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Spatial genetic structure in natural populations of the overexploited tree *Eremanthus erythropappus* (DC.) macleish (Asteraceae)

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ABSTRACT

Management and genetic conservation plans require knowledge of spatial genetic structure (SGS) to ensure the long-term maintenance of genetic variability in natural populations. This study uses spatial statistical analyses to assess the SGS in nine locations with diverse landscape characteristics where *Eremanthus erythropappus* occurs at varying densities. This species, commonly known as candeia, is widely distributed throughout Minas Gerais State, Brazil, and its wood has high economic value due to its natural durability and production of oil containing the active ingredient alpha-bisabolol. The species has undergone intense exploitation without adequate management planning. Our analyses were based on polymorphism at nine inter-simple sequence repeat (ISSR) loci. We observed SGS in five of the nine populations. The data indicate different degrees of SGS in the populations, which supports the premise that conservation plans and seed collection strategies should be informed by genetics studies.

1. Introduction

Ongoing reckless exploitation of natural resources is a critical threat to the conservation of natural tree populations. Uncontrolled logging of tree species of interest may result in changes to the genetic structure of populations with significant consequences, such as restricted gene flow and increased crossing between related individuals (Lowe et al., 2005). Spatial genetic structure (SGS) is defined as the non-random distribution of genetically similar individuals within populations (McCauley, 1997). In plants, SGS is influenced by clonal reproduction, mating systems, patterns of seed dispersal and establishment, population density of established plants (Hamrick and Nason, 1996), and microhabitat selection. Patterns of seed dispersal and establishment tend to have the greatest impact on the spatial distribution of genetic diversity within plant populations (Nason and Hamrick, 1997) and can influence the demography and spatial distribution of individuals of subsequent generations. Understanding the SGS of a species is important for defining sustainable forest management and sampling

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strategies (seed collection) in natural populations for genetic conservation or restoration, as it affects the minimum distance necessary between sampled individuals to ensure genetic variability (Cloutier et al., 2007; Melo Junior et al., 2015). Fine-scale SGS is defined as the spatial distribution of genotypes within a given population that results from processes such as selection, demographic disturbances, and environmental heterogeneity. The existence of SGS originating from restricted gene flow by seeds (Vekemans and Hardy, 2004) may be the most likely cause of fine-scale interpopulation SGS, which may affect estimates of genetic diversity (Doligez and Joly, 1997). The tree species *Eremanthus erythropappus* is subject to such effects and its populations have been exploited for decades for various commercial purposes, such as timber production for coal and chemical extraction.

Eremanthus erythropappus (DC.) MacLeish, commonly known as candeia, is a neotropical tree species that belongs to the Asteraceae family. The species is pollinated by bees, particularly *Apis mellifera* and *Trigona* sp (Vieira et al., 2010), and its seeds are dispersed by anemochory. The tree species occurs as large populations, called "candeais", in open fields and pastures in the Midwest and Southeast of Brazil (Loeuille, 2010). In Minas Gerais State, the species is managed mainly for the extraction of the essential oil alpha-bisabolol, which is widely used for cosmetic and pharmaceutical purposes. Lack of knowledge about the patterns of genetic structure of natural *E. erythropappus* populations endangers the conservation of the species over generations.

In situ and *ex situ* conservation practices rely on knowledge of ecological and genetic patterns, and understanding patterns of SGS is important for improving these strategies. In this context, the use of molecular markers has brought about significant advances in tree species population genetics studies, especially in describing the organization of genetic variability in natural plant populations (Hamrick and Loveless, 1986). While ecological and genetic information on tropical tree species are scarce, studies on SGS in neotropical tree species have shown a structured distribution of alleles and genotypes within populations (Brandão et al., 2011; Melo Junior et al., 2012, 2015).

Thus, our aim in this study was to evaluate the fine-scale spatial genetic structure in natural *E. erythropappus* populations. We seek to improve the efficiency of strategies for sustainable management and conservation of the species by demonstrating the minimum required distance between trees for seed collection for genetic conservation programs.

2. Materials and methods

2.1. Sampling sites

Individual *E. erythropappus* trees were sampled from nine locations in Minas Gerais State where the species occurs (Table 1). Leaves from 20 reproductive trees were collected at each study site, totaling 180 samples. All sampled individuals were mapped using GPS, and their leaves were collected and conserved for further analyses. All sampled populations are located in preservation areas. To avoid sampling from related trees, leaves were collected systematically from trees located between 50 and 100 m (m) apart in the Santa Rita do Sapucaí (SR), Baependi (BA), São Tomé das Letras (ST), Luminárias (LU), Carrancas (CA), Ingaí (IN), and Diamantina (DI) populations. In the Delfim Moreira (DM) and Francisco Sá (FS) populations, samples were collected randomly, without a minimum distance between individuals, due to the low density of individuals and the small fragment size.

2.2. Extraction and amplification of genomic DNA

Genomic DNA was extracted according to the CTAB protocol (Doyle and Doyle, 1990). A total of 20 inter-simple sequence repeat (ISSR) primers were tested, and the bands amplified from nine primers showed good resolution (Table 2). DNA amplification reactions were prepared on PCR microplates by applying an aliquot containing approximately 5 ng/μL of DNA from each individual. DNA samples were added to the reaction mixture containing 10X PCR buffer (500 mM Tris–HCl, pH 8.0, 200 mM KCl, 2.5 ng/mL BSA, 200 mM tartrazine, and 1% Ficoll), 1.2 μL of dNTPs + $MgCl_2$ (2.5 mM dNTPs and 25 mM $MgCl_2$), 5 U/μL of Taq polymerase, and 0.4 μM ISSR primer. PCR reactions were performed in a GeneAmp PCR System 9700 thermal cycler. The samples underwent an initial denaturation at 94 °C for two minutes, followed by 37 amplification cycles. Each

Table 1
Location, population code, and geographic coordinates of the sampled populations of *Eremanthus erythropappus*.

Location	Population code	Latitude (S)	Longitude (W)	Altitude (m)
Delfim Moreira	DM	22°18′ 26.51250″	45°10′ 40.04240″	1200
Santa Rita do Sapucaí	SR	22°11′ 17.54073″	45°35′ 31.18036″	841
Baependi	BA	21°58′ 31.34129″	44°46′ 09.38253″	895
São Tomé das Letras	ST	21°42′ 13.14629″	44°59′ 16.21553″	1314
Luminárias	LU	21°31′ 52.20951″	44°48′ 19.61931″	1160
Carrancas	CA	21°27′ 01.46192″	44°39′ 33.84086″	1139
Ingaí	IN	21°26′ 12.09065″	44°58′ 24.73977″	981
Diamantina	DI	18°12′ 03.80000″	46°24′ 53.49999″	1293
Francisco Sá	FS	16°28′ 25.68000″	43°24′ 43.2602″	658