- Bal, L. M., Meda, V., Naik, V. N. and Satya, S., Sea buckthorn berries: a potential source of valuable nutrients for nutraceuticals and cosmoceuticals. *Food Res. Int.*, 2011, 44, 1718–1727.
- 8. Zeb, A., Important therapeutic uses of sea buckthorn (*Hippophae*); a review. *J. Biol. Sci.*, 2004, **4**, 687–693.
- 9. <u>http://leh.nic.in/pages/leh.pdf</u> (accessed on 5 July 2013).
- Dafni, A., Kevan, P. G. and Husband, B. C. (eds), *Practical Pollination Biology*, Enviroquest Limited, Cambridge, Ontario, Canada, 2005.
- Heslop-Harrison, J. and Heslop-Harrison, Y., Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain Technol.*, 1970, 45, 115– 120.
- Kearns, C. A. and Inouye, D. W., *Techniques for Pollination Biologists*, University Press of Colorado, USA, 1993, pp. 77–151.
- Tandon, R., Manohara, T. N., Nijalingappa, B. H. M. and Shivanna, K. R., Pollination and pollen-pistil interaction in oil palm, *Elaeis guineensis. Ann. Bot.*, 2001, 87, 831–838.
- Mangla, Y., Tandon, R., Goel, S. and Raina, S. N., Structural organization of the gynoecium and pollen tube path in Himalayan seabuckthorn, *Hippophae rhamnoides* (Elaeagnaceae). *AoB Plants*, 2013, 5; doi: 10.1093/aobpla/plt015.
- Charlesworth, D., Why are unisexual flowers associated with wind pollination and unspecialized pollinators? *Am. Nat.*, 1993, 141, 481–490.
- 16. Charlesworth, B. and Charlesworth, D., A model for the evolution of dioecy and gynodioecy. *Am. Nat.*, 1978, **112**, 975–997.
- Friedman, J. and Barrett, S. C. H., A phylogenetic analysis of the evolution of wind pollination in the angiosperms. *Int. J. Plant Sci.*, 2008, 169, 49–58.
- Friedman, J. and Barrett, S. C. H., Wind of change: new insights on the ecology and evolution of pollination and mating in wind pollinated plants. *Ann. Bot.*, 2009, **103**, 1515–1527.
- Vamosi, J. C., Otto, S. P. and Barrett, S. C. H., Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. *J. Evol. Biol.*, 2003, 16, 1006–1018.
- Bawa, K. S., Patterns of flowering in tropical plants. In *Handbook* of *Experimental Pollination Biology* (eds Jones, C. E. and Little, R. J.), Van Nostrand, New York, 1983, pp. 394–410.
- 21. Abe, T., Flowering phenology, display size, and fruit set in an understory dioecious shrub, *Aucuba japonica* (Cornaceae). *Am. J. Bot.*, 2001, **88**, 455–461.
- Ackerman, J. D., Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. *Plant Syst. Evol.*, 2000, 222, 167–185.
- 23. Paw, U. K. T. and Hotton, C., Optimum pollen and female receptor size for anemophily. *Am. J. Bot.*, 1989, **76**, 445–453.
- 24. Llyod, D. G. and Webb, C. J., Secondary sex characteristics in plants. *Bot. Rev.*, 1977, **43**, 177–216.
- 25. Levin, D. A. and Kerster, H., Gene flow in seed plants. *Evol. Biol.*, 1974, **7**, 139–220.
- Loveless, M. D. and Hamrick, J. L., Ecological determinants of genetic structure in plant populations. *Annu. Rev. Ecol. Syst.*, 1984, 15, 65–95.
- Vallejo-Marin, M., Dorken, M. E. and Barrett, S. C. H., The ecological and evolutionary consequences of clonality for plant mating. *Annu. Rev. Ecol. Evol. Syst.*, 2010, 41, 193–213.

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Impact of climate change on the flowering of *Rhododendron arboreum* in central Himalaya, India

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Studies from different parts of the world have generated evidences of the effects of climate change on phenology and persistence of species. However, datasets or evidences are lacking for majority of the regions and species, including the climate-sensitive Himalayan biodiversity hotspot. Recognizing this gap in the information and realizing wide-ranging implications of such datasets, the present study generates evidences of changes in flowering phenology of an important trees species, Rhododendron arboreum in Indian central Himalaya. Real-time field observations (2009-2011) showed peak flowering during early February to mid-March. Analysis on long-term temperature data revealed significant (P < 0.01) increase in seasonal (winter and post-monsoon) and annual mean maximum temperature. Generalized additive model (GAM) using real-time field observations (2009-2011) and herbarium records (1893-2003) predicted 88-97 days early flowering over the last 100 years. Furthermore, GAM using long-term temperature data, real-time field observations and herbarium records depicted annual mean maximum temperature responsible for shifts in flowering dates of the target species. The study provides an important insight of species response to climate change in the Indian central Himalaya and highlights the need for further research on the subject to improve our understanding of the effects of climate change on species and consequently on ecology of the region.

Keywords: Climate change, flowering phenology, herbarium records, *Rhododendron arboreum*.

THE phenological responses of plants, particularly the early flowering ones, are considered among the prominent biological indicators of climate change^{1,2}. In this respect, various studies from different parts of the world have provided convincing evidences^{3–7}. However, most of these studies have relied on long-term datasets, created for the specific purpose of phenological measurements⁵. Unfortunately, for many regions and species such datasets/documentation are/is often not available⁶. Therefore, the need to have new source of data to build a more complete global picture is evident.

The Indian Himalayan Region (IHR), recognized amongst 34 global biodiversity hotspots (Conservation

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Locality	Elevation (m amsl)	Habitat (forest type)	2009		2010		2011	
			Date	Flowering (%)	Date	Flowering (%)	Date	Flowering (%)
Shyahidevi	2110	Mixed-oak	3 February	61.11	5 March	60.00	9 March	50.00
Binsar	2010	Pine-oak mixed	4 February	76.19	25 February	42.86	11 March	44.28
Jalana	1950	Oak	5 February	76.00	6 March	48.75	9 March	53.13
Kalika	1800	Pine	6 February	85.71	26 February	54.29	11 March	41.43

Table 1. Site characteristics and flowering intensity in Rhododendron arboreum in different localities during 2009–2011

International 2004), is known for higher sensitivity to climatic perturbations⁸, and is often referred to as datadeficient region⁹. This deficiency of information, among others, is also prevalent for phenological observations¹⁰. Therefore, the precise answer to a fundamental question about how phenological events in the region are changing as a result of climate change and what will be the future trends is lacking.

Realizing the above and considering the possibility that in absence of long-term documented records, the biological collections from museums, herbaria, zoos, botanical gardens and research stations can be utilized gainfully for determining patterns of responses to changing climate^{5,6,11}, the present study attempts to generate evidences of changes in flowering phenology of one of the common and widely known species, *Rhododendron arboreum* Smith in central Himalaya, India.

R. arboreum vern. Burans (family Ericaceae), a small evergreen tree, is known for its conspicuous deep red or pale pink flowers across the central Himalayan province of India. It commonly occurs between 1200 and 3500 m amsl, and often dominates under canopy layer of different oak (*Quercus*) forests, including *Quercus leucotrichophora* and *Quercus floribunda* forests in low to mid hills and *Quercus semecarpifolia* forests in high hill areas. Throughout the region, the species commands a high socio-cultural reverence and has been designated as the 'state tree' of Uttarakhand.

We studied the species on account of frequent media reports in recent years of its early flowering in the region, and broadly attributing this to climate change^{12,13}. The reports, however, demanded scientific evidences to prove the case.

In view of complete lack of long-term phenological records, the study considered three different but related approaches for generating evidences to explore reasonably sound trends of changes. The evidences were generated from: (i) Real-time field observations on frequency of flowering from represented sites – Four sites with abundance of *R. arboreum* were identified in Kumaun, central Himalaya (Table 1) and surveyed during the first week of February–March end (in 2009, 2010 and 2011). In each site, five quadrats $(10 \times 10 \text{ m})$ were randomly placed within a plot of $50 \times 50 \text{ m}$. The numbers of flowering trees against total trees of target species in indivi-

dual quadrats was recorded. Quadrat information was pooled to determine the percentage flowering in each site. (ii) Long-term weather data (temperature) analysis of the region - temperature data for preceding 41 years (1971-2011) were obtained from a nearby meteorological station (i.e. Vivekananda Parvatiya Krishi Anusandhan Sansthan, Hawalbagh, Almora, 1250 m amsl). Following Gaira et al.¹¹, temperature data (maximum and minimum) were analysed annually and seasonally (winter: December-February; summer: March-May; rainy: June-September; post-rainy: October-November). (iii) Synthesis of longterm historical information from herbarium records - the herbarium specimens of target species, collected between 1893 and 2003 from various parts of Uttarakhand, were examined at Botanical Survey of India (BSI), Dehradun; Forest Research Institute (FRI), Dehradun, and National Botanical Research Institute (NBRI), Lucknow, and date of collection was recorded. Assuming that the specimens are essentially collected during peak flowering phase, the information obtained was analysed for trends of flowering in the region. Towards developing a phenological model, the flowering dates were reconstructed as day of year (1 DOY = 1 January) and determined as a response variable (i.e. flowering time).

Long-term herbarium-based phenological change models require specific statistical methods for handling the complexity of data structure, i.e. non-normal and nonlinearity¹¹. In this context, the generalized additive model (GAM), which allows for choosing a wide variety of distributions for the response variable and link functions, especially to improve the quality of prediction¹⁴, was applied in the present study.

The information obtained on flowering frequency of *R. arboreum* clearly reveals greater proportion of individuals in flowering stage during February–March (early February 2009 (61–86%), late February to early March 2010 (43–60%) and early March to mid March 2011 (41–53%)) (Table 1).

While analysing the seasonal and annual temperature (maximum and minimum) data for the area using Spearman's correlation, significant increase in mean maximum temperature during winter (r = 0.561; P < 0.01) and postrainy (r = 0.398; P < 0.01) season was revealing over a time span of nearly four decades (Table 2). Also, annual mean maximum temperature exhibited significant increase

 Table 2.
 Spearman's correlation between temperature (maximum and minimum) and years

Temperature	Winter	Summer	Rainy	Post-rainy	Annual
Minimum Maximum	-0.285 0.561**	-0.114 0.129	-0.136 -0.289	-0.062 0.398**	-0.080 0.502**

**Values are significant at P < 0.01.

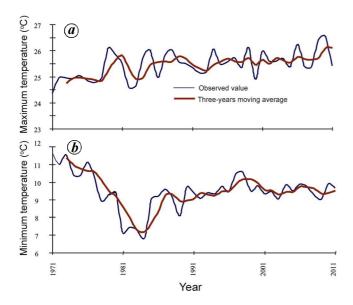


Figure 1. Long-term (1971-2011) trends of annual temperature (a) maximum and (b) minimum for Hawalbagh, Almora (1250 m amsl). Bold line indicates trends in the 3-years moving average.

(r = 0.502; P < 0.01) over the time. The three years moving average trend of annual mean maximum temperature showed warming signals (Figure 1). However, no such significant trends of change were observed in the case of minimum temperature.

Having known the current dates of flowering and overall temperature trends from the study area during the last four decades, we attempted to construct phenological change models using the herbarium records and field observations (Figure 2). When considering current flowering dates along with herbarium information (1893–2003), GAM showed significant early flowering over the last 100 years (88–97 days; GAM coefficient = -0.926, SE = 0.037, $R^2 = 37.89$, P < 0.001). Furthermore, while estimating the responses of flowering time along the increasing annual temperature (maximum), GAM showed 44–49 days early flowering (GAM coefficient = -46.23; SE = 2.27; $R^2 = 64.68$; P < 0.001) of the target species.

In general, most of the old literature indicates flowering phase for *R. arboreum* from March to May^{15} , with occasional flowering in January^{15,16}. However, the present field observations over three years revealed considerably higher frequency (47–75% trees) of bloom during February–March, which provides a strong basis to prove other observational reports of advancement in flowering events of target species from spring to winter¹³. As the observation sites (1800–2100 m) represented the midaltitude range of species distribution, it is further assumed that these dates would represent average timing of its bloom in the region.

Definite advancement in flowering time over 119 years based on herbarium and real-time field observations (88– 97 days early) further provides clear evidence of considerable advancement of flowering incidences. This advancement is, however, much more than that reported by Gaira *et al.*¹¹ in *Aconitum heterophyllum* species over the last 100 years, using herbarium records from IHR, which revealed 17–25 days advancement in flowering, attributed to increased warming during winter months. In general, more rapid advancement of flowering in different species in recent decades has been reported from other parts of the globe^{17,18}. Such advancement is most often attributed to corresponding increase in temperature^{18,19}.

Other reports, wherein herbarium records are linked with phenological observations of field data, have also established the trends of early flowering in some woody plant species owing to increased mean temperature⁶. The present study is largely in agreement with that of Miller-Rushing *et al.*⁶, which indicated an increasing annual and seasonal (i.e. winter and post-rainy season) maximum temperature. These inferences might be considered as an influencing signal for early flowering. As such, based on long-term data analysis, early flowering in plant species has been attributed to their greater responsiveness to temperature variations¹⁸.

We are, however, aware of the fact that: (i) the temperature data, as they reflect lower extreme of altitude range for the species, cannot be treated as the true reflection for the entire altitude range; (ii) the herbarium specimens also cannot be considered as absolute evidence for flowering dates. In spite of these limitations, three years of real-time field observation and GAM-generated trends would certainly confirm advancement of flowering in *R. arboreum*. Cumulative, long-term herbarium records and three years field observations based GAM provide evidence of high rates of change in flowering time (earlier), which can be attributed to increasing annual mean maximum temperature. While the estimates of change need to be treated as most conservative, the trends definitely

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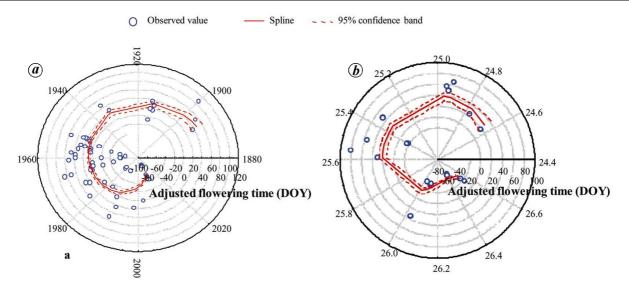


Figure 2. GAM-predicted changes in flowering period of *Rhododendron arboreum* in relation to: (*a*) using herbarium records (1893–2003) and field data (2009–11) and (*b*) annual mean maximum temperature (1971–2011).

warrant attention as an indicator of climate change impacts on flowering phenology of prominent regional species, and the likely consequences for ecosystem processes.

- Parmesan, C. and Yohe, G., A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 2003, 421, 37–42.
- Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C. and Pounds, J. A., Fingerprints of global warming on wild animals and plants. *Nature*, 2003, **421**, 57–60.
- Sparks, T. H. and Carey, P. D., The responses of species to climate over two centuries: an analysis of the Marsham phenological record, 1736–1947. J. Ecol., 1995, 83, 321–329.
- Fitter, A. H. and Fitter, R. S. R., Rapid changes in flowering time in British plants. *Science*, 2002, 296, 1689–1691.
- Primack, D., Imbres, C., Primack, R. B., Miller-Rushing, A. J., Tredici and Del, P., Herbarium specimens demonstrate earlier flowering time in response to warming in Boston. *Am. J. Bot.*, 2004, **91**, 1260–1264.
- Miller-Rushing, A. J., Primack, R. B., Primack, D. and Mukunda, S., Photographs and herbarium specimens as tools to document phenological changes in response to global warming. *Am. J. Bot.*, 2006, **93**, 1667–1674.
- Pettorelli, N., Weldgi, R. B., Holand, Ø., Mysterud, A., Breie, H. and Stenseth, N., The relative role of winter and spring conditions: linking climate and landscape-scale plant phenology to alpine reindeer body mass. *Biol. Lett.*, 2010, 1, 24–26.
- National Action Plan on Climate Change, Prime Minister's Council on Climate Change, Government of India, 2008, p. 52; <u>http://</u> pmindia.nic.in/Pg01-52.pdf
- 9. IPCC, *The Fourth Assessment Report: Climate Change, Synthesis Report*, Cambridge University Press, Cambridge, 2007.
- Badola, H. K., Phenology and climate response in Himalayan rhododendrons. In Proceedings, International Conference on Rhododendrons: Conservation and Sustainable Use (eds Marina, A., Badola, H. K. and Mohanty, B.), 2010, pp. 48–59.
- 11. Gaira, K. S., Dhar, U. and Belwal, O. K., Potential of herbarium records to sequence phenological pattern: a case study of *Aconi*-

tum heterophyllum in the Himalaya. Biodivers. Conserv., 2011, 20, 2201–2210.

- Dhyani, S. M., Spring in winter. In India Environment Portal, 14 February 2009; <u>http://www.indiaenvironmentportal.org.in/node/</u> 277525 (accessed on October 2012).
- Gusain, R., Climate change leads to early flowering in Himalayas. *India Today*, Dehradun, India, 8 February 2009; <u>http://indiatoday.intoday.in/index.php?option=com_content&task=view&id=</u> <u>27582§ionid=4&issueid=92&Itemid=1</u>.
- 14. Hastie, T. J. and Tibshirani, R. J., *Generalized Additive Models*, Chapman and Hall, New York, 1990.
- Troup, R. S., *The Silviculture of Indian Trees*, Clarendon Press, Oxford, 1921, vols I & II.
- 16. Osmoston, A. E., *A Forest Flora for Kumaun, India*, International Book Distributors, Dehradun, 1927.
- Gormsen, A. K., Hense, A., Toldam-Andersen, T. B. and Braun, P., Large scale climate variability and its effects on mean temperature and flowering time of *Prunus* and *Betula* in Denmark. *Theor. Appl. Climatol.*, 2005, 82, 41–50.
- Primack, R. B., Higuchi, H. and Miller-Rushing, A. J., The impact of climate change on cherry trees and other species in Japan. *Biol. Conserv.*, 2009, **142**, 1943–1949.
- Aono, Y. and Kazui, K., Phenological data series of cherry tree flowering in Kyoto, Japan, and its application to reconstruction of springtime temperatures since the 9th century. *Int. J. Climatol.*, 2008, 28, 905–914.

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