Genetic variation in natural populations of *Anthurium sinuatum* and *A. pentaphyllum* var. *pentaphyllum* (Araceae) from north-east Brazil using AFLP molecular markers

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Genetic variation was investigated using AFLP markers in 12 populations of *Anthurium sinuatum* and *A. pentaphyllum* var. *pentaphyllum* (Araceae) in north-east Brazil, Amazonia and the Brazilian Atlantic forest. Two unique genetic patterns characterized the populations of *A. sinuatum* as a group, but no correlation between genetic and geographical interpopulation distance was found; the Amazonian population was not separated from that in Ceará. The isolated Ceará brejo populations of *A. sinuatum* were genetically distinct, but genetic diversity levels were similar to populations elsewhere, with no evidence of genetic erosion. *Anthurium pentaphyllum* populations were significantly different from each other; Bayesian genetic structural analysis found no common genetic pattern, but revealed genetic clusters unique to subgroups and individual populations in the Atlantic forest and French Guiana. *Anthurium pentaphyllum* and *A. sinuatum* can be distinguished genetically, but individuals of both species formed intermediate genetic clusters that blurred their distinction. We suggest that genetic mixing of *A. sinuatum* and *A. pentaphyllum* has occurred in north-east Brazil, possibly connected with cycles of humid forest expansion. The weak genetic structure in *A. sinuatum* is consistent with the natural fragmentation of continuous forest areas, possibly during the Holocene. This study highlights the scientific importance of the highly threatened brejo forests for tropical American biogeography. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, **159**, 88–105.

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INTRODUCTION

The biota of the isolated, humid brejo forests of north-east Brazil have interested biologists for many years and have played a part in the continuing effort to understand the historical biogeography and possible migration routes between the major forest biomes of Amazonia and Atlantic Brazil (for example,

Andrade-Lima, 1953, 1966, 1982; Ducke, 1959; Prance, 1973; Vanzolini, 1973; Ab'Sáber, 1982; Bigarella & Andrade-Lima, 1982; Lourenço, 1988; Giulietti & Forero, 1990; Ledru, 1991, 1993; Oliveira-Filho & Ratter, 1995; Behling, 1998; Oliveira, Barreto & Suguio, 1999; Barreto, Pessenda & Suguio, 2002; Borges-Nojosa & Caramaschi, 2003; Costa, 2003; Cavalcanti & Tabarelli, 2004; Pôrto, Cabral & Tabarelli, 2004; Vasconcelos, Almeida & Costa, 2004). Despite the destructive impact of human activity, these two large forest regions are dominating features

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of South American biogeography, and continue to be the focus of baseline botanical exploration. Historical hypotheses are necessarily constrained by the patchy nature of much existing knowledge of the ranges and distinctions between species. The brejo forests are of particular interest, because they lie between the two main forest regions as a mosaic of small upland forest fragments scattered over a prevailing semi-arid landscape. Although poorly studied until recently and under severe pressure from deforestation, they provide unique opportunities for tropical American biogeographical studies.

Most studies of the brejo forests have concerned species' composition and endemicity of the brejo biota (for example, Sales, Mayo & Rodal, 1998; Pôrto *et al.*, 2004), and hitherto there have been almost no studies using genetic information to quantify relationships between populations of species common to the brejo forests, Amazonia and/or Atlantic Brazil. Carnaval (2002) studied frog phylogeography and compared populations in brejo forests and adjacent Atlantic forest in Pernambuco and Alagoas.

This article represents the first study comparing genetic variation in plant populations of the isolated brejos in the State of Ceará with populations in the Amazon and Atlantic forest regions. We report the results from two taxa of the family Araceae (Mayo, Bogner & Boyce, 1997): *Anthurium sinuatum* Benth. ex Schott and *A. pentaphyllum* (Aubl.) G.Don var. *pentaphyllum*. A parallel study of the aroid hemi-epiphyte *Monstera adansonii* Schott var. *klotzschiana* (Schott) Madison also forms part of this research project (Andrade *et al.*, 2007). *Anthurium sinuatum* is a common, usually epiphytic aroid of the most humid areas within the isolated brejo forests of Ceará, but is absent from the Brazilian Atlantic forest; it also occurs widely in the Guianas, eastern Amazonia and central Brazil. The closely related *A. pentaphyllum* does not occur in Ceará, but replaces *A. sinuatum* ecologically in the Atlantic forest, and is also found in Amazonia and further west to the Guianas and Central America; it is much more variable morphologically than *A. sinuatum* (Madison, 1978; I. M. Andrade *et al.*, pers. observ.).

TAXONOMIC CONSIDERATIONS

In the most recent taxonomic treatment of the species, Madison (1978) grouped *A. pentaphyllum* and *A. sinuatum* together with 12 other species in section *Schizoplacium* Schott. Croat & Sheffer (1983) later modified the sectional boundaries, placing them together in section *Dactylophyllium* Schott, characterized by compound, pedatisect or palmatisect leaves with the leaf segments completely free to the base, each leaflet with a distinct basal pulvinus.

Current alpha taxonomy treats these two species as distinct and there are no molecular phylogenies available. Madison (1978) considered *A. sinuatum* to be closest to *A. clavigerum* Poeppig, an extremely robust Amazonian species. The closest relatives of *A. pentaphyllum* are *A. brevipedunculatum* Madison and *A. kunthii* Poeppig (Madison, 1978).

Both the study species occur as root-climbing epiphytes, terrestrials or on rocks, with leaves divided into five to nine leaflets and the spadix turning from pale green to lavender or purple at female anthesis. Based on the taxonomic treatment by Madison (1978) and adding our own observations in the field and from herbarium specimens (Herbarium, Royal Botanic Gardens, Kew [K]; Holmgren & Holmgren, 1998), the two species can usually be distinguished as follows. *Anthurium sinuatum*: cataphylls persisting entire, leaflet margins sinuate, peduncle 14–50 cm long, flowering spadix 15–22.5 cm long, 0.4–0.9 cm thick at base, berries rich maroon (vinose). *Anthurium pentaphyllum*: cataphylls soon drying and becoming net-fibrous or deciduous, leaflet margins entire, peduncle 1–25 cm long, flowering spadix 4.3–13 cm long, 0.4–1.7 cm thick at base, berries red to purple.

Madison (1978) recognized three varieties within *A. pentaphyllum*: var. *bombacifolium* (Schott) Madison from Central America and Mexico; var. *digitatum* (Jacq.) Madison from Venezuela (now recognized as a distinct species; CATE Araceae, 2008); and var. *pentaphyllum* from the Guianas and Brazil. *Anthurium pentaphyllum* var. *pentaphyllum*, reported in this paper (and subsequently referred to as *A. pentaphyllum*), was originally described from French Guiana and circumscribed by Madison as a widespread and variable subspecies, characterized by a short erect peduncle and non-sinuate leaflets. Subsequent field-work by the first and second authors has indicated that variation in both peduncle length and degree of sinuosity of the leaflets is greater in var. *pentaphyllum* than previously thought, but with fertile material available, the morphological distinction from *A. sinuatum* is nevertheless usually quite clear.

REPRODUCTIVE BIOLOGY

Petersen (1989) reported chromosome numbers of between $2n = 30$ and $2n = 60$ for *A. pentaphyllum*, including var. *pentaphyllum*. Ramalho (1994), working with plants from Pernambuco in north-east Brazil, reported $2n = 60$ for *A. pentaphyllum* var. *pentaphyllum*; further work is needed to investigate the variation in cytology in this taxon. No detailed study of the mating system exists. Madison (1978) briefly reported the results of some hybridizations, which included *A. pentaphyllum*, finding that selfing resulted in 95% fruit set and viable seed; he con-

cluded that internal genetic barriers to hybridization between species probably exist in the palmatifid *Anthurium* species. Croat (1980) reviewed the flowering behaviour in *Anthurium* as a whole. The flowers are bisexual and tepalate, and most species are distinctly protogynous with female and male phases separated by a sterile phase (Schwerdtfeger, Gerlach & Kaiser, 2002), suggesting that outbreeding is the rule, as in Araceae generally (Mayo *et al.*, 1997). Our own field observations confirm that, in *A. sinuatum* and *A. pentaphyllum*, stigma receptivity (indicated by the presence of a conspicuous droplet) occurs prior to and separately from pollen presentation, and we have concluded that these two species are probably outbreeders. Gibernau (2003) reported bees, beetles and flies as pollinators of *Anthurium* but, as yet, there are no specific reports for *A. pentaphyllum*. The berries in both *A. pentaphyllum* and *A. sinuatum* are conspicuous and glossy red, maroon or purple, and are probably dispersed by fruit-eating birds. Levey (1988), reporting on the ecology of fruit-eating birds, cited *A. pentaphyllum* as a probable food plant.

THE BREJO FORESTS

The brejo forests of north-east Brazil are found on mountainous uplands within the semi-arid caatinga biome that occupies most of the lowlands in the interior of the region. They occur between 500 and 1110 m in altitude wherever humid prevailing winds provide annual rainfall above 1200 mm (Lima, Ferreira & Cavalcanti, 1975). The soils are well developed and, prior to human disturbance in recent times, supported forest formations with permanent water courses (Andrade-Lima, 1960, 1982; IBGE, 1985; Lins, 1989). The climate is cooler than in the surrounding caatinga, where water shortages for over 9 months of the year and high temperatures are the norm (Ab'Sáber, 1977a, b; Sampaio *et al.*, 2002). The brejo forests are currently classified as part of the Brazilian Atlantic forest (IBGE, 1985, 2008), and are considered its most threatened element, 85% having been destroyed by deforestation, despite their critical importance for regional water resources, as well as for scientific, environmental and social reasons (Rodal *et al.*, 1998; Pôrto *et al.*, 2004). Few remaining areas have effective legal protection or strategies for their preservation and recuperation, and there is an urgent need to develop science-based conservation programmes.

Various authors have proposed that the Ceará brejos represent natural fragments of a former humid forest corridor during the Pleistocene or even earlier, along the northern coast of Brazil, linking the Atlantic and Amazonian regions (for example, Ab'Sáber, 1982; Bigarella & Andrade-Lima, 1982; Borges-Nojosa & Caramaschi, 2003). In contrast with the eastern coast, this area has no littoral humid forest zone and is much drier. According to this hypothesis, the natural brejo forest fragments, prior to the large-scale human disturbance of the past 500 years, resulted from the regional spread of drier climatic conditions which restricted humid forest to higher altitudes with greater rainfall and lower temperatures. Palynological evidence is crucial to establishing the previous existence of more mesic conditions in north-east Brazil. Although Behling (1998) and Ledru (1991, 1993) have attempted reconstructions of the palaeoclimate of the vegetation of south-east Brazil in the final phase of the Quaternary, palynological studies in north-east Brazil are few. Those of Oliveira *et al.* (1999) and Barreto *et al.* (2002), on Holocene sediments in fossil dune systems of the Rio São Francisco basin, are the most interesting in the context of our work, because of their relative proximity; their study sites are located approximately 780 km south–south-west of the Ceará brejos. Oliveira *et al.* (1999) hypothesized that conditions favourable for a humid forest connection between Amazonia and the Atlantic forest could have existed in the first half of the Holocene, either along the north coast of Ceará and neighbouring states or via the gallery forests across central Brazil, or both, up to approximately 5000 BP.

The aim of our study was to investigate patterns of genetic diversity within and divergence between the studied populations, primarily to address the following questions. Do the Ceará brejo populations show genetic evidence of their geographical and ecological isolation, such as low intrapopulation diversity, and do they differ significantly in genetic marker patterns? Is the disjunct French Guiana population of *A. pentaphyllum* markedly different from the Atlantic forest populations, as might be expected in view of their great geographical separation? Is the genetic difference between the populations of the morphologically variable *A. pentaphyllum* significantly greater than in *A. sinuatum*? Do the Atlantic forest populations cluster into geographical groups matching patterns known in other biota from this region, such as the Paulista and Central Corridor centres of diversity (Aguiar *et al.*, 2003)? Can *A. sinuatum* and *A. pentaphyllum* be distinguished genetically and is there evidence of their genetic mixing in Ceará, where *A. pentaphyllum* is absent?

MATERIAL AND METHODS
SAMPLE POPULATIONS

A total of 166 individual plants was sampled for this study from 12 populations (seven populations of *A. pentaphyllum*, five populations of *A. sinuatum*) in

Table 1. Geographical details of sampled populations of *Anthurium pentaphyllum* and *Anthurium sinuatum*

Population code	Latitude and longitude	Population details	Country or Brazilian state	Sample size
Anthurium pentaphyllum var. *pentaphyllum*				
APBAR	12°46′40″S 39°03′38″W	Recôncavo, Serra da Copioba, Serra da Jibóia	Bahia	14
APBAS	15°17′49″S 39°41′27″W	Cacao Zone, Una	Bahia	19
APES	19°09′05″S 40°04′14″W	Linhares, Santa Tereza	Espírito Santo	17
APFG	04°37′26″N 52°31′58″W	Arataí	French Guiana	8
APPER	08°05′10″S 35°00′53″W	Recife, Curado	Pernambuco	9
APRJ	22°58′00″S 43°13′40″W	Rio de Janeiro (Jardim Botânico, Vista Chinesa)	Rio de Janeiro	19
APSP	23°51′14″S 46°08′20″W	Bertioga	São Paulo	8
Anthurium sinuatum				
ASCEbat	04°13′19″S 38°56′30″W	Serra de Baturité	Ceará	30
ASCEibi	03°49′58″S 40°56′23″W	Serra de Ibiapaba	Ceará	15
ASCEmar	03°54′32″S 38°43′30″W	Serra de Maranguape	Ceará	7
ASCEpac	03°57′21″S 38°37′44″W	Serra de Aratanha, Pacatuba	Ceará	14
ASPA	01°30′32″S 48°17′59″W	Acará, Marituba	Pará	6

See also map in Figure 1.

the humid forests of Ceará, eastern Amazonia, French Guiana and the Brazilian Atlantic forest (Table 1). Because of the possibility of clonal growth, care was taken when collecting to avoid sampling plants from the same tree bole, or, if terrestrial, within 10 m of each other; this reduced the scope for larger samples in some populations.

Voucher specimens of all populations sampled (Table S1, see Supporting Information) are deposited at the following herbaria: Herbário, Universidade Federal do Ceará, Fortaleza (EAC); Herbário, Universidade Estadual de Feira de Santana (HUEFS); Herbarium, Royal Botanic Gardens, Kew (K). Species names in Araceae follow CATE Araceae (2008); see Holmgren & Holmgren (1998) for herbarium codes.

MOLECULAR TECHNIQUES

Genomic DNA was extracted from leaf fragments dried in silica gel following the 2 × cetyltrimethylammonium bromide (CTAB) protocol of Doyle & Doyle (1987) with modifications (10 mL extraction with

40 μL β-mercaptoethanol, followed by precipitation in isopropanol). All samples were then cleaned using QIAQUICK columns (QIAGEN Inc., Warrington, Cheshire, UK). AFLP reactions (Vos *et al.*, 1995) were conducted according to the AFLP™ Plant Mapping Protocol of PE Applied Biosystems Inc. (Applied Biosystems, 1996) using *Eco*RI and *Mse*I. Eight primer combinations (with three and four selective bases on the *Eco*RI- and *Mse*I-based primers, respectively) were tested on a small number of samples. The two combinations which gave the best results in terms of clarity of traces, numbers of bands and numbers of polymorphic bands were primer pairs *Eco*RI-ACT and *Mse*I-CTAG, and *Eco*RI-AGG and *Mse*I-CTAG, which were then used with all samples. Fragments were separated using an ABI 3100 sequencer with Genescan and Genotyper 2.0 (PE Applied Biosystems Inc.) software. Amplified fragments between 50 and 500 base pairs (bp) were scored by visual inspection for the presence (1) or absence (0) of peaks in the output traces. Only distinct peaks were scored as present, and the manual scoring procedure was repeated three

times on separate occasions to reduce inconsistencies in scoring to a minimum. The resulting binary matrices of AFLP bands were used to carry out various kinds of genetic data analysis.

GENETIC DATA ANALYSIS

Within-population genetic diversity
Within-population genetic diversity was estimated assuming Hardy–Weinberg equilibrium using GENALEX 6 (Peakall & Smouse, 2006) to compute average expected heterozygosity (H_e, equivalent to Nei's gene diversity) for each population from the binary AFLP data matrix.

The average values of H_e calculated using GENALEX 6 were tested employing a bootstrap resampling procedure carried out using the statistical software package R (R Development Core Team, 2006). The rows of the original binary matrix were randomly permuted and the columns were then bootstrap resampled using the 'sample' function with replacement ('replace = T'). A random sample of 14 rows (the average sample size) was then made, from which a sample average H_e was calculated using the formula given in the GENALEX manual (Peakall & Smouse, 2006). This procedure was repeated 10 000 times to provide a null distribution of values for average H_e, from which P values could be calculated for the average H_e values observed in each population.

Population clustering
We used AFLP-SURV version 1.0 (Vekemans, 2002; Vekemans *et al.*, 2002) to compute F_{ST} distance matrices between populations. The program is designed for AFLP data and estimates allele frequencies at each marker locus in each population, assuming that the markers are dominant and that there are two alleles per locus (presence of the band being dominant and absence recessive). We used the default option, i.e. Bayesian method with non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999), and assumed Hardy–Weinberg equilibrium for the data. Wright's fixation index F_{ST} (Hartl & Clark, 1997) was computed using the method of Lynch & Milligan (1994), and tested by a permutation procedure of 1000 replicates, which randomly permuted individuals among the populations and then recalculated F_{ST} for each permutation; the observed value of F_{ST} was then compared with the distribution of randomized F_{ST} values. A distance matrix of pairwise F_{ST} between populations was calculated in AFLP-SURV as a measure of interpopulation genetic differentiation, from which 1000 bootstrapped replicate matrices were then computed.

To express the clustering patterns of populations visually, the bootstrapped F_{ST} matrices were used as input to compute an unweighted pair group method with arithmetic averaging (UPGMA) consensus tree using the software package PHYLIP version 3.66 (Felsenstein, 2006): first, the module NEIGHBOR was used to compute UPGMA dendrograms for all bootstrapped matrices, and then an extended majority rule consensus tree was produced using the module CONSENSE.

Significance of interpopulation genetic differences
The significance of the population pairwise differences was estimated using the software package GENALEX 6 (Peakall & Smouse, 2006). Distance matrices were prepared from the original binary matrix by computing Φ_{PT}, a metric which is analogous to F_{ST} for binary data. The significance of the interpopulation Φ_{PT} values was evaluated by permutation tests of 1000 permutations.

Test for correlation with geographical distance
Isolation-by-distance patterns were investigated by computing the correlation between geographical and genetic (Φ_{PT}) population pairwise distance matrices and applying the Mantel test, using NTSYSpc version 2.2d (Rohlf, 2005). The significance of the Mantel z statistic value was tested non-parametrically by creating a null distribution of z using 10 000 random permutations, and comparing the observed z value.

Partition of variation within and between populations
Analysis of molecular variance (AMOVA) was carried out using ARLEQUIN version 2.0 (Schneider, Roessli & Excoffier, 2000); the number of AFLP phenotypes observed in each population was computed using the setting 'Infer haplotype definitions from distance matrix'. Genetic structure and statistics were computed from a matrix of Euclidean squared distances between every pair of individuals (Schneider *et al.*, 2000). Four models were tested. The first treated all populations as a single group, i.e. a two-level analysis, to obtain a value for F_{ST} as an overall measure of population divergence. Two of the other three models treated the populations of each species separately as a single group in two-level analyses. The fourth model was a three-level hierarchical analysis treating all 12 populations in two groups, representing the two species.

Test for influence of inbreeding
Population structure was also investigated by the Bayesian software HICKORY version 1.0.4 (Holsinger, Lewis & Dey, 2002; Holsinger & Lewis, 2003), in order to assess the importance of inbreeding in the data and the assumption of Hardy–Weinberg equilibrium. HICKORY makes it possible to evaluate

departures from Hardy–Weinberg equilibrium in dominant as well as co-dominant markers. Separate analyses were carried out for the seven populations of *A. pentaphyllum* and the five populations of *A. sinuatum*. For each, the AFLP data were fitted to four models: 'full model', which allows for inbreeding; '$f = 0$' model, which implies a lack of inbreeding; '$theta = 0$' model, which implies a zero-valued F_{ST} analogue (i.e. no differentiation between populations); and 'f free' model, which decouples the estimates of f and *theta*. Computations were carried out using the default values, as recommended in the manual (Holsinger & Lewis, 2003): burn-in = 5000, number of samples = 25 000 and thinning factor = 5. To estimate the best fit of the four models, the criteria DIC (deviance information criterion), Dbar, Dhat and pD were used, with interpretations following the recommendations given in the manual.

Grouping the individuals into genetic clusters
Three methods were used to cluster individuals on the basis of their AFLP marker patterns: (1) agglomerative hierarchical cluster analysis (UPGMA); (2) principal coordinate analysis; and (3) model-based Bayesian cluster analysis.

A bootstrapped UPGMA was carried out using the software package PHYLIP version 3.66 (Felsenstein, 2006). The binary AFLP matrix was bootstrapped with 1000 replicates using the SEQBOOT module. Distance matrices for each replicate matrix were computed using the RESTDIST module, which is designed to calculate distance matrices from restriction site or fragment data, including AFLPs. The module NEIGHBOR was used to compute UPGMA dendrograms for all bootstrapped distance matrices, and an extended majority rule consensus tree was produced using the module CONSENSE. Bootstrap values were then transferred to a UPGMA dendrogram computed in NTSYSpc version 2.2 (Rohlf, 2005) from the RESTDIST distance matrix based on the original binary matrix.

Principal coordinate analysis was used to generate a two-dimensional ordination of the sampled individuals, which served primarily to illustrate the separation of the two species, based on a dissimilarity measure different from that used in UPGMA. Jaccard's similarity coefficient (Sneath & Sokal, 1973) was computed for all pairs of individuals using the SIMQUAL module in NTSYSpc version 2.2d, and then transformed into a dissimilarity measure as (1 – Jaccard's similarity). Principal coordinate analysis was carried out on this dissimilarity matrix using NTSYSpc version 2.2d (modules DCENTER, EIGEN, MXPLOT).

Model-based Bayesian analysis was carried out using the software package STRUCTURE version 2.1 (Pritchard, Stephens & Donnelly, 2000), applying a 'no admixture' model, 100 000 burn-in period length and 100 000 Markov chain Monte Carlo (MCMC) replicates after burn-in. This approach requires that the number of clusters (k) is predefined, and the analysis then assigns the individuals to the clusters probabilistically. We performed three to five runs for each value of k (2–19), and then eight runs with 500 000 MCMC replicates for the $k = 14$ model. STRUCTURE is widely used for inferring gene pool structure in genetic data.

RESULTS

The matrix of 166 individuals, from which satisfactory results were obtained for both primer combinations, was made up of 252 fragments (bands) and was used for further analysis; 100% of the scored marker bands were polymorphic. All AFLP phenotypes recorded were unique to one individual and we therefore assumed that no resampling of clones had occurred during the field collections.

WITHIN-POPULATION GENETIC DIVERSITY

In *A. pentaphyllum*, the most diverse populations, in descending order, were Bahia-Recôncavo (APBAR), Espírito Santo (APES) and Rio de Janeiro (APRJ), none of which was significantly less diverse than expected (Table 2). The southernmost population in São Paulo was much less diverse ($P < 0.0001$), and remained so when the bootstrap test was modified to take replicate samples of only $N = 8$, the sample size of this population. The northern populations in Pernambuco (APPER, $P < 0.01$) and French Guiana (APFG, $P < 0.01$) were likewise significantly less diverse than the average.

In *A. sinuatum*, the Ibiapaba population (ASCEibi), the most isolated of those studied in Ceará, was genetically the most diverse (Table 2), and Maranguape (ASCEmar) was the least diverse, measured by average expected heterozygosity (H_e). Pacatuba (ASCEpac) was the most diverse measured by percentage of polymorphic loci (P_l). The other two populations, including that at Pará (ASPA), had intermediate levels of diversity. The bootstrap analysis showed that the Ibiapaba population did not differ significantly in diversity ($P = 0.056$) from the average for pooled populations of both species taken together. The Maranguape population, however, was significantly less diverse ($P < 0.001$), and the others slightly so ($0.02 < P < 0.03$).

POPULATION CLUSTERING

UPGMA cluster analysis, based on a distance matrix of population pairwise F_{ST} (Table 3), grouped the

Table 2. Genetic diversity measures in 12 sampled populations of *Anthurium pentaphyllum* and *Anthurium sinuatum* from Brazil and French Guiana

Population code	Sample size	Percentage polymorphic loci (P_l)	Average expected heterozygosity (H_e)	SE of H_e	P value*
Anthurium pentaphyllum var. *pentaphyllum*					
APBAR	14	62.30	0.153	0.011	0.139
APBAS	19	62.30	0.144	0.010	0.051
APES	17	58.33	0.150	0.011	0.097
APFG	8	50.79	0.134	0.010	0.009
APPER	9	44.05	0.133	0.011	0.008
APRJ	19	54.76	0.146	0.011	0.065
APSP	8	30.95	0.091	0.010	0.000
Anthurium sinuatum					
ASCEbat	30	53.97	0.140	0.011	0.026
ASCEibi	15	51.98	0.145	0.011	0.056
ASCEmar	7	38.10	0.121	0.011	0.000
ASCEpac	14	57.14	0.140	0.011	0.027
ASPA	6	42.86	0.139	0.011	0.023

*The P value is the frequency at which the observed value of H_e occurred in the resample distribution of 10 000 random bootstrap replicates. If $P < 0.05$, the observed value is considered to be significantly lower than expected by chance. Diversity measures computed in GENALEX 6 (Peakall & Smouse, 2006); bootstrap resample distribution computed in R version 2.4.1. (R Development Core Team, 2006). See Table 1 for population codes.

populations into their respective species (Fig. 1), but the bootstrap support of the two species' clusters (85.8%) was weaker than between some population groups within each species. The most strongly supported cluster (96.7%) was composed of the four northern populations of *A. pentaphyllum*, in French Guiana, Pernambuco and Bahia. In *A. sinuatum*, the best supported group (93.1%) was composed of the Pará (ASPA, Amazonia) and Pacatuba (ASCEpac, Ceará) populations.

SIGNIFICANCE OF INTERPOPULATION GENETIC DIFFERENCES

Analysis of interpopulation genetic distances with GENALEX 6 showed that the 12 populations were significantly different in all possible pairs [P (probability of observed Φ_{PT}) ≤ 0.03), except in the Pará–Pacatuba population pair ($P = 0.056$).

CORRELATION WITH GEOGRAPHICAL DISTANCE

Genetic distances between populations (estimated with Φ_{PT} using GENALEX 6) and geographical distances were not significantly correlated within either species (Mantel test: *A. sinuatum*: $r = -0.26$, $P = 0.83$; *A. pentaphyllum*: $r = 0.20$, $P = 0.18$).

PARTITION OF VARIATION WITHIN AND BETWEEN POPULATIONS

The AMOVA results (Table 4) using ARLEQUIN showed that between-population variation was similar within *A. pentaphyllum* (15.82% of total variation) and within *A. sinuatum* (15.73% of total variation), and lower than that between the two species (24.80% of variation). When treated as 12 subpopulations of a single overall population, the data gave 28.7% between-population variation and 71.3% within-population variation.

INFLUENCE OF INBREEDING

Results using the Bayesian software HICKORY showed that inbreeding was unlikely to be a major driving force in determining the gene pool structure in either taxon (Table 5). In both analyses, the DIC parameter was equal or almost equal in the 'full' and '$f = 0$' models, whereas the Dbar values were lower in the '$f = 0$' model. Because the model with the lowest Dbar value is that which fits the data best, this pattern of results allows the '$f = 0$' model (inbreeding equals zero) to be considered as more likely than the 'full' model (HICKORY manual; Holsinger & Lewis, 2003).

GROUPING THE INDIVIDUALS INTO GENETIC CLUSTERS

Hierarchical cluster analysis

Agglomerative hierarchical cluster analysis (UPGMA) of individuals showed two major clusters (Fig. 2) within which the two species *A. pentaphyllum* (open bar) and *A. sinuatum* (filled bar) were recognizable,

Table 3. Pairwise genetic distance between populations of *Anthurium pentaphyllum* and *Anthurium sinuatum* (F_{ST} values in lower triangle), computed with AFLP-SURV version 1.0, and geographical distance (km) between populations (upper triangle)

	APBAR	APBAS	APES	APFG	APPER	APRJ	APSP	ASCEbat	ASCEibi	ASCEmar	ASCEpac	ASPA
APBAR	–	242	704	2425	714	1197	1418	952	1013	989	985	1600
APBAS	0.019	–	464	2620	909	973	1214	1234	1252	1231	1227	1794
APES	0.105	0.100	–	2963	1352	535	816	1712	1697	1692	1688	2148
APFG	0.027	0.048	0.141	–	2401	3216	3226	1802	1593	1802	1814	826
APPER	0.035	0.022	0.073	0.039	–	1878	2122	611	808	617	606	1646
APRJ	0.058	0.078	0.093	0.119	0.087	–	313	2135	2131	2164	2161	2436
APSP	0.238	0.253	0.176	0.297	0.258	0.161	–	2315	2285	2346	2345	2483
ASCEbat	0.238	0.265	0.334	0.245	0.265	0.315	0.453	–	226	42	46	1082
ASCEibi	0.152	0.191	0.274	0.154	0.190	0.246	0.409	0.097	–	246	257	858
ASCEmar	0.188	0.229	0.296	0.212	0.226	0.269	0.418	0.05	0.069	–	12	1097
ASCEpac	0.050	0.086	0.202	0.055	0.092	0.149	0.342	0.145	0.084	0.086	–	1109
ASPA	0.061	0.103	0.212	0.071	0.114	0.153	0.343	0.119	0.031	0.088	0.009	–

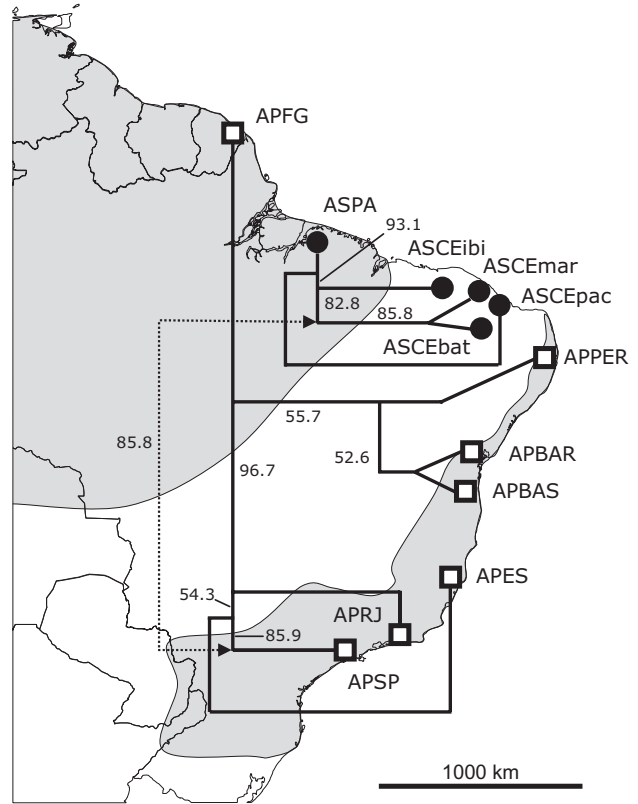See Table 1 for key to population codes.

Figure 1. Unweighted pair group method with arithmetic averaging (UPGMA) cluster analysis of populations of *Anthurium pentaphyllum* and *A. sinuatum*. Extended majority rule tree and bootstrap (computed with PHYLIP version 3.66) based on an F_{ST} distance matrix computed (with AFLP-SURV) from AFLP molecular data. Internal branches show percentage of trees (out of 1000 bootstrap replicates) in which the branch occurs; the branch connecting the subtrees of the two species is shown stippled for clarity. Filled circles are *A. sinuatum* populations; open squares are *A. pentaphyllum* populations. Approximate area of Amazon and Atlantic forests prior to European colonization shown by grey tone. See Table 1 for population codes.

but there were also smaller clusters composed of individuals from both species. Bootstrap values were generally low, especially for the larger clusters.

Principal coordinate analysis
Principal coordinate analysis produced a partial separation of the individuals of the two species, but with a central area of overlap (Fig. 3). Most of the individuals occupying an intermediate position (open symbols) were those assigned to mixed genetic clusters by the Bayesian analysis (see below). The first and second principal coordinate axes expressed 31.6% of the total variance in the Jaccard dissimilarity matrix.

Table 4. Analysis of molecular variance (AMOVA) in *Anthurium pentaphyllum* and *Anthurium sinuatum* from Brazil and French Guiana, for 166 individuals from 12 populations, using 252 AFLP molecular markers

Model	Partitioning	Variance (%)	F statistic	P
Two levels: 12 populations in one group	Among populations	28.70	$F_{ST} = 0.287$	< 0.0001
APBAR, APBAS, APES, APFG, APPER, APRJ, APSP, ASCEbat, ASCEibi, ASCEmar, ASCEpac, ASPA	Within populations	71.30		
Two levels: seven populations of *A. pentaphyllum* as one group	Among populations	15.82	$F_{ST} = 0.158$	< 0.0001
APBAR, APBAS, APES, APFG, APPER, APRJ, APSP	Within populations	84.18		
Two levels: five populations of *A. sinuatum* as one group	Among populations	15.73	$F_{ST} = 0.157$	< 0.0001
ASCEbat, ASCEibi, ASCEmar, ASCEpac, ASPA	Within populations	84.27		
Three levels: two species *A. pentaphyllum*, *A. sinuatum* as two groups	Among species	24.80	$F_{CT} = 0.248$	< 0.001
Two groups: (APBAR, APBAS, APES, APFG, APPER, APRJ, APSP); (ASCEbat, ASCEibi, ASCEmar, ASCEpac,	Among populations within species	11.90	$F_{SC} = 0.158$	< 0.0001
ASPA)	Within populations	63.29	$F_{ST} = 0.367$	< 0.0001

Computed with ARLEQUIN version 2.0. The P values represent the probability of obtaining by chance a value of F_{ST} equal to or more extreme than the observed value estimated from 10 100 permutations. See Table 1 for population codes.

Table 5. Genetic structure analysis of seven populations of *Anthurium pentaphyllum* and five populations of *Anthurium sinuatum* using HICKORY version 1.0.4

Model	Dbar	Dhat	pD	DIC	f	*Theta B*
Seven populations *Anthurium pentaphyllum*						
Full	3798.65	3065.24	733.41	4532.06	0.990	0.172
$f = 0$	3774.93	3016.94	757.99	4532.92		0.121
Theta B = 0	6309.25	6081.61	227.63	6536.88	0.991	
f free	3821.58	3059.76	761.82	4583.40	0.494	0.154
Five populations *Anthurium sinuatum*						
Full	2431.30	1971.07	460.23	2891.53	0.985	0.149
$f = 0$	2416.53	1940.13	476.40	2892.93		0.107
Theta B = 0	3790.83	3605.93	184.90	3975.72	0.983	
f free	2447.75	1966.11	481.64	2929.38	0.507	0.137

Default values for computations were used as follows: burn-in, 5000; sample, 25 000; thin, 5. See text for further explanation.

Model-based Bayesian cluster analysis

Using model-based Bayesian STRUCTURE analysis, the likelihood of the data rose to a plateau beginning at 14 clusters ($k = 14$), which was taken as the optimal cluster number following the guidelines in the program documentation (Pritchard & Wen, 2004). Above this value for k, the variability between different runs at the same k value increased, indicating a less stable structure in the data. Eight runs using 14 clusters were carried out and the result of one of these is shown in Table 6, which is a summary showing the proportion (between 0 and 1) of individuals in each population assigned to particular clusters, averaged over 500 000 replications. Figure S1 (see Supporting Information) shows the result as the probability of assignment of each individual to one of the 14 clusters. Each individual is represented in the figure as a narrow vertical line, which is coloured according to cluster. The individuals of each population are labelled and grouped together as a block, and the populations are arranged in north–south order, beginning on the left with the French Guiana *A. pentaphyllum* population, followed by the five *A. sinuatum* populations, and then the rest of the *A. pentaphyllum* populations from Pernambuco in the north to São Paulo in the south (compare Fig. 4).
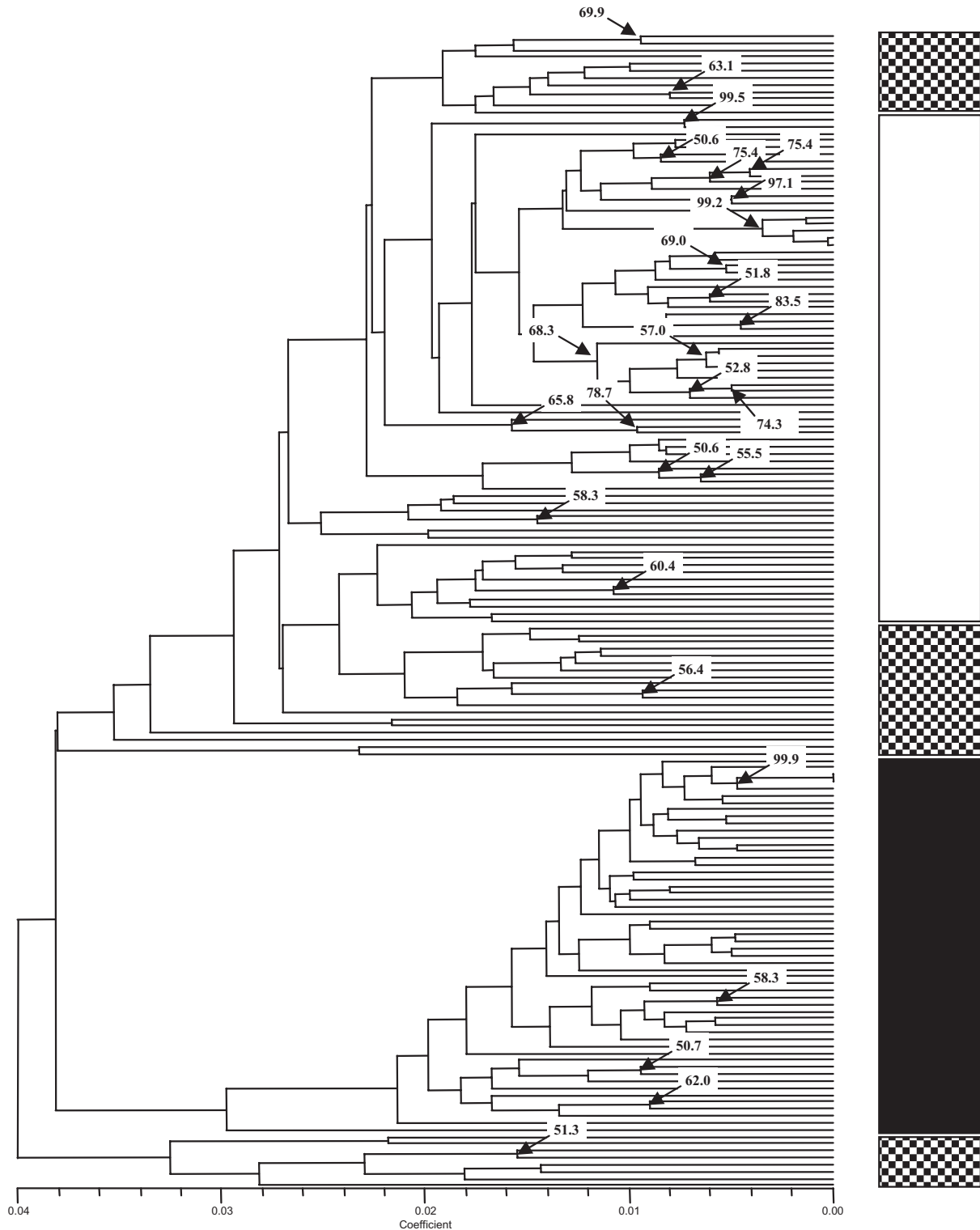
Figure 2. Unweighted pair group method with arithmetic averaging (UPGMA) cluster analysis of individuals of *Anthurium pentaphyllum* and *A. sinuatum*. Computed from AFLP molecular data with NTSYSpc version 2.2d (Rohlf, 2005) based on a distance matrix computed by RESTDIST module in PHYLIP version 3.66 (Felsenstein, 2006); bootstrap values (only those > 50% shown) derived from 1000 replicate bootstrap analysis computed in PHYLIP. Open bar, clusters containing individuals of *Anthurium pentaphyllum* only; filled bar, *Anthurium sinuatum* individuals only; chequered bars, clusters including individuals of both species.
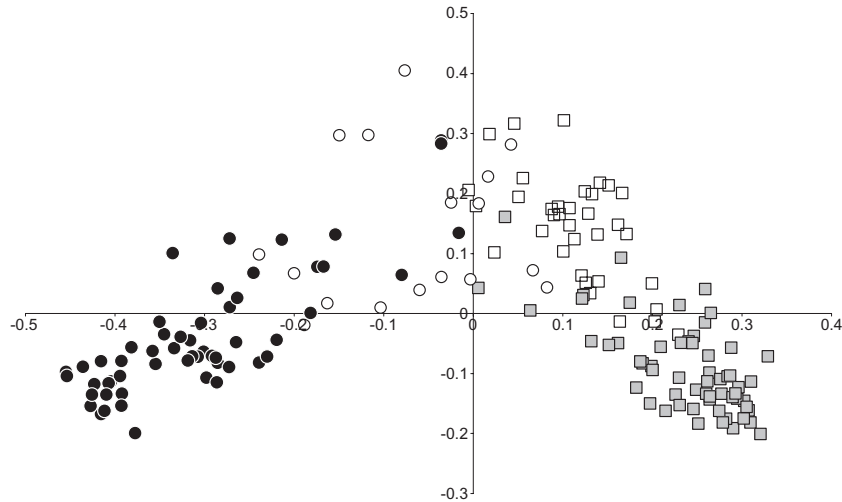
Figure 3. Genetic structure analysis based on AFLP data of 12 populations of *Anthurium pentaphyllum* (squares) and *A. sinuatum* (circles) showing individuals from mixed genetic clusters (open circles and squares) lying between individuals assigned to pure genetic clusters of *A. sinuatum* (filled circles) and *A. pentaphyllum* (grey squares). All 166 individuals are plotted in a two-dimensional principal coordinate analysis ordination (horizontal axis, first principal coordinate; vertical axis, second principal coordinate; 31.6% of total variance expressed in the space of these two axes). Principal coordinate analysis carried out using NTSYSpc version 2.2d based on Jaccard's dissimilarity matrix (see text). Genetic structure analysis carried out with STRUCTURE version 2.1 (Pritchard *et al.*, 2000).

When adjacent individuals belong to the same genetic cluster, they form a wider block of colour, and thus the patterns show the distribution of clusters in each population. There were clear differences within and between the species. The *A. sinuatum* populations showed generally more uniformity, although there were also strong contrasts, as between the well-sampled but uniform population at Baturité (ASCEbat) and the highly diverse one at Pacatuba (ASCEpac). The patterns in *A. pentaphyllum* were much more diverse; the French Guiana population (APFG) most strongly resembled that in northern Bahia (APBAR), and there were clusters which grouped the Pernambuco population, on the one hand, with those in Bahia (APPER, APBAR, APBAS) and, on the other, with that in Espírito Santo (APES). The most southerly populations in Rio de Janeiro (APRJ) and São Paulo (APSP) were distinct from one another and from the rest. Within *A. pentaphyllum*, the picture is of a gradually changing set of cluster patterns from north to south.

We examined the clusters generated over eight separate runs, looking for those consisting of individuals from only one species (pure clusters) and those combining individuals from both taxa (mixed clusters), on the assumption that these two cluster classes would provide information on patterns within species and those common to both, respectively. This procedure also showed the relative consistency of the output of the cluster patterns by the Bayesian analysis over a series of runs. Twenty-five different genetic clusters could be recognized, some of which only appeared once. In the run shown in Table 6, rows 1–13 represent the most consistent clusters, appearing in all or almost all runs; of these, eight were pure (mapped in Fig. 4) and five were mixed.

The five populations of *A. sinuatum* were grouped together by two different pure clusters (Fig. 4, Table 6, clusters 1, 2), but none of these populations had a cluster unique to itself. There was no cluster pattern linking the four Ceará populations uniquely. The Pacatuba population (ASCEpac) of *A. sinuatum* was involved in more clusters than the other populations of this species, and most of these were mixed, suggesting significant gene exchange with *A. pentaphyllum* (Table 6).

In *A. pentaphyllum*, there was no cluster which grouped all populations uniquely (Fig. 4, Table 6), but unique clusters occurred: (1) in single populations in French Guiana (cluster 3, APFG), Espírito Santo (cluster 6, APES) and Rio de Janeiro (cluster 7, APRJ); (2) in population pairs [cluster 5, Pernambuco (APPER) + Espírito Santo (APES); cluster 8, Rio (APRJ) + São Paulo (APSP)]; and (3) in the northerly Atlantic Forest [cluster 4, Pernambuco (APPER) + northern Bahia (APBAR) + southern Bahia (APBAS) + Espírito Santo (APES)].

Striking contrasts in cluster membership (and frequency) also occurred in *A. pentaphyllum* populations (Table 6). Both Bahian populations (APBAR, APBAS)

Table 6. Genetic clusters (rows) in 12 populations of *Anthurium* recovered by Bayesian analysis of AFLP data using STRUCTURE version 2.1, showing the output from a single run for a 14-cluster model ($k = 14$; 166 individuals, 252 loci, burn-in period 100 000 replications, Markov chain Monte Carlo (MCMC) 500 000 replications, no admixture model assumed

Cluster number (see Fig. 4)	*Anthurium sinuatum* populations					*Anthurium pentaphyllum* populations						
	ASPA	ASCEibi	ASCEmar	ASCEpac	ASCEbat	APFG	APPER	APBAR	APBAS	APES	APRJ	APSP
Pure clusters												
1	0.28	0.04	0.15	0.57	0.07	0	0	0	0	0	0	0
2	0.10	0.70	0.84	0.11	0.83	0	0	0	0	0	0	0
3	0	0	0	0	0	0.45	0	0.00	0	0	0	0
4	0	0	0	0	0	0	0.10	0.09	0.07	0.32	0	0
5	0	0	0	0	0	0	0.33	0	0	0.29	0	0
6	0	0	0	0	0	0	0	0	0	0.30	0	0
7	0	0	0	0	0	0	0	0	0	0	0.70	0
8	0	0	0	0	0	0	0	0	0	0	0.05	1
Mixed clusters												
9	0.22	0.19	0	0.07	0	0.22	0	0	0.04	0	0.11	0
10	0	0	0	0.02	0	0.03	0.16	0.22	0.11	0.05	0.00	0
11	0.00	0	0	0.08	0	0.22	0.40	0.30	0.42	0.00	0	0
12	0	0.00	0	0.05	0	0.08	0	0.17	0.01	0.04	0.11	0
13	0	0	0	0.06	0	0	0	0.22	0.31	0	0	0
14	*0.40*	*0.07*	*0.02*	*0.04*	*0.10*	*0*	*0*	*0.00*	*0.04*	*0*	*0.05*	*0*
Population sample size	6	15	7	14	30	8	9	14	19	17	19	8

Values show the proportion of the membership of each population in each of the 14 clusters. Of eight complete runs, clusters 1–13 were recovered in seven or eight cases, whereas cluster 14 (in italics) appeared only three times. See Table 1 for key to population codes.
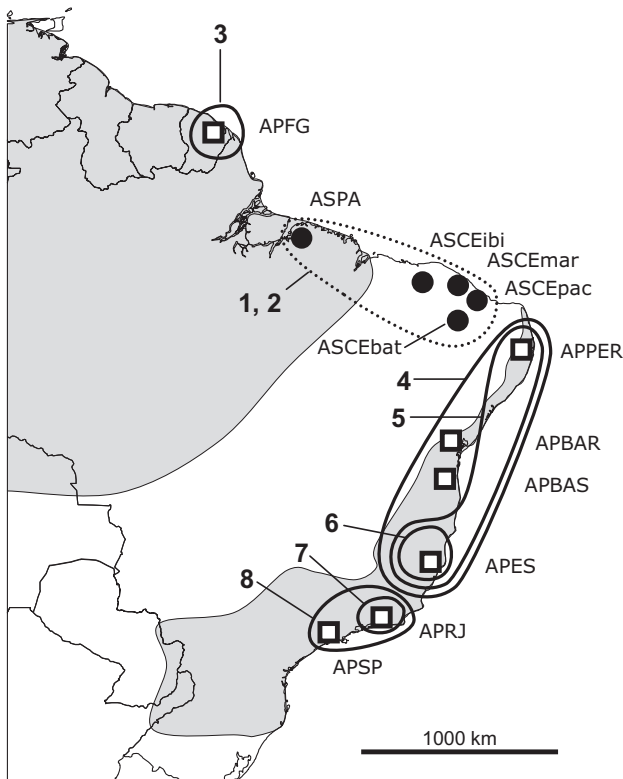
Figure 4. Genetic structure analysis of *Anthurium pentaphyllum* and *A. sinuatum* showing pure genetic clusters plotted geographically. Lines group the populations that contributed individuals to the numbered genetic clusters. The dotted line shows clusters (1, 2) consisting of *Anthurium sinuatum* individuals only. Full lines show clusters (3–8) composed of *A. pentaphyllum* individuals only. Filled circles are *A. sinuatum* populations; open squares are *A. pentaphyllum* populations. Approximate area of Amazon and Atlantic forests prior to European colonization shown by grey tone. Analysis performed with STRUCTURE version 2.1 (Pritchard *et al.*, 2000) based on AFLP molecular data. See Table 1 for population codes.

were involved in a relatively large number of mostly mixed clusters, and the great majority of their individuals were also assigned to mixed clusters. In Espírito Santo (APES), however, most individuals were in pure clusters. São Paulo (APSP) stood out as the only population composed entirely of a single genetic cluster.

DISCUSSION

Apart from our parallel work on *Monstera adansonii* (Andrade *et al.*, 2007), we know of no other study comparing genetic patterns in plants at the population level in the brejo forests of north-east Brazil. The generalization of our results and conclusions is thus limited by the absence of strictly comparable work in

the region and the small sample sizes available for some populations. There are relevant studies from the southern Atlantic forest by Barbará *et al.* (2007), C. Palma-Silva (UFRS, Porto Alegre, Brazil, unpubl. data) and G. Paggi (UFRS, Porto Alegre, Brazil, unpubl. data) on Bromeliaceae, and by Stefenon, Mailing & Finkeldey (2007) on Araucariaceae.

GENETIC DIVERSITY AND BETWEEN-POPULATION
RELATIONSHIPS IN THE BREJO POPULATIONS
OF *A. SINUATUM*

The similar levels of genetic diversity in the populations in Ceará and Amazonia (Pará) suggest that, if the Ceará brejo forest populations were ecologically isolated by the spread of a semi-arid climate throughout north-east Brazil in the Holocene (Oliveira *et al.*, 1999), this has not resulted in reduced genetic diversity, as might have been expected. Indeed, the Ceará brejo populations are, on average, slightly more diverse than those in Pará (Table 2), although distinctly less so in the Maranguape population (also observed in *Monstera adansonii* var. *klotzschiana*; Andrade *et al.*, 2007). The small sample size of this population ($N = 7$) may be partly responsible for the result and, by the same argument, the diversity of the Pará population ($N = 6$) is probably an underestimate.

The range of genetic diversity values and contrasting patterns of occurrence of the genetic clusters show that these five populations are genetically distinct from one another (Tables 2, 6) and there is no tendency for the Ceará populations to group together on the basis of genetic data, a pattern also found in *M. adansonii* var. *klotzschiana* (Andrade *et al.*, 2007). Although geographically closer to each other (Fig. 1, Table 3), the only feature shared uniquely by the four Ceará brejo populations is a tendency for more individuals to belong to pure clusters (Table 6). The distance matrices (Table 3) show that the most genetically distant populations, Pacatuba and Baturité, are relatively close geographically (46 km), whereas the most similar pairs both involve the Pará population, geographically the most distant (858 km from the nearest at Ibiapaba). There is no significant correlation of the genetic (F_{ST}) and geographical distance matrices, and the AMOVA results (Table 4) show a low overall level of genetic structure among the populations of *A. sinuatum*.

The lack of evidence for genetic erosion and the generally low level of genetic structure in the Ceará brejo populations of *A. sinuatum* could be interpreted as indirectly supporting the view (Andrade *et al.*, 2007) that these are relictual areas formed relatively recently (dating from *c*. 5000 BP) by the contraction and virtual disappearance of a previously continuous coastal humid forest, possibly connected to Amazonia.

The relatively high genetic diversity of the Ceará populations could also be explained by repeated immigration resulting from cycles of forest expansion and retraction. However, local factors, such as the occurrence of cloning (Murawski & Hamrick, 1990) and the spatial structure of the habitat, may well be more important in influencing population genetic structure in these opportunistic herbaceous epiphytes. More detailed studies within the brejo areas are needed, as well as data from *A. sinuatum* populations elsewhere in Brazil, in order to provide a more complete picture of variation in population genetic patterns.

For conservation studies and planning, this result means that each Ceará forest island population is unique and substantially variable. Our work suggests that populations only occur in association with permanent water courses, implying that they are probably highly discontinuous within each mountain area.

GENETIC DIVERSITY AND BETWEEN-POPULATION RELATIONSHIPS IN *A. PENTAPHYLLUM*

In *A. pentaphyllum*, population diversity is greatest in the central part of the Atlantic forest. Bayesian analysis shows that there are no genetic clusters that uniquely group the populations of *A. pentaphyllum* in relation to *A. sinuatum*. However, there are five pure clusters which characterize populations individually or in subgroups (Fig. 4). This result reflects the greater morphological variability of *A. pentaphyllum* (Madison, 1978), despite the AMOVA result (Table 4), which shows little difference between the two taxa in overall among-population divergence. Populations of *A. pentaphyllum* have diverged more in certain localities, for example Espírito Santo (APES), where 58% of individuals belong to clusters either unique to this population or shared only with Pernambuco (APPER). In other localities, as in Bahia (APBAR, APBAS), no individuals belong to population-specific clusters.

The genetic profiles of the two most southerly populations (Rio and São Paulo) and, especially, the distinctly lower gene diversity of the latter suggest a southward migration (compare Aide & Rivera, 1998). The relationships of the pure clusters suggest that the Espírito Santo population is closer to those of the northern part of the Atlantic forest (Bahia, Pernambuco) than to Rio de Janeiro (Fig. 4), a pattern which has been reported in various other organisms at the species' rather than the population level (for example, the Central Corridor of Aguiar *et al.*, 2003: fig. 11.2). In contrast, the UPGMA tree (Fig. 1) does not show the same patterns, presumably because of the influence of the mixed genetic clusters.

The population from French Guiana, despite its great geographical distance from the other *A. pentaphyllum* populations, does not stand apart from them

in genetic distance, and its closest genetic relationships lie with the northern Atlantic forest populations in Bahia and Pernambuco (Fig. 1).

DIFFERENCES BETWEEN *A. SINUATUM* AND *A. PENTAPHYLLUM*

In interpreting the Bayesian analysis results, we have drawn attention to the contrast in pure and mixed cluster patterns, because the analysis was based on data from two species. Our assumption is that mixed cluster patterns may indicate the footprint of historical hybridization between the two species, whereas pure cluster patterns result from gene flow events within species. It is also possible that genetic clusters involving both species represent ancestral patterns. Whatever the explanation, from a taxonomic standpoint, these two classes of molecular patterns are of interest in considering how far the two morphologically defined species can be distinguished using AFLP markers.

The two taxa, *A. pentaphyllum* and *A. sinuatum*, emerge from the genetic analyses as only partially distinct. The unsupervised UPGMA analysis (Fig. 2) failed to separate the two species completely. The mixed clusters of the optimal 14-cluster Bayesian analysis blend the two species. This is also shown by the location of mixed cluster individuals in the central portion of the principal coordinate analysis ordination space (Fig. 3), whereas those of the pure clusters are widely separated. The Pacatuba population of *A. sinuatum* shows a particularly distinctive relationship to *A. pentaphyllum*, because of the presence of small numbers of individuals belonging to clusters that are otherwise exclusive to *A. pentaphyllum* (Table 6, Fig. S1).

The AMOVA study (Table 4) shows a higher among-species variance (24.80%) than among-population variance within the species (11.9%). When treated as 12 populations of a single group, AMOVA partitions 28.7% of variance among populations and 71.3% within populations, approaching double the among-population variance within each taxon separately. This population differentiation is relatively low when compared with those of outcrossing species reviewed by Morjan & Rieseberg (2004). However, the results nevertheless provide qualified support (Fig. 3) for the morphological partition represented by the current species boundaries, based primarily on leaflet shape and peduncle length.

We have focused here especially on populations of the two taxa in north-east Brazil but, to obtain a more complete picture of the species' relationships, further work is needed. Both species have wide ranges: *A. pentaphyllum* (in the broad sense) stretches from southern Mexico to the Atlantic forest of south-east

Brazil and *A. sinuatum* as far west as Peru (TROPI-COS, 2006). *Antherium sinuatum* also includes populations in central and south-central Brazil, some of which may yet prove to be discordant with those studied here, at least judging from herbarium collections.

Our interpretations and conclusions are necessarily tentative at this stage, but the study is a useful basis from which to develop more comprehensive and detailed work in the future. Larger samples are needed to confirm the gene pool cluster patterns and population diversity levels reported here. More sampling localities are required, especially in the Atlantic forest, where genetic diversity was found to be highest, and, in Amazonia, where both species occur. AFLP markers were chosen as an efficient technique for obtaining a broad picture of molecular patterns in a previously unstudied taxon. However, such dominant markers provide no information on heterozygote frequencies, and hence no direct estimates of inbreeding. In our defence, the results of the HICKORY analysis and the pronounced protogyny typical of aroid flowers strongly suggest that inbreeding is a negligible factor in interpreting the genetic patterns studied here. Nevertheless, the use of co-dominant markers would provide the means for a more thorough understanding of the processes that have conditioned the observed genetic structure. Future work should focus on co-dominant markers, such as nuclear microsatellites. The current patchy understanding of the mating system also points to the need for new research on reproductive ecology in these taxa.

In conclusion, the populations of *A. sinuatum* share common genetic patterns which characterize them as a group. They are genetically distinct from each other, but none exhibits a genetic pattern unique to itself, and the Ceará brejo populations do not cluster together as a subgroup. There is no significant correlation between genetic and geographical interpopulation distance and no strong overall within-species pattern of relationship among the populations. There is also no evidence of a reduction in genetic diversity, which might have been expected from the geographical and ecological isolation. We interpret this low level of genetic structure as the result of historical gene flow, probably during the early Holocene, within a continuous forest which subsequently underwent natural fragmentation.

In contrast with *A. sinuatum*, we found no unifying genetic pattern for the populations of *A. pentaphyllum* and, furthermore, the populations of *A. pentaphyllum* are characterized, individually and in subgroups, by unique genetic clusters which reflect the geographical patterns recognized in many other organisms. Despite a degree of population differentia-tion, as measured by AMOVA, similar to that in *A. sinuatum*, genetic divergence appears to be greater in *A. pentaphyllum*, which is consonant with its notable morphological variability.

Anthurium pentaphyllum and *A. sinuatum* can be distinguished genetically, although many individuals of both species form intermediate genetic cluster patterns which blur the distinction between them. This is true of the Pacatuba population of *A. sinuatum* and, particularly, in the two Bahian populations of *A. pentaphyllum*, in which the great majority of individuals group into genetic clusters that mix together individuals from the two taxa. These results seem to imply that genetic mixing has occurred between *A. sinuatum* and *A. pentaphyllum* in northeast Brazil, particularly in Bahia and eastern Ceará, possibly linked to cycles of humid forest expansion. This study demonstrates that the conservation and recuperation of the biota of the highly threatened brejo forests of Ceará are of great importance for scientific, as well as environmental, economic and social reasons.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Genetic structure analysis of 12 populations of *Anthurium pentaphyllum* and *A. sinuatum*. Bar chart showing assignment of individuals of each population to one (or more) of 14 clusters as indicated by different colour patterns. Populations, species and the geographic axis are shown below the chart. The chart shows the result of a single run in STRUCTURE version 2.1 (Pritchard *et al.*, 2000); whenever adjacent individuals are assigned to the same cluster, they are shown as a single block of pattern. Population codes: *Anthurium pentaphyllum*: APBAR, Bahia Recôncavo; APBAS, southern Bahia; APES, Espírito Santo; APFG, French Guiana; APPER, Pernambuco; APRJ, Rio de Janeiro; APSP, São Paulo. *Anthurium sinuatum*: ASCEbat, Baturité Ceará; ASCEibi, Ibiapaba Ceará; ASCEmar, Maranguape Ceará; ASCEpac, Pacatuba Ceará; ASPA, Pará; see Table 1 for further details.

Appendix S1. List of voucher specimens *of Anthurium* for this study. Voucher specimens are deposited in the following herbaria: EAC, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil; HUEFS, Universidade Estadual de Feira de Santana, Bahia, Brazil; K, Royal Botanic Gardens, Kew, UK. Population codes are given in bold. See Holmgren & Holmgren (1998) for herbarium codes or search the *Index Herbariorum* website at http://sciweb.nybg.org/science2/IndexHerbariorum.asp

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