

Potential of herbarium records to sequence phenological pattern: a case study of *Aconitum heterophyllum* in the Himalaya

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Received: 27 January 2011 / Accepted: 3 June 2011 / Published online: 14 June 2011
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Abstract Several pieces of evidence indicate that global climate change is affecting biological systems all across the world. Phenology is one of the tools that may indicate changing patterns. The paper focuses on the phenological pattern of alpine/sub-alpine species *Aconitum heterophyllum*, a high-value medicinal herb of the Indian Himalayan Region (IHR), a global hotspot and known to be sensitive to climatic change. In all 117 herbarium specimens of the species collected from three provinces (Western Himalaya, North West Himalaya and Trans Himalaya) of the region were recorded. Historic herbarium records (1848–2003) were analyzed to predict the flowering patterns using Generalized Additive Model (GAM) in view of complexity in the herbarium-based data structure. GAM indicated that the flowering time responded significantly, 26 days earlier per 1,000 m ($P < 0.02$). Likewise, the model showed significantly earlier flowering (17–25 days) during the last 100 years ($P < 0.01$). Moreover, maximum temperature of winter (December–February) explained increasing trends at both elevations (lower and mid) and mean winter temperature influenced the early flowering time (19–27 days) with an increase of 1°C. The overall early flowering of *A. heterophyllum* may perhaps be considered as indicator of climate change; however, more datasets of herbarium records are required to further strengthen this premise. This study was undertaken to show that herbarium records could be utilized as a potential resource for assessing climate change using GAM.

Keywords *Aconitum heterophyllum* · Early flowering · Generalized Additive Model · Herbarium specimens · Indian Himalayan Region · Phenology · Temperature

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Introduction

Changes in the timing of phenological events are among the most important indicators of global warming (Parmesan and Yohe 2003; Root et al. 2003). Among different phenological events, flowering time is considered the most sensitive indicator in the herbaceous species (Tilman and El Haddi 1992; Walker et al. 1999) and has a potential for predicting the effect of climatic change patterns (Kittel 1998). Various studies have provided pieces of evidence that plant phenology changes due to increasing of temperature (Menzel and Fabian 1999; Bradshaw and Holzapfel 2001; Fitter and Fitter 2002). Such phenological change sequence relies on long-term datasets (Primack et al. 2004; Miller-Rushing et al. 2006; Inouye 2008; Gallagher et al. 2009). Unfortunately, for many regions and species including those occurring in Indian Himalayan Region (IHR), such data sets/documentation are often not available.

The Himalayan region, in spite of its recognition amongst 34 global biodiversity hot-spots (Conservation International 2004) and most conspicuous mountain chain having high sensitivity to climatic perturbation (NAPCC 2008), is among such data deficient regions. Alpine/sub-alpine environments are rich in endemic plants (Dhar et al. 2000) and timing of flowering plants is significantly controlled by the timing of snowfall (Inouye and Wielgolaski 2003) and its disappearance (Kudo and Suzuki 1999). Such events determine the sensitivity of the region towards the climatic change (Purohit 1991; Shrestha et al. 1999; Khanduri et al. 2008). These areas and the elements of biodiversity they hold could provide useful clues of changing patterns. Notwithstanding the deficiency in data sets, the present study was undertaken to address the following issues: (i) how phenological events in the region are changing as a result of a variety of factors including climate change and (ii) how these trends can be utilized as indicators.

In view of the non-availability of long-term written records and field-based observational data (Robbirt et al. 2011), the use of biological collections from museums, herbaria, zoos, botanical gardens and research stations for determining patterns of responses to changing climate is growing in popularity (Primack et al. 2004; Miller-Rushing et al. 2006; Gallagher et al. 2009). Herbarium data have been utilized for comparing phenological behavior (Bolmgren and Lönnberg 2005), reconstructing the phenology of plant species across large area (Lavoie and Lachance 2006) and analyzing the changing pattern, if any. Most of the herbarium-based phenological studies are based on the analysis of the group of species (Primack et al. 2004; Bolmgren and Lönnberg 2005; Miller-Rushing et al. 2006), however, a suggestion to examine the effects of climate change on a particular species is also propounded (Primack and Miller-Rushing 2009). For example, Lavoie and Lachance (2006) evaluated the early flowering pattern of *Tussilago farfara*.

In the above context, the present study focuses on *Aconitum heterophyllum* (Ranunculaceae), a perennial high-value medicinal herb, which begins above-ground growth in May and flowers (cream colour) in August (Vashistha et al. 2009) with a 20-day peak flowering time in the alpine/sub-alpine region of IHR (Nautiyal et al. 2009). Due to continuous exploitation and habitat destruction, the species is becoming rare (Nautiyal et al. 2002) and is now categorized critically endangered in India (Ved et al. 2003). The tubers of the plant contain the alkaloids aconitine, mesaconitine, hypaconitine, atisine, heteratisine, telatisine, and atidine (Khorana and Murti 1968; Mori et al. 1989), which are used in the treatment of dyspepsia, diarrhea, coughs, snake bites, fevers of contagious diseases and inflammation in the intestines (Chopra et al. 1986; Tsarong 1994). The present study was undertaken to (i) assess the usefulness of herbarium records for predicting

changes in flowering time along the elevation and over the year and (ii) analyze the relationship between flowering time and temperature.

Materials and methods

Herbarium records

Herbarium records of the target species were recorded from Botanical Survey of India (BSI), Kolkata and Dehradun; Forest Research Institute (FRI), Dehradun; National Botanical Research Institute (NBRI), Lucknow and Hemwati Nandan Bahuguna Garhwal University, Srinagar Garhwal. These institutions are the major source of herbarium records especially in the IHR. The herbarium specimens' records were collected from different provinces of IHR [i.e. Trans Himalaya (TH); North West Himalaya (NWH); and Western Himalaya (WH)] (Rodgers and Panwar 1988) (Fig. 1). However, the other provinces of IHR were not considered in the current study due to poor representation and incomplete data. We also accessed an online herbarium (i.e. Kew Herbarium) and found it incompatible due to unspecified location of the target species. The specimen numbers, collecting location, date of collection, habitat characteristics, elevational ranges and name of the collector(s) were recorded.

Of the total 117 specimens of target species, only 76 specimens were observed in the flowering condition. Specimens lacking precise information on date of collection and locations were discarded. The available specimens were observed in different phenophases (budding, flowering with bud, only flowering and flower with seeds). Only specimens with flowers with recorded collection date were considered the date of flowering of the species. For estimating and developing phenological modelling, the flowering dates were reconstructed as day of year (DOY) (1 DOY = January 1) and determined as a response variable (i.e. flowering time).



Fig. 1 Distribution of *A. heterophyllum* in the IHR and three provinces (TH, NWH and WH) included in the study

Elevation as an important explanatory variable was also included in the study. Most of the records showed a single value for elevation, while some included a range of elevation with distinct units (i.e. meters and feet). Such elevational variations were rearranged by determining the average of elevation and ensured uniformity in unit value (i.e. meter).

Temperature records

As temperature records for alpine and sub-alpine areas (elevation 2,400–4,500 m a.s.l.) of the IHR are lacking, the flowering change responses to temperature change were determined on the basis of the presumption that 6.5°C temperature decreased with increase per 1,000 m elevation (Singh 2005) and in this context, the lower elevational temperature records of Vivekananda Parvatiya Krishi Anusandhan Sansthan, Hawalbag Almora, (1,250 m a.s.l.) and mid elevational of Aryabhat Research Institute of Observational Sciences, Nainital (1,951 m a.s.l.) were utilized for comparative variability assessment. We analyzed daily-recorded temperature (maximum and minimum) of Hawalbag for 1971–2008 and Nainital 1971–1995 in a uniform manner. The temperature of both the stations was analyzed seasonally [i.e. winter (December–February), summer (March–May), monsoon (June–September) and post monsoon (October–November)] (Shrestha et al. 1999; Murty et al. 2004). Unfortunately, the temperature records for Nainital were not available consistently for the time period 1995–2008.

Statistical applications

A long time series (i.e. 155 years from 1848 to 2003) and herbarium data representing a wide range of elevations (2,400–4,800 m a.s.l.) of the target species was arranged for applying appropriate statistical methods. The herbarium-based datasets on flowering time indicated complexities due to (i) multiple data representation in a year and (ii) non-constant data series (i.e. gaps between the year of collections) (Fig. 2). Such complexities suggested non-normality and non-linearity in datasets.

Realizing the availability of non-linear and non-constant data structure, Generalized Additive Model (GAM) was selected as an appropriate approach to address these issues (McCullagh and Nelder 1989; Hastie and Tibshirani 1990; Guisan et al. 2002). Moreover,

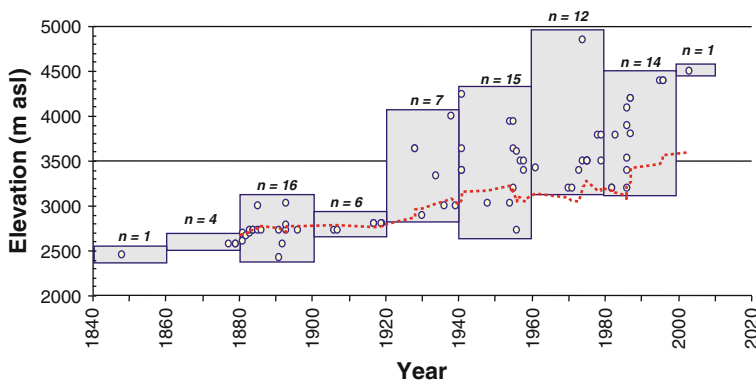


Fig. 2 Herbarium specimens collected from different elevational ranges with frequency. *Boxes* explain the variability of collection frequency between the time interval and elevation ranges. The *dotted line* represents the 5-year moving average trend for elevational variations

Hudson et al. (2009) used a Generalized Additive Model for Location, Scale and Shape (GAMLSS) approach to predict the phenological changes using long time series herbarium records and found it suitable. To illustrate, in the general linear model a response variable Y is linearly associated with values on the X explanatory variables (e.g. year, elevation and temperature) while the relationship on the Generalized Linear Model (GLM) is assumed to be as:

$$Y = g(b_0 + b_1X_1 + \cdots + b_mX_m),$$

where $g(\cdot)$ is a function and $g(\cdot)$ is called the link function, so that:

$$g_i(Y) = b_0 + b_1X_1 + \cdots + b_mX_m$$

where, Y stands for the expected value of response variable. However, the notion of additive models with GLM is derived as GAM

$$g_i(Y) = S_{if_i}(X_i)$$

Generally, GAM allows for choosing a wide variety of distributions for the response variable and linking functions to improve the effective quality of the prediction (McCullagh and Nelder 1989; Hastie and Tibshirani 1990). The GAM fit model considers the estimation of the smoothing terms in the additive model, general algorithm added in the model using any regression-type smoothers as partial residuals (i.e. R_j th set of partial residuals)

$$R_{ji} = Y - S_0 - \sum_{h \neq j} S_h(X_h)$$

The partial residuals remove the effects of all the other variables from Y , therefore Y can be used to model the effects against X_j . Such foundation algorithm provides a way for estimating each smoothing function $S_{f_i}(\cdot)$ given estimates $\left\{ \hat{S}_i(\cdot) = i \neq j \right\}$ for all.

Results

Herbarium-based observations showed that the collections frequency changed across the years. The maximum collection frequency was observed during 1880–1900 as compared to the duration from 1940 to 1960. Before the 1920s, the specimens were collected from 2,400 to 3,500 m a.s.l. elevation and after 1920s from 3,500 to 5,000 m a.s.l. Moreover, the 5-year moving average trend of collections varied towards high elevations (Fig. 2).

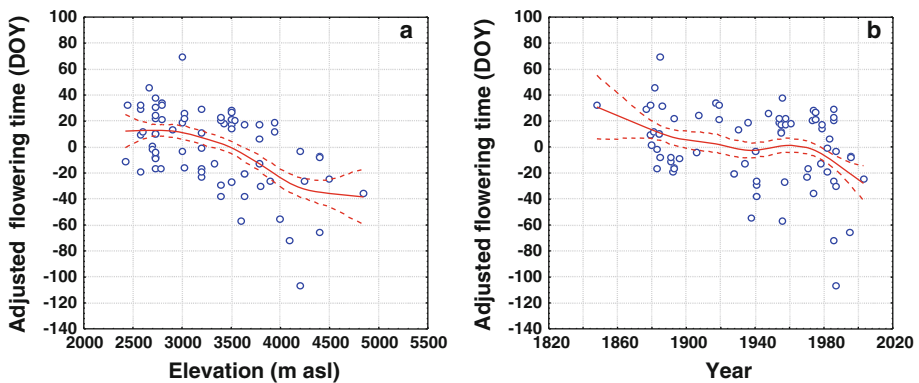
Considering the flowering pattern along the elevation, the model estimated a significant ($P < 0.02$) advancement in flowering (26 days) per 1,000 m elevation (Table 1) and showed GAM-based best smooth line with 95% confidence level (Fig. 3a). Similarly, the early-flowering trend projected (Fig. 3b), on average 17–25 days, showed flowering has become significantly earlier in the past century ($P < 0.001$).

The daily recorded temperature was analyzed (Table 2) and showed that the mean monthly maximum temperature responded increasingly over the years in the winter season at lower ($r = 0.58$) and mid ($r = 0.59$) elevation significantly ($P < 0.01$). Also, the maximum temperature increased significantly ($P < 0.05$) in the post monsoon at lower elevation ($r = 0.40$). The minimum temperature at mid elevation increased in winter—December–February ($r = 0.40$) and summer—March–May ($r = 0.49$) significantly

Table 1 Predicted early flowering of *A. heterophyllum* across the elevation and over the year using herbarium specimens using GAM

Parameters	Explanatory variables	
	Elevation	Year
Sample size	76	76
β	−0.026	−0.21
SE (β)	0.003	0.04
R^2	28.54	14.16
P -value	0.02	0.001

The significance of the smoothing best fit model generalized the changing slope as GAM coefficient (β), standard error (SE) and coefficient of determinant (R^2)

**Fig. 3** GAM-predicted early flowering of *A. heterophyllum* **a** along the elevation and **b** over the year using herbarium specimens and smoothed best fit line with 95% confidence interval**Table 2** Spearman's correlation coefficient between seasonal temperatures (maximum and minimum) and year at lower and mid elevation

Season	Temperature (°C)			
	Lower elevation ($n = 38$)		Mid elevation ($n = 25$)	
	Maximum	Minimum	Maximum	Minimum
Winter	0.58**	−0.23	0.59**	0.40*
Pre monsoon	0.07	−0.12	−0.28	0.49*
Monsoon	−0.26	−0.14	−0.19	0.25
Post monsoon	0.40*	−0.14	0.02	0.38

* $P < 0.05$, ** $P < 0.01$

($P < 0.05$). Realizing the high significance of winter maximum temperature of both elevations, the 5-year moving average trend was calculated and found to show a warming signal (Fig. 4).

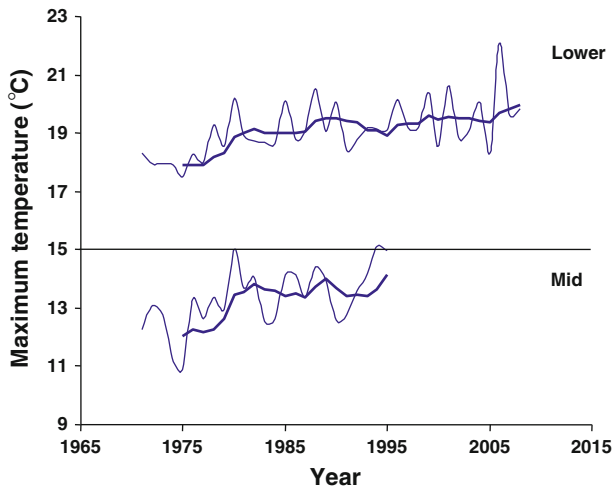


Fig. 4 Increasing trend of winter maximum temperature for two stations, i.e. Hawalbag—lower elevation from 1971 to 2008 and Nainital—mid elevation from 1971 to 1995. The **bold line** is the 5-year moving average trend

Considering the warming winter temperature, the model estimated that flowering time of *A. heterophyllum* responded significantly ($P < 0.01$) 19–27 days early (i.e. GAM coefficient = -23.16 ; SE = 4.30; $R^2 = 28.95$) with increasing 1°C temperature.

Discussion

Herbarium-based phenological assessments have gained momentum in recent years. Primack et al. (2004) utilized 372 herbarium specimens of groups of species and compared them with living plants, and detected earlier flowering over the years. Lavoie and Lachance (2006) used herbarium specimens of *T. farfara* and estimated early flowering over time. Gallagher et al. (2009) evaluated early flowering responses with respect to increasing temperature. Recently, Robbirt et al. (2011) investigated the flowering time responses by spring temperature using herbarium and field observations separately. All herbarium-based phenological studies utilized a similar response variable (i.e. flowering time) and applied routine statistical application (i.e. linear regression model). However, as a response variable, herbarium-based flowering time is beset with complexities and shows non-normality and non-linearity so application of parametric approaches may not be appropriate. This is largely due to data complexities, such as (i) wide elevational ranges, (ii) multiple records in a year, (iii) non-constant series of collections and (iv) inconsistent collection frequency in the data (Fig. 2). Therefore, GAM-based phenological modelling was found quite suitable using herbarium collections.

Herbarium collections frequency showed that the species were collected at higher elevation after the 1920s, indicating a possible shift in the range of species towards higher elevations. Several studies indicate that the species distribution ranges have shifted to higher elevations (Grabherr et al. 1994; Thuiller 2003; Pauli et al. 2007; Inouye 2008; Kelly and Goulden 2008).

In the present study we factored in flowering pattern of the target species along the elevation, which performs a significant role in the Himalaya especially for phenological activities. There is a general agreement that flowering time is delayed at higher elevation. But, we found that over the period we studied earlier flowering was observed at higher elevation (26 days earlier per 1,000 m). This may be a consequence of winter warming because warming winter results in early disappearance of snow in the alpine region and provides favorable conditions for plant growth. In this context, Shrestha et al. (1999) reports high rate of warming at higher elevation as compared to lower elevations. In addition, the snow cover suggested as a principal factor controlling the length of the growing season and phenology of the alpine plants and timing of snow release, significantly affects initiation of growth and flowering of alpine species (Kudo and Suzuki 1999; Walker et al. 1999; Inouye and Wielgolaski 2003).

Considering the flowering pattern over the year, our model reveals advancement (17–25 days) in flowering of *A. heterophyllum* in the twenty-first century. Likewise, Lavoie and Lachance (2006) observed (15–31 day) earlier flowering of *T. farfara* in the twenty-first century than around 1920 using 216 herbarium specimens. Several herbarium-based studies report earlier flowering (Bowers 2007; Miller-Rushing and Primack 2008; Gallagher et al. 2009). The early flowering pattern of *A. heterophyllum* may be linked with warming winter results, which shows that on average flowering time is earlier (19–27 days) with an increase of 1°C mean winter temperature. However, Robbirt et al. (2011), comparing herbarium and field records to examine the relationship between phenology and spring temperature, found 6 days early flowering per 1°C in *Ophrys sphegodes* in the both cases. And, Fitter and Fitter (2002) indicated that the temperature of the previous month determines the sensitivity of the flowering and most of the changes in flowering time were due to rising winter and spring temperature (Abu-Asab et al. 2001; Miller-Rushing and Primack 2008). However, *A. heterophyllum* may be considered to be sensitive towards rising temperature of winter season, which promotes earlier flowering.

This study had two major limitations: (i) the analysis of change in flowering time was based on available records of herbarium specimens and (ii) non-availability of climatic time series data of the area of occurrence of the target species. In spite of these limitations, the evidence shows that changes in winter temperature resulted in early flowering of *A. heterophyllum* at high elevations and a significant advancement of flowering over the last century. In essence, the present study opens new vistas of utilizing long-term herbarium and historical records to assess climate change responses (Lavoie and Lachance 2006; Miller-Rushing et al. 2006; Gallagher et al. 2009). Also, such studies need to be encouraged to be undertaken and we must continue survey/explorations to enrich herbarium collections without extending any further gap between the quality of past and recent biological records (Lavoie and Lachance 2006).

Acknowledgments UD acknowledges the support of National Academy of Sciences, India and KSG thanks the Director GBPIHED, for necessary facilities and to DST, New Delhi for partial financial support. We acknowledge the help rendered by BSI Kolkata and Dehradun, FRI Dehradun, NBRI Lucknow, HNBGU Srinagar Garhwal, VPKAS Almora and ARIES Nainital in collection of data. Mr. Triloki Pant is thanked for helping in data analysis. We thank anonymous referees for improving the manuscript.

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