SCIENTIA
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The genetic status of *Sorocea bonplandii* in the highly fragmented forest in southern Brazil

Estado genético de *Sorocea bonplandii* na fragmentada floresta no sul do Brasil

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Abstract

Sorocea bonplandii is a dioecious sub canopy plant species with an abundant and widespread distribution in the Atlantic Forest, Southern Brazil. It is traditionally used for art crafts and popular medicine. We have investigated the genetic status of *S. bonplandii* in one large and six small populations of the fragmented Atlantic Forest in southern Brazil. In spite of population fragmentation, the allozyme electrophoretic analysis involving 23 loci of 12 enzymes from 420 plants revealed unusually high frequencies of heterozygous genotypes and a balanced distribution, in agreement with Hardy-Weinberg proportions. Very little genetic differentiation among populations was detected. The observed excess of heterozygotes suggests that selection favors heterozygous plants. Although *S. bonplandii* is a dominant sub-canopy species which strongly depends on ombrophilous environmental conditions, its genetic structure and diversity do not appear to suffer with forest fragmentation. All of these characteristics, in addition to its medicinal uses, suggest that this species is a potential resource for sustainable management of tropical forests in southern Brazil.

Keywords: Allozyme diversity, Heterozygotes, Forest fragmentation, Subtropical Atlantic Forest

Resumo

Sorocea bonplandii é uma espécie dióica de subosque com abundante distribuição na Mata Atlântica no Sul do Brasil. É tradicionalmente usada para artesanatos e medicina popular. A estrutura genética de *S. bonplandii* foi investigada em uma população grande e seis pequenas em fragmentos florestais da Mata Atlântica no Sul do Brasil. As análises eletroforéticas de aloenzimas envolvendo 420 plantas geraram 23 locos de 12 enzimas, as quais revelaram elevada freqüência de genótipos heterozigóticos e uma distribuição equilibrada, de acordo com proporções Hardy-Weinberg. Também foi detectada baixa diferenciação genética entre as populações. O excesso de heterozigotos observado sugere que a seleção favorece plantas heterozigotas. Embora *S. bonplandii* seja uma espécie dominante no subosque, depende fortemente de condições ambientais ombrófilas; a sua estrutura genética e a diversidade não mostraram ser afetadas pela fragmentação florestal. Todas estas características, além do uso medicinal sugerem esta espécie ser um recurso potencial para a gestão sustentável das florestas tropicais do Sul do Brasil.

Palavras-chave: Diversidade alozimática, Heterozigotos, Fragmentação florestal, Floresta Subtropical Atlântica

INTRODUCTION

The Brazilian Atlantic Forest covers ca. 12% (1.2 million km²) of the total land area of Brazil, but was reduced dramatically over the past 500 years down to scattered remnants adding up to less than one tenth of its original size (FUNDAÇÃO SOS MATA ATLÂNTICA, 1998). The colonization of the western region of Santa Catarina, in southern Brazil, where the Seasonal Decidual Forest (SDF) is located, started in 1920. However, in 1940 settlement and wood exploitation became speedy, leading to more than 96% of deforestation until 1980. Currently, less than 4% of the Atlantic Forest remains in isolated fragments. These are under strong anthropogenic pressures (RUSCHEL *et al.*, 2003) and the consequences are: decreases in local biodiversity; population sizes of each species; and in gene flow. Under these conditions, inbreeding and extinction rates of the remnant species increase substantially. Researches on neotropical species have shown that habitat fragmentation increases

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genetic divergence among populations as well as inbreeding rates (CARDOSO *et al.*, 2000; AULER *et al.*, 2002; MORAES and DEBYSHIRE, 2004; SOUZA *et al.*, 2004).

Many factors can explain the gene pool and genetic identity of each species. In general, geographical factors and human actions have been claimed to cause genetic differences among populations (CARDOSO *et al.*, 2000; AULER *et al.*, 2002; SALGUEIRO *et al.*, 2004). The latter authors and Csaikl *et al.* (2002) have suggested that the differences among populations were due to different glacial refugia along the dispersion area, possibly as a result of evolutionary and ecological independence between geographically separated populations.

Management strategies to reconcile the use and conservation of a species require knowledges in botany, auto-ecology, and population genetics. However, sustainable management plans in tropical forests have revealed inconsistencies in regard to those aspects (MARTINS-da-SILVA *et al.*, 2003; UHL and VIEIRA, 1989; VAN GARDINGEN *et al.*, 2006; SIST and FERREIRA, 2007). Nevertheless, increased knowledge in those topics is essential in order to improve sustainable management strategies and to establish fundamental criteria for the sustainable forest management.

Sorocea bonplandii (Baill) W.C. Burger, Lang. & Wess. Boer (Moraceae) is a small tree of the sub-canopy layer in mature forests in southern Brazil. It is an abundant species (148 plants ha^{-1}) and comprises approximately 10% of all woody plants with DBH ≥5 cm in the SDF. An examination of all plants, independent of DBH, revealed more 6,000 plants ha^{-1} (RUSCHEL *et al.* 2006). The species is traditionally used as a food source by indigenous people, as well as for arts and crafts, and medical purposes (RUSCHEL *et al.* 2008).

Investigations in population genetics of *S. bonplandii* from the Atlantic Forest of southern Brazil by using the AFLP technique to study DNA markers surprisingly did not show genetic erosion (RUSCHEL *et al.*, 2007). We aimed to perform a similar study by using allozyme molecular markers, to quantify the diversity and genetic structure of *Sorocea bonplandii* populations in southern Brazil.

MATERIAL AND METHODS

The study area comprises a fragmented forest landscape in the subtropical Atlantic Forest in southern Brazil. The fragments are dispersed over the Uruguay River watershed (Figure 1). All these remnants were subjected to timber exploitation over the years (RUSCHEL *et al.*, 2005), and are still constantly influenced by human actions.

By chosing a species occurring in high density, allowed us to sample sufficient numbers of individuals even in the smaller forest fragments. Plant samples (leaves) were collected from seven fragments: i) TUR Parque Estadual Turvo, Derrubadas, RS; ii) CCO – Sede Capela, Itapiranga, SC; iii) SVA – São Valentin, Descanso, SC; iv) TIG Tigre, Mondaí, SC; v) BRO – Beato Roque, São João do Oeste, SC; vi) SOL Soledade, São João do Oeste, SC; and vii) SPA – São Pedro de Alcântara, SC. Only SPA is located in the Tropical Atlantic Forest, while all others are in the Subtropics. These fragments have varying exploitation histories, mainly due to logging and its features. TUR (17,491 ha) and SOL (11 ha) have not been exploited, i.e., only the larger fragment at TUR can be classified as being undisturbed, while SOL is a small fragment disturbed by a high edge influence and human effects. CCO (50 ha) and SVA (50 ha) are small fragments that were selectively exploited for high value timber species. In all others (TIG – 12 ha, BRO – 10 ha, and SPA – 30 ha) timber species were intensively exploited. Timber exploitation occurred during the last three decades (Figure 1). However, in spite of the fragmentation, the floristic structure of woody species and the natural regeneration of *S. bonplandii* were shown to be similar to those in undisturbed forests (RUSCHEL *et al.*, 2005; RUSCHEL *et al.*, 2006).

In each fragment, 80 plants larger than 25 cm in height were sampled, with the exception of SPA, where only 42 plants were sampled because of the low population density (Table 1). About six young leaves were collected from each plant. In small fragments, leaves were collected from random trees in the immediate vicinity along the largest diagonal line across the forest area. A minimum distance of 15 m was kept between sample trees. When it was necessary to complete 80 sample plants, samples were collected also from plants along lines perpendicular to the main diagonal. In the case of the largest fragment (TUR), the sampling strategy involved three parallel 750 m transects 2.5 km apart. Total height and diameter at breast height (DBH) of all sample plants were measured.

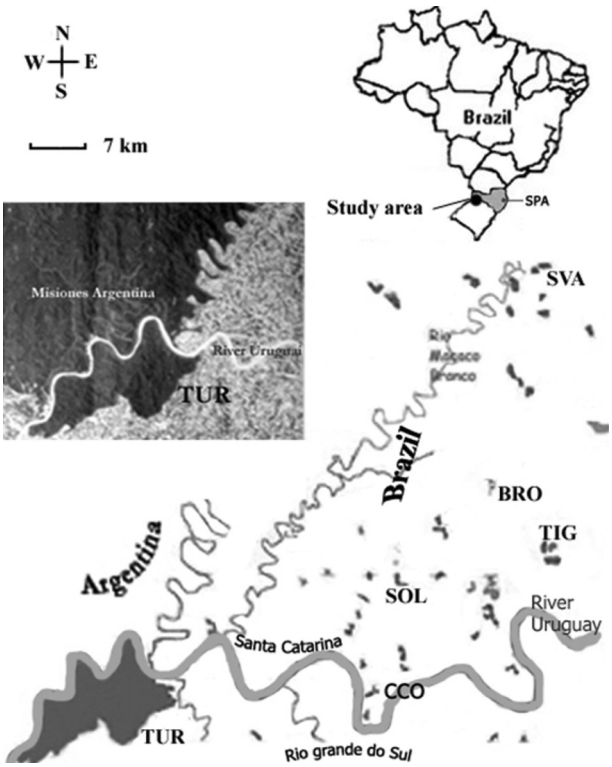

Figure 1. Location of the forest fragments of the Subtropical Atlantic Forest ecosystem of the upper Uruguay river in southern Brazil, including Park Turvo, the largest intact remainder of this ecosystem at the border to Argentina, and five smaller remnants (CCO, SOL, BRO, TIG, SVA) in Santa Catarina, in distances of ca. 20 to 50 km from Park Turvo, and of one remnant (SPA) of the Tropical Atlantic Forest ecosystem situated at the Atlantic coast, ca. 500 km away from Park Turvo (adapted from Fundação SOS Mata Atlântica, 1998).

Figura 1. Localização dos fragmentos da Floresta Subtropical Atlântica do ecossistema do rio Alto-Uruguai no sul do Brasil, incluindo o Parque Turvo, o maior remanescente intacto do ecossistema na fronteira da Argentina, e cinco remanescentes pequenos (CCO, SOL, BRO, TIG, SVA) em Santa Catarina, distantes aproximadamente 20 a 50 km do Parque Turvo, e um remanescente (SPA) da Floresta Tropical Atlântica, ecossistema da costa Atlântica, distante aproximadamente 500 km do Parque Turvo (adaptado de Fundação SOS Mata Atlântica, 1998).

Leaf samples were stored at 3° C for one week before protein was extracted from about 200 mg of leaf material in 1.5 ml of extraction buffer n° 1 (ALFENAS *et al.*, 1991) supplemented with 2% glycerol, 2% sorbitol, and about 18 mg of polyvinylpyrrolidone and stored at -60° C. After one year in storage, leaf extracts were centrifuged (13,000 rpm, 3 min, 4 °C) and subjected to horizontal gel electrophoresis with 11% (w/v) maize starch (Modified Penetrose 30 Starch - Corn Products, Brasil). Among 43 enzymes tested, 12 yielded 23 loci suitable for analysis: GOT (EC 2.6.1.1 - Aspartate Aminotransferase), α-EST (EC 3.1.1.1 - α-Esterase), FDH (EC 1.2.1.2 - Formate Dehydrogenase), G6PDH (EC 1.1.1.49 Glucose-6-Phosphate 1-Dehydrogenase), IDH (EC 1.1.1.42 - Isocitrate Dehydrogenase), MDH (EC 1.1.1.37 - Malate Dehydrogenase), PGI (EC 5.3.1.9 - Glucose-6-Phosphate Isomerase), EM (EC 1.1.1.40 - Malic Enzyme), PO (EC 1.11.1.7 - Peroxidase), PGM (EC 2.7.5.1 - Phosphoglucomutase), 6PGDH (EC 1.1.1.44 - 6-Phosphogluconate Dehydrogenase), and SOD (EC 1.15.1.1 - Superoxide Dismutase).

Zymograms were developed and interpreted as Mendelian inheritance based on band patterns, as described by Kephart (1990) and Alfenas *et al.* (1991). Only zymogram data of unambiguous quality and resolution were used for the analyses.

Data were analyzed by using the Biosys-2 software (SWOFFORD and SELANDER, 1989) to obtain the mean number of alleles per locus and to estimate the expected heterozygosity (H_e) based on Hardy-Weinberg expectation (LEVENE, 1949; NEI, 1978). The equilibrium of genotype per locus based on Hardy-Weinberg (H&W) proportion was tested by using Biosys software to calculate the Chi-Square (χ^2) value according to Levene (1949) and corrected for small sample size. The genetic structure of populations was compared by F-statistics (WRIGHT, 1965; NEI, 1977). This statistics permits the analysis of levels of inbreeding among populations (F_{ST}), within a population (F_{IS}), and the total inbreeding involving all populations (F_{IT}). Mean values of all loci for deviation from H&W equilibrium and inbreeding rates (F_{IS}, H_S G_{ST}). were compared to indicative adjusted nominal level by Bonferroni test (Rice, 1989), with the use of the FSTAT v.2.0.3.2 (GOUDET, 2008). The P-value levels of 5% for FIS within samples based on randomisations were obtained by an indicative adjustment value: if this value is greater than F_{IS} value, than F_{IS} is not in H&W equilibrium; and in H&W equilibrium, otherwise. G_{ST} Hedrick was calculated according to the formula provided by Hedrick (2005): G_{ST}-Hedrick= $[G_{ST}(1+H_S)]/(1-H_S)(H_S-1)$.

Genetic distances among populations were estimated according to Nei (1972); cluster analysis of genetic distances was performed based on the unweighted pair-group method with arithmetic averaging (UPGMA) (SNEATH and SOKAL, 1973) by using NTSYSpc (Numerical Taxonomy and Multivariate Analysis System - version 2.1) (ROHLF, 1989).

RESULTS

From 43 enzyme systems tested, 12 resulted in high quality, reproducible allozyme banding patterns. Of these, one enzyme (malic enzyme) appeared to be monomorphic, while all others showed a total of 22 polymorphic loci. Among the polymorphic loci, usually the most frequent alleles were similar in all of the seven populations analyzed (including SPA, from the Tropical Atlantic Forest). As an example, the frequency of the PGI allele "a" varied from 43% to 50% among populations. Low frequency alleles were not found in all populations, such as the allele "d" at PGM-3 locus, that was either less frequent than 5%, or absent. However, the allele "c" of 6-PGDH-2 locus was the most frequent in five populations, being the second most frequent (0.45) in population TUR, in which the frequency of the allele "b" was 0.55 (Table 1). When looking at the allele frequencies at a given locus in a given population, more than half were not in H&W equilibrium (Table 1). Loci P0 2 and SOD-1 were in H&W equilibrium in all populations, while IDH-1, MDH-2, PGI-1, and SOD-2 exhibited significant deviation from H&W equilibrium in all populations. Overall, the CCO population showed the highest number of alleles (67), while the lowest number of alleles (64) was found in the TIG population. SPA was the population which exhibited the highest percentage of genotype distributions under H&W equilibrium (55%) and SVA population presented the lowest amount of genotype distributions under H&W equilíbrium (36).

Table 1. Number of plants and the allele frequencies for the allozyme loci in *Sorocea bonplandii* among seven natural populations from Atlantic forest, southern Brazil. The F Wright fixation index per loci in, and Chi-square test for deviation from EHW for genotype distribution for each locus according to Levene (1949), and means of all plants.

Tabela 1. Número de plantas e frequência dos alelos aloenzimáticos de sete populações naturais de *Sorocea bonplandii* da Mata Atlântica, sul do Brasil. Índice de fixação F Wright por loco e teste Qui-quadrado para a distribuição de genótipos por loco em aderência ao EHW de acordo com Levene (1949), e média de todas as plantas.

Locus	TUR	SOL	CCO	SVA	TIG	BRO	SPA
ME-1	84*	76	83	78	81	75	42
a	1.0	1.0	1.0	1.0	1.0	1.0	1.0
6PGDH-1	46	49	60	47	38	54	10
a	0.14	0.22	0.28	0.21	0.36	0.29	0.10
b	0.86	0.78	0.73	0.79	0.65	0.71	0.90
F-Wright (P-value)	0.194$^{0.155}$	0.179 $^{0.180}$	0.206 $^{0.096}$	0.365$^{0.009}$	0.023 $^{0.820}$	0.231$^{0.076}$	-0.111$^{0.808}$
6PGDH-2 (N)	46	49	59	47	39	54	32
a	0.00	0.05	0.05	0.06	0.13	0.05	0.00
b	0.55	0.40	0.37	0.46	0.35	0.48	0.48
c	0.45	0.55	0.58	0.48	0.53	0.47	0.52
F-Wright (P-value)	-0.033$^{0.879}$	-0.041$^{0.232}$	0.034$^{0.191}$	-0.030$^{0.083}$	-0.004$^{0.020}$	-0.194$^{0.047}$	0.062$^{0.661}$
EST-1 (N)	52	52	58	64	61	57	32
a	0.53	0.66	0.53	0.54	0.55	0.56	0.70
b	0.47	0.34	0.47	0.46	0.45	0.44	0.30
F-Wright (P-value)	-0.363$^{0.010}$	-0.178$^{0.220}$	-0.178$^{0.197}$	0.025$^{0.791}$	-0.424$^{0.001}$	-0.282$^{0.039}$	-0.123$^{0.543}$
EST-2 (N)	65	65	68	66	61	63	42
a	0.39	0.41	0.29	0.44	0.50	0.38	0.44
b	0.61	0.59	0.71	0.56	0.50	0.62	0.56
F-Wright (P-value)	-0.206$^{0.107}$	-0.191$^{0.136}$	-0.204$^{0.104}$	-0.538$^{0.000}$	-0.344$^{0.009}$	0.125 $^{0.291}$	-0.497$^{0.002}$
FDH-1 (N)	54	53	39	42	32	51	32
a	0.20	0.18	0.40	0.19	0.27	0.27	0.06
b	0.19	0.23	0.15	0.30	0.17	0.19	0.23
c	0.54	0.58	0.41	0.46	0.56	0.51	0.53
d	0.05	0.02	0.01	0.05	0.00	0.03	0.09
e	0.03	0.00	0.03	0.00	0.00	0.01	0.08
F-Wright (P-value)	-0.229$^{0.002}$	-0.378$^{0.000}$	-0.145$^{0.487}$	-0.195 $^{0.004}$	-0.500$^{0.000}$	-0.329$^{0.001}$	-0.213$^{0.002}$
G6PDH-1 (N)	64	57	53	52	47	62	27
a	0.39	0.38	0.36	0.33	0.44	0.30	0.26
b	0.41	0.41	0.31	0.28	0.42	0.43	0.44
c	0.20	0.21	0.33	0.39	0.15	0.27	0.30
F-Wright (P-value)	0.134$^{0.220}$	-0.234$^{0.092}$	-0.219$^{0.008}$	0.126$^{0.123}$	-0.037$^{0.707}$	0.210$^{0.003}$	-0.030$^{0.320}$

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Locus	TUR	SOL	CCO	SVA	TIG	BRO	SPA
GOT-1 (N)	62	62	66	64	61	62	42
a	0.05	0.04	0.02	0.03	0.02	0.10	0.06
b	0.73	0.74	0.74	0.68	0.69	0.70	0.74
c	0.22	0.22	0.24	0.29	0.30	0.20	0.20
F-Wright (P-value)	-0.300 0.040	-0.139 0.455	-0.323 0.040	-0.413 0.003	-0.196 0.379	-0.304 0.013	-0.275 0.171
IDH-1 (N)	67	66	69	64	66	63	42
a	0.45	0.17	0.27	0.44	0.36	0.35	0.32
b	0.48	0.74	0.62	0.43	0.57	0.52	0.45
c	0.07	0.09	0.12	0.13	0.07	0.14	0.23
F-Wright (P-value)	0.538 0.000	0.499 0.000	0.404 0.000	0.356 0.002	0.369 0.003	0.626 0.000	0.517 0.000
MDH-1 (N)	72	65	71	72	72	62	41
a	0.47	0.45	0.48	0.38	0.42	0.44	0.18
b	0.53	0.55	0.52	0.62	0.58	0.56	0.82
F-Wright (P-value)	-0.393 0.001	-0.370 0.004	-0.467 0.000	-0.206 0.091	-0.109 0.386	-0.340 0.009	-0.061 0.753
MDH-2 (N)	75	66	74	73	72	64	42
a	0.41	0.46	0.43	0.42	0.44	0.40	0.30
b	0.59	0.55	0.57	0.58	0.56	0.60	0.70
F-Wright (P-value)	-0.520 0.000	-0.711 0.000	-0.762 0.000	-0.661 0.000	-0.462 0.000	-0.597 0.000	-0.424 0.007
PGI-1 (N)	80	70	78	73	73	64	42
a	0.48	0.47	0.44	0.49	0.43	0.50	0.44
b	0.20	0.22	0.27	0.19	0.22	0.20	0.32
c	0.32	0.32	0.30	0.32	0.35	0.30	0.24
F-Wright (P-value)	-0.400 0.000	-0.373 0.000	-0.360 0.000	-0.341 0.000	-0.256 0.031	-0.284 0.006	-0.401 0.001
PGM-1 (N)	71	69	75	74	67	64	42
a	0.28	0.26	0.17	0.25	0.30	0.36	0.25
b	0.37	0.33	0.31	0.37	0.34	0.28	0.39
c	0.26	0.33	0.35	0.30	0.28	0.27	0.25
d	0.10	0.08	0.17	0.08	0.08	0.09	0.11
F-Wright (P-value)	0.150 0.000	0.120 0.008	0.171 0.003	0.175 0.000	0.092 0.001	-0.079 0.000	0.194 0.000
PGM-2 (N)	71	69	75	75	69	63	42
a	0.19	0.26	0.23	0.29	0.23	0.33	0.21
b	0.39	0.37	0.41	0.41	0.38	0.34	0.62
c	0.37	0.32	0.16	0.26	0.32	0.20	0.13
d	0.06	0.05	0.20	0.05	0.07	0.13	0.04
F-Wright (P-value)	0.292 0.000	0.182 0.019	0.104 0.233	0.199 0.006	0.017 0.105	-0.085 0.294	0.181 0.000
PGM-3 (N)	71	70	75	75	69	64	42
a	0.01	0.04	0.04	0.01	0.04	0.06	0.01
b	0.54	0.57	0.47	0.51	0.56	0.56	0.75
c	0.43	0.39	0.48	0.47	0.41	0.38	0.19
d	0.02	0.00	0.01	0.01	0.00	0.00	0.05
F-Wright (P-value)	-0.333 0.075	-0.352 0.013	-0.152 0.418	-0.490 0.001	-0.331 0.022	-0.112 0.109	-0.075 0.995
PO-1 (N)	63	62	61	46	43	62	42
a	0.53	0.47	0.36	0.46	0.54	0.52	0.56
b	0.33	0.45	0.40	0.37	0.36	0.37	0.33
c	0.14	0.08	0.24	0.17	0.11	0.11	0.11
F-Wright (P-value)	-0.021 0.172	0.124 0.013	0.120 0.498	0.130 0.617	0.148 0.043	0.161 0.141	-0.055 0.435
PO-2 (N)	62	55	60	41	39	58	41
a	0.65	0.57	0.48	0.57	0.62	0.47	0.55
b	0.36	0.43	0.52	0.43	0.39	0.53	0.45
F-Wright (P-value)	0.084 0.467	-0.152 0.291	0.132 0.276	-0.047 0.825	0.025 0.812	0.030 0.769	0.163 0.262

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Locus	TUR	SOL	CCO	SVA	TIG	BRO	SPA
PO-3 (N)	55	48	50	42	34	50	37
a	0.43	0.39	0.36	0.31	0.37	0.36	0.27
b	0.26	0.31	0.37	0.46	0.35	0.37	0.31
c	0.31	0.30	0.27	0.23	0.28	0.27	0.42
F-Wright (P-value)	$0.080^{0.056}$	$0.214^{0.012}$	$0.152^{0.003}$	$0.365^{0.007}$	$0.289^{0.009}$	$0.273^{0.024}$	$0.298^{0.015}$
PO-4 (N)	52	48	52	55	44	52	35
a	0.24	0.21	0.27	0.31	0.24	0.20	0.34
b	0.66	0.69	0.62	0.56	0.71	0.71	0.56
c	0.10	0.10	0.12	0.13	0.06	0.09	0.10
F-Wright (P-value)	$0.180^{0.331}$	$-0.013^{0.526}$	$0.102^{0.008}$	$0.044^{0.123}$	$-0.025^{0.558}$	$0.180^{0.234}$	$-0.068^{0.451}$
PO-5 (N)	72	64	74	65	60	62	39
a	0.13	0.12	0.09	0.19	0.08	0.18	0.10
b	0.16	0.13	0.16	0.15	0.18	0.11	0.05
c	0.12	0.15	0.16	0.21	0.20	0.07	0.22
d	0.13	0.12	0.12	0.13	0.18	0.16	0.27
e	0.28	0.34	0.31	0.19	0.27	0.27	0.26
f	0.19	0.15	0.16	0.12	0.09	0.23	0.10
F-Wright (P-value)	$-0.057^{0.000}$	$0.060^{0.174}$	$0.008^{0.201}$	$-0.098^{0.008}$	$-0.050^{0.400}$	$-0.001^{0.000}$	$0.286^{0.082}$
SOD-1 (N)	53	67	66	66	69	46	22
a	0.01	0.04	0.01	0.01	0.00	0.02	0.05
b	0.89	0.91	0.93	0.91	0.94	0.91	0.89
c	0.10	0.05	0.06	0.08	0.07	0.07	0.07
F-Wright (P-value)	$-0.117^{0.853}$	$-0.073^{0.899}$	$-0.065^{0.958}$	$-0.092^{0.896}$	$-0.070^{0.586}$	$-0.076^{0.948}$	$-0.095^{0.963}$
SOD-2 (N)	54	65	66	68	69	45	22
a	0.64	0.71	0.70	0.65	0.65	0.69	0.66
b	0.36	0.29	0.30	0.35	0.35	0.31	0.34
F-Wright (P-value)	$-0.565^{0.000}$	$-0.413^{0.001}$	$-0.435^{0.001}$	$-0.528^{0.000}$	$-0.533^{0.000}$	$-0.452^{0.003}$	$-0.517^{0.020}$
SOD-3 (N)	41	56	49	64	47	33	16
a	0.34	0.59	0.39	0.42	0.48	0.39	0.38
b	0.66	0.41	0.61	0.58	0.52	0.61	0.63
F-Wright (P-value)	$-0.193^{0.246}$	$-0.254^{0.066}$	$-0.375^{0.011}$	$-0.409^{0.001}$	$-0.236^{0.122}$	$-0.142^{0.465}$	$-0.600^{0.022}$
Polymorphic loci in H&W (%)	50	50	50	36	45	40	55
Total allele (100%)	66	65	67	66	64	66	66

* Number of analyzed plants per locus.

On average, the number of alleles observed varied from two to six for the polymorphic loci (Table 2). On average, within the seven populations, 65.7 alleles were found in all plants (Table 2). However, the allele distributions did not vary statistically among populations. The average number of apparent alleles (A) over all plants in all populations was 2.85 and the effective number (A_e) was 2.29 (approximately 20% lower than A). This pattern was similar in all forest remnants (Table 2). The observed heterozygous genotypes (H_O) frequency of 55% was slightly higher (51.3%) than expected (H_e) under H&W equilibrium. Fixation index (F_{IS}) per population revealed that all seven populations have slight excesses of heterozygous genotypes, since the F_{IS} values varied from -0,036 to -0,099, although, all populations were in H&W equilibrium (P<0.05).

Fixation coefficients estimated with the Wright's F-statistics (F_{IS}, Hedrick-G_{ST}) revealed a large variation among allozyme loci (Table 3). The average fixation index within populations (F_{IS}= 0.082) exhibited significant variations between loci (P <0.001), although close to 60% of the loci proved to be in H&W equilibrium (P >0.05). Genetic variation among populations (F_{ST}) was close to zero over all loci, ranging from 0.003 (SOD-2) to 0.064 (IDH). However, statistically significant variations were still detected among more than half of the loci (P<0.05). When all loci were included in the analysis, the F_{ST} of 0.016 suggested statistically significant deviations from inbreeding equilibrium (P<0.001). The

comparison between inbreeding index F_{ST} to its analogous Hedrick-G_{ST} indicated small variations in 20 out of 22 loci. With the exception of GOT-1, PGM-1 PO-3, PO-4 and SOD-1, the Hedrick-G_{ST} values were higher than F_{ST}. In the locus PGM-2, while the Hedrick-G_{ST} value was 0.125, the F_{ST} was five times smaller (0.024). Overall, the average Hedrick-G_{ST} was 0.031, and F_{ST} 0.016. When populations were compared pairwise, significant differences (χ^2 test, P >0.05) were detected in all combinations including SPA and in combinations of CCO with TUR and TIG (data not shown). The largest difference was observed between SPA and CCO (F_{ST} = 0.199).

Table 2. Means and standard deviations (SD) of 23 allozyme loci for number of plants analyzed; apparent (A) and effective (Ae) number of allele per loci; percentage of polymorphic loci with higher than 95% frequency; observed heterozygosity; and expected heterozygosity for Hardy & Weinberg equilibrium (EHW) according to Nei (1978); fixation index (FIS) and the H&W equilibrium (P<0.05) tested according to the Bonferroni test (RICE, 1989) for seven natural populations of *Sorocea bonplandii* from the Atlantic Forest, southern Brazil.

Tabela 2. Média e desvio padrão (SD) em 23 locos aloenzimáticos das plantas analisadas; número de alelos aparentes (A) e efetivos (Ae) por loco; percentagem de locos polimórficos com freqüência superior a 95%, heterozigosidade observada e esperada conforme Hardy & Weinberg equilíbrio (EHW) de acordo com Nei (1978); índice de fixação (FIS) e aderência ao equílibrio de H&W (P<0.05) testada de acordo o teste Bonferroni (RICE, 1989) para sete populações naturais de *Sorocea bonplandii* da Mata Atlântica, sul do Brasil.

Fragment	Means of all loci and SD				Mean heterozygosity and SD		Index
	plants	A	Ae	P (%)	observed	expected	FIS
TUR	62.6 ± 2.4	2.87 ± 0.24	2.27 ± 0.86	95.65	0.546 ± 0.046	0.509 ± 0.037	-0.073 ns
SOL	61.3 ± 1.7	2.83 ± 0.21	2.22 ± 0.81	95.65	0.552 ± 0.046	0.503 ± 0.036	-0.099 ns
CCO	64.4 ± 2.3	2.91 ± 0.23	2.35 ± 0.89	95.65	0.561 ± 0.045	0.521 ± 0.038	-0.077 ns
SVA	61.4 ± 2.5	2.87 ± 0.22	2.37 ± 0.95	95.65	0.566 ± 0.046	0.526 ± 0.037	-0.075 ns
TIG	57.1 ± 3.1	2.74 ± 0.21	2.29 ± 0.84	95.65	0.568 ± 0.044	0.518 ± 0.036	-0.097 ns
BRO	57.8 ± 1.8	2.87 ± 0.23	2.32 ± 0.83	95.65	0.544 ± 0.046	0.520 ± 0.036	-0.045 ns
SPA	35.0 ± 2.0	2.87 ± 0.24	2.20 ± 0.82	95.65	0.510 ± 0.044	0.492 ± 0.039	-0.036 ns
Means	57.09 ± 9.3	2.85 ± 0.05	2.29 ± 0.86	95.65	0.550 ± 0.018	0.513 ± 0.011	-0.072 ± 0.021 ns
all plants	399.7 ± 13.6	2.91 ± 0.23	2.35 ± 0.95	95.65	0.552 ± 0.043	0.519 ± 0.037	-0.064 ns

ns: deviation from H&W equilibrium not statistically significative (P>0.05) based on the Bonferroni test.

Table 3. Total inbreeding (Wright's F_{IT}); inbreeding within populations (F_{IS}); and inbreeding between populations (Hedrick-G_{ST}), at 22 allozyme loci from seven natural populations of *Sorocea bonplandii* from Atlantic Forest, southern Brazil.

Tabela 3. Endogamia total da espécie (F_{IT}), engogamia populacional (F_{IS}), e endogamia entre populações (Hedrick-G_{ST}), analisados sobre 22 locos alozimáticos de sete populações naturais de *Sorocea bonplandii* da Mata Atlântica, sul do Brasil.

loci	$F_{(IT)}$	$F_{(IS)}$	$F_{(ST)}$	Hedrick-$G_{(ST)}$
6PGDH-1	0.214 P<0.001	0.194ns	0.025 P=0.020	0.043
6PGDH-2	-0.022ns	-0.037ns	0.014 ns	0.017
EST-1	-0.205 P<0.001	-0.222ns	0.015 ns	0.026
EST-2	-0.239 P<0.001	-0.258ns	0.014 ns	0.031
FDH-1	-0.254P<0.001	-0.281ns	0.021 P=0.001	0.075
G6PDH-1	0.020ns	0.001ns	0.018 P=0.010	0.048
GOT-1	-0.274 P<0.001	-0.282 P=0.023	0.006 ns	0.000
IDH-1	0.486 P<0.001	0.467 P<0.001	0.036 P<0.001	0.090
MDH-2	-0.265 P<0.001	-0.300 P=0.016	0.027 P<0.001	0.091
MDH-3	-0.592 P<0.001	-0.603 P<0.001	0.007 ns	0.020
PGI-1	-0.337 P<0.001	-0.343 P=0.001	0.005 ns	0.005
PGM-1	0.125 P<0.001	0.117ns	0.008 ns	0.006
PGM-2	0.146 P<0.001	0.125ns	0.024 P<0.001	0.105
PGM-3	-0.253 P<0.001	-0.281 P=0.015	0.022 P<0.001	0.074
PO-1	0.103 P=0.001	0.090ns	0.014 P=0.043	0.020
PO-2	0.050ns	0.035ns	0.017 ns	0.018
PO-3	0.238 P<0.001	0.228ns	0.013 ns	0.005
PO-4	0.075ns	0.064ns	0.012 ns	0.009
PO-5	0.017ns	0.004ns	0.014 P=0.001	0.078
SOD-1	-0.079 P=0.020	-0.084ns	0.005 ns	0.004
SOD-2	-0.488 P<0.001	-0.492 P<0.000	0.003 ns	0.005
SOD-3	-0.267 P<0.001	-0.302 P=0.045	0.027 P=0.012	0.046
Means	-0.065 P<0.001	-0.082 P<0.001	0.016 P<0.001	0.031
(%) of loci (P >0.05)	22.7	63.6	54.5	-

ns: not significant for statistic significance of the Chi-squre test for fixation indices according to Li and Horvitz (1953) and Workman and Niswander (1970).

When genetic distances between natural populations were analyzed, high similarities (>95%) were evident (Figure 2). SPA exhibited the largest genetic distances from all other populations.

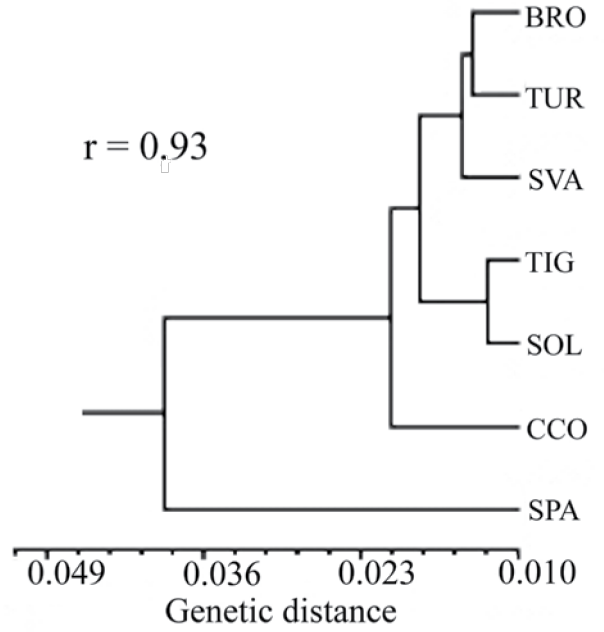

Figure 2. Cluster analysis of Nei´s (1972) unbiased genetic distances and cophenetic correlation coefficients (r) of seven populations of *Sorocea bonplandii* at the Atlantic forest remnants in southern Brazil, using a UPGMA dendrogram.

Figura 2. Dendrograma (UPGMA) da distância genética Nei´s (1972) e coeficiente de correlalção (r) de sete populações naturais de *Sorocea bonplandii* da Mata Atlântica no sul do Brasil.

DISCUSSION

Detailed knowledge of the genetic diversity of a species is crucial to determine the minimum number of plants required as a genetic stock for sustainable management and conservation of the species. The present study seems to indicate that, in spite of the extensive fragmentation of the forest, the current set of alleles in *S. bonplandii* is in equilibrium.

S. bonplandii shows characteristics indicative of high genetic diversity, such as within dioecious species (sex ratios may not be in equilibrium); and a long reproductive period. The flowering period lasts from August to November; fruit ripening from October to February, and seed shedding in all months of the year except April (VERGAMINI *et al.*, 2006; CAMPOS, 2007). Due to fruit production throughout the year, the species is an important food source for animals such as Cracidae (MIKICH, 2002) and *Alouatta guariba* (AGUIAR *et al.*, 2003). Furthermore, *S. bonplandii*, in spite of dynamic development of density and reproduction systems; its genetic diversity does not appear to suffer from forest fragmentation. Additionally, a period of less than a hundred years after fragmentation may still be too short to lead to genetic erosion. Boshier *et al.* (2004) found no impact of disturbance in an apparently undisturbed forest on fruit production in *Pachira quinata* and *Swietenia humilis*.

The slight differences in alleles between populations and between groups of diameter classes reflect the presence of rare alleles. This is evident when comparing the apparent number of alleles (A) with the effective number of alleles (Ae): the loss of alleles is evidenced by the difference in the number of effective alleles between groups of diameter classes (data no shown). This indicates that the frequency of rare alleles is greater in the group of plants with small diameter. Ruschel *et al.* (2007) using AFLPs data for the amount analyzed loci were observed high frequencies (20.7%) of rare AFLPs, in this loci (same size of AFLPs) occurs less to five percent.

In comparison to the observed allele frequencies (Ho), the expected frequency of heterozygous genotypes (He) was slightly larger and the F statistics showed a significant excess of heterozygotes in most loci. This indicates that selection may be acting against the homozygotes, as also previously observed in *Euterpe edulis* (CONTE *et al.*, 2003). Many environmental and biological factors in *S. bonplandii* populations can favour heterozygous genotypes such as: obligate outcrossing; wind pollination; flowering and fruiting over long and alternate periods; seed dispersion mainly by birds and monkeys; sapling amounts; the fact of being an opportunistic species in the forest gaps; and occurrence in high densities. Several of these characteristics potentially favour the mixing of alleles and gene flow, and contribute positively to high genetic diversity in the populations. However, the largest genetic variation among populations was due to SPA, which differentiated it from all others. That distinction can be attributed to its population density and the geographic distance from all others. In addition, in the forest fragment SPA, the forest colonization, which is a source of migrant alleles, occurred very recently, especially in the climax species *S. bonplandii* (RUSCHEL, 2009).

CONCLUSION

The genetic status of *S. bonplandii* populations were intensively analyzed by using allozyme markers. The results from the

study of these markers lead to the conclusion that the genetic status of *S. bonplandii* is similar among all forest fragments in southern Brazil. That genetic similarity among all studied populations of *S. bonplandii* was provided by all analyzed indices and genetic markers, although the SPA population is far apart from the others by more than 500 km.

These results were very similar to those obtained by using AFLP markers (RUSCHEL *et al*., 2007) on similar samples in similar populations. Thus, genetic similarity among populations can be inferred.

With these genetic characteristics, in addition to its use as a non-timber forest species (medicinal purposes), populations of *S. bonplandii* should be considered another indicator for the maintenance and protection of threatened forest remnants in future sustainable management of Brazilian tropical species.

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