

UNIVERSIDADE ESTADUAL DO CENTRO-OESTE, UNICENTRO-PR

**EFFECTS OF SELECTIVE LOGGING ON
GENETIC DIVERSITY OF *Araucaria angustifolia*
(Bertol.) Ktze**

MASTERS DISSERTATION

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EFFECTS OF SELECTIVE LOGGING ON GENETIC DIVERSITY OF
Araucaria angustifolia **(Bertol.) Ktze**

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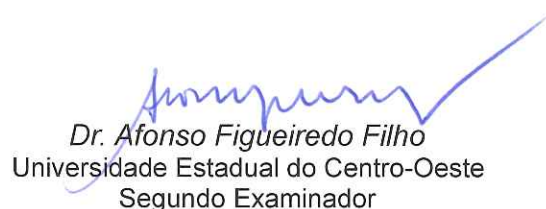
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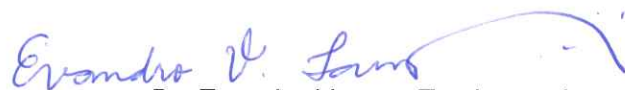
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I dedicate to my family and my entire
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"A minha própria vida científica foi uma descida de dimensões altas para baixas, conduzidas pelo desejo de entender a vida. Fui de animais a células, de células a bactérias, de bactérias a moléculas, de moléculas a elétrons. A história teve a sua ironia, porque moléculas e elétrons não possuem vida. No meu caminho, a vida escapou entre meus dedos".

(Albert Szent-Gyorgyi).

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ABSTRACT

EFFECTS OF SELECTIVE LOGGING ON GENETIC DIVERSITY OF *Araucaria angustifolia* (Bertol.) Ktze

Araucaria angustifolia is the dominant species of the Araucaria Forest and occurs in association with a diverse number of tree species and other plant types. The intense exploitation of this species, especially in the 1950's and 1970's, may have resulted in the loss of part of its genetic diversity. The purpose of this research was to evaluate the effect of selective logging on the genetic diversity and structure of a natural *A. angustifolia* population. The study was conducted in a population located in Fernandes Pinheiro county, Parana state, Brazil, where selective logging experiments were conducted with the species. We performed DNA extraction on samples from 24 logged and 24 remnant trees. For the genetic analysis we tested 14 microsatellite loci previously developed for the species and used 11 of these. From the allelic frequency analyses, we verified a total number of alleles per locus (A_T) ranging from three to 22, with a mean of 10.9. The sample of remnant trees presented higher average allelic richness ($k = 9.64$) than logged trees ($A = 8.82$). The logged trees removed from the population, on average, 3.73 rare alleles (A_r) per locus, 1.18 rare and private alleles (A_{rp}) and 1.27 private alleles (A_p). The diversity of the sampled logged trees ($H_o = 0.735$; $H_e = 0.732$) was not significantly different to the remnant trees ($H_o = 0.714$; $H_e = 0.736$). Similarly, the fixation index (F) for the logged trees ($F = -0.005$) was also not significantly different from the remnant trees ($F = 0.022$). With selective logging, there was an increase in the mean coancestry coefficient ($\bar{\theta}_{xy}$) for distance classes of 200 to 600 m. This indicates that in the post-logging scenario there is a greater probability of sampling related individuals in these classes. There was no significant difference between the diversity parameters (heterozygosity) for the logged trees and for the remnant trees. However, there was a decrease in genetic diversity for the *A. angustifolia* population submitted to selective logging, mainly due to the loss of rare and private alleles. The results of this study should be used for the development of public policies to define proposals for the sustainable management of *A. angustifolia* in the different regions of its natural occurrence.

Key-words: Araucaria forest; forest management; microsatellite markers; populations genetics.

RESUMO

EFEITO DO CORTE SELETIVO SOBRE A DIVERSIDADE GENÉTICA DE *Araucaria angustifolia* (Bertol.) Ktze

Araucaria angustifolia é a espécie dominante da Floresta Ombrófila Mista, ocorrendo de forma associada a um número diverso de espécies de árvores e outros tipos vegetais. A intensa exploração da espécie, sobretudo compreendida entre as décadas de 1950 e 1970, pode ter resultado em perda de parte da diversidade genética de populações naturais de *A. angustifolia*. O objetivo da presente pesquisa foi avaliar o efeito do corte seletivo sobre a diversidade genética e sobre a estrutura genética de populações naturais de *A. angustifolia*. Os estudos foram conduzidos em uma população localizada no município de Fernandes Pinheiro, Paraná, Brasil, que foi submetida a corte seletivo. Para extração de DNA foram coletadas amostras de 24 árvores cortadas e de 24 árvores remanescentes, com base no protocolo CTAB. Para as análises genéticas foram testados 14 locos microssatélites previamente desenvolvidos para a espécie. A partir das análises de frequências alélicas, verificou-se um número total de alelos por locos (AT) variando de três a 22, com média de 10,9. A amostra de árvores remanescentes apresentou maior riqueza alélica média ($k = 9,64$) do que as árvores cortadas ($k = 8,82$). Constatou-se que as árvores que foram cortadas retiraram da população, em média 3,73 alelos raros (A_r) por loco, 1,18 alelos raros e privados (A_{rp}) e 1,27 alelos privados (A_p). A amostra de árvores cortadas apresentou diversidade ($H_o = 0.735$; $H_e = 0.732$) significativamente similar as árvores remanescentes ($H_o = 0.714$; $H_e = 0.736$). O índice de fixação (F) também foi significativamente igual para as árvores cortadas ($F = -0,005$) e para as árvores remanescentes ($F = 0,022$). Com o corte seletivo, houve aumento do coeficiente de coancestria médio ($\bar{\theta}_{xy}$) para classes de distância de 200 a 600 m, indicando que no cenário pós-corte seletivo a probabilidade de se amostrar indivíduos aparentados dentro destas classes é maior. Não há diferença significativa entre os parâmetros de diversidade (heterozigosidade) para as árvores cortadas e árvores remanescentes. No entanto, verificou-se diminuição da diversidade genética para a população de *A. angustifolia* submetida ao corte seletivo, principalmente devido à perda de alelos raros e privados. Os resultados desta pesquisa podem ser aplicados ao desenvolvimento de políticas públicas para definição de propostas de manejo sustentável de *A. angustifolia* em áreas de ocorrência natural da espécie.

Palavras-chave: Floresta Ombrófila Mista; Manejo florestal; marcadores microssatélites; genética de populações.

1. INTRODUCTION

Araucaria angustifolia (Bertol.) Ktze (Paraná pine) is one of the most important native forest species for the southern region of Brazil, especially in regions where it occurs naturally. It is the dominant arboreal species of the Araucaria Forest. This ecosystem occurs, predominantly, in the southern region of Brazil. However, there are populations of this species in small fragments in various states of the southeast region, namely the east and south of São Paulo, the extreme south of Minas Gerais, the mountainous regions of Rio de Janeiro and Espírito Santo, and in small fragments of Argentina and Paraguay. The Araucaria Forest ecosystem was of major importance for the forestry and logging sector until the 1970's, due to its vast extension and high diversity of species, which contributes greatly to its intense exploitation (CARVALHO, 1994).

Until the 1970's, *A. angustifolia* was the native tree species of greatest economic value to Brazil and intensely exploited for high economic value of its wood. This context caused a predatory exploitation of *A. angustifolia* as well as the degradation of the Araucaria Forest, which currently occupies between 3 % and 13 % of its original area, being classified as vulnerable (GUERRA et al., 2002; RIBEIRO et al., 2009; WREGGE et al., 2015). Currently, the territory occupied by this species is reduced to a minimum fraction of its original, and the species is considered "in danger of extinction (EN)" according to the Brazilian National List of Endangered Species (BRASIL, 2016) and also as an endangered species in the IUCN (The World Conservation Union) "Red List".

According to some reports, trees containing male strobili were preferred for cutting because they did not produce seed and, phenotypically, produced more wood volume than the female trees. During the period of greatest exploitation, despite the environmental, social and economic importance of the Araucaria Forest, its conservation was not a priority, but rather the expansion of agriculture and the country's economic development (GUERRA et al., 2002; RIBEIRO et al., 2009).

The exploitation of Araucaria Forest resulted in its fragmentation as well as the isolation of remnant populations, resulting mainly in a possible genetic erosion of the natural populations. Thus, studies of genetic diversity as well as its distribution among and within populations of *A. angustifolia* should serve as a guide for forest management, being also an important basis for conservation strategies of this and other species of the Araucaria Forest.

Currently, the management of the Araucaria Forest and even commercial planting of this species is extremely limited by political and legislative terms to protect the remnants of this ecosystem, and principally *A. angustifolia*. However, such restrictions may result in a lack of interest in the species conservation. According to some authors (MELLO et al., 2003; HESS et al., 2010; TAMBARUSSI et al., 2016), the sustainable management of *A. angustifolia* as well as other native species in danger of extinction can be achieved, with the aid of technical studies that consider important factors such as cutting cycle, diameter class, and mainly, genetic information that allows minimizing the deleterious effects of inbreeding and maintenance of genetic diversity above a minimum limit.

Obtaining accurate and detailed information about native populations is extremely important for the conduct of recovery and conservation programs and for the sustainable use of the species. Access to genetic information of forest populations is essential for conducting and improving, in particular, activities such as forest management and seed collection (TAMBARUSSI, 2013). However, studies that consider the impact of forest management on forest genetic resources are scarce, especially, those related to the genetic diversity of managed populations (RATNAM et al., 2014).

Thus, this research is based on the need to obtain the bases for the sustainable use and correct management of the timber and non-timber resources of *A. angustifolia* and its genetic improvement. It is intended, with the results of this research, to contribute to the understanding of processes such as gene flow between populations, changes in effective population size and genetic diversity of populations subjected to selective logging.

1.1. OBJECTIVE

The objective of this research was to evaluate the influence of selective logging on the genetic structure and maintenance of genetic diversity in a natural population of *A. angustifolia*.

Thus, the specific objectives were to: (a) estimate diversity parameters between logged trees and remnant trees in a natural population; (b) determine the coancestry and spatial genetic structure within a population and (c) propose adequate strategies for the conservation, genetic improvement and management of natural populations of *A. angustifolia*.

1.2. HYPOTHESIS

The present study was based on the hypothesis that the genetic diversity of populations of *A. angustifolia* does not change when submitted to selective logging.

2. LITERATURE REVIEW

2.1. ARAUCARIA FOREST

Araucaria Forest is located in the South of Brazil, extending through the states of Paraná, Santa Catarina and Rio Grande do Sul. This ecosystem begins in the first plateau, immediately to the west of the Serra do Mar, and extends through the second and third plateaus of the state of Paraná to Laranjeiras do Sul, with floristic associations of *A. angustifolia*. This region is the highest part of the southern mountains, in the plateaus, where average elevations of up to 600 to 800 meters occur and exceed 1,000 meters in a few places. The lower limit of these forests is between 500 and 600 meters in the southern states, while to the north, this limit is several hundred meters higher. In these forests, representatives of the tropical and temperate flora of Brazil coexist but are dominated by the *A. angustifolia* (QUADROS, 2009).

The climate of the region is temperate, with regular rainfall and relatively well-defined seasons: the winter is usually cold with frequent frosts and even snow in some municipalities of Rio Grande do Sul, while the summer is reasonably hot. Temperatures range from 30 °C in summer to a few degrees below 0 °C in the harsh winter. The relative air humidity is related to the temperature with influence of altitude. The highest humidities are a result of oceanic influence on climate and transpiration from components of existing rain forests. The highest rainfall indices are recorded in the plateaus with rains well-distributed across the year (VELOSO et al., 1991; QUADROS, 2009).

The Araucaria Forest shows a floristic species composition from the Lauraceae family such as *Ocotea porosa* (Nees & Mart.) Barroso, *Ocotea odorifera* (Vell.) Rohwer, *Ocotea pulchella* (Nees & Mart.) Mez, besides several species popularly known as cinnamon. Also, worth mentioning are *Ilex paraguariensis* A. St.-Hil. and *Ilex theezans* Mart. ex Reissek, among other species of the Aquifoliaceae family (CARVALHO, 2006; QUADROS, 2009).

Several species from the Fabaceae (*Jacaranda* spp., *Machaerium* spp. and *Senegalia* spp.) and Myrtaceae families (*Campomanesia* spp. and *Eugenia* spp.) are also abundant in the Araucaria Forest, with conifers such as *Podocarpus lambertii* Klotzsch ex Endl. present (QUADROS, 2009).

According to Quadros (2009), there are also often species from the Rutaceae (*Balfourodendron riedelianum* (Engl.) Engl.), Euphorbiaceae (*Croton* spp.), Solanaceae

(*Solanum verbascifolium* L.) and Urticaceae families (*Boehmeria* spp. and *Urera* spp.), as well as many other species of vegetables, shrubs, lianas and herbs (QUADROS, 2009).

In the Araucaria Forest, the total area covered by forests is approximately 2.7 million hectares, which is equivalent to 24% in relative terms. Thus, it is possible to say that there are approximately 25% remaining of the original area of this ecosystem at present. In this ecosystem, the typological classes are classified as follows: 11% in the initial stage, 11.4% in the middle stage and 1.3% in the advanced stage. Thus, 12.7% of the ecosystem area is composed of relatively well-conserved forests and legally prevented from deforestation due to their relevant ecological characteristics. The natural forest cover of the state of Paraná is 18%, with about 10% with well-preserved forests. Specifically, in the Araucaria Forest the percentage of forest cover rises to 24%, with almost 13% of forests being in good conservation status. The numbers regarding forest cover in Paraná state were obtained by the Federal University of Paraná, based on technical and scientific grounds (QUADROS, 2009; ACCIOLY, 2013).

2.2. *Araucaria angustifolia*

The Araucariaceae family is the most primitive group of conifers still alive and emerged about 308 million years ago in the Paleozoic Era during the Upper Carboniferous period. Currently, the family Araucariaceae occurs exclusively in the Southern Hemisphere (South America and Oceania) and is composed of three genera. The main genus is *Araucaria*, composed of 19 species (*A. angustifolia*, *Araucaria araucana* (Molina) K. Koch, *A. bernieri* Buchh., *Araucaria biramulata* Buchh., *Araucaria columnaris* (J. R. Forst.) Hook., *Araucaria humboldtensis* Buchh., *Araucaria laubenfelsii* Corbasson, *Araucaria luxurians* Brongn. & Gris de Laub., *Araucaria montana* Brongn. & Gris, *Araucaria muelleri* Carriere Brongn. & Gris, *Araucaria nemerosa* de Laub., *Araucaria rulei* F. Muell., *Araucaria schmidii* de Laub., *Araucaria scopulorum* de Laub., *Araucaria subulata* Vieill., *Araucaria bidwillii* Hook., *Araucaria cunninghamii* Aiton ex D. Don, *Araucaria hunsteinii* Schumann, *Araucaria heterophylla* (Salisb.) Franco). In addition to *Araucaria*, the genera *Agathis* and *Wollemia* make up the family Araucariaceae, and are represented, among others, by the species *Agathis dammara* (Lamb.) Rich. and *Wollemia nobilis* Jones, respectively (ZANETTE et al., 2017; ZONNEVELD, 2012).

With three genera and 35 species, Araucariaceae are one of the smaller conifer families. Molecular phylogeny (GILMORE and HILL, 1997; QUINN et al., 2002; RAI et al., 2008; LIU

et al., 2009) found *Wollemia* to be related to both *Agathis*, and *Araucaria*, with suggestion of both increases and decreases in nuclear DNA content in the long history of the Araucariaceae.

The *Araucaria* genus is composed by 19 species and it is considered the genus of greatest diversity of the family Araucariaceae. Its distribution is limited to the Southern hemisphere in Brazil, Chile, Argentina, Australia, Papua New Guinea, New Caledonia, and Norfolk Island with the largest number of species (14) found in New Caledonia. In New Zealand, there are fossil records of species of the genus that were extinct millions of years ago (HENKEL and HOCHSTETTER, 1865; SETOGUCHI et al., 1998; SOUZA, 2015).

The main representative species of this genus native to Brazil, is *A. angustifolia*, adapted to the cold and humid mountainous regions of the south and southeast of the country, which is an area potentially vulnerable to climate change. It presents great economic, social and environmental value, since it is one of the species with the greatest potential for increase and potential of genetic gain, with excellent wood quality and seed production suitable for human and animal consumption (WREGGE et al., 2017).

Individual trees of *A. angustifolia* range in height from 10 to 35 meters, 50 to 120 centimeters in diameter, the trunks being straight, practically cylindrical, and generally without forking (CARVALHO, 1994). The bark of the trees is thick and resinous, with branch whorls that split into numerous secondary branches, densifying towards the stem apex, resulting typically in a candelabra shaped canopy, changing to an umbrella form in adult trees (REITZ et al., 1978).

The reproductive system of *A. angustifolia* is dioecious, with the presence of both male and female individuals. The sexual ratio of the species has been the objective of several studies (SOUSA et al., 2004; ZANON et al., 2009; FIGUEIREDO FILHO et al., 2015). The proportion of male and female individuals has been shown to be significantly balanced (1: 1) in different natural and planted stands (SOUSA, 2001; PALUDO et al., 2009). The female and male strobili (Figure 1) are observed from September until October. Pollen release from male strobili and their reception by female strobili occurs in the months of September to October (LORENZI, 1992; SOUSA and HATTEMER, 2003), however, pollination can extend into December, mainly by wind (MATTOS, 1994).



Figure 1 Female strobili (A), male strobili, which when mature show cylindrical form (B), pinion, the seed of the species (C), leaves (needles) of the species (D), forest dominated by *A. angustifolia* (E) and view of the trunk and crown of the species (F). Source: Compêndio Online Gerson Luiz Lopes (2018) (Figure 1A); Aguiar (2019) (Figure 1B and 1D, Acervo pessoal); Costa (2018) (Figure 1C and 1E).

Figura 1 Estróbilo feminino (A), estróbilo masculino, que quando maduro apresenta forma cilíndrica (B), pinhão, a semente da espécie (C), folhas (acículas) da espécie (D), floresta dominada por *A. angustifolia* (E) e visão do tronco e da copa da espécie (F). Fonte: Compêndio Online Gerson Luiz Lopes (2018) (Figure 1A); Aguiar (2019) (Figure 1B and 1D, Acervo pessoal); Costa (2018) (Figure 1C and 1E).

Although it shows several desirable characteristics and products of socioeconomic interest, commercial plantations of *A. angustifolia* are increasingly restricted, mainly to the Southern Brazilian states and in São Paulo state. In 2012 just over 11 thousand hectares of *A. angustifolia* planting were recorded (ABRAF, 2013).

A. angustifolia occurs naturally, exclusively, in the South American countries of Brazil, Paraguay and Argentina in the Araucaria Forest region, so called due to the abundance and dominance of this large tree species. These characteristics are responsible for representing the standard physiognomy of this forest (VELOSO et al., 1991).

Wrege et al. (2016) used the “Niche Mosaic” model to predict the regions of natural occurrence of *A. angustifolia* in Brazil (Figure 2). This model relates the occurrence of this local species to climatic variables, mainly air temperature and precipitation. Map areas highlighted in grey show the current occurrence of specie, defined by the "Mosaic Niche" model. The white areas represent areas where the species occurrence is restricted, and do not present the climatic conditions necessary for full development of the study species.

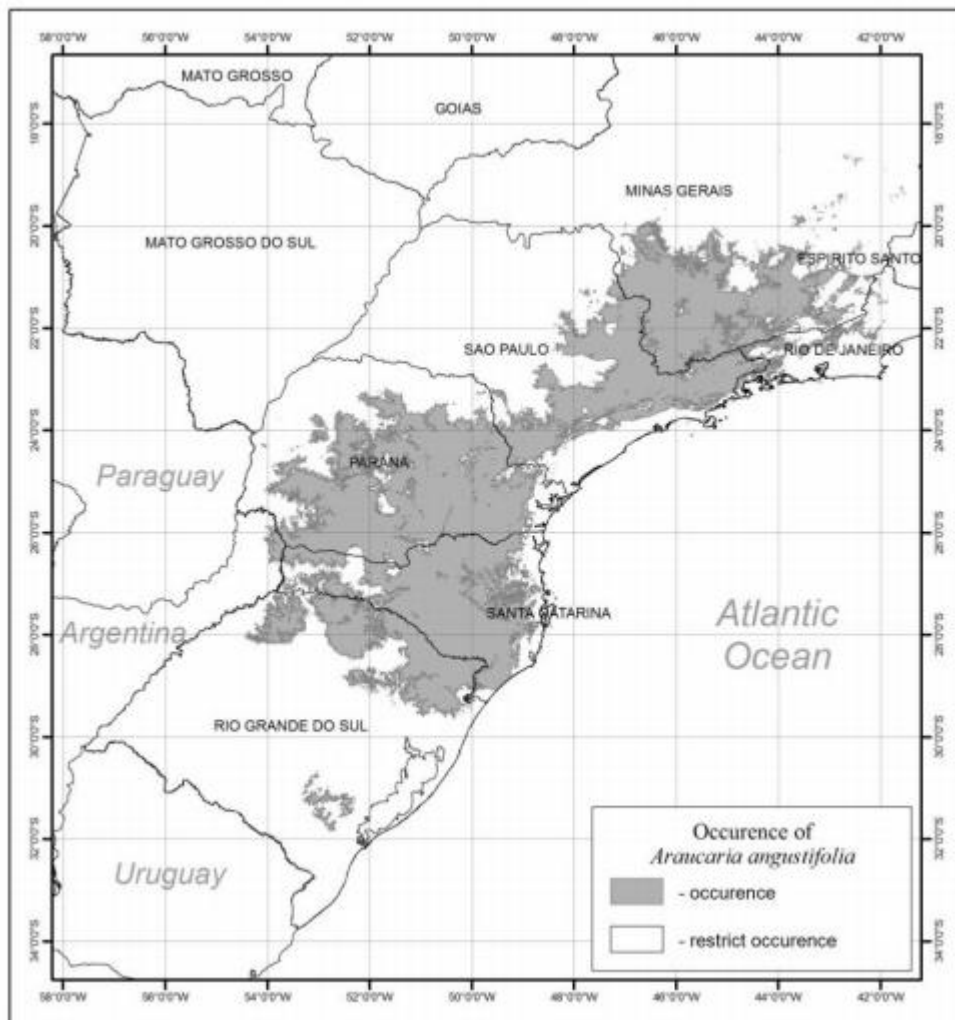


Figure 2 Estimation of the fundamental niche of *A. angustifolia* in Brazil based on the Niche mosaic model, according to Wrege et al. (2016).

Figura 2 Estimativa do nicho ecológico fundamental de *A. angustifolia* no Brasil com base no modelo Niche mosaico, de acordo com Wrege et al. (2016).

A. angustifolia is the dominant species of the Araucaria Forest, forming a continuous cover that confers an unstratified forest effect. However, under the canopy of the Araucaria Forest a diverse number of species of trees, shrubs, herbs, epiphytes and lianas occur, ranging in size and abundance, depending on the development stage of the forest. In successional stages, this species is initially classified as climax and as a light demander it spreads over fields generating new stands, benefitting from little shading in the germination and growth phase up to two years (LINDMAN, 1906; KLEIN, 1960).

The species presents low regeneration, both within the forest and disturbed environments. Its occurrence is related to genera such as *Ilex*, *Ocotea* and *Podocarpus* (CARVALHO, 1994).

In terms of fertility and soil physical conditions, *A. angustifolia* is a very demanding species. Thus, the most recommended soils for the cultivation of *A. angustifolia* are Red Latosols, with a well-developed A horizon, high levels of calcium and magnesium, high depth, friability, porosity, good drainage and water retention capacity and loam to clay texture (HOOGH, 1981).

In 1883, the extraction of *A. angustifolia* natural reserves began in southern Brazil. During the period of occupation of the territory of the states of Rio Grande do Sul, Santa Catarina and later Paraná, the species became the basis for the colonization by European immigrants, who arrived in the country at the end of the XIX century to dedicate themselves to agriculture. The exploitation of the species provided the initial capital for the establishment of several economic activities, especially with the installation of timber industries interested in the use of its high-quality wood, throughout the natural distribution of the species (ZANON, 2007).

Until the 1970s, the Araucaria Forest in Southern Brazil was considered the most important region for the economy of the Brazilian forestry and timber sector. At this time, *A. angustifolia* represented 90% of approximately one million cubic meters of wood produced and exported by Brazil. The intense exploitation, mainly between the 1950's and 1970's, has resulted in loss of part of the genetic diversity of the species natural populations, which are now divided into fragmented areas of different sizes (HUECK, 1972; LEITE and KLEIN, 1990).

Despite their importance, the remnants of the Araucaria Forest represent no more than 7% of the original area in Brazil (WREGGE et al., 2015). Today, *A. angustifolia* is in the critically endangered category at the global level (THOMAS, 2013; IUCN, 2018), because it has a habitat that is quite restricted by anthropogenic pressure and, as a result, is subject to a strong retraction in its distribution. In addition to the problems of anthropization and fragmentation due to

exploitation in the last century, climate change is a new threat, favoring the expansion of nearby forests, composed of hardwood species potentially better adapted to the hotter climate and with more efficient vegetative and reproductive development (KLEIN, 1960; KLEIN, 1984).

Currently, the management of native forests dominated by *A. angustifolia* is very restricted to protect the last existing remnants. However, there are questions as to whether this is the form of protection that will lead to the long-term desired conservation outcome. Throughout history, it has been realized that a restriction on the use of a natural resource is rarely accepted by the population, especially when there is a need for its use as a raw material or as a source of income. Protection, through the prohibition of use, results in a devaluation of natural resources, which in many cases results in loss of interest in the conservation of the raw material. Because of this, forms of management that consider both economic return and conservation are generally more efficient (NAUBER, 1995; NUTTO, 2001).

2.3. GENETIC DIVERSITY IN *Araucaria angustifolia* POPULATIONS

Biological variation is the result of evolutionary process, promoting the existence of individuals adapted to different environmental typologies. Changes in the hereditary constitution, as well as changes occurring in the environment, may result in significant genetic alterations, and may even result in the development of new species, isolated and distinct from the one that gave rise to it (PIRES et al., 2011).

The genetic diversity of a species is understood as the variation of the genes within it, and its structure corresponds to the diversity and abundance of genotypes, or even, the variation within its populations. For any species, high diversity is advantageous when the environment is in flux, allowing certain populations to adapt the natural or anthropic interventions through the selection of superior genotypes (BERG, 2001).

Genetic diversity occurs at different levels: 1) genetic diversity of species within ecosystems; 2) genetic diversity of populations within species; 3) genetic diversity of individuals within populations of a species. Genetic conservation planning for a species is highly dependent on the characterization of such different levels of diversity (KAGEYAMA, 1987).

Reductions in the size of natural populations result in the loss of genetic diversity, either by genetic drift or by the increase in homozygosis in gene loci through inbreeding, caused naturally or by carelessness in the use of natural ecosystems. As a result, populations are less

able to adapt to natural selection pressures, and inbreeding depression can occur, leaving the species more susceptible to extinction (TRAVIS et al., 1996).

The loss of genetic diversity in a species may result, as a more aggravating consequence, in the “bottleneck effect”, which consequently results in gene drift in the remaining populations, if they remain isolated for some generations. The reduction of genetic diversity leads to an increase in the probability of extinction of a population (YOUNG and BROWN, 1996).

Even in the face of the reduction of levels of genetic diversity that forest exploitation can cause, the relative importance of the different causal processes is little explored (SOUSA, 2000). Continuous gene flow between small scattered fragments can result in a secure connection network for several species, as well as the maintenance of genetic diversity, which is considered an important strategy to reduce biodiversity loss (TURNER and CORLETT, 1996).

Genetic effects in fragmented populations are observed in the different stages of the establishment of individuals, from the changes in the dynamics of regeneration and species reproduction, until the reduction of genetic diversity and maximization of genetic divergence. This is due evolutionary factors such as genetic drift, inbreeding and reduction of gene flow between populations (SHIMIZU et al., 2000; BOUZAT, 2001; AULER et al., 2002; SOUZA et al., 2009).

Knowledge of the distribution of genetic diversity within populations is extremely important for the perpetuation of species submitted to forest management, which should aim to keep levels of diversity above the minimum limit (BOTREL and CARVALHO, 2004). Genetic differentiation among and within populations is one of the main ways to study diversity within forest species (FERREIRA and GRATTAPAGLIA, 1998).

The patterns of distribution of genetic variability among and within populations is directly related to pollen and seed dispersal syndromes, as well as the reproductive system of the species and the influence of environmental factors on the distribution of individuals within the population of interest (SEBBEN et al., 2011).

Conifers have high genetic diversity, due to the long cycle and the wide geographic distribution of this plants group. In general, conifers are pollinated by the wind (anemophily), presenting a high degree of fertilization. However, *A. angustifolia* shows features that are intrinsic to its morphology that may reduce its gene flow, such as seed weight, characteristics of the pollen grain, which has dispersion hampered by its shape and by the scarcity of dispersal

vectors besides the wind. In addition, gene flow between *A. angustifolia* forest remnants is hampered by the fragmentation of these forests (SAVI, 2014).

According to Viana et al. (1992), with forest fragmentation occurs a reduction in the effective size of *A. angustifolia* populations, compromising the sustainable development not only of this species, but also of the plants and animal species associated and dependent on it.

The reduced size of forest fragments, coupled with any marked reduction in gene flow caused by the fragmentation itself, are considered as disturbing factors in the genetic structure and future evolution of *A. angustifolia*. (GUERRA et al., 1999). In addition to these factors, exploitation of the species itself constitutes a threat to its survival, making it fall into the category of vulnerable classification according to the International Union for Conservation of Nature criteria (IUCN, 2017)

The quantification of natural variability in a species, through analysis of population genetic structure, is responsible for understanding the patterns of resource allocation of variability in the evolution of each species. From this, it is possible to establish rational strategies for genetic conservation, establishing the ideal way of maintaining genetic variability and the natural evolutionary capacity of each species (DIAS, 1988).

Currently, a considerable part of the remnant of *A. angustifolia* is at risk of becoming unviable and, thus, subject to disappearance, due to the rupture of its dynamics of regeneration, maturation and reproduction. However, even completing the cycle, the reduced effective population size of the species makes the next generations more fragile due to inbreeding and loss of alleles caused, principally, by genetic drift (SHIMIZU et al., 2000).

The importance of the study of the reproductive system, spatial genetic structure and the genetic diversity of managed populations of *A. angustifolia* includes the correct application of management strategies that favor the conservation of the species, supporting, for example, the correct and genetically based selection of the trees to be removed, as well as the collection of seed from trees (DAL BEM et al., 2015).

2.4. GENETICS APPLIED TO SUSTAINABLE FOREST MANAGEMENT

Forest management should be considered as an important tool in perpetuating the survival of *A. angustifolia*, since this, if carried out sustainably, contributes to the natural regeneration of the species, with an increase in the growth rate of the remaining trees, in addition to a reduction in the natural mortality of the forest individuals (SANQUETTA, 2008).

Knowledge of the reproductive system and genetic structure of forest species, as well as their interactions within the forest system, in the case of management units or at the ecosystem level, is still scarce, making it impossible to carry out studies to promote genetic sustainability in the management of native species (VINSON, 2004).

The sustainable forest management of a species should be concerned with the conservation of a natural population, being carried out efficiently, to preserve the largest possible percentage of individuals that maintain the genetic diversity existing within the species of interest (RATNAM, 2014).

Natural forest management is an important production system in ecosystem development, and the sustainability of managed areas is a very controversial subject, especially when dealing with ecology and genetic variables, such as population density, spatial distribution, reproductive system and seed dispersal. Therefore, knowledge of these variables and the processes in which they are involved is extremely important, allowing for estimation of the impacts caused by management and exploitation activities (SILVA et al., 2004).

The relevance of genetic conservation in management plans is related to the adaptive capacity of species being proportional to genetic diversity and the maintenance of the genetic processes that, when preserved, are responsible for providing sustainability in the use of forest resources. Thus, it is recognized that the most important for sustainability in management is conservation of the processes and not the precise genetic and ecological state of population dynamics (NAMKOONG, 1998).

The reproductive system of a species can be influenced by the spatial distribution of individuals within a population, and changes in pollen and seed dispersal may have large effects on the spatial genetic structure and genetic variability of the species (TAMBARUSSI et al., 2015).

The way in which forest management is carried out today, together with fragmentation of forest populations, are responsible for reductions in population size and increases in the distance between individuals within these populations (YOUNG et al., 2001). These processes lead to loss of alleles, reduction of heterozygosity, reproductive isolation, resulting in reduced gene flow, increased inbreeding, genetic differentiation between populations and a decrease in effective population size (FRANKHAM, 2012). The spatial genetic structure and genetic variability of populations are also influenced by forest management and fragmentation, related to the mating system, through pollen and seed dispersal (EPPERSON, 2003).

Therefore, it is possible to perceive that the genetic structure of a forest population is directly related to the reproductive system and to the patterns of pollen and seeds migration seen between and within populations; factors that are affected by management of the species in question or even of other species (DEFAVARI et al., 2009; LACERDA et al., 2013).

Sebben et al. (2000), when studying the effects of forest management on the genetic structure of populations of *Tabebuia cassinoides* (Lam.) A. P. de Condolle, verified that the species, when being exploited under management, is subject to the loss of rare alleles, reduction of genetic diversity and increased inbreeding.

2.5. MICROSATELLITE MARKERS

Microsatellite markers, also called Single Sequence Repeats (SSR), were first described in 1989 by different researchers simultaneously (LITT and LUTY, 1989; WEBER and MAY, 1989; TAUTZ, 1989). This modality of molecular markers consists of repeats of small sequences of one to six nucleotides in tandem, which can be found widely distributed randomly through the genome of most eukaryotic organisms in coding or non-coding regions of proteins. However, the occurrence of SSRs in protein-coding regions is rare, which prevents them from undergoing changes, and, consequently, losing their functionality (FERREIRA and GRATAPAGLIA, 1998; VARSHNEY et al., 2005; IBANES, 2012)

SSRs are widely applied in the genetics analysis of all types of organisms, due to their high degree of information and transferability (SELKOE and TOONEN, 2006). The development of molecular markers allows the study of the genetic structure between and within populations of forest species. Molecular markers are useful for promoting the understanding of gene flow patterns, seed dispersal mechanisms and kinship between individuals of studied populations (CAVERS et al., 2005).

Microsatellite markers are the most commonly used marker for forest species, being very useful in studies related to paternity testing, mating system and genetic diversity. Their co-dominant inheritance, abundance, uniform distribution across the genome, and multiallelism are characteristics that make microsatellite markers a tool of easy applicability and high degree of information. Microsatellite markers make it possible to detect a dozen or more alleles in a population, have a high ability to differentiate genetically different individuals, even if they have close kinship or even consider if two individuals are genetically identical with high probability (FERREIRA and GRATAPAGLIA, 1998).

Molecular markers of the microsatellite type are responsible for providing information that corroborates the efficiency of genetic material collection processes, guides enrichment of the genetic base and the exploration of diversity and genetic purity, directs botanical identification studies, and also helps the planning of crosses and selection of genotypes of interest in a breeding program (FALEIRO, 2007).

Because they do not suffer any type of selective pressure, microsatellite markers are considered neutral, that is, they should not be associated with any phenotypic expression. However, the functional significance of some microsatellites has been demonstrated, as in the case of Huntington Disease, caused by an increase in the CAG motif present on chromosome four in humans (MOXON and WILLS, 1999). In addition to some diseases, microsatellites may be involved in the organization of chromatin, the regulation of metabolic DNA processes and regulation of the expression of some genes (LI et al., 2002).

The species *A. angustifolia* presents characteristics, such as limited seed production, economic and social value, which make it essential to apply molecular techniques to the characterization of genetic diversity within the species as a basis for conservation projects. Studies that make use of molecular techniques in populations of natural species are of extreme importance to obtain information about the organization of existing genetic variability, as well as factors that influence the nature and magnitude of such diversity, thus aiding the success of conservation projects and the sustainable use of forest ecosystems (SZMIDT, 1991; POWELL et al., 1995).

Since the advent of microsatellite markers, several studies have investigated the genetic variability of populations of *A. angustifolia* at the molecular level in Brazil and other South American countries. These studies have revealed high levels of polymorphism, with values of allelic richness (k) up to 106, and number of alleles per locus ranging from two to 22 (SALGUEIRO et al., 2005; STEFENON et al., 2007; BITTENCOURT and SEBBENN, 2007; BITTENCOURT and SEBBENN, 2008; BITTENCOURT and SEBBENN, 2009; PATREZE and TSAI 2010; SANT'ANNA et al., 2013; MEDINA-MACEDO et al., 2015; PÁDUA, 2015; DAL BEM et al., 2015).

3. MATERIALS AND METHODS

3.1. STUDY SITE AND SAMPLING

The present study was carried out in an experimental area of 20 hectares, located in Fernandes Pinheiro county, Paraná state, Brazil. It is located in the hydrographic microbasin of the Imbituvão river, in the South Center region of Paraná, with the coordinates 25°25'S and 50°32'W, a microregion from Prudentópolis county, bordering the municipalities of Imbituva, Teixeira Soares, São João do Triunfo, Rebouças and Irati (FERNANDES PINHEIRO, 2006). According to data from IBGE (2010), Fernandes Pinheiro occupies a surface area of 407 km² and is located at an altitude of 824 meters above sea level.

This experimental area is also studied by the Universidade Estadual do Centro-Oeste (UNICENTRO), in the “Imbituvão” project entitled “Strategies for sustainable forest management in small rural properties in South Center of Parana, Brazil” (Figure 3). One of the aims of the “Imbituvão project” was to generate a model of sustainable forest management for remnants of the Araucaria Forest, which may be replicated in Southern Brazil. In recent years, the project conducted experiments with the selective logging of *A. angustifolia*.

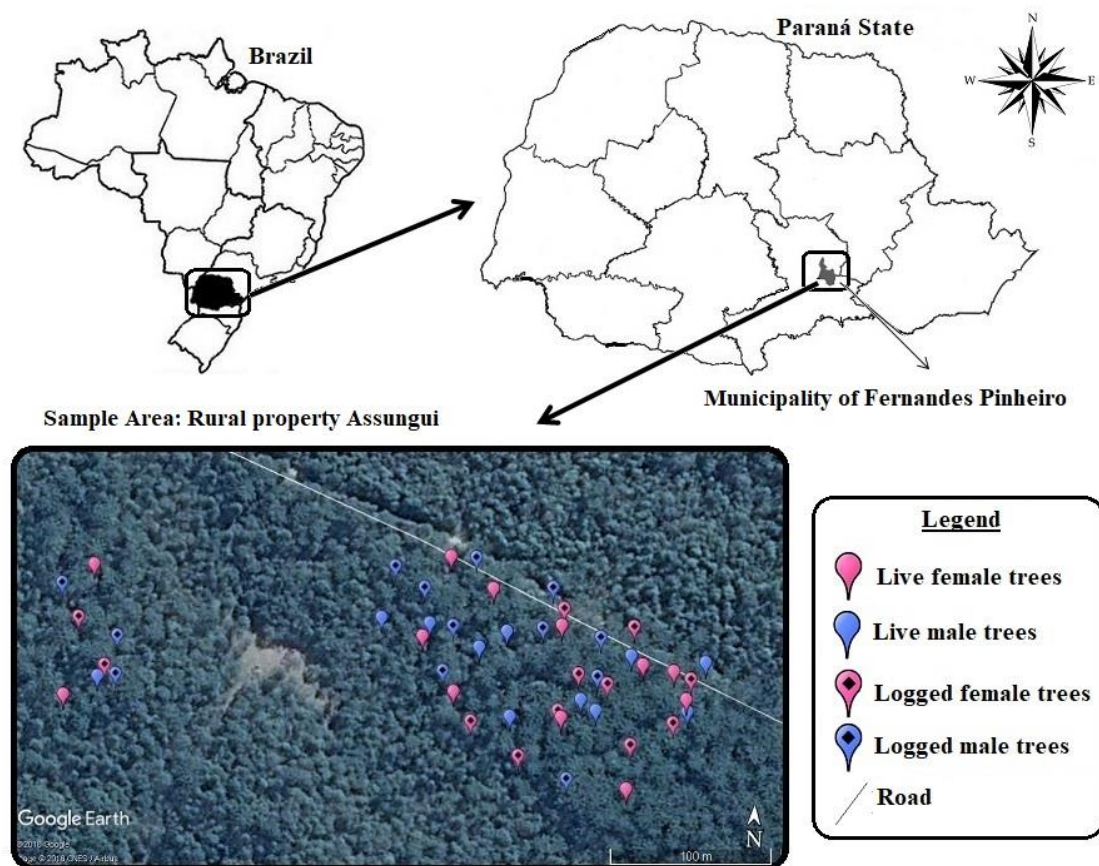


Figure 3 *A. angustifolia* population study located in Fernandes Pinheiro county, Paraná State, Brazil. Source: Homczinski (2018).

Figura 3 População de *A. angustifolia* em estudo localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil. Fonte: Homczinski (2018).

The experimental area was inventoried from a forest census, based on permanent plots. The forest inventory was carried out by the "Imbituvão" project, in order to know the dynamics of the studied forest (increment, ingrowth, mortality and floristic composition) and to assist in choosing the plots where the selective logging would be carried out. The area was subdivided into 20 plots of 1 hectare, where each hectare was divided into four quadrants of 2,500 m² each. At the center of the one-hectare plot, a smaller plot denominated a control plot with dimensions of 3,600 m² (60 mx 60 m) was allocated, respecting 20 m to minimize the border effect (Figure 4), with the purpose of monitoring the effect of different selective logging intensities. The plots were measured again after this operation and annually for the first 3 years.

The area in which the selective logging experiments were carried out covers 5.5 hectares. Initially, from the forest census conducted in 2014 year, in a total area of 20 hectares 1,000 individuals with a minimum DBH of 30 cm were identified in the area. In the selective logging experiments, 48 individuals were removed from the population. We sampled 48 *A.*

angustifolia individuals, of which 24 were logged and the other 24 were from the remaining population. We collected cambium samples from the logged trees and leaf samples from the remaining trees. Geographical coordinates were recorded for all 48 individuals (Figure 3) using GPS.

3.2. CHARACTERIZATION OF THE STUDY POPULATION'S STRUCTURE

For the distribution and spatial interaction between individuals of the species, diameter, total height and volumetric distribution and qualitative variables were analyzed using the data from the forest census to verify the structure of the study population before logging.

The quantification and analysis of the spatial distribution of the individuals of the species, used the analysis of distances between points of the univariate function of Ripley's K. This function is defined as the expected number of events within a distance (h) of an arbitrary event, divided by the intensity of points in the analyzed area. To facilitate the visualization of the results, the K function is transformed to L(s) or estimated L, which is used to graphically determine the spatial pattern of a species (SILVA et al., 2012).

According to Silva et al. (2012), the K function computes the distances between all trees of the same species on a map, to define its spatial pattern (aggregate, random or uniform). The procedure consists of a circle of radius (s) centered on each tree and the number of neighbors within this circle, thus, by varying the radius (s), one can detect the spatial pattern at different scales. In all cases, the null model (hypothesis of spatial randomness) was generated with 1,000 Monte Carlo simulations for $\alpha = 0.01$ confidence limit.

Spatial data were obtained from the Cartesian coordinates of the trees within the study area. In this analysis all the trees measured in the study area (20 hectares) were included, as well as their distribution in different categories (1: all individuals, 2: female individuals and 3: male individuals).

The analysis of the spatial dependence pattern (Ripley's bivariate function) between male and female individuals of the study species was performed using the K12 function proposed by Lotwick and Silverman (1982). The border effect was corrected by using the local weighting method (RIPLEY, 1977). The observed values of L_{12} were compared with the theoretical values obtained for a null hypothesis of independence that corresponds to the absence of interaction between two types of points. The null model (spatial independence hypothesis) was generated with 1,000 Monte Carlo simulations for $\alpha = 0.01$ confidence limit.

Both univariate and bivariate Ripley K functions were generated by software R using the statistical package "splancs" (ROWLINGSON and DIGGLE, 2017).

For analysis of the dispersion and density of the individuals of *A. angustifolia* the Kernel density estimator was used. The density along the area was estimated for the total set of trees. For this analysis, software R 3.5.2 was used with the "spatstat" package (BADDELEY and TURNER, 2005).

The diameter distribution of the species within the study area was performed by grouping the individuals into diameter classes (DBH) with 10 cm of amplitude. The number of class intervals was defined using the Sturges formula (MACHADO and FIGUEIREDO FILHO, 2014). The diameters classes were evaluated for female, male and combined, for DBH (cm), total height (m), volume and qualitative variables (plant health, stem form and crown form).

For the evaluation of qualitative variables, three categories were analyzed: Stem Form divided into sinuous stem (1), slightly sinuous stem (2) and straight stem (3) (FUPEF, 1978); Phytosanitary health divided into bad phytosanitary or attacked by pests (1), medium phytosanitary (2) and good phytosanitary (3) (FUPEF, 1978); Crown Form divided into Class 1 (unacceptable form with many irregularities across the whole crown), Class 2 (bad form, with more than 50% irregularities), Class 3 (acceptable form, with less than 50% irregularities), Class 4 (good form with small irregularity) and Class 5 (perfect form).

To verify the importance value of *A. angustifolia*, the phytosociology of the study area was evaluated according to Curtis and McIntosh (1950) methodology, based on the following indicators: Absolute Density (*AD*) and Relative Density (*RD*); Absolute dominance (*AD_o*) and Relative dominance (*RD_o*); Absolute frequency (*AF*) and Relative frequency (*RF*) and; the Importance Value (*IV%*).

3.3. CRITERIA OF FOREST MANAGEMENT

The traits considered for forest management were: diameter at breast height (DBH) > 40 cm, phytosanitary quality, stem quality, crown diameter and volume estimated according to the volume equation from FUPEF (1978). The trees of lower quality, less wood volume and phytosanitary quality were prioritized for logging. Thus, the best individuals in volume were maintained in the population. The distance between individuals was also considered to conserve the genetic variability of the population, even though it was done empirically (Figure 4).

The selective logging of *A. angustifolia* was carried out according to the Law, considering its social and scientific character (logged trees were used in studies of biomass estimation, dendrochronology, genetics, etc.), its benefit to social development. The exploited wood was auctioned, and the financial return directed to the rural owners, emphasising economic development, besides encouraging the conservation of the species through use.

For the selective logging experiments, three treatments were established. The experimental area of 5.5 hectares was divided into three blocks, with a different logging intensity applied to each. Each block was also divided into a plot, within which all *A. angustifolia* individuals with DBH above 30 cm were measured, along with identification of the sex of the individuals and the verification of rare species. In one block, a logging intensity of 20% of the basal area was applied, while in another block 30% of the basal area was cut. For the last block, it was intended to apply a logging intensity of 50% of the basal area. However, before the completion of the logging in the last block, the experiment was interrupted by order of the Public Ministry of Brazil.

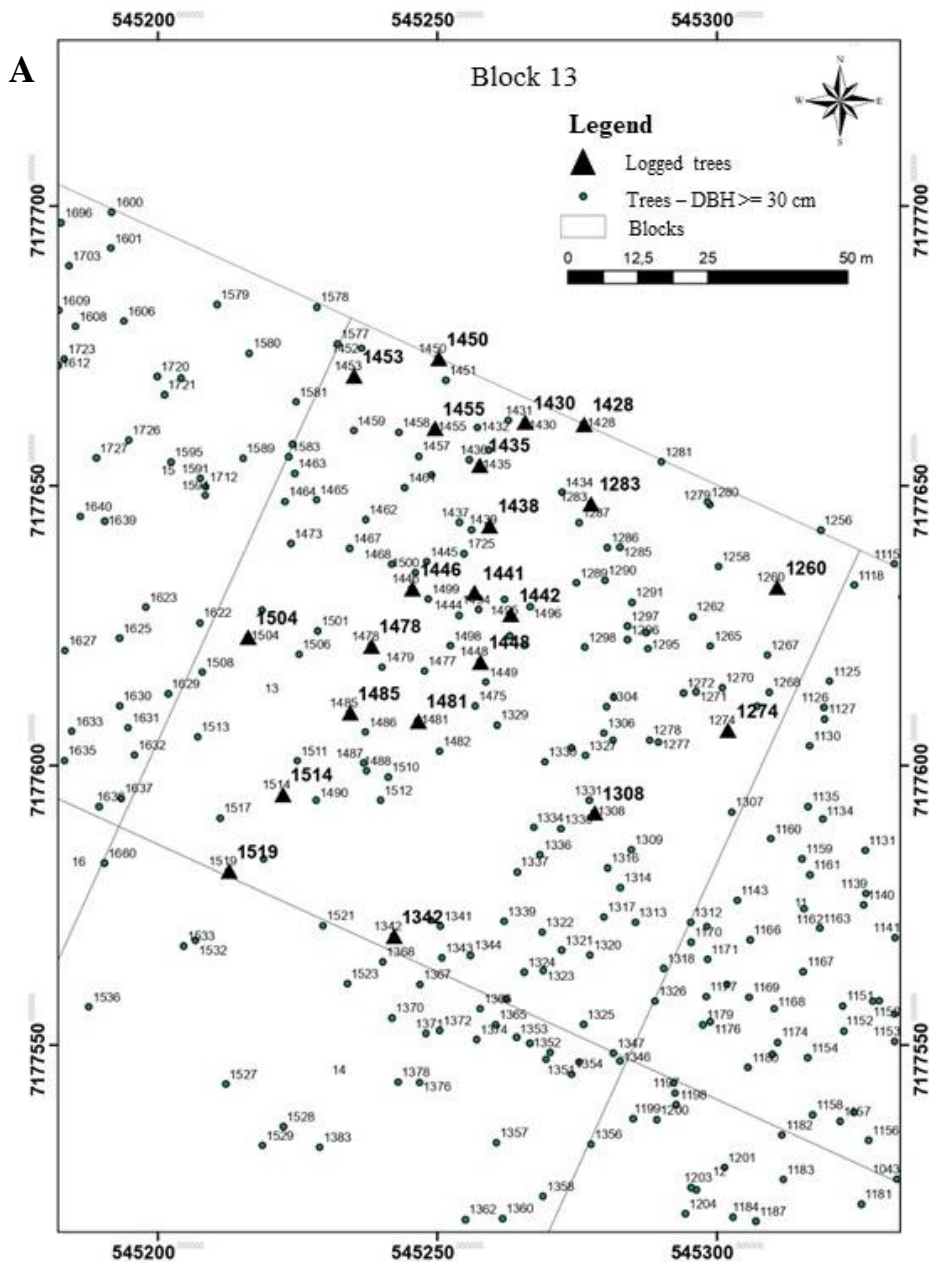


Figure 4 Sample plots established for the study of selective logging in an *A. angustifolia* population located in Fernandes Pinheiro county, Paraná State, Brazil, where (A) block in which different selective logging intensities were applied and (B) plot that subdivided the block, where logged trees and remnant trees of *A. angustifolia* and other species were identified. Source: Teleginski (2017).

Figura 4 Unidades amostrais utilizados nos estudos de corte seletivo em população de *A. angustifolia* localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil, em que (A) blocos nos quais foram aplicadas diferentes intensidades de corte seletivo e (B) parcelas que subdividiram o bloco, em que árvores cortadas e árvores remanescentes de *A. angustifolia* e outras espécies foram identificadas. Fonte: Teleginski (2017).

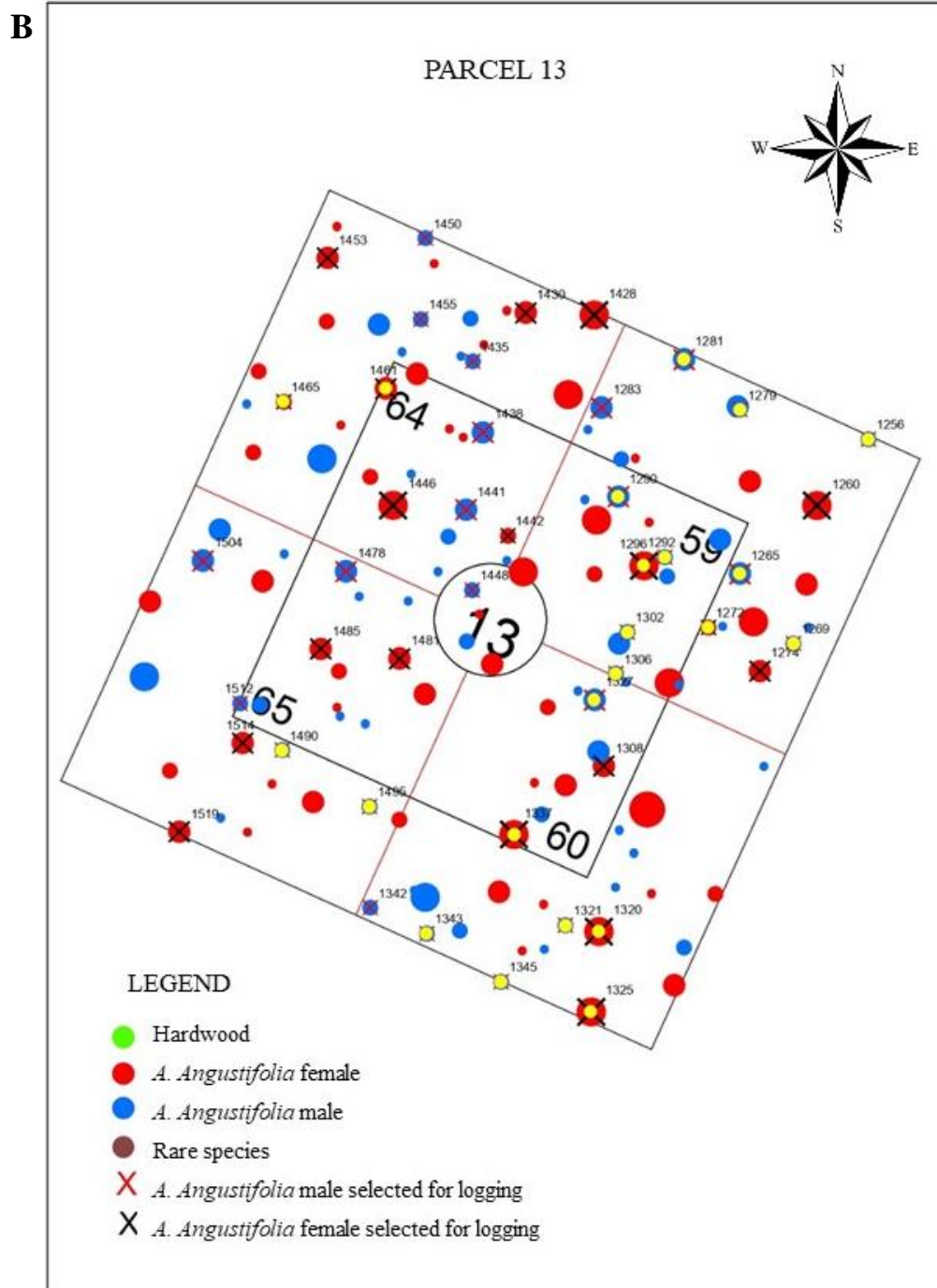


Figure 4 Continuation Sample plots established for the study of selective logging in an *A. angustifolia* population located in Fernandes Pinheiro county, Paraná State, Brazil, where (A) block in which different selective logging intensities were applied and (B) plot that subdivided the block, where logged trees and remnant trees of *A. angustifolia* and other species were identified. Source: Teleginski (2017).

Figura 4 Continuação Unidades amostrais utilizados nos estudos de corte seletivo em população de *A. angustifolia* localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil, em que (A) blocos nos quais foram aplicadas diferentes intensidades de corte seletivo e (B) parcelas que subdividiram o bloco, em que árvores cortadas e árvores remanescentes de *A. angustifolia* e outras espécies foram identificadas. Fonte: Teleginski (2017).

3.4. PLANT MATERIAL, DNA EXTRACTION AND MICROSATELLITE GENOTYPING

For the extraction of DNA and genotyping, stem samples (from the cambium region) (Figure 5A) were collected from the logged trees and leaf samples (Figure 5B) from the remnant trees in the “Imbituvão” project area. For the collection of plant materials, the remnant trees were climbed (Figure 5C).



Figure 5 Disks of *A. angustifolia* used to collect cambium samples (A); leaf (needles) samples of the species (B) and climbing of a tree to collect leaf samples (C).

Figura 5 Discos de *A. angustifolia* utilizados para coletar amostras de câmbio (A); amostra de folhas (acículas) da espécie (B) e escalada da árvore para coleta de amostras foliares (C).

After collection, the leaf and stem samples of *A. angustifolia* were stored in thermal boxes containing ice and transported to the laboratory Heréditas – Technology in DNA analysis, Brasília, Distrito Federal, Brazil. DNA was extracted, and genotyping carried out.

For DNA extraction, the protocol of Doyle and Doyle (1990) with modifications was used. The extraction of total genomic DNA from the samples was carried out from about 150 mg of fresh tissue (FERES et al., 2005), which was macerated in liquid nitrogen. We used the CTAB method described by Ferreira and Grattapaglia (1998) with modifications of the volumes of extraction buffer, chloroform-isoamyl alcohol (24:1) and isopropanol.

The protocol used the following steps: (1) to each eppendorf tube (2.0 mL) was added 900 μ L of extraction buffer (preheated to 65 °C) and 150 mg of macerated sample tissue, and then agitated and resuspended in buffer tissue; (2) it was heated in a water bath (65 °C) for 60 minutes and shaken manually for 10 minutes; (3) after removal from the water bath it was cooled to room temperature and 700 μ L of chloroform (24: 1) isomeric alcohol was added to the tube, and stirred by inverting at least 50 times to produce a homogeneous emulsion; (4) the

tubes were centrifuged for 15 minutes at 13,000 rpm; (5) the supernatant from each sample was transferred to a new labeled tube; (6) 700 μ L of cold isopropanol (kept at -20 °C) was added and homogenized gently to form a precipitate; (7) tubes were stored in a freezer (-20 °C) for 24 hours; (8) centrifuged for 10 minutes at 14,000 rpm for pellet formation; (9) the supernatant was discarded by inverting the tubes without losing the pellets; (10) the pellets were washed in 500 μ L of 70% ethanol twice and with 500 μ L of 95% ethanol once; (11) the ethanol was removed from the tubes without loss of the pellets; (12) the tubes remained at room temperature on a bench overnight for pellet drying; (13) each pellet was diluted in about 30 μ L of TE buffer (10 mM Tris-HCl, 1 mM in EDTA, pH 8.0) plus RNase (15 mg / ml) and stored at -20° C.

The DNA was quantified, determined and run on an agarose gel 1% stained with ethidium bromide and visualized under ultraviolet light.

Fourteen microsatellite markers developed and described by Schmidt et al (2007) (Aang01, Aang12, Aang14, Aang15, Aang27, Aang28, Aang37, Aang43); Bittencourt and Sebbenn (2007) (Ag20, Ag56, Ag62) and Grattapaglia, unpublished (Aa1774, Aa13140, Aa53325) were tested for the population.

3.5. ANALYSES OF LINKAGE DISEQUILIBRIUM AND GENETIC DIVERSITY

Linkage disequilibrium between pairs of loci was tested to verify the allelic association of different loci, and statistical significance assessed using Monte Carlo permutations (10,000) and Bonferroni correction ($\alpha = 0.05$).

Intrapopulation genetic diversity of the total and managed populations, was represented by the parameters: number of alleles per locus (k), observed heterozygosity (H_o) and expected heterozygosity (H_e), according to Hardy-Weinberg equilibrium ($\hat{H}_e = 1 - \sum p_i^2$) estimation. In addition, for each subpopulation, we estimated: rare alleles (A_r), which refer to alleles occurring at a frequency below 5% within the population/sample; private alleles (A_p), which are the alleles observed in only one of the populations/samples and rare and private alleles (A_{rp}), which are alleles occurring at a frequency below 5% in only one population/sample.

F statistics (F_{IS} , F_{IT} and F_{ST}) were used to represent the parameters of genetic differentiation between and within subpopulations (WRIGHT, 1951). Statistical significance was tested using the permutation method (1,000 replications).

All estimates were performed using the “R 3.3.2” (VENABLES et al., 2016) software with the package “diveRsity”. We used the software Micro-Checker 2.2.3 (VAN OOSTERHOUT et al., 2006) to estimate the frequencies of null alleles for each locus.

3.6. CORRELATION OF ALLELIC RICHNESS (k) WITH AGE AND BIOMETRIC VARIABLES

To test the hypothesis that the old trees would hold the greatest allelic richness, complete stem analysis (ANATRO) was performed to ascertain the age of the logged trees, while the remnant trees were submitted to partial stem analysis. In addition, to test if the variables used for the selection of trees for logging were associated with the allelic diversity of the individuals, age and biometric data were paired with allelic richness data (k) per individual. The association between the variables was tested using Pearson's correlation coefficient (r).

3.7. SPATIAL GENETIC STRUCTURE

Intrapopulation Spatial Genetic Structure (SGS) in the original population and managed population was studied with the software SPAGEDI version 3.1 (HARDY and VEKEMANS, 2002). The number of individual tree pairs ranged from 112 to 113 within each distance class. Seven distance classes were used, ranging from one to 1,201 m.

Coancestry of the total and managed populations were evaluated between pairs of individuals within the populations. It was represented by the mean coancestry coefficient ($\bar{\theta}_{xy}$), which in turn was estimated using the SPAGEDI software (HARDY and VEKEMANS, 2002).

Bayesian analysis was performed to identify the most probable number of genetic clusters (K) and population assignment using STRUCTURE 2.3.4 software (PRITCHARD et al., 2000; FALUSH et al., 2007). The analysis was performed under mixed ancestry and correlated allelic frequency models with 100,000 MCMC (Markov Chain Monte Carlo) repetitions after a burn-in period of 100,000. To obtain the true K value, the ΔK value (EVANNO et al., 2005), estimated from STRUCTURE Harvester (EARL and VON HOLDT, 2012) was used. This analysis was conducted for the sample of all trees.

3.8. GENETIC BOTTLENECKS

The hypothesis about the reduction in the effective population size, and, consequently reduction in genetic diversity due to forest management was tested based on allele frequencies using the Bottleneck software, version 1.2 (CORNUET and LUIKART, 1996). The models considered were the infinite allele model of mutation (IAM) and stepwise mutation model (SMM). These two models allow estimation of the expected heterozygosity of the population under mutation-drift equilibrium and were compared with the level of observed heterozygosity for the total and managed populations. The p-value of 0.05 was used to test for the existence of genetic bottlenecks based on allele frequencies.

The Bottleneck software conducts four tests for identifying populations that have recently experienced a severe reduction in effective population size (N_e) (PIRY et al., 1999). Both the measured heterozygosity (H_e) and the expected equilibrium heterozygosity (H_{eq}) refer to heterozygosity in the sense of Nei's gene diversity (1987). Heterozygosity never refers to the proportion of heterozygotes observed (H_o). Thus we are not testing for an excess of heterozygotes ($H_o > H_e$), but rather an excess of heterozygosity ($H_e > H_{eq}$).

4. RESULTS

4.1. CHARACTERIZATION OF THE STUDY POPULATION'S STRUCTURE

The cumulative values of Ripley's univariate K-function (full line), as well as the respective confidence intervals obtained from 1,000 Monte Carlo simulations (dotted lines) are presented for all 1,000 individuals (Figure 6A) for females (Figure 6B) and for male individuals (Figure 6C). For all cases, the K function showed positive values (above the confidence interval) at all distances (radius of 5 to 100 m), rejecting the hypothesis of Complete Spatial Randomness and indicating that the species presented an aggregate spatial pattern.

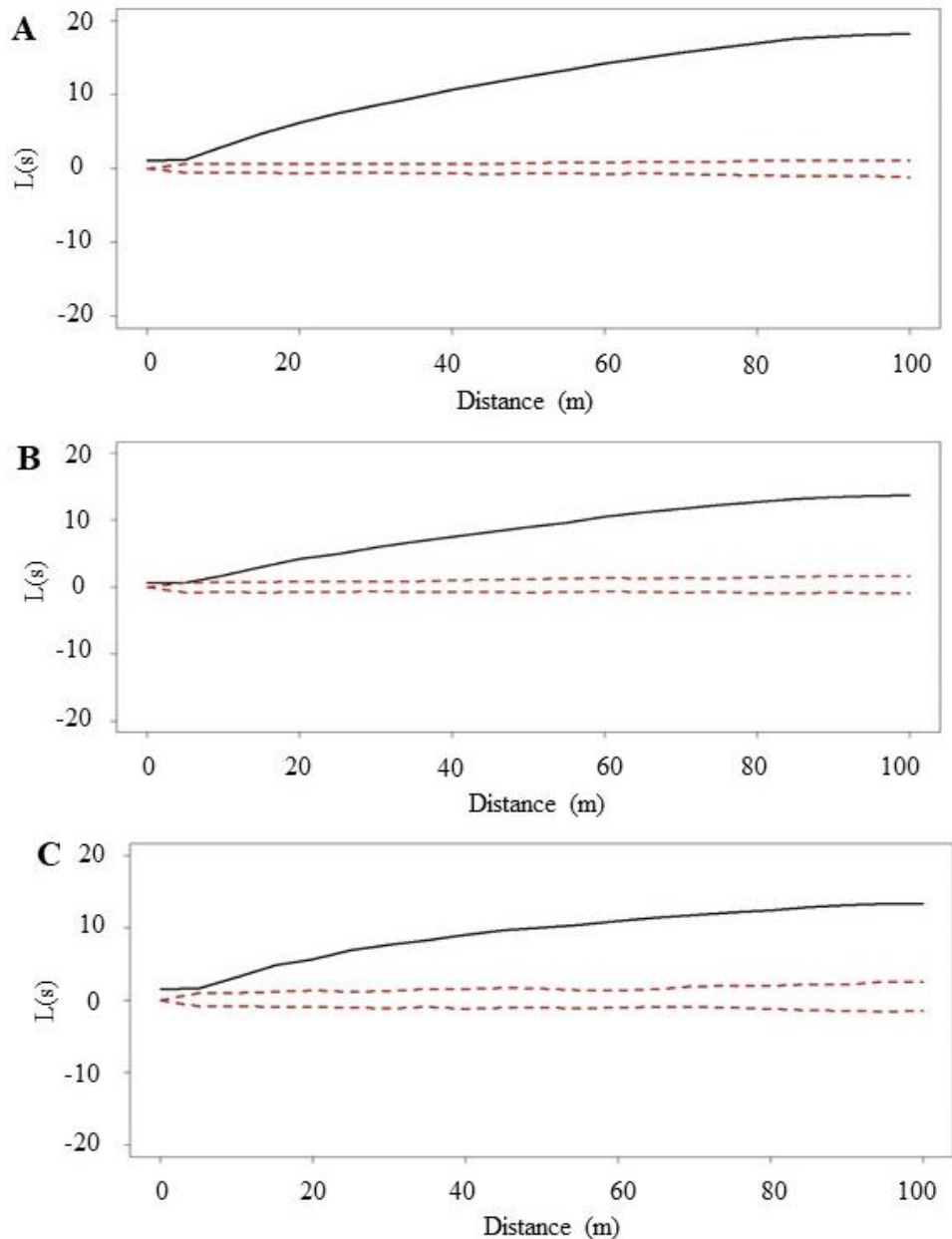


Figure 6 Analysis of Ripley's univariate K function and spatial pattern of all 1,000 individuals assessed (A), female individuals (B) and male individuals (C) of *A. angustifolia* with a minimum DBH of 30 cm in remnants of Araucaria Forest located in Fernandes Pinheiro county, Paraná state, Brazil. The full line represents the transformed K function (estimated L) and the red dotted lines represent the confidence interval with 1,000 Monte Carlo simulations for $\alpha = 0.01$.

Figura 6 Análise da função K univariada de Ripley e padrão espacial de todos os 1000 indivíduos levantadas (A), indivíduos fêmeas (B) e indivíduos machos (C) de *A. angustifolia* com DAP mínimo de 30 cm em remanescente de Floresta Ombrófila Mista localizado no município de Fernandes Pinheiro, estado do Paraná, Brasil. A linha cheia representa a função K transformada (L estimado) e as linhas pontilhadas vermelhas representam o intervalo de confiança com 1000 simulações de Monte Carlo para $\alpha = 0,01$.

One of the criteria used to select the trees to be logged, considered the intensity of occurrence of the species within the study area, prioritizing areas of more intense grouping, to avoid the opening of very close gaps. In Figure 7, it is possible to observe the sites of intense occurrence of the species, where the trees to be logged considering a distance of at least 20 m between the chosen trees.

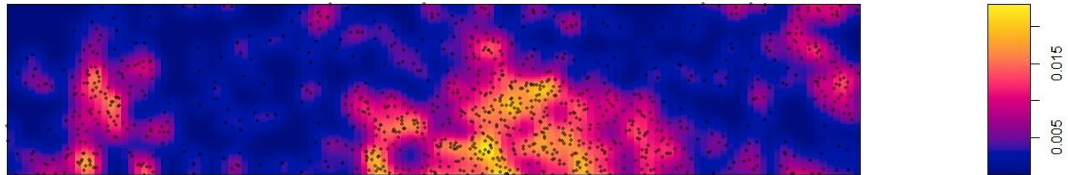


Figure 7 Visualization of the spatial pattern and density of *A. angustifolia* individuals in a remnant of Araucaria Forest located in Fernandes Pinheiro county, Paraná state, Brazil. The areas with the highest density are represented by the colors red and yellow.

Figura 7 Visualização do padrão espacial e intensidade de indivíduos de *A. angustifolia* em remanescente de Floresta Ombrófila Mista localizado no município de Fernandes Pinheiro, estado do Paraná, Brasil. As áreas com maior intensidade são representadas pelas cores vermelho e amarelo.

The bivariate K function allowed to investigate the spatial relation between female and male individuals of the study species. The species showed positive dependence between females and males from a distance of five meters (Figure 8). In 20 hectares, the proportion found was approximately 1.655 males for each female, since the number of individuals sampled in the present study resulted in 604 males and 396 females.

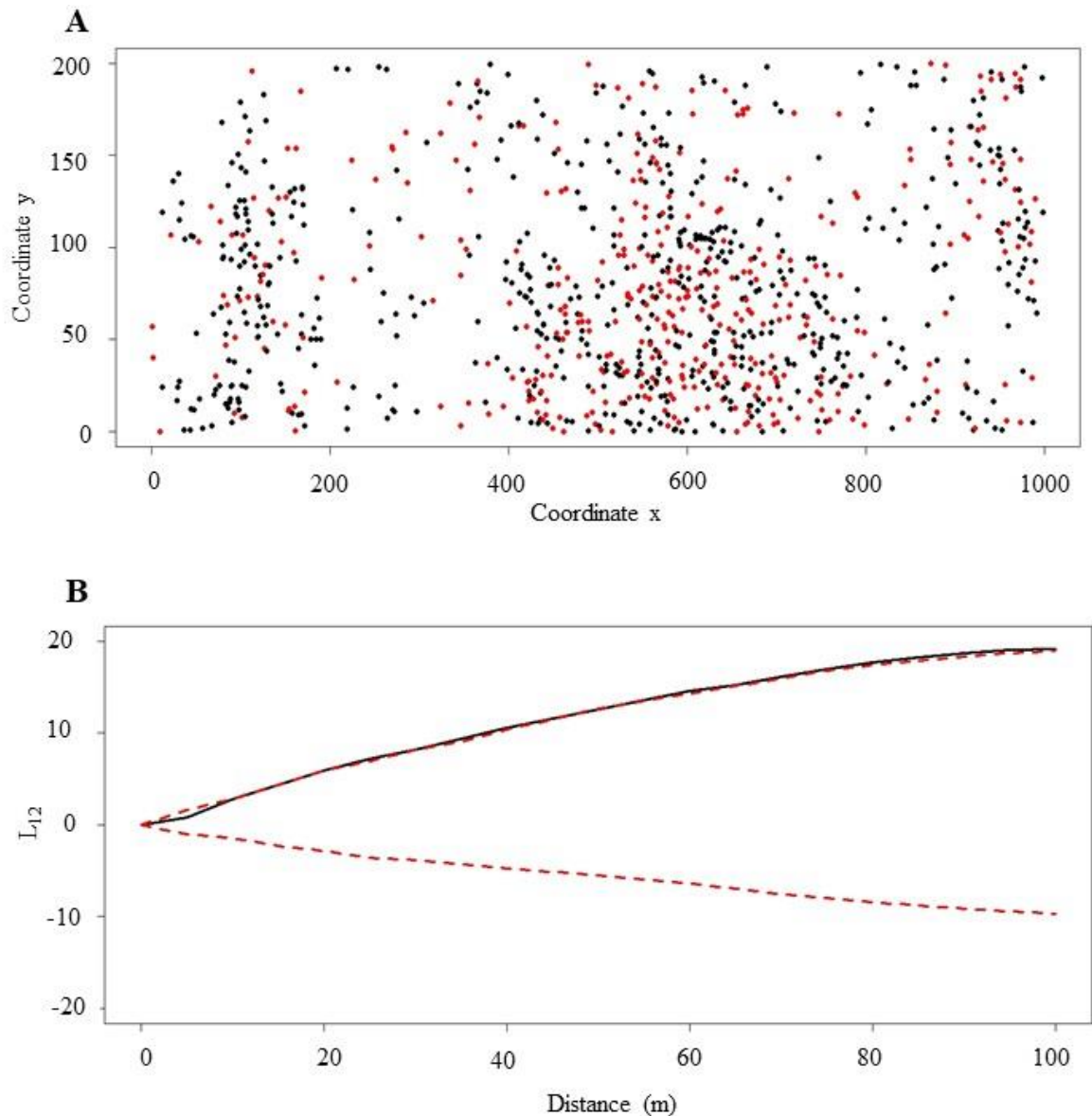


Figure 8 Spatial dependence analysis with the function K_{12} (estimated L_{12}) for male and female individuals of *A. angustifolia* in a remnant of Araucaria Forest located in Fernandes Pinheiro county, Paraná state, Brazil. In (A) the red dots represent the females and the black dots the males and in (B) the full line represents the transformed K function (estimated L) and the red dotted line represents the confidence interval with 1,000 Monte Carlo simulations for $\alpha = 0.01$.

Figura 8 Análise de dependência espacial com a função K_{12} (L_{12} estimado) para indivíduos machos e fêmeas de *A. angustifolia* em remanescente de Floresta Ombrófila Mista localizado no município de Fernandes Pinheiro, estado do Paraná, Brasil. Em (A) os pontos vermelhos representam as fêmeas e os pontos pretos representam os machos e em (B) a linha cheia representa a função K transformada (L estimado) e as linhas pontilhadas vermelhas representam o intervalo de confiança com 1000 simulações de Monte Carlo para $\alpha = 0,01$.

Although the forest census covered individuals with DBH greater than or equal to 30 cm, the population of *A. angustifolia* presented a diameter distribution with an inverted "J" format, with a decrease in the number of individuals from the lowest (30-40 cm) to the larger (> 90 cm) diameter class (Figure 9). However, females presented a unimodal distribution, with the greatest number of individuals in the 40-50 cm class (128 individuals).

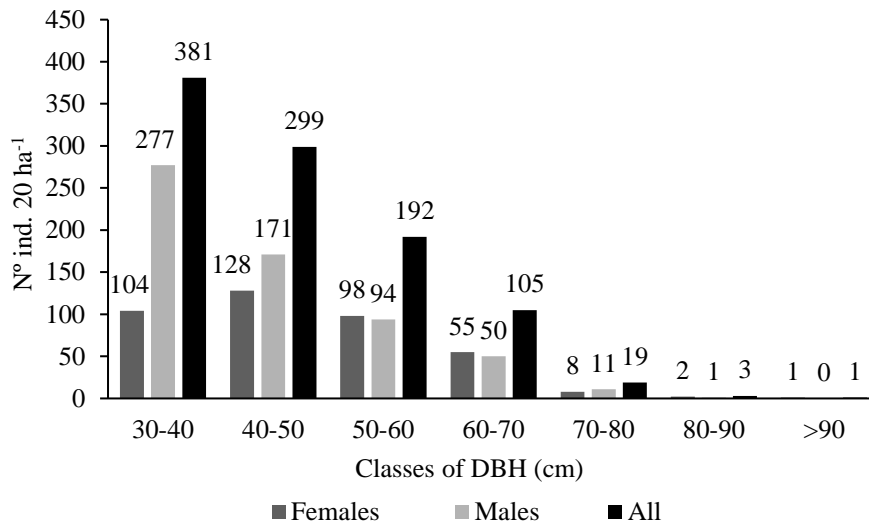


Figure 9 Diameter distribution of *A. angustifolia* trees in the year 2014 before selective logging in a remnant of Araucaria Forest located in Fernandes Pinheiro county, Paraná state, Brazil.

Figura 9 Distribuição diamétrica de *A. angustifolia* no ano de 2014 antes do corte seletivo em remanescente de Floresta Ombrófila Mista localizado no município de Fernandes Pinheiro, estado do Paraná, Brasil.

The DBH of the inventoried *A. angustifolia* population ranged from 34.79 to 98.04 cm with an overall mean of 45.55 cm. The total height ranged from 15.5 to 23 m with an overall mean of 17.1 m, while the volume ranged from 1 to 12.39 m³, with an overall mean of 2.01 m³. From the diameter class of 50-60 cm the proportion of females and males, both in number of individuals and DBH, height and volume was quite uniform with small variations between them (Table 1). It is possible to observe both in Figure 9 and Table 1 that in the diameter classes of 30-40 and 40-50 cm, the number of male individuals is larger than that of females, but the mean values of DBH, height and volume are smaller for the males than for the females.

Table 1 Mean and standard deviation (SD) of female and male individuals of *A. angustifolia* in relation to DBH (cm), total height (m) and volume (m³) in a remnant of Araucaria Forest located in Fernandes Pinheiro county, Paraná state, Brazil.

Tabela 1 Média e desvio padrão (SD) de indivíduos fêmeas e machos de *A. angustifolia* em relação ao DAP (cm), altura total (m) e volume (m³) em remanescente de Floresta Ombrófila Mista localizado no município de Fernandes Pinheiro, estado do Paraná, Brasil.

| Dioecy | Diameter class (cm) | N° of individuals | DBH (cm) | Total height (m) | Volume (m ³) |
|--------------|---------------------|-------------------|----------------------|---------------------|--------------------------|
| | | | Mean ± SD | Mean ± SD | Mean ± SD |
| Females | 30-40 | 104 | 35.50 ± 2.59 | 15.61 ± 2.31 | 1.00 ± 0.21 |
| Males | | 277 | 34.79 ± 2.94 | 15.46 ± 2.42 | 0.95 ± 0.22 |
| All | | 381 | 34.99 ± 2.86 | 15.50 ± 2.39 | 0.96 ± 0.22 |
| Females | 40-50 | 128 | 45.04 ± 2.62 | 17.20 ± 2.39 | 1.76 ± 0.30 |
| Males | | 171 | 44.19 ± 3.10 | 17.22 ± 2.26 | 1.70 ± 0.32 |
| All | | 299 | 44.55 ± 2.94 | 17.21 ± 2.32 | 1.72 ± 0.32 |
| Females | 50-60 | 98 | 54.03 ± 2.51 | 18.15 ± 2.50 | 2.73 ± 0.42 |
| Males | | 94 | 54.00 ± 2.78 | 18.68 ± 2.24 | 2.78 ± 0.41 |
| All | | 192 | 54.01 ± 2.64 | 18.41 ± 2.39 | 2.75 ± 0.42 |
| Females | 60-70 | 55 | 64.03 ± 2.56 | 19.55 ± 2.30 | 4.20 ± 0.55 |
| Males | | 50 | 64.71 ± 2.87 | 19.15 ± 2.24 | 4.25 ± 0.57 |
| All | | 105 | 64.35 ± 2.73 | 19.36 ± 2.28 | 4.22 ± 0.56 |
| Females | 70-80 | 8 | 74.37 ± 2.96 | 21.25 ± 1.09 | 6.27 ± 0.68 |
| Males | | 11 | 75.01 ± 3.13 | 19.86 ± 2.22 | 6.10 ± 0.93 |
| All | | 19 | 74.74 ± 3.07 | 20.45 ± 1.95 | 6.17 ± 0.83 |
| Females | 80-90 | 2 | 81.96 ± 0.16 | 19.00 ± 1.00 | 7.18 ± 0.25 |
| Males | | 1 | 84.99 ± 0.00 | 18.00 ± 0.00 | 7.48 ± 0.00 |
| All | | 3 | 82.97 ± 1.43 | 18.67 ± 0.94 | 7.28 ± 0.25 |
| Females | >90 | 1 | 98.04 ± 0.00 | 23.00 ± 0.00 | 12.39 ± 0.00 |
| Males | | --- | --- | --- | --- |
| All | | 1 | 98.04 ± 0.00 | 23.00 ± 0.00 | 12.39 ± 0.00 |
| Total | | 1,000 | 45.55 ± 11.17 | 17.09 ± 2.76 | 2.01 ± 1.31 |

In relation to the qualitative variables, the majority of females and males presented a straight stem (97.90%), good phytosanitary health (99.10%) and perfect crown form (51.70%) (Table 2).

Table 2 Qualitative evaluation of *A. angustifolia* trees in a remnant of Araucaria Forest located in Fernandes Pinheiro county, Paraná state, Brazil, where Stem Form: 1 – sinuous, 2 – slightly sinuous, 3 – straight; Phytosanitary health: 1 – Bad, 2 – Medium, 3 – Good; Crown Form: 1 – unacceptable form, 2 – poor form, 3 – acceptable form, 4 – good form, 5 – perfect form.

Tabela 2 Avaliação qualitativa de *A. angustifolia* em remanescente de Floresta Ombrófila Mista localizado no município de Fernandes Pinheiro, estado do Paraná, Brasil, em que Forma de tronco: 1 – tortuoso, 2 – levemente tortuoso, 3 – reto; Fitossanidade: 1 – Ruim, 2 – Média, 3 – Boa; Formato de copa: 1 – formato intolerável, 2 – formato pobre, 3 – formato tolerável, 4 – formato bom, 5 – formato perfeito.

| Dioecy | Diameter class (cm) | Stem Form (%) | | | Phytosanity (%) | | | Crown Form (%) | | | | |
|--------------|---------------------|---------------|-------------|--------------|-----------------|-------------|--------------|----------------|-------------|--------------|--------------|--------------|
| | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 5 |
| Females | 30-40 | 0.10 | 0.20 | 0.30 | 0.10 | 0.20 | 0.30 | 0.10 | 0.20 | 0.30 | 0.40 | 0.50 |
| Males | | --- | 0.20 | 10.20 | 0.10 | --- | 10.30 | --- | 1.00 | 2.20 | 4.00 | 3.20 |
| All | | --- | 1.30 | 26.40 | 0.10 | 0.20 | 27.40 | 0.40 | 4.30 | 6.00 | 8.20 | 8.80 |
| Females | 40-50 | --- | 1.50 | 36.60 | 0.20 | 0.20 | 37.70 | 0.40 | 5.30 | 8.20 | 12.20 | 12.00 |
| Males | | --- | --- | 12.80 | --- | --- | 12.80 | --- | 0.80 | 1.90 | 3.00 | 7.10 |
| All | | --- | 0.10 | 17.00 | 0.10 | 0.10 | 16.90 | --- | 0.90 | 3.60 | 5.30 | 7.30 |
| Females | 50-60 | --- | 0.10 | 29.80 | 0.10 | 0.10 | 29.70 | --- | 1.70 | 5.50 | 8.30 | 14.40 |
| Males | | 0.10 | 0.10 | 9.60 | --- | --- | 9.80 | --- | 0.20 | 0.80 | 1.70 | 7.10 |
| All | | --- | 0.20 | 9.20 | 0.10 | --- | 9.30 | --- | 0.10 | 0.40 | 3.00 | 5.90 |
| Females | 60-70 | 0.10 | 0.30 | 18.80 | 0.10 | --- | 19.10 | --- | 0.30 | 1.20 | 4.70 | 13.00 |
| Males | | --- | --- | 5.50 | --- | --- | 5.50 | --- | --- | --- | 0.10 | 5.40 |
| All | | --- | 0.10 | 4.90 | 0.10 | 0.10 | 4.80 | --- | 0.10 | --- | 0.20 | 4.70 |
| Females | 70-80 | --- | 0.10 | 10.40 | 0.10 | 0.10 | 10.30 | --- | 0.10 | --- | 0.30 | 10.10 |
| Males | | --- | --- | 0.80 | --- | --- | 0.80 | --- | --- | --- | --- | 0.80 |
| All | | --- | --- | 1.10 | --- | --- | 1.10 | --- | --- | --- | 0.10 | 1.00 |
| Females | 80-90 | --- | --- | 1.90 | --- | --- | 1.90 | --- | --- | --- | 0.10 | 1.80 |
| Males | | --- | --- | 0.20 | --- | --- | 0.20 | --- | --- | --- | --- | 0.20 |
| All | | --- | --- | 0.10 | --- | --- | 0.10 | --- | --- | --- | --- | 0.10 |
| Females | >90 | --- | --- | 0.30 | --- | --- | 0.30 | --- | --- | --- | --- | 0.30 |
| Males | | --- | --- | 0.10 | --- | --- | 0.10 | --- | --- | --- | --- | 0.10 |
| All | | --- | --- | 0.10 | --- | --- | 0.10 | --- | --- | --- | --- | 0.10 |
| Total | | 0.10 | 2.00 | 97.90 | 0.50 | 0.40 | 99.10 | 0.40 | 7.40 | 14.90 | 25.60 | 51.70 |

In the study area (20 hectares), 1,000 individuals were described for *A. angustifolia*. The study of the phytosociology of the study area showed an absolute density of 50 individuals per ha, absolute dominance (basal area) of 8.6 m².ha⁻¹ and an occurrence frequency of 99% of plots for the species. Thus, both the density, the dominance and frequency of the species in relative terms presented high values, resulting, therefore, in a percentage of importance equal to 45.04% for the year 2014. In Figure 10, in addition to *A. angustifolia*, the other nine species of greater

importance value are presented: *Cinnamomum amoenum* (Nees) Kosterm., *Myrciaria floribunda* (West ex willd.) O. Berg., *Zanthoxylum kleinii* (R. S. Cowan) P. G. Waterman, *Campomanesia xanthocarpa* O. Berg., *Lithraea molleoides* (Vell.) Engl., *Cinnamodendron dinisii* Schwacke, *Clethra scabra* Pers., *Matayba elaeagnoides* Radlk. and *Nectandra grandiflora* Nees..

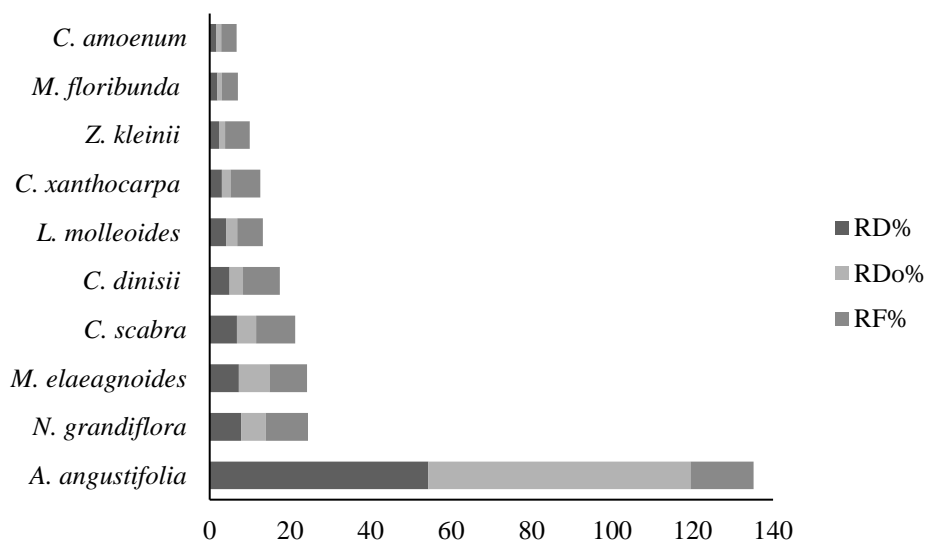


Figure 10 Relative Density (RD), Relative Dominance (RDo) and Relative Frequency of 10 species of highest Importance Value (IV) in a remnant of Araucaria Forest, located in Fernandes Pinheiro county, Paraná state, Brazil.

Figura 10 Densidade relativa (RD), Dominância relativa (RDo) e Frequência relativa de 10 espécies de maior Valor de Importância (IV) de um remanescente de Floresta Ombrófila Mista, localizado no município de Fernandes Pinheiro, estado do Paraná, Brasil.

4.2. FOREST INVENTORY AND FOREST MANAGEMENT

The *A. angustifolia* population of the fragment showed a mean DBH, total height and volume of 45.55 cm, 17.09 m and 2.00 m³ tree⁻¹, respectively (Table 3). The genotyped trees presented higher mean values for height, DBH and volume than the inventoried population.

Table 3 Descriptive statistics of the *A. angustifolia* population (A) inventoried and logged (n = 1,000) and the (B) genotyped sample (n = 48) in Fernandes Pinheiro county, Parana state, Brazil.

Tabela 3 Estatística descritiva da população de *A. angustifolia* (A) inventariada e manejada (n = 1000) e da (B) amostra genotipada (n = 48) localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil.

| (A) | | | |
|------------------------------|-----------------|-------------------------|---|
| Statistic | DBH (cm) | Total height (m) | Volume (m³ tree⁻¹) |
| Minimum | 30.08 | 8.50 | 0.53 |
| Average | 45.55 | 17.09 | 2.00 |
| Maximum | 98.04 | 25.00 | 12.39 |
| Sample variance | 124.97 | 7.61 | 1.71 |
| Standard deviation | 11.18 | 2.76 | 1.31 |
| Default error | 0.35 | 0.09 | 0.04 |
| Coefficient of Variation (%) | 24.54 | 16.15 | 65.24 |

| (B) | | | |
|------------------------------|-----------------|-------------------------|---|
| Statistic | DBH (cm) | Total height (m) | Volume (m³ tree⁻¹) |
| Minimum | 31.51 | 13.00 | 0.80 |
| Average | 48.99 | 17.91 | 2.32 |
| Maximum | 69.55 | 22.00 | 5.49 |
| Sample variance | 102.92 | 5.89 | 1.26 |
| Standard deviation | 10.14 | 2.43 | 1.12 |
| Default error | 1.46 | 0.35 | 0.16 |
| Coefficient of Variation (%) | 20.70 | 13.55 | 48.25 |

DBH = diameter at breast height (cm).

4.3. LINKAGE DISEQUILIBRIUM, GENETIC DIVERSITY, GENETIC DIFFERENTIATION AND STRUCTURE

Of 14 tested loci just 11 were used in genetic analysis of the *A. angustifolia* population (Table 2 and 3). The locus Aa13140 was monomorphic while the Aang12 and Ag62 loci did not adhere to the Hardy-Weinberg equilibrium. The linkage equilibrium test showed that there was no significant imbalance after a Bonferroni correction ($p < 0.001$) for the 11 loci used in genetic analysis. A detection test for null alleles showed no null alleles for any of the loci used.

High levels of polymorphism, 120 identified alleles, were detected in the *A. angustifolia* population. The number of alleles per locus ranged from three to 22 with a mean of 10.9 (Table 4). The sample of remnant trees showed higher mean allelic richness ($k = 9.64$) than the sample of logged trees ($k = 8.82$).

The mean number of rare alleles (4.36), private alleles (2.09) and rare and private alleles (1.91) was higher in the sample of remnant than the logged population, with values of 3.73 (A_r), 1.18 (A_{rp}) and 1.27 (A_p) respectively (Table 4). Therefore, the effect of selective management on genetic diversity in this population was negative.

Table 4 Genetic diversity for microsatellite loci analyzed in remnants and logged trees of an *A. angustifolia* population located in Fernandes Pinheiro county, Paraná state, Brazil.

Tabela 4 Diversidade genética para locos microssatelites analisados em árvores remanescentes e árvores cortadas de uma população localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil.

| Locus | AT | Populations | | | | | | | |
|--------|-------|---------------|-------|----------|-------|--------------|-------|----------|-------|
| | | Remnant trees | | | | Logged trees | | | |
| | | k | A_r | A_{rp} | A_p | K | A_r | A_{rp} | A_p |
| Aang01 | 20 | 16 | 6 | 3 | 3 | 17 | 9 | 4 | 4 |
| Aang14 | 9 | 7 | 1 | 1 | 1 | 8 | 2 | 1 | 2 |
| Aang15 | 22 | 19 | 14 | 4 | 4 | 18 | 9 | 3 | 3 |
| Aang27 | 7 | 7 | 2 | 0 | 0 | 7 | 0 | 0 | 0 |
| Aang28 | 7 | 6 | 2 | 2 | 2 | 5 | 1 | 1 | 1 |
| Aang37 | 3 | 3 | 1 | 0 | 0 | 3 | 1 | 0 | 0 |
| Aang43 | 13 | 12 | 7 | 5 | 5 | 8 | 4 | 1 | 1 |
| Aa1774 | 3 | 3 | 1 | 0 | 0 | 3 | 1 | 0 | 0 |
| Aa5332 | 20 | 18 | 10 | 4 | 6 | 14 | 9 | 2 | 2 |
| Ag20 | 9 | 8 | 2 | 2 | 2 | 7 | 2 | 1 | 1 |
| Ag56 | 7 | 7 | 2 | 0 | 0 | 7 | 3 | 0 | 0 |
| Mean | 10.91 | 9.64 | 4.36 | 1.91 | 2.09 | 8.82 | 3.73 | 1.18 | 1.27 |
| SD | 6.86 | 5.73 | 4.37 | 1.87 | 2.17 | 5.21 | 3.55 | 1.33 | 1.35 |
| All | 120 | 106 | 48 | 21 | 23 | 97 | 41 | 13 | 14 |

Total number of alleles per locus for entire sample (AT), number of alleles per locus within each population (k), rare alleles (A_r), rare and private alleles (A_{rp}), and private alleles (A_p).

The analysis of the geographical distribution of the alleles per individual verified that both the rare alleles and the common alleles are distributed in a dispersed way along the population located in the area (5.5 hectares) of the selective logging experiments (Figure 11). Considering all loci, 70.83% of individuals that were genotyped have rare alleles. This value in the logged and remnant trees was 62.5% and 79.16% of genotyped trees, respectively.

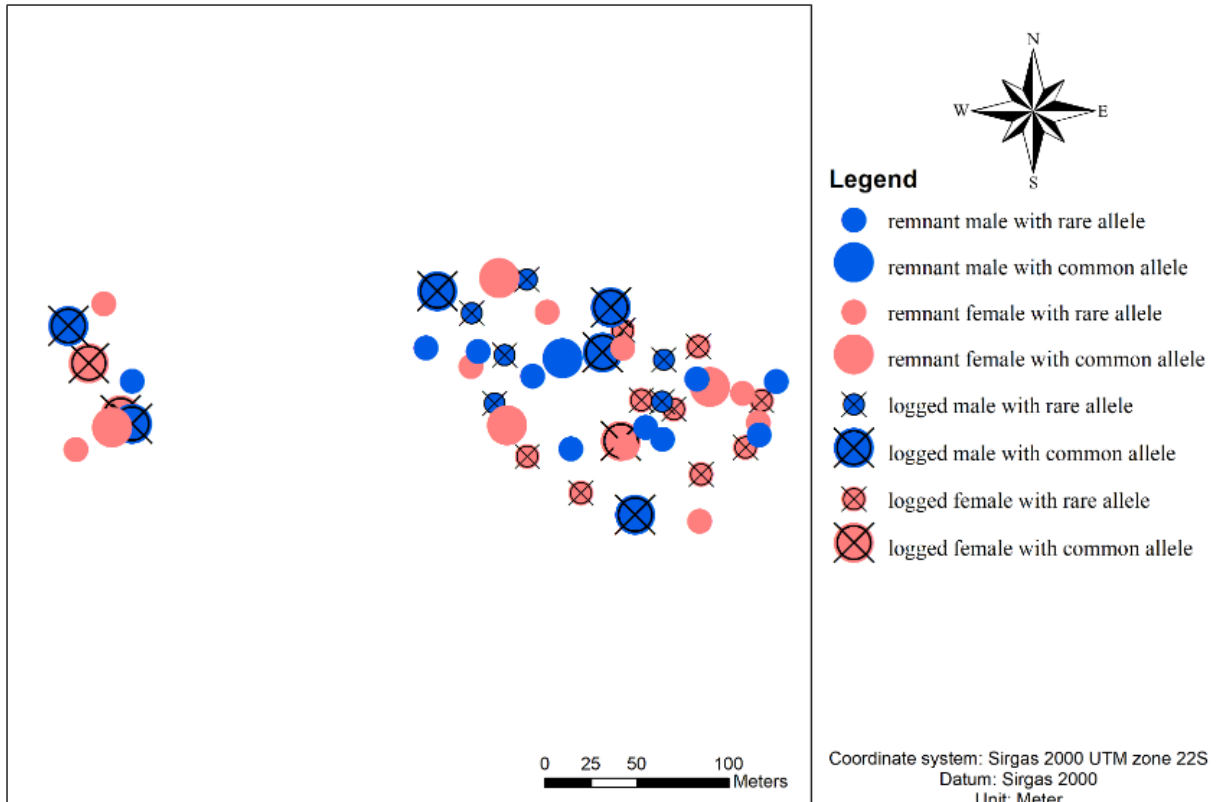


Figure 11 Geographic distribution of common alleles and rare alleles for logged and remnant trees in an *A. angustifolia* population located in Fernandes Pinheiro county, Parana state, Brazil.

Figura 11 Distribuição geográfica de alelos comuns e alelos raros para árvores cortadas e árvores remanescentes em população de *A. angustifolia* localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil.

Regarding diversity indexes (Table 5), the total sample of trees ($n = 48$) showed observed heterozygosity (H_o) of 0.724 and expected heterozygosity (H_e) of 0.743. The sample of remnant trees ($n = 24$) showed observed heterozygosity (H_o) of 0.714 and expected heterozygosity (H_e) of 0.748. For the sample of logged trees ($n = 24$) the genetic diversity were $H_o = 0.735$ and $H_e = 0.752$. The heterozygosity parameters did not present significant statistical difference between the subsamples.

Table 5 Genetic parameters estimated for eleven microsatellite loci for *A. angustifolia* for All trees, Remnant trees and Logged trees in Fernandes Pinheiro county, Parana state, Brazil. Sample size for each subpopulation (n); observed heterozygosity (H_o); expected heterozygosity (H_e) and fixation index (F) with confidence intervals at 95% (CI 95%) probability.

Tabela 5 Parâmetros genéticos estimados para onze locos microssatélites de *A. angustifolia* para amostras de árvores Total, Árvores Remanescentes e Árvores Cortadas no município de Fernandes Pinheiro, estado do Paraná, Brasil. Tamanho amostral para cada subpopulação (n); heterozigosidade observada (H_o); heterozigosidade esperada e índice de fixação (F) com intervalo de confiança de 95% (IC 95%) de probabilidade.

| Locus | All trees (n = 48) | | | Remnant trees (n = 24) | | | Logged trees (n = 24) | | |
|---------|--------------------|-------|-------------------------|------------------------|-------|-------------------------|-----------------------|-------|-------------------------|
| | H_o | H_e | F (CI 95%) | H_o | H_e | F (CI 95%) | H_o | H_e | F (CI 95%) |
| Aang01 | 0.740 | 0.930 | 0.204 (0.06 to 0.33) | 0.710 | 0.920 | 0.228 (0.02 to 0.41) | 0.770 | 0.920 | 0.163 (-0.04 to 0.33) |
| Aang14 | 0.880 | 0.810 | -0.086 (-0.18 to 0.03) | 0.790 | 0.780 | -0.013 (-0.21 to 0.15) | 0.960 | 0.830 | -0.157 (-0.27 to -0.06) |
| Aang15 | 0.930 | 0.930 | 0.000 (-0.8 to 0.05) | 0.880 | 0.910 | 0.033 (0.09 to 0.15) | 1.000 | 0.930 | -0.075 (-0.13 to -0.08) |
| Aang27 | 0.880 | 0.820 | -0.073 (-0.18 to 0.03) | 0.830 | 0.800 | -0.037 (-0.21 to 0.13) | 0.920 | 0.820 | -0.122 (-0.26 to 0.00) |
| Aang28 | 0.830 | 0.720 | -0.153 (-0.32 to -0.01) | 0.880 | 0.730 | -0.205 (-0.42 to -0.01) | 0.790 | 0.690 | -0.145 (-0.39 to 0.07) |
| Aang37 | 0.400 | 0.460 | 0.130 (-0.14 to 0.38) | 0.380 | 0.410 | 0.073 (-0.30 to 0.43) | 0.420 | 0.500 | 0.160 (-0.23 to 0.49) |
| Aang43 | 0.580 | 0.630 | 0.079 (-0.09 to 0.26) | 0.620 | 0.700 | 0.114 (-0.14 to 0.32) | 0.540 | 0.550 | 0.018 (-0.25 to 0.27) |
| Aa1774 | 0.460 | 0.410 | -0.122 (-0.32 to 0.09) | 0.380 | 0.340 | -0.118 (-0.31 to 0.13) | 0.540 | 0.460 | -0.174 (-0.48 to 0.12) |
| Aa53325 | 0.890 | 0.900 | 0.011 (-0.07 to 0.09) | 0.960 | 0.920 | -0.043 (-0.12 to 0.02) | 0.820 | 0.850 | 0.035 (-0.12 to 0.15) |
| Ag20 | 0.660 | 0.780 | 0.154 (0.00 to 0.30) | 0.750 | 0.820 | 0.085 (-0.12 to 0.25) | 0.570 | 0.730 | 0.219 (-0.03 to 0.44) |
| Ag56 | 0.710 | 0.780 | 0.090 (-0.09 to 0.25) | 0.670 | 0.770 | 0.130 (-0.13 to 0.41) | 0.750 | 0.770 | 0.026 (-0.21 to 0.24) |
| Mean | 0.724 | 0.743 | 0.021 (-0.04 to 0.06) | 0.714 | 0.736 | 0.022 (-0.06 to 0.08) | 0.735 | 0.732 | -0.005 (-0.09 to 0.04) |

The mean fixation index (F) within population was 0.021 (ranged from -0.04 to 0.06) for all trees ($n = 48$). For some loci (Aang14, Aang27, Aang28, Aa1774), H_o was higher than H_e , leading to negative F values. For the sample of remnant trees, the mean fixation index was $F = 0.022$ (-0.06 to 0.08), while for the logged trees $F = -0.005$ (-0.09 to 0.04), indicating an excess of heterozygotes within the sample of logged trees. The higher fixation index (F) values for the whole sample and the sample of remnant trees indicate that the trees within these are more related than the trees comprising the logged trees sample (Figure 12).

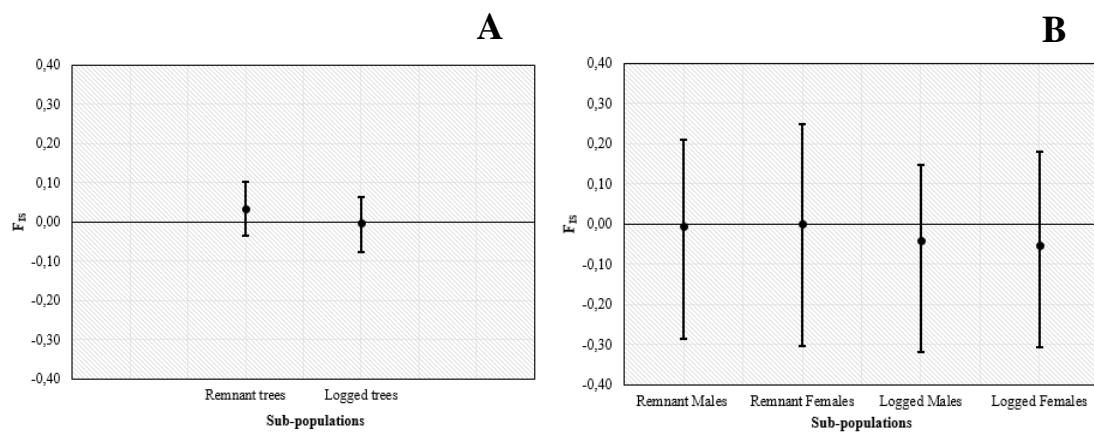


Figure 12 Fixation index (F_{IS}) parameter estimated with eleven microsatellite loci for (A) sub-populations of remnant trees and logged trees and for (B) sub-populations of remnant males, remnant females, logged males and logged females in an *A. angustifolia* population located in Fernandes Pinheiro county, Parana state, Brazil. Analyses considered a 95% confidence interval (CI).

Figura 12 Parâmetro índice de fixação (F_{IS}) estimado com onze locos microssatélites para (A) subpopulações de árvores remanescentes e árvores cortadas e para (B) subpopulações de árvores machos remanescentes, fêmeas remanescentes, machos cortados e fêmeas cortadas em uma população de *A. angustifolia* localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil. Análises realizadas com intervalo de confiança (IC) de 95%.

Observed heterozygosity (H_o) as well as expected heterozygosity (H_e) did not show significant differences between the samples of logged trees and remnant trees (Figure 13A and 13B). These parameters also did not present significant differences when the samples were subdivided into remnant females and logged females, remnant males and logged males (Figure 13C and 13D).

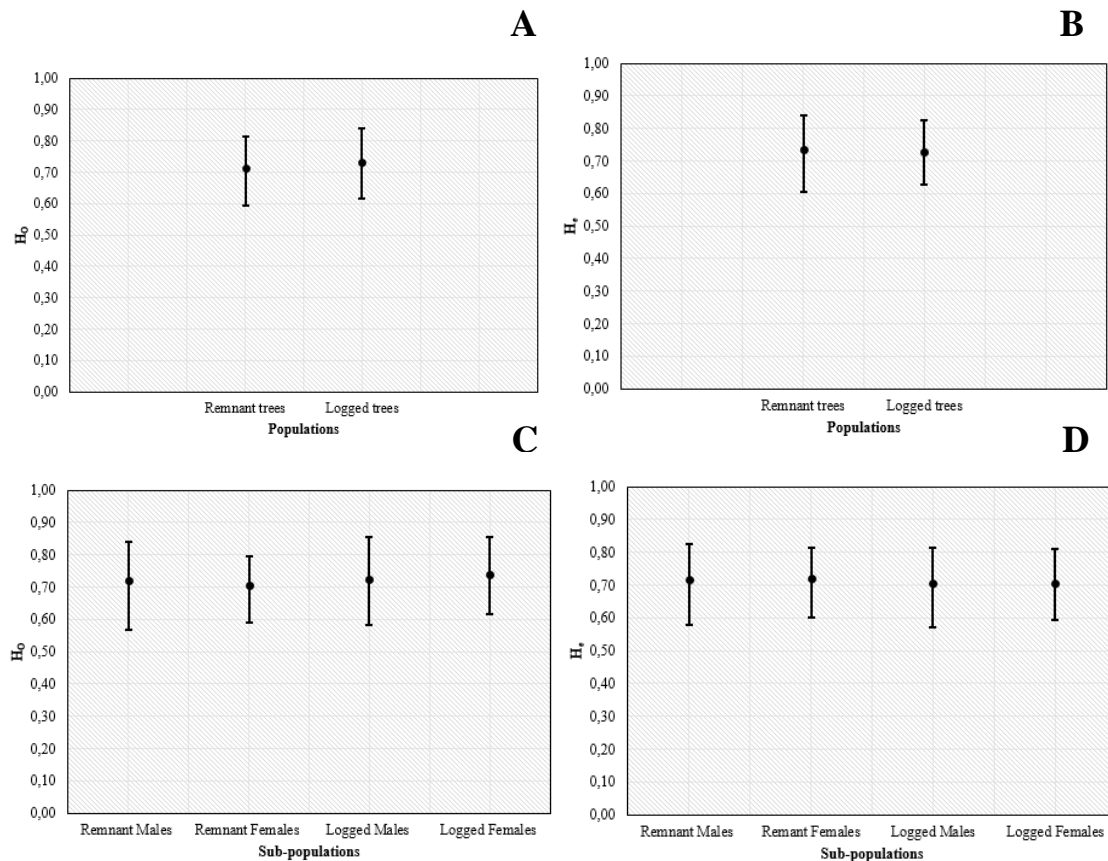


Figure 13 Parameters estimated with eleven microsatellite loci for remnant males, remnant females, logged males and logged females in an *A. angustifolia* population located in Fernandes Pinheiro county, Paraná state, Brazil, where (A) Observed heterozygosity (H_o) estimated for sub-populations of remnant trees and logged trees, (B) Expected heterozygosity (H_e) estimated for sub-populations of remnant trees and logged trees, (C) Observed heterozygosity (H_o) estimated for sub-populations of remnant males, remnant females, logged males and logged females and (D) Expected heterozygosity (H_e) for each sub-population. Analyses considered a 95% confidence interval (CI).

Figura 13 Parâmetros estimados com onze locos microssatélites para machos remanescentes, fêmeas remanescentes, machos cortados e fêmeas cortadas em uma população de *A. angustifolia* localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil, em que (A) heterozigosidade observada (H_o) estimada para subpopulações de árvores remanescentes e árvores cortadas, (B) heterozigosidade esperada (H_e) estimada para subpopulações de árvores remanescentes e árvores cortadas, (C) heterozigosidade observada (H_o) estimada para subpopulações de árvores machos remanescentes, fêmeas remanescentes, machos cortados e fêmeas cortadas e (D) heterozigosidade esperada (H_e) para cada subpopulação. Análises realizadas com intervalo de confiança (IC) de 95%.

4.4. CORRELATION OF ALLELIC RICHNESS (k) WITH AGE AND BIOMETRIC VARIABLES

The age of the remnant trees which were submitted to partial stem analysis ranged from 50 to 115 years, while for logged trees, the age ranged from 64 to 188 years. The variables tree age and individual allelic richness were not correlated, with only 0.3% of the observed variations in tree age explaining the variation observed in allelic richness (Figure 14).

After analyzing the correlation of allelic richness with dendrometric variables, a low correlation was observed. These variables were used to select the trees. In genetic terms, the dendrometric variables are not sufficient to explain the genetic diversity of the study population.

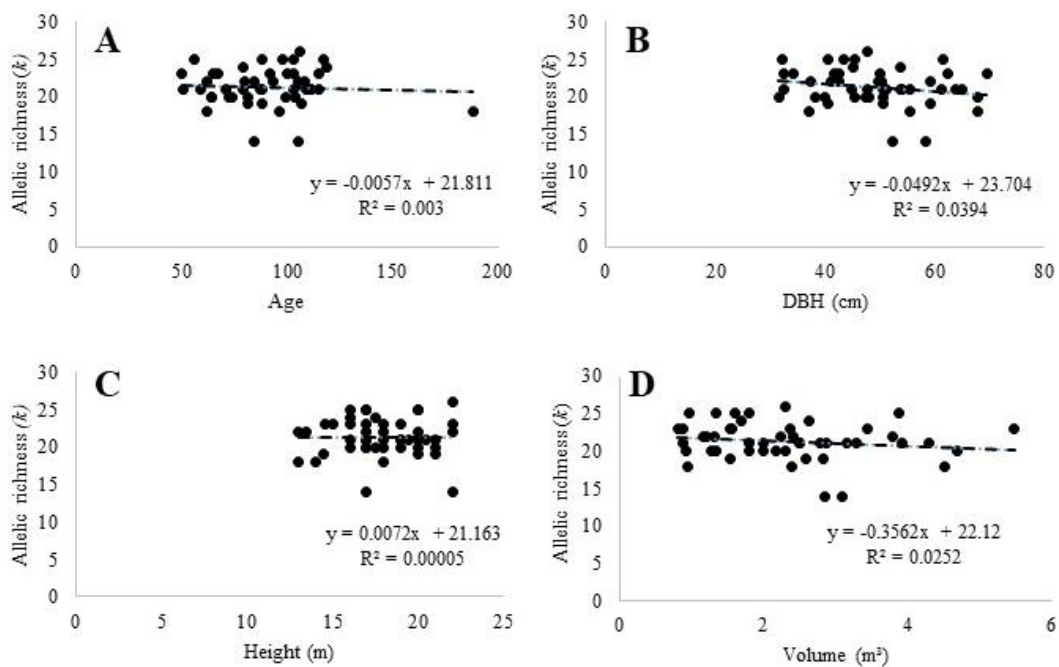


Figure 14 Relationship between individual allelic richness with age (A), DBH (cm) (B), height (cm) (C) and volume (m^3) (D) of the remnant trees and logged trees in an *A. angustifolia* population located in Fernandes Pinheiro county, Parana state, Brazil.

Figura 14 Relação entre riqueza alélica individual com as variáveis idade (A), DAP (cm) (B), altura (m) (C) e volume (m^3) (D) de árvores remanescentes e árvores cortadas em uma população de *A. angustifolia* localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil.

4.5. SPATIAL GENETIC STRUCTURE

The STRUCTURE analysis revealed that the most probable number of real populations was nine ($K = 9$) (Figure 15). It is possible to observe that individuals 17, 23, 45 and 46 were the most distinct of the population and the most uniform among them, with most of the contribution to their genome coming from a single population.

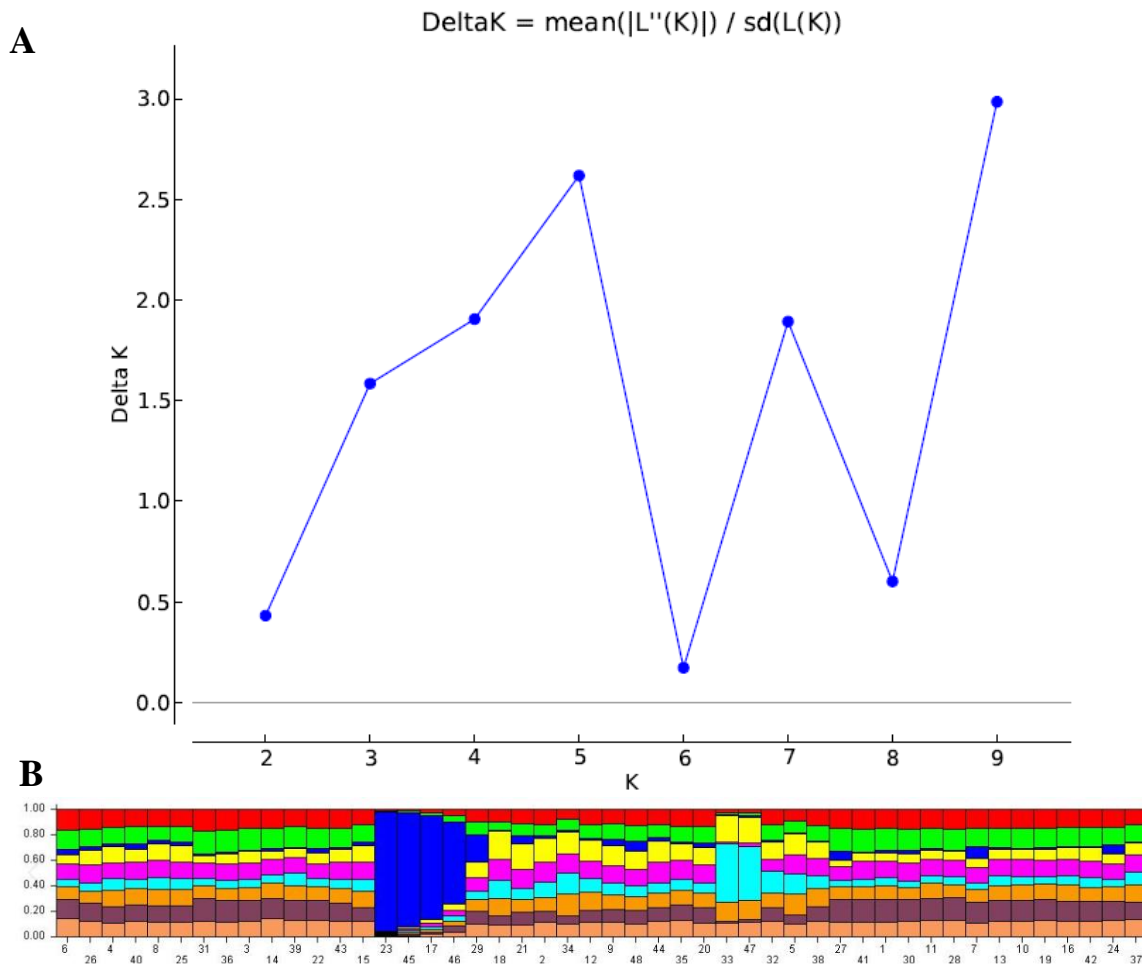


Figure 15 Genetic structure of an *A. angustifolia* population located in Fernandes Pinheiro county, Paraná state, Brazil. (A) Plot based on delta-K calculated according to Evanno et al. (2005) to estimate the actual number of clusters for the 48 sampled individuals. (B) Assignment of 48 *A. angustifolia* individuals from one population into nine ($K = 9$) clusters using a Bayesian-based population genetic structure analysis. Each solid bar represents a single individual, while colored areas correspond to distinct genetic clusters. Bars with two or more colors denote admixed genomes.

Figura 15 Estrutura genética de uma população de *A. angustifolia* localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil. (A) Gráfico com base em delta-K calculado de acordo com Evanno et al. (2005) para estimar o número real de clusters para os 48 indivíduos amostrados. (B) Atribuição de 48 indivíduos de *A. angustifolia* de uma população dentro de nove ($K = 9$) clusters utilizando análise de estrutura genética populacional Bayesiana. Cada barra sólida representa um indivíduo,

em que as áreas coloridas correspondem a clusters genéticos distintos. Barras com duas ou mais cores denotam genomas misturados.

The mean observed coancestry ($\bar{\theta}_{xy}$) by distance class for the total population (48 individuals) and for the managed population (24 individuals) after logging was very similar (Figure 16). After logging, there was an increase in the mean coancestry for the distance classes of 200-400 m and 400-600 m.

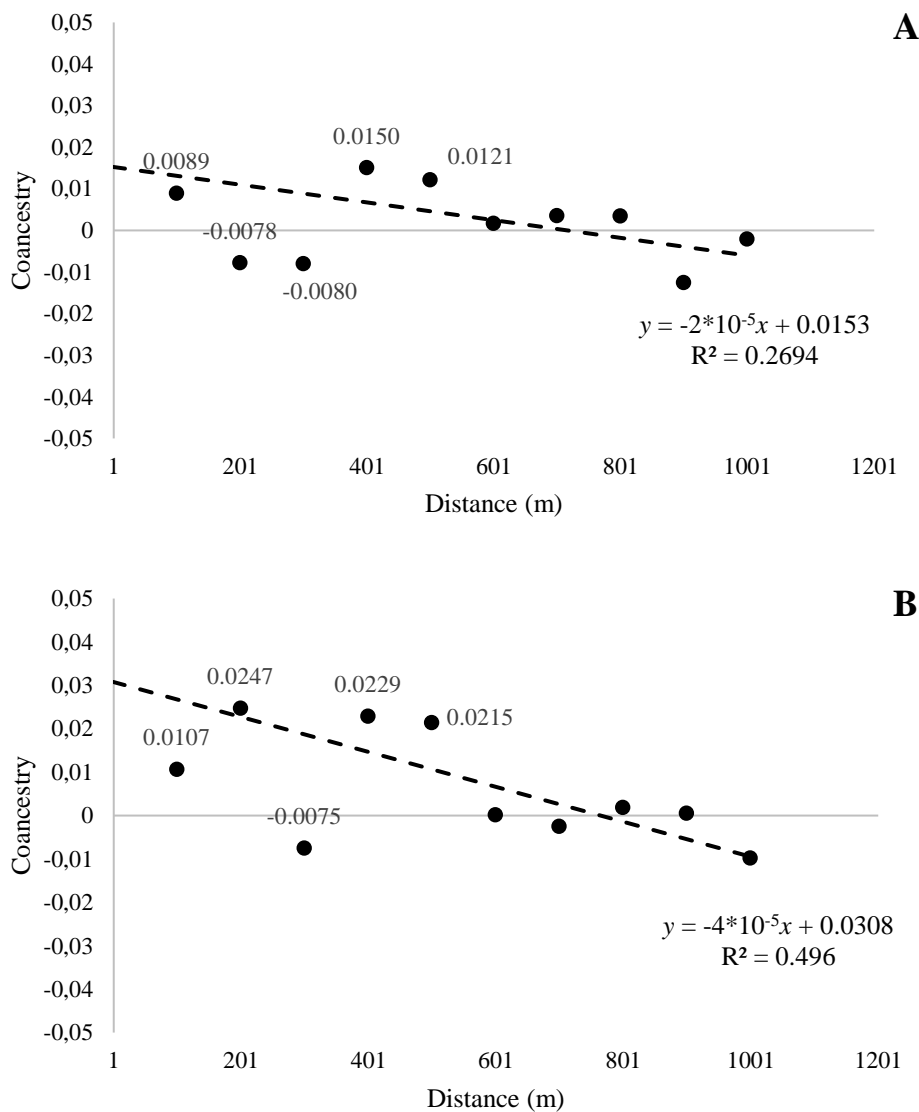


Figure 16 Mean observed coancestry by distance class for (A) total sample and (B) remnant individuals of an *A. angustifolia* population located in Fernandes Pinheiro county, Parana state, Brazil. The number of pairs of individuals ranged from 112 to 113 within each distance class.

Figura 16 Coancestria observada média em função de classes de distância para (A) amostra total e (B) indivíduos remanescentes de uma população de *A. angustifolia*

localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil. O número de pares de indivíduos variou de 112 até 113 dentro de cada classe de distância.

The frequency distribution of trees as a function of coancestry classes showed that the total population ($n = 48$) had higher levels of coancestry, and higher frequency values when compared to the remnant population sample ($n = 24$) (Figure 17).

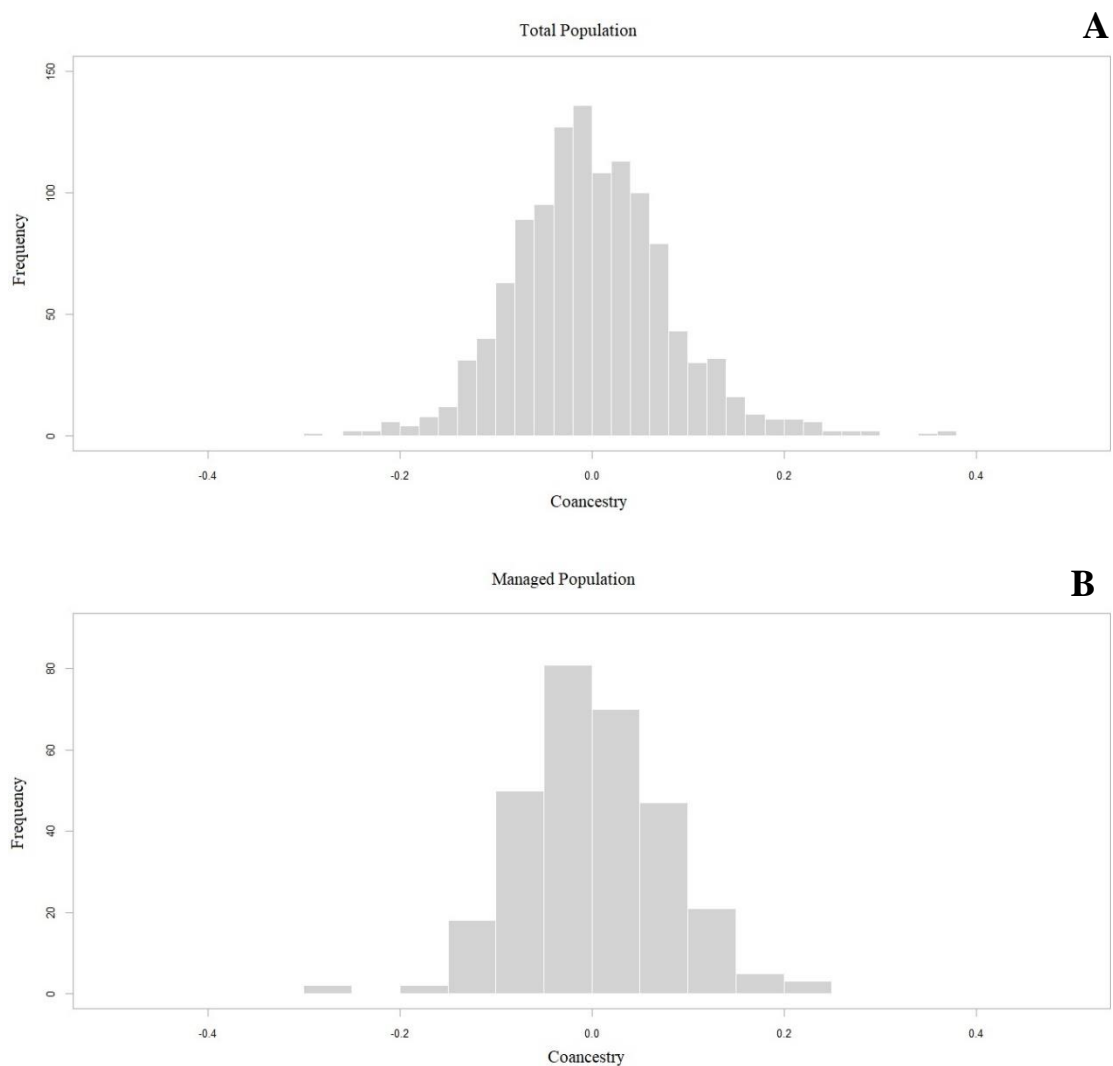


Figure 17 Frequency distribution of *A. angustifolia* trees as a function of coancestry classes for (A) the total population sample ($n = 48$) and (B) the logged population sample ($n = 24$). Population located in Fernandes Pinheiro county, Parana state, Brazil.

Figura 17 Distribuição de frequência de indivíduos de *A. angustifolia* em função de classes de coancestria para (A) amostra total ($n = 48$) e (B) amostra de árvores cortadas

($n = 24$). População localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil.

When the total population was subdivided in two samples, remnant and logged trees, the frequency distribution as a function of coancestry showed similar values. However, the sample of logged trees showed more coancestry classes and higher frequency of positive coancestry values than the sample of remnant trees (Figure 18).

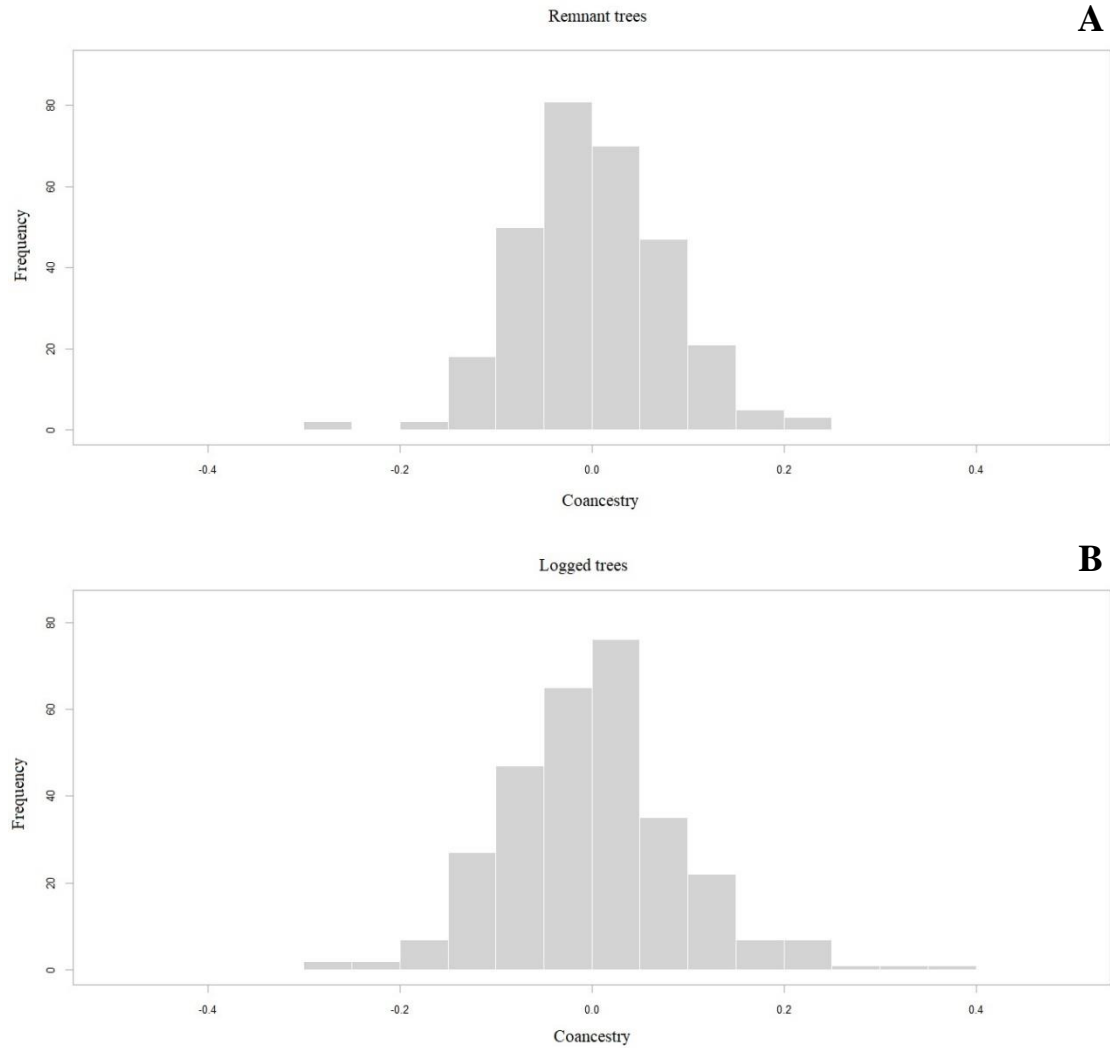


Figure 18 Frequency distribution of *A. angustifolia* trees as a function of coancestry classes for (A) the sample of remnant trees ($n = 24$) and (B) the sample of logged trees ($n = 24$). Population located in Fernandes Pinheiro county, Parana state, Brazil.

Figura 18 Distribuição de frequência de indivíduos de *A. angustifolia* em função de classes de coancestria para (A) amostra de árvores remanescentes ($n = 24$) e (B) amostra de árvores cortadas ($n = 24$). População localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil.

The analysis of the density distribution of *A. angustifolia* individuals as a function of coancestry classes for the samples of remnant trees and logged trees, both composed of 24 trees, showed a higher kurtosis for the logged trees in relation to the Gaussian distribution curve (Figure 19).

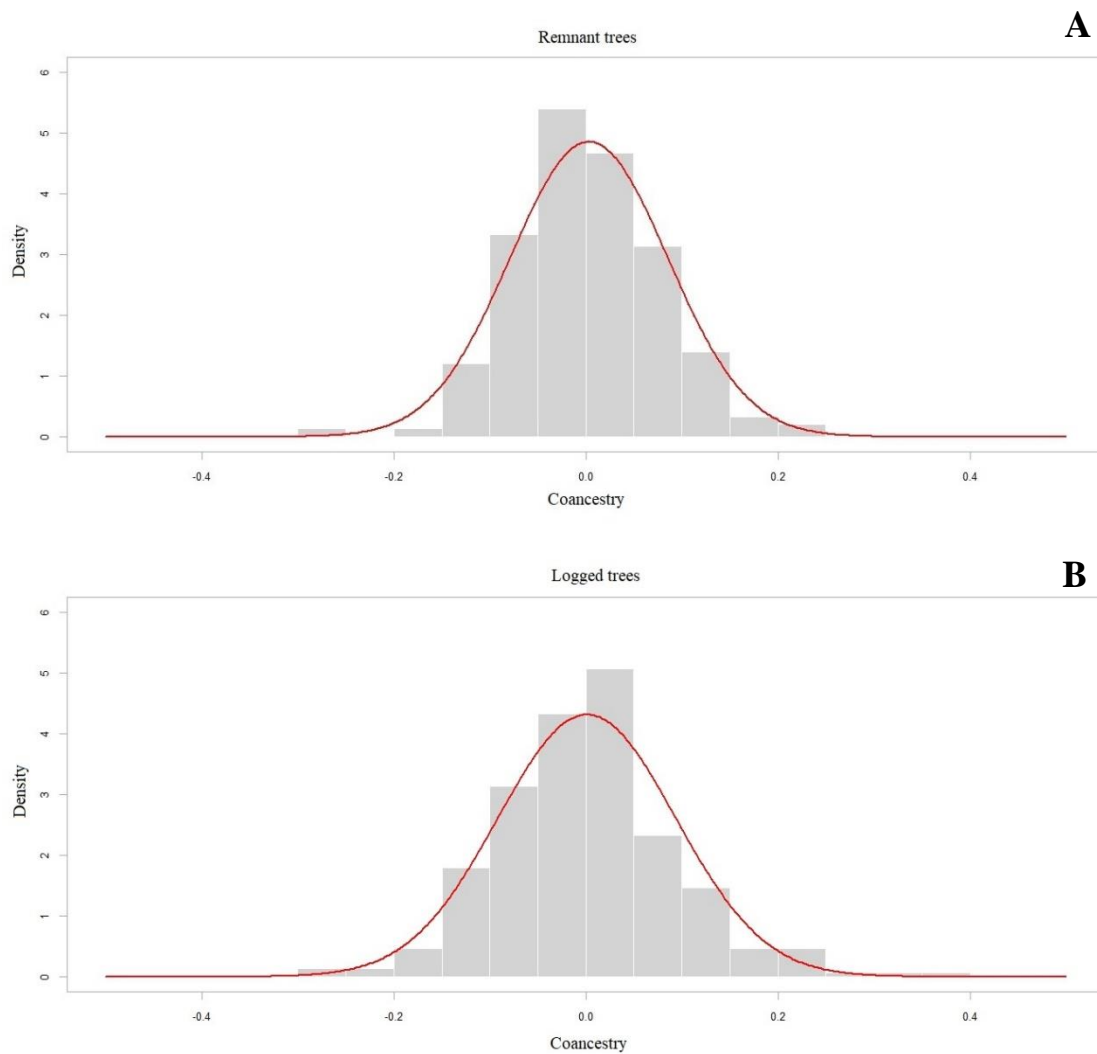


Figure 19 Density distribution of *A. angustifolia* individuals as a function of coancestry classes for (A) the sample of remnant trees ($n = 24$) and for (B) the sample of logged trees ($n = 24$). Population located in Fernandes Pinheiro county, Parana state, Brazil.

Figura 19 Distribuição de densidade de indivíduos de *A. angustifolia* em função de classes de coancestria para (A) amostra de árvores remanescentes ($n = 24$) e para (B) amostra de árvores cortadas ($n = 24$). População localizada no município de Fernandes Pinheiro, estado do Paraná, Brasil.

4.6. GENETIC BOTTLENECKS

For the remnant sample, the infinite allele model of mutation (IAM), showed 5.64 loci with a significant ($p < 0.00715$) excess of heterozygosity expected from 11. Considering all loci fit a stepwise mutation model SMM, in mutation-drift equilibrium, the analysis showed 6.24 loci with excess heterozygosity expected, however, observed heterozygosity was similar (8 loci) and not statistically significant ($p < 0.22360$).

For the logged sample the expected number of loci with heterozygosity excess was 5.60, whereas, the results showed 10 loci with heterozygosity excess and one locus with heterozygosity deficiency in the IAM analysis. The observed value of loci was higher than the expected value and significant ($p < 0.00647$). For the stepwise mutation model (SMM), the expected value of loci with heterozygosity excess was 6.0; however, the observed value was eight loci and was not statistically significant ($p < 0.18044$). The results for SMM showed that three loci have heterozygosity deficiency.

5. DISCUSSION

5.1. CHARACTERIZATION OF STUDY POPULATION STRUCTURE

According to Silva et al. (2012) in tropical species, an aggregated distribution pattern is common, however, several physical and biological factors may be related to the distribution observed for the species in the study population. The typology of soils, the availability of nutrients, competition among others, are some of these factors. An aggregated population implies that individuals cluster in spots, leaving spaces relatively unoccupied. Limited dispersion and habitat specialization are related to spotting or clustering, so individuals are in the most favorable parts of the habitat (HAY et al., 2000). An aggregated pattern was also reported for *A. angustifolia* by Anjos et al. (2004) in study sites in Parana state, however, the authors observed variation in behavior (aggregated and random) along the area at different distances. In the present study, this variation was not observed, and the aggregated behavior was verified in all analyzes (general, female and male) and at all distances evaluated (5 to 100 m).

For *A. angustifolia*, the aggregated distribution pattern can be explained by the way in which gene flow occurs between populations, pollination of individuals and seed dispersal. The seeds of *A. angustifolia* are dispersed primarily by barochory and secondarily by birds, being consumed by many species of fauna, among which *Dasyprocta* spp. and *Cyanocorax caeruleus* (Vieillot) bury the seed to eat them later, acting as dispersers (ANJOS, 1991; WREGGE et al., 2017).

The intraspecific relation between female and male was positive, suggesting a spatial dependence between them, presenting a ratio of 1: 1.6 (396 females and 604 males). This value was higher than those previously reported for *A. angustifolia* (1: 1, PALUDO et al., 2009, 1: 1.2, MANTOVANI et al., 2004, BITTENCOURT and SEBBENN 2007 and 1: 1.5, MANTOVANI et al., 2001). According to Vieira et al. (2008) populations of dioecious plant species often exhibit deviations from the sex ratio, commonly associated with the life history characteristics of the species or spatial segregation of the sexes in heterogeneous environments. This fact suggests a possible intervention in the area which resulted in a low proportion of females.

Regarding the diameter distribution the population presents a decreasing distribution, typical of native forest, which indicates that the population is still growing. In the evaluation at female and male level, the females presented a unimodal diameter

distribution and the males a decreased distribution. According to Bailey and Dell (1973) the distribution of diameter classes is a simple and powerful way of describing the structure of a forest, since the diameter is generally highly correlated with other important variables such as height and volume. The diameter distribution is important in the description of the structural profile and evaluates the growing stock and can be used to identify the classes with a deficit or surplus of trees (SCOLFORO et al., 1997).

The qualitative variables provided important information about the state of the study population, suggesting that it presented good development over the years with little influence of the environment. This is because the species presented straight stems, without phytopathogenic problems and with perfect crown form, suggesting little or no competition for light, since this species usually dominates the forest canopy with this forest typology.

In relation to phytosociology, the study species was considered the most important of the fragment, with a percentage of 45.09% of importance value, a result quite typical of this type of forest formation. According to Oliveira and Amaral (2004), the estimated Import Value Index (IV) for plant species in undisturbed areas can be used in management plans as an indicator of ecological importance due to the influence of the most frequent and dominant species in the basic processes of flora balance and wildlife maintenance, providing shelter and food. To carry out interventions in a forest area, it is necessary to know its characteristics, such as the richness of existing species and their phytosociological value. This information is important to characterize the region, to carry out forest interventions, as well as to know the economic and floristic potential of the region (ATTANASIO, 2008).

5.2. GENETIC DIVERSITY

Null alleles in microsatellite markers are frequent, since these alleles may be the result of amplification failures or when a mutation sequence changes one or both flanking regions (CALLEN et al., 1993). Despite this, in the current study, in general, we did not find evidence of null alleles in all analyzed loci. The detection of null alleles is important because their presence above 25% may alter the results of parameter estimates, such as number of alleles, heterozygosity, inbreeding index (DEWOOD; NASON; HIPKINS, 2006) and genetic distances (CHAPUIS and ESTOUP, 2007).

In populations of *A. angustifolia* in the state of Minas Gerais, Brazil, the presence of null alleles was reported for two loci from the eight tested, in a proportion of 8% to 28%, and these null alleles were observed in four of nine of studied populations (PÁDUA, 2015). Kelly et al. (2011) pointed out that null alleles with frequencies less than or equal to 25% do not significantly affect the evaluation of population structure, and this was the result observed in the present research (null alleles = 0%).

It was found that both allelic richness and number of rare alleles (A_r), private alleles (A_p), and rare and private alleles (A_{rp}) were higher for the remnant trees sample than for the logged trees sample. For the Aang27, Aang37, Aa1774 e Ag56 loci, both the remnant tree sample and the logged tree sample showed all alleles in common, with no private alleles present in either sample. The loss of rare alleles is detrimental to a population, since rare alleles are believed to offer unique responses to future evolutionary challenges (MEFFE and CARROLL, 1997). The evolutionary potential of populations might be influenced by the occurrence of rare alleles (HAWLEY et al., 1999). In addition, rare alleles are of interest to sampling strategy and gene flow theory (SJÖGREN and WYÖNI, 1994), and their maintenance is extremely important when wanting to aid the future management of natural populations.

The loss of low frequency alleles due to the direct removal of individuals or to early processes of genetic drift has a large impact in the reduction of gene flow between fragmented populations. This effect is most striking for lower frequencies of private alleles detected in more disturbed populations (INZA et al., 2018). Similarly, using microsatellite markers, Bittencourt and Sebbenn (2009) observed the loss of rare alleles in fragmented populations when compared with continuous populations of *A. angustifolia* in Brazil. Some authors report that constant or intense forest disturbance may result in a decrease in heterozygosity, leading to a greater risk of extinction of the local population (YOUNG and BOYLE, 2000; BITTENCOURT and SEBBENN, 2007; INZA et al., 2012).

For a better understanding of our results, we surveyed genetic diversity studies using microsatellite markers for *A. angustifolia* in continuous forests and fragments in the Central-West, South and Southeast regions of Brazil (Table 6). The number of alleles (120) found in the present study is considered high, especially when compared to other microsatellite studies for *A. angustifolia* (SANT'ANNA et al., 2013; STEFENON et al., 2007; BITTENCOURT and SEBBENN, 2009). The number of alleles per loci ranged

from three (loci Aang 37 and Aa1774) to 22 (locus Aang15), which are similar to those found in the other studies presented in Table 6.

Table 6 Studies of genetic diversity with microsatellite markers in *A. angustifolia*.

Tabela 6 Estudos sobre diversidade genética com marcadores microssatélites de *A. angustifolia*.

| Authors | Pop | Loci | Ind. | <i>k</i> | <i>A_e</i> (M) | <i>H_e</i> | <i>H_o</i> | <i>F</i> |
|--------------------------------|----------|-----------|-----------|------------|--------------------------|----------------------|----------------------|--------------|
| Salgueiro et al. (2005) | 3 | 8 | 60 | 49 | 3-11 (7.12) | 0.34 | 0.31 | 0.146 |
| Stefenon et al. (2007) | 6 | 5 | 384 | 73 | 7-21 (14.6) | 0.71 | 0.58 | 0.110 |
| Bittencourt and Sebbenn (2007) | 1 | 8 | 617 | 77 | 4-16 (9.6) | 0.59 | 0.51 | 0.158 |
| Bittencourt and Sebbenn (2008) | 1 | 8 | 298 | 62 | 3-15 (7.75) | 0.58 | 0.55 | 0.058 |
| Bittencourt and Sebbenn (2009) | 1 | 8 | 1179 | 106 | 4-20 (13.25) | 0.61 | 0.54 | 0.119 |
| Patreze and Tsai (2010) | 1 | 6 | 105 | 70 | 5-18 (11.7) | 0.74 | 0.81 | -0.089 |
| Sant'anna et al. (2013) | 1 | 9 | 513 | 86 | 3-22 (9.56) | 0.63 | 0.56 | 0.130 |
| Medina-Macedo et al. (2015) | 2 | 10 | 295 | 81 | 4-16 (8.1) | 0.70 | 0.91 | -0.310 |
| Pádua (2015) | 9 | 8 | 450 | 64 | 2-15 (8) | 0.77 | 0.82 | -0.067 |
| Dal Bem et al. (2015) | 1 | 8 | 158 | 77 | - | 0.54 | 0.54 | 0.069 |
| Present study | 1 | 11 | 48 | 120 | 3-22 (10.91) | 0.74 | 0.72 | 0.021 |

Number of populations studied (Pop.), number of loci (Loci), number of individuals (Ind.), allelic richness (*k*), range of alleles per locus (mean of alleles per locus) (*A_e* (M)), expected heterozygosity (*H_e*), observed heterozygosity (*H_o*) and fixation index (*F*).

In studies of other species, the authors observed loss of alleles due to the selective logging of trees based on simulations using the Ecogene model (SEBBENN et al., 2008; LACERDA et al., 2013; VINSON et al., 2014), and also in empirical studies comparing populations before and after selective logging of trees (SEBBENN et al., 2001; LACERDA et al., 2008; SILVA et al., 2008). On other hand, simulations to evaluate the effect of different selective logging scenarios on the genetic diversity and basal area of populations of *A. angustifolia*, detected the loss of alleles in all scenarios tested, with greater losses for scenarios with smaller minimum diameter cut and highest exploitation rates (DAL BEM et al., 2015). These suggest that studies with higher density of markers and related to growth and adaptive traits should be performed for logged populations. These results can provide guidance for adequate thinning in natural and planted populations to avoid losses of genetic variability.

According to Marshall and Brown's (1975) classification of the geographic distribution of alleles (Figure 20), the population of *A. angustifolia* shows individuals that have both rare alleles and common alleles, which are distributed throughout the study area. Marshall and Brown (1975) considered the question of optimal sampling strategies

for genetic conservation through the sustainable use of plants, and the application of these concepts is crucial in achieving sustainable forest management.

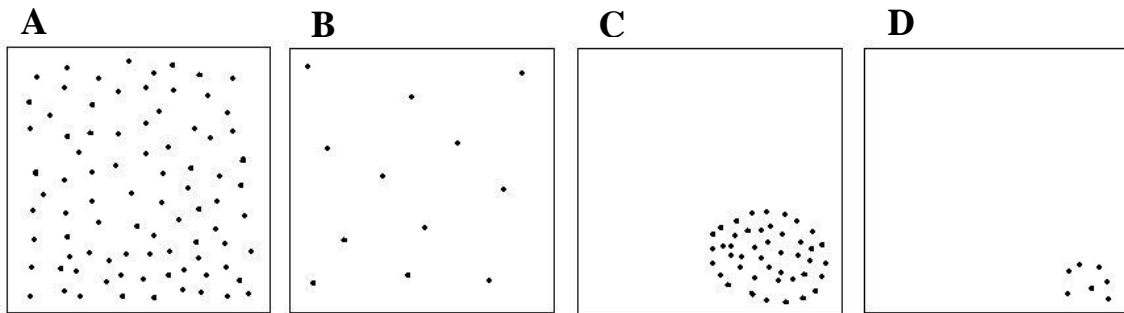


Figure 20 Geographical distribution of alleles adapted from Marshal and Brown (1975), where (A) common and widespread alleles; (B) rare and widespread alleles; (C) common and localized alleles and (D) rare and localized alleles.

Figura 20 Distribuição geográfica de alelos adaptada de Marshal e Brown (1975), em que (A) alelos comuns e dispersos; (B) alelos raros e dispersos; (C) alelos comuns e localizados e (D) alelos raros e localizados.

5.3. SPATIAL GENETIC STRUCTURE

The selective logging of trees resulted in an increase in mean coancestry ($\bar{\theta}_{xy}$) for distance classes between 200 and 600 m. This result indicates that, under the current logging scenario (managed population) there is a greater probability of sampled trees being related within these distance classes. This directly influences activities such as seed collection and forest management, for which, among other factors, a minimum distance between individuals should be respected to avoid processes such as inbreeding and maintain an adequate effective population size.

The results obtained with regard to genetic drift, inbreeding and coancestry can directly influence relatively small and isolated populations, as is the case for the present study, altering patterns of genetic diversity and adaptation, and consequently having an effect on the genetic conservation of a species (SIMBERLOFF, 2009). The capacity of this population to resist pathogens and pests, for example, may also be affected by the increase in the mean coancestry as a function of the selective logging (SPIELMAN et al., 2004).

Defavari et al. (2009) argued that, processes such as the founder effect (population originated by only one or very few genotypes), genetic drift (random changes in allele frequencies over time) and limited seed dispersal can generate significant spatial genetic

structure (SGS). In this case, it is important to highlight the history of the population, which has previously been submitted to selective logging, besides the introduction of new individuals through the planting of seedlings from different provenances.

SGS is influenced by mating system, gene flow, population density caused by the dispersal of seeds and pollen near to the mother tree, and overlapping generations (SEBBENN et al., 2011; SILVA et al., 2011). In the case of *A. angustifolia*, pollination is by wind (BITTENCOURT and SEBBENN, 2009), which implies crosses between different individuals at long distances, more gene flow between populations and, consequently, more genetic differentiation within populations. Such factors are of fundamental importance and should be considered in achieving sustainable forest management of the species.

5.4. GENETIC BOTTLENECKS

Populations that have experienced a recent reduction in their effective population size exhibit a correlative reduction in allele number and heterozygosity at polymorphic loci. But the allele number is reduced faster than heterozygosity (H_e). Thus, H_e becomes larger than the expected heterozygosity at mutation-drift equilibrium (H_{eq}) because H_{eq} is calculated from the allele number and the sample size (COURNET and LUIKART, 1996). For both models tested in this study, there was no evidence of a genetic bottleneck effect in the studied population.

Significant deviations from population mutation-drift equilibrium (e.g. bottlenecks) are important to detect because equilibrium is an assumption required for numerous analysis of population genetics data (NEI, 1987). Bottlenecks are important to detect in conservation biology because they can increase the risk of population extinction and may play a role in some modes of speciation (HARRISON, 1991; HOWARD, 1993).

Bottleneck events or founder effects may also lead to a drop in the genetic diversity of a population. In a study of populations of *A. angustifolia* in southern and southeastern Brazil, evidence of genetic bottlenecks was found in all southeastern populations analyzed, but in only three out of 13 southern populations (STEFENON et al., 2008). Anthropogenic activities such as selective logging may result in intensification of events such as the bottleneck effect. It has been verified in recent studies that selective exploitation results in a reduction in population size, leading to a loss of rare alleles, a decrease in heterozygosity and an increase in inbreeding of the Araucaria Forest

population (SOUZA et al., 2009). Despite this, in the present study, selective logging had greater effects only on the occurrence of rare and private alleles within the population.

However, even after drastic exploitation in the last century, the populations of southern Brazil *A. angustifolia* still conserve their genetic diversity. This is possibly due to recent fragmentation of the forest while *A. angustifolia*, is long lived up to 500 years. Due to the high-quality wood of the species (CARVALHO, 2003) and the importance of araucaria seeds as a source of nutrition for various human populations (REITZ et al., 1988), the transport of seeds by natives or other more recent human populations cannot be ignored. Thus, the current distribution of *A. angustifolia* and genetic traits may have been influenced by human interference. However, the observed reduction in genetic diversity in the southeast populations and the genetic differentiation between the southern and southeastern populations are primarily due to bottleneck events since the last glacial period, followed by genetic drift, and not to human exploration (LOWE et al., 2004, FRANKHAM et al., 2005).

6. CONCLUSIONS AND RECOMMENDATIONS FOR MANAGEMENT of *A. angustifolia* POPULATIONS

There were no significant difference between the diversity parameters for the logged trees and remnant trees. However, there was a decrease in genetic diversity for the population of *A. angustifolia* submitted to selective logging, mainly due to the loss of rare and private alleles, which may be of fundamental importance for the species survival.

The conclusions of this study are based on a small sample size, compared to other studies of genetic diversity with microsatellites in *A. angustifolia*. In addition, it is emphasized that the selective logging experiments were carried out with much more rigid criteria in comparison with the way that forest management is traditionally carried out for native species.

Given the degree of genetic diversity found in this population, it should be conserved, if possible both *in situ* and *ex situ*. It is possible to affirm that the high degree of polymorphism of this population would also make management feasible, if rules are established for the conservation of allelic diversity of individuals. Before future interventions and selective logging are carried out on this population, it is imperative that sampling of the logged individuals for conservation and vegetative propagation is made.

It is suggested that for future selective loggings, the spatial genetic structure of the population is considered. Ideally, the most closely related individuals should be logged to remove less genetic diversity from the population. It is very important to monitor this population, regarding the entry of new individuals and the regrowth of logged individuals. The monitoring of these events is fundamental for planning the maintenance of rare and private alleles in the population.

The results of this study show the importance of molecular genetic analysis as an auxiliary tool to forest management, particularly regarding the selection of guidelines for logging, aiming at the conservation of the genetic diversity of *A. angustifolia*.

Historically, the genetic diversity of small natural populations of *A. angustifolia* located in Brazil has tended to decrease, mainly due to a series of events that have reduced heterozygosity and increased loss of alleles. Such events may lead to extinction, and it is extremely important that management and conservation strategies consider the genetic differences between populations and between individuals within populations.

The results of this study should be used for the development of public policy to define proposals for the sustainable management of *A. angustifolia* in the different regions of its natural occurrence.

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