

Herbarium specimens show contrasting phenological responses to Himalayan climate

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Responses by flowering plants to climate change are complex and only beginning to be understood. Through analyses of 10,295 herbarium specimens of Himalayan *Rhododendron* collected by plant hunters and botanists since 1884, we were able to separate these responses into significant components. We found a lack of directional change in mean flowering time over the past 45 y of rapid warming. However, over the full 125 y of collections, mean flowering time shows a significant response to year-to-year changes in temperature, and this response varies with season of warming. Mean flowering advances with annual warming (2.27 d earlier per 1 °C warming), and also is delayed with fall warming (2.54 d later per 1 °C warming). Annual warming may advance flowering through positive effects on overwintering bud formation, whereas fall warming may delay flowering through an impact on chilling requirements. The lack of a directional response suggests that contrasting phenological responses to temperature changes may obscure temperature sensitivity in plants. By drawing on large collections from multiple herbaria, made over more than a century, we show how these data may inform studies even of remote localities, and we highlight the increasing value of these and other natural history collections in understanding long-term change.

phenology | global warming

In an era of ongoing climate change (1), shifts in seasonal timing of life history events (phenology) are among the first and the most important responses seen in biological systems (2–5). Changes in phenology potentially impact organism reproduction, population survival, species boundaries, and ecosystem service (6–8). However, despite the importance of phenological changes (9, 10), data sources are limited (11). Satellite imagery (12), experimental studies (13), and modern observational records of phenology (11, 14) are temporally restricted to the last few decades. Although historical phenological records kept by scientists, amateur naturalists, or for cultural reasons (15–17) may extend much further, these are often limited in geographic range, and tend to focus on North America and Europe (but see ref. 18).

Such records have not been found for the Himalayan region, an area of particular concern when considering climate change. Rapid temperature increases and changes in precipitation, in combination with the importance of Himalayan snowpack and glaciers to water supply and monsoon cycles, make the region one of the most threatened nonpolar areas of the world (1, 19). Recent climate change is impacting Himalayan biological systems, including those upon which humans rely (20–23).

Despite its remoteness, the botanical richness of Yulong Mountain (27°N, 100.2°E), at the eastern limit of the Himalayan region, has made it a center of botanical collection since the late 19th century. Yulong Mountain was home to the prolific plant hunters George Forrest (collecting 1904–1930) and Joseph Rock (collecting 1918–1948). Other early collectors in the area included Jean Marie Delavayi, Heinrich Handel-Mazzetti, Frank Kingdon Ward, George Ludlow and Frank Sheriff, Yu Dejun

(T. T. Yu), and Feng Goumei (K. M. Feng). One of the most collected taxa was *Rhododendron*, a genus of particular ecological, cultural, and economic importance in the Himalaya (24, 25). Especially during the early part of the 20th century, *Rhododendron* was also of great horticultural value in Europe and North America (26). Originally gathered for species delimitation, historical herbarium collections have been used to impute changes in species ranges (27, 28) and in traits (29, 30). Specimens were usually collected in flower and with data on time and place of collection. Although now dispersed among different herbaria, when compiled, these collections and their associated data constitute a sizable body of knowledge on historical plant distributions and phenologies.

We used 10,295 *Rhododendron* herbarium specimens from this remote but well-sampled area of the Himalaya to infer flowering time response to temperature from 1884 to 2009.

Methods

We located collections of the 36 *Rhododendron* species that occur in Lijiang County, preserved in the herbaria of Royal Botanic Garden Edinburgh (E); Royal Botanic Gardens (K); The Natural History Museum (BM); Kunming Institute of Botany, Chinese Academy of Sciences (KUN); Institute of Botany, Chinese Academy of Sciences (PE); Harvard University (A, GH); Missouri Botanical Garden (MO); Muséum National d'Histoire Naturelle (P); and Universität Wien (WU). In all, 10,295 specimens were digitally imaged, and their label information was compiled into databases, including date, elevation, and location of collection. Collection data from duplicate collections (those made at the same time and from the same plant, but held by different herbaria) were combined, and, where available, collection information was supplemented with information from collector field books, diaries, and maps.

Significance

Natural events in temperate ecosystems are triggered by seasonal temperature changes. Climate change may shift the timing of these events. We use a century of herbarium collections of Himalayan rhododendrons to investigate climate-driven change in flowering time. Although increased annual temperatures are associated with earlier flowering, increased fall temperatures are associated with delayed flowering. Annual warming may advance flowering through positive effects on overwintering bud formation, whereas fall warming may delay flowering through an impact on chilling requirements. These contrasting effects have resulted in opposing changes in flowering time, even as temperatures have warmed rapidly in the past 45 y. This study demonstrates the value of natural history collections to inform ecological questions, especially regarding climate change.

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We treated duplicates as a single collection, and removed collections without information on date and elevation, collections without flowers, and collections made outside the geographic bounds of our climate data. Finally, because *Rhododendron* species can exhibit off-season flowering in early winter (*remontance*), we removed collections made more than 80 d after the species mean flowering date (67 collections) to focus our analysis on peak flowering time. The final data set used for the analyses comprised 1,147 specimens that satisfied all criteria. For a supplementary analysis, we considered an additional 1,199 specimens that lacked information on day of collection but had information on month of collection (Table S1).

Mean elevations and mean flowering seasons differ among Yulong *Rhododendron* spp. To combine our analysis across species, flowering time (day) and elevation (meters above sea level) of each collection were converted to within-species deviation from the mean as *flowering*, i.e., days after (+) or before (−) the species mean collection date, and *elevation*, i.e., meters above (+) or below (−) the species mean elevation. Analyses presented herein are conducted on deviations for all collections.

Two sources of historical weather information are available for Yulong Mountain and the surrounding areas of the eastern Himalaya where *Rhododendron* specimens were collected. The China Meteorological Administration weather station [i.e., Lijiang weather station (LWS)] in Lijiang, 25 km south of Yulong Mountain, offers measured, daily temperature and precipitation information, but is only available beginning in 1952. The Global Historical Climate Network (GHCN) (31) provides a longer temporal range of climate data over a broader spatial scale: monthly temperature deviations above or below the mean for the observational period are available for 5° gridded cells. For GHCN data, we averaged the three eastern Himalayan grid cells (90°E to 105°E and 25°N to 30°N) that encompassed the specimen collection area. For the period of overlapping data 1952–2009, GHCN data were strongly correlated with LWS data (Pearson $r = 0.76$).

We used LWS data (1952–2009) and the 460 herbarium collections from the period to look for the effects of recent climate change on phenology. We used linear regression to test for change over year in average annual temperature of the year preceding collection (*annual.temp.LWS*) and precipitation (*precip*) and change in *flowering* over the same period. Simple linear regression analyses were carried out with base R functions (32).

With all 1,146 herbarium collections and GHCN data (1884–2009), we examined year-to-year response of *flowering* to average annual temperature of the year preceding collection (*annual.temp*), and average seasonal temperatures. The 12 mo preceding the main *Rhododendron* flowering peak in May were divided into four 3-mo periods: *spring.temp*, February to April; *winter.temp*, November to January; *fall.temp*, August to October; and *summer.temp*, May to July. We used backward stepwise selection to test which variables (*elevation*, *annual.temp*, *spring.temp*, *winter.temp*, *fall.temp*, and *summer.temp*) best explained variation in *flowering*. By using the MASS package in R (32, 33), we selected the multiple linear regression model that minimized Akaike information criterion. The *flowering* data were then broken into individual species, data from which were tested against the model selected by the stepwise regression.

Additionally, we examined robustness of our model by testing it against an expanded data set for *flowering* and *elevation* at a coarser temporal scale (all collections for which month of collection data were available; Table S1) and against a different environmental data set for *annual.temp* and *fall.temp* (temperature variables based on LWS data; Table S2). By using the LWS data set, we also tested for precipitation as a significant factor. To avoid problems with inflated P values caused by multiple collections within years, we tested the generic model against average annual *flowering*, weighted by number of collections (Table S3). We also used linear regression to test for an effect of differential collection intensity across years and across decades on annual and decadal mean *flowering* (Fig. S1), and for directional change in *flowering* over year during periods of warming and cooling (Fig. S2).

Results

Over the recent period 1952–2009 (LWS data), mean annual temperature (*annual.temp.LWS*) significantly increased (0.13 °C per decade, $P = 0.0001$, $r^2 = 0.22$; Fig. 1). Over this period, *flowering* showed no significant change ($P = 0.14$). Average annual precipitation (*precip*) also showed no significant change ($P = 0.26$).

Over the past 125 y (1884–2009, GHCN data), for the full stepwise regression model considering all variables in explaining year-to-year change in *flowering*, the model that minimized AIC showed that *flowering* responds to *annual.temp* (2.27 d earlier per 1 °C), *fall.temp* (2.54 d later per 1 °C), and *elevation* (1.4 d later per 100 m); henceforth the “generic model” (Table 1). Considered

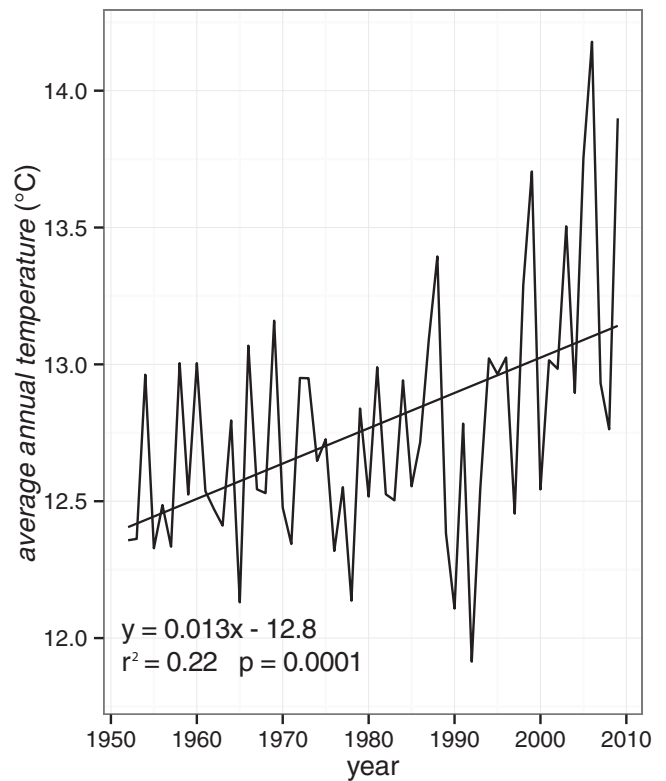


Fig. 1. Annual average temperatures recorded by the LWS (*annual.temp.LWS*) have significantly increased during the past 57 y (0.13 °C per decade, $P = 0.0001$, $r^2 = 0.22$).

separately as simple linear regressions, each of these three variables remained similar in effect size, sign, and significance (Fig. 2).

The generic model (*flowering*~*annual.temp*+*fall.temp*+*elevation*) was applied to collections of each species individually (Table 2). In general, species model term coefficients remained similar in effect size and sign to generic model coefficients (although not always significant given reduced sample size). Only one species, *Rhododendron virgatum*, showed a significant model coefficient that differed in sign from the generic model coefficients.

Applied to an expanded data set of coarser temporal scale (all collections for which month of collection data were available), the generic model coefficients remained significant, and similar in effect size and sign (Table S1). A model analogous to the generic model but with temperature terms based on LWS data rather than GHCN data (*flowering*~*annual.temp.LWS*+*fall.temp.LWS*+*elevation*) remained significant, and similar in sign (Table S2). The LWS data also included precipitation, but precipitation metrics added to this model were nonsignificant. A second analogous model with *flowering* and *elevation* treated as annual averages

Table 1. Flowering time significantly responds to weather

Parameter	Estimate	SE	P value
Intercept	4.11	0.84	1.23×10^{-06}
<i>annual.temp</i>	−2.27 (d/°C)	0.31	6.68×10^{-13}
<i>fall.temp</i>	2.54 (d/°C)	0.32	1.42×10^{-14}
<i>elevation</i>	0.014 (d/m)	0.001	1.46×10^{-15}

The variable *flowering* (i.e., day of collection deviation from the species mean) responds significantly to the additive effects of *annual.temp* (i.e., annual average temperature deviation of the year preceding collection), *fall.temp* (i.e., temperature deviation of the fall preceding collection) and *elevation* (i.e., elevation of collection deviation from the species mean) (adjusted $r^2 = 0.11$).

Table 2. Flowering time of *Rhododendron* spp.

Species	annual.temp, d/°C	fall.temp, d/°C	elevation, d/m	N
<i>Rhododendron adenogynum</i>	-10*	8*	0.01	35
<i>Rhododendron anthosphaerum</i>	-2.5	-9	0.02	36
<i>Rhododendron balfourianum</i>	-4.4	5	0.04*	14
<i>Rhododendron beesianum</i>	-2.2*	2*	0.01	60
<i>Rhododendron bureavii</i>	-0.5	1.9	0.01	25
<i>Rhododendron cephalanthum</i>	0.57	1.4	0.02*	40
<i>Rhododendron cuneatum</i>	-4.4*	0.56	0.02*	36
<i>Rhododendron decorum</i>	-3.4*	3.2*	0.01	100
<i>Rhododendron delavayi</i>	-6	3.9	0.03	15
<i>Rhododendron edgeworthii</i>	-1.4	4.8*	0	31
<i>Rhododendron fastigiatum</i>	-7.7*	4.1*	0.02*	15
<i>Rhododendron heliolepis</i>	2.1	0.41	0	23
<i>Rhododendron hippophaeoides</i>	-7*	3.6	0.02	33
<i>Rhododendron impeditum</i>	-9.6*	9.6*	-0.03	15
<i>Rhododendron irroratum</i>	-4.6*	2.3	-0.01	28
<i>Rhododendron lepidotum</i>	-0.54	3.3*	0	69
<i>Rhododendron oreotrephe</i>	-1.1	1.5	0.03*	44
<i>Rhododendron phaeochrysum</i>	-1.7	2.2*	0.02*	119
<i>Rhododendron primuliflorum</i>	0.31	3.6*	0.02*	75
<i>Rhododendron racemosum</i>	-2.6	1.6	0.02*	53
<i>Rhododendron rex</i>	-3.5	-0.8	0.01	22
<i>Rhododendron rubiginosum</i>	-8.9*	8.3*	0.02	46
<i>Rhododendron rupicola</i>	-1.9	5*	0.02*	49
<i>Rhododendron saluenense</i>	-1.6	-4	0.01	24
<i>Rhododendron uvariifolium</i>	-8	0.75	0.04*	18
<i>Rhododendron virgatum</i>	2.1	-4.5*	0.03*	28
<i>Rhododendron yunnanense</i>	-3.8*	5.8*	-0.01	32

Flowering time of *Rhododendron* spp. is determined by the same variables as the generic model (Table 1), $flowering \sim annual.temp + fall.temp + elevation$, where date of collection deviation from the species mean (*flowering*) is the dependent variable and previous annual average temperature deviation (*annual.temp*), previous fall average temperature deviation (*fall.temp*), and elevation of collection deviation from the species mean (*elevation*) are independent variables. Species coefficients are mostly of the same sign as the generic model. Species with a number of collections <13 included in the generic analysis but not considered separately here are: *Rhododendron genestierianum*, *Rhododendron mollicomum*, *Rhododendron orthocladum*, *Rhododendron scaberrifolium*, *Rhododendron tatsienense*, *Rhododendron telmateium*, *Rhododendron traillianum*, *Rhododendron trichostomum*, and *Rhododendron vernicosum*.

*Significant coefficients at $P < 0.05$.

$(flowering_{YEAR} \sim annual.temp + fall.temp + elevation_{YEAR})$ was significant, and similar in effect size and sign (Table S3), indicating that multiple subsamples (specimens) within year did not affect significance. Additionally, we did not find differential collection intensity across years to be significantly related to annual or decadal mean *flowering* (Fig. S1), indicating that mean collection time (*flowering*) is an unbiased estimate.

Discussion

The significant warming trend we found 1952–2009 (Fig. 1) is in accord with global trends (1) and with other studies focused on this area of the eastern Himalaya (20, 21, 34, 35). The lack of significant directional change in *Rhododendron* flowering time over this warming period might initially suggest that the genus is nonresponsive to temperature. Other multispecies studies have found some taxa not to exhibit response to climate, and a lack of response may lead studies to be discontinued or remain unpublished (14, 36, 37). However, a lack of phenological shift in response to climate may be a real biological effect with important consequences (38–40). In this study, despite the lack of directional change observed in *Rhododendron* during the warming period 1952–2009, we show that the genus is sensitive to year-to-year temperature changes (Table 1). The opposite and nearly equal effects of annual warming advancing flowering and fall warming delaying flowering may be responsible for this lack of directional change.

The effect of warmer average annual temperature advancing *Rhododendron* flowering may be caused by positive effects of temperature on the formation and growth of overwintering buds (41), which will become the following year’s flowers, and agrees with a body of literature showing warming resulting in advancing phenology (4, 15, 42, 43). The effect of warmer fall temperatures delaying *Rhododendron* flowering may be caused by a chilling requirement, which must be reached before overwintering buds will break dormancy and begin their spring growth (12, 14, 44). These results agree with recent landscape-level analysis of the Tibetan Plateau that suggested cold-season warming resulted in delayed phenology (12). Although that study and others (45) found chilling requirements were met in winter, *Rhododendron* is an alpine genus, and the first sustained freezing temperatures occur in their habitats in the fall. An ecosystem analysis (21) across the Himalayan region found that, even though most locations showed advanced phenology with spring warming, many also showed delayed phenology with fall warming. The role of chilling in driving phenological response to climate change extends beyond the Himalaya (37, 46,47), and it has been suggested to be a factor in taxa that previously were considered to be nonresponsive to climate change (14, 37).

The effect sizes seen in annual average warming on phenology (-2.27 d/°C) and fall warming on phenology (2.54 d/°C) are similar to effect sizes seen in similar studies (10, 48), although some studies have shown considerably greater effect sizes (11).

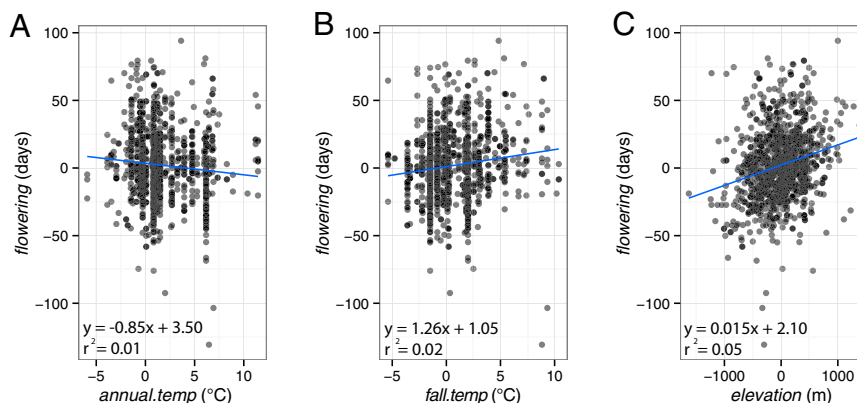


Fig. 2. Flowering time of Himalayan *Rhododendron* responds to annual temperature, fall temperature, and elevation. (A) *flowering* (i.e., day of collection deviation from the species mean) regresses negatively (-0.85 d/°C) and significantly ($P = 0.002$) with increasing *annual.temp* (i.e., annual average temperature deviation of the year preceding collection). (B) *flowering* regresses positively (1.26 d/°C) and significantly ($P < 0.001$) with increasing *fall.temp* (i.e., temperature deviation of the fall preceding collection). (C) *flowering* regresses positively (0.15 d per 100 m) and significantly ($P < 0.001$) with increasing *elevation* (i.e., elevation of collection deviation from the species mean). For visual clarity, points are 50% transparent; fully black points represent two or more collections. The model chosen by stepwise selection includes the additive effects of these three variables (Table 1).

Differences among species in the balance of these two contrasting phenological responses may account for the smaller generic effect size. Although we see general congruence between generic and species analyses, there are also likely to be real differences between species. One species for which we did not find significant effects, *Rhododendron delavayi*, has also not exhibited a chilling requirement in field studies (24). *R. virgatum* was the only species for which we found significant effects of a different sign than the generic model, with increasing *fall.temp* associated significantly with decreasing *flowering* (advanced phenology). *R. virgatum* and *R. delavayi* are among the lowest-elevation species in our sample. As chilling requirements have been proposed as an adaptation to cold climates (19, 24, 37), the lack of chilling requirement we observed in these two species could be related to the milder climatic conditions experienced by low-altitude rhododendron species.

The effects on phenology of warmer temperatures, delayed winter chilling, and other cues such as photoperiod are only beginning to be understood (49, 50). In this context, the validation of phenological models (50) and robust inference through combined methods (39) are important. Our model of contrasting effects in Himalayan *Rhododendron* is applicable to different data sets for *Rhododendron* collections (Table S1) and for temperature measurements (Table S2). Although phenological analyses of herbarium data offer unique insights into past responses, they model past responses only, and long-term phenological observation is necessary to fully understand present responses and model those in the future. To complement the herbarium data and test our models, we are directly monitoring *Rhododendron* phenology on Yulong Mountain, conducting artificial warming experiments, and documenting indigenous peoples' observations of change.

This study joins other work from the past decade showing the value of herbarium collections to infer long-term phenology (10, 11, 43, 48, 51–63). These have increasingly shown that not only can the “messy” data from herbarium collections be used to infer phenology, but that these data can reveal the complex effects on phenology of geography (43), pollination (62), morphological traits (48, 52), and, in this study, the contrasting response of warming across different seasons. By drawing together historical collections dispersed across many herbaria, we show that herbarium records have the ability to provide information beyond systematics, and further afield than eastern North America and Europe. In addition, our analysis of specimens for which at least month of collection was available yielded similar results to those for which day of collection was available. This suggests that even “incomplete” data such as these, which are often discarded from analysis (11, 54, 56), may merit examination.

Finally, a decline in botanical collection in recent years noted by other authors (10, 54) is also reflected in our data (Fig. S1). In an era of rapid climate change, botanical and other natural history collections hold increasingly valuable data for understanding long-term change and supporting conservation (64). Strengthening specimen collection, curation, and data availability should be a priority.

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