Chloroplast microsatellites reveal genetic diversity and population structure in natural populations of Himalayan Cedar (*Cedrus deodara* (Roxb.) G. Don) in India

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Abstract

Himalayan cedar (*Cedrus deodara*) is one of the most important temperate timber species of Western Himalayas and is considered to be among the endangered conifer species in the region. Knowledge of genetic diversity and population structure will help guide gene conservation strategies for this species. Ten polymorphic chloroplast microsatellites (cpSSR) were used to study genetic diversity and population structure in twenty one natural populations of *C. deodara* throughout its entire distribution range in Western Himalayas. When alleles at each of the 10 loci were jointly analysed, 254 different haplotypes were identified among 1050 individuals. The cpSSRs indicate that *C. deodara* forests maintain a moderately high level of genetic diversity (mean $h = 0.79$). AMOVA analysis showed that most of the diversity in *C. deodara* occurs within populations. Bayesian analysis for population structure (BAPS) revealed spatial structuring of the variation (22 % of the total variation) and sub-structuring captured nineteen genetic clusters in the entire divisions of the populations. Most of the populations were clustered independently with minor admixtures. The distribution of genetic diversity and sub-structuring of *C. deodara* may be due to restricted gene flow due to geographic isolation, genetic drift, and natural selection. These findings indicated existence of genetically distinct and different high diversity and low diversity clusters, which are potential groups of populations that require attention for their conservation and management. The results are interpreted in context of future conservation plans for *C. deodara*.

Keywords: : *Cedrus deodara*, chloroplast microsatellites, genetic diversity, haplotype

Introduction

The genus *Cedrus* is believed to have been widely but discontinuously distributed in the Old World during pre quaternary times, according to Pons (1998). Presently, the natural geographic distribution of this genus is limited to Morocco and Algeria for *Cedrus atlantica* (Atlas cedar); to Lebanon, Syria, and Turkey for *Cedrus libani* (Lebanon cedar); to Cyprus for *Cedrus brevifolia* (Cyprus cedar); and to the Himalayan Mountains for *Cedrus deodara* (Himalayan cedar; Debazac, 1964; Vidakovic 1991) (Dagher-Kharat et al., 2006). Of these four species, *Cedrus deodara* commonly referred to as deodar, is one of the most important and endangered temperate timber species of Western Himalayas. The species is found growing right from Afghanistan to Garhwal up to the Valley of Dhaul River below Niti pass at an elevation ranging from 1200 m to 3050 mamsl (Tewari, 1994). The altitudinal range of deodar is usually higher on the southern than on the northern slopes and the trees grows best and reaches its largest dimensions under cool site conditions on northern aspect. It covers an area of about 0.2 million hectares in India mainly in the states of Jammu and Kashmir, Himachal Pradesh and Uttarakhand.

C. deodara is a large conifer tree of conical shape with spreading horizontal branches. The species is in great demand as building material because of its durability, rot-resistant

characters, fragrance and fine close grain which is capable of taking a high polish. Due to its multifarious uses it had been subjected to exploitation for centuries. During the British colonial period in India, Deodar wood was extensively used for the construction of barracks, public buildings, bridges, canals and railway cars. Presently natural deodar forests are under great pressure not only due to human impact but also due to the changing climatic conditions. Steady increase in human population, over-exploitation of natural resources, extensive clearing of forests for developmental activity, widespread logging/lopping and grazing have been responsible for the loss of deodar forests in the Himalayan region. Conservation of this valuable species is immediately required for the sustenance of fragile Himalayan ecosystems.

Long term survival of a species depends on the sustenance of adequate genetic variability within and among populations to accommodate new selection pressure brought about by environmental changes. Genetic diversity is fundamental component of biodiversity and is as critical to sustainability of our natural resources as are species diversity and ecosystem.

Within family Pinaceae, opposite uni-parental inheritance of mitochondrial and chloroplast genomes provides opportunities to assess discontinuity within an effectively haploid genome. The alleles of loci in an organelle genome can be collectively considered as a haplotype because they are not recombining (Terrab et al., 2006). Paternal inheritance of chloroplast genomes in most conifers (Neale and Sederoff, 1989) makes chloroplast microsatellites particularly effective markers for studying mating systems, uni-parental lineages, and gene flow via pollen. Predominant paternal inheritance of chloroplast DNA has been demonstrated in European *Abies* with previously characterized *Pinus thunbergii* primers (Vendramin et al., 1996) at two highly variable microsatellite loci (Vendramin and Ziegenhagen, 1997; Ziegenhagen et al., 1998; Vendramin et al., 1999). Intraspecific diversity and population structure in heterologous amplification with these primers have been demonstrated in many other pine species as well (Cato and Richardson, 1996; Morgante et al., 1998).

Knowledge of the variation and distribution of genetic diversity can guide efficient gene conservation sampling designs (Richards et al., 2007). Genetic information is lacking for many species that provide important ecosystem services yet have not had immediate economic value (Schoettle et al., 2012). In spite of the biological and economic importance of *C. deodara* and the existence of severe threat perception on the species, no information is known about its genetic diversity distribution and population structure. In this paper, we have used chloroplast microsatellites to study 1) the levels of haplotypic diversity existing in populations and (2) the population genetic structure of the existing populations of *C. deodara* along the whole distributional area in Himalayas (India). Ten cpSSR loci from 1050 trees from 21 sites in the core distribution range of the species across Himalayan Mountains covering three states viz. Jammu and Kashmir, Himachal Pradesh and Uttarakhand were analyzed. The information will be useful to guide gene conservation strategies for the species

Materials and Methods

Plant Material

A total of twenty-one large contiguous deodar populations from three Himalayan states of India were selected for the study that included nine forests from Uttarakhand, ten from Himachal Pradesh and two from Jammu and Kashmir (Table 2, Fig. 1). In order to capture as much as genetic variation as possible within the population, 50 trees were sampled from each population. Selection of the trees was done randomly. The trees considered for collections were well spaced and separated from each other with at least 100 m apart. The geographical location viz. latitude, longitude and altitude of the sampled trees/populations were recorded. Needles from each of the 50 randomly selected standing adult trees per population were collected and were used for DNA isolation and genotyping.

Fig.1
Map showing location of 21 *C. deodara* populations sampled for genetic diversity analysis in three states in Himalayan mountain range

DNA Extraction and PCR amplification

Genomic DNA was extracted following protocols by Doyle and Doyle (1990) with some modifications by the addition of 5mM Ascorbic acid, 2 % PVP and 0.3 % 2-mercaptoethanol. The washed and dried needles were ground in liquid nitrogen and incubated at 60°C for 40 minutes in the modified extraction buffer. Chloroform-isoamyl alcohol (24:1) was added and the g-DNA was precipitated by addition of chilled isopropanol. The g-DNA pellet obtained was washed with 76 % alcohol and 10 mM ammonium acetate. The pellet was rewashed in 70 % ethanol centrifuged at 13,000 rpm for 15 minutes, vacuum dried

and re-suspended in 100µl of autoclaved 10mM Tris-EDTA buffer (pH 8.0). The extracted DNA was quantified and diluted to 15ng/µl for further use in amplification reactions.

A total of 80 microsatellite primers from *P. thunbergii*, *P. sylvestris*, *P. merkussi*, *P. densiflora*, *P. resinosa* and *P. taeda* were tested by Ginwal et al. (2011) on *Cedrus deodara* for cross-genus amplification. The primers showing positive amplification were then screened for polymorphism. Of the 80 primers tested on *C. deodara*, ten chloroplast microsatellite primer pairs showing consistent polymorphism were selected for studying the population genetic structure of twenty one populations of *C. deodara* (Table 1). PCR amplifications were performed by the method of Ginwal et al. (2011), and the PCR-amplified fragments were electrophoresed along with GeneScan ROX 400 internal size standard on an ABI prism 3100 Genetic Analyser (Applied Biosystems). Allele sizes were assigned against the internal size standard and individuals were genotyped using GeneScan analysis software and Genotyper software version 3.7 (Applied Biosystems).

Statistical analyses

Here we have used the terms “locus” to refer to a cpSSR site, and “allele” to refer to a size-variant at a given cpSSR site. As chloroplast has haploid genomes that are paternally inherited (in most gymnosperms) (Neale and Sederoff, 1989; Watano et al., 1996), hence for the diversity measure, the chloroplast genome was regarded as a single nonrecombining locus and repeat length differences between individuals were scored as haplotypic variants over all cpSSR sites (Parducci et al., 2001). Allele size information at the ten cpSSR loci analysed was used to construct haplotypes. The population genetic parameters viz. effective number of haplotypes (n), and Nei's unbiased haplotypic diversity (h) were computed as $n_e = 1 / (\sum p_i^2)$, where p is the frequency of the i -th haplotype in a population, and by estimating the unbiased genetic diversity (h), which accounts for small population sizes was computed as $h = [n / (n-1)] * (1 - \sum p_i^2)$, where n is the number of individuals analyzed in a population and p_i is the frequency of i -th haplotype in a population (Nei, 1987). Unique number of haplotypes (P_H) was calculated using Haplotype Analysis software version 1.05 (Eliades and Eliades, 2009). Gene flow (N_m) which is a measure of effective number of migrants per population per generation, was estimated indirectly from the G_{ST} values at each locus and from the average values over all the loci by applying McDermott and McDonald's (1993) formula $N_m = 0.5(1-G_{ST})/G_{ST}$ for the studied populations using POPGENE 1.32 (Yeh et al., 1999).

An unbiased estimate of Wright's fixation index F_{ST} (an indicator of the degree of differentiation among populations) was calculated as a measure of genetic differentiation in FSTAT 2.9.3 (Goudet, 2002). The standard error of F_{ST} was calculated using unbiased jackknife analysis. Analysis of molecular variance was carried out with ARLEQUIN 3.0 software (Excoffier et al., 2005). A hierarchical analysis of variance was used to partition the total variance into covariance components consisting of percentage variation among populations and within populations.

The Bayesian model based approach implemented in BAPS software version 6 (Corander et al., 2013) was used to estimate the hidden population substructure by clustering sample populations into panmictic groups (Corander et al., 2003). We carried out 10 repetitions of the algorithm for each K ranging between 5 and 21.

Results

The selected ten cpSSR primers consistently amplified all the twenty one populations of *C. deodara* under standard conditions and were polymorphic in all the populations investigated. The detail of these 10 primers has been presented in Table 1.

Table 1

The detail of chloroplast microsatellite primers that were used for the study.

Primer	Sequence (5'-3')	No. of repeat units	bp	T _m	Product size	Genetic characteristics of primers	
						n_a	n_e
Pt15169	CTTGATGGAATAGCAGCC	(C) ₈ (T) ₈ A(T) ₈	19	58.3	118	5.0	3.08
	GGAAGGGCATTAAAGTCATTA	(C) ₈ (T) ₁₄	21				
Pt26081	CCCGTATCCAGATATACTTCCA	(T) ₁₄	22	58	112	5.0	3.16
	TGGTTTGATTTCATTCGTCAT	(T) ₁₁	21				
Pt30204	TCATAGCGGAAGATCCTCTTT	(A) ₁₂ (G) ₁₀	21	58	145	4.0	3.13
	CGGATTGATCCTAACCATACC	(A) ₈ (G) ₈	21				
Pt36480	TTTTGCTTACAAAATAAAAGAGG	(T) ₁₁	24	58.1	147	5.0	3.07
	AAATTCCTAAAGAAGGAAGAGCA	(T) ₉	23				
Pt71936	TTTATTGGAAATACACTAGCCC	(T) ₁₁	22	58.1	148	5.0	3.36
	AAAACCGTACATGAGATTCCC	(T) ₁₄	21				
PCP9434	AAACTGACGTAGATGCCATGG	(A) ₁₀	21	58.5	131	5.0	2.59
	GCGGTATGAGGGAAGAAGC		19				
PCP26106	AATCCGACAAAAAAGATTGGG	(A) ₁₄	21	58.5	149	5.0	3.56
	GCTCCATTTCACGTGGTTG		19				
PCP36567	AAAAGAGGAGGAAAAACACCTT	(T) ₁₁	22	58.5	115	4.0	3.02
	AAGAGCAGACAAGTAAGGGGC		21				
PCP45071	ACTGGTCTGATCGACCCAAT	(T) ₁₅	20	58.5	149	5.0	3.47
	TTCTACACTTGCAGAAACCC		20				
PCP100842	TCAATACAAATGATGGGAGTCG	(A) ₁₂	22	58.5	146	4.0	2.85
	TTTTGCCATATCTGAAACTCC		22				
Mean						4.7	3.13

n_a = observed number of alleles; n_e = effective number of alleles;

The most variable cpSSR loci across 21 studies populations were Pt15169, Pt26081, Pt36480, Pt71936, PCP9434, PCP26106 and PCP45071 with 5 alleles each (Table 1). The number of alleles per polymorphic locus ranged from 4-5 for all the 10 primer pairs with a mean of 4.7. The effective number of alleles ranged from 2.59 to 3.56 with a mean of 3.13. Gene flow N_m calculated was 1.951, indicating moderate levels of gene flow.

Haplotype distribution

When alleles at each of the 10 loci were jointly analyzed, 254 different haplotypes were identified among 1050 individuals. Table 2 shows the genetic characteristic of the chloroplast haplotypes based on the 10 cpSSR loci in 21 *C. deodara* populations. The lowest value of haplotypic diversity ($h = 0.71$) was observed in population 17 from Churah Forest Division (HP) and the highest value of haplotypic diversity ($h = 0.89$) was

observed in population 21 from Batote (JK). Estimates of the effective number of haplotypes and haplotypic diversity averaged across all the populations were 4.99 and 0.79 respectively. In intra population analysis, an average of twelve private haplotypes was detected. The population 1 from Theog Forest Division (H.P.) contained the highest number (23) of private haplotypes, while population 10 from Chopal Forest Division (H.P.) had the lowest (2) (Table 2). Inter population analysis revealed high levels of total genetic diversity (H_T) and consequently, high levels within each populations (H_S). However, diversity due to genetic differentiation (D_{ST}) was comparatively low in all the populations (Figure 2).

Table 2

Haplotypic distribution of twenty one populations of *C. deodara*

Code	Population detail	Geographical coordinates	Alt (m)	Effective number of Haplotypes (ne)	Haplotypic diversity (h)	Number of private haplotypes (P _u)
	Forest area	Location	Latitude (°N) Longitude (°E)	amsl		
1	Theog Forest Division (HP)	Vintage point bifurcation of road rainfall	31° 03'45"N 77° 19'62"E	2212-2478	6.596	0.849
2	Chopal Forest Division (HP)	Sairan	30° 53'99"N 77° 31'56"E	2162-2415	6.545	0.848
3	Chakrata Forest Division (UK)	Kathyan Beat and Motta range	30° 53'07"N 77° 56' 47"E	2074-2294	4.513	0.779
4	Jageswar Forest Division (UK)	Purvipanwanula, Jageswar 2A, 2B and 3	29° 38'29"N 79° 51' 25"E	1783-1992	4.417	0.774
5	Champawat Forest Division (UK)	Malaharaswar, Ghatudedarwani	29° 20'28"N 80° 07' 46"E	1555-1667	4.153	0.760
6	Lohaghat Forest Division (UK)	Lohaghat reserve and chidiadhunga	29° 21'28"N 80° 06' 46"E	1533-1680	4.132	0.759
7	Anni Forest Division (HP)	Luhri and Chowai	31° 27'48"N 77° 27' 20"E	1938-2120	5.198	0.808
8	Karsog Forest Division (HP)	Seri	31° 20' 31"N 77° 18' 29"E	1909-2391	4.808	0.793
9	Rampur Forest Division (HP)	Bhurja, Punan	31° 18' 52"N 77° 35' 09"E	2267-2520	4.266	0.766
10	Chopal Forest Division (HP)	Chopal	31° 56' 96"N 77° 34' 92"E	2313-2503	5.040	0.802
11	Barkot Forest Division (UK)	Beyali	33° 05' 27"N 75° 19' 47"E	1847-2017	3.597	0.723
12	Uttarkashi Forest Division (UK)	Harshil	30° 53' 07"N 77° 56' 47"E	2414-2685	3.698	0.730
13	Uttarkashi Forest Division (UK)	Gangotri	30° 59' 07"N 78° 53' 54"E	2887-3087	4.902	0.797
14	Kullu Forest Division (HP)	Naggajhir and Phetavan	32° 06' 24"N 77° 09' 74"E	1618-1942	5.459	0.818
15	Wild life Shimla Forest Division (HP)	Binoo (C2 and C8)	30° 58' 89"N 77° 12' 17"E	1952-2089	5.507	0.819
16	Wildlife Chamba Forest Division (HP)	Kalatoop	32° 33' 04"N 76° 01' 08"E	2181-2461	3.731	0.733
17	Chaurah Forest Division (HP)	Khani Reserve forest	32° 47' 42"N 75° 55' 78"E	1785-2090	3.492	0.714
18	Tons Forest Division (UK)	Gokul beat	31° 04' 19"N 77° 54' 47"E	2291-2462	5.230	0.810
19	Tons Forest Division (UK)	Sankri (Purti block and Saurnd)	31° 04' 17"N 78° 12' 32"E	1963-2092	5.353	0.814
20	Patnitop (JK)	Udhampur	33° 04' 24"N 75° 19' 46"E	1942-2076	5.020	0.802
21	Batote (JK)	Batote	33° 05' 34"N 75° 19' 50"E	1989-2017	9.259	0.893
	Mean			4.996	0.790	12.095

HP-Himachal Pradesh, UK-Uttarakhand, JK-Jammu and Kashmir

AMOVA analysis

The twenty one populations of *C. deodara* showed significant levels of genetic differentiation quantified using ARLEQUIN. The hierarchical level of molecular variance among populations was confirmed by analysis of molecular variance (AMOVA). The total molecular variance was portioned into two levels i.e. among populations and within populations (Table 3). AMOVA analysis revealed that 77.29 % of the variation was present among individuals within populations, 22.71 % was due to differences among populations. Variance estimates were based

Fig.2

Inter population genetic parameters

Fig. 3

Inferred clusters of individual genotypes with highest posterior probabilities of likelihood values obtained at K=19. The colours represent the most likely ancestry of the cluster from which the genotype or partial genotype was drawn. The populations (P1 to P21) are separated by black vertical lines.

Fig. 4

Log-marginal likelihood distribution of K genetic clusters using BAPS for all populations of *Cedrus deodara*.

on 1000 permutations. Accordingly, the differences between individuals variation within the populations was statistically significant ($P < 0.001$). The populations revealed relatively high genetic differentiation ($F_{ST} = 0.22$) and inter-population diversity (Table 3).

Population Structure analysis

BAPS analysis identified nineteen different clusters as the best partition of the whole sample (all the 10 best marginal log-likelihood values were for K=19, with the most optimal value of -21992.1578) (Figure 4). The distribution of the populations into different clusters identified by the BAPS analysis has been presented in Table 5. The 21 populations of *C. deodara* were

Table 3
Hierarchical analysis of molecular variance (AMOVA) partitioning variance into among population and within populations

Source of variation	df	SS	MSS	Variance components	Percentage variation
Among population	20	877.066	101.289	0.858	22.709 $F_{SC} = 0.17$
Within populations	1029	3004.860	2.920	2.920	77.290 $F_{ST} = 0.22$
Total	1049	3881.927	104.209	3.778	

df = Degree of freedom, SS = sum of squares, MSS = mean sum of squares

Table 4
Distribution of the populations into the nineteen cpSSR clusters identified by the BAPS analysis.

Population Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-
2	-	-	-	-	-	-	49	-	-	-	-	-	-	-	1	-	-	-	-
3	-	-	-	-	-	-	-	-	50	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	49	-	-	-	1	-	-	-	-	-
5	-	-	-	-	-	-	-	1	-	-	-	-	-	48	1	-	-	-	-
6	-	-	1	-	-	-	-	-	-	-	1	-	47	1	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	49	1	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-	-	-	-	-
10	-	-	4	-	-	-	-	-	-	-	-	-	-	45	1	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50	-
12	-	-	-	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	45	-	-	-	1	-	-	1	-	-	-	-	-	2	-	1	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	48	-	2	-	-	-	-	-
17	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	3	47	-	-	-	-
20	-	-	-	-	-	-	48	-	-	-	-	2	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	48	-	-	-	-	-	1	1	-	-	-	-
Over all	45	50	55	50	51	50	49	48	49	50	49	49	52	50	150	51	51	50	50

divided into 19 clusters for both the individual and admixture models (Figure 3). For K=19, the clusters formed were biologically most consistent with populations 5 and 6 falling under the same cluster 15 both belonging to Uttarakhand, similarly clusters 8 and 9 are characterized by the populations of Jammu and Kashmir (J.K.). The results showed that most of the populations were clustered independently with higher levels of admixtures occurring in populations belonging to Uttarakhand.

Discussion

Our results clearly showed that *C. deodara* maintains a high level of genetic diversity in Western Himalayas. This is in accordance with the general observation in most coniferous species (Vendramin et al., 1998; Clark et al., 2000; Parducci et al., 2001; Ribeiro et al., 2001; Gomez et al., 2003; Hansen et al., 2005). The high genetic diversity in *C. deodara* in the present study is in accordance with the very high genetic diversity of 0.95 reported earlier in *Cedrus atlantica* with the help of

chloroplast microsatellite markers. The highest level of haplotypic diversity was concentrated in population 21 from Batote, Jammu and Kashmir, which should therefore deserve particular attention in genetic conservation programs for Himalayan cedar. Apart from maintaining higher genetic diversity, the populations of *C. deodara* are also significantly structured. As expected, owing to the greater degree of isolation, population divergence or isolation is higher for *C. deodara* ($F_{ST} = 0.22$), which was higher than that reported for *C. atlantica* ($F_{ST} = 0.107$) (Terrab et al., 2006) and *P. radiata* (Wu et al., 1998), but was lower in comparison to Himalayan Chir pine (*P. roxburghii*) ($F_{ST} = 0.32$, Chauhan 2011).

The main reason for the high genetic diversity observed in *C. deodara* may be associated with its long life cycle outcrossing mating system, wind pollination behaviour and high regeneration potential (Mehta et al., 2015; Tiwari et al., 2018). Another factor that has an important bearing is its large geographic distribution range, which had major differences in geography, climate and habitat conditions. Himalayan ridge top ecosystems are considered to be more sensitive to climate change as they are characterized by uniform sunlight exposure, but variation in altitude give birth to climatic differences and subsequent vegetation changes (Tiwari et al., 2018). Most of the variation in *C. deodara* lies within the populations as the species is a temperate cross pollinated with wind and gravity-dispersed seeds, this finding may fit with the general observation that woody, perennial and outbreeding species maintain most of their variation within populations. These results are compatible with those involving other woody perennial, out breeding plant species, especially conifers (Hamrick et al., 1992). In a review, Nybom and Bartish (2000) assembled AMOVA derived F_{ST} values for about 100 plant species and confirmed the tendency of gymnosperms to have lower values of population differentiation than angiosperms. The F_{ST} value for *C. deodara* in this study is comparatively lower than other conifer species with discontinuous distributions and large populations. For instance, *P. cembra* has an F_{ST} value of 0.32 (Szmidt, 1982), Scottish *P. sylvestris* populations recorded F_{ST} value of 0.37 (Sinclair et al., 1997), and *P. torreyana* has a value of 1.0 with different alleles fixed in each one of its two populations (Ledig and Conkle, 1983). Several studies have shown that differentiation generally increases with distance (Dumolin-Lapègue et al., 1997; Grivet & Petit, 2002a; Palmé and Vendramin 2002; Heuertzet et al., 2004).

The Bayesian analysis methodology takes the number of populations as well as the allele frequencies in each population as random variables thereby detecting the hidden population substructure by clustering sampled populations into a panmictic group (Corander et al., 2004; Elaidés et al., 2011). The Bayesian analysis identified 19 independent ancestries and some of them appeared within populations as well. Furthermore, the clusters showed very limited and in some populations no admixture indicating that they were highly differentiated probably due to weak pollen-mediated gene flow between populations. Since the populations collected were geographically far apart it is possible that the populations have not admixed in recent history. Gene flow is the factor that is usually

supported by effective cross-pollination, seed and pollen dispersal mechanism (Lee et al., 2000) and presence of strong supporters for gene exchange. The levels of gene flow in out crossing, wind pollinated tree species such as *C. deodara* are expected to be high, but the value ($N_m = 1.95$) is considerably lower than reported for conifer species, such as *Larix lyallii* ($N_m = 3.5$; Khasa et al., 2006), *P. taeda* ($N_m = 1.87-6.71$; Al-Rababah and Williams, 2002) and *P. strobus* ($N_m = 3.10$, Mehes et al., 2009). Gene flow and genetic differentiation are a vice-versa phenomenon and are co-dependent, if one component increases the other decreases. It appears there are limited restrictions in the gene flow in the natural range of distribution of this species resulting in heterogeneous genetic structures. Restricted gene flow among populations may limit migration of genes or genotypes to new suitable habitats as the climate continues to change (Schoettle et al., 2012). Furthermore, if climate suitable for *C. deodara* no longer exists, genetic diversity of the species will be deteriorated by the loss of the more genetically diverse high-elevation populations.

The genetic diversity mainly came from individuals in different populations. Results suggest a lack of genetic connectivity across the distribution range regardless of site type. Geographical isolation is the main reason for the differences among the populations. The Himalayan vegetation landscape particularly the north-western and central Himalayan region have highly fragmented forest patches as compared to the eastern Himalayan region (Reddy et al., 2013). The conversion of vegetation cover to non-forest area by human activity further increased forest fragmentation (Sharma and Roy 2007). Expansion in agriculture and the built-up area are the major drivers of forest cover change and fragmentation resulting in forest degradation with implications for biodiversity, habitat ecosystem services, and people's livelihoods (Batar et al., 2017). One of the primary consequences of habitat fragmentation is the loss of connection among fragments with similar characteristics. Many species are therefore disconnected and this may lead to genetic isolation, minimizing the possibility of exchanging alleles, which means a low gene flow (N_m) among populations. This creates local population structure and tends to increase genetic divergence among fragments (Konzen, 2014).

The investigation revealed that the deodar forests of Jammu and Kashmir and majority of the deodar forests of Himachal Pradesh are rich in haplotypic diversity (values > 8) particularly the populations 1, 2, and 21. On the other hand, the forests originating from Uttarakhand state possess a lower level of diversity, particularly in two populations viz. 11 and 17. The total area of deodar forests in India is estimated around 203,263 ha (Himachal Pradesh 69,872 ha, Jammu and Kashmir 113,000 ha and Uttarakhand 20,391 ha) (Tewari, 1994). Looking at its range of distribution, it is naturally found in the north-western Himalayas from Kashmir to Garhwal (part of Uttarakhand) covering the states of Jammu and Kashmir, Himachal Pradesh and north western part of Uttarakhand. In Uttarakhand, only the Tons forest division (populations 18 and 19) of Tehri Garhwal Himalayas forms the eastern natural limit in the sampling range, hence it is obvious that these regions

would have higher haplotypic diversity as is evident. Beside this, *C. deodara* has become naturalized beyond this limit in Uttarakhand in many patches at Lohaghat (population 6), Champawat (population 5), Jageshwar (population 4) etc. These populations fall in one genetic cluster and encompass high-admixed ancestry of diverse genotypes as compared to other populations. This is possibly due to human mediated movement of genotypes in these populations and seems more like plantation rather than natural population. Due to geographical distances these forests were unable to receive the gene movement from other high diversity forests.

Conservation implications

Conservation priority should be given to populations with 1) high level of genetic diversity and 2) possessing unique haplotypes. A distinct high diversity group evident here is represented by eight populations (1, 2, 7, 10, 18, 19, 20, 21) wherein the haplotypic diversity levels are > 0.80. Second, some of the populations (1, 8, 9, 12, 17, 18) possess highest private or rare haplotypes. Such populations are more likely to be in need of genetic rescue and/or restoration. If these populations become extinct, the diversity of *C. deodara* would be diminished /deteriorated. Combining these two categories, there will be eleven populations viz. 1, 2, 7, 8, 9, 10, 12, 17, 18, 19 and 21 that would require special attention for their conservation. Augmented gene flow from these genetically diverse and distinct populations needs to be considered as a way of increasing fitness and the adaptive potential of populations. Among these populations, some appear to have higher priority than others based on both the uniqueness and diversity level. Populations 1 and 18 had the highest contribution to the total gene diversity as well as rare haplotypes. Therefore, these two populations should have the top priority in conservation planning and implementation. Timber harvesting may be prohibited in these populations and sustainable forest management strategies should be implemented in order to maintain the genetic diversity of these populations. An in situ/ex-situ conservation strategy would be effective to protect these habitats. In order to make best possible use of the high genetic diversity populations, it will be important to harvest any available seed from these populations and be used for plantations and infusing diversity in less diversity populations and for their ex situ conservation.

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