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Region-specific phenological sensitivities and rates of climate warming generate divergent temporal shifts in flowering date across a species' range

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Abstract:	Premise of study: Forecasting how species will respond phenologically to future changes in climate is a major challenge. Many studies have focused on estimating species- and community-wide phenological sensitivities to climate to make such predictions, but sensitivities may vary within species, which could result in divergent phenological responses to climate change. Methods: We used 743 herbarium specimens of the mountain jewelflower (Streptanthus tortuosus, Brassicaceae)collected over 112 years to investigate whether individuals sampled from relatively warm vs. cool regions differ in their sensitivity to climate, and whether this has resulted in divergent phenological shifts in response to climate warming. Key results: During the past century, individuals sampled from warm regions exhibited a 20-day advancement in flowering date; individuals in cool regions showed no evidence of a shift. We evaluate two potential drivers of these divergent responses: differences between regions in (1) the degree of phenological sensitivity to climate and (2) the magnitude of climate change experienced by plants, or (3) both. Plants sampled from warm regions exhibit higher sensitivities to temperature-related variables and experienced a greater degree of climate warming than those from cool regions; thus our results suggest that the greater temporal shift in flowering date in warm regions is driven by both of these factors. Conclusions: Our results are among the first to demonstrate that species exhibit intraspecific variation in sensitivity to climate and that this variation can contribute to divergent responses to climate change. Future studies attempting to forecast temporal shifts in phenology should consider intraspecific variation.
Keywords:	Phenological shifts, flowering time, herbarium specimens, climate change, montane, Streptanthus tortuosus, Brassicaceae, California
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	Susan J. Mazer (Conceptualization; Formal analysis; Funding acquisition; Methodology; Writing – review & editing)

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[Last revised 27 October 2020.]

Dr. Pamela Diggle
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May 25, 2021

Dear Dr. Diggle,

Thank you for over-seeing the review of our manuscript titled “Region-specific phenological sensitivities and rates of climate warming generate divergent temporal shifts in flowering date across a species’ range” (AJB-D-21-00008R1). We have carefully addressed all of the Editorial Office Comments regarding formatting and figure size. We would also like to address the Associate Editor’s comment regarding how the uncertainty radius was used in the analysis. We would like to clarify that yes, the uncertainty radius was only used to exclude specimens from the final, cleaned dataset - those specimens with greater than 4,000 meter uncertainty radius were excluded. This is clarified on line 208-209 and it is also included as an exclusion criteria in Appendix S3.

We hope you consider that this revised manuscript merits publication in the *American Journal of Botany.*

We look forward to hearing from you.

Sincerely,
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Region-specific phenological sensitivities and rates of climate warming generate divergent temporal shifts in flowering date across a species' range

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ABSTRACT

Premise of study: Forecasting how species will respond phenologically to future changes in climate is a major challenge. Many studies have focused on estimating species- and community-wide phenological sensitivities to climate to make such predictions, but sensitivities may vary ***within*** species, which could result in divergent phenological responses to climate change.

Methods: We used 743 herbarium specimens of the mountain jewelflower (*Streptanthus tortuosus*, Brassicaceae) collected over 112 years to investigate whether individuals sampled from relatively warm vs. cool regions differ in their sensitivity to climate, and whether this has resulted in divergent phenological shifts in response to climate warming.

Key results: During the past century, individuals sampled from warm regions exhibited a 20-day advancement in flowering date; individuals in cool regions showed no evidence of a shift. We evaluate two potential drivers of these divergent responses: differences between regions in (1) the degree of phenological sensitivity to climate and (2) the magnitude of climate change experienced by plants, or (3) both. Plants sampled from warm regions exhibit higher sensitivities to temperature-related variables ***and*** experienced a greater degree of climate warming than those from cool regions; thus our results suggest that the greater temporal shift in flowering date in warm regions is driven by both of these factors.

Conclusions: Our results are among the first to demonstrate that species exhibit intraspecific variation in sensitivity to climate and that this variation can contribute to divergent responses to climate change. Future studies attempting to forecast temporal shifts in phenology should consider intraspecific variation.

Keywords: Phenological shifts, flowering time, herbarium specimens, climate change, montane, *Streptanthus tortuosus*, Brassicaceae, California

INTRODUCTION

Evaluating changes in plant phenology is a powerful way to assess the impact of climate change on terrestrial ecosystems because climate and phenology are intimately linked (Menzel et al., 2006, 2020; Cleland et al., 2007). Shifts in flowering and fruiting phenology in response to directional climate change during the past century have been reported in many taxa and ecosystems (Chmielewski and Rötzer, 2001; Fitter and Fitter, 2002; Primack et al., 2004; Forrest et al., 2010; Beaubien and Hamann, 2011; CaraDonna et al., 2014). These shifts can have ecosystem-wide consequences by altering or disrupting plant-pollinator interactions (Miller-Rushing et al., 2010; Huang and Hao, 2018; Kehrberger and Holzschuh, 2019; Kudo and Cooper, 2019), and by influencing competitive interactions among co-flowering plants (such as competition for resources such as pollinators; Forrest et al., 2010; CaraDonna et al., 2014).

Forecasting how species will respond to future changes in climate and predicting the ecosystem-wide consequences of these changes are still major challenges (Ibáñez et al., 2010; Pau et al., 2011; Wolkovich et al., 2014). However, several recent discoveries indicate that such predictions eventually may be within reach. For example, estimates of many species' phenological sensitivity to local climate conditions (i.e., the absolute change in the flowering onset date in response to each one-degree increase in temperature) indicate that species differ with respect to how climate influences the timing of their reproduction (Cook et al., 2012; Wolkovich et al., 2012; Park and Mazer, 2018). Generating a sufficient number of regional and site-specific estimates of phenological sensitivity to temperature for a wide range of taxa would improve our ability to predict how the flowering onset dates of entire communities will respond to climate change (Hufft et al., 2018). Similarly, studies of climate-induced phenological mismatches between plants and their pollinators show that mutualistic species interactions can be disrupted by climate change (Kudo and Ida, 2013; Inouye, 2019; Kehrberger and Holzschuh, 2019; but see Iler et al., 2013). Given estimates of the phenological sensitivities of mutualistic (plant-pollinator or plant-disperser) or antagonistic (e.g., plant-herbivore) species pairs or networks, we may be able to forecast the effects of future climatic conditions on these species' associations. Estimating phenological sensitivity within and among taxa is a critical first step towards predicting how future changes in climate will affect the direction and magnitude of phenological shifts and forecasting how these changes may affect ecosystem-wide processes (Menzel et al., 2006; Forrest and Miller-Rushing, 2010; Forrest et al., 2010; Ibáñez et al., 2010; Mazer et al., 2013; Park et al., 2019).

Previous studies investigating phenological sensitivity to climate have largely focused on estimating species-wide sensitivities (Menzel et al., 2006; Cook et al., 2012; Wolkovich et al., 2012; Zhang et al., 2015); however, few studies have been designed to determine whether sensitivities vary ***within*** species (but see Wang et al., 2015; Prevéy et al., 2017; Park et al., 2018; Rafferty et al., 2020; Song et al., 2020) and the consequences of such intraspecific variation in sensitivity to climate are not well understood. Variation in phenological sensitivity within species may be as high as variation in sensitivity among species (as demonstrated by Park et al., 2018), and this could result in unequal shifts in the timing of reproduction across a species' range whereby some populations advance or delay their flowering date more quickly than others (Park et al., 2018; Rafferty et al., 2020). Population-specific responses to climate change could reduce phenological synchrony among populations, altering gene flow patterns as well as disrupting beneficial and antagonistic interspecific relationships (Rafferty et al., 2020). Therefore, determining whether species exhibit intraspecific variability in sensitivity to climate is likely to improve our ability to predict both short- and long-term effects of climate change on the phenology of plant populations and communities. In addition, such studies could help land managers to identify regions within species' ranges that are predicted to experience greater (or lesser) phenological shifts in response to climate change and manage these areas accordingly (Morellato et al., 2016; Olliff- Yang et al., 2020).

Recently, the availability and demonstrated reliability of digitized herbarium records has sparked interest in the use of herbarium-based data to estimate species' phenological sensitivities to a myriad of climate variables including temperature (Robbirt et al., 2011; Davis et al., 2015; Rawal et al., 2015; Park et al., 2018; Daru et al., 2019), the number of frost-free days (Park and Mazer, 2018), precipitation as snow (Park et al., 2018), growing degree days (Mulder et al., 2017; Hufft et al., 2018), and precipitation (Matthews and Mazer, 2016; Love et al., 2019). Given the deep temporal record and broad spatial sampling provided by herbarium records for some of the more well-collected species, these records offer an unprecedented opportunity to explore whether species exhibit intraspecific, regional variation in phenological sensitivity to climate and, if so, whether they exhibit geographic variation in the rate of phenological change over time. In one example, Park et al. (2018) used over 7000 herbarium records representing 30 flowering species distributed across a broad latitudinal range (~30-48°N) in the Eastern United States and found that, within species, populations experiencing chronically warmer conditions at lower latitudes are more sensitive to spring temperature than those experiencing chronically cooler conditions at higher latitudes. Due to these regional differences in sensitivity, Park et al. (2018) predicted that populations in chronically warmer regions of a given species' range will advance their phenology more rapidly than populations in chronically cooler regions in response to large-scale warming.

Despite the potential consequences of intraspecific variation in climate sensitivity, few studies have explicitly assessed whether species exhibit differences in sensitivity among populations (Park et al., 2018; Rafferty et al., 2020; Song et al., 2020), and none have used the spatial breadth and temporal depth represented by herbarium specimens to test whether differences in sensitivity may have already resulted in divergent temporal shifts in response to climate change during the past century. In the current study, we aim to fill this gap and also to test the prediction (derived from Park et al.'s observations) that individuals sampled from relatively warm regions of a species' range exhibit greater phenological advancement in response to historical temperature increases than those sampled from cooler regions. We accomplished this using 743 herbarium records representing a 112-year collection period of the mountain jewelflower (*Streptanthus tortuosus* Kellogg; Brassicaceae), a montane wildflower species that spans a wide geographic and climatic range in California. First, we evaluated whether individuals sampled from sites characterized by chronically warm vs. cool conditions differ with respect to their temporal shifts in phenology during the past century. This analysis detected that individuals sampled from warm regions of this species' range exhibited greater phenological advancement than those sampled from cooler regions. Given this observed difference, we then evaluated the relative importance of two, non-mutually exclusive potential drivers of these divergent temporal responses: (1) regional differences in the degree of phenological sensitivity to climate among individuals sampled from warm vs. cool regions, (2) regional differences in the magnitude of climate change experienced by individuals sampled from warm vs. cool regions during the period of collection, or (3) both. To evaluate the potential role of these mechanisms in driving the observed divergent responses, we used these herbarium records to assess whether sensitivity to climate differs between individuals sampled from warm vs. cool regions, and whether climate change occurred uniformly across chronically warm and cool regions of the mountain jewelflower's range.

METHODS

***Study system*—** The mountain jewelflower (*Streptanthus tortuosus* Kellogg; Brassicaceae) is a forb that is native to the California Floristic Province. The species flowers between early spring and late summer, depending on its location (e.g., high or low elevation). Vernalization during the winter months is required to induce flowering in *S. tortuosus* (Preston, 1991; Gremer et al., 2019). Plant size, flower size and flower color (yellow-white, purple, or intermediate) are variable across its range (Preston, 1994; Baldwin et al., 2012; Love, personal observation). The species is pollinated predominately by bees, but a wide variety of pollinators have been documented visiting flowers, including wasps, flies, butterflies, and beetles. Although *S. tortuosus* is self-compatible, few seeds are set in the absence of effective pollinators (Preston, 1994).

Streptanthus tortuosus has three features that make it a highly suitable species with which to assess intraspecific variation in phenological sensitivity to climate and its potential causes and consequences. First, the species spans a wide variety of climatic conditions in California—from low-elevation, relatively hot and dry foothills to high-elevation, relatively cold and mesic environments (based on its distribution documented in the California Consortium of Herbaria, www.cch2.org, and in the Global Biodiversity Information Facility, www.gbif.org); consequently, we may expect that different environmental cues may induce the timing of cyclical life events in different habitats, generating intraspecific, regional variation in climate sensitivities. Indeed, it has been demonstrated that, among 21 populations of *S. tortuosus* across an elevation gradient, populations differ with respect to the environmental cues that induce seed germination (Gremer et al., 2019) We may similarly expect flowering phenology to respond to distinct environmental cues across climate gradients. Second, *S. tortuosus* is phenologically sensitive to temperature and precipitation (Love et al., 2019); however, it is unknown whether the degree of sensitivity varies within the species. Third, *S. tortuosus* is well represented by herbarium records that provide a spatially and temporally robust dataset with which to detect, if present, regional variation in the magnitude of climate change experienced during the past century and in phenological sensitivity to climate (California Consortium of Herbaria; www.cch2.org).

***Phenological scoring*—**To address our objectives, we assembled 1,322 herbarium records from seven herbaria (CAS, CHSC, DAV, OBI, RSA, SFV, and UCJEPS) that represent the spatial range of *Streptanthus tortuosus* (Appendix S1; see the Supplementary Data with this article). Herbarium specimens from CAS, CHSC, OBI, and SFV were imaged using an ORTECH Photo e- Box Plus 1419 imaging station (ORTECH Professional Lighting, Chula Vista, California, USA) at UC Santa Barbara’s Cheadle Center for Biodiversity and Ecological Restoration. Imaged herbarium specimens from DAV, RSA, and UCJEPS were downloaded from the California Consortium of Herbaria 2 portal (CCH2; www.CCH2.org). Prior to the quantitative phenological scoring of herbarium specimens (described below), we excluded 13 specimens with blurry images, 98 specimens with a recorded collection date that spanned a range greater than three days (e.g., May, 1898 or May 1-15th, 1898), 20 specimens with no reproductive structures, and 53 specimens with highly overlapping reproductive structures, which preclude obtaining an accurate score. Other criteria for excluding specimens are described below.

To score the phenological status of the remaining 1,138 herbarium specimens, we counted the number of buds, flowers, immature, and mature fruits borne by each plant on a given specimen sheet and then used these counts to calculate a quantitative metric of phenological progression – the phenological index (PI) – for each plant according to the phenophase definitions and the ImageJ protocol presented by Love et al. (2019; Equation 1). The PI represents the degree of phenological advancement of each plant on a given herbarium specimen and recording this value enables the statistical control for variation in phenological stage among specimen sheets when included in phenoclimatic models (Love et al., 2019). To calculate the PI, each class of reproductive unit was assigned a specific value from 1–4 that represents its phenological advancement relative to other classes (buds = 1, flowers = 2, immature fruits = 3, and mature fruits = 4). The proportions of reproductive units in each class were then used to calculate a phenological index using the following equation:

Equation 1:

$$Phenological\ index\ (PI)\ =\ \sum_{i\,=\,1}^{4}(P_x)(i)$$

Where *Px* represents the proportion of reproductive units in class *x* and *i* represents the index value associated with that class (e.g., buds have a value of 1). A given plant with a PI of 1 would represent a plant displaying only buds while a plant with a PI of 4 would represent a plant bearing only mature fruits. For example, a plant with four buds, nine flowers, 11 immature fruits, and 43 mature fruits would have a PI of 3.38, which would indicate that the plant is relatively late in its phenological advancement. For herbarium specimens with more than one reproductive plant on a single sheet, the phenological indices of all reproductive plants on a given sheet were averaged to produce a mean PI value for the specimen (Appendix S2).

After scoring, each herbarium specimen was georeferenced by either (1) downloading the latitude and longitude from the California Consortium of Herbaria (www.cch2.org) or (2) by extracting coordinates from the written location description on the specimen label using GeoLocate (www.geo-locate.org). Each set of coordinates was associated with a radius of uncertainty in meters to indicate the precision of the specimen location. We excluded 104 specimens that we were unable to georeference because the location description provided on the label was too general (e.g., Yosemite National Park), 94 specimens with an uncertainty radius greater than 4,000 meters, and 164 duplicate specimen records. A summary of the exclusion criteria and number of specimens excluded with each criterion can be found in Appendix S3.

The collection date of each remaining specimen was converted into a day of year of collection (DOY) from 1 to 365. The resulting dataset included 776 herbarium specimens collected between July 4th, 1863 and August 9th, 2013 and represented the geographic range of *S. tortuosus* well (Fig. 1).

Climate data**—**We evaluated phenological sensitivity to a set of climate variables that have been found to be important for predicting flowering onset in other taxa (Cleland et al., 2007; Anderson et al., 2012; Park and Mazer, 2018). Climatic conditions (both long-term mean climate and climate during the year of specimen collection) at the collection location of each specimen were characterized by extracting site-specific climate data from Climate NA, a climate data source that downscales gridded PRISM data to scale-free point locations (Wang, Hamann, et al., 2016). To characterize the temperature during the year in which each specimen was collected (YOC), we extracted the site- and year-specific minimum (Tmin), maximum (Tmax), and mean (Tave) temperatures during winter (average between December and February, when buds are developing) and spring/summer (average between March and August, when flowers are opening and being pollinated). We focused on winter temperatures because *S. tortuosus* requires vernalization to induce flowering (Preston, 1991; Gremer et al., 2019), and thus we might expect it to be sensitive to winter temperatures. We also focused on the spring/summer temperatures because the onset of flowering for this species occurs between March and August, so plants may use temperature as a cue during this period to induce the onset of flowering.

To characterize the onset and length of the growing season during the YOC, we downloaded the day of year of onset of the frost-free period (bFFP) and the number of frost-free days (NFFD) at each specimen collection location (Table 1). To characterize precipitation during the YOC, we downloaded the cumulative annual precipitation as snow (PAS) and the cumulative annual precipitation (MAP, which includes PAS; Table 1). The earliest historical climate data available through ClimateNA is 1901, thus those specimens collected before 1901 (33 specimens) were excluded from the final dataset (Appendix S3). The site-specific YOC data were extracted for 743 specimens with collection dates ranging from May 17th, 1902 to August 9th, 2013. To characterize the long-term mean or chronic temperature conditions at each collection site, we extracted the long-term mean (90-year; 1901-1990) of mean annual temperature (MAT).

Delimiting cool vs. warm regions—To delimit which specimens were collected in chronically cool vs. chronically warm regions of the mountain jewelflower's range, we first used the distribution of long-term mean MAT among all collection sites to calculate ten quantiles (i.e., deciles). Then, in order to create a more explicit comparison between cool vs. warm regions, the central two deciles (deciles 5 and 6) were removed from the dataset (n = 150, Appendix S4). Below, we refer to the dataset from which these two deciles were removed as the "reduced data set" (n = 593). Specimens representing the lower four deciles (1-4) were considered to be collected from chronically cool regions (n = 299) while the upper four deciles (7-10) were considered to be collected from chronically warm regions (n = 294; Appendix S4). The cool vs. warm regions were defined by the chronic conditions at each given specimen collection site (Fig. 1); however, these regions align well with geographic subregions within the borders of California (Jepson Flora Project, 2021). In this study, warm regions correspond geographically to the Klamath and North Coast Ranges, and the Sierra Nevada Foothills. Cool regions correspond to the High Sierra Nevada. The mean DOY between regions was compared using a Welch two-sample t-test in R version 3.6.1 (R Core Team, 2020).

Detecting temporal shifts in phenology— To assess phenological shifts during the 112-year collection period (1902-2013), we constructed two multiple linear regressions designed to detect the effect of collection year on DOY. The first model was designed to detect a shift among all specimens pooled (n = 743) and included the phenological index (PI), year of collection, and geographic variables (elevation, latitude, and longitude) as predictor variables (Equation 2).

Equation 2:

$$\text{DOY} = b_0 + b_1(\text{Phenological Index}) + b_2(\text{Year of collection}) + b_3(\text{Latitude}) + b_4(\text{Longitude}) + b_5(\text{Elevation}) + \varepsilon$$

The second model was designed to determine whether the magnitude and/or direction of phenological shifts depends on the long-term mean climatic conditions to which collection sites were exposed (i.e., cool vs. warm regions). This model was constructed using the reduced dataset and included DOY as the response variable and the PI, year of collection, geographic variables, temperature region (cool or warm), and the year x temperature region interaction as predictors. The geographic variables were included in order to control for the possibility that non-random sampling across the collection period could confound the detection of the temporal shifts in phenology (Equation 3).

Equation 3:

$$\text{DOY} = b_0 + b_1(\text{Phenological Index}) + b_2(\text{Year of Collection}) + b_3(\text{Latitude}) + b_4(\text{Longitude}) + b_5(\text{Elevation}) + b_6(\text{Temperature Region}) + b_7(\text{Year of Collection}) \times (\text{Temperature Region}) + \varepsilon$$

Estimating region-specific sensitivities to climate—To determine whether individuals sampled from cool vs. warm regions collectively differ in their estimated sensitivities to climate (i.e., the absolute change in the DOY in response to each one-unit increase in the climate variable), we constructed 11 multiple linear regressions (one for each climate variable listed in

Table 2) designed to detect the effect of climate during the YOC on DOY (Equation 4). Each model included DOY as the response variable, while the temperature region (cool vs. warm), the PI (to control for variation in phenological stage among specimens), and one of the 11 climate variables of interest were included as the predictors. In addition, the models designed to estimate sensitivity to temperature, bFFP, or NFFD included the YOC MAP as an independent variable; these models therefore controlled for variation in MAP when detecting the sensitivity of DOY to temperature-based variables. Similarly, the models designed to estimate the sensitivity to MAP or PAS included YOC MAT as an independent variable. The two-way interaction between temperature region (cool vs. warm) and the YOC climate variable of interest was also included to determine whether sensitivity of DOY to the climate variable differed between regions (Equation 4).

Equation 4:

$$\text{DOY} = b_0 + b_1(\text{Temperature Region}) + b_2(\text{Phenological Index}) + b_3(\text{Climate Variable}_{\text{YOC}}) +$$
$$b_4(\text{MAP}_{\text{YOC}} \text{ or } \text{MAT}_{\text{YOC}}) + b_5(\text{Temperature Region}) \times (\text{Climate Variable}_{\text{YOC}}) + \varepsilon$$

Estimating region-specific changes in climate through time—To determine whether the magnitude of climate change experienced by individuals sampled from cool vs. warm regions differed during the 112-year collection period, we constructed 11 multiple linear regressions (one for each climate variable listed in Table 2) designed to detect the effect of specimen collection year on the YOC climate at a given specimen’s collection location (Equation 5). Each model included the climate variable of interest as the response variable, the temperature region (cool vs. warm), the YOC, the geographic variables (latitude, longitude, and elevation), and the YOC

MAP (for models estimating changes in temperature, bFFP, or NFFD) or YOC MAT (for models estimating changes in MAP or PAS) as main effects. The geographic variables were included in order to control for the possibility that spatially non-random sampling across the collection period could confound the detection of the direct relationship between climate and year. The YOC x temperature region (cool vs. warm) interaction was also included in the model to determine whether the estimated magnitude of climate change over the 112-year collection period differed between regions (Equation 5).

Equation 5:

$$\text{Climate Variable}_{\text{YOC}} = b_0 + b_1(\text{Temperature Region}) + b_2(\text{Year of Collection}) + b_3(\text{Latitude}) + b_4(\text{Longitude}) + b_5(\text{Elevation}) + b_6(\text{MAP}_{\text{YOC}} \text{ or } \text{MAT}_{\text{YOC}}) + b_7(\text{Temperature Region}) \times (\text{Year of Collection}) + \varepsilon$$

We chose to seek evidence of region-specific temporal phenological shifts, sensitivity to climate, and climate change by assessing these effects in discrete warm vs. cool regions rather than by assessing the interaction between long-term mean MAT (as a continuous variable) and year or seasonal temperature because long-term mean MAT is highly correlated with some of the 11 climatic variables of interest (e.g., correlation with winter Tmin: r=0.95; with spring/summer Tmin: r=0.97) and with some of the geographic variables (e.g., correlation with elevation: r=-0.95). To avoid difficulties in interpreting the output of multiple regressions when there is multicollinearity among predictor variables, and to maintain consistency and comparability among all models, we chose to assess region-specific phenological responses in warm vs. cool regions.

All multiple linear regressions conducted for this study were constructed using OLS regression and type III sum of squares with the *lm()* function and *car* package in R (Fox and Weisberg, 2019). All analyses were performed in R version 3.6.1 (R Core Team, 2020).

Relative importance of regional differences in sensitivity vs. differences in the magnitude of climate change**—**To evaluate the relative importance of regional differences in sensitivity vs. regional differences in the magnitude of climate change in driving the observed differences in the phenological advancement through time between regions, we calculated the ratio between estimates of both climate change and sensitivity to climate in cool vs. warm regions. If, for example, the ratio between the magnitudes of climate change in warm vs. cool regions (e.g., Δ winter Tmin in warm regions/Δ winter Tmin in cool region) was consistently higher than the ratio between the sensitivity to climate variables in warm vs. cool regions (e.g., warm region sensitivity to winter Tmin/cool region sensitivity to winter Tmin), then we may infer that regional differences in the magnitude of climate change are more important than regional differences in sensitivity in explaining the observed difference between regions in temporal phenological shifts. Conversely, if the ratio between sensitivities in warm vs. cool regions was higher than that between the magnitudes of climate change in cool vs. warm regions, then differences in the degree of sensitivity may be more important in driving observed differences in temporal phenological shifts.

RESULTS

Our dataset spanned a 112-year collection period from 1902-2013 (Appendix S5). The mean DOY among all specimens (n = 743) was 182 (July 1st; SD=35.04, range: 76-256; Appendix S6). Specimens sampled from cool regions were, on average, collected 50 days later than those sampled from warm regions (95% CI: 45.04-54.96 days, t = 22.23, P < 0.001; Fig. 2). The mean DOY among specimens sampled from cool regions (n = 299; 90-year MAT range: -0.5-6.46°C) was 205 (July 24th; SD = 23.59, range: 148-256) and from warm regions (n = 294; 90-year MAT range: 9.46-16.9°C) was 155 (June 4th; SD = 31.12, range: 76-222; Fig. 2).

Temporal shifts in flowering date**—**Among all specimens (n = 743), we detected a 10-day advancement in flowering DOY over the past 100 years (estimate = -0.10±0.03 days/year, t = -3.75, df = 1, P<0.001) independent of phenological status and geographic location (Table 1a; Fig. 3a). This temporal shift, however, differed significantly between warm and cool regions. We detected a 19-day advancement in flowering date among specimens sampled from warm regions (estimate = -0.19±0.04 days/year, t = -4.61, df = 1, P < 0.001), but we found no evidence of a temporal shift among specimens sampled from cool regions (t = 0.29, df = 1, P = 0.77; Table 1b; Fig. 3b). The model designed to detect a temporal shift in flowering DOY among all specimens explained 70% of the variance in DOY while the model designed to detect temporal shifts in warm vs. cool regions using the reduced dataset accounted for 74% of the variance in DOY (Table 1).

Region-specific sensitivity to climate—Increased annual and seasonal temperatures advanced flowering DOY among all specimens in the reduced dataset, but those sampled from warm regions were more sensitive than those from cold regions to all of the temperature-related climate variables (MAT; winter Tmin, Tave, Tmax; and spring/summer Tmin, Tave, Tmax) tested in this study independent of geographic location and phenological status (Table 2). For example, among specimens sampled from warm regions, flowering DOY advanced 5.84±0.47 days per 1°C increase in winter Tmin while among specimens sampled from cool regions, flowering DOY advanced only 2.91±0.49 days per 1°C increase in winter Tmin (temperature region x winter Tmin: $t = 4.33$, $df = 1$, $P < 0.001$; Fig. 4a; Table 2b; Appendix S7). Similarly, among specimens sampled from warm regions, flowering DOY advanced 7.08±0.50 days per 1°C increase in the mean minimum temperature during spring and summer while, among specimens sampled from cool regions, flowering DOY advanced only 3.64±0.57 days per 1°C increase in spring/summer Tmin (temperature region x spring/summer Tmin: $t = 4.46$, $df = 1$, $P < 0.001$; Fig. 4b; Table 2b; Appendix S7). The seven models designed to estimate the sensitivity of *S. tortuosus* to temperature-related variables in cool vs. warm regions explained 72-77% of the variance in DOY (Appendix S7).

Similarly, specimens sampled from warm regions were more sensitive to the onset of the growing season (as estimated by the bFFP) and the length of the growing season (as estimated by the NFFD). Earlier onset and longer growing seasons advanced flowering DOY among all specimens in the reduced dataset, but these effects were strongest among specimens sampled from warm regions (Table 2). Among specimens sampled from warm regions, a one-day advance in the onset of the growing season advanced flowering DOY by 0.52±0.04 days while in cool regions a one-day advance in the onset of the growing season advanced flowering DOY by only 0.27±0.07 days (temperature region x bFFP: t = -3.27, df = 1, P=0.001; Fig. 3c; Table 2b; Appendix S7). The models designed to estimate the sensitivity of flowering DOY to the bFFP and NFFD explained 73% and 72% of the variance in DOY, respectively (Appendix S7).

Among all specimens in the reduced dataset, increased precipitation (as measured by MAP and PAS) delayed flowering DOY. The two-way interaction between MAP and temperature region was not significant, indicating that the effect of MAP on flowering DOY did not significantly differ between cool and warm regions (temperature region x MAP: t = 1.81, df = 1, P = 0.07; Table 2b; Appendix S7). In both cool and warm regions, flowering DOY was delayed 0.92 days per 100-millimeter increase in MAP (MAP: estimate = 0.0092 days/mm; t = 7.21, df=1, P < 0.001; Table 2b; Appendix S7). Specimens sampled from warm regions were more sensitive to PAS than those from cool regions (warm: estimate = 0.14±0.02 days/mm; cool: estimate = 0.016±0.003 days/mm; temperature region x PAS: t = -5.82, df = 1, P < 0.001; Table 2b; Appendix S7). Both models designed to estimate the sensitivity of flowering DOY to MAP and PAS explained 75% of the variance in DOY (Appendix S7).

Region-specific changes in climate through time—Among temperature-related variables, we detected long-term temporal increases in MAT, winter Tmin, winter Tave, winter Tmax, spring/summer Tmin, and spring/summer Tave independent of geographic location and MAP during the YOC (Table 2a; Appendix S8). Warm regions experienced a greater degree of temporal change in winter and spring/summer minimum temperatures than cool regions. During the past century, winter minimum temperatures increased an estimated 2.3±0.41°C in warm regions vs. 0.93±0.42°C in cool regions (temperature region x year: t = -2.35, df = 1, P = 0.019;

Fig. 4d; Table 2a; Appendix S8). Similarly, spring/summer minimum temperatures increased an estimated 3.19±0.35°C in warm regions vs. 1.89±0.36°C in cool regions during the past century (temperature region x year: t = -2.57, df = 1, P = 0.01; Fig. 4e; Table 2a; Appendix S8). The degree of temporal change in MAT, winter Tmax, winter Tave, and spring/summer Tave was similar between cool and warm regions (i.e., the year x temperature region interaction was not significant; Table 2a). We detected no evidence that the maximum temperature during the spring/summer growing season had changed in either temperature region (t = 1.52, df = 1, P = 0.13; Table 2a; Appendix S8). The seven models designed to detect changes in annual and seasonal temperature among sampled locations during the past century explained 84-93% of the variance in temperature (Appendix S8).

Similarly, we detected long-term temporal changes in the day of year of onset of the growing season (as estimated by bFFP) and the length of the growing season (as estimated by NFFD) independent of geography and MAP in the YOC. In the case of both variables, the degree of change experienced by specimens sampled from warm regions was greater than that experienced by specimens sampled from cool regions. The beginning of the frost-free period has advanced an estimated 32.98±4.48 days in warm regions vs. 12.78±4.55 days in cool regions during the past century (temperature region x year: t = 3.16, df = 1, P = 0.0017; Fig. 4f; Table 2a; Appendix S8). The growing season has lengthened by an estimated 54.42±6.86 days in warm regions vs. 31.47±6.96 days in cool regions during the past century (temperature region x year: t = -2.35, df = 1, P = 0.019; Table 2a; Appendix S8).

We detected a long-term mean increase in MAP in warm regions but no evidence of a change in MAP in cool regions during the past century (Table 2). Specimens sampled from warm regions experienced an increase of 428±118 mm of cumulative MAP during the past 100 years while those sampled from cool regions experienced no significant change in MAP (temperature region x year: t = -3.87, df = 1, P<0.001; Table 2a; Appendix S8). We detected a decrease of 146±47 mm in PAS in cool regions (temperature region x year: t = -2.92, df = 1, P = 0.004; Table 2a; Appendix S8), but no significant change in warm regions during the past century. The models designed to detect temporal changes in precipitation independent of geography and MAT in the YOC explained 30% of the variance in MAP and 90% of the variance in PAS among all sampled locations during the 112-year collection period (Appendix S8).

Relative importance of regional differences in sensitivity vs. regional differences in the magnitude of climate change**—** Cool and warm regions differ with respect to *both* the magnitude of climate change experienced by individuals as well as estimated phenological sensitivity to climate among individuals (Figure 4, Table 2). With respect to minimum temperatures, the bFFP, the NFFD, and MAP, individuals sampled from warm regions have experienced a greater degree of change and also exhibit a higher degree of phenological sensitivity to these variables (Table 2). For example, relative to cool regions, warm regions have experienced a 2.47x and 1.68x greater increase in winter Tmin and spring/summer Tmin, respectively, and individuals sampled from warm regions are 2.01x and 1.94x more sensitive to winter Tmin and spring/summer Tmin, respectively, than those sampled from cool regions (Figure 4a, 4b, 4d, and 4e). Similarly, warm regions have experienced a 2.58x greater advancement in the bFFP and individuals sampled from warm regions are 1.93x more sensitive to the bFFP than those sampled from cool regions (Fig. 4c, Fig. 4f).

DISCUSSION

This study demonstrates that, based on data derived from herbarium specimens collected across a species' range, the degree of phenological sensitivity to climate and the magnitude of phenological shifts through time in response to climate change can vary within species. During the 112-year collection period examined here, flowering date advanced significantly among individuals of *Streptanthus tortuosus* collected in relatively warm regions but exhibited no evidence of advancement among those collected in cool regions. The evidence presented here suggests that the difference in temporal shifts in flowering date between cool and warm regions is likely driven by *both* (1) intraspecific variation in phenological sensitivity to climate variables and (2) differences in the magnitude of climate change experienced by individuals and populations in cool vs. warm regions. These results and their implications are discussed in detail below.

Regional differences in phenological advancement—The results presented here add to the mounting evidence that plants have responded to climate change (specifically, increases in temperature) by advancing their flowering date (Parmesan and Yohe, 2003; Cleland et al., 2007; but see Banaszak et al., 2020); however, this is one of the few studies to detect intra-specific variation in the magnitude of temporal shifts within a wide-ranging species (Prevéy et al., 2017; Rafferty et al., 2020). Among all individuals of *S. tortuosus* sampled in this study, flowering date advanced 10 days during the past century, but this pattern was driven by the advancement of individuals collected in warm regions, which exhibit a 20-day advancement in flowering date

(Fig. 3, Table 1). Individuals collected in relatively cool regions exhibited no evidence of advancement in flowering date (Fig. 3b, Table 1b).

Biases inherent in herbarium-based phenological data may influence estimates of phenological shifts through time (Lavoie, 2013; Daru et al., 2018). For example, specimens are collected non-randomly through space and time; therefore, it is possible that temporal shifts in phenology detected in herbarium-based studies reflect changes in sampling locations through time rather than true responses to climate change. For example, if more recent collections represent specimens in warmer locations where flowering occurs earlier, then this would result in an advancement in collection date through time that may be wrongfully interpreted as an advancement in phenology. In addition, reproductive specimens may be collected at any phenological stage between budding and fruiting, limiting our ability to reliably use the DOY as an estimate of flowering date (Love et al., 2019). Moreover, this variation could potentially introduce noise or bias into models estimating phenological shifts. In this study, we addressed these potential biases by controlling for both collection location and phenological stage (as estimated by the phenological index) in models designed to estimate phenological shifts, providing us with greater confidence in our estimates of phenological shifts through time.

By seeking evidence for intraspecific, regional variation in phenological advancement, studies may detect non-uniform shifts in phenology across species' ranges; and such divergent phenologies may have several ecological consequences that merit investigation. First, intraspecific differences in phenological advancement among populations may reduce flowering synchrony and pollen-mediated gene flow, thereby affecting processes such as local adaptation or population divergence (Ison et al., 2014; Wang, Tang, et al., 2016; Rafferty et al., 2020).

Second, non-uniform shifts in phenology may alter population- or region-specific interactions with other organisms (e.g., pollinators, herbivores, competitors; Elzinga et al., 2007; Kudo and Ida, 2013). Third, divergent shifts in phenology may expose some populations to novel conditions (e.g., increased exposure to frost or drought; Franks et al., 2007; Inouye, 2008) or to novel interactions (e.g., plant-plant, plant-herbivore, plant-pollinator interactions; Fabina et al., 2010; Forrest et al., 2010; Theobald et al., 2017). Given these consequences and their potential impact on plant survival, fitness, and population persistence (Fabina et al., 2010; Forrest and Miller-Rushing, 2010; Anderson et al., 2012; Springate and Kover, 2014), assessing the spatial complexity of temporal phenological shifts will help us to forecast the ecological consequences of climate change.

Regional differences in phenological sensitivity to climate—Higher temperature during the year of specimen collection advances flowering date (DOY) in both cool and warm regions; however, relative to individuals sampled from cool regions, individuals in warm regions were about twice as phenologically sensitive to all of the temperature and growing season length variables (i.e., bFFP and NFFD) examined in this study (Fig. 4, Table 2). In addition, individuals in warm regions flower earlier than those in cool regions (Fig. 2). This intraspecific pattern – where individuals in relatively warm regions both flower earlier and are more phenologically sensitive to temperature – is consistent with the few other studies that have investigated regional variation in temperature sensitivity within species (Menzel et al., 2006; Wang et al., 2015; Park et al., 2018; Song et al., 2020). Moreover, the intraspecific pattern detected here is consistent with the interspecific patterns of temperature sensitivity estimated from many other herbarium-based and field-based studies (i.e., *species* or *communities* that flower earlier and/or occur in warm climates are more sensitive to temperature than those that flower later and/or occur in cooler climates; Menzel et al., 2006; Rutishauser et al., 2009; Cook et al., 2012; Wolkovich et al., 2012; Park et al., 2018). The consistent patterns at various taxonomic and ecological scales suggest that the underlying drivers may be similar and could reflect variation in phenological sensitivity due to differences in life-history strategies (Kudoh et al., 1995; Caffarra and Donnelly, 2011; Li et al., 2014), differences in the reliability of temperature cues (Lapenis et al., 2014; Park et al., 2018), or differences in the abiotic drivers of selection on plant phenology (Theobald et al., 2017).

Contrary to the intraspecific pattern detected in this study, where individuals in warm regions are more sensitive to temperature than those in cool regions, Prevéy et al. (2017) found that, among 47 plant species occurring in the Arctic tundra, conspecific populations occupying relatively cool regions at higher latitudes were more sensitive to temperature than those occupying warm regions at lower latitudes. These contrasting patterns suggest that spatial variation in temperature sensitivity may differ among biomes (Ernakovich et al., 2014; Carbognani et al., 2018). However, despite detecting a pattern that contrasts with the findings presented here, Prevéy et al. (2017) also found that interspecific and intraspecific patterns in temperature sensitivity matched – species, as well as conspecific populations, in warmer regions were less sensitive to temperature than those in cooler regions, again suggesting that drivers underlying inter vs. intraspecific variation in phenological sensitivity may be similar.

Our study highlights the importance of considering intraspecific variation when estimating temperature sensitivity, especially when using these estimates to predict a species’ phenological responses to future climate change (Forrest and Miller-Rushing, 2010; Pau et al., 2011; Wolkovich et al., 2014). For example, if a species' sensitivity is estimated by assessing its response to interannual variation in climate at a single (or even a few, spatially close) study site(s), it may not accurately capture the spatial complexity of temperature sensitivity across that species' range. Predictions derived from these estimates could potentially over- or underestimate temporal phenological shifts, and consequently, could result in inaccurate predictions regarding the impact of climate change on species and their interactions with other organisms.

Temporal shifts in phenology are explained by sensitivity to climate and the magnitude of climate change in warm regions—Because of their spatial and temporal sampling breadth, herbarium-based data are uniquely suited to test how well estimated phenological sensitivities explain temporal shifts in phenology that have already occurred in response to climate change during the past century. For example, in the present study, minimum temperature during the flowering period (spring/summer) of *S. tortuosus* in warm regions has increased an estimated 3.2±0.3°C during the past century and, based on the sensitivity of flowering time to spring/summer Tmin exhibited by individuals in warm regions, we would predict the increase in spring/summer Tmin to result in a 18.60-26.83 day advancement of DOY among individuals in warm regions over the past century (Fig. 4b, Fig. 4e, Table 2). This predicted phenological shift is similar to the observed 19.0±3.0 day advancement among individuals in warm regions during the past century that was detected in this study. In addition to spring/summer Tmin, the predicted temporal advancement of DOY in warm regions in response to increases in winter Tmin (10.15-17.1 days) and advances in the onset of the bFFP (13.68-20.98 days) are also similar to the observed advancement (Table 2). The 428±118 mm increase in MAP in warm regions during the past century is predicted to delay flowering date by 2.85-5.02 days and may have slightly counteracted the advance caused by increasing temperatures and advancing bFFP.

Given that (1) individuals in cool regions are sensitive to temperature and other temperature-related variables (e.g., bFFP and NFFD), and that (2) the temperature has changed during the 112-year observation period – why are we unable to detect any temporal shift in phenology among individuals sampled from cool regions? One possibility is that individuals in cool vs. warm parts of species ranges may rely on different cues to induce flowering. For example, many alpine wildflowers are phenologically sensitive to the date of snowmelt (Totland and Alatalo, 2002; Kudo and Hirao, 2006; Inouye, 2008; Carbognani et al., 2018). Because individuals of *S. tortuosus* in cool regions primarily occur in high elevation, alpine environments, the date of snowmelt may be an important cue to induce flowering (Fig. 1). While we did not test for the direct effects of snowmelt date on DOY in this study, it is likely strongly correlated with the bFFP (the date on which temperatures are consistently above 0°C). In cool regions, the predicted temporal advancement of flowering date based on the sensitivity of individuals to the bFFP in response to the 12.8±4.3 day advancement in the bFFP during the past century is 1.7-5.8 days (Table 2). Given that the standard error of the estimated temporal shift in flowering date is ±3 days, the slight advancement of flowering date predicted by the advance in the bFFP may be too small to detect due to background variation in flowering date in response to interannual variation in climate (Fig. 3b, Table 1b). Moreover, compared to specimens collected in warm regions, those collected in cool regions are underrepresented in more recent years, when the effects of warming may have been expressed more strongly (due to accelerating climate change), and this may have hindered our ability to detect a significant phenological shift (Appendix S5).

We found that the greater temporal advancement of flowering date among specimens in warm regions vs. those collected in cool regions is explained by both (1) a higher sensitivity to phenologically important climate variables and (2) a greater increase in temperature (especially minimum temperatures), and consequently, a greater advance in the onset of spring (as measured by the bFFP) experienced by individuals collected in warm regions (Fig. 3b, Fig. 4, Table 2). This is one of the first studies to demonstrate that differences in both sensitivity and the degree of climate change experienced among populations contribute to regional differences in the magnitude of temporal shifts in phenology (Prevéy et al., 2017). Given these results, future studies using models to predict changes in phenology and species interactions should consider both differences in the degree of sensitivity to climate and the expected magnitude of climate change when forecasting impacts (Cleland et al., 2007; Forrest and Miller-Rushing, 2010; Pau et al., 2011). Additionally, by hindcasting temporal shifts in phenology using estimated sensitivities to various climate variables, we can assess the predictive capacity of these sensitivities and thus may be able to improve the accuracy of predictions regarding future shifts in phenology in response to continuing climate change.

Relative importance of regional differences in sensitivity vs. the magnitude of climate change— One of the goals of this study was to evaluate which of the potential drivers – regional differences in the magnitude of climate change vs. regional differences in the degree of sensitivity to climate – may be more important in explaining differences in the observed phenological advancement through time between the regions. We found that, among the five climate variables (winter Tmin, spring/summer Tmin, bFFP, and NFFD) that differed between regions with respect to both the magnitude of change in that variable during the past century and the degree of phenological sensitivity to that variable, warmer regions exhibited about twice the magnitude of climate change as cool regions, and the plants sampled from warm regions were approximately twice as sensitive as those sampled from cool regions (Table 2). For example, spring/summer Tmin increased 1.68 times as fast in warm regions than cool regions, and individuals in warm regions are about 1.94 times as sensitive to spring/summer Tmin (Table 2) as individuals in cool regions. Moreover, neither the ratio between the regional magnitudes of climate change nor between regional degrees of sensitivity was consistently higher than the other (Table 2). Based on the evidence presented here, the two factors may be similarly important in driving divergent phenological shifts during the past century. To our knowledge, this is the first study to assess the relative importance of the magnitude of climate change vs. the degree of phenological sensitivity in driving temporal shifts in phenology and this comparative framework may be useful to assess the relative importance of these two factors for the many species that are well represented by herbarium specimens.

CONCLUSION

This is one of the few studies to demonstrate that herbarium-based data can be used to detect regionally-distinct phenological patterns when incorporating fine-scale phenological scoring (Park et al., 2018; Song et al., 2020). The results presented here directly support the prediction, first introduced by Park et al. (2018), that, collectively, plants sampled from regions that exhibit higher sensitivity to temperature will advance their phenology more rapidly in response to warming than those sampled from regions which collectively exhibit lower sensitivity to temperature. Furthermore, we found that this divergent response was also driven by regional differences in the magnitude of climate change. Our findings highlight the need to measure **both** phenological sensitivity to climate and the magnitude of climate change experienced over a given time period when trying to explain intra- or interspecific variation in the magnitude of phenological change in response to directional changes in climate. Our study also adds to the mounting evidence that herbarium records are useful sources of phenological data (Davis et al., 2015; Willis et al., 2017; Jones and Daehler, 2018), and reinforces the need to preserve these valuable natural history collections while also expanding our capacity to extract high-quality and meaningful phenological data from imaged specimens (e.g., through using machine learning methods; Blagoderov et al., 2012; Lorieul et al., 2019; Goëau et al., 2020; Pearson et al., 2020).

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AUTHOR CONTRIBUTIONS

N.L.L.R and S.J.M. both conceived of this project. N.L.L.R led the effort to collect and analyze the data. Both N.L.L.R and S.J.M. contributed to writing the paper

DATA AVAILABILITY

The full (n = 743) and reduced (n = 593) datasets are available at https://doi.org/10.5281/zenodo.4299389 and the R scripts used to construct all models conducted here can be found at https://github.com/natalielrlove/region-specific-pheno-responses.git

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of this article

Appendix S1. Numbers of specimens from each herbarium included in the final dataset and in the reduced dataset with the middle 20% of the temperature range of *S. tortuosus* removed (n = 150 specimens) that was used for cool vs. warm region comparisons.

Appendix S2. The distribution of phenological indices (PIs) among (a) sites of collection of all specimens (n = 743) and (b) sites where specimens were collected in cool (shown in blue; n = 299) and warm (shown in red; n = 294) regions.

Appendix S3. Criteria for excluding herbarium specimens from the final dataset and the number of specimens removed based on each criterion.

Appendix S4. The distribution of mean long-term (90-year) mean annual temperatures (MAT) among (a) sites of collection of all specimens (n = 743) and (b) sites where specimens were collected in cool (shown in blue; n = 299) and warm (shown in red; n = 294) regions.

Appendix S5. The distribution of specimen collection years among (a) sites of collection of all specimens (n = 743) and (b) sites where specimens were collected in cool (shown in blue; n = 299) and warm (shown in red; n = 294) regions.

Appendix S6. The distribution of the day of year of specimen collection (DOY) among (a) sites of collection of all specimens (n = 743) and (b) sites where specimens were collected in cool (shown in blue; n = 299) and warm (shown in red; n = 294) regions.

Appendix S7. Parameter estimates and summary statistics for the multiple linear regressions conducted to detect the effect of each climate variable of interest on the specimen day of year of collection (DOY) in cool vs. warm regions while controlling for the phenological index and the mean annual precipitation (MAP) or mean annual temperature (MAT) among specimens in the reduced dataset (n=593).

Appendix S8. Parameter estimates and summary statistics for the multiple linear regressions conducted to detect the effect of the year on the each climate variables of interest in cool vs. warm regions (year x temperature region) while controlling for geographic variables and the mean annual precipitation (MAP) or mean annual temperature (MAT) among specimens in the reduced dataset (n=593).

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TABLES

Table 1. Summary of the linear regression conducted to detect the effect of collection year on the day of year of collection (DOY) while controlling for geographic variables (latitude, longitude, and elevation) among (a) all specimens (n = 743) and (b) among specimens in the reduced dataset (n = 593) to compare shifts in cool (n = 299) vs. warm (n =294) regions.

a.

Independent Variable	**Estimate**	**SE**	***t* ratio**	***P* >|t|**
Intercept	216.89	127.57	1.70	0.09
Phenological index	16.88	1.02	16.57	**<0.001**
Year	-0.10	0.027	-3.75	**<0.001**
Elevation	0.029	0.0010	27.94	**<0.001**
Latitude	5.02	1.07	4.68	**<0.001**
Longitude	1.02	1.22	0.84	0.40

Source of Variation	**Df**	**SS**	**F Ratio**	***P* value**
Intercept	1	1073	2.89	0.09
Phenological index	1	101899	274.45	**<0.001**
Year	1	5219	14.06	**<0.001**
Elevation	1	289774	780.45	**<0.001**
Latitude	1	8121	21.87	**<0.001**
Longitude	1	259	0.70	0.40
Error	737	273642		
R^2				0.70

b.

Term	**Estimate**	**SE**	***t* ratio**	***P* >|t|**
Intercept	-116.07	133.99	-0.87	0.39
Phenological index	17.33	1.13	15.35	**<0.001**
Year	-0.090	0.030	-2.99	**0.0029**
Temperature region [C]	1.06	1.78	0.59	0.55
Elevation	0.029	0.0017	17.17	**<0.001**

Latitude	4.31	1.28	3.37	<**0.001**
Longitude	-0.33	1.46	-0.23	0.82
Year x temperature region [C]	0.10	0.030	3.43	<**0.001**

Analysis of Variance Source	**Df**	**SS**	**F Ratio**	***P* value**
Intercept	1	270	0.75	0.39
Phenological index	1	84698	235.56	<**0.001**
Year	1	3224	8.97	**0.0029**
Temperature region	1	127	0.35	0.55
Elevation	1	105952	294.68	<**0.001**
Latitude	1	4087	11.37	<**0.001**
Longitude	1	19	0.05	0.82
Year x temperature region	1	4226	11.75	<**0.001**
Error	585	210340		
R^2				0.74

944

Table 2. Regression coefficients (a) representing the estimated change in the climate variables during the 112-year collection period in cool vs. warm regions independent of model covariates (latitude, longitude, elevation, and cumulative mean annual precipitation (MAP) or mean annual temperature (MAT)), and (b) the estimated sensitivity of *S. tortuosus* to each climate variable in cool versus warm regions independent of model covariates (cumulative mean annual precipitation and the phenological index). When the two-way interaction between the climate variable and temperature region (cool vs. warm) is significant at **α** = 0.95 (indicating that the estimates differ between cool vs. warm regions), an estimate for each region is reported. When the interaction is not significant, a single estimate for both regions is reported. The ratio of the warm to cool region coefficient estimate is listed when the coefficient differs significantly between regions. Full model summaries for estimating the magnitude of climate change during the past century and the estimated sensitivities to climate are presented in Appendix S7 and S8, respectively.

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

	a. Estimated change during the past century			**b. Sensitivity to climate**		
Climate variable	**Cool**	**Warm**	**Warm:Cool Ratio**	**Cool**	**Warm**	**Warm:Cool Ratio**
MAT (°C)	1.34±0.20***			-4.46±0.58***	-8.31±0.51***	1.86
Winter Tmin (°C)	0.93±0.42*	2.3±0.41***	2.47	-2.91±0.49***	-5.84±0.47***	2.01
Winter Tmax (°C)	1.15±0.27***			-2.91±0.53***	-5.97±0.44***	2.05
Winter Tave (°C)	1.38±0.26***			-3.55±0.56***	-6.56±0.47***	1.84
Spring/summer Tmin (°C)	1.89±0.36***	3.19±0.35***	1.68	-3.64±0.57***	-7.08±0.50***	1.94
Spring/summer Tmax (°C)	NS			-4.02±0.44***	-6.22±0.40***	1.55
Spring/summer Tave (°C)	1.47±0.22***			-4.42±0.51***	-7.85±0.46***	1.78
bFFP (days)	-12.78±4.55***	-32.98±4.48***	2.58	0.27±0.07***	0.52±0.04***	1.93
NFFD (days)	31.47±6.96***	54.52±6.86***	1.73	-0.16±0.04***	-0.32±0.02***	2.00
MAP (mm)	NS	428±118***		0.0092±0.0013***		
PAS (mm)	-145.87**	NS		0.016±0.0036***	0.142±0.022***	8.88

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$

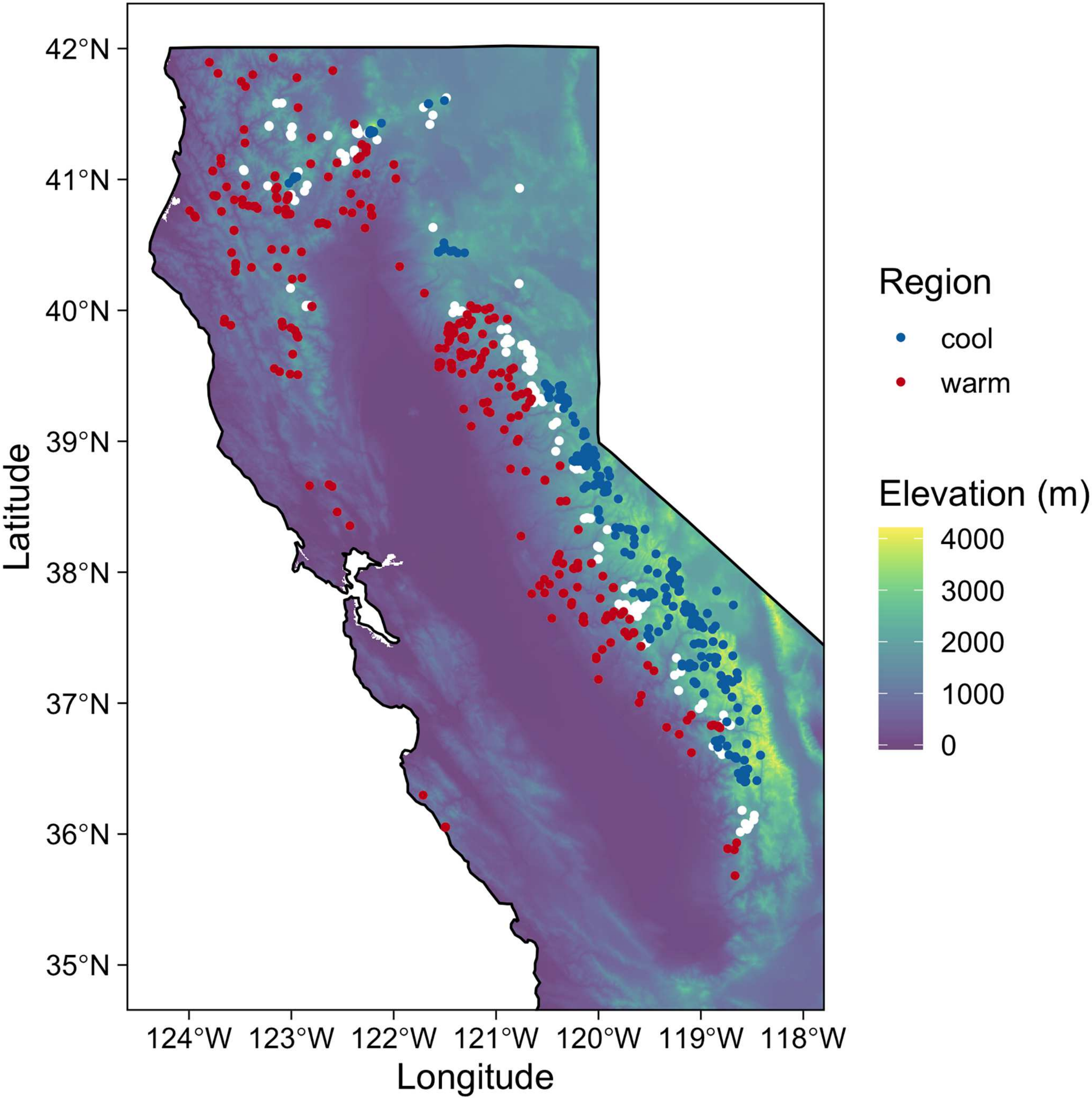
FIGURE CAPTIONS

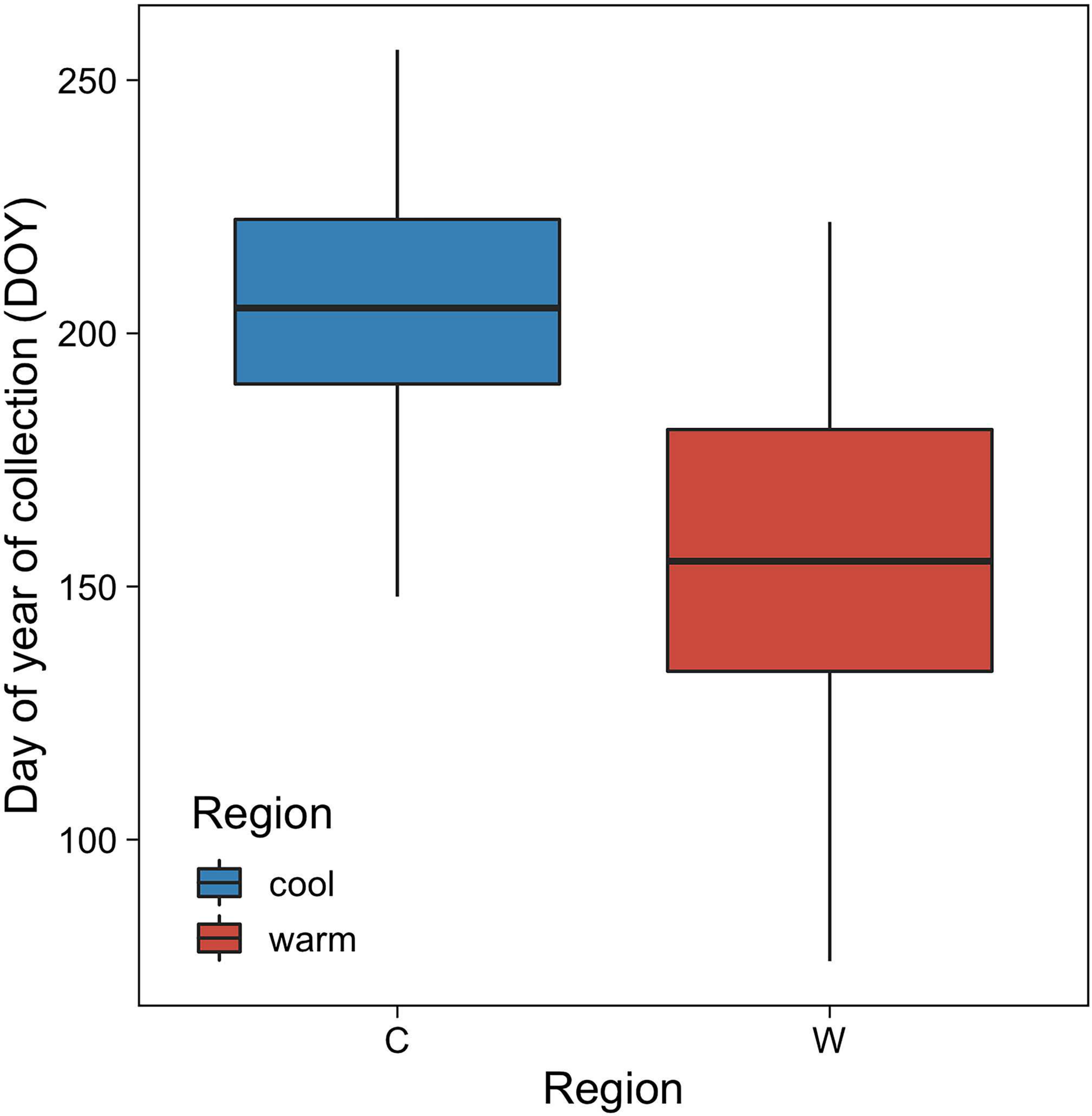
Figure 1. Locations of *Streptanthus tortuosus* specimens collected in cool regions (blue circles; n = 299) and warm regions (red circles; n = 294) of the species' range. The white circles are those records for which the collection site represents the middle 20% of the temperature range of *S. tortuosus* (n = 150). These records were removed prior to the warm vs. cool region comparisons.

Figure 2. Box and whisker plots representing the distribution of the day of year (DOY) of specimens collected in cool (blue) and warm (red) regions. The horizontal line within each box represents the median and the lower and upper hinges correspond to the first and third quartiles, respectively. The lower and upper whiskers extend to the smallest and largest values of DOY.

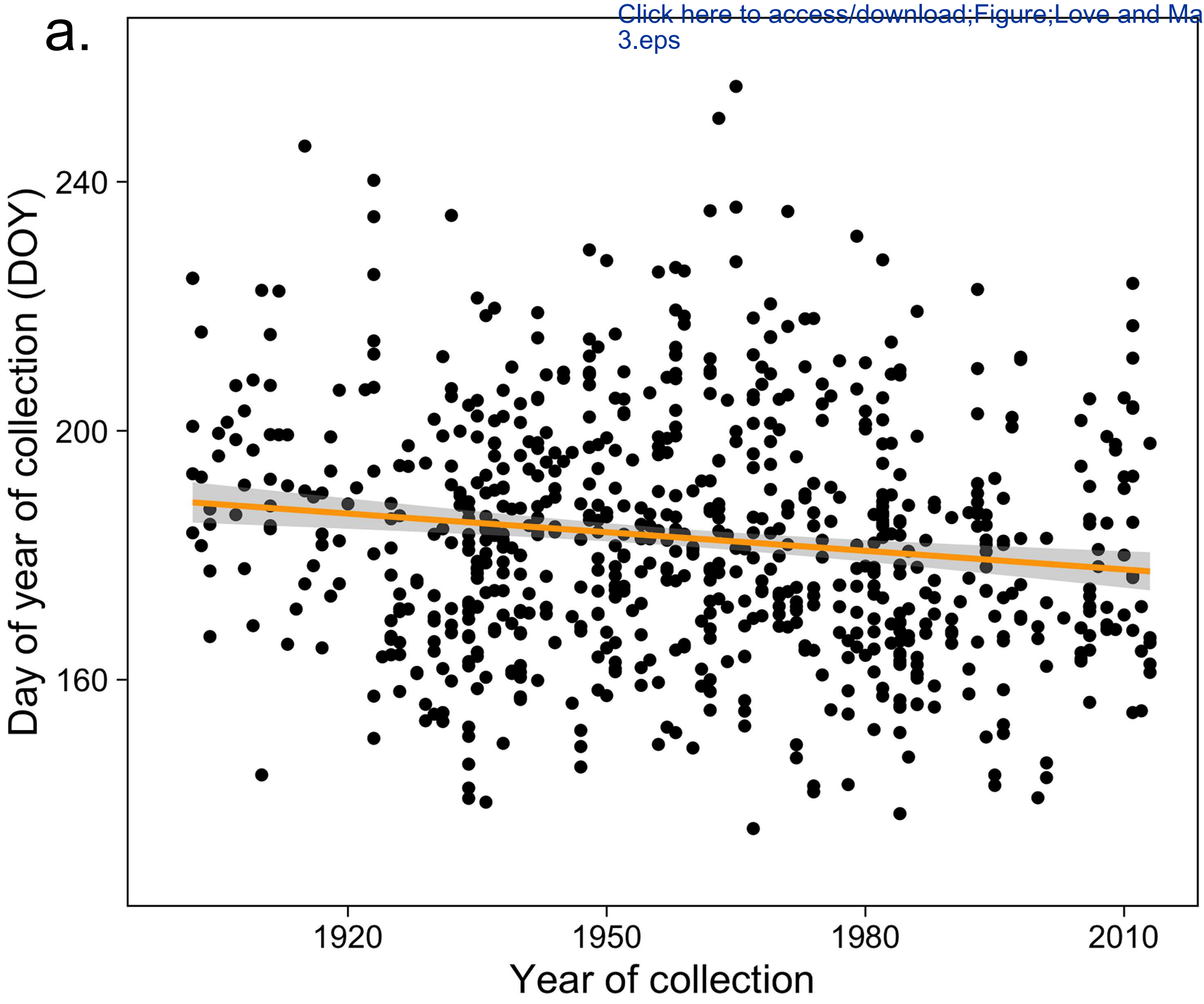
Figure 3. The relationship between the day of year of collection (DOY) and year for (a) all specimens in the final dataset (n = 743) and (b) those specimens collected in cool (blue points; n = 299) and warm (red points; n = 294) regions.

Figure 4. Partial regression plots representing the sensitivity of *S. tortuosus* to (a) minimum winter temperature, (b) minimum temperature during spring and summer, and (c) the day of year of the beginning of the frost-free period independent of model covariates (cumulative mean annual precipitation and the phenological index) in cool versus warm regions. The remaining plots (d-f) show how these same variables have shifted during the 112-year collection period independent of model covariates (latitude, longitude, elevation, and cumulative mean annual precipitation). Individual slopes for each of these relationships are listed in Table 2.

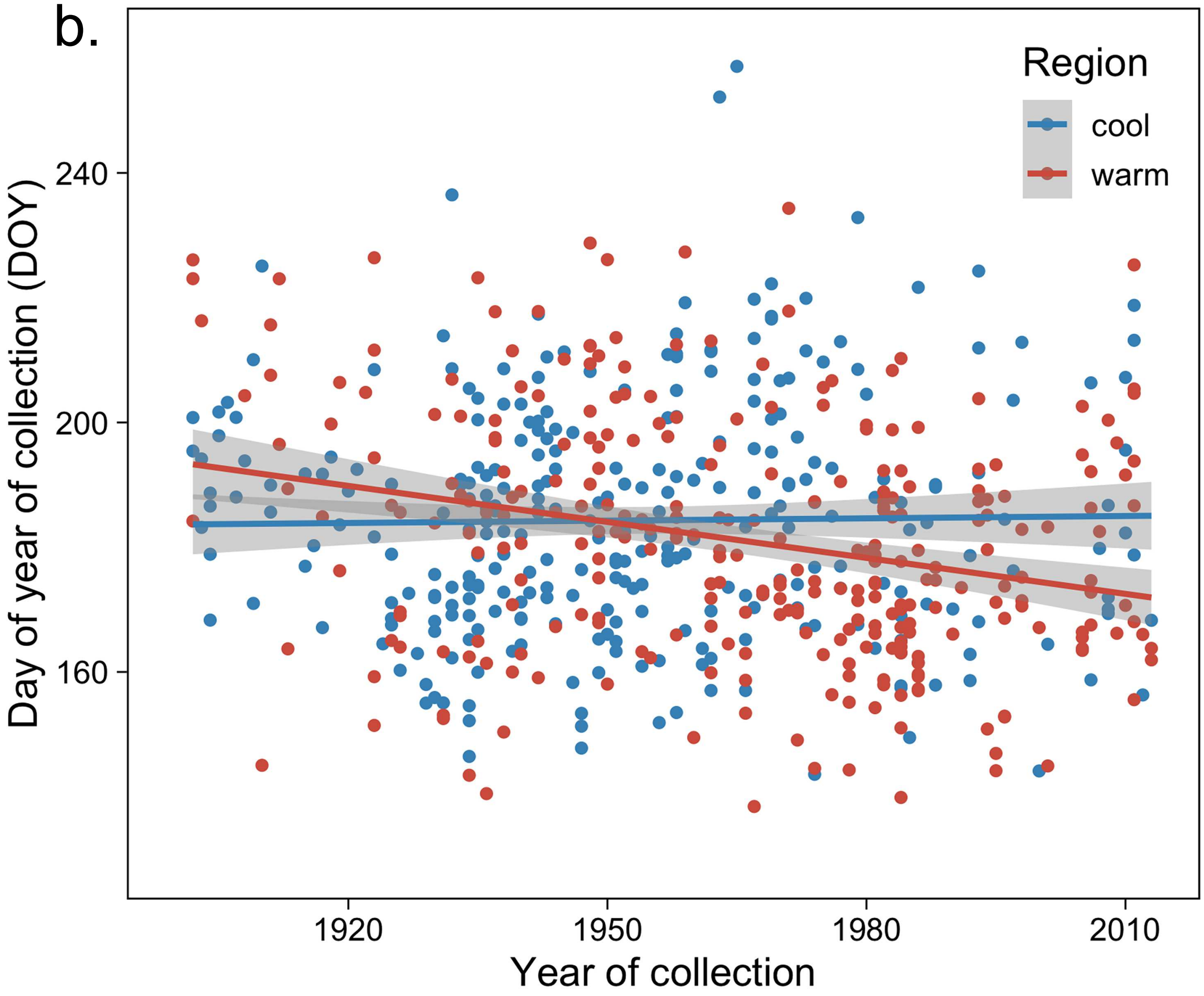

Region
cool
warm
Elevation (m)
4000
3000
2000
1000
0
42°N
41°N
40°N
39°N
38°N
37°N
36°N
35°N
Latitude
124°W
123°W
122°W
121°W
120°W
119°W
118°W
Longitude

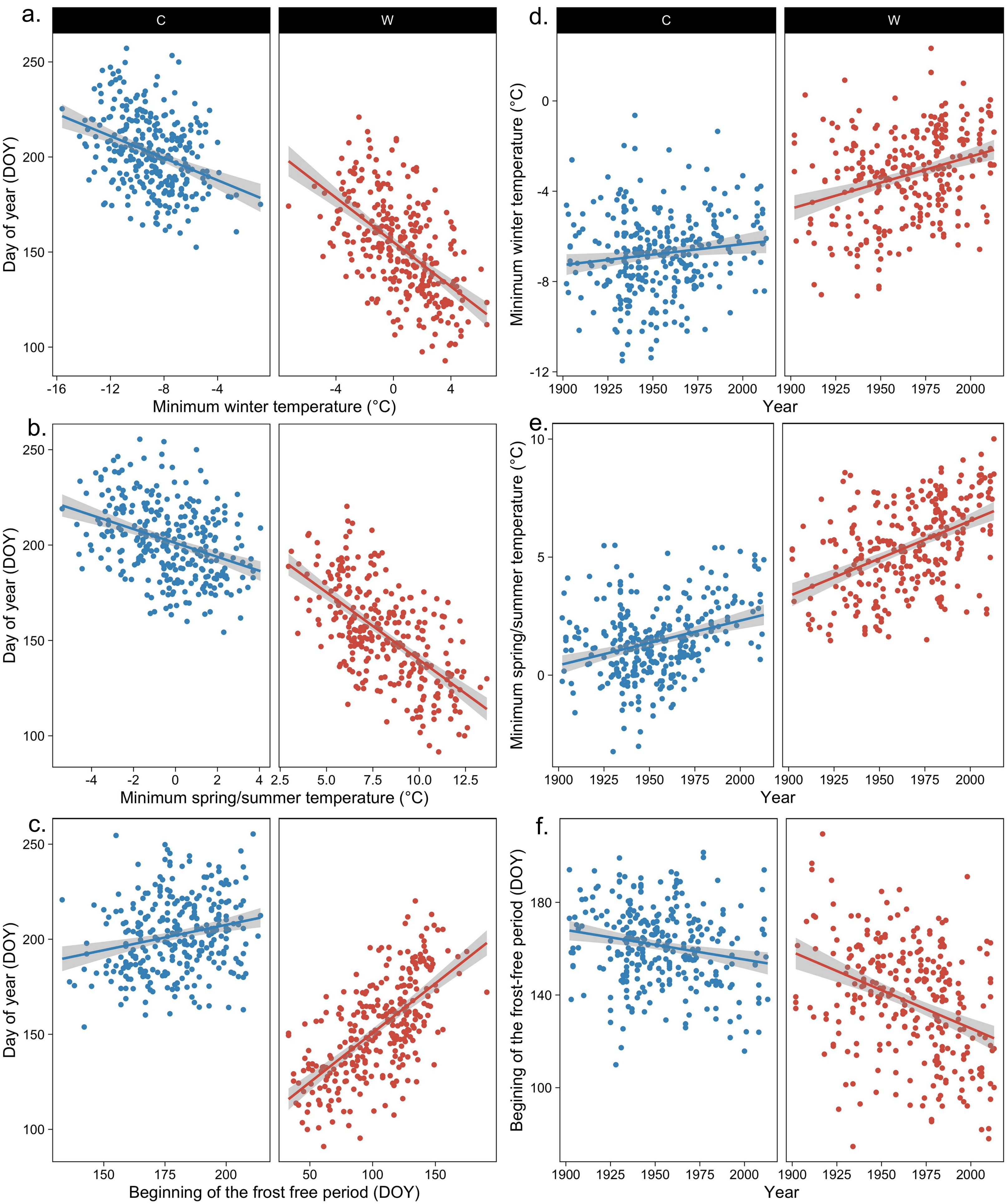

250
200
150
100
Day of year of collection (DOY)
Region
cool
warm
C
W
Region

a.


240
200
160
Day of year of collection (DOY)
1920
1950
1980
2010
Year of collection

b.


Region
cool
warm
240
200
160
Day of year of collection (DOY)
1920
1950
1980
2010
Year of collection


a.
C
W
Day of year (DOY)
250
200
150
100
-16
-12
-8
-4
-4
0
4
Minimum winter temperature (°C)
b.
Day of year (DOY)
250
200
150
100
-4
-2
0
2
4
2.5
5.0
7.5
10.0
12.5
Minimum spring/summer temperature (°C)
c.
Day of year (DOY)
250
200
150
100
150
175
200
50
100
150
Beginning of the frost free period (DOY)
d.
C
W
Minimum winter temperature (°C)
0
-4
-8
-12
1900
1925
1950
1975
2000
Year
e.
Minimum spring/summer temperature (°C)
10
5
0
1900
1925
1950
1975
2000
Year
f.
Begining of the frost-free period (DOY)
180
140
100
1900
1925
1950
1975
2000
Year

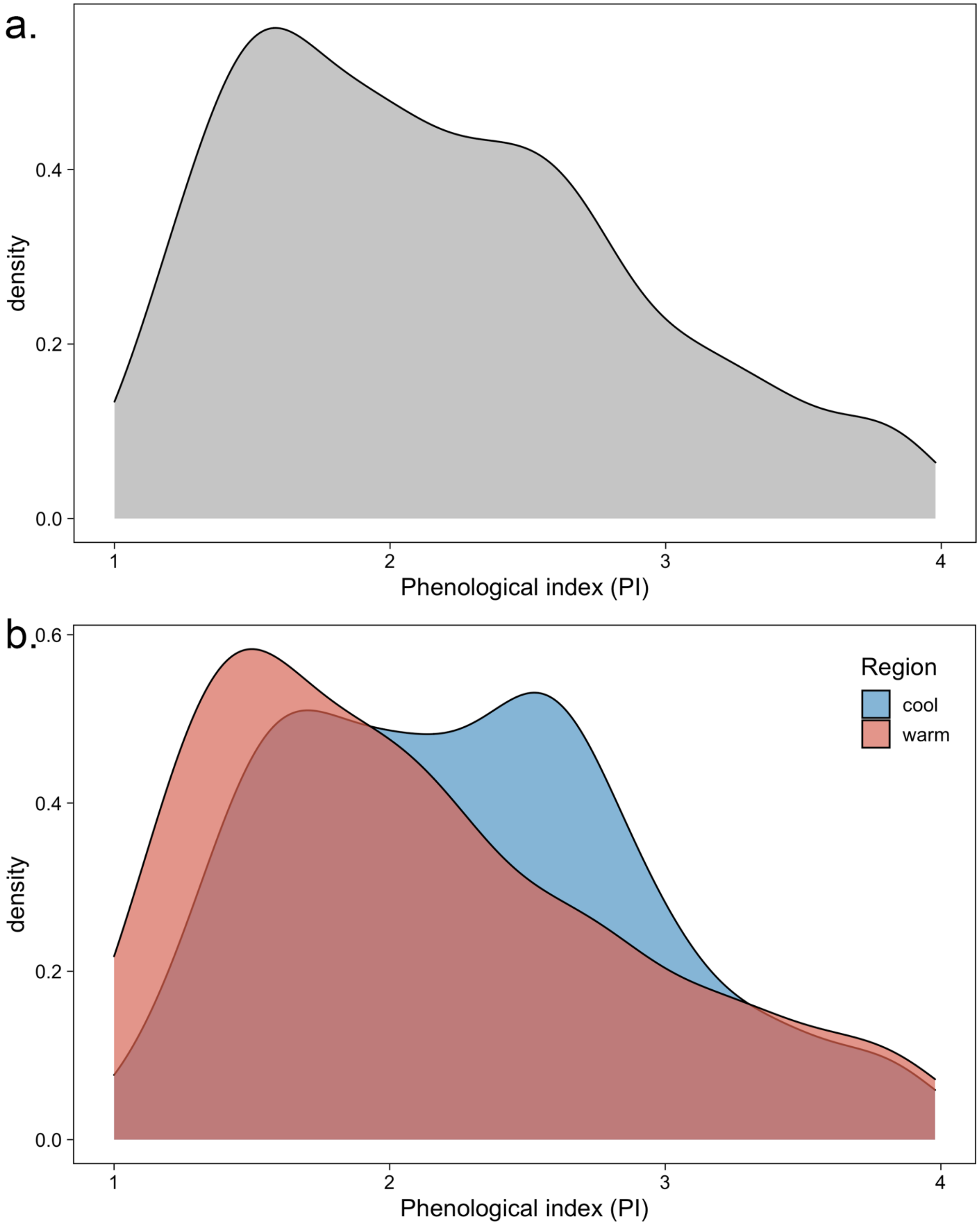
Love and Mazer - American Journal of Botany 2021 - Appendix S1

Appendix S1. Numbers of specimens from each herbarium included in the final datas the middle 20% of the temperature range of *S. tortuosus* removed (n = 150 specimens region comparisons.

Herbarium	Herbarium Code	# specimens in final dataset
University of California, Berkeley	UC/JEPS	154
California Academy of Sciences	CAS	306
California State University, Chico	CHSC	84
University of California, Davis	DAV	69
California Polytechnic State University, San Luis Obispo	OBI	9
Rancho Santa Ana Botanic Garden	RSA	118
California State University, Northridge	SFV	3
	Total	743

et and in the reduced dataset with
) that was used for cool vs. warm

specimens in the reduced dataset (warm vs. cool regions)
128
236
65
59
7
96
2
593



Appendix S2. The distribution of phenological indices (PIs) among (a) sites of collection of all specimens (n = 743) and (b) sites where specimens were collected in cool (shown in blue; n = 299) and warm (shown in red; n = 294) regions.

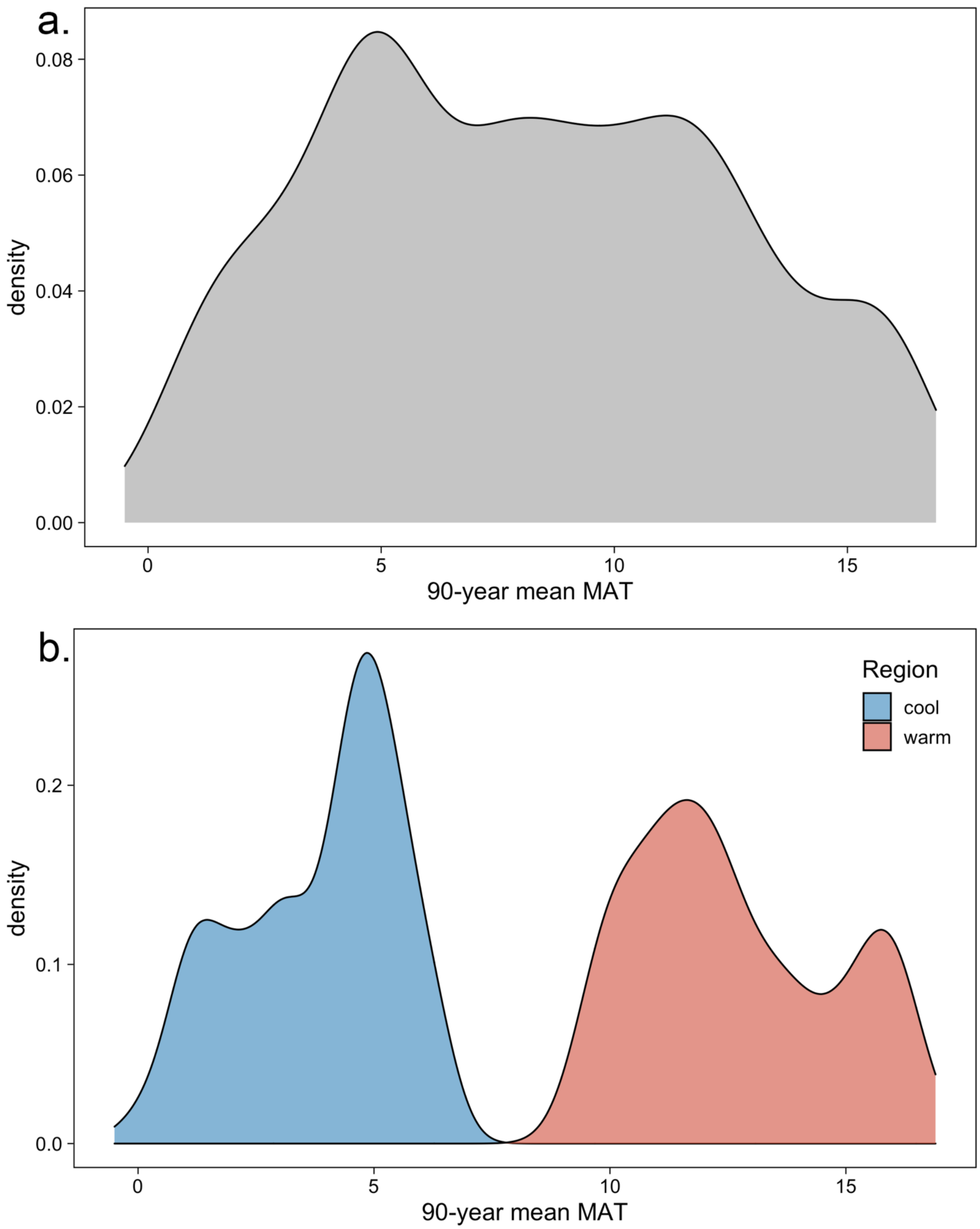
Appendix S3. Criteria for excluding herbarium specimens from the final dataset and the number o

Criteria for exclusion	**# specimens removed**
Blury image	13
Collection date spans > 3 days	98
No reproductive structures present	20
Highly overlapping reproductive structures	53
Unable to georeference	104
Georeference uncertainty radius >4km	94
Duplicate record	164
Collected earlier than available climate data	33

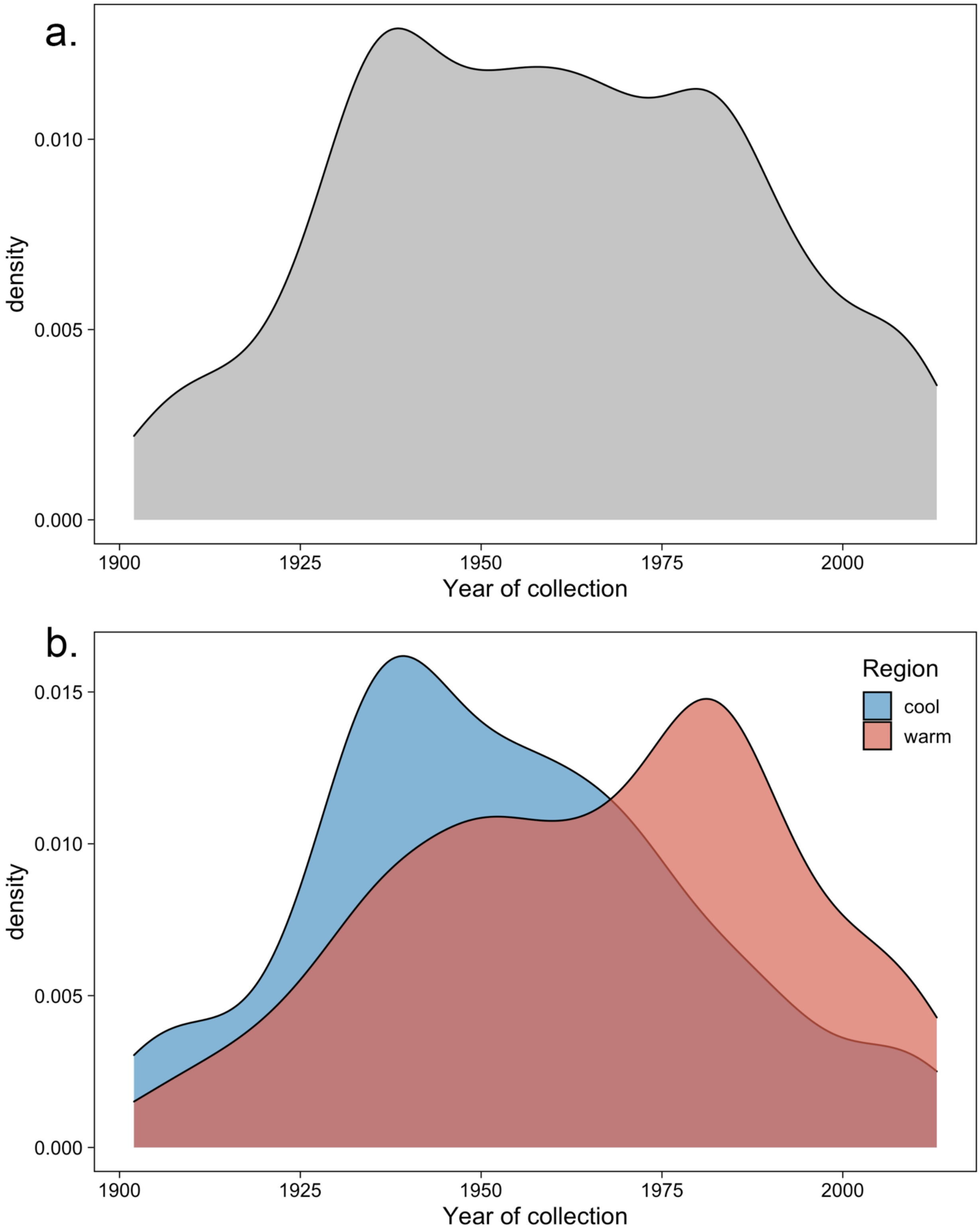
Total specimens assembled for study	1,322
Total specimens excluded	579
Total specimens retained for analysis	743

of specimens removed based on each criterion.

Love and Mazer – American Journal of Botany – Appendix S4



Appendix S4. The distribution of mean long-term (90-year) mean annual temperatures (MAT) among (a) sites of collection of all specimens (n = 743) and (b) sites where specimens were collected in cool (shown in blue; n = 299) and warm (shown in red; n = 294) regions.



Appendix S5. The distribution of specimen collection years among (a) sites of collection of all specimens (n = 743) and (b) sites where specimens were collected in cool (shown in blue; n = 299) and warm (shown in red; n = 294) regions.

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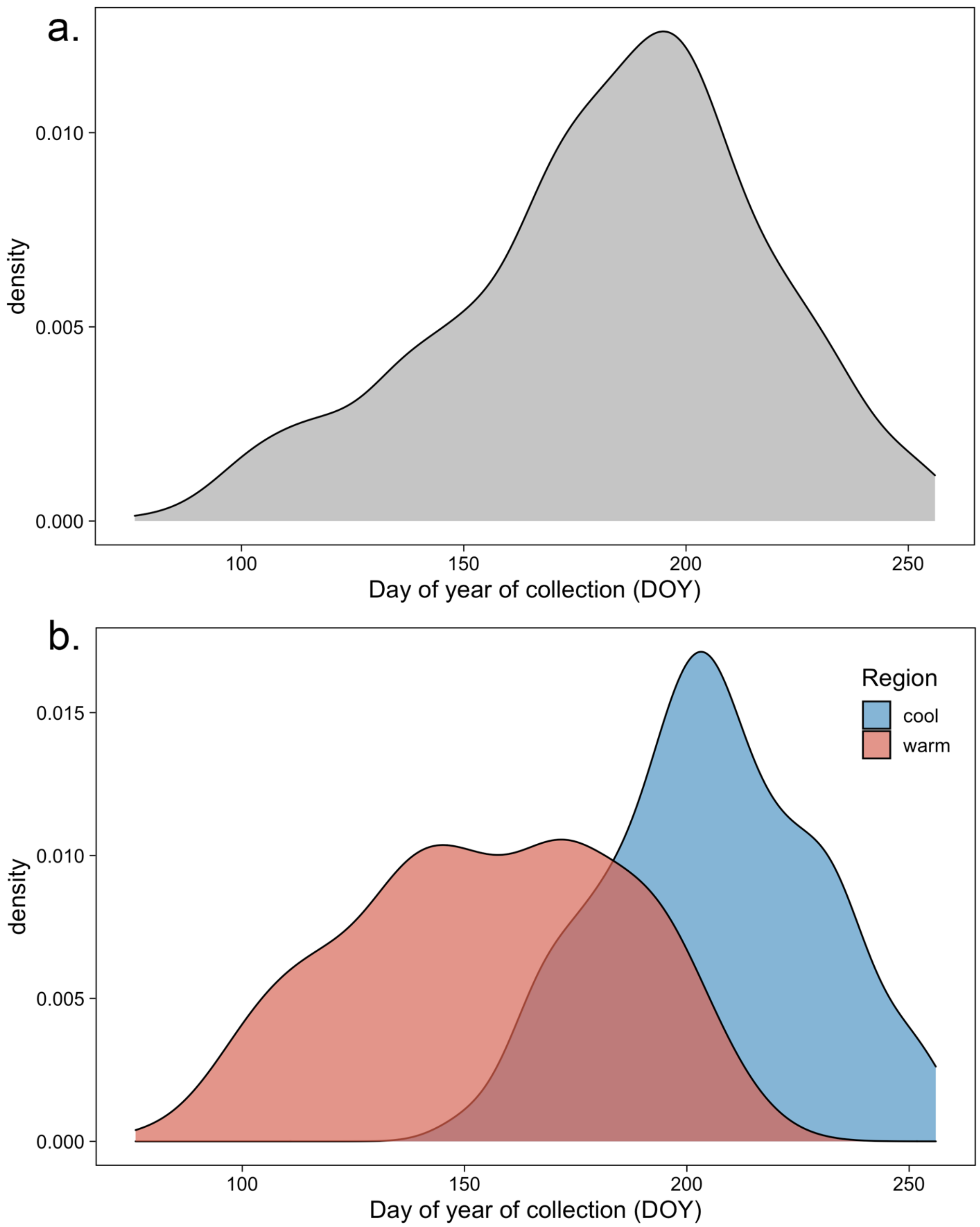

Appendix S6. The distribution of the day of year of specimen collection (DOY) among (a) sites of collection of all specimens (n = 743) and (b) sites where specimens were collected in cool (shown in blue; n = 299) and warm (shown in red; n = 294) regions.

bFFP. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted beginning of the frost-free period (bFFP) on the specimen day of year of collection (DOY) in cool vs. region x bFFP) while controlling for the phenological index and the mean annual precipitation (MAP) reduced dataset (n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	132.81	3.39	39.15	<0.001
Phenological Index (PI)	17.87	1.13	15.76	<0.001
Temperature region	9.74	1.65	5.92	<0.001
bFFP	0.39	0.039	10.22	<0.001
MAP	0.010	0.0013	7.95	<0.001
Temperature region x bFFP	-0.13	0.039	-3.27	0.001

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	575169.55	1	1532.35	<0.001
Phenological Index (PI)	93244.72	1	248.42	<0.001
Temperature region	13136.36	1	35.00	<0.001
bFFP	39172.55	1	104.36	<0.001
MAP	23744.80	1	63.26	<0.001
Temperature region x bFFP	4025.37	1	10.72	0.001
Residuals	220331.36	587		
Adjusted R^2	0.73			

to detect the effect of the
warm regions (temperature
among specimens in the

NFFD. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to detec frost free days in the year of collection (NFFD) on the specimen day of year of collection (DOY) in cool vs. wa region x NFFD) while controlling for the phenological index and the mean annual precipitation (MAP) among dataset (n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	131.90	3.55	37.17	<0.001
Phenological Index (PI)	17.75	1.15	15.42	<0.001
Temperature region	8.18	1.73	4.73	<0.001
NFFD	-0.24	0.023	-10.65	<0.001
MAP	0.012	0.0013	8.75	<0.001
Temperature region x NFFD	0.079	0.023	3.42	<0.001

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	533857.05	1	1381.89	<0.001
Phenological Index (PI)	91865.17	1	237.79	<0.001
Temperature region	8654.09	1	22.40	<0.001
NFFD	43799.85	1	113.38	<0.001
MAP	29608.86	1	76.64	<0.001
Temperature region x NFFD	4522.31	1	11.71	<0.001
Residuals	226771.73	587		
Adjusted R^2	0.72			

ct the effect of the number of
arm regions (temperature
specimens in the reduced

MAP. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to detect precipitation (MAP) on the specimen day of year of collection (DOY) in cool vs. warm regions (temperature re controlling for the phenological index and the mean annual temperature (MAT) among specimens in the reduce

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	200.39	4.35	46.07	<0.001
Phenological Index (PI)	17.12	1.08	15.82	<0.001
Temperature region	-5.27	1.89	-2.79	0.005
MAP	0.0092	0.0013	7.21	<0.001
MAT	-6.82	0.40	-17.24	<0.001
Temperature region x MAP	0.0024	0.0013	1.81	0.071

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	722410.12	1	2122.65	<0.001
Phenological Index (PI)	85165.96	1	250.24	<0.001
Temperature region	2644.01	1	7.77	0.005
MAP	17682.80	1	51.96	<0.001
MAT	101103.33	1	297.07	<0.001
Temperature region x MAP	1112.88	1	3.27	0.071
Residuals	199776.08	587		
Adjusted R^2	0.76			

the effect of mean annual
gion x MAP) while
d dataset (n=593).

MAT. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to detec temperature (MAT) on the specimen day of year of collection (DOY) in cool vs. warm regions (temperature r controlling for the phenological index and the mean annual precipitation (MAP) among specimens in the redu

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	142.82	3.54	40.29	<0.001
Phenological Index (PI)	16.87	1.06	15.85	<0.001
Temperature region	-3.60	1.83	-1.97	0.050
MAT	-6.39	0.38	-16.74	<0.001
MAP	0.0073	0.0013	5.76	<0.001
Temperature region x MAT	1.93	0.39	4.94	<0.001

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	533496.764	1	1623.59992	<0.001
Phenological Index (PI)	82568.92702	1	251.283442	<0.001
Temperature region	1270.811793	1	3.86748348	0.050
MAT	92051.05947	1	280.140579	<0.001
MAP	10894.65727	1	33.1558986	<0.001
Temperature region x MAT	8007.330503	1	24.3688472	<0.001
Residuals	192881.6312	587		
Adjusted R^2	0.76			

ct the effect of mean annual
egion x MAT) while
ced dataset (n=593).

PAS. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to det
as snow (PAS) on the specimen day of year of collection (DOY) in cool vs. warm regions (temperature reg
for the phenological index and the mean annual precipitation (MAP) among specimens in the reduced data

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	198.15	4.57	43.36	<0.001
Phenological Index (PI)	17.98	1.08	16.68	<0.001
Temperature region	-19.32	2.70	-7.16	<0.001
PAS	0.079	0.012	6.85	<0.001
MAT	-4.65	0.50	-9.33	<0.001
Temperature region x PAS	-0.063	0.011	-5.82	<0.001

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	640411.41	1	1880.12	<0.001
Phenological Index (PI)	94823.02	1	278.38	<0.001
Temperature region	17443.20	1	51.21	<0.001
PAS	15967.82	1	46.88	<0.001
MAT	29657.82	1	87.07	<0.001
Temperature region x PAS	11544.46	1	33.89	<0.001
Residuals	199945.42	587		
Adjusted R^2	0.76			

ect the effect of precipitation
ion x PAS) while controlling
set (n=593).

Spring/summer Tave. Parameter estimates (a) and summary statistics (b) for the multiple linear regression cond
temperature (Tave) during the spring and summer on the specimen day of year of collection (DOY) in cool vs. wa
spring/summer Tave) while controlling for the phenological index and the mean annual precipitation (MAP) amo
(n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	143.61	3.45	41.68	<0.001
Phenological Index (PI)	16.98	1.05	16.22	<0.001
Temperature region	-3.36	1.72	-1.95	0.0512
Spring/summer Tave	-6.14	0.34	-17.83	<0.001
MAP	0.0059	0.0013	4.73	<0.001
Temperature region x spring/summer Tave	1.72	0.35	4.91	<0.001

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	553446.62	1	1737.47	<0.001
Phenological Index (PI)	83756.58	1	262.94	<0.001
Temperature region	1215.64	1	3.82	0.0512
Spring/summer Tave	101270.08	1	317.92	<0.001
MAP	7126.26	1	22.37	<0.001
Temperature region x spring/summer Tave	7664.22	1	24.06	<0.001
Residuals	186980.34	587		
Adjusted R^2	0.77			

ucted to detect the effect of average
rm regions (temperature region x
ng specimens in the reduced dataset

Spring/summer Tmax. Parameter estimates (a) and summary statistics (b) for the multiple linear regression co temperature (Tmax) during the spring and summer on the specimen day of year of collection (DOY) in cool vs. spring/summer Tmax) while controlling for the phenological index and the mean annual precipitation (MAP) an (n=593).

a.

Independent variable	Estimate	SE	*t* ratio	P>|t|
Intercept	143.46	3.42	41.91	<0.001
Phenological Index (PI)	17.37	1.07	16.29	<0.001
Temperature region	-0.38	1.62	-0.23	0.815
Spring/summer Tmax	-5.12	0.30	-17.22	<0.001
MAP	0.0034	0.0013	2.61	0.009
Temperature region x spring/summer Tmax	1.10	0.30	3.70	<0.001

b.

Source of variation	Sum Sq	df	F value	Pr(>F)
Intercept	581475	1	1756.09	<0.001
Phenological Index (PI)	87911	1	265.49	<0.001
Temperature region	18	1	0.05	0.815
Spring/summer Tmax	98187	1	296.53	<0.001
MAP	2257	1	6.81	0.009
Temperature region x spring/summer Tmax	4537	1	13.70	<0.001
Residuals	194367	587		
Adjusted R^2	0.76			

nducted to detect the effect of maximum
warm regions (temperature region x
nong specimens in the reduced dataset

Spring/summer Tmin. Parameter estimates (a) and summary statistics (b) for the multiple linear regression c temperature (Tmin) during the spring and summer on the specimen day of year of collection (DOY) in cool vs spring/summer Tmin) while controlling for the phenological index and the mean annual precipitation (MAP) a (n=593).

a.

Independent variable	Estimate	SE	*t* ratio	P>|t|
Intercept	136.94	3.57	38.37	<0.001
Phenological Index (PI)	17.18	1.12	15.29	<0.001
Temperature region	1.91	1.78	1.07	0.284
Spring/summer Tmin	-5.36	0.38	-14.13	<0.001
MAP	0.01	0.0013	7.92	<0.001
Temperature region x spring/summer Tmin	1.72	0.38	4.46	<0.001

b.

Source of variation	Sum Sq	df	F value	Pr(>F)
Intercept	538789.02	1	1472.12827	4.16E-162
Phenological Index (PI)	85612.83	1	233.919138	1.09E-44
Temperature region	420.16	1	1.14801187	0.28440605
Spring/summer Tmin	73051.58	1	199.598146	3.20E-39
MAP	22947.06	1	62.6980304	1.21E-14
Temperature region x spring/summer Tmin	7280.28	1	19.8918438	9.82E-06
	214838.04	587		
Adjusted R^2	0.74			

onducted to detect the effect of minimum
. warm regions (temperature region x
among specimens in the reduced dataset

Winter Tave. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted t (Tave) during winter on the specimen day of year of collection (DOY) in cool vs. warm regions (temperature re the phenological index and the mean annual precipitation (MAP) among specimens in the reduced dataset (n=5

a.

Independent variable	Estimate	SE	*t* ratio	P>|t|
Intercept	135.89	3.49	38.93	<0.001
Phenological Index (PI)	17.31	1.13	15.39	<0.001
Temperature region	3.20	1.73	1.85	0.0642
Winter Tave	-5.06	0.37	-13.85	<0.001
MAP	0.010	0.0013	7.83	<0.001
Temperature region x winter Tave	1.50	0.37	4.08	<0.001

b.

Source of variation	Sum Sq	df	F value	Pr(>F)
Intercept	557777.4205	1	1515.45902	<0.001
Phenological Index (PI)	87125.54372	1	236.716629	<0.001
Temperature region	1265.144571	1	3.43734737	0.0642
Winter Tave	70572.92164	1	191.743815	<0.001
MAP	22539.18892	1	61.2380779	<0.001
Temperature region x winter Tave	6114.628339	1	16.6132015	<0.001
Residuals	216050.2804	587		
Adjusted R^2	0.74			

detect the effect of the average temperature
gion x winter Tave) while controlling for
93).

Winter Tmax. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to maximum temperature (Tmax) during winter on the specimen day of year of collection (DOY) in cool vs. warm re winter Tmax) while controlling for the phenological index and the mean annual precipitation (MAP) among speci (n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	139.61	3.40	41.08	<0.001
Phenological Index (PI)	17.38	1.14	15.24	<0.001
Temperature region	7.62	1.54	4.96	<0.001
Winter Tmax	-4.44	0.35	-12.85	<0.001
MAP	0.0067	0.0013	5.03	<0.001
Temperature region x winter Tmax	1.53	0.34	4.48	<0.001

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	637645.88	1	1687.79	<0.001
Phenological Index (PI)	87765.14	1	232.31	<0.001
Temperature region	9295.26	1	24.60	<0.001
Winter Tmax	62377.67	1	165.11	<0.001
MAP	9555.27	1	25.29	<0.001
Temperature region x winter Tmax	7580.10	1	20.06	<0.001
Residuals	221768.33	587		
Adjusted R^2	0.73			

detect the effect of the
egions (temperature region x
mens in the reduced dataset

Winter Tmin. Parameter estimates (a) and summary statistics (b) for the multiple linear regression minimum temperature (Tmin) during winter on the specimen day of year of collection (DOY) in coo winter Tmin) while controlling for the phenological index and the mean annual precipitation (MAP) (n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	131.25	3.56	36.85	<0.001
Phenological Index (PI)	17.65	1.16	15.22	<0.001
Temperature region	4.26	1.78	2.39	0.0172
Winter Tmin	-4.37	0.34	-12.76	<0.001
MAP	0.014	0.0014	9.92	<0.001
Temperature region x winter Tmin	1.47	0.34	4.33	<0.001

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	532071.51	1	1357.55	<0.001
Phenological Index (PI)	90736.10	1	231.51	<0.001
Temperature region	2238.96	1	5.71	0.0172
Winter Tmin	63817.64	1	162.83	<0.001
MAP	38600.15	1	98.49	<0.001
Temperature region x winter Tmin	7332.79	1	18.71	<0.001
Residuals	230065.13	587		
Adjusted R^2	0.72			

conducted to detect the effect of the
l vs. warm regions (temperature region x
among specimens in the reduced dataset

bFFP. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to dete the beginning of the frost-free period (bFFP) in cool vs. warm regions (year x temperature region) while conti variables and the mean annual precipitation (MAP) among specimens in the reduced dataset (n=593).

a.

Independent variable	Estimate	SE	*t* ratio	P>|t|
Intercept	588.44	144.53	4.07	<0.001
Year	-0.23	0.032	-7.18	<0.001
Temperature region	10.74	1.89	5.67	<0.001
Elevation	0.028	0.0019	15.13	<0.001
Latitude	9.83	1.36	7.20	<0.001
Longitude	7.23	1.55	4.66	<0.001
MAP	-0.0042	0.0016	-2.64	0.0086
Year x temperature region	0.10	0.032	3.16	0.0017

b.

Source of variation	Sum Sq	df	F value	Pr(>F)
Intercept	6683.91	1	16.58	<0.001
Year	20809.94	1	51.61	<0.001
Temperature region	12955.70	1	32.13	<0.001
Elevation	92270.40	1	228.83	<0.001
Latitude	20920.96	1	51.88	<0.001
Longitude	8762.31	1	21.73	<0.001
MAP	2803.02	1	6.95	0.0086
Year x temperature region	4025.21	1	9.98	0.0017
Residuals	235892.57	585		
Adjusted R^2	0.8			

ct the effect of the year on
rolling for geographic

NFFP. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to dete
the number of frost-free days in a given year (NFFP) in cool vs. warm regions (year x temperature region) wh
variables and the mean annual precipitation (MAP) among specimens in the reduced dataset (n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	-963.67	221.24	-4.36	<0.001
Year	0.43	0.049	8.82	<0.001
Temperature region	-20.32	2.90	-7.01	<0.001
Elevation	-0.046	0.0029	-16.16	<0.001
Latitude	-16.18	2.09	-7.74	<0.001
Longitude	-14.92	2.37	-6.29	<0.001
MAP	0.010	0.0024	4.19	<0.001
Year x temperature region	-0.12	0.048932898	-2.35	0.0189

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	17926.21	1	18.97	<0.001
Year	73458.94	1	77.75	<0.001
Temperature region	46411.81	1	49.12	<0.001
Elevation	246773.55	1	261.18	<0.001
Latitude	56656.83	1	59.96	<0.001
Longitude	37360.72	1	39.54	<0.001
MAP	16551.63	1	17.52	<0.001
Year x temperature region	5237.73	1	5.54	0.0189
Residuals	552740.32	585		
Adjusted R^2	0.85			

ct the effect of the year on
ile controlling for geographic

MAP. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to detect th annual precipitation (MAP) in cool vs. warm regions (year x temperature region) while controlling for geographi annual temperature (MAT) among specimens in the reduced dataset (n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	-21826.85	3636.64	-6.00	<0.001
Year	1.47	0.85	1.73	0.085
Temperature region	-92.71	55.89	-1.66	0.098
Elevation	0.40	0.07	5.54	<0.001
Latitude	123.18	35.68	3.45	<0.001
Longitude	-144.12	39.77	-3.62	<0.001
MAT	28.48	16.83	1.69	0.091
Year x temperature region	-2.81	0.82	-3.43	<0.001

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	9734275.19	1	36.02	<0.001
Year	805135.22	1	2.98	0.085
Temperature region	743566.15	1	2.75	0.098
Elevation	8303757.68	1	30.73	<0.001
Latitude	3221042.92	1	11.92	<0.001
Longitude	3548715.11	1	13.13	<0.001
MAT	773680.50	1	2.86	0.091
Year x temperature region	3170257.37	1	11.73	<0.001
Residuals	158080275.52	585		
Adjusted R^2	0.3			

e effect of the year on mean
variables and the mean

MAT. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to dete
mean annual temperature (MAT) in cool vs. warm regions (year x temperature region) while controlling for g
mean annual precipitation (MAP) among specimens in the reduced dataset (n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	11.94	9.17	1.30	0.193
Year	0.013	0.0020	6.64	<0.001
Temperature region	-1.61	0.12	-13.36	<0.001
Elevation	-0.0033	0.00012	-27.65	<0.001
Latitude	-0.42	0.087	-4.88	<0.001
Longitude	-0.15	0.098	-1.55	0.122
MAP	0.00017	0.00010	1.69	0.091
Year x temperature region	-0.0030	0.0020	-1.48	0.138

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	2.75	1	1.70	0.193
Year	71.46	1	44.04	<0.001
Temperature region	289.72	1	178.55	<0.001
Elevation	1240.41	1	764.46	<0.001
Latitude	38.71	1	23.86	<0.001
Longitude	3.89	1	2.39	0.122
MAP	4.65	1	2.86	0.091
Year x temperature region	3.57	1	2.20	0.138
Residuals	949.22	585		
Adjusted R^2	0.93			

ct the effect of the year on
eographic variables and the

PAS. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted to detect t
precipitation as snow (PAS) in cool vs. warm regions (year x temperature region) while controlling for geograp
annual temperature (MAT) among specimens in the reduced dataset (n=593).

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	-341.90	1451.19	-0.24	0.814
Year	-0.50	0.34	-1.47	0.141
Temperature region	93.47	22.30	4.19	<0.001
Elevation	15.35	14.24	1.08	0.281
Latitude	-3.11	15.87	-0.20	0.845
Longitude	0.025	0.029	0.88	0.381
MAT	-40.56	6.72	-6.04	<0.001
Year x temperature region	-0.96	0.33	-2.92	0.004

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	2388.49	1	0.056	0.814
Year	93419.79	1	2.17	0.141
Temperature region	755823.50	1	17.57	<0.001
Elevation	50044.78	1	1.16	0.281
Latitude	1654.24	1	0.038	0.845
Longitude	33016.71	1	0.77	0.381
MAT	1569563.76	1	36.48	<0.001
Year x temperature region	367374.22	1	8.54	0.004
Residuals	25172524.65	585		
Adjusted R^2	0.68			

the effect of the year on
hic variables and the mean

Spring/summer Tave. Parameter estimates (a) and summary statistics (b) for the multiple linear regressio effect of the year on the average temperature (Tave) during spring and summer in cool vs. warm regions (y while controlling for geographic variables and the mean annual precipitation (MAP) among specimens in t

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	32.38	10.20	3.17	0.002
Year	0.015	0.0022	6.52	<0.001
Temperature region	-1.67	0.13	-12.48	<0.001
Elevation	-0.0034	0.00013	-26.04	<0.001
Latitude	-0.13	0.096	-1.37	0.171
Longitude	0.081	0.11	0.74	0.457
MAP	-0.00013	0.00011	-1.16	0.246
Year x temperature region	-0.0034	0.0023	-1.52	0.129

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	20.23	1	10.07	0.002
Year	85.42	1	42.50	<0.001
Temperature region	312.91	1	155.69	<0.001
Elevation	1363.17	1	678.26	<0.001
Latitude	3.78	1	1.88	0.171
Longitude	1.11	1	0.55	0.457
MAP	2.71	1	1.35	0.246
Year x temperature region	4.64	1	2.31	0.129
Residuals	1175.74	585		
Adjusted R^2	0.92			

n conducted to detect the
year x temperature region)
ne reduced dataset (n=593).

Spring/summer Tmax. Parameter estimates (a) and summary statistics (b) for the multiple linear regressi effect of the year on the maximum temperature (Tmax) during spring and summer in cool vs. warm regior while controlling for geographic variables and the mean annual precipitation (MAP) among specimens in t

a.

Independent variable	Estimate	SE	*t* ratio	P>|t|
Intercept	70.77	11.65	6.07	<0.001
Year	0.0039	0.0026	1.53	0.128
Temperature region	-1.49	0.15	-9.78	<0.001
Elevation	-0.0043	0.00015	-28.37	<0.001
Latitude	0.11	0.11	0.97	0.330
Longitude	0.40	0.13	3.19	0.002
MAP	-0.00060	0.00013	-4.69	<0.001
Year x temperature region	-0.00032	0.0026	-0.13	0.900

b.

Source of variation	Sum Sq	df	F value	Pr(>F)
Intercept	96.68	1	36.87	<0.001
Year	6.10	1	2.33	0.128
Temperature region	250.68	1	95.60	<0.001
Elevation	2110.86	1	805.03	<0.001
Latitude	2.49	1	0.95	0.330
Longitude	26.64	1	10.16	0.002
MAP	57.77	1	22.03	<0.001
Year x temperature region	0.041	1	0.016	0.900
Residuals	1533.92	585		
Adjusted R^2	0.91			

on conducted to detect the ns (year x temperature region) the reduced dataset (n=593).

Spring/summer Tmin. Parameter estimates (a) and summary statistics (b) for the multiple linear regression
of the year on the minimum temperature (Tmin) during spring and summer in cool vs. warm regions (year x
controlling for geographic variables and the mean annual precipitation (MAP) among specimens in the reduc

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	-6.00	11.42	-0.52	0.600
Year	0.025	0.0025	10.09	<0.001
Temperature region	-1.85	0.15	-12.34	<0.001
Elevation	-0.0026	0.00015	-17.55	<0.001
Latitude	-0.37	0.11	-3.43	<0.001
Longitude	-0.23	0.12	-1.92	0.056
MAP	0.00034	0.00013	2.70	0.007
Year x temperature region	-0.0065	0.0025	-2.57	0.010

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	0.69	1	0.28	0.600
Year	256.54	1	101.82	<0.001
Temperature region	383.89	1	152.37	<0.001
Elevation	776.32	1	308.13	<0.001
Latitude	29.58	1	11.74	<0.001
Longitude	9.25	1	3.67	0.056
MAP	18.32	1	7.27	0.007
Year x temperature region	16.66	1	6.61	0.010
Residuals	1473.89	585		
Adjusted R^2	0.89			

conducted to detect the effect
temperature region) while
ced dataset (n=593).

Winter Tave. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conducted
year on the average temperature (Tave) during winter in cool vs. warm regions (year x temperature region) w
geographic variables and the mean annual precipitation (MAP) among specimens in the reduced dataset (n=5

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	-9.87	11.70	-0.84	0.399
Year	0.014	0.0026	5.35	<0.001
Temperature region	-1.48	0.15	-9.64	<0.001
Elevation	-0.0032	0.00015	-21.02	<0.001
Latitude	-0.79	0.11	-7.16	<0.001
Longitude	-0.39	0.13	-3.07	0.002
MAP	0.00068	0.00013	5.25	<0.001
Year x temperature region	-0.0048	0.0026	-1.84	0.066

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	1.88	1	0.71	0.399
Year	75.56	1	28.61	<0.001
Temperature region	245.68	1	93.02	<0.001
Elevation	1167.36	1	441.97	<0.001
Latitude	135.37	1	51.25	<0.001
Longitude	24.90	1	9.43	0.002
MAP	72.94	1	27.61	<0.001
Year x temperature region	8.96	1	3.39	0.066
Residuals	1545.12	585		
Adjusted R^2	0.88			

to detect the effect of the
hile controlling for
93).

Winter Tmax. Parameter estimates (a) and summary statistics (b) for the multiple linear regression condu year on the maximum temperature (Tmaximum) during winter in cool vs. warm regions (year x temperatu for geographic variables and the mean annual precipitation (MAP) among specimens in the reduced datase

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	38.96	12.47	3.12	0.0019
Year	0.011	0.0027	4.17	<0.001
Temperature region	-1.30	0.16	-7.96	<0.001
Elevation	-0.0033	0.00016	-20.43	<0.001
Latitude	-0.93	0.12	-7.90	<0.001
Longitude	-0.076	0.13	-0.57	0.571
MAP	0.00033	0.00014	2.40	0.017
Year x temperature region	-0.0026	0.0028	-0.95	0.341

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	29.31	1	9.76	0.0019
Year	52.31	1	17.43	<0.001
Temperature region	190.20	1	63.37	<0.001
Elevation	1252.57	1	417.34	<0.001
Latitude	187.35	1	62.42	<0.001
Longitude	0.97	1	0.32	0.571
MAP	17.35	1	5.78	0.017
Year x temperature region	2.73	1	0.91	0.341
Residuals	1755.79	585		
Adjusted R^2	0.85			

cted to detect the effect of the
e region) while controlling
t (n=593).

Winter Tmin. Parameter estimates (a) and summary statistics (b) for the multiple linear regression conduc
year on the minimum temperature (Tmin) during winter in cool vs. warm regions (year x temperature regio
geographic variables and the mean annual precipitation (MAP) among specimens in the reduced dataset (n

a.

Independent variable	**Estimate**	**SE**	***t* ratio**	**P>|t|**
Intercept	-58.30	13.31	-4.38	<0.001
Year	0.016	0.0029	5.51	<0.001
Temperature region	-1.66	0.17	-9.50	<0.001
Elevation	-0.0031	0.00017	-17.81	<0.001
Latitude	-0.65	0.13	-5.13	<0.001
Longitude	-0.69	0.14	-4.83	<0.001
MAP	0.0010	0.00015	6.99	<0.001
Year x temperature region	-0.0069	0.0029	-2.35	0.0192

b.

Source of variation	**Sum Sq**	**df**	**F value**	**Pr(>F)**
Intercept	65.60	1	19.17	<0.001
Year	104.07	1	30.41	<0.001
Temperature region	308.75	1	90.23	<0.001
Elevation	1085.12	1	317.13	<0.001
Latitude	90.12	1	26.34	<0.001
Longitude	79.71	1	23.30	<0.001
MAP	167.34	1	48.91	<0.001
Year x temperature region	18.86	1	5.51	0.0192
Residuals	2001.68	585		
Adjusted R^2	0.88			

cted to detect the effect of the
on) while controlling for
=593).