UNIVERSIDADE FEDERAL DO PAMPA

CAMPUS SÃO GABRIEL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

RAFAEL PLÁ MATIELO LEMOS

PADRÕES DE DISTRIBUIÇÃO E ESTRUTURA GENÉTICA DE *Schinus molle* L. NA REGIÃO DO PAMPA BRASILEIRO

DISSERTAÇÃO DE MESTRADO

SÃO GABRIEL

2014

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Dissertação apresentada ao Programa de Pós-Graduação *Stricto sensu* em Ciências Biológicas da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Mestre em Ciências Biológicas.

Orientador: Prof. Dr. Valdir Marcos Stefenon

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Elaborada por:

RAFAEL PLÁ MATIELO LEMOS

Dissertação defendida e aprovada em 08 de agosto de 2014

Banca Examinadora:

_____

Prof. Dr. Valdir Marcos Stefenon

Orientador - UNIPAMPA

_____

Prof. Dr. Filipe de Carvalho Victoria

Membro Titular - UNIPAMPA

_____

Prof. Dr. Luiz Fernando Würdig Roesch

Membro Titular - UNIPAMPA

_____

Prof. Dr. Frederico Costa Beber Vieira

Suplente - UNIPAMPA

AGRADECIMENTOS

RESUMO

Um dos principais aspectos para a ecologia de populações e evolução é o entendimento da conectividade entre os indivíduos e seus grupos. O bioma Pampa apresenta diversos componentes florísticos de grande importância ecológica, e sua estrutura de campo, estépico-savana, vem sendo fragmentada e impactada pelo sistema de produção e pela falta de manejo desse bioma naturalmente frágil. A partir da informação da diversidade genética de populações nativas é possível entender o atual estado de fragmentação ambiental, esclarecer se há fluxo-gênico entre populações, e sugerir formas de manejo que possam garantir a sobrevivência da biodiversidade local. Nesse estudo, *Schinus molle* L. (Anacardiaceae) foi empregada para avaliar a dinâmica ecológica, a diversidade e a estrutura genética em espécies arbóreas no bioma Pampa. A dinâmica de expansão prevista para a espécie foi determinada através de modelagem de nichos ecológicos e estrutura e diversidade genética foram avaliadas em nove populações amostradas dentro do bioma Pampa *stricto sensu*, utilizando marcadores microssatélite e AFLP. O mapa da modelagem de nichos ecológicos de *S. molle* sugere a expansão da espécie sobre o campo, como um fenômeno natural da dinâmica ecológica do bioma. A estrutura genética intra- e inter-populacional sugere limitações ao fluxo gênico e, a diversidade genética intra-populacional é baixa se comparada a espécies com as mesmas características biológicas. O isolamento entre populações e o pequeno tamanho destas parece ser o principal fator interferindo negativamente no ambiente. A manutenção de conexões entre as populações é a ação imediata sugerida para preservar a espécie e o bioma.

Palavras-chave: expansão florestal, campo, diversidade genética, fluxo-gênico, AFLPs.

ABSTRACT

One of the main aspects for the population ecology and evolution is the understanding of the connectivity among individuals and their groups. The Pampa biome presents several floristic elements of high ecological importance and its grassy structure, steppic-savanna, has been fragmented and impacted by the production system and by the absence of management of this naturally fragile biome. From the information about genetic diversity of native populations, it is possible to understand the current status of the environment degradation, highlighting the presence of gene flow among populations and to suggest management strategies that could guarantee the survival of the local biodiversity. In this study, *Schinus molle* L. (Anacardiaceae) was employed to evaluate the ecological dynamic, the genetic diversity and structure in tree species of the Pampa biome. The expected expansion dynamic for this species was determined through ecological niche modeling and the genetic diversity and structure were evaluated in nine populations sampled within the Pampa *stricto sensu*, using microsatellite and AFLP markers. The ecological niche modeling map of *S. molle* suggests the species expansion over the grassland as a natural phenomenon of the biomes ecological dynamic. The intra- and inter-population genetic structure suggests limitations to the gene flow and the intra-population genetic structure is low in comparison to species with the same biological traits. The isolation among populations and their small size seems to be the main factor negatively interfering in the environment. The maintenance of connections among the populations is the immediate action suggested to safeguard the species and the biome.

Keywords: forest expansion, grassland, genetic diversity, gene-flow, AFLPs.

SUMÁRIO

INTRODUÇÃO GERAL

O bioma Pampa brasileiro é representado principalmente por um ambiente característico de campo/estepe e savana-estépica (Cordeiro e Hasenack, 2009). Estas formações são dominadas por gramíneas, porém apresentam importantes áreas com formações arbóreas na forma de capões de mato e de mata ciliar (Roesch et al., 2009). Ocupa cerca de 2% do território brasileiro sendo restrito ao estado do Rio Grande do Sul onde estende-se por 63% da área deste estado e caracteriza a fisionomia da metade sul. De acordo com Boldrini et. al. (2010) e Deble (2011), esse bioma, situado ao sul do paralelo 30ºS, está na fronteira entre as zonas climáticas tropical e subtropical onde é evidenciada a interposição de campos e florestas em seu território geográfico, o que garante a riqueza de espécies e uma grande biodiversidade sendo grande parte endêmica a essa região e amaçada de extinção segundo os critérios da União Internacional da Conservação da Natureza (IUCN, 2010).

Recentes alterações globais nos ecossistemas tem gerado a necessidade de conservação do patrimônio genético de diversas espécies. O bioma Pampa tem sofrido grande impacto do sistema de produção agrosilvipastoril, sua paisagem vem sendo alterada em larga escala e segundo o Relatório Técnico: "Cobertura vegetal do bioma Pampa" (PROBIO, 2007) apenas 42% da cobertura natural do bioma Pampa permanecia inalterada o que já deve ter diminuído consideravelmente nesses últimos sete anos. Levando em consideração a fragilidade natural deste bioma, caracterizada no trabalho de Roesch et al. (2009), existe uma tendência de continuidade e consequente ampliação do processo de degradação ambiental e sociocultural da região. A destruição das áreas de formação florestal no Pampa (matas de galeria, capões de mato e etc.) acarreta em drástica redução da biodiversidade local tendo em vista que essas formações são refúgio e habitat para diversas espécies da fauna e flora inclusive endêmicas da região (Deble, 2011).

Embora nenhuma expansão expressiva de populações arbóreas ser observada neste bioma (Bredekamp et al., 2002; Pillar, 2003; Roesch et al., 2009), as condições climáticas atuais, a profundidade e os tipos de solos do Pampa brasileiro são considerados favoráveis para o desenvolvimento de florestas. Iniciativas tomadas nos últimos anos, visando gerar conhecimento sobre a atual biodiversidade do Pampa gaúcho, têm centrado esforços em inventários taxonômicos, como o Inventário Florestal Contínuo do Rio Grande do Sul (UFSM/SEMA-RS, 2007), e levantamentos fitossociológicos de áreas de campo (Boldrini et al., 1998; Pillar et al. 2009; Freitas et al., 2010). Apesar da importância incontestável destes trabalhos para o conhecimento da diversidade biológica do bioma Pampa, existe ainda uma

carência em relação a inventários genéticos, que permitam determinar áreas de especial atenção para a conservação dos recursos genéticos florestais da região.

Considerando que grande parte da riqueza e da biodiversidade da região Pampeana do estado do Rio Grande do Sul carece de informações cientificamente sistematizadas, a estimativa adequada da diversidade em nível genético representa um passo importante para o reconhecimento de áreas prioritárias para a conservação dos recursos genéticos florestais deste bioma. A continuada erosão dos recursos genéticos florestais também demanda uma precisa prospecção da diversidade genética desse ecossistema, propiciando ferramentas para a reavaliação das formas de uso dos recursos florestais.

Para ecologia de populações e evolução, um dos aspectos mais importantes é o entendimento da conectividade entre indivíduos e seus grupos. O fluxo gênico é um fator vital para a estrutura das populações ao longo do tempo. Enquanto alto fluxo gênico leva à homogeneidade de estrutura genética, o baixo fluxo gênico não permite a distribuição aleatória de alelos e genótipos (Stefenon et al., 2008) e a estrutura genética do componente arbóreo demonstra o verdadeiro estado de fragmentação do ambiente em que ocorre. A exposição das populações aos problemas ecológicos e genéticos através de fragmentação florestal pode aumentar a endogamia, a perda de variação gênica e diminuir a capacidade reprodutiva e resistência à doenças o que faz com que essas populações sejam mais suscetíveis a mudanças ambientais e torna a adaptação mais difícil (Souza et al., 2013). É essencial que as populações nativas mantenham diversidade genética suficiente para serem capazes de responder às rápidas alterações climáticas e impactos externos, reduzindo assim a probabilidade de extinções locais (Federman et al., 2014).

Marcadores moleculares têm sido amplamente utilizados para avaliar os níveis e padrões de fluxo gênico entre e dentro de populações de espécies arbóreas. Pesquisas com o objetivo de destacar a estrutura genética espacial, ou seja, dentro do fluxo gênico da população, são baseadas principalmente na computação de coeficientes de parentesco, comparando os valores observados com o esperado em cruzamentos aleatórios (Hardy et al., 2006). Para tais estudos, os marcadores moleculares devem ser tão variáveis e polimórficos quanto possível. O marcador AFLP (*Amplified fragment length polymorphism*/Polimorfismo de fragmento de tamanho amplificado) não necessita de conhecimento anterior da sequência nucleotídica, é altamente polimórfico e têm um alto nível de reprodutibilidade. Têm sido amplamente utilizado para estimar relações genéticas e avaliações de diversidade (Vuylsteke et al. 2008), sendo extremamente confiável na análise de diversidade genética espacial (Jump e Peñuelas, 2007; Stefenon et al., 2008).

Diversos estudos têm investigado os padrões de estrutura genética espacial em populações naturais de espécies de árvores da Floresta Amazônica, Mata Atlântica e Cerrado no Brasil (ver Stefenon et al., 2008, Baldauf et al., 2014). No entanto, estudos sobre estrutura genética de espécies arbóreas nativas para o bioma Pampa têm sido negligenciados.

Como modelo para este estudo optamos pela utilização do *Schinus molle* L. (Anacardiaceae) também conhecida por Aroeira-salsa, Aroeira-piriquita ou Anacahuita. *S. molle* é uma árvore de porte mediano, com 3 a 10 metros de altura e expressiva dispersão pelo estado do Rio Grande do Sul, sendo encontrada em beiras de estrada do bioma Pampa. Possui ampla distribuição global sendo naturalmente encontrada no sul do Brasil, Argentina, Uruguai, Paraguai, Bolívia e Peru (Sobral e Jarenkow, 2006) além de relatos como invasora ou naturalizada na África do Sul (Henderson, 2007; Milton, 2007; Iponga, Milton e Richardson, 2008, 2009a, b, c; Richardson et al, 2010 e Iponga de 2010), Havaí (Asner et al., 2008) e na Califórnia (Howard e Minnich, 1989). Apresenta madeira dura e fácil de trabalhar, de alta durabilidade quando exposta às intempéries. Na região, tem sido geralmente utilizada para moirões de cerca devido a esta durabilidade (Reitz et al., 1988). Por ser heliófita e seletiva xerófita, frequente em solos arenosos dos campos da Depressão Central, apresenta-se como uma potencial espécie para ser empregada no controle da expansão de áreas de arenização. Os óleos essenciais encontrados nas folhas, frutos e casca desta planta possuem propriedades antimicrobianas, antifúngicas, antiespasmódicas, antipiréticas, anti-inflamatórias e cicatrizantes sendo amplamente utilizados na medicina popular (Fernandes e Favero, 2014).

HIPÓTESES

- *Schinus molle* L. tem as características necessárias para abrigar expansão florestal sobre os campos do bioma Pampa além de possuir alta diversidade genética, permitindo elevado potencial adaptativo.

- As populações de *S. molle* têm uma diferenciação genética consistente, devido ao seu isolamento geográfico dentro do Pampa brasileiro, devido a um processo de isolamento-por-distância.

OBJETIVO GERAL

Realizar o levantamento da distribuição geográfica de populações naturais de *Schinus molle* L. no bioma Pampa Brasileiro, verificar a existência de um padrão para a ocorrência dessa espécie nesse ambiente, identificar os padrões de diversidade e estrutura genética dentro e entre suas populações além de constatar a possível necessidade de conservação desse recurso genético.

OBJETIVOS ESPECÍFICOS

- Identificar e mapear populações naturais de *Schinus molle* L. no Pampa *stricto sensu* (região da Campanha) e coletar amostras foliares de ao menos 30 indivíduos em 10 destas populações, para estudos de genética populacional.

- Caracterizar a diversidade genética intra e interpopulacional de *S. molle*, através de marcadores microssatélites e/ou AFLPs e correlacionar as análises com a fragmentação ambiental e a possível expansão florestal sobre o campo.

METODOLOGIA EMPREGADA

Amostras foliares de indivíduos adultos (n = 278) foram coletadas em nove populações naturais de *Schinus molle* L. na Região da Fronteira Oeste e Sul do Estado do Rio Grande do Sul (Pampa *stricto sensu*). Para as análises de distribuição da espécie, foram registrados, durante o trabalho de campo, os pontos de ocorrência de populações naturais, enquanto os dados climáticos foram obtidos em bancos de dados específicos. Para as análises genéticas, inicialmente testou-se a transferência de marcadores microssatélites desenvolvidos para a espécie *Schinus terebinthifolius* Raddi. Devido à baixa transferência dos locos microssatélites, marcadores AFLP foram utilizados. O detalhamento sobre os materiais e métodos utilizados são descritos nos capítulos a seguir.

REFERÊNCIAS

Asner, G. P.; Jones, M. O.; Martin, R. E.; Knapp, D. E.; Hughes, R. F. (2008). Remote sensing of native and invasive species in Hawaiian forests. Remote Sensing of Environment 112:1912–1926.

Baldauf, C.; Guillardi, M. C.; Aguirra, T. J.; Correa, C. E.; Santos, F. A. M.; Souza, A. P.; Sebbenn, A. M. (2014) Genetic diversity, spatial genetic structure and realized seed and pollen dispersal of *Himatanthus drasticus* (Apocynaceae) in the Brazilian savanna. Conserv Genet 1-11.

Boldrini, I. I.; Ferreira, P. M. A.; Andrade, B. O.; Schneider, A. A.; Setubal, R. B.; Trevisan, R; Freitas, E. M. (2010) Bioma Pampa: diversidade florística e fisionômica. Porto Alegre: Pallotti.

Boldrini, I. I.; Miotto, S. T. S.; Longhi-Wagner, H. M.; Pillar, V. D.; Marzall, K. (1998) Aspectos florísticos e ecológicos da vegetação campestre do Morro da Polícia, Porto Alegre, RS, Brasil. Acta Botanica Brasilica. 12:89-100.

Bredekamp, G. J.; Spada, F.; Kazmierczak, E. (2002) On the origin of northern and southern hemisphere grasslands. Plant Ecology 163:209–229.

Cordeiro, J. L. P.; Hasenack, H. (2009) Cobertura Vegetal Atual do Rio Grande do Sul. In: Pillar, V. P.; Müller, S. C.; Castilhos, Z. M. S.; Jacques, A. V. (eds.) Campos Sulinos: conservação e uso sustentável da biodiversidade. v 2. Brasília MMA. p 285-299.

Deble, L. P. (2011) A Vegetação Campestre no Bioma Pampa. In: Deble, L. P.; Deble, A. S. O.; Leão, A. L. S. (Org.). O Bioma Pampa: contribuições científicas. Bagé, RS: Ediurcamp.

Federman, S.; Hyseni, C.; Clement, W.; Oatham, M. P.; Caccone, A. (2014) Habitat fragmentation and the genetic structure of the Amazonian palm *Mauritia flexuosa* L. f. (Arecaceae) on the island of Trinidad. Conserv Genet. 15:355–362.

Fernandes, E. T.; Favero, S. (2014) Óleo essencial de *Schinus molle* L. para o controle de *Sitophilus zeamais* Most.1855 (Coleoptera:Curculionidae) em milho. Rev. Bras. de Agroecologia. 9(1):225-231.

Freitas, E. M.; Trevisan, R.; Schneider, A. A.; Boldrini, I. I. (2010) Floristic diversity in areas of sandy soil grasslands in Southwestern Rio Grande do Sul, Brazil. Revista Brasileira de Biociências 8:112-130.

Hardy, O. J.; Maggia, L.; Bandou, E.; Breyne, P.; Caron, H.; Chevallier, M. E.; Doligez, A.; Dutech, C.; Kremer, A.; Hallé, C. L.; Troispoux, V.; Veron, V.; Degen, B. (2006)

Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. Mol Ecol 15:559–571.

Henderson, L. (2007) Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37(2):215–248.

Howard, L. F.; Minnich, R. A.; (1989) The introduction and Naturalization of *Schinus molle* (Pepper Tree) in Riverside, California. Landscape and Urban Planning 18:77-95.

Iponga, D. M. (2010) Seed set of the invasive tree *Schinus molle* (Anacardiaceae) in semi-arid savanna, South Africa: The role of pollinators and selfing. Journal of Arid Environments. 74:414–416.

Iponga, D. M.; Milton, S. J.; Richardson, D. M. (2008) Superiority in competition for light: A crucial attribute defining the impact of the invasive alien tree *Schinus molle* (Anacardiaceae) in South African savanna. Journal of Arid Environments. 72:612–623.

Iponga, D. M.; Milton, S. J.; Richardson, D. M. (2009a) Performance of seedlings of the invasive alien tree *Schinus molle* L. under indigenous and alien host trees in semi-arid savanna. African Journal of Ecology. 48:155–158.

Iponga, D. M.; Milton, S. J.; Richardson, D. M. (2009b) Reproductive potential and seedling establishment of the invasive alien tree *Schinus molle* (Anacardiaceae) in South Africa. Austral Ecology. 34:678–687.

Iponga, D. M.; Milton, S. J.; Richardson, D. M. (2009c) Soil type, microsite, and herbivory influence growth and survival of *Schinus molle* (Peruvian pepper tree) invading semi-arid African savanna. Biol Invasions. 11:159–169.

IUCN (2010) Guidelines for Using the IUCN Red List Categories and Criteria. Version 8.1. Prepared by the Standards and Petitions Sub–Committee in March 2010.

Jump, A. S.; Peñuelas, J. (2007) Extensive spatial genetic structure revealed by AFLP but not SSR molecular markers in the Wind-pollinated tree, Fagus sylvatica. Mol Ecol 16:925-936.

Milton, S. J.; Wilson, J. R. U.; Richardson, D. M.; Seymour, C. L.; Dean, W. R. J.; Iponga, D. M.; Proche, S. (2007) Invasive alien plants infiltrate bird-mediated shrub nucleation processes in arid savanna. Journal of Ecology. 95:648–661.

Pillar, V. D. (2003) Dinâmica da expansão florestal em mosaicos de floresta e campos no sul do Brasil. In: Claudino-Sales, V. (Ed.) Ecossistemas Brasileiros: Manejo e Conservação, Fortaleza: Expressão Gráfica e Editora, pp. 209-216.

Pillar, V. P.; Müller, S. C.; Castilhos, Z. M. S.; Jacques, A. V. (2009) Campos Sulinos: conservação e uso sustentável da biodiversidade. v 1. Brasília MMA. 403 p.

PROBIO (2007). Cobertura Vegetal do Bioma Pampa. Relatório Técnico. Ministério do Meio Ambiente.

Reitz, R.; Klein, R. M.; Reis, A. (1988) Projeto Medeira do Rio Grande do Sul. SUDESUL, Herbário Barbosa Rodrigues.

Richardson, D. M.; Iponga, D.M.; Roura-Pascual, N.; Krug, R. M.; Milton, S. J.; Hughes, G. O.; Thuiller, W. (2010) Accommodating scenarios of climate change and management in modelling the distribution of the invasive tree *Schinus molle* in South Africa. Ecography. 3:1049- 1061.

Roesch, L. F. W.; Vieira, F. C. B.; Pereira, V. A.; Schünemann, A. L.; Teixeira, I. F.; Senna, A. J. T.; Stefenon, V. M. (2009) The Brazilian Pampa: A Fragile Biome. Diversity 1:182-198.

Sobral, M.; Jarenkow, J. A. (2006) Flora Arbórea e Arborescente do Rio Grande do Sul, Brasil. São Carlos: RiMa: Novo Ambiente.

Souza, L. B.; Ruas, E. A.; Rodrigues, L. A.; Ruas, C. F.; Ruas, P. M. (2013) AFLP marker analysis revealing genetic structure of the tree *Parapiptadenia rigida* (Benth.) Brenan (Leguminosae-Mimosoideae) in the southern Brazilian Tropical Rainforest. Genetics and Molecular Biology. 36(4):533-539.

Stefenon, V. M.; Gailing, O.; Finkeldey, R. (2008) The role of gene flow in shaping genetic structures of the subtropical conifer species *Araucaria angustifolia*. Plant Biol 10:356-364.

UFSM/SEMA-RS (2007) Inventário Florestal Contínuo do Rio Grande do Sul. http://w3.ufsm.br/ifcrs/frame.htm.

Vuylsteke, M.; Peleman, J. D.; Eijk, M. J. T. (2008) AFLP technology for DNA fingerprinting. Nat Protoc. 2(6):1387-1398.

CAPÍTULO 1. Modeling distribution of *Schinus molle* L. in the Brazilian Pampa: insights on vegetation dynamic and conservation of the biome.

(Artigo submetido ao Periódico *Annals of the Forest Research* sob autoria de Rafael Plá Matielo Lemos, Cristiane Barbosa D'Oliveira, Carla Roberta Rodrigues, Luiz Fernando Würdig Roesch, Valdir Marcos Stefenon)

ABSTRACT

Several researchers have proposed the development of natural forests in the Brazilian Pampa biome should occur, although no significant forest expansion over grassland has been noticed. In this study, we used niche-modeling distribution of the tree species *Schinus molle* L. to discuss the vegetation dynamic of the Brazilian Pampa and strategies that assure the conservation of this biome. Based on climatic variables, we show that a broad area of the Brazilian Pampa is suitable for expansion of *S. molle* populations, supporting the forest expansion over grassland as a natural process in the Pampa biome. We propose that the nonexistence of tree species expansion over the grassland is probably effect of the resilience of the grassland and the human interference through expansion of agriculture, ranching and forestry with exotic species. Therefore, conservationist actions should focus on establishing preservation unities that include forest populations and grassland, while the environmental management should be applied just in grazing areas with historical human interference. Such actions will respect the ecological dynamic of the Pampa and value the forest formations in this grassland-dominated environment.

Key words: Forest expansion; Peruvian pepper tree; maximum entropy; vegetation dynamic

Introduction

Schinus molle L. (Anacardiaceae) is an evergreen dioecious tree species (Figure 1A) with natural occurrence in the Pampa Biome. It is recognized as an invasive species in South Africa (Iponga et al. 2009), Hawaii (Asner et al. 2008) and California (Howard & Minnich 1989), with high dispersion capacity and large ability to survive into new regions. These characteristics offer good opportunities for gaining insights regarding the ecological dynamic of tree species populations in the Brazilian Pampa and understanding the needs for the conservation of this biome.

The Brazilian Pampa is restricted to the southernmost region of the country, where it occupies about 176,496 km2 comprising a mosaic of different vegetation physiognomies, dominated by grassland environments (Roesch et al. 2009). The current climatic conditions and the depth of the soils from the Brazilian Pampa have been considered favorable to the development of forests, although no expressive expansion of tree populations is observed in this biome (Bredekamp et al. 2002; Pillar 2003; Roesch et al. 2009). Consequently, remains the question: which features are determinant in precluding the expansion of the tree populations over the grassland in the Pampa Biome? Although it is an old question (e.g. Rambo 1956; Hueck 1966; Klein 1975), few studies tried to answer it through empirical research of ecological dynamic of tree species.

Although forests are not the most significant plant component of the Pampa biome, woody formations are quite important in this region. Forest formations are crucial for the protection of watercourses, as housing and nesting area for several birds, mammals and epiphyte plants, as well as being a source of high biodiversity. However, conserving regional biodiversity is directly associated to the species occurring in a region and the several biotic and abiotic features that comprise an ecosystem (Myers et al., 2000).

Aiming to have rapid information towards taking decisions about nature preservation, conservationists have increasingly relied on predictive models to provide decision-makers with information on species distribution. In addition, modeling the species distribution over ecosystems have become a strategic tool in documenting biodiversity on the planet and are crucial to understanding the effect of multiple stresses caused by climate and human-induced changes (Saatchi et al. 2008).

The present study aimed to highlight the natural dynamic of tree populations in the Pampa biome concerning ecological succession of the vegetation, by determining likely occurrence areas of *S. molle* populations in the Pampa biome in Southern Brazil, using maximum entropy and GIS-based modeling. Considering the pioneer nature of *S. molle*, the

large dispersion capacity and the high adaptability, we intend to answer two main questions: (1) is the expansion of tree species over grassland an expected process in the Brazilian Pampa? (2) Which strategies should be used to guarantee the natural dynamic and conservation of this biome?

Material and Methods

Studied species and data collection

Schinus molle (Peruvian pepper tree) is an important tree species naturally occurring in the Pampa biome (Sobral & Jarenkow 2006). It can flower at any time of the year and female trees bear a mixture of ripe and unripe fruits (Figure 1B) most of the time, making their seeds available for dispersal throughout the year (Milton et al. 2007). Seeds are dispersed by birds, mammals and in running water, while seedlings become established in washes, ravines, old fields and rock outcrops (Howard & Minnich 1989). Schinus molle is recognized by its use in the folks' medicine (Dikshit et al., 1986), by the pharmaceutical uses of its essential oils (Marongiu et al., 2004) and by its use as the delicacy pink pepper (Goldstein and Coleman, 2004).

Data about occurrence of S. molle populations were collected in the savanna, steppic-savanna and steppe portions of the Brazilian Pampa, hereafter called Pampa stricto sensu (29000'–32000'S, 53030'–58000'W). The Pampa stricto sensu (Figure 2A) is characterized by large extensions of grassy vegetation with occurrence of tree species as gallery forests and scattered forest formations across the grasslands (Roesch et al. 2009). Because S. molle is a heliophyte species that usually occurs in small to median sized populations along disturbed environments, we conducted a search throughout all the Pampa stricto sensu for such locations, as well as for primary and secondary forest formations, where populations of S. molle are expected to be found. Since this species is sometimes planted by landowners in the Pampa biome, just clusters formed by at least 10 individuals with random distribution were classified as a population of S. molle, characterizing natural dispersion of the species.

Distribution model simulations

To estimate the extent of occurrence and the most probable natural locations of S. molle occupation in the Pampa biome we employed the maximum entropy distribution model algorithm, using the software MaxEnt version 3.3.3 (Phillips et al. 2006). This algorithm estimates the probability distribution for a species' occurrence as function of environmental limitations, based on species presence data and environmental variable layers of the study

area. The model includes a deterministic outline, which enables performing confident analyses with information about presence-only point occurrences and high performance with few point localities (Saatchi et al. 2008). The actual occurrence of *S. molle* within the Pampa stricto sensu was registered as latitude and longitude in the middle point of each population.

Climatic data were extracted from the WorldClim database (http://www.worldclim.org; Hijmans et al. 2005). These metrics are derived from monthly temperature and rainfall values (11 temperature and eight precipitation metrics), representing biologically meaningful variables for characterizing species distribution (Nix, 1986).

The Geographic Information System DIVA-GIS (http://www.diva-gis.org) was used to compile the MaxEnt results interpolating climatic surfaces for global land areas in 2.5 arc minutes maps (about 5 km² resolution). The predictive power of the model was determined by the area under the curve (AUC) as excellent (AUC > 0.90), good (0.80 > AUC ≤ 0.90), acceptable (0.70 > AUC ≤ 0.80), bad (0.60 > AUC ≤ 0.70) and invalid (0.50 > AUC ≤ 0.60), according to Araújo et al. (2005). A Jackknife test was employed to determine the prediction power of each variable by setting the variable aside from the test and generating its percentage of contribution to the model.

Results

Fifteen sites that fitted the environmental requirements of the species in the Pampa stricto sensu were visited, covering all the main savanna, steppic-savanna and steppe areas of the Brazilian Pampa (Figure 2B). Among these sites, nine populations of S. mole were identified, composed by more than 50 individuals with random distribution, characterizing natural dispersion. Since the MaxEnt analysis performs satisfactorily even with few point locations, this coverage of the biome can be considered sufficient to simulate the occurrence area expected for *S. molle* in the Brazilian Pampa. These occurrence data and nineteen bioclimatic variables were employed for the distribution modeling analysis, which presented an excellent predictive power (AUC = 0.99). The three most important bioclimatic variables, which explaining more than 60% of the model, were precipitation seasonality, precipitation of the driest month and temperature seasonality (37.7%, 13.4% and 13.3% of contribution, respectively; Table 1).

For the Pampa stricto sensu, three populations of *S. molle* (SG, VN and CS) occur within the region with 100% probability of the species occurrence, five (MV, AL, SL, DP and BG) within the region with 50% probability and one population (QI) in the region with 25% probability, near the 50% zone (Figure 2B). Among the sites where no population was found,

four (BQ, UR, HN, AC) are located in regions with less than 25% predictive probability of *S. molle* occurrence. However, two sites lacking populations (CQ and RS) are located within the region of 100% probability of species occurrence (Figure 2B).

Discussion

Reviewing the diversity and conservation of the Pampa biome, Roesch et al. (2009) proposed that more comprehensive inventories of the biological diversity and distribution of animal and plant species and their interactions should be a key priority in future studies. Our study focused in simulating the distribution occurrence area correlated to climatic traits for a pioneer tree species, aiming to foresee the natural dynamic of the vegetation with emphasis in the forest expansion over grasslands, as well as to propose strategies for conservation of the Brazilian Pampa.

Lehmann et al. (2011) simulated the predictive occurrence area of forest and savanna environments in the South Hemisphere based on effective rainfall, rainfall seasonality, soil fertility and topographic complexity. Although using fewer climatic variables and covering just part of the Brazilian Pampa (from 29°S to 30°S Latitude), the results obtained by Lehmann et al. (2011) were congruent with our findings, revealing this portion of the Pampa with >50% chance of developing forests and presenting the precipitation as the main variable in the simulation model. In addition to work with occurrence data from all the Pampa stricto sensu, our analysis have a geographical resolution near 5 km², enabling the determination of areas ranging from 6.2% to 100.0% of probability of tree occurrence, refining the previous study. The potential distribution area of S. *molle* predicted in our study with more than 50% probability covers basically all the seven different physiographic formations of the Brazilian Pampa. Just a large extension of the coastal region and the westernmost part of the steppic-savanna environment within the Brazilian Pampa does not figure as occurrence area of *S. molle*. Based on the occurrence of economically important forest tree species, the westernmost part of the Brazilian Pampa has the lowest similarity to the other environments of the biome (Roesch et al. 2009), emphasizing the physiographic uniqueness of this region. This result also expresses the high biodiversity of the Brazilian Pampa (see Roesch et al. 2009 for a discussion about this issue). The Uruguayan Pampa revealed a smaller proportion of predicted areas with 100% probability of *S. molle* occurrence, confirming the Brazilian Pampa as a more feasible region for development of forest formations, as proposed by Rambo (1956), Hueck (1966) and Klein (1975).

Top-down control (predation and herbivory) is usually considered of minor

importance in guiding population expansion in terrestrial ecosystems (Bond 2008). Therefore, abiotic factors (bottom-up control) should be the main aspect in suppressing woody recruitment and expansion of tree populations over grassy areas of the Pampa biome. The prediction of forest occurrence over much larger areas than the actual occurrence has been obtained for different regions of the world based on dynamic global vegetation models grounded on climatic and soil elements only (Bond et al. 2003, 2005). Including fire in the model, a better fit was obtained for African grassy environments, suggesting that this is an important element in shaping current forest-grassland limits and impeding forest expansion (Bond 2008). However, natural fire is not an element of vegetation dynamic control in the Brazilian Pampa, as indicated by palynological records from nearby regions (Behling and Pillar 2007), the physiological dynamic of seed germination in the Pampa's grassland, which are not directly stimulated to germinate by fire (Overbeck et al. 2006), and the absence of the characteristic traits of fire-resistant species in trees growing in the forest formations in this biome. Thus, expansion of *S. molle* populations over grasslands in the Brazilian Pampa seems to be the natural dynamic of this biome, extending the actual occurrence vicinity of forests. As such demographic growth is not widely observed, we propose that ecological and anthropogenic elements are responsible by precluding the expansion of tree species populations over grasslands in the Pampa biome, despite the favorable conditions.

The ecological element is related to the natural succession of the environment. The grasslands of the Pampa biome are resilient formations in a region where the cold and dry climate changed to warm and humid faster than the ecological dynamic of the environmental succession could evolve. According to the palynological register (Behling et al. 2004, Behling & Pillar 2007), the emergence of the actual climate in southern Brazil occurred recently at both geological and ecological scales. Therefore, the time to the vegetation transition from grassland to forest formations did not elapse.

The anthropogenic element is linked to the historical land use in the Pampa biome. Much probably the expansion of agriculture and grazing lands and the establishment of exotic-tree plantations is an important factor maintaining the grassland dominance and precluding the forest expansion. The absence of *S. molle* populations in regions with 100% predicted conditions for the species occurrence likely is effect of human interference. Using mathematic models for the grassland-Araucaria forest dynamic in southern Brazil, Innes et al. (2013), proposed that human-environment interactions qualitatively alter the composition of forest-grassland mosaic ecosystems.

Conclusions

The use of "proper management strategies where grasslands are subject to shrub encroachment and forest expansion" maintaining the grassy vegetation and avoiding forest expansion has been proposed (Overbeck et al. 2007) as a policy for the conservation of the Brazilian Pampa. On the other hand, Roesch et al. (2009) suggested that priority areas for the conservation of the flora of the Brazilian Pampa must take into consideration the fact that the natural plant communities are both highly fragmented and probably quite divergent. The present study suggests the natural ecological dynamic of plant communities implicates the expansion of tree populations over grasslands in the majority of the Brazilian Pampa vicinity.

Thus, the forest expansion over grassland has to be considered a natural process in the biome and the conservationist actions should focus on establishing conservation unities that include forest populations and grassland, allowing the expansion of forest species over the grassy areas. The grassland management should be used just in grazing areas with historical human interference. These actions will allow the natural succession in the Pampa biome, respecting its ecological dynamic and valuing the forest formations in this grassland-dominated environment.

REFERENCES

Araújo M.B., Pearson R.G., Thuiller W., Erhard, M., 2005. Validation of species–climate impact models under climate change. Global Change Biology 11: 1504-1513.

Asner G.P., Jones M.O., Martin R.E., Knapp D.E., Hughes R.F., 2008. Remote sensing of native and invasive species in Hawaiian forests. Remote Sensing of Environment 112: 1912–1926.

Behling H., Pillar V.D., 2007. Late Quaternary vegetation, biodiversity and fire dynamics on the southern Brazilian highland and their implication for conservation and management of modern Araucaria forest and grassland ecosystems. Philosophical Transactions of the Royal Society of London B 362: 243–251.

Behling H., Pillar V.D., Orlóci L., Bauermann S.G., 2004. Late Quaternary Araucaria forest, grassland (Campos), fire and climate dynamics, studied by high-resolution pollen,

charcoal and multivariate analysis of the Cambará do Sul core in southern Brazil. Palaeogeography, Palaeoclimatology and Palaeoecology 203: 277-297.

Bond W.J., 2008. What Limits Trees in C4 Grasslands and Savannas? Annual Reviews of Ecology, Evolution and Systematics 39: 641–59.

Bond, W.J., Midgley, G.F., Woodward, F.I., 2003. What controls South African vegetation - climate or fire? South African Journal of Botany 69: 79–91.

Bond W.J., Woodward F.I., Midgley G.F., 2005. The global distribution of ecosystems in a world without fire. New Phytologist 165: 525–538.

Bredekamp G.J., Spada F., Kazmierczak E., 2002. On the origin of northern and southern hemisphere grasslands. Plant Ecology 163: 209–229.

Dikshit A., Naqvi A.A., Husain A., 1986. *Schinus molle*: a new source of natural fungitoxicant. Applied ad Environmental Microbiology 51: 1085-1088.

Goldstein D.J., Coleman R.C., 2004. *Schinus molle* L. (Anacardiaceae) Chicha Production In The Central Andes. Economic Botany 58: 523-529.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., Jarvis A., 2005. Very High Resolution Interpolated Climate Surfaces For Global Land Areas. International Journal of Climatology 25: 1965–1978.

Howard L.F., Minnich R.A., 1989. The introduction and Naturalization of *Schinus molle* (Pepper Tree) in Riverside, California. Landscape and Urban Planning 18: 77-95.

Hueck K., 1966. Die Wälder Südamerikas. Stuttgart: G. Fischer, 422 p.

IBGE, 2004. Mapa de Biomas do Brasil. Rio de Janeiro, Brazil. [Map of the biomes of Brazil]

Innes C., Anand M., Bauch C.T., 2013. The impact of human-environment interactions on the stability of forest-grassland mosaic ecosystems. Science Report 3: 2689-2699

Iponga D.M., Milton S.J., Richardson D.M., 2009. Reproductive potential and seedling establishment of the invasive alien tree *Schinus molle* (Anacardiaceae) in South Africa. Austral Ecology 34: 678–687.

Klein R.M., 1975. Southern Brazilian phytogeographic features and the probable influence of Upper Quaternary climate changes in the floristic distribution. Boletim Paranaense de Geociências 33: 67-88.

Lehmann C.E.R., Archibald S.A., Hoffmann W.A., Bond W.J., 2011. Deciphering the distribution of the savanna biome. New Phytologist 191: 197–209.

Marongiu B., Porcedda A.P.S., Casu R., Pierucci P., 2004. Chemical composition of the oil and supercritical CO2 extract of *Schinus molle* L. Flavour And Fragrance Journal 19: 554–558.

Milton S.J., Wilson J.R.U. Richardson D.M., Seymour C.L., Dean W.R.J., Iponga D.M., Proche S., 2007. Invasive alien plants infiltrate bird-mediated shrub nucleation processes in arid savanna. Journal of Ecology 95: 648–661.

Myers N., Mittermeier R.A., Mittermeier C.G., da Fonseca G.A.B., Kent J., 2000. Biodiversity hotspots for conservation priorities. Nature 403 853−858.

Overbeck G.E., Muller S.C., Pillar V.D., Pfadenhauer J., 2006. No heat-stimulated germination found in herbaceous species from burned subtropical grassland. Plant Ecology 184: 237–243.

Overbeck G.E., Muller S.C., Fidelis A., Pfadenhauer J., Pillar V.D., Blanco C.C., et al., 2007. Brazil's neglected biome: the South Brazilian Campos. Perspectives in Plant Ecology, Evolution and Systematics 9: 101-116.

Phillips S.J., Anderson R.P., Schapire R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190: 231–259

Pillar V.D., 2003. Dinâmica da expansão florestal em mosaicos de floresta e campos no sul do Brasil. In: Claudino-Sales, V. (Ed.) Ecossistemas Brasileiros: Manejo e Conservação, Fortaleza: Expressão Gráfica e Editora, pp. 209-216. [Dynamic of the forest expansion in mosaics of forest and grasslands in South Brazil]

Rambo B., 1956. A fisionomia do Rio Grande do Sul. Porto Alegre: Selbach, 456 p. [The physiognomy of the Rio Grande do Sul]

Roesch L.F.W., Vieira F.C.B., Pereira V.A., Schünemann A.L., Teixeira I.F., Senna A.J.T., Stefenon V.M., 2009. The Brazilian Pampa: A Fragile Biome. Diversity 1: 182-198.

Saatchi S., Buermann W., ter Steege H., Mori S., Smith T.B., 2008. Modeling distribution of Amazonian tree species and diversity using remote sensing measurements. Remote Sensing of Environment 112: 2000–2017

Sobral M., Jarenkow J.A., 2006. Flora Arbórea e Arborescente do Rio Grande do Sul, Brasil. São Carlos: RiMa, 350 p. [Arboreal and Shrub flora of the Rio Grande do Sul, Brazil]

Vélez E., Chomenko L., Schaffer W., Madeira M., 2009. Um panorama sobre as iniciativas de conservação dos Campos Sulinos. In: Pillar V.D., et al. (eds.) Campos Sulinos: conservação e uso sustentável da biodiversidade. pp. 356-379. [An overview about the conservation initiatives of the Southern grasslands]
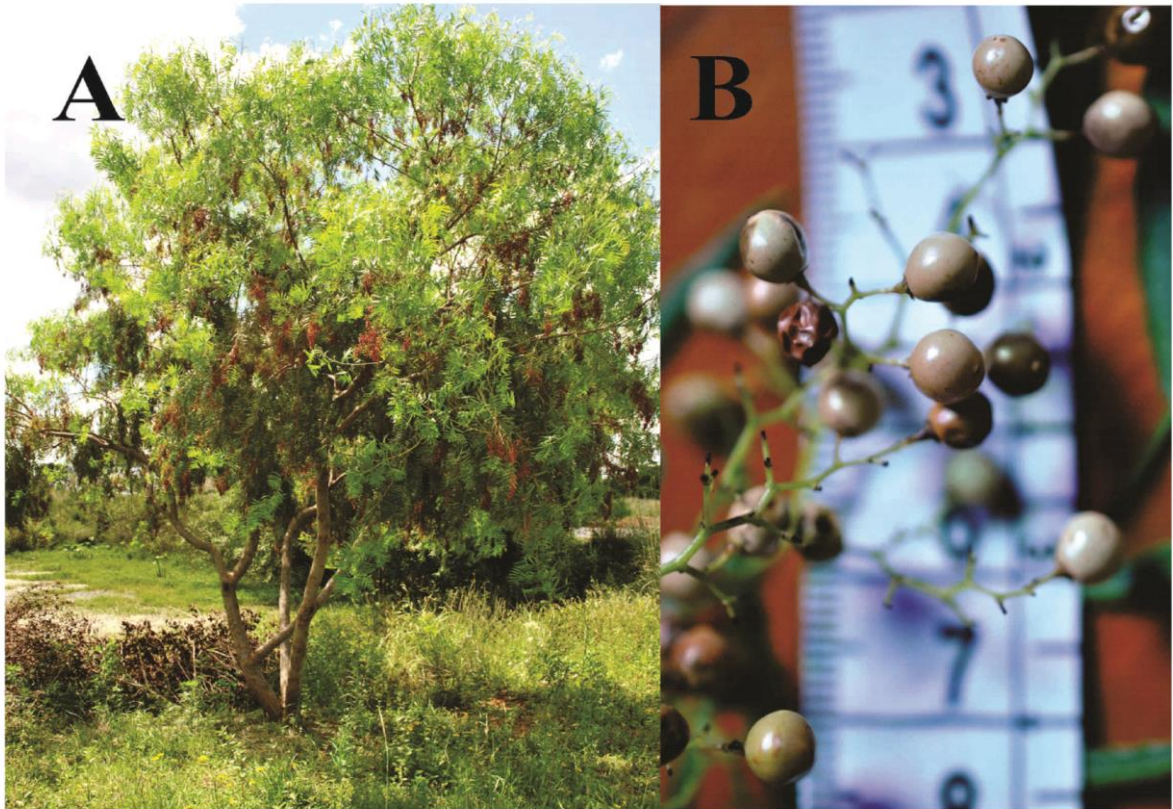
Figure 1 *Schinus molle* in the Pampa *stricto sensu*. (A) Female individual of *S. molle* in its natural habitat. (B) Ripe fruits of *S. molle*.
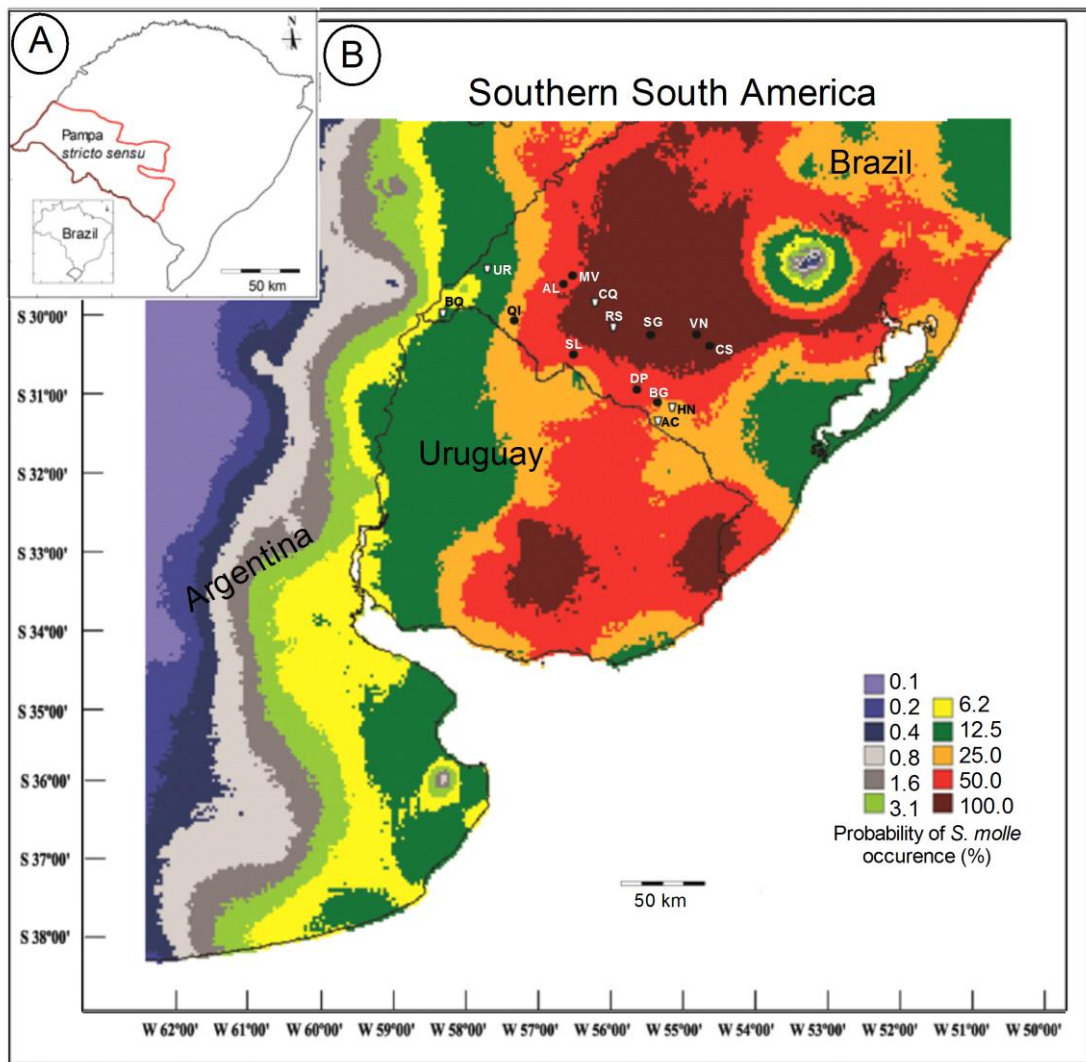
Figure 2 Map of the studied area in Southern Brazil, including the Uruguayan territory and part of Argentina. (A) Geographic range of the Pampa *stricto sensu* in the southernmost Brazilian state Rio Grande do Sul. (B) Predicted distribution of *Schinus molle* within the Pampa biome in South Brazil, Uruguay and part of Argentina. Colors represent the different probability of species occurrence based on the climatic variables evaluated. Natural populations of *Schinus molle* were found in Alegrete (AL), Bagé (BG), Caçapava do Sul (CS), Dom Pedrito (DP), Manoel Viana (MV), Quaraí (QI), Santana do Livramento (SL), São Gabriel (SG) and Vila Nova do Sul (VN). No populations were observed in Aceguá (AC), Cacequi (CQ), Hulha Negra (HN), Barra do Quaraí (BQ), Rosário do Sul (RS) and Uruguaiana (UR).

Table 1: Percent contribution of the bioclimatic environmental variables used in the maximum entropy analysis for the predictive distribution of *Schinus molle* L. in the Pampa biome.

BioClim Legend	Environmental Variables	Percent Contribution
BIO 15	Precipitation of Seasonality (coefficient of variation)	37.7
BIO 14	Precipitation of Driest Month	13.4
BIO 4	Temperature Seasonality (standard deviation*100)	13.3
BIO 6	Min Temperature of Coldest Month	7.5
BIO 3	Isothermality (BIO2/BIO7)*(100)	6.8
BIO 11	Mean Temperature of Coldest Quarter	6.1
BIO 12	Annual Precipitation	5.9
BIO 19	Precipitation of Coldest Quarter	2.9
BIO 17	Precipitation of Driest Quarter	2.4
BIO 5	Max Temperature of Warmest Month	1.9
ALT	Altitude	0.9
BIO 8	Mean Temperature of Wettest Quarter	0.8
BIO 1	Annual Mean Temperature	0.3
BIO 2	Mean Diurnal Range [Mean of monthly (max temp – min temp)]	0.2
BIO 7	Temperature Annual Range (BIO5-BIO6)	0
BIO 9	Mean Temperature of Driest Quarter	0
BIO 13	Precipitation of Wettest Month	0
BIO 10	Mean Temperature of Warmest Quarter	0
BIO 16	Precipitation of Wettest Quarter	0
BIO 18	Precipitation of Warmest Quarter	0

CAPÍTULO 2. Genetic diversity, population structure and internal gene flow in populations of *Schinus molle* (Anacardiaceae) from the Brazilian Pampa

(Manuscrito submetido ao Periódico ***Tree Genetics & Genomes*** sob autoria de Rafael Plá Matielo Lemos, Cristiane Barbosa D'Oliveira, Valdir Marcos Stefenon)

Abstract

One of the most important aspects for population ecology and evolution is the understanding of the connectivity between individual and their groups. In this study, AFLP markers were employed in order to examine the fine-scale spatial genetic structure (SGS) and the internal gene flow of nine natural populations of *Schinus molle* across the Brazilian Pampa biome. The dispersal capacity of *S. molle* in the Brazilian Pampa conditions, the implications of the forest fragmentation to SGS of the species in this biome and the impact of the actual SGS of the species to its conservation and management were investigated. A total of 248 polymorphic AFLP loci were evaluated, revealing significant SGS at short distances in all populations. Estimations of gene dispersal distance did not converge in eight out of the nine populations, suggesting that the dispersion capacity of this species is larger than the area covered by the studied populations. The management of *S. molle* natural occurrence in this biome appears to be simple taken into account this species plasticity and the area of its expansion through the grassland. Maintaining the existent forest patches to ensure its biodiversity, this important wood component might survive the impact of anthropic economic interests.

Keywords: SGS, Brazilian Pampa, internal gene flow, within population gene dispersal

Introduction

The actual spatial distribution of genotypes in Nature is the result of biotic and abiotic factors such as capacity of seed and pollen dispersal (e.g. Jump and Peñuelas 2007, Stefenon et al. 2007, 2008) and demographic history (e.g. Ledig et al. 1999, Stefenon et al. 2009). In addition, evolutionary forces acting on populations over the years have considerable consequences for current plant populations, since the multiplicity of genetic frequencies increases the adaptive evolution potential (Heywood 1991). Thus, the gene flow is a vital factor for populations` structure over time. While high gene dispersion guides to the homogeneity of populations genetic structure, low gene flow leads to non-random distribution of alleles and genotypes. Within forest tree populations, genetic diversity is usually distributed heterogeneously, with individuals regularly becoming less similar at genetic level as the geographical distance between them increases.

Several studies have investigated patterns of fine-scale spatial genetic structure (SGS) and the within population gene flow in natural populations of tree species of the Amazon Forest and Atlantic Forest biomes in Brazil (e.g. Stefenon et al. 2008, Baldauf et al. 2014). However, tree species natives to the Pampa biome in southern Brazil have been neglected concerning genetic structure surveys. The Brazilian Pampa is composed by steppic-savanna flora associated to forest vegetation (Roesch et al. 2009, Deble 2011), covering an area of about 176,500 km^2 in Southern Brazil (Fig. 1). The biological uniqueness of the Pampa biome may be highlighted by the debate concerning the classification of its vegetation (Roesch et al. 2009). Independent of this discussion, the official classification of the ecological domains in the Brazilian Pampa (MMA 2002, UFSM/SEMA-RS 2007) considers the existence of a coastal vegetation along the Atlantic shore, and steppic-savanna formations across the remainder of this biome (Pampa *stricto sensu*, Fig. 1). Despite the predominance of grassland formations in the Pampa *stricto sensu*, this region presents important natural tree formations as riparian forest as well as scattered small to medium sized forest nucleations within the grassy environment. Although the natural dynamics of the vegetation in this biome seems to be the expansion of the forest formations (Lehmann et al. 2011, Lemos et al. unpublished data), the growth of the agriculture and pasture areas and the establishment of exotic forest plantations have precluded such natural dynamics and made difficult the preservation of the genetic resources of tree species in this biome.

Schinus molle L. (Anacardiaceae) is a native tree species from the Pampa biome, occurring as scattered non-connected forest formations, usually with small demographic size. It can flower at any time of the year and female trees bear a mixture of ripe and unripe fruits

most of the time making their seeds available for dispersal throughout the year (Milton et al. 2007). Seeds are dispersed by birds, mammals and in running water, while seedlings become established in washes, ravines, old fields and rock outcrops (Howard and Minnich 1989). *Schinus molle* is a pioneer species with high adaptability and capability to spread and survive into new habitats, as demonstrated by the large areas occupied by this species in South Africa (Iponga et al. 2009), Hawaii (Asner et al. 2008) and California (Howard and Minnich 1989), where it was introduced as an ornamental plant. Due to this high adaptability, *S. molle* is already considered a naturalized species in both countries (Howard and Minnich 1989, Iponga et al. 2009, Richardson et al. 2010), colonizing a large variety of environments. Considering these features, *S. molle* may support the forest expansion over the Pampean grasslands in southern Brazil, through the natural establishment of forest formations in non-forested areas, transposing anthropic and environmental resilience barriers. However, the patterns of genetic structure and gene flow of tree species growing in the Pampa biome are still a query.

In this study, we employed AFLP markers in order to examine the fine-scale spatial genetic structure and internal gene flow in nine natural populations of *S. molle* growing in the Brazilian Pampa biome. Molecular markers have been widely used to assess the levels and patterns of historical gene dispersal within populations of tree species. Surveys aiming to highlight SGS are mainly based on computing kinship coefficients and comparing the observed values with the expected at random mating (Hardy et al. 2006). For such studies, molecular markers should be as variable as possible. AFLP markers have often been used to estimate genetic relationships and diversity (Vuylsteke et al. 2008), being highly reliable in analysis of fine-scale spatial genetic structure (Jump and Peñuelas 2007, Stefenon et al. 2008). Using this procedure and considering the previous studies concerning the invasiveness capacity of *S. molle* in South Africa and USA, we intended to answer three main questions: (1) How large is the gene dispersal capacity of *S. molle* within populations in steppic-savanna conditions? (2) Which are the implications of the forest fragmentation to the fine-scale spatial genetic structure of *S. molle* in the Brazilian Pampa? (3) Does the actual fine-scale spatial genetic structure of this species leads to a need of imperative conservation and management plans?

Materials and Methods

Sampling strategy and DNA extraction

Plant material from populations of *S. molle* was collected across the steppic-savanna portion of the Brazilian Pampa ($29^000'–32^000'$S, $53^030'–58^000'$W; Fig. 1 and Table 1).

Because *S. molle* is a heliophyte species usually occurring in small to medium-sized populations along disturbed environments, we conducted a search throughout all the region for such locations, as well as for primary and secondary forest formations, where populations of this species could be found. Since this species is sometimes planted by landowners in the Pampa biome, just clusters formed by at least 10 individuals with random distribution within an area of about 3 km range were classified as a population of *S. molle*, characterizing natural dispersion of the species. A total of nine populations were found and at least 30 adult individuals were collected in each site, totalizing 278 trees sampled. Given the small size of the populations, this sample size covered about 70% of the individuals from each site. The spatial position for each sampled tree (altitude, longitude and latitude) was recorded using a GPS equipment (Garmim® e-trex). For the fine-scale spatial genetic structure analysis, longitude and latitude were transformed into the Universal Transverse Mercator projection (UTM coordinates). The main distribution of the trees followed a linear orientation within the sampled sites, although six populations revealed some aggregations of trees along the occurrence area. Two compound healthy leaves were sampled from each tree, deposited in plastic bags with silica gel and maintained at room temperature until DNA isolation. About 100 mg of plant material was washed with 70% ethanol and distilled water, disrupted with TissueLyser II (Qiagen®), and total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen®), following the instructions of the manufacturer. The quality and the amount of the extracted DNA were evaluated on a NanoVue™ Plus Spectrophotometer (GE Healthcare).

AFLP analysis

AFLP reactions were performed as described by Vos et al. (1995), with slight modifications. For the restriction/ligation reaction, about 100 ng of total genomic DNA were incubated with the restriction enzymes *Pst*I and *Mse*I and the corresponding *Pst*I and *Mse*I adapters at 37 ℃ for 15 hours followed by 72 ℃ for 15 minutes in a single reaction (2.5X *T4* DNA ligase reaction buffer, 2.5 U *Mse*I, 2.5 U *Pst*I, 2.5 µg BSA, 25 pmol of each adapter, 1.0 U *T4* DNA ligase). The reaction mixture was diluted four-fold and used as template for the PCR selective amplification with the primer combinations *Pst*-GAG/*M*-GAA and *Pst*-GAG/*M*-GCT (4 µl template DNA, 25 pmol of fluorescent *Pst* primer, 25 pmol of *Mse* primer, 1.0 U Taq polymerase, 2X PCR buffer, 0.25 mM of each dNTP). The *Pst*-GAG primer was labeled with AlexaFluor® 680 nm (Invitrogen). The PCR conditions for the selective reaction were an initial denaturation step at 95 ℃ for 2 minutes followed by 26 cycles at 95℃, 56 ℃, 72 ℃ for 1 minute each for each cycle and of a final extension step at

72 ºC for 5 minutes. All PCR reactions were carried out in an Eppendorf Thermal Cycler. AFLP fragments were electrophoretically separated using a Li-Cor 4300L automated DNA Sequencer® (Li-Cor Inc.) and automatically scored and analyzed in SAGA[GT] Software®. An additional visual check of each scored gel was made in order to correct possible mismarked bands. For each population the presence/absence of AFLP bands was transformed into a binary matrix (0, 1) for posterior analysis. The complete AFLP analysis (from the restriction reaction to the band scoring) was repeated twice for two samples from each population in order to select only the repeatable bands for the analysis.

Linkage and neutrality of the AFLP markers

Since AFLP fragments originated with the enzymes *Pst*I/*Mse*I are usually distributed uniformly throughout the genome (Castiglioni et al., 1999; Young et al., 1999; Scott-Saintagne et al., 2004), some loci may be linked to each other or even to genes under selection. In order to evaluate the overall genomic coverage of the AFLP markers, the presence of linked AFLP loci was determined using the software AFLPOP (Duchesne and Bernatchez 2002). Clusters of possibly linked loci were reduced to a single locus eliminating redundant data.

Population genetic diversity

Population genetic diversity was assessed assuming random mating, using the software AFLP-SURV (Vekemans, 2002). Allelic frequencies of AFLP markers were estimated using a Bayesian approach with non-uniform prior distribution of allele frequencies (Zhivotovsky, 1999), which gives an unbiased estimate of allele frequencies (Zhivotovsky, 1999; Kraus, 2000). Percentage of polymorphic loci (PPL) and genetic diversity (H_j) were computed following the approach of Lynch and Milligan (1994), i.e., pruning loci with null allele frequency higher than $1-3/n$, where n is the population sample size. Band richness (B_R; Coart et al., 2005) expected at each AFLP locus was computed using the rarefaction approach, based on a sample size of 27 trees using AFLPDIV 1.0 (Petit, 2007).

Analysis of population structure

The overall and pairwise population differentiation (Φ_{ST}) was calculated in ARLEQUIN 3.5 software through a non-hierarchical analysis of molecular variance (AMOVA) approach (Excoffier et al. 2005). The statistical significance was determined using 10000 permutations of genotypes among populations.

To determine the population structure, a Bayesian model-based cluster analysis of population structure was performed in STRUCTURE program version 3.2 (Pritchard et al. 2000), using the frequency independent alleles models with 200000 Markov Chain Monte Carlo steps and 100000 burn-in periods. In this analysis, individual multilocus genotypes are assigned probabilistically to a defined number K of clusters, according to a particular membership coefficient, or into multiple groups with membership coefficients summed up to one across groups. The non-admixture model was used, with correlated allele frequencies. K was set at two to fifteen and 20 replicates were run for each K. The optimum number of clusters K was selected using the approach suggested by Evanno et al. (2005).

In order to further evaluate the pattern of isolation by distance (IBD), the correlation between genetic differentiation and geographical distance among populations was evaluated by regressing the population pairwise genetic differentiation matrix (Φ_{ST}) against the pairwise geographical distance matrix (in km), using a Mantel test with 1000 permutations performed in the software NTSYS-PC 2.0 (Rohlf 1998).

Analysis of fine-scale spatial genetic structure and internal gene flow

Fine-scale spatial genetic structure (SGS) was analyzed in each population through kinship coefficients (F_{ij}) using the approach described by Hardy (2003). The number of distance classes (distance intervals within which all pairs of sampling points are considered) was fixed in eight for each population, allowing displaying a minimum of 40 pairs of individuals in each distance class. The relationship between geographic distance classes and genetic similarity was acquired for each population as the regression slope of kinship coefficients on log-transformed distances (b_F). Statistical significance of F_1 and b_F was determined through the upper and lower bounds of 95% confidence interval of F_{ij} defined after 20,000 permutations of locations among individuals. Sp-statistic (Vekemans and Hardy 2004) was computed for each population based on regression slope of kinship coefficients, as $Sp = -b_F/(1 - F_1)$, where F_1 is the mean kinship coefficient between individuals belonging to the first distance class. The Sp-statistic is expected to summarize the intensity of SGS, allowing a quantitative comparison among species and/or populations.

An indirect estimation of gene flow from the SGS estimates was performed assuming equilibrium of isolation-by-distance in the fine-scale genetic structure. In such a case, the extent of gene flow can be expressed in terms of Wright's neighbourhood size as $\hat{N}_b \equiv 4\pi D\sigma^2$, where D is the effective population density and σ^2 represents the physical

distance between parent and offspring (Fenster et al. 2003). Estimations of $D\sigma^2$ were obtained from the regression of the pairwise F_{ij} values on geographical distance as $\hat{N}_b = (F_1 - 1)/b_F$ and gene dispersal was estimated through the procedure described by Vekemans and Hardy (2004), using one-fourth of the census density (in trees/hectare) as the predicted effective population density. All estimations of SGS and internal gene flow were performed using the software SPAGEDI (Hardy and Vekemans 2002).

Results

Linkage of AFLP markers

A total of 250 polymorphic AFLP loci ranging from 60 to 250 bp were evaluated. The primer combination *Pst*-GAG/*M*-GAA revealed 150 polymorphic reproducible loci, while the combination *Pst*-GAG/*M*-GCT revealed 100 polymorphic reproducible loci. According to the analysis of loci linkage, out of the 250 AFLP markers obtained from the two primer combinations, just two loci from the *Pst*-GAG/*M*-GCT combination revealed significant association, resulting in a total of 248 polymorphic AFLP loci employed in the analyses. The very low number of putatively linked AFLP loci suggests a random representation of the genome and reliability of the genetic information obtained.

Genetic diversity and population structure

The percentage of polymorphic loci (PPL) ranged from 16% in population VN to 68% in population BG with a mean of 51% over the nine populations. Estimations of genetic diversity varied from H_j = 0.06 (VN) to H_j = 0.23 (BG) with a mean of H_j = 0.15. Band richness ranged from B_R = 1.40 in population VN to B_R = 1.85 in population MV (Table 2).

While the overall population differentiation was Φ_{ST} = 0.29, pairwise genetic differentiation ranged from Φ_{ST} = 0.12 between populations SG and SL to Φ_{ST} = 0.45 between VN and QI (Table 3).

In the Bayesian analysis of population structure, the values of ln(X/K) increased progressively with the number of clusters. Using the analysis of ΔK, AFLP data suggested population structure at K = 7 (Fig 2). At K= 7, five populations revealed higher membership at single clusters: DP (99.9%), VN (99.8%), BG (96.7%), QI (93.7%) and SL (92.4%). Populations SG, MV, AL and CS revealed different levels of genetic admixture, with membership < 82% in a single cluster (Fig. 3)

In terms of isolation by distance among populations, the Mantel test showed that the degree of genetic differentiation between sample locations slightly increased with geographical distance of populations ($r = 0.3$; $p = 0.029$).

Estimates of SGS and gene dispersal within populations

Among populations, the number of analyzed pairs of trees in the SGS analysis ranged from 43 (population SG) to 64 (population AL) across distance classes, with a participation of 53% (population MV) to 80% (population DP) of the individuals in each distance class, ensuring statistical robustness for the analysis. Negative values were reported for the regression slope of F_{ij} (b_F; Table 4) in all populations indicating an isolation-by-distance process where individuals spatially close are genetically more similar to each other than individuals separated by larger distances. A pattern of positive F_{ij} in short distance classes (i.e. <30 m) and negative in large distance classes (i.e. >100 m) in most populations is clear. A near monotonic decrease in the mean kinship coefficient with the increase of distance is observed in all populations except for VN population (Fig. 4). The average F_{ij} between individuals at the first distance class (F_1) ranged from 0.034 to 0.097 (Table 4). Significant SGS was detected at short distances in all populations (Fig. 4).

Significant fine-scale SGS was revealed comparing populations according to *Sp*-statistic (Table 4). Populations QR and VN showed the strongest SGS probably due to the higher kinship value found in the first and second distance classes and the strongest negative log slope (b_F).

The estimations of gene dispersal distance converged just for population SL, with $\sigma = 338.93$ m. For all the other eight populations there was no convergence, suggesting that the dispersion capacity of this species is larger than the area covered by the studied populations.

Discussion

Genetic diversity and population structure

AFLP data revealed moderate to low level of genetic diversity and significant population structure in the Pampean populations of *Schinus molle* studied. In comparison to data compiled by Nybom (2004) and Nybom and Bartish (2000) for dominant markers, the gene diversity assessed by AFLPs in the present study suggests moderate to low level of genetic variation among the Pampean populations of *Schinus molle* (mean $H_j = 0.15$), considering the means for long-lived perennial species, outcrossing species and species with seeds dispersed by animals (mean $H > 0.23$).

Concerning the levels of population genetic differentiation ($\Phi_{ST} = 0.29$), the gene flow is within the range reported by Nybon and Bartish (2000) for long-lived perennial species, outcrossing species and seed dispersed by animals species (mean Φ_{ST} from 0.25 to 0.29), but higher than species with early-successional status ($\Phi_{ST} = 0.45$). Accordingly, the genetic structure revealed by the Bayesian analysis suggests the existence of seven genetic clusters with very low gene flow into four out of the nine populations. On the other hand, low significantly positive correlation ($r = 0.3$) between pairwise genetic differentiation and geographic distance was revealed. Although this correlation is low, the level of differentiation reveals that the gene flow among populations is limited, mainly if we consider the high capacity of dispersal expected for *S. molle* as an invasive species (Henderson 2007; Iponga 2010).

Within population gene flow

In South African savannas, the range of seed dispersal of *S. molle* is estimated to reach about 320 meters only (Iponga et al. 2009). This range matches the estimated gene dispersal for population SL ($\sigma = 338.93$ m) in our study. On the other hand, the pollination through honeybees in the Brazilian Pampa may reach up to 5,000 meters (our unpublished data). The failure of the gene dispersal estimations σ in the other eight populations confirms such a long extent capacity of dispersal, since the computing model tends to collapse when the estimation of the dispersal distance becomes larger than the distance d_{ij} for all ij pairs of individuals (Vekemans and Hardy 2004). This non-convergence may happens due to three factors: (i) the information from the genetic markers is insufficient to get reliable estimates, (ii) SGS is not representative of an isolation-by-distance process, and/or (iii) the spatial scale of the sample was not adequate (Vekemans and Hardy 2004).

The first factor, concerning the insufficiency of the marker informative power, can be discarded. AFLPs have been shown to provide reliable information for SGS analysis due to the large genome coverage of the marker (e.g. Jump and Peñuelas 2007, Stefenon et al. 2008). In some situations, this marker may even be more informative than microsatellite markers (Jump and Peñuelas 2007, Stefenon et al. 2008). In our study, the large number of neutral uncorrelated AFLP loci evaluated allowed wide genome coverage and provided high informative capacity for getting reliable estimations of SGS. Therefore, this may not be the cause of the estimations failure.

Considering the trend revealed in the plots of kinship coefficient *versus* geographic distance (Fig. 4) and the significantly negative values of the slopes for all populations, the

second factor can also be excluded or considered of minor importance. The observed tendency of the correlograms reveals the existence of an isolation-by-distance process within populations, with individuals geographically more distant revealing lower genetic similarity (Fig. 4). Pondering the size of the studied populations and consequent short distance among individuals, the spatial scale of the sample, i.e. the geographic coverage of the sampling, may be the factor causing the non-convergence of the computations. Thus, the expected extent of dispersal is larger than the more distant pair of individuals in all populations reaching more than 4,500 meters, the maximal distance observed in our sample (population BG, supplementary Fig. 1).

Forest fragmentation and conservation of S. molle genetic resources

The actual distribution of forest formations in the Brazilian Pampa is clearly fragmented, with grassland, agricultural or urban areas disconnecting the tree populations. Generally, tree species with limited gene flow capacity presents significant genetic structure among populations in such fragmented environments, as effect of an isolation-by-distance process. The significant fine-scale spatial genetic structure observed in all studied populations of *S. molle* from the Brazilian Pampa represents the isolation by distance hypothesis at intra-population level. Concerning the species life trait, the mean *Sp*-statistic estimated for *S. molle* populations ($Sp = 0.021$; 95% CI = 0.0117-0.0308) are within the mean values compiled by Vekemans and Hardy (2004) for outcrossing species ($Sp = 0.0126$), species with pollen dispersed through animals ($Sp = 0.0171$) and species with wind-dispersed seeds ($Sp = 0.0120$). In addition, the estimations of *Sp*-statistic in *S. molle* populations are among the higher values estimated for several other forest tree species (Vekemans and Hardy 2004, Hardy et al 2006, Jump and Peñuelas 2007, Stefenon et al. 2008, Ismail et al. 2014). Population density may significantly interfere in the level of SGS, with higher values of *Sp*-statistic estimations observed in low-density populations (Vekemans and Hardy 2004).

Considering the predicted large distance gene flow of *S. molle* (see above) and the fact that larger populations tend to present lower levels of SGS (e.g. Heuertz et al. 2003), the main impact over the fine-scale genetic structure within *S. molle* populations seems to be not the isolation due to the forest fragmentation, but the small size of these forest formations, caused by the forest fragmentation. Even inside its dispersion range, this study evidenced that the gene flow is compromised and the island-effect might take place in this biome, as the

expansion of natural areas of tree species occurrence has been precluded, impeding the connection among populations.

Remarks on conservation and management of S. molle genetic resources

The Brazilian Pampa is notorious by the large grassland fields. In function of such large dispersion capacity and adaptive potential under the current climatic conditions, the expansion of *S. molle* over grassland is expected to occur through all the South American Pampa (Lemos et al. unpublished data). However, the resilience of the grassland and the human interference through expansion of agriculture, pasture and forestry with exotic species may represent a very intense impact leading to the isolation and fragmentation of populations of forest tree species in this biome (Roesch et al. 2009).

The ecological and economic importance of *S. molle* and other tree species for the Brazilian Pampa is irrefutable. The wood component in this biome serves as a barrier in riverbanks, refugee for many wild animals and as substrate for native plant species, some of them endemic to this biome (Guadagnin et al. 2009, Roesch et al. 2009, Deble 2011). In addition, *S. molle* has medicinal properties (Dikshit et al., 1986), pharmaceutical uses for its essential oils (Marongiu et al., 2004) and is appreciated in the culinary because the spice pink pepper (Goldstein and Coleman, 2004). So, conservation strategies should be discussed towards finding ways for managing and protecting these native forest formations.

Comparisons of fine-scale SGS of tree species have been made through *Sp*-statistic to ensure that this data could be standardized across studies, although such estimations are affected by specific characteristics such as breeding system and life form. *Schinus molle* is recognized by its dispersion plasticity and capacity to survive in different environments (Asner et al. 2008, Iponga et al. 2010). However, the within-population genetic structure of its populations is strongly affected by the fragmentation and consequent small size of the forest formations. Following the International Union of Conservation Nature rules for classifying endangered species (IUCN 2010), *S. molle* is not considered threatened, although the SGS observed for its populations in the Brazilian Pampa may lead the species to undergo the effects of inbreeding depression and genetic drift within a few generations. At least some populations of *S. molle* and other tree species are highly isolated genetic unities, as expressed in the structure analysis. This in turn leads to a new query: "how fragile are the IUCN red list species for SGS?"

The conservation of the *S. molle* genetic resources in the Brazilian Pampa seems to be simple taken into account the species plasticity and the area of its expansion through the

grassland. Maintaining the existent forest patches and allowing the connection among populations, this important wood component might survive the impact of anthropic economic interests and harbor the forest expansion over the Pampean grassland as a natural process.

References

Asner GP, Jones MO, Martin RE, Knapp DE, Hughes RF (2008) Remote sensing of native and invasive species in Hawaiian forests. Remote Sens Environ 112: 1912–1926.

Baldauf C, Guillardi MC, Aguirra TJ, Correa CE, Santos FAM, Souza AP, Sebbenn AM (2014) Genetic diversity, spatial genetic structure and realized seed and pollen dispersal of *Himatanthus drasticus* (Apocynaceae) in the Brazilian savanna. Conserv Genet 1-11.

Deble LP (2011) A Vegetação Campestre no Bioma Pampa. In: Deble LP, Deble ASO, Leão ALS (Org.). O Bioma Pampa: contribuições científicas. Bagé.

Dikshit A, Naqvi AA, Husain A (1986) *Schinus molle*: a new source of natural fungitoxicant. Appl Environ Microbiol 51:1085-1088.

Duchesne P, Bernatchez L (2002) AFLPOP: a computer program for simulated and real population allocation, based on AFLP data. Mol Ecol Notes 2(3):380–383.

Fenster CB, Vekemans X, Hardy OJ (2003) Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaecrista fasciculate* (Leguminosae). Evolution 57:995–1007.

Guadagnin DL, Zalba SM, Górriz BC, Fonseca CR, Nebbia AJ, Cuevas YA, Emer C, Germain P, Wendland EMR, Perello LFC, Bastos MCS, Sanhueza CC, Bálsamo SM, Villalobos AE (2009) Árvores e arbustos exóticos invasores no Pampa: questões ecológicas, culturais e socioeconômicas de um desafio crescente. In: Pillar VP, Müller SC, Castilhos ZMS, Jacques AVA (ed) Campos Sulinos: conservação e uso sustentável da biodiversidade. Brasilia: MMA. p 403.

Goldstein DJ, Coleman RC (2004) *Schinus molle* L. (Anacardiaceae) Chicha Production In The Central Andes. Econ Bot 58: 523-529.

Hardy OJ (2003) Estimation of pairwise relatedness between individuals and characterization of isolation-by-distance processes using dominant genetic markers. Mol Ecol 12:1577-1588.

Hardy OJ, Maggia L, Bandou E, Breyne P, Caron H, Chevallier ME, Doligez A, Dutech C, Kremer A, Hallé CL, Troispoux V, Veron V, Degen B (2006) Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. Mol Ecol 15:559–571.

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2(4):618-620.

Henderson L (2007) Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia 37(2):215–248.

Heywood JS (1991) Spatial analysis of genetic variation in plant populations. Annu Rev Ecol Syst 22:335–355.

Heuertz M, Vekemans X, Hausman J-F, Palada M, Hardy OJ (2003) Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. Mol Ecol 12: 2483-2495.

Howard LF, Minnich RA (1989) The introduction and Naturalization of *Schinus molle* (Pepper Tree) in Riverside, California. Landscape Urban Plan 18:77-95.

Iponga DM, Milton SJ, Richardson DM (2009) Reproductive potential and seedling establishment of the invasive alien tree Schinus molle (Anacardiaceae) in South Africa. Austral Ecol 34:678–687.

Iponga DM (2010) Seed set of the invasive tree Schinus molle (Anacardiaceae) in semi-arid savanna, South Africa: The role of pollinators and selfing. J Arid Environ 74:414–416.

Ismail SA, Ghazoul J, Ravikanth G, Kushalappa CG, Uma-Shaanker R, Kettle CJ (2014) Fragmentation Genetics of *Vateria indica*: implications for management of forest genetic resources of an endemic dipterocarp. Conserv Genet 15:533–545.

IUCN (2010) Guidelines for Using the IUCN Red List Categories and Criteria. Version 8.1. Prepared by the Standards and Petitions Sub–Committee in March 2010.

Jump AS, Peñuelas J (2007) Extensive spatial genetic structure revealed by AFLP but not SSR molecular markers in the Wind-pollinated tree, *Fagus sylvatica*. Mol Ecol 16:925-936.

Lehmann CER, Archibald SA, Hoffmann WA, Bond WJ (2011) Deciphering the distribution of the savanna biome. New Phytol 191:197-209.

Marongiu B, Porcedda APS, Casu R, Pierucci P (2004) Chemical Composition Of The Oil And Supercritical CO_2 Extract Of *Schinus molle* L. Flav Frag J 19: 554–558.

Milton SJ, Wilson JRU, Richardson DM, Seymour CL, Dean WRJ, Iponga DM, Proche S (2007) Invasive alien plants infiltrate bird-mediated shrub nucleation processes in arid savanna. J Ecol 95: 648–661.

Richardson DM, Iponga DM, Roura-Pascual N, Krug RM, Milton SJ, Hughes GO, Thuiller W (2010) Accommodating scenarios of climate change and management in modelling the distribution of the invasive tree *Schinus molle* in South Africa. Ecography 3:1049-1061.

Roesch LFW, Vieira FCB, Pereira VA, Schünemann AL, Teixeira IF, Senna AJT, Stefenon VM (2009) The Brazilian Pampa: A Fragile Biome. Diversity 182-198.

Stefenon VM, Gailing O, Finkeldey R (2008) The role of gene flow in shaping genetic structures of the subtropical conifer species *Araucaria angustifolia*. Plant Biol 10:356-364.

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. Mol Ecol. 13:921-935.

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

Vuylsteke M, Peleman JD, Eijk MJT (2008) AFLP technology for DNA fingerprinting. Nat Protoc. 2(6):1387-1398.
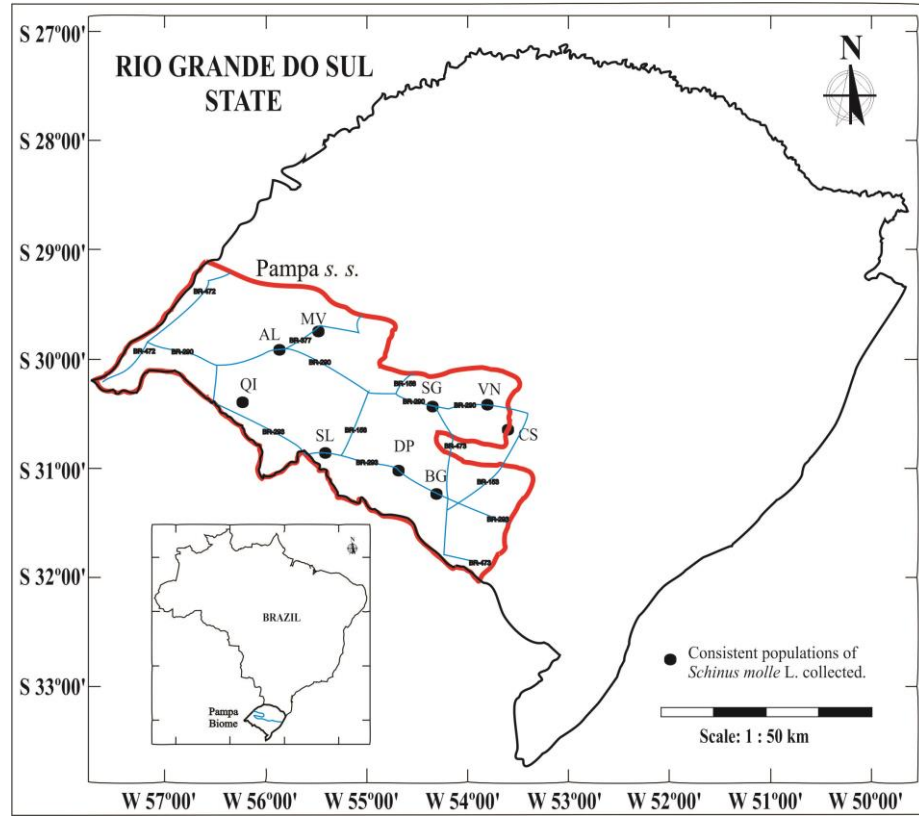
Fig. 1: Map of the studied area in the Pampa *stricto sensu*, southern Brazil, highlighting each sampled population.
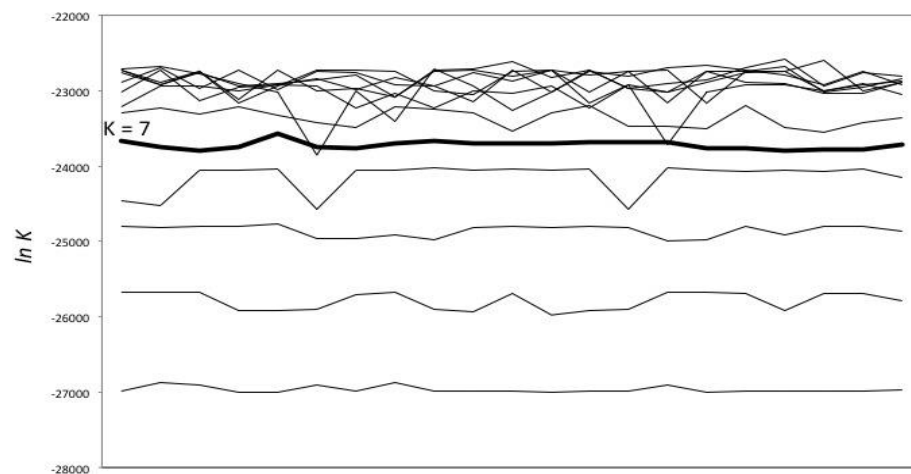
Fig. 2: Determination of population structure based on Bayesian clustering analysis. Estimated values of log likelihood In($X|K$) as function of the number of clusters (*K*) of population structure for the non-admixture model. *ΔK* was determined following Evanno et al. (2005).
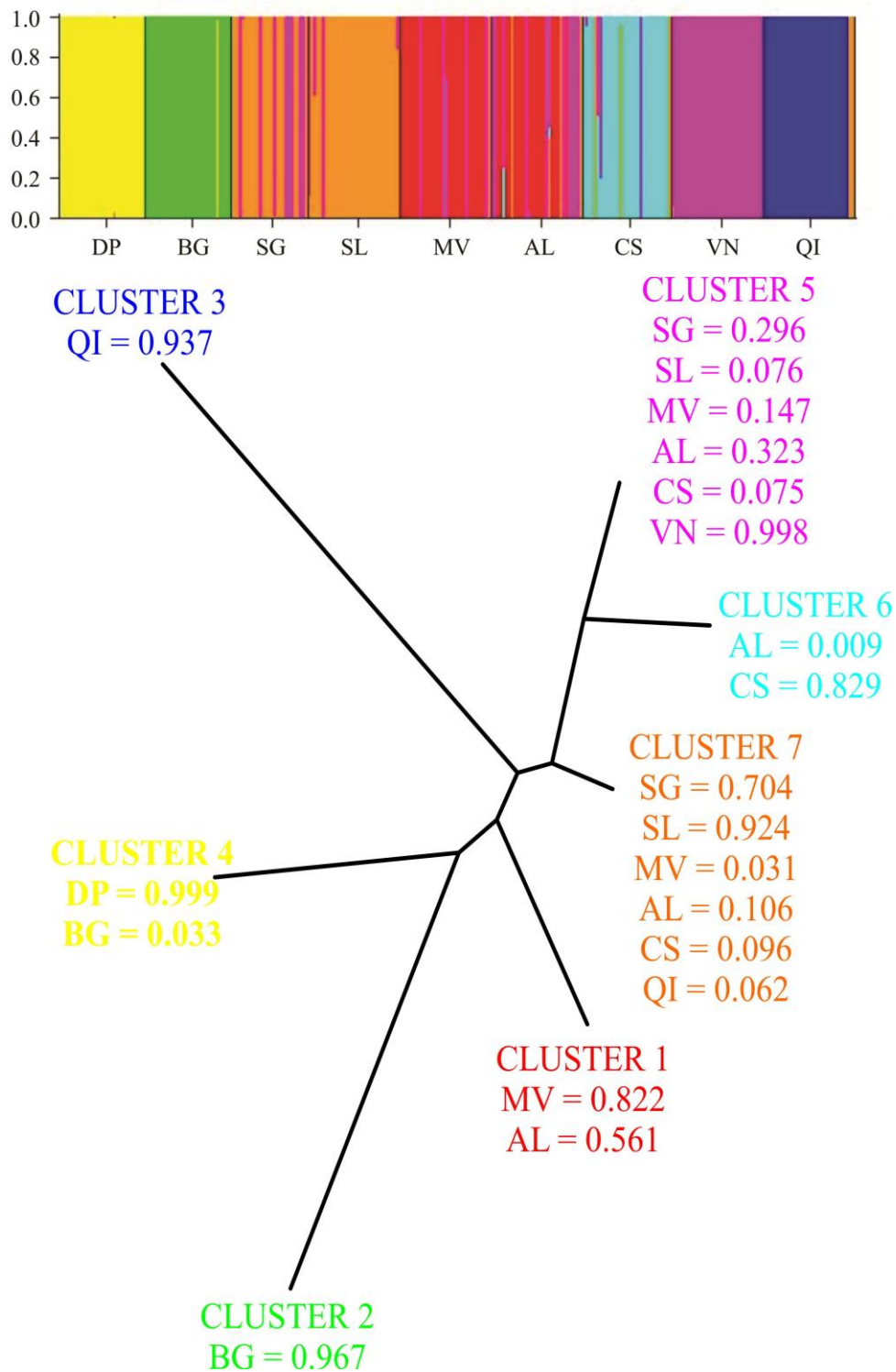
Fig. 3: Membership of the nine populations of *Schinus molle* in each cluster of the Bayesian analysis of population structure considering existence of seven clusters (K = 7) and the relation between populations. The membership determines the proportion of individuals from each population that was designed to a particular cluster as function of their genetic composition.

Fig. 4. Correlograms of kinship coefficient measures (F_{ij}) plotted against the distance based on AFLP data. Filled symbols are statistically significant at the 5% level.

Supplementary Fig. 1: Geographic position of each tree of *S. molle* within the sampled populations. Note that the distance scale is different for each population.

Table 1 Population density, spatial distribution, and location of the sampled area of *Schinus molle* in the Brazilian Pampa.

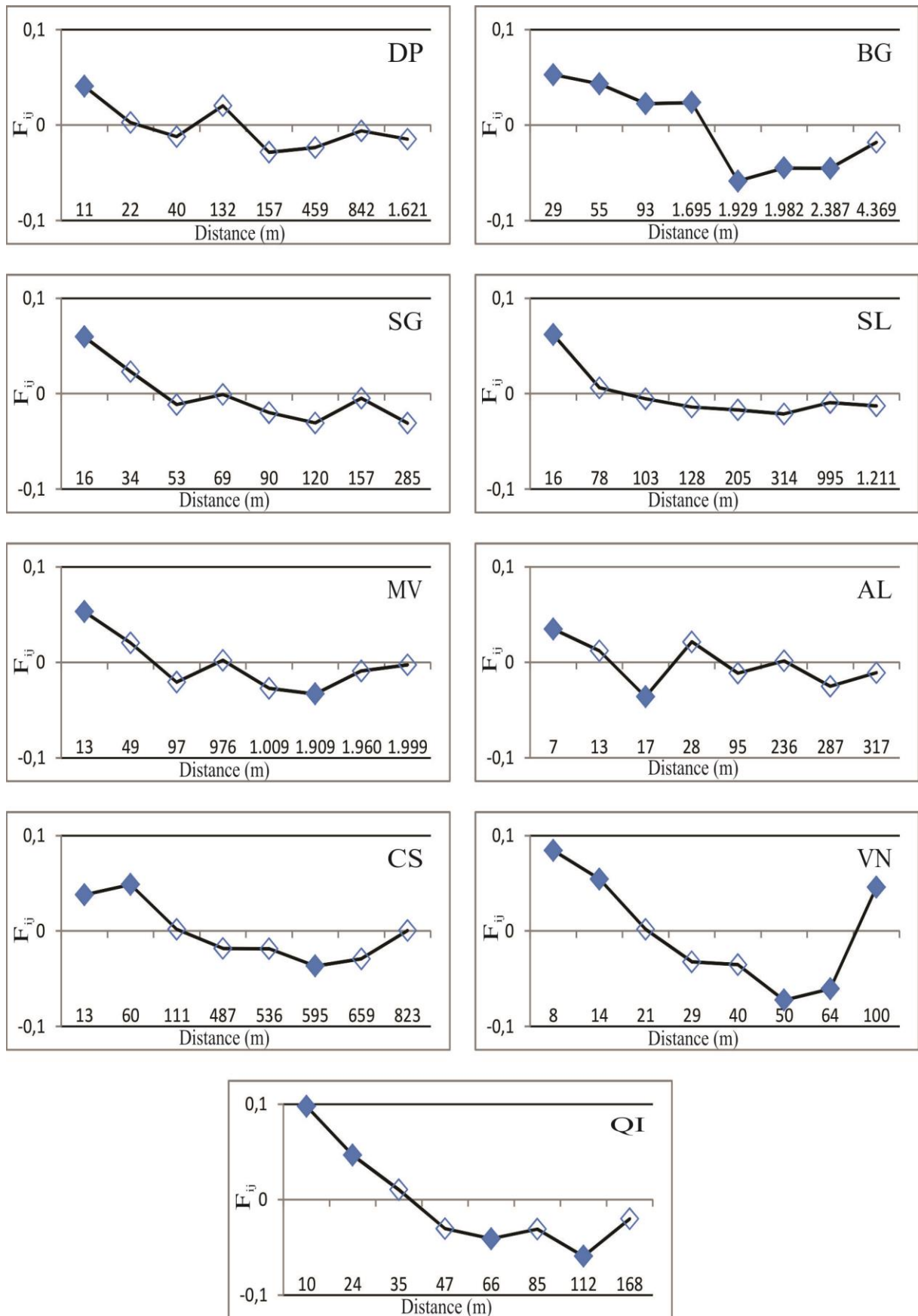| Populations	Census Density[1]	Linear distribution (m)	Sampled Area (ha)	Latitude (S)	Longitude (W)	Altitude (m)
Dom Pedrito (DP)	1.55	1615	19.38	31º01'25''	54º35'28''	155
Bagé (BG)	0.16	4366	189.92	31º16'09''	54º07'21''	285
São Gabriel (SG)	32.67	285	0.826	30º21'39''	54º18'35''	99
Santana do Livramento (SL)	3.69	1221	8.669	30º50'45''	55º27'31''	185
Manoel Viana (MV)	0.76	1988	42.344	29º37'07''	55º29'37''	100
Alegrete (AL)	31.75	315	1.008	29º49'15''	55º43'30''	130
Caçapava do Sul (CS)	2.52	821	12.315	30º36'83''	53º23'49''	149
Vila Nova do Sul (VN)	198.02	101	0.162	30º20'40''	53º54'12''	237
Quaraí (QI)	90.17	169	0.355	30º24'21''	56º10'47''	161

[1] Census density determined as trees/hectare (1 ha = 10,000 m^2)

Table 2. Summary of total individuals sampled (n), number of polymorphic loci (#loc_P), percentage of polymorphic loci (PLP), gene diversity (H_j) and Fragment Richness (B_R) values assessed overall AFLP loci in each natural population of *Schinus molle.*

Population	n	#loc_P	PLP	H_j	B_R
DP	30	144	57.6	0.18	1.711
BG	30	170	68.0	0.23	1.791
SG	27	140	56.0	0.16	1.760
SL	32	143	57.2	0.14	1.743
MV	32	156	62.4	0.17	1.855
AL	32	130	52.0	0.13	1.731
CS	31	89	35.6	0.10	1.630
VN	32	42	16.8	0.06	1.404
QI	32	142	56.8	0.20	1.743
Mean		128	51.3	0.15	1.708

Table 3. Summary of pairwise Φ_{ST} (below diagonal) assessed overall AFLP loci in natural populations of *Schinus molle*. The above diagonal shows the statistical significance.

	DP	BG	SG	SL	MV	AL	CS	VN	QI
DP		***	***	***	***	***	***	***	***
BG	0.2825		***	***	***	***	***	***	***
SG	0.2082	0.2844		***	***	***	***	***	***
SL	0.2570	0.3134	0.1237		***	***	***	***	***
MV	0.3040	0.2825	0.2311	0.2234		***	***	***	***
AL	0.2365	0.3007	0.1887	0.1752	0.1856		***	***	***
CS	0.3138	0.3887	0.2165	0.2050	0.2768	0.2463		***	***
VN	0.3591	0.4540	0.2664	0.2529	0.3189	0.2562	0.2451		***
QI	0.3459	0.3514	0.2743	0.2977	0.3394	0.3263	0.3511	0.4562	

Table 4. Estimation of fine-scale spatial genetic structure in populations of *Schinus molle* growing within the Brazilian Pampa.

Population	b_F	F_1	*Sp*-statistics	Pairs	%
Dom Pedrito (DP)	-0.0086	0.0405	0.0089	53 - 55	80
Bagé (BG)	-0.0175	0.0526	0.0185	53 - 55	70
São Gabriel (SG)	-0.0257	0.0594	0.0273	43 - 45	74.1
Santana do Livramento (SL)	-0.0144	0.0618	0.0154	62	78.1
Manoel Viana (MV)	-0.0091	0.0530	0.0096	62	53.1
Alegrete (AL)	-0.0085	0.0347	0.0089	60 - 64	75
Caçapava do Sul (CS)	-0.0141	0.0377	0.0146	58 - 59	58.1
Vila Nova do Sul (VN)	-0.0360	0.0842	0.0393	61 - 63	65.6
Quaraí (QI)	-0.0440	0.0977	0.0487	61 - 62	75

b_F = regression slope of F_{ij} on log distance; F_1 = multilocus kinship coefficient between individuals of the first distance class; *Sp*-statistic = quantification of SGS; *Pairs* = range of minimum and maximum number of individual pairs on the distance classes; % = minimum percentage of participation of individuals on distance classes.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

O presente estudo sugere que a dinâmica natural das comunidades vegetais implica a expansão do componente arbóreo sobre o campo no bioma Pampa. A variabilidade genética e a capacidade de dispersão demonstrou que *Schinus molle* L. pode ser uma espécie-chave, capaz de colonizar diferentes locais, proporcionando ambiente para a expansão das formações florestais. No entanto, considerando o número reduzido de indivíduos, os baixos níveis de diversidade e estrutura genética das populações estudadas, a manutenção da capacidade de adaptação não é assegurada no Pampa brasileiro, dados os efeitos negativos da deriva genética e endogamia nessas pequenas populações isoladas.

Embora *S. molle* não seja considerada uma espécie em extinção de acordo com os documentos oficiais, a baixa diversidade genética e significativa diferenciação entre as populações, sugerem que esta espécie sofre os graves efeitos da fragmentação florestal no Pampa brasileiro.

Essa foi uma pesquisa pioneira para o bioma Pampa, tendo em vista que trabalhos sobre a dispersão, diversidade e estrutura genética de componentes vegetais não foram registrados para esse ambiente. Trabalhos futuros aliando novas tecnologias como sequenciamento de nova geração, análises de estrutura de solo, mapeamento das áreas degradadas, identificação local dos impactos devem ser utilizados para contribuir com essa perspectiva. Acreditamos que, a partir do levantamento da estrutura e diversidade genética de espécies nativas, é possível diagnosticar os impactos sofridos nesses biomas e que esses dados podem servir de ferramentas precisas e confiáveis para propostas de manejo e conservação ambiental.