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RESEARCH ARTICLE

Genetic structure of the rattan *Calamus thwaitesii* in core, buffer and peripheral regions of three protected areas in central Western Ghats, India: do protected areas serve as refugia for genetic resources of economically important plants?

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Abstract

Given the increasing anthropogenic pressures on forests, the various protected areas—national parks, sanctuaries, and biosphere reserves—serve as the last footholds for conserving biological diversity. However, because protected areas are often targeted for the conservation of selected species, particularly charismatic animals, concerns have been raised about their effectiveness in conserving nontarget taxa and their genetic resources. In this paper, we evaluate whether protected areas can serve as refugia for genetic resources of economically important plants that are threatened due to extraction pressures. We examine the population structure and genetic diversity of an economically important rattan, *Calamus thwaitesii*, in the core, buffer and peripheral regions of three protected areas in the central Western Ghats, southern India. Our results indicate that in all the three protected areas, the core and buffer regions maintain a better population structure, as well as higher genetic diversity, than the peripheral regions of the protected area. Thus, despite the escalating pressures of extraction, the protected areas are effective in conserving the genetic resources of rattan. These results underscore the importance of protected areas in conservation of nontarget species and emphasize the need to further strengthen the protected-area network to offer refugia for economically important plant species.

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Introduction

Protected areas (PAs) are believed to be the cornerstones for biodiversity conservation, and the safest wilderness strongholds around the globe (Pimm and Lawton 1998; Bruner *et al*. 2001). In the face of ever-increasing threats to forests, the network of PAs offers the best possible approach to conserve biological diversity and the genetic resources of economically important species (Hogbin *et al*. 2000; Woodford 2000; Bruner *et al*. 2001; Theilade *et al*. 2001). For example, in Thailand, a large number of important timber species, which have been extensively harvested from the native forests, are now found only in PAs (Changtragoon 2001). Nevertheless, several concerns have been raised about the effectiveness of PAs in conservation. For example, PAs are often established based on the presence of large charismatic mammal species and, hence, might not address the conservation concerns of nontarget taxa (Rodgers and Panwar 1988). It has also been argued that PAs (a) are generally too small to

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host viable populations, (b) act as insular and isolated habitats that do not allow gene mixing across populations, and (c) can be costly and demanding in terms of logistics to secure from extraneous pressures and human encroachment (Chapman *et al*. 2003; Uma Shaanker *et al*. 2003).

In recent years, several attempts have been made to identify the gaps, if any, and determine the effectiveness of PAs in conserving biological diversity (Wickneshwari and Boyle 2000; Kutty and Kothari 2001; Nageswara Rao *et al*. 2001a; Uma Shaanker *et al*. 2003). Nageswara Rao *et al*. (2001b) showed that populations of sandal, a tree treasured for its heartwood oil in India and has been extensively poached, have higher genetic diversity in PAs such as national parks and sanctuaries than outside.

In this paper, we examine the effectiveness of PAs in conserving the genetic resources of a species of rattan (trailing or climbing palms) in the Western Ghats, a megadiversity centre in South India. Popularly also known as canes, rattans are used in the furniture industry and form an important component of the livelihood of forest-fringe communities in South and South-East Asia. A number of economically important species of rattans are extensively harvested, often leading to a severe lack of regeneration (Ravikanth *et al*. 2002; Narwade *et al*. 2003). We compared the population structure and genetic diversity of *Calamus thwaitesii*, an economically important cane, occurring in core, buffer and peripheral regions of three PAs in the central Western Ghats, India. The results show that PAs can play a significant role in conservation of nontarget species, particularly economically important species. Based on the results, we discuss the role of PAs in conserving the genetic resources of rattans in particular, and of other nontimber forest product species in general, and argue that PAs could form an important conservation approach for species that are highly threatened.

Materials and methods

Study sites and design

The study was conducted in the Western Ghats, India, one of the 25 biodiversity hotspots of the world (Myers *et al*. 2000). The PAs in the country comprise about 4.6% of the total geographical area (NWDC 1999). The Western Ghats includes 34 national parks and wildlife sanctuaries, together covering about 7300 km^2 (NWDC 1999). Based on the availability of rattan species within the PA network of the Western Ghats, three study sites, namely Anshi National Park (ANP; 14°54′ to 15°07′N latitude and 74°16′ to 74°30′E longitude), Kollur Mookambika Wildlife Sanctuary (KWS; 13°54′ to 13°38′N latitude and 74°38′ to 74°56′E longitude), and Sharavathi Wildlife Sanctuary (SWS; 13°54′ to 14°12′N latitude and 74°38′ to 75°00′E longitude) were selected for the study (figure 1). The two wildlife sanctuaries (Kollur Mookambika and Sharavathi) were established in the year 1974, and the Anshi National Park was established in 1987 (Kutty and Kothari 2001). However, much before it was declared as a

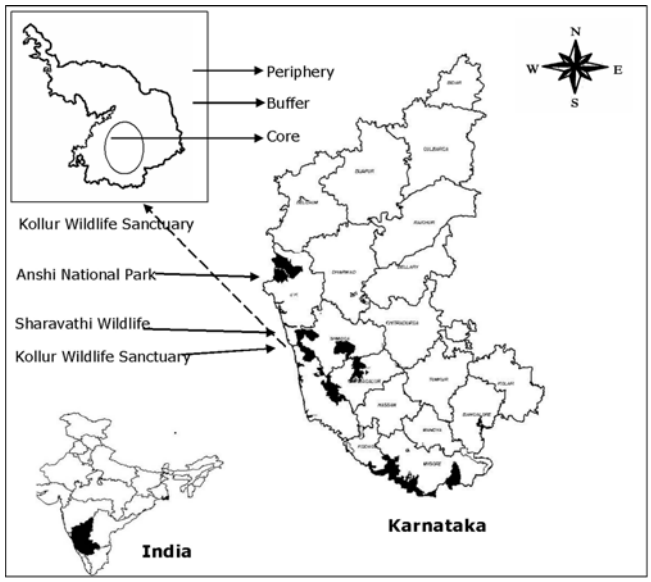

Figure 1. Map of the study sites at the central Western Ghats, India. The protected areas are all located in the central Western Ghats in the state of Karnataka (shaded). The arrows indicate the three protected areas studied.

protected area, ANP was part of the Dandeli Wildlife Sanctuary that was also established in the year 1974.

Extraction of rattans has preceded the formation of the PAs and our own studies indicate that, in most parts of the Western Ghats, extraction of rattans has been recorded by the forest departments since the last 70 years (Uma Shaanker *et al*. 2004c). Harvesting of rattans is done each year. Based on the administrative boundary of each of the PAs, three zones, namely core, buffer and periphery, were identified, radiating outwards from the PA. Although PAs are supposed to be insulated from humans and cattle, often the peripheral and buffer regions tend to be more open to disturbance than the core of the PA because of the heavy pressures. Thus, the zones from core to periphery offer decreasing levels of protection against disturbance due to human interferences, cattle grazing, etc. As a measure of the extent of disturbance in each of the zones, the percentage of rattan stems harvested or cut was recorded (see details below). In each PA, the three zones were separated by approximately 2 to 3 km in a linear direction (core to buffer to periphery).

Study species

The study was carried out on an economically important species of rattan, *Calamus thwaitesii*, distributed in the Western Ghats (Lakshmana 1993; Ravikanth *et al*. 2002). This rattan is extensively used in the manufacture of furniture, umbrella handles, walking sticks, and sports goods, and forms the predominant rattan extracted from the Western Ghats (Lakshmana 1993; Renuka 1999; Ravikanth *et al*. 2001, 2002). The rattan is dioecious, and flowers between October and January, and fruits between April and May. Beetles and bees are the major pollinators of this species, whereas birds,

herbivores and water are the major seed dispersal agents. Propagation of the rattan is entirely by seeds. The rattans take an average of 10–14 years to flower and fruit. Harvestable canes of length 4 to 6 metres feet are obtained in 8–10 years. The species is restricted to the mid-altitude and wetter zones of the Western Ghats (Lakshmana 1993; Uma Shaanker *et al*. 2004c).

Population structure

In each of the three zones within a given PA, 10 quadrats (10 m × 10 m) were laid out randomly, and data on the number of clumps per quadrat, number of culms per clump, height of all individuals >1 m, girth of all individuals >3 m tall at collar region, number of flowering individuals, and number of culms harvested were recorded. The height of the culms was estimated visually.

As an index of regeneration, the numbers of seedlings and saplings (<1 m height) were recorded in each of the quadrats. For each quadrat, the number of regenerants was divided by the number of adults to obtain an index of the regeneration per adult. The numbers of cut and broken stems of *C. thwaitesii* were recorded and expressed as a percentage of the total number of stems harvested for each quadrat (Ganeshaiah *et al*. 1998; Uma Shaanker *et al*. 2004b). The differences in the various parameters across the three zones (core, buffer and periphery) were analysed using Student's *t*-test and analysis of variance (ANOVA). All the parameters with per cent values were arcsine transformed before subjecting to statistical analysis.

The height-class and girth-class distributions of the individual stems in each zone were determined. The frequency distribution of the height-class of stems across the three zones within a PA was statistically evaluated using the non-parametric Kolmogorov–Smirnov test (Siegel and Castellan 1988).

Genetic variability

DNA extraction and PCR protocol: Leaf samples were collected from 15 (or as many as could be found, whichever was lower) randomly selected individuals from the core, buffer and peripheral regions in each of the three PAs. In each zone, the area sampled was approximately 0.05 km^2. Samples were air-dried and preserved in silica gel until DNA isolation. Total genomic DNA was extracted using a modified CTAB (cetyltrimethylammonium bromide) protocol (Doyle and Doyle 1987). The DNA was quantified spectrophotometrically at 260 nm and visualized by ethidium bromide staining on a 0.8% agarose gel. The DNA was then diluted to a concentration of 10 ng/μl. PCR amplification was carried out in a 25-μl-volume reaction mixture containing 25 ng template DNA, 2.5 μl 10× reaction buffer containing 15 μM $MgCl_2$, 3 μM of each dNTP, 0.25 μM primer and 0.5 unit *Taq* DNA polymerase (Bangalore Genei, India).

PCR was conducted using intersimple sequence repeat (ISSR) primers. In recent years, ISSRs have been widely used to investigate clonal diversity and population genetic structure (Zietkiewicz *et al*. 1994; Tani *et al*. 1998; Wolfe and Liston 1998; Esselman *et al*. 1999; Rossetto *et al*. 1999). As a PCR-based marker, ISSRs have several advantages over other, conventional markers. ISSR primers anneal directly to simple sequence repeats and, thus, unlike SSR markers, no prior knowledge of target sequences is required for ISSRs (Godwin *et al*. 1997). Moreover, the sequences that ISSRs target are abundant throughout eukaryotic genomes and evolve rapidly. Consequently ISSRs may reveal high number of polymorphic fragments per primer (Fang and Roose 1997; Esselman *et al*. 1999). In addition, studies have indicated that ISSRs produce reliable and reproducible bands because of the higher annealing temperature and longer sequence of ISSR primers (Tsumura *et al*. 1996; Nagaoka and Ogihara 1997; Qian *et al*. 2001).

Twentyfive ISSR primers from the UBC set (Sigma, USA) were screened, and finally 10 of them (UBC 890, UBC 841, UBC 835, UBC 834, UBC 868, UBC 880, UBC 855, UBC 848, ISSR 4, ISSR 5) were used in the PCR amplification. The thermocycler program for PCR was set for 3 min at 94°C, followed by 35 cycles of 45 s at 94°C, 45 s annealing at 45°C and 2 min extension at 72°C, and a final extension cycle of 8 min at 72°C. Amplification products were resolved electrophoretically on 1.5% agarose gels in 1× TBE (pH 8.0) buffer by loading the entire reaction volume into prepared wells. Gels were run until a bromophenol blue indicator dye ran 10 cm from the well. Gels were stained with ethidium bromide, and bands were visualized and photographed under UV light.

Data analysis: The ISSR-amplified products were scored as either present (1) or absent (0) (Wendel and Weeden 1989) and analysis was done using the population-genetic software POPGENE version 1.32 (Yeh and Boyle 1997). Population-genetic parameters such as per cent polymorphism (an amplified product was considered polymorphic if the frequency of the most frequent ISSR-amplified product was <95%) and Nei (1973) gene diversity were computed.

For each population collected in individual zones, Dice dissimilarity index based on the amplification products was computed (Sorenson 1948; Deshpande *et al*. 2001). Dice dissimilarity matrix was generated by computing the Dice dissimilarity coefficient as $1 - (2N_{ab}/N_a + N_b)$, where N_a is the total number of bands present in lane a, N_b the total number of bands in lane b, and N_{ab} the number of bands common to lanes a and b (Nei and Li 1979). The Dice coefficients were used for UPGMA analysis and to construct a cluster diagram. To evaluate the robustness of the grouping formed, the binary data matrix was subjected to bootstrapping using the WinBoot program (Yap and Nelson 1996). The phenogram was reconstructed 1000 times by repeating sampling with replacement, and the frequency with which the groups were formed was used to indicate the strength of the group.

The frequency distribution of the Dice dissimilarity index was developed for each zone within a given PA, and compared across zones by a Kolmogorov–Smirnov test (Siegel and Castellan 1988). A two-way ANOVA was performed to analyse differences in the dissimilarity indices among zones (core, buffer and periphery) within the three study sites.

Results

ISSR PCR products

Over the three sites, amplification products from 10 ISSR primers (UBC 890, UBC 841, UBC 835, UBC 834, UBC 868, UBC 880, UBC 855, UBC 848, ISSR 4 and ISSR 5) were analysed. The number of bands scored and the per cent polymorphism obtained for the various populations across the sites is given in table 1. On an average, over the 10 primers, about 45% of the 93 to 109 amplification products were polymorphic. Among the three sites, site-specific ISSR amplification products were obtained for Anshi National Park (ANP) and Kollur Wildlife Sanctuary (KWS) only. The Sharavathi Wildlife Sanctuary (SWS) had no site-specific amplification (table 2).

Table 1. Number of bands scored and per cent polymorphism over the 10 ISSR primers used in *Calamus thwaitesii*.

Site	Zone	No. of bands scored	% Polymorphism
ANP	Core	109	47.71
	Buffer	109	39.45
	Periphery	109	42.20
KWS	Core	95	46.32
	Buffer	95	44.21
	Periphery	95	43.16
SWS	Core	93	47.31
	Buffer	93	51.61
	Periphery	93	45.16

Table 2. Site-specific ISSR product amplification in *Calamus thwaitesii*, and their frequencies in core, buffer and peripheral zones of the protected areas.

Site	Primer	Band	Core	Buffer	Periphery
ANP	UBC880	1	0.071	0	0
	UBC848	2	0	0.2143	0
KWS	UBC841	1	0	0	0.21429
	UBC868	2	0.0833	0	0
	UBC848	3	0	0.1429	0
	UBC848	4	0	0	0.21429
	UBC848	5	0	0	0.14286
	UBC848	6	0	0	0.42857
	UBC890		0	0.2857	0
SWS		0	0	0	0

Table 3. Population parameters of *Calamus thwaitesii* in core, buffer and peripheral zones of the three protected areas in central Western Ghats, India.

Parameter	Zone	ANP Mean±SD	KWS Mean±SD	SWS Mean±SD	All 3 sites Mean±SD
Mean regenerants per quadrat	Core	5.4±3.29^{a}	13.5±7.79^{a}	0.2±0.42^{ab}	6.1±6.5^{a}
	Buffer	1.0±1.25^{b}	4.7±5.35^{b}	0.7±0.82^{a}	1.7±3.2^{b}
	Periphery	0.95±0.85^{b}	0.4±0.84^{c}	0.1±0.31^{b}	0.57±0.84^{c}
Mean regenerants per adult	Core	2.55±2.17^{a}	2.75±2.49^{a}	0.1±0.31^{a}	1.99±2.23^{a}
	Buffer	0.64±1.02^{b}	1.50±2.20^{ab}	0.6±0.84^{a}	0.84±1.42^{b}
	Periphery	0.2±0.61^{b}	0.27±0.64^{b}	0.1±0.31^{a}	0.19±0.55^{c}
Mean density (clumps per 100 sq.m)	Core	5.5±2.01^{a}	14.3±5.27^{a}	3.1±2.33^{a}	7.52±4.95^{a}
	Buffer	2.35±0.87^{b}	9.9±5.21^{b}	2.7±1.56^{a}	4.3±4.22^{b}
	Periphery	2.4±1.66^{b}	2.5±1.26^{c}	1.4±1.5^{a}	2.4±1.49^{c}
Mean culms per clump	Core	4.02±1.65^{a}	8.14±1.23^{a}	2.12±2.29^{a}	4.83±2.4^{a}
	Buffer	3.13±1.17^{b}	5.09±1.73^{b}	2.32±1.37^{a}	3.53±1.69^{ab}
	Periphery	3.05±1.33^{b}	4.76±2.52^{c}	2.0±2.06^{a}	3.38±1.95^{ab}
Proportion of culms harvested	Core	0.05±0.049^{a}	0.01±0.01^{a}	0.12±0.17^{a}	0.043±0.05^{a}
	Buffer	0.086±0.15^{a}	0.02±0.03^{b}	0.10±0.13^{a}	0.089±0.14^{a}
	Periphery	0.069±0.17^{a}	0.10±0.12^{c}	0.18±0.36^{a}	0.11±0.20^{ab}
No. of flowered individuals	Core	ND	0.6±0.84^{a}	ND	
	Buffer	ND	0.1±0.31^{a}	ND	
	Periphery	ND	0.1±0.31^{a}	ND	

For each parameter, values with dissimilar letters in superscript are significantly different at the 0.05 level (*t*-test). ND, Data not recorded. ANP, Anshi National Park; KWS, Kollur Mookambika Wildlife Sanctuary; SWS, Sharavathi Wildlife Sanctuary.

Population structure

In ANP and KWS, the mean density of rattan was highest in the core, followed by that in buffer and periphery populations (*t*-test, $P < 0.05$; table 3). The core populations in ANP and KWS had significantly higher average number of regenerants per adult compared to the buffer and peripheral populations (*t*-test, $P < 0.05$; table 3). Moreover, there was a relatively larger representation at both sites of higher height-class of stems in the core compared to the periphery, indicating a better stand of rattans in general (Kolmogorov–Smirnov test, $P < 0.05$; data not given). In contrast to these two sites, in SWS the population in the buffer zone was found to be the most dense, with a relatively higher regeneration per adult, compared to the populations in the core and peripheral regions (table 3).

Table 4. One-way ANOVA for different population parameters among the three zones (core, buffer and periphery) in all the three protected areas in central Western Ghats, India. For all parameters except per cent harvesting, the absolute values were used. For per cent harvesting, the data was arcsine transformed before analysis.

Population parameter	d.f.	*F*	*P*
Regeneration per adult	2	13.64	0.0001
Per cent harvest	2	1.21	0.300
Density	2	15.06	0.000
Culms per clump	2	4.27	0.016

One-way ANOVA for the various population parameters across the three zones for the three PAs indicated a significant zone effect for density, culms per clump, and regeneration per adult, but not for per cent stems harvested (table 4).

Genetic diversity and structure

Nei's gene diversity, calculated based on the amplified product, did not show significant differences among the zones, although the mean diversity index for both ANP and KWS was highest in the core compared to the buffer and peripheral regions; in SWS, the diversity index was highest for the buffer population (table 5).

The mean Dice dissimilarity index decreased significantly from core to buffer and peripheral populations in ANP and KWS. In SWS, individuals from the core were significantly less dissimilar among themselves compared to those among buffer and peripheral populations. These results were also reflected in the frequency distribution of the Dice dissimilarity indices within the respective zones. In both ANP and KWS, the dissimilarity indices of core population were more widely distributed with a larger mean compared to the population from the periphery (figure 2,a&b). In SWS, there was no significant difference in the frequency distribution between the core and peripheral populations (figure 2,c). Over all the populations, the Dice dissimilarity index decreased significantly from core to periphery. In other words, on an average, the populations in the core of the sanctuary or protected area were genetically more diverse than those in the periphery. A two-way ANOVA on dissimilarity index indicated significant effects of zone, site (PA) ($P = 0.001$) and zone × site interaction ($P = 0.034$) (table 6).

Dendrograms of the clustering of individuals from the three regions within a site indicated that for all the three sites the individuals did not significantly segregate based on the regions from where they were sampled (figure not shown).

Discussion

In many parts of the world, such as in India, forest-dwelling communities depend upon a variety of non-timber forest product (NTFP) species for their livelihood (NCHSE 1987). In these communities, the NTFPs can easily account for about 60% of the cash income of the people (Murali *et al*. 1996; Uma Shaanker *et al*. 2003). Such high level of

Table 5. Mean gene diversity, per cent polymorphism, and mean Dice dissimilarity index of *Calamus thwaitesii* in core, buffer and peripheral zones in three protected areas in central Western Ghats, India.

Site	Zone	*n*	*h* (±SD)	h^* (±SD)	Per cent polymorphism	Mean Dice dissimilarity index (±SD)
ANP	Core	14	0.152 (0.187)^a	0.319 (0.141)^a	47.71	0.096 (0.030)^a
	Buffer	14	0.124 (0.174)^a	0.309 (0.136)^a	39.45	0.074 (0.021)^b
	Periphery	14	0.124 (0.167)^a	0.297 (0.130)^a	42.20	0.075 (0.023)^b
KWS	Core	12	0.148 (0.182)^a	0.319 (0.130)^a	46.32	0.107 (0.002)^a
	Buffer	14	0.137 (0.181)^a	0.311 (0.143)^a	44.21	0.093 (0.007)^b
	Periphery	14	0.137 (0.186)^a	0.318 (0.149)^a	43.16	0.092 (0.000)^b
SWS	Core	14	0.129 (0.163)^a	0.272 (0.132)^a	47.31	0.085 (0.032)^a
	Buffer	14	0.172 (0.186)^b	0.333 (0.115)^b	51.61	0.114 (0.032)^b
	Periphery	14	0.146 (0.183)^{ab}	0.323 (0.131)^{ab}	45.16	0.097 (0.044)^c

n, Number of individuals assessed; *h*, mean gene diversity at all amplified gene products; h^*, mean gene diversity for polymorphic amplified products.
For each parameter, values with dissimilar letters in superscript are significantly different at the 0.05 level (*t*-test).

Table 6. Two-way ANOVA for Dice dissimilarity index across all the zones (core, buffer and periphery) and protected areas.

Source of variation	SS	d.f.	MS	*F*	*P*
Site (protected areas)	0.043	2	0.021	19.75	0.00001
Zone (core, buffer and periphery)	0.007	2	0.003	3.37	0.034
Interaction sites × zones	0.066	4	0.016	15.13	0.000061
Error	0.884	810	0.001		
Total	1.00	818			

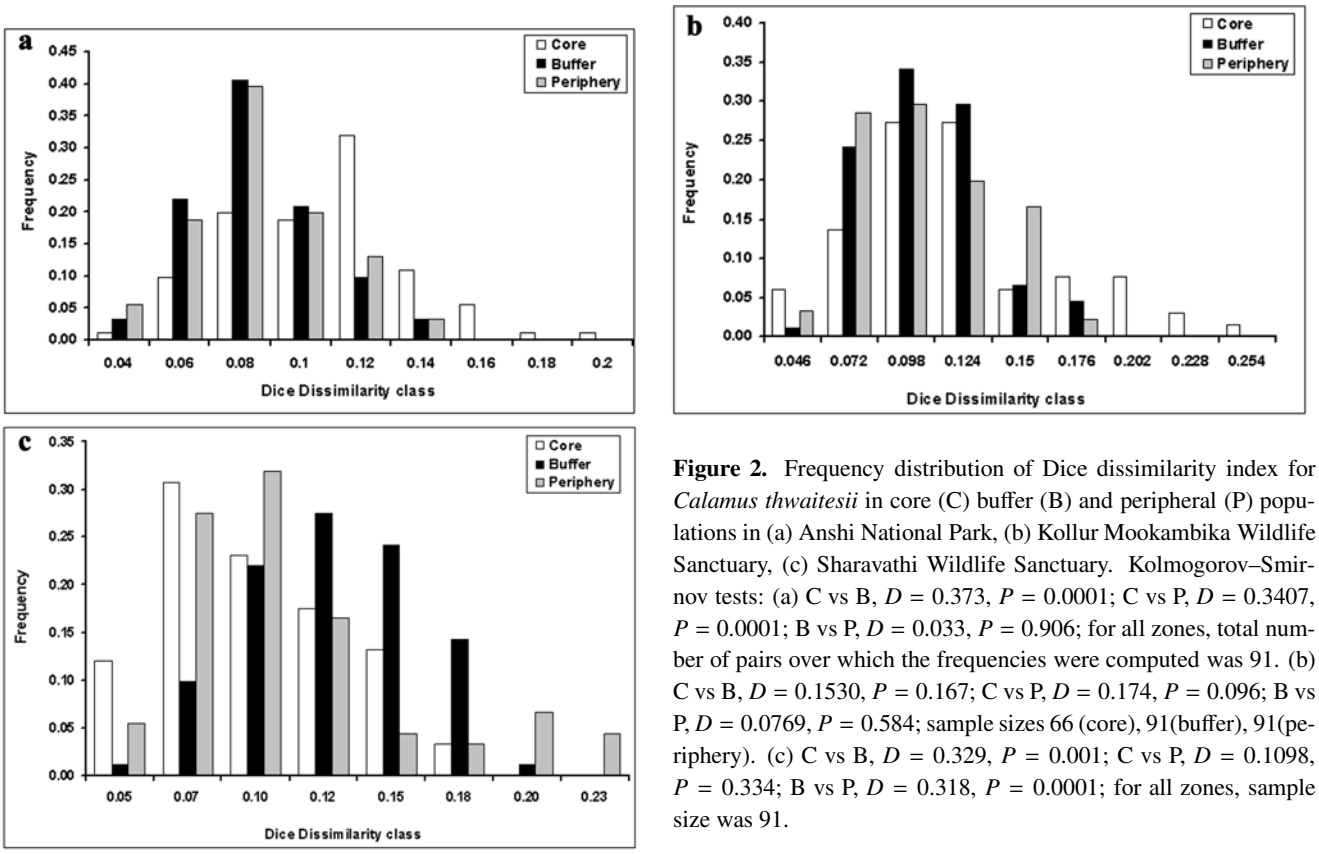

Figure 2. Frequency distribution of Dice dissimilarity index for *Calamus thwaitesii* in core (C) buffer (B) and peripheral (P) populations in (a) Anshi National Park, (b) Kollur Mookambika Wildlife Sanctuary, (c) Sharavathi Wildlife Sanctuary. Kolmogorov–Smirnov tests: (a) C vs B, $D = 0.373$, $P = 0.0001$; C vs P, $D = 0.3407$, $P = 0.0001$; B vs P, $D = 0.033$, $P = 0.906$; for all zones, total number of pairs over which the frequencies were computed was 91. (b) C vs B, $D = 0.1530$, $P = 0.167$; C vs P, $D = 0.174$, $P = 0.096$; B vs P, $D = 0.0769$, $P = 0.584$; sample sizes 66 (core), 91(buffer), 91(periphery). (c) C vs B, $D = 0.329$, $P = 0.001$; C vs P, $D = 0.1098$, $P = 0.334$; B vs P, $D = 0.318$, $P = 0.0001$; for all zones, sample size was 91.

dependency has led to an increasing pressure on the forest resources (Uma Shaanker *et al*. 2004a). Evidence of this threat is already reflected in (a) the dwindling resource status of several economically important plants such as sandal, rattans and bamboos (Chalvaraju *et al*. 2001; Nageswara Rao *et al*. 2001a; Ravikanth *et al*. 2002; Uma Shaanker *et al*. 2004b), (b) the endangerment of populations of several scores of economically important plants, especially the medicinal plants (Ved 2003), and (c) the local extinctions of populations of several species (Ved *et al*. 2001). With increasing population pressures and changes in land use systems, the threat to these species is expected to increase further. Several models and approaches to counter the threats have been proposed and addressed from time to time, including the possibility of (a) an NTFP working plan (to regulate the harvest), (b) models for sustainable harvesting of populations, and (c) community management of forest resources (Uma Shaanker *et al*. 2004b). A major lacuna of many of these approaches, however, is in their implementability and, thus, their lack of effectiveness.

In contrast to these approaches, the formal PA model offers an alternative and perhaps a relatively more robust prescription for averting the threat that many of the NTFP species face in human-dominated forested landscapes (Uma Shaanker *et al*. 2004c). By legislation, PAs are excluded from human interventions and, thus, expected to represent islands of conserved forest genetic resources. In fact, these represent perhaps the frontiers of conservation of genetic and biological diversity in an otherwise human-ravaged landscape (Uma Shaanker *et al*. 2004a). In the recent past, several studies have tried to identify the effectiveness of PAs. A recent study that collated information from over 93 PAs from 22 countries reiterated the usefulness of PAs in conserving biological diversity (Bruner *et al*. 2001).

Few studies, however, have addressed the role of PAs in maintaining the population structure and genetic diversity of focal species that are otherwise subject to intense harvesting pressures (Nageswara Rao *et al*. 2001b). The present study examines the role played by PAs in conserving the population structure and genetic diversity of rattans, an intensively extracted NTFP from the Western Ghats, South India (Ravikanth *et al*. 2002).

Population structure

Our study showed that in two of the three PAs evaluated, critical population parameters are better maintained in the core of the PAs, relative to the buffer and peripheral regions. For example, the mean density of clumps, mean number of regenerants per adult (an index of reproductive turnover), and mean number of culms per clump were all significantly higher in the core zones of ANP and KWS compared to those in the peripheral zones. The results could be attributed to the underlying pattern of differences in the harvesting of canes across the different zones of a PA. The core of the PAs generally experienced less harvesting pressure compared to the buffer or the peripheral regions. This could be due to the fact that the core zone is often further away from human settlements than are the buffer and peripheral regions; consequently, the former are less accessible to human interventions. Our results also indicate that, although the PAs are supposed to be sacrosanct from human interventions in principle, in practice they are not. However, in the context of the overarching pressures that the NTFP resources are generally put to in habitats such as the Western Ghats, it is interesting to note that the core areas of the PAs are still effective in retaining a reasonable population structure compared to the buffer and peripheral regions.

In contrast to ANP and KWS, however, the buffer region of SWS maintained a better population status than did the core and peripheral regions. Interestingly, in this PA the buffer region had the least extent of human pressure with significantly smaller proportion of culms harvested compared to the other two zones. This intriguing deviation from the other two PAs (KWS and ANP) is perhaps due to the large-scale habitat destruction and changes brought in the core zone of SWS following the recent working of the forest for the establishment of a hydroelectric dam (P. Hegde, 'Unceasing struggle to save environment', *Deccan Herald* (Bangalore), 5 June 2003; B. N. Mogata, 'Shrinking green cover', *Deccan Herald* (Bangalore), 14 February 2003). Nonetheless, data from this site also support the notion that the peripheral regions of the PAs are indeed more vulnerable to harvesting pressures (as expected) compared to the buffer and core regions of the PAs. Furthermore, the results from this site signal the need for, and importance of, close monitoring of the PAs for them to be effective in conserving the genetic resources of important NTFP species.

Rearranging the data based on the degree of human impact (per cent cut and broken stems) rather than by region (core, buffer and periphery), we found that for all the three sites, the regions with the least human impacts were the most genetically diverse (figure 3). Though not causal in nature, these results do indicate that disturbance to regions in protected areas could lead to loss of genetic diversity of populations.

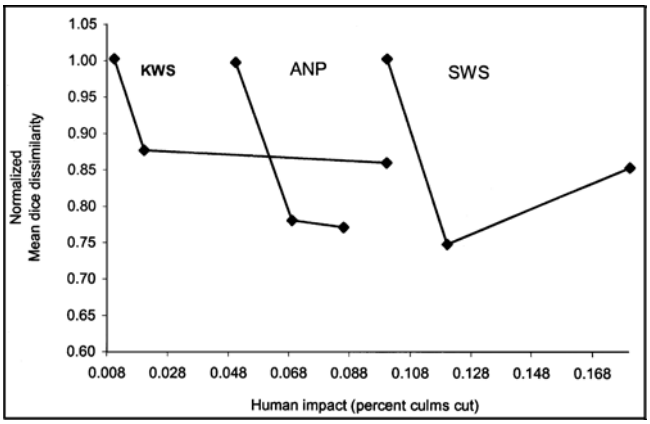

Figure 3. Normalized mean Dice dissimilarity index and human impact (per cent culms cut of *Calamus thwaitesii*) across the core, buffer and peripheral zones in the three protected areas.

Genetic diversity and structure

In agreement with the reduced harvesting pressures and better population stand in the core of the PAs in ANS and KWS, the populations here also had a higher gene diversity index compared to the populations in the buffer and peripheral regions, though the differences were not significant. Dice dissimilarity index computed based on the differences in the ISSR amplification products indicated that the core populations of ANS and KWS were significantly more genetically diverse than were the populations in the respective buffer and peripheral regions of these PAs. Indeed, this result was also indicated by a significant zone × site effect for Dice dissimilarity coefficients. Finally, as obtained for the population parameters, in SWS, the gene diversity and Dice dissimilarity coefficients were higher for the buffer than the core and the peripheral populations.

Thus, from both the population structure and genetic diversity estimates, it appears that, with the exception of SWS, the core regions of the PAs conserve genetic resources of rattans better than do the buffer and peripheral regions. The relatively higher levels of genetic diversity in the core compared to the peripheral regions could be partly attributed to a better population stand in the former. With a significantly lower level of harvesting of rattans in the core than in the periphery, more stems could be expected to contribute to gene flow in the core. In fact, data for KWS indicate that core regions have a greater mean number of flowering individuals than do the buffer and peripheral regions.

The relatively higher densities of rattan in the core compared to the periphery might be due to both a lower level

of disturbance and the intrinsic spatial distribution gradients of the species. If core populations represent the geometrical central populations, they could also be expected to experience a greater gene flow from surrounding populations than would the peripheral populations (Lonn and Prentice 2002). In the present context, it is important to note that the core populations are able to maintain higher densities of the species and, therefore, by default have implications for the maintenance of genetic diversity. Over all PAs, the normalized mean Dice dissimilarity index of the populations was significantly positively correlated with density of rattan clumps (figure 4; $P < 0.05$). Positive correlations between gene diversity and density have earlier been

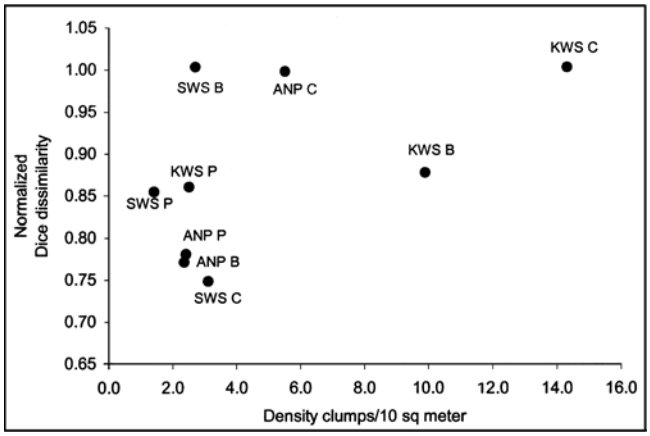

Figure 4. Normalized mean Dice dissimilarity index as a function of density of *Calamus thwaitesii* from all the three protected areas across the core (C), buffer (B) and peripheral (P) zones ($y = 0.0698\ln x + 0.7862$; $R^2 = 0.265$, $P < 0.05$).

reported for several tree species and have underlined the importance of demography in maintaining population genetic variability (Nageswara Rao *et al*. 2001b; Lonn and Prentice 2002). Population density could be especially crucial in determining the levels of gene diversity in rattan, as these are dioecious plants; small populations could constrain mating partners and hence lead to greater levels of inbreeding. The higher genetic diversity in the core could also be expected to result in higher fitness of such populations, compared to those at the periphery, as has been demonstrated in many other systems (Oostermeijer *et al*. 1994). Across the PAs, the regeneration success of rattans (as a measure of fitness) was found to be positively correlated with the mean Dice dissimilarity index (figure 5).

Conclusions

Human pressures due to large extent of subsistence on forest products have severely threatened a large part of the tropical forests. Several workers have reported the decline in abundance as also the decline in levels of genetic diversity of populations subjected to extraction (Frankham and Ralls 1998; Gillies *et al*. 1999; Wickneshwari and Boyle 2000; Changtragoon 2001; Uma Shaanker *et al*. 2001a). A number of species have been rendered rare, endangered, or threatened, with a high risk of going locally if not globally extinct (Vasudeva *et al*. 2001; Cole 2003). Clearly, for such species, PAs, including national parks and sanctuaries, seem to be the only last

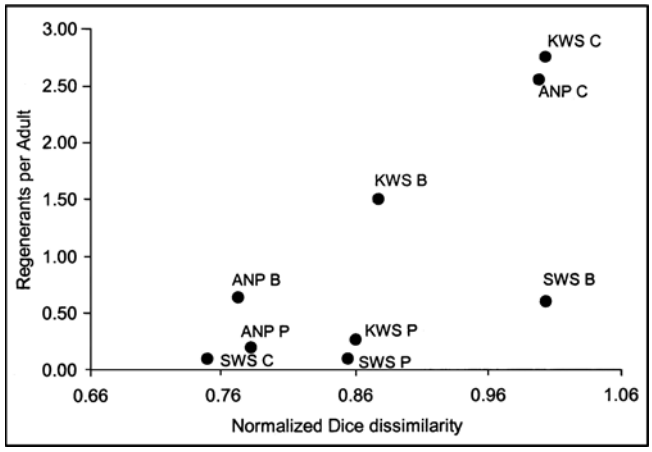

Figure 5. Mean regeneration per adult as a function of normalized mean Dice dissimilarity index for *Calamus thwaitesii* from all the three protected areas across the core (C), buffer (B) and peripheral (P) zones ($y = 0.002e^{8.9244x}$; $R^2 = 0.5054$, $P < 0.01$).

refugia to hold not only viable populations but also relatively higher levels of genetic diversity. This study represents one of the few attempts to address whether the protected areas indeed conserve the genetic diversity of economically important plants. The findings open the possibility of examining the relevance of PAs further with an expanded list of species and sites. Such studies could lay a strong foundation for further strengthening the PA network, especially in human-dominated forested landscapes around the world (Noss 1996; Bruner *et al*. 2001).

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