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Gender plasticity uncovers multiple sexual morphs in natural populations of *Cedrus deodara* (Roxb.) G. Don

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Abstract

Background: The expression of gender in gymnosperms is challenging because the extent of variability in gender segregations in tree crowns and selection pressure of nature can modify the gender through time.

Methods: An in-depth investigation on spatial segregation of genders in tree crowns and sex expression of a total of 500 trees in five different natural populations of *Cedrus deodara* was carried out and verified the occurrence of subdioecious (co-existence of male, female, and monoecious) genders in *C. deodara*.

Results: Five different sexual morphs were apparent among the 500 selected individuals as (1) pure male (M): bearing only male strobili in the whole crown with 22.2%; (2) pure female (F): bearing only female strobili in the whole crown with 12.4%; (3) mixed-monoecious (MM): bearing both male and female strobili with 13.6%; (4) predominantly female (PF) with 25.6%; and (5) predominantly male (PM) with 26.2%. The occurrence of multiple sexual morphs resulted from the complex selection pressure, which increased the stability of the populations. The segregation of genders in crown layers deemed to increase the fitness that may be a mechanism for accelerating outcrossing. The results of the study suggest that the subdioecious gender expression in *C. deodara* is evolved through the monoecy–paradioecy pathway. The production of male strobili revealed non-significant effect of tree sizes whereas a significant effect was observed for the production of female strobili. Our study established that the total pollen and seed outputs in *C. deodara* changed frequently according to gender expression.

Conclusions: The size of the tree, resource availability, sex allocation, plant architecture, gender segregation in crowns, and habitat conditions are the prominent causes for gender plasticity.

Keywords: Gender, Sex expression, Gender segregation, Strobili, Pre-fertilisation, Monoecy, Dioecy

Background

Gender represents the relative genetic contribution of an individual as a female and/or a male parent in the next generation (Lloyd 1979). Gender plasticity within populations of angiosperms has received considerable theoretical interests (e.g., Bawa 1980; Freeman et al. 1980; Rottenberg 1998; Khanduri et al. 2019; Blake-Mahmud and Struwe 2019). In contrast, few studies have been reported to show the extent and causes of gender variations in gymnosperms (Owens and Hardev 1990; Walas et al. 2018), such as *Cupressus* (Lev-Yadun and Liphschitz 1987), *Pinus* (Fouler 1965; Smith 1981), *Taxus* (Allison 1991), *Juniperus* (Jordano 1991), and *Abies* (Arista and Talavera 1997). Even in monomorphic populations (hermaphrodites or monoecious), a continuous variation exists in the functional male and female expressions (Lloyd 1980; Primack and Lloyd 1980; Bawa and Webb 1984; Case and Barrett 2001). Such variations can arise from phenotypic plasticity (often associated with differences in plant size), age (Lloyd and Bawa 1984; Freeman et al. 1997), or from the genetic

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determination of phenotypes (Westergaard 1958; Meagher 1988; Charlesworth 1999).

Sex expression in plants is a quantitative phenomenon, which depends on the relative proportion of reproductive units of both sexes within an individual plant (Borges 1998). The plasticity of sex expression in higher plants indicates the existence of different sex-determining factors. In general, these variations are the product of a complex interplay of genetics and environmental factors (Lloyd and Bawa 1984; Sarkissian et al. 2001). Such gender plasticity is particularly common in monoecious plants because the production of separate male and female flowers enables greater freedom to respond to specific environmental conditions (Sarkissian et al. 2001). The gymnosperms belong to family Picaceae, viz. *Abies*, *Cathaya*, *Cedrus*, *Larix*, *Keteleeria*, *Nothotsuga*, *Picea*, *Pseudolarix*, *Pseudotsuga*, *Tsuga*, and *Pinus*, and have monoecious mating system with wind-dispersed winged seeds (Givnish 1980; Eckenwalder 2009). However, all species in Gingkoaceae, Cycadaceae, and Gnetaceae are dioecious (Ming et al. 2011; Walas et al. 2018) and dioecy in gymnosperms is evolved from monoecy (Leslie et al. 2013). Mixed sexual system is very rare in gymnosperms (Walas et al. 2018) as compared to angiosperms (Vaughton and Ramsey 2012; Blake-Mahmud and Struwe 2020), which has paid attention to this phenomenon (Floyd 1983; Goldman and Willson 1986; Blake-Mahmud and Struwe 2020). Therefore, a comprehensive study on sex expression in monoecious trees is needed to understand (i) the differential return of investment in reproduction relating to both sexes and (ii) the occurrence of frequency of the leaky morphs (intermediate phenotypes between monoecy and dioecy) as compared to the “stable” distinct male and female morphs, which is so far largely unevaluated.

The phenotypic expression of gender depends on the distribution of resources to male and/or female functions in each reproductive episode (Goldman and Willson 1986). Sex changes in plants have been considered as a strategy of sex allocation to enhance fitness over the lifetime (Policansky 1981). According to sex allocation theory (Freeman et al. 1981; Charnov 1982), monoecious plants have the advantage of being flexible in their sex allocation, because of spatial differences in microhabitat quality or temporal changes during the years (Wolfe and Shmida 1997). A widely accepted explanations for the sex change have been offered by the size advantage model (Charnov 1982), which predicts that the plants change their sexual expression when size or age-specific reproductive success differs between males and females.

Plant architecture is also known to influence reproductive effort (Veillon 1978, Lechowicz 1984, Schmitt 1993). Most conifers, including pines, have an apparent gender segregation pattern in their crowns. Female cones are located on the main vertical branches of the upper part of the crown, whereas male cones are produced mostly in the middle and lower parts. This kind of gender segregation studies within crown layers are very limited and has been reported in *Cupressus* (Lev-Yadun and Liphschitz 1987), *Araucaria* (Veillon 1978), *Abies* (Arista and Talavera 1997), and *Picea* (Schwab et al. 2005).

Cedrus deodara (Roxb.) G. Don (Himalayan cedar) is a precious timber resource tree of western Himalaya, which grows in pure stands or in association with other Himalayan conifers between 1800 and 2600 m asl (occasionally between 1200 and 3000 m asl) altitudes. However, Sharma et al. (2018) have reported that in Himalayan temperate zone the broad-leaved forests are being gradually replaced by *C. deodara* forests. The tree looks pyramidal with pendulous branches at the young stage (Dirr 1990; Tewari 1994), which afterwards grows as a giant tree. The deodar is a wind-pollinated monoecious species that grows to a height of 15 to 50 m. The pollination occurs in autumn and the seed cones mature after 12 months. The specific aims of the study were to (i) document the phenotypic variation in gender and sex-expression in *C. deodara*, (ii) describe the spatial patterns of gender segregations within the canopies of *C. deodara*, and (iii) depict the effect of tree size, gender segregation, and sex-expression on the production of male and female strobili in this species.

Methods

Study site

The study was conducted on a total of 500 trees in five natural populations of *C. deodara* from March 2016 to October 2017 during one reproductive season. The flowering period within a population was up to 150 days from June to October. Deodar was the only species in the canopy layer (pure forest) and the canopy of trees were about 10–40 m in height in all selected populations. The climate of the selected populations is cold temperate type, where the summer temperature goes around 15 to 25°C and the winter temperature dropped below the freezing point.

Gender segregation in tree crowns and sex expression

One hundred individuals in each population were selected randomly and observed carefully during the field study. Tree parameters such as tree height, crown length (the length of the crown between tip and lower green branches forming green crown), and bole length (distance between ground level and first lower green branch) were measured using Blume-Leiss altimeter model BL7. Field studies were conducted during the flowering season, when the female and male strobili were clearly visible. The gender of the strobili was carefully examined using binoculars (model 37056 Super Zenith Prismatic) in case of large trees. The gender classes were determined on the basis of the presence and absence of male and female strobili within the tree crowns. Forked and non-flowered trees were excluded to avoid biases.

All the sampled individuals in each population were observed for sex expression during the study period. The crowns of all the selected trees were examined closely and gender occupancy in each crown was measured on the basis of their crown length and position of male and female strobili. Trees were categorized into three groups on the basis of the location of male and female strobili in the crown viz; (i) trees in which lower crown layer was male and upper crown layer was female (LC: M and UC: F), (ii) trees in which lower crown layer was female and upper crown layer was male (LC: F and UC: M), and (iii) trees in which lower and upper crown layers were male and the middle crown layer was female (LC: M, UC: M, MC: F). The first two categories were again divided into two subgroups on the basis of gender occurrence in each crown layer, i.e., males or females cover >¼th (25%) but <3/4th (75%) of the crown length were termed as (a) predominantly male (PM) and (b) predominantly female (PF). Detailed representation and categorization of sexual morphs according to gender segregation in tree crowns are depicted in Fig. 1a–j.

There was a continuum in sex expression from pure monoecious individuals to pure males or pure females. Thus, five sexual phenotypes were grouped as (a) pure male (M) bearing only male strobili in the whole crown, (b) pure female (F) bearing only female strobili in the whole crown, (c) mixed-monoecious (MM) bearing both male and female strobili, (d) predominantly male (PM), and (e) predominantly female (PF).

Strobili vs pollen production

Total numbers of male/female branches, male and female strobili were determined both in canopy layers and in whole trees. The productions of female and male strobili within the crowns were examined manually by careful observations with the help of binoculars. The effect of tree size classes on sex expression and reproduction was examined by measuring the diameter of the sampled trees using tree calliper. The production of pollen grains per microsporophyll was estimated following the method of Khanduri and Sharma (2010). Total 20 strobili were sampled on each tree, and the number of microsporophylls was counted manually. The pollen production per tree was calculated using the equation; $P = A \times Mi \times S$; where, P is total pollen production per tree, A is the average pollen production per microsporophyll, Mi is the average number of microsporophylls per male strobili, and S is the total male strobili production per tree.

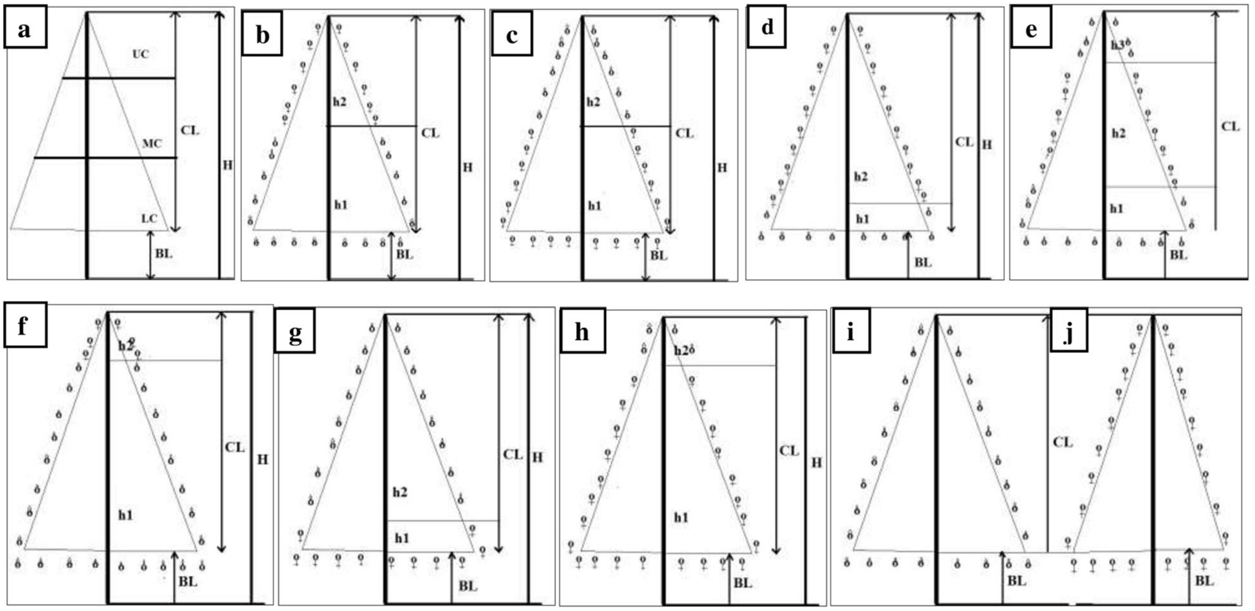

Fig. 1 Schematic representations of gender plasticity and sex expression in natural populations of *Cedrus deodara*. **a** Crown differentiation (LC: lover crown layer, MC: middle crown layer, UC: upper crown layer, CL: total crown length, BL: bole length, and H: total height), **b** mixed-monoecious ($h1 \approx h2$, h1: length of LC, h2: length of UC) in which UC is female (♀) and LC is male (♂), **c** mixed-monoecious ($h1 \approx h2$) in which UC is male and LC is female, **d** predominantly female ($h2 > h1$, $h2 \geq 3/4$ CL) in which UC is female and LC is male, **e** monoecious ($h2 \geq$ or $\leq$ $(h1 + h3)$, h2: length of MC, h3 length of UC, h1 is the length of LC) in which LC is male, UC is male, and MC is female, **f** predominantly male ($h1 > h2$, $h1 \geq 3/4$ CL) in which LC is male and UC is female, **g** predominantly male ($h1 < h2$, $h2 \geq 3/4$ CL) in which UC is male and LC is female; **h** predominantly female ($h1 > h2$, $h1 \geq 3/4$ CL) in which LC is female and UC is male, **i**–**j** dioecious, **i** male tree, and **j** female tree

For the estimation of male strobili production, the number of apical branches bearing male strobili was counted first and then multiplied by the mean number of male strobili per branch.

Statistical analyses

Gender plasticity within the selected individual trees from all populations was analyzed with the help of *G* test. Percent occurrences of gender and sex expression were calculated separately for the entire sampled individuals of populations, which were calculated by dividing the population of a gender with total sampled population and multiplied by one hundred. One-way ANOVA was used to assess the gender variation among populations and in different tree sizes. Chi-square (χ^2) test was performed to assess the variability of sex expression in various size classes and in populations. One-way ANOVA was also used to assess the variability in the production of male and female strobili with the effect of sex expressions, size-classes, and gender segregations in each crown layer as fixed effects. Single factor ANOVA was also used to examine the effect of sex expression on pollen production, variation in pollen production among strobili, and the variability of gender segregation in crown layers.

Results

Spatial segregation of genders in tree crowns and sex expression

There was a great variation in the distribution of genders in all the selected populations. The selected 500 individuals were classified into three gender classes i.e., (i) male (111 trees, Fig. 2A), (ii) female (62 trees, Fig. 2B), and (iii) monoecious (327 trees, Fig. 2C). The monoecious groups appeared in three different classes was based on the extent of gender occurrence in crown layers such as (a) mixed-monoecious (MM), (b) predominantly female (PF), and (c) predominantly male (PM). Of the 327 monoecious individuals, (i) 206 were categorized in the group of lower crown female (LC: F) and upper crown male (UC: M), (ii) 82 individuals were in the group of lower crown male (LC: M) and upper crown female (UC: F), and (iii) remaining 39 individuals were in the third group of lower crown male (LC: M), upper crown male (UC: M), and middle crown female (MC: F) (Table 1). Similarly, out of 206 individuals of the first group, i.e., lower crown female (LC: F) and upper crown male (UC: M); (ia) 93 individuals fell in the subgroup predominantly female (PF), (ib) 26 individuals in the predominantly male (PM), and (ic) 87 individuals in mixed-monoecious (MM) subgroup. Further, out of 82 individuals of the second group, i.e., LC: M and UC: F, (iia) 38 individuals were belonging to PF, (iib) 6 individuals to PM, and 41 individuals to MM subgroup. In the same way, out of 39 individuals of the third group, i.e., LC: M, UC: M, and MC: F, (iiia) 36 individuals were in the subgroup PM and 3 individuals in PF. Our observations have shown that the male strobili developed mainly on the lower and upper branches of the crowns, while female strobili appeared more in middle branches of the crown layers (F=13.409, p<0.0001, df= 2). In addition, the male strobili were largely developed on the lateral branches which were often attached to the primary or secondary branches. By contrast, the female strobili frequently appeared on the basal part of the primary or secondary branches, which were emerged from the main stems.

The sex expression was differed significantly among populations (χ^2 = 152.6, df = 4, p < 0.0001). The occurrence of sexual morphs in percentile among selected individuals was male 22.2%, female 12.4%, and monoecious 65.4% (includes mixed-monoecious 13.6%, predominantly female 25.6%, and predominantly male 26.2%). When females and predominantly female individuals were grouped, the average was around 38.0% of the population, whereas males and predominantly males were averaged at 48.4%. The sexual morphs differed markedly in size classes (χ^2 = 44.36, df = 4, p < 0.0001), more number of females and predominantly females were recorded in higher diameter classes, whereas males and predominantly males were distributed unevenly among the size classes (Table 2).

The results of *G* test have shown that there was a significant variation in the gender categories (Male *vs* Female; PF vs PM; and Dioecy vs Monoecy) within the selected individuals and also in the populations (Table 3). However, difference between populations on gender expression was not significant (F= 0, p=1, df= 4), and the variability of genders in the population was found significant (F=48.03, p< 0.0001, df=2). The maximum occurrence of male genders/strobili was recorded in Jwarna population, followed by Dandachali population, whereas maximum occurrence of female genders/strobili was recorded in Ranichauri population, followed by Kaudia population. The occurrence of monoecious individuals was found highest in Kaudia population, followed by Badsahithaul population (Table 4).

Strobili vs pollen production

The sexual phenotypes varied markedly for the production of male and female strobili in the selected populations. Male and predominantly male sexual morphs were found producing more number of male strobili than monoecious and predominantly female morphs (F = 49.45, p<0.00001, df= 4). Similarly, the female and predominantly female morphs were observed to produce more female strobili than monoecious and predominately male morphs (F=31.45, p<0.00001, df=4).

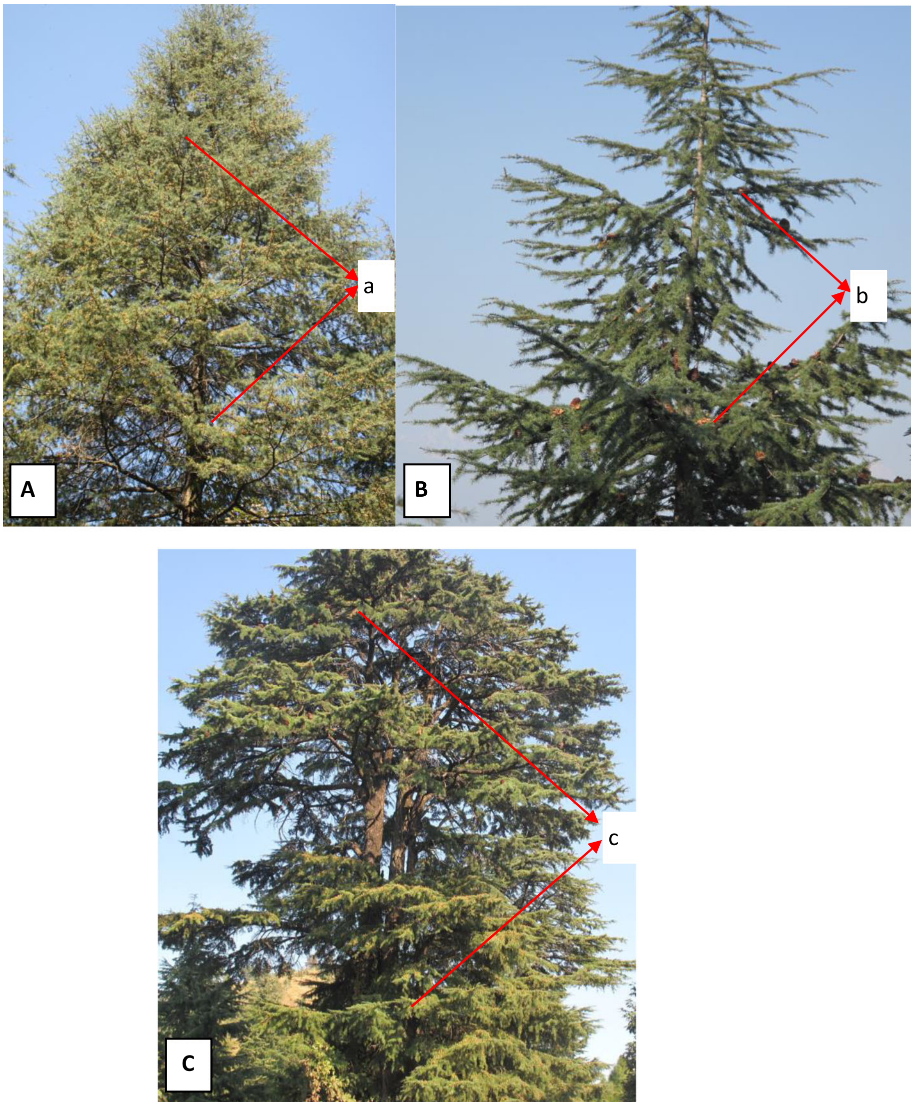

Fig. 2 Sex expression in *Cedrus deodara*. **A** Male tree (a: Cluster of male cones developed all over the canopy layers). **B** Female tree (b: Female cone development all over the canopy layers). **C** Monoecious tree (c: Male and female cone development within a tree)

Table 1 Sexual-segregation among crown layers within the populations of *Cedrus deodara* (n=500), LC: lover crown layer, MC: middle crown layer, UC: upper crown layer, M: male, and F: female

Sex-segregation in crown layers	Number of individuals
LC: F, UC: M (Monoecious)	206
LC: M, UC: F (Monoecious)	82
LC:M, UC:M, MC:F (Monoecious)	39
(Dioecious)	
Male	111
Female	62

Moreover, the effect of tree sizes on the production of male strobili was non-significant (F = 0.739, p = 0.5373, df= 4), whereas there was a significant effect of tree sizes on the production of female strobili (F=15.50, p< 0.00001, df=4). On average, the pre-fertilization stages took 5–6 months from cone initiation to pollination (Fig. 3a–f).

The total pollen output per tree significantly varied according to the sexual phenotypes (F= 49.458, p < 0.0001, df= 4), although pollen production per male strobili (F= 0.325, p=0.807, df= 3), number of microsporophylls per strobili (F= 0.418, p=0.764, df= 3) and mean number of pollen grains per microsporophyll (F= 0.586, p=0.878,

Table 2 Occurrence of sexual-morphs (in percentage) within size classes and among genders in *Cedrus deodara* populations (*d*: diameter, *n* = 500)

Gender classification	***d* (cm)**	**Occurrence of sexual-morphs (%) within size classes**				
		M	**F**	**MM**	**PM**	**PF**
Male (M)	20–30	1.4	0.4	0.6	0.4	0.2
Female (F)	30–40	5.6	2.0	4.8	5.0	5.6
Monoecious						
a) Mixed-monoecious (MM)	40–50	8.0	3.6	2.4	4.8	8.8
b) Predominantly male (PM)	50–60	4.2	2.4	4.0	8.2	6.0
c) Predominantly female (PF)	>60	3.0	4.0	1.8	7.8	5.0
Total occurrence (%) among genders		22.2	12.4	13.6	26.2	25.6

M male, *F* female, *MM* mixed-monoecious, *PM* predominantly male, *PF* predominantly female

df= 3) were not significant among sexual morphs. Thus, pollen output from a tree is directly related to the production of male strobili. The mean number of microsporophylls per strobili was 419.6 ± 51, the mean number of pollen grains per microsporophyll was 9571 ± 715, the mean number of pollen grains per strobili was 4016257 ± 299839, and the total pollen output per sexual morph was 9.3 ± 6.3 × 10^9 (MM), 22.5 ± 9.0 × 10^9 (M), 16.9 ± 6.7 × 10^9 (PM), and 2.9 ± 1.5 × 10^9 (PF). The total production of pollen among the sexual morphs was in the order of M> PM > MM> PF.

Discussion

The present study is the first investigation on gender plasticity in natural populations of *C. deodara*. Monoecy is common in deodar. Surprisingly, we found three phenotypic genders in all the studied populations of *C. deodara*. Thus, changes in gender expressions in the sampled trees gave rise to male, female, and monoecious genders, thereby producing sub-dioecious populations. The similar type of gender expression was also witnessed in some other monoecious trees (Allison 1991; Jordano 1991; Arista and Talavera 1996, 1997; Leslie et al. 2013). There was a negligible effect of population differences on gender expression; however, marked variations among genders were observed in all the populations. Several factors which have been reported transforming gender expression in monoecious plants are (i) sex-allocation (Charlesworth and Charlesworth 1981; Charnov 1982; Lovett-Doust 1989; de Jong et al. 2008; Pannell 2017), (ii) environmental stress (Freeman and Vitale 1985, Vials and Pannell 2012; Walas et al. 2018), (iii) adaptation to wind pollination (Frankel and Galun 1977; Masaka and Takada 2006; Friedman and Barrett 2009; Walas et al. 2018), and (iv) selective pressure for increasing outcrossing (Arista and Talavera 1996;

Table 3 Gender plasticity within the populations of *Cedrus deodara G* test (*significant, NS non-significant)

Category	**Observed frequency**	***G* value**	***P* value**	**Populations**	**Observed frequency**	***G* value**	***P* value**
Male vs female	111/62	14.07	0.0002	Ranichauri	14/26	3.65^{*}	0.0564
				Badsahithaul	25/5	14.55^{*}	0.0001
				Dandachali	30/5	19.81^{*}	<0.0001
				Kaudia	8/20	5.31^{*}	0.0213
				Jwarna	34/6	21.63^{*}	<0.0001
PF *vs* PM	131/68	20.29	<0.0001	Ranichauri	10/8	0.22^{NS}	0.6374
				Badsahithaul	35/15	8.22^{*}	0.0041
				Dandachali	25/10	6.64^{*}	0.0099
				Kaudia	52/4	48.82^{*}	<0.0001
				Jwarna	9/31	12.79^{*}	0.0003
Monoecy vs dioecy	327/173	48.21	<0.0001	Ranichauri	60/40	4.03^{*}	0.0455
				Badsahithaul	70/30	16.45^{*}	<0.0001
				Dandachali	65/35	9.14^{*}	0.0025
				Kaudia	72/28	20.03^{*}	<0.0001
				Jwarna	60/40	4.02^{*}	0.0452

Table 4 Locality and occurrence of sexual-morphs (in percentage) studied in the natural populations of *C. deodara*, M: male, F: female, MM: mixed-monoecious, PM: predominantly male, PF: predominantly female

Population/ provenance	Altitude (m)	Latitude/ longitude	Occurrence of sexual-morphs (%) (*n*=500)				
			M	F	PF	PM	MM
Ranichauri	1800	30° 18″ N/78° 24″ E	14	26	10	8	42
Badsahithaul	1676	30° 08″ N/78° 61″ E	25	5	35	15	20
Dandachali	1950	30° 31″ N/78° 42″ E	30	5	25	10	30
Kaudia	2323	30° 41″ N/78° 36″ E	8	20	52	4	16
Jwarna	2500	30° 42″ N/78° 31″ E	34	6	9	31	20

Meagher 2007). The effect of tree size classes on gender expression was significant. The females and monoecious genders were often found in higher diameter classes, which would be likely to compensate the higher reproductive investment (Lloyd and Bawa 1984; Ne'eman et al. 2011; Khanduri et al. 2019).

A stable coexistence of male, female, predominantly male, predominantly female, and mixed-monoecious was recorded in the sampled populations. We have observed that the distribution of sexual-morphs within the populations varied markedly. One possible interpretation of multiple sexual morphs in *C. deodara* is probably due to the reproductive resource trade-off between genders within the crown and existence of sex-instability in both the genders (female and male), resulting because of the complex interplay of genetics and environmental factors. Aggregations of females and predominantly females in *C. deodara* were encountered largely under shady or low light acquiring sites and males were often distributed in the surroundings or open free zones in intense light availability conditions. Similar results were also obtained in case of *Cedrus atlantica* for male strobili (Saouab and Amraoui 2020). Moreover, specific studies on the availability of environmental factors are needed for discussing the possible impact of environmental factors varying between the populations (light intensity, altitude, temperature, soil) on these sexual changes. In western Himalaya, India, high frequencies of female trees occurred in mast seeding year in *C. deodara* forests at four different locations (Sharma and Bhondge 2016). Subdioecious sexual-system is assumed to be the advancement towards the unisexuality, where a portion of the population has reached to a stable sex expression (Geetha et al. 2006; Ehlers and Bataillon 2007).

Our observations might support that the evolution of subdioecy to dioecy from monoecy in *C. deodara* would be through paradioecy pathway rather than gynodioecy, because of inconstancy in both female and male sex

Fig. 3 Pre-fertilization phenological events in *Cedrus deodara*. **a**–**b** Male cone initiation and development (**a**, June - **b**, October). **c** Explosive pollen release assisted by wind (October). **d** Initial stage of female cone (October) and **e** Development of female cone (Next season June) **f** Developed/ mature female cone (October)

expressions. According to Delph and Wolf (2005), subdioecy is assumed to evolve through the gynodioecious pathway, with inconstancy only in the male individuals. In contrast, Lloyd (1972) stated that paradioecious populations have inconstant females and males, with inconstancies being of similar magnitude in both sexes. Paradioecy is an intermediate state of evolution from monoecy to dioecy (Lloyd 1972; Sakai and Weller 1999; Renner and Won 2001). In angiosperms, numerous studies have indicated the gynodioecy pathway (Lloyd 1976; Charlesworth and Charlesworth 1987; Ehlers and Bataillon 2007), and the monoecy–paradioecy pathway was little acknowledged (Renner and Ricklefs 1995; Dorken and Barrett 2004; Ehlers and Bataillon 2007). However, the prevalent occurrence of liability in sexual morphs in gymnosperms through monoecy–paradioecy pathway has been supported by number of workers such as Allison (1991) in *Taxus canadensis*, Jordano (1991) in *Juniperus phoenica* and Flores-Renteria et al. (2012) in *Pinus johannis*.

Additionally, we have observed that sex expression differed among tree size classes. More number of females and predominantly females arose in higher diameter classes, whereas males, predominantly males and mixed-monoecious were found distributed independently. We have speculated that the minimum threshold energy cost for initiation of female reproduction is often higher than the male; therefore, there was more prevalence of male gender. Our findings are well in support of the Size Advantage Hypothesis (Bonser and Aarssen 2003; Revel et al. 2012). As the amount of available resources increases, plants often respond by increasing their sizes. In areas with high nutrient availability, plants also respond by favoring an increase in the number of more energetically expensive female gender, making plant size potentially a strong predictor of female bias in gender expression (Toivonen and Mutikainen 2012; Hasegawa and Takeda 2005).

In *C. deodara*, the production of cones (male or female) increased with an increase in sex expression (maleness or femaleness) and relative size of individual trees. The production of female cones has a linear relation with increasing tree sizes, accompanied by proportional increase in femaleness over the crown. The production of male cones was high in monoecious individuals; however, it was not increased with tree size classes. The pollen production in male cones did not vary significantly among sexual morphs, indicating that the total pollen output from a tree is proportional to the total male strobili production per tree. The average pollen output of individual trees was on par with the results of Khanduri and Sharma (2002, 2009). Due to variation in the production of male strobili per tree, the predominantly male trees produced high pollen per tree and predominantly female trees had low pollen output per tree. Indeed, trees growing in resource-rich location often produce large sized trees with higher number of cones per tree (Arista and Talavera 1997). Further, the total pollen output in *Cedrus* is functionally related to the extent of maleness over the crown, which ultimately augment the efficiency of wind pollination and seed set in neighboring females through pollen dispersion. Our findings support that the male fitness in wind-pollinated plants increase seed-siring success through wider dispersal of pollen (Charlesworth and Charlesworth 1981; Charnov 1982; Burd and Allen 1988; Charlesworth and Morgan 1991; de Jong and Klinkhamer 1994; Klinkhamer et al. 1997; Hesse and Pannell 2011).

C. deodara is an anemophilous tree with segregated male and female strobili in crown layers. We predict that the segregation of strobili in tree crowns is an adaptive strategy in monoecious species, which is induced by changes in hormonal concentrations along the stems in response to the natural selection pressure to increase its fitness. The segregations of strobili would give some selective advantages under circumstances such as plasticity in the allocation of male and female functions (Freeman et al. 1981; Miglia and Freeman 1996), and specialization of males in pollen dispersal and females in capturing the pollen (Mirov 1967; Shmida et al. 2000). It has been suggested that the location of females in top canopy might reduce the rate of self-pollination (Faegri and Van der Pijl 1971; Charnov 1987) and increase the rate of seed dispersal (Shmida et al. 2000). The female cones may also serve as the efficient sinks for photosynthetic resources (Lev-Yadun and Liphschitz 1987; Owens 1991). Nevertheless, the allocation of males on the top can increase their fitness through long-distance dispersal of pollen (Lloyd and Bawa 1984) and also can enhance the geitonogamous self-pollination within the crown (Khanduri and Sharma 2010). Additionally, segregation of strobili/genders has a marked effect on the production of male and female cones. Female cones are more developed in the lower crown layers likely due to high resource availability and greater chances to get pollinated from distant high trees (Arista and Talavera 1997). Furthermore, the increased diameter growth of branches in lower crown layers also provides more mechanical support to the female cones (Wilson 2000).

Conclusions

The evidence presented in this study apparently indicates that the natural populations of *C. deodara* possess a subdioecious gender expression (coexistence of male, female, and monoecious). The wide array of sexual morphs and the stability of unisexual individuals make *C. deodara* a potential model to evaluate hypotheses of the evolution of dioecy. Natural existence of predominantly females and predominantly males indicates the instability in both the genders, strongly supporting that the subdioecious populations of *C. deodara* must have been evolved through the paradioecious pathway. The sympatric, monomorphic, and dimorphic sexual systems allow testing of ecological differentiations and habitat specializations between the sexes that would suggest an important role for resource availability in the evolution and maintenance of gender variation. Our study strongly suggests that the total male and female cone production within an individual is the function of gender expression. However, gender in trees is strongly influenced by age, resource trade-off within the gender, plant architecture, density, and environmental pressure. The plasticity of gender is an evolutionary advantage for attaining the gender stability, thereby maximizing the percentage of outcrossing. Long-term research over the years and repeated innovative works on many wild species are required for full understanding of the phenomenon of reproductive advantage through sex morphing in wild monoecious populations, particularly in gymnosperms.

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Authors' contributions
VPK and AS conceived the study, set up the experiment, conducted the field work, and drafted the initial manuscript. VPK revised the manuscript. CMS helped in designing the field experiment and improved the drafts. The authors read and approved the manuscript.

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Declarations

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NA.

Competing interests
The authors declare that they have no competing interests.

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