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Original article

Increasing temperature causes flowering onset time changes of alpine ginger *Roscoea* in the Central Himalayas

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ABSTRACT

Recent herbarium-based phenology assessments of many plant species have found significant responses to global climate change over the previous century. In this study, we investigate how the flowering phenology of three alpine ginger *Roscoea* species responses to climate change over the century from 1913 to 2011, by comparing between herbarium-based phenology records and direct flowering observations. According to the observations, flowering onset of the three alpine ginger species occurred either 22 days earlier or was delayed by 8–30 days when comparing the mean peak flowering date between herbarium-based phenology records and direct flowering observations. It is likely that this significant change in flowering onset is due to increased annual minimum and maximum temperatures and mean annual temperature by about 0.053°C per year. Our results also show that flowering time changes occurred due to an increasing winter–spring minimum temperature and monsoon minimum temperature, suggesting that these *Roscoea* species respond greatly to climate warming resulting in changes on flowering times.

Introduction

Historical herbarium records of plant phenology data have been used as a tool to determine climatic changes in tropical and temperate regions over the past century (Calinger et al 2013; Gaira et al 2011; Hart et al 2014; Holle et al 2010; Primack et al 2004; Robbirt et al 2011; Schwartz 1999). However, comparing direct flowering observation of current individual species with historic herbarium phenology records has rarely been used in either tropical or temperate regions (Hart et al 2014; Rawal et al 2014; Robbirt et al 2011). It has been suggested (Miller-Rushing et al 2006) that assessing long-term datasets of a single species flowering dates may be an unreliable way of assessing flowering time changes by climate change (Amano et al 2010; Bock et al 2014; Robbirt et al 2011). Conversely, using long-term herbarium records of the change in flowering times of multiple species has been suggested as an appropriate predictor for the analysis of flowering time changes in response to climate change (Gaira et al 2011; Miller-Rushing et al 2006; Primack et al 2004). Additionally, phenological changes have recently been shown to impact interspecific interactions and evolutionary processes (Bradshaw and Holzapfel 2001; Harrington et al 1999; Inouye et al 2000; Primack et al 2004; Visser and Holleman 2001), with flowering time considered the most sensitive indicator of these in herbaceous species (Tilman and El Haddi 1992; Walker et al 1999). Here we focus on Himalayan alpine ginger species, comparing herbarium-based historic data and direct flowering observations. An earlier study of Himalayan alpine gingers in the Trans Himalayas has occurred on individual species; however, they only compared historic herbarium records and did not focus on direct flowering observation due to the lack of such data sets (Gaira et al 2011).

In highland forests, significantly increased temperatures due to climate change are known to affect herbaceous forest species (Bertrand et al 2011). Therefore, some highland mountainous forest species may be more sensitive to climate change than their lowland forest species equivalents. Moreover, a trend of significantly increased warming has been found in higher elevations when

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compared with lower elevations in the Himalayas and Tibet Plateau (Liu and Chen 2000). According to the Intergovernmental Panel on Climate Change, the global temperature will rise to 1–3.5°C by 2100 (IPCC 1996). Additionally, temperature trends in Nepal have shown a general warming over the period 1977–1994 (Shrestha et al 1999); however, the significant temperature differences were found during the winter season with less difference in the monsoon season. Furthermore, recent studies have found average temperature increases at higher elevations of the Himalayas (Sharma et al 2009; Shrestha et al 2012).

In the Central Himalayas, alpine plant communities are found in high elevation temperate regions, which are dominated by alpine pine and *Quercus*. Within these alpine plant communities, *Roscoea* is the only alpine ginger genus and is distributed from Kashmir through the Himalayas to Vietnam, extending northwards into China (Cowley 1982; Cowley 2007; Wu et al 1994), with some species found in north-eastern India (Cowley 2007). There are about 23 recognized species of *Roscoea*, eight of which are endemic to China (Wu et al 1994), three endemic to Nepal, and one each to Bhutan and India (Cowley 2007; Mao and Bhaumik 2007). *Roscoea* likely dispersed from northern India to Kashmir and then to the Eastern Himalayas and Sikkim, Bhutan and then on to Tibet, Yunnan, and Sichuan Province. Surprisingly, there are no records of populations in-between Bhutan and China. This disjunction in distribution is likely a dearth of favorable climatic conditions in this region due to the uplift taking place as a result of the collision between the Indian and Asian tectonic plates (Ngamriabsakul et al 2000; Zhao et al 2015).

A number of studies have detailed the taxonomy, history, evolution and phylogeny, and reproductive biology of *Roscoea* in the Himalayas and China (Cowley 1982; Fan and Li 2012; Ngamriabsakul et al 2000; Wu et al 1994; Zhang and Li 2008; Zhang et al 2011). This genus is highly specialized in its microhabitat requirements and therefore threatened due to human disturbance factors such as clearing the forest for road formation and habitat destruction for mine production. These disturbances are major threats to the population and may lead to the extinction of some species.

Roscoea generally occur in the alpine montane belt within a colder climatic regime with the genera likely originating from the current Himalayan and Chinese Clade (Ngamriabsakul et al 2000). Thus, it has been suggested that *Roscoea* flowering is adapted to occur under low temperatures and high humidity in the higher elevation regions of the alpine zone. Another reason for this suggestion is that, they occur in *Quercus*, pine and Rhododendron-oak forest and display plant growth, phenology, and shade tolerance conditions adapted to these environments. Therefore, we predict that *Roscoea* might be highly sensitive to changes in the annual minimum temperature and seasonal minimum temperature. Previous studies in recent years have indicated changes in minimum temperature may cause a delay in the onset of flowering time (Holle et al 2010). In Nepal, temperature has displayed an increasing trend, with air temperature increasing by 1.5°C over 25 years and the mean annual temperature increasing 0.06°C per year (Shrestha et al 2012). It is therefore important to understand how *Roscoea* flowering responds to climate warming through long-term herbarium phenology records (HPR) and through direct flowering observations (DFO).

As mentioned, in recent times, studies using HPR have shown significant changes in plant flowering times due to climatic change (Gaira et al 2011; Holle et al 2010; Lavoie and Lachance 2006; Miller-Rushing et al 2006; Primack et al 2004). Some of these studies have demonstrated flowering events are occurring earlier, whilst others have shown a delayed onset due to climate change over the past century (Gaira et al 2011; Holle et al 2010; Primack et al 2004). Additionally, one of these studies inferred that decreasing spring temperature and precipitation caused delayed flowering times (Crimmins et al 2010). As such, in this study we investigate whether flowering times in three *Roscoea* species of the Central Himalayas have changed due to increasing maximum, minimum temperature, and seasonal temperature (Bradshaw and Holzapfel 2001; Fitter and Fitter 2002; Gaira et al 2011; Holle et al 2010; Menzel and Fabian 1999). For this study, we examined flowering data from HPR dated 1913 to 2007 and compared them with direct observations of flowering times in 2009 and 2011. However, this study only focused on the phenology of the herbarium specimens by examining mean flowering dates across the herbarium specimens. We tested the following predictions and questions: (1) does the *Roscoea* flowering time change in response to increasing temperature, including minimum, maximum, average temperature, and seasonal temperatures?; and (2) how does the relationship between *Roscoea* flowering dates, months, and year vary based on a comparison between the HPR and DFO records?

Materials and methods

Study areas and species

The Central Himalayas are located in the central part of north and south-western Nepal and cover the area from 26°59'N and 84°52'E in the south to 28°18'N and 85°33'E in the north (Figure 1). The mean annual temperature of the study area is 19.84 ± 0.08°C and the mean maximum temperature is 27.52 ± 0.30°C and the mean minimum annual temperature is 12.9 ± 0.19°C. The average winter and spring temperatures are 11.58 ± 0.15°C over 40 years, from 1970 to 2011. The mean annual rainfall of the area is 1572 mm with 80% of the total rainfall occurring in the period June–September (Figure 2). In the central Himalayas, different seasons such as spring (February–April), summer (May–mid-June), rainy (mid-June–mid-September), autumn (mid-September–November), winter (December–January), and monsoon types were classified as pre-monsoon, monsoon, and postmonsoon according to Nepal temperature reconstruction data in Kathmandu, Nepal (Cook et al 2003; Shrestha et al 1999). Two vegetation types are common in the study area; oak forest and pine forest and both exist in sandy, silty, and clay loam soil types. The soils found under both vegetation types are slightly acidic in nature, but oak forests are found on areas with higher nutrients compare to those of pine forests (Sheikh and Kumar 2010).

The study focused on three Roscoea species: *Roscoea alpinia Royle, Roscoea capitata Sm, and Roscoea purpurea Sm. belongs to family Zingiberaceae.*

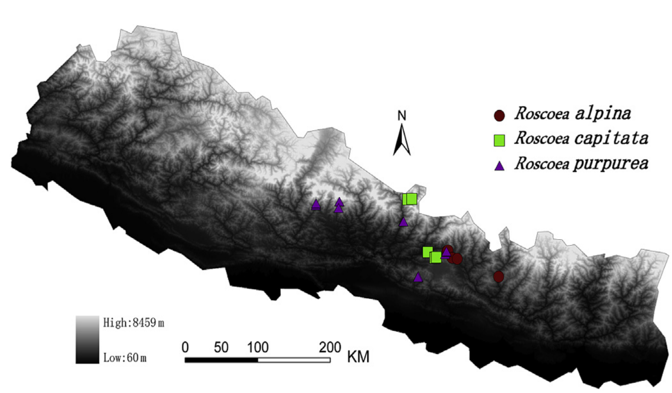

Figure 1. Map of the study sites surveyed during direct flowering observation in the year 2009 and 2011 in the Central Himalayas.

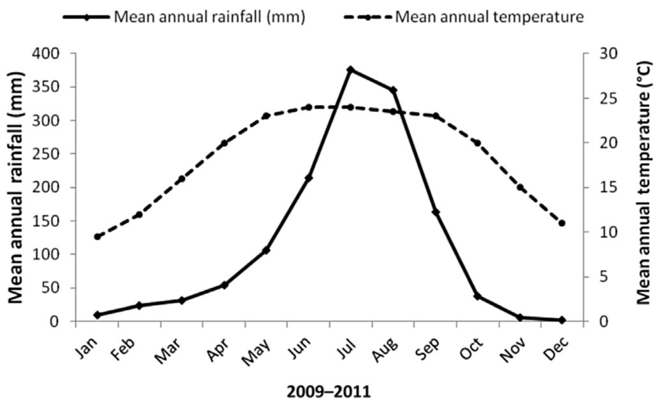

Figure 2. Mean annual rainfall and temperature over the period 2009–2011 for the study area in the Central Himalayas.

Roscoea alpinia: Perennial, rhizomatous, and tuberous rooted herb from 6–12 cm to 20–30 cm tall. Leaves at flowering time usually one to two and underdeveloped. Distributions: India, Kashmir, 2440 m, Nepal 2400–3960 m. General flowering period in Nepal: May–June (Cowley 1982, 2007).
Roscoea capitata: Perennial, rhizomatous, and tuberous rooted herb to 45 cm tall. Distributions; India and Nepal, 1220–2600 m. Flowering period from June to September (Cowley 1982, 2007).
Roscoea purpurea: Perennial, 25–38 cm tall, flowers light purple, mauve, lilac, pink, red, white, or white with purple markings, usually flower opens in the morning. Distribution: India and Nepal, 1700–2700 m. Flowering period from end of June to September (Cowley 1982, 2007). All three *Roscoea* species were photographed (Figure 3) through DFO.

Many of the sites sampled for flowering observation were disturbed by various human activities such as construction and extension of roads, agricultural land establishment, land use changes, and ecotourism development. Preferential *Roscoea* habitat is mostly south-north facing slopes of *Rhododendron*-oak forest where they are often found near forest edges due to shade tolerance; however, they do also occur nearby roadsides in grasslands at higher elevations (≥ 2600 m).

Data collection

Field surveys of DFO periods were carried out from July 2009 to August 2009 and from June to July 2011 at 13 sites in the Central Himalayas. From these sites, different flowering populations were observed, and altitude, latitude, and longitude were recorded using a Geographical Positioning System (see Appendix 1). Prior to field observations, we collected the herbarium records information of *Roscoea* distributions from the National Herbarium and Plant Laboratories, Godavari, near Kathmandu, whose collections date back to 1913. For example, some of the oldest specimens of *Roscoea* species were deposited by R.E. Cooper, A.K. Bulley Esq. Cheshire, on July 30, 1913. We gathered information on the long-term history of herbarium phenological records through qualitative assessments of the *Roscoea* herbarium collections over the period dated 1913–2007. However, we did not collect herbarium specimens during this study. We utilized as a tool the HPR, which is a well-known comparison tool for climate change and has previously be used in several recent studies (Holle et al 2010; Gaira et al 2011; Miller-Rushing et al 2006; Primack et al 2004). Using HPR, we recorded: the distribution of altitude ranges, date of flowering, place of specimen collection, accession number, and the locality of *Roscoea* species from the herbarium records. As mentioned, our DFO field survey occurred during two periods in the years 2009 and 2011, and was performed at many different locations across the central Himalayas.

A total of 134 herbarium records were analyzed, covering 54 sites, and a time period of 1913–2007. *Roscoea* flowering dates, place of collection, accession number, and altitude were recorded from these herbarium records. Twenty-one flowering populations were observed (DFO) over 13 sites during 2009 and 2011, whilst an additional 24 populations were observed in a vegetative state (not flowering) during June and July in five sites and samples were collected and photographed with flowering condition noted. Three *Roscoea* species were identified and confirmed through floral key characters using the National Herbarium and Plant Laboratories, Kathmandu and the review—The genus *Roscoea* (Cowley 2007).

The number of HPR from 1913–2007 and the number of DFO from 2009 and 2011 were used for further analysis. We calculated the frequency of HPR and DFO records in order to show the distribution of flowering records over a century. We gathered mean annual maximum and minimum temperature from the period 1970 to 2011 and rainfall data from 1975 to 2011 using the online climate data provided at http://www.ncdc.noaa.gov/cdo-web and http://www.geodata.us/weather.

Data analysis

The mean annual temperature, mean maximum annual temperature, and mean minimum annual temperature were regressed with year from 1970 to 2011 (total 41 years) to see whether a warming signal occurred in the Central Himalayas. The relationship between flowering season of blooming dates and mean spring temperature were tested using simple linear regression. Flowering months and dates based on HPR and DFO were regressed with *Roscoea* flowering year over a century from 1913 to 2011. Flowering dates based on HPR and DFO was regressed with *Roscoea* flowering year over a century. We also regressed between flowering dates and months based on HPR and DFO, in order to find out current initial flowering time. Mean annual precipitation and seasonal precipitation were regressed for 37 years to examine whether the precipitation had changed over that time period. The maximum and minimum temperature (°C) of various seasons such as winter and spring, premonsoon, monsoon, and postmonsoon was tested using a Spearman's Correlation Coefficient. In order to examine variation in the number of blooming days this variable was compared with HPR and DFO using mean flowering dates over a century from 1913 to 2011. The normal flowering dates (Gregorian calendar) were converted into Julian date (Holle et al 2010) to test for statistical differences in the mean value (*t* test) and standard deviation (*f* statistic) for each of the three *Roscoea* species. Julian dates were used to prevent differences that may occur in the herbarium records due to the calendars used (as occurs for computer programming (Ohms 1986). One-way analysis of variance was used to test for significant differences between *Roscoea* flowering distributions at different sites, years, and flowering dates. This statistical tests were performed using the software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Flowering records of HPR and DFO

Three *Roscoea* species were analyzed using both HPR and DFO records from 176 collections over a century. Of these records, 134 were HPR and 45 DFO (as observed from various sites; Figure 4; Appendix 1). Herbarium-based records showed that the frequency

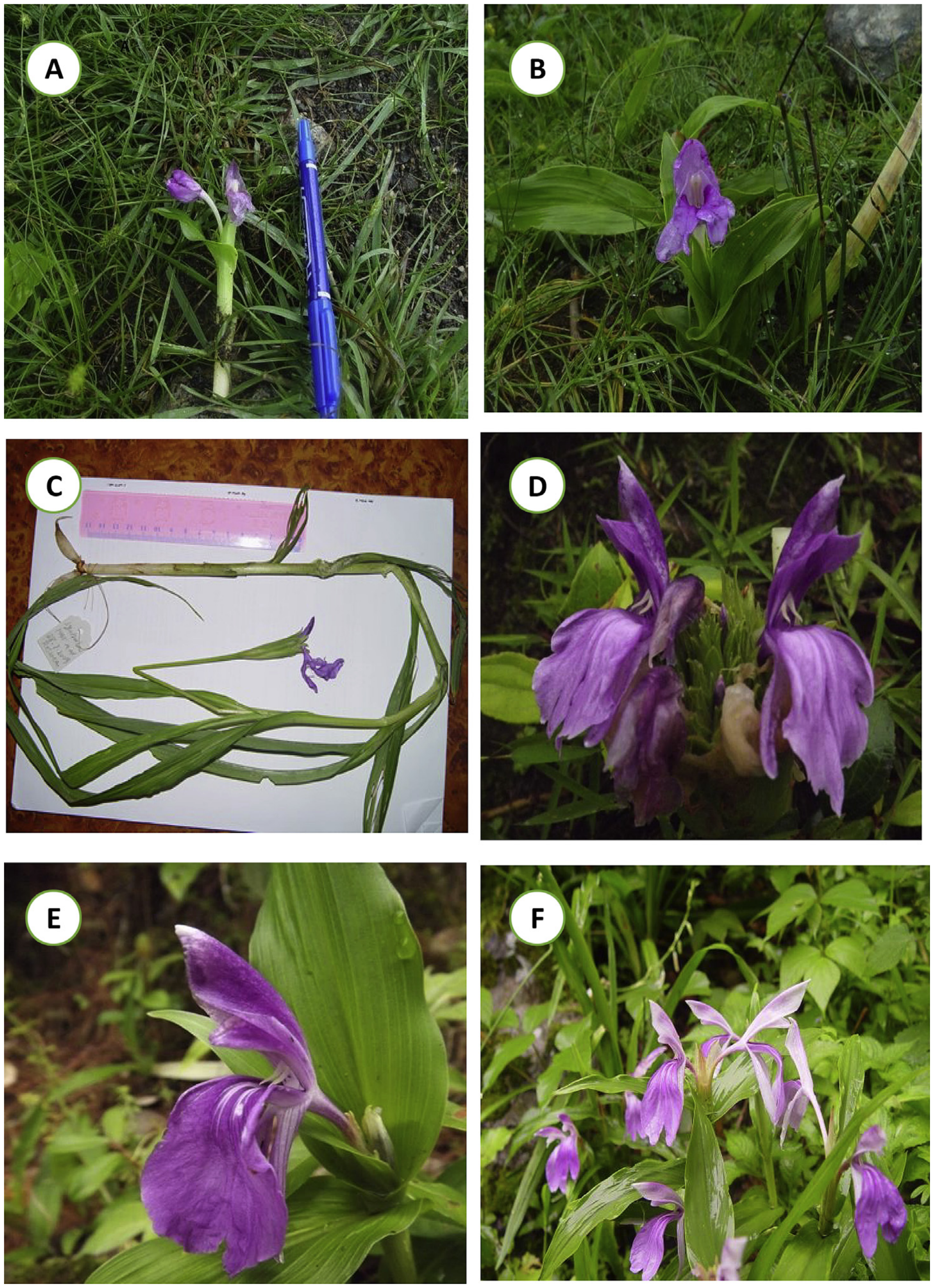

Figure 3. Photographs of early and delay flowering *Roscoea* taken during the field surveys during years 2009 and 2011. A, B, *Roscoea alpinia* plant; B, *R. alpinia* flower; C. herbarium collection of *Roscoea capitata*; D, *R. capitata* with flowers; E, early flowering date of *Roscoea purpurea*; and F, peak flowering date of *R. purpurea*.

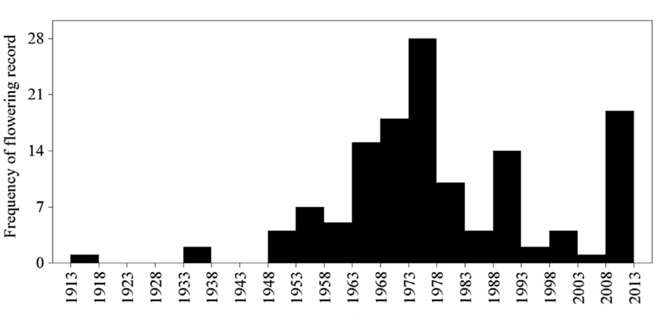

Figure 4. Frequency of flowering records for the three examined *Roscoea* species over the years 1913–2011 based on herbarium phenology records and direct flowering observations.

of collection at the different sites changed across the period 1913–2007 and that DFO occurred at 15 sites in 2009 and 30 sites in 2011. The maximum herbarium collection occurred over the period 1964–1993 during which almost 66% (89 records) of the herbarium collections were made. Most of the specimen collection occurred across an elevational ranges from 1500 m to 4000 m above sea level. Additionally, there was no significant change in the elevational distribution of the *Roscoea* collections ($R^2 = 0.001$, $df = 144$, nonsignificant) over a century, with collections consistently occurring at medium and higher elevations.

Effect of temperature and rainfall

Mean annual temperature increased significantly over the period 1970–2011 ($R^2 = 0.30$, $df = 40$, $p = 0.0002$; Figure 5), the

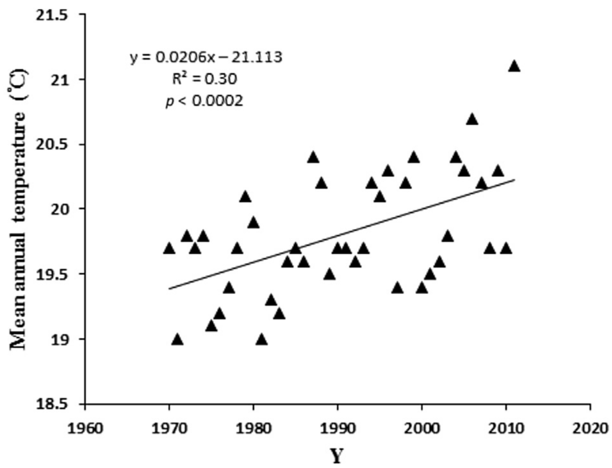

Figure 5. Relationship between mean annual temperature and year over the period 1970–2011 in the Central Himalayas. A significant positive trends shows temperature occurring over the examined time period ($R^2 = 0.30$, df = 40, $p < 0.0002$).

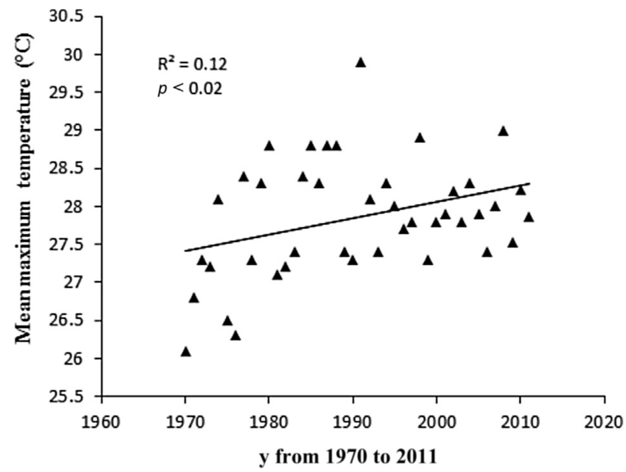

Figure 7. Relationship between mean maximum annual temperature and year over the period 1970–2011 in the Central Himalayas. A significant positive trends shows temperature increasing over the examined time periods ($R^2 = 0.12$, df = 40, $p < 0.02$).

warming trend showed average temperature increases of 0.053°C per year from 1970–2011. Moreover, minimum temperature increased significantly over the study period by ∼0.8°C ($R^2 = 0.20$, $df = 40$, $p = 0.001$; Figure 6). Analogously, maximum temperature displayed a significant warming signal over the study period, increasing by 0.7°C ($R^2 = 0.12$, $df = 40$, $p = 0.02$, Figure 7).

Daily temperature, as analyzed by seasons, showed a significant mean monthly minimum temperature increase over the study period for the seasons of winter and spring ($r = 0.44$, $p < 0.005$) as it did for the monsoon minimum temperature ($r = 0.44$, $p < 0.004$; Table 1). This finding indicates a significant increase in the minimum temperature during the winter and spring seasons; which could delay the flowering. However, *Roscoea* usually flowers during the monsoon period, but the increased minimum temperature during the monsoon could delay the onset of flowering.

There was a significant increase in the mean spring temperature along with the initial *Roscoea* flowering date (from May to October; $R^2 = 0.08$, $df = 90$, $p = 0.004$; Figure 8), indicating that *Roscoea* exhibited a delayed flowering onset across the examined century.

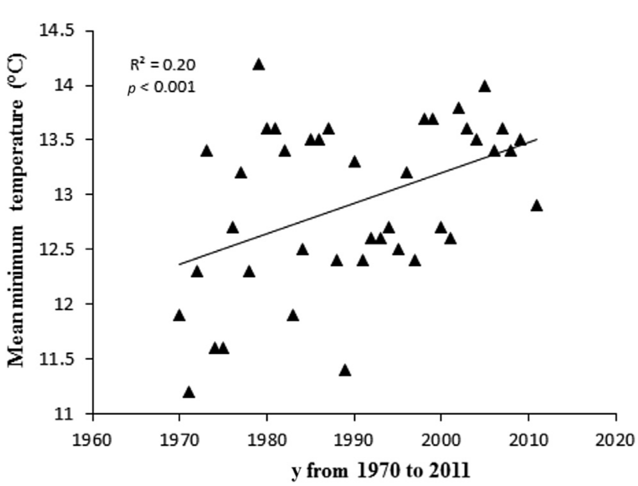

Figure 6. Relationship between mean minimum annual temperature and year for the studied period 1970–2011 in the Central Himalayas. A significant positive trends shows minimum annual temperature increased over the examined time period ($R^2 = 0.20$, df = 40, $p < 0.001$).

Mean annual rainfall significantly increased over the examined 37 years ($R^2 = 0.17$, $df = 36$, $p = 0.01$); however, this appears to be driven by the significant increase in monsoonal precipitation (from June to September) over the years from 1975–2011 (regression fit with adjusted $R^2 = 0.11$, $df = 36$, $p = 0.04$). The percentage of annual rainfall received during the monsoon ranged from 68% to 86% over the examined 37 years.

Flowering onset time changes based on HPR and DFO

According to our observation, the mean peak flowering date of *Roscoea* species differed between the HPR and DFO analyses across the three species. Among these, the flowering onset of *R. alpinia* differed by a delay of 30 days between HPR and DFO, whereas *R. capitata* blooming time had a difference of an 8-day delay (Table 2). The peak flowering period of *R. purpurea* was not observed during DFO; however, the first day of flowering for this species in Daman was observed to occur on June 2, 2011 (although only 2 individuals were observed flowering from the 7 populations) and peak flowering occurred in July 2011. Therefore, two species, *R. alpinia* and *R. capitata*, showed a delayed flowering onset trend ranging from 8 days to 30 days across an examined century. However, *R. purpurea* was found to exhibit an earlier flowering onset of 22 days across the examined century. Additionally, the Julian date of flowering for each species showed significant variation when comparing HPR and DFO records (Table 3). Therefore, the flowering date of each species significantly varied over the examined century.

Table 1. Spearman's correlation coefficient between seasonal temperatures (maximum and minimum) at high elevations (> 1500 m above sea level) over the years 1975–2011. Significant correlations are associated with early and delay onset of flowering in *Roscoea*.

Season	Temperature (°C)	
	Maximum $N = 37$	Minimum $N = 37$
Winter & spring	−0.20	0.44*
Premonsoon	−0.39†	0.11
Monsoon	−0.31‡	0.44*
Postmonsoon	0.001	−0.41*

* $p < 0.01$.
† $p < 0.001$.
‡ $p < 0.05$.

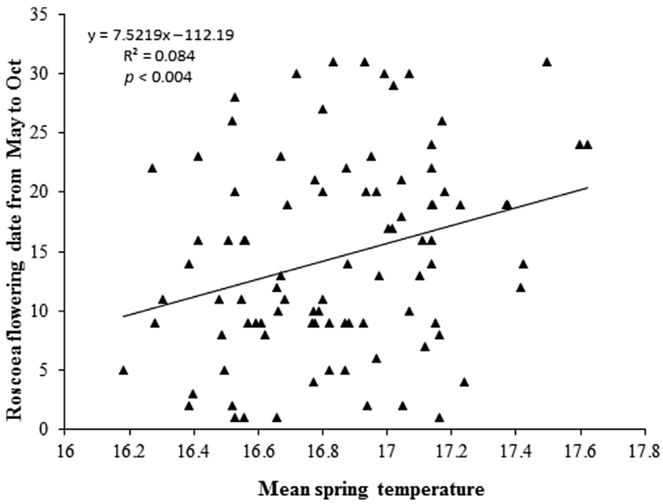

Figure 8. Relationship between initial flowering date of flowering season and mean spring temperature 1970–2011 in the Central Himalayas. A significant positive trends shows delayed flowering occurring as mean spring temperature increased ($R^2 = 0.084$, df = 91, $p < 0.004$).

Variation of flowering sequence among sites

According to HPR and DFO records, the observation of the initial flowering time for the examined *Roscoea* species differed significantly between the examined sites ($F = 9.04$, $df = 5$, $p < 0.05$) and their year of collection ($F = 12.57$, $df = 5$, $p < 0.01$). Moreover, flowering time (Julian date of flowering) varied significantly over the examined century ($F = 7.92$, $df = 5$, $p < 0.05$; Table 4).

Flowering time changes on Roscoea species

The initial flowering months and dates did not significantly changes over the period 1913–2011, however delayed blooming did occur over the last decade (Figure 9). The flowering dates of HPR and DFO records significantly changed with number of years from 1913 to 2011($R^2 = 0.05$, $df = 106$, $p < 0.03$; Figure 10), indicating a delay in flowering dates across the examined century. The initial flowering dates of HPR and DFO records significantly declined along with consecutive months of flowering over the examined time period ($R^2 = 0.15$, $df = 106$, $p < 0.0001$; Figure 11), thus, showing a delay in flowering onset time period.

Table 2. Mean peak flowering date of herbarium phenology records (1913–2007) and direct flowering observation (2009–2011) of the three examined *Roscoea* species in the Central Himalayas.

Species names	Mean peak flowering date		Mean number of flowering d earlier (+) and delayed (−)
	Herbarium phenology records	Direct flowering observation	
Roscoea alpinia	6th July	4th August	−30
Roscoea capitata	15th July	22nd July	−8
Roscoea purpurea	12th August	20th July	+22

Table 3. Comparison of mean and standard deviation of Julian date difference between herbarium phenology records (1913–2007) and direct flowering observation (2009–2011) indicating significant differences in flowering time over the examined time period for the three *Roscoea* species in the Central Himalayas.

Species names	Julian date difference	*t* test	*N*	*p*	*F* statistic	*p*
Roscoea alpinia	12797.14	4.17	36	0.0002	438.590	0.001
Roscoea capitata	14584.92	6.41	20	0.0001	345.618	0.001
Roscoea purpurea	13453.41	7.70	62	0.0001	181.484	0.001

Table 4. Analysis of variance was used to compare the different sources of variation between the herbarium phenology records and direct flowering observation in three *Roscoea* species from Central Himalayas.

Source of variation	df	SS	*F*	p^*
Site	5	1819.5	9.04	0.040
Year	5	764.83	12.57	0.024
Flowering time (Jd)	5	2169.5	7.92	0.048
Site × y	8	2057.56	5.31	0.047
Site × flowering time (Jd)	8	2350.22	5.61	0.042
Y × flowering time (Jd)	8	1954.89	5.81	0.039

Df = degree of freedom; Jd = Julian date of flowering between 1913–2011; SS = sum of squares.
* All *p*-values are significant level at an alpha level of < 0.05.

Discussion

Flowering time changes due to increasing temperature

Recent studies using herbarium-based flowering assessments have shown that flowering time in a variety of plant species have changed significantly due to climate change (Gaira et al 2011; Holle et al 2010; Miller-Rushing et al 2006; Primack et al 2004). In particular, increasing temperature has created rapid changes in different types of weather events over the past 50 years at the global level (Jump et al 2009). Holle et al (2010) suggested that increased temperature changed the timing of flowering frequency, characterized either by earlier or delayed flowering. Primack et al (2004) used 372 herbarium species and found earlier flowering responses to climate change, with similar results from other studies suggesting that this early-flowering is due to increasing temperature over time (Gaira et al 2011; Gallagher et al 2009; Lavoie and Lachance 2006; Robbirt et al 2011). Recent studies have found a mean warming trend increases in the Himalayas 1.5°C and at an average rate of 0.06°C per year; however, the highest temperature increases were recorded in the winter season 1.7°C and lowest in the summer 0.75°C (Shrestha et al 2012). The findings of this study

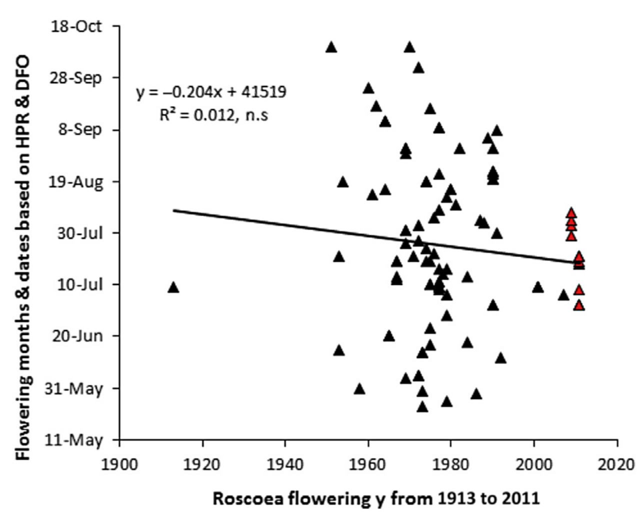

Figure 9. The relationship between initial flowering (months and dates) based on herbarium phenology records and direct flowering observation in the Central Himalayas. A nonsignificant negative relationship occurs between initial flowering and year examined with no flowering record found during May and June after the year 2000. The black triangle shape denotes herbarium phenology records and the red triangle shape denotes direct flowering observation records. DFO = direct flowering observation; HPR = herbarium phenology records; n.s. = nonsignificant.

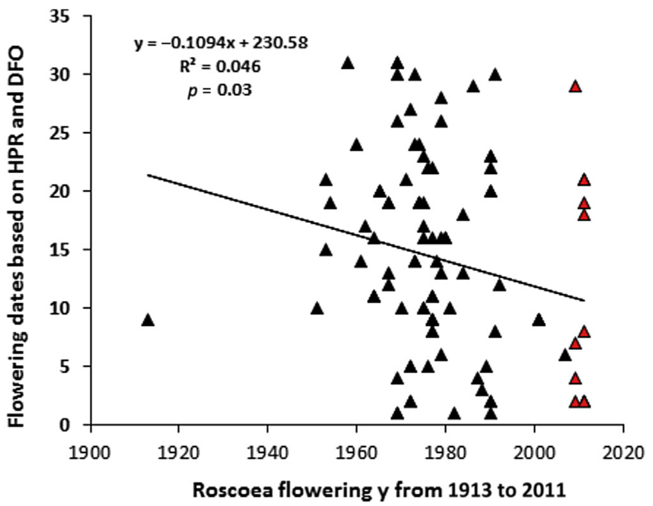

Figure 10. The relationship between initial flowering dates and year examined based on herbarium phenology records and direct flowering observation in the Central Himalayas. Overall, the initial flowering time is significantly delayed over the examined time period for the three examined *Roscoea* species. The black triangle shapes denotes herbarium phenology records and red triangle shapes denotes direct flowering observation records. DFO = direct flowering observation; HPR = herbarium phenology records.

indicate that *Roscoea* flowering times have changed due to increasing seasonal temperature. Our results also suggest that winter and spring temperature increases are the major causes of flowering time changes (earlier or delayed).

Shrestha et al (2012) demonstrated that when the timing of the "start of the seasons" exhibited a negative trend, flowering occurred earlier and when they exhibited a positive trends flowering was delayed. According to our results, flowering seasons showed a significant positive trend, with mean spring temperature, resulting in a delayed pattern of *Roscoea* flowering season when examining the period mid-July in 2009 and 2011 compared with herbarium records from 1913 to 1999, of when flowering starts from mid-May to June.

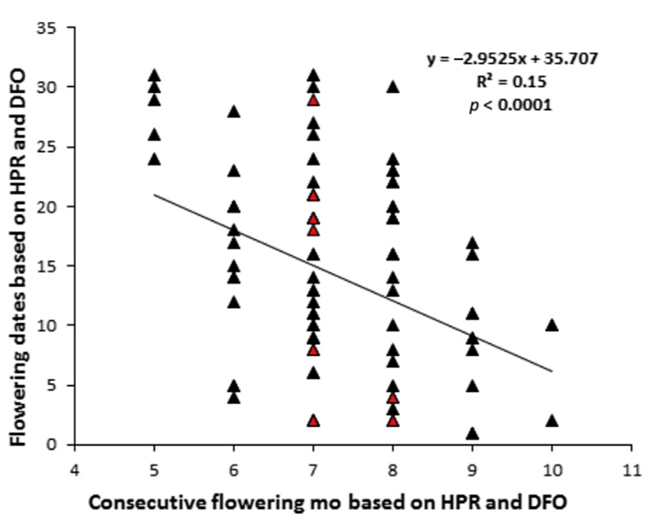

Figure 11. The relationship between consecutive flowering months and initial flowering dates based on herbarium phenology records and direct flowering observation (DFO). A significantly delay in the initial flowering time of three *Roscoea* has resulted in a decline in the number of months consecutively flowering particularly at DFO sites ($R^2 = 0.15$, df = 106, $p = 0.0001$). Additionally, no flowering has occurred in May and June in recent years. The black triangle shapes denotes herbarium phenology records and red triangle shapes denotes DFOs. DFO = direct flowering observation; HPR = herbarium phenology records.

Flowering time changes based on HPR and DFO

The present study utilized 134 herbarium specimens from 36 years (1913–2007) across a century, and DFO from 2 years (2009 and 2011) and found that flowering time varied significantly, with an earlier and delayed flowering signal due to increasing minimum temperature and spring temperature. Notably, *Roscoea* species significantly responded to increased minimum temperature and mean spring temperature rather than maximum temperature under the local environmental conditions. Recent studies indicate that increased spring temperatures either advance or delay the date of first flowering (Crimmins et al 2010; Dunnell and Travers 2011). Additionally, Holle et al (2010) noted that flowering time was strongly delayed by variable minimum temperature across 345 species and also found that Julian date of flowering significantly increased in response to a rise in average temperature. In the Trans-Himalayas, *Aconitum heterophyllum* flowered earlier (19–27 days) with increased overall temperature of ~1°C (Gaira et al 2011), whereas we found that *Roscoea*'s flowering time occurred earlier (22 days) and delayed (8–30 days) with increasing average temperature 0.053°C per year (1970–2011). However, it must be noted that higher elevations flowering may also be delayed due to local environmental disturbance and severe drought (Gaira et al 2011). Therefore, ongoing increasing temperature linked to current climatic changes may also increase drought stress which may cause a negative impact on *Roscoea* species distributions, with elevated mortality and declining plant reproduction.

Furthermore, Körner (2003) has suggested that some alpine plant groups such as *Primulaceae*, *Ranunculaceae*, and *Cyperaceae* species are flowering earlier, and species of *Asteraceae* and *Campanulaceae* are experiencing a flowering delay in response to climate change. Previous studies have suggested that a delay in flowering may result in plants producing high seed output but the seeds have a reduced rate of maturing because of time constraints; therefore those plants experience higher levels of selfing and apomixis (Körner 2003). Similarly, recent *Roscoea* reproductive biology studies indicates several species breeds through self-pollination under natural conditions (Zhang and Li 2008, Zhang et al 2011), whilst a recent new discovery found a delayed pollination mechanism on *Roscoea debilis* through self-pollination (Fan and Li 2012). However, long-term studies are necessary to understand whether *Roscoea* delay flowering affects reproductive success through seed germination and reproductive success.

Alpine gingers in the genera *Roscoea* are highly specialized, as are the alpine plant communities within which they exist. *Roscoea* flowering time has changed due to climate warming, in particular, increasing minimum annual temperatures and increasing spring temperatures. As a consequence their reproductive success and population size may vary over the next few decades, due to potential reproductive declines caused by alterations of plant-pollinator interactions. However, the long term observation of *Roscoea* phenology, along with local and global climatic variables would enable a deeper understanding of the likely response of the herbaceous alpine floral community of the central Himalayas to future climatic conditions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.japb.2015.08.003.

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