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# Climate change-driven shifts in elevation and ecophysiological traits of Himalayan plants during the past century

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**As broad-scale distributions of plants are shaped by climatic conditions, changes of climate necessarily result in shifts of distributional limits. These shifts are closely coupled with changes in plant ecophysiology, growth and productivity. Among environments subjected to the highest increase in temperature in the last decade and the greatest expected warming predicted for the future, high-mountain biomes belong to the most frequently considered. Evidence for distributional shifts has been mostly documented in European and American mountains, while the largest and highest mountainous areas are located in Asia. The present study aims to detect climate change-driven shifts in elevation and ecophysiological traits of endemic herb species of Himalaya with the help of herbarium specimens as potential tool. We observed significant rapid upward elevational shift of 55.2 m/decade compared to average global shifting of 6.1 m/decade and impulsive variations in secondary metabolite concentration. Significant negative relationship was found for stomatal density, $\delta^{13}$C with the lapse of years. Analysis of instrumental temperature data reveals an increase of 0.31°C in mean maximum and 0.79°C in mean minimum temperature during the last century.**

**Keywords:** Climate change, distributional shift, $\delta^{13}$C, Himalayan plants, metabolites, stomatal density.

LONG-TERM observations, experiments and modelling studies have demonstrated significant changes in patterns of global biodiversity in response to climate change[1–4]. In particular, mountainous regions are predicted to be more vulnerable for biodiversity loss due to fragmented ecosystems[5]. Recently, upward shifting of vegetation zones,

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range expansion and contraction, as well as shifts in phenology and eco-physiological characters, manifested by climate change have been observed in these ecologically fragile regions[6–9]. Concatenation of species distributional pattern changes, regional climate trends and physiological mechanism are needed to ascertain climate induced species shift[10]. Based on the assumption of 0.5°C decline in temperature per 100 m gain in altitude, climate change is estimated to cause an upward shift of vegetation belts at the rate of 8–10 m per decade[1], with risk of extinction of plants restricted to mountain summits[11]. In other words, upward shifting of vegetation will push the upper vegetation zone towards much higher altitudes in mountains[12]. In mountain environment, structural changes in plants along the altitudes may influence their physiology, which in turn may alter the metabolite content. Among climatic variables, $CO_2$ is considered as a principal causal factor for changes in physiological characters such as stomatal density[13], water use efficiency[8] and also secondary metabolism[14]. In general, stomatal parameters are under genetic control[15] and their relative stomatal density remains unaffected under given environmental conditions but variations such as in carbon dioxide[16], temperature[17], $\delta^{13}$C (ref. 18) and environmental factors may influence stomatal parameters. Secondary metabolites are important constituents of plants due to their medicinal values[10]. The concentration of secondary metabolites is influenced by several environmental factors[19] prevalent at different altitudes. Elevated $CO_2$ concentration in the atmosphere increases the carbon supply excess to need for growth and maintenance could be partitioned to c-based secondary metabolites such as fatty acids, sterols, flavonoides and other phenolic compounds.

Himalaya, being a global biodiversity hotspot and the source of the eight largest Asian rivers fed by 15,000 glaciers, is crucial to the sustenance of more than two billion people[20]. Himalaya is experiencing rapid climate change and its effect on biodiversity, ecosystem services and human well-being is more severe than any other parts of the world[21]. Moreover, climate trends at regional level are disparate from climate trends at the global level[22]. On the top of it, with a fillip for rapid economic development in India during the last two decades, the natural environment of Himalaya has been substantially degraded. Anthropogenic pressure in the form of development of modern infrastructure has led to large-scale deforestation, submergence of human settlements, accelerated erosion of topsoil, land-slides, siltation and pollution of water bodies[23]. These mountains are fast losing their resilience capacity to sustain local climatic fluctuations[24]. Thus, local flora and fauna are incapable of responding to rapid climate fluctuations associated with climate change.

With the exception of a few local observations[25], there are no systematic studies of climate change impact on the endemic flora and fauna of Himalaya. There is thus a paucity of studies on climate-induced shifting of elevation ranges for the Himalayan flora. Fortunately, the Himalaya has a long history of botanical exploration and collection. This has prompted us to explore herbarium specimens of three representative plants, namely, *Geranium nepalense* Sweet (Geraniaceae), *Inula racemosa* Hook. f. (Asteraceae) and *Lavatera kashmiriana* Camb. (Malvaceae) collected during the past century to unravel the response of Himalayan plants to climate change. The older herbarium data (1900–1960) was compared with data generated from recent herbarium records (1961–2010) and supplemented with measurements of stomatal density, $\delta^{13}$C and metabolite content of the herbarium specimens.

We studied whether the climate change has had an impact on elevational distribution pattern and eco-physiological traits of plants in Kashmir Himalaya during the past 110 years and suggest that an increase in temperature and atmospheric $CO_2$ concentration altered the altitudinal distribution pattern, stomatal density, $\delta^{13}$C and concentration of c-based secondary metabolites over time within individual herbaceous species.

The present study was conducted in the temperate and alpine regions of Kashmir Himalaya along an altitudinal gradient of 1200–3700 m (33°20′–34°54′N and 73°55′–75°33′E) that covers an area approximately 15,948 sq. km. We selected three herbaceous plant species, *Lavatera kashmiriana*, *Geranium nepalense* and *Inula racemosa*, which serve as model plants for identified ecological region and are well represented in the herbarium collections. The collections housed in Indian herbaria dating back to last 110 years were used for the study. We obtained all relevant information recorded on the herbarium sheets for the selected three species from eight major herbaria of India: Herbaria of Botanical Survey of India, Howrah (CAL) and Dehradun (BSD), ICFRE, Dehradun (DD), CSIR-NBRI, Lucknow (LWG), CSIR-CIMAP, Lucknow (CIMAP), CSIR-CDRI, Lucknow (CDRI), CSIR-IIIM, Jammu (RRLH), and University of Kashmir, Srinagar (KASH). In total, we had 466 herbarium specimens with *G. nepalense*, *I. racemosa* and *L. kashmiriana* having 156, 136 and 174 specimens respectively. The study period of 110 years was divided into two: 1900–1960 and 1961–2010.

From large scale floristic inventories made over the last 100 years, we extracted two sub-samples, the first sub-sample included surveys carried out during 1900–1960 and the other one during 1961–2010. We studied changes in species distributional elevation over the last 110 years. We analysed data and provided means of altitudinal distribution for each species in both the periods. The difference in distributional elevation of both periods was taken into consideration as the altitudinal shift during last 110 years.

We obtained temperature and precipitation records from the India Meteorological Department (Pune) for the time period 1900–2010 for the stations index no. 42027

(Srinagar), 09214 (Kokernag) and 42026 (Gulmarg), the meteorological stations nearest to the localities of herbarium collections sites. From average monthly values of minimum and maximum temperatures, precipitation, mean minimum and mean maximum temperature with mean precipitation were calculated for each year. We have followed altitude-for-latitude temperature model for calculating the temperature at Kokernag and Gulmarg stations which were not available prior to 1970. According to this model, the decline in temperature by 1°C occurs with an increase of ~167 m altitude or ~145 km latitude[26]. We obtained published data for atmospheric $CO_2$ concentration for 1960 and 2010 from annual collections of Mauna-Loa, Hawaii[27].

To measure stomatal density, epidermal peels of at least two healthy leaves of herbarium samples were analysed to assess the changes in stomatal densities during the past 110 years. The standard deviation was calculated on the basis of these counts and an average value was taken for each of the two time periods, i.e. 1900–1960 and 1961–2010. The stomatal density was determined from mature leaf samples of 452 herbarium specimens taking 1 × 1 cm middle portion under light microscope (Olympus monocular) equipped with a scaled grid. The samples were soaked in boiling water and dipped in 5% KOH solution for peeling off. The peelings were stained with 1% safranin for 2 min, dehydrated in upgrading series of ethyl alcohol from 30% to absolute, treated with xylene and mounted in Canada balsam. Observations were made under light microscope using a camera lucida (Nikkon, Tokyo). Stomatal density is expressed as SD = SC/$A_g$, where SC is stomatal count and $A_g$ is the area of grid measured through microscope. Amphistomatic studies were made for all the samples and stomatal densities were determined on the basis of average of stomatal counts for both abaxial and adaxial surfaces.

The *n*-hexane extracts of leaf samples of herbarium specimens were analysed by GC–MS to find out the composition of individual fatty acids and sterols. Similarly dried leaves (1.0 g) of the herbarium samples were extracted with hexane using tissue homogenizer (Kinematica Polytron Homogenizer PT 6100) and concentrated for phytochemical analysis. The resulting oily mass containing fatty acids and sterols was stored at –20°C till analysis. The lipid profile was monitored using Thermo Trace GC ultra coupled with Thermo fisher DSQ II mass spectrometers with electron impact ionization at 70 eV for generating mass spectra. Chromatographic separation of metabolites was done on 30 mm × 0.25 mm Thermo TR50 column (polysiloxane column coated with 50% methyl and 50% phenyl groups). X-calibur software was used to process the chromatographic and mass spectrometric data. The resulting GC–MS profile was analysed using WILLY and NIST mass spectral library and chromatogram was matched with commercially available standards and were further processed to TMS derivatization. AMDIS32 was used to perform component peak identification and spectral deconvolution at low sensitivity and medium resolution.

We sub-sampled the leaves of the same herbarium specimens as those measured for stomatal density for analysis of $\delta^{13}$ carbon isotope ratio. Approximately 0.60 mg fine powder was combusted in tin capsule in a Flash EA 1112 series elemental analyser (Thermo Finnigan). $CO_2$ thus produced was channelled into a Delta V plus IRMS isotope ratio mass spectrometer (Thermo Electron, Germany) via a TC/EA gas control unit (Thermo Finnigan, Germany). Output of carbon isotope ratio (13C/12C) was then monitored relative to the Pee dee Belemnite standard. $\delta^{13}$C was determined using the following equation[28]

$$\delta^{13}\mathrm{C}(‰) = \frac{R_{\text{Standard}} - R_{\text{Standard}}}{R_{\text{Standard}}} \times 1000.$$

We used two methods for analysis; first, a general linear model for regression analysis was used to determine the extent of variation in stomatal density and $\delta^{13}$C with the passage of time and increasing altitude for each individual species. To find out the variation in SD, $\delta^{13}$C and altitudinal changes (dependent variables), we calculated the mean changes in SD, $\delta^{13}$C and altitudinal changes of the herbarium specimens collected during the last 110 years (independent variables). Second, we used one-way ANOVA to test the significance difference in SD and $\delta^{13}$C during the last 110 years for each species. Statistical analysis of variance was done through INDOSTAT software (INDOSTAT Services, Hyderabad, India).

Significant altitudinal shift was observed in all the three herbaceous plant species on the basis of herbarium records of the past 110 years. The species moved at a different pace although all three species grew in more or less same habitat and exhibited a similar pattern of altitudinal range shift (Table S1, see Supplementary Information online). Scattered plots provided marked altitudinal shift in Himalayan species (Figure 1). When all the three plant species were considered, the mean elevation of these species showed an average increase of 55.2 m/decade between 1900 and 2010, much larger than the average global rate of 6.1 m/decade[29]. Larger shifts were observed in maximum elevation for *G. nepalense* and *I. racemosa* than for *L. kashmiriana*, which is a high-altitude species.

In Kashmir Himalaya, between the years 1900 and 2010, the mean annual average maximum temperature increased by 0.31°C/100 years and the mean annual average minimum temperature increased by 0.79°C/100 years. Here, we expect the upward shifting and changes in ecophysiological traits if the Kashmir Himalaya climate has warmed on the basis of its minimum temperature limits. During the past six decades, precipitation increased by 8.15 mm in the Kashmir Himalaya (Figure S1, see Supplementary Information online).

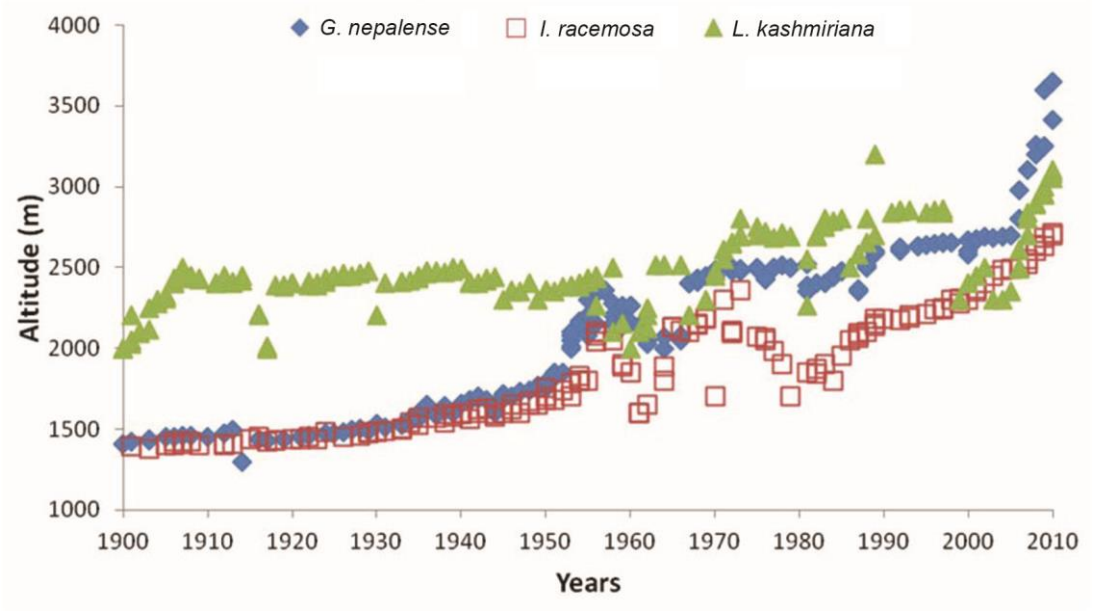


**Figure 1.** Scattered plots illustrating the temporal altitudinal distribution of 3 species for different periods (1900–1960) and (1961–2010).

Our results demonstrate a highly significant decline in stomatal density ($P < 0.01$) in all the three plant species from the beginning of 20th century. Results of 452 observations show an average reduction of 21.64%, 17.58% and 16.84% in *G. nepalense*, *I. racemosa* and *L. kashmiriana* respectively. The decreasing trend in stomatal density for these plant species between 1900–1960 and 1961–2010 is statistically significant ($P < 0.01$) with mean difference of 90.157/mm$^2$ for *G. nepalense*, 76.906/mm$^2$ for *I. racemosa* and 107.998/mm$^2$ for *L. kashmiriana*, leading to an average of 91.687/mm$^2$ reduction in stomatal density. Stomatal density was inversely related to the elapse of years in all the three species. The values were for *G. nepalense* ($y = -1.859x + 4088$, $R^2 = 0.954$, $P < 0.05$), *I. racemosa* ($y = -1.646x + 3692$, $R^2 = 0.907$, $P \leq 0.05$, CI = 95%) and *L. kashmiriana* ($y = -1.310x + 2984$, $R^2 = 0.963$, $P < 0.05$) respectively. The $\delta^{13}$C was significantly negatively correlated with the elapse of years in all three species, with values for *G. nepalense* ($y = -0.048x + 74.89$, $R^2 = 0.872$, $P < 0.05$, CI = 95%), *I. racemosa* ($y = -0.017x + 15.94$, $R^2 = 0.803$, $P < 0.05$, CI = 95%) and for *L. kashmiriana* ($y = -0.044x + 70.62$, $R^2 = 0.919$, $P < 0.05$) (Figure 2).

A positive correlation between stable carbon isotope values and stomatal density was found in all three species. The overall decrease of average $\delta^{13}$C from 1900–1960 to 1961–2010 is for *G. nepalense* from –18.0039 (± SE 0.1478, $n = 24$) to –21.0199 (± SE 0.048, $n = 28$), for *I. racemosa* from –17.387 (±SE 0.0652, $n = 25$) to –18.384 (± SE 0.083, $n = 25$) and for *L. kashmiriana* from –15.907 (± SE 0.1133, $n = 33$) to –18.528 (± SE 0.215, $n = 28$). The values are highly significant ($P < 0.01$, CI = 99%) as summarized in Table 1.

Percentage peak area of the GC chromatograms suggested that palmitic acid, stearic acid and linolenic acid were the dominant fatty acids found in leaves, while other fatty acids were present in small amounts (Table 2). Per cent peak area of all the fatty acids varied considerably between herbarium specimens of two periods (1900–1960 and 1961–2010). The per cent peak area of palmitic acid was higher for 1900–1960 than for 1961–2010 for these plant species. Stearic acid and linoleic acid showed similar trends. However, polyunsaturated fatty acid (18 : 3) showed a reverse trend to that of monounsaturated (18 : 1) and saturated fatty acids (16 : 0 and 18 : 0) (Figure S2, see Supplementary Information online).

The Himalayan mountain region represents the longest bioclimatic elevation gradient in the world and rich resource of plant diversity. In such regions, the herbarium specimens collected in the past seem to provide invaluable data source for studying climate change induced plant responses. In the present study we used herbarium specimens from individual herbaceous species from the natural habitat to examine eco-physiological changes they produced over the last 110 years. We assessed responses of the three model species to the climate change by measuring shifts in their altitudinal ranges in montane zone (ca. 2500 m elevation gradient between 1200 and 3700 m amsl) of Kashmir Himalaya for 110 years. The plants, *G. nepalense* play an important role in checking soil erosion and water loss, *I. racemosa* is an important medicinal plant mainly used in heart diseases[31] and *L. kashmiriana* is endemic to this Himalayan region. Climate change seems to have triggered a shift in the distribution of some mountain species (e.g. *I. racemosa*), which otherwise show restricted altitudinal distribution

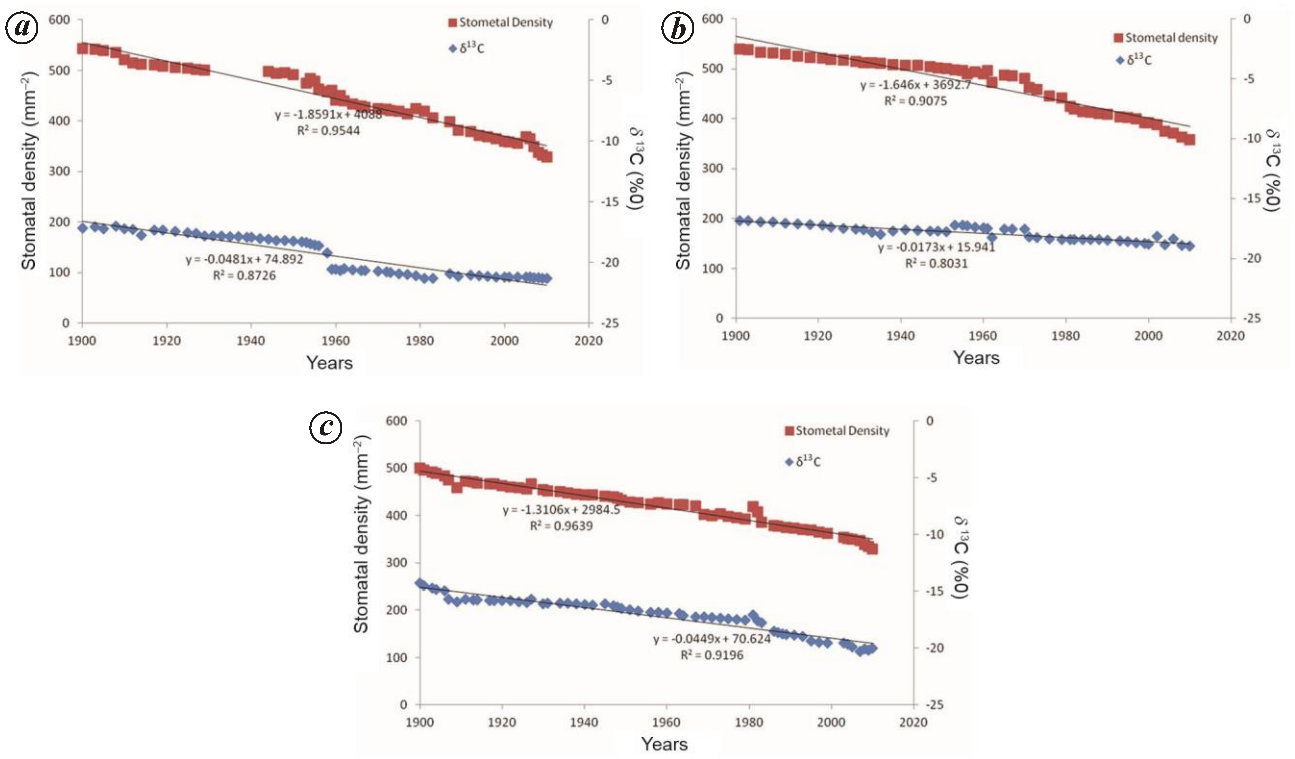


**Figure 2.** Changes in stomatal density (®) and $\delta^{13}$C (©) during last 110 years. ***a***, *Geranium nepalense*; ***b***, *Inula racemosa*; ***c***, *Lavatera kashmiriana*.

**Table 1.** Variation in maximum temperature, minimum temperature, precipitation, $CO_2$ concentration, stomatal density, $\delta^{13}$C and metabolites within two periods, i.e. 1900–1960 and 1961–2010

| Years | 1900–1960 | | 1961–2010 | |
|---|---|---|---|---|
| Max. temp. (°C) | 16.37 | | 16.68 | |
| Min. temp. (°C) | 4.50 | | 5.29 | |
| Precipitation (mm) | 88.19 | | 96.34 | |
| $CO_2$ | 316.90 | | 389.84 | |
| Plant name | Stomatal density | $\delta^{13}$C | Stomatal density | $\delta^{13}$C |
| *G. nepalense* | 499.116** ± 5.556 | –18.0039** ± 0.1478 | 391.118** ± 6.791 | –21.0199** ± 0.048 |
| *I. racemosa* | 512.749** ± 2.927 | –17.387** ± 0.0652 | 422.592** ± 8.255 | –18.384** ± 0.083 |
| *L. kashmiriana* | 456.592** ± 3.678 | –15.907** ± 0.1133 | 379.686** ± 5.379 | –18.528** ± 0.215 |

**$P < 0.01$.

(1700–1800 m amsl) and microhabitat preferences. The observed changes in maximum elevation and distribution range suggest that both the maximum and minimum altitudes for these species may have shifted upward and this effect is more pronounced in herbaceous species than in woody plants due to their shorter life cycle, faster maturation and narrow adaptability to tolerate rapid changes in climatic conditions. Considerable shift of 0.51°C/100 years has been observed for the Indian subcontinent during the period 1901–2007. Most of physiological climate research addressed species maximum temperature limits[32] and not the minimum temperature limits. An increase of 72.94 ppm in the atmospheric global $CO_2$ concentration (316.90–389.84 ppm) has been recorded in the last 50 years[26], which significantly affected the eco-physiology of plants, particularly of herbaceous species (Table 1). Atmospheric $CO_2$ concentrations and temperature can influence stomatal density and $\delta^{13}$C values through influencing cell differentiation and physiological metabolism[33]. Montane flora faces lower partial pressure of $CO_2$ and are more responsive to the change in $CO_2$ partial pressure than the flora from the higher $CO_2$ environment. This suggests that enhanced levels of $CO_2$ will do greater reduction in stomatal density and carbon isotope discrimination value at higher altitude. Strong negative relationship between lapse of years which is positively related with atmospheric $CO_2$ concentration and stomatal density indicates that during high $CO_2$ concentration,

**Table 2.** Secondary metabolites (fatty acid and sterol) content of the model plant species within two periods, i.e. 1900–1960 and 1961–2010

| Secondary metabolites | | tR (min.) | Mass fragmentation | Concentration (% peak area) in model plant species | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *G. nepalense* (1900–1960) | (1961–2010) | *I. racemosa* 1900–1960) | (1961–2010) | *L. kashmiriana* (1900–1960) | (1961–2010) |
| Fatty acids | Plamitic acid | 32.57 | *m/z* 328 ($M^+$), 313 ($M^+$–$CH_3$), 285, 269, 243, 117, 73 | 3.73 | 2.36 | 11.81 | 4.7 | 3.94 | 0.92 |
| | Stearic acid | 36.11 | *m/z* 356 ($M^+$), 341 ($M^+$-$CH_3$), 328, 297, 147, 145, 132, 117, 97, 73, 43 | 4.13 | 3.04 | 9.16 | – | – | – |
| | Linoleic acid | 36.36 | *m/z* 352 ($M^+$), 337 ($M^+$–$CH_3$), 262, 129, 95, 73 | – | – | 10.66 | – | 30.44 | 27.34 |
| | Linolenic acid | 36.4 | *m/z* 350 ($M^+$), 335 ($M^+$–$CH_3$), 280, 149, 129, 95, 73 | 0.93 | 1.51 | – | 1.06 | – | – |
| | Behenic acid | 42.45 | *m/z* 458 ($M^+$), 397 ($M^+$–$CH_3$), 218, 145, 132, 117, 73 | – | – | 2.43 | – | 0.19 | – |
| Sterols | Cholesterol | 50.73 | *m/z* 458 ($M^+$), 369, 329, 255, 147, 73 | – | – | 0.54 | 0.29 | 0.25 | 0.06 |
| | Stigmasterol | 52.62 | *m/z* 484 ($M^+$), 394, 255, 217, 147, 129, 73 | – | – | 0.12 | 0.12 | – | – |
| | $\beta$-sitosterol | 53.07 | *m/z* 486 ($M^+$), 471 ($M^+$–$CH_3$), 396, 280, 217, 147, 73 | 0.69 | 5.19 | 1.14 | – | – | – |

leaves tend to reduce stomatal density and vice versa, this relationship may be advantageous allowing plants to retain more water when $CO_2$ is abundant and increase the stomatal conductance when $CO_2$ is less abundant. On the basis of 452 observations, we found altitude rising with passage of time (Figure 1), strong positive relationship between stomatal density and $\delta^{13}C$ and both were inversely proportional to rising $CO_2$ and altitude. This relationship was maintained with each individuals studied over time.

Metabolic pathways involved in secondary metabolites produce a spectrum of compounds and some of them may be pharmaceutically important, such as gallic acid and quercitin isolated from *G. nepalense*[34] and inulin from *I. racemosa*[35]. Variations in secondary metabolite composition are influenced by environmental factors including temperature, water availability and $CO_2$ concentration[36] and also with increasing altitude[37]. We have observed significant reduction in fatty acids content during last 100 years with rising altitude. Changes in metabolite concentration over the last century indicate that phytosterols have been affected by climatic factors particularly increase in temperature, $CO_2$ concentration and precipitation in the Himalaya, the plants thereby losing their pharmacological activity that is reported to be beneficial to the human health[38].

In conclusion, Himalayan plants are moving at a much faster pace (55.2 m/decade) towards higher altitude than the general assumption of altitudinal shifting of 8–10 m/decade in the world wide mountain ecosystems. Regional climate changes have affected the ecophysiology of plants in general. Enhanced levels of $CO_2$ and increasing temperature cause greater reduction in stomatal density at higher altitude and considerable variations in stable carbon isotope value and secondary metabolites production indicate the defensive capability of the plant to adapt to environmental changes. Mean temperature (minimum and maximum) in Kashmir Himalaya increased over the past 110 years and has induced an upward altitudinal shifting of the plant species. Therefore, it is concluded that the rate of upward shifting of the mountainous plants depends upon the extent of changes in the local climatic conditions and may be influenced by other factors including anthropogenic pressures that operate differently in each mountain systems of the world.

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