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NOTE

Spatial Pattern and Fine-Scale Genetic Structure Indicating Recent Colonization of the Palm *Euterpe edulis* in a Brazilian Atlantic Forest Fragment

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Introduction

Rainforests worldwide are subject to increasing amounts of external pressure and degradation that dissect continuous species distributions into small and isolated fragments (Whitmore 1997). This spatial isolation and reduction of natural populations negatively affects the reproductive success of many tropical plants (Fuchs et al. 2003; Quesada et al. 2004). A reduction in the number of reproductive individuals in a population represents a decrease in the number of pollen/ovary donors and in the quantity of pollen deposited (Aizen and Feinsinger 1994). This may be accompanied by a decrease in the abundance of pollinators and selection for self-compatibility (Stephenson et al. 2000). Subsequently, such impacts can lead to

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genetic bottlenecks, increased random genetic drift, and inbreeding depression, which can ultimately result in a loss of genetic variation (Ellstrand and Elam 1993; Vieira and Carvalho 2008).

In plant populations the spatial distribution pattern is influenced by various ecological and evolutionary processes, such as seed dispersal, intra- and interspecific competition, and environmental heterogeneity, which take place during the life history of a plant (Condit et al. 2000). The degree to which individuals are aggregated affects plant mating systems (Byrne et al. 2007) and also how plant species use resources (Getzin et al. 2008) or can be used as resources (Itoh et al. 2003). In addition to the ecological and evolutionary processes affecting spatial distribution patterns, the fine-scale genetic structure within plant populations can be influenced by limited pollen dispersal, local genetic drift, inbreeding, and selection favoring the same or different genotypes (Heywood 1991; Epperson and Alvarez-Buylla 1997). Studies of fine-scale genetic structure of nonwoody plants from tropical rainforests are still scarce, particularly for palms (Luna et al. 2005; Sezen et al. 2005), despite their substantial contribution to tropical rainforest diversity.

Because information is relatively scarce on the genetic composition of founding populations, the aim of this study is to verify the occurrence of fine-scale genetic structure in a second-growth population of *Euterpe edulis* Mart., an insect-pollinated and bird/mammal-dispersed native palm of the Brazilian Atlantic forest. We hypothesized that patterns of genetic variation should reflect the expectation for a second-growth forest in regeneration, namely, low levels of genetic variation (Aldrich and Hamrick 1998; Sezen et al. 2005). If the population was founded by a reduced number of immigrants, our expectation is of a genetic bottleneck due to recent colonization. Finally, considering that reproductive dominance is common in early successional forests (Sezen et al. 2005), we expected a radial distribution of the density and significant family structure over short distances.

Materials and Methods

The study was carried out in a forest fragment of c. 8.5 ha, located at 21°13′17″S and 44°57′47″W, on the campus of the Federal University of Lavras, southeastern Brazil. The tree flora of this area is characteristic of a tropical montane semideciduous forest. The presence of *Euterpe edulis* in this seasonal tropical fragment is the result of secondary succession, being represented mostly by saplings with the youngest leaf on the exposed stem up to 2-m height. We studied the spatial pattern and genetic structure of all mapped and identified trees of *E. edulis* in this fragment, totaling 29 individuals (Fig. 1).

Leaf samples were cut and crushed with a mortar and pestle in a phosphate-polyvinylpyrrolidone extraction buffer with the addition of β-mercaptoethanol. Discontinuous system vertical allozyme electrophoresis in polyacrylamide gel was performed using 7.5% PAGE gels and carried out at 4°C over 3 h (at 80 mA and 300 V). Five enzyme systems showed banding patterns that could be reliably scored. These enzymes were acid phosphatase (EC 3.1.3.2, loci *Acp*1 and *Acp*2), α-esterase (EC 3.1.1.1, α-*Est*), β-esterase (EC 3.1.1.1, β-*Est*), malate dehydrogenase

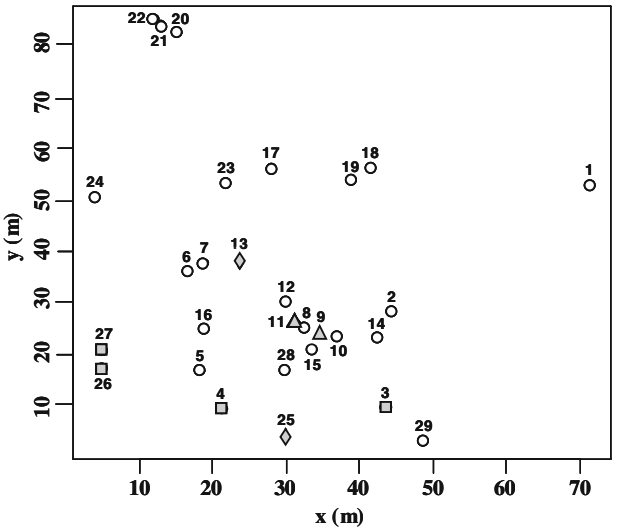

Fig. 1 Spatial distribution of all individuals of *Euterpe edulis* sampled in a fragment of Brazilian Atlantic forest. The individuals are differentiated by Nei's genetic identity: *open circle*, 100%, $n = 21$ (six clusters at the UPGMA); *filled diamond*, 96–99%, $n = 2$; *filled square*, 83–96%, $n = 4$; *filled triangle*, 63–83%, $n = 2$

(EC 1.1.1.37, *Mdh*1, *Mdh*2, and *Mdh*3), and peroxidase (EC 1.11.1.7, *Per*1, *Per*2, and *Per*3). Staining protocols and the genetic basis of allozyme banding patterns were inferred from segregation patterns with reference to typical subunit structure and conceptual methods (Wendel and Weeden 1989).

The following genetic diversity parameters were estimated using the program Popgene 1.31 (Yeh et al. 1999): proportion of polymorphic loci (P_L; 0.95 criterion), mean number of alleles per locus (A), observed heterozygosity (H_o), and Nei's gene diversity (H_e). An estimate of inbreeding levels was obtained using Wright's fixation index ($f = 1 - H_o/H_e$). Significance of f was assessed through estimation of the 95% confidence interval (CI) after 1,000 bootstraps. Fisher's exact tests were used to check if frequencies of homozygotes and heterozygotes per locus deviated from those expected under Hardy–Weinberg equilibrium. Genetic identity among individuals was assessed using Nei (1978) and then analyzed using an unweighted pair group method arithmetic average (UPGMA). The possibility of founder effects due to recent colonization (genetic bottlenecks) was tested using the Bottleneck 1.2.02 program (Cornuet and Luikart 1996). All enzyme loci were assumed to fit an infinite allele model of mutation (IAM), and the significance was assessed using the Wilcoxon signed rank test, based on 1,000 replications.

The neighborhood density function described by Condit et al. (2000) was used to investigate the spatial pattern of individuals. This function is similar to Ripley's K-function, except that it is noncumulative. The 99% CI for the statistic was estimated by performing a Monte Carlo procedure with 499 replicates for $\alpha = 0.01$. These calculations were analyzed with SpPack 1.38 (Perry 2004). Nason's kinship F_{ij} estimator (Loiselle et al. 1995) was used in the analysis of fine-scale genetic

structure. This coefficient can estimate between pairs of mapped individuals, using the *xy* coordinates, a ratio of differences between probabilities of identity by descent between homologous genes (Rousset 2002). The absence of fine-scale genetic structure was tested within each class using 1,000 permutations. These calculations were performed using the program SPAGeDi 1.2g (Hardy and Vekemans 2002).

Results

Genetic variation from 10 polymorphic allozyme loci was surveyed ($P_L = 100.0\%$), and 20 alleles were scored. The average number of alleles per locus was 2.0. High heterozygosities and the absence of allele fixation were observed ($f = -0.265$, $P < 0.05$). The significant negative f value might suggest an excess of heterozygotes. The observed mean heterozygosity and standard error were $H_o = 0.588 \pm 0.039$ and the gene diversity was $H_e = 0.467 \pm 0.013$. Probabilities of the genotype frequency deviation from Hardy–Weinberg equilibrium were calculated for all polymorphic loci, and significant deviations from Hardy–Weinberg expectations were detected for *Acp*1 and *Acp*2. The Wilcoxon sign-rank test for recent population bottlenecks was significant for the population ($P = 0.004$).

The spatial pattern analysis indicates a radial distribution of the density. At distance t (6 m), the measured value of the neighborhood density function was significantly higher than the upper value of the CI, under the hypothesis of complete spatial randomness (Fig. 2). The genetic structure at the 95% confidence level showed a significantly positive coancestry value ($F_{ij} = 0.040$, $P = 0.019$) at 15 m and indicates the tendency of increased structure among trees that are spatially closer to each other (Fig. 2). Conversely, trees that are more distantly spaced showed less genetic similarity.

Discussion

A high proportion of polymorphic loci and gene diversity was observed for *E. edulis* in this Brazilian Atlantic forest fragment. At the species level, high gene diversity has been related to life history and ecological characteristics such as a wide geographic range, primarily outcrossing, and animal-seed dispersal mechanisms (Hamrick and Loveless 1989). Considering that flowers of *E. edulis* are insect pollinated, with protandrous dicogamy (Mantovani and Morellato 2000), the opening of male flowers before female flowers in the same panicles seems to benefit outcrossing. However, high gene diversity does not necessarily mean high genetic diversity. *E. edulis* individuals in this fragment showed high gene diversity (i.e., high heterozygosity), but there were only two alleles at each locus in the whole population. Likewise, we observed that most of the individuals of this population consisted entirely of clones (Fig. 1), derived possibly from one plant with high heterozygosity.

Inbreeding may partly explain the lower within-population genetic variation (Hamrick and Loveless 1989). Considering that this species has a long flowering period and produces one or three flowerings per reproductive cycle, this may

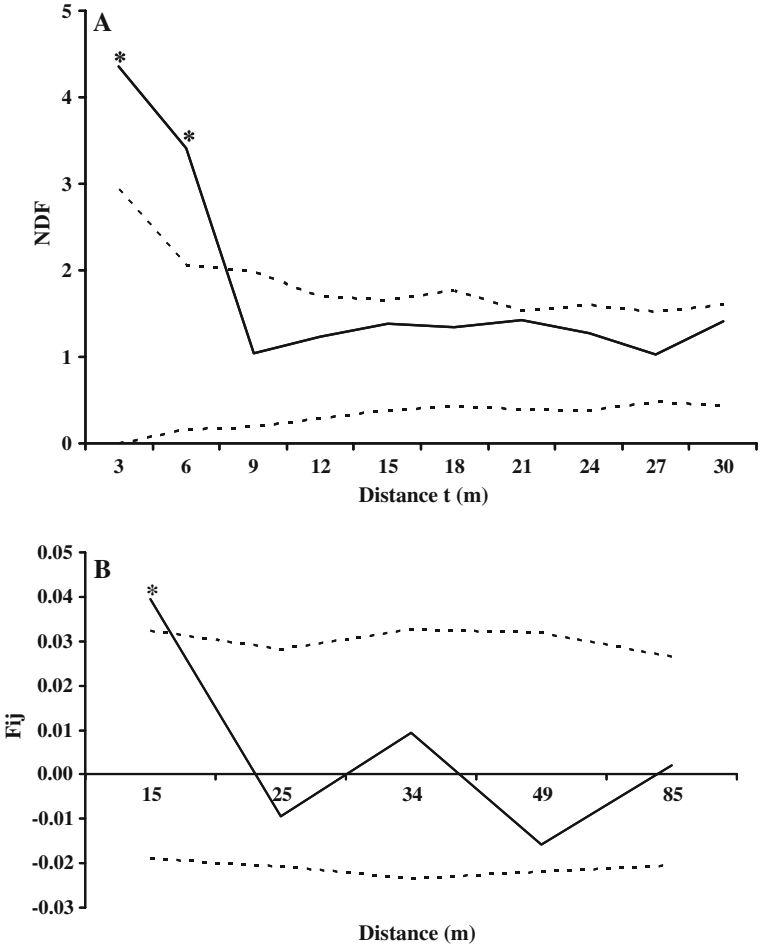

Fig. 2 Spatial pattern analysis (**A**) and estimated coancestry (F_{ij}) (**B**) for *Euterpe edulis* individuals sampled in a fragment of Brazilian Atlantic forest. *Broken lines* correspond to the CI for the null hypothesis of complete spatial randomness of trees or genotypes. *Asterisks* indicate significant levels

increase the possibility of inbreeding by male and female flowers from different inflorescences on the same plant (Reis 1996). This may be explained also by the recent genetic bottleneck detected in this population, which is the result of secondary succession by a reduced number of immigrants. In fact, for second-growth forests, population genetic studies indicate bottlenecks through reproductive dominance, reduced genetic diversity of a founder population, and increased levels of inbreeding (Aldrich and Hamrick 1998; Sezen et al. 2005).

We found a radial distribution of the density, suggesting dispersion distance from a single source. The possible mechanisms of clumping have been discussed mainly by other groups from the viewpoint of seed dispersal (Plotkin et al. 2000) and gap recruitment (Itoh et al. 1997; Plotkin et al. 2000). Indeed, the presence of *E. edulis*

in this fragment is the result of secondary succession. Moreover, the low density of maternal seed sources and, consequently, limited seed dispersal can explain the radial distribution. As a result, nonrandom spatial genetic patterns within a natural population of *E. edulis* were measured in this study. The spatial distribution of the genetic variation within plant populations has been interpreted as a result of evolutionary forces. For example, recent colonization events, limited seed and pollen dispersal, and low density of maternal seed sources can act singly or in concert to create high levels of genetic structure within populations (Jones et al. 2005).

Considering the small size of the sampled population of *E. edulis*, reproductively mature individuals should constitute only a small proportion of the total number of palms. Thus, limited gamete flow may restrict genetic variation due to decreased numbers of mating partners. Restricted gene dispersion (resulting from the limited capacity of pollen migration, as well as seed dispersion at short distances) has been considered the main reason for spatial genetic structuring in plant populations (Latta et al. 1998). Furthermore, considering the small population size and the nonoccurrence of the species near fragments, the gravity-dispersed seeds from maternal palms in the surrounding area can explain such recruitment. Spatial aggregation of related genotypes can also promote biparental inbreeding, leading to a reduced fitness due to inbreeding depression (Stacy 2001). Finally, in a situation where few compatible mates are available, selection for self-compatibility may be strong (Stephenson et al. 2000).

In conclusion, our results show that this population of *E. edulis* presents a relatively low genetic diversity. If these individuals continue to breed only among themselves (no immigration), this would lead to severe inbreeding depression. Thus, the conservation implications for the species are evident in the region of Lavras, since the species has been observed only in this fragment. We suggest that the preservation of the extant population of *E. edulis* through the creation of a nature reserve would be the ideal solution. In addition, we would recommend the implementation of in situ strategies by introducing allochthonous individuals to increase the effective population size and by minimizing adverse effects (e.g., outbreeding depression), to reinforce conservation of the species.

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References

Aizen MA, Feinsinger P (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75(2):330–351

Aldrich PR, Hamrick JL (1998) Reproductive dominance of pasture trees in a fragmented tropical forest mosaic. Science 281:103–105

Byrne M, Elliott CP, Yates C, Coates DJ (2007) Extensive pollen dispersal in a bird-pollinated shrub, *Calothamnus quadrifidus*, in a fragmented landscape. Mol Ecol 16:1303–1314

Condit R, Ashton PS, Baker P, Bunyavejchewin S, Gunatilleke S, Gunatilleke N, Hubbell SP, Losos E, Manokaran N, Sukumar R, Yamakura T (2000) Spatial patterns in the distribution of tropical tree species. Science 288:1414–1418

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Ellstrand N, Elam E (1993) Population genetic consequences of small population size: implications for plant conservation. Ann Rev Ecol Syst 24:217–241

Epperson BK, Alvarez-Buylla E (1997) Spatial autocorrelation analysis of family structure in multiple life stages of *Cecropia obtusifolia*. Evolution 51:275–282

Fuchs EJ, Lobo JA, Quesada M (2003) Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. Cons Biol 17(1):149–157

Getzin S, Wiegand T, Wiegand K, He F (2008) Heterogeneity influences spatial patterns and demographics in forest stands. J Ecol 96:807–820

Hamrick JL, Loveless MD (1989) The genetic structure of tropical tree populations: associations with reproductive biology. In: Bock JH, Linhart YB (eds) The evolutionary ecology of plants. Westview Press, Boulder, pp 129–146

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620

Heywood JS (1991) Spatial analysis of genetic variation in plant populations. Ann Rev Ecol Syst 22:335–355

Itoh A, Yamakura T, Ogino K, Lee HS, Ashton PS (1997) Spatial distribution patterns of two predominant emergent trees in a tropical rainforest in Sarawak, Malaysia. Plant Ecol 132:121–136

Itoh A, Yamakura T, Ohkubo T, Kanzaki M, Palmiotto P, Tan S, Lee HS (2003) Spatially aggregated fruiting in an emergent Bornean tree. J Trop Ecol 19:531–538

Jones FA, Chen J, Weng GJ, Hubbell SP (2005) A genetic evaluation of seed dispersal in the Neotropical tree *Jacaranda copaia* (Bignoniaceae). Am Nat 166:543–555

Latta RG, Linhart YB, Fleck D, Elliot M (1998) Direct and indirect estimates of seed versus pollen movement within a population of ponderosa pine. Evolution 52(1):61–67

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot 82(11):1420–1425

Luna R, Epperson BK, Oyama K (2005) Spatial genetic structure of two sympatric neotropical palms with contrasting life histories. Heredity 95:298–305

Mantovani A, Morellato P (2000) Fenologia da floração, frutificação, mudança foliar e aspectos da biologia floral. Sellowia 49(52):23–38

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 87(3):583–590

Perry GLW (2004) SpPack: spatial point pattern analysis in Excel using Visual Basic for Applications (VBA). Environ Model Softw 19:559–569

Plotkin JB, Potts M, Leslie N, Manokaran N, Lafrankie J, Ashton PS (2000) Species-area curves, spatial aggregation, and habitat specialization in tropical forests. J Theor Biol 207:81–99

Quesada M, Stoner KE, Lobo JA, Herrerías-Diego Y, Palacios-Guevara C, Murguía-Rosas MA, Salazar KAO (2004) Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat pollinated bombacaceous trees. Biotropica 36:131–138

Reis MS (1996) Distribuição e dinâmica da variabilidade genética em populações naturais de palmiteiro (*Euterpe edulis* Martius). PhD Thesis, Universidade de São Paulo

Rousset F (2002) Inbreeding and relatedness coefficients: what do they measure? Heredity 88:371–380

Sezen UU, Chazdon RL, Holsinger KE (2005) Genetic consequences of tropical second-growth forest regeneration. Science 307:11

Stacy EA (2001) Cross-fertility in two tropical tree species: evidence of inbreeding depression within populations and genetic divergence among populations. Am J Bot 88:1041–1051

Stephenson AG, Good SV, Vogler DW (2000) Interrelationships among inbreeding depression, plasticity in the self-incompatibility system, and the breeding system of *Campanula rapunculoides* L. (Campanulaceae). Ann Bot 85:211–219

Vieira FA, Carvalho D (2008) Genetic structure of an insect-pollinated and bird dispersed tropical tree in vegetation fragments and corridors: implications for conservation. Biodivers Conserv 17:2305–2321

Wendel JF, Weeden NF (1989) Visualization and interpretation of plant isozymes. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Dioscorides Press, Portland, pp 5–45

Whitmore TC (1997) Tropical forest disturbance, disappearance, and species loss. In: Laurence WL, Bierregaard RO (eds) Tropical Forest Remnants - Ecology, Management, and Conservation of Fragmented Communities. The University of Chicago Press, Chicago, pp 3–12

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX (1999) POPGENE, the user friendly shareware for population genetic analysis, version 1.31. University of Alberta and Centre for International Forestry Research