



RESEARCH ARTICLE

Can species distribution models and molecular tools help unravel disjunct distribution of *Rhododendron arboreum*?

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Abstract. The apparent absence of Himalayan low-elevation taxa in the central Indian region and resumption of their distribution in the high elevation of Western Ghats has puzzled biogeographers for several decades. Many theories have been proposed to explain this but attempts remain futile owing to insufficient empirical support. Here, we have employed a montane tree species, *Rhododendron arboreum* to investigate this pattern by integrating past ecological niche modelling with molecular signatures. Reconstruction of paleo-ecological niche from interglacial to Last Glacial Maxima (LGM) portrayed a gradual depletion of vegetation cover with extreme impoverishment in the Holocene. A similar pattern was also reflected from genetic signatures; population history revealed a very recent split between the Himalayas and Western Ghats in the late Quaternary. A few other tree species exhibiting the same disjunction demonstrated a similar modification of paleo-ecological niche from last interglacial. The study clearly indicated that the populations in the Western Ghats to be a relictual remnants of a once continuous distribution of *R. arboreum*.

Keywords. disjunction; satpura hypothesis; Himalaya; Western Ghats; quaternary climate; India; *Rhododendron arboreum*.

Introduction

Biotic disjunctions are intriguing spatial patterns created by past vicariant movements (Raven and Axelrod 1972; Mairal *et al.* 2015), dispersal (Renner 2004), and/or climatic oscillations (Hewitt 2004; Wiens and Donoghue 2004). In the Indian subcontinent, one such striking formation is an apparent absence of a large number of Himalayan low-elevation taxa in central Indian region, and their abundance in and around mid elevation of the Western Ghats (WG). This baffling discontinuity of biotic distribution is known as

Central Indian Disjunction (CID) which has long tempted past biogeographers to propose a plausible cause. Historically important and foremost was the Satpura hypothesis by Hora (1949) that sought to explain this pattern. Hora (1949) postulated a southward colonization from the eastern Himalayas via Vindhya–Satpura ranges to the WG. Following this, several other prominent hypotheses have been proposed that offered alternative routes of colonization and diversification, e.g. via the Eastern Ghats (Abdulali 1949), the Brij area of northern India (Dilger 1952), and the Aravalli range (Mani 1974), all of which demonstrated dispersals tracing different corridors. On the other hand, the relictual distribution hypothesis has also been proposed that considers the present disjunct distributions as remnants of past continuous distributions (Ali 1935; Das 1996; Ripley *et al.* 1986). In support of this, climatic perturbations were

AR and GR conceived the study. AR drafted the project design, collected field specimens, and carried out the molecular lab work. GR facilitated the lab and field work. RR and RMS assisted AR in the molecular work. AR, RR, RMS and GR participated in data analysis, and drafted the manuscript. RR conducted the Paleo-ENM analysis.

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cited as drivers, which was brought about by the northward movement of the Indian plate. The tectonic movement has resulted in drier spells and a replacement of tropical evergreen forests by deciduous types in mid Miocene (Meher-Homji 1983). This followed further deterioration during late Miocene and more drastic alterations during Quaternary period caused a major biotic reshuffling.

Earlier researchers have attempted to resolve this striking pattern by examining a range of plants with molecular markers (Bahulikar et al. 2004; Apte et al. 2006; Bano et al. 2014; Kuttapetty et al. 2015). However, their efforts to unravel this perplexing distribution have been frustrated by scant sampling, and their inability to date the divergence or to model demographic events. As a consequence, the current understanding still lacks precise information on the timing of the event and other correlated factors that have sculpted this distinct biotic disjunction.

In this study, we have studied a montane tree, *Rhododendron arboreum* of family Ericaceae, exhibiting similar disjunct distribution to understand this pattern (biotic disjunction). In a phylogeographic framework, we integrate paleo-ecological niche distribution models with molecular tools and aim to address the following questions, why and when did the central Indian disjunction happen? In doing so, we test the hypotheses: (i) whether this disjunct distribution corroborates to dispersal or to Pleistocene relict, and also (ii) if the timing of this event falls in the Quaternary period, or alternatively is it relatively ancient dating back to Miocene?

Materials and methods

Sampling, DNA extraction, PCR, sequencing and genotyping

The distribution of *R. arboreum* is mostly restricted in low to mid elevation of the Himalayas and the WG, i.e. on northern and southern sides of central India (figure 1). Since, our hypothesis demanded sampling from the populations around these two broader regions, the collections were made from the WG as well as from the Eastern Himalayas. We attempted to test the two possible hypotheses (i) that the population found in the WG are the relicts of the once continuous distribution and (ii) that the populations found in the WG possibly migrated from the Eastern Himalayas.

To test these two hypothesis, we collected fresh leaf samples from *R. arboreum* populations across various locations of eastern Himalaya and the WG (table 1). We sampled five populations, namely Barsey Wildlife Sanctuary (BWS), Yumthang (Y), Kanchenjunga National Park (KNP), Singalila (S) and Tawang (T) across eastern Himalaya and two populations Ooty (O) and Munnar (M) in WG. The identity of the species was confirmed by Dr R. Ganesan, taxonomist at ATREE and the herbarium of the species deposited at ATREE herbaria (voucher numbers ATREE/CG/5565 to ATREE/CG/5567). Within each population, six to ten individuals were sampled taking care that each individual was at least 10–15 m apart. A sample of 50 mg of leaves was used to extract total

genomic DNA according to a modified (CTAB) procedure (Doyle and Doyle 1987). Both nuclear and chloroplast markers were sequenced to characterize the genetic variability. We amplified and sequenced two cpDNA fragments (*trnS-trnG* and *trnC-trnF*) and nuclear ITS region using a standard protocol. Sequences were edited, aligned with ClustalW as implemented in MEGA 5.1. Individuals were also genotyped for eight fluorescent-labelled microsatellite primers (namely R25, R98, R346, R422, R460, RF29, RA50 and RA254) selected from a panel of microsatellites reported by Choudhary et al. (2014). All the sequence generated in this study have been deposited in the GenBank with the corresponding accession numbers (MF687750–MF687844, MF683730–MF683824, and MF679651–MF679827).

Genetic diversity, population structure, haplotype network construction

We estimated the parameters at various levels, for e.g. larger groups/regions (e.g. the Himalayas and WG) and populations within larger groups (BWS, Y, KNP, S and T within the Himalaya; and O and M within the WG) (tables 1, 2a). Sequences were used to estimate the basic population genetic parameters, including the number of segregating sites (S), Watterson's parameter (θ_w), haplotype number (h), and diversity (H_d) in the DnaSP v. 5.00.04 (Librado and Rozas 2009). For both chloroplast and nuclear DNA, evolutionary relationships among the individuals were drawn using NETWORK V4.5.1.6 (www.fluxus-engineering.com) (Bandelt et al. 1999). We employed GENODIVE to estimate summary statistics such as allelic diversity, allele range, fixation indices such as G_{ST} and standardized G_{ST} and analogues for microsatellites (Meirmans and Van Tienderen 2004). To understand the hierarchical distribution of genetic diversity within the population, among populations within the group, and between groups; an AMOVA was performed in ARLEQUIN v. 3.1.1 (Excoffier and Lischer 2010). In addition to population structure, we estimated the number of putative genetic clusters (k) in the microsatellite dataset using a maximum likelihood-based method implemented in FLOCK program (Duchesne and Turgeon 2009).

Inference of demographic histories

To understand the population history of *R. arboreum*, we employed an approximate Bayesian computation (ABC) framework in DIYABC 2.0.4 based on nuclear microsatellites (Cornuet et al. 2014). We assumed the generation time of 10–15 years considering that the tree takes 10–15 years to bear fruits. We have selected divergence model after initial runs with the following scenarios: (i) divergence, (ii) divergence with bottleneck, and (iii) divergence with expansion, where divergence model outperformed others. Following this, to gain insight on the temporal location of the event, we

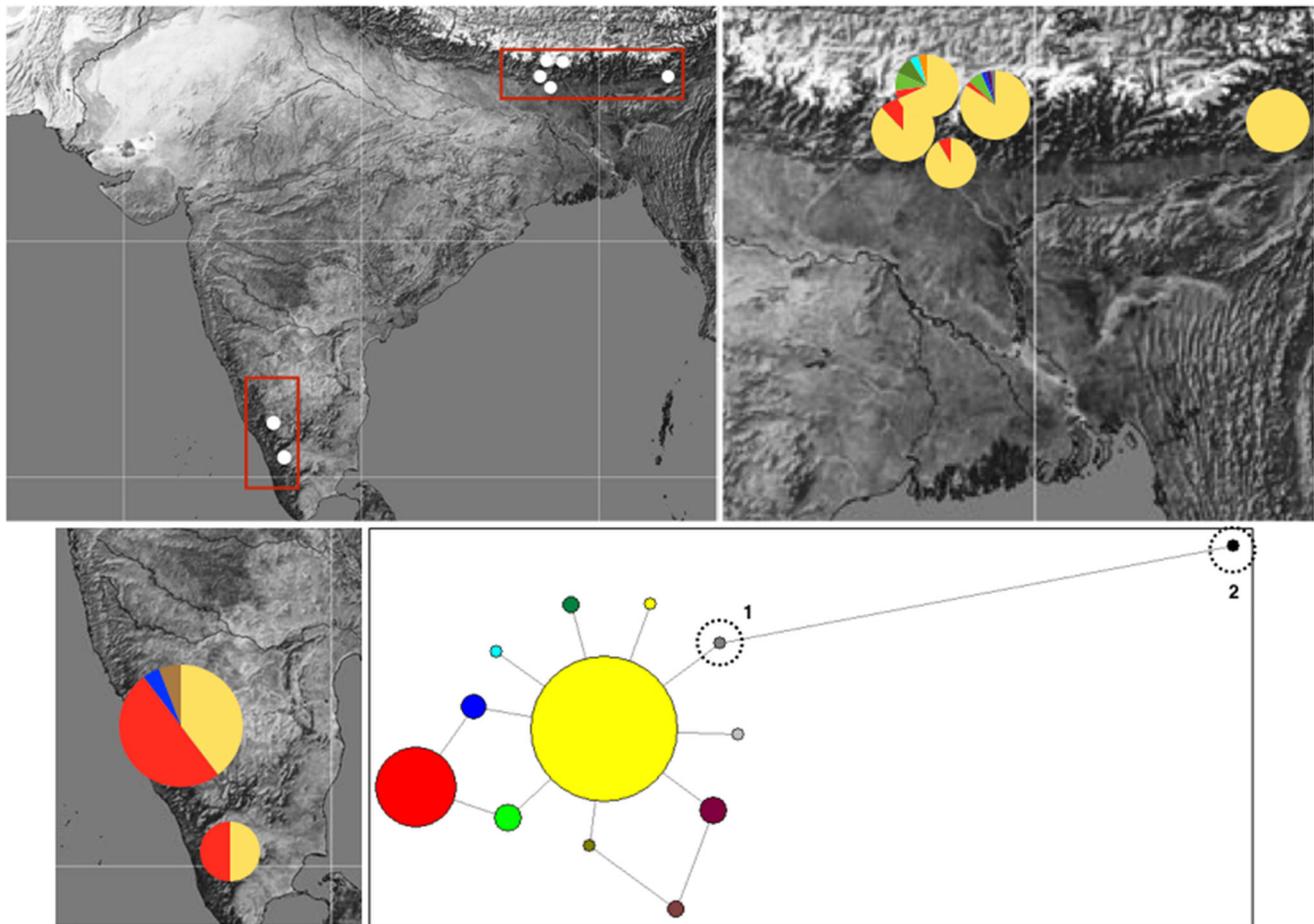


Figure 1. (i) A broader view of the geographic regions (Himalaya and WG) with six major areas magnified / localities of *R. arboreum* populations where sampling was carried out, and the frequency of nuclear DNA haplotypes (the size of the circle roughly proportional to the sample size); (ii) nuclear haplotype network showing all the haplotypes and two outgroups (encircled and labelled as 1 and 2) represented by *R. delavayi* and *Empetrum*, respectively.

performed (i) very old divergence (middle to late Miocene), (ii) old divergence (early to middle Quaternary), and (iii) recent divergence (middle Quaternary to the Holocene) (figure 1 in electronic supplementary material at <http://www.ias.ac.in/jgenet/>). We simulated 1×10^6 datasets for each of the above competing scenarios, selected the best possible one, and estimated the parameters under the best possible scenario only.

Paleo-ecological niche modelling

We employed ecological niche modelling (ENM) to predict the paleo-distribution of the *Rhododendron* during four periods: at the present time, mid Holocene (~ 6000 BP), the Last Glacial Maxima (LGM, $\sim 22,000$ BP) and at the last interglacial period (LIG, $\sim 120,000$ – $140,000$ BP). To understand whether the underlying driver of disjunction is common across species or in other words, whether Quaternary climatic oscillation is the predominant causal agent of discontinuity, we employed ENM for three other species (*Eurya nitida*, *Gaultheria fragmentissima* and *Symplocos laurina*) demonstrating similar disjunction in distribution.

The distribution information of these three species was obtained from the previously published studies (Bahulikar *et al.* 2004; Apte *et al.* 2006; Bano *et al.* 2014; Kuttapetty *et al.* 2015). For details on the determination of genetic clusters, the inference of demographic histories, and the paleo-ecological niche modelling, see methods in electronic supplementary material.

We have received required permission from Sikkim Forest Department (letter number 42/GOS/FEWMD-BD-R-2013/CF(WL)/03 dated 13/08/2013) for collecting specimens. However, in most cases specimens were collected from fringe areas and other anthropogenic landscapes (as in West Bengal, Arunachal Pradesh and Tamil Nadu) which were well outside the sanctuaries or national parks.

Results

Genetic diversity

Nuclear ITS revealed a total of 10 haplotypes with the central haplotype representing more than 68% of the

Table 1. Genetic diversity within populations of *R. arboreum* based on chloroplast and nuclear haplotypes, and nuclear microsatellite DNA data.

| Region/group | State | Population | Location | | Chloroplast N cpDNA (haplotypes) | ITS N nDNA (haplotypes) | Microsatellites | | | | | |
|---|--|----------------------------------|-----------------|------------------|--|--|-----------------|----------------|----------------|----------------|----------------|----------------|
| | | | Latitude (N) | Longitude (E) | | | N | N _p | A _R | A _E | H _o | H _T |
| Eastern and north-eastern India Himalaya (Himalaya) | Sikkim | Barsey Wildlife Sanctuary (BWS) | 27.1906° | 88.1219° | H1(9), H3(1), H5(1) | H1(22), H2(3) | 20 | 3 | 9.750 | 6.197 | 0.635 | 0.853 |
| | | Yumthang (Y) | 27.6686° | 88.7314° | H1(13), H2(2), H4(1) | H1(38), H2(1), H3(3), H4(1), H7(1), H8(1) | 15 | 0 | 9.000 | 5.848 | 0.769 | 0.860 |
| | | Kanchenjunga National Park (KNP) | 27.4246° | 88.1913° | H1(10), H2(1) | H1(15), H2(1), H3(2), H6(2), H9(1), H10(1) | 20 | 0 | 9.500 | 6.686 | 0.650 | 0.863 |
| WG | West Bengal Arunachal Pradesh Tamil Nadu | Singalila (S) | 26.9986° | 88.04° | H1(9) | H1(11), H2(1) | 10 | 0 | 6.37 | 5.127 | 0.738 | 0.867 |
| | | Tawang (T) | 27.5919° | 91.9125° | H1(12) | H1(15) | 12 | 1 | 8.000 | 5.229 | 0.646 | 0.822 |
| | | Ooty (O) | 11.4097° | 76.7241° | H1(30), H6(1), H7(1), H8(1) | H1(27), H2(34), H4(3), H5(4) | 38 | 6 | 11.87 | 5.135 | 0.647 | 0.782 |
| | Kerala | Munnar (M) | 10.0405° | 77.0223° | H1(3) | H1(1), H2(1) | 5 | 0 | 4.250 | 3.371 | 0.600 | 0.783 |

N, number of individuals sequenced/genotyped; *N_p*, number of private alleles; *A_R*, allelic richness; *A_E*, number of effective alleles; *H_o*, observed heterozygosity; *H_T*, total heterozygosity (gene diversity).

individuals (table 1; figure 1). The haplotype network has a star shape with a high-frequency central haplotype linked to low-frequency ones, which marks a signature of population expansion. It is also attached to other out-groups (*R. delavayi* and *Empetrum* species) implying the high-frequency central haplotype to the oldest haplotype amongst all. Himalaya harboured five private haplotypes of a total of nine; whereas WG possessed only one private haplotype in a total of four. Similarly, the combined sequence of chloroplasts yielded eight haplotypes with more than 90% individuals belonging to the commonest central haplotype resulting in a star network (figure 2 in electronic supplementary material). In ITS data, Himalaya appeared to be genetically more diverse than WG in terms of a suite of indices (*S*, *H_D*, θ_w), but chloroplast diversity places the Himalaya only little higher.

Eight microsatellites characterized by higher polymorphism yielded 122 alleles with an average of 15.2 alleles per locus. The two larger groups, i.e. Himalaya and WG possessed 9.7 and 10.2 alleles per locus, and four and six private alleles in total, respectively (tables 1, 2a). Other measures of diversity (*H_o*, *G_{ST}*, etc.) also differ minimally between the groups. It reflects that the microsatellite genetic diversity of Himalaya is only marginally different from WG unlike diversity at ITS region (table 2a).

Genetic structure: Hierarchical genetic structure analyses revealed the varying degree of differentiation with ITS and microsatellites. With ITS, only 56.62% and 43.52% of the variance is harboured within and among the populations, respectively. In contrast, most (90.31%) of the variance for microsatellites is within-population (table 2b).

To determine the optimum number of genetic clusters, a plateau length, $n \geq 6$ was never reached, even after running several iterations. But, at $k=2$, multiple short plateau lengths were obtained ($n=2$) and it implied a low statistical support for two not-so-distinct genetic clusters (table 1 in electronic supplementary material).

Inference of demographic histories

Among the three final models of demographic history, a recent divergence during late Quaternary outperformed others (table 3; table 2 in electronic supplementary material). The gradual disappearance of vegetation was perhaps initiated during early Pleistocene and that continued during last interglacial and LGM. It is the time-period when two populations, namely the Himalaya and the WG underwent a split 1590–2810 generation time before present (c.a. 15.9–42.15 Kya assuming generation time = 10–15 years) according to genetic data. The situation worsened in the Holocene due to acidification, which ensured further shrinkage of remnant patches; the impoverished condition continued in recent times.

Table 2. (a) Genetic diversity and genetic differentiation of 7 populations of *Rhododendron arboreum* at the species and group/region levels. (b) Results of the analysis of molecular variance (AMOVA) for nuclear ITS data and nuclear microsatellite data of seven *R. arboreum* populations belonging to two groups (regions).

| (a) | | | | | | | | | | |
|---|---|----------------|---------------------|------------|-------|-------------------------|-------|----------|------------|-----------|
| Region/group | Nuclear ITS | | | | | Microsatellite | | | | |
| | Haplotypes (<i>h</i>) | <i>s</i> | H_d | θ_w | N_p | A_R | H_o | G_{ST} | G''_{ST} | D_{est} |
| Eastern and north-eastern India Himalaya (Himalaya) | 9 (H1, H2, H3, H4, H6, H7, H8, H9, H10) | 7 | 0.273 | 0.0021 | 4 | 9.7 | 0.687 | 0.040 | 0.339 | 0.305 |
| Western Ghats (WG) | 4 (H1, H2, H4, H5) | 4 | 0.593 | 0.0007 | 6 | 10.2 | 0.623 | 0.049 | 0.428 | 0.369 |
| (b) | | | | | | | | | | |
| ITS | d.f. | Sum of squares | Variance components | | | Percentage of variation | | | | |
| Source of variation | | | | | | | | | | |
| Among groups | 1 | 21.937 | 0.24396 | | | 43.52 | | | | |
| Among populations | | | | | | | | | | |
| Within groups | 4 | 1.344 | 0.0009 | | | 0.16 | | | | |
| Within populations | 184 | 58.082 | 0.31567 | | | 56.3 | | | | |
| Total | 189 | 81.363 | 0.56053 | | | | | | | |
| $F_{ST} = 0.436, P = 0.0000$ | | | | | | | | | | |
| Microsatellites | | | | | | | | | | |
| Among groups | 1 | 31.435 | 0.15637 Va | | | 4.23 | | | | |
| Among populations | | | | | | | | | | |
| Within groups | 5 | 43.127 | 0.20217 Vb | | | 5.46 | | | | |
| Within populations | 225 | 752.012 | 3.34227 Vc | | | 90.31 | | | | |
| Total | 231 | 826.573 | 3.70081 | | | | | | | |
| Fixation indices | | | | | | | | | | |
| $F_{ST} = 0.096, P = 0.0000$ | | | | | | | | | | |

s, Number of segregating sites; θ_w , Watterson's parameter; H_d , gene diversity; N_p , number of private alleles; H_o , observed heterozygosity; G_{ST} , fixation index, G''_{ST} , corrected standardized fixation index; D_{est} , Jost differentiation index.

Table 3. Posterior values of the parameters.

| Model | N_{Him} | | N_{WG} | | t | |
|---|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| | Mean | Mode | Mean | Mode | Mean | Mode |
| Recent divergence (middle Quaternary to Holocene) | 3.17×10^4 | 1.77×10^4 | 2.19×10^4 | 1.09×10^4 | 2.81×10^3 | 1.59×10^3 |

N_{Him} , effective population size of Himalaya; N_{WG} , effective population size of WG; t , time since two populations have diverged (in terms of generation time).

Paleo-ecological niche modelling

The paleo distribution map developed using the ecological niche modelling of *Rhododendron* clearly portrayed a gradual reduction of its potential distribution area initiated during the LIG (figure 2). During temporal stretch spanning from the LIG to LGM, the potential area of distribution in southern India was extended up to central and northern WG. In northern India, the distribution was extended up to the central Indian plateau. However, from the mid Holocene onwards, the tree underwent range contraction, thus

restricting the distribution in the south Indian montane region, north, east and northeast Indian Himalaya but with a low probability in northern WG region. Nearly similar pattern of change was found in *Gaultheria* and *Symplocos*, where high probability regions were gradually contracted from the LIG onwards (figure 3 in electronic supplementary material). However, central Indian plateau still exhibited the areas with low to medium probability until the Holocene. On the contrary, the range expansion happened instead of contraction in *Eurya*. From LIG onwards, north Deccan plateau was marked with low to moderate probabilities, which in

course of time has extended towards the Gangetic plain. A closer look revealed a crucial role of temperature (both bioclim 6 and 7), which has maximum effect on model development in all studied species. Apart from this, other contributing variables also included precipitation seasonality and amount received at the wettest quarter.

Discussion

The genetic diversity of the two larger populations (across Himalayas and WG) was carried out using both the nuclear as well as chloroplast markers. The nuclear genome is inherited biparentally and reflects both seed and pollen gene flow. The chloroplast genome of angiosperms, however, is mainly transmitted through seeds and reveals maternal lineages. Microsatellite markers are classically used to

investigate population genetic structures while the chloroplast DNA (cpDNA) is useful for understanding intraspecific phylogeography in plants. Although the chloroplast genome is conservative in its evolution, intraspecific cpDNA variation has been reported in many plant species (Schaal *et al.* 1998). The chloroplast region allows us to infer historical recolonization routes as a consequence of its maternal inheritance (Okaura and Harada 2002).

In the current study, while ITS revealed that the Himalayan populations to be genetically more diverse than WG, chloroplast data revealed that the Himalaya are only marginally higher in diversity compared to the WG. Similarly, the genetic diversity analysis using the microsatellite markers also reflected a similar pattern that of Himalayas being marginally different from the WG (table 2a). The higher diversity patterns in both the chloroplast as well as microsatellite markers of the WG populations clearly

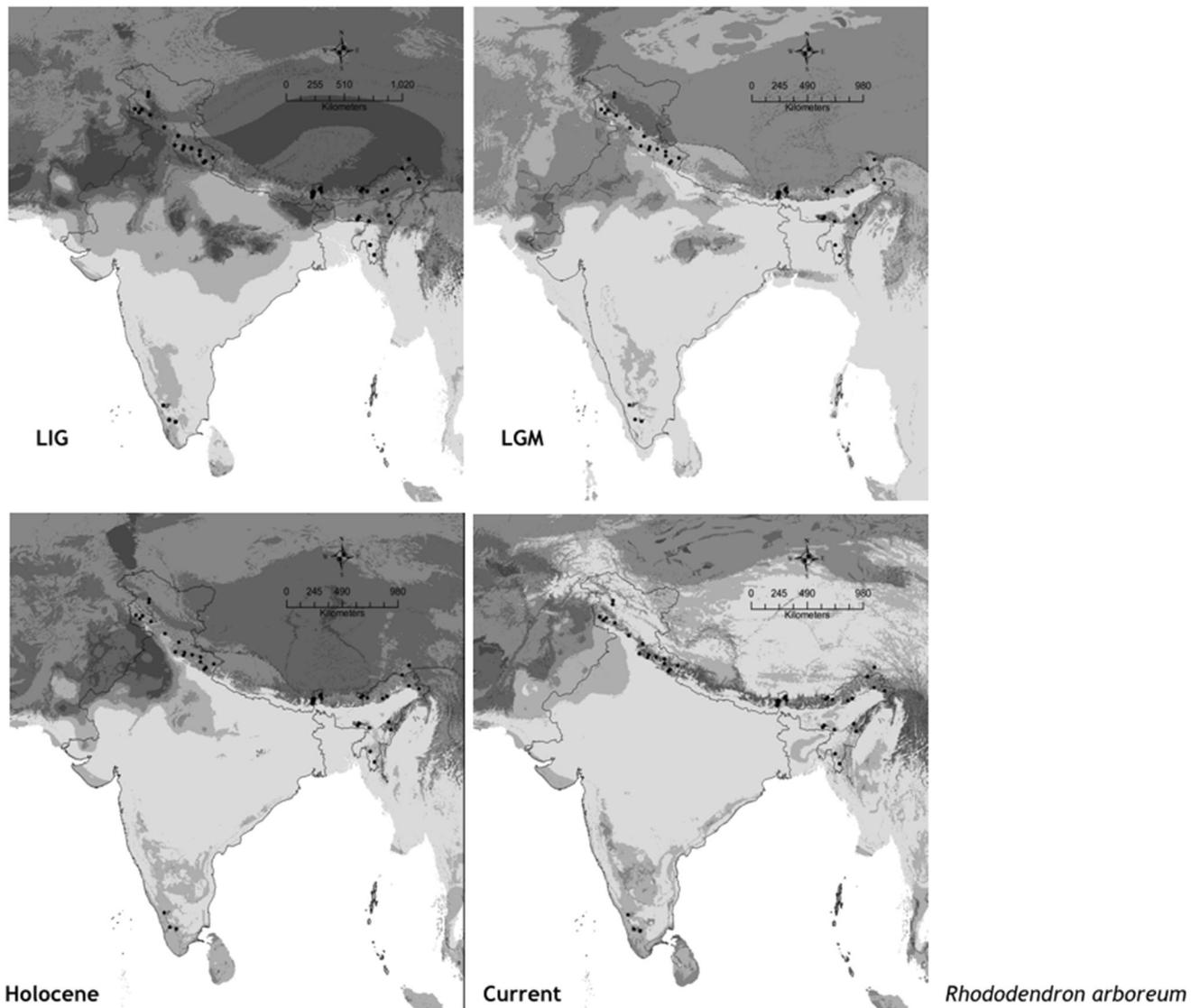


Figure 2. Potential distribution of *R. arboreum* in Indian subcontinent at different time periods: (i) LIG; (ii) LGM; (iii) the Holocene; and (iv) the current time.

indicate that the populations in the WG to be a relictual remnants of a once continuous distribution of *R. arboreum*. Thus, the other possibility of long-distance dispersal of *R. arboreum* may not hold water considering that the populations in WG were equally diverse. Long distance dispersal would result in very low levels of diversity due to genetic bottlenecks or founder events (Pannell and Charlesworth 2000; Jacquemyn *et al.* 2009).

Our findings using population history (paleo-ecological niche) models revealed a recent divergence during the late Quaternary that stood in agreement with the gradual shrinkage in paleo-ecological niche distribution of *Rhododendron*. This fact is also supported from similar paleo-ecological distribution change of other two species, i.e. *Gaultheria* and *Symplocos* spanning over the same-time frame. Therefore, a dramatic rearrangement of biota operative over last million years, in light of which the disjunction appeared to represent, a relictual distribution crafted by Quaternary climatic oscillations.

The riddle of central Indian disjunction has provoked many early researchers; the initial investigations to unravel the underlying agents has been done by Hora employing fishes of WG (Hora 1949); which later followed a diverse group of organisms, for e.g., birds (Ripley and Beehler 1990), mammals (Karanth 2000), amphibia (Bossuyt and Milinkovitch 2000) and plants (Apte *et al.* 2006; Bahulikar *et al.* 2004). Hora's arguments have invited severe criticism. For e.g., Menon (1980) pointed out the geological evidence, especially Garo-Rajmahal gap as a major obstacle, he also suggested reframing the problem in terms of the Pleistocene glaciation. Later, Daniels (2001) ruled out this possibility and instead attributed the origin of the puzzle to incorrect taxonomy.

The wrong taxonomic description is also cited in genus *Trachypithecus* that was termed as a case of false disjunct (Karanth 2003). In addition to wrong taxonomy, other confounding issues, i.e. multiple independent origins and local diversification generating evolutionary convergence across disparate geographic regions have been recorded in ranid frogs from Madagascar and Asia (Bossuyt and Milinkovitch 2000). On the other hand, disjunct distribution of the flying lizards (genus *Draco*) of Asia caused by range fragmentation supports relictual distribution hypothesis (Honda *et al.* 1999).

Summarizing, a diverse group of taxa seems to exhibit an absence of occurrence across central Indian region and the causation essentially relates to their distinctive phylogeographic history. Evolutionary trajectories of these taxa seem to be temporally isolated implying that they have undergone diversification during a different period of geological time but attained a convergence in disjunction pattern; and thus, we argue that the drivers of their spatial divergence may not necessarily be due to similar geologic or climatic events. In other words, we emphasize that Quaternary climate may be a common driver of intraspecific or population divergence across central Indian region, for e.g. as recorded in *R.*

arboreum (Jain *et al.* 2000; Kuttapetty *et al.* 2014), *Eurya nitida* (Bahulikar *et al.* 2004), *Gaultheria fragmentissima* (Apte *et al.* 2006) and *Symplocos laurina* (Banu *et al.* 2010). Our interpretation of the genetic data also supports the fact that the timing and the underlying drivers of divergence could relate to Quaternary climate.

While the indices of genetic diversity and structure have offered information on the current pattern (i.e., the medium to high differentiation, two genetic clusters and high diversity), they remain silent on the underlying historical drivers. However, population history reconstruction employing genetic polymorphism has proved to be an effective tool. Although a large number of taxa show such patterns, robust phylogeographic studies are scant in this direction. Moreover, earlier studies mostly relied on genetic diversity analyses from WG and NE Himalaya, which provided no further insight into historical events of causation (Jain *et al.* 2000). To resolve this, along with elucidating population history, we have recreated paleo-ecological niche of multiple unrelated taxa to test whether climatic variability during the ice age was a supreme driver to cause interpopulation divergence; which broadly appeared to be the case. Paleo-ecological niche of the selected taxa revealed an almost similar contraction in distribution initiating from interglacial maxima with a drastic change in post-LGM and in the mid Holocene; except *Eurya* that demonstrated a range expansion LGM onwards. The lower altitudinal requirement could explain this expansion of *Eurya* around central Indian plateau where the temperature remains ambient throughout the year. However, the gradual range contraction is most pronounced in *Rhododendron*. Inferences from genetic data reflect the notion obtained from paleo-ecological climatic niche; the timing of population split of *Rhododendron* appears to be very recent, c.a 15.9–42.15 kyr which correlates well with the time period of climatic oscillation instigating rapid biotic reshuffle.

Although the WG and the Himalaya are seemingly at moderate to high differentiation and represent two genetic clusters, the genetic diversity of Himalayan population is marginally higher than WG; the fact weakens the chance of the former acting as the founder to the latter, in other words, dispersal hypothesis does not seem to be supported by genetics. Altogether, it strengthens the crucial role of Quaternary climate in the creation of this enigmatic discontinuous distribution. While information from past vegetation and climatic reconstruction could have been very illuminating, currently it is constrained due to scanty records.

Drawing on evidence from the effect of late Miocene, it could be stated that a prevailing warmer and wetter condition spurred tropical forest growth in the Indian subcontinent (Pound *et al.* 2012). It gradually followed a replacement of the tropical forests by deciduous types grossly over the Indian peninsula due to the onset of a drier climate owing to the uplifting of the Himalayas and the WG (Meher-Homji 1983). Post-Miocene, climatic conditions deteriorated considerably with an abundance of various vegetation types

over millions of years from early Quaternary (Sukumar *et al.* 1993; Rajagopalan *et al.* 1997) and a culmination with recent Holocene aridification (Ponton *et al.* 2012). Likewise, the notions from past climatic niche have also hinted at a key role of temperature-related variables; together, it grossly lends support to the reorganization of past climatic niche and population split owing to such climatic change.

On the other end, interspecific divergence and speciation of biota portraying this biogeographic pattern depict relatively ancient biotic change. These congeneric members perhaps underwent diversification much earlier, responding to the climatic, ecological and geological milieu. The only molecular evidence stems from caecilian amphibian *Gege-neophis* where the divergence between Eastern and WG lineages was too ancient in origin prior to Miocene (Gower *et al.* 2016). This appears to be rather in agreement with the recent research on the effect of Quaternary on speciation (Barnosky 2005); which suggests that the effect of climatic fluctuations on biota is mostly at the population level and undermines its role in the formation of species.

Conclusion

Our results based on both CpDNA and microsatellite markers, as well as paleo-ecological niche models clearly indicate that the populations in WG are relictual remnants of a once continuous distribution. The central Indian disjunction is a quite common pattern displayed by a large number of taxa. Our interpretation using molecular tools and niche model elucidated that the disjunction exhibited by *R. arboreum* was created during Quaternary period when the climatic perturbation caused a radical change in the vegetation. The fact is echoed from the paleo-ecological niche of other taxa that underwent similar change following the last interglacial.

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