Climate-driven change in Himalayan Rhododendron phenology

Robert Evan Hart  
*University of Missouri-St. Louis*

Follow this and additional works at: [https://irl.umsl.edu/dissertation](https://irl.umsl.edu/dissertation)

Recommended Citation  
[https://irl.umsl.edu/dissertation/174](https://irl.umsl.edu/dissertation/174)
Climate-driven change in Himalayan *Rhododendron* phenology

by

Robbie Hart
B.A. Linguistics, Swarthmore College, 2004

A Dissertation Submitted to The Graduate School
at the University of Missouri-St. Louis in partial fulfillment of the requirements
for the degree Doctor of Philosophy in Biology (Ecology, Evolution and Systematics)

May 2015

Advisory Committee

Jan Salick, Ph.D. (Thesis advisor)

Robert Marquis, Ph.D. (Co-advisor)

Robert Ricklefs, Ph.D.

Tiffany Knight, Ph.D.
**Dissertation Abstract**

Phenology – the seasonal timing of life-history events – is a critical dimension of natural history. In plants, reproductive phenology is particularly important, affecting gene flow, population persistence and species boundaries. Phenology is also one of the earliest and most noticeable traits by which organisms respond to climate change. However, these responses are complex, and only beginning to be understood, especially in the montane and alpine environments that are among the ecosystems most vulnerable to climate change. Drawing from diverse data sets and employing multiple methodologies, I examined how climate affects phenology in *Rhododendron* spp. of Mt. Yulong, Yunnan, China.

Mt. Yulong is a center of diversity for *Rhododendron*, home to a suite of species that are diverse, dominant, and ecologically and culturally salient. They are also extraordinarily well represented by a unique set of historical plant collections. In Chapter 1, I reassemble these historical collections to build a proxy record of *Rhododendron* phenology, and use this record to show that although increased annual temperatures are associated with earlier flowering, increased fall temperatures are associated with delayed flowering. These contrasting effects have resulted in opposing changes in flowering time, even during rapid recent anthropogenic warming.

In Chapter 2, I comprehensively monitor flowering phenology in the most common *Rhododendron* species on Mt. Yulong over gradients of season and elevation. I measure individual response to inter-annual temperature change, contextualize the results within the herbarium record established in Chapter 1, and characterize the effects of phenology on reproductive success. Mt. Yulong *Rhododendron* spp. show an elaborate sequenced progression of flowering over season and elevation. Phenological
response to temperature varies among species, elevation, and season. Both phenology and response to temperature directly impact reproductive success, making this progression vulnerable to future climate change.

In Chapter 3, I build a community phylogeny and test phylogenetic signal to examine how phylogeny affects this phenological progression. Uniquely among phenological traits, last flowering day shows a phylogenetic signal, and is associated with flower size and fruit size. I suggest that this pattern may be driven by the limited time for fruit development before the onset of cold temperatures in autumn and highlight the unique and asymmetric pressures on phenology at seasonal extremes.

In chapter 4, I explore the dynamics of ecological knowledge of *Rhododendron* phenology around Mt. Yulong in two ethnic groups: the indigenous Naxi and immigrant Nuosu Yi. Local knowledge is rich and local understandings of changes and drivers parallels results from Chapter 1 and 2. While these knowledge systems are dynamic and able to adapt to change, they are also threatened by urbanization and changing lifeways.

In combination, these studies reveal the complexity of phenological response to climate change. Our multiple methodologies allow a deeper exploration than simple ‘earlier spring’ models of phenological response, while similar patterns seen among the diverse data sources increases our confidence in each. Mt. Yulong *Rhododendron* spp. flower in a progression over season and elevation, constrained to some degree by phylogeny. Both phenology and response to temperature directly impact reproductive success. Their responses to temperature vary among species and across elevations, and the effects of warming in different seasons drive contrasting responses.
Table of Contents

Chapter 1 Herbarium specimens show contrasting phenological responses to Himalayan climate.....................................................................................................................1

Chapter 2 Phenological progressions of Rhododendron flowering over season and elevation..........................................................................................................................32

Chapter 3 Phylogeny determines phenology of last flowering in Himalayan Rhododendron..........................................................................................................................81

Chapter 4 Dynamic ecological knowledge systems amid changing place and climate: Mt. Yulong rhododendrons..........................................................................................112
Chapter 1

Herbarium specimens show contrasting phenological responses to Himalayan climate

Published as Hart, Robbie, Jan Salick, Sailesh Ranjitkar, and Jianchu Xu. Herbarium specimens show contrasting phenological responses to Himalayan climate. *PNAS*.

Abstract

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 years of rapid warming. However, over the full 125 years 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 days earlier/$^\circ$C warming), and also is delayed with fall warming (2.54 days later/$^\circ$C warming). Annual warming may advance flowering through positive effects upon overwintering bud formation, while 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.
Introduction

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. While 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 (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 among the most threatened non-polar 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). While 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 1884-2009.

**Methods**

We located collections of the 36 *Rhododendron* species (Table 2) 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), Beijing Institute of Botany, Chinese Academy of Sciences (PE), Harvard University (A, GH), Missouri Botanical Garden (MO), Muséum National d'Histoire Naturelle (P), and Universitäti Wien (WU). In all, 10,295 specimens were digitally imaged, and their label information databased, 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.

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 a small off-season flowering in early winter (remontance) we removed collections made more than 80 days 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. In order to combine our analysis across species, flowering time (day) and elevation (masl) of each collection were converted to within-species deviation from the mean as *flowering*: days after (+) or before (-) the species mean collection date, and *elevation*: meters above (+) or below (-) the species mean elevation. Analyses presented below 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 (LWS) in Lijiang, 25 km south of Yulong Mountain, offers raw, 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 - 105°E and 25°N - 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’s 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 \(\text{annual.temp}_{\text{LWS}}\) and precipitation \(\text{precip}\) 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 \(\text{annual.temp}\), and average seasonal temperatures. The twelve months preceding the main \textit{Rhododendron} flowering peak in May were divided into four three-month periods: \textit{spring.temp}\text{ February-April, winter.temp}\text{ November-January, fall.temp}\text{ August-October, and summer.temp}\text{ May-July}. We used backwards stepwise selection to test which variables \(\text{elevation, annual.temp, spring.temp, winter.temp, fall.temp, summer.temp}\) best explained variation in flowering. Using the MASS package in R (32, 33), we selected the multiple linear regression model that minimized AIC. 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 flower\_rising 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). Using the LWS data set we also tested for precipitation as a significant factor. To avoid problems with inflated p-values due to multiple collections within years, we tested the generic model against average annual flower\_rising, 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 flower\_rising (Table S4), and for directional change in flower\_rising over year during periods of warming and cooling (Figure S2).

**Results**

Over the recent period 1952-2009 (Lijiang weather station data), mean annual temperature (annual\_temp\_LWS) significantly increased (0.13 C°/decade, p=0.0001, \( r^2=0.22 \)) (Figure 1). Over this period, flower\_rising showed no significant change (p=0.14). Average annual precipitation (precip) also shows no significant change (p=0.26).

Over the past 125 years (1884-2009, GHCN data), for the full stepwise regression model considering all variables in explaining year to year change in flower\_rising, the model that minimized AIC shows that flower\_rising responds to annual\_temp (2.26 days earlier/C°), fall\_temp (2.54 days later/C°), and elevation (1.4 days later / 100 meters)
(henceforth, the “generic model”, Table 1). Considered separately as simple linear regressions, each of these three variables remained similar in effect size, sign, and significance (Figure 2).

The generic model \((\text{flowering} \sim \text{annual.temp} + \text{fall.temp} + \text{elevation})\) 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, \(R.\ virgatum\), 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 \((\text{flowering} \sim \text{annual.temp}_{LWS} + \text{fall.temp}_{LWS} + \text{elevation})\) remained significant, and similar in sign (Table S2). The LWS data also included precipitation, but precipitation metrics added to this model were non-significant. A second analogous model with \(\text{flowering}\) and \(\text{elevation}\) treated as annual averages \((\text{flowering}_{\text{YEAR}} \sim \text{annual.temp} + \text{fall.temp} + \text{elevation}_{\text{YEAR}})\) 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 \(\text{flowering}\) (Figure S1), indicating that mean collection time \((\text{flowering})\) is an unbiased estimate.
**Discussion**

The significant warming trend we found 1952-2009 (Figure 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 non-responsive to temperature. Other multi-species 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 are in line 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.
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 although 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 non-responsive to climate change (14, 37).

The effect sizes seen in annual average warming on phenology (-2.27 days/C°) and fall warming on phenology (2.54 days/C°) are similar to effect sizes seen in similar studies (10, 48) although some studies have shown considerably greater effect sizes (11). 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). *Rhododendron 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 (24, 37, 19), 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 suggesting contrasting effects in Himalayan *Rhododendron* is applicable to different data sets both 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 last 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 collection be used to infer phenology, but that these data can reveal 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 (Figure 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.

Acknowledgements

We thank staff at all herbaria consulted, and undergraduates at University of Missouri–St. Louis, who provided essential aid to this research. Research was supported by the Biodiversity Conservation and Sustainable Development in Southwest China NSF-IGERT (DGE#0549369), by the National Key Basic Research Program of China (Grant No. 2014CB954100), and by the Whitney R. Harris World Ecology Center. SR was supported by National Science Foundation China (Grant No. 31270524).

References


studies: a case study with the orchid Ophrys sphegodes. J Ecol.


27. Loiselle BA, Jørgensen PM, Consiglio T (2008) Predicting species distributions


35. Li Z-S, Zhang Q-B, Ma K (2011) Tree-ring reconstruction of summer temperature for A.D. 1475-2003 in the central Hengduan Mountains,


44. Pope KS et al. (2013) Detecting nonlinear response of spring phenology to
climate change by Bayesian analysis. Global Change Biol.


Table 1. Flowering (day of collection deviation from the species mean) responds significantly to the additive effects of annual.temp (annual average temperature deviation of the year preceding collection), fall.temp (temperature deviation of the fall preceding collection) and elevation (elevation of collection deviation from the species mean).

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>4.11</td>
<td>0.84</td>
<td>1.23 x</td>
</tr>
<tr>
<td>annual.temp</td>
<td>-2.27</td>
<td>0.31</td>
<td>6.68 x</td>
</tr>
<tr>
<td>fall.temp</td>
<td>2.54</td>
<td>0.32</td>
<td>1.42 x</td>
</tr>
<tr>
<td>elevation</td>
<td>0.014</td>
<td>0.001</td>
<td>1.46 x</td>
</tr>
<tr>
<td>Adjusted r²:</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 (following page). Flowering time of *Rhododendron* spp. is determined by the same variables as the generic model (Table 1), $\text{flowering} \sim \text{annual.temp} + \text{fall.temp} + \text{elevation}$, where date of collection deviation from the species mean ($\text{flowering}$) is the dependent variable and previous annual average temperature deviation ($\text{annual.temp}$), previous fall average temperature deviation ($\text{fall.temp}$), and elevation of collection deviation from the species mean ($\text{elevation}$) are independent variables. Species coefficients are mostly of the same sign as the generic model. Significant coefficients ($p<0.05$) are indicated with stars. Species with N (number of collections) <13 included in the generic analysis but not considered separately here are: *R. genestierianum*, *R. mollicomum*, *R. orthocladum*, *R. scabrifolium*, *R. tatsienense*, *R. telmateium*, *R. traillianum*, *R. trichostomum* and *R. vernicosum*. 
<table>
<thead>
<tr>
<th>species</th>
<th>annual temp (days/C°)</th>
<th>fall. temp (days/C°)</th>
<th>elevation (days/m)</th>
<th>N</th>
<th>N (&gt;1952)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. adenogynum</em></td>
<td>-10*</td>
<td>8*</td>
<td>0.01</td>
<td>35</td>
<td>10</td>
</tr>
<tr>
<td><em>R. anthosphaerum</em></td>
<td>-2.5</td>
<td>-9</td>
<td>0.02</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td><em>R. balfourianum</em></td>
<td>-4.4</td>
<td>5</td>
<td>0.04*</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td><em>R. beeianum</em></td>
<td>-2.2*</td>
<td>2*</td>
<td>0.01</td>
<td>60</td>
<td>35</td>
</tr>
<tr>
<td><em>R. bureavii</em></td>
<td>-0.5</td>
<td>1.9</td>
<td>0.01</td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td><em>R. cephalanthum</em></td>
<td>0.57</td>
<td>1.4</td>
<td>0.02*</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td><em>R. cuneatum</em></td>
<td>-4.4*</td>
<td>0.56</td>
<td>0.02*</td>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td><em>R. decorum</em></td>
<td>-3.4*</td>
<td>3.2*</td>
<td>0.01</td>
<td>100</td>
<td>61</td>
</tr>
<tr>
<td><em>R. delavayi</em></td>
<td>-6</td>
<td>3.9</td>
<td>0.03</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td><em>R. edgeworthii</em></td>
<td>-1.4</td>
<td>4.8*</td>
<td>0</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td><em>R. fastigiatum</em></td>
<td>-7.7*</td>
<td>4.1*</td>
<td>0.02*</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td><em>R. heliolepis</em></td>
<td>2.1</td>
<td>0.41</td>
<td>0</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td><em>R. hippoc描oides</em></td>
<td>-7*</td>
<td>3.6</td>
<td>0.02</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td><em>R. impeditum</em></td>
<td>-9.6*</td>
<td>9.6*</td>
<td>-0.03</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td><em>R. irroratum</em></td>
<td>-4.6*</td>
<td>2.3</td>
<td>-0.01</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td><em>R. lepidotum</em></td>
<td>-0.54</td>
<td>3.3*</td>
<td>0.003</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td><em>R. ooretraphes</em></td>
<td>-1.1</td>
<td>1.5</td>
<td>0.03*</td>
<td>44</td>
<td>14</td>
</tr>
<tr>
<td><em>R. phaeochrysum</em></td>
<td>-1.7</td>
<td>2.2*</td>
<td>0.02*</td>
<td>119</td>
<td>50</td>
</tr>
<tr>
<td><em>R. primuliflorum</em></td>
<td>0.31</td>
<td>3.6*</td>
<td>0.02*</td>
<td>75</td>
<td>47</td>
</tr>
<tr>
<td><em>R. racemosum</em></td>
<td>-2.6</td>
<td>1.6</td>
<td>0.02*</td>
<td>53</td>
<td>16</td>
</tr>
<tr>
<td><em>R. rex</em></td>
<td>-3.5</td>
<td>-0.8</td>
<td>0.01</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td><em>R. rubiginosum</em></td>
<td>-8.9*</td>
<td>8.3*</td>
<td>0.02</td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td><em>R. rupeicola</em></td>
<td>-1.9</td>
<td>5*</td>
<td>0.02*</td>
<td>49</td>
<td>18</td>
</tr>
<tr>
<td><em>R. saluenense</em></td>
<td>-1.6</td>
<td>-4</td>
<td>0.01</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td><em>R. warifolium</em></td>
<td>-8</td>
<td>0.75</td>
<td>0.04*</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td><em>R. virgatum</em></td>
<td>2.1</td>
<td>-4.5*</td>
<td>0.03*</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td><em>R. yunnanense</em></td>
<td>-3.8*</td>
<td>5.8*</td>
<td>-0.01</td>
<td>32</td>
<td>14</td>
</tr>
</tbody>
</table>
Table S1. Month of flowering responds significantly to average annual temperatures, fall temperatures, and elevation. The model

$\text{flowering.month} \sim \text{annual.temp} + \text{fall.temp} + \text{elevation}$

was tested for this larger data set of coarser temporal scale by considering month of collection rather than day of collection as our metric for flowering time. The 1,147 collections with day of collection were converted to month of collection (for example, every collection recorded as being made in June was coded as June 15, or day 165), and an additional 1,199 collections for which only information about month of collection could be recovered were added to the dataset. As with $\text{flowering}$, $\text{flowering.month}$ is expressed as deviation from the species mean, and in day units.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.11</td>
<td>0.74</td>
<td>0.88</td>
</tr>
<tr>
<td>$\text{annual.temp}$</td>
<td>-2.10</td>
<td>0.22</td>
<td>$&lt;2 \times 10^{-16}$</td>
</tr>
<tr>
<td>$\text{fall.temp}$</td>
<td>3.16</td>
<td>0.28</td>
<td>$&lt;2 \times 10^{-16}$</td>
</tr>
<tr>
<td>$\text{elevation}$</td>
<td>0.015</td>
<td>0.002</td>
<td>$&lt;2 \times 10^{-16}$</td>
</tr>
</tbody>
</table>

Adjusted $r^2$: 0.09
Table S2. Annual and fall warming remain significant, and similar in sign to the
generic model, when tested against Lijiang weather station (LWS) data for the past 57
years. Here, we used average daily LWS temperatures to compute seasonal and
annual temperature averages as in the generic analysis, and tested for change in
flowering of the 460 specimens collected 1952-2009. Precipitation measures added to
the model, including total previous year precipitation and total spring precipitation,
were not significant.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>9.72</td>
<td>51.8</td>
<td>0.85</td>
</tr>
<tr>
<td>annual.temp.LWS</td>
<td>-0.10</td>
<td>0.37</td>
<td>0.02</td>
</tr>
<tr>
<td>fall.temp.LWS</td>
<td>0.66</td>
<td>0.21</td>
<td>0.002</td>
</tr>
<tr>
<td>elevation</td>
<td>0.017</td>
<td>0.003</td>
<td>5.00 x 10^{-9}</td>
</tr>
<tr>
<td>Adjusted r^2: 0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table S3.** The generic model remains significant when collection time is treated as a weighted mean. To avoid problems with inflated significance values due to multiple collections within years, we tested the generic model against the annual mean flowering, weighted by annual number of collections.

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Std. Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.04</td>
<td>0.85</td>
<td>0.02</td>
</tr>
<tr>
<td>annual.temp</td>
<td>-2.30</td>
<td>0.53</td>
<td>0.00004</td>
</tr>
<tr>
<td>fall.temp</td>
<td>2.57</td>
<td>0.56</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

Adjusted $r^2$: 0.21
Figure 1. Annual average temperatures recorded by the Lijiang weather station (\textit{annual.temp.LWS}) have significantly increased over the past 57 years (0.13 C$^\circ$/decade, $p=0.0001$, $r^2=0.22$).
Figure 2. Flowering time of Himalayan *Rhododendron* responds to annual temperature, fall temperature and elevation. **A)** *flowering* (day of collection deviation from the species mean) regresses negatively (-0.85 days/°C) and significantly (p=0.002) with increasing *annual.temp* (annual average temperature deviation of the year preceding collection). **B)** *flowering* regresses positively (1.26 days/°C) and significantly (p<0.001) with increasing *fall.temp* (temperature deviation of the fall preceding collection). **C)** *flowering* regresses positively (0.15 days/100 meters) and significantly (p<0.001) with increasing *elevation* (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).
Figure S1 (following page). Day of collection deviation from the species mean (flowering) averaged by year (A) and by decade (B) shows no significant relationship with number of collections. This means that while collection intensity varies by year (C) and by decade (D), the variation does not bias flowering. Collections are highest in the 1920s when several prolific early plant hunters exhaustively sampled the area, and decline in recent decades.
Figure S2 (following page). During periods of significant cooling and warming, day of collection deviation (*flowing*) did not change over year although it did significantly respond to average annual temperatures, fall temperatures, and elevation. Average annual temperature deviation (**A**) and average fall temperature deviation (**B**) for the period 1881-1959 decreased, while average annual temperature deviation (**D**) and average fall temperature deviation (**E**) for the period 1960-2009 increased. Flowering time (**C**, **F**) shows no directional change over year during these periods (1881-1959: \( p=0.43 \); 1960-2009: \( p=0.54 \)). For each period, the generic model (**Table 1**), *flowing ~ annual.temp + fall.temp + elevation* is significant, and coefficients are similar in size and direction (1884-1959: \( \text{flowering} = -2.75 \text{annual.temp} + 2.83 \text{fall.temp} + 0.012 \text{elevation}, r^2=0.09, p<0.0001 \); 1960-2009: \( \text{flowering} = -1.46 \text{annual.temp} + 2.15 \text{fall.temp} + 0.017 \text{elevation}, r^2=0.09, p<0.0001 \).
Chapter 2

Phenological progressions of *Rhododendron* flowering over season and elevation

Abstract

Seasonal timing (phenology) of reproduction is a critical dimension of life-history, affecting ecological and evolutionary processes including individual fitness, community interactions, species boundaries and climate change adaptation. Staggered phenological sequences in flowering plants have long been a topic of interest but multi-dimensional progressions have received little attention, especially in the montane and alpine environments that are among the ecosystems most vulnerable to climate change.

We comprehensively monitored flowering phenology in an assemblage of ten co-occurring Himalayan *Rhododendron* species over gradients of 1400 m elevation and 7 months of flowering, measured individual response to weather in 2 years, contextualized the results within a 125-yr model of flowering time derived from herbarium specimens and characterized the effects of phenology on reproductive success.

Phenology is affected by elevation (4.4 – 5.5 days later/100m). Within this general trend, co-occurring *Rhododendron* species are overdispersed in elevation and season of flowering and have less overlap in flowering niche (2.9% overlap) than expected by chance (5.8% – 11.7% overlap). Although higher elevations have the greatest number of co-occurring species, they also evidence greater abiotic constraints, with the shortest flowering period and the greatest intra- and inter-specific synchrony. For both generic and specific models, observed flowering time was predicted by the herbarium-derived
model (>95% of plants flowering within prediction intervals). Flower and fruit quantities were greater in plants which flowered slightly earlier than their population mean, and in plants that responded to warmer weather with slightly earlier flowering, suggesting selection for climate change adaptation. In sum, Himalayan *Rhododendron* show an elaborate sequenced progression of flowering over season and elevation. Phenological response to temperature varies among species, elevation, and season, and both phenology and response to temperature directly impact reproductive success, making this progression vulnerable to future climate change.

**Introduction**

Phenology is a critical dimension of natural history. The abiotic and biotic environments in which key life history stages occur are defined by phenological distribution in time as much as by geographical distribution in space. In plants, reproductive phenology is particularly important, affecting gene flow, population persistence, and species boundaries (Antonovics 2006; Kameyama & Kudo 2009; Devaux & Lande 2009; Ladinig et al. 2013). The staggered sequence of flowering and fruiting among species assemblages has long been a topic of interest (Clarke 1893; Robertson 1895). The community implications of this phenological progression include resource partitioning and the continuous provisioning of pollinators (Waser & Real 1979; Kochmer & Handel 1986; Sherry et al. 2007a) and dispersers (Wheelwright 1985; González-Castro et al. 2012).

Phenology is one of the earliest and most noticeable traits by which organisms respond to climate change (Parmesan & Yohe 2003; Parmesan 2006; Bertin 2009; Wolkovich et al. 2014). There is increasing evidence that temperature affects range limits through
phenology as well as through growth (Chuine 2010; Lenz et al. 2014). Across the globe, plant growth and reproductive phenologies, as well as ranges, have advanced with warming temperatures (Cleland et al. 2007; Wolkovich et al. 2012; Ellwood et al. 2013). However, certain species may not have sufficient plasticity or rate of adaptation to keep pace with changing climate (Visser et al. 2010; Cook, Wolkovich & Parmesan 2012). Insufficient adaptive phenological response has been associated with declines in plant species (Willis et al. 2008; 2010). Among species that do respond, responses are variable and differences are associated with phylogeny (Levin 2006; Lessard-Therrien, Davies & Bolmgren 2014) and invasiveness (Polgar & Primack 2013; Ellwood et al. 2013).

Mechanistically, species respond to varying phenological cues including not only growing season temperatures, but chilling requirements (Schwartz & Hanes 2010; Cook et al. 2012), photoperiod (Keller & Körner 2014) and snowmelt (Price & Waser 2008). In the context of phenological progressions, differential responses to change among species may lead to mismatches among plant species or among plants and animal pollinators, seed dispersers or herbivores (Parmesan 2006, Post et al. 2008, Miller-Rushing et al. 2010, Forrest and Thomson 2011). While a phenological response to these temperature cues ('phenological tracking' or 'temperature sensitivity') can be adaptive, it also may expose species to abiotic threats, including increased variability in temperature (e.g., late frosts) at phenological extremes (Inouye & McGuire 1991; Chuine 2010; Augspurger 2013).

These abiotic constraints are particularly salient in alpine environments (Inouye 2008) where late spring and early fall bracket a short period of time for growth and reproduction (Levesque, Henry & Svoboda 1997; Körner 2003). With the close association of elevation and temperature, gradients of elevation, like those of latitude,
have been used as proxy for climate change (Wang et al. 2014b; a) and as an important element in predictive models of phenology (Krinner et al. 2005; Allen et al. 2014).

However, just as populations at varying latitudes may respond to different selective pressures on phenology (Chuine 2010), so may populations along an elevational gradient. In spite of this, phenological observations explicitly integrating elevational gradients remain limited to a few sites (Inouye 2008; Wolkovich et al. 2012; Cornelius et al. 2013). Similarly, recent attention to fall phenology suggests that seasonal gradients also condition response to warming temperatures, with fall-flowering species showing different responses than spring-flowering species (Sherry et al. 2007a; Fridley 2012). These contrasts are only visible in studies that prioritize whole-season measures of phenology rather than relying solely on first flowering date (Miller-Rushing, Inouye & Primack 2008; CaraDonna, Iler & Inouye 2014) and to a great extent remain unaddressed (Polgar & Primack 2013; Laube et al. 2014).

The eastern Himalaya are the center of distribution for *Rhododendron* and offer an ideal opportunity to explore phenological progressions across seasonal and elevational gradients. *Rhododendron* species are dominant from middle to high elevations and flower from early spring to late summer. *Rhododendron* phenology has implications both to pollinators (including the endemic honeybee *Apis cerana*) and to local people, for whom the ritual importance of *Rhododendron* is tied to its status as the earliest and highest of flowers (Hart & Salick in prep). Eastern Himalayan *Rhododendron* are also extraordinarily well represented in historical plant collections dating to the late 19th century, from which Hart et al. (2014) developed a model of phenological response to temperature variations. Here, we quantitatively monitor flowering phenology over two years in an assemblage of
Himalayan *Rhododendron* species over elevation and season, including interspecific differences, responses to temperature, and the resulting effects on reproductive success.

**Materials and Methods**

**Study site:**
At the far eastern edge of the Himalaya, Mt. Yulong (玉龙雪山, 27.0° N, 100.1° E) is a geographical nexus among the tropical lowlands of SE Asia, the northern temperate vegetation of China, and the alpine Tibetan Plateau (Fig. 1A). It is the southernmost glaciated mountain in Eurasia and within the 'Mountains of Southwest China', a global hotspot of biological diversity (Mittermeier et al. 2004). This area is the world's richest in temperate plant species (Kier et al. 2005), and is the center of diversity for many plant genera (Wen et al. 2014) including *Rhododendron* (Fig. 1A). More than 30% of the world's *Rhododendron* species occur in the area and half of them are endemic (Wu, Raven & Hong 2005). Their species richness is further concentrated in Northwest Yunnan, where Mt. Yulong (Fig. 1B) and other ranges (collectively called the Hengduan Mountains) are separated by the deep gorges carved by the Yangtze, Mekong, and Salween Rivers. The resulting biogeographic isolation creates the high beta-diversity (Salick et al. 2004; Wen et al. 2014) that is an important component of the Hengduan Mountains' species richness.

Mt. Yulong was the home base of the plant-hunters George Forrest and Joseph Rock, whose collections, along with those of later botanists, provide a deep historical context.

On Mt. Yulong (Fig. 2), lower elevations are mostly pine-oak forest with *Rhododendron* understory. With increasing elevation, vegetation grades to oak scrub, alpine meadows, and finally rock cliffs and retreating glaciers (Wang et al. 2007; Kong et al. 2009). At all
elevations, *Rhododendron* species make up a substantial portion of the plant communities, although their life-forms vary depending on elevation and species: shrub understory in forest, small-tree canopy at middle and higher elevations, and dwarf shrub patches in open alpine areas.

We monitored phenological progressions in *Rhododendron* flowering over elevation and time, including interspecific differences, changes with weather and effects on reproductive success.

**Transect structure:**
*Rhododendron* populations to be monitored along the elevational gradient were sampled in a stratified random pattern. Two transects on the eastern slopes of the mountain and two transects on the western slopes were laid from the lowest elevation forests at the edge of cultivated land (2760 masl on the east and 3060 masl on the west) to the local elevational limit of *Rhododendron* species (4060 masl). At every 100 m of elevation along the transects, two monitoring points were set, one directly north and one directly south. Each was offset a random distance between 5 and 20 meters from the transect. This produced 60 points on the east slopes and 44 points on the west slopes (Fig. 1C), at 15 elevations. From each of these points, we calculated density, monitored phenology, and recorded fruit-set for each *Rhododendron* species present (henceforth each ‘population’).

Density was calculated by the variable area transect method (Parker 1979), a powerful and efficient plotless density estimator which performs well with spatially aggregated distributions (Engeman et al. 1994) such as *Rhododendron* populations. From each transect point, we continued directly north or south (approximately along the elevational contour),
establishing a 1.5 m wide subtransect of variable length. For each *Rhododendron* species encountered, the distance along the transect, height and maximum horizontal canopy cover of each plant was measured for the first 4 plants. The density parameter of a given species, in terms of plants per square meter, was calculated as $D=(nr-1)/(w\Sigma l_i)$, where $n$ is the number of subtransects, $r$ is the $n$th plant (here, 4), $w$ is the search width (here, 1.5 m), and $\Sigma l_i$ is the sum of lengths (distances from point) to the $r$th plant. If 50 m along the subtransect were searched encountering 1 – 3 plants, the species was recorded as low density ($D=0.005$), and if no plants were encountered, the species was recorded as absent. Species were identified in the field according to the local plant checklist (Wang et al. 2007). Voucher specimens of each species were collected, identifications confirmed at the herbaria of the Kunming Institute of Botany-Chinese Academy of Sciences (KUN) and the Missouri Botanical Garden (MO) with reference to collections and the Flora of China (Wu et al. 2005). Duplicate specimens were deposited at KUN, MO and the herbaria of the Royal Botanic Garden Edinburgh (E) and Harvard University (A, GH).

For phenological monitoring of *Rhododendron* populations at each point, the nearest 12 plants of each species present were given unique identification tags for repeat monitoring. For the entire flowering period (February to August) in 2012 and 2013, the phenological state of each plant was recorded twice monthly (13 – 17 day intervals). Phenological state was quantified by counting the number of inflorescences on each plant bearing flower buds showing color (hereafter, "budding"), the number of inflorescences bearing flowers in anthesis ("flowering"), the number of inflorescences bearing flowers past anthesis ("past") and, in 2013 only, the number of inflorescences bearing mature fruits ("fruiting").
For each plant, we computed flowering day as the mean of observation dates weighted by number of inflorescences $\sum o_i(f_i/\Sigma f_i)$, where $o_i$ is the day number of each observation, $f_i$ is the number of inflorescences observed on that day for an individual $i$ and $f_i/\Sigma f_i$ is the normalized number of inflorescences. Infrequently, we observed past inflorescences but no flowering inflorescences; for these plants we assigned flowering day as the midpoint between the date when past flowers were observed and the previous observation date.

**Flowering across elevation and season:**
To test for the effects of elevation on phenology, we constructed linear regressions of plant flowering day with elevation across the genus and within each species.

To test for overdispersion of flowering among species, we constructed a Poisson family generalized linear model (glm) of the number of species flowering as a response to elevation and month, and tested for a significant difference from equidispersion. To further elaborate dispersion, we measure niche overlap following the methods of Albrecht and Gotelli (2001). We categorized species by season as early-season (mean flowering before Julian day 130), mid-season (between days 130 and 160) and late-season (after day 160). In each combination of the 14 elevations and three seasons, we calculated a Czechanowski overlap index (Feinsinger, Spears & Poole 1981) for each pair of species as $1-0.5\Sigma (p_1; p_2)$, where $p_1; p_2$ are the proportions of populations of species 1 and 2, respectively, with their mean flowering at an elevation-season combination $i$. We then used a permutation test to assess significance on the basis of 1,000 randomizations in which records were reassigned within species across elevation-season combinations (Albrecht & Gotelli 2001).
To examine how elevation affects variability, we used linear regression to model response to elevation of the standard deviation of flowering day within populations and among species at the same elevations. Individual variance was calculated as the deviation of the flowering day of an individual from the mean flowering day for the population:

$$\sigma(f_i) = f_i - \bar{f}/12$$

where $f_i$ is the flowering day of the individual and $\bar{f}/12$ is the mean flowering day for the 12 plants within the population.

**Phenology and temperature:**

We compared observed flowering times against our model for temperature effects on *Rhododendron* phenology (Hart et al. 2014). In this generic model, derived from herbarium specimens collected between 1885 – 2009, flowering day responds to deviations in:

- preceding-year mean annual temperature (warming effect: 2.26 days earlier/°C),
- preceding-year mean fall temperature (chilling effect: 2.54 days later/°C), and elevation (1.4 days later/100 meters). We applied the model to weather data from the Lijiang weather station of the China Meteorological Administration to predict flowering day on Mt. Yulong. Hart et al. (2014) also report specific models with their own parameters for flowering day for nine of the ten species monitored here. For the genus, and for each of these species, we compared the model predictions for flowering day in 2012 and for 2013 with the observed mean flowering day in those years. As a measure of fit, we calculated the percentage of plants whose flowering day was within the 95% prediction interval of the models.

We calculated variation between the years as the difference between the flowering day of an individual plant between the years 2012 and 2013 ($f_{2013} - f_{2012}$). This inter-annual...
difference was taken as a measurement of temperature sensitivity. Because these responses to inter-annual temperature change are relatively small compared to differences among species and elevations, we used a paired Wilcoxon analysis to test for significant differences in flowering day of individuals in 2012 and 2013. We also tested if the amount of temperature sensitivity responded to elevation by constructing a hierarchical linear mixed model of temperature sensitivity over elevation, blocked within species.

**Reproductive effects:**
To explore the reproductive effects of phenological deviation, we constructed Poisson glms of two reproductive measures: total inflorescences (the number of past inflorescences observed on an individual at the end of anthesis) and fruiting inflorescences. Each were modeled in response to the phenological deviation in 2012 and 2013 of each individual flowering day from the population mean flowering day. To test the effects of temperature sensitivity on reproduction, we constructed similar Poisson glms to explain total inflorescences and total fruits with temperature sensitivity as an explanatory variable. We modeled both phenological deviation and temperature sensitivity as linear and quadratic terms to account for potential effects of earlier and later variance.

Linear and glm modeling and permutation analyses used the base package of the statistical framework R 3.1.1 (Venables & Ripley 2002) and dispersion testing used the R package AER (Kleiber & Zeileis 2008).
Results

There is strong evidence for sequenced flowering progressions of *Rhododendron* species on Mt. Yulong, structured by season and elevation. Flowering time responds to inter-annual temperature differences, and is well predicted by a specimen-derived model of temperature effects on phenology. Both phenology and response to inter-annual temperature differences affect individual reproductive fitness.

Flowering across elevation and season:

At the 104 monitoring points along the transects, 113 populations, represented by 1,356 plants (12/population), were measured in each year (Table 1). Ten *Rhododendron* species were found on the transects (Fig. 3), and the number of *Rhododendron* species co-occurring around a point ranged from 0 to 4. The number of co-occurring species increased with elevation \( y=0.0007x-0.6959, \ p=0.006, \ r^2=0.09 \). Simple linear regression of the genus showed a significant relationship of later flowering (increasing Julian day) at higher elevations (2012: \( y=0.055x-53.87, \ p<0.001, \ r^2=0.36 \); 2013: \( y=0.044x-17.99, \ p<0.001, \ r^2=0.33 \)) as well as for most species in both years (Fig. 6).

*Rhododendron* species and flowering were widely distributed in space and time (Table 1). Mean flowering among *Rhododendron* populations ranged from February to August (Julian day 88 – 218) and the elevations at which species had their highest densities ranged from 3360 – 3960 m asl. A fitted Poisson glm for number of species flowering by elevation, month and their interaction \( y=-1.543x-0.002z+0.0004xz \) was significantly overdispersed (\( \alpha=0.37, \ p=0.01 \), confirming greater spread among species in elevation and season than expected by chance. Each seasonal cohort had high-elevation
species, mid-elevation species, and low-elevation species (Fig. 4). The observed overlap among flowering niches defined by elevation and season was 2.9%, significantly lower than the expected overlap based on a randomized null model, which was 8.5% (confidence interval 5.8% – 11.7%) (Fig. 5). These patterns create an elaborate phenological progression over time and elevation.

Three measures of synchrony in flowering increased with elevation. First, higher elevations were significantly associated with smaller standard deviations of flowering day within populations (y=-0.002x+13.7, p=0.013, r²=0.03) (Fig. 7A). Second, standard deviation among populations of different species around the same monitoring point also decreased with increasing elevation (y=-0.029x+122.4, p<0.001, r²=0.43) (Fig. 7B). Third, the absolute value of individual deviation from the population mean decreased with increasing elevation (linear mixed effects model across-species fit values 2012: y=-0.004x+18.92, p=0.001; 2013: y=-0.004x+21.47, p<0.001).

**Phenology and temperature:**
In both 2012 and 2013, observed phenology was comparable to that predicted from the herbarium-derived model. Lijiang weather station data showed preceding-year mean annual temperature (to account for overall warming) and preceding-year mean fall temperature (to account for chilling) respectively for 2012 to be 13.04 °C and 15.90 °C, and for 2013 to be 13.81 °C and 16.04 °C. As deviations from the long-term mean, these are +2.43 °C and +2.51 °C in 2012 and +3.20 °C and +2.64 °C in 2013. Using these temperature deviations, flowering day was predicted for each elevation with the generic model, and for each species model (Table 2, Fig. S1). In both years, nearly all plants observed had a flowering day within the 95% model prediction intervals (99% of plants
within the prediction intervals in 2012 and 96% in 2013). Within these margins, plants tended to flower slightly earlier than predicted: in 2012 the mean observed generic flowering time was 6 days earlier than the predicted flowering time and in 2013 it was 8 days earlier. This trend across the genus was also evidenced for most species (Table 2), with *R. yunnanense* a notable exception.

Among individuals that flowered in both years, flowering day in 2013 was significantly earlier (Wilcoxon paired analysis p<0.001) by -2.36 ± 0.91 days. This response to temperature decreased with elevation: the phenological response to inter-annual temperature differences was significantly less at higher elevations (linear mixed effects model fit values y=-0.007x+33.98, p=0.001). Within species, flowering was earlier in 2013 (Wilcoxon paired p<0.05): for *R. beesianum* (-8.1), *R. oreotrephes* (-3.4), *R. adenogynum* (-8.0), and *R. lepidotum* (-26) days. Only *R. yunnanense* flowered significantly later in 2013 (by +2.0 days).

**Reproductive effects:**
The number of flowering inflorescences and fruiting inflorescences was greater in plants with flowering day slightly earlier than their population mean flowering, and in plants that flowered slightly earlier in 2013 than 2012. Poisson glms showed reproductive metrics to respond significantly both to the deviation of a plant from the mean of the population and to phenological differences between years. Later flowering day, as well as deviation from the population mean flowering day in either direction, reduced quantity of both flowering inflorescences (y=-0.032x-0.001x^2-0.071, p=0.02; Fig. 8A) and fruiting inflorescences (y=-0.041x-0.002x^2-0.327, p=0.005; Fig. 8B). Similarly, later flowering day
of a plant in 2013 than in 2012 as well as greater difference between years in either direction reduced quantity of both flowering inflorescences \( (y=-0.022x-0.001x^2-0.055, \quad p=0.009; \quad \text{Fig. 8C}) \) and fruiting inflorescences \( (y=-0.029x-0.001x^2-0.136, \quad p<0.001; \quad \text{Fig. 8D}) \).

**Discussion**

**Flowering across elevation and season:**
In the easternmost Himalaya, *Rhododendron* spp. flower throughout the warmer months from February to August. Their density and dominance create masses of flowers with a salience that extends beyond *Rhododendron* reproduction to impact both pollinator provisioning and human culture. This flowering profusion is structured by elevation, season and species, creating an elaborate phenological progression. Species flower in distinct seasons, with a set of three early-season species, four mid-season species, and three late flowering species. Simultaneously, these species are divided into a progression of elevations (Fig. 4). At lower elevations (~3000 m), we see the early-season species followed by mid-season species. Intermediate elevations (~3500m), have the longest sequence of flowering, from early-season to late-season species. This likely reflects the greatest duration of appropriate temperatures, although temporal mid-domain effects have been proposed in other studies (Morales, Dodge & Inouye 2005). High elevations (~4000 m), with greater abiotic constraints, have the most truncated flowering season (Fig. 7), but still evidence a distinct seasonal progression. The changing bands of flower color over the season that show this coordinated phenological progression has been noted in the past. Looking at Mt. Yulong from a distance one can distinguish: Victorian plant-hunter Frank Kingdon-Ward wrote eloquently of the *Rhododendron* spp. that cover these
mountains in “a tidal wave of dense colours which gleam and glow in leagues [and] leap at you as you climb” (Kingdon-Ward 1923). This impressive display inspired Kingdon-Ward and successive generations of collectors (Wagner 1992; Mueggler 2005; Glover et al. 2011; Mueggler 2011) to make the super-numerous collections that make comparative studies today so fruitful (Hart et al. 2014). It also inspires the local Yi and Naxi peoples to attribute spiritual import and practical agricultural timing to *Rhododendron* phenology (Hart & Salick in prep).

Flowering progressions such as these have been suggested to reflect evolutionary and ecological pressures, including temporal and spatial barriers to hybridization (Stiles 1979, Antonovics 2006; Levin 2006; Devaux & Lande 2009) and/or the partitioning of flowering across time as a resource (i.e., competition for pollination service) (Waser & Real 1979; Kochmer & Handel 1986; Sherry et al. 2007a). Indeed, we found the overlap among species in season-elevation flowering niche to be much less than that expected by chance (Fig. 5).

However, at higher elevations, we found greater interspecific flowering synchrony (Fig. 7) and more co-occurring species, despite the shorter flowering period. This is further evidence for the increased importance of abiotic constraints at these elevations, which is also supported by the reduced response of phenology to inter-annual temperature differences at higher elevations. Other studies have also suggested reduced biotic pressures such as competition for pollinators at higher elevations (Callaway et al. 2002; Ghazoul 2006).
**Phenology and temperature:**

Elevation is a common proxy for temperature (‘adiabatic lapse rate’, generally reported as 0.6°C/100 m, e.g., Nagy and Grabherr 2010), and we found that lower-elevation populations of *Rhododendron* species flower earlier. Additionally, *Rhododendron* as a genus and most species flowered earlier with warmer temperatures in 2013. In our study, the change in flowering observed over elevation (Fig. 6) was proportional to the modeled and observed changes in flowering with weather (Table 2). As measured here, both the changes in phenology over elevation and across years are greater than predicted from the herbarium-derived model. Likewise, although observed flowering was within the prediction interval of the herbarium model, across the genus and for most species observed flowering was slightly earlier than the predicted dates (Table 2). Potential causes of this additionally advanced flowering are several: the greater power available from comprehensive ecological sampling, temporal scale effects (Wolkovich et al. 2014), or a nonlinear response to temperature increases as climate change takes its toll (Iler et al. 2013).

The role of local adaptation to elevation is also suggested by our results. If the phenological differences along the elevational gradient are taken as a response to temperature differences of 0.6 °C/100 m, then they represent a change of 6.5 – 9.5 days/1 °C, greater than either the observed or modeled responses to weather. This parallels other studies that have shown differences across elevations to exceed those expected calculated by adiabatic lapse rate (Cornelius et al. 2013), as well as reciprocal transplant experiments that show differential responses in plants from high and low-elevation populations (Wang et al. 2014a), and genetic studies showing adaptive variation
across elevation in *Rhododendron* populations (Hsieh et al. 2013). With the previous results on temperature, perhaps together these argue that a non-linear response to temperature is the most likely explanation for additionally advanced flowering.

For most of these generic patterns, *R. yunnanense* may be the exception that proves the rule. *R. yunnanense* flowered later than predicted by the specimen-derived model; flowered later, rather than earlier, with warmer temperatures in 2013; and did not show a significant change in flowering time with elevation (Fig. 6). Unique among the *Rhododendron* species on Mt. Yulong, *R. yunnanense* has fully deciduous foliar phenology and flowers before leafing Deciduousness has been connected with leaf-out phenology (Panchen et al. 2014), and this distinct life-history may be connected to its unusual responses to temperature seen here. Similarly, *R. yunnanense* also flowered later with warmer temperatures in studies done at Royal Botanic Gardens Edinburgh 2002 – 2009 (Harper 2010).

**Reproductive effects:**
Flowering phenology may directly affect plant fitness through biotic pressures on reproduction (e.g., pollination success or seed predation, Augspurger 1981) as well as abiotic pressures (e.g., fruit development). Our results show that plants that flowered much earlier or later than the population mean had fewer flowers and fewer fruits. These results may reflect biotic limitations if pollinators are unavailable, or abiotic limitations to phenology, as expected with normalizing selection (Devaux & Lande 2008; Koenig et al. 2012). Similarly, plants that respond to inter-annual differences in temperature in the direction that might be expected, flowering earlier with the warmer temperatures in 2013,
had greater reproductive success than those that flowered later. This supports the interpretation of 2012 – 2013 phenological changes as adaptive sensitivity to temperature. However, just as large deviations from the population mean were associated with lower reproductive success, so were extreme responses to inter-annual changes in temperature in either direction, suggesting that only a measured phenological response to temperature is adaptive. For *Rhododendron*, reproductive success is enhanced by temperature sensitivity but can also be reduced by too much variability from the phenological progression over season and elevation. Similar effects have been seen in studies where flowering time is modified through elevational transplanting (Scheepens & Stöcklin 2013). Alternatively, this could reflect the influence of biotic forces, as is the case when plants, and not their pollinators, respond to inter-annual temperature changes (Cleland et al. 2007), or physiological pressures, as when different phenological cues or different adaptive pressures drive contrasting responses (Sherry et al. 2007b; Cook et al. 2012; Hart et al. 2014).

**Conclusion:**

Despite the importance of phenology, phenological records often rely on simple measures such as first flowering date (Fitter & Fitter 2002; Cook et al. 2012; Wang et al. 2014a). More rigorous exploration of phenological distributions within individuals and within populations is lacking (Miller-Rushing et al. 2008; Forrest, Inouye & Thomson 2010; Tooke & Battey 2010), but often reveals complex responses (CaraDonna & Inouye 2015). Little attention has been paid to the importance of differential changes along elevational gradients, even while comparisons between early-season and late-season phenology has
become increasingly of interest (Sherry et al. 2007a; Wolkovich et al. 2012; Cornelius et al. 2013). Our attention to means and variations, and to gradients of elevation and season allowed exploration of differences in synchrony and of differential pressures on high-elevation and late-season flowering. Likewise, studies that are able to bring both rigorous field observation and long-term herbarium records to bear on phenological response to inter-annual temperature differences remain rare (Robbirt et al. 2011). In our work (Hart et al. 2014) and herein, similar patterns between two distinct data sources and temporal scales increases our confidence in each. Especially important in predicting the effects of future climate change, studies showing direct connections between phenology and reproductive success are comparatively rare, and tend to reveal very different patterns in different ecosystems (Chuine 2010). In Himalayan Rhododendron, we see a sequenced progression of Rhododendron species widely dispersed along gradients of season and elevation. Individuals respond to inter-annual changes in temperature in a manner that parallels that inferred from herbarium collections. Variations in both phenological progression and in response to inter-annual temperature directly impact reproductive success.

**Acknowledgements**

We thank the staff of the Jade Dragon Field Station and associated Lijiang Forest Ecosystem Research Station, and acknowledge gratefully the foresight of the Kunming Institute of Botany-Chinese Academy of Sciences and the Royal Botanic Garden Edinburgh who jointly established these facilities. Research was supported by the Biodiversity Conservation and Sustainable Development in Southwest China United
States National Science Foundation–Integrative Graduate Education and Research Traineeship DGE 0549369, the Whitney R. Harris World Ecology Center, and the Explorers Club.
References


Table 1 (following 2 pages). Summary metrics by elevation and species: density (plants/m²); number of populations monitored (each population consists of 12 plants), and for 2012 and 2013: percentage of plants flowering and the mean and standard deviation of flowering day of individuals.
<table>
<thead>
<tr>
<th>Elevation</th>
<th>Density (plants/m²)</th>
<th>N</th>
<th>2012 % Plants flowering</th>
<th>2012 mean flowering day ± sd</th>
<th>2013 % Plants flowering</th>
<th>2013 mean flowering day ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>R. racemosum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2760</td>
<td>0.29</td>
<td>48</td>
<td>44</td>
<td>88 ±7</td>
<td>15</td>
<td>90 ±8</td>
</tr>
<tr>
<td>2860</td>
<td>0.03</td>
<td>48</td>
<td>42</td>
<td>93 ±8</td>
<td>42</td>
<td>124 ±15</td>
</tr>
<tr>
<td>2960</td>
<td>0.34</td>
<td>24</td>
<td>46</td>
<td>93 ±7</td>
<td>21</td>
<td>86 ±7</td>
</tr>
<tr>
<td>3060</td>
<td>0.12</td>
<td>48</td>
<td>56</td>
<td>91 ±9</td>
<td>42</td>
<td>98 ±28</td>
</tr>
<tr>
<td>3160</td>
<td>0.005</td>
<td>48</td>
<td>48</td>
<td>115 ±34</td>
<td>65</td>
<td>109 ±28</td>
</tr>
<tr>
<td>3260</td>
<td>0.10</td>
<td>72</td>
<td>42</td>
<td>113 ±8</td>
<td>63</td>
<td>134 ±14</td>
</tr>
<tr>
<td>3360</td>
<td>0.82</td>
<td>48</td>
<td>92</td>
<td>118 ±4</td>
<td>85</td>
<td>112 ±5</td>
</tr>
<tr>
<td>3460</td>
<td>0.005</td>
<td>36</td>
<td>72</td>
<td>134 ±7</td>
<td>44</td>
<td>130 ±9</td>
</tr>
<tr>
<td><strong>R. rubiginosum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3160</td>
<td>0.005</td>
<td>48</td>
<td>52</td>
<td>107 ±7</td>
<td>50</td>
<td>111 ±10</td>
</tr>
<tr>
<td>3260</td>
<td>0.005</td>
<td>48</td>
<td>25</td>
<td>103 ±6</td>
<td>58</td>
<td>113 ±15</td>
</tr>
<tr>
<td>3360</td>
<td>0.005</td>
<td>24</td>
<td>58</td>
<td>118 ±6</td>
<td>75</td>
<td>113 ±8</td>
</tr>
<tr>
<td>3460</td>
<td>0.04</td>
<td>60</td>
<td>50</td>
<td>131 ±7</td>
<td>55</td>
<td>134 ±11</td>
</tr>
<tr>
<td>3560</td>
<td>0.48</td>
<td>72</td>
<td>63</td>
<td>130 ±5</td>
<td>83</td>
<td>124 ±7</td>
</tr>
<tr>
<td>3660</td>
<td>0.25</td>
<td>36</td>
<td>28</td>
<td>137 ±9</td>
<td>75</td>
<td>138 ±11</td>
</tr>
<tr>
<td><strong>R. beesianum (C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3760</td>
<td>0.04</td>
<td>24</td>
<td>79</td>
<td>127 ±6</td>
<td>75</td>
<td>115 ±6</td>
</tr>
<tr>
<td>3860</td>
<td>0.005</td>
<td>24</td>
<td>63</td>
<td>132 ±4</td>
<td>75</td>
<td>136 ±6</td>
</tr>
<tr>
<td><strong>R. yunnanense</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3060</td>
<td>0.005</td>
<td>24</td>
<td>71</td>
<td>132 ±5</td>
<td>38</td>
<td>139 ±8</td>
</tr>
<tr>
<td>3160</td>
<td>0.005</td>
<td>24</td>
<td>63</td>
<td>158 ±19</td>
<td>42</td>
<td>150 ±6</td>
</tr>
<tr>
<td>3260</td>
<td>0.04</td>
<td>24</td>
<td>71</td>
<td>142 ±6</td>
<td>79</td>
<td>144 ±6</td>
</tr>
<tr>
<td>3360</td>
<td>0.34</td>
<td>24</td>
<td>75</td>
<td>142 ±8</td>
<td>88</td>
<td>145 ±7</td>
</tr>
<tr>
<td>elevation</td>
<td>density (plants/m²)</td>
<td>N</td>
<td>2012 % flowering</td>
<td>2012 mean flowering day +/− sd</td>
<td>2013 % flowering</td>
<td>2013 mean flowering day +/− sd</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td>---</td>
<td>------------------</td>
<td>-------------------------------</td>
<td>------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td><strong>R. oreotrephes (E)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3460</td>
<td>0.50</td>
<td>48</td>
<td>77</td>
<td>151 +8</td>
<td>58</td>
<td>146 +5</td>
</tr>
<tr>
<td>3560</td>
<td>0.38</td>
<td>24</td>
<td>67</td>
<td>154 +7</td>
<td>50</td>
<td>147 +5</td>
</tr>
<tr>
<td>3660</td>
<td>0.03</td>
<td>36</td>
<td>33</td>
<td>151 +7</td>
<td>47</td>
<td>155 +5</td>
</tr>
<tr>
<td>3760</td>
<td>0.005</td>
<td>12</td>
<td>100</td>
<td>148 +3</td>
<td>100</td>
<td>152 +4</td>
</tr>
<tr>
<td><strong>R. traillianum (F)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3560</td>
<td>0.005</td>
<td>12</td>
<td>75</td>
<td>129 +2</td>
<td>58</td>
<td>130 +3</td>
</tr>
<tr>
<td>3660</td>
<td>0.005</td>
<td>36</td>
<td>75</td>
<td>138 +4</td>
<td>83</td>
<td>133 +5</td>
</tr>
<tr>
<td>3760</td>
<td>0.05</td>
<td>24</td>
<td>67</td>
<td>141 +3</td>
<td>75</td>
<td>143 +4</td>
</tr>
<tr>
<td>3860</td>
<td>0.005</td>
<td>24</td>
<td>25</td>
<td>142 +3</td>
<td>79</td>
<td>148 +2</td>
</tr>
<tr>
<td>4060</td>
<td>0.005</td>
<td>12</td>
<td>58</td>
<td>151 +4</td>
<td>92</td>
<td>150 +2</td>
</tr>
<tr>
<td><strong>R. adenogynum (G)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3960</td>
<td>0.27</td>
<td>36</td>
<td>81</td>
<td>158 +6</td>
<td>86</td>
<td>156 +5</td>
</tr>
<tr>
<td>4060</td>
<td>0.01</td>
<td>36</td>
<td>75</td>
<td>161 +5</td>
<td>75</td>
<td>160 +6</td>
</tr>
<tr>
<td><strong>R. lepidotum (H)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3160</td>
<td>0.52</td>
<td>24</td>
<td>100</td>
<td>209 +7</td>
<td>67</td>
<td>184 +6</td>
</tr>
<tr>
<td>3460</td>
<td>0.89</td>
<td>24</td>
<td>63</td>
<td>218 +3</td>
<td>42</td>
<td>197 +7</td>
</tr>
<tr>
<td><strong>R. impeditum (I)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3560</td>
<td>0.005</td>
<td>12</td>
<td>92</td>
<td>144 +11</td>
<td>92</td>
<td>153 +7</td>
</tr>
<tr>
<td>3660</td>
<td>0.37</td>
<td>36</td>
<td>97</td>
<td>167 +25</td>
<td>92</td>
<td>156 +9</td>
</tr>
<tr>
<td>3760</td>
<td>0.005</td>
<td>12</td>
<td>100</td>
<td>165 +9</td>
<td>83</td>
<td>158 +8</td>
</tr>
<tr>
<td>3860</td>
<td>0.23</td>
<td>36</td>
<td>92</td>
<td>165 +12</td>
<td>100</td>
<td>163 +8</td>
</tr>
<tr>
<td>3960</td>
<td>0.13</td>
<td>24</td>
<td>88</td>
<td>171 +10</td>
<td>75</td>
<td>167 +7</td>
</tr>
<tr>
<td><strong>R. primuliflorum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3960</td>
<td>0.81</td>
<td>36</td>
<td>92</td>
<td>165 +7</td>
<td>92</td>
<td>167 +7</td>
</tr>
<tr>
<td>4060</td>
<td>0.01</td>
<td>36</td>
<td>98</td>
<td>165 +7</td>
<td>85</td>
<td>168 +8</td>
</tr>
</tbody>
</table>
Table 2. Modeled and observed phenologies are comparable.

Predicted flowering day is derived from the generic and specific models derived from herbarium specimens (Hart et al. 2014), parameterized with preceding-year annual and fall temperatures. The mean of the predictions made for each elevation (Fig. S1) is similar to mean flowering observed in the transects. The percentage of plants with an observed flowering day within the 95% prediction intervals of the herbarium-derived model (% fit) is close to 100% with the exception of *R. beesianum* in 2013.

<table>
<thead>
<tr>
<th>Rhododendron species</th>
<th>Predicted flowering day 2012</th>
<th>Observed flowering day 2012</th>
<th>% fit 2012</th>
<th>Predicted flowering day 2013</th>
<th>Observed flowering day 2013</th>
<th>% fit 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus</td>
<td>156</td>
<td>150</td>
<td>96%</td>
<td>154</td>
<td>146</td>
<td>99%</td>
</tr>
<tr>
<td><em>R. racemosum</em> (A)</td>
<td>129</td>
<td>102</td>
<td>95%</td>
<td>127</td>
<td>110</td>
<td>93%</td>
</tr>
<tr>
<td><em>R. rubiginosum</em> (B)</td>
<td>148</td>
<td>118</td>
<td>100%</td>
<td>142</td>
<td>120</td>
<td>100%</td>
</tr>
<tr>
<td><em>R. beesianum</em> (C)</td>
<td>155</td>
<td>131</td>
<td>100%</td>
<td>154</td>
<td>125</td>
<td>50%</td>
</tr>
<tr>
<td><em>R. yunnanense</em> (D)</td>
<td>140</td>
<td>143</td>
<td>100%</td>
<td>138</td>
<td>144</td>
<td>100%</td>
</tr>
<tr>
<td><em>R. oreotrephes</em> (E)</td>
<td>164</td>
<td>153</td>
<td>100%</td>
<td>163</td>
<td>150</td>
<td>100%</td>
</tr>
<tr>
<td><em>R. adenogynum</em> (G)</td>
<td>174</td>
<td>164</td>
<td>100%</td>
<td>168</td>
<td>156</td>
<td>100%</td>
</tr>
<tr>
<td><em>R. lepidotum</em> (H)</td>
<td>195</td>
<td>213</td>
<td>100%</td>
<td>195</td>
<td>186</td>
<td>100%</td>
</tr>
<tr>
<td><em>R. impeditum</em> (I)</td>
<td>168</td>
<td>163</td>
<td>99%</td>
<td>162</td>
<td>160</td>
<td>100%</td>
</tr>
<tr>
<td><em>R. primuliflorum</em> (J)</td>
<td>181</td>
<td>167</td>
<td>100%</td>
<td>181</td>
<td>166</td>
<td>100%</td>
</tr>
</tbody>
</table>
Figure 1 (following page). Transects on Mt. Yulong sample a hotspot of Rhododendron species richness.

A) *Rhododendron* species richness is concentrated in Southwest China, particularly in the Hengduan Mtns. where northwest Yunnan meets southeast Tibet and southwest Sichuan (compiled from species location data in *Flora of China* (Wu et al. 2005). B) Mt. Yulong is located in Northwest Yunnan at the center of this hotspot of *Rhododendron* species richness. C) Two transects each were constructed on the east and west aspects of Mt. Yulong from the lowest elevation forests at the edge of cultivated land (2760 masl on the east and 3060 m asl on the west) to the local elevational limit of *Rhododendron* species (4060 masl).
Figure 2. Elevation structures vegetation communities on Mt. Yulong.

High agricultural fields grade into pine-oak forest, oak scrub, alpine meadows and limestone outcroppings. Pictured here are the eastern slopes, which range from the Lijiang Basin (2600 masl) in the foreground to the receding Yanggong Glacier (~5000 masl) and Satseto Peak (5596 masl) in the background.
Figure 3 (following pag). Ten *Rhododendron species* were monitored along gradients of season and elevation: **A)** *R. racemosum* Franch., **B)** *R. rubiginosum* Franch., **C)** *R. beesianum* Diels, **D)** *R. yunnanense* Franch., **E)** *R. oreotrephes* W.W. Sm., **F)** *R. traillianum* Forrest & W.W. Sm., **G)** *R. adenogynum* Diels, **H)** *R. lepidotum* Wall. Ex G. Don, **I)** *R. impeditum* Ball. f. & W.W. Sm., **J)** *R. primuliflorum* Bureau & Franch.
Figure 4 (following page). Phenological progression over season and elevation. In both 2012 and 2013, flowering of Rhododendron species at a given season are dispersed over elevation, and species within an elevational band flower sequentially. Each pane contains populations (12 individuals each, total N given in Table 1) of one Rhododendron species with letters coding species as in Fig. 3. Colours indicate seasonal cohorts based on species mean flowering time: yellow: early-season (before Julian day 130), purple: mid-season (between day 130 and 160), and green: late-season (after day 160).
Figure 5. *Rhododendrons* overlap less than expected by chance. The mean overlap between elevation-season flowering niche (the proportion of populations of a species flowering in each combination of the 14 elevations and three seasons) for all observed species pairs was only 2.9%. This is significantly lower than the overlaps for 1,000 simulated randomized populations (black histogram, mean 8.5%, confidence interval 5.8 – 11.7%) for which records were reassigned within species.
**Figure 6. Time of *Rhododendron* flowering is delayed with higher elevation.**

Linear regressions for all *Rhododendron* species combined (bold lines) show significantly later mean flowering day at higher elevations. Separate regressions for most species (regression lines with letters coding species as in Fig. 3) show a similarly later mean flowering day at higher elevations (nonsignificant species regressions shown as dotted lines, full regression equations for all spp. in Table S1).
Figure 7. With higher elevations, flowering synchrony increases — a decrease in standard deviation (sd) of flowering day — both within and among species. A) Within populations of a species, flowering day sd decreased with increasing elevation ($y = -0.002x + 13.70, p = 0.013, r^2 = 0.025$). B) At sites with more than one species, mean flowering day sd among species significantly decreased with increasing elevation ($y = -0.029x + 122.4, p < 0.001, r^2 = 0.432$).
Figure 8 (following page). Reproductive advantage of earlier flowering and fruiting. Number of flowers and fruits is greater in plants with flowering day slightly earlier than their population mean flowering, and in plants that flowered slightly earlier in 2013 than 2012. Poisson glms (blue line) showed that plants with positive deviation (flowering day later than the population mean) and plants with large deviation in either direction showed A) reduced quantity of flowering inflorescences (normalized by species; \( p=0.02 \)) and B) reduced quantity of fruiting inflorescences (normalized by species; \( p=0.005 \)). Plants with positive interannual difference (later flowering day of a plant in 2013 than 2012) and plants with large inter-annual difference in either direction showed C) reduced quantity of flowering inflorescences (\( p=0.009 \)) and D) reduced fruiting inflorescences (\( p<0.001 \)).
Figure S1 (following page). Herbarium-derived model predicts observed phenology. We applied models for temperature effects on *Rhododendron* species phenology derived from historical herbarium collections between 1885 – 2009 (Hart et al. 2014) to weather data at each elevation for 2012 and 2013 (horizontal panes) and for each species (vertical panes, with letters coding species as in Fig. 3). Model predictions (black regression lines) are comparable with observed flowering (blue circles and blue regression lines) (Table 2). Although observations are slightly earlier than predicted, only *R. beesianum* (C) in 2013 was significantly outside the model prediction interval, flowering much earlier.
Chapter 3

Phylogeny constrains phenology of last flowering in Himalayan Rhododendron

Abstract

How and to what degree phylogeny affects phenology and phenological temperature sensitivity remain open questions, with implications for understanding community assembly and predicting responses to climate change. We explored phylogenetic signal in phenology of Himalayan Rhododendron spp. at the center of their distribution, on Mt. Yulong, Yunnan Province, China. We constructed a community phylogeny based on trnL-trnL-trnF, atpH-I, RPB2-I (3F-4R) and ITS 4-5, and gathered trait data along a 1400 m elevational transect. Uniquely among phenological traits, last flowering day showed a phylogenetic signal. Last flowering day was significantly clustered (Blomberg’s K=1.29, p=0.03), as were the morphological traits flower size (K=1.2, p=0.03) and fruit size (K= 1.63, p=0.01). The clade representing species on Mt. Yulong with the latest last flowering day also has the smallest flowers and fruits. Across the phylogeny, smaller fruit sizes are significantly correlated with a later last flowering day (p=0.046), but not with mean flowering day (p=0.16) or first flowering day (p=0.29), and we suggest that this pattern may be driven by the limited time for fruit development before the onset of cold temperatures in autumn. Further evidence for this is provided by data from the Flora of China for 160 Yunnan Rhododendron spp., for which later last flowering month was significantly correlated with a shorter span of time between flowering and fruiting (p<0.001, $r^2 = 0.35$) and with smaller flowers ($-0.5$ cm/month, p<0.001, $r^2 = 0.10$) and smaller fruits ($-0.4$ cm/month, p<0.001, $r^2 = 0.10$).
0.09). First flower and peak flower, common measures of phenology, did not show phylogenetic signal in our *Rhododendron* community, nor did measures of temperature sensitivity. The finding of phylogenetic signal and morphological correlates in last, but not first flowering day underscores the importance of quantifying phenology as a series of dates, rather than with a single measure, and illustrates the unique pressures on the beginning and end of phenology both within species and across the season.

**Introduction**

mechanistic interpretations as phylogenetic constraint (Bolmgren and Cowan 2008),
they can help us to understand how species may be able, or unable, to change
phenology.

Historically, early-season measures of salient spring phenology such as first
flowering day have been common (Fitter and Fitter 2002, Parmesan and Yohe 2003,
Primack et al. 2009), but there is an increasing recognition of the importance of more
comprehensive measures of phenological components (Miller Rushing et al. 2008,
CaraDonna et al. 2014). These include the beginning, mean, and end of phenological
events within individuals and species (e.g., first flowering day, mean flowering day, last
flowering day) and across species (e.g., spring, summer, and autumn flowering), and
across sequenced phenological events in ecosystems (e.g., flowering, leafout, leaf
dehiscence, fruiting) (Fridley 2012, Wolkovich and Cleland 2014, Gallinat et al.
2015). Few studies have looked comprehensively at these components of phenology in
relation to phylogeny (but see CaraDonna and Inouye 2015), even as it is understood
that both intra-specific and intra-annual components may be affected by different

We investigated phylogenetic patterns in reproductive phenology and associated
traits in Himalayan *Rhododendron*, a large genus of Ericaceae with ~1000 spp.
worldwide. *Rhododendron* diversity is concentrated in the eastern Himalayas, where the
major subgenera *Rhododendron* and *Hymenanthes* are centered (Chamberlain 1982, Wu
et al. 2005). These subgenera have been divided on the basis of morphological
characters into numerous sections and subsections which are commonly used for
Rhododendron spp. make up an important component of plant communities at all elevations (2700 – 4100 m asl). They flower in a determined sequence across gradients of season and elevation, and their reproductive phenology responds to annual changes in temperature (Hart et al. 2014, Hart et al. in press). Further, Rhododendron spp. of the eastern Himalaya and in particular of Mt. Yulong have a uniquely deep historical collection record which allowed us to reconstruct their sensitivity to annual temperature changes across the last 125 yr (Hart et al. 2014). Using a community phylogeny and trait data from observations along a 1400 m elevational transect and the extensive herbarium record, we examine whether, and how, phylogeny constrains phenology in Himalayan Rhododendron.

**Methods**

We made 31 collections of 17 species that occur on Mt. Yulong (Table 1) 2700 – 4100 m asl. With each collection, leaf material for DNA analyses was collected into silica. Species were identified in the field with the local plant checklist (Wang et al. 2007) and confirmed at the herbarium of the Missouri Botanical Garden (MO) with reference to the Flora of China (Wu et al. 2005). Vouchers were deposited at MO and the herbaria of the Kunming Institute of Botany – Chinese Academy of Sciences (KUN), and the Royal Botanic Garden Edinburgh (E).

DNA extraction and sequencing were conducted at the Department of Botany, University of Wisconsin-Madison in 2010 – 2013. Four gene regions were targeted: two chloroplast regions: *tabC-F* and *atpH-I*, and two nuclear regions: *RPB2-I (3F-4R)* and *ITS 4-5*. Total genomic DNA was extracted from leaves dried on silica gel using
DNeasy-like protocol of Alexander et al. (2007) with Epoch (Sugar Land, Texas, USA) columns (for subgenus *Rhododendron*) and the CTAB method (Doyle and Doyle 1987), modified by using 700 μL - 1000 μL of CTAB buffer initially due to the difficulty of pulverizing the leathery leaves, and 91% isopropanol rather than 100% (for the subgenus *Hymenanthes*). Polymerase chain reactions were conducted for *RPB2-I* with RTG PCR beads (GE Healthcare, Buckinghamshire, UK) and for the other three gene regions using 16.5μL water, 2.5 μL ThermoPol Buffer (New England Biolabs, Ipswich, MA), 2.5 μL dNTPs, 1.25 μL BSA (New England Biolabs), 0.8 μL forward primer, 0.8 μL reverse primer, 1 μL DNA template, and 0.5 μL *Taq* Polymerase (New England Biolabs). After observation by gel electrophoresis, PCR products were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, California, USA). PCR products were cycle sequenced and sequencing products submitted to the University of Wisconsin-Madison DNA Sequencing Center. Extraction and sequencing methods are described in complete detail in Georgian (2014).

Sequences from the cranberry *Vaccinium macrocarpon* were added from genbank (accession JQ248601.1) to serve as an outgroup. Sequences were automatically aligned using the Geneious alignment algorithm in Geneious R6 (BioMatters, San Francisco, California), and then checked and edited manually. A tree for all four gene regions concatenated was produced in MrBayes and BEAST 2.1.3 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003, Bouckaert et al. 2014). Analyses were run for 1,000,000 generations, with sampling at 1,000 generation intervals, with the GTR model with gamma-distributed rate variation across sites, partitioned by gene region. We estimated date ranges of bifurcation of the outgroup
vs. the genus *Rhododendron* and the bifurcation of subgenera *Hymenanthes* and *Rhododendron* based on fossil records (Popp et al. 2011) and matK datings (Milne 2004). Using these estimates, we dated our phylogram with the package APE (Paradis et al. 2004, Paradis 2011) in the R statistical framework v. 3.1.1 (Venables and Ripley 2002).

For the nine most common *Rhododendron* species on Mt. Yulong, we measured traits of reproductive phenology, including seasonal and elevational flowering ranges, phenological sensitivity, and reproductive fitness. These ecological and phenological traits were recorded on Mt. Yulong along 8 transects 2760 – 4060 m asl. At every 100 m of elevation, 12 plants of each *Rhododendron* species present were permanently marked and their flowering quantified at 15-day intervals during 2012 and 2013. Density of each species (plants/m²) was calculated at each site with the variable area transect method (Parker 1979), and for 2013, fruit-set was quantified for each of the marked plants. Hart et al. (in press) give full details for these monitoring methods.

From these data, we summarized the following traits within species: elevational niche, measured by the mean, lowest and highest elevational distributions; phenology, measured by mean, first and last flowering day and flowering duration; phenological sensitivity to temperature, measured by the change in mean flowering time between the years 2012 and 2013 (days) and the change in mean flowering time over elevation (days/100 m); and reproductive fitness, measured by mean flowers/plant, mean fruits/plant, proportion of flowers fruiting, and calculated flowering density (flowers/m², calculated as density x mean flowers/plant).
As an additional measure of temperature sensitivity, we included the modeled response to temperature (days/°C) for each species based on herbarium collections 1885 – 2009 (Hart et al. 2014). We consulted the Flora of China (Wu et al. 2005) for reproductive morphology of the Mt. Yulong species: flower size (diameter) and fruit size (length).

We used these traits in combination with the dated phylogenetic tree, pruned to these nine Mt. Yulong species monitored, to compute Blomberg’s $K$ statistic of phylogenetic signal and an associated p-value test which compares the observed pattern of similarity among relatives to a null model based on tip-shuffling (Blomberg et al. 2003) using the R package picante (Kembel et al. 2010). Traits with a significant phylogenetic signal ($p > 0.05$) were mapped onto the tree using the R package phytools (Revell 2012, 2013).

To contextualize these results within seasonal temperature patterns on Mt. Yulong, we compared flowering times within and across Mt. Yulong species to daily average temperatures from the Lijiang weather station (China Meteorological Administration), adjusted -7°C (to account for an adiabatic lapse rate of 0.7°C / 100 m elevation) to estimate temperatures at 3400 m asl, the center of *Rhododendron* elevational distribution on Mt. Yulong.

To complement these local-scale analyses with broader data, data from the *Flora of China* (Wu et al. 2005) on the first flowering month, last flowering month, mean flower size and mean fruit size were collated for 160 *Rhododendron* spp. (all Yunnan province spp. for which these data were available). Data on first and last fruiting month were also available, allowing estimation of fruit development time for each
species as the number of months between the mean flowering month and the mean fruiting month. We tested correlations among these traits, analogous to the phenological and morphological metrics for Mt Yulong *Rhododendron* spp., with linear regressions run in the base R package (Venables and Ripley 2002).

**Results**

Concatenated, the four gene regions comprised 3,737 base pairs for the 17 *Rhododendron* species and 1 outgroup. The tree (Figure 1) shows strong support for two clades, A and B (0.99 posterior probability support), which correspond to the subgenera *Rhododendron* and *Hymenanthes*, and subclades which generally match previous classifications by section and subsections. A novel, but well-supported feature of this tree is Clade A1, which groups into A 1.1 two species of section *Pogonatha* (*R. trichostomum* and *R. primuliflorum*), as well as *R. lepidotum*, which has been classified into section *Lepidota* and into A1.2 includes species of section *Rhododendron* subsection *Lapponica*. Sections *Pogonatha* and *Lepidota* and section *Rhododendron* subsection *Lapponica* have not previously been closely associated (Cullen 1980, Wu et al. 2005) but they do share morphological and phenological similarities.

All nodes relevant to the pruned tree containing the Mt. Yulong spp. (Figure 2) were well resolved with the exception of the placement of the two accessions of *R. yunnanense* into two different clades. We considered two topologies, one in which *R. yunnanense* is included in A2.2 (as in Figure 1) and one in which it is included in A2.1. Phylogenetic signal results were similar with both topologies, and we base further
analyses and figures on the first topology, which retains the integrity of subsection *Triflora*.

Out of the 14 traits considered for the Mt. Yulong spp., only three showed a significant phylogenetic signal: last flowering day ($K=1.29$, $p=0.03$), flower size ($K=1.63$, $p=0.01$) and fruit size ($K=1.2$, $p=0.03$) (Table 2). Each of these traits was significantly clustered in the phylogeny, indicated by $K>1$ (Figure 2). The remaining traits all had $K<1$, but none were found to be significantly more dispersed than expected by chance. The latest last flowering day occurred in A1 (mean last flowering on Julian day 226), with earlier last flowering day in A2 and B (mean last flowering day 172.5 and 179; Figure 2A). Both flowers and fruits were also smallest in A1 (mean flower 1.33 cm, mean fruit 0.52 cm; Figure 2B-C). Clade A2.1 (*R. racemosum*) also had small flowers (1.15 cm), while A2.2 (*R. rubiginosum* + subsection *Triflora*) had midsized flowers (mean 2.68 cm) and B had the largest flowers (mean 3.88 cm; Figure 2B). The largest fruits occurred in A2 (mean 1.15 cm), with smaller fruits in B (mean 0.63 cm) despite the large flowers (Figure 2C). Among the Mt. Yulong spp., last flowering day was negatively correlated with fruit size ($y=-0.09x+0.04$, $p=0.047$, $r^2=0.45$), although not significantly with flower size ($y=-0.024x+0.013$, $p=0.09$, $r^2=0.35$). Fruit and flower size did not significantly correlate with mean flowering day (fruit size: $p=0.16$, flower size: $p=0.63$) or first flowering day (fruit size: $p=0.29$, flower size: $p=0.85$).

Last flowering day across Mt. Yulong *Rhododendron* spp. ranges from Julian day 108 – 195. Daily average temperatures for these dates estimated for 3400 m asl are $6°$ C – $16°$, with a mean daily average temperature of $11.5°$ C. Similarly warm temperatures continue for > 1.5 months after this period, and daily average
temperatures do not dip below 6° C until day 250. Even considering the broader
distribution of last flowering dates implied by a kernel density estimation curve of
individual plant last flowering day, virtually all flowering is complete before autumn,
despite these warm and consistent autumn temperatures (Figure 3).

In our regional analysis of 160 Yunnan spp., last flowering month was
significantly correlated with flower size \(y=-0.4x+5.8, p=4 \times 10^{-5}, r^2=0.10\), fruit size
\(y=-0.3x+3.6, p=0.0002, r^2=0.09\), and fruit development time \(y=-0.9x+8.9, p<2 \times 10^{-16}, r^2=0.35\)\. First flowering month was not significant as an additive term and did not
substantially improve the \(r^2\) value when added to the model for flower size \(p=0.48, r^2=0.10\), flower size \(p=0.78, r^2=0.09\), or fruit development time \(p=0.14, r^2=0.36\). Fruit development time was correlated with larger flowers \(y=0.4x+1.8, p=3 \times 10^{-10}, r^2=0.22\) and larger fruits \(y=0.3x+0.8, p=3 \times 10^{-6}, r^2=0.13;\ Figure 4\).

**Discussion**

For Mt. Yulong *Rhododendron* spp. last flowering day is phylogenetically clustered while
first and mean flowering day show no significant phylogenetic signal. This trend is
especially pronounced in clade A1 (Figure 2A), which consists of three species with the
latest last flowering day. Although there is increasing support for the importance of
measuring phenology as a compound event with a beginning, peak, duration and end
2015), few studies on phylogeny have explicitly considered these measures.

CaraDonna and Inouye (2015), one exception, found phylogenetic signal only in first
and peak flowering day, and not in last flowering day, in an alpine plant community
in the Rocky Mountains. Possibly, this difference is related to local seasonal regimes –
with no persistent snowpack on Mt. Yulong, the beginning of the season (and the
related first flowering day of early species) may be under looser constraints than in the
Rockies. Despite this difference, both studies underscore the importance of
comprehensive phenology measures in gaining full understanding of phylogenetic
patterns in phenology, and of the unique pressures on the beginning and end of
phenological events.

Why should the last flowering day show phylogenetic clustering and first flowering
day not show such signal? Panchen et al. (2014) suggest that phylogenetic signal in
phenological traits may be driven to some extent by correlations with other conserved
traits. We present evidence that last flowering day is correlated with fruit size in Mt.
Yulong Rhododendron spp., and that last flowering month is correlated with fruit size
among the 160 Yunnan Rhododendron spp. (Table 3). We suggest that this correlation
and common phylogenetic clustering may be driven by the reduced fruit development
time available to species with a later last flowering in autumn. Among the Yunnan
Rhododendron spp., fruit development times are significantly correlated with last, but
not first flowering month, and shorter development times are associated with smaller
fruits and flowers.

As with many other phenological events (Wolkovich and Ettinger 2014), fruiting
and last flowering day are inextricably related by simple temporal sequence. Although
Rhododendron spp. flower across a broad range of seasons and are overdispersed in
elevation-season niche (Hart et al. in press), the phenological distribution of the genus
is significantly skewed towards the spring, and a period of early-fall warm
temperatures remains with no species flowering (Figure 4). This ‘empty’ period reflects fruit development time, and is in accord with general predictions for phenological sequences affected by spring and fall cold-stress (Wolkovich and Cleland 2014). Thus, the pressure exerted by the onset of cold fall temperatures upon fruit development (and so on the last flowering day) may help to explain this asymmetrical relationship between temperature and *Rhododendron* phenology on Mt. Yulong (Figure 4). *Rhododendron* spp. must flower in spring and summer so that fruits can develop in autumn before the onset of cold weather. The species with the latest last flowering dates are under particular autumn constraint, and only small-fruited and small-flowered species occupy this phenological period. In turn these constraints are evidenced in their phylogenetic relatedness.

Our quantitative data for *Rhododendron* support Primack’s (1987) proposition that phenology could be determined by fruit size if larger-fruited species require a longer period for fruit development before cold fall temperatures. Other studies report related results between late flowering and low seed mass (Mazer 1989, Vile et al. 2006, Catorci et al. 2012), including montane and alpine plant communities on the Tibetan Plateau (Du and Qi 2010, Peng et al. 2011, Wang et al. 2014a). However, studies do not always agree on the phylogenetic patterns around these correlations (Bolmgren et al. 2003, Du and Qi 2010). Wang et al. (2014b) studied 49 south-eastern Tibetan *Rhododendron* spp., and although fruit/seed development time was not directly measured, the authors did find seed mass to decrease with elevation, similarly suggesting constraints of the shorter season and earlier onset of cold temperatures at higher elevations.
A contrast between spring-flowering species with a quick return on investments and later-flowering species with more robust tissues is a classic and widely supported trade-off in reproductive phenology and morphology (Lechowicz 1984, Bolmgren and Cowan 2008, Wokovich and Ettinger 2014, Wolkovich and Cleland 2014). Our results suggest another dimension to this trait axis: for Himalayan *Rhododendron*, ‘fast and cheap’ species have unique access to late-season flowering. A renewed consideration of this strategy may help to explain previous, apparently conflicting results, if other species also are fast and cheap in the fall (Bolmgren and Cowan 2009, Sun and Frelich 2011, Catorci et al. 2012, Wolkovich and Cleland 2014).

The selective pressures on late flowering are shown in our previous work (Hart et al. in press), where we present evidence that later flowering results in reduced quantity of flowers and of fruits within Mt. Yulong *Rhododendron* species. Although these shared patterns between inter-specific and intra-specific comparisons are not necessarily a general rule (Clausen et al. 1948, Bolgren and Cowan 2008), similar selective pressures for small and quickly-ripening fruits, have been seen, particularly in montane and alpine temperature regimes. Kudo (1991, 1993) found that later flowering in *Rhododendron aureum* in Japanese mountains enhanced pollination success but decreased fitness, as a higher proportion of fruits were unable to mature before the onset of cold weather. Spira and Pollak (1986) showed this effect both within and across alpine gentian species.

Above, we make the case that phylogeny affects phenology, with related species occupying similar areas of a reproductive investment / phenology trade-off. An alternative hypothesis that could explain the tree we derived (Fig. 3) is that phenology
may structure genetic relatedness. Mt. Yulong *Rhododendron* spp. co-flower, share similar pollinator species and are known to hybridize (Milne et al. 2003, 2010, Georgian 2014, Hart et al. in press). That phylogenetic patterns in phenology are most obvious in clade A1 (Figure 2A), which is novel in this study, could support the interpretation of greater gene flow among species that share flowering time. However, were this the case, we might expect species that share similar mean flowering day and first flowering day to be equally as related as those that share last flowering day, which is not the case. Further, it is not entirely surprising that our molecular phylogeny differs from previous *Rhododendron* infrageneric classification – other molecular work has called many of the classical relationships between *Rhododendron* subsections into question (Georgian 2014, Goetsch et al. 2009). Modern population genetic methods (Kameyama and Kudo 2009) and the wealth of historical collections available (Hart et al. 2014) for Himalayan *Rhododendron* spp. make contemporary and historical patterns of hybridization and phenology a promising area for future research.

We found no significant phylogenetic signal in phenological sensitivity to temperature. This contrasts with previous studies (Willis et al. 2008, Davis et al. 2010, Mazer et al. 2013) which propose that phylogenetic relatedness may signal individual species ability to respond to shifts in temperature and future responses to climate change. Previous studies have often been conducted across broad taxonomic spectra, and it may be that these patterns simply do not apply to infrageneric differences. However, as in Rocky Mountains plant communities (CaraDonna and Inouye 2015) we found some phenological components, but not phenological sensitivity, to be phylogenetically conserved. Perhaps in these systems, phenologies have been able to
respond to temperatures experienced so far without constraint from phenological extremes. For Himalayan *Rhododendron*, the correlation of last flowering day with reproductive morphology and fitness suggest a threshold beyond which phenological sensitivity will not be possible with ongoing climate change.

**References**


L. Betancourt, K. Bolmgren, E. E. Cleland, B. I. Cook, T. M. Crimmins, S. J.
Mazer, G. J. McCabe, S. Pau, J. Regetz, M. D. Schwartz, and S. E. Travers.
1530.

Davis, C. C., C. G. Willis, R. B. Primack, and A. J. Miller-Rushing. 2010. The
importance of phylogeny to the study of phenological response to global climate
change. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**:3202–
3213.

Devaux, C., and R. Lande. 2008. Incipient allochronic speciation due to non-selective
assortative mating by flowering time, mutation and genetic drift. *Proceedings of the
Royal Society B: Biological Sciences* 275:2723–2732.

Du, G., and W. Qi. 2010. Trade-offs between flowering time, plant height, and seed
size within and across 11 communities of a QingHai-Tibetan flora. *Plant Ecology*
**209**:321–333.


Forrest, J., D. W. Inouye, and J. D. Thomson. 2010. Flowering phenology in
subalpine meadows: Does climate variation influence community co-flowering

Fridley, J. D. 2012. Extended leaf phenology and the autumn niche in deciduous


Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic


Table 1. Thirty-two accessions of 17 *Rhododendron* species were sequenced. Specimens collected on Mt. Yulong represent the major temperate subgenera of *Rhododendron*: *Rhododendron* and *Hymenanthes*. *R. trichostomum* and *R. primuliflorum* grouped in section *Pogonantha*; all other subgenus *Rhododendron* species are grouped in section *Rhododendron* and further into subsections. We follow Cullen (1980) in placing *R. racemosum* into subsection *Scabrifolia*. Species indicated with * were those for which trait data was collected on phenological transects. Multiple accessions of a species are listed in sequence according to Figure 1. For voucher numbers, H indicates specimens with Robbie E. Hart as collector, G with Elizabeth Georgian as collector, and HG vouchers with both.

<table>
<thead>
<tr>
<th>Subgenus</th>
<th>(Sub)section</th>
<th>Species</th>
<th>Voucher Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhododendron</em></td>
<td>section <em>Pogonantha</em></td>
<td><em>R. trichostomum</em> Franch.</td>
<td>HG6</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. primuliflorum</em> Bureau &amp; Franch. *</td>
<td>H112</td>
</tr>
<tr>
<td><em>Lepidota</em></td>
<td></td>
<td><em>R. lepidotum</em> Wall. ex G. Don *</td>
<td>HG5</td>
</tr>
<tr>
<td><em>Lapponica</em></td>
<td></td>
<td><em>R. telmateium</em> Balf. f. &amp; W. W. Sm.</td>
<td>H115, H63</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. rupestris</em> W. W. Sm.</td>
<td>H113</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. fastigiatum</em> Franch.</td>
<td>G58</td>
</tr>
<tr>
<td><em>Scabrifolia</em></td>
<td></td>
<td><em>R. racemosum</em> Franch. *</td>
<td>HG7, G57, H54</td>
</tr>
<tr>
<td><em>Triflora</em></td>
<td></td>
<td><em>R. yunnanense</em> Franch. *</td>
<td>HG1, G60</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. oreotrephes</em> W. W. Sm. *</td>
<td>H26, H53, G62</td>
</tr>
<tr>
<td><em>Heliolepida</em></td>
<td></td>
<td><em>R. rubiginosum</em> Franch. *</td>
<td>HG5, HG4, H30</td>
</tr>
<tr>
<td><em>Hymenanthes</em></td>
<td><em>Taliensia</em></td>
<td><em>R. traillianum</em> Forrest &amp; W. W. Sm. *</td>
<td>H111, H61</td>
</tr>
<tr>
<td></td>
<td><em>Adenogynum</em> Diels *</td>
<td></td>
<td>H114</td>
</tr>
<tr>
<td><em>Fulva</em></td>
<td></td>
<td><em>R. warriolatum</em> Diels</td>
<td>H93, H94</td>
</tr>
<tr>
<td><em>Irrorata</em></td>
<td></td>
<td><em>R. irroratum</em> Franch.</td>
<td>H97, H96</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>R. anthropophorum</em> Diels</td>
<td>H92</td>
</tr>
<tr>
<td><em>Fortunea</em></td>
<td></td>
<td><em>R. decorum</em> Franch.</td>
<td>G61, HG3, G56</td>
</tr>
</tbody>
</table>
**Table 2. Last flowering day, flower size and fruit size all show significant phylogenetic signals.** Each of these three traits is phylogenetically clustered (K<1) within the Mt. Yulong *Rhododendron* spp. (Figure 2).

<table>
<thead>
<tr>
<th>trait</th>
<th>K</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>elevation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean elevation</td>
<td>0.47</td>
<td>0.39</td>
</tr>
<tr>
<td>lowest elevation</td>
<td>0.48</td>
<td>0.39</td>
</tr>
<tr>
<td>highest elevation</td>
<td>0.46</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>phenology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean flowering</td>
<td>0.68</td>
<td>0.11</td>
</tr>
<tr>
<td>first flowering day</td>
<td>0.75</td>
<td>0.08</td>
</tr>
<tr>
<td>last flowering day</td>
<td>1.29</td>
<td>0.03 *</td>
</tr>
<tr>
<td>duration of flowering</td>
<td>0.32</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>phenological sensitivity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flowering change 2012–2013</td>
<td>0.30</td>
<td>0.83</td>
</tr>
<tr>
<td>flowering change/100 m</td>
<td>0.31</td>
<td>0.86</td>
</tr>
<tr>
<td>flowering change/1°C</td>
<td>0.74</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>reproductive fitness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flower quantity</td>
<td>0.39</td>
<td>0.68</td>
</tr>
<tr>
<td>fruit quantity</td>
<td>0.44</td>
<td>0.48</td>
</tr>
<tr>
<td>fructification proportion</td>
<td>0.35</td>
<td>0.77</td>
</tr>
<tr>
<td>flowers/m²</td>
<td>0.40</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>morphology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flower size</td>
<td>1.63</td>
<td>0.01 *</td>
</tr>
<tr>
<td>fruit size</td>
<td>1.20</td>
<td>0.03 *</td>
</tr>
</tbody>
</table>
Figure 1 (following page). Phylogeny of 17 Mt. Yulong *Rhododendron* species, based on concatenated sequences for *trnL-trnL-trnF, atpH-I, RPB2-I (3F-4R)* and *ITS 4-5*. Posterior probability support is given for each node to the level of species. Clade A equates to subgenus *Rhododendron* and clade B to subgenus *Hymenanthes*. Temporal scale is based on estimated date ranges for the first and second bifurcation based on fossil records (Popp et al. 2011) and matK datings (Milne 2004).
Figure 2. Last flowering day, flower size and fruit size are phylogenetically clustered. For each commonly occurring *Rhododendron* species on Mt. Yulong, trait values for A) last flowering day (Julian day), B) flower size and C) fruit size show clustering when mapped on to a phylogram pruned from the dated consensus tree (Figure 1).
Figure 3. *Rhododendron* flowering phenology is skewed towards spring.

(A) Daily average temperatures for 2012 from the Lijiang weather station, adjusted -7°C to estimate temperatures at 3400 m on Mt. Yulong, the center of *Rhododendron* elevational distribution. A locally weighted scatterplot smoothing (loess) curve is fitted through the 2012 temperatures (heavy line). B) Kernel density estimation curve of *Rhododendron* last flower on Mt. Yulong in 2012. Although flowering time is widespread among *Rhododendron* spp. and the earliest flowering occurs during cold temperatures, virtually all flowering is complete before autumn, despite warm and consistent autumn temperatures. This fall period allows fruit development.
Figure 4. Longer fruit development time allows larger fruits and flowers.

Fruit development time (the number of months between the mean fruiting month and mean flowering month) correlates with fruit size (A) and flower size (B) across 160 Yunnan *Rhododendron* species.
Chapter 4

Dynamic ecological knowledge systems amid changing place and climate: Mt. Yulong rhododendrons

Abstract

How dynamic is place-based traditional ecological knowledge (TEK)? How can generative and conservative processes allow its adaption to a changed environment? How do different cultures mediate TEK of a shared place? We address these questions with an in depth study of TEK of the diverse and socio-ecologically salient genus Rhododendron among the indigenous Naxi and immigrant Nuosu Yi of Mt. Yulong, NW Yunnan, China. TEK in both cultures is rich and intimately connected to the seasonal and elevational progression of Rhododendron spp. Naxi and Yi knowledge of trends and drivers of change parallel those in ecological studies.

Knowledge richness was connected with place (urban vs. rural dwelling and elevation of village), and the immigrant Yi had a knowledge base as rich as or richer than that of the indigenous Naxi. Both Yi and Naxi informants credited this knowledge equality to a combination of generative processes (Yi villages were higher in elevation and Yi livelihoods made more use of mountain resources, which enabled them to acquire knowledge of plants quickly), and conservative processes (Yi migrated from an equally diverse mountain region in which the genus is also salient and retained their system of TEK even as its elements were adapted). Among rural Naxi, cultural systems (seasonal festivals and ethnotaxonomy) conserved knowledge, even while their direct use of rhododendrons decreased with changing lifeways. Both Naxi and Yi, with urbanization, evidenced dramatic changes in TEK.
Introduction

The dynamics and demographic structure of Traditional Ecological Knowledge (TEK) are central to ethnobotany (Reyes-García 2009). How do conservative processes and generative processes (Parlee et al. 2006) in TEK interact? How does TEK, as place-based knowledge (Menzies 2006), respond to a change in place? What are the similarities and differences between TEK in a culture that spans different environments, TEK across cultures sharing an environment (Ceuterick et al. 2011, Quave and Pieroni 2015), and TEK in indigenous and migrant cultures (Reyes-García 2009)? If TEK is knowledge accreted over generations, how does it adapt to and allow adaptation to environmental changes such as climate change (Salick and Byg 2007, Salick and Ross 2009)? These issues draw into question even the term ‘traditional ecological knowledge’, although they equally make problematic the alternatives ‘indigenous knowledge’ and ‘local knowledge’ (Thaman et al. 2014).

We address these questions in Northwest Yunnan, at once a hotspot of biological and cultural diversity (Gorenflo et al. 2012) and an area experiencing dramatic social and ecological changes. These changes include unprecedented shifts in lifeways and rapid urbanization (Ye et al. 2013), added to the effects of climate change (Baker and Moseley 2007, Xu et al. 2009, Fan et al. 2011). With more than three hundred species (many endemic), Rhododendron is one of the richest genera in Northwest Yunnan (Wu et al. 2005, Wen et al. 2014). For the indigenous peoples of Yunnan, Rhododendron spp. have utilitarian and cultural significance (Jernigan 2012, Georgian 2014), they are documented in historical healing and ritual texts (Li and Luo 2003) as well as in
contemporary Tibetan Medicine (Salick 2006), and they even appear in indigenous creation myths (Mathieu 2003).

Mt. Yulong (玉龙雪山, 27.1°N 100.1°E) is at the center of Yunnan’s rhododendron diversity. The mountain is equally central to the indigenous Naxi, who are uniquely and anciently associated with the Mt. Yulong area (Rock 1963, Mathieu 2003, Mueggler 2011). Over the past 50 years, many Nuosu Yi have migrated from the Yunnan-Sichuan border and Southern Sichuan Province to the Mt. Yulong area, where they tend to occupy marginal, higher-elevation areas, have less access to urban areas and farmland, and rely more on mountain resources (Harrell et al. 2000, Harrell and Li 2003, Huber et al. 2015).

Mt. Yulong is also a center of historical collections of *Rhododendron* (Hart et al. 2014), due largely to the pioneering plant-hunters George Forrest (collecting 1904–1930) and Joseph Rock (collecting 1918–1948), and to the tireless efforts of the Naxi collectors with whom they collaborated (Glover et al. 2011, Mueggler 2011). These historical collections, in combination with recent ecological monitoring and artificial warming experiments, document climate-driven change in *Rhododendron* flowering time (Hart et al. 2014, in prep). Traditional phenological knowledge (Lantz and Turner 2003, Nabhan 2010) is one way that TEK has been used to understand the effects of climate change. In the Mt. Yulong area, phenological knowledge offers 1) a long-term set of observations comparable to historical data and 2) direct and detailed observations comparable to ecological monitoring.

Working in rural communities of Naxi and Yi around Mt. Yulong and in neighboring urban Lijiang, we examined the effects of culture and of place between
and within cultures. We explored how generative and conservative knowledge processes create dynamic systems of traditional ecological knowledge among peoples who have experienced and are experiencing a changed geographic place, changing lifeways that affect their ecological interaction with that place, and a changing climate.

**Methods**

Interviews were conducted 2009 – 2013 in 8 villages surrounding Mt. Yulong and in Lijiang City. The villages were the highest and closest to Mt. Yulong’s southern and eastern slopes, and comprised the predominantly Naxi areas of gerater Baisha, Yulong, Yuhu and Wenhai; and the predominantly Yi areas of Xuehua, Yunshanping, “23 km” and “61 km”. Structured interviews were conducted predominantly in Yi language or Naxi language (depending on the fluency of the interviewee) by functionally tri-lingual field assistants of Yi and Naxi ethnicity, with the first author recording data. Prior informed consent was obtained before each interview.

*Rhododendron* spp.¹ flower in progressions across season and elevation (Hart et al. in prep), so in order to present species simultaneously, we used photo elicitation (Thomas et al. 2007) rather then methods requiring a living plant. These photographs depicted 11 common species on Mt. Yulong (Table 1). On one face the species

---

¹ Here and below “species” refers to the taxonomy of *Rhododendron* according to the Flora of China (Wu et al. 2005), with which we determined species and we chose photographs. This may or may not be congruent to emic ‘ethnotaxa’ (Berlin et al. 1966, Hunn 1982, Harrison 2007), as we discuss below.
growth habit was depicted, with a person for scale, and on the other face a close-up of leaf and flower morphology was depicted. Informants were first asked to name each species, and then to provide names and descriptions of any additional species or similar plants. Then they were asked to complete three sorting tasks. In the first, the ‘pile sort’ (Martin 2010), they were asked to sort the photographs into piles according to which were the most similar. Second, they were asked to sequence the photographs in order of species flowering time across the season. Finally, they were asked to sequence the photographs in the order of species elevational distribution. With reference to their sequences, they were asked about annual changes in flowering time, about long-term changes in flowering time and elevational range, and about what they perceived to be drivers of change. In sequencing tasks, informants could express uncertainty by leaving photographs combined in piles with tied rank. After the sorting tasks, informants were asked with reference to the photographs about species used for food, fodder, honey sources, medicine, decoration, ritual, wood or other uses, and which featured in songs, stories, or sayings (which they were asked to sing or tell).

Interview data from rural Yi (N=32), rural Naxi (N=29), Lijiang Yi (N=9) and Lijiang Naxi (N=11) was analyzed separately. Pile-sort data was analyzed by ordination, constructing an aggregate distance matrix for each of the four interview groups. In this matrix, cell i,j represented the proportion of informants who grouped the species in row i together with the species in column j. A non-metric multidimensional scaling (NMDS) was run on the Bray distances of this matrix with the package vegan (Oksanen et al. 2013). To aggregate phenology or elevation sequences across individuals (who had varying numbers of tied scores or items sorted),
the raw sequence ranks were centered and scaled by subtracting column means and
dividing by standard deviations using the scale function of the base R package version
3.1.1 (Venables and Ripley 2002). Within each group, we then calculated mean scaled
phenology and elevation ranks for each species. These mean scores, and the
ethnotaxon to which each species belonged, were fit as environmental variables to the
NMDS ordination, and their significance in explaining this ordination tested with a
permutation test (999 permutations, \( \alpha = 0.05 \)) in vegan (Oksanen et al. 2013).

We extracted summary metrics for each individual that included the number of
ethnotaxon tiers (ethnogenus, ethnospecies, ethnovariety; Berlin et al. 1966, Hunn
1982), the total number of ethnotaxa; the number of species with uses and the total
number of uses reported; and the number of distinct (non-tied) groups identified in
the phenology and elevation sequencing tasks. Significant differences across the
different groups for each of these metrics were assessed with Kruskal-Wallis and post-
hoc Dunn tests (de Mendiburu 2014, Dinno 2015).

**Results**

By rural Yi informants, all *Rhododendron* spp. were grouped into the Yi language
ethnogenus *shuoxma* (ꁁꄀ), with the exception of one informant who distinguished *R.*
racemosum and *R. primuliflorum* as *hmaxvie* (ꀄꁀ). For each species, the most frequent Yi
language report was one of two ethnospecies: 1) *shuoxma axge* (ꀉꇰ) ‘large’, which
included *R. oreotrephes, R. beesianum, R. decorum, R. vernicosum, R. rubiginosum, R.*
adenogynum, *R. traillianum*; and 2) *shuoxma axyi* (ꁀꄀ) ‘child’, or *shuoxma etzyr* (ꁀꄀ)
‘small’, which included *R. yunnanense, R. racemosum, R. impeditum* and *R. primuliflorum*. In
contrast to the Yi language word *shouxma*, and to the Mandarin Chinese word *dujuanhua* (杜鹃花) used by urban Yi and Naxi, Naxi language speakers did not report a single ethnogenus that equated to the genus *Rhododendron*. For rural Naxi informants, the most frequent Naxi language report for each species was one of three ethnogenera: 1) *shuo*, which included *R. yunnanense*, *R. rubiginosum*, *R. oreotrephes*; 2) *mun*, which included *R. decorum*, *R. adenogynum*, *R. beesianum*, *R. traillianum* and *R. vernicosum*; or 3) *adzegedze*, which included *R. racemosum* and *R. impeditum*. For both Yi language and Naxi language ethnotaxa, alternate classifications were common (Table 1). The most common alternate classification was the Yi language ethnospecies *shouxma mgepqy* (ꑪꐔ) ‘buckwheat rhododendron’ (*R. racemosum* and *R. impeditum*). Occasionally, a third tier of ethnotaxonomy (ethnovariety) was given (e.g. Yi language *shouxma axge aqu ꑬꐔꐃ‘large white rhododendron’). Most name reports (and all Naxi languages name reports) referred to a plant’s morphology (size, habit, flower / leaf color, etc.). For Yi, ‘buckwheat’ rhododendron *shouxma mgepqy* was a major exception, as were other more occasional name reports that made reference to habitat (e.g., *shouxma ggu hxo* (ꑬꐔ‘high mountain’) or use (e.g., *shouxma jji yy* (ꑬ‘nectar’ and *shouxma yo* (ꑮ‘sheep’). Additional ethnotaxa (usually 1 – 2) which were not represented in the photographs were mentioned by 20% of Naxi and 44% of Yi informants. These included a yellow-flowered species (possibly *R. wardii*) mentioned by both Naxi and Yi informants as very rare; and a red-flowered species (likely *R. delavayi*) that did not

---

2 One informant had only heard of it, another had seen it in a photograph by Joseph Rock that his father had once possessed; a third said "you can seek the yellow flower but will never find it"
occur in the Mt. Yulong area but was mentioned by many Yi informants as an important species to the Yi core area in Sichuan.

Few informants sequenced all 11 species. On average, rural informants grouped sequenced photographs into 3 – 6 piles, within which species ranks were tied (Figure 1). Within both rural groups, there were significant overlaps between species, but distinct aggregate phenological and elevational sequences emerged (Figure 2). These were broadly similar across Naxi and Yi informants, and included for both groups *R. racemosum* as distinctively early-flowering and low in elevation. Naxi and Yi informants also agreed on *R. impeditum* as an early-flowering species, and *R. primuliflorum* as late. The groups differed on *R. yunnanense*, which Yi informants reported as lower and earlier flowering, and on *R. beesianum*, which Naxi informants reported to be the latest flowering and highest species.

No informants reported changes in elevational sequence or elevation ranges, since “rhododendrons can’t get up and walk”. In contrast, although very few informants noted long-term directional changes in phenology, 52% of Naxi informants and 66% of Yi informants reported confusing changes to the phenological sequence, with species flowering unpredictably in any given year. Of those who reported changes, 24% related them to variation in annual temperature (most often earlier flowering with warming), and 73% to variation in rainfall (most often earlier flowering with heavier rains / earlier monsoon).

Photographs were generally sorted by rural informants into 5 – 7 piles of similar species. Although there was a large variance among which species occurred in which piles with each other, and although informants volunteered different reasons for why
they had chosen to sort species into the piles they did (habitat, morphology, ethnotaxa, phenology, and mixed criteria), certain species were grouped more often with one another. These clusters are similar between rural groups, but with distinct features (Figure 3). *R. racemosum* and *R. impeditum* formed the most differentiated cluster across both Naxi and Yi informants, although for Yi, *R. yunnanense* was a peripheral member of this group. The environmental fitting function showed phenology, elevation, and ethnotaxon to describe the actual ordination significantly better than 999 permutations (Table 2).

Despite an initial disclaimer of *Rhododendron* spp. as ‘useless’ plants by many informants, multiple uses were reported for every species. Often, these uses were specific or ethnotaxonomically specific. Many use categories had similar report frequencies among Naxi and Yi, although particular uses and species used often differed among the groups. The use of rhododendrons as food was the most common use reported by both Yi (80%) and Naxi (43%) informants. This category was predominantly represented for both groups by *R. yunnanense* (mentioned by 83% of informants who singled out a specific food species), the flowers of which are eaten fresh. Several Naxi informants contrasted *R. yunnanense* and *R. decorum*, the latter of which they reported as poisonous. In contrast, Yi informants reported eating nectar from *R. decorum* flowers. Similarly, rhododendrons as a foraging source for honeybees (*Apis cerana* and *Apis mellifera* were both managed) were reported by ~25% of Naxi and Yi informants, while several Naxi informants cautioned that only certain ethnotaxa or species that flowered in certain seasons could safely be used. Both Naxi and Yi informants reported that some species, particularly *R. racemosum* and *R. impeditum*,
could safely be used as fodder for sheep and goats, but some disagreed and singled those species out as harmful. Similarly, several informants warned that *R. beesianum* was especially toxic to cows and yak, but one reported that the leaves in fact could be boiled as a stimulant for those animals. No human medical uses were reported by Naxi informants. Species-specific medical uses of the leaves, flowers, roots, and parasites (*Balanophora involucrata*) were reported by a few Yi informants, most commonly for skin-related ailments. Use of the wood of various *Rhododendron* spp. for fuel (all species) and for carving (especially *R. traillianum* and other large species) was also commonly reported by both Yi (64%) and Naxi (43%) informants. Naxi tended to mention carving small things such as pipes, weaving shuttles, or pieces of horse tack. Yi often mentioned the use of rhododendron to carve the distinctive Yi lacquerware (Figure 4), although this art was reported to be rare in the Lijiang area. Religious and ritual uses of *Rhododendron* spp. were more widely reported by Yi (68%) than by Naxi (33%) informants. Naxi informants most often mentioned the importance of rhododendrons (particularly *adzegedze*) in connection with the seasonal springtime festivals Qingming and Sanduojie, and several informants with specialized knowledge mentioned the place of rhododendrons in *dongba* ritual (Figure 5). In contrast, a more informal and active ritual use was mentioned by Yi informants: *shouxma axge* banishing malignant spirits, ritual use of *R. racemosum* for ensuring a good harvest when the first buckwheat crop is planted, and of various other species for hunting sacrifices and constructing ritual implements. The use of rhododendrons for decoration was reported by both Yi (42%) and Naxi (43%) informants, although this too differed in detail. Cut flowers were commonly used by Yi people as decoration, while for Naxi people, potted or planted rhododendrons were an important component of lush
courtyard gardens. Naxi informants prized rare and large specimens – some bought species from other areas at floral markets, while others would mount expeditions to Mt. Yulong in order to collect wild plants.

Across all interview sections, the seasonal progression of rhododendrons and their importance as seasonal indicators emerged as a crosscutting theme. The flowering of the ‘buckwheat’ rhododendron *shouxma mgepqy* signaled to Yi informants the correct time to plant buckwheat and the flowering of *R. decorum* the correct time to plant potatoes, while *R. yunnanense*, had changed from flowering with the latter to the former. *R. impeditum* was reported to flower at the time of *Ophiocordyceps sinensis* harvest. *R. racemosum* always flowered before the Sanduojie festival, but one informant was worried that the recent drought could result in it not flowering at all. Rhododendrons were reported to flower at the same time the cuckoos started calling. One Naxi Dongba scholar reported that the importance of *mun* to purification rituals (Figure 5, 6C) was directly tied to gradients of phenology and elevation – the status of *mun* as the earliest and highest of flowers to bloom lent it purity. Songs and sayings that were reported invariably related to seasonality, including many in which rhododendron flowers were a harbinger of spring, and one which matched each ethnotaxon to a season.

A second crosscutting theme that emerged was that of knowledge dynamics and preservation. Many of the songs and sayings were imperfectly remembered by those

---

3 A valuable fungus which is wild-harvest for sale. Mt. Yulong is at its southeast range limit, so this is likely to be a new indicator for the Yi.

4 Cuckoo is also an important indicator of spring near Mt. Yulong, as it is elsewhere in the world. Interestingly, the connection is also suggested also by the Mandarin Chinese name for rhododendron, *dujuanhua* (杜鹃花) ‘cuckoo-flower’.
who reported them. As one informant said, “only the old people know the stories”. Two Naxi informants referred me to nearby Yi villages with the thought that they might remember more of the old songs. Both Yi and Naxi reported using rhododendrons less for fuel, as roads and vehicles made it easy to obtain better wood. Yi reported previously cutting and burning rhododendrons to expand subalpine pasture, a practice that has been reduced due to government policy. Naxi informants reported less frequent use in general, except horticultural. Many Yi informants excused their knowledge because the ‘true’ Yi people who held authentic knowledge remained in the core Yi areas in Sichuan. At the same time, they also repeatedly stressed the importance of preservation of the cultural knowledge they did have, which needed to be stored, in the words of one informant, ‘in the heart’. Several Yi informants reported an additional rhododendron species not included in the photographs, which flowered in April, had deep red flowers and could be used for dying. This was likely *R. delavayi*, which does not grow in the immediate vicinity of Mt. Yulong but does further east in the mountains closer to the core Yi areas. For both Naxi and Yi informants living in Lijiang, knowledge and practice both were greatly changed: urban Naxi and Yi informants generally did not differentiate rhododendron species linguistically (using the Mandarin Chinese *dujuanhua* for all species) or in pile sorts or sequencing tasks, and reported very few uses (Figure 1).

5 Another Yi informant explained this importance with reference to religion: while Han Chinese customs may seem very relevant here and now, they will be of little use in the Yi afterlife.
Discussion

TEK of *Rhododendron* spp. was strongly associated with rural peoples in both ethnic groups (Figure 1). The immigrant Yi people had a knowledge base about the indigenous Mt. Yulong rhododendrons as elaborate or in some cases more elaborate than that of the indigenous Naxi (Figure 1). This speaks to the importance of ecological place to TEK, and both Yi and Naxi informants themselves credited a generative processes: the Yi people spent more time in the mountains and so quickly learned about its plants. However, geographic origin and cultural ‘place’ was also important. Yi preserved a strong orientation toward Sichuan as a core cultural area, even while accumulating knowledge about the new species they encountered on Mt. Yulong. Some elements were conserved: species (*R. delavayi*) and uses (lacquerware) not connected to Mt. Yulong were still reported, although they were no longer immediately relevant. Other elements were adapted. The use of *shuoxma mgepqy* as an indicator of buckwheat planting season is a practice that the Mt. Yulong Yi share with those who still reside in the core Yi area, although an indigenous Mt. Yulong species was given the name and cultural utility of the original Sichuan species (S. Harrell, pers. comm.). In general, this suggests the importance of both conservative and generative processes for systems of TEK. The Yi people conserved a TEK system that valorized rhododendrons from their original geographic place, while specific elements within the system change (new species on Mt. Yulong substituted for their ecological analogues).

---

6 It may be an unremarked feature of these “utilitarian” (Hunn 1982) ethnotaxonomies that classification systems are more adaptable if identity is defined by ecological function (or ethnobotanical utility) rather than by common ancestry.
Likewise, for the Naxi, cultural systems acted to conserve TEK. Although direct uses of rhododendrons were fewer in the lower and more accessible Naxi villages, TEK was conserved through the importance of rhododendrons to seasonal festivals and their place in Naxi spoken and written languages (e.g. Figure 6). *R. racemosum* was one of the strongest examples of this conservation process. Still important to both the traditional Naxi Sanduojie festival (which propitiates the resident god of Mt. Yulong) and to the syncretic Naxi practice of the Chinese festival Qingming, *R. racemosum* was also the most frequently mentioned species and the species most consistently differentiated in pile sorting, elevational and phenological sequencing (Figures 2, 3).

As one of the most salient plant genera to Yunnan, rhododendrons have been documented as foci of TEK previously (Pei 1989), and as a genus used in different ways by different ethnic groups (Georgian 2014). In Eurasia, knowledge of the toxic and therapeutic effects of rhododendrons extends from ancient Greek sources (Murphy 2004, Xenophon 2009) to modern cases of both poisoning and folk-medicinal uses (Koca and Koca 2007, Demircan et al. 2009). In China, early medieval records of rhododendrons refer to the plant’s toxicity to sheep (Lo and Cullen 2005). This toxicity provides resistance to grazing – one reason that Yunnan rhododendrons have been successful invaders of alpine pasture areas in response to burn bans and climate change (Brandt et al. 2013). Our study underscores the deep ecological and utilitarian knowledge of rhododendrons across cultures in relation to particular rhododendron species and ethnotaxa. Finely delineated and specific knowledge may be especially important given the great diversity of *Rhododendron*
species and their interspecific variation in toxicity (as above, and as reported in our results).

This rich and specific knowledge among Naxi and Yi adapts not only to changing geographic place and to changing lifeways but also to changing climate. Even as TEK acts as a baseline from which indigenous people can perceive climate changes (Salick and Byg 2007), it also responds to those changes. One example is *R. yunnanense*, which was reported to once flower at buckwheat planting time, and now at potato planting time. Similarly, both Naxi and Yi people reported rhododendron phenology to be driven by annual changes in weather. However, people have not noticed long-term directional changes in flowering time. These observation match with results from long-term phenological records derived from herbarium collections (Hart et al. 2014) and ecological monitoring (Hart et al. in prep).

Knowledge systems may allow adaptation to a changing geographic place, and conservation of knowledge through changing lifeways. However, the synchronous effects of urbanization and cultural assimilation occurring at a rapid pace throughout China (and around the globe) threaten these systems (Berkes et al. 2000, Harrison 2007, Ye et al. 2013). In Lijiang, the changed ecological place and changing culture associated with urban environments coincided with dramatic reduction in TEK among both Naxi and Yi. In an era of rapid change, combined conservation of cultural and ecological diversity is necessary to preserve knowledge systems and the adaptive capacity they offer.
Acknowledgements

We thank our 4 field assistants, who put an immense amount of time and energy into this work, and many patient and generous Naxi and Yi informants without whom the study would not have been possible. This research was supported by the “Biodiversity Conservation and Sustainable Development in Southwest China” NSF IGERT grant # 0549369, the Whitney R. Harris World Ecology Center, the Explorers Club, and the Anne S. Chatham Fellowship in Medicinal Botany of the Garden Club of America.
References


de Mendiburu, F. 2014. agricolae: Statistical Procedures for Agricultural Research. <cran.r-project.org/package=agricolae>


Dinno, A. 2015. dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. <cran.r-project.org/package=dunn.test>


Hart, R., J. Salick, S. Ranjitkar, and J. Xu. 2014. Herbarium specimens show


Table 1. Ethnotaxa applied to *Rhododendron* species in Naxi and Yi languages. The first name given was the one applied by the majority of informants, and alternate or variety names follow.

<table>
<thead>
<tr>
<th>Species</th>
<th>Naxi</th>
<th>Yi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. oreotrephes</em></td>
<td>shuo</td>
<td>shouxma axge ꜑ꐙ Ꚇ ‘large’</td>
</tr>
<tr>
<td></td>
<td>mun</td>
<td></td>
</tr>
<tr>
<td><em>R. yunnanense</em></td>
<td>shuo</td>
<td>shouxma axyi ꜑ꑳ ‘child’</td>
</tr>
<tr>
<td></td>
<td>shuo par ‘white’</td>
<td></td>
</tr>
<tr>
<td><em>R. impeditum</em></td>
<td>adzegedze</td>
<td>shouxma axyi ꜑ꑳ ‘buckwheat’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>shouxma etzyr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>shouxma ddebbip ꜑ ‘short’</td>
</tr>
<tr>
<td><em>R. traillianum</em></td>
<td>mun</td>
<td>shouxma axge</td>
</tr>
<tr>
<td></td>
<td>shuo</td>
<td>shouxma axge anmu ꜑ ‘tall’</td>
</tr>
<tr>
<td></td>
<td>mun lego par ‘white hair’</td>
<td></td>
</tr>
<tr>
<td><em>R. vernicosum</em></td>
<td>mun</td>
<td>shouxma axge</td>
</tr>
<tr>
<td></td>
<td>shuo</td>
<td></td>
</tr>
<tr>
<td><em>R. adenogynum</em></td>
<td>mun</td>
<td>shouxma axge</td>
</tr>
<tr>
<td><em>R. decorum</em></td>
<td>mun</td>
<td>shouxma axge</td>
</tr>
<tr>
<td></td>
<td></td>
<td>shouxma axge aqu ꜑ Ꚇ ‘white’</td>
</tr>
<tr>
<td><em>R. primuliflorum</em></td>
<td>shuo</td>
<td>shouxma axyi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>shouxma etzyr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>shouxma jiyy ꜑ ‘nectar’</td>
</tr>
<tr>
<td><em>R. racemosum</em></td>
<td>adzegedze</td>
<td>shouxma axyi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>shouxma mgepqy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>shouxma etzyr</td>
</tr>
<tr>
<td><em>R. rubiginosum</em></td>
<td>shuo</td>
<td>shouxma axge</td>
</tr>
<tr>
<td><em>R. beesianum</em></td>
<td>mun</td>
<td>shouxma axge</td>
</tr>
</tbody>
</table>
Table 2. Ethnotaxon and TEK of phenology and elevation explain pile sort groupings. Ethnotaxon, mean scaled rank and mean phenological rank as assigned by informants to each species were fit the ordination of pile-sort scores (Figure 3). P-values are based on a permutation test (999 permutations).

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>r²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural Yi</td>
<td>Mean phenological rank</td>
<td>0.71</td>
<td>0.009**</td>
</tr>
<tr>
<td></td>
<td>Mean elevation rank</td>
<td>0.77</td>
<td>0.005**</td>
</tr>
<tr>
<td></td>
<td>Ethnospecies</td>
<td>0.71</td>
<td>0.004**</td>
</tr>
<tr>
<td>Rural Naxi</td>
<td>Mean phenological rank</td>
<td>0.59</td>
<td>0.04*</td>
</tr>
<tr>
<td></td>
<td>Mean elevation rank</td>
<td>0.63</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td>Ethnogenus</td>
<td>0.59</td>
<td>0.0*</td>
</tr>
</tbody>
</table>
Figure 1 (following page). Knowledge metrics vary with ecological place and culture. For the 11 *Rhododendron* spp., urban Naxi and urban Yi knowledge metrics were significantly lower than rural Naxi and rural Yi metrics, including: number of ethnotaxonomic tiers (ethnogenus, ethnospecies, ethnovariety) and the total number of ethnotaxa; the number of species used and the total number of uses reported; and the number of distinct groups identified in the phenology and elevation sequencing tasks. Rural Yi scores were significantly higher than rural Naxi for ethnotaxonomic tiers, useful species and total uses, and phenology groups. Within each metric, different letters indicate groups that are significantly different from one another according to Kolmogorov-Smirnov post-hoc tests (Dunn test, α=0.05).
Figure 2. Traditional ecological knowledge of elevation and phenology sequences. Scaled rank values of rhododendron species sequenced by A) phenology and B) elevation by rural Naxi (pink) and rural Yi (blue) informants.
Figure 3. Pile-sorts of *Rhododendron* spp. Rural Naxi and rural Yi informants sorted photographs into piles according to which were most similar. Non-metric multidimensional scaling (based on a distance matrix of the proportion of times in which each species pair were placed into the same group) allows a visual analysis of clustering patterns. The most notable cluster is that of *R. racemosum* and *R. impeditum* (+ *R. yunnanense* for Yi) as a distinct group.
Figure 4. Yi lacquerware. Smaller pieces, including the goblet and ladles here, are made from rhododendron wood.
Figure 5. **Purification ceremony.** A Naxi *dongba* performing a seasonal purification ritual with *Artemisia* and *Rhododendron*; likely *mun bbue* (c.f. Figure 6C)
Figure 6. Naxi pictographs for *Rhododendron* recorded by Joseph Rock from dongba ritual specialists in the early 20th century. A) A tree of the ethnogenus *mun*, B) *Mun* depicted in the glyph for a sacred mountain, C) *Mun* and *bbue* ‘artemisia’ used in cleansing ritual. This pictographic/ideographic script remains the most commonly orthography for the Naxi language.