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Schinus terebinthifolius: Population structure and implications for its conservation

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ABSTRACT

The high demand for the fruit of *Schinus terebinthifolius*, known as the Brazilian Pepper Tree, has generated attention for vegetation studies in the São Francisco River Basin, Sergipe State, Brazil. The fruit is heavily exploited and the genetic consequences of the exploitation are currently unknown. The objective of this study was to estimate the genetic diversity of three distinct populations of *S. terebinthifolius* from Caatinga, Atlantic Forest, and Ecotone, and to define priority areas between them for conservation in the river basin. The studied area is located along 81.52 km of the São Francisco River Basin. The sample of 162 individuals was studied by ISSR molecular markers. There are recent genetic bottlenecks in studied populations, and the genetic differentiation among populations was Fst = 0.27. The populations from the Caatinga and Atlantic Forest biomes presented a low level of genetic divergence (0.14). There was no correlation between the genetic and spatial distance between the populations. We detected genetic barriers and nine distinct genetic groups ($K = 9$). The presence of exclusive loci in each studied population provides evidence to support the definition of these populations as potential management units for conservation.

1. Introduction

The effects of habitat loss on a population's viability are significant both for biodiversity and conservation studies. Research has shown that changes in habitat and climate cause negative impacts on ecological and genetic traits of natural plant populations. Such changes are considered to be the main drivers behind the loss of diversity (Lindenmayer and Fischer, 2007; Lindenmayer et al., 2008).

The key challenge in recent years has been to assess population viability and the role of environmental heterogeneity in the functional connectivity of populations (Lindenmayer and Fischer, 2007; Revilla and Wiegand, 2008). Therefore, current

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studies assess the response to landscape heterogeneity based on the life-history traits of a species. Depending on the environmental pressures experienced by the species, and if the population size is decreasing, the species may be severely threatened (Miyaki, 2009). To ensure the conservation and long-term viability in the environment, it is necessary to identify if the populations have retained a set of distinct genetic characteristics (Avise, 2000).

Evolutionary Significant Units (ESU) are populations which demonstrate a significant divergence in allele frequencies (Moritz, 1994). ESU have emerged as important aspect of conservation, and they are understood to be a key target for conservationist strategies that coincide (or not) with recognized interspecific limits (Mace, 2004). ESU are also appropriate for defining *in situ* conservation strategies, or even in the sampling of individuals for germplasm banks. In order to ensure the management and the viability of ESU, strategies must be optimized through the identification of Management Units (MU). In this approach, MU are geographically distinct populations which demonstrate divergence in the allele frequencies, thus assuring the maintenance of ESU (Diniz-Filho and Telles, 2002; Manel et al., 2003).

In Sergipe State, located in the Northeast of Brazil, about 90% of the region's natural ecosystems have been converted into grassland, with intensive agricultural activities. These regions have been severely devastated, with only a few remaining areas of original coastal forest, restinga, and riparian vegetation, arboreal/shrub, and dry Caatinga forest, all of which continue to experience pressure from anthropogenic activities (MOPEC, 2008). Currently, some cities located on the São Francisco River banks (the main river in the Brazilian Northeast), such as Santana do São Francisco, Brejo Grande, Ilha das Flores, Pacatuba in Sergipe, and Piaçabuçu in Alagoas State, have been the target of an intense exploitation of *Schinus terebinthifolius* Raddi. The fruit of this species, popularly known as Brazilian pepper, is exported to Europe, USA, Canada, and Argentina, and it is used as culinary seasoning and the production of essential oils. This species occurs naturally, but the remaining natural populations have been heavily exploited for the past ten years.

The pressure of extractivism on this species is worrisome, because fruit harvesting, which reduces the amount of seed-seedling in the soil bank, is thus hampering its natural distribution and propagation, leading to differential seedling distribution over time. Furthermore, the creation of seed-seedling banks, with a low genetic variability, may arise, due to autogamy, resulting in a decrement in the effective number of individuals (Peters, 1996). Consequently, the long-term reduction of adaptation of new allele combinations, and an increased incidence of less vigorous individuals could occur (Primack and Efraim, 2001), due to endogamy and genetic drift.

Information about the levels of genetic diversity of natural populations, allows us to obtain an understanding of ecology, and the distribution of genetic variability. Our study hypothesizes that the populations are located within geographical proximity, and that genetic differentiation is expected to be limited, as result of the high levels of gene flow. Moreover, the populations are located on the banks of the São Francisco River; consequently, the river could act as a secondary disperser of Brazilian pepper seeds, which would result in homogenization of the gene flow, and decreasing genetic differentiation. Therefore, the aim of this study was to estimate the genetic diversity of three populations of *S. terebinthifolius*, and to define priority areas for conservation along the São Francisco River Basin.

2. Materials and methods

The studied area covers a range of 81.52 km and is located in the lower São Francisco River region, in the Northeast of Brazil (Fig. 1).

The area was chosen due to the high demand for Brazilian pepper. Were sampled and geo-referenced, 30 Schinus terebinthifolius individuals from Caatinga Domain, 47 from Atlantic Forest, and 85 from Ecotone, between both regions.

Genomic DNA was extracted from young leaves (2.0 g) by the optimized CTAB method and added with 0.2% b-mercaptoethanol (v/v) (Nienhuis et al., 1995). The estimative number of polymorphic fragments was performed using GENES software (Cruz, 2006). The genetic analysis was carried out with AFLP-SURV version 1.0 (Vekemans, 2002), using Bayesian analysis, for the non-uniform distribution of estimative frequencies based on Zhivotovsky (1999). The binary data, obtained by the presence or absence of amplified fragments was used to estimate the allele frequencies, the number of loci, the number of polymorphic loci, Nei's genetic diversity ($\hat{H}e$) (Nei, 1973), and genetic differentiation between the populations (Fst). Gene flow was obtained by POPGENE 1.31 software analysis (Yeh et al., 1997) and we used BOTTLENECK 1.2.02 software (Cornuet and Luikart, 1996) to verify a significant recent decrement for the effective population size (N_e), based on a severe and recent genetic bottleneck. The number of exclusive loci and the analysis of molecular variance (AMOVA) were obtained by GenAlEx software (Peakall and Smouse, 2006).

The spatial patterns of genetic variability, in a multivariate context, were identified by geographical distance classes (the first class with an upper limit of 3.7 m and the final distance class with limits of 33.6–40.4 m), clustered in matrices of spatial connectivity, and correlated with genetic distance. The significance of the matrix correlation coefficients was evaluated by Mantel's *Z* statistic (Mantel, 1967) using NTSYS 2.0 (Rohlf, 2000). The intercept of spatial correlograms was used as a parameter to define the minimum distance between populations which were genetically independent, and subsequently, were used to define management units (MU) (Diniz-Filho and Telles, 2002).

Genetic diversity, θ^B, was estimated by Bayesian analysis, performed by HICKORY v. 1.1 (Holsinger and Lewis, 2003), where the value of θ^B was obtained by using the average of the four different models used in HICKORY, with Markov Chain Monte Carlo simulation. The four models include the full model, which estimates the values for θ^B and f; models θ^B and f both of which assume θ^B and f are equal to zero; and the *free f* model, which chooses random values for f (Holsinger et al., 2002). The identification of discontinuity in genetic data across geographical space was performed by BARRIER software (Manni et al.,

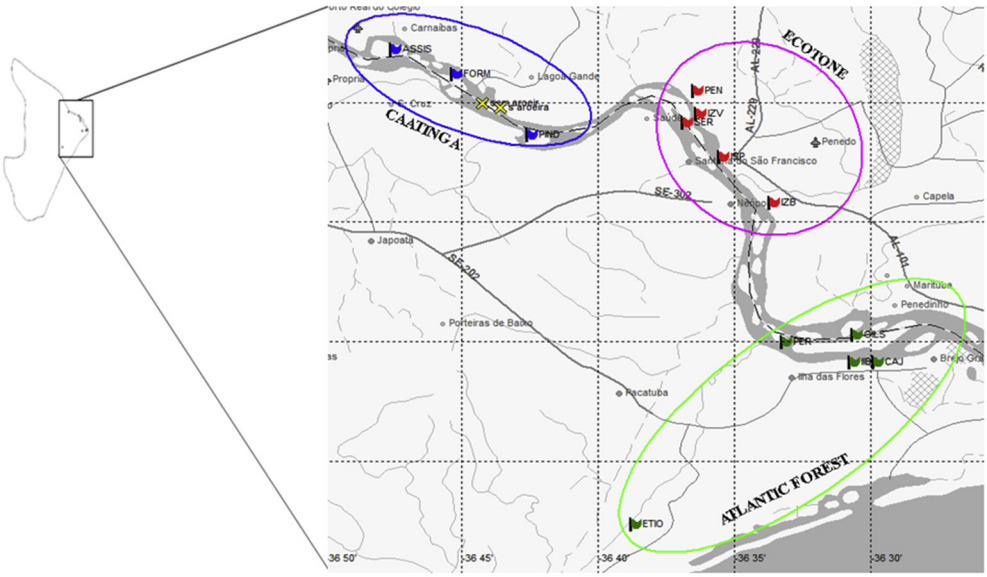

Fig. 1. Study area of *Schinus terebinthifolius* populations; blue represents the Caatinga; red represents the Ecotone; and green represents the Atlantic Forest. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2004). The sampled populations were connected by the Delaunay triangulation method, according to the geographical coordinates, and the barriers were identified using the Monmonier algorithm (Manni et al., 2004). Based on Bayesian modeling, the analysis of the population's genetic structure was performed by STRUCTURE v. 2.3 (Pritchard et al., 2000; Hubisz et al., 2009). This model is able to identify the structure and the proportion of genotypes from other groups following the Evanno et al. (2005) method ($\Delta K = m|L''(K)|/s[L(K)]$) and by using Structure Harvester software (Earl and VonHoldt, 2012).

The effective population size (N_e) was calculated according to the methodology presented by Vencovsky (1997) for simple and multiple populations.

Management Units were defined based on the intercept of spatial correlograms, genetic discontinuities, and the effective population size, aiming to maintain the genetic variability assessed by the ISSR markers.

3. Results

Using the sampled 162 individuals of Brazilian Pepper Tree, the amplification of genomic DNA with 11 ISSR primers resulted in 181 fragments of which 157 were polymorphic. The number of fragments per studied area was: 145 for Caatinga, 150 for Ecotone, and 153 for Atlantic Forest. Thirty exclusive loci were identified across all populations, of which 19 were detected only for the Ecotone population with frequencies ranging from 0.01 to 1.0. However, for the Ecotone population, two fixed loci were observed and five loci presented frequencies of less than 5%. For the Atlantic Forest population, we found nine exclusive loci with frequencies ranging from 0.04 to 0.68. For the Caatinga population, two exclusive loci were identified, one with a frequency of 0.10 (present in three individuals) and other with a frequency of 0.43 (found in 12 individuals). Considering both the Ecotone and Atlantic Forest populations, the majority of exclusive loci occurred at high frequencies (higher than 10%). Common loci in the sampled areas were not detected.

The genetic diversity ($\hat{H}e$) presented an average of 0.20 (0.20–0.21) and the Atlantic Forest population presented the highest diversity index (0.21) when in comparison with the other sampled populations. According to AMOVA, the majority of the genetic variability occurred within the populations (61%).

The genetic differentiation between populations (Fst) was 0.27 and the greatest differentiation was found between the Ecotone and Atlantic Forest populations (0.33), with a rate of gene flow of 1.7, and between the Ecotone and Caatinga populations (0.33), with a rate of gene flow of 1.6. The genetic differentiation between the Caatinga and Atlantic Forest populations was 0.14 and the gene flow was higher than 4.0, even though the populations are distant, being approximately 29.56 km away. There was no significant correlation between the genetic and geographic distance (Fig. 2).

The Bayesian analysis using the $f = 0$ model was the most adequate for an estimative of the genetic distance between the pairs of populations due to the lower DIC value (3831.23). The map of genetic distance (θ^B), through Delaunay triangulation, confirmed the existence of genetic discontinuity among the sampled Brazilian pepper populations.

Furthermore, *a posteriori* probabilities, estimated by the Bayesian cluster method, implemented in STRUCTURE, identified nine genetic clusters.

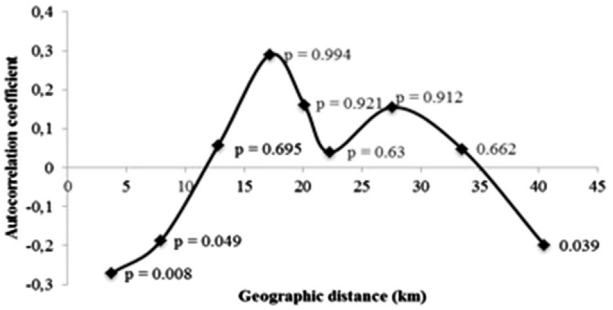

Fig. 2. Mantel correlograms based on the correlation between θ^B (Bayesian distances) and geographical distance classes found for *S. terebinthifolius* populations located in the São Francisco River Basin.

Tests of adherence to the modeling of infinite alleles, resulting from mutation, presented no equilibrium, which indicated the occurrence of recent bottlenecks ($p < 0.05$, Wilcoxon signed-rank test). The three sampled populations presented in excess of heterozygosity resulting in a loss of effective population size.

The effective population size (N_e) was 15 individuals in the Caatinga population, 42 in the Ecotone population, and 23 in the Atlantic Forest population; the N_e/n ratio for the three populations was 0.5.

4. Discussion

Most of the genetic diversity is distributed within the populations (61%). This pattern to be expected, as the diversity values within the populations are generally higher in perennial and outcrossing species, than in annual and autogamous species (Hu et al., 2010; Shao et al., 2009). However, this level of genetic diversity (61%) is considered low when compared to other studies on neotropical species. The same species in two riparian fragment forests along the Tibagi River Basin in Paraná State, Brazil, had estimated the genetic diversity of 86.3% within the populations and was estimated the genetic differentiation between populations of Fst = 0.137, which is considered to be a moderate level of divergence (Ruas et al., 2011).

According to Wright (1965), our results suggest that the sampled Brazilian pepper populations present high genetic differentiation. Fst value (0.27) from of between 0.22 and 0.36 are often found, and are obtained for outcrossing species, or for those species classified as pioneers (Nybom, 2004).

The occurrence of exclusive loci in each population contributed to the increased differentiation among the populations (Fst). Although the number of exclusive loci is significant, the species may experience an immediate reduction in exclusive loci, if the genotypes with these loci are removed from the population.

The similarity between the Caatinga and Atlantic Forest populations (86%) was higher than between the Ecotone and Caatinga populations, and between the Ecotone and Atlantic Forest populations (both with 67%), which can be explained by the high number of exclusive loci found in the Ecotone population (Table 1), and the presence of exclusive loci indicates a restricted gene flow (Seoane et al., 2000).

The high frequencies of exclusive loci (up to 30%) indicate the observed migration rates reflect a long-term gene flow, therefore these studied populations could be consider as metapopulations (Astolfi et al., 2012). This fact is also demonstrated

Table 1
Frequency of exclusive loci in the natural habitat of *S. terebinthifolius* in the São Francisco River Basin.

Locus	Areas of natural occurrence			Locus	Areas of natural occurrence		
	Caatinga	Ecotone	Atlantic Forest		Caatinga	Ecotone	Atlantic Forest
Locus014	0.000	0.120	0.000	Locus076	0.433	0.000	0.000
Locus018	0.000	0.060	0.000	Locus088	0.000	0.000	0.277
Locus021	0.000	0.131	0.000	Locus090	0.000	0.000	0.128
Locus031	0.000	0.000	0.383	Locus095	0.000	1.000	0.000
Locus034	0.000	0.000	0.319	Locus104	0.000	0.047	0.000
Locus035	0.000	0.012	0.000	Locus111	0.000	0.000	0.340
Locus038	0.000	0.000	0.681	Locus117	0.000	0.012	0.000
Locus046	0.000	0.435	0.000	Locus120	0.000	0.071	0.000
Locus047	0.000	0.094	0.000	Locus121	0.000	0.282	0.000
Locus052	0.000	0.118	0.000	Locus137	0.000	0.459	0.000
Locus061	0.000	0.000	0.106	Locus142	0.000	0.024	0.000
Locus064	0.000	0.435	0.000	Locus150	0.000	0.000	0.277
Locus065	0.000	0.141	0.000	Locus162	0.000	0.035	0.000
Locus070	0.100	0.000	0.000	Locus176	0.000	0.012	0.000
Locus073	0.000	0.000	0.000	Locus181	0.000	1.000	0.000

by the Fst value (0.14) between the Caatinga and Atlantic Forest populations, which indicates a low subdivision between them, and results in rates of gene flow equal to 4.0. Therefore, the gene flow was approximately four times the rate required to avoid divergence due to genetic drift (Wang, 2004). Our data does not support the hypothesis of the proximity of populations, because the migration of pollen and seeds, is high for the populations that are close with lowest rate of gene flow, while the populations separated by greater distances, present high rates of gene flow.

There was no significant correlation between the genetic distance and the geographic distance (Fig. 2); therefore, there is not a spatial pattern of genetic distance and gene flow. The lack of a spatial pattern indicates that the existence of geographical barriers leads to the absence of correlation between the analyzed parameters (Mohsen and Ali, 2008). This fact was demonstrated through Delaunay triangulation, where the map of genetic distance, θ^B, showed the existence of genetic discontinuity (barriers), separating the studied populations. The first two barriers isolated the three populations (16.5 km); while a third barrier isolated the population located in Alagoas State with the Zé Viana island population (located at a distance of 1.7 km). Therefore, the existing genetic discontinuities are the result of factors beyond the geographical framework. In a study performed to verify the effect of fragmentation on the genetic structure in populations of *Chorisia speciosa*, Souza et al. (2004) observed that the Nei genetic distance, and the geographical distance, were not associated. Souza suggested that the most probable cause for the difference between populations, it is not explained by geographic distance, but by genetic drift. This is similar to the results found herein for *S. terebinthifolius*, We found a significant number of loci with excess heterozygosity, in relation to the expected heterozygosity, which was based on the assumption of the equilibrium between mutation and drift (Cornuet and Luikart, 1996), suggesting a bottleneck. Furthermore, for *Spondias lutea* L., which is located in the same region there were similar results (Gois et al., 2009). The *S. lutea* population presented a tendency toward an excess of heterozygotes relative to the Hardy–Weinberg equilibrium (EHW) ($f = -0.065$).

Although the nine genetic groups identified, by Structure program, is significant, and the alleles homogeneity at each collection point, since the sample areas are on islands located along the river, and each group is essentially restricted to each island, with few representative alleles from between the island groups. The effective population size (N_e) showed a high degree of relatedness between the individuals, demonstrating that the conservation requires special attention. Based on the results obtained from the Mantel test, genetic discontinuities, bottlenecks, and effective size of the populations, it is necessary to delimit the three studied populations as MU, since must be capable of maintaining minimum viable populations, thus avoiding the loss of genetic variability due to drift or endogamy. Therefore, the N_e obtained for the species, a minimum of 300 individuals is necessary in the short-term, and 3000 in the long-term, to conserve these particular species in the study area.

According to Garza and Williamson (2001), if the population experiences a drastic and continuous decline, it may take hundreds of generations to recover. Thus, the current unsustainable exploitation of the Brazilian Pepper Tree is making a long-term recovery unfeasible for the studied populations.

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