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Genetic variability of an endangered Bromeliaceae species (*Pitcairnia albiflos*) from the Brazilian Atlantic rainforest

R. Domingues[1,2], M.A. Machado[2], R.C. Forzza[3], T.D. Melo[1], S. Wohlres-Viana[2] and L.F. Viccini[1]

[1]Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil
[2]Embrapa Gado de Leite, Juiz de Fora, MG, Brazil
[3]Jardim Botânico do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Corresponding author: L.F. Viccini
E-mail: lyderson.viccini@ufjf.edu.br

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ABSTRACT. *Pitcairnia albiflos* is a Bromeliaceae species endemic to Brazil that has been included as data-deficient in the extinction risk list of Brazilian flora. We analyzed genetic variability in *P. albiflos* populations using RAPD markers to investigate population structure and reproductive mechanisms and also to evaluate the actual extinction risk level of this species. Leaves of 56 individuals of *P. albiflos* from three populations were collected: Urca Hill (UH, 20 individuals), Chacrinha State Park (CSP, 24 individuals) and Tijuca National Park (TNP, 12 individuals). The RAPD technique was effective in characterizing the genetic diversity in the *P. albiflos* populations since it was possible to differentiate the populations and to identify exclusive bands for at least two of them. Even if there is low genetic diversity among them (CSP-UH = 0.463; CSP-TNP = 0.440; UH-TNP = 0.524), the populations seem to be isolated according to the low genetic diversity observed within them (H_{pop} CSP = 0.060; H_{pop} UH = 0.042; H_{pop} TNP = 0.130). This fact might be the result of clonal and self-reproduction predominance and also from environmental degradation around the collection areas. Consequently, it would be important to protect

all populations both *in situ* and *ex situ* to prevent the decrease of genetic variability. The low genetic variability among individuals of the same population confirms the inclusion of this species as critically endangered in the risk list for Brazilian flora.

Key words: *Pitcairnia albiflos*; RAPD markers; Clonal reproduction; Genetic diversity

INTRODUCTION

Over the last decade, the use of Brazilian native plants for landscaping and the accelerated devastation of some ecosystems have caused irreparable loss of a large number of species, including some Bromeliaceae species.

Several species of Bromeliaceae were added to the extinction risk list for Brazilian flora as data deficient (Ministério do Meio Ambiente, 2010). Among them, *Pitcairnia albiflos*, an endemic species from Rio de Janeiro rocky outcrops (Wendt, 1994; Wendt and Chamas, 1997), occurs in Gávea Stone, Urca Hill, Sugar Loaf Mountain, Corcovado Hill, and some rocks in Tijuca Forest. The populations have suffered with exotic grass invasion, trampling by climbers and annual burning. These areas belong to the Brazilian Atlantic Rainforest, one of the world's most important and vulnerable biodiversity "hotspots" (Myers et al., 2000). Therefore, an understanding of genetic diversity can help in the evaluation of extinction risk level.

Small population size, isolation among populations and habitat disturbance are factors frequently associated with the increase of extinction risk of species (Liu et al., 2006). As a consequence of habitat fragmentation, the reduction of distinct ecological areas and the decrease of inter- and intrapopulation genetic variability, in addition to inbreeding, have reduced, over the years, the capacity of species to adapt to environmental changes (Young and Boyle, 2000).

The environmental conditions that plant populations are subjected to can differ substantially. These conditions affect the dynamic of the population, gene flow, selection, and adaptation processes (Stanton et al., 1997; Wade and Goodnight, 1998; Thompson, 1999).

Factors that affect reproduction and dispersion are special determinants of the genetic structure (Loveless and Hamrick, 1984). Currently, the knowledge of the structure is known to be a fundamental step in elaborating conservation programs (Sharma et al., 2000; Mamuris et al., 2001). The genetic structure of clonal populations can be complex, reflecting the spatial distribution in genets and ramets, due to vegetative and sexual reproduction (Xie et al., 2001; Auge et al., 2001; Ruggiero et al., 2005).

One important tool to understanding the population dynamics of clonal species is the use of molecular techniques that can determine the size and dispersion of genets (McGlaughlin and Friar, 2007). Increasingly, the estimation of genetic variability has been based on information obtained at the DNA level (Sgorbati et al., 2004). Among others, RAPD markers have been widely used to characterize the genetic diversity of natural plant populations (Allnutt et al., 2003; Viccini et al., 2004; Cavallari et al., 2006). For poorly studied species, the RAPD technique is of great value because it does not require previous knowledge about their DNA sequence since arbitrary primers are used (Ercan et al., 2004).

The use of RAPD markers has been criticized due to the lack of reproducibility and repeatability; however, these features can be avoided with improved laboratory techniques

and scoring procedures (Skroch and Nienhuis 1995; Weising et al., 1995; Bussell, 1999). Additionally, some authors emphasize that these markers are highly stable and reproducible in controlled experimental conditions (Parani et al., 1997).

As part of studies related to preservation and characterization of Brazilian genetic plant resources, the aim of this study was to access the genetic variability of *P. albiflos* populations using the RAPD technique to elucidate aspects of population structure, evaluate the actual extinction risk level of the species and also to contribute to conservation strategies designed for *P. albiflos*.

MATERIAL AND METHODS

Sample strategy and DNA extraction

Leaves of 56 individuals of *P. albiflos* from three populations were collected: Urca Hill (UH), Chacrinha State Park (CSP) and Tijuca National Park (TNP) (Figure 1). The 24 individuals collected at CSP were subdivided into 6 groups, each one with 4 individuals. The groups were called CSPa, CSPb, CSPc, CSPd, CSPe, and CSPf. The individuals were identified from 1 to 4 in each group. From UH, 20 individuals were subdivided into five groups, with four individuals each (also numbered from 1 to 4 in each group). The groups were called UHa, UHb, UHc, UHd, and UHe (Figure 2). The last 12 individuals, from the TNP population, were identified from 1 to 12 and were considered as a unique group. The subdivision of each population has been done according to the spatial distribution of each group. Approximately 300 mg young leaf tissue was collected from each individual. The samples were maintained in plastic bags with silica gel until DNA extraction. Genomic DNA was extracted using a modified phenol/chloroform method shortly described as follows. N2 ground tissues were transferred to 2-mL tubes containing CTAB-based saline-proteinase K buffer. Proteins were removed by phenol-chloroform treatment. Quality and concentration of DNA were determined with the GeneQuant Pro spectrophotometer (GE Healthcare, Buckinghamshire, United Kingdom).

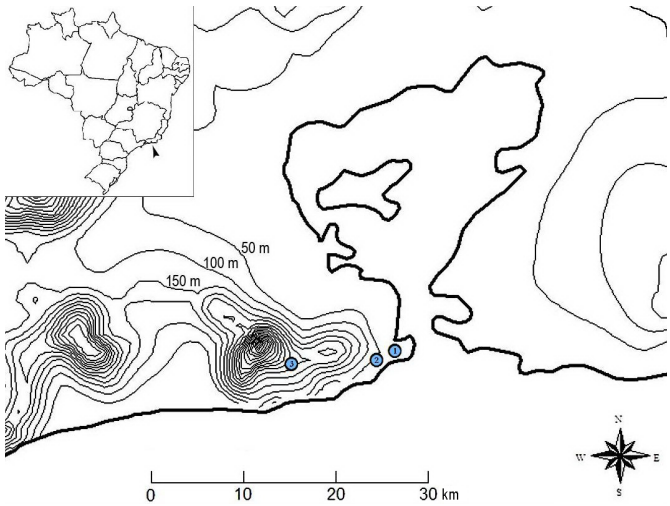

Figure 1. Collection points. The blue circles indicate the location of each population. 1 = Urca Hill - UH (22° 57' 13" S/43° 09' 47" W); 2 = Chacrinha State Park - CSP (22° 57' 46" S/43° 10' 52" W), and 3 = Tijuca National Park - TNP (22° 57' 60" S/43° 15' 49" W).

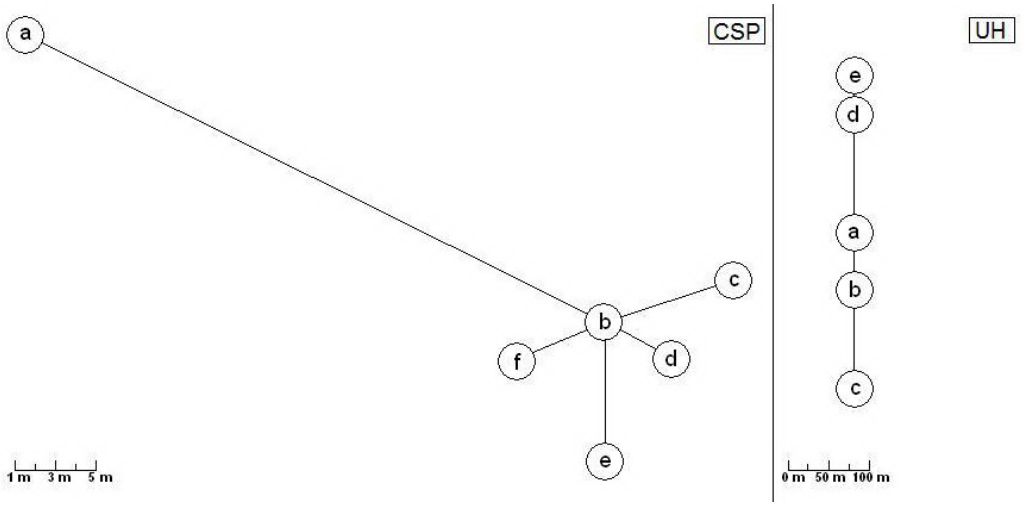

Figure 2. Groups of individuals (lower case letters) in the Chacrinha State Park (CSP) and Urca Hill (UH) populations.

Primer selection and RAPD amplification

Initially, 200 ten-mer primers (Operon Technologies, Alameda, USA) were tested to identify those capable of producing clear and reproducible marks. Thirty-five primers were selected and used for RAPD-PCR amplification.

The reaction was performed using 25 µL Tris-HCl, pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1% Triton X-100, 0.4 µM primer, 0.1 mM of each dNTP, 1 U Taq DNA polymerase, and 45 ng genomic DNA.

Cycling was performed in a GENEAMP PCR System 9700 (Applied Biosystems) under the following conditions: an initial denaturation step at 95°C for 5 min followed by 45 cycles of 1 min at 95°C, 1 min at 36°C and 2 min at 72°C, and a final elongation step of 10 min at 72°C.

Amplified RAPD products were resolved on 1.5% agarose gels stained with ethidium bromide and visualized under UV light. DNA markers were visually classified according to their intensity (resolution and amplification level) and only the consistent markers with high intensity were analyzed. They were scored using the presence (1) or absence (0) of homologous DNA bands among samples and a matrix with the RAPD phenotypes was constructed.

Statistical analysis

The relative genetic distance between each pair of individuals was estimated using the arithmetic complement of Jaccard's coefficient (Jaccard, 1901) and group analysis was derived from the unweighted pair group method with arithmetic mean (UPGMA). Jaccard's coefficient was used to estimate the genetic similarity following the formula:

$$S_{ij} = a / a + b + c \qquad \text{(Equation 1)}$$

where *a* is the number of marks present in both individuals, *b* is the number of exclusive marks of individual *i*, and *c* is the number of exclusive markers of individual *j*.

Using the values of genetic distance among individuals, the average genetic distance (AGD) between groups within the same population and between the populations was calculated.

The analyses of molecular variances (AMOVAs) were used to verify the partition of genetic variability among different levels (Table 1). Four AMOVAs were performed according to spatial distribution of populations and subpopulations: at the population level (AM1); at population and subpopulation levels (AM2); only the individuals from "CSP" subdivided into subpopulations (AM3), and only the individuals from "UH" subdivided into subpopulations (AM4). The gene flows among the populations (Table 2) were estimated using the following formula:

$$Nm = [(1 / \Phi st) - 1] / 4 \quad \text{(Equation 2)}$$

where *Φst* represents the differentiation level among the populations.

The analyses were done using the Genes software v. 2007.0.0 (Cruz, 2008).

The Shannon-Wiener diversity index (H) was calculated for each mark according to the formula:

$$H'_0 = - \sum p_i \log_2 p_i \quad \text{(Equation 3)}$$

where p_i is the frequency of presence or absence of RAPD markers among individuals.

The components of the Shannon-Wiener diversity index, calculated with the POPGENE software (Yeh et al., 1997), were used to estimate the variability in population and also within the species (Table 3). Percentage of polymorphic bands (PPBs) within populations and species were also calculated.

RESULTS

The set of 35 primers generated 88 RAPD markers among the 56 *P. albiflos* individuals. From these, 31 were polymorphic (PPB = 0.35) and two exclusive bands were detected for CSP and 9 exclusive bands for the TNP population.

The genetic distance among the individuals ranged from 0 to 0.065 (mean = 0.024). In the CSP, the AGD among all individuals was 0.013. Among the individuals from the same group of the CSP population the AGD ranged from 0 to 0.025 at CSPc and CSPd, respectively. The lowest AGD between groups was 0.011 (between CSPb and CSPd) and the highest was 0.019 (between CSPa and CSPf).

In the UH population, the AGD among all individuals was 0.010 and among individuals from the same group ranged from 0.004 to 0.010, for UHb and UHc, respectively. The lowest AGD between the groups was 0.006 (between UHb and UHc) and the highest was 0.013 (between UHa and UHe). Regarding TNP, the AGD of the unique group was 0.038.

The AGDs between the populations were 0.022 for CSP-UH, 0.034 for CSP-TNP and 0.036 for UH-TNP. The partitions of the estimated genetic variability (Table 1) indicate that there is a relatively high percentage of variability among the populations and also among the groups (mainly in the UH population). Regarding the gene flow values between the populations (Table 2), a small variation among them was observed, the smallest one being between TNP and UH, which are the most distant populations.

Table 1. Distribution of genetic variability (AMOVA).

	AM1	AM2	AM3	AM4
Intra-groups	0.534**	0.484**	0.839**	0.629**
Among groups within a population	-	0.087*	0.161**	0.371**
Among populations	0.466**	0.429**	-	-

AM1 = at population level; AM2 = at population and subpopulation levels; AM3 = only the individuals from Chacrinha State Park subdivided into subpopulations, and AM4 = only the individuals from Urca Hill subdivided into subpopulations. *P < 0.05; **P < 0.01.

Table 2. Φst estimated from AMOVA (below diagonal) and gene flow index (above diagonal).

Population	CSP	UH	TNP
CSP	-	0.290	0.319
UH	0.463	-	0.227
TNP	0.440	0.524	-

CSP = Chacrinha State Park; UH = Urca Hill; TNP = Tijuca National Park.

All individuals from UH were grouped together (Figure 3) and almost all individuals from CSP were placed closely. A high number of individuals identified as potential clones (individuals without genetic difference) in the populations UH and CSP contrast with the absence of potential clones among the TNP individuals. Considering the Shannon index, the TNP population showed the highest genetic diversity (0.13, Table 3).

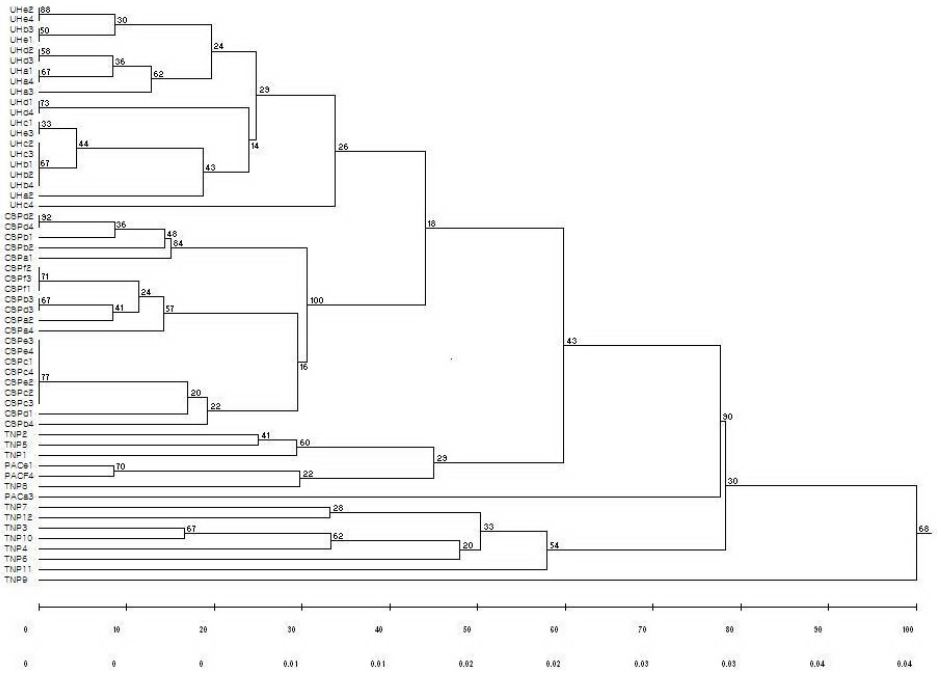

Figure 3. UPGMA dendrogram generated from relative genetic distances among *Pitcairnia albiflos* individuals. CSP = Chacrinha State Park; UH = Urca Hill; TNP = Tijuca National Park.

Table 3. Genetic diversity by Shannon index.

Group	Genetic diversity
H_{sp} *Pitcairnia albiflos*	0.112
H_{pop} CSP	0.060
H_{pop} UH	0.042
H_{pop} TNP	0.130

CSP = Chacrinha State Park; UH = Urca Hill; TNP = Tijuca National Park.

DISCUSSION

The PPB value, AGD and the diversity calculated with the Shannon index for *P. albiflos* indicate low genetic variability within the species. According to Tansley and Brown (2000), small and isolated populations are particularly subject to inbreeding and gene drift. As a consequence, low genetic diversity is expected when compared to large populations. In the same way, rare and endemic species typically show low levels of genetic variability (Hamrick and Godt, 1989). Wendt et al. (2001) used the restricted distribution of the *P. albiflos* population to explain the evolution of self-compatibility in this species. Previous studies also support that restricted species tend to be more self-compatible and to have less genetic variation than widespread ones (Kruckeberg and Rabinowitz, 1985; Wyatt et al., 1992).

Regarding the genetic diversity estimated by Shannon's index, the diversity in the TNP population was the highest. The fact that clones have not been detected reinforces the high genetic diversity observed for this population. Considering all populations of *P. albiflos* investigated here, this population is perhaps the one least affected by human action probably due to the surrounding vegetation, which provides better nesting and feeding for pollinators. The larger number of individuals, which increase the probability of outcrossing, and the smaller proportion of highly similar individuals also support this conclusion.

The partition of genetic variability by AMOVA indicates the occurrence of genetically structured populations (Table 1). Wendt et al. (2002) attributed self-pollination as the major reproductive mechanism in *P. albiflos*, mainly due to the unfavorable conditions for pollination, such as lack of flowering and the small population size. The absence of gene flow (less than 1) and the high proportion of variability among the populations (46.61%) corroborate the hypothesis of the predominance of self-reproduction. Barbara et al. (2009) also observed low gene flow (ranging from 0.54 to 1.91) among populations of *Alcantarea glaziouana* (Bromeliaceae), which occurs in the same region as *P. albiflos*. Habitat fragmentation might contribute to increasing genetic differentiation as a result of genetic drift (Young et al., 1996; Dayanandan et al., 1999). This fragmentation is even worse once the surroundings are partially or totally degraded or urbanized, which hampers the nesting and feeding of pollinators.

Regarding the groups within populations, UH showed a higher proportion of genetic variation than CSP. Perhaps the higher average distances of UH groups over the CSP groups (Figure 2) contribute to this scenario.

According to Loveless and Hamrick (1984), pollinators such as bats and hummingbirds, with their behavior and flight capacity, can visit several plants, increasing variability within the populations. On the other hand, this behavior may also contribute to decreasing the divergence among populations, since pollen dispersion over long distances maintains the populations connected by gene flow. Although bats and hummingbirds can visit *P. albiflos*

flowers (Wendt et al., 2002) it seems that the outcrossing is not frequent and also an inefficient event, which could explain the higher structure in UH populations.

According to the dendrogram, the individuals from UH can be seen clustered with some individuals from CSP. This fact could be a consequence of the smaller distance between these two populations compared to the distance among them and TNP. Another hypothesis is a common genetic origin of CSP and UH, indicated by the junction of the two groups without individuals from TNP in the dendrogram (Figure 3).

Three individuals from CSP are grouped outside of the major CSP group. Seeds with high dispersion capacity, carried by the wind, promote greater displacement of genotypes inside a population and, eventually, between populations. In addition, human actions may also be responsible for the lack of correlation between the genetic and geographic distances in some cases (Stankiewicz et al., 2001). Besides, these individuals increase the gene flow estimated between CSP and TNP.

Considering the low genetic diversity detected among the individuals of the same population and the high proportion of the total diversity among the populations, the best way to collect samples to make a germplasm bank is to collect samples from all populations, but not necessarily using a large number of individuals. The presence of exclusive alleles in the populations of *P. albiflos* reinforces the importance of sampling all populations. Besides, the extinction of a unique population could lead to a significant loss of genetic variation and also of potentially important alleles. Regarding *in situ* conservation and considering the high proportion of total diversity among populations, it is important to protect the individuals from Urca Hill and from other outcrops due to the constant presence of tourists and climbers.

CONCLUSIONS

The RAPD markers were effective in characterizing the genetic diversity in *P. albiflos* populations and may be used to establish the best strategy to develop conservation programs. The low genetic variability among individuals of the same population confirms the inclusion of this species as critically endangered in the risk list for Brazilian flora.

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REFERENCES

Allnutt TR, Newton AC, Premoli A and Lara A (2003). Genetic variation in the threatened South American conifer *Pilgerodendron uviferum* (Cupressaceae), detected using RAPD markers. *Biol. Conserv.* 114: 245-253.

Auge H, Neuffer B, Erlinghagen F, Grupe R, et al. (2001). Demographic and random amplified polymorphic DNA analyses reveal high levels of genetic diversity in a clonal violet. *Mol. Ecol.* 10: 1811-1819.

Barbara T, Martinelli G, Palma-Silva C, Fay MF, et al. (2009). Genetic relationships and variation in reproductive strategies in four closely related bromeliads adapted to neotropical 'inselbergs': *Alcantarea glaziouana*, *A. regina*, *A. geniculata* and *A. imperialis* (Bromeliaceae). *Ann. Bot.* 103: 65-77.

Bussell JD (1999). The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of *Isotoma petraea* (Lobeliaceae). *Mol. Ecol.* 8: 775-789.

Cavallari MM, Forzza R, Veasey EA, Zucchi MI, et al. (2006). Genetic variation in three endangered species of

Encholirium (Bromeliaceae) from Cadeia do Espinhaço, Brazil, detected using RAPD markers. *Biodivers. Conserv.* 15: 4357-4373.

Cruz CD (2008). Programa Genes Diversidade Genética. Editora UFV, Viçosa.

Dayanandan S, Dole J, Bawa K and Kesseli R (1999). Population structure delineated with microsatellite markers in fragmented populations of a tropical tree, *Carapa guianensis* (Meliaceae). *Mol. Ecol.* 8: 1585-1592.

Ercan AG, Taskin M and Turgut K (2004). Analysis of genetic diversity in *Turkish sesame* (*Sesamum indicum* L.) populations using RAPD markers. *Genet. Res. Crop Evol.* 51: 599-607.

Hamrick JL and Godt MJW (1989). Allozyme Diversity in Plant Species. In: Plant Population Genetics, Breeding and Genetic Resources (Brown AHD, Clegg MT, Kahler AL and Weir BS, eds.). Sinauer, Sunderland, 43-63.

Jaccard P (1901). Édute Comparative de la Distribution Florale Dans une Portion des Alpes et des Jura. Bulletin de la Société Vaudoise des Sciences Naturelles, Lausanne, 547-579.

Kruckeberg AR and Rabinowitz D (1985). Biological aspects of endemism in higher plants. *Annu. Rev. Ecol. Syst.* 16: 447-479.

Liu P, Yang YS, Xu G and Hao C (2006). Physiological response of rare and endangered seven-son-flower (*Heptacodium miconioides*) to light stress under habitat fragmentation. *Environ. Exp. Bot.* 57: 32-40.

Loveless MD and Hamrick JL (1984). Ecological determinants of genetic structure in plant populations. *Ann. Rev. Ecol. Syst.* 15: 65-95.

Mamuris Z, Sfougaris AI and Stamatis C (2001). Genetic structure of Greek brown hare (*Lepus europaeus*) populations as revealed by mtDBNA RFLP-PCR analysis: implications for conserving genetic diversity. *Biol. Conserv.* 101: 187-196.

McGlaughlin ME and Friar EA (2007). Clonality in the endangered *Ambrosia pumila* (Asteraceae) inferred from RAPD markers; Implications for conservation and management. *Conserv. Genet.* 8: 319-330.

Ministério do Meio Ambiente (2010). Available at [http://www.mma.gov.br/estruturas/ascom_boletins/_arquivos/83_19092008034949.pdf]. Accessed December 16, 2010.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, et al. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.

Parani M, Singh KN, Rangasamy S and Ramalingam RS (1997). Identification of *Sesamum alatum* x *Sesamum indicum* hybrid using protein, isozyme and RAPD markers. *Indian J. Genet.* 57: 381-388.

Ruggiero MV, Reusch TB and Procaccini G (2005). Local genetic structure in a clonal dioecious angiosperm. *Mol. Ecol.* 14: 957-967.

Sgorbati S, Labra M, Grugni E, Barcaccia G, et al. (2004). A survey of genetic diversity and reproductive biology of *Puya raimondii* (Bromeliaceae), the endangered queen of the Andes. *Plant Biol.* 6: 222-230.

Sharma IK, Clements MA and Jones DL (2000). Observations of high genetic variability in the endangered Australian terrestrial orchid *Pterostylis gibbosa* R. Br. (Orchidaceae). *Biochem. Syst. Ecol.* 28: 651-663.

Skroch PW and Nienhuis J (1995). Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. *Theor. Appl. Genet.* 91: 1086-1091.

Stankiewicz M, Gadamski G and Gawronski SW (2001). Genetic variation and phylogenetic relationships of triazine-resistant and triazine-susceptible biotypes of *Solanum nigrum*- using RAPD markers. *Weed Res.* 41: 287-300.

Stanton ML, Galen C and Shore J (1997). Population structure along a steep environmental gradient: consequences of flowering time and habitat variation in the snow buttercup, *Ranunculus adoneus*. *Evolution* 51: 79-94.

Tansley SA and Brown CR (2000). RAPD variation in the rare and endangered *Leucadendron elimense* (Proteaceae): implications for their conservation. *Biol. Conserv.* 95: 39-48.

Thompson JN (1999). Specific hypothesis on the geographic mosaic of coevolution. *Am. Nat.* 153: 156-174.

Viccini LF, Costa DSC, Machado MA and Campos AL (2004). Genetic diversity among nine species of *Lippia* (Verbenaceae) based on RAPD markers. *Plant Syst. Evol.* 246: 1-8.

Wade M and Goodnight CJ (1998). The theories of Fisher and Wright in the context of metapopulations: when nature does many small experiments. *Evolution* 52: 1537-1553.

Weising K, Nybom H, Wolff K and Meyer W (1995). DNA Fingerprinting in Plants and Fungi, CRC Press, Boca Raton.

Wendt T (1994). *Pitcairnia* L'Heritier (Bromeliaceae) of Rio de Janeiro State. *Selbyana* 15: 66-78.

Wendt T and Chamas CC (1997). As Pitcairnias do leste do Brasil - suas verdadeiras identidades. *Bromelia* 4: 12-18.

Wendt T, Canela MBF, Faria APG and Rios RI (2001). Reproductive biology and natural hybridization between two endemic species of *Pitcairnia* (Bromeliaceae). *Am. J. Bot.* 88: 1760-1767.

Wendt T, Canela MBF, Klein DE and Rios RI (2002). Selfing facilitates reproductive isolation among three sympatric species of *Pitcairnia* (Bromeliaceae). *Plant Syst. Evol.* 232: 201-212.

Wyatt R, Evans EA and Sorenson JC (1992). The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). VI. Electrophoretically detectable genetic variation. *Syst. Bot.* 17: 201-209.

Xie ZZ, Lu YY, Ge S, Hong DD, et al. (2001). Clonality in wild rice (*Oryza rufipogon*, Poaceae) and its implications for conservation management. *Am. J. Bot.* 88: 1058-1064.

Yeh FC, Yang RC and Boyle T (1997). POPGENE Version 1.3 (32-bit). Population Genetic Analysis. University of Alberta and Center for International Forestry Research, Alberta.

Young AG and Boyle TJ (2000). Forest Fragmentation. In: Forest Conservation Genetics (Young A, Boshier D and Boyle T, eds.). CSIRO Publishing, Melbourne, 123-135.

Young A, Boyle T and Brown T (1996). The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* 11: 413-418.