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Published in **Issue No. 132**, page 17 to 21 - (20447) characters

Identification of hot spots of species richness and genetic variability in rattans: an approach using geographical information systems (GIS) and molecular tools

[G. Ravikanth](#) [K.N. Ganeshaiyah](#) [Uma Shaanker](#) **Introduction**

Rattans or canes are a group of trailing or climbing palms with characteristic scaly fruits. They belong to the genus *Calamus* of the *Arecaceae* (*Palmae*) family. A few other genera like *Daemonorops*, *Plectocomia*, *Korthalsia* also yield rattans of commercial importance; however, all the rattans in south India belong to the genus *Calamus*. They comprise more than 50% of the total palm taxa found in India (Basu 1985) and form typical components of the moist forests in the Western Ghats, sub-Himalayan hills, valleys of Eastern and North-eastern India and in the Andaman and Nicobar Islands. India harbours about 60 species of rattans and is one of the richest sources of rattans in the world, next only to China (Renuka 1999). The rattans are a very important source of livelihood for the economically and socially weaker sections of the community (Uma Shaanker et al. 2000). It is estimated that more than half a million people are directly employed in harvesting and processing of rattans in the rural areas of Southeast Asia. In India, over 300 000 people are involved in cane-based industries (Anonymous 1983).

As the world's demand for rattan and rattan products is increasing, there is a tremendous pressure on the natural population of rattans. Besides the extraction pressures, the rattans are also severely threatened by changes in land use patterns (Biswas 1991). The threat is accentuated by the fact that rattans are dioecious and because of their premature harvest, they rarely come to flowering and fruiting (Lakshmana 1993). These have resulted in extremely poor regeneration of many of the economically important species of rattans. In recent years, there has been an increasing concern over the loss of natural populations of rattans in South and Southeast Asia. A number of endemic species are already on the verge of being highly threatened and endangered (Lakshmana 1993). It is estimated that of the 21 species identified in the Western Ghats, nearly 10 are under threat with one, *Calamus rheedi*, already extinct (Renuka 1999). The status of the different species of rattans is far less clear in the Eastern Himalayan forests and the Andaman and Nicobar Islands. It is feared that unless urgent measures are taken to protect the natural stands of rattans, the genetic resources of these endangered palms would be irreversibly lost. Unfortunately efforts aimed at conserving the genetic resources of rattans suffer from want of precise information on the hot spots of species richness as well as their genetic diversity. In this paper, using geographical information systems (GIS) and molecular tools, we have attempted to identify the hot-spots of species richness and genetic variability of an economically important rattan species in the Western Ghats. Based on the results, we propose strategies that could lead to a

Key words / Descriptors

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comprehensive conservation effort of the rattan genetic resources.

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Materials and methods

Geographical focus of the study

The study was conducted in the Western Ghats, a mountain chain running parallel to the West Coast of south India and one of the 18-biodiversity hotspots of the world (Myers 1988). The Western Ghats contains some of the last remaining forests in peninsular India and is characterized by high levels of biodiversity and endemism. Though they cover only 5% of the land area, they contain 27% of the country's total plant species. Over 21 species of rattans have been reported from the Western Ghats, with many of them highly threatened and endangered (Renuka 1999).

Identification of hot-spots of species richness

Data on the distribution of rattan species in the Western Ghats were obtained from flora, herbaria, books and other published sources including forest department records and archival material. The latitude and longitude of the places of occurrence were obtained and digitized using GIS MapInfo software. Based on the number of records of occurrence of a species at a place, contours of species richness were constructed using a 3-D Mapper software in the GIS MapInfo frame and the hot-spots of species richness identified (Ganeshaiyah et al. 2000). Since the contours of species richness could guide conservation efforts to areas with high concentration of species, we refer them as 'species richness contours' (Ganeshaiyah and Uma Shaanker 1998).

Identification of hot-spots of genetic variability of economically important rattan (Calamus thwaitesii) in the Western Ghats

We assessed the intra-specific genetic variability of *Calamus thwaitesii*, one of the intensively extracted species of rattans in the forests of Central Western Ghats in the state of Karnataka, covering the districts of Coorg, Dakshina Kannada, Chickmagalur,

Hassan, Shimoga and Uttara Kannada. At each of the sites, based on the information obtained from the local forest division and ranges, the approximate spatial location and distribution of rattans was marked on the map. After a preliminary field survey of the sites, 15 distinct populations were identified and sampled for the study using isozyme analysis. From each population or site approximately 40 individuals were sampled and genetic analysis performed over 12 loci from eight enzyme systems [phospho gluco mutase (PGM), phospho gluco isomerase (PGI), phospho gluco dehydrogenase (PGDH), isocitrate dehydrogenase (IDH), malic enzyme (ME), malate dehydrogenase (MDH), shikimate dehydrogenase (SKDH) and glucose-6-phosphate dehydrogenase (G6PDH)] using POPGENE software (Yeh and Boyle 1997). The hot spot of genetic variability, as reflected by the per cent observed heterozygosity, was identified.

Population genetic analysis: The data was subjected to population genetic analysis following the software POPGENE (Yeh and Boyle 1997). The following genetic parameters were estimated/determined.

Allele and genotype frequencies: At each locus, the frequency of each allele and the genotype frequency were computed.

Mean and effective number of alleles: The mean number of alleles per locus for each population was determined. The number of alleles needed within a locus to maintain the current level of heterozygosity was determined as $\frac{1}{\sum P_i^2}$, where P_i is the frequency of the i th allele in each population.

Polymorphic percentage: The proportion of polymorphic loci was estimated for each population. For purposes of analysis we derived two estimates of percent polymorphism. A locus was considered polymorphic if the frequency of the most frequent allele was below 95%. A locus was also considered polymorphic if it had more than one allele, irrespective of the frequency of the occurrence of the alleles (no criterion)

Shannon information index: The allele diversity within a locus was estimated as the Shannon diversity index (H'), where P_i is the frequency of the i th allele at a locus. The H' was averaged over all loci to refer to the diversity of alleles at all loci.

Percent observed and expected heterozygosity: For each locus the number of heterozygotes were recorded and expressed as percent observed heterozygosity. The percent heterozygosity was then pooled and averaged over all loci to arrive at the percent observed heterozygosity for a population. The percent heterozygosity was computed for both (a) overall loci and (b) polymorphic loci only. The expected heterozygosity for each locus and overall loci for each population in the Hardy–Weinberg equilibrium was computed as $\sum P_i^2$, where P_i refers to the allele frequency.

Population differentiation statistics: Differentiation of populations was analyzed following Wright's (1978) F-statistics. The F-statistics measures the degree of genetic differentiation among populations relative to the total population variance. This statistic is sensitive to the total number of populations included in the analysis. F-statistics are given by:

where, F_{it} is the total deviation from expected frequencies under the Hardy–Weinberg equilibrium, F_{st} is the deviation due to population subdivision and F_{is} is the deviation within population subdivisions.

Gene flow (Nm) among populations: Gene flow of a population or the estimated number of migrants exchanged between populations per generation was calculated using

Wright's method (1951). This method is influenced by the distribution of common alleles and is given as where G_{st} =mean proportion of total gene diversity at polymorphic loci due to differences between populations.

Results and discussion

Hot-spots of species richness

The Western Ghats of India account for nearly 21 species of rattans belonging to the genus *Calamus*. Based on the contours of species richness, there appears to be three centers of species richness of rattans in the Western Ghats, namely south and north of Palghat gap and in the Western Ghats of Southern Karnataka (Figure 1). In the Western Ghats of Southern Karnataka there are as many as nine different species located in a small district of Kodagu; south and north of the Palghat gap there are as many as six different species.

In other words, these regions could be considered as hot spots of rattan species richness in the Western Ghats. Accordingly, efforts aimed at conserving the rattan genetic resources at the species level could concentrate on these regions for maximizing conservation gains (Ravikanth et al. 1999). However, for a more comprehensive program of conservation it might be necessary to overlay other layers of information such as the levels of vulnerability of the sites to human pressure, land use changes etc. Based on such information conservation efforts could be prioritized to focus on sites with a high concentration of endemic species but which are threatened and vulnerable.

Hot-spots of genetic variability in *Calamus thwaitesii*

The analysis of 15 populations of *C. thwaitesii* populations involving 12 loci yielded an overall percent heterozygosity of $11.63 \pm 2.92\%$, with a range between 6.62 and 15.56%. These estimates compare well with those reported for tropical woody and shrubby species (Hamrick and Loveless 1986). However, the estimates are low when compared with those reported by Bon et al. (1995) for *C. manan* (37%) and for *C. subinermis* (42%). It is important to note that the latter study included a large number of plantation rattans and perhaps selected clones as well, which could have contributed to a higher estimate of the percent heterozygosity. Over all the populations, there was a heterozygote deficiency to an extent of 59.8%, indicating that there might be substantial inbreeding of the rattans. In their study of endemic rattans, Bon et al. (1995) showed that *C. manan*, a solitary stemmed species of rattans, seemed to suffer from heterozygote deficiency (around 21.27%). A high degree of heterozygote deficiency has been attributed to several reasons including (a) the experience of severe bottlenecks in the evolutionary past of the species, which reduce the genetic variability within the populations (Ledig 1988) and (b) genetic drift. Unfortunately, however, we have no information on the evolutionary history of the rattans in the Western Ghats to evaluate if indeed the species has experienced any bottlenecks.

The low levels of heterozygosity in the species are intriguing despite the fact that the rattans are dioecious and are expected to be obligate out-crossers. However, because of the poor regeneration owing to both premature and indiscriminate harvest, it is likely that reproduction in rattans is constrained by limited mating partners leading progressively to greater levels of homozygosity. Of the 15 populations studied, we observed fruiting in only 4–5 populations and even in these the fruiting was sparse; both of these factors over time could lead to a loss of genetic variability in the population. Though not unequivocal, there is evidence to suggest that the Western Ghats have experienced a long history of extraction of rattans and that the regeneration is severely affected.

There was a strong spatial pattern in the distribution of genetic variability with populations at the southern latitudes and the eastern longitudes having a higher level of heterozygosity compared with those at northern latitudes and western longitudes (Figure 2). A multiple regression analysis wherein the percent observed heterozygosity was regressed on the variables latitude, longitude and rainfall indicated a moderately good predictability of the dependent variable (percent heterozygosity, $p < 0.0933$). A stepwise regression of the percent heterozygosity on the variables longitude, latitude and rainfall was performed. Of the three independent variables longitude seemed to explain the variation in the percent heterozygosity (incremental $R^2 = 0.424$, $p < 0.01$; Table 1). The other two variables latitude and rainfall contributed an incremental R^2 of only 0.034 and 0.064, respectively. The observed pattern could in part be explained by the distributional limits of the species; populations at the northern latitudes and western longitudes appear to be at the limits of distribution and hence may have a restricted exchange of gene flow with the rest of the populations.

The low levels of heterozygosity in *C. thwaitesii* as revealed by our study raises concern on the status of the genetic diversity of other species of rattans that are endemic, with highly restricted distribution and furthermore on those species that are highly threatened. It would be worthwhile to examine the patterns of genetic variation in species of rattans that are endemic or otherwise highly threatened and compare them with the results obtained in this study.

Conservation and management implications

It is estimated that 90% of the world's rattan is harvested from the natural forests. In India, almost all of the current requirements of rattans are obtained from the natural forests. The enormous pressure of such extraction has had its impact in terms of lowered stand of the rattans, reduced regeneration (in some sites with no regeneration at all), lack of fruit and seed set etc. Despite these pressures, there has been no cogent plan to develop strategies for the conservation of the rattan genetic resources in the country. Most often conservation efforts, much as it is for other species, have been handicapped by lack of information on the criteria to be adopted to identify populations and sites where the genetic resources could be conserved.

Using GIS and molecular tools, our study has provided a geographic perspective of the genetic resources of rattans at the species level and for an economically important species at the population level. Information on the spatial distribution of genetic resources is crucial to formulate strategies on the in situ conservation of the resources as well as to arrive at sampling protocols for germplasm collections. Our study demonstrates that for conservation purposes, it might be useful to concentrate efforts at two levels, namely, species richness and intra-specific genetic variability of populations. It is clear from the study that the contour of species richness is steepest at Kodagu and north and south of Palghat gap while that for the genetic variability of *C. thwaitesii* at sites in southern latitudes and eastern longitudes. We hence suggest that for any long-term conservation of rattans at the species level, efforts should be aimed at Kodagu and south and north of Palghat gap to conserve the maximum number of species. On the other hand, to conserve the genetic resources at the intra-specific level of *C. thwaitesii*, greater emphasis should be laid on populations from southern Karnataka.

Acknowledgements

The work is supported by grants from the International Plant Genetic Resources Institute, Asia-Pacific-Oceania Office, Malaysia. We acknowledge the encouragement and support received from Ramanatha Rao, IPGRI, Malaysia. We would like to thank

Narayani Barve and ATREE, Bangalore, for the help rendered in developing the maps. We acknowledge the cooperation of the various forest officers of the Karnataka Forest Department in providing the necessary permission to visit the forest divisions in the Western Ghats.

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