Plant Syst Evol (2015) 301:1207–1213
DOI 10.1007/s00606-014-1144-3

ORIGINAL ARTICLE

Fine-scale genetic structure in *Tibouchina papyrus* (Pohl) Toledo (Melastomataceae), an endemic and habitat-restricted species from Central Brazil

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Received: 27 December 2012 / Accepted: 25 July 2014 / Published online: 9 September 2014

Abstract Spatial genetic structure (SGS) is the non-random distribution of genotypes in space, which is usually correlated with life history traits and is driven by ecological and demographic processes. This study aimed to use spatial statistical analysis methods to assess genetic diversity and spatial genetic structure in three known disjunct localities where *Tibouchina papyrus* occurs, varying in aggregation and density of individuals and in landscape characteristics. This small tree species is endemic to the "Cerrado" (savannas) region of Central Brazil and is also restricted to elevation rock fields ("campo rupestre"). Therefore, it can be considered a model species to help guide studies on Cerrado endemic plants that are isolated with geographic distributions restricted to this highland habitat. The analyses were based on the polymorphisms at ten microsatellite loci. Our working hypothesis was that wind-dispersed species, such as *T. papyrus*, present no or low SGS because of the long distance seed gene flow. The spatial genetic structure was indeed weak for two of the three populations. The intensity and shape of the SGS are related to density and aggregation (defined by Ripley's *K* statistic of spatial aggregation) of individuals in the three localities. Low SGS occurs in populations with aggregation patterns, corroborating the overall hypothesis, although in this species, this pattern depends on the landscape characteristics of each local population.

Keywords Cerrado biome · Gene flow · Microsatellite · Pau-papel · SGS

Introduction

Spatial genetic structure (SGS) is the non-random distribution of genotypes within a population (Vekemans and Hardy 2004) and can result from different ecological and microevolutionary processes driving molecular patterns of variation in natural populations (Vekemans and Hardy 2004). Issues related to the seed and pollen dispersal mode, for instance, have been considered a major determinant of genetic structure in local plant populations because they can directly affect the spatial distribution of individuals within a population and consequently their genetic structure (Loveless and Hamrick 1984; Loiselle et al. 1995; Oddou-Muratorio et al. 2003). On the other hand, the magnitude of the effect of these factors is obviously related to ecological and landscape characteristics, which can limit dispersal paths within a region.

It is important to note, however, that understanding these ecological and life history patterns and their consequent demographic processes is not a trivial task, because direct estimates of seed and pollen dispersal require complex methodologies and experimentation, usually involving a considerable field effort. Therefore, indirect approaches to understand the association of these patterns and processes with the SGS based on molecular methods have

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been proposed and successfully used to estimate gene flow within populations (Epperson 1993; Smouse et al. 2001; Smouse and Sork 2004; Burczyk and Koralewski 2005; Hardy et al. 2006; Pandey and Rajora 2012). At the same time, knowing these SGS parameters is important for developing more efficient in situ and ex situ conservation strategies (e.g., Diniz-Filho and Telles 2002, 2006). The molecular techniques, combined with spatial autocorrelation analyses, enabled the refinement of methods of analysis and allowed the estimation of apparent gene flow within and between populations (Fenster et al. 2003; Vekemans and Hardy 2004; Diniz-Filho et al. 2008). In this context, the microsatellites are powerful molecular markers for these analyses because of their high level of genetic information, which makes them useful for estimating population genetic parameters and understanding detailed patterns of gene flow and parentage composition (Morgante and Olivieri 1993).

The Cerrado biome covers 22 % of Brazil's land area (Silva et al. 2006) and is considered one of the biodiversity hotspots (Myers et al. 2000), with more than 50 % of the natural vegetation being lost in the past 35 years (Klink and Machado 2005). *Tibouchina papyrus* (Pohl) Toledo (Melastomataceae), known as "pau-papel," is an endemic tree species that occurs in the vegetation subtype known as "campo rupestre" of the Cerrado biome (Montoro and Santos 2007). The species has a restricted and disjunct distribution, and it is known to occur in a few localities, mainly in Serra de Natividade, in the Southeast Tocantins State, and in the Serra Dourada and Serra dos Pirineus in western Goiás State (Telles et al. 2011; Maracahipes et al. 2012). Due to its restricted distribution, this species was framed by the criterion D2 IUCN (2001) and categorized as vulnerable in the list of endangered plant species in Brazil ("Biodiversitas" in 2005). According to Collevatti et al. (2012), these three populations are genetically different ($F_{ST} = 0.684$) based on phylogeographical analyses with chloroplast and microsatellite markers, and they can each be considered highly differentiated evolutionarily significant units (ESUs) (sensu Moritz 1994). Besides the endemism and rarity, the species is threatened because of the highly unstable habitat with high levels of disturbance caused by fire during the dry season as well as sandstone and quartzite disruption mainly during the rainy season (Collevatti et al. 2010). The species is self-compatible, flowers are hermaphroditic, and buzz pollination is performed mainly by large bees, such as *Xylocopa* spp., *Bombus* spp. and *Centris* spp. The very small seeds are wind dispersed (Montoro and Santos 2007).

Thus, our goal here is to analyze fine-scale SGS in the three known populations of the species. Based on the ecological and life history characteristics of *T. papyrus* described above, our working hypothesis was that wind-dispersed species present no or low SGS because of long distance seed gene flow, as observed in comparative analyses among species (e.g., Vekemans and Hardy 2004; Hardy et al. 2006; Collevatti et al. 2010). However, these overall patterns can be disrupted by landscape and demographic characteristics particular to a given population. We then used spatial autocorrelation analyses of microsatellite markers to estimate the neighborhood area and report the SGS of *T. papyrus*, trying to relate the observed patterns to the life history of the species and landscape and demographic characteristics of each of the three localities analyzed.

Materials and methods

Sampling and genotyping

Individual *T. papyrus* trees were sampled in three known localities of species occurrence: Serra dos Pirineus, Pirenópolis municipality, Goiás State (16° 03′ 58″ S and 50° 10′ 22″ W), Serra Dourada, Cidade de Goiás municipality, Goiás State (15° 47′ 44″ S and 48° 49′ 57″ W), and Serra de Natividade, Natividade municipality, Tocantins State (11° 40′ 08″ S and 47° 41′ 51″ W) (Fig. 1). We sampled all individuals found in Serra dos Pirineus (216) and Serra Dourada (66). In Serra de Natividade, we randomly sampled 192 individuals because this population was the largest, with $\sim$1,500 individual trees. In this last locality, it is possible to observe the formation of two groups of individuals related to the altitudinal variation of the landscape in this region (one located at a mean altitude of ca. 320 m and another at a mean altitude of ca. 560 m). All individuals sampled were mapped using GPS, and their leaves were sampled and conserved for further analyses.

Total genomic DNA was extracted from expanded leaves following the standard CTAB 2 % procedure (Doyle and Doyle 1987). The amplifications were based on polymorphisms at ten microsatellite loci (see Telles et al. 2011) and performed in a 15-µl volume containing 0.26 µM of each primer, 1U Taq DNA polymerase (Phoneutria, BR), 216 µM of each dNTP, 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 2.16 mg of BSA and 12.5 ng of template DNA. Amplifications were performed using a thermal controller (Applied Biosystems, CA) under the following conditions: 94 °C for 5 min (one cycle), 94 °C for 1 min, primer-specific temperature for 1 min, 72 °C for 1 min (30 cycles) and 72 °C for 7 min (one cycle). Polymorphisms were detected on 6 % denaturing polyacrylamide gels stained with silver nitrate (Creste et al. 2001) and sized by comparison to a 10-bp DNA ladder standard (Invitrogen®). The confirmation of each of the alleles was performed from a new gel containing a sample with all existing alleles.

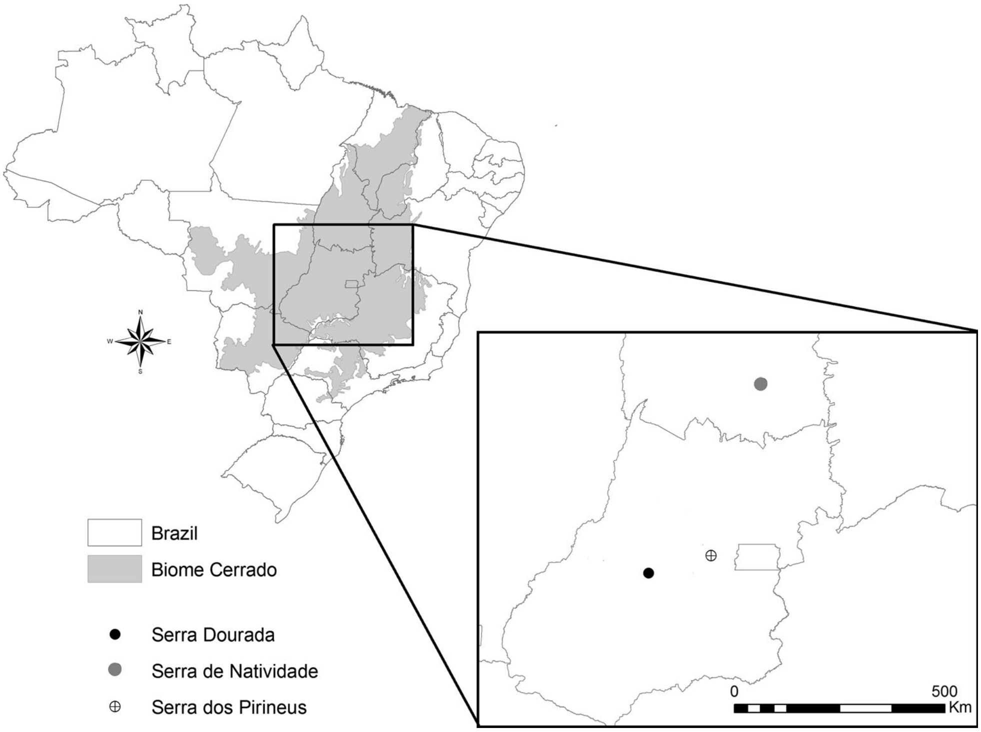

Fig. 1 Sampling localities: the three known places where *T. papyrus* can be found in rocky, high-altitude fields in the Cerrado biome

Spatial genetic structure

The number of alleles per locus, allelic richness, observed and expected heterozygosities under Hardy-Weinberg equilibrium (Nei 1978), and inbreeding coefficients (*f*) were analyzed with FSTAT 2.9.3.2 (Goudet 2002). Randomization-based tests with Bonferroni correction were performed for testing deviations from Hardy-Weinberg expectations and linkage equilibrium (Goudet et al. 1996).

Autocorrelation analyses (see Sokal and Oden 1978 for initial applications and Diniz-Filho and Bini 2012 for a recent historical account) were performed to verify the kinship structure in the adult population and estimate seed dispersal using the Nason estimator F_{ij} (Loiselle et al. 1995), given as:

$$F_{ij} = \sum_{1} \left[\sum_{a} \left(\sum_{ci} \sum_{cj} (x_{lcia} - p_{la})(x_{lcja} - p_{la}) \Big/ \sum_{ci} \sum_{cj} 1 \right) + \sum_{a} (p_{la}(1 - p_{la})/(n_1 - 1))\right] \Bigg/ \sum_{1} \sum_{a} (p_{la}(1 - p_{la}))$$

where x_{lcia} is an indicator variable ($x_{lcia} = 1$ if the allele on chromosome *c* at locus *l* for individual *i* is *a*; otherwise, $x_{lcia} = 0$), p_{la} is the allele frequency *a* at locus *l* in the reference sample, n_1 is the number of genes defined in the sample at locus *l* (the number of individuals times the ploidy level minus the number missing alleles), and $\sum_{ci}$ stands for the sum over the homologous chromosomes of individual *i*. Here, the term involving ($n_1 - 1$) is a sampling bias correction.

The relationship between F_{ij} and the distance class is informative about dispersal processes. For example, a positive relationship between F_{ij} and distance is expected when seed dispersal is restricted so that siblings or parents and offspring reside near each other, i.e., individuals that are physically close present a higher kinship. Here we computed F_{ij} values between all pairs of adult individuals using the SPAGeDI 1.2 (Spatial Pattern Analysis of Genetic Diversity) software (Hardy and Vekemans 2002). To test for kinship structure, the F_{ij} values were regressed on the natural logarithm of the spatial distance between individuals. Also F_{ij} values were averaged over a set of distance classes and plotted against these distance classes. The distance classes were defined to keep the number of pairwise comparisons within each distance interval approximately constant. Permutation tests (10,000 permutations) were used to verify the deviation of observed kinship for each distance class from null expectation and of regression. Standard errors (SEs) were estimated by jackknife over loci.

The strength of the spatial genetic structure in the locality was quantified using the parameter $S_p = b/(F_1 - 1)$, where F_1 is the average kinship coefficient between individuals of the first distance class and *b* is the slope of the regression. Seed dispersal was estimated based on the genetic neighborhood (N_b) using the regression slope *b* and the intra-group F_{ij} ($N_b = (F_1 - 1)/b$ or $N_b = 1/S_p$) (see

Vekemans and Hardy 2004 for details); the effective neighborhood size is equivalent to the number of reproducing individuals in a 2σ circle of radius (Wright 1946).

To evaluate whether the spatial structure of individuals differs in the three populations, the distance frequency distributions of the populations were compared using the Kolmogorov-Smirnov test (Sokal and Rohlf 1995). We also analyzed the spatial distribution of individuals (point pattern) for each locality to help interpret the SGS patterns. These patterns were evaluated by the distribution of pairwise distances (in meters) and by computing Ripley's *K* function (see Ledo et al. 2012) among individuals. Ripley's *K* statistic was tested against the expected values using 1,000 permutations in SAM 4.0 (Rangel et al. 2010).

Results

All pairs of microsatellite loci were in linkage equilibrium (all $P > 0.05$), revealing that they were suitable for use in population genetic analysis. Serra de Natividade shows higher genetic diversity, and Serra dos Pirineus and Serra Dourada display a statistically lower heterozygosity than expected under Hardy-Weinberg equilibrium (Table 1).

Spatial distribution of adult individuals differed in the three populations (Fig. 2), although they were aggregated ($P < 0.01$) according to Ripley's *K* function in all localities. For Serra dos Pirineus, the mean distance between pairs of individuals was 1,775 m (SD = 1,235 m), and most (i.e., more than 70 %) individuals were distant from each other by less than 2,000 m, showing a wider distribution of individuals. For the Serra Dourada, the mean distance was equal to 1,030 m (SD = 840 m). On the other hand, in Serra de Natividade, the individuals were much more clumped than in the other two populations, with a mean distance equal to 650 m (SD = 487.5 m).

The autocorrelation analysis for Serra dos Pirineus (Fig. 3a) showed a clinal pattern with a significant spatial autocorrelation up to 90 m, coupled with significant positive autocorrelation of about 3,000 m. Kinship coefficients were weakly related to the logarithm of the center of the distance class ($R^2 = 0.002$; $P < 0.001$). For this population, the size of the genetic neighborhood (N_b) was 72.9 individuals, less than half of the individuals sampled (Table 2).

Table 1 Genetic diversity of the three populations of *Tibouchina papyrus*

Population	*N*	Ar	H_e	H_o	*f*
Serra dos Pirineus	216	1.5	0.205	0.155	0.246
Serra Dourada	66	1.5	0.357	0.280	0.216
Serra de Natividade	192	2.1	0.409	0.386	0.057^{ns}

Values followed by ns are nonsignificant, for $P = 0.00167$, Bonferroni correction for a nominal value of 0.05

N number of individuals sampled, *Ar* allelic richness, H_e expected heterozygosity, H_o observed heterozygosity, *f* inbreeding coefficient

Analysis of the SGS in Serra Dourada revealed that the kinship decreases significantly after 70 m, and after this distance class, no further significant coefficients were found, so the autocorrelation profile did not display a clear clinal pattern (Fig. 3b). Kinship was also weakly related to the logarithm of distance ($R^2 = 0.003$; $P < 0.05$). Neighborhood size was not estimated for this population because of the lack of significant spatial genetic structure (Table 2).

Finally, the correlogram of individuals from Serra de Natividade exhibited a clinal pattern of variation, with positive and significant correlation between kinship, decreasing strongly after 220 m, and also showing a negative correlation in the last distance classes (Fig. 3c). The autocorrelation analysis showed that kinship in this locality was slightly more related to the logarithm of distance ($R^2 = 0.191$; $P < 0.001$) than in the other two localities, and the genetic neighborhood was much lower than in the other two localities and equal to 11.9 (Table 2).

The spatial genetic structure was thus much stronger in the Serra de Natividade, with a higher $S_p = 0.0841$ than that observed for the two populations from Goiás state (Serra dos Pirineus = 0.0137; Serra Dourada = 0.0095) (see Table 2).

When dividing Serra de Natividade into two groups of individuals (one located at a mean altitude of ca. 320 m and another at a mean altitude of ca. 560 m), we found low values of S_p (0.0392 and 0.0087, respectively) and no clinal pattern of variation in the correlogram.

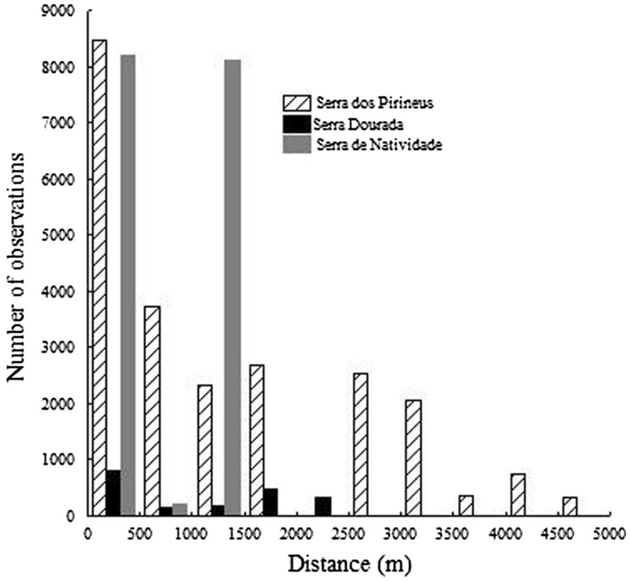

Fig. 2 Pairwise distance distribution of the individuals in the three populations of *T. papyrus*. Individuals were considered aggregated in all localities according to the randomization test based on Ripley's *K* function (Kolmogorov-Smirnov test $P < 0.001$)

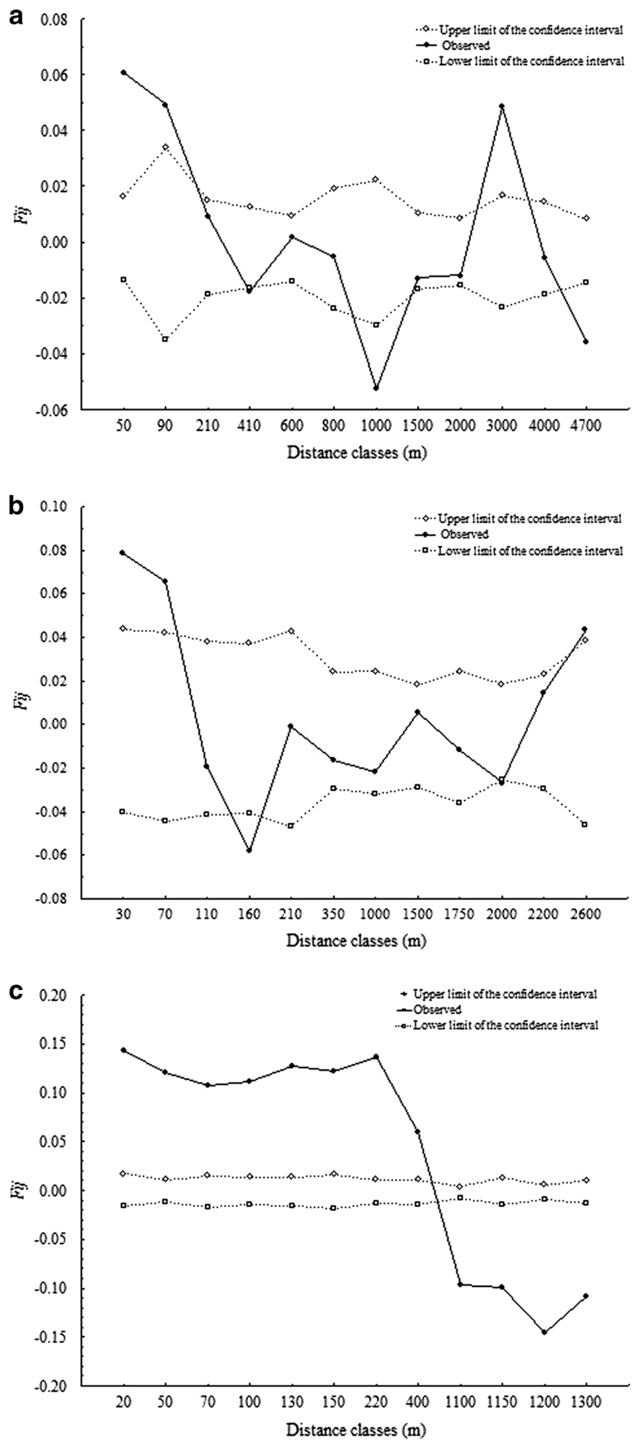

Fig. 3 Relationship between kinship (F_{ij}) and distance classes in Serra dos Pirineus (**a**), Serra Dourada (**b**) and Serra de Natividade (**c**) populations with respective 95 % confidence intervals (*dotted lines*)

Table 2 Comparison of spatial genetic structure (SGS) and genetic neighborhood among the three populations

Population	F_1*	b*	S_p	N_b
Serra dos Pirineus	0.060 ± 0.069	−0.0129	0.0137	72.93
Serra Dourada	0.072 ± 0.030	−0.0089	0.0095	–
Serra de Natividade	0.143 ± 0.064	−0.0721	0.0841	11.88

F_1 intra-group F_{ij}; b regression slope for the relationship between kinship and logarithmic spatial distance; S_p strength of the spatial genetic structure parameter; N_b genetic neighborhood (m). *SE* standard error

*Values are statistically significant for $P < 0.05$

Discussion

Spatial genetic structure is influenced by numerous ecological and evolutionary factors (Loveless and Hamrick 1984; Hamrick and Loveless 1986; Wang et al. 2013), including the mating system, dispersal of pollen and seed, neighborhood size and demographic processes (Nathan and Muller-Landau 2000; Buerki et al. 2009; Colling et al. 2010; Unger et al. 2011). Thus, patterns of SGS can be used to interpret the extent and pattern of seed dispersal (Epperson 1993; Kalisz et al. 2001; Vekemans and Hardy 2004; Chung et al. 2005). The existence of a weak SGS can be a direct consequence of the dispersal and reproductive dynamics in open environments (Horn et al. 2001; Hamrick 2004). According to Kalisz et al. (2001). When pollen dispersal is localized but the seed is dispersed over long distances, a weak SGS is expected, as in *T. papyrus*, which occurs in “campo rupestre,” for which pollen dispersal takes place (by bees) and seeds are dispersed by the wind. This is the case for two populations of *T. papyrus,* supporting the above statements overall. However, it is important to highlight that a different pattern of SGS is observed in Serra de Natividade, reflecting other components that explain them. The presence of SGS in Serra de Natividade is probably related to its particular landscape characteristics, which form a geographical division of individuals, separating them into two groups of individuals. Thus, the wind dispersal is not sufficient to mix the alleles across the entire local population because of the altitude difference of the two groups. Indeed, dividing this population into two groups, low S_p values were observed, supporting the existence of a weak SGS in wind-dispersed plants.

The SGS of populations of *T. papyrus*, measured by the S_p statistic (mean value across populations equal to 0.035), was within the expected values observed for plant species (see Vekemans and Hardy 2004), especially wind-dispersed species (Collevatti et al. 2014a).This value was lower than that of previously studied animal-dispersed Cerrado tree species, such as *Caryocar brasiliense* (Collevatti et al. 2010; Collevatti and Hay 2011) and *Dipteryx alata* (Collevatti et al. 2010); however, it presented a stronger SGS than *A. crassiflora* species, which show lower values than for other animal-dispersed species (Collevatti et al. 2014b). This lower S_p value is expected because trees with small seeds dispersed by the wind are favored over animal-dispersed trees with large fruits (Cordeiro and Howe 2003), and the wind may promote long distance seed dispersal (Horn et al. 2001). Thus, patterns of SGS observed for *T. papyrus* match the expectations based on its ecological and life history traits, with coherent values for other plant species in general and for Cerrado species in particular.

Because *T. papyrus* possess a geographically narrow range, with a small number of known occurrences and restricted to isolated, high-altitude habitats in the Cerrado region, which is one of the biodiversity hotspots (see Myers et al. 2000), it is also important to discuss the implications of the SGS observed for the species for its conservation. Previous theoretical and empirical evaluations (i.e., Diniz-Filho and Telles 2002, 2006; Escudero et al. 2003; Diniz-Filho et al. 2012) suggested that, for conservation purposes, autocorrelation analyses may be useful to establish criteria to maximize genetic diversity, to both represent the species variability when sampling for germplasm collection and minimize the extinction risk due to the low variability and capacity to adapt to environmental changes. Thus, it is important to conserve individuals from different populations and even patches within populations at a finer spatial scale. This fine-scale evaluation can then be important when spatial structure is observed within populations, as observed here, and parameters estimated using SGS autocorrelation analyses can be useful for establishing guidelines for more effective conservation strategies (Collevatti et al. 2010). For example, Diniz-Filho and Telles (2002, 2006) advise using the intercept of the autocorrelogram to define the size of the interval distance for sampling germplasm, avoiding sampling individuals of the same genetic neighborhood area, thus minimizing redundancy of genetic representation and minimizing costs in ex situ conservation programs.

Despite the weak SGS found in two of the *T. papyrus* populations, the autocorrelation analysis of the microsatellite genetic variation showed autocorrelation in the first distance classes, indicating some degree of relatedness between individuals that are closer spatially. The distances represented by these classes provide important information for the management of these populations, because they indicate a minimum distance between individuals to be sampled in each one (Diniz-Filho and Telles 2002, 2006). For example, based on the SGS and maps of individuals, it may be interesting to keep a distance of 90 m among individuals in Serra dos Pirineus, whereas for Serra Dourada a distance of around 70 m must be used to guide sampling.

Thus, in summary, knowing the SGS for *T. papyrus* provides a fine guide for replicating sampling within localities to better represent genetic diversity of the species (i.e., an important step for further ex situ conservation programs). At the same time, our study comparing SGS in distinct localities revealed that, beyond the overall expectations for the SGS, it is important to better understand how the balance between intrinsic ecological and life history characteristics related to seed and pollen dispersal and extrinsic landscape characteristics creates the SGS within localities. Further studies may be important for understanding how these variable SGSs are stable in time and how further molecular (adaptive) and phenotypic variations are related to the neutral patterns observed here. In a last instance, it may be possible to understand how individual fitness varies among the localities, allowing the establishment of efficient conservation strategies, which is very important for endemic and habitat-restricted species such as *T. papyrus*.

Acknowledgments This work was supported by several grants and fellowships to the research network GENPAC (Geographical Genetics and Regional Planning for Natural Resources in Brazilian Cerrado) from CNPq/MCT/CAPES (projects 471492/2007-8, 564717/2010-0 and 563727/2010-1) and by the Núcleo de Excelência em Genética e Conservação de Espécies do Cerrado-GECER (PRONEX/FAPEG/CNPq CP 07-2009). The field work was supported by Systema Naturae Consultoria Ambiental LTDA. Work by M.P.C.T., T.N.S. and R.G.C. has been continuously supported by productivity fellowships from CNPq.

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