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# Evaluation of the extent of genetic variation in mahoganies (Meliaceae) using RAPD markers

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**Abstract** Despite the economic importance of mahoganies (Meliaceae) little is known of the pattern of genetic variation within this family of tropical trees. We describe the application of a polymerase chain reaction (PCR)-based polymorphic DNA assay procedure random amplified polymorphic DNAs (RAPDs) to assess the extent of genetic variation between eight mahogany species from four genera. Pronounced genetic differentiation was found between the species and genera. There was a clear separation of *Cedrela odorata* from the other species, with 95% of the variable amplification products differing, whereas *Lovoa trichilioides*, *Khaya* spp. and *Swietenia* spp. were more closely grouped. These results are consistent with the current taxonomic viewpoint. A number of markers were found to be diagnostic for particular species, which could be of value in determining the status of putative hybrids. The application of RAPDs to the study of genetic variation in mahoganies is discussed in the context of developing genetic conservation and improvement strategies for these species.

**Key words** RAPDs · Mahoganies · Genetic variation
Conservation · Genetic Improvement

## Introduction

Mahoganies (Meliaceae) are amongst the most commercially important tropical timber tree species, dominating international trade in the areas where they are native (Lamb 1966; Read 1990). Despite this, the pattern and distribution of genetic variation within this family is virtually unknown. Most information has been gained from the analysis of material from different geographical origins in provenance and progeny tests (Newton et al. 1993a,b). Although the number of such field trials is extremely limited, results have indicated that a significant amount of intraspecific genetic variation exists within key economic genera such as *Cedrela* and *Swietenia* (Newton et al. 1993b,c). Information on other mahogany species, such as members of the African genera *Khaya* and *Lovoa*, is even more scant (Newton et al. 1993c).

Recently, concern has increased about the genetic conservation of mahogany species as a result of the high rates of deforestation in the areas where these species are native (Knees and Gardner 1983; Newton et al. 1993b; Read 1990; Rodan et al. 1992), and this is reflected in the inclusion of *Swietenia* in Appendix II of the Convention on International Trade in Endangered Species (CITES) (Rodan et al. 1992). A means of assessing genetic diversity and the distribution of variability in mahoganies is therefore of crucial importance for: (1) defining more accurately the conservation status of particular populations; (2) quantifying the effects of logging on the genepools of mahogany species; and (3) developing integrated scientifically-based genetic improvement and conservation strategies. Recently, DNA-based procedures such as restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs) have been applied to the detection of genetic polymorphisms in plants (Hadrys et al. 1992; Hu and Quiros 1991; Newbury and Ford-Lloyd 1993; Waugh and Powell 1992; Welsh and McClelland 1990; Welsh et al. 1991; Williams et al. 1990). The latter approach overcomes many of the limitations of RFLPs and has been used for clone identification in cocoa (Wilde et al. 1992), coffee (Orozco-Castillo et al. 1994) and banana (Kaemmer et al. 1992), and population differentiation in *Gliricidia* spp. (Chalmers et al. 1992). In this report we have used RAPD markers to estimate the level of genetic variation between 29 mahogany accessions from eight different species and four genera.

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## Materials and methods

### Plant material

Plants used in this study (Table 1) were either collected as seed from the field by staff of the Institute of Terrestrial Ecology (A. C. Newton, R. R. B. Leakey) or obtained from one of a range of institutions (see Newton et al. 1991, 1992) and raised at the Institute of Terrestrial Ecology, Edinburgh.

### Polymorphic assay procedures

DNA was isolated from fresh leaf material using a modification of the method of Gawel and Jarret (1991) exactly as described in Orozco-Castillo et al. (1994). Polymerase chain reactions (PCR), agarose gel electrophoresis and data recording were performed exactly as described previously by Chalmers et al. (1992). The sequences of the primers (5′-3′) used are as follows: SC10-70, TTGGCCGCGA; SC10-72, TGGGACCATG; SC10-88, TGAGATGGGC; SC10-89, ACGCGTCATC and SC10-90, TGGTGTCCGG. Primers were synthesised on an Applied Bio-systems 391 PCR-mate oligonucleotide synthesiser.

### Data analysis

Principal co-ordinate analysis and single linkage cluster analysis (Kempton and McNicol 1990) were performed with the GENSTAT 5 statistical package.

## Results

The level of polymorphism detected with RAPD markers was assayed in the mahogany species listed in Table 1. Each of the five primers used detected polymorphism, with an

**Table 1** List of accessions of mahogany (Meliaceae) analysed using RAPDs

| Accession no. | Name | ITE code no. | Origin |
|---|---|---|---|
| 1 | *Cedrela odorata* | 1016 | Nicaragua |
| 2 | *Cedrela odorata* | 1/90 | Costa Rica |
| 3 | *Cedrela odorata* | 42/79 | Guatemala |
| 4 | *Cedrela odorata* | 42/79 | Guatemala |
| 5 | *Swietenia humilis* | 58/90 | Costa Rica |
| 6 | *Swietenia humilis* | 58/90 | Costa Rica |
| 7 | *Swietenia humilis* | 56/87 | Honduras |
| 8 | *Swietenia humilis* | 56/87 | Honduras |
| 9 | *Swietenia humilis* | 8014 | Guatemala |
| 10 | *Khaya senegalensis* | 8002 | Nigeria |
| 11 | *Swietenia macrophylla* | 408/89 | Puerto Rico |
| 12 | *Swietenia macrophylla* | 8000 | Puerto Rico |
| 13 | *Swietenia macrophylla* | 8012 | Puerto Rico |
| 14 | *Swietenia macrophylla* | 8004 | Haiti |
| 15 | *Swietenia macrophylla* | 8005 | Haiti |
| 16 | *Swietenia macrophylla* | 8013 | Puerto Rico |
| 17 | *Swietenia macrophylla* | 8009 | Honduras |
| 18 | *Swietenia macrophylla* | 8007 | Honduras |
| 19 | *Swietenia macrophylla* | 8011 | Puerto Rico |
| 20 | *Swietenia macrophylla* | 8006 | Haiti |
| 21 | *Swietenia mahagoni* | 76/7 | Florida |
| 22 | *Khaya anthotheca* | K5/85 | Ivory Coast |
| 23 | *Khaya senegalensis* | 8000 | Nigeria |
| 24 | *Khaya senegalensis* | 8001 | Nigeria |
| 25 | *Khaya ivorensis* | 8012 | Nigeria |
| 26 | *Khaya ivorensis* | 8017 | Nigeria |
| 27 | *Khaya ivorensis* | 8002 | Nigeria |
| 28 | *Lovoa trichoiloides* | 8015 | Cameroon |
| 29 | *Lovoa trichoiloides* | 8012 | Cameroon |

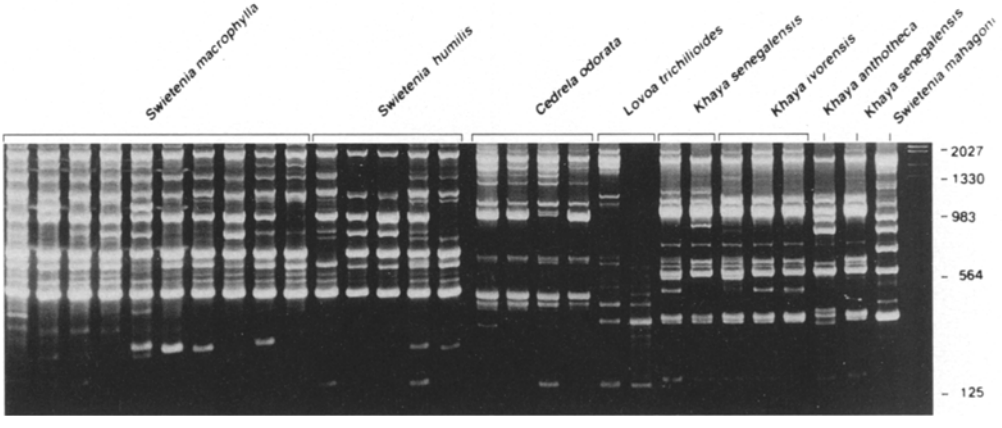


**Fig. 1** Amplification products generated from 29 individuals of eight species of Meliaceae using primer SC10-89. The products were generated and resolved as described in the Materials and methods

average of 13 polymorphic loci per primer. An example of the polymorphism detected with primer SC10-89 is shown in Fig. 1. A dendrogram displaying hierarchial associations is given in Fig. 2. The dendrogram is generated by group-average clustering where the similarity between two groups is defined as the average similarity of all loci scored in each group. There is a clear separation of *Cedrela* sp. from the other species with 95% of the variable products differing. *Lovoa trichilioides* is clearly distinguished from the *Khaya* and *Swietenia* spp., and the African *Khaya* and the American *Swietenia* spp. are also separated from each other. Importantly, a number of RAPD products were unique and could be considered diagnostic for a given species. In order to assess whether the clustering of populations based on RAPDs could be further resolved, principal co-ordinate analysis was used to analyse the shared fragment data available for the 29 accessions (Fig. 3). The first two principal components of this analysis account for 57% of the total variation. The clear separation of *Cedrela odorata* from the other species and the distinct groupings of the other genera reflects their geographical distribution.

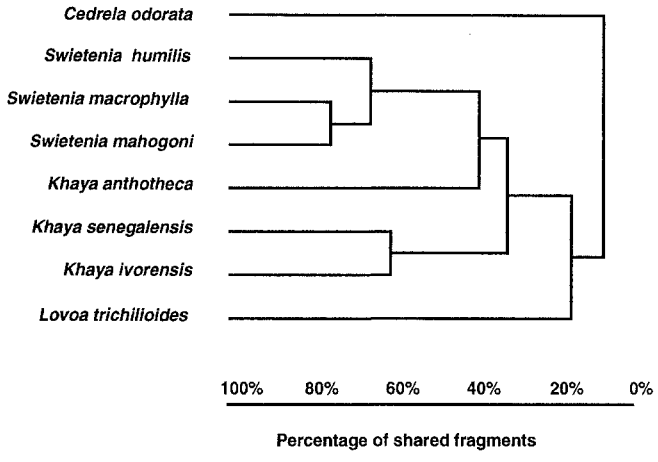


**Fig. 2** Dendrogram of different species of Meliaceae generated by group average clustering analysis

## Discussion

In this investigation, DNA from eight species from four genera of Meliaceae was examined using RAPDs. Intra- and interspecific polymorphism was detected in the samples analysed. The value of the RAPD approach is supported by the close similarity between the dendrogram based on RAPD results (Fig. 2) and the taxonomic relationships between the different genera based on morphological characteristics (B. T. Styles, personal communication). In particular, the fact that *Swietenia* and *Khaya* were more closely grouped with each other than either genus was with *Cedrela* or *Lovoa* is directly supported by traditional taxonomic evidence.

The respective geographic distributions of these genera are of interest in the context of their taxonomic relationships. Whereas *Lovoa* and *Khaya* are native to Africa, both *Cedrela* and *Swietenia* are restricted to tropical America. Evidence from both morphological and genetic characteristics support the notion that *Swietenia* and *Khaya* may have had a common ancestor prior to the rifting of Gondwanaland in the Lower Cretaceous (Whitmore 1990). *Swietenia macrophylla* is not native to Puerto Rico, although it has been widely established there through forestry operations (Weaver and Bauer 1986). *S. mahagoni* is also present on the island, and the two species clearly hybridise freely, based on the spectrum of morphological variation observed (Whitmore and Hinojosa 1977). A similar situation exists on Haiti, where *S. macrophylla* has been introduced, but *S. mahagoni* also occurs. It may well be that the accessions of *S. macrophylla* from both Puerto Rico and Haiti, while from a clearly identifiable parent tree, may be of hybrid origin. Conversely, while *S. mahagoni* is native to Florida, where *S. macrophylla* has been introduced, putative hybrid progeny have been recorded (Howard et al. 1988). In addition, the Costa Rican population of *Swietenia* shows a continuum of morphological variation between *S. macrophylla* and *S. humilis*, which are known to be interfertile (Whitmore and Hinojosa 1977). The accessions from these localities may therefore also include hybrid material. However, it is interesting to note that the *S. humilis* accessions

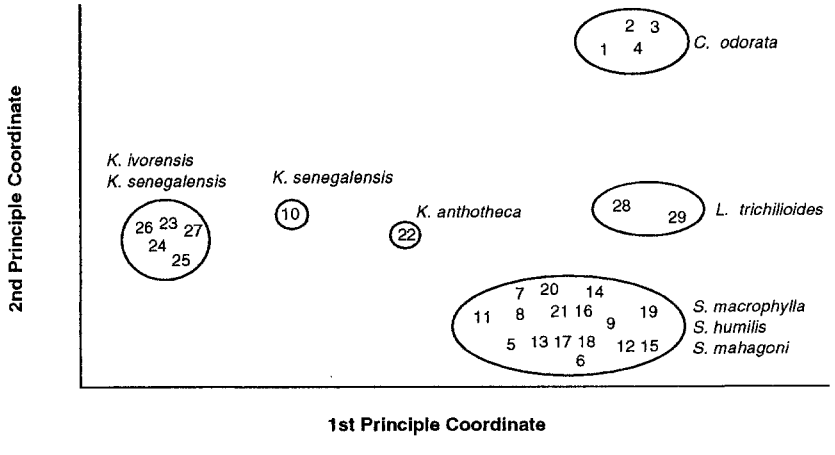


**Fig. 3** Principal co-ordinates analysis of the different Meliaceae accessions studied

were so distinct in the analyses presented here. It is therefore possible that these may represent pure *S. humilis*.

Clearly the complex pattern of genetic variation in *Swietenia* requires further investigation using genetically pure accessions and larger sample sizes to derive species-specific markers. If such markers were identified, the status of the different putative hybrid populations could be accurately assessed. This would be of great value from a genetic conservation standpoint. All three *Khaya* species analysed have broad distributions in West Africa, *K. ivorensis* being restricted to higher rainfall areas nearer the coast. Although ecologically distinct, it is possible that a similar situation might exist to that in *Swietenia*, with a complex pattern of variation within each species and a propensity to hybridise. Genetic variation in *Cedrela* species has been investigated in some detail through an international series of provenance trials co-ordinated by the Oxford Forestry Institute (Chaplin 1980). Differences in growth rate and in susceptibility to pest attack have been recorded at both the individual tree and provenance level (Burley and Nikles 1973; Nikles *et al.* 1978; Newton et al. 1993b). Investigation of the molecular basis of these differences would be useful, particularly if molecular markers associated with these characteristics could be developed. This species produces a valuable timber and has been widely planted across the tropics (Styles 1981). Molecular approaches might also be usefully applied to defining the identity of *Cedrela angustifolia*, a species of uncertain taxonomic status (Styles 1981) but with a particularly high economic potential (Chaplin 1980).

Results obtained using RAPDs with another tropical tree species, *Gliricidia sepium*, indicate that this technique may be used to quantify accurately the extent of genetic diversity between and within populations (Chalmers et al. 1992; Waugh and Powell 1992). RAPDs could therefore be used to select priority areas for conservation and to provide vital information for the development of genetic sampling, conservation and improvement strategies (Waugh and Powell 1992; Newbury and Ford-Lloyd 1993). The development of such strategies for mahogany is an urgent requirement, given the current high rate of deforestation of wild populations (Newton et al. 1993a,b,c). We envisage that RAPDs could have a useful role in developing such a strategy for mahogany.

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