Habitat fragmentation decreased the genetic variability of *Trichilia elegans* A. Juss. (Meliaceae) in southern Brazil

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(Received 3rd July 2013)

Abstract

Habitat fragmentation and the creation of so-called edge effects may have different implications on flora and fauna, including complex genetic responses. This study aimed to analyze the genetic diversity in a local population of the shade tolerant tree *Trichilia elegans* A. Juss (Meliaceae), with emphasis to the evaluation of genetic variation towards an edge-interior gradient. The results of isoenzymes assays showed that the edge subpopulation experienced the highest allele loss, while fixed alleles increased towards the interior. The total polymorphic loci percentage was 76.67%, being higher in the Middle subpopulation, whilethe average sample size for a locus (N) and the mean number of alleles for a locus (N_a) were significantly lower in the Edge subpopulation. The indices H_o, H_e and *f* showed good heterozygosity in the total population, indicating high genetic variability. The genetic distance Fs_t and *Nm* followed the same pattern, with Middle and Interior subpopulations showing higher similarity and the Edge as the farthest one, also showing less gene flow in relation to the others. Principal Coordinates Analysis (PCoA) allowed us to separate the three subpopulations with the first two axes explaining 65% of total variation, confirming that forest fragmentation affects the genetics of *Trichilia elegans* within the analyzed fragment.

Key words: Isoenzymes, habitat fragmentation, allele frequency, gene flow, araucaria forest.

Introduction

Throughout the last five centuries Brazilian Atlantic forest has been exploited and destroyed by timber trade, agriculture and urban expansion (Schwarcz et al., 2010). In these sense, the habitat fragmentation and the creation of so-called edge effects may have several implications on fauna and flora, which varies according to life histories and regeneration modes (Sugiyama and Peterson, 2013). The genetic implications may be complex (Rivero-Guerra, 2008; Shapcott et al., 2008; Andrade et al., 2009), including rare alleles loss, increased inbreeding (Vranckx et al., 2012) and reduced reproductive fitness (Finger et al., 2011), leading to genetic differentiation in subpopulations created by fragmentation (Finger et al., 2012).

The landscape surrounding the fragments exerts influence upon the responses to the forest fragmentation (Schwarcz et al., 2010). To insect-pollinated species, habitat fragmentation plays a negative role by restricting insect movement, while for wind and self-pollinated species, fragmentation may increase the abundance (Kolb and Diekmann, 2005). Regarding plant dispersal, zoochoric species are more likely to be negatively impacted by fragmented landscapes than species with abiotic dispersal, which in general tends to increase their abundance these habitats (Terborgh et al., 2008).

Meliaceae has a pantropical distribution with near 100 native species from Brazil. Among the genera occurring in Brazil, *Trichilia* includes several species of understory tree, shade-tolerant, typical from Atlantic seasonal (Souza and Lorenzi, 2005), Araucaria mixed (Barddal et al., 2004) and Atlantic rain forests (Oliveira-Filho et al., 2006). The *Trichilia elegans* A. Juss. is a common component of seasonal forests, that occurs from Venezuela to Uruguay. This specie is shade-tolerant and shows zoochoric dispersion (Lehn et al., 2008), which make it susceptible to the fragmentation and the edge effects as well can have negative impact to its genetic conservation (Moura et al., 2012).

High levels of genetic diversity is quite essential to biodiversity maintenance in forest remnants (Boyle, 2000). In these sense, understanding how fragmentation affect the genetic profile of plant populations is an important tool for conservation species. In this work we investigated the effect of edge gradient upon genetic diversity of *Trichilia elegans* (Meliaceae) using the isoenzymes profile.

Our hypothesis is that fragmentation produced by agricultural activity provokes alterations on the structure and phytosociology of forest fragments, generating the typical characteristics of edge effect that includes changes in the composition of pollinators and dispersing agents (Honnay et al., 2002; Gonzalez et al., 2010). This situation has a negative effect upon pioneers species with zoochoric dispersion (as *T. elegans*). As consequence, these species can have loss of genetic variability, by reduction of genic flow, especially in allogamous plants.

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DOI:10.1515/sg-2014-0018
edited by Thünen Institute of Forest Genetics

Materials and Methods

We conducted our study at the "*Parque Natural Municipal of Sertão*", a 590 ha conservation unit, located in northern Rio Grande do Sul (Brazil) (central point coordinates 28°02'31"S, 52°13'28"W). The area has a sloped landscape with an altitude of approximately 650 m. Subtropical regional climate shows mean annual temperature of 17.6°C, with mean annual rainfall of 1,900 mm well-distributed throughout the year. Typical vegetation is an Atlantic Forest Domain extension (Oliveira-Filho et al., 2006) with a transition between seasonal semideciduous and Mixed *Araucaria* rain forests (Budke et al., 2010).

This conservation unit is inside of an agricultural matrix, characterized by presence of soybean, corn and wheat, since 60 years ago. The area not presents ecological corridors linked to other green areas with significant size. The edge of this fragment contains wealth of tree regenerate components, with great proportion of both pioneers and invasive species, and a visible reduction on the height and diameter of adult plants in relation to the population located at the interior of the fragment.

T. elegans was choice to this study because it is an allogamous plant, has zoochoric dispersal and is abundant in all the extension of edge-interior gradient analyzed. Due the time elapse from fragmentation, only regenerating samples were analyzed, since the adult plants can be reaming of the period previous to the fragmentation.

Samples of 90 regenerating *T. elegans* plants (height ≥ 1.0 m and diameter at ground height ≤ 4.7 cm) were collected along a 450 m transect across an edge-interior gradient. Three sampling units (different representative transects) were plotted into each of three groups, also considered as subpopulations: Edge – E (located in the first 50 m starting at the edge), Middle – M (starting 150 m away from the E group) and Interior – I (starting 150 m away from the M group). In each one of 3 sampling units of different subpopulations, plant collections began from the center of each sample with five (05) plants to the right side of the transect and five (05) to the left, parallel to the forest edge and with 10 m distance among collected plants.

All collected material was kept in liquid nitrogen before being stored in an ultra-freezer (–85°C). Enzyme extractions were performed using approximately 1 g of leaf tissue.mL^{-1} of extraction solution 1 (Alfenas et al., 1998), adding approximately 150 mg of PVP-40 during grinding for removal of phenolic compounds and to increase their stability.

Solution preparation and procedures for developing and staining followed the method of Alfenas et al. (1998). A vertical double vat was used for electrophoresis to which was applied 50 µL of sample plus 3.5 µL of TA (0.4 g.mL^{-1} sucrose + 0.0025 g.ml^{-1} bromophenol blue). The enzymes were separated on 12% polyacrylamide gels in 1.5 M Tris-HCl as a buffer solution, with pH 8.8 for the running gel and pH 6.8 for the stacking gel. The buffer appliedin the tank and electrodes was the Tris-glycine (14 g.L^{-1} glycine + 3 g.L^{-1} Trisma base) with pH 8.9, diluted 10 times. The electric current for the electrophoretic run was 220 V and 40 mA.

Thus, we applied six isoenzyme systems (Aconitase (ACO) EC-Enzyme-Code 4.2.1.3, alcohol dehydrogenase (ADH) EC-1.1.1.1, malic enzyme (ME) EC-1.1.1.40, glucose dehydrogenase (GDH) EC-1.1.1.47, superoxide dismutase (SOD) and EC-1.15.1.1 sorbitol dehydrogenase (SDH) EC-1.1.1.14). Result analyzeswerebased on the presence, sharpness, number and location of bands.

For data analysis, we calculated the allele frequencies, genetic diversity indices [percentage of polymorphic loci (P), apparent number (N_a) and effective number (N_e) of alleles per population, heterozygosity observed (H_o) and expected (H_e) (Nei, 1973), genetic divergence of Wright

Table 1. – Allele frequencies in 20 allozyme loci into three subpopulations of *Trichilia elegans* A. Juss. the Araucaria Forest fragment, southern Brazil.

Loci	Alleles	Subpopulations		
		E	M	I
SDH 2	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
SDH 3	1	0.000	0.000	1.000
SDH 4	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
ADH 1	1	0.300	0.500	0.500
	2	0.700	0.500	0.500
ADH 2	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
ADH 3	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
GDH 2	1	0.500	0.500	0.813
	2	0.500	0.500	0.188
GDH 3	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
GDH 4	1	0.000	1.000	1.000
GDH 5	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
GDH 6	1	0.000	1.000	1.000
SOD 1	1	0.000	0.500	1.000
	2	0.000	0.500	0.000
SOD 2	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
SOD 3	1	1.000	1.000	1.000
SOD 4	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
ACO 2	1	0.867	0.875	0.833
	2	0.133	0.125	0.167
ACO 3	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
ACO 4	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
EM 2	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
EM 3	1	0.500	0.500	0.500
	2	0.500	0.500	0.500
Alleles Total	36	31	35	35

E – Edge; M – Middle; I – Interior.

DOI:10.1515/sg-2014-0018
edited by Thünen Institute of Forest Genetics

(F_{st}), gene flow (*Nm*) and genetic distances (Nei, 1972) by using the GenAlEx 6.4 software (Peakall and SMOUSE, 2006). The analysis of molecular variance AMOVA and Principal Coordinates Analysis (PCoA) were generated by the same program.

Results

From the six isoenzyme systems analyzed in the subpopulations of *T. elegans*, were generated 27 loci, 20 of which were able to interpretation and present 36 different alleles. Results about allele frequencies are summarized in *Table 1*, which shows that this parameter was not different between subpopulations M and I, but some allele losses and fixation were identified. In subpopulation E, five alleles were lost (SDH-3, GDH-4, GDH-6, and 2 SOD-1)and only one allele was lost in subpopulationM (SDH-3), while the allele 2SOD-1 was lost in subpopulation I. The subpopulation I had higher allele fixation (05) and an exclusive allele (1 SDH-3),while M and E have 03 and 01 fixed alleles, respectively. The M subpopulation also showed one exclusive allele (2 SOD-1).

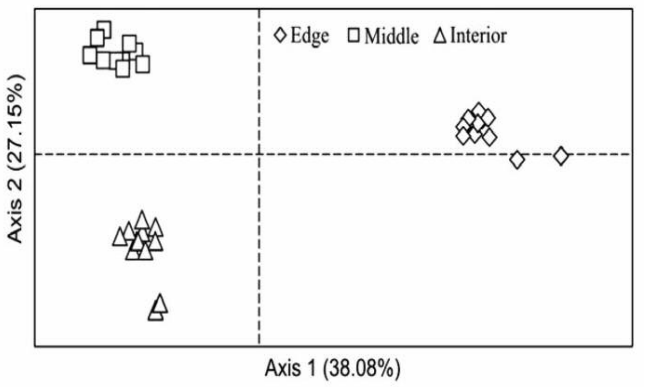

Figure 1. – Principal Coordinates Analysis of three *Trichilia elegans* A. Juss. subpopulations in an edge-interior gradient inside a Araucaria Forest fragment, southern Brazil.

Table 2. – Genetic diversity indices in three subpopulations of *Trichilia elegans* A. Juss., in edge-interior gradient the Araucaria Forest fragment in southern Brazil.

Genetic Diversity Indices	Subpopulations		
	Edge	Middle	Interior
N	21.0	27.6	26.9
N_a	1.55±0.18	1.75±0.12	1.75±0.09
P	75	80	75
H_o	0.67±0.09	0.76±0.09	0.67±0.10
H_e	0.35±0.05	0.38±0.04	0.35±0.04
f	-0.85	-0.94	-0.86

N = average sample size per locus; N_a = average number of alleles per locus, *P* = polymorphic loci percentage, H_o = average observed heterozygosity, H_e = expected heterozygosity values *f* = Index Fixation; ± = standard error.

Table 3. – Genetic divergence index (F_{st}), Nei's genetic distance and estimated values of gene flow (*Nm*) between subpopulations of *Trichilia elegans* A. Juss. considering a edge-interior gradient of a Araucaria Forest fragment in southern Brazil.

		Subpopulations		
		Edge	Middle	Interior
F_{st}	Edge	0.000		
	Middle	0.198	0.000	
	Interior	0.219	0.085	0.000
Nei's Genetic Distance	Edge	0.000		
	Middle	0.130	0.000	
	Interior	0.201	0.070	0.000
Nm	Edge	0.000		
	Middle	1.015	0.000	
	Interior	0.894	2.691	0.000

The estimated genetic variability for the three subpopulations is shown in *Table 2*. The percentage of total polymorphic loci was 76.67%, with higher polymorphism in the M subpopulation. The average number of apparent alleles (N_a) per loci was lower in the E subpopulation with 1.55 and equal in subpopulations M and I. As for the effective number of alleles (N_e), the values were slightly lower than N_a with 1.50, 1.71 and 1.69, respectively for E, M and I subpopulations.

The three subpopulations showedhigh rates of observed heterozygosity (H_o), and almost double the values expected for heterozygosities under Hardy-Weinberg equilibrium (H_e). H_o was equivalent in the E and I subpopulations (0.67) and lower as compared with M subpopulation (0.76) (*Table 3*).

The genetic distances (Nei, 1972) and the rate of genetic divergence among populations (F_{st}), showed the same pattern, with subpopulation I closer to M and both divergent from E. The estimated gene flow (Nm) values were around one (01), featuring sufficient gene flow between three subpopulations, which is higher between M and I, following the same pattern showed by F_{st}.

Regarding to molecular variance, AMOVA revealed that most variation is located among subpopulations (60%) and the lowest part is encountered inside them (40%). Consistent with all other results, Principal Coordinates Analysis (*Fig. 1*) split all three subpopulations according to the distance from edge-interior gradient, with 65% of total variance explained into the first two ordination axes.

Discussion

Direct analysis of subpopulations to study allele frequencies is of paramount importance because some parameters commonly used in genetic variability interpretation may not clearly demonstrate allele loss

DOI:10.1515/sg-2014-0018
edited by Thünen Institute of Forest Genetics

(Botrel and Carvalho, 2004). In our study, allele frequencies differed from E in relation to M and I subpopulations of *T. elegans*. The E subpopulation showed higher allele loss, while the number of fixed alleles increased toward M and I subpopulations. These results are in according with the hypothesis of the negative edge effect upon genetic variability due loss of rare alleles (Vranckx et al., 2012). The observed changes in allele frequencies among subpopulations may be indicative of initial genetic drift, as evidenced in edge gradient analysis, as well encountered in other fragmented areas that have modified pollen dispersal (Klein et al., 2008; Ismail et al., 2012). This phenomenon may occur by migration, which alters the allele frequencies in the population by introducing alleles from other ones (Botrel and Carvalho, 2004). We found only two exclusive alleles, in two different subpopulations, which are not indicative of progressive genetic erosion for the species (Ruschel et al., 2008). In any case, we emphasize the importance of the preservation of the entire *T. elegans* local population, because the maintenance of greater number of exclusive alleles is related to higher genetic diversity (Ismail et al., 2012).

The lowest values of average sample size per locus (N) and average number of alleles per locus (N_a) found for the E subpopulation (*Table 2*) reinforce the idea that edge effect is acting on the *T. elegans* population. Botrel et al. (2006), with similar analysis in two populations of *Calophyllum brasiliense* Camb. (Calophyllaceae) found similar amounts of N_a and suggested that such values are quite low, which agreesto our hypothesis of marked allele loss at E subpopulation.

A low effective population size is one of potential causes of heterozygote excess (Reyes-Zepeda et al., 2013), shown by fixation index *f* within subpopulations (negative values). A high heterozygosity in allogamous species as *T. elegans*, is of great value to the specific evolution, since it allows to several genotypic recombinations, which contributes to genetic variability (Botrel and Carvalho, 2004; Souza et al., 2004). Heterozygosity is favored by natural selection acting in order to avoid the genetic drift effects (Reyes-Zepeda et al., 2013).

The high rates of heterozygosity observed from all subpopulations suggest that, in general, the local population of *T. elegans* has a good evolutionary adaptive potential due to high genetic variability, enabling a large number of new genotypic combinations (Sebbenn et al., 2000).

The levels of genetic variation found in this study were higher than those found for other understory tree species. Hamrick and Loveless (1986) found a H_e of 0.13 and P of 32.2% in *Psychotria tenuinervis* (Rubiaceae). Ramos et al. (2010) studied a local *Psychotria tenuinervis* population and found a H_e of 0.35 and P of 71% for plants near the anthropogenic edge (50 m) and a H_e of 0.25 and P of 50% for plants of this population inside the fragment (200 m from the edge).

Recently it has been argued that several genetic studies of plant populations in fragmented locations found no decrease in genetic variability (Kramer et al., 2008). However, in this study, it was found that the edge effect, resulting from habitat fragmentation, at least, caused variation in the genetic variability of all subpopulations, since most of the indices of diversity, genetic distance and divergence were influenced by the edge-interior gradient.

The divergence index F_{st} is used both to observe the proportion of genetic variability found among populations and to measure the frequencies of homozygous genotypes compared to heterozygotes (Yang et al., 1996; Matioli, 2001). By the F_{st} index, we found that the E subpopulation has a divergent structure of moderately high shape for the other subpopulations, since the observed values were between 0.15 and 0.25. This scenario configures a frame initial differentiation of genetic variability in the proportions found among subpopulations (Zucchi et al., 2004). The genetic distances of *Nei* repeat the same F_{st} pattern, and even if they are considered relatively low values (Yeh, 2000) they vary such that as the larger the proximity of the edge forest and distance between subpopulations, the greater the genetic distance between them.

High genetic divergences indicate, in principle, lower levels of gene flow (Moraes et al., 2005). *Nm* is an estimate of gene flow based on F_{st} and refers to the number of migrants per generation, but gene flow is not necessarily equal to *Nm*. So, $Nm > 1$ is equivalent to great gene flow, able to prevent genetic differentiation by drift or natural selection, because it acts by homogenizing a spatial genetic variation (Hamrick and Nason, 2000). If $Nm < 1$, chances of genetic differentiation occurrence between populations are larger (Tremblay and Ackerman, 2001). In this sense, the estimated values of gene flow found in *Trichilia elegans* indicate that genetic differentiation in the subpopulations analyzed is being prevented. However, even though a few meters away from each other, the lower gene flow between plants fromedge to the others analyzed areas was clear, showing that, with the advance in edge gradient to the interior, the gene flow rates are higher, confirming the edge effect on the genetics of the analyzed population.

Factors such as the population size, inbreeding, limited dispersal of pollen and seeds and, therefore, fall in the gene flow levels, reduce the variability within the population via genetic drift and thereby contribute to the development of genetic heterogeneity among populations (Hamrick and Nason, 2000; Zanettini and Cavalli, 2003). The highest percentage of molecular variance found between subpopulations studied corroborate this assertion.

The Principal Coordinates Analysis clearly shows the edge effect derived from the habitat fragmentation, acting as specifically as possible, influencing the genetic diversity of the *Trichilia elegans* local population. Finally, the results confirm the negative effect of forest fragmentation on genetic variability towards the edge-interior gradient.

Acknowledgement

We thank the managers of the Parque Natural Municipal Sertão hat permitted the collect of *T. elegans* sam-

DOI:10.1515/sg-2014-0018
edited by Thünen Institute of Forest Genetics

ples at UC. We also thank CNPq, Secretaria de Ciência e Tecnologia-RS and FAPERGS for the financial support.

References

ALFENAS, C. A., W. BRUNE, J. R. OLIVEIRA, S. K. ALONSO and M. SCORTICNHINI (1998): Extração de proteínas para eletroforese. *In:* Eletroforese de isoenzimas e proteínas afins: fundamentos e aplicações em plantas e microrganismos (Org. Alfenas). Viçosa UFV, pp 85–114.

ANDRADE, I. M., S. J. MAYO, C. VAN DEN BERG, M. F. FAY, M. CHESTER, C. LEXER and D. KIRKUP (2009): Genetic variation in natural populations of *Anthurium sinuatum* and *A. pentaphyllum* var. *pentaphyllum* (Araceae) from north-east Brazil using AFLP molecular markers. Botanical Journal of the Linnean Society **159**: 88–105.

BARDDAL, M. L., C. V. RODERJAN, F. GALVÃO and G. R. CURCIO (2004): Phytosociology of the understory of a Floodplain Forest, at Araucaria, PR. Ciência Florestal **14**: 35–45.

BOTREL, M. C. G. and D. CARVALHO (2004): Isozymes variability in natural populations of „jacarandá paulista" (*Machaerium villosum* Vog.). Brazilian Journal of Botany **27**: 621–627.

BOTREL, M. C. G., A. M. SOUZA, D. CARVALHO, S. I. C. PINTO, M. C. O. MOURA and R. A. ESTOPA (2006): Genetic characterization of *Calophyllum brasiliensis* Camb. in two populations of riparian forest. Revista Árvore **30**: 821–827.

BOYLE, T. J. (2000): Criteria and Indicators for the Conservation of Genetic Diversity. *In:* Forest Conservation Genetics: Principles and Practice (Eds. Young, Boshier and Boyle). CSIRO Publishing, pp 239–251.

BUDKE, J. C., M. S. ALBERTI, C. ZANARDI, C. BARRATO and E. M. ZANIN (2010): Bamboo dieback and tree regeneration responses in a subtropical forest of South America. Forest Ecology and Management **260**: 1345–1349.

FINGER, A., C. J. KETTLE, C. N. KAISER-BUNBURY, T. VALENTIN, D. DOUDEE, D. MATATIKEN and J. GHAZOUL (2011): Back from the brink: potential for genetic rescue in a critically endangered tree. Molecular Ecology **20**: 3773–3784.

FINGER, A., C. J. KETTLE, C. N. KAISER-BUNBURY, T. VALENTIN, J. MOUGAL and J. GHAZOUL (2012): Forest fragmentation genetics in a formerly widespread island endemic tree: *Vateriopsis seychellarum* (Dipterocarpaceae). Molecular Ecology **21**: 2369–2382.

GONZALEZ, M., S. LADET, M. DECONCHAT, A. CABANETTES, D. ALARD and G. BALENT (2010): Relative contribution of edge and interior zones to patch size effect on species richness: An example for woody plants. Forest Ecology and Management **259**: 266–274.

HAMRICK, J. L. and M. D. LOVELESS (1986): Isozyme variation in tropical trees: procedures and preliminary results. Biotropica **18**: 201–207.

HAMRICK, J. L. and J. D. NASON (2000): Gene flow in forest trees. *In:* Forest conservation genetics: principles and practice. (Eds. YOUNG, BOSHIER and BOYLE) CSIRO Publishing 06 pp 81–89.

HONNAY, O., K. VERHEYEN and M. HERMY (2002): Permeability of ancient forest edges for weedy plant species invasion. Forest Ecology and Management **161**: 109-122.

ISMAIL, S. A., J. GHAZOUL, G. RAVIKANTH, R. U. SHAANKER, C. G. KUSHALAPPA and C. J. KETTLE (2012): Does long-distance pollen dispersal preclude inbreeding in tropical trees? Fragmentation genetics of *Dysoxylum malabaricum* in an agro-forest landscape. Molecular Ecology **21**: 5484–5496.

KLEIN E. K., N. DESASSIS and S. ODDOU-MURATORIO (2008): Pollen flow in the wildservice tree, *Sorbus torminalis* (L.) Crantz. IV. Whole interindividual variance of male fecundity estimated jointly with the dispersal kernel. Molecular Ecology **17**: 3323–3336.

KOLB, A. and M. DIEKMANN (2005): Effects of Life-History Traits on Responses of Plant Species to Forest Fragmentation. Conservation Biology **19**: 929–938.

KRAMER, A. T., J. L. ISON, M. V. ASHLEY and H. HOWE (2008): The paradox of forest fragmentation genetics. Conservation Biology **22**: 878–885.

LEHN, C. R., S. M. SALIS, P. P. MATTOS and G. A. DAMASCENO-JUNIOR (2008): Estrutura e distribuição espacial de *Trichilia elegans* A. Juss. (Meliaceae) em uma floresta semidecídua no Pantanal da Nhecolândia, Mato Grosso do Sul. Revista Brasileira de Biologia Neotropical **5**: 1–9.

MATIOLI, S. R. (2001): Biologia molecular e evolução. Holos, Ribeirão Preto/SP. 202 pp.

MORAES, M. L. T., P. Y. KAGEYAMA and M. A. SEBBENN (2005): Genetic diversity and effective size in two *Myracrodruon urundeuva* Fr. All., population under ex situ conservation. Revista Árvore **29**: 281–289.

MOURA, T. M., K. MARTINS, P. S. SUJII, A. M. SEBBENN and L. J. CHAVES (2012): Genetic structure in fragmented populations of *Solanum lycocarpum* A. St.-Hil. with distinct anthropogenic histories in a Cerrado region of Brazil. Genetic and Molecular Research **11**: 2674–2682.

NEI, M. (1973): Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the USA pp. 3321–3323.

NEI, M. (1972): Genetic distance between populations. The American Naturalist **106**: 283–292.

OLIVEIRA-FILHO, A. T., J. A. JARENKOW and M. J. N. RODAL (2006): Floristic relationships of seasonally dry forests of eastern South America based on tree species distribution patterns. *In:* Neotropical Savannas and Dry forests: Plant Diversity, Biogeography and Conservation. (Eds. PENNINGTON, LEWIS and RATTER). CRC Press pp. 159–192.

PEAKALL, R. and P. E. SMOUSE (2006): GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**: 288–295.

RAMOS, F. N., P. F. LIMA, M. I. ZUCCHI, C. A. COLOMBO and V. N. SOLFERINI (2010): Genetic structure of tree and shrubby species among anthropogenic edges, natural edges, and interior of an Atlantic forest fragment. Biochemical Genetics **48**: 215–228.

REYES-ZEPEDA, F., J. GONZÁLEZ-ASTORGA and C. MONTAÑA (2013): Heterozygote excess through life history stages in *Cestrum miradorense* Francey (Solanaceae), an endemic shrub in a fragmented cloud forest habitat. Plant Biology **15**: 176–185.

RIVERO-GUERRA, A. O. (2008): Phenotypic differentiation of peripheral populations of *Santolina rosmarinifolia* (Asteraceae). Botanical Journal of the Linnean Society **158**: 650–668.

DOI:10.1515/sg-2014-0018
edited by Thünen Institute of Forest Genetics

RUSCHEL, A. R., J. PEDRO and R. O. NODARI (2008): Genetic diversity in anthropogenized populations of fumo brabo (*Solanum mauritianum*) in Santa Catarina, Brazil. Scientia Forestalis **36**: 63–72.

SCHWARCZ, K. D., C. L. PATACA, A. G. ABREU, J. M. BARIANI, C. M. T. MACRINI and V. N. SOLFERINI (2010): Genetic diversity in Atlantic Forest trees: fragmentation effects on *Astronium graveolens* (Anacardiaceae) and *Metrodorea nigra* (Rutaceae), species with distinct seed dispersal strategies. Botanical Journal of the Linnean Society **164**: 326–336.

SEBBENN, A. M., P. Y. KAGEYAMA, A. C. M. F. SIQUEIRA and A. C. E. ZANATTO (2000): Taxa de cruzamento em populações de *Cariniana legalis* (Mart.) O. Ktze.: implicações para a conservação e melhoramento genético. Scientia Forestalis **58**: 25–40.

SHAPCOTT, A., B. BAU and P. KATIK (2008): The potential implications of rainforest history, hybridization, and climate change on the phylogenetics of a rare genus of herbs *Romnalda* (Dasypogonaceae) from New Guinea and Australia. Botanical Journal of the Linnean Society **157**: 455–474.

SOUZA, L. M. F. I., P. Y. KAGEYAMA and A. M. SEBBENN (2004): Genetic structure in fragmented populations of *Chorisia speciosa* St. Hil. Scientia forestalis **65**: 70–79.

SOUZA, V. C. and H. LORENZI (2005): Botânica Sistemática. Editora Instituto Plantarum. Nova Odessa. pp 640.

SUGIYAMA, A. and C. J. PETERSON (2013): Edge Effects Act Differentially on Multiple Early Regeneration Stages of a Shade-tolerant Tree *Tapirira mexicana*. Biotropica **45**: 37–44.

TERBORGH, J., G. NUÑEZ-ITURRI, N. C. PITMAN, F. H. VALVERDE, P. ALVAREZ, V. SWAMY, E. G. PRINGLE and C. E. PAINE (2008): Tree recruitment in an empty forest. Ecology **89**: 1757–1768.

TREMBLAY, R. L. and J. D. ACKERMAN (2001): Gene flow and effective population size in *Lepanthes* (Orchidaceae): a case for genetic drift. Biological Journal of the Linnean Society **72**: 47–62.

VRANCKX G., H. JACQUEMYN, B. MUYS and O. HONNAY (2012): Meta-analysis of susceptibility of woody plants to loss of genetic diversity through habitat fragmentation. Conservation Biology **26**: 228–237.

YANG, R. C., F. C. YEH and A. D. YANCHUKT (1996): A Comparison of Isozyme and Quantitative Genetic Variation in *Pinus contorta* ssp. *latifolia* by F_{st}. Genetics **142**: 1045–1052.

YEH, F. (2000): Population genetics. *In:* Forest conservation genetics: principles and practice. (Eds. YOUNG, BOSHIER and BOYLE). CSIRO Publishing pp 352. Melbourne.

ZANETTINI, M. H. B. and S. S. CAVALLI (2003): Variabilidade genética em função do modo de reprodução. *In:* Freitas, L. B.; Bered, F. Genética e evolução vegetal. Editora UFRGS pp 177–187. Porto Alegre/RS.

ZUCCHI, M. I., J. B. PINHEIRO, A. V. AGUIAR, L. J. CHAVES, A. S. G. COELHO and R. VENCOVSKY (2004): Spatial autocorrelation of divergence in populations of *Eugenia dysenterica* DC. using ssr markers. Floresta e Ambiente **11**: 29–38.

DOI:10.1515/sg-2014-0018
edited by Thünen Institute of Forest Genetics