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Research Article

The genetics and conservation of *Araucaria angustifolia*: I. Genetic structure and diversity of natural populations by means of non-adaptive variation in the state of Santa Catarina, Brazil

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Abstract

The objective of this study was to generate information relative to the allele distribution and dynamics within and among populations of *Araucaria angustifolia*, a naturally-occurring conifer in the south of Brazil, being known popularly as "pinheiro-do-Paraná", "araucaria" or pine tree. In order to elucidate the levels and the distribution of the genetic variability, the population's genetic structure and the genetic distance among natural populations of this species with different levels of disturbance in different geographical areas were studied in detail. For this, samples of leaf tissue were collected from 328 adult individuals in nine natural populations in Santa Catarina State. To analyze the samples, the allozyme technique was applied in starch gel electrophoresis (penetrose 13%), with citrate/morfholine buffer. Nine enzymatic systems (PGM, PGI MDH, PRX, SKDH, 6PGDH, ACP, IDH and G6PDH) revealed 15 loci. The analysis provided values for H_e and H_o of 0.084 and 0.072, respectively. The general average of polymorphic loci was 73% in the species and 26.6% in the studied populations and the allele number per locus was 1.6. Wright's F-statistical estimates indicated the existence of inbreeding in populations (F_{IS} = 0.148) and a low divergence among populations (F_{ST} = 0.044). However, the inbreeding values were variable in different populations. Taken together, the results indicated that the greater part of the genetic variability is contained within populations. The working hypothesis that originally there was greater genetic diversity can be supported by these results which indicate that in the degraded populations the diversity indexes are lower in the degraded populations than those found in better-conserved populations. Thus the fragmentation of the forest followed by "araucaria" exploitation could have contributed to the genetic differentiation expressed through the allele frequency of the studied population.

Key words: pinheiro, forest degradation, genetic diversity, genetic erosion.

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Introduction

Araucaria angustifolia (Bert.) O. Kuntze is a native species of the Tropical Atlantic Forest (Veloso and Goes Filho, 1982; Veloso *et al*., 1991). Natural populations or plantations can be found mainly in the three southernmost states of Brazil (Paraná, Santa Catarina and Rio Grande do Sul) where it is called "pinheiro-do-Paraná", "araucaria" or "pinheiro brasileiro". This species has also spread throughout other states such as São Paulo and Minas Gerais and in Argentina, particularly in the Province of Missiones (Klein, 1960; Carvalho, 1994).

In its region of natural occurrence, It is one of most important trees in its region of natural occurrence due to its relevant ecological, economic and social functions. As a dominant tree, populations of adult individuals create a microcosmic environment where shade-tolerant plant species of other taxa can grow and develop. Naturally, the seeds feed the wild fauna, including birds and rodents, which are the main araucaria seed dispersors. As the seeds have a high nutritious value, humans also use them for food. Seed collection and trading constitutes a source of income to an undetermined number of families living near the natural araucaria populations of araucaria. Its wood high quality wood, which gives it its main economic value, can be used for almost everything, especially housing, furniture, and pulp (Carvalho, 1994, Reitz *et al.,* 1978).

Prior to European contact, the araucaria forest size was estimated to be 200,000 km^2. Uncontrolled exploitation, mainly in the 20th century, led the species to the vulnerable category (IUCN), although some scientists are now aware of its imminent extinction. Most of the remaining araucarias, about 1 to 3% (Guerra *et al*., 2000), are still under pressure of exploitation pressure by the timber industry. Therefore, conservation and sustainable management strategies for this species should be implemented as soon as possible. A knowledge of the population's genetic structure is crucial to define such strategies properly, a knowledge of the population's genetic structure is crucial.

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The genetic structure of natural populations has been studied more intensely since the development and utilization of non-adaptive markers, since they provided the means to interpret observations at the gene level. These studies have characterized how genetic variation is distributed among and within populations and which factors are associated with, or determinant of, the structure itself (Lewontin and Hubby, 1966; Hamrick *et al*., 1979; Hamrick and Godt, 1989; Reis *et al*., 1998). Information about the existing extent and organization of genetic variation at species level in their ecosystems helps the definition of strategies not only for breeding, but also for management and genetic conservation.

In this context, isozyme electrophoresis has been widely used in more than a thousand species (Lewontin, 1991) because of its favorable attributes: simplicity of methodology, low cost, simple molecular basis used for polymorphism, and absence of environmental effects, almost without exceptions. Allozymic loci can be effectively identified in a large number of individuals in a short period of time. Since 1980, several studies have been carried out to characterize the genetic structure of natural populations of tropical species by the use of isozyme markers (Hamrick and Loveless, 1986; Loveless and Hamrick, 1987; Buckley *et al*., 1988; Murawsky and Hamrick, 1991; Hamrick and Murawsky, 1991; Eguiarte *et al*., 1992; Reis *et al*., 1998).

Several authors have noted genotypic variation within the araucaria species. The large distribution probably contributes to its differentiation in botanical varieties or ecotypes (Carvalho, 1994). Reitz and Klein (1964) described nine botanical varieties of *Araucaria angustifolia*: 1) *elegans;* 2) *sancti josephi;* 3) *angustifolia;* 4) *caiova*; 5) *indehiscens*; 6) *nigra;* 7) *striata*; 8) *semi-alba*; and 9) *alba.* The two main criteria used to distinguish them were ripening time and seed color. An additional variety, *catarinensis*, has an uncovered seed ventral face uncovered (Mattos, 1994).

The existence of geographical races was also demonstrated by Gurgel and Gurgel Filho (1964), who based their inference on the differences shown by distinct provenances. Kageyama and Jacob (1980) detected the existence of genetic variations within and among three natural populations, whose amounts were distinct from those of populations established at different altitudes. The differences in the wood volume from 24 provenances collected in five Brazilian States (MG, SP, PR, SC, and RS) were statistically significant, indicating the existence of genetic variation in this trait (Monteiro and Speltz, 1989).

However, a few studies have been carried out with biochemical and molecular markers in araucaria. Mazza (1997), using RAPDs markers was able to associate lower genetic similarities with larger geographical distances among populations. Later, Shimizu *et al*. (2000) established the values of 0.240 and 0.248 for the observed and expected heterozygosity, respectively. In this study, the authors detected 2.4 alleles per allozymic locus in 120 individuals from The Parque Nacional do Iguaçu. Using PCR-AFLP of chloroplast DNA, Schogl (2000) detected 12 haplotypes, 72% of which were common to all eight Santa Catarina araucaria populations. In this study, the total diversity (H_T) was 0.612 and the average difference among populations (G_{ST}) was 0.28.

The objective of this study was to characterize the genetic structure of natural fragmented populations with isozyme markers to provide support for the development of species conservation, sustainable management, and breeding strategies. In addition, inferences about the effects of the fragmentation on the genetic structure of natural populations were made.

Materials and Methods

Population sampling

Leaf samples were randomly collected from 328 adult individuals of nine naturally-occurring populations in different regions of Santa Catarina: FLONA of Três Barras (FTB), Fazenda Rancho Alegre (FRA), Fazenda Antonio Carlos (RAC), Fazenda Amola Faca (FAF), Fazenda Guamirim Gateados (FGG), Parque Municipal de Lajes (PML), Estação de Caçador (ECA), Fazenda Urupema (URU), and ARIE of Victor Meirelles (AVM) (Figure 1). The geographic location, altitude, area size, population size, forest type, and a partial history of the use of the nine populations are included in Table I. The leaf samples (needles) were taken from adult plants that had a diameter at breast height

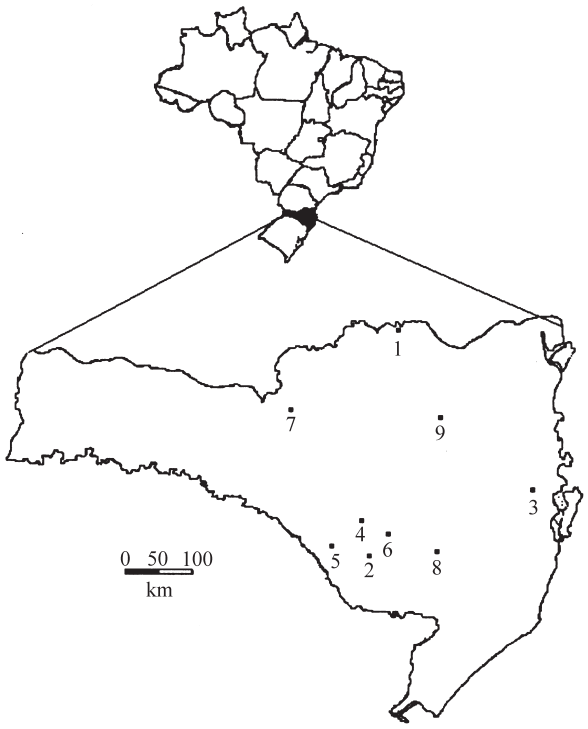

Figure 1 - Geographic location of the sampled populations in Santa Catarina state (Brazil): 1-FTB, 2-FRA, 3-RAC, 4-FAF, 5-FGG, 6-PML, 7-ECA, 8-URU and 9-AVM.

Table I - Geographic location, altitude, area size and population size, forest type, and area history of the nine *Araucaria Angustifolia* populations.

Population	Location	Conservation Unit Type/Institution[1]	Geographic coordenates Lat. / Long.	Altitude (m)	Area (ha)/ Population size	Forest type	History of use
FTB	Três Barras	FLONA/IBAMA	26º06'23" 59º19'20"	802	500/27,000	Araucaria Mixed Forest	Selective cut - 50 years ago. Today: secondary forest.
FRA	Lages	Reserve/ Private property	2753'18" 5015'18"	918	30/2,700	Araucaria Mixed Forest and Grassland	Adults plants with 80 years. Conserved area.
RAC	Antonio Carlos	Reserve/ Private property	2725'84" 4850'98"	600-910	5,000/500	Ombrophyl Dense Forest / Araucaria Mixed Forest	Timber exploitation in the 60s. Grazing in the period 1960-1989. Since that, forest reserve.
FAF	Lages	Reserve/EPAGRI	27°48'58" 50°19'34"	918	20/1,700	Araucaria Mixed Forest and Grassland	Anthropic intense perturbation 50 years ago: selective cut and grazing.
FGG	Campo Belo do Sul	Reserve/ Private property	27°53'57" 50°43'39"	960	1,000/40,000	Araucaria Mixed Forest and Grassland	Wild conserved area with abundant fauna and araucaria seeds.
PML	Lages	Park/Municipality	2747'52" 50°22'3"	918	20/400	Araucaria Mixed Forest	Very degraded area.
ECA	Caçador	Reserve/EPAGRI	26°46'31" 26°46'31"	920	772/10,000	Araucaria Mixed Forest	Conserved since 1948, with primary forest composition.
URU	Urupema	Private Property	27°57'25" 49°53'93"	980	50/3,000	Araucaria Mixed Forest	Selective cut in the past, but conserved afterwards.
AVM	Victor Meirelles	ARIE/Municipality	26°40'26" 49°49'50"	400-800	3,440/4,000	Ombrophyl Dense Forest / Araucaria Mixed Forest	Degraded area with selective cut in the 1980's.

[1]FLONA- National Forest; ARIE - Area of Relevant Ecological Interest; IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis; EPAGRI-Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina S.A.

(DBH) greater than 35 cm, after which they were transported in insulated boxes to the laboratory and stored in a refrigerator (5 to 10 °C) until use.

Isozyme electrophoresis

Isozyme electrophoresis was performed with starch gels 13% (w/v) composed of corn Penetrose 30 (Cornproducts, Mogi Guaçu-SP). Four gel/electrode buffer systems were tested: 1) morpholine-citrate (citric acid 0,04 M and N-[3-amino-propyl] morpholine, pH 6.1; Cheliac and Pittel,1984), 2) tris-citrate (Tris - Citric acid, pH 6.6) / histidine [L-Histidine 0,05 M pH 6.0]; Paiva, 1992), 3) Lithium (tris-citrate [Tris - Citric acid 0,05 M pH 8.5] / Lithium hydroxide 0.06 M pH 8.1; Alfenas *et al*., 1991) and 4) Histidine (L-Histidine-HCl 0,05 M pH 7,0 / Tris 0.125 M pH 7.0; Alfenas *et al*., 1991). Electrophoretic buffer systems were used to assay 20 enzymatic systems (Auler, 2000). Nine enzymatic systems were selected to perform the population analyses: PGM, PGI, MDH, PRX, SKDH, 6PGDH, ACP, IDH, and G6PDH.

Genetic indexes

The allozymic data were utilized to estimate the genetic indexes: 1) allele number per locus (total number of alleles/number of loci); 2) percentage of polymorphic loci (total number of polymorphic loci x 100/number of loci); 3) allelic frequencies (Weir, 1990); 4) expected heterozygosity ($He = 1-\Sigma p_i^2$, p_i being the mean frequency of the *i* allele in a locus; Nei, 1973); 5) observed heterozygosity (direct counting); and 6) Wright's fixation index. To address the population's genetic structure it was estimated the genetic diversity among and within populations (Nei, 1973, 1977, 1978) and the statistics F of Wright (Wright, 1951, 1965) were estimated. The chi-squares (χ^2) for the Hardy-Weinberg equilibrium deviation, based on observed and expected frequencies, grouped or not, and for the inbreeding equilibrium, were estimated as suggested by Li and Horvitz (1953). A locus was considered polymorphic when the frequency of the most common allele did not reach a frequency value higher than 0.95. The BIOSYS-1 computer program (Swofford and Selander, 1989) was used to estimate the allelic frequencies and the mentioned genetic indexes.

According to Nei (1973), the variation in gene frequency among subpopulations may be analyzed by the F-statistics of Wright: $[1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})]$, where F_{IT} and F_{IS} are correlations between two uniting gametes to produce the individuals relative to the total population and relative to the subpopulations, respectively, and F_{ST} is the correlation between two gametes drawn at random from each subpopulation. These three indices can then be taken as a deviation from Hardy-Weinberg equilibrium and F_{ST}

can be interpreted as a genetic index of divergence among subpopulations. Using Wright's statistics, the author found that the gene diversity in the total population or total heterozygosity (H_T) can be distributed within (H_S) and among subpopulations (D_{ST}), and the gene differentiation among subpopulations (G_{ST}) is estimated by the proportion D_{ST}/H_T.

Genetic similarities

Genetic similarities and genetic distances among populations were estimated according to Nei (1972, 1973, 1978). The obtained values were utilized to construct a dendrogram based on the unweighted paired group method (UPGMA method; Sneath and Sokal, 1973).

Results

Selection of the isozyme systems

Nine out of 80 combinations of electrophoretic buffers/isozyme systems, which showed better band color intensity and separation, were selected to perform this study. Among them, eight selected systems (PGI, PGM, MDH, SKDH, IDH, 6PGDH, ACP, and G6PDH) showed the best resolution in the morpholine-citrate buffer. Although the color intensity for PRX was also obtained with tris ci-

Table II - Allelic frequencies for 15 allozymic loci analyzed in 328 individuals of nine populations of *Araucaria angustifolia* in Santa Catarina.

		Populations/Sample size								
Locus	Allele	FTB	FRA	RAC	FAF	FGG	PML	ECA	URU	AVM
		33	25	34	42	41	28	45	37	43
PGI-1	1	1.000	1.000	0.971	1.000	0.902	0.893	1.000	1.000	1.000
	2	0	0	0.029	0	0.098	0	0	0	0
	3	0	0	0	0	0	0.107	0	0	0
PGI-2	1	0.883	0.760	0.882	1.000	0.817	0.796	0.878	0.889	0.940
	2	0.100	0.240	0.059	0	0.122	0.204	0.078	0.083	0.060
	3	0.017	0	0	0	0.037	0	0	0	0
	4	0	0	0.059	0	0.024	0	0.044	0.028	0
PGM-1	1	0.967	0.960	0.956	1.000	0.939	0.982	0.967	0.973	0.988
	2	0	0	0	0	0.061	0	0	0	0
	3	0.033	0.040	0.044	0	0	0.018	0.033	0.027	0.012
MDH-1	1	1.000	1.000	1.000	1.000	0.976	1.000	1.000	1.000	1.000
	2	0	0	0	0	0.024	0	0	0	0
MD-2	1	1.000	1.000	1.000	1.000	0.939	1.000	0.978	1.000	1.000
	2	0	0	0	0	0.061	0	0.022	0	0
6PG-1	1	0.064	0.609	0.556	0.414	0.610	0.696	0.656	0.392	0.577
	2	0.019	0.043	0.019	0.029	0.085	0.089	0.056	0.108	0.038
	3	0.327	0.348	0.407	0.529	0.305	0.214	0.289	0.500	0.385
	4	0	0	0.019	0.029	0	0	0	0	0
PRX-1	1	0.833	1.000	0.941	0.952	1.000	1.000	0.856	0.931	0.953
	2	0.167	0	0.059	0.049	0	0	0.144	0.069	0.047
PRX-3	1	1.000	1.000	1.000	1.000	1.000	1.000	0.932	0.986	0.965
	2	0	0	0	0	0	0	0.068	0.014	0.035
PRX-4	1	0.883	1.000	1.000	0.857	0.863	1.000	0.909	0.919	0.986
	2	0.117	0	0	0.143	0.138	0	0.091	0.081	0.014
PRX-5	1	1.000	1.000	1.000	1.000	0.939	1.000	0.976	0.986	0.988
	2	0	0	0	0	0.061	0	0.024	0.014	0.012
SKDH-1	1	1.000	1.000	0.971	1.000	0.988	0.982	0.911	0.919	0.988
	2	0	0	0.029	0	0.012	0.018	0.089	0.081	0.012
SKDH-2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G6P-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDH-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ACP-1	1	0.833	1.000	0.956	0.963	0.975	1.000	0.978	0.944	1.000
	2	0.167	0	0.044	0.037	0.025	0	0.022	0.056	0

trate/histidine buffer, this system was equally run under morpholine-citrate buffer because a higher number of activity regions of activity (four against three loci) were detected, without losing any evaluation quality. Overall, 15 putative loci were revealed by these nine systems (Auler, 2000).

Genetic diversity

The average number of alleles per locus, taking into account the entire set of the 15 putative loci, was 1.6 and ranged from 1.3 (FRA) to 1.9 (FGG). Among loci, The number of alleles detected among the loci ranged from one (loci IDH-1, G6PDH-1 and SKDH-1) to four (loci PGI-2 and 6PG-1). A total of 33 alleles were found in these nine populations, an average of 23.7 per population (Table II)

Allelic frequencies from different loci ranged from zero to 1.0, depending on the locus and the population (Table II). For all loci with two detected alleles in all populations, one of them was usually at a frequency which was 4 to 30 times higher than the frequency of the second allele. Within a population, the percentage of polymorphic loci ranged from 13.3% (FRA, FAF, and AVM) to 46.7% (FGG), and the average was 26.6% (Table III). In fact, populations FGG and ECA exhibited at least two alleles in 10 out of 15 loci. However, at species level this value reached 73.3%. When only the polymorphic loci were analyzed, the average value for the percentage of polymorphic loci was 33.3%.

Among populations and across loci, the mean expected heterozygosity (H_e) ranged from 0.060 (AVM) to 0.116 (FGG), average 0.084, and the mean observed heterozygosity (H_o) ranged from 0.044 (AVM) to 0.104 (URU), average 0,073 (Table III). The H_e average value was 0.105 when only the polymorphic loci were analyzed.

Among the nine populations, ECA and URU fitted the panmitic equilibrium (Table IV). However, by grouping the classes with the least frequent alleles, adherence to the HW equilibrium was reached by all nine populations. The adherence to the inbreeding equilibrium was performed for all loci when sufficient genotypic classes (or degree of freedom) were available. The results indicated that all populations were in inbreeding equilibrium (Table IV).

Population genetic structure

The mean total heterozygosity (H_T) for all nine populations, estimated according to Nei, was 0.089, and for sub-populations (H_S) it was 0.084. The G_{ST}, which indicates how much of the genetic diversity is among populations, reached the value of 0.056. Similar analysis through Wright F-statistics of Wright revealed different values of F_{IS}, F_{IT} and F_{ST} values for different loci (Table V). While a value of 1.000 was found for F_{IS} in the loci PGI-1 and MDH-1 loci, a value of -0.088 was obtained with locus PRX-4. For F_{IT}, similar results were found (Table V). The greatest genetic diversity among populations, F_{ST} = 0.075, was revealed by the locus PGI-1. Although the F_{ST} values were variable from locus to locus, the mean value of 0.044 was similar to thevalue of G_{ST} (0.056) value. In addition, these statistics revealed the existence of a certain degree of inbreeding, not only within population populations but also at species level.

The F_{ST} values for selected associations of populations are included in Table VI. The lowest value of F_{ST} value (0.005) was revealed when RAC and AVM were

Table III - Genetic diversity indexes for nine *Araucaria angustifolia* populations based on 15 loci.

Populations	N	A*	P**	H_o	H_e***	F
FTB	28.9	1.5	33.3	0.061 ± 0.031	0.102 ± 0.039	0.402
FRA	21.6	1.3	13.3	0.061 ± 0.039	0.065 ± 0.041	0.061
RAC	33.2	1.7	20.0	0.061 ± 0.032	0.077 ± 0.036	0.208
FAF	39.5	1.4	13.3	0.049 ± 0.030	0.064 ± 0.039	0.234
FGG	39.7	1.9	46.7	0.092 ± 0.037	0.116 ± 0.039	0.207
PML	26.7	1.4	20.0	0.058 ± 0.038	0.071 ± 0.037	0.183
ECA	44.0	1.8	40.0	0.121 ± 0.039	0.108 ± 0.035	-0.120
URU	35.6	1.7	40.0	0.104 ± 0.040	0.096 ± 0.040	-0.083
AVM	41.0	1.6	13.3	0.044 ± 0.020	0.060 ± 0.034	0.267
Populations mean	34.5	1.6	26.6	0.073	0.084	0.143
Species mean	-	2.0	73.3			
No degraded	41.8	1.8	43.3	0.106	0.112	0.054
Degraded	31.5	1.4	19.9	0.053	0.059	0.102
PNI	120.0	2.3	80.0	0.240	0.248	0.030

*Standard mean error = 0.2; ** If the 95% criterion for frequency of the most common allele is used; *** Unbiased estimate of NEI (1978) ± standard mean error.
PNI = Parque Nacional do Iguaçu (Shimizu *et al.*, 2000).

Table IV - Chi-square (χ^2) for the Hardy-Weinberg equilibrium deviation based on observed and expected frequencies, grouped or not, and inbreeding equilibrium, of nine natural *Araucaria angustifolia* populations.

	Hardy-Weinberg equilibrium						
	Classes not grouped			Classes grouped*			Inbreeding equilibrium
Populations	GL	χ^2	Phw	GL	χ^2	Pp	$F_{(P)}$
FTB	10	56.611	< 1	2	1.111	70-50	50-30
FRA	5	24.153	< 1	1	0.208	70-50	20-10
RAC	14	85.766	< 1	2	0.874	70-50	90-80
FAF	9	83.884	< 1	1	4.775	4-2.5	90-95
FGG	17	139.396	< 1	2	0.457	80-70	80-70
PML	7	36.006	< 1	1	1.996	20-10	50-30
ECA	14	6.942	95-90	2	1.653	50-30	70-50
URU	13	9.716	70-50	2	1.887	50-30	50-30
AVM	10	24.637	< 1	1	6.953	1-0.2	50-30

*Grouped alleles: number of homozygotes for the most common allele, number of heterozygotes among common and rare alleles, number of rare homozygotes, and other heterozygotes.

Table V - Estimates of Wright F-statistics of Wright of 328 adult individuals from nine populations of *Araucaria angustifolia*.

Locus	$F_{(IS)}$	$F_{(IT)}$	$F_{(ST)}$
PGI-1	1.000^{ns}	1.000^{ns}	0.075**
PGI-2	0.032^{ns}	0.076^{ns}	0.045^{ns}
PGM-1	0.073^{ns}	0.087^{ns}	0.016^{ns}
MDH-1	1.000^{ns}	1.000^{ns}	0.022^{ns}
MDH-2	0.564^{ns}	0.582**	0.042**
6PGD-1	0.111*	0.144**	0.038^{ns}
PRX-1	0.324 **	0.364**	0.059**
PRX-3	-0.055^{n}	-0.013^{ns}	0.039*
PRX-4	-0.088^{ns}	-0.027^{ns}	0.056**
PRX-5	-0.044^{ns}	-0.013^{ns}	0.030^{ns}
SKD-1	0.060^{ns}	0.098^{ns}	0.040**
ACP-1	0.417**	0.455**	0.064**
Mean	0.148 *	0.186**	0.044**

ns = not significant.
* and ** = Significant at p 0.01and at p 0.05 by χ^2 test, respectively.

Table VI - Selected estimates of F_{IS} , F_{IT} and F_{ST} estimates of contrasting individual or grouped populations of *Araucaria angustifolia* based on 15 loci.

Populations	F_{IS}	F_{IT}	F_{ST}
All populations	0.148*	0.186**	0.044**
PML x FRA x FAF x FGG x URU	0.120	0.165	0.052**
ECA x FTB x AVM x RAC	0.181	0.200	0.023**
PML x FAF x FRA	0.146	0.203	0.067**
FGG x ECA	0.076	0.091	0.016**
FTB x URU	0.183	0.206	0.028*
FGG x AVM	0.218	0.234	0.020**
RAC x AVM	0.218	0.222	0.005**
ECA+FGG x AVM+RAC	0.139	0.148	0.011**
ECA+FGG x AVM+PML	0.138	0.146	0.009**
PML x FAF+ FRA	0.183	0.215	0.039*

ns = not significant, * and ** significant at p 0.01and p 0.05 by χ^2 test, respectively.

compared, both located in areas with ecological tension. The value of this parameter jumped to 0.028 when very distant (±200 km) populations were compared (FTB and URU) and to 0.067 when populations PML, FAF and FRA were put together. Although the values of the F-statistics are low, they are statistically different from zero, as indicated by the heterogeneity (contingency) tests.

Genetic similarity among populations

The similarity among populations ranged from 0.992 to 0.999 (data not shown). In three cases, the genetic distance between two populations was near zero: RAC and AVM, FRA and PML, and FAF and URU.

Two main groups were formed by UPGMA with the Nei unbiased genetic distance of Nei (Figure 2). The first of these includes populations FAF and URU. Two sub-groups

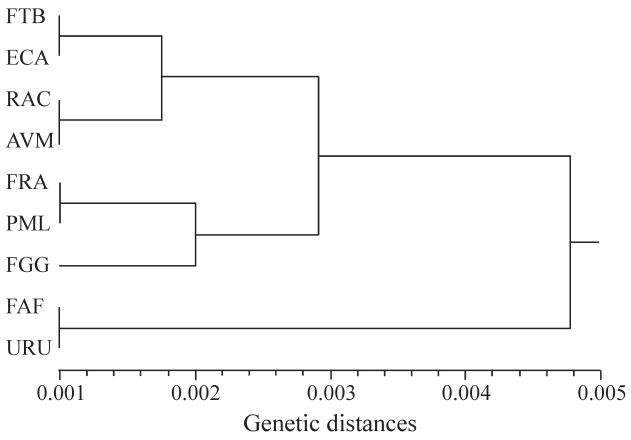

Figure 2 - UPGMA dendrogram reflecting the genetic similarities among nine populations of *Araucaria angustifolia* based on allelic frequencies of 15 loci.

form the second: a) populations FTB, ECA, RAC and AVM and b) populations FRA, PML and FGG. While populations of the first sub-group are located in the north of Santa Catarina, the others from the second sub-group are located in the southern region of the state. Although they do not overlap geographically, the populations of each group are included in areas with similar climatic conditions and soil.

Discussion

This study demonstrated that nine out of 80 electrophoretic buffer/isozyme system combinations could be used routinely, because they provided high resolution and unambiguous zimogram patterns. Some of the selected systems, such as tris-citrate/histidine, also showed also their potential for use with other gel/electrode buffers, depending on further adjustment in their protocols.

Since no genetic studies of allozymic loci have been carried out on this species, the inference of the existence of a locus was based on studies of other plant species. Nevertheless, several genetic indexes were estimated to describe the genetic structure of the remaining natural populations in Santa Catarina state, based on these non-adaptive markers.

As expected, with the increase in the number of analyzed individuals, the number of alleles per locus increased. At populational level, the number of alleles ranged from 19 to 28 in 15 loci, when 25 to 45 plants were assayed. However, at species level, in the same loci, 33 alleles were captured when the sample reached 328 plants.

Unique alleles were detected in FGG and PML, the most preserved and degraded areas, respectively, both in the Lages region. Rare alleles were shared by populations the RAC and FGG, FTB and FGG, ECA and FGG, and FAF and RAC populations. It is intriguing that unique or rare alleles were found in six of the nine populations. However, populations located in the Lages region maintain most of them. Thus, it is reasonable to advocate that any plan for conservation should include this region.

From the amount of survey of different plants sampled across the state of Santa Catarina State (328), three out of the 15 loci were monomorphic. The other 12 loci were polymorphic at species level, but not all of them were polymorphic at populational level. In addition, for polymorphic loci, it is relevant to mention that the proportion between the frequencies of the most frequent allele and its companion allele, in all loci with two alleles, was very high, usually higher than 4.

The percentages of polymorphic loci (mean 43.3%, Table III) and numbers of alleles per locus (mean 1.8) were substantially higher in the populations FGG and ECA populations, both under good conservation status, in comparison with the values (mean 20.0% and 1.4, respectively) revealed by FTB, FAF, PML and AVM, the most degraded populations. This comparison is very useful, since all populations are fragments of the same forest, with different degrees of degradation and conservation. In fact, the indiscriminate exploitation of pinheiro-do-Paraná is responsible for a significant proportion of the genetic erosion of this species.

The two most conserved populations, FGG and ECA, also showed the highest values for observed (H_o = 106) and expected (H_e = 0.112) heterozygosity. The opposite (H_o = 0,053; H_e = 0,059) was verified with the populations FTB, FAF, PML and AVM populations, the most exploited among the surveyed populations. Shiraishi (1983) classified the conifers as the taxa with one of the greatest values for H_e values (= 0.207) in comparison with other taxa. An exception was indicated by Hamrick *et al*. (1989), since they found the lowest value for genetic diversity in *Pinus longeava* among 653 reports on 449 species from 165 genera. More recently, the mean heterozygosity values estimated for species of the genus *Larix* (a *Pinaceae*) ranged from 0.048 to 0.170 (Semerikov *et al*., 1999). All heterozygosity values estimated for pinheiro-do-Paraná, in the present study, are included in this interval.

For a group of tropical trees and a wind-pollinated species group, Hamrick and Godt (1989) obtained mean heterozygosities of 0.109 and 0.123, respectively. Taking into account the fact that pinheiro-do-Paraná is a wind-pollinated tree, its heterozygosity values are lower than those estimated by Hamrick and Godt (1989). Additionally, for some Atlantic Rain Forest species such as *Euterpe edulis* (Reis *et al*., 1998), *Cedrela fissilis* (Gandara, 1996) and *Myracrodruon* urundeuva (Moraes, 1992; Lacerda, 1997) the mean heterozygosity values ranged from 0.378 to 0.570; 0.222 to 0.222, and from 0.076 to 0.160, respectively.

In seven of the nine populations, The observed heterozygosity values were lower than expected in seven of the nine populations, indicating the presence of a certain level of inbreeding (F ranged from 0.061 to 0.402). In fact, the adherence of the nine populations to the inbreeding equilibrium model reinforced the existence of inbreeding in this species. This inbreeding could in part have arisen from non-random mating, since it is possible that the surveyed populations still represent the population structure's pre-fragmentation structure, and that insufficient time has passed for the reconstruction of an entire structure. Additionally, genetic drift could have played a role in this case. However, it cannot be ruled out that a small proportion of new individuals could have appeared after the forest fragmentation in some of the populations. In the remaining two populations, ECA and URU, an excess of heterozygotes was found.

Both approaches to address the populational structure features indicated similar patterns. Thus, among populations, the degree of genetic diversity was not so great, varying from 0.044 (F_{ST}) to 0.056 (G_{ST}). This low amount of genetic diversity among populations was also revealed by the estimates of genetic distances. However, this kind of

data should be taken with caution because the variation under analysis is of a non-adaptive type. To further define conservation strategies, other parameters, such as adaptiveness (*e.g.*: fitness and development) or agronomic interest (*e.g.*: woody quality) should be taken into account.

Among gymnosperms, there is a strong variation in the population structure among gymnosperms. The values of the *Araucaria angustifolia* genetic indexes are either higher or lower than the values obtained in other species of this taxa group, depending on the comparison. Hence, Ge *et al.* (1998) found a very high divergence (F_{ST} = 0.441) among eight populations of *Cathaya argyrophylla* (a subtropical conifer from China). Kitamura and Rahman (1992) also obtained higher values with natural populations of *Agathis borneensis* (*Araucariaceae)* from southeast Asia (H_T = 0.122, H_S = 0.106 and G_{ST} = 0.140). In contrast, Hamrick and Smith (1987) obtained a value of G_{ST} = 0.016 for 17 populations of *Pinus contorta*, a temperate climate wind-pollinated conifer, which was three times smaller than the value (G_{ST} = 0.056) estimated for *Araucaria angustifolia*. In fact, the value of 5.6% is very close to 6.8%, a G_{ST} average for wind-pollinated conifers based on isozyme markers (Hamrick and Godt, 1989).

Although polyzygotic polyembryogenesis occurs in pinheiro-do-Paraná (Guerra *et al.*, 2000), only one embryo reached maturity within the magagamephophyte, a maternal tissue responsible for feeding the germinating embryo and the development of the new plant. If it is a filter, a stabilizing selection could be conservative in this case. But the role of this event in the amount of genetic diversity of the species, whether adaptive or non-adaptive, is still not known.

The geographically closed populations FAF, PML, and FRA populations showed discrepant levels of variation. When they were taken as a whole the F_{ST} was 0.067, higher than the average (0.044). This value is considerably higher than that exhibited by comparing two distant populations, FTB and URU (0.028). This and other comparisons are not in agreement with the hypothesis that geographically close populations located near one another show low genetic divergence due to the intense gene flow. The pinheiro-do-Paraná is a typical dioecious species, with a low frequency of monoecious plants, indicating the obligatory pollen movement. However, it not known how far the pollen travels. In addition, before European contact, the araucaria forest was a continuum forest with many natural seed dispersers. Thus, it is reasonable to invoke genetic drift as a cause of those discrepancies, as a consequence of the intense timber exploitation. This clue was given by the difference between the genetic diversity indexes for FGG (the most conserved) and the nearby populations FRA, PML, and FAF populations (most degraded ones), which could be attributed to anthropoid action.

Concerns raised about genetic erosion are pertinent under such an exploitation rate, notably in small populations. Hall *et al*. (1996) examined the genetic diversity and population differentiation of *Pithecellobium elegans*, a neotropical rain forest canopy tree from Costa Rica. Eight forest fragments and a large reserve (1,500 ha) were compared for several parameters of population genetics. Allozyme heterozygosity (0.13), polymorphism (35%) and effective number of alleles (1.24) were all similar to the values reported for other tropical tree species that also occur at densities of less than one individual per hectare. However, these measures of genetic variation were lowest in populations of the smallest size, farthest from the reserve, and more isolated from other populations. In addition, differentiation among samples collected in small forest fragments and the reserve population accounted for 10% of the total genetic variation observed. The authors found a positive relationship between the level of differentiation of populations from the reserve population and their distance from the reserve. Thus, the fragmentation of what was once a large, continuous forested area is now resulting in the genetic erosion of small, isolated populations of *Pithecellobium elegans*.

In fact, the results presented here are dependent on both fragmentation and degradation. The trend 'the lower the fragment size, the lower the genetic diversity' has been tested at various different opportunities. Prober and Brown (1994) detected a significant correlation among the population size and the genetic diversity values for *Eucalyptus albens* in Australia. The more far-apart populations and those with less than 500 individuals were the ones that showed the least genetic variation. A similar correlation was found by Billington (1991) in *Halocarpus bidwillii*, a dioecious conifer native to New Zealand. The fragmentation of *Pithecellobium elegans* populations of *Pithecellobium elegans* was the cause of the genetic erosion according to Hall *et al.* (1996).

In the case of pinheiro-do-Paraná, the highest values for numbers of alleles per locus, percentages of polymorphic loci, and observed heterozygosity were verified in the most conserved populations, even though they were isolated by fragmentation. The history of use of these populations revealed that they had not been exploited at all or the exploitation level was very low in comparison with that of the populations FAF, PML, AVM and FTB populations, which are fragments that were highly exploited before the second half of the 20th century, and showed a low level of genetic diversity.

Conclusion and Remarks on the Future

At this point we can summarize the pinheiro-do-Paraná population diversity features: rare alleles in few populations, alleles with low frequencies at most loci, a variable percentage of polymorphic loci, and low genetic divergence among populations. How can this be explained? The first idea is that these features are intrinsic to the species. Alternatively, the effects of fragmentation followed

by exploitation or tree removal, which can be called `degradation', caused major genetic erosion in this species. The fact that a rare allele was found in The Parque Municipal de Lages (PML), a degraded population, and a higher number of alleles was found on the Fazenda Guamirim Gateados (FGG) suggests that genetic diversity was higher prior to European contact. But how much higher it was than it is today has not been determined yet.

Besides gene flow and generation overlap, which favor genetic exchange, the isolation process could contribute to the low level of inter-populational diversity. The forest fragmentation has occurred within the last 100 years, a recent event in this long-living species' history. Thus, the time after fragmentation has so far been insufficient to allow a more substantial differentiation among populations.

By combining this low amount of genetic variation found among populations with a small-forested area, not bigger than 3% of the original area (Guerra *et al*., 2000), we could claim the existence of a fragile state of polymorphism in the pinheiro-do-Paraná. From a biological point of view, the larger populations are far apart in most cases. Beneath them, the regeneration is very sparse. The species does not spread as it did in the past. The situation is fragile not only in terms of the amount of genetic variation, but also in terms of areas of conservation. The danger is that any further exploitation of these already threatened populations could reduce the currently fragile level of polymorphism by chance alone.

Although it is often possible to establish the exact status of genetically degraded species, there are populations such as those at Fazenda Guamirim Gateados and EPAGRI Caçador that show greater polymorphism than other populations. Moreover, populations located in transition areas such as ARIE of Victor Meirelles and The Antonio Carlos Reserve should be included in conservation and breeding programs.

The different indexes obtained in this study suggest that any kind of indiscriminate exploitation should be halted immediately to avoid further genetic erosion or increase in the genetic vulnerability of the species. Furthermore, it is necessary to complement and associate this data with that related to the species' auto-ecology to better design adequate conservation and breeding strategies for the sustainable management of the *Araucaria angustifolia*.

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