fit

287   to either the field observational data or the herbarium data in the "climate" model.

288   We tested the relationship between the slopes generated by these two models in

289   two ways. First, we did a simple paired *t*-test on the slopes (paired by species).

290   Failure to reject the null hypothesis of no difference would suggest that the

291   observed and herbarium data are recording similar responses to climate. We also fit

292   a Model-II regression to the paired slopes (Model-II, or reduced major-axis

293   regression makes no assumption about the "independent" or "dependent" variable

294   (Gotelli and Ellison, 2012). The slope of this regression tests whether the two sets of

295   data vary in parallel, and the intercept is an estimate of how the expected shift in

296   flowering date differs between the two datasets.

297   In the main text, we report data only for the 600 field observations from

298   Concord combined with 297 herbarium records from Middlesex County (where the

299   town of Concord is located). The results were qualitatively identical when we

300   combined the Concord observations with the 680 herbarium records from four

301   nearby counties (results in Supplementary Online Material). Raw data and model

302   code are publicly accessible from the Harvard Forest Data archive

303   (http://harvardforest.fas.harvard.edu/data-archive), dataset HF-XXX (will provide

304   DOI in galley).

305

306   **RESULTS AND DISCUSSION**

307   *Data density of field observations and herbarium records*–Field observations (which

308   we refer to henceforth as "observational data") of early flowering dates have been

309   highly episodic (Fig. 1c). Thoreau recorded dates of first flowering in Concord

310   annually from 1852 to 1858; Hosmer recorded first flowering in 1875, and then

311   annually between 1878 and 1903; Primack and Miller-Rushing's data span 2003–

312   2006, and our own observational data include 2011–2013. In contrast, we have

313   1108 herbarium records (which we refer to henceforth as "herbarium data") of

314   flowering occurrences in the state of Massachusetts collected between 1852 and

315   2012 (Fig. 1d) with 297 records from the same county as Concord (Middlesex), and

316   680 remaining records from four nearby counties (Supplementary Online Material,

317   Fig. S1).

318   During the combined data interval (1852–2013), mean spring temperatures

319   varied widely, ranging from < 1 to > 8 °C. Similarly, mean annual temperatures

320   ranged from < 6 to > 11 °C. To characterize this variation in temperature, we defined

321   the "climatic space" of Concord since 1852 as the region encompassed by the range

322   of mean spring temperatures and mean annual temperatures (Fig. 1e). Three points

323   are worth emphasizing about the sampling coverage of this climatic space. First,

324   herbarium data covered a much larger percentage of this climatic space than

325   observational data (91% versus 76%, respectively). Second, observational data

326   were notably lacking in years with unusually cool springs (i.e., those below the

327   regression line in Fig. 1e). Third, despite broad inter-annual variability for both

328   mean spring and mean annual temperatures (Figs. 1a, 1b), the historical and

329   contemporary observational data represent extreme endpoints in climatic space.

330   Note that the historical data amassed by Thoreau and Hosmer were collected during

331   a relatively cold period, whereas the more contemporary data were collected during

332   a relatively warm period (i.e., Miller-Rushing & Primack, Davis & Connolly) (Figs. 1a,

333   1b). This sampling artifact could bias inference about potential long-term secular

334   trends on phenology. However, the statistical bias of observational records caused

335   by this lack of overlap across the climate data is potentially ameliorated by the

336   herbarium data, which is distributed randomly across the climatic space (Fig. 1e).

337

338   *Herbarium data parallel field observations, but reduce long-term estimates of*

339   *phenological advancement attributed to climate change*–Overall, earliest recorded

340   flowering dates in Middlesex County were negatively associated with mean spring

341   temperatures for all species (i.e., flowering was earlier; Fig. 2; overall slope = –3.8

342   days/°C; $F_{1,696}$ = 96.4, P < 0.001); results were similar for the four nearby counties

343   (Supplemental Online Material, Fig. S2). There was no significant interaction

344   between mean spring temperature and observation type on earliest flowering date

345   ($F_{1,696} = 0.5$, $P = 0.47$), suggesting that that the overall relationship (i.e., slope)

346   between mean spring temperature and earliest flowering date did not differ

347   between field observations and herbarium data.

348   The paired *t*-test comparing the slopes of the species-specific regression lines

349   for observed and herbarium data shown in Fig. 2 found no significant differences

350   ($t_{18} = 0.45$, $P = 0.65$). Although the slopes of observed and herbarium data do not fall

351   on a 1:1 line (Fig. 3), the *y*-intercept of the plot, –2.8 days, suggests that observed

352   first flowering dates are, on average, just under 3 days earlier than estimated

353   earliest flowering date of herbarium specimens. Thus, we were confident (contra

354   CaraDonna, Iler, and Inouye, 2014), that we could fit a common climate model to

355   these data as a whole, combining herbarium data to fill the gaps in the field

356   observational data (black lines in Fig. 2). To minimize effects of outliers, however,

357   we fit this common slope using robust linear models (Venables and Ripley, 2002).

358   Overall, our results support a single previous study that has looked at the

359   fidelity of herbarium records with respect to field observations, but for a greatly

360   reduced number of species and phylogenetic diversity. Robbirt et al. (2011)

361   compared an abundance of field and herbarium data from across Europe for the

362   single terrestrial orchid *Ophrys sphegodes*, and found no significant difference

363   between the two data types for estimates of peak flowering time as a function of

364   spring temperature.

365   Although the response of earliest flowering date was similar both for field

366   and herbarium data, the intercepts differed by 2.8 days. This result should not be

367   surprising because the observational data that we used were collected with the

368   explicit purpose of capturing the earliest flowering day. The natural historians and

369   ecologists who collected these data routinely sampled several, often consecutive

370   days before flowering occurred and consulted local residents and experts to

371   increase the likelihood of identifying first flowering events. In contrast, the

372   collections represented by the herbarium data that we used rarely were made

373   expressly to capture first flowering events, but rather to document interesting,

374   frequently abundant plants in an area at multiple developmental stages (e.g.,

375   flowering, fruiting), usually meant for systematic and floristic research. These

376   samples often were obtained after the time that the first flowers appeared.

377   Furthermore, our more conservative scoring of flowering time for specimens (≥

378   75% open flowers) potentially contributed to the overall later date among

379   herbarium records. Nonetheless, our results provide the first broad validation, for a

380   region in central New England, that herbarium records can be used to address

381   spatial and temporal trends in phenology when and where field observational data

382   are unavailable. These results underscore the enormous promise of leveraging

383   herbarium records for understanding the impacts of climate change in New England,

384   and perhaps more broadly.

385   We also demonstrated that the inter-annual variability in climate covered by

386   the herbarium data fully encompassed and was substantially larger than the range

387   of climate space encompassed by observational data (Fig. 1e). This was true despite

388   the potential biases observed in sampling temporal variability, including episodic

389   field observations, and herbarium specimens collected predominantly before 1960.

390   This result demonstrates for the first time to our knowledge that herbarium records

391   represent key sources of data for filling those parts of the climatic space for which

392   direct field observations are unavailable, and for determining how species

393   dynamically adjust their flowering time to inter-annual temperature variation.

394   Analysis of our combined field and herbarium data suggested an earlier flowering

395   by 3.5 days/°C (Fig. 2), similar to estimates from larger-scale studies that have used

396   herbarium records to assess phenological effects of climatic change. Calinger et al.,

397   (2013), for example, reported an average of change of 2.4 days/°C for flowering in

398   141 species in the Midwestern United States. Similarly, Everill et al., (2014)

399   reported an advancement of leaf out by 2 days/°C for 27 common deciduous woody

400   species in the northeastern United States.

401   Importantly, however, concluding that plants flower earlier following

402   warmer springs (or in warmer years) is not the same as saying that these plants

403   flower earlier now than they did in the 1850s or early 1900s (cf. Miller-Rushing and

404   Primack, 2008; Ellwood et al., 2013). When we regressed earliest flowering date on

405   calendar year, no significant effect was observed ($F_{1,696} = 3.28$, $P = 0.07$; Fig. 4), nor

406   was there an interaction between data type (field observation vs. herbarium) and

407   calendar year ($F_{1,696}$ = 0.01, P = 0.75). Even though both mean spring and mean

408   annual temperatures are clearly rising (Figs. 1a, 1b), inter-annual variation in both

409   spring or annual temperatures (> 7°C) far exceed the long-term trend in

410   temperature (1.5 °C/century): in fact, spring of 2012 was the warmest (8.3 °C) on

411   record, but the spring of 2013 was nearly as cold (5.9 °C) as some of the warmest

412   springs during Hosmer's observations more than a century ago (1898: 5.2 °C; 1903:

413   6.7 °C; Fig. 1b). Phenological events in recent years illustrate this point remarkably

414   well. For example, in 2012, most species flowered early in the year (mean observed

415   first flowering date of all 20 species was 27 April), but in 2013, most species

416   flowered much later (mean observed first flowering date was 23 May). Thus, our

417   findings indicate that researchers should approach long-term phenological

418   assessments using field or observational data with caution given the high degree of

419   inter-annual variability in temperature.

420   There are two likely explanations for the discrepancies in long-term

421   phenological trends we observed between our own results and past studies for New

422   England (Primack et al., 2004; Miller-Rushing et al., 2006; Willis et al., 2008;

423   Panchen et al., 2012). First, historical and contemporary observational data were

424   collected in non-overlapping regions of climatic space (Fig. 1e). Despite pronounced

425   inter-annual variability in annual and spring temperatures (Figs. 1a, 1b), historical

426   field observations were made during relatively cool periods with late springs, while

427   more recent observations have been made during a record-setting warm period

428   with early springs (Fig. 1e). Consequently, the use of field observational data alone

429   is biased towards finding strong shifts in flowering over the last century. As we have

430   indicated above, herbarium data greatly help to alleviate this sampling bias in

431   climatic space. Second, because spring flowering species are thought to be on

432   average more responsive to temperature, other studies of phenological

433   advancement have focused on these species with the premise that they would likely

434   exhibit the greatest long-term response, which indeed they do (Miller-Rushing &

435   Primack, 2008). Our analyses, however, demonstrate that the inclusion of later-

436   flowering species (summer and early fall) results in this long-term trend being non-

437   significant, and thus far less dramatic when the seasonal variation of flowering

438 across the flora is considered (Fig. 5). This is not to say that climatic change has not

439 impacted or will not continue to impact spring ephemeral communities. However,

440 we caution against making long-term phenological predictions based only on short-

441 term trends especially where inter-annual variability is high (regression lines in

442 Figs. 1a, 1b).

443   We obtained qualitatively similar results when we included herbarium data

444 from the four adjacent counties in our analysis (Supplemental Online Material, Fig.

445 S3). However, with the inclusion of additional herbarium data, the interaction term

446 between calendar year and observation type was significant (Supplemental Online

447 Material, Table S2). In other words, not only the intercepts (as in the Middlesex

448 County data alone) but also the slopes of the regression lines relating flowering date

449 to calendar year differed between observational and herbarium data. Although the

450 common slope fitted to each species was essentially flat, the slopes fit to the

451 observational data and the herbarium data were not parallel to one another. This

452 result illustrates that inter-annual temperature variability among locations is

453 substantial and suggests potential limitations in using herbarium data from areas

454 that are not closely co-located with observational data. Specifically, the lack of co-

455 located observational data may lead to potentially spurious interpretation of

456 phenological change across larger areas where only herbarium records are

457 available.

458   Previous studies of the Concord flora have drawn a clear link between short-

459 term phenological sensitivity to temperature and declining abundance (Willis *et al.*,

460 2008). Our revised estimates of phenological sensitivity to inter-annual spring

461 temperature raise questions about one recently hypothesized mechanism driving

462 this decline—phenological mismatch. Bartomeous et al. (2011) found that several

463 common New England insect pollinators were sensitive to spring temperature,

464 advancing their flight times by 3.6 days/°C . This is remarkably similar to our own

465 flowering phenology results of 3.5 days/°C. In contrast, Bartomeous et al. (2011)

466 also found that these same pollinators had advanced their phenology over the last

467 century by ~10 days, which is on par with previous studies of plant phenology in

468 New England (Miller-Rushing and Primack 2008). Their interpretation of these

469   results was that ecological mismatches in plant-pollinator mutualisms were unlikely

470   to explain the decline among plants in the region. Our results, however,

471   demonstrated no significant trend in long-term flowering shifts among New England

472   plants (Figs. 4, S4, Table S2). This indicates that pollinators may be emerging

473   significantly earlier than their plant hosts for all but the most temperature-sensitive

474   plant species. This re-interpretation of Bartomeous et al.'s conclusions reopens the

475   question of the importance of pollinator mismatches to the decline of those less-

476   temperature-sensitive species in New England.

477

478   *Using herbarium records to assess phenological cueing mechanisms*–Finally, we

479   suggest that analysis of herbarium data can be used to identify variability in

480   physiological mechanisms that cue phenological events (Fig. 5). It is clear that

481   species vary in their flowering response to spring temperatures (Figs. 2, 4).

482   Including the random effects term (i.e., the species effect) in the model substantially

483   improved model fit (AIC full model = 5584, AIC model without species = 6902).

484   Regression of observed phenological advancement (days/°C) on the random effects

485   term for each species (i.e., the change in y intercept relative to a common model)

486   revealed several interesting patterns (Fig. 5). First, spring-blooming species have

487   much less variability in their phenological responses to mean spring temperatures

488   than do summer- or fall-blooming species. This suggests that flowering in spring-

489   blooming species (i.e., those that bloom before early June) is strongly controlled by

490   temperature. In contrast, the large variability in response of summer-, and fall-

491   blooming species suggests that flowering in these species is controlled by a variety

492   of different factors, including photoperiod and winter chilling (Körner and Basler,

493   2010). When we included herbarium data from nearby counties in this analysis

494   (Supplementary Online Material, Fig. S4), the variability in response of later-

495   blooming species was somewhat reduced and the relationship between the species-

496   specific random effect size and advancement of flowering time was more

497   pronounced. Nonetheless, the variability in response of later-blooming species still

498   exceeded that of spring-blooming species by more than two-fold.

499   In either case, distinguishing more precisely between different phenological

500   cues, at least at broad scales, might now be possible with the greatly expanded

501   geographic and temporal sampling available from herbarium records. This approach

502   can further guide more focused experiments to establish cues for different species,

503   but even the correlative associations we have identified between climate and

504   phenology are valuable. This is likely to be especially so for species whose cueing

505   mechanisms are simpler and restricted primarily to a single variable. Such data are

506   in great demand, yet are seldom available for a large diversity of species across a

507   region. A recent review of flowering cues by Pau et al., (2011) underscores this

508   demand. Their meta-analysis summarized 115 studies from field observational data.

509   From these studies, they identified sufficient data for only 325 species. While

510   several tropical and boreal species were included, the majority were from

511   temperate regions, primarily in the United States and Western Europe (Pau et al.,

512   2011). Furthermore, all of these studies were restricted to single sites, and thus

513   failed to capture the potential geographic variation within species. These findings

514   greatly emphasize the limited taxonomic and geographic scope of field

515   observational data available for large-scale phenological research (Wolkovich, Cook,

516   and Davies, 2014).

517   In contrast, herbarium data hold great promise for overcoming this impasse

518   and improving assessments of how species will respond to future climate change. In

519   particular, our results could be used in process-based models to distinguish the

520   relative important of temperature, chilling, and photoperiod across a wide diversity

521   of species (Richardson et al., 2006; Morisette et al., 2008; Migliavacca et al., 2012;

522   Archetti et al., 2013; Siniscalco et al., 2014). Previous studies have been limited to a

523   few dozen species with sufficient inter-annual sampling, typically derived from a

524   small number of well-documented, long-term ecological study sites. The reliability

525   of herbarium data, however, offers the promise of greatly expanding these studies to

526   understand how species will respond to recent climatic change and the potential to

527   untangle the relative importance of multiple cues (e.g., photoperiod, temperature)

528   and how they vary across space. Finally, the ability to study a broader diversity of

529 species could greatly expand our knowledge of deeper phylogenetic patterns

530 involving phenological response mechanisms (Davies et al., 2013).

531

532 *Future directions*–Our results indicate that herbarium data represent a valuable

533 resource for studying both temporal trends *and* mechanisms of phenological

534 change. The next challenge is to scale-up our assessments of phenological responses

535 and mechanisms to include the thousands of species on the landscape that are also

536 represented in herbarium collections. These spatially and temporally explicit

537 records of biodiversity are increasingly becoming available digitally as a result of

538 investment in high-throughput digital imaging, GIS, and rigorous spatial analyses.

539 The Harvard University Herbaria, along with several collaborating institutions, are

540 presently enhancing the digital infrastructure for the flora of New England by

541 capturing specimen-level metadata and images into digital form. Alongside this

542 effort, co-authors Davis and Willis have created a crowdsourcing platform ("Curio")

543 with Edith Law (University of Waterloo) to engage volunteer botanists in detecting

544 flowers, buds, and fruits on herbarium records. We intend to use this platform to

545 capture phenological data from the ~1 million digitized specimens from New

546 England and use these data to understand how plants have responded, and will

547 respond, to climatic change in this region. Future studies focused on species that

548 differ in their flowering season and may respond differently to climatic change (e.g.,

549 *Vaccinium angustifolium* versus *Daucus carota*) but have large geographic ranges,

550 are well represented in herbaria, and can be identified easily by amateur botanists,

551 making them especially valuable for these efforts. Moreover, by taking advantage of

552 crowdsourcing, we will be able to assess all of the relevant stages of plant phenology

553 critical to climate change, including leaf-out, transitions from bud to flower, peak

554 flowering time, and transition to fruiting.

555

556

557   **LITERATURE CITED**

558

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698

699   **Table 1**. Summary of study species. Scientific names, common names, native/introduced status, and growth habit from USDA

700   PLANTS (http://plants.usda.gov/java/).

Species	Common Name	Family	Date range	No. of years of data	Native status
Daucus carota L.	Queen Anne's lace	Apiaceae	1853-2008	35	Introduced
Aralia nudicalis L.	wild sarsaparilla	Araliaceae	1858-2012	47	Native
Barbarea vulgaris W.T. Aiton	garden yellowrocket	Brassicaceae	1877-2005	51	Introduced
Gaultheria procumbens L.	eastern teaberry	Ericaceae	1877-2011	32	Native
Gaylussacia baccata (Wangenh.) K. Koch	black huckleberry	Ericaceae	1858-2011	36	Native
Vaccinium angustifolium Aiton	lowbush blueberry	Ericaceae	1878-2012	46	Native
Vicia cracca L.	bird vetch	Fabaceae	1877-2006	21	Introduced
Iris prismatica Pursh ex Ker Gawl.	slender blue iris	Iridaceae	1877-1934	24	Native
Arethusa bulbosa L.	dragon's mouth	Orchidaceae	1861-1980	30	Native
Calopogon tuberosus (L.) Britton, Sterns & Poggenb.	tuberous grasspink	Orchidaceae	1857-1984	19	Native
Corallorhiza maculata (Raf.) Raf.	summer coralroot	Orchidaceae	1854-1930	22	Native
Cypripedium acaule Aiton	moccasin flower	Orchidaceae	1861-2012	51	Native

Platanthera grandiflora (Bigelow) Lindl.	greater purple fringed orchid	Orchidaceae	1861-1960	25	Native
Platanthera lacera (Michx.) G. Don	green fringed orchid	Orchidaceae	1854-1949	39	Native
Platanthera psycodes (L.) Lindl.	lesser purple fringed orchid	Orchidaceae	1854-1958	21	Native
Pogonia ophioglossoides (L.) Ker Gawl.	snakemouth orchid	Orchidaceae	1852-1962	44	Native
Chelidonium majus L.	celandine	Papaveraceae	1877-2011	21	Introduced
Aquilegia canadensis L.	red columbine	Ranunculaceae	1882-2012	39	Native
Ranunculus acris L.	tall buttercup	Ranunculaceae	1858-2011	34	Native/Introduced

701

702 Figure legends

703

704 **Fig. 1.** Climatic and phenological data. **(A)** Mean annual temperatures (°C) and **(B)**

705 mean monthly temperatures recorded at Great Blue Hill, Massachusetts (1885-

706 present) and reconstructed by Miller-Rushing and Primack (1852-1884). **(C)**

707 Observed first flowering dates in Concord, MA and **(D**) earliest flowering dates on

708 herbarium sheets from Middlesex County of the 20 species listed in Table 1. **(E)**

709 Coverage of the climatic space (1852-2013; all boxes) by herbarium data (magenta

710 boxes and magenta convex hull), Thoreau's observations (orange dots and orange

711 convex hull), Hosmer's observations (blue dots and blue convex hull), and

712 contemporary observations (black dots and black convex hull). Unsampled points in

713 the climate space are represented by grey boxes without colored dots. Convex hulls

714 encompass the outer boundaries of the climate space defined by the most extreme

715 observations; they were fit using the "chull" function in R (base graphics). The grey

716 line is the best-fit regression line relating mean spring temperature to mean annual

717 temperature.

718

719 **Fig. 2.** Relationship between mean spring temperature and earliest flowering date

720 from field observations in Concord, MA or recorded on herbarium specimens from

721 Middlesex County, MA. The blue points and lines are data and associated linear

722 regressions for field observations; the red points and lines are data and associated

723 linear regressions for herbarium specimens (fit using linear mixed effect models);

724 and the black lines are common species-specific robust linear regressions.

725

726 **Fig. 3.** Relationship between phenological responses to climate estimated from

727 herbarium specimens and observed in the field. The values on the *x*-axis are the

728 slopes estimated for herbarium specimens (red lines in Fig. 2) and the values on the

729 *y*-axis are the slopes estimated for observational data (blue lines in Fig. 2). The

730 dashed grey line is a 1:1 reference line.
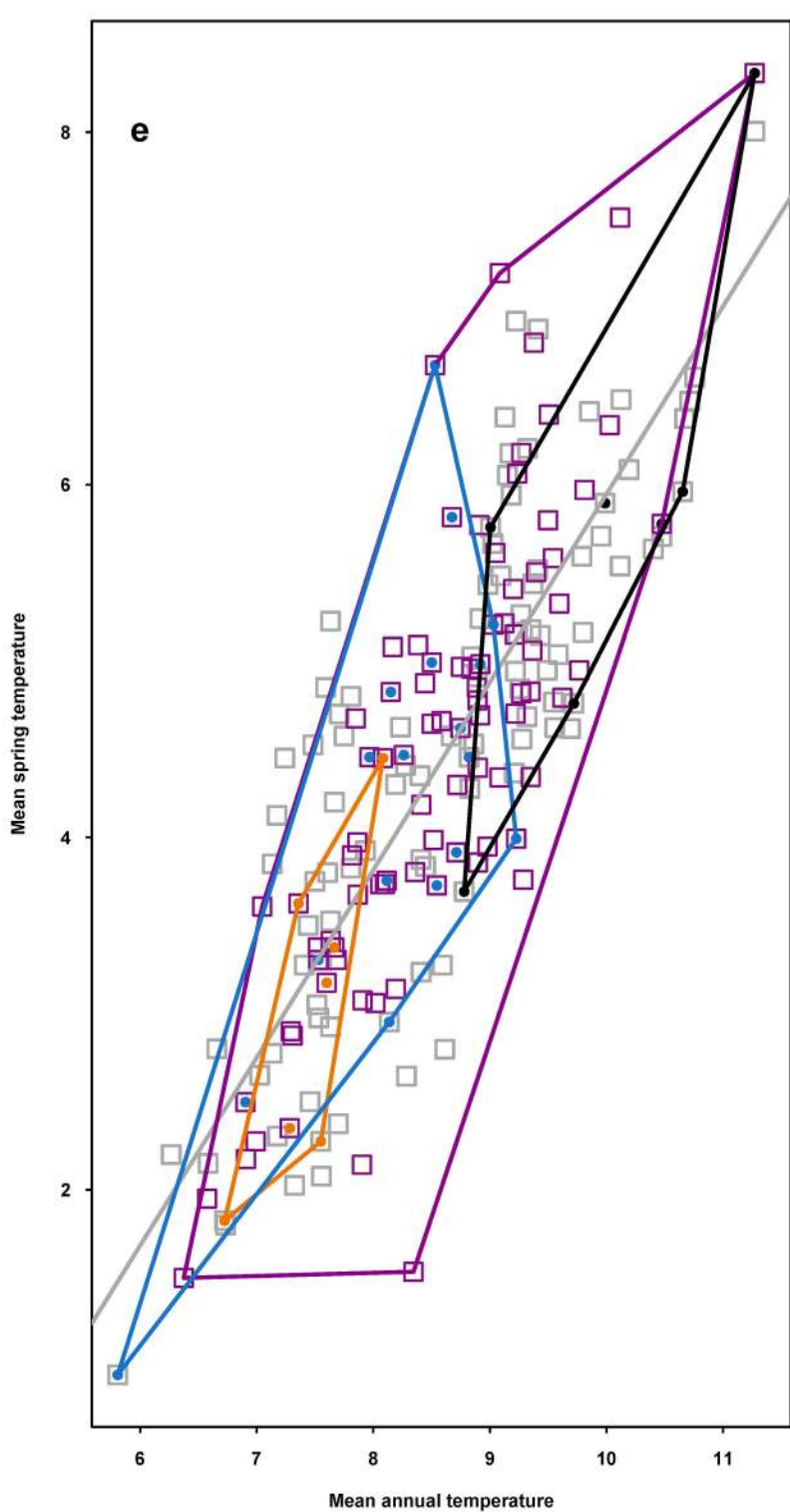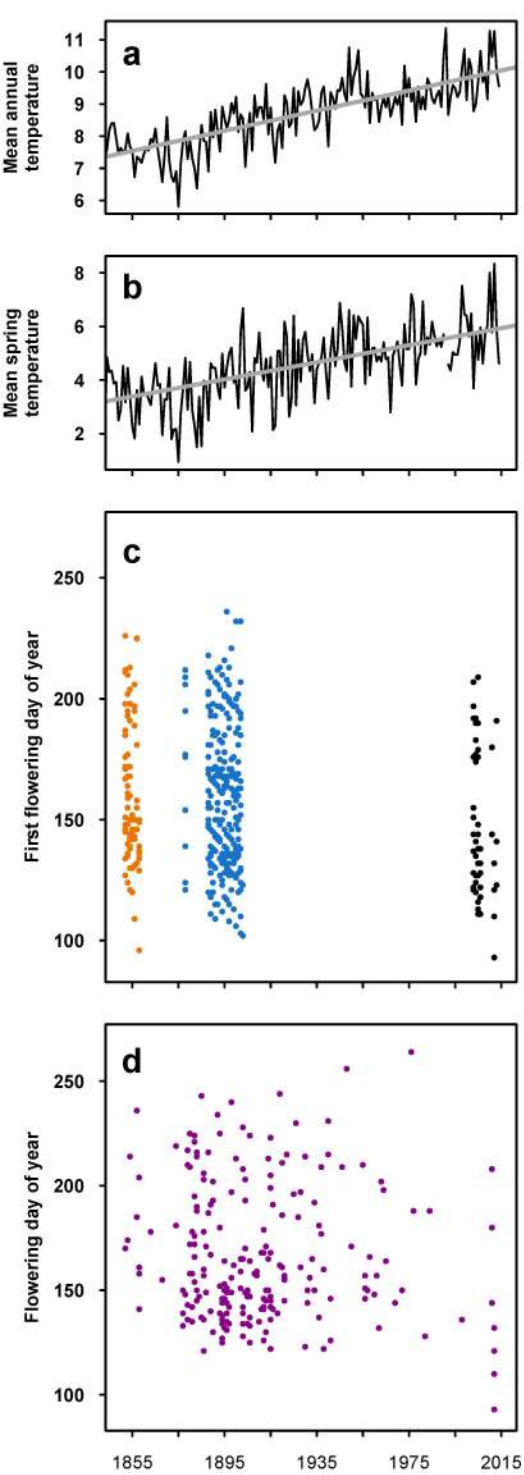
731

732   **Fig. 4.** Relationship between calendar year and earliest flowering date observed in

733   the field or recorded on herbarium specimens from Middlesex County. The blue

734   points and lines are data and associated linear regressions for field observations;

735   the red points and lines are data and associated linear regressions for herbarium

736   specimens; and the black lines are common species-specific robust linear
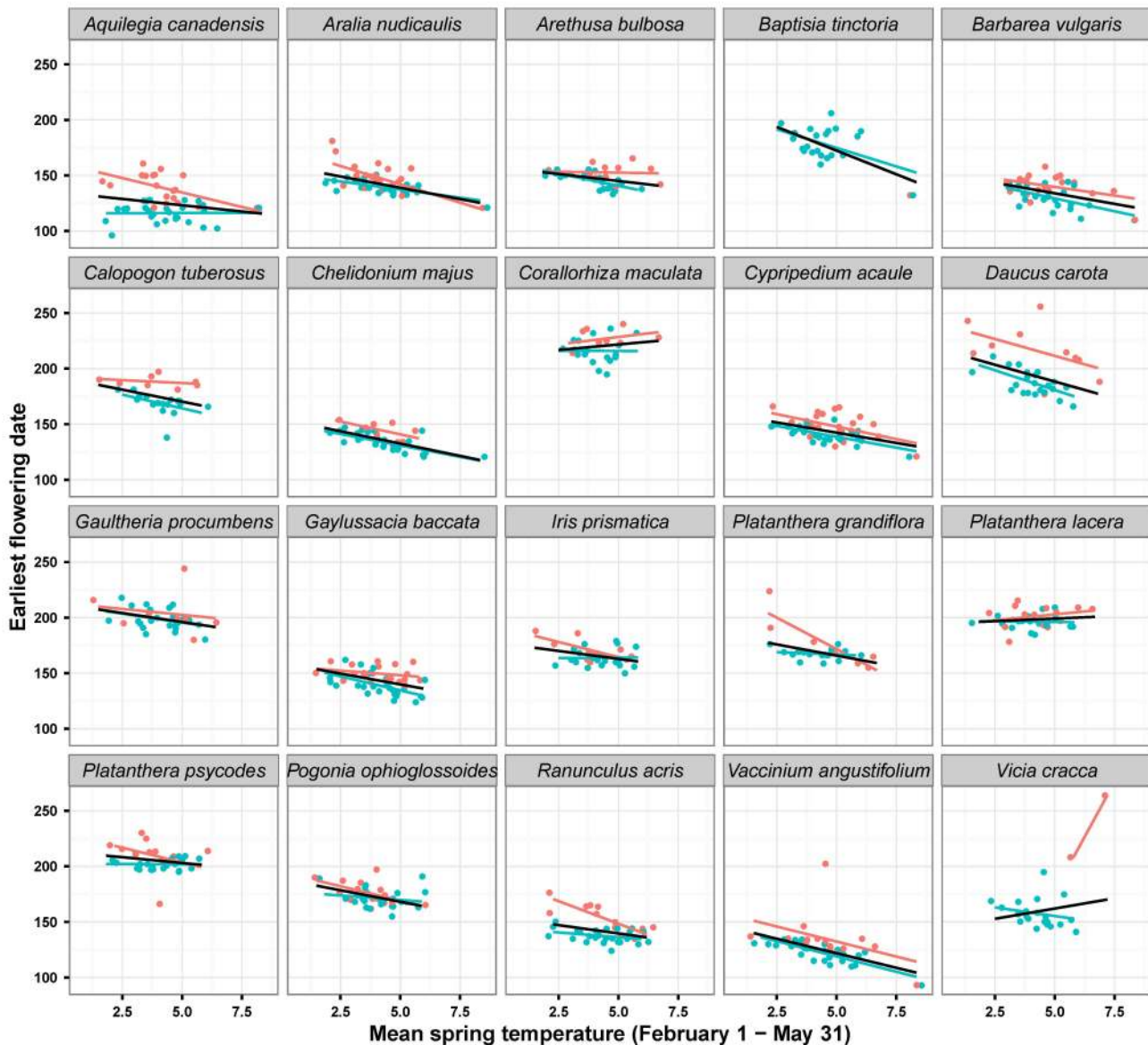
737   regressions.

738

739

740   **Fig. 5.** Relationship between species-specific random effect size and the acceleration

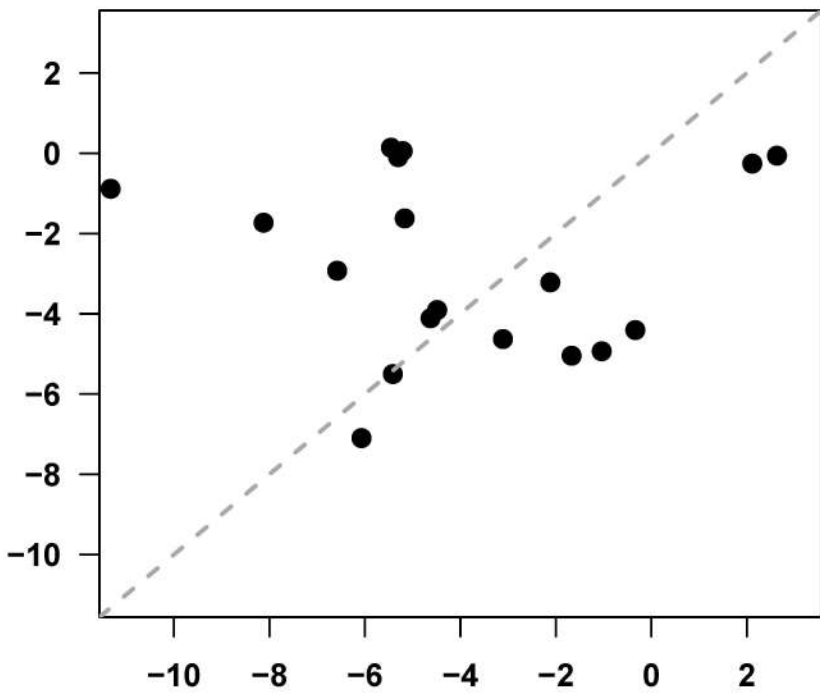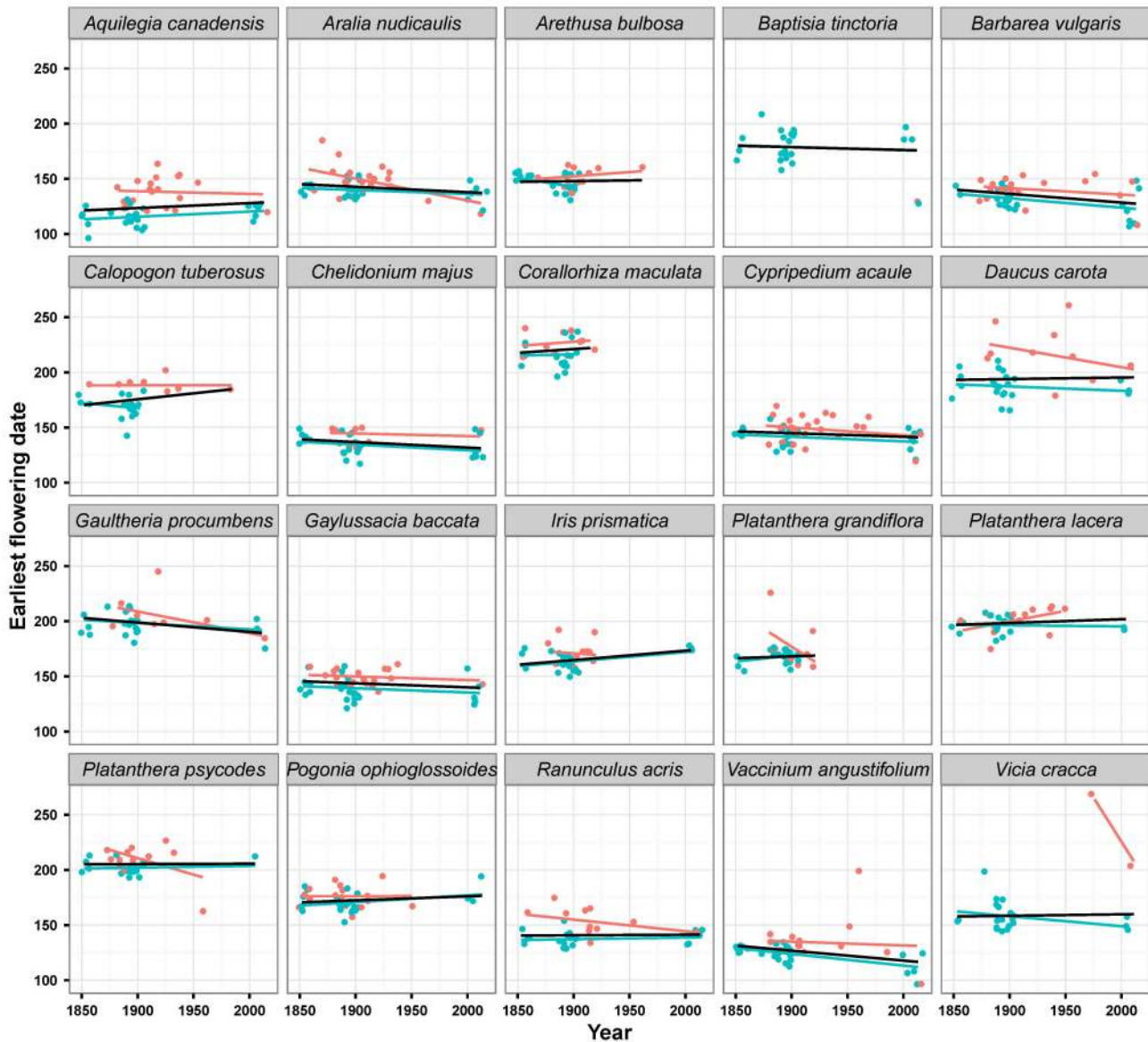741   of flowering (i.e., the values of the common slopes fit in Fig. 2).

742

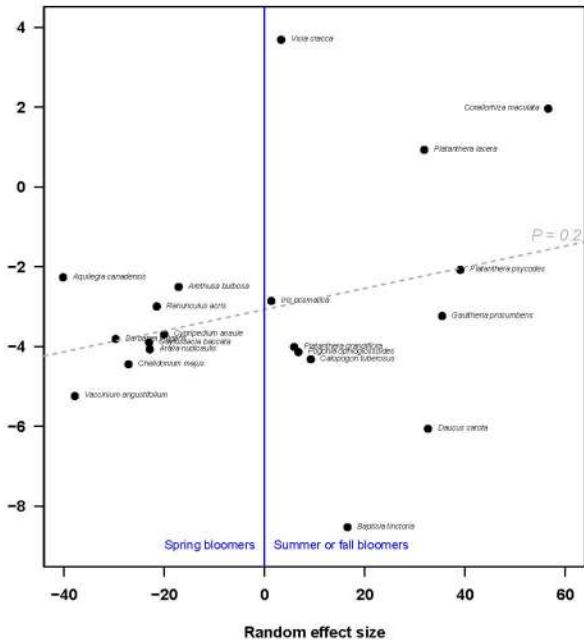Herbarium specimens   Field observations

Earliest flowering date vs. Year for 20 plant species, comparing Herbarium specimens and Field observations.

Aquilegia canadensis · *Aralia nudicaulis* · *Arethusa bulbosa* · *Baptisia tinctoria* · *Barbarea vulgaris* · *Calopogon tuberosus* · *Chelidonium majus* · *Corallorhiza maculata* · *Cypripedium acaule* · *Daucus carota* · *Gaultheria procumbens* · *Gaylussacia baccata* · *Iris prismatica* · *Platanthera grandiflora* · *Platanthera lacera* · *Platanthera psycodes* · *Pogonia ophioglossoides* · *Ranunculus acris* · *Vaccinium angustifolium* · *Vicia cracca*

Herbarium specimens — Field observations

Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms

Charles C. Davis, Charles G. Willis, Bryan Connolly, Courtland Kelly, and Aaron M. Ellison
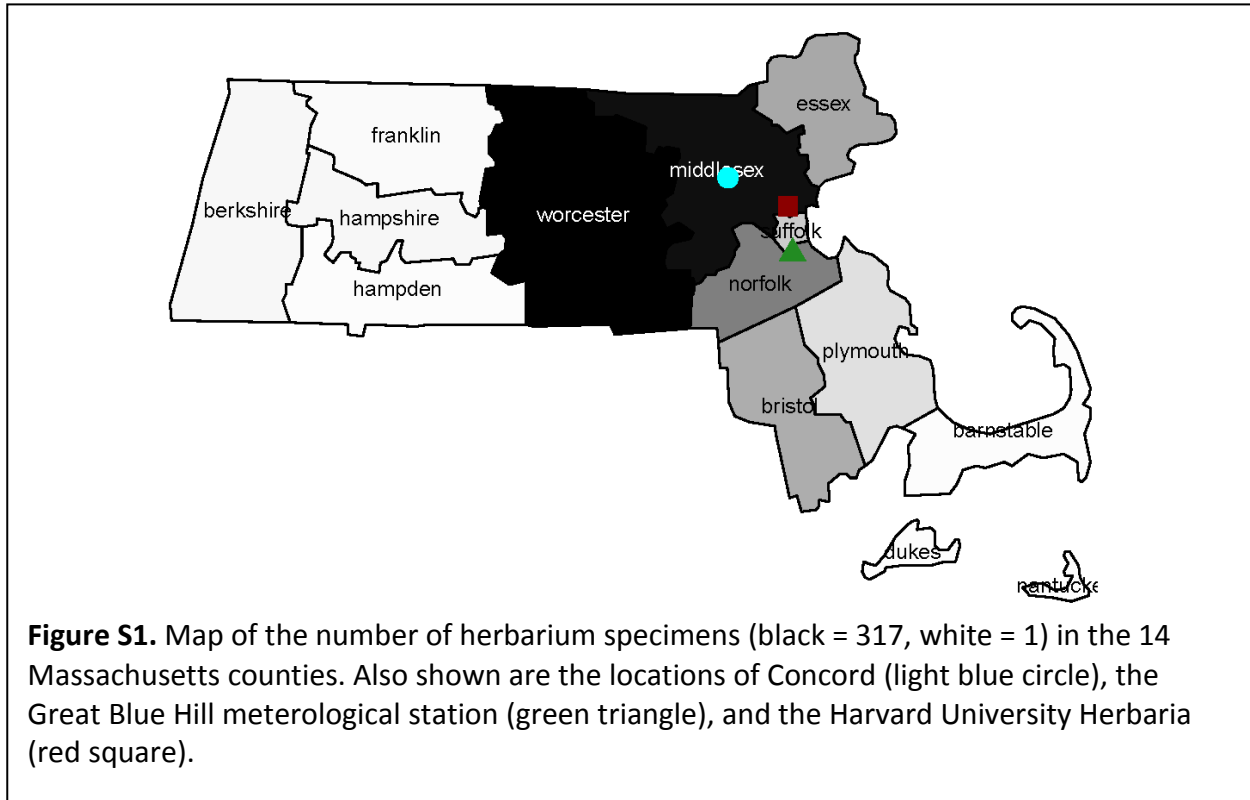
Supplementary Online Information

Table of contents

1. Geographic distribution of herbarium data

Most of the 1103 herbarium specimens of our 20 target species from Massachusetts (1852-2012) were collected from Worcester County (317) and Middlesex County (297) (Fig. S1). Among the 14 Massachusetts counties, only three other counties, Bristol, Essex, and Norfolk, had > 100 herbarium specimens.

Figure S1. Map of the number of herbarium specimens (black = 317, white = 1) in the 14 Massachusetts counties. Also shown are the locations of Concord (light blue circle), the Great Blue Hill meterological station (green triangle), and the Harvard University Herbaria (red square).

2. Climatic effects on phenology from the five counties

In the main text, we present analyses of the effects of mean spring temperatures on plants observed in Concord and collected from Concord and other towns in Middlesex County. Inclusion of data from the other four counties (Worcester, Norfolk, Bristol, and Essex) for which we have reasonable sample sizes yielded similar patterns (Fig. S2) to what we observed for Middlesex County alone (main text, Fig. 2).
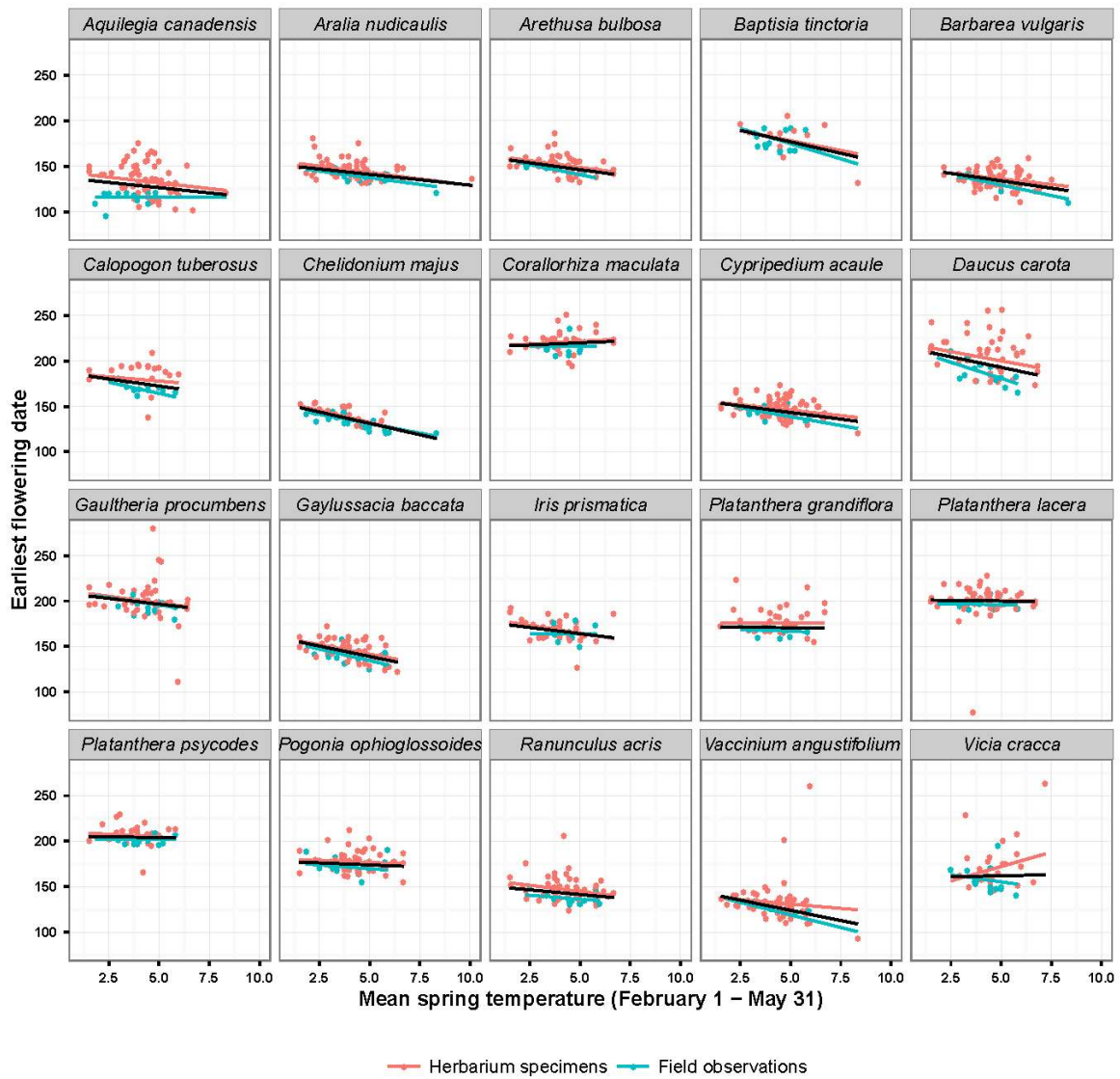
Figure S2. Relationship between mean spring temperature and earliest flowering date observed in the field or recorded on herbarium specimens. The blue points and lines are data and associated linear regressions for field observations; the red points and lines are data and associated linear regressions for herbarium specimens; and the black lines are common species-specific robust linear regressions.

As with the Middlesex County data alone, the main effects (mean spring temperature) and datum type (observed or herbarium) were significant, but their interaction was not (Table S1). Inclusion of the species-specific random effect also improved the fit of the model (AIC full model = 12,761; AIC of reduced model = 15,248).

Table S1. ANOVA table for effects of fixed variables (mean spring temperature) and datum type (herbarium or field observation) and their interaction on earliest flowering date (as day of year) for five Massachusetts counties. The model specification in R was:

```
library(nlme)
model.temps <- lme(Date ~spring.mean.temp*DatumType, random= −1|Species)
```

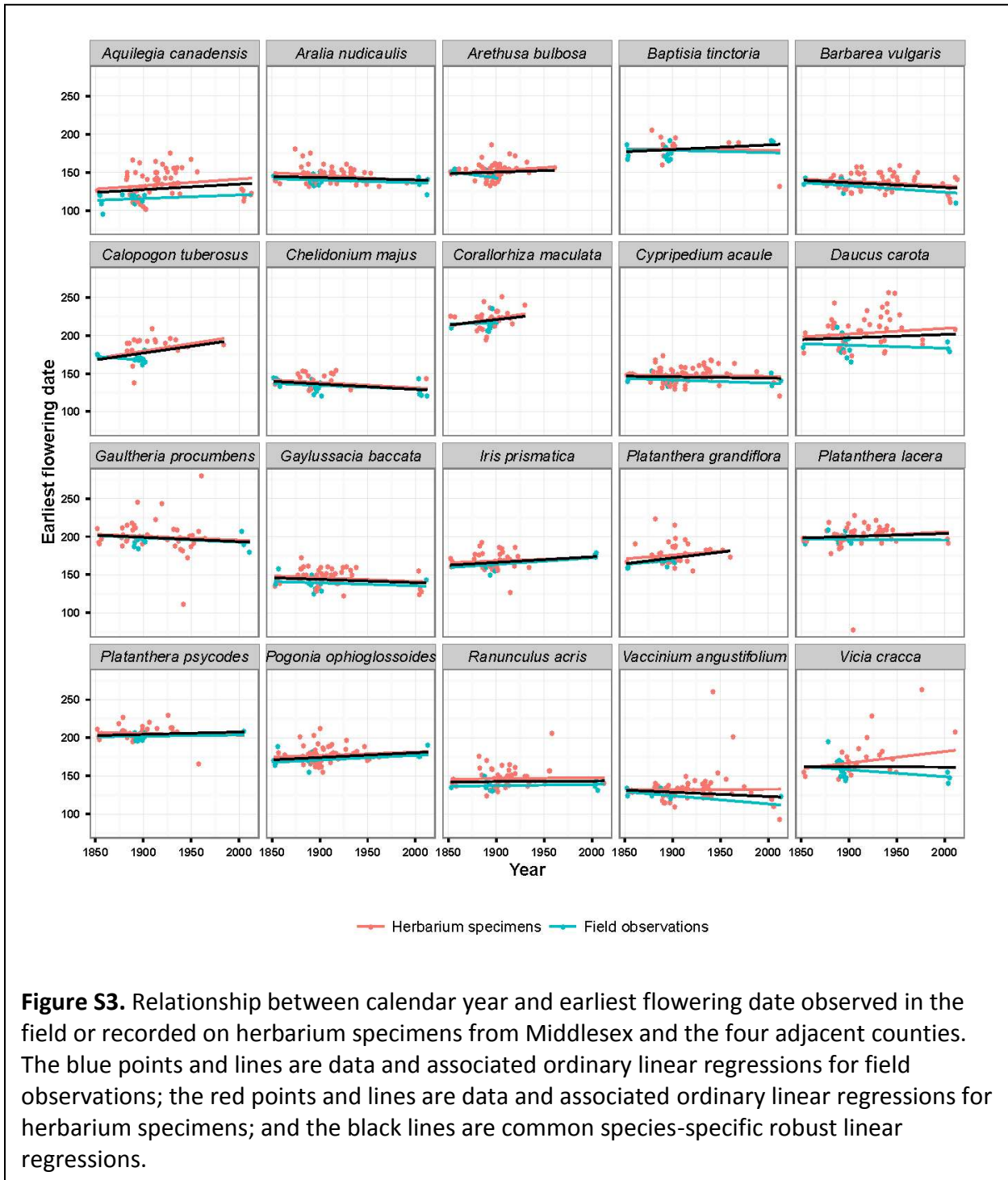|	Estimate	df	F	*P*
Intercept	177.2	1, 1560	712.3	< 0.001
Mean spring temperature	−2.2	1, 1560	78.0	< 0.001
Datum type	−2.8	1, 1560	101.7	< 0.001
Temp × Datum type	−1.6	1, 1560	2.3	0.09

3. Climatic effects are not the same as annual changes

As we pointed out in the main text, concluding that plants flower earlier following warmer springs or in warmer years is not the same as saying that these plants flower earlier now than they did 100 years ago. This can be observed when we regress earliest flowering date on calendar year for either Middlesex County data alone (main text Fig. 4), or for data from all five counties with adequate herbarium specimens (Fig. S3; Table S2).

Table S2 - ANOVA table for effects of fixed variables (year) and datum type (herbarium or field observation) and their interaction on earliest flowering date (as day of year) of plants observed in Concord and collected from Middlesex and the four adjacent counties (Figure S4). The model specification in R was:

```
library(nlme)
model.temps <- lme(Date ~Year*DatumType, random= −1|Species)
```

	Estimate	df	F	*P*
Intercept	146.2	1, 1560	706.5	< 0.001
Year	0.01	1, 1560	0.004	0.95
Datum type	68.9	1, 1560	97.4	< 0.001
Year × Datum type	−0.04	1, 1560	5.23	0.02

Figure S3. Relationship between calendar year and earliest flowering date observed in the field or recorded on herbarium specimens from Middlesex and the four adjacent counties. The blue points and lines are data and associated ordinary linear regressions for field observations; the red points and lines are data and associated ordinary linear regressions for herbarium specimens; and the black lines are common species-specific robust linear regressions.

4. Using herbarium data to identify physiological mechanisms for cueing phenological events

Including the herbarium data from all five counties in analyzing the species-specific relationship of phenological advancement (days/°C) substantially improved the fit of the model (main text Fig. 5). In particular, we observed less variability in estimates of phenological advance of late flowering species (compare main text Fig. 5 with Fig. S4), and the overall relationship between the species-specific random effect size and advancement of flowering was statistically significant ($P = 0.03$). Nonetheless, the amount of variation explained by this regression remained small ($r^2 = 0.23$ for the regression using data from all five counties; $r^2 = 0.07$ for the regression using data only from Middlesex County).

Figure S4. Relationship between species-specific random effect size and the advancement of flowering (common slopes fit in Fig. S2).