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Influence of phylogeny and abiotic factors varies across early and late reproductive phenology of Himalayan *Rhododendrons*

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Abstract. Understanding the influence of evolutionary history on species-specific phenological events of high-altitude plants and their sensitivity to the abiotic factors has gained importance mainly in the context of climate change. However, the majority of phenology studies across altitudinal gradient are carried out on flowering, whereas other reproductive phenology events are seldom considered. We tested the role of abiotic factors and evolutionary history on the reproductive phenology traits of high-altitude *Rhododendron* community which comprised of 10 locally aggregated species in Kyongnosla Alpine Sanctuary, Sikkim Himalaya. The study was carried out from 2013 to 2015 across an altitude gradient of 3400–4230 m a.s.l. We generated dated phylogenetic hypotheses to test for phylogenetic signal in reproductive phenology events, and its durations across 10 *Rhododendron* species and also among groups of species distributed at every 100 m altitude. Comparative phylogenetic methods were used to explore the relationship between phenology traits and abiotic variables such as daylength and temperature. The early phenology events such as budding, flowering, and initial fruiting, which occurred during the favorable month of the year, exhibited strong phylogenetic signal and were mainly associated with daylength and temperature. In contrast, the later events such as immature fruiting, mature fruiting, and fruit dehiscence, which occurred during the later months of the year, showed a weak phylogenetic signal and were mostly associated with daylength. With the increase in altitude, we found a decreasing trend of phylogenetic signal for the early phenology events and later events did not show a significant trend. Our results suggest that only early events are constrained by evolutionary history; thus, the closely related species share the similar timing of the early phenology events. Also, the role of shared evolutionary history in phenological trait sensitivity to the abiotic factors reduces from early to the late phenology events. This approach can be extended to other representative plant families of the Himalayan region to better understand the response of reproductive traits to abiotic factors in deep evolutionary time.

Key words: climate change; duration; flowering; fruiting; Himalaya; phenology; phylogenetic conservatism; *Rhododendron*.

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INTRODUCTION

Montane communities are susceptible to changes in climate, due to their occurrence at high altitudes, exposure to low temperatures, short growing season, and restricted range boundaries (Parmesan 2006, Hülber et al. 2010). Compared to other regions, mountain floras are considered highly sensitive to future climate change as their reproductive phenology events are often strongly associated with climate-related ecological factors. Over the last few decades with the growing concern of climate change, there is an increasing interest to understand the role of ecological factors in the evolution of mountain plant communities (Forrest and Miller-Rushing 2010). In particular, plant reproductive phenology has received enormous attention because of the potential for the climate to alter flowering timing and thereby pollination (Hegland et al. 2009), seed dispersal (Johnson 1992), and the long-term persistence of populations (Rathcke and Lacey 1985). The Himalayan montane ecosystems represent one of the major global biomes and critical ecosystems where climate change impact is profound. Particularly, mountain ecosystems in the Eastern Himalayan region of India are considered extremely sensitive to climate change (Shrestha et al. 2012, Telwala et al. 2013, Manish et al. 2016). Despite the growing importance of phenology as one of the most reliable bioindicators of climate change (CaraDonna et al. 2014), there is inadequate literature available on the reproductive flowering phenology of high-altitude plants from this region (but see Ranjitkar et al. 2013, Hart et al. 2016).

In high mountain regions, flowering phenology changes with altitude (Bertiller et al. 1990). There is strong evidence that environmental cues such as temperature, daylength (Ranjitkar et al. 2013, Li et al. 2016), and snowfall (Forrest and Miller-Rushing 2010) are the crucial factors influencing the timing of phenological events. Biotic factors such as the presence of pollinators and seed dispersers also serve as an important driver for staggering (Pleasants 1980) and aggregation of phenology events (Rathcke 1983).

Apart from the importance of various environmental and ecological factors that determine the response of reproductive phenology events, phylogenetically related species may also share similar phenology events at similar time. The strong genetic constraints on timing of phenology events and limited time for divergence may promote closely related species to share similar phenological traits (Lessard-Therrien et al. 2014), a phenomenon called phylogenetic conservatism (Harvey and Pagel 1991). Incorporating phylogenetic information into predictive modeling of phenological response to abiotic variation not only allows us to account for shared evolutionary history of traits, but it is also critical for robust prediction of phenological sensitivity to climate change (Cleland et al. 2012, Wolkovich et al. 2014).

The phylogenetic signal in plant phenology may vary in stressful environments. For instance, the strong phylogenetic conservatism in flowering phenology at high altitude (Li et al. 2016) might be associated with the facilitative process that is known to increase with environmental stress (Callaway et al. 2002). So, the overlap in the timing of flowering of closely related species might be a strategy that high-altitude plants adopt to attract more pollinators. Contrastingly, convergent evolution due to strong abiotic selection pressure may override any shared evolutionary history, and that might lead to convergent traits that may weaken the phylogenetic conservatism (Lessard-Therrien et al. 2014). Therefore, understanding the phylogenetic signal in phenology events in high-altitude plant communities is challenging; and due to limited study on few phenology traits, their generality still remains unclear. The recent studies also highlight the importance of measuring the first, peak, and duration of phenology events (CaraDonna and Inouye 2015, Hart et al. 2016), which have been shown to shift independently of one another under climate change (CaraDonna et al. 2014).

The duration of the reproductive phenology events in plants also forms an important component of their reproductive success. The fruit development time might as well affect the flowering timing in the community, for instance, species with larger fruits might flower much earlier as they may require longer maturation time (Primack 1987) compared to species with smaller fruits. However, the relationship of duration with abiotic factors and evolutionary history has been seldom tested (but see Staggemeier et al. 2010)

The lack of long-term phenological records poses constraints to assessing phenological response to climate change. However, studies designed along an altitudinal gradient can provide baseline information on phenological response to a change in climatic conditions (Ranjitkar et al. 2013). Mountains have therefore been regarded as ideal natural laboratories for research related to climate change (Thuiller 2007).

Here, for the first time in the Himalayan mountain plant community, we attempt to understand the phenological response of entire reproductive traits to abiotic factors by accounting for species shared evolutionary history. Our study focusses on 10 locally aggregated high-altitude *Rhododendron* taxa. *Rhododendrons* serve as a keystone species of high altitude (Singh et al. 2009) in the Himalayan region. The large variation in its flowering timing and distribution along a large altitude gradient makes it a model plant community to understand the sensitivity of reproductive traits to change in abiotic factors. Specifically, our objectives are to (1) examine whether there is phylogenetic signal in reproductive phenology events, and its durations across *Rhododendron* species and within the species of *Rhododendron* community at each altitude band, (2) assess the response of various reproductive phenological traits of *Rhododendron* community distributed across the altitude gradient to variation in abiotic variables, and (3) finally, to determine whether the sensitivity of phenological traits to abiotic variable exhibits phylogenetic constraint.

METHODS

Study area

The study was conducted in the Kyongnosla Alpine Sanctuary (here onwards, referred to as KAS) in East district of Sikkim (27° N, 88° E), India, from 2013 to 2015 across an altitude gradient of 2843–4230 m a.s.l. KAS comprises an area of 31 km^2 (Fig. 1). *Rhododendron* species are an essential component of plant communities at every altitude. The lower altitude from 3000 to 3800 m is characterized by tall trees and shrubs of mixed *Rhododendron* and conifer forests with *R. campanulatum, R. thomsonii, R. hodgsonii,* and *R. campylocarpum* as the dominant species of *Rhododendron*. The higher altitude from 3900 to 4200 m comprises scrubby *Rhododendron* species, conifers, and many other alpine herbs. The common *Rhododendron* species in this altitude includes *R. aeruginosum, R. setosum, R. lepidotum,* and *R. anthopogon. R. cinnabarinum* and *R. wightii* are the two less abundant species found in this region. The study was carried out from 3400 m to avoid anthropogenic disturbances at the fringes. In addition, a ban since 1998 on any human use of protected areas of Sikkim makes the area inside the sanctuary free of any anthropogenic disturbances such as grazing, human settlements, and other anthropogenic activities.

Measuring phenological traits

*Reproductive phenology events.—*To select the species for the phenology monitoring, we laid three transects of $50 \times 20\ \text{m}^2$ at every 100 m altitude, and a total of 27 transects were laid across the study site. We encountered 10 *Rhododendron* species inside the vegetation plots and all 10 were selected as a focal species. Ten trees were randomly selected, and a total of 30 inflorescences per species were monitored at every 100 m. We monitored the same individuals from 2013 to 2015. Phenology was recorded at every fifteen-day interval from May when the earliest bud opened until December by which time all fruits had dehisced.

Reproductive phenology events were scored as budding–fully closed bud (Fig. 2a, b), flowering–open flowers (Fig. 2c–f), initial fruiting–first fruit (Fig. 2g), immature fruiting–immature green fruits (Fig. 2h, i), mature fruiting–mature brown fruits (Fig. 2j), and fruit dehiscence (Fig. 2k, l). Each event was recorded for three levels start, peak, and end which refers to the percentages of the number of first, maximum, and last florets remaining in the study at that time. However, end day did not show any significant association with either abiotic factors or shared evolutionary history; hence, we did not include it for further analysis.

*Phenology duration.—*Duration of budding, flowering, initial fruiting, immature fruiting, mature fruiting, and fruit dehiscence was calculated for all 10 *Rhododendron* species. We considered the duration at population level based on the differences in the day of the year (DOY) of start and end event.

Phylogenetic tree construction

We collected leaf samples and voucher specimens for the 10 focal *Rhododendron* species, from KAS. Vouchers were identified using the *Rhododendrons of Sikkim* (Pradhan 2010), the *Flora of*

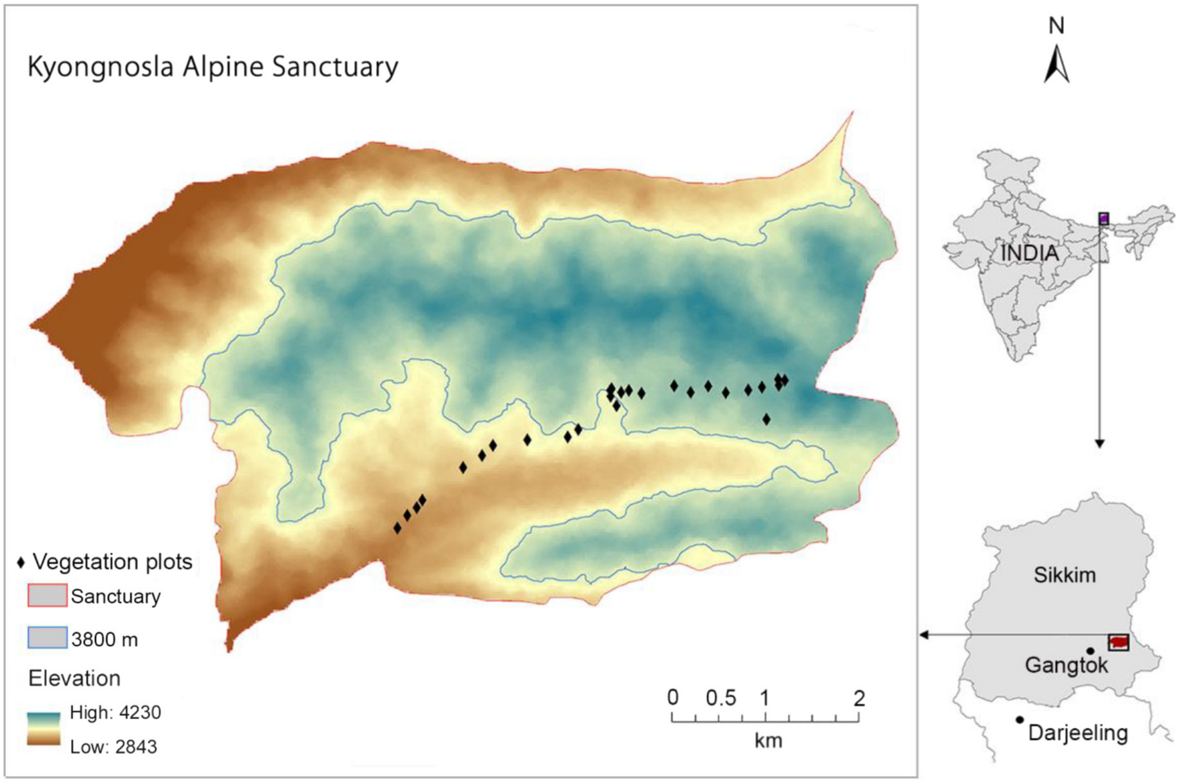

Fig. 1. Sampling location of 10 *Rhododendron* species in the Kyongnosla Alpine Sanctuary in Sikkim Himalaya, India. Black diamond sign signifies the vegetation plot sites along an altitudinal gradient.

China (Wu et al. 2005), and the *Flora of Bhutan* (Grierson and Long 1983). A voucher specimen is deposited in the herbarium at ATREE, Bangalore, India. To reconstruct a dated phylogenetic tree for 10 *Rhododendron* species in the study, we first identified candidate loci that have been successfully used in Ericaceae systematics. Based on this prior information, we selected three loci from chloroplast and nuclear genomes, which included maturase K gene (*mat*K), *psb*A-*trn*H intergenic spacer, and internal transcribed spacer (*ITS*). First, for 10 *Rhododendron* species in the study, we generated DNA sequences for the two loci using the following procedure: We extracted the total genomic DNA from leaf samples using modified CTAB method (Sambrook and Russell 2001). Then, the DNA was subjected to PCR reaction with loci *psb*A-*trn*H and *ITS* (see Appendix S1 for complete laboratory protocol) and for *mat*K we used the sequences from GenBank. In addition, we collected sequence data for 118 species belonging to family Ericaceae and outgroup Actinidiaceae (Appendix S1: Table S1). We inferred Ericaceae-wide phylogenetic relationship using maximum likelihood (ML) and Bayesian inference methods. Finally, the dated phylogenetic tree was obtained using the Bayesian method (Sanderson 2002) as implemented in the program BEAST v.1.8.2 (Drummond and Rambaut 2007). All phylogenetic analyses were performed using the CIPRES Science Gateway v.3.3 (Miller et al. 2011; see Appendix S1 for complete analytical details of dated phylogeny construction).

Abiotic data

To record daily temperature, 10 IButtons were deployed along the altitude gradient at every 200 m and recorded at 1-h interval. IButtons in every site were set for recording soil and the atmospheric temperature. For soil temperature, the IButton temperature sensors were installed at a depth of 10–12 cm in the soil, and for atmospheric temperature, they were located in an

Fig. 2. Examples of buds, flowers, fruits, and seed dehiscence of *Rhododendron* species studied in the Kyongnosla Alpine Sanctuary. Flower buds of *Rhododendron campanulatum* (a) and *Rhododendron hodgsonii* (b). Peak flowering of *Rhododendron thomsonii* (c), *R. campanulatum* (d), *Rhododendron cinnabarinum* (e), and *Rhododendron setosum* (f). Initial fruit of *R. thomsonii* (g). Immature fruit of *R. thomsonii* (h) and *Rhododendron lepidotum* (i). Mature fruit of *Rhododendron aeruginosum* (j) and fruit dehiscence of *R. aeruginosum* (k) and *R. setosum* (l). Photo credits: S. Basnett.

open area. The daylength was calculated with the geosphere package (Hijmans et al. 2017) in R.

Statistical analyses

Phylogenetic signal and mapping of phenological traits.—The phylogenetic signal for the mean of all phenological traits across 10 *Rhododendron* species was evaluated using two metrics of phylogenetic signal Pagel's lambda (Pagel 1999) and Blomberg's *K* (Blomberg et al. 2003). To measure the phylogenetic signal among the species across each 100 m altitude band, we used only Blomberg's *K*. Blomberg's *K* corresponds to the observed degree of similarity among phylogenetically related species compared with expectations obtained from the Brownian motion of model of evolution, in the picante package (Kembel et al. 2010) in R. Blomberg's *K* values close to one indicate trait evolution consistent with the Brownian motion model of evolution, while *K* values close to zero indicate a random distribution of trait values with respect to the phylogeny (Blomberg et al. 2003). The significance of Blomberg's *K* was tested by comparing the observed *K* value to a null distribution generated by comparing 1000 randomizations of trait values across the tips of the phylogenetic tree (Kembel et al. 2010). Pagel's lambda can range between 0 (no PS) and 1 (high PS) and is determined by ML. Intermediate values of Pagel's lambda signify that the trait evolution is phylogenetically correlated, but do not follow a fully Brownian motion model (Pagel 1999). We used stochastic character mapping (Huelsenbeck et al. 2003) method to visualize and reconstruct the phylogenetic pattern of reproductive phenology events. We used the package phytools (Revell 2012) to reconstruct the trait evolution. Phytools uses a Markov chain Monte Carlo approach to model the evolution of a continuous trait on a phylogenetic tree (Huelsenbeck et al. 2003).

Abiotic factors and phenological traits

To test for the spatial autocorrelation among the temperature values of five altitudinal sites for every month, we calculated Morans *I* using the R package Ape (Paradis et al. 2004). For all the statistical analysis, we considered day and night combined temperatures. The R package psych (Revelle 2011) was used to check the multicollinearity correlation coefficients among the predictor variables. To evaluate how the phenological events are influenced by the climatic factors, a generalized linear model (GLM) postulating Gaussian distribution was applied. Phenology is affected by the cumulative climatic changes rather than instantaneous variation; therefore, we report bimonthly mean temperature in our statistical analysis. For the response variable, we considered mean DOY (first, peak) of each phenological event and the mean duration of each phenology event. We calculated the response variable for every 100 m altitude averaged across three years. We used a Kruskal–Wallis test to explore annual variation in each phenological trait. A best-fit model (most probable combination of the predictor variables) was selected for each phenological trait based on the Akaike's information criteria with the lowest Akaike's information criterion (AIC; Akaike 1981) and highest Pseudo R^2 (McFadden 1974). To minimize inter-annual sources of variability, we used the three-year average to represent each species flowering phenology events at every 100 m altitude.

Influence of phylogeny

To account for the effect of abiotic variables independent of shared evolutionary history on phenological traits, two different models using the mean value per species of all phenology events and phenology duration were considered. Firstly, we evaluated this relationship using a simple linear model. Then, to account for the effect of shared evolutionary history, we used phylogenetic generalized least square (PGLS) with a λ parameter estimated with ML as a measure of the phylogenetic signal. This analysis was performed in "caper" package (Orme 2013) in R. Phylogenetic generalized least square takes into account phylogenetic non-independence in the data (Freckleton et al. 2002), and we determined the species level correlation in the phenology traits to the climatic variables. Phylogenetic generalized least square provides a more general and flexible approach to the widely used independent contrast methods (Felsenstein 1985) for assessing correlations between traits independent of phylogenetic divergence. This analysis was performed for all the phenological traits with only those climatic variables obtained from the GLM best-fit model. All comparative phylogenetic analysis including phylogenetic signal test was repeated across the 1000 pruned (included only 10 *Rhododendron* species of interest) tree topology obtained from BEAST analysis to account for phylogenetic and branch length uncertainty.

RESULTS

As the results involve multiple events of flowering and fruiting phenophase, they were coded appropriately for further use in the figures and tables. The events of phenophases were coded as bud (B), flower (F), initial fruiting (IFr), immature fruit (IMFr), mature (MFr), and fruit dehiscence (FrD). The day of the first and peak flowering was coded in italicized lower case and prefixed to the events as *f*B, *f*F, *f*IFr, *f*IMFr, *f*MFr, *f*FrD and *p*B, *p*F, *p*lFr, *p*IMFr, *p*MFr, *p*FrD. Duration was postfixed to event as du (Bdu, Fdu, IFrdu, IMFrdu, MFrdu, FrDdu).

Henceforth, phenology events such as first and peak budding, flowering, and initial fruiting which occurs from 123 to 215 DOY are considered as early events, whereas events such as first and peak immature fruiting, mature fruiting, and fruit dehiscence which occurs from 180 to 352 DOY are regarded as late events (Appendix S2: Fig. S1). In the case of duration, the budding and flowering duration were similar across species and ranged from 21 to nearly 50 d. However, with the initial, immature, and mature fruiting events, the duration was longer and ranged from 45 to 135 d, whereas the fruit dehiscence duration was shorter and ranged from 24 to 62 d.

Phylogenetic signal in phenological traits

In the case of flowering phenology event, the values of Blomberg's K ranged from 0.251 to 0.893, and Pagel's lambda ranged from 0.000 to 0.954. Both Blomberg's K ($P < 0.01$) and Pagel's lambda ($P < 0.05$) showed significant phylogenetic signal in the DOY of the early phenology events from budding event to peak initial fruiting day (Table 1, Fig. 3). In the case of duration, value of Blomberg's K ranged from 0.256 to 1.080, and Pagel's lambda from 0.00 to 0.885. However, only fruit dehiscence duration showed a significant phylogenetic signal ($P < 0.01$) for both the metrics (Blomberg's $K = 1.080$, Pagel's lambda = 0.885) and the duration of flowering and fruiting showed a significant phylogenetic signal only for Blomberg's K. There was no apparent phylogenetic signal in durations of other phenological events (Table 1). Within each altitude band, we did not detect significant phylogenetic signal in any of the phenology traits. However, for most of the events from first budding up to peak immature event, we noticed a difference in the strength of the phylogenetic signal across the altitude bands. A decrease in Blomberg's K from the lower to higher altitude bands was detected, although the K value was still lower than predicted by a Brownian motion model ($K = 1$) of trait evolution (Appendix S2: Fig. S2).

Table 1. Strength and significance of phylogenetic signal in the three-year mean day of the year for *f*B, *p*B, *f*F, *p*F, *f*IFr, *p*IFr, *f*IMFr, *p*IMFr, *f*MFr, *p*MFr, *f*FrD, and *p*FrD followed by three-year mean of Bdu, Fdu, IFrdu, IMFrdu, MFrdu, and FrDdu of *Rhododendron* community (N = 10) estimated using BEAST tree.

Phenology events	Blomberg's K	Pagel's λ
*f*B	0.893**	0.954*
*p*B	0.838**	0.910*
*f*F	0.853**	0.913*
*p*F	0.778**	0.849*
*f*IFr	0.862**	0.901*
*p*IFr	0.790**	0.956*
*f*IMFr	0.386	0.000
*p*IMFr	0.337	0.000
*f*MFr	0.251	0.000
*p*MFr	0.340	0.000
*f*FrD	0.551*	0.511
*p*FrD	0.272	0.000
Bdu	0.256	0.000
Fdu	0.588*	0.567
IFrdu	0.584*	0.606
IMFrdu	0.454	0.202
MFrdu	0.259	0.000
FrDdu	1.080**	0.885**

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Influence of abiotic factors on phenology traits

We did not detect spatial autocorrelation in temperature across the five elevation gradient ($P > 0.05$) for all the months. A strong correlation of elevation with atmospheric temperature ($r^2 = -0.88$) and soil temperature ($r^2 = -0.94$) and also between atmospheric and soil temperature ($r^2 = 0.91$) was found (Appendix S2: Fig. S3). We did not detect significant annual variation in any early phenology events and durations (Appendix S2: Table S2).

Results of a GLM (lowest AIC and highest Pseudo R^2) for the phenology events indicate that first budding day, peak budding day, and first flowering day were influenced by daylength. For peak flowering day and first initial fruiting day, temperature showed a significant influence. For later phenology events, daylength was the best-fit predictors. After peak flowering day onwards, daylength showed a significant negative effect on all other phenology events (Appendix S2: Table S3). The result of a GLM for reproductive events duration showed that temperature explained flowering and immature fruit duration, whereas dehiscent duration was associated with daylength (Appendix S2: Table S4).

Phylogenetic pattern of phenological sensitivity to abiotic variables

The simple linear (Appendix S2: Table S5) and pgls regression model (Appendix S2: Table S6) approaches tested on mean species values of phenology traits were not significantly different (Table 2).

We observed a significant effect of daylength on first budding ($R^2 = 0.50$, $P < 0.05$, $\lambda = 0.95$; Fig. 4a), peak budding ($R^2 = 0.45$, $P < 0.05$, $\lambda = 0.90$; Fig. 4b), and peak initial fruiting day ($R^2 = 0.68$, $P < 0.01$, $\lambda = 0.95$; Fig. 4c). We found that estimated Pagel's λ for the PGLS models was typically much greater than zero for early events such as start and peak flowering and first initial fruiting day. We observed a significant effect of temperature on peak immature fruiting ($R^2 = 0.47$, $P < 0.05$, $\lambda = 0.0001$; Fig. 4d) but they were

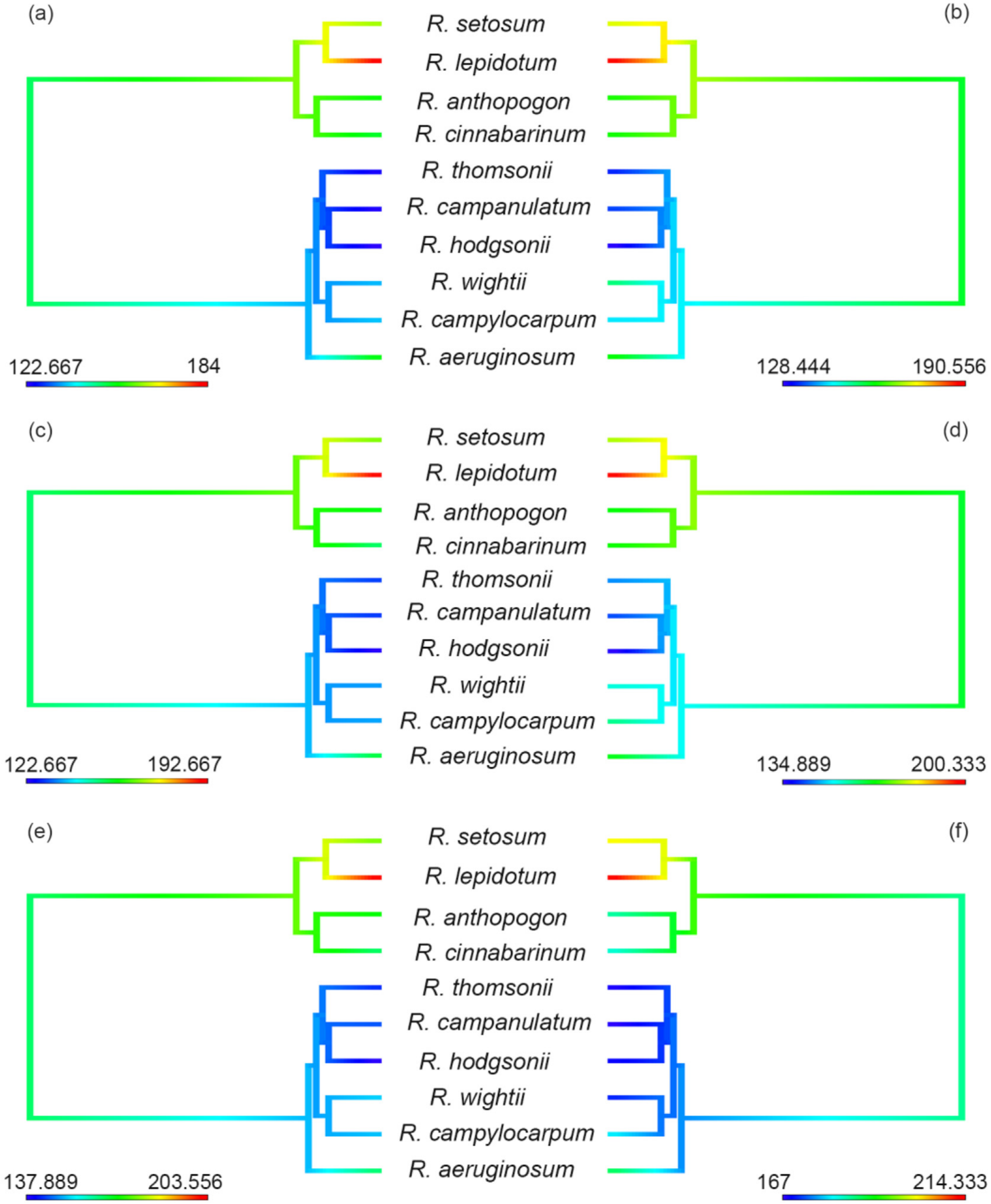

Fig. 3. Stochastic mapping of day of the year (DOY) trait values of 10 *Rhododendron* species first budding day (a), peak budding day (b), first flowering day (c), peak flowering day (d), first initial fruiting day (e), and peak initial fruiting day (f). The DOY trait values are visualized as different colors along nodes and branches of the phylogeny.

statistically independent of phylogenetic relationship. Daylength more significantly explained the later events from first and peak immature and mature fruiting ($R^2 = 0.69$, $P < 0.01$, $\lambda = 0.0001$; Fig. 4e) to first ($R^2 = 0.83$, $P < 0.001$, $\lambda = 0.0001$; Fig. 4f) and peak dehiscence event. However, in contrast to some of the earlier events, we did not detect any phylogenetic dependence in the later events and the Pagel's λ values were equal to zero ($\lambda = 0.0001$; Table 2).

Initial fruiting duration was significantly associated with daylength ($R^2 = 0.56$, $P < 0.01$, $\lambda = 0.0006$), whereas immature fruiting duration was significantly explained by temperature ($R^2 = 0.66$, $P < 0.01$, $\lambda = 0.206$). The effect of climatic variables on any other phenology duration traits was not significant. We did not notice strong phylogenetic dependence in any duration traits. However, high Pagel's λ values ($\lambda = 0.888$) were found for the dehiscence duration (Table 2; Appendix S2: Table S6).

DISCUSSION

Our result highlights that phylogenetic conservatism among *Rhododendron* species is not consistent across the various reproductive events. We found phylogenetic signal for the early events such as budding, flowering, initial fruiting, and the dehiscence duration. Along the altitude gradient, we observed an overall decrease in the strength of the phylogenetic signal on early phenological events. Daylength and temperature influenced the early phenology events, whereas later events were influenced by daylength. The strength of phylogenetic dependence in phenological trait sensitivity to the abiotic factors reduced from early to the late events. The later events were strongly associated with abiotic factors, and the relationship was not constrained by phylogenetic relationship of taxa.

Phylogenetic constraints on early phenology events

Consistent with the earlier study on the alpine plant community of Tibetan plateau, we found that budding, flowering, and initial fruiting were significantly aggregated across phylogenetically closely related *Rhododendron* species (Li et al. 2016). The opening of buds at the right time might consequently help plants to flower and fruit at favorable conditions when the pollinator availability is high, and also meet the required

Table 2. Summary and comparison of simple linear regression and phylogenetic generalized least square between phenology traits and abiotic variables on mean species values (N = 10).

Traits	Predictors	Lm adjusted R^2	Pgls adjusted R^2	Pagel's λ	*t*-Test	*t*-Test for slope *P*-value
*f*B	Daylength	0.746***	0.506*	0.955	1.265	0.223
*p*B	Daylength	0.612**	0.456*	0.902	1.268	0.222
*f*F	Daylength	0.274*	0.021	0.675	1.165	0.251
*p*F	Temperature	−0.057	−0.112	0.851	0.768	0.452
*f*IFr	Temperature	0.033	−0.096	0.922	1.471	0.158
*p*IFr	Temperature	0.265*	0.011	0.956	1.277	0.217
	Daylength	0.844***	0.687**	0.956	1.511	0.157
*f*IMFr	Daylength	0.888***	0.888***	0.0001	0.001	0.991
	Temperature	−0.055	0.055	0.0001	0.000	1.000
*p*IMFr	Daylength	0.836***	0.833***	0.0001	0.000	1.000
	Temperature	0.471*	0.471*	0.00001	0.000	1.000
*f*MFr	Daylength	0.672**	0.672**	0.0001	0.000	1.000
*p*MFr	Daylength	0.693**	0.693**	0.0001	0.000	1.000
	Temperature	−0.124	−0.124	0.0001	0.000	1.000
*f*FrD	Daylength	0.834**	0.834***	0.0001	0.000	1.000
*p*FrD	Daylength	0.746***	0.746***	0.0001	0.000	1.000
Fdu	Temperature	−0.006	0.051	0.607	0.000	1.000
IFrdu	Daylength	0.567**	0.567**	0.0006	0.000	1.000
IMFrdu	Temperature	0.666**	0.668**	0.206	0.000	1.000
FrDdu	Daylength	0.267	−0.121	0.888	1.937	0.073

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

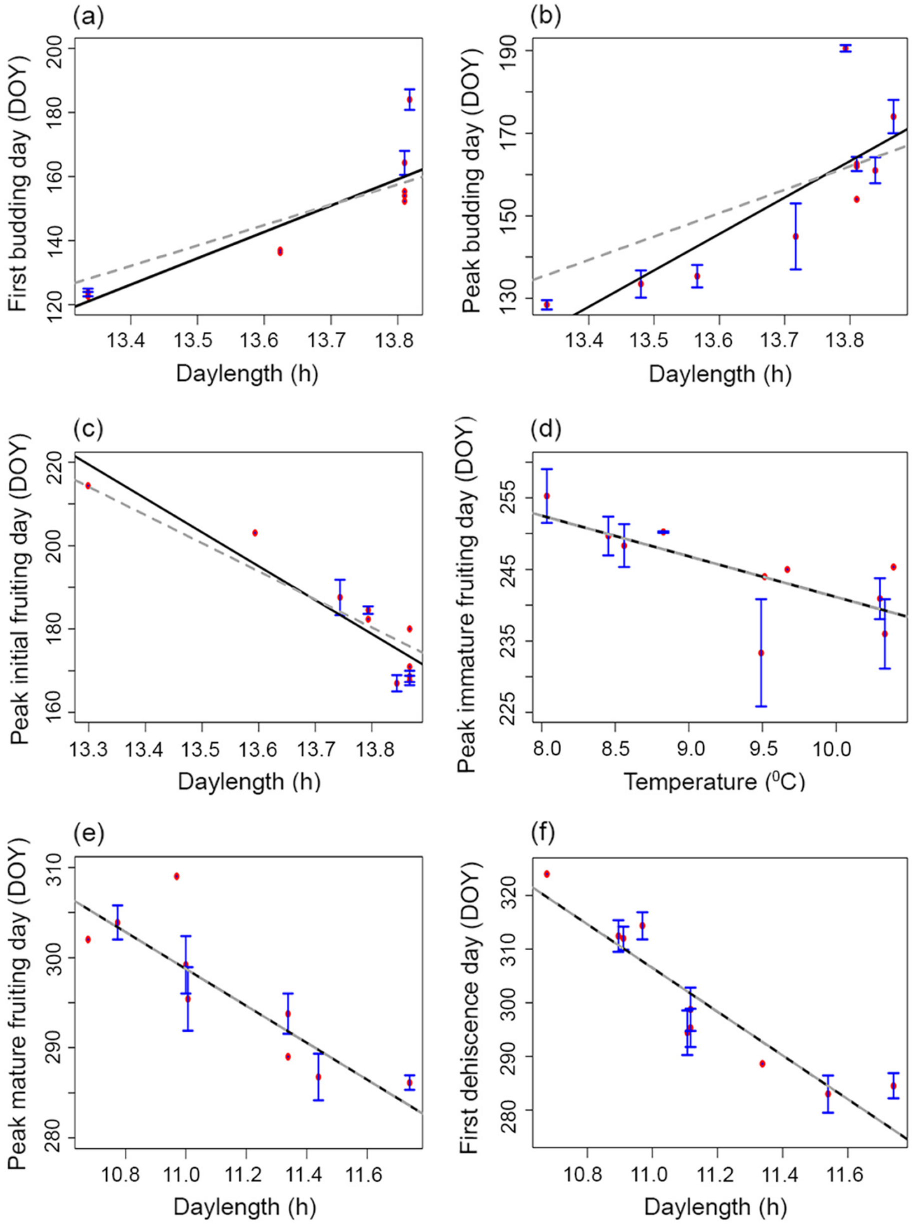

Fig. 4. Relationship between reproductive phenology events and best explained abiotic variables. Solid red symbols are species mean with ± standard error for each reproductive phenology event across altitude first budding day (a), peak budding day (b), peak initial fruiting day (c), peak immature fruiting day (d), peak mature fruiting day (e), and first dehiscence day (f). Black solid line shows trend in abiotic variables with phenology events as shown by simple linear regression, and gray dashed line shows trend in abiotic variables with phenology events as shown by phylogenetic generalized least square regression.

climatic conditions. In the case of flowering phenology contradicting to our result, a study on 10 *Rhododendron* species in Mount Yunnan of China (Hart et al. 2016) did not detect a phylogenetic signal. This contradicting results might be associated with the difference in the *Rhododendron* species composition. Despite having an overlap in the altitudinal range, these two study sites

(Sikkim and Yunan) differ in *Rhododendron* species composition.

We found the strongest and the most consistent pattern of phylogenetic signal in fruit dehiscence durations using both indices (Pagel's lambda and Blomberg's *K*). The phylogenetic conservatism in dehiscence duration might explain that related species share similar timing of reproductive events as a result of a conserved evolutionary response to specific environmental cues. A study from the Tibetan plateau has reported a negative correlation between seed mass and altitude across 42 species of *Rhododendron* (Ericaceae), which could be due to the short growing season and decline in resource availability (Guo et al. 2010). Fruits of *Rhododendron* species located at higher altitude are also known to mature faster than species found in the lower altitude because of their smaller size (Hart et al. 2016). We noticed a similar pattern, where most of the lower altitude species flower early, have larger fruits, and take an extended period to mature and dehisce their seeds. However, the higher altitude communities flower late, have small fruits, and complete their fruit maturation and seed dehiscence within a short period.

The signal in budding and flowering duration was detected only by Blomberg's *K*. The discrepancy in flowering and fruiting duration using Pagel's lambda might be due to the following reasons as reported by CaraDonna and Inouye (2015). Firstly, compared to Blomberg's *K*, Pagel's lambda is considered as a conservative way of measuring phylogenetic signal, which performs better than Blomberg's *K* under Brownian motion model of trait evolution (Münkemüller et al. 2012). Secondly, for the duration, we considered the start and end of the flowering individuals, which is often represented by a few individuals than a cumulative effect of the population (Miller-Rushing et al. 2008). The absence of phylogenetic signal among species within the same altitude might be due to the small number of species, which limits the understanding of the correlation across related species. Also, to calculate the average value per species, we considered many individuals exposed to different microhabitats across altitudes (Ranjitkar et al. 2013). The variations across these individuals might also affect the phylogenetic signal within bands. Although there was no significant phylogenetic signal across altitudes, there was a trend that is still consistent with the earlier study on flowering phenology of subarctic plant species (Lessard-Therrien et al. 2014), where high phylogenetic signal in the lower altitude was detected compared to the higher altitude.

In the Himalayan region at higher altitude, species are exposed to a much harsher environment such as low temperature, heavy snowfall, and strong winds and the land surface is more barren and rocky as we move toward higher elevation. The community present in this environment may be more distantly related as convergent evolution due to strong abiotic selection pressure may override any common evolutionary history (Lessard-Therrien et al. 2014) and show less phylogenetic signal compare to lower altitude plant community.

Influence of abiotic factors varied across different phenology events

Our result suggests that at a community level, daylength is the primary abiotic factor associated with the budding event. A similar influence of daylength on budding events was observed on budding of 34 Myrteae species in an Atlantic rain forest (Staggemeier et al. 2010). Daylength is associated with breaking the bud dormancy in several plant species (Calle et al. 2010). The first opening of bud starts during May which is when the plants start receiving most extended daylength of 13.33–13.62 h. Hence, daylength could be one of the reliable factors that high-altitude plants could depend on for the opening of the bud, when the surface is covered with snow, and the average temperature ranges from 6°C to 9°C. The effect of temperature on flowering phenology is consistent with another study on *Rhododendron arboreum* across the altitude gradient of Nepal and China (Ranjitkar et al. 2013). However, the author also reported that apart from temperature, soil moisture is the most important variable for explaining initial flowering dates followed by low pH soil, organic matter, and soil nitrogen for peak flowering.

In the case of immature and mature fruiting, our findings coincide with the earlier study on Myrteae (Staggemeier et al. 2010) followed by another study on an Alpine plant where the fruiting reached a maximum when the daylength declined (Keller and Körner 2003). These events also last for the longest among all other events from 50 to almost 135 d, and daylength seems to be the only factor significantly influencing it.

We detected a strong association of temperature with flowering duration. In the case of flowering, the warmer temperature may initiate more nutrient uptake, producing a higher number of flowers leading to the overall extension in the flowering timing (Campbell and Halama 1993). Warm temperatures may also be beneficial for pollinators, and it has also been suggested that biotically pollinated plants flower for a longer duration than abiotically pollinated plants (Rabinowitz et al. 1981). However, other factors such as life history traits, and seed development strategy, may also have a stronger influence on flowering duration (Jia et al. 2011). We also report for the first time that in harsh environmental conditions, the later phenology events such as dehiscence duration are strongly explained by daylength. These events occur in the months when the temperature starts to drop, and it may be, then the daylength becomes the only available cues for fruit dehiscence. Apart from daylength, the fruit dehiscence duration might also be constraint by the extreme factors such as snow and wind. Completion of fruit dehiscence before the onset of snowfall might be critical for plants survival. As *Rhododendron* species are wind dispersed, it is possible that fruit dehiscence duration is also related to wind velocity. During our field observation, we noticed that the wind velocity in this region increases toward the later months such as November and December. However, it requires further research to confirm this relationship.

Weak phylogenetic signal in phenological sensitivity to abiotic variables

Our study did not exhibit any phylogenetic dependence in species phenological response to temperature, which was in contrast to the earlier study carried out on plant community (Li et al. 2016, Du et al. 2017) in Tibetan plateau and across different sites in Northern Hemisphere (Davies et al. 2013). However, our results are consistent with another study on a group of *Rhododendron* species in Yunnan, China (Hart et al. 2016), and the Colorado Rocky Mountains plant communities (CaraDonna and Inouye 2015). They also found flowering phenology, but not phenological sensitivity to temperature to be phylogenetically conserved.

We found strong phylogenetic dependence of species in budding day and peak initial fruiting day response to daylength. The absence of phylogenetic dependence in the later events from the first immature fruiting day to peak dehiscence day indicates that the abiotic factors strongly influence the later phenology events in extreme environmental regions like Himalaya.

In the case of duration, we found that its sensitivity to daylength and temperature was not phylogenetically conserved. However, the higher lambda value in the case of dehiscence duration indicates that it might be more phylogenetically constrained. The absence of other studies on the influence of phylogeny on duration leaves no scope to draw inferences from this study and also to identify the underlying mechanism that is responsible for the phylogenetic dependence in durations of different phenology events.

CONCLUSIONS

We demonstrate the importance of studying the complete reproductive phenology events to decipher the role played by abiotic factors and the shared evolutionary history of taxa. Studying selective phenology events might give an incomplete understanding of the role played by various drivers in shaping different reproductive phenology events. Our study shows that the influence of abiotic factors and phylogenetic conservatism tends to vary across different reproductive phenology events. Stronger phylogenetic conservatism was noticed across *Rhododendron* species for the early phenology events such as budding, flowering, and initial fruiting. However, the role of abiotic factors was more important for the later phenology events such as immature fruiting, mature fruiting, and fruit dehiscence. Our study for the first time also presents the detailed research on duration of reproductive phenology events. A similar study can be employed for more extensive set of species and even among representative families of the higher altitudes for a better understanding of general trends and the drivers associated with reproductive phenology of the high-altitude plants of the Himalayan region.

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