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Conservation genetics of bush mango from central/west Africa: implications from random amplified polymorphic DNA analysis

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Abstract

Genetic variation was assessed in the two bush mango species, *Irvingia gabonensis* and *I. wombolu*, valuable multipurpose fruit trees from central and west Africa that are currently undergoing domestication. A total of 130 individuals sampled from Cameroon, Nigeria and Gabon were analysed using 74 random amplified polymorphic DNAs (RAPDs). Significant genetic integrity was found in the two morphologically similar species (among-species analysis of molecular variance [AMOVA] variance component 25.8%, $P < 0.001$), with no evidence of hybridization, even between individuals from areas of sympatry where hybridization was considered probable. Results suggest that large-scale transplantation of either species into new habitats will probably not lead to genetic introgression from or into the other species. Therefore, subsequent cultivation of the two species should not be hindered by this consideration, although further studies on the potential for hybridization/introgression between these species would be prudent. Significant genetic differentiation of both species (among-countries within species, nested AMOVA variance component 9.8%, $P < 0.001$) was observed over the sampled regions, and genetic similarity of samples decreased significantly with increasing geographical distance, according to number of alleles in common (NAC) analysis. 'Hot spots' of genetic diversity were found clustered in southern Nigeria and southern Cameroon for *I. wombolu*, and in southern Nigeria, southern Cameroon and central Gabon for *I. gabonensis*. The possible reasons for this distribution of genetic variation are discussed, but it may reflect evolutionary history, as these populations occur in areas of postulated Pleistocene refugia. The application of these results to domestication programmes and, in the light of extensive deforestation in the region, conservation approaches, is discussed.

Keywords: Africa, bush mango, conservation genetics, genetic diversity, indigenous fruit tree, *Irvingia gabonensis*, *Irvingia wombolu*, moist tropical forest, RAPD

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Introduction

Irvingia gabonensis Aubry-Lecomte ex O'Rorke and *I. wombolu* Vermoesen (Irvingiaceae), collectively known as bush mango or dika nut, are economically important fruit trees native to moist lowland tropical forest in central and west Africa (Mollet *et al*. 1995; Harris 1996). The fruit mesocarp of *I. gabonensis*, sweet bush mango, is appreciated as a snack or fresh fruit. Ground kernels of both species are used to thicken and flavour soups, although those of *I. wombolu*, bitter bush mango, are most valued and fetch high prices in cross-border trade, contributing significantly to the economy of the region as a whole (Ndoye 1995; Ayuk *et al*. 1999). Despite being economically valuable, bush mango is not widely planted in most regions and fruits are still harvested mainly from wild forest trees. Currently both species are the subject of research in Cameroon and Nigeria for domestication as potential components of agroforestry systems (Ladipo *et al*. 1996).

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Indeed, in a recent survey of subsistence farmers from Cameroon, Nigeria, Gabon and Ghana, 86% listed these *Irvingia* species as the most important trees for domestication in the humid lowlands of west Africa (Mollet *et al*. 1995; Franzel *et al*. 1996). As part of this research, and owing to concern over the high rate of deforestation across their native range, particularly in the moist lowland forests of Nigeria, extensive collections of both species have been made in Cameroon and Nigeria, and collections of *I. gabonensis* have also been undertaken in Gabon (Leakey & Simons 1998). Collections are currently under evaluation in field trials, which also function as *ex situ* conservation stands (Ladipo *et al*. 1996).

Few studies have been conducted on the reproductive ecology of *I. gabonensis* and *I. wombolu*, but both species are believed to be predominantly outcrossing and insect pollinated (by small wasps, flies and ants), with seed dispersed by humans during migration and through the digestive system of large mammals, particularly elephants (Ujor 1995; Harris 1996). The overall range and ecology of the two species are distinct (Harris 1996): *I. wombolu* is found in forest on dry or seasonally flooded soils (beside streams and in gallery forest), across sub-Saharan Africa from Sierra Leone to Uganda. *I. gabonensis* inhabits central African forest, mainly on dry soil (Nigeria, Cameroon, Central African Republic, Gabon and Congo). Their distributions overlap significantly in Cameroon and Nigeria, where they occur sympatrically, but the phenology of the two taxa differs. For example, in Cameroon, *I. gabonensis* generally flowers early in the rainy season (April) and fruits at the end (August), whereas *I. wombolu* flowers at the beginning of the dry season (October) and fruits in April (Harris 1996). Depending on conditions, however, flowering time overlaps in some years and regions (Ladipo *et al*. 1996) and, whilst the potential for intertaxon crossing has not been evaluated, it is possible that hybridization may occur in regions of sympatry. The potential threat of interspecific hybridization, particularly where large-scale cultivated introductions of one taxon are made to an area where the other is native, has led to concerns over the future genetic integrity and conservation status of natural stands.

An understanding of the inter- and intraspecific distribution of genetic variation within the native ranges of *I. gabonensis* and *I. wombolu* is essential for producing appropriate conservation and sustainable utilization strategies. Random amplified polymorphic DNA (RAPD) analysis (Williams *et al*. 1990), a technique based on the polymerase chain reaction (PCR), has been employed widely for assessing genetic variation in a range of tropical tree species (Dawson *et al*. 1995; Nesbitt *et al*. 1995; Gillies *et al*. 1997; Maguire & Sedgley 1997; Cardoso *et al*. 1998; Dawson & Powell 1999). RAPD analysis is quick and, once optimized, methodologically straightforward and may be applied to previously unstudied taxa as no DNA sequence information is required (Williams *et al*. 1990). Despite its wide application, and in common with other techniques that randomly target genomic regions (e.g. amplified fragment length polymorphism [AFLP] analysis; Vos *et al*. 1995), RAPD analysis suffers from a number of limitations if used to address ecological questions (Lynch & Milligan 1994). First, markers are mostly dominant and not applicable for direct estimation of heterozygosity. Second, co-migrating bands may be nonhomologous, particularly where comparisons are made between species (Rieseberg 1996; Gillies & Abbott 1998), and shared product absences are especially problematic, owing to the wide variety of mutations that can prevent amplification (Black 1993). However, the impact of these limitations can be reduced by scoring an appropriate number of RAPD fragments (usually > 30) and using appropriate techniques for analysis (Lynch & Milligan 1994). The main advantage of the RAPD technique is that it provides large numbers of markers, allowing resolution of complex patterns of genetic variation (Huff *et al*. 1993; Peakall *et al*. 1995).

In this study we used RAPD analysis to assess the partitioning of genetic variation within and between *I. gabonensis* and *I. wombolu* from central/west Africa. Data were used to provide information for: (i) prioritizing methods and regions for future germplasm collection and evaluation of both species; (ii) the potential interaction between taxa and the extent of interspecific hybridization; and (iii) development of optimal approaches for *ex situ* and *in situ* conservation. Finally, very few studies have examined genetic variation in indigenous African trees (Brain 1989; Joly *et al*. 1992; Chevallier *et al*. 1994; Dyer 1994; Dulloo *et al*. 1997; Harris *et al*. 1997; Dawson & Powell 1999). This is the first significant DNA-based assessment of nuclear genetic variation in a forest tree species from central/west Africa and may therefore provide data for more general application to species of similar natural history and distribution in this unique and understudied region.

Materials and methods

Plant material

In collaboration with local communities, germplasm collections of *Irvingia gabonensis* Aubry-Lecomte ex O'Rorke and *I. wombolu* Vermoesen were conducted by the Nigerian Institute of Horticulture (NIHORT), Institut de la Recherche Agricole pour le Développement (IRAD), national agricultural research institutes and the International Centre for Research in Agroforestry (ICRAF) in Nigeria, Cameroon and Gabon during 1994 and 1995. Trees were collected from natural forest or as remnants on farm land. In the latter case, planting of trees could not always be excluded by interviewing farmers, but was considered

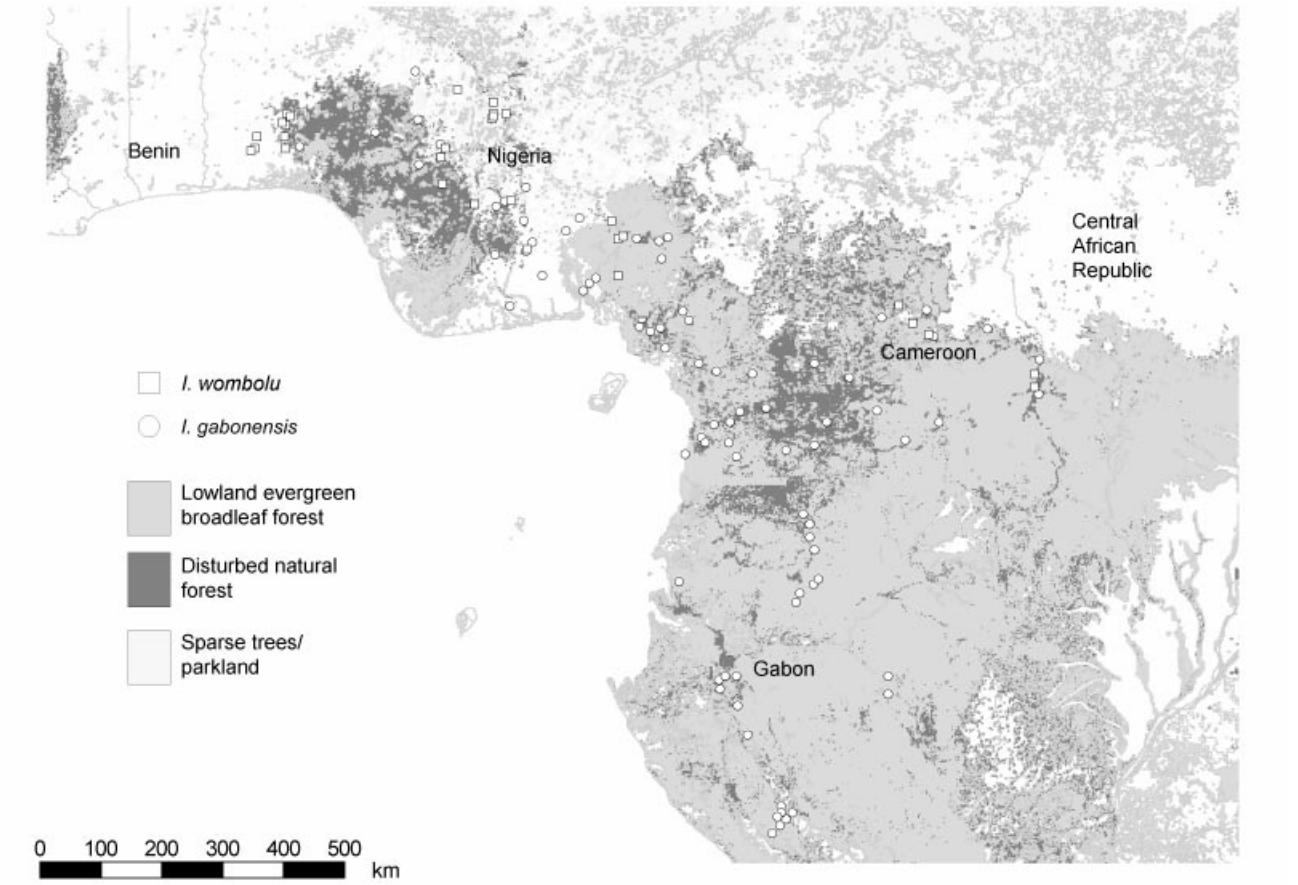

Fig. 1 Locations of samples identified during collection as *Irvingia gabonensis* (circles) and *I. wombolu* (squares) on a map of the natural vegetation distribution of central/west Africa (N.B. some sample points are coincident). Data were taken from a database on the global extent of forest (Iremonger *et al*. 1997) published by the World Conservation Monitoring Centre (WCMC) and the Centre for International Forest Research (CIFOR).

unlikely. Open pollinated fruit from single trees were collected at each location and established in progeny trials/genebanks in Nigeria and Cameroon in 1995 and 1996. Leaf material was collected in 1995 and 1996 (by I. K. Dawson) from field trials, and silica gel used to dry and preserve samples (Weising *et al*. 1995). In each case, leaf material was sampled from a single tree of a field-pollinated progeny array. In total, 87 individuals, initially identified during collection as *I. gabonensis*, were sampled from Cameroon (37), Gabon (25) and Nigeria (25), whilst 43 individuals identified during collection as *I. wombolu* were sampled from Cameroon (five) and Nigeria (38) (Fig. 1). Samples were chosen to assess macrogeographical genetic structure across the region.

DNA extraction and RAPD analysis

Total genomic DNA was extracted and purified using a modified hexadecyltrimethylammonium bromide (CTAB) mini-preparation method of Doyle & Doyle (1987). DNA concentrations were determined, relative to uncut lambda DNA, on 1% agarose gels. The conditions for RAPD–PCR were identical to those used by Dawson *et al*. (1996), and fragments were resolved on 1.4% agarose gels and sized against a 1-kb ladder (Gibco). After an initial survey of 20 RAPD primers (all from Operon Techologies, kit B), using a subsample of material to test for levels of polymorphism and reproducibility, 10 primers were chosen for the full study (OPB1, 4, 6, 8, 11, 12, 14, 16, 17, 18).

Data analysis

The collection represents a sample of geo-referenced individuals across a broad area of the range of both species, rather than a collection of population samples. Therefore, analysis did not examine population genetic structure, but

rather the overall pattern and partitioning of genetic variation across the sampled range. The presence/absence of RAPD fragments was scored for each individual, and Jaccard's distance was calculated between pairwise combinations, based on the shared presence of fragments (Weising *et al*. 1995), using PCO3D (Adams 1995). The first two principal coordinates were calculated from distance estimates and plotted graphically. At this point, six individuals were identified that did not cluster with individuals of the taxon of which they had been initially identified during collection. The species-diagnostic RAPD fragment profile was examined for these individuals and they were removed from further analyses. The extent and significance of hierarchical partitioning of genetic variation amongst species and countries was calculated from the nested genetic distance variance components (based on $1 - S$, where S = the distance estimate of Jaccard) using the program WIN AMOVA 1.55 (analysis of molecular variation; Excoffier *et al*. 1992). Although originally developed for use with Euclidean metric distances, in practice AMOVA can be used with other genetic distance indices with little adverse impact (Huff *et al*. 1993). Significance values were assigned to variance components based on 1000 random permutations of individuals, assuming no genetic structure. To examine geographical structuring of genetic variation in more detail, an autocorrelation between geographical and genetic distance was calculated using number of alleles in common (NAC; Surles *et al*. 1990; Hamrick *et al*. 1993) analysis. Only RAPD fragments with a frequency between 0.2 and 0.5, considered for each species separately, were utilized for the NAC analysis, as such fragments were assumed to be heterozygous for analysis. To examine local genetic diversity, the distribution of rare RAPD fragments (i.e. those with a frequency < 0.2 for a given species) was plotted, and Shannon's estimate (Russell *et al*. 1993) was calculated for all samples in a 100-km radius around each sample point. To assess the significance of diversity estimates, the data sets of each species were resampled at random 100 times for the range of sample sizes occurring within the 100-km radius subsamples (two to 16 individuals). To determine whether sample diversity estimates were significantly higher (or lower) than expected, estimates were compared with the 95% confidence interval (CI) around the mean of the random sample calculated for sample size.

Results

In total, 74 RAPDs were reliably amplified using 10 primers. RAPD analysis clearly distinguished *Irvingia gabonensis* and *I. wombolu* as distinct genetic entities. These results were reflected in the plot of the first two principal coordinates (Fig. 2), which clearly distinguishes individuals of each taxa into two well-differentiated groups. Six individuals were placed into a species grouping that was different from that identified during collection and are indicated in Fig. 2; four originally identified as *I. wombolu* (A, B, C, D; Table 1) grouped with *I. gabonensis* and two originally identified as *I. gabonensis* (E, F; Table 1) grouped with *I. wombolu*. Excluding these six individuals, RAPD fragments with a frequency difference

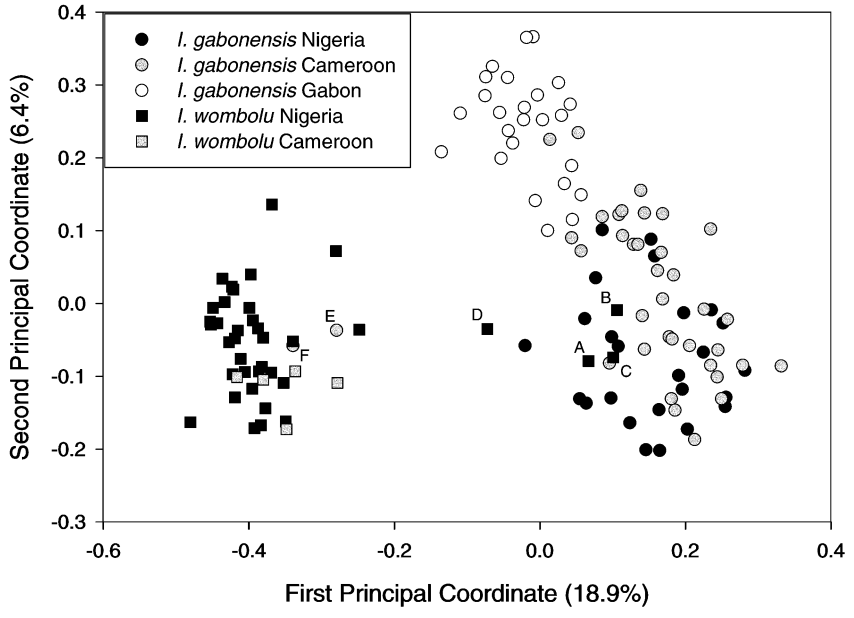

Fig. 2 Plot of first two principal coordinates calculated from Jaccard's distance estimate of presence/absence data for 74 random amplified polymorphic DNA (RAPD) fragments of *Irvingia gabonensis* and *I. wombolu*. Individuals whose placement did not correspond with species designation made at the time of collection are labelled (A, B, C and D for *I. wombolu*, E and F for *I. gabonensis*; see Table 1).

Table 1 Frequencies of random amplified polymorphic DNA (RAPD) fragments with a between-species difference of ≥ 0.5 (calculated for the whole sample of each species excluding the six unusual/misidentified individuals). Nuclear index values were generated by calculating the number of *Irvingia gabonensis*-diagnostic markers present as a proportion of all diagnostic polymorphisms scored. 'Pure' *I. gabonensis* individuals are therefore expected to have a value approaching 1 and 'pure' *I. wombolu* a value approaching 0. Six individuals (A to F) occupying unusual positions in principal coordinate analysis (Fig. 2), which were excluded from frequency estimates, had index values within the range of the alternate species and were apparently misclassified during collection (classification during collection given in parentheses)

	I. gabonensis	*I. wombolu*
Frequency of polymorphisms		
RAPD fragment	*N* = 85	*N* = 39
OPB1-I250	0.56*	0.00
OPB1-I400	0.93*	0.03
OPB4-I1600	0.74*	0.08
OPB8-I1100	0.85*	0.21
OPB11-I1050	0.09	0.62*
OPB11-I1400	0.09	0.92*
OPB11-I2000	0.02	0.69*
OPB12-I480	0.53*	0.03
OPB12-I800	0.99*	0.31
OPB17-I1100	0.96*	0.05
OPB18-I1450	0.92*	0.05
Nuclear index		
Average for species	0.94	0.10
Range for species	0.53–1.00	0.00–0.43
A (*I. wombolu*)	0.65	
B (*I. wombolu*)	1.00	
C (*I. wombolu*)	1.00	
D (*I. wombolu*)	1.00	
E (*I. gabonensis*)		0.36
F (*I. gabonensis*)		0.27

*These fragments are considered diagnostic for the species indicated.

between species of at least 0.5, and therefore useful for species discrimination, are presented in Table 1. Eight were present at high frequency in *I. gabonensis* and three in *I. wombolu*. When the frequencies of species-diagnostic RAPDs were examined for the six unusual individuals, all six fell within the range of variation for their reclassified species group (Table 1) and there was no evidence to indicate that they represented interspecific hybrids. However, the diagnostic RAPD frequency scores (Table 1) for one of the reclassified *I. gabonensis* individuals (A, 0.65) and both of the reclassified *I. wombolu* individuals (E, 0.36 and F, 0.27), were close to the extremes of their species range (0.53–1.00 and 0.00–0.43, respectively). Excluding these six individuals, AMOVA found individuals of both species to be significantly differentiated from each other ($P < 0.001$, PhiST = 0.357; Table 2) with 25.8% of the total variance component attributable to that between species.

Distinct genetic differentiation was also obvious by country within both species (Fig. 2). In particular, Gabon individuals of *I. gabonensis* were distinct from those from Cameroon and Nigeria, whilst Cameroon individuals of *I. wombolu* were differentiated from those from Nigeria. Examination of the second level of hierarchical partitioning of genetic variation by AMOVA demonstrated that genetic differentiation amongst countries nested within species was significant ($P < 0.001$, PhiSC = 0.133; Table 2), with a variance component of 9.9%. The influence of geographical distance on genetic similarity, separate from that related to national boundaries, was examined further using NAC analysis (Fig. 3). For *I. gabonensis*, genetic similarity declined steadily from an initial high of 1.72 for individuals separated by < 100 km, down to 1.55 for individuals separated by ≥ 1100 km. For *I. wombolu*, there was a similar, although lower magnitude, decrease in genetic similarity over distance, from a high of 1.77 for individuals separated by < 100 km, down to a minimum of 1.67 for individuals separated by 900 km. The much higher variation

Table 2 Results of nested analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) for 85 individuals of *Irvingia gabonensis* (35 from Cameroon, 25 from Gabon and 25 from Nigeria) and 39 individuals of *I. wombolu* (five from Cameroon and 34 from Nigeria), employing 74 random amplified polymorphic DNA (RAPD) fragments

Source of variation	d.f.	MSD	Variance component	*P*-value	% of total
Nested analysis					
Among species	2	4.550	0.067	< 0.001	25.8
Among countries	5	0.724	0.026	< 0.001	9.9
Among individuals within countries	123	0.168	0.168	< 0.001	64.3

PhiST, 0.357; PhiSC, 0.133; PhiCT, 0.258.
Nested analysis was carried out on all individuals by species and country. Degrees of freedom (d.f.), mean deviations (MSDs) and the significance (*P*) of the variance components are shown.

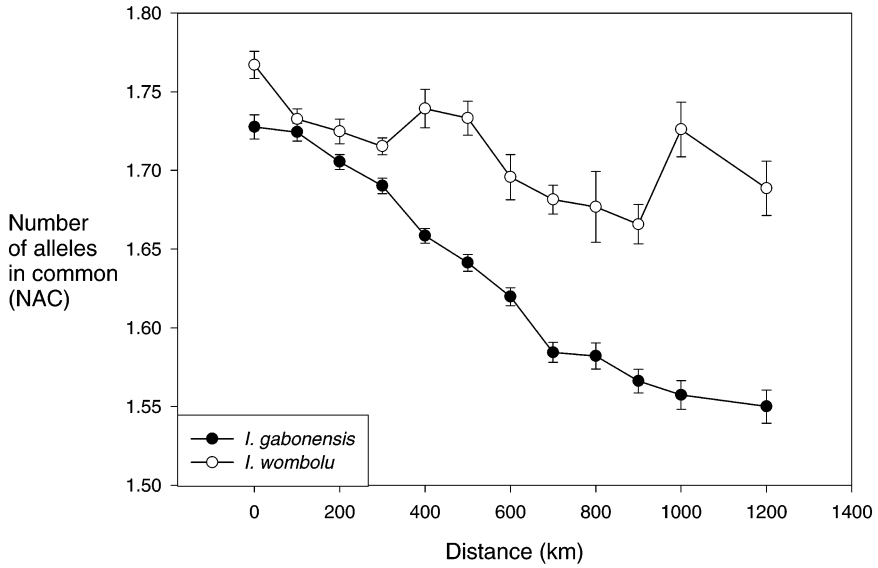

Fig. 3 Plot of genetic similarity by distance from number of alleles in common (NAC) analysis for *Irvingia gabonensis* and *I. wombolu* in central/west Africa.

in the similarity estimates for more distantly separated individuals of *I. wombolu* is a consequence of the small Cameroon sample ($n = 5$).

A study of the rare RAPD fragments (Fig. 4) clearly identified southern Nigeria and southern Cameroon as areas harbouring rare alleles and significantly higher levels of genetic diversity for *I. wombolu*. For *I. gabonensis*, southern Nigeria and southern Cameroon were areas that harboured significantly higher levels of genetic diversity. In addition, taken together, southwestern Cameroon and Gabon also harboured a relatively large proportion of rare alleles for *I. gabonensis* (13 out of 17).

Discussion

Study of the distribution of genetic variation in bush mango has provided information useful in the active domestication of these species and has prioritized areas for future collection and conservation. Genetic diversity data are scarce for central/west African tropical tree species and therefore these data may be of more general relevance for providing insights into the extent and partitioning of genetic variation expected for species with a similar life history and habitat range.

Interspecific variation: species integrity

Owing to the morphological similarity of *Irvingia gabonensis* and *I. wombolu*, difficulties are encountered distinguishing herbarium specimens. Okafor (1975) considered these two taxa to be conspecific and treated them both as varieties of *I. gabonensis* (var. *gabonensis* and var. *excelsa*, respectively). More recently, however, Harris (1996), in an extensive taxonomic revision of the Irvingiaceae, concluded that on the basis of additional field characters (in particular, fruit taste), sweet and bitter bush mango types should be classed as separate species (*I. gabonensis* and *I. wombolu*, respectively). Our data, which indicate significant differences among sweet and bitter bush mango genotypes (among-species AMOVA, 25.8%, $P < 0.001$), support the conclusion of Harris (1996) that these taxa are distinct genetic entities.

Close taxonomic relationship, very similar morphology (Harris 1996) and range overlap suggest that potential exists for hybridization between *I. gabonensis* and *I. wombolu*. Although Harris (1996), based on his morphological characterization, found no evidence of hybridization, during survey work by ICRAF and national partners in 1994 and 1995, a small number of trees were identified that had morphological characteristics intermediate to both species (David Ladipo, personal communication). Data obtained in this work, employing 74 RAPDs, allowed more detailed testing for possible interspecific hybridization. In common with Harris (1996), no evidence was found for hybridization between the taxa in samples taken from Cameroon and Nigeria, the main zone of range overlap between the two species. Rather, individuals could be unambiguously assigned to defined species groups during analysis. In addition, the molecular analysis indicated that six individuals had been misidentified during collection, but rather than representing any genetic overlap between the two species, these individuals fell within the range of RAPD variation of their reclassified group. However, in three cases, individual index values were close to the limit of distribution for their reclassified species and may represent extreme variants within the species

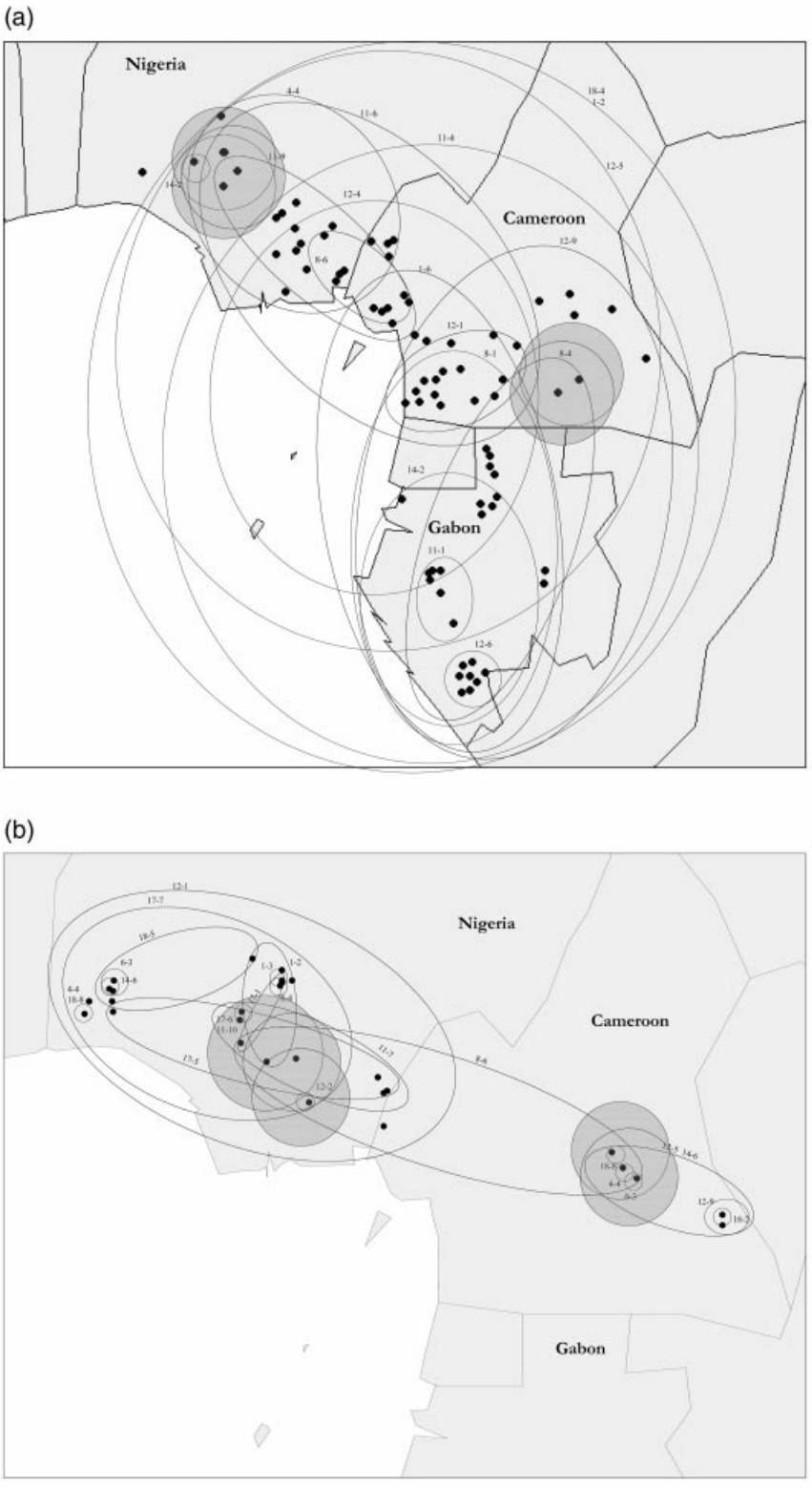

Fig. 4 Plot of geographical distribution of rare random amplified polymorphic DNA (RAPD) fragments (with frequency < 0.2) and areas of significantly higher than expected genetic diversity for (a) *Irvingia gabonensis* and (b) *I. wombolu* in central/west Africa. Open circles describe the distribution range of individual RAPD fragments (labelled, e.g. 12–5 is primer OPB12, fragment number 5) and shaded circles indicate areas of significant genetic diversity as estimated by Shannon's index. Genetic diversity was calculated for all samples within a 100-km radius around each sample point and the significance of the estimate was compared with the 95% confidence limit around the mean for 100 resamplings (with replacement) of the data set for the range of sample sizes (two to 16 individuals) for each species. N.B. misclassified individuals were omitted and some sample coordinates are coincident.

range. Such a level of field misidentification between two closely related taxa is not unusual and, indeed, is relatively low, indicating that field identification of germplasm was generally accurate. The misidentification of these individuals confirms that there is variation for flowering time in natural populations, which was the main criterion used for field identification. The lack of evidence found, within this study, for interspecific hybridization between *I. wombolu*

and *I. gabonensis* has implications for their use and distribution during cultivation programmes. Whilst further studies need to be conducted to examine breeding isolation, our data would indicate that the two species do not readily hybridize, even when sympatric, and could therefore be cultivated in new areas without fear of introgression to, or from, the other species native to that area. However, in the future it is recommended that additional criteria are used for species identification (e.g. fruit taste), and further studies on the potential for hybridization/introgression between these species would be prudent.

Intraspecific variation: isolation-by-distance and diversity 'hot spots'

Field observations and floral morphology suggest that both *Irvingia* species are predominantly outcrossing (Harris 1996). Indeed, a survey of RAPD and cleaved amplified polymorphic sequence (CAPS) variation in five to nine individuals of 10 progeny arrays of *I. gabonensis* indicated up to 100% outcrossing in sib families (A. J. Lowe, unpublished). Such a high level of outcrossing is expected to lead to a relatively homogenous distribution of nuclear-encoded genetic variation among natural populations, with the majority of variation present being maintained within populations. This is the typical situation for outcrossing temperate tree species with effective gene dispersal mechanisms (Hamrick 1994) (e.g. *Fagus sylvatica*, Müller-Starck 1991; European *Quercus* species, Zanetto & Kremer 1995; Bodénès *et al*. 1997). The current study, however, found that RAPD variation (which is predominantly composed of nuclear-encoded loci; Rieseberg 1996) was not distributed homogeneously across the sample range of bush mango, and significant regional differentiation was observed by country (nested AMOVA amongst countries within species, 9.9%, $P < 0.001$). The results of an autocorrelation between genetic and geographical distance (NAC analysis) also indicated that there was significant isolation-by-distance for both species. As an increased number of range-wide diversity studies are conducted within tropical regions, it appears that relatively high levels of partitioning of genetic variation are not uncommon in tropical tree species with wide geographical ranges (e.g. Gillies *et al*. 1997; Chamberlain 1998; Dawson & Powell 1999; Newton *et al*. 1999). The basis for this structure may include isolation, ecotypic differentiation or poorly known taxon delimitation. In the case of bush mango, more pronounced regional differentiation may result from limited gene flow and the subsequent processes of isolation and drift. Seed dispersal is predominantly by large mammals and humans and so there is great potential for long-distance gene flow (White *et al*. 1993). However, in practice, seeds are large and recalcitrant, remaining viable for a maximum of 4 weeks (Harris 1996), so actual successful seed colonization events by these mechanisms will probably be quite limited. Gene flow by pollen will also probably be restricted owing to the limited range of pollinators (ants, small wasps and flies; Harris 1996).

Genetic diversity was also found to be concentrated within specific areas (southern Nigeria and southern Cameroon for *I. wombolu* and *I. gabonensis* and also central Gabon for *I. gabonensis*, on the basis of rare alleles), although the location of areas of highest diversity for *I. wombolu* should be treated with some caution owing to the small sample size in Cameroon ($n = 5$). Knowledge of the pollen- and seed-dispersal mechanisms of bush mango does not explain the occurrence of these genetic diversity 'hot spots'. To investigate this phenomenon further, a number of factors were examined that may have influence on regional partitioning of genetic variation (i.e. altitude, mean rainfall, mean temperature, position in relation to the Cameroon mountains, habitat type, forest disturbance). A multiple regression test (not shown) found that none of these factors were responsible for the observed partitioning of diversity. These genetic diversity 'hot spots' do, however, coincide closely with postulated Pleistocene forest refugia (i.e. southern Cameroon, southwestern Nigeria and central Gabon) proposed by Hamilton (1981) and Sosef (1994), based on species diversity studies. In the case of many temperate tree species, it appears that Pleistocene dynamics have not only had an influence on the distribution of taxa, but also on the intraspecific partitioning of genetic diversity (Comes & Kadereit 1998). As more work is conducted in the tropics, it appears that historical events may also have had a strong influence on the intraspecific partitioning of diversity for long-lived tree species (Newton *et al*. 1999).

Conservation priorities

As bush mango seed loses viability within 4 weeks of falling (Harris 1996), it cannot be conserved in *ex situ* seed banks and therefore has to be conserved as living trees. Strategies for prioritizing the conservation of genetic diversity need to consider not only the level of diversity in an area but also the current threat to, and condition of, a particular region. Forest cover maps of west Africa (Fig. 1) indicate that the lowland evergreen broadleaf rain forest of Nigeria is particularly at threat from deforestation, and a significant area of natural forest has already been cleared or disturbed. Our data indicate that areas of southern Nigeria harbour a significant amount of the genetic diversity within *I. wombolu* and *I. gabonensis* and that this area should be a priority for conservation action. For economically valuable taxa such as bush mango, a *circa situ* conservation strategy (Simons 1996) can be pursued for areas under imminent threat. The value of fruit and

kernels can be promoted to encourage farmers to plant wildings of local, native stock as field borders or alternatively to enrich their cocoa plantations. Or, an *ex situ* approach can be adopted where the remaining natural populations are intensively collected and conserved as living orchards. In this situation, a co-ordinated effort between countries in the region is required because a significant proportion of variation crosses national boundaries. The option of *ex situ* conservation is one of last resort, and should be undertaken only if all other conservation routes have been exhausted and decimation of the remaining natural populations is imminent. Whilst *ex situ* conservation can support domestication programmes and is useful for preserving genes and economically important characteristics, this type of collection freezes variation in time and populations are no longer evolutionary viable units. In southern Cameroon and central Gabon, where the extent of lowland evergreen broadleaf rain forest is less disturbed, an *in situ* habitat conservation policy would be preferential for both species.

In this study, we have used anonymous nuclear-encoded markers to assess genetic variation in bush mango. It has been questioned whether such markers provide any useful insight into the levels of adaptive variation within a species, which should be a primary focus of a conservation or domestication programme. The present study was undertaken in conjunction with field trial assessment of material, and early results indicate significant differences in growth rate and tree form amongst accessions of both *I. gabonensis* and *I. wombolu* that correspond with RAPD data (Zacharie Tchoundjeu, personal communication). It is anticipated that the results from both field and molecular analyses will be used synergistically to identify suitable material for domestication and conservation programmes.

The specific characteristics of the RAPD method (random, uncharacterized multiple genomic loci; dominant nature of markers; and possibility of co-migrating, nonhomologous bands) result in limitations for population genetic studies (Lynch & Milligan 1994). Despite this, RAPD analysis can be used effectively for the initial assessment of levels and partitioning of genetic variation within plant species, particularly in tropical tree species for which there is very little other genetic diversity information available. RAPD analysis has been usefully applied to bush mango to provide valuable information on species integrity, misidentification of germplasm collections and regional partitioning of genetic diversity. On the strength of these initial findings, further molecular studies of bush mango using additional marker systems, such as CAPS (Lowe *et al*. 1998), are underway. Specifically, these studies include an examination of genetic diversity in a larger proportion of the natural range of *I. wombolu*, the partitioning of genetic variation within and between natural populations, and the field outcrossing rates. The value of bush mango and the importance of the moist tropical forests of Africa as reservoirs of biodiversity indicate that continued research of this nature should be assigned a high priority. Finally, data presented here represent the first geographically extensive study of nuclear genetic variation in any central/west African, moist lowland tropical forest tree. Further and more detailed studies on other species are required before general models for moist lowland forest structure in the region can be developed.

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