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FORESTRY SCIENCE

Variability and genetic structure in fragments of *Eugenia involucrata* De Candolle established through microsatellite markers

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ABSCTRACT: *Eugenia involucrata DC. is a forest species with high environmental and economic potential. The objective of this study was to quantify the genetic variability and analyzed the genetic structure of three natural fragments located in the central region of the Rio Grande do Sul state, Brazil. We used four microsatellite loci developed for the congener species Eugenia uniflora and using GenAlEx 6.5 software, parameters of genetic variability and its partition among and within fragments were estimated for each locus. We observed high levels of genetic variability (3.67 alleles per locus; H_O = 0.815; H_E = 0.625; F_{IS} = −0.294), most of which (93%) were distributed within the fragments, suggesting that these individuals came from a single original population. Gene flow between fragments was high (2.35 to 4.56 migrants per generation), resulting in low genetic differentiation indexes (F_{ST} values ranging from 0.052 to 0.096). The fragments showed high genetic variability, distributed within the remnants themselves, and low genetic differentiation. Our results have repercussions for planning locally adapted germplasm collections for forest restoration programs, thereby avoiding the implantation of populations with an exogamous depression.*
Key words: *forest species, gene flow, population genetics.*

Variabilidade e estruturação genética em fragmentos de *Eugenia involucrata* de Candolle utilizando marcadores microssatélites

RESUMO: *Eugenia involucrata DC. é uma espécie florestal com elevado potencial ambiental e econômico. O presente trabalho teve por objetivo quantificar a variabilidade e analisar a estruturação genética em três fragmentos naturais localizados na região central do estado do Rio Grande do Sul, Brasil. Com o emprego de quatro locos microssatélites desenvolvidos para a espécie congênere Eugenia uniflora e usando-se o software GenAlEx 6.5, foram estimados parâmetros de variabilidade genética, para cada loco, e sua partição entre e dentro dos fragmentos. Foram observados altos índices de variabilidade genética (3,67 alelos por loco; H_O = 0,815; H_E = 0,625; F_{IS} = -0,294), com a maior proporção (93%) distribuída dentro dos fragmentos, sugerindo que os indivíduos estudados são provenientes de uma única população original. O fluxo gênico foi elevado entre os fragmentos, variando de 2,35 a 4,56 migrantes por geração, resultando em baixo índice de diferenciação genética (F_{ST}) entre 0,052 a 0,096. Os fragmentos estudados apresentam elevada variabilidade genética, a maior parte distribuída dentro dos próprios remanescentes, e baixa diferenciação genética. Os resultados observados têm repercussões no planejamento de coleta de germoplasma com adaptação local para programas de restauração florestal, assim evitando a implantação de populações com depressão exogâmica.*
Palavras-chave: *espécie florestal, fluxo gênico, genética de populações.*

Eugenia involucrata (Myrtaceae) is a tree species native to Brazil and is classified as autogamous. It reaches reproductive age between 6 and 7 years of age, being pollinated by bees (*Apis mellifera*) and dispersed by different birds. Its flowers are melliferous and can be used in beekeeping. Its fruits are used in animal and human food and can be consumed fresh or used in the preparation of sweets, jellies and liquors (CARVALHO, 2009; LISBÔA et al., 2011). The wood is compact, elastic, highly resistant, and highly durable. It can be used in civil construction to make tool handles, slats, and agricultural tools, as well as general use as firewood and charcoal of excellent quality (CARVALHO, 2009).

Its natural occurrence in Brazil occurs in the Atlantic Forest biome, more specifically in the ecosystems of Dense Ombrophilous, Semideciduous Seasonal, and Mixed Ombrophilous forests. It can be found in the states of Rio de Janeiro, São Paulo, Minas Gerais, Paraná, Santa Catarina and Rio Grande do Sul (LISBÔA et al., 2011).

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However, the perpetuation of this important forest resource is threatened because of the growing fragmentation that the biome suffered, primarily during the last 60 years, by anthropic activities, such as logging, construction of cities and highways, agricultural practices, and pollution. This conversion of a continuous area into small fragments leads to the spatial isolation of individuals and under these circumstances the performance of genetic processes, such as inbreeding and drift, can affect the viability of natural plant populations, leading to their extinction (CARVALHO, 2009; MATESANZ et al., 2017).

Studies related to the quantification of genetic variability and analyses of the genetic structure of their natural populations using DNA markers can provide subsidies for the conservation of forest resources, providing the appropriate selection of management strategies (CARVALHO, 2009; CONSON et al., 2013). In this study, microsatellite primers developed for the congener species *Eugenia uniflora* were used to quantify the variability and analyze the genetic structure in three natural fragments of *E. involucrata* located in the central region of the state of Rio Grande do Sul, Brazil.

Leaf samples were collected from 24 adult individuals from the three fragments, located in the municipalities of Quevedos (29°21′09″S; 54°04′18″W), Santa Maria (29°41′03″S; 53°4825″W), and Silveira Martins (29°38′33″S; 53°35′08″W). According to reports by residents in nearby places (data not shown), individual trees from Quevedos were approximately 100 years old, whereas those from Santa Maria and Silveira Martins, were approximately 60 years old. Genomic DNA was obtained using the CTAB protocol (Doyle and Doyle, 1990). Working solutions at 50 ng μL^{-1} were amplified via polymerase chain reaction (PCR), and 11 microsatellite loci developed by SARZI et al. (2019) were initially evaluated (Pit 26, Pit 32, Pit 34, Pit 38, Pit 48, Pit 53, Pit 57, Pit 64, Pit 66, Pit 71, and Pit 72).

The amplification reactions were performed in a BIO-RAD C1000 Touch® thermocycler (BioRad Co., Hercules, CA, USA) in a final volume of 12.5 µL, which were prepared using Eppendorf epMotion® 5070 (Eppendorf AG, Hamburg, Germany), containing 50 ng of DNA, 0.25 µM of buffer, 0.5 µM of $MgCl_2$, 1 U of Taq DNA polymerase, 0.05 µM of each dNTP, 0.125 µM of forward primer, 0.125 µM of reverse primer, and 0.125 µM of the M-13 primer (50-TGTAAAACGACGGCCAGT-30) labeled with AlexaFluor 680 fluorescence (Invitrogen®). PCR reactions consisted of an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C (Pit 26, 34, 48, and 57) or 52 °C (the other primers) for 30 s, an extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. Subsequently, the fragments were separated by electrophoresis on a 6% (w/v) polyacrylamide gel using a Li-Cor 4300S® automatic DNA sequencer (LiCor Inc., Lincoln, NB, USA) and were analyzed using SAGA-GT Software® (LiCor Inc.). Population genetic parameters were estimated using GenAlEx 6.5 software (PEAKALL & SMOUSE, 2012). For each locus, the total number of alleles (*A*), observed heterozygosity (H_O), expected heterozygosity (H_E), population fixation index (F_{IS}), genetic differentiation index (F_{ST}), and Hardy–Weinberg equilibrium (HWE) were tested. To quantify the distribution of genetic variability between and within fragments, an analysis of molecular variance (AMOVA) was performed. Gene flow was also estimated for each pair of fragments, considered to be the number of migrants by generation (*Nm*), based on the equation:

$Nm = \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right)$ where *Nm* is the number of migrants per generation and F_{ST} is the index of genetic differentiation (WRIGHT, 1949). Of the tested loci, only four (Pit 26, Pit 57, Pit 64 and Pit 66) were amplified in all studied individuals, indicating a low transferability rate between the two species. Polymorphism was observed, with Pit 57 producing the highest number of alleles (5) in Quevedos, whereas Pit 26, Pit 57, and Pit 66 in Santa Maria, and Pit 64 and Pit 66 in Silveira Martins exhibited the lowest number (three alleles). Similarly, 3.5 alleles per locus were recorded in *E. brasiliensis*, whereas higher numbers were detected in other similar species: eight in *E. uniflora*, 7.9 in *E. piriformis*, and 8.3 in *E. francavilleana* (FERREIRA-RAMOS et al., 2014). Quevedos, which in general presented the highest levels of genetic diversity (A = 4.250; H_O = 0.850, H_E = 0.690; Table 1), also indicated the presence of exclusive alleles (Table 1). Values close to diversity indices were observed in *E. uniflora* in the Pampa biome, also with microsatellites (SARZI et al., 2019).

The largest proportion of genetic variability was distributed within (93%) the studied remnants, suggesting that the individuals studied came from a single original population. This result also indicated that crosses in this population would be predominant, which is contrary to the classification of the species as autogamous (CARVALHO, 2009). Thus, the fragments studied in the present work were classified as mixed with a predominance of crossings (DESTRO & MONTALVÁN, 1999). Corroborating this result, Golle (2010) recorded greater variability within five populations of *E. involucrata*.

Table 1 - Estimation of genetic parameters by microsatellite locus and fragment of *Eugenia involucrata*. *A* is the average number of alleles per locus per fragment; H_O is the heterozygosity observed, H_E is the heterozygosity expected, F_{IS} is the Fixation Index, *n* is the number of individuals analyzed, *At* is the total number of alleles, and A_{excl} is the number of unique alleles.

Locus	*A*	H_O	H_E	F_{IS}
Pit 26	3.67	0.933	0.667	-0.408 ns
Pit 57	4.00	1	0.667	-0.510 ns
Pit 64	3.67	0.475	0.564	0.182 *
Pit 66	3.33	0.850	0.604	-0.440 ns
Média	3.67	0.815	0.625	-0.294
Parameters	----------Fragment----------			
	Quevedos		Santa Maria	Silveira Martins
n	10		6	8
A_t	17		13	14
A	4.25		3.25	3.5
A_{excl}	0.5		0	0
H_O	0.850		0.875	0.719
H_E	0.690		0.580	0.605
F_{IS}	-0.232		-0.509	-0.188

ns = Hardy–Weinberg equilibrium (P>0.05); * = absence of Hardy-Weinberg equilibrium (P<0.05).

The analyzed loci revealed average observed heterozygosity (H_O) that did not differ significantly from the expected heterozygosity (H_E), with values of 0.815 and 0.625 (Table 1), respectively, indicating that they were in HWE. As a result, the fixation index did not differ from zero (F_{IS} = −0.294; Table 1). This behavior, observed in three of the four loci being studied, was the opposite of that expected in autogamous population and even more so in populations in the process of fragmentation, suggesting that the time in which fragmentation occurred has not been long enough to promote genetic differentiation between fragments. Pit 64 was an exception, in that its heterozygosity estimates indicated a deficiency (H_O = 0.475 versus H_E = 0.564; Table 1), demonstrating that this locus was not in HWE, which suggested the existence of some evolutionary factor.

Gene flow estimates were high (2.35 for Quevedos and Santa Maria, 3.85 for Quevedos and Silveira Martins, and 4.56 for Silveira Martins and Santa Maria), and subsequently, resulted in low F_{ST} values, ranging from 0.052 (Silveira Martins and Santa Maria) to 0.096 (Quevedos and Santa Maria). Among populations of the same species in the South, Southeast, and Northeast regions of Brazil, moderate genetic differentiation was obtained (F_{ST} = 0.211) (SALGUEIRO et al., 2004). Our research revealed high genetic variability in the studied fragments, a greater proportion of which was distributed within the fragments as a result of crosses between individuals. There was also high gene flow, and consequently, low genetic differentiation among the remnants, which guarantees the maintenance of high levels of genetic variability, which is important to the survival of populations facing changes in their environment. The observed results also have consequences in the planning of germplasm collection for local adaptation for forest restoration programs, thereby avoiding the implantation of populations with an exogamous depression.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHOR CONTRIBUTIONS

The authors contributed equally to the manuscript.

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