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Landscape conservation genetics of *Dipteryx alata* ("baru" tree: Fabaceae) from Cerrado region of central Brazil

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Abstract In this paper random amplified polymorphic DNA (RAPD) was used to evaluate the degree of among-population differentiation and associated spatial patterns of genetic divergence for *Dipteryx alata* Vogel populations from Cerrado region of central Brazil, furnishing support for future programs of conservation of this species. We analyzed patterns of genetic and spatial population structure using 45 RAPD loci scored for 309 trees, sampled from five different regions with two populations each. Genetic structure analysis suggested that panmixia null hypothesis can be rejected, with significant among-population components of 15%. Hierarchical partition by Analysis of Molecular Variance (AMOVA) shows that 5% of genetic variation is within regions, whereas 10% of variation is among regions, and these results were confirmed by a Bayesian analyses on HICKORY. The Mantel correlogram revealed that this divergence is spatially structured, so that local populations situated at short geographic distances could not be considered independent units for conservation and management. However, genetic discontinuities among populations were found in the northwest and southeast parts of the study area, corresponding to regions of recent socio-economic expansion and high population density, respectively. Taking both geographic distances and genetic discontinuities into account it is possible to establish a group of population to be conserved, covering most of *D. alata* geographic distribution and congruent with previously established priority areas for conservation in the Cerrado region.

Keywords Biodiversity · *Dipteryx alata* Vogel · Cerrado · Conservation genetics · Genetic structure · Operational units · RAPD · Spatial autocorrelation

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Introduction

The recent advances in using molecular markers allows a better understanding of microevolutionary processes generating population differentiation in space and time, which in turn can be of great value to conservation efforts (Newton et al. 1999; Crandall et al. 2000; Hedrick 2001; Anne 2006). Despite difficulties in distinguishing processes of population differentiation, they sometimes generate similar spatial patterns of intraspecific genetic similarity and can be used by conservation geneticists to establish evolutionary significant units (ESU), management units (MU) or operational units (OU) (Moritz 1994; Avise and Hamrick 1996; Avise 2000; Diniz-Filho and Telles

2002, 2006; Telles et al. 2006). Despite some disagreements about these concepts and the empirical recognition of these units (Paetkau 1999; Newton et al. 1999; Crandall et al. 2000; Fraser and Bernatchez 2001; Telles et al. 2003a), it is now clear that the analysis of population structure is important to establish conservation and management programs to better assess genetic diversity (Gurdebeke et al. 2003).

Landscape genetics is a new discipline, which aims to provide information on how landscape and environmental features influence population genetic structure (Manel et al. 2003; Pearse and Crandall 2004, Guillot et al. 2005; Sork and Smouse 2006; Kidd and Ritchie 2006). The first step is the analysis of spatial patterns of genetic variation and the detection of genetic discontinuities among local populations, which could be associated to landscape level features, especially those related to recent human occupation (see Manel et al. 2003). For example, it is well known that habitat fragmentation, loss or conversion, generated by different types of human activities, can decrease levels of gene flow, and reduce effective population sizes of a species in a region (Fahring 2003). Consequently, there will be an increase in the genetic divergence between these more isolated populations in the regions (despite they are close in geographic space) (e.g., Collevatti et al. 2003). Thus, landscape level effects caused by human habitat conversion must be analyzed simultaneously with ''natural'' processes of genetic divergence, such as isolation-by-distance (Epperson 2003).

The Cerrado biome is one of the richest savanna biomes in the world and harbors an immense floral and faunal diversity (Ratter et al. 1997). Plant endemism is very high, estimated at as high as 4,400 species of higher plants by Myers et al. (2000), who ranked the Brazilian Cerrado among the 25 global biodiversity ''hotspots'' conservation. This is also due to high levels of habitat conversion, since about 40% of the original Cerrado biome area has been converted to ''anthropic landscape.'' Thus, there is urgent need for implementation of conservation plans based on best possible available information on biodiversity patterns (Klink and Moreira 2002; Oliveira-Filho and Ratter 2002; Klink and Machado 2005).

Many of the plant species found in the Cerrado region possess a strong economic importance and have been utilized unsustainably by local human populations, adding a strong exploitation component that has accelerated loss of genetic diversity by habitat destruction. The main problem for conservation and management of these plant populations in the Cerrado is that many life history and demographic traits that regulate population density are correlated with patterns of genetic population structure, such as degree of among population differentiation and within population fixation indexes (inbreeding) (Hamrick et al. 1979; Hamrick and Godt 1990; Geburek 1997). Both unsustainable exploitation and habitat loss can quickly change population structure and, consequently, may cause reduction in genetic diversity. These local populations can then be driven to extinction in near future, so it is now imperative to better understand patterns of population structure.

Thus, understanding patterns of genetic variation within and among local populations may be critical to establish conservation programs and to plan sustainable exploitation activities in natural plant populations. A better planned management of wild populations is also important because there are still many difficulties in the domestication and cultivation of many Cerrado species, due to their long life cycles, low germination rates in artificial conditions, low initial productivity and high-variable fruit quality (Almeida et al. 1987; Fonseca and Muniz 1992; Borém 1998).

Among Cerrado plants with a wide potential of exploitation, we can highlight *Dipteryx alata*, the ''barueiro.'' This species is a native fruit plant of the family Fabaceae, widely distributed in the Cerrado biome, mainly in the dense savannic formations (strict sensu ''Cerrados'' and the ''Cerradões'') and in the dry forests. It is the only species of *Dipteryx* in the Cerrado, despite other species of the genus occur in the Amazon forests (Ribeiro et al. 2000). The species has a potential use for human and animal food, in the medicinal industry and as wood for building (Almeida et al. 1998). Siqueira et al. (1993) suggested that the ''barueiro'' has an allogamic reproductive system. Its flower is hermafrodite, and the polinization is performed by insects (Thum and Costa 1999). Dispersion of fruits can occur by gravity (short distance) and by animals, mainly by bats (perhaps long distances) (Ribeiro et al. 2000).

In this paper, we used random amplified polymorphic DNA (RAPD) to evaluate spatial patterns of genetic variation in populations of *D. alata* from Cerrado region of central Brazil, based on samples taken from most of the species' geographic range. More specifically, we analyzed the degree of among-population differentiation and the spatial patterns of genetic divergence, which can be used to define units for future programs of in situ and ex situ conservation for this species.

Materials and methods

Sampling, DNA extraction, primer selection and RAPD-PCR amplification

The material for this study consisted of a total of 309 trees obtained from sampling in ten localities (local populations hereafter) of natural occurrence of *D. alata* in the Brazilian Cerrado (Fig. 1, Table 1), covering most of the geographic range of the species.

The DNA was extracted from young leaves, using the CTAB (cetyltrime thylammonium bromide) method described by Ferreira and Grattapaglia (1998). The quality of the extracted DNA was evaluated in agarose gels (1%) stained with ethidium bromide and quantified by comparison with ladder DNA (low DNA mass of Invitrogen^{TM}, Carlsbad, CA, USA). DNA was diluted to a working concentration of approximately 2.5 ng/μL.

The primers (Operon technologies, Inc.; Alameda, CA, USA) were selected analyzing a total of 120 primers (kits A, B, C, H, M, and P; 20 primers per kit) for three individuals of *D. alata*, to identify primers that yielded a reproducible and scoreable banding pattern. The polymerase chain reaction (PCR) was done in a PCT-100, MJ Research PCR system with a total volume of 13 μL containing 5.5 μL of water, 3 μL of genomic DNA (2.5 ng/μL), 1.52 μL of 10× PCR Buffer (Invitrogen^{TM}) 1.22 μL of dNTPs (2.5 mM), 0.46 μL of $MgCl_2$ (2.5 mM), 1.18 μL of primer, 0.12 μL of Taq Polymerase (5U-Invitrogen^{TM}). PCR profiles comprised of a initial denaturation of DNA for 3 min at 96°C, 40 cycles at of 1 min denaturation at 92°C, 1 min annealing at 37°C, 1 min extension at 72°C, and one 3 min cycle at 72°C for final extension.

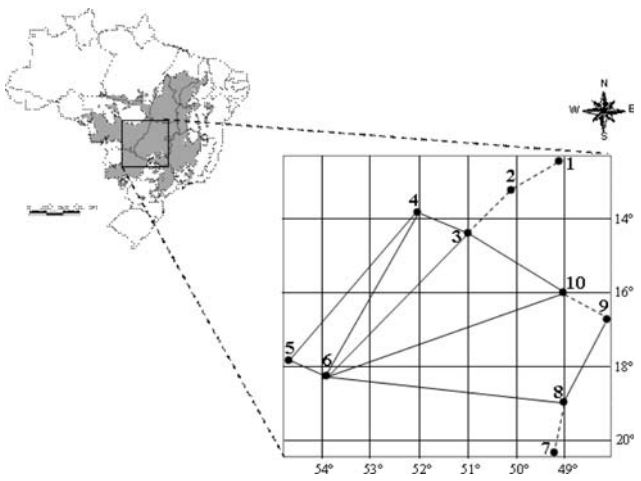

Fig. 1 Distribution of ten sampled population of *Dipteryx alata* in the Cerrado region of central Brazil (see also Table 1). The network linking the sampled population is obtained by Gabriel Criterion and the *dashed lines* indicates boundaries

The amplification products were separated on 1.5% agarose gels stained with ethidium bromide, run in 1× TBE buffer at 120 V for 4 h, in groups with 23 samples (individuals) separated by 100 bp DNA ladder (Amersham Pharmacia Biotech^{TM}, Piscataway, NJ, USA). Digital images of gels were capture using EDAS120-KODAK and individual profiles for all gels were scored for the presence (1) or absence (0) of bands (loci), using the 100 bp DNA ladder as a reference for aligning bands of different gels to different loci and to minimize ambiguity in the coding procedure.

Statistical analyses

Molecular variation obtained by scoring RAPD bands was first analyzed using an Analysis of Molecular Variance (AMOVA). The AMOVA allows one to partition multivariate Euclidian distances calculated between pairs of individuals using RAPD bands into among and within-regions and among and within-populations variance components. The proportion of genetic variation among populations is then estimated by the Φ_{ST} statistics (analogous to the classical F_{ST} value), an estimate of the among-population component of genetic divergence, and the significance of this estimate against the null hypothesis of no among-population variation was tested by 5,000 random permutations using Arlequin Version 2.000 (Schneider et al. 2000).

A more direct estimate of the relative magnitude of population divergence from dominant markers was also obtained using the Bayesian approach proposed by Holsinger (1999) (see Telles et al. 2006, for a recent application). The ''a posteriori'' distribution of the estimator θ^B (also analogous to the F_{ST}) was approximated numerically through a Markov Chain Monte Carlo (MCMC) simulation, and tends to converge to a beta distribution. The software HICKORY Version 1.0 (Holsinger and Lewis 2003) allows the estimation of four different models. The first is a full model, in which both θ^B and f (i.e., the F_{IS}, the inbreeding coefficient) are estimated. Alternatively, models can assume θ^B or f equal to zero. Finally, because estimates of f based on dominant markers are usually strongly biased (especially at low sample sizes, i.e., $n < 10$), HICKORY allows one to estimate a final model in which f is free to vary. The sampler will not attempt to estimate f and instead it will choose values of f at random from its prior distribution while estimating other parameters during the MCMC run. Estimates of θ^B and other

Table 1 The ten local populations analyzed, sample sizes, municipalities in Cerrado region of central Brazil, the geographical coordinates (latitude and longitude), and altitude (m)

Population	Locality (municipalities)	*n*	Latitude	Longitude	Altitude (m)
1	Alvorada-TO	32	12°26.928	49°06.880	301
2	São Miguel do Araguaia-GO	31	13°13.479	50°06.157	342
3	Cocalinho-MT	32	14°23.817	50°59.733	241
4	Água Boa-MT	32	13°50.333	52°02.500	315
5	Sonora-MS	31	17°51.181	54°42.211	454
6	Alcinópolis-MS	32	18°16.079	53°55.563	387
7	Icém-SP	32	20°20.816	49°12.931	469
8	Monte Alegre de Minas-MG	31	18°58.671	49°01.434	696
9	Luziânia-GO	27	16° 43.935	48°07.829	808
10	Pirenópolis-GO	29	15°59.829	49°02.069	756

parameters obtained in this way incorporate all of the uncertainty in the prior of *f*. These models were compared using the Deviant Information Criterion (DIC) (Holsinger 1999; Holsinger et al. 2002; Spiegelhalter et al. 2002; Holsinger and Wallace 2004). The region divergence was estimated utilizing HICKORY, considering each region as one population, because this software does not calculate genetic divergence from hierarchical groups.

In this paper, the θ^B was also estimated between pairs of local populations, providing then an estimate of genetic divergence between pairs of local populations to be further used in explicit analyses of spatial patterns in genetic data. This is important because it is now accepted that a single coefficient of population structure is usually not enough to describe the complex patterns of population divergence (Pearse and Crandall 2004). A first approach to evaluate patterns in genetic distances was to apply clustering and ordination techniques. A UPGMA (Legendre and Legendre 1998) was used to generate a hierarchical classification of populations, whereas a non-metric multidimensional scaling (NMDS) (Lessa 1990; Legendre and Legendre 1998) was used to allocate the populations in a reduced two or 3D genetic space. Cophenetic correlation was used to check for representativeness of clustering procedure, and badness of fit of NMDS in 2D and 3D were assessed by the stress index.

Spatial patterns in genetic distances estimated by θ^B were evaluated using different forms of Mantel tests of matrix correspondence (Smouse et al. 1986; Manly 1985, 1986). A first possibility is to obtain a single standardized Mantel's *Z* coefficient, which is related to a Pearson correlation between pairwise geographic and genetic distances. Positive values of matrix correlation indicate that populations close in geographic space tend to be genetically similar, whereas the null expectation is that matrix correlation is zero. In this case, the two matrices are independent (i.e., there is no spatial patterns in genetic distances). Because pairwise genetic or geographic distances are not independent, a randomization procedure is needed to test the statistical significance of the matrix correlation coefficient. In this paper, we used 10,000 random permutations of genetic distances between populations to construct the null distribution of standardized Mantel's *Z* (see Manly 1997).

A second approach is to decompose the relationship between geographic and genetic distances, evaluating then more complex relationships between them. For example, in an isolation-by-distance process, populations close in space tend to be genetically similar, although at larger geographic distances there is no clear pattern and it is difficult to predict the similarity among populations (Epperson 2003). Thus, we also obtained a Mantel correlogram based on genetic distances (Sokal and Oden 1978a, b; Diniz-Filho and Telles 2002; Epperson 2003), by comparing the pairwise θ^B genetic distances with five binary connectivity matrices, in which elements equal to 1.0 indicate that pairs of local populations compared are situated within the same geographic distance class, and zero otherwise. In this paper, correlograms were built using five geographic distances classes, whose upper limits were 285, 450, 550, 690, and 880 km. These intervals were established in such a way that approximately the same number of connections are assigned to each matrix. All Mantel tests were performed using the NTSYS Version 1.8 (Numerical Taxonomy and Multivariate Analysis System) (Rohlf 1989).

The *X*-intercept of a Mantel correlogram is the geographic distance at which a line linking or interpolating among the Mantel's *Z* statistics crosses the axis of geographic distances. This *X*-intercept can be interpreted as the geographic distance at which matrix correlation becomes non-significant and is an indicator of the genetic patch, i.e., the geographic distance below which it is possible to define groups of genetically self-dependent local populations. This distance can then be used to establish sampling strategies for conservation

and management of the genetic variability, by using the so-called ''OU'' for conservation (see Diniz-Filho and Telles 2002; Telles et al. 2003a).

The pairwise genetic distances obtained using θ^B statistics were also used to apply a simple approach to assess discontinuities in multivariate genetic data across geographic space (see Manel et al. 2003; Telles et al. 2003a). Local populations were linked by a Gabriel graph (Gabriel and Sokal 1969; Arnaud 2003), in which connections between two populations will be established if, and only if, the circumscribed circle passing through their geographical coordinates includes no other population. We mapped the 30% of the highest ratios between genetic and geographic distances along the Gabriel network, allowing a simple evaluation of the genetic discontinuities (Legendre and Legendre 1998; Manel et al. 2003; Telles et al. 2003a). The network linking was performed using the SAM Version 1.0 (*Spatial Analysis in Macroecology*), available at www.ecoevol.ufg.br/sam (Rangel et al. 2006).

Results

We selected five that yielded clearer band patterns, after testing for a total of 120 primers. These primers produced a total of 45 scorable bands (i.e., loci), varying from 7 to 12 loci by primer (Table 2). Out of the 45 loci analyzed, 88.9% (considering the polymorphism criterion of 99%) were polymorphic when all local populations were considered simultaneously, with values ranging from 48.9 to 73.3% for each of the ten local populations (Table 3).

The Φ_{ST} from AMOVA was equal to 0.155 ($P < 0.0002$, with 5,000 permutations), out of this, around 5% are within regions and 10% are among regions, so that 84.5% of the genetic variation is within local populations. On the other hand, of the three models obtained using the Bayesian approach (Table 4), the one with smaller DIC was the full model (DIC = 1,421.8), in which θ^B value was equal to 0.161 (95% confidence interval: 0.135–0.196). In this model, the f value is very low ($f = 0.067$). The $f = 0$ model is the second best one according to DIC (1,426.0), with $\theta^B = 0.152$. The differences among the values of DIC of two models are very small, so that the two values of θ^B are equally probable. The free-f model is the one with larger DIC (DIC = 1,448.6), in which θ^B was equal to 0.193 and $f = 0.289$. Thus, the Bayesian approach is coherent with AMOVA results, suggesting a significant among-population component of genetic variation around 15%. The region divergence of Bayesian estimate was also similar (~11%). Despite difficulties in estimating inbreeding in these local populations, the Bayesian approach modeling suggests that it might be small, by considering the similarity between DIC values from the models with $f = 0.067$ and $f = 0$. Further investigations about within-population inbreeding levels and its effect on life-history and demographic parameters are still necessary.

Genetic distances among pairwise populations, evaluated by θ^B parameter, ranged from 0.056 to 0.197. Within each region the distances, ranged from 0.056 to 0.147, and among regions they ranged from 0.074 to 0.197. The UPGMA had a relatively low cophenetic correlation of 0.77, suggesting a difficulty in representing among-population genetic components (see Rodrigues and Diniz-Filho 1998). A much better solution was achieved by NMDS. The stress of the 2D solution was equal to 0.11 and a correlation between distance in NMDS space and original genetic distances equal to 0.96. So, representation of genetic distances in the reduced genetic space of NMDS is adequate (Fig. 2) and suggests that populations 3, 4, and 10 form a distinct group in relation to other populations.

The Mantel test revealed no significant overall correlation between pairwise θ^B and geographic distances ($r = 0.12$; $P = 0.443$ with 10,000 permutations). However, the multivariate correlogram (Fig. 3) showed a low but significant matrix correlation in the first geographic distance class ($r = 0.258$; $P = 0.048$), indicating that genetic similarity among local populations close in geographic space tends to be slightly larger than expected by chance alone. Matrix correlation then

Table 2 RAPD primers, their sequences and their respective number of bands, number of polymorphic bands, and percentage of polymorphic bands analyzed in the ten local populations of *Dipteryx alata*

Primer	Sequence 5′–3′										Total bands	Polymorphic bands	Percentage of polymorphic bands
OPC-19	G	T	T	G	C	C	A	G	C	C	7	6	85.71
OPP-19	G	G	G	A	A	G	G	A	C	A	10	7	70.00
OPP-15	G	G	A	A	G	C	C	A	A	C	9	7	77.78
OPP-07	G	T	C	C	A	T	G	C	C	A	7	3	42.86
OPP-08	A	C	A	T	C	G	C	C	C	A	12	11	91.67
Average											9	6.8	–

Table 3 Proportion of polymorphic bands and percentage of polymorphic bands in ten sampled populations of *Dipteryx alata* in Cerrado of Central Brazil

Population	Polymorphic bands	Percentage of polymorphic bands
1	23	51.11
2	25	55.56
3	32	71.11
4	33	73.33
5	24	53.33
6	24	53.33
7	33	73.33
8	24	53.33
9	22	48.89
10	31	68.89
Total	40	88.89

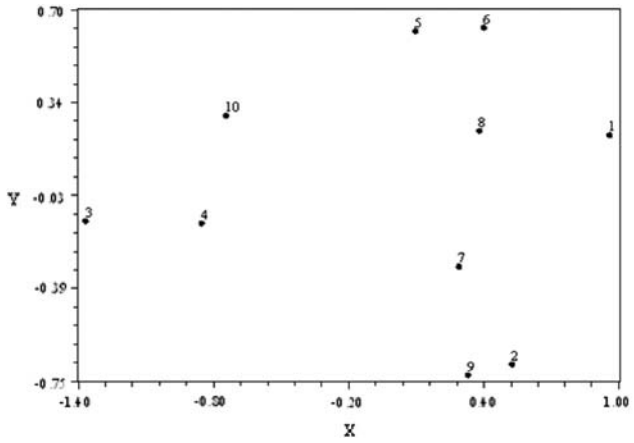

Fig. 2 Distribution of local populations in the 2D solution of non-metric multidimensional scaling (*NMDS*) based on between pairwise θ^B genetic distances from 45 RAPD loci. Stress value was equal to 0.11

decreases to non-significant values up to the fifth distance class and then tends to stabilize, revealing a patch size of around 400 km.

Mapping the 30% of the highest values of the ratio between genetic and geographic distances suggest that some pairs of populations along Gabriel network are more genetically dissimilar than expected by their small geographic distances. We could detect some discontinuity between populations 1–2, 2–3, 7–8, and 9–10 (Fig. 1), which are then clustered in southeast and northwest parts of central Brazil.

Discussion

Population divergence

Results of both AMOVA and Bayesian analysis suggest that panmixia null hypothesis can be rejected, since statistically significant among-population components of around 15–16% were obtained. This value is within the observed interval obtained when analyzing other tree populations from Cerrado, previously studied (see Collevatti et al. 2001; Zucchi et al. 2003, 2005; Telles et al. 2003a, b; Gonçalves-Alvim et al. 2004), including *Cariocar brasiliense* and *Eugenia dysenterica*, which have similar mating systems (preferential allogamy) and common agents of dispersion and polinization (Ribeiro et al. 2000; see below). Considering only papers that used RAPD markers in Brazilian species, Araújo (2001) and Zucchi et al. (2005) found higher Φ_{ST} values of 0.26 and 0.27, for *C. brasiliense* and *E. dysenterica*, respectively. Trindade and Chaves (2005) found a much smaller value of 0.08 for *E. dysenterica*, which can be attributed to the smallest geographic distance among studied populations. Zimback et al. (2004) found a G_{ST} of 0.125 for genetic variation among populations of *Trichillia pallida*, a species from Atlantic forest.

These values of F_{ST} and related statistics are in accordance with expectations under life history and ecological characteristics of the species. Thus, despite some discussion of validity of RAPD markers due to low reproducibility and the current possibility of potential application of more complex molecular markers (see Rabouam 1999; Kjolner et al. 2004; Nybom 2004), our results seem to be robust to some of these problems and, thus, can furnish an initial description of the genetic variability in an relatively unknown species. This is because we used a careful coding scheme and primer selection procedure, and performed an

Table 4 Posterior means, standard deviation (SD), and 95% credible intervals (CI) of f and θ^B for RAPD analyze of population divergence among ten local populations of *Dipteryx alata* under three alternative models, including the Deviant Information Criterion (DIC)

Model	θ^B			F			DIC
	Average	SD	CI (95%)	Average	SD	CI (95%)	
Full	0.161	0.016	(0.135, 0.196)	0.067	0.055	(0.002, 0.202)	**1,421.7791**
$f = 0$	0.152	0.014	(0.127, 0.181)	0	–	–	1,426.0059
f livre	0.193	0.018	(0.160, 0.230)	0.503	0.289	(0.027, 0.972)	1,448.6550

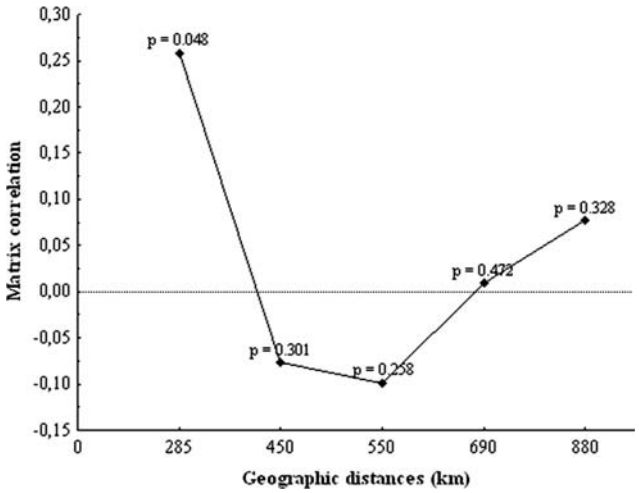

Fig. 3 Mantel correlogram for genetic divergence estimated for θ^B among ten local populations of *Dipteryx alata*, in five geographic distance classes. The *dashed-line* indicated the expected matrix correlation under the null hypothesis of absence of spatial patterns. *Numbers* indicate the Type I error obtained using 10,000 random permutations by Mantel test, so only the coefficient in the first distance class is significant at 5% level

evaluation of bands reproducibility and uniform laboratorial conditions prior to all analyses. Indeed, many papers reinforced the advantages of RAPD approach applied to population level when these conditions are met (Parker et al. 1998; Sunnucks 2000; Kjolner et al. 2004; Cavallari et al. 2006).

Ecological and life history traits of ''barueiro'' indeed allow a relatively high level of gene flow among local populations, reducing the relative magnitude of population structure, and generating among-population components of genetic divergence coherent with those found in this study. Siqueira et al. (1993) indeed suggested that the ''barueiro'' has an allogamic reproductive system, with polinization by insects and dispersal by gravity (short distance) and by animals, mainly by bats. More importantly, there are also significant spatial patterns of genetic divergence at small distance class, suggesting moderate levels of gene flow between populations situated at smaller geographic distances (see Hutchison and Templeton 1999). This reveals a spatially structured lack of equilibrium between dispersal and gene flow, which also causes the difficult in detecting spatial patterns using overall Mantel tests.

However, the relatively low magnitude of spatial patterns according to Mantel test at smallest geographic distance class (r = 0.258) suggests that other factors, such as habitat fragmentation and landscape level processes, might also affect genetic diversity among populations. Under a simple model of spatially structured gene flow and drift within local populations, a much stronger spatial patterns and genetic similarity among close local populations would be expected. This interpretation is reinforced by the presence of some genetic discontinuities among geographically adjacent populations, as discussed below (see also Fig. 3).

Spatial patterns of genetic differentiation

The observed spatial pattern of genetic differentiation detected by Mantel correlograms and by multivariate techniques (UPGMA and NMDS), in which geographically close populations are similar, is a frequent outcome of stochastic processes of population differentiation, such as stepping stones and isolation-by-distances (see Epperson 2003; Escudero et al. 2003). However, the spatial patterns observed here for *D. alata* are not too strong, and at least in part the lower levels of gene flow could be explained by the presence of barriers caused by anthropogenic impacts, because of the mix between natural processes and anthropogenic effects (Telles et al. 2003a). This reduction in the magnitude of spatial patterns would arise, for example, if close populations in geographic space, which should be more genetically similar under ''natural'' processes such as isolation-by-distance, become dissimilar when habitat fragmentation reduces the level of current gene flow between them.

The two groups of genetic discontinuities found in this study coincide with regions of more intense recent human occupation in Central Brazil, characterized by successive wave fronts of colonization coming from the south and southeastern parts of the country since the 18th century. There was a clear acceleration of these processes after the transference of the national capital from Rio de Janeiro to Brasilia, in 1960. Besides, many governmental programs were created to attract modern and highly technological agriculture (see Klink and Moreira 2002; Cavalcanti and Joly 2002). Actually, the southern part of the Cerrado Biome in Central Brazil contains in the present day the highest levels of human population density (see Rangel et al. 2007 for an extensive discussion of regional patterns of human occupation in Central Brazil). We found in this area the genetic discontinuities involving populations 7, 8, 9, and 10 and indeed human population density around these four local populations (measured using a 1° grid cell covering the Cerrado biome) is significantly higher (t = 5.56; P = 0.032) than in other parts of the study area.

In the northwest part of the Cerrado biome, in which we found genetic discontinuities among local populations 1, 2, and 3, human occupation is

characterized by agricultural and cattle ranching recent expansions. Different from what is observed for the other group of discontinuities, the human population in this northwest part of biome is relatively low, since the agriculture is based on modern and highly technological extensive practices (Klink and Machado 2005). Coherent with this explanation, we found higher levels of agricultural activities around these three local populations, when compared to the others in the study area, expressed by the average percentage of farming by municipalities (t = 4.9; P = 0.001) and average percentage of land occupied by cattle ranching (t = 3.6; P = 0.004).

Thus, the two groups of genetic discontinuities detected in this study were explained by different sets of human activities, which in turn are congruent with the definitions used by Brazilian Government to classify the two parts of the Cerrado biome. The first group of discontinuities, local populations 7, 8, 9, and 10, is located in a region characterized by ''high anthropic pression,'' whereas the second group, formed by local populations 1, 2, and 3, is located in the region of ''socio-economic expansion'' (see Brasil 1999, available at www.bdt.org.br/workshop/cerrado/br/).

Operational units for conservation

Independently of the microevolutionary processes and landscape ecological factors (including anthropogenic ones) involved in the origin and maintenance of spatial genetic structure of *D. alata*, the spatial patterns revealed that populations situated at a small spatial distance tend to be slightly more similar than expected by chance alone, and thus that 400 km is the minimum distance between samples needed to conserve or assess genetic diversity with maximum efficiency (i.e., minimizing redundancy) and with lower costs (Diniz-Filho and Telles 2002; Manel et al. 2003; Chung et al. 2005). This strategy could be used either to define units for conservation programs or to establish sampling strategies for ex situ conservation programs, under the following reasoning discussed in detail by Telles et al. (2003a).

In theory, collecting samples situated around this minimum patch distance maximizes the possibility of assessing more genetic heterogeneity and thus avoiding pseudoreplication of the genetic variability (Diniz-Filho and Telles 2002), although further tests of the efficiency of this approach are still necessary (Manel et al. 2003). Conversely, if a researcher or manager is interested only in replicating a small fraction of the genetic variability (for example, to get samples of a local genetic variant that is needed for a specific breeding program), the optimum distance between samples must always be lower than the intercept of the correlogram. In fact, all samples situated at a geographic distance lower than the X-intercept, particularly in a correlogram that follows the expectation under stochastic processes of genetic divergence and tend to stabilize in the last distance classes, could be considered a single genetic unit for conservation or management.

Based on this theoretical reasoning, it would be possible to use the patch size estimated by the spatial correlograms based on RAPD divergence among *D. alata* populations (i.e., around 400 km) to provide one possible scheme of OU for conservation (sensu Diniz-Filho and Telles 2002). We could establish four groups of populations, in which the geographic distances within groups tend to be smaller than 400 km, whereas distances between groups centers is higher than this value. Based on samples within these four groups, we could select four populations to establish a network (i.e., a set of local populations to be defined as ''OU.'' For example, based on relatively high values of genetic diversity within populations, we could trigger a network in the population 4 and then sequentially choose populations 10, 6, and 7 and get a minimum number of populations that represent, with minimum redundancy, the overall genetic diversity analyzed for the species.

However, this first network did not take into account the genetic discontinuities previously discussed. So, although some local populations analyzed here are close in geographic space (at a distance smaller than 400 km) and could be considered in principle ''redundant,'' if there is a discontinuity among them they could be considered as independent OU. Thus, adding the four barriers to the previously defined network (with populations 4, 6, 7, and 10), we could get an alternative scheme with seven populations as our proposal for OU for the ''barueiro'' in Central Brazil (Fig. 4). Its is interesting to note that these seven units are situated within regions that were recently considered as priority areas for conservation in Brazilian Cerrado (see Cavalcanti and Joly 2002), which can improve the potential persistence of these populations, under a more general systematic planning for conserving Cerrado biodiversity.

Concluding remarks

Our analysis of RAPD divergence in Cerrado populations of *D. alata* shows relatively high levels of population divergence. Also, this divergence is spatially structured following simultaneously processes of stochastic genetic divergence and patterns of human occupation, characterized by successive wave fronts of

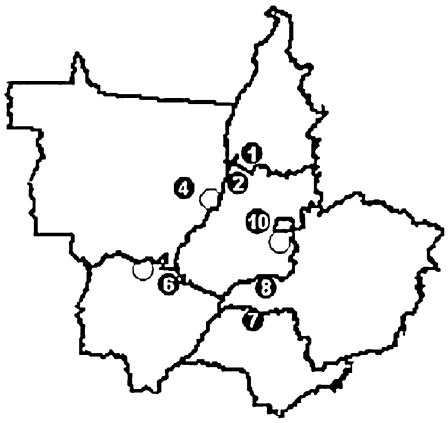

Fig. 4 Possible definition of operational units for conservation (sensu Diniz-Filho and Telles 2002) based on Mantel multivariate correlogram and using a 400 km *X*-intercept and, at the same time, taking into account the analysis of genetic discontinuities

colonization coming from the south and southeastern parts of the country since the 18th century. In general, local populations situated at small geographic distances could be in principle not considered independent units for conservation and management, but some genetic discontinuities disturb this pattern. Thus, taking into account both geographic distances and genetic discontinuities allowed us to establish a set of populations covering most of *D. alata* geographic distribution, and situated within regions that were recently considered as priority areas for conservation in Brazilian Cerrado.

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References

Almeida SP, Proença CEB, Sano SM, et al (1998) Cerrado: espécies vegetais úteis. Embrapa-CPAC, Planaltina

Almeida SP, Silva JA, Ribeiro JF (1987) Aproveitamento alimentar de espécies nativas dos cerrados: araticum, barú, cagaita e jatobá. Embrapa-CPAC, Planaltina

Anne C (2006) Choosing the right molecular genetic markers for studying biodiversity: from molecular evolution to practical aspects. Genetica 127:101–120

Araújo TCC (2001) Estrutura da variabilidade genética e estrutura populacional de Caryocar brasiliense Camb. no Estado de Goiás utilizando marcadores RAPD. Thesis, Universidade Federal de Goiás, Brazil

Arnaud JF (2003) Metapopulation genetic structure and migration pathways in the land snail *Helix aspersa*: influence of landscape heterogeneity. Landsc Ecol 18:333–346

Avise JC (2000) Phylogeography. Harvard University press, Cambridge

Avise JC, Hamrick JL (1996) Conservation genetics: case histories from nature. Chapman and Hall, London

Borém A (1998) Melhoramento de Plantas, 2nd edn. Editora da UFV, Viçosa

Brasil (1999) Ações prioritárias para a conservação da biodiversidade do Cerrado e Pantanal. Brasília. MMA, Funatura, Conservation International, Fundação Biodiversitas, Universidade de Brasília

Cavalcanti RB, Joly CA (2002) Biodiversity and conservation priorities in the Cerrado region. In: Oliveira OS, Marquis RJ (eds) The Cerrados of Brazil: ecology and natural history of a neotropical savanna. Columbia University Press, New York

Cavallari MM, Forzza RC, Veasey EA et al (2006) Genetic variation in three endangered species of *Encholirium* (Bromeliaceae) from Cadeia do Espinhaço, Brazil, Setected using RAPD Markers. Biodivers Conserv (Online first) DOI: 10.1007/s10531-005-3741-5

Chung MY, Nason JD, Chung MG (2005) Patterns of hybridization and population genetic structure in the terrestrial orchids *Liparis kumokiri* and *Liparis makinoana* (Orchidaceae) in sympatric populations. Mol Ecol 14:4389–4402

Collevatti RG, Grattapaglia D, Hay JD (2001) Population genetic structure of the endangered tropical tree species *Caryocar brasiliense*, based on variability at microsatellite loci. Mol Ecol 10:349–356

Collevatti RG, Grattapaglia D, Hay JD (2003) Evidences for multiple maternal lineages of *Caryocar brasiliense* populations in the Brazilian Cerrado based on the analysis of chloroplast DNA sequences and microsatellite haplotype variation. Mol Ecol 12:105–115

Crandall KA, Bininda-Emonds ORP, Mace GM, et al (2000) Considering evolutionary processes in conservation biology. Trends Ecol Evol 15:290–295

Diniz-Filho JAF, Telles MPC (2002) Spatial autocorrelation analysis and the identification of operational units for conservation in continuous populations. Conserv Biol 16:924–935

Diniz-Filho JAF, Telles MPC (2006) Optimization procedures for establishing reserve networks for biodiversity conservation taking into account population genetic structure. Genet Mol Biol 29(2):207–214

Epperson BK (2003) Geographical Genetics. Princeton University Press, NJ

Escudero A, Iriondo JM, Torres E (2003) Spatial analysis of genetic diversity as a tool for plant conservation. Biol Conserv 113:351–365

Fahring L (2003) Effects of fragmentation on biodiversity. Annu Rev Ecol Syst 34:487–515

Ferreira ME, Grattapaglia D (1998) Introdução ao uso de marcadores moleculares em análise genética. Embrapa/Cenargen, Brasília

Fonseca AG, Muniz IAF (1992) Informações sobre a cultura de espécies frutíferas nativas da região de cerrado. Infor Agropec 173:12–17

Fraser DJ, Bernatchez L (2001) Adaptative evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol 10:2741–2752

Gabriel KR, Sokal RR (1969) A new statistical approach to geographic variation analysis. Syst Zool 18:259–278
Geburek T (1997) Isozymes and DNA markers in gene conservation of forest trees. Biodivers Conserv 6:1639–1654
Gonçalves-Alvim SJ, Collevatti RG, Fernandes GW (2004) Effects of genetic variability and habitat of *Qualea parviflora* (Vochysiaceae) on Herbivory by Free-feeding and Gall-forming insects. Ann Bot 94:259–268
Guillot G, Estoup A, Mortier F, Cosson JF (2005) A spatial statistical model for landscape genetics. Genetics 170:1261–1280
Gurdebeke S, Maelfait J-P, Backeljau T (2003) Contrasting allozyme and RAPD variation in spider populations from patchy forest habitats. Genetica 119:27–34
Hamrick JL, Godt JW (1990) Allozyme diversity in plant species. In: Brown ADH, Cleg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding and genetic resources. Sinauer, Suderland
Hamrick JL, Linhart YB, Mitton JB (1979) Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annu Rev Ecol Syst 10:173–200
Hedrick PW (2001) Conservation genetics: where are we now? Trends Ecol Evol 16:629–638
Holsinger KE (1999) Analysis of genetic diversity in geographically structured populations: a bayesian perspective. Hereditas 130:245–255
Holsinger KE, Lewis PO (2003) HICKORY V. 1.0. (software) www/eeb.uconn.edu/ Cited 12 Sep. 2006
Holsinger KE, Lewis PO, Dey DK (2002) A bayesian method for analysis of genetic population structure with dominant marker data. Mol Ecol 11:1157–1164
Holsinger KE, Wallace LE (2004) Bayesian approaches for the analysis of population genetic structure: an example from *Platanthera leucophaea* (Orchidaceae). Mol Ecol 13:887–894
Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914
Kidd DM, Ritchie MG (2006) Phylogeographic information systems: putting the geography into phylogeography. J Biogeogr 33:1851–1865
Kjolner S, Sastad SM, Taberlet P, et al (2004) Amplified fragment length polymorphism versus random amplified polymorphic DNA markers: clonal diversity in *Saxifraga cernua*. Mol Ecol 13:81–86
Klink CA, Machado RB (2005) Conservation of the Brazilian Cerrado. Conserv Biol 19:707–713
Klink CA, Moreira AG (2002) Past and current human occupation, and land use. In: Oliveira OS, Marquis RJ (eds) The cerrados of Brazil: ecology and natural history of a neotropical savanna. Columbia University Press, New York
Legendre P, Legendre L (1998) Numerical ecology. Elsevier, Amsterdan
Lessa E (1990) Multidimensional analysis of geographic genetic structure. Syst Zool 39:242–252
Manel S, Schwartz MK, Luikart G, et al (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18:189–197
Manly BFJ (1985) The statistics of natural selection. Chapman and Hall, London
Manly BFJ (1986) Randomization and regression methods for testing for associations with geographical, environmental and biological distances between populations. Res Popul Ecol 28:201–218
Manly BFJ (1997) Randomization, bootstrap and Monte Carlo methods in biology. Chapman and Hall, London
Moritz C (1994) Defining ''evolutionarily significant units'' for conservation. Trends Ecol Evol 9:373–375
Myers N, Mittermeier RA, Mittermeier CG, et al (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858
Newton AC, Allnutt TR, Gillies ACM, et al (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. Trends Ecol Evol 14:140–145
Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143–1155
Oliveira-Filho AT, Ratter JA (2002) Vegetation physionomies and woody flora of the Cerrado biome. In: Oliveira OS, Marquis RJ (eds) The cerrados of Brazil: ecology and natural history of a neotropical savanna. Columbia University Press, New York
Paetkau D (1999) Using genetic to identify intraspecific conservation units: a critique of current proposals. Conserv Biol 13:1507–1509
Parker PG, Snow AA, Schug MD, et al (1998) What molecules can tell us about populations: choosing and using a molecular marker. Ecology 79:361–382
Pearse DE, Crandall KA (2004) Beyond F_{ST}: analysis of population genetic data for conservation. Conserv Genet 5:585–602
Rabouam C, Comes AM, Bretagnolle V, et al (1999) Features of DNA fragments obtained by random amplified polymorphic DNA (RAPD) assays. Mol Ecol 8:493–503
Rangel TFLVB, Diniz-Filho JAF, Bini LM (2006) Towards an integrated computational tool for spatial analysis in macroecology and biogeography. Glob Ecol Biogeogr 15:321–327
Rangel TFLVB, Bini LM, Diniz-Filho JAF, Pinto MP, Carvalho P, Bastos RP (2007) Human development and biodiversity conservation in Brazilian Cerrado. Appl Geogr 27:14–27
Ratter JA, Ribeiro JF, Bridgewater S (1997) The Brazilian cerrado vegetation and threats to its biodiversity. Ann Bot 80:223–230
Ribeiro JF, Sano SM, Brito MA, et al (2000) Baru (*Dipteryx alata* Vog.). Funep, Jaboticabal
Rodrigues FM, Diniz-Filho JAF (1998) Hierarchical structure of genetic distances: effects of matrix size, spatial distribution and correlation structure among gene frequencies. Genet Mol Biol 21:233–240
Rohlf FJ (1989) NTSYS-Pc: numerical taxonomy and multivariate analysis system. Exeter Publishers, New York
Schneider S, Roessli D, Excoffier L (2000) Arlequin Ver. 2.000: a software for population genetic data analysis. (software) http://anthro.unige.ch/arlequin. Cited 12 Sep. 2006
Siqueira ACMdeF, Nogueira JCB, Kageyama PY (1993) Conservação dos recursos genéticos ex situ do cumaru (*Dipteryx alata*) Vog. Leguminoseae. Rev Inst Flor 5:231–243
Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the mantel test of matrix correspondence. Syst Zool 35:627–632
Sokal RR, Oden NL (1978a) Spatial autocorrelation in biology. 1. Methodology. Biol J Linn Soc 10:199–228
Sork VL, Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations. Landsc Ecol 21(6):821–836
Sokal RR, Oden NL (1978b) Spatial autocorrelation in biology. 2. Some biological implications and four applications of evolutionary and ecological interest. Biol J Linn Soc 10:229–249

Spiegelhalter DJ, Best NG, Carlin BP, et al (2002) Bayesian measures of model complexity and fit. J R Stat Soc B 64:483–689

Sunnucks P (2000) Efficient genetic markers for population biology. Trends Ecol Evol 15:199–203

Telles MPC, Bastos RP, Soares TN, et al (2006) RAPD variation and population genetic structure of *Physalaemus cuvieri* (Anura: Leptodactylidae) in central Brazil. Genetica 128:323–332

Telles MPC, Coelho ASG, Chaves LJ, et al (2003a) Genetic diversity and population structure of Eugenia dysenterica DC ('cagaiteira'—Myrtacea) in central Brazil: implications for conservation and management. Conserv Genet 4:685–695

Telles MPC, Valva FD'A, Bandeira LF, et al (2003b) Caracterização genética de populações naturais e araticunzeiro (*Annona crassiflora* Mart.—Annonaceae) no Estado de Goiás. Rev Bras Botânica 26:123–129

Thum AB, Costa EC (1999) Entomofauna visitante das inflorescencias de *Syagrus romanzoffiana* (Cham.) Glassm. (Palmae). Rev Fac Zootech Vet Agron 5/6:43–47

Trindade MG, Chaves LJ (2005) Genetic structure of natural *Eugenia dysenterica DC* (Myrtaceae) populations in northeastern Goiás, Brazil, accessed by morphological traits and RAPD markers. Genet Mol Biol 28:407–413

Zimback L, Mori ES, Kageyama PY, et al (2004) Genetic structure of *Trichilia pallida* Swartz (Meliaceae) population by RAPD marks. Sci Forestalis 65:114–119

Zucchi M, Brondani RV, Pinheiro JB, et al (2003) Genetic structure and gene flow in Eugenia dysentericaDC in the Brazilian cerrado utilizing SSR markers. Genet Mol Biol 26:449–457

Zucchi M, Pinheiro JB, Chaves LJ, et al (2005) Genetic structure and gene flow of *Eugenia dysenterica* natural populations. Pesqui Agropecu Bras 40:975–980