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RESEARCH ARTICLE

Fine- and local- scale genetic structure of *Dysoxylum malabaricum*, a late-successional canopy tree species in disturbed forest patches in the Western Ghats, India

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Abstract *Dysoxylum malabaricum* (white cedar) is an economically important tree species, endemic to the Western Ghats, India, which is the world's most densely populated biodiversity hotspot. In this study, we used variation at ten nuclear simple sequence repeat loci to investigate genetic diversity and fine scale spatial genetic structure (FSGS) in seedlings and adults of *D. malabaricum* from four forest patches in the northern part of the Western Ghats. When genetic variation was compared between seedlings and adults across locations, significant differences were detected in allelic richness, observed heterozygosity, fixation index (F_{IS}), and relatedness ($P < 0.05$). Reduced genetic diversity and increased relatedness at the seedling stage might be due to fragmentation and disturbance. There was no FSGS at the adult stage and FSGS was limited to shorter distance classes at the seedling stage. However, there was clear spatial genetic structure at the landscape level (<50 km), regardless of age class, due to limited gene flow between forest patches. A comparison of the distributions of size classes in the four locations with published data from a more southern area, showed that large trees (diameter at breast height, DBH, >130 cm) are present in the southern sacred forests but not in the northern forest reserves. This pattern is likely due to stronger harvesting pressure in the north compared to the south, because in the north there are no cultural taboos regulating the extraction of natural resources. The implications for forest conservation in this biodiversity hotspot are discussed.

Keywords *Dysoxylum malabaricum* · Fragmentation · Disturbance · Land use · Spatial genetic structure · Western Ghats

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Introduction

Tropical forests harbor large amounts of biodiversity, but human activities over the last few decades have caused severe deforestation, fragmentation and disturbance (Wright and Muller-Landau 2006; Abdullah and Nakagoshi 2007). As a consequence, many forests that were once continuous now exist as isolated and disturbed patches or meta-populations (Sork and Smouse 2006). Natural and artificial habitat disturbances, such as forest fires, tsunamis, and land use, and their effects on the genetic diversity of species are important topics in ecology and conservation biology (Banks et al. 2013; Iwaizumi et al. 2013). In many cases of habitat disturbance, decreases in genetic diversity and increases in population differentiation are expected, especially when disturbances induce genetic drift and restrict gene flow among populations (Vellend 2004; Evanno et al. 2009; Struebig et al. 2011). Currently, one of the most prevalent environmental changes, especially in tropical forests, is anthropogenic disturbance, including land use change and deforestation, which results in loss of continuous habitat and the creation of smaller, spatially isolated, forest fragments. While habitat loss and overharvesting is an immediate threat to many forest tree species, disturbance may also be detrimental to forest ecosystems in the long term. In particular, following disturbance late-successional canopy species, which constitute a large portion of the biomass and provide shade and habitat to other species, are replaced by pioneer species, which may undermine the integrity of the ecosystem. Furthermore, recent studies demonstrate that both genetic and species diversities can decrease following fragmentation and disturbance (Vellend and Geber 2005; Evanno et al. 2009; Struebig et al. 2011). Because genetic diversity is an integral part of biodiversity and the basis of a species' adaptive potential, it is important to assess the genetic diversity of late-successional trees to ensure their long term persistence in disturbed tropical forests. The present study aims to address this problem by combining genetic and demographic approaches for investigating genetic diversity and genetic structure across age classes.

Measuring spatial genetic structure provides a way to assess how pollen and seed flow changes over time. Spatial genetic structure (SGS) measures the nonrandom geographical distribution of genotypes, and results in increased relatedness among geographically close individuals compared to more distant ones (Epperson 1992; Vekemans and Hardy 2004). Fine scale SGS (FSGS) has been observed in plant species (Vekemans and Hardy 2004) and measures of FSGS have been suggested to be more sensitive than clustering algorithms and standard inbreeding statistics to detect the early genetic effects of environmental changes (Wang et al. 2011). Although SGS can be affected by several factors, including colonization events and micro-environmental selection, its main determinants are believed to be mating system, dispersal mode and adult density (Vekemans and Hardy 2004). Moreover, as SGS patterns are scale dependent, it is important to evaluate genetic structure at several geographic levels, i.e., broad scale (e.g. several hundred to thousands of kilometers), fine scale (e.g. over tens to several hundred meters) and also at an intermediate landscape scale (Manel et al. 2003; Tsuda et al. 2010), to understand how SGS is formed over a species distribution range.

Dysoxylum malabaricum Bedd. Ex C.DC. (white cedar) is an economically important and endangered species, endemic to the Western Ghats region of India, one of the world's eight hottest hotspots of biodiversity (Myers et al. 2000) and by far the most densely human populated of the biodiversity hotspots (Cicotta et al. 2000). *Dysoxylum malabaricum* is considered a sacred tree and is harvested for religious purposes, including the construction of temples (Manjunath 2003), as well as for commercial and personal uses (Kumar 2009). While the degradation of forests in the present study area has been occurring for about 200 years, the most severe usage has occurred more recently, e.g. from the forest reserves in Northern Karnataka in the Western Ghats (Gokhale 2004). Over the last few decades, overharvesting and land use have reduced and fragmented the remaining populations; most notably the stock of juvenile and young adult individuals has been reduced in some locations, with potential long-term effects on the reproductive success and survival of populations (Shivanna et al. 2003; Ismail et al. 2014). In particular, the species density in sacred groves and forest reserves distributed throughout the Western Ghats region is at risk (Boraiah et al. 2003; Gunaga et al. 2013). Sacred groves and forest reserves have traditionally been sustainably managed by local communities (Chandrakanth et al. 2004; Tambat et al. 2005). However, in recent years, due to changes in socio-economic conditions and decreasing social norms regulating the extraction of natural resources from these forests, they are now largely a source of income and are exposed to non-sustainable harvesting (Tambat et al. 2005; Gunaga et al. 2013). As an old-growth and large canopy tree depending on large birds for dispersal, *D. malabaricum* provides an ideal system for studying the ability of economically and ecologically important late-successional tree species to survive in disturbed habitats.

Previous conservation genetics studies on *D. malabaricum* have shown the presence of genetic structure on a range-wide and local scale. In the range-wide study, genetic clusters were found to stretch over large as well as small geographic distances, with more clustering and a higher fixation index (F_{IS}) in the northern part of the

species distribution range (Bodare et al. 2013). One FSGS study in a moderately disturbed southern location (Kodagu, Fig. 1) suggested the aggregation of genotypes over small distances, which intensified in younger cohorts (Ismail et al. 2012). However, it is not known whether this pattern is site-specific, or common in other disturbed forest patches throughout the Western Ghats. Moreover, Ismail et al. (2014) found that although seedlings are plentiful, juveniles are extremely rare and small-sized trees below 10 cm DBH are completely absent in Kodagu. This demographic imbalance indicates a failure to recruit from early life stages to the adult life stage. Thus, it is important to compare the genetic structure of *D. malarbaricum* in northern and southern populations and discuss the potential underlying mechanisms of SGS, for example contrasting demographic structure and more patchy distribution in the northern marginal populations.

In the present study, we employed nuclear simple sequence repeat (nSSR) markers to assess genetic diversity and population structure in four forest reserves at the northern edge of the range of *D. malabaricum*. We compared SGS among age classes to determine whether disturbance over the last few decades has led to genetic isolation in this species. Furthermore, we collected data on the demographic structure of the populations and the perceived level of disturbance, based on the intensity of human land use activities. The specific aims of the present study were to: (i) assess genetic diversity and SGS in four locations and among cohorts (age classes) within locations, (ii) estimate the overall population structure using a clustering algorithm and landscape-scale SGS approach, (iii) evaluate the impact of disturbance and fragmentation on genetic diversity and population demography, and (iv) discuss implications for conservation efforts.

Materials and methods

Study species and site

Dysoxylum malabaricum Bedd. Ex C.DC. is an evergreen, tall canopy tree, with adults reaching heights of 30–40 m. It is an almost fully outcrossing species in which seeds are dispersed primarily via the Malabar grey hornbill (*Ocyceros griserus*) and Malabar pied hornbill (*Anthracoceros coronatus*), but occasionally by Mountain imperial pigeons (*Ducula badia*) as well as by gravity. The species is thought to be pollinated by insects, such as small beetles and thrips (Shivanna et al. 2003). *Dysoxylum malabaricum* is generally found at low densities in mixed-forests and

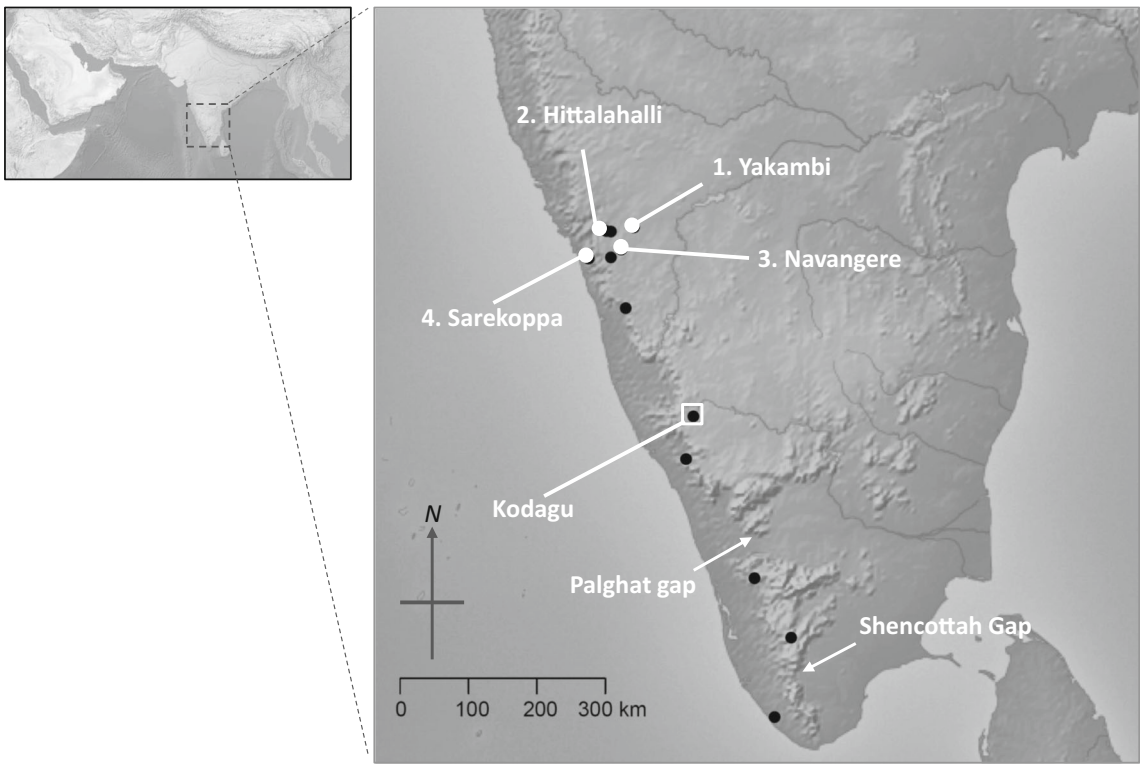

Fig. 1 The locations of the four populations examined in the current fine-scale study (*white circles*). The locations of the other 8 populations examined in the previous broad-scale genetic structure analysis by Bodare et al. (2013) (*black circles*). The *white square marks* Kodagu where Ismail et al. (2012, 2014) conducted a study on fine-scale genetic structure and parentage analysis

Table 1 Location, patchy size, sample size (n), density, values of genetic diversity paramters; allelic richness ($A_{[20]}$), gene diversity (h), fixation index (F_{IS}) and estimate of F_{IS} corrected for null alleles, and Sp value from the Sp statistics in 4 locations of *Dysoxylum malabaricum*

Location (population code)	Latitude (°N)	Longitude (°E)	Patch size (ha)	n	Density (ha)	$A_{[20]}$	h	F_{IS}	Corrected F_{IS}	Sp (±SE)
Yakambi	14.85	75.11	5.32							
1. Adult (YKA)				13	2.44	4.32	0.609	0.201	0.000	–
2. Juvenile (YKJ)				4	0.75	–	0.671	–	–	–
3. Seedling (YKS)				73		4.30	0.610	0.063	0.007	0.0138 (±0.0039)
Hittalahalli	14.79	74.80	126							
4. Adult (HTA)				51	0.40	5.20	0.641	−0.06	0.000	0.0017 (±0.0043)
5. Juvenile (HTJ)				26	0.21	4.93	0.616	0.030	0.000	–
6. Seedling (HTS)				59		4.83	0.626	0.084	0.000	0.0104 (±0.0047)
Navangere	14.56	74.94	3.72							
7. Adult (NGA)				60	16.13	5.07	0.625	0.410	0.342	−0.0167 (±0.0052)
8. Juvenile (NGJ)				2	0.54	–	0.500	–	–	–
9. Seedling (NGS)				75		4.23	0.552	0.021	0.000	0.0003 (±0.0022)
Sarekoppa	14.44	74.51	17.92							
10. Adult (SKA)				59	3.29	5.05	0.593	0.319	0.290	0.0037 (±0.0084)
11. Juvenile (SKJ)				9	0.50	–	0.574	–	–	–
12. Seedling (SKS)				103		4.29	0.558	0.024	0.002	0.0036 (±0.0009)

In each population individuals were allocated to 3 different age classes based on height and DBH

does not form pure dominant stands, as is common for other late successional canopy trees in tropical forests. The forest floor of mixed forests containing *D. malabaricum* is covered by herbaceous plants as well as seedlings or juveniles of tree species, and occasionally there are several open gaps due to logging of canopy trees. *Dysoxylum malabaricum* is a threatened species protected by law in state forests, and is categorized as Endangered (EN) under the Indian National Threat Assessment, which uses the same criteria as the IUCN (Ravikumar and Ved 2000). However, in private forests, locals may harvest trees after obtaining permission from the Forest Department. Unfortunately, illegal logging is not uncommon. Trees are logged for their valuable timber, usually when they reach a girth of more than 180 cm (Menon and Balasubramanyan 2006).

As SGS patterns often vary significantly over a species distribution range, it is recommended to examine several populations using the same sampling strategy in each (Jump et al. 2012). Thus, the present study focused on four locations: Yakambi, Hittalahalli, Navangere and Sarekoppa, at the northern margin of the distribution range of *D. malabaricum* in the Western Ghats, India (around 14.5°N, Fig. 1; Table 1). While there is no data available on the historical population sizes and ranges of *D. malabaricum* in these four locations, fragmentation of the landscape suggests an impact of recent human activities. According to a previous study on the range-wide genetic structure of *D. malabaricum* (Bodare et al. 2013), Yakambi and Hittalahalli, and Navangere and Sarekoppa belong to two different genetic clusters respectively (inferred by a STRUCTURE analysis Pritchard et al. 2000). Based on the following evidence: (i) many cut or broken stems and branches, (ii) presence of cattle or dung pads, (iii) a lot of paths running through the forests, and (iv) presence of invasive species, all investigated forest patches were obviously heavily disturbed, with Navengere slightly less affected.

Sample collection

Tissue samples of *D. malabaricum* were collected and individuals were categorized into three age classes based on height and diameter at breast height (DBH): adults (DBH >10 cm), juveniles (height ≥1 m but DBH <10 cm), or seedlings (height <1 m). Trees with a DBH of approximately 10 cm are 12–15 years of age, the time

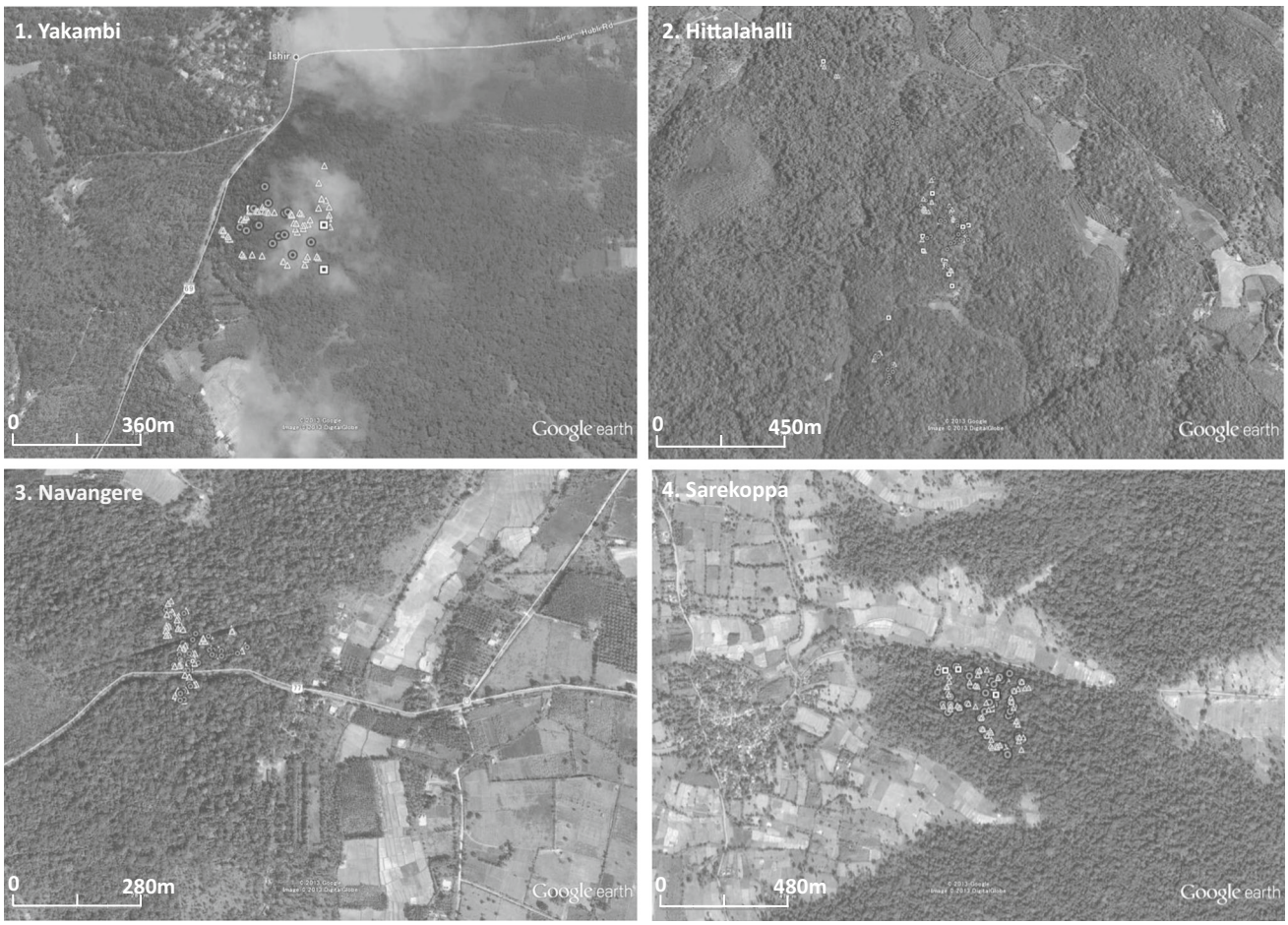

Fig. 2 Satellite maps over the four sampling locations by Google Earth (Google). Adult trees are represented by *circles*, juveniles by *squares* and seedlings by *triangles*

when this species first bears fruit. However, trees only reach the canopy after approximately 25 years, at which stage they acquire their full reproductive potential (Bodare et al. 2013). Adults and juveniles were sampled exhaustively in the examined forest patches during a thorough field survey (Fig. 2). Seedlings were plentiful and therefore generally collected randomly within the same range as the adults. When possible, seedlings both close to and far away from adult trees were collected. The four chosen locations are clearly distinct stands but additional single trees may have been scattered in nearby forests. A total of 534 individuals were sampled from the three age classes at the four locations: 13–60 adults and 59–103 seedlings were collected in each location, only one population had more than 10 juveniles (Hittalahalli, Table 1; Fig. 2). The geographical coordinates for each individual were recorded using a Garmin 60CSx handheld GPS. Although the four locations sampled in this study were also examined in our previous study (Bodare et al. 2013), for this study individuals from these four sites were re-sampled in January 2013 at a much greater density. The density of individuals per hectare was calculated for adults and juveniles at each location, based on patch size. Inner bark samples were collected for adults while leaf samples were collected for juveniles and seedlings. All samples were stored at −20 °C until DNA extraction.

Evaluation of demographic structure

Individual DBH was recorded for all adults. Mean DBH and standard deviation (SD) were calculated for each location. To test for pairwise differences between locations, mean DBH were compared using a Tukey's Honest Significant Difference test with a 95 % confidence interval (CI). The DBH distributions of the four locations were compared with a recent study (Ismail et al. 2014) that examined DBH of 235 *D. malabaricum* adults in sacred forests near Kodagu, a location further south in the species distribution range (Fig. 1).

DNA extraction, amplification and genotyping

DNA was extracted using a CTAB (cetyltrimethylammonium bromide) method (Sambrook et al. 1989). The DNA samples were genotyped at 11 nuclear simple sequence repeat (nSSR) markers (Dysmal 1, 2, 3, 7, 9, 13, 14, 18, 22 and 26; Hemmilä et al. 2010). Fluorescent-dyed primer pairs were mixed into three multiplex primer sets and amplified by polymerase chain reaction (PCR) in mixtures containing 1.2 μL of 1–10 ng DNA, 3.0 μL of master mix buffer (Type-it Microsatellite PCR kit, Qiagen), 1.2 μL of H_2O, and 0.6 μL of primer mix (with the concentration of each primer pair adjusted to 1–2 μM). Amplification was carried out using a DNA Thermal Cycler (Takara) with the following program: hot-start DNA polymerase and denaturation at 95 °C for 15 min; 32 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s; and a final 30 min extension step at 72 °C. The amplified PCR products were loaded onto an ABI 3500 autosequencer (Applied Biosystems), and their sizes and genotypes were determined using GeneMapper software (Applied Biosystems).

Genetic diversity within populations and among age classes

The twelve sample categories (three age classes × four locations) were regarded as twelve "populations" in this study. Since the sample size in three of the juvenile groups (Yakambi, Navangere and Sarekoppa) was small, they were removed from the analyses, except for gene diversity estimation and STRUCTURE analysis. To check for the presence of null alleles, data were analyzed with FreeNA (Chapuis and Estoup 2007) and INEST (Chybicki and Burczik 2009). Linkage disequilibrium was tested for locus pairs in each population and over all populations using Genepop v. 4.2 (Raymond and Rousset 1995; Rousset 2008) and the *p*-value was adjusted with the implemented Bonferroni correction. Allelic richness (A) based on 10 diploid individuals (El Mousadik and Petit 1996) and gene diversity (*h*) (Nei 1987) were calculated in FSTAT v. 2.9.3 (hereafter FSTAT, Goudet 1995, 2001). Fixation index (F_{IS}) and its unbiased values after correction for null alleles were calculated in INEST (Chybicki and Burczik 2009). To evaluate whether the cohorts examined in this study had experienced recent bottlenecks, we employed the BOTTLENECK 1.2.02 software (hereafter, BOTTLENECK analysis; Piry et al. 1999; Cornuet and Luikart 1996) under both the infinite allele mutation model (IAM) and the two-phase model (TPM; 30 % of multistep mutation and 70 % single-step mutation) assumptions.

To assess whether genetic diversity and population differentiation differ among age classes (seedlings and adults), average values of observed heterozygosity (H_O), gene diversity (*h*), allelic richness, relatedness (Queller and Goodnight 1989), and F_{ST} were compared, treating the two age classes as two groups. Differences in these parameters between the age classes were tested for significance using a permutation test in FSTAT. We employed one-sided *P*-values to test whether the value in one group was significantly larger than the other. Due to the limited sample size in the juvenile age class, juveniles were not considered in this test.

Population differentiation and structure

Genetic differentiation among populations was evaluated by calculating the overall and pairwise fixation index (F_{ST}; Weir and Cockerham 1984) and its confidence intervals (95 and 99 %), determined on the basis of 1000 bootstrapping replicates using FSTAT. The F_{ST} values corrected for null alleles were calculated with the software FreeNA (Chapuis and Estoup 2007). The standardized values of G_{ST}, G'_{ST} (Hedrick 2005) were also calculated. For the estimation of these statistics, the nine groups with sample sizes >10 (i.e., excluding the three small juvenile groups) were used. The genetic relationships among populations were evaluated by generating a Neighbor-joining (NJ) tree based on D_A genetic distances (Nei et al. 1983), using Populations 1.2.30 beta software (Langella 2007). The statistical confidence in the topology of the tree was evaluated by 1000 bootstraps using the same software.

To evaluate whether there was a different pattern in genetic differentiation and admixture among age classes and/or locations, genetic structure was also investigated with the model-based clustering algorithm implemented in the software STRUCTURE v. 2.3.3 (Pritchard et al. 2000; Hubisz et al. 2009). All data were included in the analysis, i.e., all three age classes were considered in each of the four populations. A number of clusters (*K*) varying from 1 to 12, was evaluated under the correlated allele frequencies model by running 100,000 burn-in Markov Chain Monte Carlo (MCMC) repetitions and 100,000 subsequent repetitions. The probabilities of the data for each *K* were averaged over 20 runs. To help determine the optimal *K*, STRUCTURE HARVESTER (Earl and vonHoldt 2012) was employed to visualize the probability of the data for each *K* and to calculate ΔK according to the method described by Evanno et al. (2005). Bar charts representing the proportion of cluster membership in each individual were produced using the software DISTRUCT (Rosenberg 2004).

Spatial genetic structure

Individual-based spatial genetic structure at a fine scale (FSGS) was examined by calculating the multi-locus genotypic distances between individuals (Peakall et al.

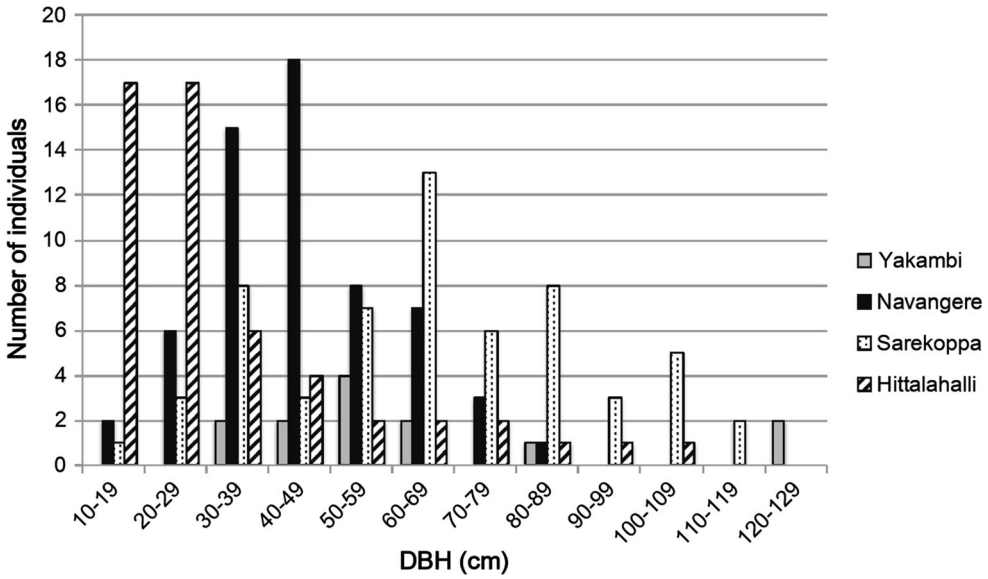

Fig. 3 Distribution of size classes of *D. malabaricum* adult trees at the four locations. Size is given as diameter at breast height (DBH) in cm

1995) and translating them into spatial autocorrelation (r; Smouse and Peakall 1999) based on individual GPS coordinates. The analysis was carried out using GenAlEx 6.4 (hereafter, GenAlEx, Peakall and Smouse 2006). The spatial autocorrelation of each cohort was tested using two approaches. First, the upper and lower 95 % confidence intervals around r were determined with 999 permutations, by the random shuffling of all individuals among their geographic locations. Second, one thousand bootstraps were conducted to define the 95 % confidence interval for each distance class. When the bootstrap confidence interval does not straddle $r = 0$, significant spatial genetic structure is inferred (Peakall and Smouse 2012). The FSGS analysis was conducted for seven groups, excluding the adult age class of Yakambi and the three juvenile groups with low sample sizes. Nine distance classes were retained for the entire dataset and the "even sample size" option in GenAlEx was employed to analyze even sample sizes across the different distance classes. This setting of the FSGS analysis allowed us to retain more than 120 individual-pairs in each distance class in all cohorts (Table S1). To analyze the heterogeneity of the SGS among distance classes, we used the method described by Smouse et al. (2008). Fisher's combined probability criterion, ω, was used as a gauge of the departure of the entire correlogram from the null hypothesis of no spatial structure for any distance class; values were computed using the tail probabilities (P-values) for each distance class. The significance of the criterion was determined on the basis of 999 permutations. The heterogeneity tests were performed using GenAlEx. This heterogeneity test is considered significant when $P < 0.01$, taking into account type I errors as suggested by Banks and Peakall (2012). Furthermore, to quantify and compare the intensity of FSGS among age classes and location, we calculated the Sp statistic: $Sp = -b/(1 - F_1)$ where b is the slope of the regression of multilocus kinship coefficients F_{ij} (Loiselle et al. 1995) on ln(distance), and F_1 is the mean F_{ij} between individuals in the first distance class, following Vekemans and Hardy (2004) and Hardy et al. (2006). The standard error of the Sp values were calculated using the formula: (SE b)/$(1 - F_1)$ where SE b is the standard error of b (Finger et al. 2012). For all datasets we used the distance classes provided by GenAlEx. The Sp statistics were calculated using SPAGeDi 1.4 (hereafter, SPAGeDi, Hardy and Vekemans 2002). Although FSGS was also evaluated based on F_{ij} using Spagedi, since the main results were quite similar to the ones from GenAlEx (result not shown), we simply employed Spagedi to obtain the Sp statistics in this study. In addition, to evaluate SGS at the intermediate landscape level (Manel et al. 2003; Tsuda et al. 2010), SGS analyses and heterogeneity tests (using GenAlEx and SPAGeDi) were conducted between seedlings and adults over a landscape scale of 50 km, pooling samples from the same age class into one dataset. For this landscape scale analysis, five distance classes with 10 km intervals were used for both seedling and adult datasets.

Results

Evaluation of demographic structure and population density

In three out of the four populations (Navangere, Yakambi and Sarekoppa), adult individuals were confined to the mid-range size classes (DBH 30–69 cm) and very few individuals below 30 cm were observed (Fig. 3). Only in

the Hittalahalli population, were young adults more common. Old-growth trees were scarce in all populations and no trees had a DBH above 124 cm (Fig. 3). Mean DBH and SD were 31.9 ± 29.5 cm (Hittalahalli), 45.3 ± 14.9 (Navangere), 64.5 ± 29.5 (Yakambi) and 66.2 ± 24.9 (Sarekoppa). Mean DBH differed significantly between all population pairs except Yakambi-Sarekoppa. In the more southern Kodagu population, the mean DBH was 81.2 ± 31.7 cm (Ismail et al. 2014), significantly larger than in three of the four northern populations examined here (all except Yakambi). The DBH class distribution was similar in the northern and southern populations although more old-growth trees were found in Kodagu (Fig. S1). Patch size ranged from 3.72 ha (Navangere) to 126 ha (Hittalahalli). The density of adult trees (number of individuals per hectare) was 0.40 (Hittalahalli), 2.44 (Yakambi), 3.29 (Sarekoppa) and 16.13 (Navangere) (Table 1). The density of juveniles (number of individuals per hectare) ranged from 0.21 (Hittalahalli) to 0.75 (Yakambi) and was generally low (Table 1).

Genetic diversity within populations and among age classes

Although null alleles were detected by FreeNA at several loci, the frequencies of most of the null alleles were lower than 0.10. In the few cases where higher null allele frequencies were detected, they were population, not locus, specific. Thus, all loci were retained for subsequent analysis and allele frequencies were corrected for null alleles. Linkage disequilibrium between locus pairs was not significant (after Bonferroni correction) in either individual populations or over all samples. Allelic richness and gene diversity were higher in adults than in seedlings at all locations, except for gene diversity in Yakambi (Table 1). When genetic variation was compared between seedlings and adults (averaging by age class across locations), significant differences were detected in allelic richness, observed heterozygosity, F_{IS}, and relatedness ($P < 0.05$, Table 2). Highly positive values of F_{IS} were detected in the adults from three locations (Yakambi, Navangere and Sarekoppa), and the values remained high even after null allele correction in Navangere and Sarekoppa (Table 1). In the BOTTLENECK analysis, the only significant H_E excess ($P < 0.05$) was detected in adults from Hittalahalli (HTA) using the IAM.

Population differentiation and structure

Overall F_{ST} among the nine groups was 0.078, similar to the null allele corrected value (0.075, Table S2). Although the F_{ST} value among seedling populations (0.109) was higher than among adult populations (0.058), the difference was not significant ($P = 0.061$, Table 2). Pairwise F_{ST} values were significant in all population pairs except for a few comparisons between age class groups within the same location: adults-seedlings in Yakambi and all age classes in Hittalahalli. The overall value of G'_{ST} was 0.233. The NJ tree showed clear genetic differentiation among the four locations and genetic similarity among age classes within locations. Each node was well supported by bootstrap values higher than 95 % (Fig. 4a). In the STRUCTURE analysis, the mean probability of the data (LnP(D)) increased steadily up to $K = 4$ (Fig. 4b). ΔK suggested $K = 2$ as optimal, although it peaked also for $K = 4$ (Fig. 4b). At $K = 2$, all age classes for Yakambi and Hittalahalli formed one cluster and Sarekoppa and Navangere formed the other. At $K = 4$, each geographical location formed a separate cluster containing all their respective age classes with some degree of admixture, most notably in the adults of Sarekoppa (Fig. 4c).

Spatial genetic structure

The 95 % CIs of the FSGS analysis revealed significant positive autocorrelations at shorter distance classes for all seedling groups except Navangere (Fig. 5). This pattern was not detected in any of the four adult groups (Fig. 5). In addition, the heterogeneity tests based on ω showed that the correlograms of all seedling groups except Navangere were significant ($P < 0.01$), but significant ω values were not detected in adults (Fig. 5). Similarly, although a higher *Sp* value was detected in seedlings than adults in Hittalahali, (*Sp*, 0.0003 ± 0.022SE in seedlings and −0.0167 ± 0.0052SE in adults), the *Sp* values were generally low in all datasets and the difference of *Sp* values among seedlings and adults did not show any trend

Table 2 Group comparison of genetic variation between seedlings and adults evaluated for allelic richness, observed heterozygosity (H_O), gene diversity (h), fixation index (F_{IS}), relatedness and F_{ST}

Age class	Allelic richness	H_O	h	F_{IS}	Relatedness	F_{ST}
Seedlings	4.409	0.540	0.582	0.072	0.185	0.109
Adults	4.912	0.415	0.620	0.331	0.085	0.058
P-value	0.034	0.020	0.156	0.016	0.045	0.061

P-values are based on the permutation test implemented in FSTAT

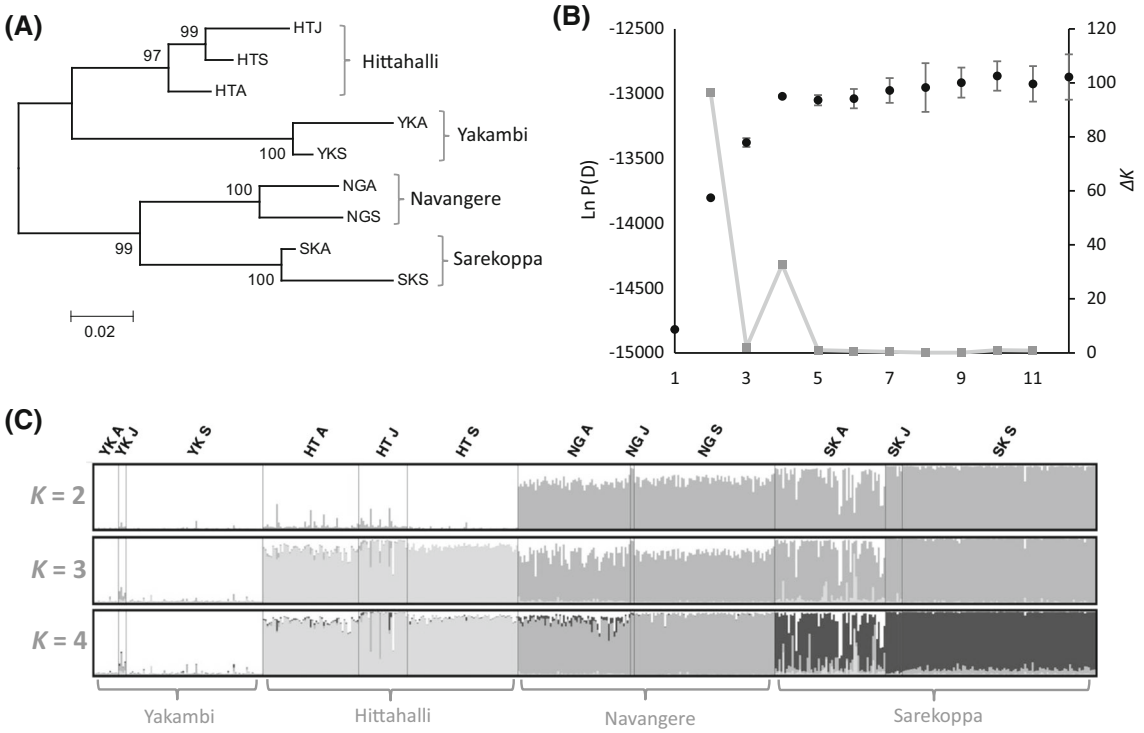

Fig. 4 **a** Neighbor-joining (NJ) tree of 9 cohorts based on D_A distance (Nei et al. 1983).**b** The values of LnP (**d**) from 20 runs for each value of K (1–12; *left axis*) and ΔK (*right axis*). **c** Ancestry estimates from STRUCTURE for $K = 2$ through $K = 4$. Each K is represented by the STRUCTURE run with the highest likelihood and shows the individual's estimated proportion of membership to each cluster. Abbreviations for population codes are as in Table 1

(Table 1). More pronounced spatial genetic patterns were detected in both seedlings and adults at the landscape level (up to 50 km), with significant positive autocorrelations at the 1st distance class (10 km) and significant negative autocorrelations from the 2nd or 3rd distance classes to the 5th distance class. The ω criterion revealed that correlograms for both seedlings and adults were significant ($P < 0.001$, Fig. 6). The *Sp* value for seedlings (0.0363 ± 0.0107SE) was higher than adults (0.0154 ± 0.0024SE) at the landscape scale.

Discussion

Comparison of genetic diversity among age classes

Allelic richness and gene diversity were similar across the four locations for any given age class. This is in line with the lack of large-scale geographical pattern previously detected for these two diversity parameters (Bodare et al. 2013). However, allelic richness was significantly lower in seedlings than in adults, while gene diversity did not show a significant difference among age classes. As allelic richness is reduced faster than gene diversity during a bottleneck (Nei et al. 1975; Maruyama and Fuerst 1985), this might be the first signal of the impact of a recent or ongoing bottleneck caused by fragmentation and disturbance, although the BOTTLENECK analysis only found evidence for a bottleneck in adults from one population. F_{IS} was significantly higher in adults than in seedlings. This pattern remained even after correction for null alleles in the adults of two of the four populations (Navangere and Sarekoppa), suggesting a deviation from Hardy–Weinberg equilibrium (HWE). In contrast to this result, many studies on tropical trees have demonstrated that effective selection against inbred offspring at very early life stages can lead to similarly low levels of inbreeding in seedlings and adult trees (e.g. Hufford and Hamrick 2003; Naito et al. 2005). Marginal populations of widespread species are often expected to be more strongly affected by genetic drift than central ones and this could lead to decreased genetic diversity and increased inbreeding and genetic differentiation (Hampe and Petit 2005; Eckert et al. 2008). Indeed, this pattern has been detected in empirical data (e.g. Arnaud-Haond et al. 2006). The significant excess of H_E detected in adults in Hittalahali could be due to a significant departure from mutation-drift equilibrium (MDE) in this population. The marginality of our four study

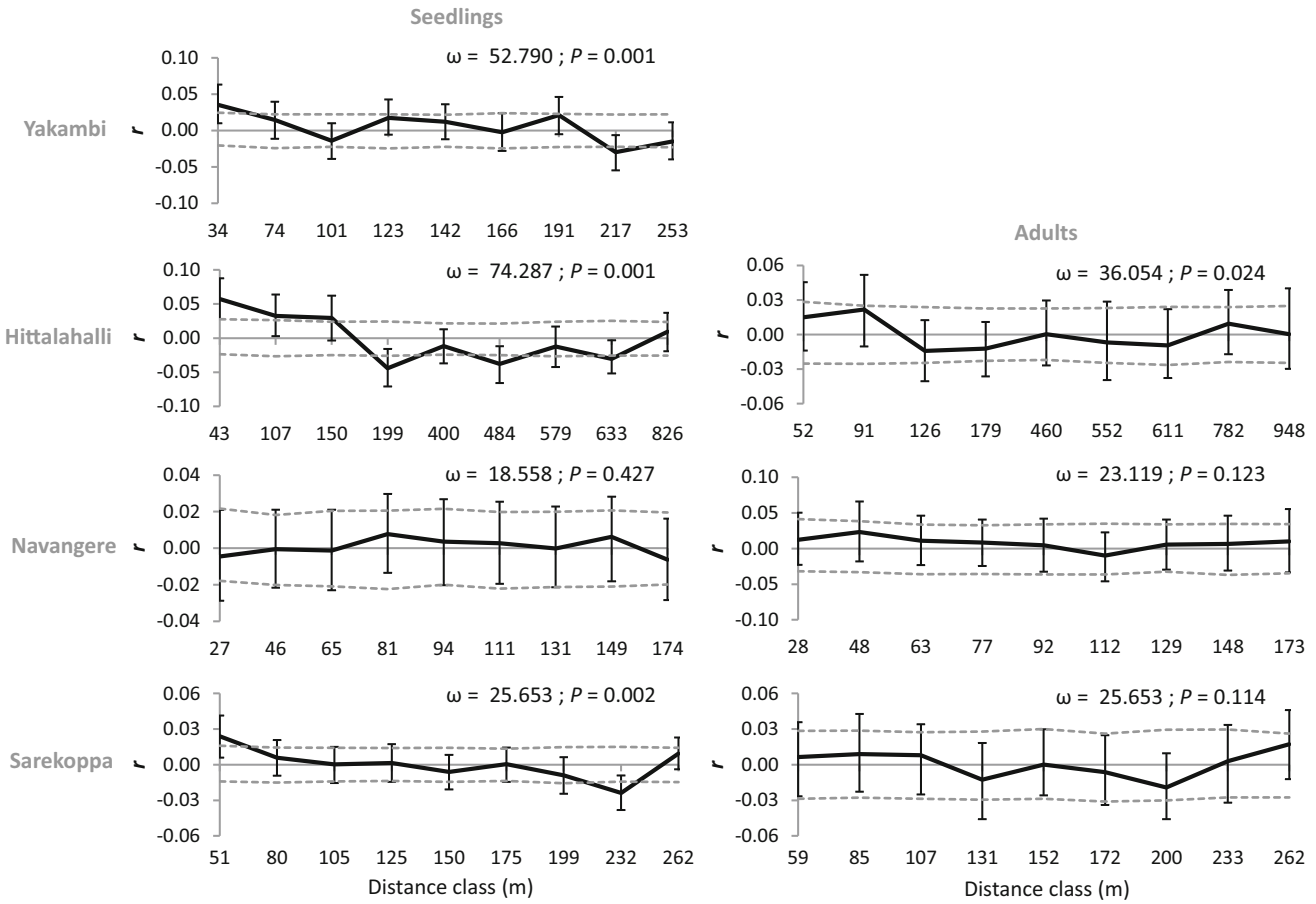

Fig. 5 Fine-scale spatial genetic structure estimated by the autocorrelation coefficient, *r* (Smouse and Peakall 1999). Upper and lower error bars bound the 95 % confidence interval about *r* as determined by bootstrap resampling. Upper and lower confidence limits bound the 95 % confidence interval about the null hypothesis of no spatial structure for the combined data set as determined by permutation

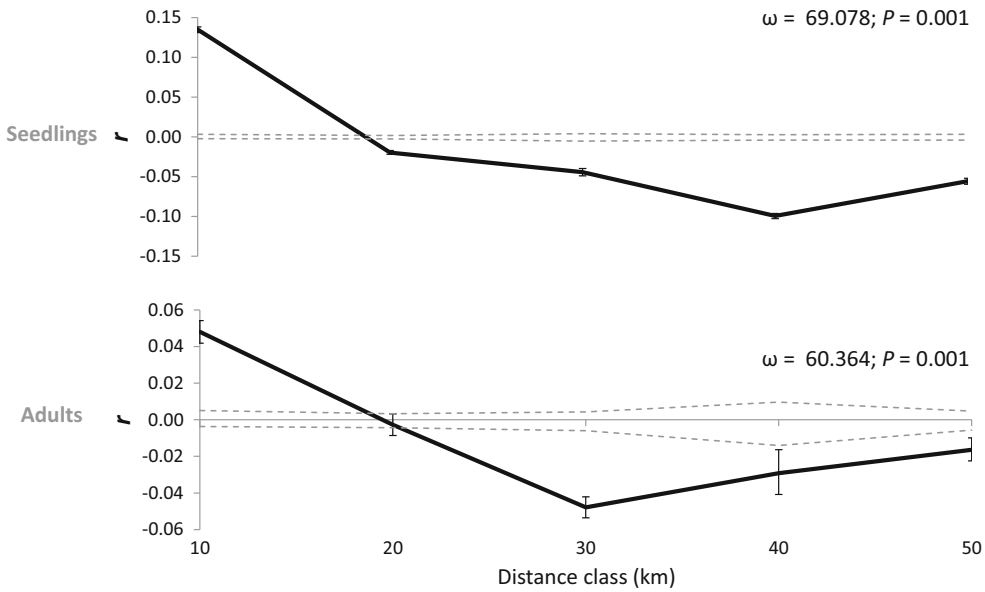

Fig. 6 Landscape spatial genetic structure estimated by the autocorrelation coefficient, *r* (Smouse and Peakall 1999)

populations, located at the northern edge of the species' distribution range, could possibly explain the observed departures from HWE and MDE. However, if so, we would expect to observe higher F_{IS} values or significant bottlenecks in all four locations. Thus, although the marginality of the populations could still be a contributing factor, local factors like past demographic events, i.e., a Wahlund effect, are also plausible. This may be particularly true in Sarekoppa, where some amount of admixture was detected in adults but not in juveniles and seedlings (STRUCTURE analysis, Fig. 4c). In Navangere, adults also showed some amount of admixture, but little admixture was detected in seedlings (for $K = 4$). This admixture in adult classes could be generated by gene flow among populations, suggesting a higher connectivity among populations in the past. Thus, substructure within adults could be one of the reasons for the high F_{IS} value in adults. Similarly, Mathiasen et al. (2007) detected higher values of F_{IS} in adults than seedlings of *Embothirum coccineum*, a tree species in fragmented forests in Chile, and suggested that Wahlund effects prior to fragmentation could explain this pattern.

Fine scale genetic structure in seedlings but not in adults

The general pattern detected in this study shows that seedlings are genetically more structured than adults. Significant FSGS, significant positive autocorrelations at the shortest distance classes and significant correlograms, was observed for seedlings but not adults, in all locations except Navangere. As discussed above, the density of adults was quite high and the patch size small in Navangere, so gene dispersal via both pollen and seeds seems to occur randomly in this small area and thus, FSGS might not be generated in seedlings. In support of the FSGS analysis, the mean relatedness of seedling populations was significantly higher than that of adult populations, and a more pronounced SGS in seedlings compared to adults was also clear at the landscape level. A similar pattern was detected by Ismail et al. (2012) in the Southern region (Kodagu area), where over a 216 km^2 area, seedlings showed a higher *Sp* value than adults (0.0238 and 0.0107, respectively). As the same pattern was observed in two geographical areas with different landscape characteristics, this pattern could be a common phenomenon occurring naturally, regardless of the level of anthropogenic disturbance. One aspect in common to both areas, which obviously affects gene flow and population genetic structure, is the mode of seed dispersal. *Dysoxylum malabaricum* seeds are primarily dispersed by hornbills. Field observations in the studied area (Vasudeva R, pers. observation) indicate that hornbills pick seeds one by one, ingest their lipid-rich seed coat and regurgitate the seeds, without the seed coat, nearby. When a group of birds visit a tree, the seeds get dispersed around the mother tree in a clumped manner (Manjunath 2003). Greenhouse experiments have demonstrated that seeds with intact coats tend to rot and fail to germinate (Shivanna et al. 2003). Restricted and clumped seed dispersal could therefore explain the presence of FSGS at small scales in seedlings. Moreover, restricted pollination due to habitat fragmentation might also promote FSGS. The lack of FSGS among adult trees could be due to severe mortality in sibs that are dispersed in aggregation (e.g. clumped seeds), reducing the pairwise relatedness at short distances. The observed sharp decrease of seedling to juvenile abundances would support this hypothesis, although it should be noted that current mortality rates may likely go beyond those of the historical, sustainable populations. Such density-dependent mortality due to predation, diseases or competition was initially proposed by the Janzen-Connell hypothesis (Janzen 1970; Connell 1971) to explain the diversity of tree species in tropical forests, but has also been shown to offer a good explanation for the spatial genetic structure of individual tree species in temperate forests (e.g. Steinitz et al. 2011).

We acknowledge that sample sizes and the number of loci examined can influence SGS analyses. Hardy and Vekemans (2002, the manual of SPAGeDi) recommended employing more than 100 individual-pairs in each distance class. In addition, Cavers et al. (2005) studied an optimal sampling strategy based on simulations and recommended employing at least one hundred individuals with 10 SSR loci. Jump and Peñuelas (2007) discussed that 150–200 individuals and six SSR loci might be too few to provide a good estimation of SGS in European beech (*Fagus sylvatica*). On the other hand, Epperson (2005) suggested a minimum sample size of 50, and noted that it might be adequate for most purposes if the values of the number of individuals (n) $\times$ total number of allele across loci (k) are over two thousand (Epperson and Li 1996). In this study, as we examined more than 120 individual-pairs in each distance class in all cohorts, the sample size in each distance class is adequate for SGS analysis according to Hardy and Vekemans (2002). But the number of individuals in each cohort ($n = 51$–103) in this study was lower than the recommendation by Cavers et al. (2005). However, their re-sampled data of 50 individuals with 10 SSR loci, similar to this study, still showed a correlation (ca. 0.7) with the "real data" (even though they considered a correlation threshold of 0.9). In addition, the nk values of our data range from 3825 (51 individuals with 75 alleles in the adult class of Hittalahali) to 7519 (103 individuals with 73 alleles in the seedling class of Sarekoppa) and thus, are within the recommendations of Epperson and Li (1996) and Epperson (2005). Therefore, although we need to be aware of several sources of bias in SGS analysis, our

sample sizes should be adequate to determine SGS patterns and be informative for understanding the ecology and evolution of *D. malarbaricum*.

Strong population structure at the landscape level

Considering that G'_{ST} was 0.33 over the whole species range of 730 km in Bodare et al. (2013), the corresponding value of 0.23 in this study over ca 50 km is relatively high. The STRUCTURE analysis also supported a clear genetic clustering of populations which, for $K = 2$, was the same as in the range-wide survey by Bodare et al. (2013), with Yakambi and Hittalahalli, and Navangere and Sarekoppa belonging to different clusters. As previously observed (Fogelqvist et al. 2010), more intense sampling can unravel further clustering; in the present study the four populations constituted four separate clusters (at $K = 4$), whereas with the smaller sample sizes of our previous study (Bodare et al. 2013), only two clusters were detected. The strong genetic structure among the four locations, but not among age classes, was also clearly shown by the NJ tree. This indicates that seed and pollen dispersal dynamics, in the examined landscape in this study, are more limited than previously inferred from the range-wide study (Bodare et al. 2013), where geographical gaps of 30–40 km did not constitute reproductive barriers; supporting results from a local study in the southern part of the species range (Ismail et al. 2012), which showed a low frequency of long-distance pollen flow up to 24 km.

Demographic patterns and the impact of harvesting pressure on DBH distributions

This study and Ismail et al. (2014) found similar demographic patterns, regarding the absence of juveniles and small-sized trees below 10 cm DBH. The density of juveniles was less than one individual per hectare in any location. This demographic imbalance indicates recruitment failure from the early life stages into the adult life stage. If recruitment success in this species cannot be increased, the species will likely go extinct in such fragmented landscapes. The direct cause of the lack of juveniles and young adults is not known, but could be due to seedling mortality caused by decreased canopy cover after logging, increased competition from early successional species (Ismail et al. 2014), or pests and predation. This recruitment failure contributes to the commonly observed shifts in tree species compositions after fragmentation, where late successional tree species are replaced by early successional species (Tabarelli et al. 2012).

Mean DBH values in the examined locations ranged from 31.9 (Hittalahalli) to 66.2 cm (Sarekoppa) and these values correspond to ages of at least 43 and 89 years, respectively. In contrast, the mean DBH of adults in the southern population, Kodagu was 81.2 ± 31.7 cm (Ismail et al. 2014), corresponding to at least 110 years, larger and older than in any of the four northern populations. Moreover, although frequencies were low, older and larger *D. malabaricum* trees whose DBH ranged from 130 to 199 cm were still found in the Kodagu population (Ismail et al. 2014, Fig. S1), while in the present study no large size classes were observed. The difference in the distributions of DBH between regions can be explained by a difference in the treatments of the sacred groves and forest reserve patches by local people. In India, sacred groves are traditionally managed forest patches that functionally link social life and forest management systems of a region, and play an important role in the conservation of local biodiversity (Boraiah et al. 2003; Bhagwat et al. 2005). However, the traditions and spiritual focus of the local people have been changing together with land use (Chandrakanth et al. 2004). In fact, the time scale of the age of the examined adult populations in this study roughly coincides with the period of rapid expansion of agriculture and land use related to the independence of India in 1947. Moreover, the DBH of more than half of the adults were between 30 and 49 cm in Navangere, suggesting that this adult cohort was established over a limited area and in a rather short period of time. These results suggest an episode of human-mediated vegetation change by intense logging in the last several decades. As mentioned in the introduction, sacred trees such as *D. malabaricum* have recently been harvested more extensively in Northern Karnataka, within the examined area of this study. In contrast, although there are exceptions, valuable trees in sacred groves in Kodagu are predominantly harvested by local temple committees with the permission of the Forest Department. Moreover, allelic richness values were quite similar among age classes in sacred groves in this area (Ismail et al. 2012). Therefore, this management practice, which is still adopted in the Southern Western Ghats, may have contributed to not only preserving large individual trees but also in maintaining the genetic diversity of the species. Thus, the traditional restrictions on harvesting *D. malarbaricum* only for temples have likely helped to maintain a larger span of overlapping generations and consequently higher genetic diversity. This finding implies that the common practice of harvesting all large trees based on size thresholds might undermine long term persistence of economically and ecologically important old growth tree species, especially in small fragmented tree populations.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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