Sousa Silva, Mirian de; Almeida Vieira, Fábio de; Carvalho, Dulcinéia de
Diversity and genetic structure in natural populations of Geonoma schottiana Mart (Arecaceae):
implications for conservation
Universidade Federal de Lavras
Lavras, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=74418601006
DIVERSITY AND GENETIC STRUCTURE IN NATURAL POPULATIONS OF Geonoma schottiana Mart (ARECACEAE): IMPLICATIONS FOR CONSERVATION

Mirian de Sousa Silva¹, Fábio de Almeida Vieira², Dulcinéia de Carvalho³

(received: February 10, 2010; accepted: February 25, 2011)

ABSTRACT: Geonoma schottiana is an underbrush palm which is found in high densities in tropical forests. This species is known for having an asynchronous fruit producing pattern, over all seasons of the year, thus being an important food source for frugivores. This work aims to determine the diversity and spatial genetic structure of two natural populations, referred to as MC I and MC II, of which 60 individuals were sampled, in Poço Bonito Biological Reserve, Lavras, Minas Gerais state. Results of 10 polymorphic isozyme loci indicated a high genetic diversity for the species ($\hat{H} = 0.428$ and $H = 0.570$), with an mean number of alleles per locus of 2.0. Estimates of Cockerham’s coancestry coefficients indicated an absence of intrapopulation ($f = -0.343$) and interpopulation inbreeding ($F = -0.161$), suggesting that on average populations are not endogamous. A high genetic divergence was found between populations ($\theta_p = 13.5\%$), in comparison to most tropical species (<5%). Consequently, the estimated historical gene flow was low ($Nm = 0.40$). The analysis of spatial distribution of G. schottiana genotypes in MCI revealed a random distribution of genotypes. The high genetic diversity indices found suggest that the populations in question favor in situ genetic conservation, consequently favoring the conservation of riparian environments.

Key words: Allozymes, spatial genetic structure, genetic conservation.

DIVERSIDADE E ESTRUTURA GENÉTICA EM POPULAÇÕES NATURAIS DE Geonoma schottiana Mart (ARECACEAE): IMPLICAÇÕES PARA A CONSERVAÇÃO

RESUMO: Geonoma schottiana é uma palmeira de sub-bosque com alta densidade em florestas tropicais. A espécie se destaca pela assincronia na produção de frutos durante todas as estações, sendo uma importante fonte de alimento para animais frugívoros. Objetivou-se, com este trabalho, determinar a diversidade e a estrutura genética espacial de duas populações naturais, MC I e MC II, em que foram amostrados 60 indivíduos, na Reserva Biológica do Poço Bonito, em Lavras, Minas Gerais. Os resultados dos 10 locos isoenzimáticos polimórficos indicaram alta diversidade genética para a espécie ($\hat{H} = 0.428$ e $H = 0.570$), com número médio de alelos por loco igual a 2.0. As estimativas dos coeficientes de coancestralidade de Cockerham indicaram ausência de endogamia dentro ($f = -0.343$) e também para o conjunto das populações ($F = -0.161$), sugerindo que, em média, as populações não são endogâmicas. Foi encontrada uma alta divergência genética entre populações ($\theta_p = 13.5\%$), quando comparada com a maioria das espécies tropicais (<5%). Consequentemente, o fluxo gênico histórico estimado foi baixo ($Nm = 0.40$). A análise da distribuição espacial dos genótipos de G. schottiana na MCI revelou que a distribuição dos genótipos é aleatória. Os altos índices de diversidade genética detectados sugerem que as populações estudadas são favoráveis à conservação genética in situ e, consequentemente, dos ambientes ciliares.

Palavras-chave: Aloenzimas, estrutura genética espacial, conservação genética.

1 INTRODUCTION

Tropical biomes are highly fragmented, which leads to a reduction in the size of several ecosystems and thus to local species extinction. The main consequence is evolutionary implications on account of the loss of genetic variability, which reduces the ability of natural populations to adapt to environmental changes (YOUNG; BOYLE, 2000).

Regardless of the various effects of habitat fragmentation, remaining fragments play a crucial ecological role for the local and regional landscape, constituting a potential source of propagules and habitats for dispersers and pollinators. Preserved vegetation patches can act as corridors for fauna species and for dispersion of flora species, thus securing biological diversity and gene flow among populations (TEWKSBURY et al., 2002; VIEIRA; CARVALHO, 2008).
Inserted in this context, in the Alto Rio Grande region, south Minas Gerais state, is the original vegetation cover which once consisted of miscellaneous patches of forest, savannah, high altitude grassland and rocky grassland but today is reduced to fragments of primitive vegetation (Seasonal Semideciduous Forest), most of which subjected to serious disturbances from fire, extensive cattle raising or selective timber removal (OLIVEIRA-FILHO; MACHADO, 1993).

*Geonoma schottiana* Mart. (Arecaceae) is an underbrush palm species found in high densities in tropical forests. It has a well defined supporting stalk, although it may shoot out to form a thicket structure, having a frequently twisted stipe, between 1.6 m and 6 m in length (HENDERSON et al., 1995). This species is known for its extended fruiting periods throughout virtually every month of the year, revealing its potential importance for the conservation of fruit eating birds (SAMPAIO; SCARIOT, 2008). Characteristics relating to the biometry of *G. schottiana* fruits are reported by Silva et al. (2007), who reinforce the importance of this species as a food source for the fauna.

However, research studies are required with several arboreal species and ecological conditions in order to provide information to fully implement management and genetic conservation strategies. A very peculiar ecological condition is provided by riparian areas, as they fulfill important functions that include not only maintaining water quality and quantity but also contributing to biodiversity conservation by acting as ecological corridors for many species.

Therefore, investigating the levels of genetic variability and their distribution among and within natural populations, and also gene flow, is critical to fully implement any genetic conservation program. Bearing in mind ecology concepts and population genetics, allozyme markers have been used as a tool to obtain information and evaluate genetic variability in natural plant populations (PINTO et al., 2004; VIEIRA; CARVALHO, 2008; VIEIRA et al., 2010).

This work aims to evaluate and understand the patterns of inter- and intrapopulation genetic diversity, in order to determine the gene flow and study the spatial distribution of genotypes in two populations of *Geonoma schottiana*. The reason for selecting this species for the genetic studies of this work is because it is considered to be a key species in plant communities and because it is abundant in riparian environments.

## 2 MATERIAL AND METHODS

### 2.1 Study site and sampling

Individuals were sampled in Quedas do Rio Bonito forest park (PFQRB), which is located south of the municipality of Lavras - MG (21°19’ South and 44°59’ West), at an altitude that ranges between 950 and 1,200 m. The region is a transition zone between the savannas of Central Brazil and semideciduous forests of Southeast and South Brazil (OLIVEIRA-FILHO; FLUMINHAN FILHO, 1999). The local climate, according to Köppen classification, is transitional between Cwb and Cwa, in other words, temperate with dry winters, with average annual precipitation of 1,529.7 mm and average annual temperature of 19.4°C. The PFQRB park constitutes a valuable sample of existing primitive vegetation in the Alto Rio Grande region, as its five main vegetation types – forest, savannah, high altitude grassland, rocky grassland and candeal – are well represented and reasonably preserved (OLIVEIRA-FILHO; FLUMINHAN FILHO, 1999).

Two populations of *G. schottiana* were analyzed adjacent to a body of water, which is abundant due to the convergence of the drainage basin. The sampled *G. schottiana* individuals are thus situated in the riparian zone of Córrego dos Vilas Boas which, according to IBGE’s Brazilian vegetation classification, is referred to as Alluvial Semideciduous Seasonal Forest with an emerging canopy (VELOSO et al., 1991). The populations are referred to as MC I and MC II. Unlike MC I, which boasts beaten paths along the body of water, MC II is in better conservation conditions. The relevant populations are approximately 660.0 m apart, at varying altitudes (Table 1).

### Table 1 – Coordinates, altitude and total patch sampled of *Geonoma schottiana* populations.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (m)*</th>
<th>Patch (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC I</td>
<td>21°19’53”</td>
<td>44°59’09”</td>
<td>1,011 to 1,045</td>
<td>138</td>
</tr>
<tr>
<td>MC II</td>
<td>21°19’56”</td>
<td>44°58’34”</td>
<td>1,131 to 1,145</td>
<td>391</td>
</tr>
</tbody>
</table>

*Altitude at actual sampling location.

Leaf tissues were collected from 30 reproductive individuals in each population, as evidenced by the presence of inflorescences. MC I individuals were randomly collected along the existing trails that ran parallel to the body of water. MC II individuals were
Diversity and genetic structure in natural populations ...

randomly collected along the body of water but in an area of compact canopy and difficult access, which prevented plant mapping. Individuals sampled in MC I were georeferenced using a GPS, for a subsequent spatial autocorrelation analysis. The leaf samples were taken to the Laboratory of Genetic Conservation of Arboreal Species of the Department of Forest Sciences/UFLA, where they were deep frozen at -80°C, so as to prevent enzyme degradation.

2.2 Allozyme extraction and electrophoresis

For enzyme extraction, 200 mg of leaf tissue were used per 1 mL of buffer solution (buffer no.1) (ALFENAS et al., 1998). The extracts were then centrifuged at 12,000 rpm at 4°C for 10 minutes. After centrifugation, 35 µL of the supernatant were applied to the gels in a vertical electrophoresis system, as conducted in a polyacrylamide gel supporting medium, using a 4% concentration gel and a 12.5% separation gel. For the electrophoresis run, an amperage of 10 mA per gel was used, and 300 Volts, set to run for about three and a half hours, at 4°C. Ten enzyme systems were selected as a function of the good resolution of the band patterns: alcohol dehydrogenase (ADH), α-esterase (α-EST), β-esterase (β-EST), acid phosphatase (ACP), glucose dehydrogenase (GDH), glutamate dehydrogenase (GTDH), glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH), sorbitol dehydrogenase (SDH), and shikimate dehydrogenase (SKDH). The zymograms were interpreted according to the enzyme pattern definition available in literature (ALFENAS et al., 1998).

2.3 Statistical analyses

The interpretation of zymograms enabled determination of the genotypes of each individual and estimation of several genetic parameters. Allele frequencies were estimated by a direct count of the number of alleles per locus, divided by the total number of alleles in the locus: \( \hat{P}_i = \frac{n_i}{n} \), where \( \hat{P}_i \) is the frequency of allele \( i \), \( n_i \) is the number of occurrence of allele \( i \) and \( n \) is the total number of alleles sampled. Based on the allele frequencies, genetic diversity indices were estimated: percentage of polymorphic loci \( (P_s) \), criterion 0.95), mean number of alleles per locus \( (A) \), mean observed heterozygosity \( (\hat{H}_o) \), mean expected heterozygosity \( (\hat{H}_e) \) and Wright’s fixation indices \( (f) \), using program BIOSYS-2 (SWOFFORD; SELANDER, 1997).

The genetic structure was assessed based on Cockerham’s coancestry coefficients (COCKERHAM, 1969). Parameters being estimated included: \( \hat{f}_i \), mean fixation index within populations; \( \hat{F} \), fixation index between populations; and \( \theta_p \) genetic divergence between populations. These parameters correspond to the estimates of Wright’s \( F_{IS} \), \( F_{IT} \) and \( F_{ST} \) respectively. The analysis of variance was run using program GDA (LEWIS; ZAYKIN, 2000). Estimates of historical gene flow between populations were obtained using the equation proposed by Crow and Aoki (1984): \( \hat{Nm} = \left[ (1/ \hat{F}_{ST}) - 1 \right] / 4\alpha \), where: \( \alpha = [n / (n - 1)]^2 \), \( \hat{Nm} \) being the number of migrants and \( n \), the number of populations. The neighborhood size was estimated by \( \hat{N}_b = 2\pi . \hat{Nm} \) (SLATKIN; BARTON, 1989).

For analysis of the spatial genetic structure (EGE) of G. schottiana, in MC I, distance class intervals among individuals were determined by testing the rules for each interval, as suggested by Hardy and Vekemans (2002). As for MC II, the spatial genetic structure was not estimated on account of the difficulty in mapping the individuals of this population. The multilocus coancestry coefficient \( (F_p) \) according to Loiselle et al. (1995) was estimated among plants for each of the distance classes using program SPAGeDi version 1.2g (HARDY; VEKEMANS, 2002). This coefficient estimates the probability of identity of alleles of two homologous genes as sampled in two individuals. The EGE intensity was calculated by \( Sp = -b_{log} \) \( (1 - F_{log}) \), where \( b_{log} \) is the slope of the regression curve of coefficient \( F \) on the logarithm of distance and \( F_{log} \) is the mean coancestry coefficient between individuals in the first distance class. When \( b_{log} = 0 \) the null hypothesis of random EGE is accepted. The standard error of estimated mean was obtained using jackknife resampling among loci, then confidence intervals at the 95% probability level were constructed of the estimated mean coancestry coefficient for each distance class. Absence of spatial genetic structure was tested within each distance class using 1,000 permutations.

3 RESULTS AND DISCUSSION

3.1 Allele frequencies

Frequencies were obtained of 20 alleles distributed in 10 loci for the two populations (Table 2). For individuals sampled in MC I and II, the highest allele frequencies were found in the second allele, in 50% and 90% of the loci respectively. In MC I, 20% of the loci showed the same
allele frequency, while in MC II only the GTDH allozyme system showed the same allele frequencies. Considering that loci showing genic similarity (low variation range) will present allele frequencies between 0.350 and 0.650 (FRANKEL et al., 1995), it was noted that for MC I and MC II this proportion was 60% and 80% respectively. According to Frankel et al. (1995), higher similarity in allele frequencies for a population is an indicator of higher genetic diversity and less susceptibility to the effects of genetic drift, in comparison to dissimilar allele frequencies. These high values of heterozygosity suggest the existence of selective mechanisms acting in favor of heterozygotes. Similar results were found in other works, including the work of Hamrick et al. (1993) and Reis (1996).

### 3.2 Intrapopulation genetic variability

The number of alleles per locus (\(\hat{A}\)) was equal to 2.0 (Table 3), similar to results found for several palm species (EGUIARTE et al., 1992; VIEIRA et al., 2010). The percentage of polymorphic loci (\(P\)) was 100% for both populations. The observed heterozygosity values were higher than the expected, revealing a tendency toward excess heterozygotes in relation to the Hardy-Weinberg equilibrium. The high genetic diversity detected can be explained by an absence of rare alleles and allele frequencies in similarity. These high values of heterozygosity suggest the existence of selective mechanisms acting in favor of heterozygotes. Similar results were found in other works, including the work of Hamrick et al. (1993) and Reis (1996).

### 3.3 Genetic structure

Estimates of Cockerham’s coancestry coefficients indicated absence of intrapopulation (\(f = -0.343\)) and interpopulation inbreeding (\(F = -0.161\)), suggesting that on average the populations are not endogamous (Table 4). The estimate of genetic differentiation (\(\theta_s\)) between populations indicated that 13.5% of the genetic variability is among populations. This estimate is high if compared to most tropical species (< 5%). This is probably due to the reproductive isolation of the populations in question. Considering the neutral nature of the allozyme marker, the divergence between the populations could be the result of a structured spatial distribution of the genotypes; the effect, for instance, of a short or locally restricted seed dispersion or even of formation of thickets and possibility of self-fertilization. This would possibly explain the high genetic divergence found, considering that the measure being used (\(\hat{\theta}_s\)) calculates the difference among populations as a function of the mean coancestry within populations. Thus, a mean coancestry of 0.135 indicates that within groups (subpopulations) the coancestry was within the expected range in half sibs (0.125) and full sibs (0.25).

The number of migrants per generation (\(\hat{N}_m\)) was estimated at 0.40, indicating low historical gene flow. The neighborhood size (\(N_h\)) was 2.51 individuals, indicating that the number of parent individuals randomly exchanging alleles may be in the range of 2 to 3. These values can be considered relatively low if compared to results obtained by Reis (1996) for Euterpe edulis: \(\hat{N}_m = 10.7\) and \(N_h = 67\). Several factors may affect gene flow in natural populations, including the reproductive system of the relevant species and ecological relations between plants and their pollinators and dispersers (DICK et al., 2003).

### Table 2 – Allele frequencies in 10 isozyme loci in two populations of *G. schottiana*.

<table>
<thead>
<tr>
<th>Loci</th>
<th>Allele</th>
<th>Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MC I</td>
</tr>
<tr>
<td>ADH</td>
<td>1</td>
<td>0.446</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.554</td>
</tr>
<tr>
<td>α-EST</td>
<td>1</td>
<td>0.589</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.411</td>
</tr>
<tr>
<td>β-EST</td>
<td>1</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.550</td>
</tr>
<tr>
<td>ACP</td>
<td>1</td>
<td>0.609</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.391</td>
</tr>
<tr>
<td>GDH</td>
<td>1</td>
<td>0.060</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.940</td>
</tr>
<tr>
<td>GTDH</td>
<td>1</td>
<td>0.269</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.731</td>
</tr>
<tr>
<td>GOT</td>
<td>1</td>
<td>0.500</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.500</td>
</tr>
<tr>
<td>MDH</td>
<td>1</td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.759</td>
</tr>
<tr>
<td>SDH</td>
<td>1</td>
<td>0.500</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.500</td>
</tr>
<tr>
<td>SKDH</td>
<td>1</td>
<td>0.867</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.133</td>
</tr>
</tbody>
</table>
According to Wright (1951), when the estimated gene flow is less than one ($\hat{N}m < 1$), the effects of migration are insufficient to counteract the effects of drift, thus favoring divergence between populations. It should be noted that the calculated gene flow value based on genetic divergence reflects flow over a long time span, in the past. The estimate, rather than indicating occurrence of gene flow in a given reproductive event, it calculates the levels that may have occurred to produce the observed patterns of genetic structure (SMOUSE; SORK, 2004). Therefore, the current gene flow between the two populations could be even lower. In addition, it could be implicated by anthropic intervention via trail opening, as is the case with MC I. This could be repelling local fauna species, which constitute the main dispersers of this plant species. An analysis of open pollinated progenies may help understand the gene flow pattern as a function of the local anthropization process.

### 3.4 Spatial genetic structure

The analysis of spatial distribution of genotypes revealed absence of a genetic structure in MC I, in other words, a random distribution of *G. schottiana* genotypes in all distance classes (Figure 1). The values of coancestry coefficients ($\hat{F}_{ij}$) for the relevant population were close to zero or negative and nonsignificant. This suggests that spatially close individuals of *G. schottiana* are not any more related than more distanced individuals. The null hypothesis of a random distribution of the genetic structure ($\theta = 0$) was rejected in the relevant population, with the slope value of the regression curve being approximately $-0.006$ ($P < 0.001$), nonsignificant. The $Sp$ value of 0.006 revealed a weak and nonsignificant spatial genetic structure for the population, as was expected, since the values of coancestry coefficients were close to zero.
The absence of spatial structure observed indicates that local selection and, or, local genetic drift are weak enough not to produce a significant structure in the presence of gene flow (DOLIGEZ; JOLY, 1997). However, it should be noted that the lack of spatial genetic structure may well be a sampling artifact. The sample was small (30 plants per population) and randomly collected, therefore closely related individuals were not always sampled and that reduced the chance of detecting spatial genetic structure. As suggested by Cavers et al. (2005), ideally one should use samples of at least 150 genotypes, from within a well defined area, such as a square plot.

4 CONCLUSIONS

The diversity detected was high in relation to the Hardy-Weinberg equilibrium, indicating absence of inbreeding. These populations were found to be potential tolls for in situ genetic conservation and forest management.

Most of the genetic variability of the species being studied occurs within natural populations.

The gene flow found between the populations was low, characterizing a high genetic divergence between them.

The analysis of spatial distribution of genotypes revealed absence of genetic structure in MC I.

Studies concerning the biology of the relevant species and correlations with biotic and abiotic factors are important, because only by accumulating information will more sustainable strategies be suggested for management, understanding of the dynamics and also development of conservation policies.

5 ACKNOWLEDGMENTS

The authors wish to thank CNPq for granting the scholarship and financing the research project, and Parque Florestal Quedas do Rio Bonito for making access available to plant populations and for supporting field work.

6 REFERENCES


Diversity and genetic structure in natural populations...


*Cerne, Lavras, v. 17, n. 2, p. 195-201, abr./jun. 2011*