Alison Gonçalves Nazareno

CONSERVAÇÃO DE *Butia eriospatha* (MARTIUS EX DRUDE) BECCARI (ARECACEAE): UMA ESPÉCIE DA FLORA BRASILEIRA AMEAÇADA DE EXTINÇÃO

Tese submetida ao Programa de Pós-Graduação em Recursos Genéticos Vegetais da Universidade Federal de Santa Catarina para a obtenção do Grau de Doutor em Ciências. Orientador: Prof. Maurício Sedrez dos Reis

Florianópolis

2013

Ficha de identificação da obra elaborada pelo autor,
através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Nazareno, Alison Gonçalves
Conservação de Butia eriospatha (Martius ex Drude) Beccari (Arecaceae): uma espécie da flora brasileira ameaçada de extinção / Alison Gonçalves Nazareno ; orientador, Maurício Sedrez dos Reis - Florianópolis, SC, 2013.
141 p.

Tese (doutorado) - Universidade Federal de Santa Catarina, Centro de Ciências Agrárias. Programa de Pós-Graduação em Recursos Genéticos Vegetais.

Inclui referências

1. Recursos Genéticos Vegetais. 2. Conservação genética. 3. Diversidade Genética. 4. Biologia reprodutiva. 5. Demografia. I. Reis, Maurício Sedrez dos. II. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Recursos Genéticos Vegetais. III. Título.

AGRADECIMENTOS

A tentativa de agradecer às pessoas importantes que cruzaram e fortaleceram meu caminho até esta etapa poderia render inúmeras páginas. No entanto, este será um agradecimento simbólico. Seria incapaz de expressar minha toda gratidão, mesmo assim, realizarei essa tarefa, compreendendo sua incompletude e possível injustiça. A toda minha família, em especial a minha mãe – Maria da Fé – e aos meus irmãos – Tales, Rafael e Ana Carolina – agradeço pelo apoio incondicional. Ao meu orientador, Maurício Sedrez dos Reis, agradeço pela oportunidade, confiança, ensinamentos e por seu apoio em todas as etapas deste trabalho. Agradeço-lhe também por ter me permitido participar de projetos relevantes, como o Inventário Florístico Florestal de Santa Catarina (IFFSC) e a Lista de Espécies da Flora Catarinense ameaçadas de extinção. Aos membros da banca examinadora – Professores Dulcinéia de Carvalho, Miguel Pedro Guerra, Maria Imaculada Zucchi, Mariana Telles e Nivaldo Peroni – agradeço pelo olhar crítico e construtivo. Ao Programa de Pós Graduação em Recursos Genéticos da UFSC: professores, funcionários (em especial à Bernadete Ribas) e aos amigos que aqui encontrei, em especial, agradeço à Morgana Lopes, Karina Vanessa Hmeljevski, Ramon Scherer, Eva Regina, Carolina Cristofolini e Glauco Schussler pelo agradável convívio. Aos eternos amigos: Gustavo Moreira, Helena Aguiar, Juliana Ferez, Marcela Guidugli, Bruna Carolina, Rosangela Costa e Dulcinéia de Carvalho. A Dr[a]. Evelyn R. Nimmo pela edição dos textos em língua inglesa. A Karina Vanessa Hmeljevski e Renata Duzzioni pela ajuda com as ilustrações. Ao Prof. Adelar, pela indicação das primeiras populações de *Butia eriospatha*. Ao Laboratório de Fisiologia do Desenvolvimento e Genética Vegetal (LFDGV) pelo suporte oferecido. A todos do Núcleo de Pesquisas em Florestas Tropicais (NPFT) pela imensurável ajuda em campo. Pelo apoio financeiro, agradeço à CAPES e ao CNPq pela bolsa de estudo e, ao PRONEX (FAPÈSC/ CNPq), IFFSC (FAPESC) e CNPq que tornaram possíveis as viagens de campo e análises de laboratório.

"The population problem has no technical solution; it requires a fundamental extension in morality."

Garret Hardin, 1968

SUMÁRIO EXECUTIVO

Butia eriospatha (Martius ex Drude) Beccari (Arecaceae) é uma palmeira nativa do Sul do Brasil que está sob grande pressão antrópica. A espécie encontra-se ameaçada de extinção devido à venda ilegal de plantas adultas nos mercados local e internacional, à exploração insustentável de seus frutos, à presença do gado nas áreas de ocorrência e, à degradação e redução de seu hábitat devido aos reflorestamentos com espécies exóticas. Informações sobre os níveis de diversidade genética, bem como o conhecimento da ecologia de *B. eriospatha* são fundamentais e devem ser utilizadas em planos de conservação. Neste contexto, o objetivo geral deste trabalho foi estabelecer estratégias de conservação a partir de informações genéticas e ecológicas de *B. eriospatha.* Com base em análises de DNA nuclear, utilizando-se nove marcadores microssatélites, o presente estudo avaliou o sistema reprodutivo, a diversidade e a estrutura genética de oito populações naturais de *B. eriospatha* (n=920) localizadas no Estado de Santa Catarina. Análise adicional em uma coleção de indivíduos de *B. eriospatha* (n=50) oriundos de tráfico ilegal, localizados em residências na cidade de Florianópolis/SC, foi realizada com o intuito de comparar os níveis de diversidade genética com os observados nas populações naturais. Além das análises genéticas, aspectos ecológicos como fenologia, demografia, herbivoria e predação foram também analisados em algumas das populações de *B. eriospatha*. Análises do sistema reprodutivo indicaram que *B. eriospatha* é uma espécie que se reproduz, preferencialmente, por cruzamentos ($\hat{t}_m$=0,96). A espécie é auto-compatível e a reprodução pode ocorrer por geitonogamia, indicando a capacidade de populações ou mesmo de indivíduos isolados sobreviver e persisitir. Os níveis de diversidade genética foram intermediários e variaram entre as populações de *B. eriosptaha* (H_E=0,40–0,53; H_O=0,22–0,51, A_R=2,67–3,67). A presença de alelos raros e privativos foram observados na maioria das populações estudadas. A divergência genética entre as populações foi elevada e significativa (F_{ST}=0,16–0,25, p<0,005), indicando existirem diferenças genéticas importantes entre as populações ao longo do Estado. A presença de alelos raros e privativos e a diferenciação genética entre as populações de *B. eriospatha* atestam a necessidade de conservação *in situ* da variabilidade genética que ainda existe. Quando comparados, os níveis de diversidade genética foram maiores nos indivíduos que foram ilegalmente comercializados

(H_E=0,62, A_R=5,11). A presença de alelos privativos e os índices de diversidade genética observados na coleção de indivíduos em Florianópolis indicam alto potencial para a coleta de sementes e uso de mudas em projetos de restauração e enriquecimento das populações naturais de *B. eriospatha*. Além disto, baseado em análises Bayesianas, a maior parte dos indivíduos que foram ilegalmente comercializados e plantados em Florianópolis tiveram diferente origens. Em relação a fenologia reprodutiva, a espécie floresce entre outubro e março, frutificando entre novembro e julho. No entanto, a floração e a frutificação foi sazonal entre eventos reprodutivos. As populações de *B. eriospatha*, exceto uma, apresentaram estrutura demográfica bimodal, constituída por plantas adultas e plântulas. Altas taxas de herbivoria foram registradas, indicando que a estrutura demográfica observada é resultado da ação do gado sobre o componente regenerante. Isto é corroborado pela ocorrência de outros estádios ontogenéticos em uma população onde o gado não está presente. Todos esses resultados, somados ao uso insustentável dos frutos e da alta taxa de predação de sementes, refletem o grau de ameaça em que se encontram as populações de *B. eriospatha.* Além da relevância de se considerar várias populações em ações de conservação da espécie, mapear e monitorar as populações remanescentes, combater o comércio ilegal e impedir a entrada do gado nas populações de *B. eriospatha* são ações imprescindíveis para evitar que, de fato, a espécie seja perdida.

Palavras-chave: comércio ilegal, demografia, diversidade genética, herbivoria, fenologia reprodutiva, marcadores microssatélites, sistema reprodutivo

EXECUTIVE SUMMARY

Butia eriospatha (Martius ex Drude) Beccari (Arecaceae), a palm species native to southern Brazil, is under severe pressure due to anthropogenic factors. The species is threatened with extinction because of the illegal sale of adult plants in local and international markets, unsustainable exploitation of its fruit, livestock grazing in its habitat, and the reduction and degradation of its habitat due to reforestation with exotic species. Information on the genetic diversity levels, as well as ecological knowledge of *B. eriospatha*, are fundamental and an essential part of conservation planning for this species. Thus, the aim of this study was to establish conservation strategies from genetic and ecological information of *B. eriospatha*. Based on nuclear DNA analyzes using nine microsatellite markers, the present study evaluates the reproductive system, genetic diversity and structure of eight natural populations of *B. eriospatha* (n = 920) located in the state of Santa Catarina, Brazil. An additional analysis of a sample of illegally-trafficked *B. eriospatha* (n = 50) individuals, located in residences of the city of Florianópolis, Santa Catarina, was performed in order to compare levels of genetic diversity with those observed in natural populations. Along with genetic analysis, ecological information, such as phenology, demography, herbivory and predation, were also analyzed in four of the sample populations. Analysis of the reproductive system indicates that *B. eriospatha* is a species that reproduces primarily through out-crossing ($\hat{t}_m$=0.96). However, the species is self-compatible and reproduction can occur through geitonogamy, indicating the ability of populations, or even isolated individuals, to survive and persist. The levels of genetic diversity found in this study were intermediate and ranged between populations of *B. eriosptaha* (H_E=0.40–0.53; H_O=0.22–0.51, A_R=2.67–3.67). Rare alleles were observed in most of the studied populations and genetic divergence among populations was high and significant (F_{ST}=0.16–0.25, p<0.005), indicating important genetic differences between populations. The presence of rare alleles and genetic differentiation among populations of *B. eriospatha* attests to the need for *in situ* conservation of the genetic variability that remains. When compared with the natural populations, the levels of genetic diversity were greater in the individuals that were illegally marketed (H_E=0.62, A_R=5.11). The presence of rare alleles and high rates of genetic diversity observed in the sample from Florianópolis indicates a high potential for

seed collection and the use of seedlings in restoration projects and enrichment of natural populations of *B. eriospatha*. Moreover, based on Bayesian analysis, the illegally-purchased individuals planted in Florianópolis come from a variety of source populations. Regarding reproductive phenology, the species blooms between October and March, and fruiting occurs from November to July. However, flowering and fruiting were seasonal between observed reproductive events. All the studied natural populations of *B. eriospatha*, except one, showed a bimodal population structure, consisting of mature plants and seedlings. High rates of herbivory were recorded, indicating that the observed demographic structure is mainly the result of cattle grazing on the regeneration. This conclusion is supported by the occurrence of various ontogenic stages in a population where cattle are not present. Along with the unsustainable use of the fruit and high rates of seed predation, the results discussed herein reflect the level of risk facing *B. eriospatha* populations. Besides the necessary conservation of the species in a variety of populations, it is important to consider activities that will help prevent the loss of this species, such as mapping and monitoring the remaining populations, combating illegal trade, and preventing cattle grazing in *B. eriospatha* habitats.

Keywords: demographics, genetic diversity, herbivory, illegal trade, mating system, microsatellite markers, reproductive phenology

LISTA DE FIGURAS

CAPÍTULO 4

CAPÍTULO 5

LISTA DE TABELAS

CAPÍTULO 5

SUMÁRIO

INTRODUÇÃO

Da intrigante trama entre o cuco (*Cuculus canouros*) e o rouxinol (*Luscinia megarhynchos*) (DAVIES et al., 1998) à tragédia das áreas dos comuns (HARDIN, 1968) – em que prejuízos são coletivizados e os benefícios individualizados –, a mesma lógica existe: a da trapaça. O cuco nunca choca seus ovos ou cuida de seus filhotes. Em vez disso, bota um ovo idêntico ao do rouxinol e, para evitar que ele o reconheça falso, joga o do rouxinol para fora. Da mesma forma que a estratégia reprodutiva do cuco pode ser considerada cruel do ponto de vista ético, a tragédia das áreas dos comuns também o é. No entanto, as consequências destas trapaças têm direcionamentos distintos. Enquanto há um processo recíproco de mudança evolutiva entre o cuco e o rouxinol, o existente entre o homem e a natureza é unívoco: a resiliência da natureza é perdida diante das constantes atrocidades causadas pela ação antrópica. As consequências da trapaça do homem sobre a natureza são imensuráveis, mas entre elas destaca-se a perda de diversidade biológica, a erosão e desertificação dos solos, a homogeneização da fauna e da flora, e a diminuição da qualidade da água e do ar. Mas será que somos mesmo espertos ao trapacearmos? Quem ganha quando uma espécie é extinta? Quem se beneficia com a destruição e degradação das florestas? Por que para alguns a conservação da diversidade biológica é importante e para outros não? Por que conservar é preciso? Por que conservar a espécie A e não a B? Estas são perguntas inquietantes que vão além do escopo deste estudo, mas que de certo modo foram motivadores para a sua realização.

Mesmo que muitas vezes ideológica, a conservação dos recursos biológicos tem se tornado uma prioridade, principalmente pelos impactos negativos na funcionalidade e composição dos ecossistemas ocasionados pelo aumento da população humana e por conta de suas atividades econômicas (LAURANCE et al., 2006). Processos determinísticos como a introdução de espécies exóticas, a exploração demasiada dos recursos naturais, o comércio ilegal de espécies, a perda e a fragmentação de hábitats são, hoje, as causas primárias que têm culminado na extinção de espécies (LANDE, 1999; LADLE & WHITTAKER, 2011).

No Brasil, cerca de 1,7 milhões de hectares de florestas são perdidos anualmente (WRIGHT, 2005) e, com a aprovação do novo código florestal em 2012, as projeções são ainda mais alarmantes.

Sparovek et al. (2010) estimaram que 22 milhões de hectares de florestas podem ser legalmente perdidos sobre a proposta do novo código florestal. A degradação e a redução de hábitat, resultando em remanescentes florestais menores e isolados, vêm representando uma séria ameaça à diversidade biológica (YOUNG et al., 1996).

Em vista das taxas de destruição e perda de florestas, as populações vegetais estão experimentando contrações em seus tamanhos populacionais e algumas espécies podem ser mais susceptíveis as estocasticidades demográfica e genética do que outras (DICK et al., 2008). Para estas espécies, a compreensão de processos genéticos e demográficos é necessária para que estratégias efetivas de conservação possam ser estabelecidas. Neste contexto, espécies ameaçadas de extinção como a palmeira *Butia eriospatha* (Martius ex Drude) Beccari, necessitam de ações mitigadoras para sua conservação.

O gênero *Butia* (família Arecaceae, subfamília Arecoideae, subtribo Buttinae; DRANSFIELD et al., 2005) compreende 18 espécies que estão distribuídas exclusivamente no sul da América do Sul com populações no Brasil, Paraguai, Uruguai e Argentina (NOBLICK, 2010). Dentre as espécies de *Butia*, *Butia eriospatha* (Figura 1A), popularmente conhecida como butiá-da-serra, é uma espécie que ocorre no Brasil em zonas de campos no Paraná, Santa Catarina e Rio Grande do Sul (REITZ, 1974). De acordo com Reitz (1974) e Henderson et al. (1995), *B. eriospatha* caracteriza-se por apresentar estipe ereto, com 3 a 6 metros de altura e diâmetro de aproximadamente 50 cm. As frondes são pinadas, azul-esverdeadas, apresentando 1 metro ou mais de comprimento, e os pecíolos munidos de dentes ou espinhos relativamente fracos ou estreitos (REITZ, 1974). As inflorescências são densamente ramificadas, com flores masculinas e femininas (Figura 1C). Os frutos são globosos, com mesocarpo carnoso e adocicado, amarelos, contendo de 1-3 sementes (Figure 1 D). Como em outras espécies de *Butia*, os frutos maduros podem ser consumidos *in natura* ou usados na elaboração de sucos, geléias e bebidas alcoólicas (BUTTOW et al., 2009; SAMPAIO, 2011). Da semente, extrai-se um tipo de azeite comestível (BUTTOW et al., 2009). Seu estipe é usado em construções rústicas; as fibras das frondes podem ser usadas para fabricação de chapéus, cestos, cordas e enchimentos de colchões e estofados (MAURMANN, 2010; SAMPAIO, 2011). A espécie, *B. eriospatha* também é muito apreciada no paisagismo, inclusive pelo mercado internacional de plantas ornamentais (FISCHER et al., 2007).

Populações naturais de *B. eriospatha* estão sofrendo as consequências da perda de hábitats e de atividades antrópicas

relacionadas ao uso da terra. A espécie encontra-se sobre ameaça de extinção (Instrução Normativa 06, MMA 2008) e de acordo com os critérios da Lista Vermelha da IUCN (2010), *B. eriospatha* está vulnerável à extinção devido à exploração insustentável de seus recursos, ao comércio ilegal das plantas adultas, à degradação e redução de seus ambientes naturais, à introdução de espécies exóticas (e.g. plantios extensivos com *Pinus* spp.) e à presença do gado em sua área de ocorrência. O pastoreio nas populações de *Butia* vem agravando a regeneração natural (CHEBATAROFF, 1971; CARDOSO, 1995; AZAMBUJA, 2009) e as populações de *B. eriospatha* são constituídas, aparentemente, apenas por indivíduos centenários. Devido à presença do gado e de outros fatores antrópicos, outras espécies do gênero *Butia* também estão sob a ameaça de extinção (BRUSSA & GRELA, 2007; GAIEIRO et al., 2011; REIS et al., 2012). Embora pouco se conheça sobre a ecologia de *B. eriospatha*, há um consenso de que as populações silvestres da espécie estão em declínio, e a taxa de recrutamento de indivíduos reprodutivos das populações parece ser nula.

Como as outras espécies do gênero *Butia*, os indivíduos de *B. eriospatha* apresentam distribuição espacial agregada, às vezes densa e extensa, na forma de "populações-ilhas" conhecidas como butiazais (Figura 1B). A maioria dos butiazais ocorre em áreas de Campos de Altitude – um subtipo do Domínio da Mata Atlântica – (REITZ, 1974), que vêem sendo progressivamente transformadas ou degradadas por atividades relacionadas ao uso do solo (BILENCA & MIÑARRO, 2004; PILLAR, 2006). Em comparação com as florestas, a conservação dos Campos de Altitude tem sido negligenciada pela legislação ambiental, havendo poucas unidades de conservação que os contemplem (OVERBECK et al., 2007). Haja vista a realidade de conservação dos Campos de Altitude, o risco de extinção de *B. eriospatha* aumenta, sendo necessárias estratégias eficazes de conservação para a espécie. No entanto, para a definição de estratégias de conservação são imprescindíveis estudos genéticos, demográficos e biológicos, em nível populacional, das espécies que compõem tais ecossistemas. Neste contexto, o objetivo geral deste trabalho foi o de estabelecer estratégias de conservação a partir de informações ecológicas e da organização e distribuição da variabilidade genética de *Butia eriospatha*.

Estudos de genética molecular e ecologia têm se tornado parte integrante de diversos estudos de conservação. Informações quanto ao grau de organização e dinâmica da variabilidade genética em populações, bem como o entendimento da demografia e biologia reprodutiva das espécies são fundamentais para o delineamento de

estratégias de conservação. No entanto, há carência de trabalhos científicos que investiguem sobre a biologia de *B. eriospatha*, bem como sobre as consequências que as ameaças (e.g., presença do gado e o comércio ilegal) acarretam para a espécie. Para que um programa efetivo de conservação seja estabelecido para *B. eriospatha*, indicar as consequências das principais ameaças sobre a espécie, bem como o entendimento do grau e da organização da diversidade genética existente, o modo pelo qual a espécie se reproduz e a sua estrutura demográfica, são fundamentais para a tomada de ações efetivas, permitindo assim a continuidade do seu processo evolutivo. Assim, este trabalho buscou compreender a situação atual das populações de *B. eriopatha*, em termos biológicos e de ameça. Com base nas informações geradas, ações e políticas visando a conservação das populações da espécie na natureza foram propostas. Dessa forma, ao longo deste trabalho – dividido em 5 capítulos – subsídios são fornecidos para que planos de conservação possam ser estabelecidos para a espécie. Na sequência, são apresentados os objetivos e os principais resultados obtidos em cada um dos capítulos.

Os microssatélites (Sequências Simples Repetidas – SSR) são marcadores ideais para estudos genéticos por possuírem uma série de características desejáveis, tais como: distribuição ao acaso e ocorrência em grande quantidade em genomas de eucariotos; codominância dos alelos; facilidade de detecção via reação em cadeia da polimerase (PCR) e alta diversidade alélica (CHASE et al., 1996; DAYANANDAN et al., 1997). Neste sentido, o primeiro e o segundo capítulos apresentam os resultados da abordagem realizada para acessar e padronizar métodos de caracterização de marcadores microssatélites para estudos genéticos com a espécie *B. eriospatha*. No primeiro capítulo, que trata do desenvolvimento de marcadores microssatélites, obteve-se um total de 14 marcadores moleculares, dentre os quais, nove apresentaram polimorfismo em indivíduos de *B. eriospatha*. No entanto, em função do baixo polimorfismo apresentado, estes marcadores são limitantes em estudos cujo intuito é determinar o fluxo gênico contemporâneo. Por outro lado, independente do grau de polimorfismo apresentado, a aplicabilidade desses marcadores em estudos genéticos com outras espécies do gênero *Butia* é claramente possível e recomendada.

O segundo capítulo ilustra uma forma alternativa de aplicabilidade de marcadores microssatélites que são, aparentemente, pouco informativos. A variação no comprimento (i.e., número de nucleotídeos com repetições em sequência) é, geralmente, o único critério utilizado para caracterizar a diversidade alélica em *loci* que

exibem polimorfismo. Infelizmente, locos SSR, que são monomórficos em comprimento, são excluídos dos estudos genéticos devido à sua aparente falta de variabilidade genética. Desta forma, a variação de nucleotídeos em um marcador microssatélite sem polimorfismo entre seus alelos (i.e., monomórfico) foi avaliada com o intuito de fornecer uma nova e informativa ferramenta para análises genéticas. A espécie *B. eriospatha* foi considerada como uma espécie modelo para elucidar esta questão, visto que as palmeiras parecem ter, entre as angiospermas, as menores taxas de mutação (SMITH & DONOGHUE, 2009). Devido aos níveis de informação e de diversidade nas sequências de um marcador microssatélite monomórfico, este estudo demonstrou que marcadores monomórficos podem ser uma ferramenta útil para estudos filogenéticos para muitas espécies de plantas.

No terceiro capítulo, a abordagem foi dada sobre os aspectos reprodutivos da espécie. Com o objetivo de determinar quando e como *B. eriospatha* se reproduz, dados genéticos baseados em sete *loci* microssatélites foram combinados com dados de fenologia reprodutiva. A espécie floresce entre outubro e março, frutificando entre novembro e julho. No entanto, a floração e a frutificação foi sazonal entre eventos reprodutivos. Foi demonstrado que *B. eriospatha* é uma espécie protândrica, autocompatível e que se reproduz, principalmente, por cruzamentos, embora a reprodução possa ocorrer por geitonogamia (i.e., autofecundação que ocorre entre flores de uma mesma planta). Embora este estudo tenha elucidado alguns aspectos da biologia reprodutiva da espécie, indicando também possíveis agentes polinizadores (Figura 1E-F), estudos sobre a biologia da polinização da espécie é uma lacuna ainda não explorada.

Buscando estimar o risco de declínio populacional de *B. eriospatha*, no quarto capítulo, foram investigados a demografia, a regeneração natural, as taxas de herbivoria e os níveis de diversidade genética de plantas adultas e regenerantes em quatro populações com tamanhos populacionais distintos. Com base nestas informações, estimativas populacionais foram obtidas para a espécie. Os resultados deste estudo indicaram que a presença do gado nas populações de *B. eriospatha* afeta severamente sua estrutura demográfica. Das populações estudadas, três apresentaram estrutura demográfica bimodal (i.e., com apenas plantas adultas e plântulas), com altas taxas de herbivoria sobre o componente regenerante. Para uma população, onde não há a presença do gado, foi descrita e quantificada pela primeira vez a ocorrência de outros quatro estádios ontogenéticos para a espécie (Apêndice 1). Em relação à variação genética, as populações de *B. eriospatha*

apresentaram índices moderados de diversidade, sendo as populações com tamanhos populacionais reduzidos as mais susceptíveis às estocasticidades genéticas. Além disto, estimativas de tamanhos populacionais indicaram que *B. eriospatha* está em iminente risco de extinção local, podendo ter seus tamanhos efetivos populacionais reduzidos de 50% nas próximas quatro décadas.

No quinto capítulo, foi abordado a questão do comércio ilegal da espécie. As duas principais questões deste estudo foram (1) investigar se indivíduos que foram ilegalmente comercializados apresentam maior diversidade genética em relação aos indivíduos das populações naturais e (2) identificar a população de origem dos indivíduos comercializados. Demonstrou-se que há maior diversidade genética nos indivíduos que foram ilegalmente comercializados. Além disto, a maior parte dos indivíduos que foram ilegalmente comercializados e plantados em Florianópolis era proveniente de diversas populações. Neste trabalho, demonstrou-se também que o uso de técnicas de investigação forense pode auxiliar no controle do comércio ilegal de *B. eriospatha*. Em adição, são apresentadas evidências do comércio ilegal da espécie (Apêndice 2), ilustrando-se o modo como *B. eriospatha* é retirada das populações e os locais onde a espécie é plantada (Apêndice 3).

Ao final, conclusões e encaminhamentos que podem ser realizados em um futuro próximo são apresentados.

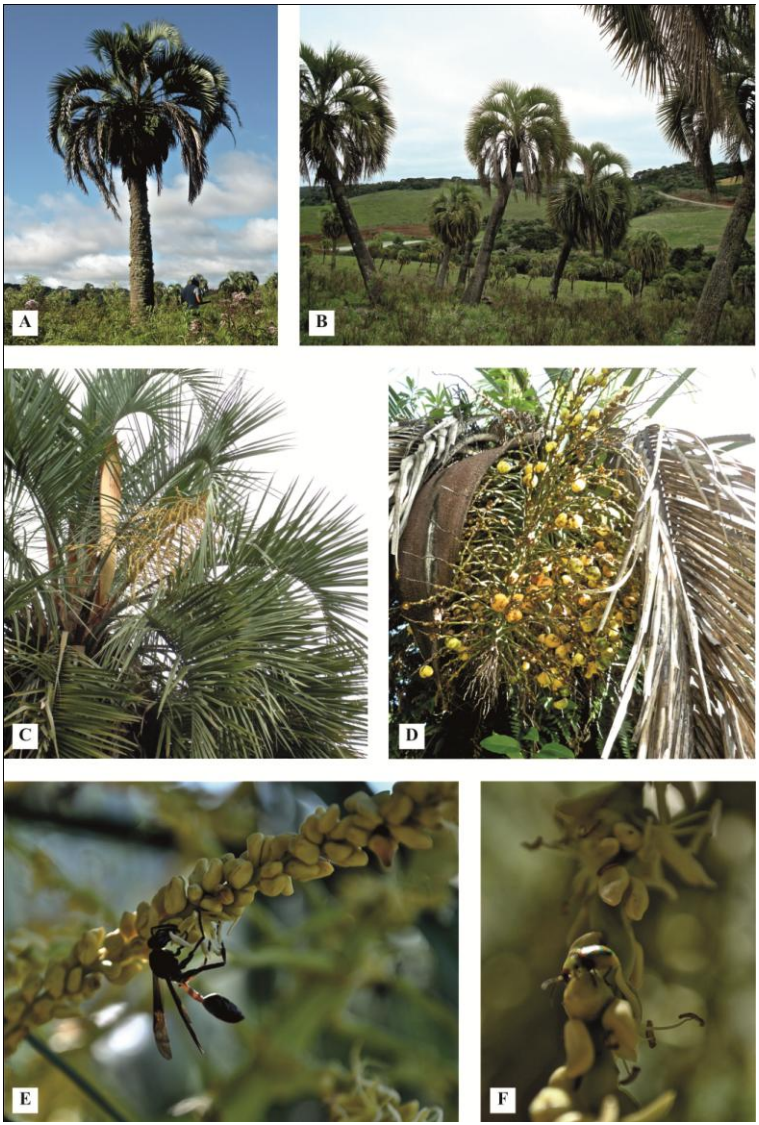


Figura 1. Indivíduo (A) e população (B) de *Butia eriospatha* (Martius ex Drude) Beccari. Em C e D, observam-se a inflorescência e a infrutescência, respectivamente, de *B. eriospatha*. Alguns dos visitantes florais observados forrageando em flores de *B. eriospatha* estão indicados nas figuras E e F.

REFERÊNCIAS

Azambuja, A.C. (2009) Demografia e fenologia reprodutiva de *Butia capitata* (Mart.) Becc. (Arecaceae) em Arambaré, Rio Grande do Sul. Dissertaçao de Mestrado, Universidade Federal do Rio Grande do Sul. 47 p.

Bilenca, D. & Miñarro, F. (2004) Identificación de Áreas Valiosas de Pastizal (AVPs) en las Pampas y Campos da Argentina, Uruguay y Sur de Brasil. Buenos Aires: Fundación Vida Silvestre, 323 p.

Brussa, C.A. & Grela, I.A. (2007) Flora Arbórea del Uruguay. Con énfasis en las especies de Rivera y Tacuarembó. COFUSA, Uruguay.

Buttow, M.V., Barbieri, R.L., Neitzke, R.S. & Heiden, G. (2009) Conhecimento tradicional associado ao uso de butiás (*Butia* spp., Arecaceae) no Sul do Brasil. Revista Brasileira de Fruticultura, 31, 1069-1075.

Cardoso, M.C.L. (1995) El palmar, la palma y el butiá. PROBIDES. Fichas didácticas, 4(1) 23p.

Chase, M., Kesseli, R. & BAWA, K. (1996) Microssatellite markers for population and conservation genetics of tropical trees. American Journal of Botany**,** 83, 51-57.

Chebataroff, J. (1971) Condiciones ecológicas que influyen en la distribución de las palmeras del Uruguay. Facultad de Humanidades y Ciencias, Montevideo, Uruguay. 24p.

Davies, N.B., Kilner, R.M. & Noble, D.G. (1998) Nestling cuckoos, *Cuculus canorus*, exploit hosts with begging calls that mimic a brood. Proceedings of the Royal Society B, 265, 673-678.

Dayanandan, S., Bawa, K.S. & Kerseli, R. (1997) Conservation of microsatellites among tropical trees (Leguminosae). American Journal of Botany, 84, 1658-1663.

Dick, C.W., Hardy, O.J., Jones, F.A. & Petit, R.J. (2008) Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. Tropical Plant Biology, 1, 20-33.

Dransfield J., Uhl N.W., Asmussen C.B., Baker W.J., Harley, M.M. & Lewis, C.E. (2005) A new phylogenetic classification of the palm family, Arecaceae. Kew Bulletin, 60, 559-569.

Fischer, S.Z, Stumpf, E.R.T., Heiden, G., Barbieri, R.L. & Wasun, R.A. (2007) Plantas da flora brasileira no mercado internacional de floricultura. Revista Brasileira de Biociências, 5(1), 510-512.

Gaieiro, P., Mazzella, C., Agostini, G., Bertolazzi, S. & Rossato, M. (2011) Genetic diversity among endangered Uruguayan populations of *Butia* Becc. species based on ISSR. Plant Systematic and Evolution, 292, 105-116.

Hardin, G. (1968) The tragedy of the commons. Science, 162, 1243-1248.

Henderson, A., Galeano, G. & Bernal, R. (1995) Field guide to the palms of the Americas. New Jersey: Princeton University Press.

IUCN World Conservation Union. (2010) Red List of Threatened Species. Version 2010.1. [cited 2012 January 15]. Available from: .http://www.iucnredlist.org.

Ladle, R.J. & Whittaker, R.J. (2011) Conservation biogeography. Blackwell Publishing Ltd.

Lande, R. (1999) Extinction risks from anthropogenic, ecological, and genetic factors. In: Genetics and the extinction of species. Eds (Landweber LF, Dobson AP). Princeton University Press, Princeton, New Jersey pp. 1-22.

Laurance, W.F. et al. (2006) Rapid decay of tree-community composition in Amazonian forest fragments. Proceedings of National American Science, 103, 19010-19014.

Maurmann, K. (2010) Como o manejo tradicional da folha de *Butia capitata* (Martius) Beccari pode promover a conservação dos butiazais? Trabalho de conclusão de curso, Instituto de Biociências, Universidade Federal do Rio Grande do Sul.

MMA, Ministério do Meio Ambiente. (2008) In: Lista oficial de espécies da Flora brasileira ameaçada de extinção. Diário Oficial da União de 24 de setembro de 2008, nº 185. Seção 1, p. 75-83.

Noblick, L.R. (2010) *Butia*. In: Lorenzi H, Noblick LR, Kahn F, Ferreira E, editors. Flora brasileira Lorenzi: Arecaceae (palmeiras). Instituto Plantarum, Nova Odessa, Brazil.

Overbeck, G.E., Muller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C.C., Boldrini, I.I., Both, R. & Forneck, E.D. (2007) Brazil's neglected biome: The South Brazilian Campos. Perspectives in Plant Ecology, Evolution and Systematics, 9, 101-116.

Pillar, V.P. (2006) "WORKSHOP: Estado Atual e Desafios para a Conservação dos Campos". 24 p.

Reis, M.S., Mantovani, A., Silva, J.Z., Mariot, A., Bittencourt, R., Nazareno, A.G., Ferreira, S.K., Steiner, F., Montagna, T., Silva, A.A.L.S., Fernandes, C.D., Altrak, G. & Figueredo, L.G. (2012) Distribuição da diversidade genética e conservação de espécies arbóreas em remanescentes florestais de Santa Catarina. In: Vibrans, A.C., Sevegnani, L., Gasper, A.L. & Lingner, D.V., editors. Inventário florístico florestal de Santa Catarina. v. 1 Blumenau: Edifurb.

Reitz, R. (1974) Palmeiras. *In*: Flora Ilustrada Catarinense. Itajaí, SC: Herbário Barbosa Rodrigues.

Sampaio, L.K.A. (2011) Etnobotânica e estrutura populacional de butiá, *Butia catarinenses* Noblick & Lorenzi (Arecaceae) na comunidade dos Areais da Ribanceira de Imbituba/SC. Dissertação apresentada no Programa de Pós-Graduação em Biologia Vegetal, Universidade Federal de Santa Catarina.

Smith, S.A. & Donoghue, M.J. (2009) Rates of molecular evolution are linked to life history in flowering plants. Science, 322, 86-89.

Sparovek, G., Berndes, G., Klug, I.L.F. & Barreto, A.G.O.P. (2010) Brazilian agriculture and environmental legislation: status and future challenges. Environmental Science and Technology, 44, 6046–6053.

Wright, S.J. (2005) Tropical forests in a changing environment. Trends in Ecology and Evolution, 20(10), 553-560.

Young, A., Boyle, T. & Brown, T. (1996) The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution, 11(10), 413-418.

CAPÍTULO 1

Este manuscrito encontra-se publicado no período *American Journal of Botany*

Microsatellite markers for *Butia eriospatha* (Arecaceae), a vulnerable palm species from the Atlantic Rainforest of Brazil

Nazareno AG, Zucchi MI, Reis MS. 2011. Microsatellite markers for *Butia eriospatha* (Arecaceae), a vulnerable palm species from the Atlantic Rainforest of Brazil. American Journal of Botany 98: e198 – e200.

ABSTRACT

Premise of the study: Microsatellite markers were developed for the vulnerable palm species *Butia eriospatha* (Mart. ex Drude) Becc. in order to investigate genetic diversity, spatial genetic structure, mating system, and population dynamics.

Methods and Results: From a genomic library enriched for GA/CA repeats, 14 sets of primers were isolated and characterized for 50 *B. eriospatha* samples from two populations. The number of alleles per locus ranged from 2 to 6 (with amplified dinucleotide repeat-based primers); the observed and expected heterozygosities ranged from 0.000 to 1.000 and from 0.120 to 0.690, respectively. At least 86% primers were also amplified for *Butia catarinensis* Noblick & Lorenzi, another threatened palm species from the Atlantic Rainforest in Brazil.

Conclusions: The new marker set described here will be useful for studies of population genetics of *B. eriospatha* and they have been shown to be applicable for other species from the *Butia* genus.

Key Words: conservation genetics; simple sequence repeats; transferability

INTRODUCTION

The Brazilian palm flora is very rich, with estimates of 387 species (Glassman, 1972). However, more research regarding palm flora and the specific threats facing each species is needed in order to design and implement urgently required management plans to guarantee the conservation of these genetic resources. Among the genera threatened in Brazil, the palms of the *Butia* genus deserve special attention due to the high risk of their becoming a threatened species as a result of human activities. *Butia eriospatha* (Mart. ex Drude) Becc. is a long-living palm species native to the Atlantic Rainforest region, with a natural range from Southern Brazil to Uruguay (Reitz, 1974). The species also grows in high altitude grassland. The remaining populations of vulnerable *B. ertiospatha* mostly consist of mature individuals aged one hundred years or older. The species has suffered severe population decline due to the reforestation of exotic tree species, illegal sale of adult plants in both local and international trade, overexploitation of fruit and herbivory due to local livestock farming.

Microsatellite markers (simple sequence repeats - SSR) have been developed (Arnold et al., 2002; Li et al., 2010) and used as a very effective tool to generate useful information for species conservation (Nazareno et al., 2009). Although microsatellite markers constitute informative systems for ecological genetics, they have not yet been developed for any *Butia* species. In order to investigate the population structure and genetic diversity in the remaining populations, we developed microsatellite markers for *B. eriospatha*. Furthermore, we investigated the transferability of these loci in *Butia catarinensis* Noblick & Lorenzi to other threatened tropical palm species from the Atlantic Rainforest.

METHODS AND RESULTS

A $(GA)_n$ and $(CA)_n$ microsatellite-enriched library was constructed according to the method employed by Billotte *et al.* (1999), using a biotin-labeled microsatellite oligoprobe and streptavidin-coated magnetic beads. Total genomic DNA was extracted from the leaf tissue of one individual of *B. eriospatha* according to Doyle and Doyle (1990) and was digested with RsaI restriction enzyme. The fragments were then linked to adapters (Rsa21 5'-CTCTTGCTTACGCGTGGACTA-3' and Rsa25 5'-TAGTCCACGCGTAAGCAAGAGCACA-3'). The library was enriched for dinucleotide sequences, using biotinylated $(CT)_8$ and

$(GT)_8$. Streptavidin-coated paramagnetic beads and a magnetic rack were employed to recover the fragments containing microsatellites. Selected fragments were amplified by polymerase chain reaction (PCR), using the Rsa21 adapter as a primer, and were then linked to the pGEM-T vector (Promega, Madison, Wisconsin, USA). Plasmids were introduced into *Escherichia coli* XL-1 Blue strains. Transformed cells were grown on Petri dishes with Luria–Bertani (LB) agar medium containing ampicilin (100 μg ml^{-1}) and X-galactosidase (5-bromo-4-chloro-indolyl-ß-D-galactoside) (50 μg ml^{-1}). A total of 192 positive clones were sequenced using an ABI 377 and the Big Dye Terminator Kit (Applied Biosystems, Vienna, Austria), 30 of which presented microsatellite sequences with at least five tandem repeats. Primers were designed with the software PRIMER3 (Rozen and Skaletsky, 2000) by setting product size ranges from 100 to 300 base pairs (bp), primer size from 18 to 22 bp, GC% from 40 to 60, and melting temperature from 57 to 60 °C.

PCR reactions specific for the amplification of each microsatellite locus were set up. Every reaction consisted of a final volume of 10 μl containing 0.3 μM of each primer, 1 U Taq DNA polymerase, 0.25 mM each of dNTP and 1x $MgCl_2$- free reaction buffer [75 mM Tris–HCl pH 9.0, 50 mM KCl and 20 mM $(NH_4)_2SO_4$], 1.5 mM $MgCl_2$ and 2.5 ng of template DNA. The optimal melting temperature was assessed separately for each primer pair. In total, 17 temperatures (between 46-62 °C) were tested using 10 individuals of the *Butia eriospatha*. The PCR profile used to amplify the microsatellites was 96°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, melting at *Tm* °C (Table 1) for 45 s, 72°C for 1 min, and a final elongation step at 72°C for 5 min. Amplifications were performed with a C1000^{TM} Thermal Cycler (Bio-Rad, Singapore). PCR products were denatured and separated on 10% denaturing polyacrylamide gels stained with silver nitrate. Allele sizes were estimated by comparing to a 10 bp DNA ladder standard (Invitrogen). For the polymorphism evaluation, we sampled 50 reproductive individuals of *B. eriospatha* from two natural populations (25 individuals per population), 200 km apart, located in Santa Catarina State, Southern Brazil. We do not provide exact locations for natural populations in order to reduce the risk of illegal harvesting and trade of adult individuals. Genetic diversity parameters and probabilities of paternity exclusion were estimated using CERVUS version 3.0 (Kalinowski et al., 2007).

From these 30 primer pairs, 14 amplified to the expected size fragment (Table 1) while the others showed no amplification,

multibanding patterns or pronounced stutters. For this microsattelite set, five loci were monomorphic with fixed alleles in both *B. eriospatha* populations surveyed. A total of 34 alleles were identified and the mean number of alleles ranged from 2.25 (Population 1) to 3.11 (Population 2). The observed and expected heterozygosities ranged from 0.000 to 1.000 and from 0.120 to 0.690, respectively. The paternity exclusion probability reached 0.47728 for the first parent and 0.17710 for the second parent for all polymorphic loci. Because of the low probability values, these microsatellite markers will not yet allow accurate parentage studies in natural populations of *B. eriospatha* even in situations where maternity or paternity is known.

Additionally, transferability of the 14 primer pairs was tested in 10 individuals of *Butia catarinensis* using the same PCR protocol described above. Most primers (86%) also were successfully amplified for *B. catarinensis* (Table 2). The results indicate that there is a high potential for transferring microsatellite markers between species of the same genus in the Arecaceae family.

CONCLUSIONS

The microsatellite set developed are useful in investigations of genetic diversity, spatial genetic structure, mating system, and population dynamics of *B. eriospatha*. Future studies with these microsatellite loci will be possible in genetic analyses of related taxa. We plan to use these markers to evaluate the *B. eriospatha* genetic status in all existing populations in Santa Catarina State, the native region of this vulnerable palm species.

Taxon; *Voucher specimen*, Collection locale; Herbarium.

Butia eriospatha (Mart. ex Drude) Becc.; Stival-Santos et al. 20624, Brazil, SC; FURB.

Butia catarinensis Noblick & Lorenzi; Korte 33601, Brazil, SC; FURB.

Table 1. Characteristics of 14 dinucleotide microsatellite loci developed for *Butia eriospatha* (Mart. ex Drude) Becc. from Santa Catarina State, Southern Brazil. Locus name, primer sequence (F: foward, R: reverse), repeat motif, fragment size in base pair (bp), melting temperature (*Tm*), and GenBank accession numbers are show.

SSR	Sequence 5' – 3'	Repeat motif	Size pb	*Tm* ºC	GenBank Access nº.
but01	F: CCTATGCTTACCTTCCAAAGC	$(AT)_9$	190	56	HQ588787
	R: CAAGAGTGGTGGATCATGGTA				
but04	F: GCCTAGCTAGACAACCCAAGA	$(CA)_6$	202	54	HQ588788
	R: TTGCTTAGGGAGTGAACGTG				
but06	F: CCATAACAGCCGGAGTTTGT	$(GA)_{15}$	179	54	HQ588789
	R: GAAGGATGCTGTTCTTGTTGG				
but07	F: ACGTCATCCCATACCAAGAAA	$(CA)_{16}(GA)_{13}$	280	56	HQ588790
	R: TGCCCATTGTAACACGTTATG				
but08	F: GCGAAATCCAAACCATACG	$(CA)_9(TA)_5$	194	56	HQ588791
	R: GCATCATACGAGGGAGGAAT				
but09	F:GGCTAAGTTTTCAAAGGGAAGA	$(CT)_{17}$	161	54	HQ588780
	R: GGGACCTGGTAAAGGAATGA				
but10	F: AAGAGTGAGCTTGGGGAGTG	$(GT)_5$	132	53	HQ588781
	R: TGATGCTGTGCTGTGAGAGA				
but11	F: TGCACCCGAACCATATAAACT	$(TG)_5$	149	53	HQ588782
	R: CAAGAGGGTGGGGTAAATTG				
but14	F: TACCAATTGTTGCCCAGCTA	$(CA)_6$	188	51	HQ588783
	R: TGTGCACCGAACCACTATAAA				
but16	F: AGGCTCTACCCCTTTCTTGG	$(TA)_{10}$	139	54	HQ588784
	R: CTCTCTCACACACACGCACA				
but17	F: AGAGCAGAGCTTTGGGAGAA	$(CT)_9(AT)_5(AC)_9$	204	54	HQ588785
	R: GCCATTCATGGACTCCAAA				
but18	F: GATTCTTGCCAGCACACAAA	$(AT)_7$	246	48	HQ588786
	R: AGTCAATGGTTTTGGACATGG				
but20	F: CCAAACAACCCAAGATAGAGC	$(AG)_8$	147	54	HQ588792
	R:CAAGGAGAGACATTCTTCAAGC				
but23	F: AGAGGATGGGGCATTTGAT	$(CT)_{12}$	168	55	HQ588793
	R: CCCTTCCATAAGCATGTTCC				

Table 2. Loci names, allele frequencies, allelic richness (*A)*, and observed heterozygosity (H_O) for *Butia eriospatha* (Mart. ex Drude) Becc. populations in Santa Catarina State, Southern Brazil, are shown. The success of transferability (T) for *Butia catarinensis* Noblick & Lorenzi is also show.

		Butia eriospatha						*Butia catarinensis*	
		Population 1 (N=25)			**Population 2 (N=25)**				
Loci	Alleles	Frequency	*A*	H_O	Frequency	*A*	H_O	T	*Tm* (ºC)
but01	190	1.00	01		1.00	01		-	
but04	202	1.00	01		1.00	01		+	54
but06	179	0.00	03	0.333	0.16	04	0.080	+	54
	181	0.00			0.04				
	183	0.29			0.04				
	185	0.66			0.76				
	187	0.05			0.00				
but07	270	0.00	02	0.167	0.02	04	0.520	+	54
	278	0.00			0.14				
	280	0.92			0.62				
	284	0.08			0.22				
but08	194	0.50	02	1.000	0.39	06	1.000	+	54
	196	0.00			0.06				
	198	0.50			0.39				
	200	0.00			0.06				
	206	0.00			0.05				
	210	0.00			0.05				
but09	161	0.02	03	0.240	0.04	04	0.583	+	54
	163	0.10			0.44				
	165	0.00			0.02				
	167	0.88			0.50				
but10	132	1.00	01		1.00	01		+	54
but11	149	0.94	02	0.125	0.94	02	0.125	+	54
	151	0.06			0.06				

but14	188	1.00	01		1.00	01		+	54
but16	139	1.00	01		0.54	02	0.920	+	54
	141	0.00			0.46				
but17	202	0.87	02	0.000	0.87	02	0.000	+	54
	204	0.13			0.13				
but18	246	0.92	02	0.000	0.84	02	0.000	+	54
	250	0.08			0.16				
but20	147	1.00	01		1.00	01		-	
but23	168	0.33	02	0.667	0.46	02	0.920	+	56
	172	0.67			0.54				
Mean			2.25	0.316		3.11	0.460		

Monomorphic loci are in bold letters; microsatellite loci lacking amplification success for *Butia catarinensis* are indicated by (-), and those showing a successful amplification are marked with (+); melting temperature (*Tm*).

LITERATURE CITED

ARNOLD, C., M. ROSSETTO, J. MCNALLY, AND R. J. HENRY. 2002. The application of SSRs characterized for grape (*Vitis vinifera*) to conservation studies in Vitaceae. *American Journal of Botany* 89: 22–28.

BILLOTTE, N., P. J. L. LAGODA, A. M. RISTERUCCI, AND F. C. BAURENS. 1999. Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops. *Fruits* 54: 277–288.

DOYLE J. J., AND J. L. DOYLE. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.

GLASSMAN, S. F. 1972. A revision of B.E. Dahlgren's Index of American Palms. Lehre: J. Cramer.

KALINOWSKI, S. T., M. L. TAPER, AND T. C. MARSHALL. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16: 1099–1106.

LI, X-Y., Z-F. WANG, P. ZHU, W-H. YE, X-F. HUANG, L-F. WU, S-F. CHAI, AND H-L. CAO. 2010. Ten microsatellite markers in endangered species *Sauvagesia rhodoleuca* (Ochnaceae). *American Journal of Botany* 97: e61–e62.

NAZARENO, A. G., R. A. S. PEREIRA, J. M. FERES, M. A. MESTRINER, AND A. L. ALZATE-MARIN. 2009. Transferability and characterization of microsatellite markers in two Neotropical *Ficus* species. *Genetics and Molecular Biology* 32: 568–571.

REITZ, R. 1974. Palmeiras. In: Reitz R (ed) Flora ilustrada catarinense. Herbário Barbosa Rodrigues, Itajaí, Brazil.

ROZEN, S., AND H. J. SKALETSKY. 2000. Primer3 on the www for general users and for biologist programmers. *In* S. Krawetz and S. Misener [eds.], Bioinformatics methods and protocols: methods in molecular biology, 365–386. Humana Press, Totowa, New Jersey, USA.

CAPÍTULO 2

Este manuscrito encontra-se publicado no período *American Journal of Botany*

The same but different: monomorphic microsatellite markers as a new tool for genetic analysis

Nazareno AG, Reis MS. 2011. The same but different: monomorphic microsatellite markers as a new tool for genetic analysis. American Journal of Botany 98: e265 – e267.

ABSTRACT

Premise of the study: The nucleotide variation at a microsatellite locus lacking length polymorphisms among its alleles was assessed in order to generate an informative tool for genetic analysis.

Methods and Results: From a set of microsatellite markers, a monomorphic microsatellite locus developed for the palm species *Butia eriospatha* was used to elucidate if there are polymorphic sites in its flanking regions. DNA sequences ≈133 bp long were obtained. Aligned sequences show variation at 17 polymorphic sites with both insertions and nucleotide substitutions. Fourteen distinct sequences (alleles) among 22 individuals were identified. The percent sequence difference varied from 0.0 to 5% indicating that there is significant variation among sequences.

Conclusions: Due to significant levels of information and sequence diversity on a SSR locus of identical size, our study highlights that this molecular marker class can be an useful tool for population genetics and evolutionary studies for many plant species.

Key Words: *Butia eriospatha*; monomorphic SSR; sequence variation

INTRODUCTION

Many different methods can be employed to reveal molecular markers. Polymerase chain reaction (PCR)-based assays of simple sequence repeat loci (SSRs or microsatellites) have become the most popular and powerful of the current methods for identifying highly polymorphic genetic markers. Polymorphisms in SSRs, which are abundant in the genomes of many taxa, are thought to result from saltatory replication, unequal crossovers or possibly gene conversion (Lewin, 1997) and often exhibit high levels of heritable variation in the number of repetitions of a particular motif. Traditional genetic surveys of microsatellite loci capitalize on SSR variation because it is easy to score DNA fragments by size. Length variation is usually the sole and most conspicuous criterion employed to characterize allelic diversity at loci displaying variable numbers of tandem repeats. Unfortunately, SSR loci that are monomorphic in length have been excluded from both evolutionary and population genetics approaches due to their apparent lack of genetic variability. Furthermore, since microsatellite loci were discovered (Litt and Luty, 1989; Tautz 1989), only recently have periodicals (e.g., *American Journal of Botany*) begun to accept publications that include monomorphic loci.

Beyond variation by size at SSR loci, previous reports of DNA sequence analysis have noted that nucleotide sequence variation in flanking regions of polymorphic loci have hinted at the mutational complexity and evolutionary diversity that can underlie conventionally detected size differences among microsatellite alleles (Estoup et al., 1995; Ortí et al., 1997). From this point of view, published monomorphic loci can be used in addition to length variation. Therefore, the goals of this study are to address some of the questions that have arisen regarding the use of monomorphic loci: How much variation remains hidden at a monomorphic SSR locus? Is this molecular marker class suitable for genetic analysis? The vulnerable palm *Butia eriospatha* (Mart. ex Drude) Becc. was considered as a model species to elucidate the answers to these questions as palms appear to have lower rates of molecular change (Smith and Donoghue, 2009).

METHODS AND RESULTS

From a set of microsatellite markers previously isolated for the palm species *Butia eriospatha* (Nazareno et al. 2011), a dinucleotide $(GT)_5$ monomorphic microsatellite locus (*BUT10*) was used to elucidate if

there are polymorphic sites in its flanking regions. In order to ratify the status of the monomorphic locus, the present study initially characterized 100 individuals from four populations for the *BUT10* locus. The PCR and profile used to amplify the *BUT10* locus are described at Nazareno et al. (2011). PCR products were denatured and separated with 10% denaturing polyacrylamide (39:1 acrylamide to bisacrylamide) gels stained with silver nitrate. Gels were run with 1x TBE buffer (90 mM Tris, 92 mM boric acid, and 2.5 mM EDTA) on a vertical electrophoresis at a constant electric current (21 mA for each gel) for six hours. Allele sizes were estimated by comparison with a 10 base pairs (bp) DNA ladder standard (Invitrogen, Carlsbad, California, USA). All samples contained fragments with the same length (≈132 bp).

In order to detect sequence variation, we selected all 29 *B. eriospatha* plants from one natural population out of the original sample. Furthermore, one adult plant from a population (designed as P) 200 km away, located in Santa Catarina State, Southern Brazil, was added to the analysis. For sequence procedures, the PCR products of *BUT10* locus were stained with Gel-Red, and the presence of a single clear band was verified in 1.5% agarose gel. The gel was run with 1x TBE buffer on a horizontal electrophoresis at a constant voltage (120 V) for one hour, and was viewed under UV light. The bands were extracted from the gel and purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, Wisconsin, USA) and ligated into pGEM-T Easy Vector System I (Promega). The ligation reaction consisted of a final volume of 20 µl containing 3U T4 DNA Ligase (Promega), 10x ligase buffer (300 mM Tris–HCl pH 7.8, 100 mM $MgCl_2$, 100 mM DDT and 10 mM ATP), 50 ng of plasmids, and 20 ng of amplified fragments. Plasmids were introduced into chemically competent *Escherichia coli* OmniMAX™ 2-T1 strains (Invitrogen). The transformation reactions consisted of a final volume of 65 µl [32 µl transfobuffer (10mM KCl, 3mM CaCl2, 5mM MgCl2, 15mM Polyethylene glycol 1450), 8 µl of the transformation reaction, and 25 µl OmniMAX™ 2-T1 cells]. Transformed cells were grown on Petri dishes with Luria-Bertani (LB) agar medium containing ampicilin (100 µg ml^{-1}) and X-galactosidase [(5-bromo-4-chloro-indolyl-ß-D-galactoside), (50 µg ml^{-1})]. Plasmids containing the insert were sequenced. At least four clones of each sample were sequenced to obtain a consensus sequence using the DYEnamic ET Dye Terminator kit (GE Healthcare, Buckinghamshire, UK) in a MEGA BACE sequencer (GE Healthcare). Samples without consensus were excluded from analysis. DNA sequences were aligned

visually and the percent sequence difference was calculated following the equation $p = z_d/z_t$ proposed by Avise (2004), where z_d is the number of nucleotides that differ between two sequences, and z_t is the total number of nucleotides compared.

The nucleotide sequence of the *BUT10* locus was determined for 22 individuals that showed sequence consensus. DNA sequences varied from 133 to 134 bp long. Aligned sequences showed variation at 17 polymorphic sites of the monomorphic locus *BUT10* in both flanking regions and microsatellite motif (Figure 1). Alignments of this genomic region indicate that variations at sequence level included both insertions and nucleotide substitutions. There were two nucleotide insertions at position 33 in two different sequences (Figure 1). This resulted in two alleles with a length of 134 bp that the acrylamide gel did not distinguish from alleles of 133 bp in length. Irrespective of nucleotide insertions, the DNA sequences of SSR alleles of identical size (133 bp) revealed considerable variation resulting in 12 alleles due to nucleotide substitutions. The frequency of 14 alleles ranged from 0.05 to 0.27. The percent sequence difference varied from 0.0 to 5% indicating that there is significant variation among sequences.

CONCLUSIONS

Due to the observed sequence variation within a population of *B. eriospatha,*a taxon with apparently low evolutionary rate, this study demonstrates that monomorphic molecular markers can be suitable for genetic population and phylogenetic analyses in many plant species. We plan to use this molecular marker to evaluate the genetic divergence of *B. eriospatha* among populations in the Atlantic Rainforest, the native region of this vulnerable palm species.

Taxon; *Voucher specimen*, Collection locale; Herbarium, GenBank accession numbers.

Butia eriospatha (Mart. ex Drude) Becc.; Stival-Santos et al. 20624, Brazil, SC; FURB, JF748761, JF748762, JF748763, JF748764, JF748765, JF748766, JF748767, JF748768, JF748769, JF748770, JF748771, JF748772, JF748773, JF748774, JF748775, JF748776, JF748777, JF748778, JF748779, JF748780, JF748781, JF748782.

LITERATURE CITED

AVISE, J. C. [ed.], 2004. Molecular markers, natural history, and evolution, 2nd ed. Sinauer Associations, Suderland, MA, USA.

ESTOUP, A., C. TAILLIEZ, J.-M. CORNUET, AND M. SOLIGNAC. 1995. Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). *Molecular Biology and Evolution* 12: 1074–1084.

LEWIN, B. 1997. Genes VI. Oxford University Press, New York.

LITT, M., AND J. A. LUTY. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics* 44: 387–401.

NAZARENO A. G., M. I. ZUCCHI, AND M. S. DOS REIS. 2011. Microsatellite markers for *Butia eriospatha* (Arecaceae), a vulnerable palm species from the Atlantic Rainforest of Brazil. *American Journal of Botany* in press.

ORTÍ, G., D. E. PEARSE, AND J. C. AVISE. 1997. Phylogenetic assessment of length variation at a microsatellite locus. *Proceedings of the National Academy of Sciences of the United States of America* 94: 10745-10749.

SMITH, S. A., AND M. J. DONOGHUE. 2009. Rates of molecular evolution are linked to life history in flowering plants. *Science* 322: 86-89.

TAUTZ, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Resources* 17: 6463–647

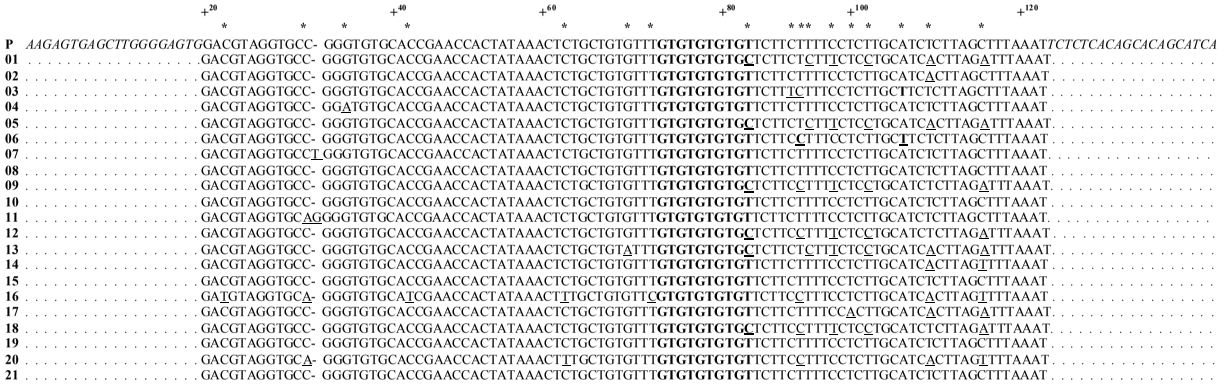


Figure 1. Aligned sequences showing the variation at 17 polymorphic sites (*) from the monomorphic locus *BUT10* isolated from *Butia eriospatha* (Mart. ex Drude) Becc. Primer sequences are in italics or represented by dots. Insertions or nucleotide substitutions are underlined and repeat motif is in bold. The samples from one to 21 were obtained from one population. The sample indicated as P was obtained from a *B. eriospatha* plant from a population 200 km away, in Santa Catarina State, Southern Brazil. All DNA sequences were deposited in GenBank under accession numbers JF748761-JF748782.

CAPÍTULO 3

Este manuscrito encontra-se publicado no período *Journal of Heredity*

Linking phenology to mating system: exploring the reproductive biology of the threatened palm species *Butia eriospatha*

Nazareno AG, Reis MS. 2012. Linking phenology to matin system: exploring the reproductive biology of the threatened palm species *Butia eriospatha*. Journal of Heredity 103: 842-852.

ABSTRACT

The reproductive biology of the vulnerable palm species *Butia eriospatha* was studied to provide important information that contributes to our understanding and conservation of the species. In order to determine when and how *B. erisopatha* reproduces, we combined data from seven nuclear microsatellite loci with ecological data on flowering and fruiting phenology collected between 2009 and 2011 from a population (N=515) in the Atlantic Rainforest, Southern Brazil. Periods of flowering and fruit production were seasonal and variable across reproductive events. Mating system analyses indicate that *B. eriospatha* is a predominantly outcrossing species, ($\hat{t}_m$=0.961), since a certain degree of biparental inbreeding does occur. The species is self-compatible and reproduction may also occur by geitonogamy, indicating the ability of isolated populations to survive and persist. Open-pollinated seeds varied in relatedness, including mainly half-sibs and full-sibs. The effective population size was lower than that expected for panmictic populations. Hence, seeds for conservation programs must be collected from a large number of seed-trees to ensure an adequate effective population in the sample. The collection of germplasm is a high priority strategy that should be employed to maintain the genetic variability that remains.

Key words: circular statistics, conservation genetics, mating system estimation, microsatellites, outcrossing

The palm family (Arecaceae) has about 2,450 species distributed in many of the world's tropical forests and it is one of the better studied tropical families of angiosperms (Barfod et al. 2011). Although many important advances have been made in palm evolutionary biology concerning life history (Henderson 2002; Rodrígues-Buriticá et al. 2005; Eiserhardt et al. 2011), molecular evolution (Chase 2004; Smith and Donoghue 2008), population genetics (Luna et al. 2005; Shapcott et al. 2009), and pollination mechanisms (Barfod et al. 2011), our knowledge is still somewhat limited with respect to reproductive biology, specifically relating to the mating system (Bovi et al. 2003; Conte et al. 2008; Ramos et al. 2011; Abreu et al. 2012).

In plants, mating systems that employ cross- and/or self-fertilization control the patterns of genetic transmission within and among populations. While self-fertilization (i.e., selfing) restricts gene migration through pollen flow, outcrossing promotes pollen flow and reduces the likelihood of micro-geographical differentiation and population sub-structuring. However, even in the presence of long distance pollen flow, population sub-structuring can occur due to localized seed dispersal. Mating patterns are determined by reproductive and environmental features such as self-incompatibility mechanisms (Goodwillie et al. 2005), gender and degree of dichogamy in flowering plants, foraging behaviour of pollinators (Hirao et al. 2006), flowering phenology (Oddou-Muratorio et al. 2006), spatial isolation of trees (Fuchs et al. 2003), and plant density (Murawski and Hamrick 1991).

Tropical trees are mainly outcrossing (Ward et al. 2005); however, mixed mating systems—which include both selfing and outcrossing—frequently occur in a wide variety of plants (Goodwillie et al. 2005), including palms (Conte et al. 2008; Ramos et al. 2011; Abreu et al. 2012). In addition, reproductive biology studies have shown that self-compatibility in plant species is also frequent (Goodwillie 1999, 2001). Although it is generally accepted that palm families are predominantly self-compatible (Read 1975; Scariot et al. 1991; Ashburner et al. 2001; Barfod et al. 2003; Bovi et al. 2003; Chan et al. 2011), few studies have tested the self-compatibility of specific palm species.

Self-compatibility within a species is advantageous when no pollinator vectors are available and could improve the chances of reproductive success when mates are scarce or when the population is small. As reductions in population sizes become common due to

anthropogenic disturbances, such as land conversion, self-compatible species would be able to survive and persist more than a few generations in fragmented habitats or at small population sizes. Hence, understanding the reproductive biology of a plant species is a key element in conservation programs and is particularly important for those species that are at risk of extinction.

Natural populations of the neotropical palm species *Butia eriospatha* (Martius ex Drude) Beccari are suffering the consequences of human intervention especially through loss of habitat due to exotic tree species reforestation, illegal sale of specimens in both local and international trade, overexploitation of fruit, and cattle farming. Furthermore, the remaining populations of *B. ertiospatha* mainly consist of mature individuals aged one hundred years or older (Nazareno et al. 2011). This vulnerable species (IUCN 2010) is a long-lived palm (subfamily Arecoideae, subtribe Buttinae; Dransfield et al. 2005) locally known as *butiá-da-serra*. It is native to the Atlantic Forest, which ranges from Southern Brazil to Uruguay (Reitz 1974) and grows in high-altitude grasslands (a subtype of the Atlantic Forest Domain). Previous observations indicate that *B. eriospatha* (2n = 32; Correa et al. 2009) is monoecious (Reitz 1974). *B. eriospatha* is a palm species with numerous male and female yellow flowers arranged in distinct parts of the same inflorescence (i.e., androgynous). The inflorescences are protandrous in which male flowers come into anthesis before female flowers. Since there may be several inflorescences on a single individual, selfing (i.e., geitonogamy) can occur due to pollen flow from one inflorescence to another one with female receptive flowers. The fleshy fruit of the species is approximately 2.0 cm in diameter and is eaten by both local human populations and frugivores, such as birds and squirrels. No published study has examined the details of the reproductive biology of *B. ertiospatha* and therefore the goal of this study is to shed light on this important aspect of the species.

In order to elucidate when and how *B. eriospatha* reproduces, we coupled genetic data with ecological data on individual size, flowering, and fruiting. Specifically, we address the following questions: (a) Is there a pattern of seasonality in flowering and fruiting? (b) Is the species strictly outcrossing? (c) Is there some mechanism to avoid self-fertilization? Our results will influence long-term decisions for in situ and ex situ conservation and breeding of this threatened palm species.

Materials and Methods

Study site

We sampled one population of *B. eriospatha* located near the municipality of Curitibanos (27º16' S, 50º34' W), in Santa Catarina State, Western Plateau, Southern Brazil. This population covers an area of about 16 hectares with individuals in a clustered distribution (Figure 1). In order to reduce the risk of illegal poaching and trade of mature individuals from this population, we do not provide the exact location of the study site. Historically, the study region was the first area on the plateau of Santa Catarina State colonized by Europeans and the area has consistently been occupied by mainly livestock herders. Cattle have been present for at least 90 years in this *B. eriospatha* population's habitat.

Flowering and fruiting phenology

Field measurements

Phenological observations were carried out during two consecutive reproductive events (October 2009 to May 2010 and October 2010 to May 2011). In the studied population (Figure 1), all individuals (N=515) were tagged with a numbered aluminium plate, and the diameter at breast height (DBH) was recorded. Due to mortality, the number of monitored palm trees in 2010-2011 was 504 individuals. The following phenological stages (i.e., phenophases) were recorded and data collected at 15-day intervals: (1) inflorescence with male flowers; (2) inflorescence with female flowers; (3) infructescence with unripe fruit; and (4) infrutescence with ripe fruit. For every palm, we recorded the number of reproductive structures (i.e., inflorescence and/or infructescence) and their specific phenophase during each phenological observation. In order to detect evidence of geitonogamy, flowering overlap was also assessed (i.e., the presence of both male and female flowers during inflorescence in a same plant).

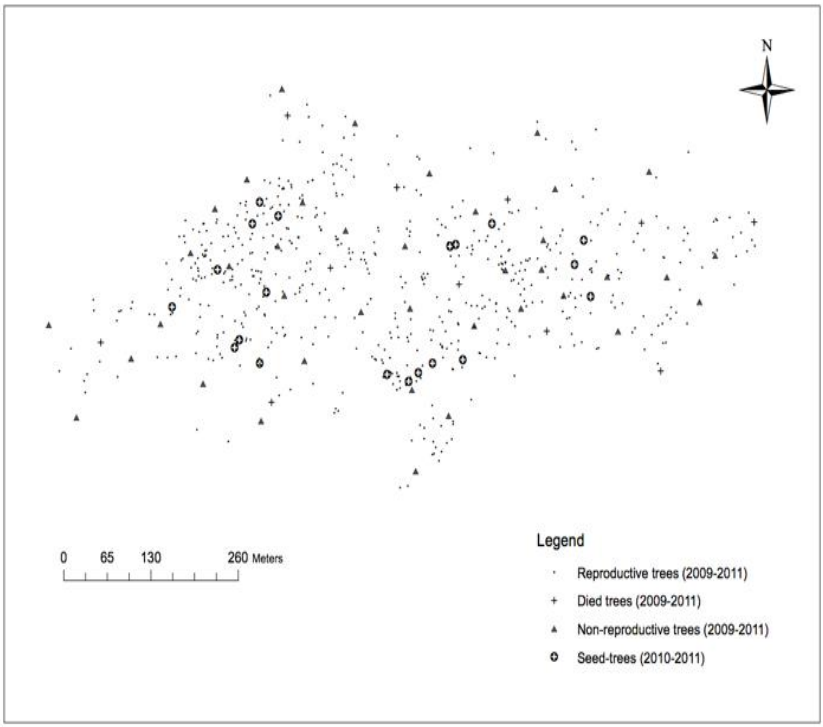


Figure 1 Map of the study population showing the spatial distribution of all studied *Butia eriospatha* (Martius ex Drude) Beccari individuals in the Atlantic Rainforest, Santa Catarina State, Southern Brazil. Reproductive and non-reproductive trees, died trees, and seed-trees are showed.

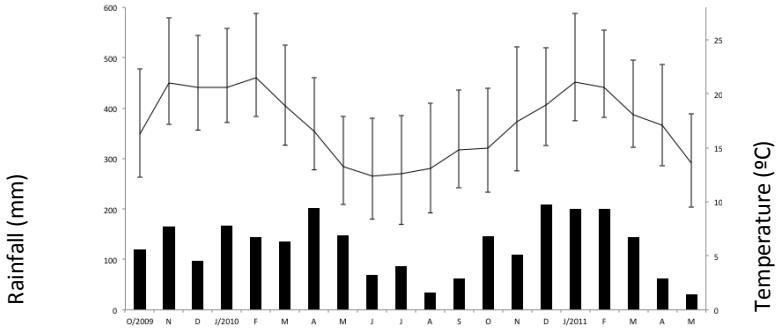


Figure 2 Annual distribution of average monthly temperatures (ºC) including maximum and minimum temperatures, and rainfall distribution (mm) for the municipality of Curitibanos, in the Santa Catarina State Western Plateau, Southern Brazil. Bars represent rainfall data and line represents temperature data.

Phenological statistical analyses

The analyses were based on the period of open flowers (male and female) and (un)ripe fruits, hereafter called flowering and fruiting. The size of the reproductive *B. eriospatha* population was calculated as the number of individuals that reproduced during the 2009-2011 period. We analysed differences in reproductive effort (number of inflorescence per palm tree) with Kolmogorov-Smirnov test between the reproductive events. Additionally, the effect of DBH on flowering (yes/no) was inferred by an unpaired *t*-test (Zar 1996) under the H_0 hypothesis: where the mean DBH of reproductive palm trees is the same as the mean DBH of non-reproductive palm trees.

Circular statistics were used to determine seasonality, as proposed by Morellato et al. (2000). To calculate the circular statistic parameters, months were converted to angles, from 0º January (no. 1) to 330º December (no. 12) at intervals of 30º. The frequency of reproductive structure in a specific phenophase within each angle was calculated and the following parameters estimated for each reproductive event: mean angle *a*, and the length of vector *r*, a measure of concentration around the mean angle that ranges from zero, when an equal number of

phenological records occur at each angle, to one, when all records occur at one single angle or month. The significance of the mean angle was estimated using the Rayleigh(z) test (Zar 1996). The mean date for each phenophase was determined by converting the mean angle to corresponding mean dates.

The seasonality hypothesis also followed Morellato et al. (2000), by defining H_0 as the condition where phenological variables are distributed uniformly throughout the year, thus suggesting no seasonality. If H_0 is rejected, the phenological variables are not uniformly distributed and there is a significant mean angle or date for the phenological variable or seasonal pattern. The intensity of concentration around the mean angle denoted by *r*, can be considered a measure of the degree of seasonality (Morellato et al. 2000). When the mean angle was significantly different from zero, a two sample Watson-Williams test (*F*) was performed to compare the mean dates between the reproductive events. Possible climatic conditions such as temperature and rainfall were correlated with the phenological events (Spearman's correlation). All analyses followed Zar (1996) and were performed with ORIANA software (Kovach 2004).

Genetic analysis

Sampling, DNA extraction and microsatellite marker amplification

In order to determine the mating system, we analysed 20 open-pollinated progeny from 20 seed-trees sampled randomly in the final reproductive event (2010-2011; Figure 1). Genomic DNA extraction from leaves of seed-trees followed the standard Cetyltrimethylammonium bromide (CTAB) procedure (Alzate-Marin et al. 2009). For the progeny arrays, DNA was extracted directly from the embryo due to the low germination potential of dormant seeds. For DNA extraction from embryos we used the NucleoSpin® kit (MACHEREY-NAGEL GmbH & Co. KG), according to manufacturers' instructions. The PCR and profile used to amplify the seven microsatellite loci (But06, But07, But08, But09, But11, But16, and But23) are described in Nazareno et al. (2011). PCR products were denatured and separated with 10% denaturing polyacrylamide gels (39:1 acrylamide to bisacrylamide) stained with silver nitrate. Gels were run with 1 X TBE buffer (90 mM Tris, 92 mM boric acid, and 2.5 mM EDTA) on a vertical electrophoresis at a constant electric current (21

mA for each gel). Allele sizes were estimated by comparison with a 10 base pair DNA ladder standard (Invitrogen, Carlsbad, California, USA).

Prior genetic analysis

We tested for Mendelian inheritance of alleles and gametic disequilibrium before including the microsatellite set in our study. Loci that are included in analyses despite gross violations of these assumptions or high rates of error could lead to inaccurate and biased genetic estimates (Selkoe and Toonen 2006). Since the mating system analysis presupposes that alleles at different loci segregate independently, we undertook Mendelian inheritance analyses for each locus, based on the mother tree and their open-pollinated family as proposed by Gillet and Hattemer (1989). As gametic disequilibrium creates pseudo-replication for analyses in which loci are assumed to be independent samples of the genome, we used the FSTAT software (Goudet 2002) to test all loci for linkage disequilibrium, applying the Bonferroni correction for multiple comparisons.

Determination of the mating system

The mating system of *B. eriospatha* was analyzed under the mixed-mating and correlated mating models, using the Multilocus mating system program MLTR version 3.2 (Ritland 2008). Since the null alleles can reduce the power to estimate mating systems, the analysis was performed taking into account that all loci may contain null alleles even if there are none. The parameters estimated were: multilocus outcrossing rate ($\hat{t}_m$); single-locus outcrossing rate ($\hat{t}_s$); selfing correlation ($\hat{r}_s$); biparental inbreeding rate ($\hat{t}_m - \hat{t}_s$); and multilocus paternity correlation ($\hat{r}_{p(m)}$) or proportion of full sibs among outcrossed progeny. The inbreeding coefficient of maternal parents ($\hat{F}_m$) was also calculated. Analyses at the population level were carried out using the probabilities of Expectation Maximization (EM) numerical method and at the individual level using the method of moments (MME) according to Ritland (2004). The standard error for each parameter was calculated from 1,000 bootstrap replicates with resampling among families. To determine whether the values were significantly lower than one ($\hat{t}_m$ and $\hat{t}_s$) or greater than zero ($\hat{F}_m$, $\hat{t}_m - \hat{t}_s$, and $\hat{r}_{p(m)}$), a 95% confidence interval was calculated.

From the mating system parameters, other demographic and

genetic parameters were assessed. The neighborhood size ($\widehat{N}_{ep}$), i.e., the number of pollen donors contributing to each family, was estimated as $1/\hat{r}_{p(m)}$ (Ritland 1989). The average proportion of self-sibs ($\hat{P}_{ss}$), half-sibs ($\hat{P}_{hs}$), full-sibs ($\hat{P}_{fs}$), and self-half-sibs ($\hat{P}_{shs}$) within families was estimated as: $\hat{P}_{ss} = \hat{s}^2$; $\hat{P}_{hs}=\hat{t}_m{}^2(1-\hat{r}_{p(m)})$; $\hat{P}_{fs}=\hat{t}_m{}^2\ \hat{r}_{p(m)}$; and $\hat{P}_{shs}=2\hat{s}\hat{t}_m$, where $\hat{s}$ $(=1-\hat{t}_m)$ is the selfing rate. The confidence intervals for these parameters were calculated based on both upper and lower confidence limits estimated from mating system parameters using the MLTR program.

Additionally, we calculated the coancestry coefficient among plants within progenies ($\hat{\theta}_{xy}$) from the correlation coefficient of relatedness among plants within progenies ($\hat{r}_{xy}$), as proposed by Ritland (1989): $\hat{r}_{xy}=0.25(1+\hat{F}_m)[4\hat{s}+(\hat{t}_m{}^2+\hat{t}_m\hat{s}\hat{r}_s)(1+\hat{r}_{p(m)})$. Based on the coancestry coefficient, we estimated the effective size of variance $\widehat{N}_{e(v)}=0.5/\hat{\theta}_{xy}$ (Cockerham 1969), considering that for diploid species the coefficient $\hat{\theta}_{xy}$ is half the coefficient $\hat{r}_{xy}$ (Lynch and Walsh 1998). It is important to note that the minimum coancestry coefficient expected is 0.125 for half-sibs. Further, this parameter is expected to accommodate different levels of relatedness within families: self-sibs, half-sibs, full-sibs and self-half-sibs. Thus, in open-pollinated offspring incorporating different kinds of relatives, $\hat{\theta}$ is expected to have values between 0.125 and 1 (the coancestry expected between two self-sib individuals from an autogamous species). Additionally, the Nason's estimator of kinship coefficient (f_{ij} or coefficient of coancestory) described in Loiselle et al. (1995) was performed for maternal trees using the software SPAGeDi (Hardy and Vekemans 2002).

The coefficient of inbreeding in embryos ($\hat{F}_o$) was inferred by calculating the fixation index using the FSTAT program (Goudet 2002). To test if $\hat{F}_o$ was significantly different from zero, 10,000 permutations of alleles among individuals were performed. The total coefficient of inbreeding $\hat{F}_o$ $(=\hat{F}_s+\hat{F}_{tm-ts})$ in embryos was split into its components resulting from self-fertilization $\hat{F}_s$ $(=0.5\hat{s}(1+\hat{F}_m)$, Barrett and Kohn 1991), and mating among relatives, $\hat{F}_{tm-ts}$.

We also estimated the number of seed-trees ($\hat{m}$) from which it would be necessary to collect seeds in order to retain the reference effective population size ($N_{e(reference)}$) of 500 (Nunney and Campbell 1993). This was calculated following the method of Sebbenn (2006), based on the relationship between the desired effective population size

of the conservation program ($N_{e(reference)}$) and the average variance effective population size estimated for plants within progenies $\widehat{N}_{e(v)}$.

In order to determine if the mating events of the *B. eriospatha* population occurred due to random mating, we estimated the coefficient of pollen pool structure (Φ_{ft}) from two-generation analyses (TWOGENER, Smouse et al. 2001). It is an estimator of genetic differentiation among pollen pools (ranges from 0 to 1) which is an analogue of Wright's F_{ST}. The standard error for Φ_{ft} was calculated using the jackknife procedure over loci. Calculations were performed using the R language for TWOGENER analysis as was written by A. S. Hirao (available from http://hosho.ees.hokudai.ac.jp/~hirao/TWOGENER/TwoGener.html).

Results

Flowering and fruiting phenology

The reproductive population of the sampled *B. eriospatha* population ranged from 474 ($N_{2009\text{-}2010}$=515) to 459 individuals ($N_{2010\text{-}2011}$=504). Approximately 8% of the total population did not exhibit reproductive activity over the 2009-2011 study periods (Figure 1). The flowering intensity was significantly different between reproductive events (D=0.69, P<0.05). In the 2010-2011 study period, the number of inflorescence (N=868, mean per plant=2.93, minimum=1, maximum=6) was 34% lower than during the 2009-2010 period (N=1316, mean per plant =3.15, minimum=1, maximum=7). The smallest individual that flowered had a DBH of 6.68 cm. Furthermore, some palm trees with a large diameter did not flower during the study period. In agreement, there is no significant difference in the mean DBH for reproductive and non-reproductive palm trees ($t_{2009\text{-}2010}$=2.311, P>0.05; $t_{2010\text{-}2011}$=1.961, P>0.05). In both reproductive events, there was an overlap in flowering for 18% ($N_{2009\text{-}2010}$=85) to 19% ($N_{2010\text{-}2011}$=87) of individuals, indicating that geitonogamy can occur in *B. eriospatha*.

We also found that both the periods of flowering and fruit production were variable between reproductive events. The peak in reproduction during the 2010-2011 study period occurred about one month later than that observed during the 2009-2010 reproductive event. Flowering (male and female) was concentrated in the wet season, between October and March (Figure 1). The *B. eriospatha* population produced fruit for a long period of time, from November through July,

with a peak of unripe fruit one month later than that reported in 2009-2010 period (Table 1). The period of ripe fruiting activity for animal-dispersal occurred from February to July with the peak amount of ripe fruit variable between reproductive events (Table 1).

Table 1 Results of circular statistical analyses testing for seasonality in phenological behavior for *Butia eriospatha* (Martius ex Drude) Beccari in the Atlantic Rainforest, Santa Catarina State, Southern Brazil. Significant values $P<0.05$. Mean angle for each phenophase do not differ by the Watson-Williams test ($P<0.001$) if it is followed by the same letter.

	Phenological variables							
	Male flowers		Female flowers		Unripe Fruits		Ripe fruits	
	2009–2010	2010–2011	2009–2010	2010–2011	2009–2010	2010–2011	2009–2010	2010–2011
Observations (*N*)	1316	868	1295	849	3406	3207	1202	845
Mean angle (*a*)	318.11º [a]	342.45º [b]	350.54º [a]	0.73º [b]	24.99º [a]	46.74º [b]	61.43º [a]	86.45º [b]
Mean date	21/Nov	14/Dec	22/Dec	01/Jan	25/Jan	16/Feb	03/Mar	29/Mar
Length of mean vector (*r*)	0.95	0.76	0.79	0.76	0.83	0.81	0.96	0.94
Rayleigh(z) test of uniformity (*p*)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

For each reproductive event, the mean date of flowering and fruiting was significant (Table 1), indicating that phenological variables are not uniformly distributed throughout the year. The *r* values, ranging from 0.76 (male and female flowers at 2010-2011) to 0.96 (ripe fruits at 2010-2011; Table 1), denote a high degree of seasonality. When phenophases were compared between reproductive events, the mean angle or mean date was significantly different between male flowers ($F=303.5$, $P<0.001$), female flowers ($F=30.8$, $P<0.001$), unripe fruit ($F=559.9$, $P<0.001$) and ripe fruit ($F=737.6$, $P<0.001$). Therefore, these variables seem to exhibit uneven seasonal patterns.

We found significant correlations between climate variables and

flowering and fruiting in both study periods. Flowering (female) was both positively correlated with mean temperature (r_s=0.76, P=0.0001) and precipitation (r_s=0.54, P=0.014). However, the same correlation was not found for male flowers (r_s=0.42, P=0.063) which was minimally but significantly correlated with mean temperature (r_s=0.58, P=0.007). For fruiting, significant correlations were found only for unripe fruit: mean temperature (r_s=0.91, P<0.0001) and precipitation (r_s=0.54, P=0.013).

Mating system

The microsatellite set for *B. eriospatha* had a Mendelian inheritance (Supplementary Appendix Table 1) and no significant linkage disequilibrium (P>0.001) was found between loci for seed-trees and progenies. Therefore, the microsatellite markers used are appropriate for investigating the mating system of this palm tree species.

As the multilocus ($\hat{t}_m$=0.961) and single-locus ($\hat{t}_s$=0.909) outcrossing rates (Table 2) differ statistically from unity, the *B. eriospatha* is a predominantly outcrossed species. The individual multilocus and single-locus outcrossing rates were variable, ranging from 0.746 to 1.136, and from 0.629 to 1.044, respectively (Table 3). Estimates of outcrossing rates greater than unity are an artifact of the algorithm used in the computer analysis; however, the individual estimates for ($\hat{t}_m$) and ($\hat{t}_s$) did not differ statistically from unity (Table 3). The difference between the multilocus and single-locus outcrossing rate was low but significantly different from zero ($\hat{t}_m$–$\hat{t}_s$=0.052), suggesting a low proportion of mating among relatives (Table 2). However, individual differences in $\hat{t}_m$–$\hat{t}_s$ show significant and high values among the seed-trees, ranging from 0.155 to 0.328 (Table 3). Also, the individual estimates of selfing were high and significantly different from zero for two families (7 and 19, Table 3). Estimates of selfing and the presence of overlap during flowering episodes (Table 3) indicate that *B. eriospatha* is a self-compatible palm species. The estimate of the correlation of selfing ($\hat{r}_s$) was low and significantly different from zero ($\hat{r}_s$=0.104, P < 0.05) indicating low variation in the individual self-fertilization rate in this population.

The multilocus paternity correlation was significantly different from zero ($\hat{r}_{p(m)}$=0.442, Table 2). This $\hat{r}_{p(m)}$ value indicates that less than three fathers ($\widehat{N}_{ep}$=2.26) contributed to individual progeny arrays in the *B. eriospatha* population. Furthermore, the coefficient of pollen pool

structure of maternal trees was high and statically different from zero ($\widehat{\Phi}_{fi}$=0.225, P<0.05), indicating a non-random mating pattern probably due to the restriction of pollen distribution in the population. Additionally, combining the estimate of the paternity correlation with the estimated multilocus outcrossing rate, it is possible to determine the proportion of different kinship types within families (Table 2). In *B. eriospatha* populations, the offspring within families were composed predominantly of half-sibs (51.54%), followed by full-sibs (40.80%). The fixation index of mother plants ($\hat{F}_m$) estimated for the progenies was nil (Table 2), indicating an absence of inbreeding in the sampled mother plants. Although inbreeding was low in progenies as result of the kinship coefficient of their parental trees ($\hat{f}_{ij}$=0.04, 95% confidence interval after 10,000 permutations over loci ranging from -0.16 to 0.14), biparental inbreeding was the main cause of the observed inbreeding (Table 2). The mean coefficient of coancestry within progenies ($\hat{\theta}_{xy}$) was 0.187. This value was lower than expected for full-sib families (=0.25). The estimate of effective population size ($\widehat{N}_{e(v)}$=2.67) was also lower than that expected for half-sib families (=4). The number of seed-trees ($\hat{m}$) from which it is necessary to collect seeds, aiming to retain an effective population size of 500, is 187 palm trees (Table 2).

Table 2 Mating system parameters, estimates of inbreeding and relatedness of a *Butia eriospatha* (Martius ex Drude) Beccari population in the Atlantic Rainforest, Santa Catarina State, Southern Brazil. CI is the confidence interval calculated by 1,000 bootstraps.

Mating system		95% CI
Multilocus outcrossing rate: $\hat{t}_m$	0.961	0.956 - 0.966
Single-locus outcrossing rate: $\hat{t}_s$	0.909	0.906 – 0.912
Selfing rate: $\hat{s} = 1-\hat{t}_m$	0.039	0.034 – 0.044
Mating among relatives: $\hat{t}_m-\hat{t}_s$	0.052	0.048 – 0.056
Correlation of selfing: $\hat{r}_s$	0.104	0.103 – 0.105
Multilocus paternity correlation: $\hat{r}_{p(m)}$	0.442	0.439 – 0.445
Effective number of pollen donors: $\hat{N}_{ep}$	2.260	2.241 – 2.283
Inbreeding and genetic structure		
Inbreeding coefficient of maternal parents: $\hat{F}_m$	0.000	0.000 – 0.000
Inbreeding coefficient of progeny: $\hat{F}_o$	0.050	0.019 – 0.073
Inbreeding in progeny from selfing: $\hat{F}_s$	0.020	0.008 – 0.054
Inbreeding in progeny from mating among relatives: $\hat{F}_{tm-ts}$	0.030	0.017 – 0.069
Proportion (%) of self-sibs pairs: $\hat{P}_{ss}$	0.160	0.120 – 0.190
Proportion (%) of half-sibs pairs: $\hat{P}_{hs}$	51.54	50.55 – 55.21
Proportion (%) of full-sibs pairs: $\hat{P}_{fs}$	40.80	40.02 – 44.06
Proportion (%) of self-half-sibs pairs: $\hat{P}_{shs}$	7.500	6.531 – 8.460
Coancestry within offspring: $\hat{\theta}_{xy}$	0.187	0.173 – 0.192
Effective size of variance: $\hat{N}_{e(v)}$	2.670	2.632 – 2.793
Number of seed-trees for seed collection: $\hat{m}$	187.0	179.0 – 189.0
Sample size		
Number of seed-trees	20.0	
Average number of offspring for seed-tree	19.5	

Table 3 Estimates of the mating system and flowering overlap (FO) for each *Butia eriospatha* (Martius ex Drude) Beccari seed-trees in the Atlantic Rainforest, Santa Catarina State, Southern Brazil. CI is the confidence interval calculated by 1,000 bootstraps. n, number of progenies; $\hat{t}_m$, multilocus outcrossing rate; $\hat{t}_s$, single-locus outcrossing rate; $\hat{s}$, selfing rate; $\hat{t}_m - \hat{t}_s$, outcrossing rate among relatives; (+) indicates flowering overlap; confidence intervals that fall within one (for $\hat{t}_m$ and $\hat{t}_s$ estimates) or zero (for $\hat{s}$ and $\hat{t}_m - \hat{t}_s$ estimates) are not significant.

Family/[n]	$\hat{t}_m$	CI 95%	$\hat{t}_s$	CI 95%	$\hat{s}$	CI 95%	$\hat{t}_m - \hat{t}_s$	CI 95%	FO
01 [20]	1.001	0.950 – 1.051	0.997	0.756 – 1.238	0.000	-0.052 – 0.049	0.004	-0.186 – 0.194	-
02 [20]	0.957	0.894 – 1.019	0.629	0.413 – 0.845	0.043	-0.019 – 0.105	0.328	0.175 – 0.480	-
03 [20]	0.866	0.691 – 1.040	0.857	0.672 – 1.041	0.134	-0.040 – 0.308	0.009	-0.001 – 0.018	+
04 [20]	1.069	0.931 – 1.206	1.011	0.805 – 1.216	0.000	-0.206 – 0.068	0.058	-0.010 – 0.127	-
05 [20]	0.992	0.896 – 1.088	0.923	0.750 – 1.095	0.008	-0.088 – 0.104	0.069	-0.007 – 0.145	-
06 [20]	0.983	0.891 – 1.075	0.877	0.614 – 1.139	0.017	-0.075 – 0.109	0.106	-0.064 – 0.276	-
07 [20]	0.746	0.514 – 0.977	0.582	0.229 – 0.934	0.254	0.022 – 0.485	0.164	0.042 – 0.285	+
08 [20]	1.063	0.967 – 1.159	1.044	0.732 – 1.356	0.000	-0.159 – 0.033	0.019	-0.197 – 0.234	-
09 [20]	1.057	0.912 – 1.202	1.002	0.759 – 1.245	0.000	-0.202 – 0.088	0.055	-0.043 – 0.153	-
10 [19]	1.136	0.961 – 1.131	0.976	0.567 – 1.495	0.000	-0.310 – 0.038	0.160	-0.185 – 0.504	-
11 [20]	1.028	0.985 – 1.071	1.010	0.859 – 1.161	0.000	-0.071 – 0.015	0.018	-0.089 – 0.126	-
12 [16]	1.034	0.856 – 1.212	1.002	0.774 – 1.229	0.000	-0.212 – 0.144	0.032	-0.017 – 0.081	-
13 [20]	0.997	0.875 – 1.118	0.993	0.798 – 1.187	0.003	-0.118 – 0.124	0.004	-0.068 – 0.076	-
14 [20]	1.059	0.919 – 1.198	0.970	0.674 – 1.266	0.000	-0.198 – 0.080	0.089	-0.067 – 0.246	-
15 [19]	1.086	0.917 – 1.254	0.963	0.669 – 1.257	0.000	-0.254 – 0.082	0.123	-0.002 – 0.248	-
16 [20]	1.019	0.868 – 1.169	1.005	0.783 – 1.226	0.000	-0.169 – 0.132	0.014	-0.056 – 0.084	-
17 [20]	1.048	0.912 – 1.183	1.023	0.682 – 1.360	0.000	-0.183 – 0.087	0.025	-0.180 – 0.230	-
18 [20]	1.008	0.847 – 1.168	0.999	0.774 – 1.224	0.000	-0.168 – 0.152	0.009	-0.055 – 0.073	-
19 [19]	0.873	0.767 – 0.978	0.718	0.477 – 0.959	0.127	0.021 – 0.232	0.155	0.019 – 0.290	+
20 [20]	1.041	0.862 – 1.219	1.003	0.650 – 1.356	0.000	-0.219 – 0.137	0.038	-0.136 – 0.212	-

Discussion

Flowering and fruiting phenology

We found considerable variation in flowering and fruiting between reproductive events in the studied *B. eriospatha* population. The number of flowering trees, flowering intensity and fruiting were all greater during the 2009-2010 study period than during the 2010-2011 period. Further, we did not find any impact of diameter at breast height (DBH), a common measurement of tree size, on reproductive success. The strategy of converting growth into fitness through the correlation of size and reproductive success is a documented strategy for plants which has been reported for several trees species (Weiner et al. 2009), including palms (e.g., *Butia capitata*, Castellani et al. 1998). In addition, the breeding population of *B. eriospatha* seems to be diminishing, probably because the population consists of mature individuals aged one hundred years or older. Furthermore, it is known that the location of individuals in the landscape and local conditions affects flowering intensity (Oddou-Muratorio et al. 2005); for example, the availability of light promotes flowering. As the *B. eriospatha* inhabits open environments, the availability of light is likely not a factor. Nevertheless, the spatial distribution of individuals might contribute to reproductive success because plants located in proximity might compete for resources (e.g., availability of soil nutrients). In Southern Brazil, isolated *Butia capitata* individuals are more successful in reproduction than those located in clusters (AC Azambuja, personal communication).

We also found that flowering was more synchronous among individuals during the 2009-2010 reproductive event in comparison to the 2010-2011 period (see *r* values for male and female flowers in Table 1). However, *B. eriospatha* individuals displayed a significant degree of overlap in sexual phases. Hence, sexual reproduction may then occur by geitonogamy. A similar pattern was also documented for the congeneric palm species *Butia capitata* in the Atlantic Rainforest (L Rosa, personal communication), and for other palms species (Borchsenius 1997; Martén and Quesada 2001).

Butia eriospatha also shows seasonality of flowering and fruiting with significant correlations for flowering and unripe fruit production with the wettest and warmest periods. Unlike the data reported in Reitz (1974) for the same region, flowering of *B. eriospatha* occurred from October to March and fruits ripened from February to July. Several

studies have shown that reproductive phenological behavior in palms is extremely variable (Henderson 2002); flowering can be restricted to a particular season, and fruiting is frequently non-seasonal (De Steven et al. 1987; Peres 1994; Henderson et al. 2000; Genini et al. 2009).

Although *B. eriospatha* does not provide sufficient resources for all vertebrate frugivores throughout the year, their importance as a major resource in this habit is supported by the production of ripe fruit for about six months of the year. Terborgh (1986) stressed that tropical forest plants, such as palms and figs, sustain a large proportion of the vertebrate animal community during annual seasons of food scarcity. Further studies at the community level are essential in order to determine if *B. eriospatha* is a key plant resource for frugivores in the high-altitude grasslands.

Mating system

This study represents the first analysis of mating system parameters of the *Butia* genus. We found that *B. eriospatha* is a predominately outcrossing species and seems to be self-compatible. The self-compatibility of this palm species can be supported by three lines of evidence: (1) significant and high selfing rates observed in individual families, although the number of progeny analyzed per maternal tree was low; (2) flowering overlap between inflorescence of individual palms; and (3) the production of viable seeds from an isolated individual which were germinated and grown into seedlings at the Botanical Garden of São Paulo, Sao Paulo State, Brazil (personal observation). This evidence indicates the potential of *B. eriospatha* to reproduce in the absence of pollinators or in isolation.

Despite the advantages of this reproductive strategy, selfing can incur negative consequences because through autogamy a recessive allele can increase its incidence by up to 50% in the population (Fisher 1941). Furthermore, self-fertilization is detrimental when selfed offspring suffer reduced viability due to inbreeding depression (Herlihyand and Eckert 2002; Goodwillie et al. 2005) brought on by increases in the frequency of homozygotes. Like *B. eriospatha*, most palms studied to date are self-compatible, including *Thrinax parviflora* (Read 1975), *Acanthococos emensis* (Silberbauer-Gottsberger 1990), *Bactris gasipaes* (Clement and Arkcoll 1991), *Acrocomia aculeata* (Scariot et al. 1991), *Butia capitata* (L Rosa, personal communication), *Cocos nucifera* (Ashburner et al. 2001), *Licuala spinosa* (Barfod et al.

2003), *Archontophoenix* spp. (Bovi et al. 2003), *Syagrus coronata* (KMR Rocha, personal communication), *Johannesteijsmannia lanceolata* (Chan et al. 2011), *J. magnifica* (Chan and Saw 2011); however, the compatibility among many major groups of palms is still unknown (Henderson 2002).

Even though self-compatibility seems to be a prevailing breeding strategy in palms, different mechanisms to promote outcrossing have developed. In species with hermaphrodite flowers, self-pollination is avoided by a temporal separation of female and male phases, for instance, by protandry (*Thrinax*) or protogyny (*Cryosophila*) (Silberbauer-Gottsberger 1990). Palms with androgynous inflorescence likewise may be protandrous (e.g., *Butia* and *Syagrus*), or protogynous such as the genera *Bactris* and *Acrocomia* (Silberbauer-Gottsberger 1990). For *B. eriospatha*, outcrossing is predominantly promoted by protandry. The high multilocus outcrossing rate in *B. eriospatha*, which confirms its allogamy, is comparable with rates found for other palm species, including *Astrocarium mexicanum* (Eguiarte et al. 1992), *Euterpe edulis* (Reis et al. 2000; Gaiotto et al. 2003; Conte et al. 2008), *Astrocaryum aculeatum* (Ramos et al. 2011), and other mainly outcrossing tropical tree species such as *Symphonia globulifera* (Degen et al. 2004), *Solanum lycocarpum* (Martins et al. 2006), *Carapa guianensis* (Cloutier et al. 2007), *Ficus arpazusa* (Nazareno and Carvalho 2009).

Nevertheless, *B. eriospatha* shows a certain level of biparental inbreeding, indicating that outcrossing occurs between relatives. Further, the paternity correlation, which indicates the proportion of plants generated by biparental crosses, was high, suggesting a non-random process of cross-pollination. Similarly, the high value of the coefficient of pollen pool structure indicates that assortative mating may have occurred in the *B. eriospatha* population studied. High differentiation in pollen gene pool implicates a restriction in the effective number of pollen donors as was observed for *B. eriospatha* (Table 2) and also reported for other tree species (Lacerda et al. 2008; Llorens et al. 2012). One assumption of the mixed-mating model is that the allele frequencies in the pollen and ovule pools are homogeneous due to random mating (Ritland and Jain 1981). Several factors could account for the departure from this model, including: unequal male and female contributions among adult trees within the population; pollen coming from outside of the population; selection between the time of pollination and progeny

sampling; and/or non-random mating of genotypes during outcrossing events (Murawski and Hamrick 1992; Doligez and Joly 1997; Lee et al. 2000). In *B. eriospatha*, assortative mating may have occurred through non-random mating and/or due to the limited flight range of pollinators.

Although there is currently no information regarding the behavior of pollinators for this species, we predict that *B. eriospatha* is likely pollinated by beetles. This is based on the high predation of seeds (25%, N=4367; unpublished data) and by Silberbauer-Gottsberger's (1990) argument that the palm-beetle relationship has evolved to a balance between parasitism and successful pollination. Additionally, for the nearest relative of *B. eriospatha*, the palm species *Butia leiospatha* (Barb. Rodr.) Becc, two beetle species (Curculionidae and Nitidulidae) breed in different parts of the inflorescence and flowers. Some of them are effective pollinators (e.g., *Anchylorhynchus* sp.), and their females oviposit on the gynoeceum after having passed over the stigmas of various flowers (Silberbauer-Gottsberger 1990). And in both *B. eriospatha* and *B. leiospatha*, larvae of beetles break out from the fruit (Silberbauer-Gottsberger 1990). Future studies on the pollination biology of *B. erisopatha* are necessary and can help clarify the reproductive biology of the species.

The coancestry in the progenies of the study population means there is an 18.7% probability that two alleles sampled in two plants of the same progeny are identical by descent. The coancestry coefficient is also important in estimating the effective size of variance, which measures the genetic representativeness of progenies compared to the reference population. For *B. eriospatha*, the effective size of variance was estimated at 2.67, indicating that part of progenies are full-sibs. Mating among relatives was the main cause of the observed inbreeding in offspring. On the other hand, the remaining inbreeding detected in offspring could be attributed to selfing. While we did not observe inbreeding among maternal parents, a previous study indicated that there is significant inbreeding among adult plants in this population (unpublished data), which may be an artifact of the sampled seed-trees.

Although this study provides important information regarding aspects of *B. eriospatha* reproductive biology, the sample is only one population with data from a limited time period. As mating patterns may vary substantially among populations and from one flowering event to another (Reis 1996; Hoebee et al. 2007), our study cannot be replicated at the population level. As well as providing preliminary data on *B.*

eriospatha biology, the conclusions of this study indicate that the studied population is a suitable source from which to sample seeds for *B. eriospatha* conservation programs. Clearly more long-term studies of this palm are needed, particularly those that monitor individual palms and their reproductive structures. A detailed picture of contemporary pollination processes and investigations of the floral biology will strengthen the guidelines for effective conservation of the *B. eriospatha* species.

Implications for conservation

Recently, the studied *B. eriospatha* population was reduced to 490 mature individuals due to natural mortality. According to our observations, about 8% of all individuals are non-reproductive, thus suggesting a current breeding population of 450. Considering a mortality rate of 2% (mean calculated across the 2009-2011 period), this population could be at risk of local extinction in the short-term, and the number of seed-trees (=187, estimated through the mating system) necessary for seed collection to retain the effective population size of 500 may be scarce in the near future. Beyond the size of the reproductive population, about 19% of all individuals are capable of selfing by geitonogamy. Although selfing would be advantageous to *B. eriospatha* when no pollinator vectors are available, the effects of inbreeding can lead to a decrease in genotype diversity. Further, if the population is small, the inbred plants may be in danger when there is an adverse change in habitat or environment due to low levels of genetic diversity, or if deleterious genes persist in progenies in the long-term. Hence, ex situ conservation based on estimates of effective size of variance for *B. eriospatha* is a high priority strategy that will help ensure the conservation of the genetic variability that remains.

While ex situ conservation programs and monitoring of the remaining populations are essential strategies in protecting *B. eriospatha*, combating illegal trade is necessary to improve the current state of this species. Given the amount of trade of *B. eriospatha* abroad, it is also appropriate to include this species in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix I, which prohibits the international trade of threatened species. Another possible step in preserving the species is related to cattle herding. Placing enclosures around some reproductive plants could encourage seedling survival. This relatively simple technique has

been used effectively for similarly threatened palms in other parts of the world (e.g., *Rhopalostylis baueri*, Walls 2000; *Ptychosperma macarthurii*, Liddle et al. 2006). However, such a strategy requires effective management as restricting the area can lead to genetic structuring within populations.

Funding

AGN was supported by a doctoral fellowship from CNPq, and MSR by CNPq (M.S.R. #304724/210-6).

Acknowledgements

This study is part of the Doctoral Thesis (Plant Genetic Resources Program – Federal University of Santa Catarina) of the first author. We thank Dr. James L Hamrick and two anonymous reviewers for their many helpful comments that improved the paper. The authors thank the Physiology of Development and Plant Genetics Laboratory of the Federal University of Santa Catarina (UFSC) for logistical support. We thank Dr. Evelyn Nimmo for her English language editing of the manuscript. We wish to thank the researches of Rainforest Research (NPFT) for their kind help in field work.

Supplementary Material

Table S1 *G* statistic for the hypothesis of Mendelian segregation of microsatellite markers for *Butia eriospatha* (Martius ex Drude) Beccari from the Atlantic Rainforest, Santa Catarina State, Southern Brazil. N_{ij} e N_{ii} +N_{jj} are observed genotypic numbers of heterozygotes and homozygotes respectively; n, is the number of analysed plants; a, includes all heterozygotes plants at a locus; b, excluded all plants with segregation deviation. *, Significant values $P<0.05$. The hypothesis of heterogeneity segregation between the progenies was tested through a *G* test ($G_{heterogeneity}$), subtracting the pooled *G* test ($G_{1:1pooled}$) from total *G* test ($G_{hypothesis1:1}$). These statistics were additive, so that $\sum G_{hypothesis1:1} = \sum G_{heterogeneity} + \sum G_{1:1pooled}$ (Weir 1996).

Locus	Parental genotipic	N	N_{ij}:N_{ii}+N_{ij}	$\Sigma G_{hypothesis\ 1:1}$	$G_{heterogeneity}$	$G_{1:1\ pooled}$
But06	181183[a]	03	26:27	2.423	2.405	0.019
	183185[a]	14	88:153	58.177*	40.427*	17.750*
	183185[b]	09	68:93	13.718	9.821	3.897
But07	278280[a]	07	25:82	49.292*	17.298*	31.994*
	278280[b]	02	14:15	3.254	3.220	0.034
	280282[a]	06	21:83	48.428*	8.889	39.538*
	280282[b]	03	14:35	9.524	0.226	9.298*
But08	194198[a]	14	80:167	50.575*	19.264*	31.311*
	194198[b]	11	73:120	15.242	3.681	11.561*
But09	163165[a]	03	9:40	9.752*	0.000	21.191*
	163165[b]	01	5:12	2.969	2.969	0.000
	165167[a]	11	43:75	13.005	4.217	18.787*
	165167[b]	09	40:55	7.222	4.844	2.378
But11	149151[a]	11	105:108	38.224*	38.182*	0.042
	149151[b]	07	65:69	3.013	2.893	0.119
But16	139141[a]	10	48:141	43.349*	0.000	47.814*
	139141[b]	04	28:44	6.053	2.467	3.585
But23	168172[a]	10	144:48	62.864*	12.633	50.232*
	168172[b]	03	33:23	3.111	1.316	1.795

References

Abreu AG, Priolli RHG, Azevedo-Filho JA, Nucci SM, Zucchi MI, Coelho RM, Colombo CA. 2012. The genetic structure and mating system of *Acrocomia aculeata* (Arecaceae). Genet Mol Biol. 35:116-121.

Alzate-Marin AL, Guidugli MC, Soriani HH, Martinez CA, Mestriner MA. 2009. An efficient and rapid DNA minipreparation procedure suitable for PCR/SSR and RAPD analyses in tropical forest tree species. Braz Arch Biol Technol. 52:1217–1224.

Ashburner GR, Faure MG, James EA, Thompson WK, Halloran GM. 2001. Pollination and breeding system of a population of Tall Coconut Palm *Cocos nucifera* L. (Arecaceae) on the Gazelle Peninsula of Papua New Guinea. Pacific Conserv Biol. 6:344-351.

Barfod AS, Burholt T, Borchsenius F. 2003. Contrasting pollination modes in three species of *Licuala* (Arecaceae: Coryphoideae). Telopea 10:207–223.

Barfod AS, Hagen M, Borchsenius F. 2011. Twenty-five years of progress in understanding pollination mechanisms in palms (Arecaceae). Ann Bot. 108:1503-1516.

Barrett SCH, Kohn J. 1991. The genetic and evolutionary consequences of small population size in plant: implications for conservation. In: Falk D, Holsinger KE, editors. Genetics and conservation of rare plants. Oxford University Press, New York, p. 3–30.

Bovi MLA, Resende MDV, Spiering SH. 2003. Genetic parameters estimation in King palm through a mixed mating system model. Horticultura Brasileira 21:93-98.

Borchsenius F. 1997. Flowering biology of *Geonoma irena* and *G. cuneata* var. *sodiroi* (Arecaceae). Plant Syst Evol. 208:187–196.

Castellani RL, Tarabini T, Reis A. 1998. Biologia reprodutiva de *Butia capitata* (Martius) Beccari var. Odorata (Palmae) na resting do município de Laguna, SC. Rev Bras Bot. 21:281-287.

Clement CR, Arkcoll DB. 1991. The pejibaye (*Bactris gasipaes* H.B.K., Palmae) as an oil crop: potential and breeding strategy. Oleagineux 46:293-299.

Chan YM, Lim AL, Saw LG. 2011. Reproductive biology of the endangered and endemic palm *Johannesteijsmannia lanceolata* (Arecaceae). J Trop Forest

Sci. 23:213-221.

Chan YM, Saw LG. 2011. Notes on the pollination ecology of the palm genus *Johannesteijsmannia* (Arecaceae). J Pollin Ecol. 6:108-117.

Chase MW. 2004. Monocot relationships: an overview. Am J Bot. 91:1645–1655.

Cloutier D, Kanashiro M, Ciampi AY, Schoen DJ. 2007. Impact of selective logging on inbreeding and gene dispersal in an Amazonian tree population of *Carapa guianensis* Aubl. Mol Ecol. 16:797–809.

Cockerham CC. 1969. Variance of gene frequencies. Evolution 23:72–84.

Conte R, Mantovani A, Reis MS, Vencovsky R. 2008. Genetic structure and mating system of *Euterpe edulis* Mart. Populations: a comparative analysis using microsatellite and allozyme markers. J Hered. 99:476-482.

Correa LB, Barbieri RL, Rossato M, Buttow MV, Heiden G. 2009. Caracterização cariológica de palmeiras do genero *Butia* (Arecaceae). Rev Bras Frutic. 31:1111-1116.

De Steven D, Windsord DM, Putz FE, De Leon B. 1987. Vegetative and reproductive phenologies of a palm assemblage in Panama. Biotropica 19:342–356.

Degen B, Bandou E, Caron H. 2004. Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana. Heredity 93:585–591.

Doligez A, Joly HI. 1997. Mating system of *Carapa procera* (Meliaceae) in the French Guiana tropical forest. Am J Bot. 84:461-470.

Dransfield J, Uhl NW, Asmussen CB, Baker WJ, Harley MM, Lewis CE. 2005. A new phylogenetic classification of the palm family, Arecaceae. Kew Bulletin 60:559-569.

Eguiarte LE, Perez-Nasser N, Piñero D. 1992. Genetic structure, outcrossing rate and heterosis in *Astrocaryum mexicanum* (Tropical Palm): implications for evolution and conservation. Heredity 69:217-228.

Eiserhardt WL, Svenning J-C, Kissling WD, Balslev H. 2011. Geographical ecology of the palms (Arecaceae): determinants of diversity and distributions across spatial sclaes. Ann Bot. 1-26.

Fisher RA. 1941. Average excess and average effect of a gene substitution. Ann Eugenics 11:53-63.

Fuchs EJ, Lobo JA, Quesada M. 2003. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. Conserv Biol. 17:149–157.

Gaiotto FA, Grattapaglia D, Vencovsky R. 2003. Genetic structure, mating system and long-distance gene flow in heart of palm (*Euterpe edulis* Mart.). J Hered. 94:399-406.

Genini J, Galetti M, Morellato LPC. 2009. Fruiting phenology of palms and trees in an Atlantic rainforest land-bridge island. Flora 204:131-145.

Gillet E, Hattemer HH. 1989. Genetic analysis of isoenzyme phenotypes using single tree progenies. Heredity 63:135–141.

Goodwillie C. 1999. Multiple origins of self-compatibility in *Linanthus* section *Leptosiphon* (Polemoniaceae): phylogenetic evidence from internal-transcribed-spacer sequence data. Evolution 53:1387–1395.

Goodwillie C. 2001. Pollen limitation and the evolution of self-compatibility in *Linanthus* (Polemoniaceae). Int J Plant Sci. 162:1283–92.

Goodwillie C, Kalisz S, Eckert CG. 2005. The evolutionary enigma of mixed mating systems in plants: Occurrence, Theoretical Explanations, and Empirical Evidence. Annu Rev Ecol Evol Syst. 36: 47-79.

Goudet J. 2002. FSTAT: a program to estimate and test gene diversities and fixation indices. Version 2.9.3.2. [cited 2012 February 12]. Available from: http://www.unil.ch/izea/softwares/fstat.html.

Hardy O, Vekemans X. 2002. SPAGeDi 1. 2: a versatile computer program to analyze spatial genetic structure at the individual or population levels. Mol Ecol. Notes. 2:618-620. [cited 2012 June 10]. Available from: http://www.ulb.ac.be/sciences/lagev/software.html.

Henderson A. 2002. Evolution and Ecology of Palms. The New York Botanical Garden Press, New York.

Henderson A, Fischer B, Scariot A, Pacheco MAW, Pardini R. 2000. Flowering phenology of a palm community in a central Amazon forest. Brittonia 52:149–159.

Herlihy CR and Eckert CG. 2002. Genetic cost of reproductive assurance in a self-fertilizing plant. Nature 416:320-322.

Hirao AS, Kameyama Y, Ohara M, Isagi Y, Kudo G. 2006. Seasonal changes in pollinator activity influence pollen dispersal and seed production of the alpine shrub *Rhododendron aureum* (Ericaceae). Mol Ecol. 15:1165–1173.

Hoebee SE, Arnold U, Duggelin C, Gugerli F, Brodbeck S, Rotach P, Holderegger R. 2007. Mating patterns and contemporary gene flow by pollen in a large continuous and a small isolated population of the scattered forest tree *Sorbus torminalis*. Heredity 99:47-55.

IUCN World Conservation Union. 2010. Red List of Threatened Species. Version 2010.1. [cited 2012 January 15]. Available from: .http://www.iucnredlist.org.

Köppen W. 1948. Climatologia: con un estudio de los climas de la tierra. Fondo de Cultura Económica, México.

Kovach WL. 2004. Oriana for Windows. Kovach Computer Services, Pentraeth, Wales, UK.

Lacerda AEB, Kanashiro M, Sebbenn AM. 2008. Long-pollen movement and deviation of random mating in a low-density continuous population of a tropical tree *Hymenaea courbaril* in the Brazilian Amazon. Biotropica 40:462-470.

Lee SL, Wickneswari R, Mahani MC and Zakri AH. 2000. Mating system parameters in a tropical tree species, *Shorea leprosula* Miq. (Dipterocarpaceae), from Malysian lowland dipterocarp forest. Biotropica 32:693-702.

Liddle DT, Brook BW, Matthews J, Taylor SM, Caley P. 2006. Threat and response: A decade of decline in a regionally endangered rainforest palm affected by fire and introduced animals. Biol Conserv. 132:362-375.

Llorens TM, Byrne M, Yates CJ, Nistelberger HM, Coates DJ. 2012. Evaluating the influence of different aspects of habitat fragmentation on mating patterns and pollen dispersal in the bird-pollinated *Banksia sphaerocarpa* var. *caesia*. Mol Ecol. 21:314-328.

Loiselle BA, Sork VL, Nason J, Graham C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot. 82:1420-1425.

Luna R, Epperson BK, Oyama K. 2005. Spatial genetic structure of two

sympatric neotropical palms with contrasting life histories. Heredity 95:298-305.

Lynch M, Walsh B. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland.

Martén S, Quesada M. 2001. Phenology, sexual expression, and reproductive success of the rare Neotropical palm *Geonoma epetiolata*. Biotropica 33:596–605.

Martins K, Chaves LJ, Buso GSC, Kageyama PY. 2006. Mating system and fine-scale spatial genetic structure of *Solanum lycocarpum* St.Hil. (Solanaceae) in the Brazilian Cerrado. Conserv Genet. 7:957–969.

Morellato LPC, Talora CD, Takahasi A, Bencke CC, Romera CEE, Zipparo V. 2000. Phenology of Atlantic rainforest trees: a comparative study. Biotropica 32:811–823.

Murawski DA, Hamrick JL. 1991. The effect of the density of flowering individuals on the mating systems of nine tropical tree species. Heredity 67:167-174.

Murawski DA, Hamrick JL. 1992. The mating system of *Cavanillesia platanifolia* under extremes of flowering-tree density: a test of predictions. Biotropica 24:99–101.

Nazareno AG, Carvalho D. 2009. What the reasons for no inbreeding and high genetic diversity of the neotropical fig tree *Ficus arpazusa*? Conserv Genet. 10:1789-1793.

Nazareno AG, Zucchi MI, Reis MS. 2011. Microsatellite markers for *Butia eriospatha* (Arecaceae), a vulnerable palm species from the Atlantic Rainforest of Brazil. Am J Bot. 98: e198 – e200.

Nunney L, Campbell KA. 1993. Assessing minimum viable population size: demography meets population genetics. Tree 8:234–239.

Oddou-Muratorio S, Klein EK, Austerlitz F. 2005. Pollen flow in the wild service tree, *Sorbus torminalis* (L.) Crantz. II. Pollen dispersal and heterogeneity in mating success inferred from parent-offspring analysis. Mol Ecol. 14:4441–4452.

Oddou-Muratorio S, Klein EK, Demesure-Mush B, Austerlitz F. 2006. Real-time patterns of pollen flow in the wild-service tree, *Sorbus torminalis*

(Rosaceae). III. Mating patterns and the ecological maternal neighborhood. Am J Bot. 93:1650–1659.

Peres CA. 1994. Composition, density, and fruiting phenology of arborescent palms in an Amazonian Terra Firme forest. Biotropica 26:285–294.

Ramos SLF, Lopes MTG, Lopes R, Cunha RNV, Macêdo JLV, Contim LAS, Clement CR, Rodrigues DP, Bernardes LG. 2011. Determination of the mating system of Tucumã palm using microsatellite markers. Crop Breed Appl Biotech. 11:181-185.

Read RW. 1975. The genus *Thrinax* (Palmae: Coryphoideae). Smithsonian Contributions to Botany 19:1–98.

Reis, M.S. 1996. Dinâmica da movimentação de alelos: subsídios para conservação e manejo de populações naturais em plantas. Braz J Genet. 19:37-47.

Reis MS, Fantini AC, Nodari RO, Reis A, Guerra MP, Mantovani A. 2000. Management and conservation of natural populations in Atlantic Rain Forest: the case study of Palm Heart (*Euterpe edulis* Martius). Biotropica 32: 894-902.

Reitz R. 1974. Palmeiras – Parte 1. Flora ilustrada catarinense, Herbário Barbosa Rodrigues, Brazil.

Ritland K. 1989. Correlated matings in the partial selfer *Mimulus guttatus*. Evolution 43:848-859.

Ritland K. 2008. Multilocus mating system program – MLTR version 3.2. University of British Colombia, Vancouver. [cited 2012 June 11]. Available from: http://genetics.forestry.ubc.ca/ritland/programs.

Ritland K, Jain SK. 1981. A model for the estimation of outcrossing rate and gene frequencies using n-independent loci. Heredity 47:35-52.

Rodrígues-Buriticá S, Orjuela MA, Galeano G. 2005. Demography and life history of *Geonoma orbignyana*: An understory palm used as foliage in Colombia. Forest Ecol Manag. 211:329-340.

Scariot AO, Lleras E, Hay JD. 1991. Reproductive biology of the palm *Acrocomia aculeata* in Central Brazil. Biotropica 23:12-22.

Sebbenn AM. 2006. Sistema de reprodução em espécies arbóreas tropicais e suas implicaçõess para a seleção de árvores matrizes para reflorestamentos

ambientais. In: Higa AR, Silva LD, editors. Pomares de sementes de espécies nativas. Curitiba, FUPEF, p. 193–138.

Selkoe KA, Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett. 9:615-629.

Shapcott A, Dowe JL, Ford H. 2009. Low genetic diversity and recovery implications of the vulnerable Bankoualé Palm *Livistona carinensis* (Arecaceae), from North-eastern Africa and Southern Arabian Peninsula. Conserv Genet. 10:317-327.

Silberbauer-Gottsberger I. 1990. Pollination and evolution in palms. Phyton 30:231-233.

Smith SA, Donoghue MJ. 2008. Rates of molecular evolution are linked to life history in flowering plants. Science 322:86-89.

Smouse PE, Dyer RJ, Westfall RD, Sork VL. 2001. Two-generation analysis of pollen flow across a landscape. I. Male gamete heterogeneity among females. Evolution 55:260–271.

Terborgh J. 1986. Keystone plant resources in the tropical forest. In: Soulé, ME, editor. Conservation Biology II: The Science of Scarcity and Diversity. Sinauer, Sunderland, p. 330–344.

Walls G. 2000. Love them or lose them... Palm forests of Pitt Island and their wildlife. An ecological assessment of management needs, with comment from Pitt Islanders. Conservation Advisory Science Notes No. 283, Department of Conservation, Wellington.

Ward M, Dick CW, Gribel R, Lowe AJ. 2005. To self, or not to self. A review of outcrossing and pollen-mediated gene flow in Neotropical trees. Heredity 95:246–254.

Weiner J, Campbell LG, Pino J, Echartes L. 2009. The allometry of reproduction within plant populations. J Ecol. 97:1220-1233.

Weir BS. 1996. Genetic Data Analysis. Sinauer Associates, Sunderland, USA.

Zar JH. 1996. Biostatistical analysis. Prentice-Hall, Upper Saddle River, New Jersey.

CAPÍTULO 4

Este manuscrito segue as normas de formatação do periódico *Journal of Heredity*

At risk of population decline? An ecological and genetic approach to the threatened palm species *Butia eriospatha* (Arecaceae)

Núcleo de Pesquisas em Florestas Tropicais, Federal University of Santa Catarina, CP 476, 88040-900, Florianópolis, Santa Catarina, Brazil.

Address correspondence to Alison G. Nazareno at the above address, or e-mail: alison_nazareno@yahoo.com.br

ABSTRACT

Wild populations of the vulnerable palm species *Butia eriospatha* are suffering the consequences of human intervention through loss of habitat due to exotic tree species' reforestation, illegal trade, overexploitation of fruit, and cattle farming. In order to estimate the risk of population decline for *B. eriospatha*, we investigate the patterns of demography, natural regeneration, herbivory, and the levels of genetic diversity using nine microsatellite markers from both adults and seedlings sampled from four populations of varying sizes in Southern Brazil. Three *B. eriospatha* populations showed a bimodal age structure made up of adult plants and seedlings and high rates (>77%) of livestock herbivory. For one population, we describe and quantify for the first time the occurrence of six ontogenetic stages for this threatened palm species. Our results indicate that cattle grazing in *B. eriospatha* population areas severely affect their demographic structure. Populations of *B. eriospatha* showed moderate genetic diversity and high levels of genetic differentiation ($F_{ST\ adult\ plants}$ = 0.287, $F_{ST\ seedlings}$ = 0.175). The amount of observed heterozygosity differed significantly between small (H_O = 0.329) and large populations (H_O = 0.461), indicating that small populations have a reduced ability to respond adaptively to environmental changes. The effective population size (N_e) indicates that *B. eriospatha* individuals have low genetic similarity, with N_e/N ratio ranging from 0.58 to 0.89. The estimated population size indicates that *B. eriospatha* is at a high risk of becoming an endangered species. With nil turnover rates and a mortality rate of 2.0%, we show that the populations investigated in this study would be at an extremely high risk of local extinction, with a greater than 50% reduction in the effective population size, in the next 40 years. Although this study highlights the importance of analysing both population ecology parameters and genetic data to better understand the level of risk facing threatened species, we emphasize that policy actions are urgently needed for effective conservation of this vulnerable biological resource.

Key words: Atlantic Rainforest, cattle farming, conservation biology, effective population size

The ongoing decline of plant diversity will have a greater impact on human populations than any other type of biodiversity loss (Schatz 2009). The present biodiversity crisis is the result of four major anthropogenic processes: the fragmentation of habitats; the introduction of exotic species; the overexploitation of species; and the deterioration of environments leading to decreased habitat quality (Honnay et al. 2005; Laurance et al. 2012). Deforestation of tropical areas has resulted in diminishing populations of many plant species (Saunders et al. 1991; Turner 1996; Matos and Bovi 2002; Schatz 2009), including palms (Johnson 1996; Thebaud and Strasberg 1997; Manohara et al. 2010). One of the many dramatic examples comes from the Brazilian Atlantic Rainforest where rapid habitat loss has caused the extinction of the palm species *Trithrinax schyzophylla* (Glassman 1987). After loss of habitat, the second major threat to palm species is the introduction of free-roaming exotic and domestic animal species into wild population areas. Cattle, pigs, chickens, goats and other exotic animal species have been allowed to forage in these areas, which affects the natural regeneration of native plant populations (Lorence and Sussman 1986; Grove 1995). In the Caribbean, the endangered palm species *Coccothrinax barbadensis* has been decimated by livestock that eat not only the leaves of most seedlings, but the seedlings as well (Morici 1997). The decline of the *C. barbadensis* populations due to animal grazing is not an isolated example. It is consistent with other palm floras, such as *Latania loddigesii* (North et al. 1994), *Butia capitata* (Baéz and Jaurena 2000), *Geonoma interrupta* (James and Maidman 2003), *Pritchardia* spp. (Chapin et al. 2004), *Ptychosperma macarthurii* (Liddle et al. 2006), *Butia lallemantii*, *B. paraguayensis*, and *B. yatay* (Gaieiro et al. 2011).

Palms (Arecaceae) were among the first plant groups to receive attention regarding the risks of becoming endangered (Moore 1979) and to have conservation action plans (Johnson 1996). The interest in palm conservation is due to both their economic and ecological importance (Balick and Beck 1990; Zambrana et al. 2007). Despite this early intervention, many palm species are heading towards population collapse (Moore 1979). The IUCN Red List of Threatened Species (2012) includes about 31% of the world's 1150 palm species (Moore 1973). In the Neotropical ecozone, approximately eight percent of palm species are under threat. According to IUCN (2012), Brazil has 21 threatened palm species, represented in 11 genera. Although the Brazilian palm flora is very rich, with estimates of 387 species

(Glassman 1972), more research regarding the palm flora and specific threats to each species is needed in order to design and implement urgently required management plans to ensure the conservation of these genetic resources.

The *Butia* genus (Arecaceae, subfamily Arecoideae, subtribe Buttinae; Dransfield et al. 2005) is one of the genera threatened in Brazil. This genus is comprised of 18 species that are distributed exclusively throughout South America, across Brazil, Paraguay, Argentina and Uruguay (Noblick 2010). The majority of these species require special attention due to serious demographic issues which may have significant implications for their viability in the near future. Of all species, however, only two [*Butia purpuracens* Glassman and *Butia eriospatha* (Martius ex Drude) Beccari] are listed as vulnerable on the IUCN Red List (IUCN 2012). While two of the ten species with populations in Brazil are on the Brazilian Endangered Species list (Instrução Normativa 06, MMA 2008): *B. catarinenses* Noblick & Lorenzi and *B. eriospatha*.

Butia eriospatha is a monoecious, slow-growing, palm species locally known as *butiá-da-serra*. It is endemic to the Atlantic Forest and grows in highlands (or *campos de altitude*, a subtype of the Atlantic Forest Domain; Reitz 1974). The current populations of *B. eriospatha* generally consist of mature individuals aged one hundred years or older, in clustered distributions, known as *butiazais,* which are sometimes dense and extensive. Analyses of the species' reproductive biology indicate that *B. eriospatha* (2n = 32; Correa 2009) is a self-compatible and predominantly outcrossing species, although reproduction can occur by geitonogamy (Nazareno and Reis 2012). Pollinators have not yet been identified for the species; however, during field research in 2011 bees, wasps, beetles and ants were observed visiting the flowers. The fleshy fruit is about 2.0 cm in diameter and is edible by both humans and frugivores, such as birds and squirrels.

Wild populations of *B. eriospatha* are constantly suffering the consequences of human intervention especially through the loss of habitat due to exotic tree species reforestation (e.g., *Pinus* species), the illegal sale of adult plants in both local and international trade, and the overexploitation of their fruits. In addition, cattle-grazing continues to alter the natural regeneration of *B. eriospatha* populations, which may be contributing to population decline. Although little is known about the ecology of *B. eriospatha*, there is a general consensus that wild

populations are declining and the turnover rate of mature individuals seems to be nil. Considering our current understanding of the species, and despite a lack of quantitative studies, the risk of extinction for *B. eriospatha* appears to be high and effective conservation strategies are necessary.

The conservation of individual species requires a species-level approach with an understanding of population biology and genetics in order to make effective conservation decisions. From this perspective, understanding population dynamics and existing genetic diversity are fundamental components that could maximise the success of *B. eriospatha* conservation plans. In order to investigate the risks of population decline for *B. eriospatha*, we integrated data on demographic structure, natural regeneration, herbivory, and the levels and distribution of genetic diversity from four remaining *B. eriospatha* populations. Specifically, we address the following question: Are the *B. eriospatha* populations declining?

This study is part of a larger project that examines the population structure and genetic diversity of threatened plant species in Southern Brazil with the goal of influencing long-term conservation strategies and policies. Furthermore, we believe that this study may prove useful as a starting point for conservation and management activities for other threatened *Butia* species.

Materials and Methods

Study area

Four populations of *B. eriospatha* (named A, B, C, and D) located in the Western Plateau of Santa Catarina State, Southern Brazil, were sampled. Two of these populations (A and B) are considered 'large', with more than 450 individuals, and the other two (C and D) are 'small', with less than 50 individuals. While this study focused on quantitatively investigating four populations, another ten populations were mapped across the range of their distribution in Santa Catarina State in order to estimate the distance from the nearest population. Population D is isolated from other *B. eriospatha* populations by a distance of at least 10 km. Among the sampled populations, A and B are the most proximal (≈3.00 Km) while populations A and D and C and D are the most distant (≈100.00 Km). We do not provide the exact locations of the wild populations discussed in this study in order to reduce the risk of illegal

poaching of mature individuals from these stands. The study region was the first area to be populated on the plateau of Santa Catarina and the first colonisers were mainly livestock herders. In the immediate surrounding area of populations A, B, and C, cattle have been present for at least 90 years and cultivation of invasive *Pinus* species began in the 1970s (a practice that was introduced after the massive depletion of *Araucaria angustifolia* due to over-exploitation). Of all *B. eriospatha* populations mapped in the Santa Catarina State, only one *B. eriospatha* population was found without cattle grazing in the surrounding area (D population). Field data collection was undertaken between 2009 and 2011.

Demography, Regeneration and Herbivory

Based on field observations, we described six ontogenetic stages related to morphologic and morphometric traits. In each *B. eriospatha* population, all individuals were tagged with a numbered aluminium plate, mapped (GPS Garmin Model 76S), and the diameter at 1.30 meters breast height (DBH) and total height (H) were recorded. To accurately estimate the size of the reproductive population, the effective number of breeders (N_b) was calculated as the number of individuals that reproduced in the observed event plus those that had reproduced in a previous event. Reproductive individuals in the previous event were identified by the presence of dried reproductive structures and/or seeds under the plant.

To study regeneration and herbivory, data was collected from 30 circular plots (radius of 2 m) installed in each of the studied *B. eriospatha* population. Fifteen plots were randomly placed around *B. eriospatha* adult individuals and 15 plots were placed in grazing areas (i.e., plots were installed inside the population area but without influence of the palm canopy). Plots were installed as such in order to avoid an overestimation of the number of seedlings (defined here as plants having from one to three eophyll). In order to verify whether there are differences between seedling densities among *B. eriospatha* populations, the Kruskal-Wallis test was performed. In each circular plot, all plants (including both damaged and undamaged) were counted and assigned to one of six ontogenetic stages. The height of undamaged plants (e.g., seedlings) was measured to the closest centimetre using a ruler. Data were analysed using a contingency table and statistical

significance was tested with chi-square to verify whether there are differences in the number of damaged individuals among the populations. To test if herbivory is density-dependent, the Spearman's non-parametric correlation was carried out using R software, version 2.9.0 (2009).

Genotyping

Genetic samples were collected from the same populations where the demographic studies were conducted. In order to investigate whether there are genetic differences between generations within each population, samples were taken from leaf material from two ontogenetic stages: adult plants and seedlings (see Table 2 for sample or census sizes for A, B, C and D populations). Samples were kept cool in individually labelled plastic bags while in the field. Genomic DNA extraction from leaves was conducted using the NucleoSpin® kit (MACHEREY-NAGEL GmbH & Co. KG), according to the manufacturer's instructions. The PCR and profile used to amplify the nine microsatellite loci (*But06, But07, But08, But09, But11, But16, But17, But18*, and *But23*) are described in Nazareno et al. (2011). PCR products were denatured and separated with 10% denaturing polyacrylamide gels (39:1 acrylamide to bisacrylamide) stained with silver nitrate. Gels were run with 1 X TBE buffer (90 mM Tris, 92 mM boric acid, and 2.5 mM EDTA) on a vertical electrophoresis at a constant electric current (21 mA for each gel). Allele sizes were estimated by comparison with a 10 base pair DNA ladder standard (Invitrogen, Carlsbad, California, USA).

Genetic analysis

As an initial analysis, for each *B. eriospatha* population (both adults and seedlings) we tested the hypothesis that each of the nine loci used in this study are independent samples of the genome. The linkage disequilibrium test was carried out in the FSTAT 2.9.3.2 software (Goudet 2002) applying the Bonferroni correction for multiple comparisons (Rice 1989).

Allele frequencies and the following parameters were calculated: number of alleles (K), allelic richness (A), number of private (A_P) and rare (R) alleles (defined here as alleles with a frequency of less than 5%), observed heterozygosity (H_O), and expected heterozygosity (H_E,

Nei 1978). In order to make the allelic richness independent from sample size, the rarefaction method was used to standardize it to the smallest sample size (El Mousadick and Petit 1996). Genetic differentiation (F_{ST}) for the two ontogenetic stages (adult plants and seedlings) was estimated following Weir and Cockerham (1984). Furthermore, significance of the deviation from the Hardy-Weinberg equilibrium was tested. These analyses were run using the FSTAT 2.9.3.2 program (Goudet 2002). The inbreeding index (F_{IS}) and Nason's estimator of coancestry coefficient (F_{ij}), described in Loiselle et al. (1995), were estimated for each population and its significance (determined by 10,000 permutations across loci) tested using the SPAGeDi program (Hardy and Vekemans 2002). Null allele frequencies were assessed for the *B. eriospatha* populations using the Microchecker software V 2.2.0 (van Oosterhout et al. 2004). If significant homozygosity was detected at a given locus, it was dropped and a modified average F_{IS} across all loci was calculated; its significance was calculated using a jackknife across all loci. Estimates of genetic differentiation was also calculated using the ENA method (10,000 permutations) implemented in the FreeNA program (Chapius and Estoup 2007) which corrects for the presence of null alleles. In addition, from the inbreeding index (excluding the loci segregating for null alleles) and coancestry coefficient, we calculated the effective population size (N_e) for adult and seedling plants as proposed by Cockerham (1969). In order to verify the extent of genetic variation expected in *B. eriospatha* populations, we calculated the ratio of the N_e to N (samples or census). When applicable, analysis of variance (ANOVA) was performed in order to compare the genetic parameters between 'small' and 'large' populations and between ontogenic stages. Because some genetic estimates (e.g., F_{IS}, *Ne*/N) are proportions, they were treated as binomial observations using an arcsine transformation before performing ANOVA.

Estimates of Population size

We assumed that the risk of *B. eriospatha* becoming endangered depends on: 1) the intrinsic rate of natural increase (r), expressed here as a difference between the annual turnover rate of breeding individuals from the regenerative stage (R) and the annual mortality rate (m); and 2) the effective population size (N_e), a key parameter in evolutionary genetics generally defined as the size of an ideal population that results in a given variance in allele frequency or amount of inbreeding (Crow

and Kimura 1970, Jamieson and Allendorf 2012). For this study, risks of population decline were examined using the stochastic model of exponential growth adapted from Malthus (1993): $N_t = N_e e^{rt}$, where N is the population size through time (t), N_e is the effective population size, e = 2.718... (base of natural logs), and r is the intrinsic rate of natural increase. As the first principle of population dynamics, the Malthusian growth model has been widely regarded in the field of population ecology (Wissel and Stocker 1991, Turchin 2000). For estimates of population size, the N_e values were obtained using the following equation: $N_e = (N_e/N)N_b$. Based on information obtained from farmers residing near the study populations, it takes approximately 20 years for a *B. eriospatha* individual to reach reproductive maturity. In addition, one criterion adopted by IUCN (2001) to assess the risk of a species becoming threatened is based on 10 years or three generations. Our field observations show that at least one individual is lost annually in populations of *B. eriospatha*. Furthermore, because our study was conducted over a short period and because of the intrinsic slow growth rate of this palm species, we have not been able to assess the demographic dynamics of *B. eriospatha* plants in areas unaffected by cattle grazing. Thus, scenarios were simulated for populations A, B, and C (where the cattle has been present at least 90 years) with t varying from 10 to 100 years, and m varying from 0.2 to 2.0% (a mortality rate of 2%; mean calculated over the 2009-2011 study period). This range in mortality rate allows for at least one individual to be lost annually in each of the populations studied.

Results

Demography, Regeneration and Herbivory

The demographic pattern for three of the four *B. eriospatha* populations (A, B, and C) was represented by two classes of individuals: adult and seedling plants, with no evidence of recruitment to later stages of maturity. In the D population, where no cattle-grazing has been recorded, we quantified six ontogenetic stages (Figure 1).

For most of these stages, both the number of individuals and the density were low: Infant (n=5, d=0.01 ind.m^{-2}), Juvenile (n=1, d=0.005 ind.m^{-2}), Immature (n=18, d=0.05 ind.m^{-2}), and Reproductive (n=11,

d=0.03 ind.m^{-2}). For all 1,211 adult plants across all populations, mean DBH and height (H) showed a low standard deviation (SD; Table 1), indicating homogeneity in the demographic patterns of the assessed populations.

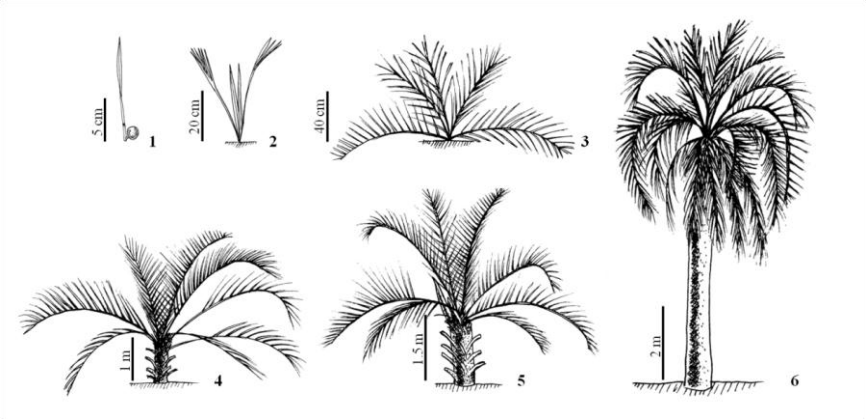


FIGURE 1 Characterization of the six ontogenic stages for *Butia eriospatha* (Martius ex Drude) Beccari based on morphologic and morphometric traits. **1** *Seedling* - plants having from one to three eophylls; **2** *Infant* - plants with bifid leaf, incompletely segmented ones or completely segmented ones; **3** *Juvenile* - plants with leaf blades completely segmented, with a measurable diameter but stemless; **4** *Immature* - plants with leaf blades completely segmented, apparent stem with height of up to 150 cm; **5** *Reproductive* - recognized by the production of flowers and fruits, apparent stem with minimum height of 150 cm; **6** *Adult* – reproductive or senescent individual, stem with height greater than 2.5 meters.

For all but one *B. eriospatha* population, the effective number of breeders (N_b) was lower than census size (N) and ranged between 40 and

630 (Table 1). The ratio N_b to N ranged from 0.83 to 1.00 (Table 1) indicating that most individuals are reproductive. Seedlings of *B. eriospatha* had a density that varied from 0.001 ind.m^{-2} in the C population to 0.62 ind.m^{-2} in the A population (Table 1), and differed significantly between populations (n=4, p<0.001, df=3, H=48.29, Kruskal Wallis test). In the C population we found the lowest number of seedlings (n=29, census size), which is related to the small number of adult plants but also indicates susceptibly to stochastic processes. The mean height of undamaged seedlings and the mean percentage of seedlings damaged in grazing areas, in plots under the palm canopy, and in all the plots, are summarised in Table 1. The number of seedlings damaged was different among populations (χ^2 = 9.78; df=3; p=0.005). However, there is no significant correlation between density and rate of damaged plants (r_A = 0.09, r_B = -0.09, r_C = -0.11; p>0.05).

Genetic diversity

Our analysis of the independent segregation hypothesis in all samples (both adult and seedlings) found that 41 of 288 locus combinations showed significant deviation at p=0.05, but no pairs of loci were found to be in significant genotypic disequilibrium after Bonferroni correction (p<0.001). Hence, the nine loci analysed were used for all genetic analyses for *B. eriospatha*.

From the DNA analysis of nine loci and the total sample of 330 genotypes (adults and seedlings), 40 alleles were detected. The number of alleles per locus ranged from two (*But11, But17*, and *But18*) to nine (*But08*), with an average of 4.44 alleles per locus. For adult plants, the observed heterozygosity ranged from 0.22 (C population) to 0.47 (D population), and the expected heterozygosity ranged from 0.40 (C population) to 0.49 (A and D populations, Table 2). For seedlings, the observed heterozygosity ranged from 0.32 (D population) to 0.51 (A population), and the expected heterozygosity ranged from 0.49 (C and D populations) to 0.53 (A and B populations, Table 2). Rare and private alleles were also detected in both ontogenetic stages (Table 2). The presence of few rare alleles in all populations and the low allelic polymorphism contributed to the moderate values of expected heterozygosity (Table 2). For populations A and B, some alleles were found exclusively in their seedlings.

TABLE 1 Population ecology parameters of adult and seedling *Butia eriospatha* (Martius ex Drude) Beccari plants from the studied populations located on the Western Plateau, Santa Catarina State, Southern Brazil.

		Populations			
Adults/Reproductives		A	B	C	D
N		490	651	41	40
N_b		450	630	34	40
N_b/N		0.92	0.97	0.83	1.00
A (ha)		12.46	11.20	2.50	1.00
D_P (km)		2.0	2.2	1.5	10.0
DBH (cm)	Mean ± SD	34.1 ± 4.2	32.7 ± 4.5	47.7 ± 5.9	30.1 ± 3.9
	Min	21	22	34	22
	Max	48	46	58	42
H (m)	Mean ± SD	5.06 ±0.94	4.96 ±1.01	5.27 ±0.91	5.15 ±0.89
	Min	2.5	2.5	3.0	3.0
	Max	10.5	8.5	7.0	8.0
Seedlings					
n		234	200	29	64
Density (ind.m^{-2})		0.62	0.53	0.001	0.17
H (cm)	Mean ± SD	11.2 ± 3.3	9.7 ± 3.1	9.5 ± 2.5	20.0 ±11.2
	Min	2.0	2.8	2.0	6.0
	Max	12.6	11.8	10.0	50.0
Herbivory					
Grazing area	Mean ± SD	73±15.6	84±4.4	76±9.1	0
Around palm tree	Mean ± SD	83 ± 2.8	90±4.1	84±7.3	0
All plots	Mean ± SD	78±11.9	87±5.1	80±8.9	0

Number of adult plants (N); number of seedlings sampled (n), except for the C population where (n) represents the census; effective number of breeders (N_b); coverage area (A) and distance from the nearest population (D_P); mean, standard deviations (SD), minimum and maximum values for diameter at breast height (DBH) and height (H). The seedling density (d) was calculated based in the sampled area at each population (≈ 377 m^{-2}), except for the C population.

Furthermore, two seedlings of the C population showed the presence of one allele (frequency of 0.0345) that was not found in the adult plants (Table 2), indicating that this small population is not isolated. The average allele richness, estimated by rarefaction, and both the observed and expected heterozygosities were not significantly different between the adults and seedlings.

However, the observed heterozygosity did significantly differ between 'small' and 'large' populations. The one-tailed *P*-values after 5000 permutations for the hypothesis (a) that genetic diversity is higher among adults than seedlings are as follow: allelic richness (adults = 2.937, seedlings = 3.100, p=0.621), observed heterozygosity (adults = 0.377, seedlings = 0.440, p=0.836), and gene diversity (adults = 0.463, seedlings = 0.515, p=0.923); and (b) that genetic diversity is higher in large populations than in small populations, considering adults and seedlings, are as follow: allelic richness (large population = 3.295, small population = 2.742, p=0.197), observed heterozygosity (large population = 0.461, small population = 0.329, p=0.033), and genetic diversity (large population = 0.507, small population = 0.460, p=0.230).

The studied *B. eriospatha* populations showed high levels of genetic differentiation, significantly different from zero for adults and seedlings, indicating that genetic diversity was distributed mainly within populations at 72.0% ($F_{ST\text{ - adult plants}}$ = 0.28, p<0.05), and 83% ($F_{ST\text{ - seedlings}}$ = 0.17, p<0.05). The overall estimate of F_{ST} obtained after the correction for null alleles remained highly significant for adults (F_{ST}=0.25, p<0.05) and for seedlings (F_{ST}=0.16, p<0.05).

For all *B. eriospatha* populations (both adults and seedlings), the Hardy-Weinberg equilibrium test found that of 144 locus–population combinations, 36, 27 and 19 or 25.0%, 19.0% and 13.0% showed significant deviation at p=0.05, 0.01 and 0.001, respectively. As the F_{IS} was positive and significantly different from zero for almost all *B. eriospatha* populations (Table 2), the pattern of excess homozygosity can be related to the presence of null alleles (Table S1). When loci with significant null alleles were omitted, the mean F_{IS} values remained positive and significantly different from zero for three (A, B, and C) of the four populations (adult plants, Table 2). Among the seedlings in the small populations (C and D), there was an excess of homozygotes indicating that small populations, as expected, are more susceptible to genetic drift. However, there was no difference of F_{IS} between small and large populations (n=8, p=0.189, df=1, F=2.195, one-way ANOVA).

The presence of null alleles in the loci *But06, But07, But09, But17*, and *But18* in almost all populations (Table S1) strengthened the effect of null alleles on the F_{IS} estimates even if species-specific microsatellite loci are used.

The average coancestry coefficients for adult and seedling plants were assumed to be zero (Table 2), because in random mating a low level of biparental inbreeding is expected. The *Ne* values indicated that the individuals sampled in each population have low genetic similarity. For instance, of the 50 samples from A population, 38 individuals were neither inbred, nor related (N_e/N=0.75). In this same population, 45 of the 52 seedlings were neither inbred, nor related (N_e/N=0.87). Although the N_e/N seems to be lower in the small populations, no significant differences for populations of different sizes were observed (n=8, p=0.9097, df=1, F=0.014, one-way ANOVA). However, for the smallest and apparently isolated D population, the relationship N_e/N between ontogenetic stages (Table 2) suggests that the frequency of related individuals is increasing in new generations of the population.

Population size

The estimated population size for all three populations as a function of effective population size (*Ne*) and intrinsic rate of natural increase (*r*) indicates that *B. eriospatha* is at a high risk of becoming an endangered species. Due to the historical use of the populations, and considering the continued presence of cattle, the annual turnover rate of breeding individuals from the regenerative stage (*R*) was estimated at zero. The estimated values for *Ne* were 338, 473 and 20 for A, B, and C population, respectively. With nil turnover rates and a mortality rate of 2.0%, the estimates indicate that all populations investigated in this study are under extremely high risk of extinction (a reduction of more than 50% of effective population size) in the next 40 years (Table 3).

TABLE 2 Population genetics estimates for adult plants and seedlings of *Butia eriospatha* (Martius ex Drude) Beccari sampled from four populations in Santa Catarina State, Southern Brazil. N, sample (s) or census sizes (c); *K*, number of alleles; A_R, allelic richness by rarefaction based on the minimum sample size of 29 individuals; A_P, number of private alleles; *R*, number of rare alleles (defined here as alleles with a frequency of less than 5%); H_E and H_O, expected and observed heterozygosity, respectively; F_{IS}, inbreeding index; F_{IS}^1 inbreeding index excluding the loci segregating for null alleles. F_{ij}, Nason's estimator of coancestry coefficient; N_e, effective population size and the ratio between N_e and N.

Populations	N	*K*	A_R	A_P/R	H_E/H_O	F_{IS}	F_{IS}^1	F_{ij}	N_e	N_e/N
Adults										
A	50[s]	26	2.89	0/3	0.49/0.43	0.13*	0.08*	0.0018	37.7	0.75
B	50[s]	30	3.33	0/5	0.47/0.42	0.12*	0.08*	0.0014	37.3	0.75
C	41[c]	23	2.56	0/1	0.40/0.22	0.46*	0.12*	0.0068	23.7	0.58
D	29[s]	28	3.11	0/4	0.49/0.47	0.04	0.00	0.0008	25.9	0.89
Seedlings										
A	52[s]	33	3.67	1/5	0.53/0.51	0.01	0.09*	0.0001	45.4	0.87
B	50[s]	34	3.78	1/5	0.53/047	0.11*	-0.05	0.0013	41.0	0.82
C	29[c]	24	2.67	0/1	0.49/036	0.26*	0.20*	0.0068	19.6	0.67
D	29[s]	28	2.67	0/1	0.49/0.32	0.36*	0.11*	0.0047	18.7	0.64

* Significant at $P > 0.05$.

TABLE 3 Estimates of population size (N) for three *Butia eriospatha* (Martius ex Drude) Beccari populations from Southern Brazil, with time (t), as function of the effective population size (N_e) and of the intrinsic rate of natural increase (r) expressed as a difference between the annual turnover rate of breeding individuals from the regenerative stage (R) and the annual mortality rate (m). The *Ne* values, showed in parenthesis, are results of the product between N_b (see Table 1) and N_e/N (see Table 2).

	Populations of *Butia eriospatha*											
	A Population (N_e=338)				**B** Population (N_e=473)				**C** Population (N_e=20)			
t/m	**0.2**	**0.6**	**1.2**	**2.0**	**0.2**	**0.6**	**1.2**	**2.0**	**0.2**	**0.6**	**1.2**	**2.0**
10	331	318	300	277	464	445	420	387	19	19	18	16
20	325	300	266	227	454	420	372	317	19	18	16	13
30	318	282	236	186	445	395	330	260	18	17	14	11
40	312	266	209	152	437	372	293	213	18	16	12	9
50	306	250	186	124	428	350	260	174	18	15	11	7
60	300	236	165	102	420	330	230	142	17	14	10	6
70	294	222	146	83	411	311	204	117	17	13	9	5
80	288	209	129	68	403	293	181	96	17	12	8	4
90	282	197	115	56	395	276	161	78	16	12	7	3
100	277	186	102	46	387	260	142	64	15	11	6	3

Discussion

The current status of wild populations of the vulnerable palm *B. eriospatha* illustrates an example of a species at high risk of extinction at the local level. This scenario seems to be the result of livestock herbivory, which can lead to demographic decline, as well as high susceptibility of *B. eriospatha* to environmental changes resulting from their amount of genetic diversity, mainly among small populations.

The demographic results showed a bimodal age structure made up of adult plants and seedlings for three of the four populations studied. Although this study surveyed four populations, ten other populations across the range of their distribution have a similar demographic

structure consisting of only adult plants and seedlings (Reis et al. 2012). In this study we described and quantified for the first time the occurrence of other ontogenetic stages of this threatened palm species for one population which to our knowledge has not been affected by cattle grazing. These results strongly indicate that cattle foraging among *B. eriospatha* populations severely affects their demographic structure. The detected high rates of herbivory (at least 77%, Table 1) prevented the survival of most seedlings and reduced the likelihood of young individuals progressing through the size class structure over time. The discrepancy between the number of seedlings and adult plants in the three *B. eriospatha* populations affected by cattle-grazing can be dated back at least 90 years to the introduction of cattle. However, this view could be underestimated if compared with the *Butia* populations of Uruguay. The Uruguayan populations of *Butia* palms have suffered greatly since the introduction of cattle over 300 years ago (Cardoso 1995). Baéz and Jaurena's (2000) study of the palm *Butia capitata* in Bañados del Este Biosphere Reserve (Uruguay) reported a similar demographic structure to the results discussed in the current study among populations in cattle and sheep farming areas. Likewise, *B. lallemanti* and *B. paraguayensis* are also experiencing demographic threats such as aging populations and lack of recruitment (Brussa and Grela 2007).

To date, although we have found only one *B. eriospatha* population not affected by cattle-grazing, our results are encouraging and should be used to guide future conservation and management planning. Furthermore, there have been some positive results relating to the management of cattle grazing for *Butia* populations in Uruguay. Baéz and Jaurena (2000) analysed the demographic structure of *B. capitata* populations after the removal of cattle. They showed that within eight years there was a restructuring of the population, with seedlings being able to establish and develop into higher size classes. Such improvements resulting from the removal of grazing animals in wild populations has also been reported for other species (North et al. 1994; Liddle et al. 2006). Considering the number of *B. eriospatha* seedlings found in the studied populations, it seems likely that the threats posed by livestock grazing can be reversed for this palm species. However, other adverse factors continue to threaten *B. eriospatha,* such as the loss of habitat due to *Pinus* reforestation and the illegal harvesting and sale of adult plants. *B. eriospatha* has high ornamental value; in

Brazil, one adult individual can cost approximately $3000US, while in Europe or North America, the price varies depending on stem size. The trade of *B. eriospatha* is not an isolated case; illegal trading is a global concern that has contributed significantly to the extirpation of many fauna and flora species (Redford 1992; Wilkie et al. 2011). The factors discussed above, along with the lack of seedling recruitment due to the cattle grazing, have considerably decreased the *B. eriospatha* population size putting it at high risk of local extinction. However, it is important to ascertain the causes of deterministic declines of *B. eriospatha* populations in order to develop effective restoration and conservation strategies that can help to reverse the population decline.

The ongoing decrease in population size of *B. eriospatha* also raises concerns regarding the existing genetic diversity. Loss of genetic diversity by genetic drift or habitat loss and habitat fragmentation can threaten the ability of populations to adapt to environmental changes (Lande 1999). Although our study found moderate genetic diversity across the investigated *B. eriospatha* populations, the smallest populations, as theoretically expected (Wright 1943; Crow and Kimura 1970; Nei et al. 1975), were more susceptible to loss of genetic diversity by genetic drift than the larger ones. Moderate to low genetic diversity was also reported for *B. catarinensis* (Reis et al. 2012) and for other endangered palm species. For example, Dowe et al. (1997) reported very low genetic diversity within the remaining wild populations of the endangered palm *Carpoxylon macrospermum*. Similarly, an extremely low genetic diversity was documented for the threatened palms *Ptychosperma macarthurii* (Shapcott 1998), *Phoenix canariensis* (Gonzáles-Pérez et al. 2004), and *Livistona carinensis* (Shapcott et al. 2009). Extremely low genetic variation was also reported by Jian et al. (2010) among the widespread mangrove species *Nypa fruticans*. However, high levels of genetic diversity was found in small populations of the endangered palm *Voaniola gerardii* as the result of historically larger populations or populations linked by gene flow (Shapcott et al. 2012). The decline of population size for a species with moderate to low genetic diversity seems to be the common factor for low genetic variation. Several authors (Brown 1989; Boyce 1992; Ellstrand and Elam 1993; Honnay and Jacquemyn 2007) have suggested that a decrease in population size can lead to increasing homozygosity due to higher levels of effective inbreeding among closely related individuals, ultimately causing reduced viability and species extinction.

We further suggest that the observed pattern of genetic diversity in *B. eriospatha* is likely the result of long-term genetic consequences related to the reduced numbers of populations and smaller (sometimes isolated) populations, thereby favouring inbreeding and exacerbating the loss of alleles by genetic drift. These factors, which are associated with the spatial distribution of populations, corroborate the high genetic differentiation values observed for *B. eriospatha* populations. Using allozyme markers to study 14 populations, Reis et al. (2012) found a high differentiation index (F_{ST}=0.36) among *B. eriospatha* populations. Similar results of high genetic structure were also found between populations of other palm species, including *Lemurophoenix halleuxii*, an endangered and endemic palm species to Madagascar, for which Shapcott et al. (2012) reported that about 20% of the genetic diversity is distributed between populations.

Although there are few archaeological, palynological or botanical records for *B. eriospatha* (Bauermann et al. 2010), we hypothesize that the moderate diversity pattern observed in *B. eriospatha* populations may be associated with the dispersal of human populations. Like other palm species, the fruit of *B. eriospatha* is considered an important component in the human diet. Thus, in the past, hunter-gatherers may have contributed to seed dispersal and planting of this species. Anthropogenic effects on the expansion of *Araucaria angustifolia*–a tree species that also occurs in highlands in southern Brazil and produces seeds widely consumed by humans–was reported by some authors (Bitencourt and Krauspenhar 2006; Reis et al. *in press*). Although the genetic diversity existing in *B. eriospatha* populations may have been due to processes of anthropogenic dispersal, it is difficult to identify a single factor that explains the genetic diversity for this vulnerable palm species. On the other hand, moderate to low genetic diversity levels in palms can be related to lower rates of evolution, as suggested by Smith and Donoghue (2009).

With regards to conservation, the present study found rare and private alleles in some populations of *B. eriospatha* indicating the need for *in situ* and *ex situ* conservation of the remaining genetic diversity. However, specific measures are urgently required to conserve the genetic resources of small populations. *B. eriospatha* is protected by Brazilian law (Instrução Normativa 06, MMA 2008); however, enforcement has been ineffective in protecting the species. As noted in a previous study (Nazareno and Reis 2012), mapping and monitoring of

the remaining populations and combating illegal trade can help change the current state of this species. Given the ongoing trade of *B. eriospatha* abroad, it is also prudent to include this species in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix I, which prohibits the international trade of threatened species. Furthermore, some threat abatement actions can be undertaken to support the recovery of *B. eriospatha*, including: (a) identifying populations of high conservation priority, specifically small populations (less than 100 individuals) that are likely to decline due to demographic processes; (b) recovering small populations [although the germination rate of this palm species is very low, a high rate of germination of in vitro cultures of zygotic embryos has been successfully demonstrated (Claumann 2009, Minardi et al. 2011)]; (c) identifying illegal trade activities in order to control and manage access to wild areas of *B. eriospatha*; and (d) raising awareness within the local community, particularly for landholders, flower shop owners which sell the species, and landscape architects who suggest this species for residential and public gardens.

Another possible step in preserving the species is related to cattle herding. In this study, the estimates of population size indicate that in cattle grazing areas, *B. eriospatha* is likely to experience a population depletion of over 50% in the next 40 years. Ensuring that population areas are inaccessible to cattle by fencing the area is possible. However, this is not a simple solution. The *B. eriospatha* populations surveyed in this study are located on private land where cattle farming is the main economic activity. Creating protected areas in these locations and providing alternatives for the farmers are essential to reducing the risk of local extinction. Placing enclosures around some reproductive plants could also help seedling survival. However, this solution requires special care and monitoring since it can lead to genetic structuring.

Clearly, our results indicate the urgent need for action and more long-term studies to preserve *B. eriospatha* and conserve the remaining genetic diversity. However, there are several issues that raise concerns about the future of *B. eriospatha*. Collecting information about the number and size of populations throughout the species distribution area should be a priority as this data is necessary to design effective conservation strategies. In addition, while the recruitment of new individuals has been unsuccessful due to high rates of herbivory, it is critical that we understand the demographic dynamics of *B. eriospatha*

without the influence of livestock so that recovery activities within small populations can serve as a guideline for preventing the extinction of species of the *Butia* genus. Although this study highlights the importance of analysing both population ecology parameters and genetic data to better understand the risks facing threatened species, we emphasize that policy actions are urgently needed and should be based on our recommendations for conservation effectiveness of this vulnerable biological resource.

Funding

National Counsel of Technological and Scientific Development (CNPq) (to A.G.N.); CNPq (304724/210-6) to M.S.R. Financial support by FAPESC/IFFSC (Santa Catarina State Research Council, Brazil/Santa Catarina's Floristic and Forest Inventory) is gratefully acknowledge.

Acknowledgments

We are grateful to the Núcleo de Pesquisas em Florestas Tropicais (Rainforest Research Department) of the Federal University of Santa Catarina (UFSC) for assistance during field work. We would also like to thank the Physiology of Development and Plant Genetics Laboratory at the Federal University of Santa Catarina (UFSC) for providing the infrastructure required for microsatellite analysis. The authors thank Dr. Evelyn R. Nimmo for editing the English of the manuscript and Karina Vanessa Hmeljevski for help with the Figure 1. Alison Gonçalves Nazareno was supported by a doctoral fellowship from CNPq (National Council of Technological and Scientific Development). This study is part of Alison Gonçalves Nazareno's graduate work in Plant Genetic Resources at UFSC.

Supplementary Material

TABLE S1 Estimates of null allele frequency for adult and seedling plants of *Butia eriospatha* (Martius ex Drude) Beccari, from Santa Catarina State, Southern Brazil. * Presence of null allele based on Oosterhout's method.

	Adults				Seedlings			
Locus	A	B	C	D	A	B	C	D
But06	0.2153*	0.0462	0.2545*	0.1415*	0.1106	0.1711*	0.1686*	0.2367*
But07	-0.0370	-0.0331	0.1987*	0.1159	0.1551*	0.2219*	0.1383*	0.2247*
But08	0.0716	0.0271	0.1956*	-0.4008	-0.1224	-0.0837	0.1193	0.0216
But09	0.2409*	0.2247*	0.2204*	0.1089	0.2008*	0.2211*	0.1392*	0.1619*
But11	0.0784	-0.0547	0.1189	-0.2327	-0.0099	-0.1429	0.0403	-0.0679
But16	0.1109	0.1145	0.2291*	0.1065	-0.1472	0.1117	0.1678*	0.1370
But17	0.1218	0.0889	0.2988*	-0.0318	0.0096	0.0871	0.2218*	0.1948*
But18	0.0131	0.1262	0.3389*	0.0345	0.0444	0.0019	0.2390*	0.2909*
But23	-0.5725	-0.2095	-0.1760	-0.1025	-0.5203	-0.3277	-0.0895	0.0587

References

Báez F, Jaurena M. 2000. Regeneración del palmar de butiá (*Butia capitata*) en condiciones de pastoreo. Relevamiento de establecimientos rurales de Rocha. Probides, Uruguay.

Balick MJ, Beck HT. 1990. Useful Palms of the World: A Synoptic Bibliography. Columbia University Press, New York.

Bauermann SG, Evaldt ACP, Zanchin JR, Bordignon SAL. 2010. Diferenciação polínica de Butia, Euterpe, Geonoma, Syagrus e Thritrinax e implicações palioecológicas de Arecaceae para o Rio Grande do Sul. Iheringia Sér Bot. 65:35-46.

Bitencourt ALV, Krauspenhar PM. 2006. Possible prehistoric anthropogenic effect on *Araucaria angustifolia* (Bert.) O. Kuntze expansion during the late Holocene. Rev Bras Paleont. 9:109-116.

Boyce MS. 1992. Population viability analysis. Ann Rev Ecol Syst. 23:481-506.

Brown AHD. 1989. Genetic characterization of plant mating systems. In: Brown ADH, Clegg MT, Kahler AL, Weir BS, editors. Plant population genetics, breeding and genetic resources. Sunderland (MA): Sinaueuer Associates. p. 145-162.

Brussa CA, Grela IA. 2007. Flora Arbórea del Uruguay. Con énfasis en las especies de Rivera y Tacuarembó. COFUSA, Uruguay.

Cardoso MC. 1995. El palmar, la palma y el butiá. Probides, Uruguay.

Chapin MH, Wood KR, Perlman SP, Maunder M. 2004. A review of the conservation status of the endemic *Pritchardia* palms of Hawaii. Oryx. 38:273-281.

Chapuis MP, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol. 24:621-631.

Claumann AD. 2009. Embriogenese somática de *Butia eryospatha*. Dissertação (Mestrado em Recursos Genéticos Vegetais) - Universidade Federal de Santa Catarina.

Cockerham CC. 1969. Variance of gene frequencies. Evolution. 23:72-84.

Correa LB, Barbieri RL, Rossato M, Buttow MW, Heiden G. 2009.

Caracterização cariológica de palmeiras do genero *Butia* (Arecaceae). Rev Bras Frutic. 31:1111-1116.

Crow J, Kimura M. 1970. An Introduction to Population Genetics Theory. Harper and Row, New York.

Dowe JL, Benzie J, Ballment E. 1997. Ecology and genetics of *Carpoxylon macrospermum* H. Wendl. & Drude (Arecaceae), an endangered palm from Vanuatu. Biol Conserv. 79:205-219.

Dransfield J, Uhl NW, Asmussen CB, Baker WJ, Harley MM, Lewis CE. 2005. A new phylogenetic classification of the palm family, Arecaceae. Kew Bull. 60:559-569.

Ellstrand NC, Elam DR. 1993. Population genetic consequences of small population size: Implications for plant conservation. Ann Rev Ecol Syst. 24:217-242.

El Mousadik A, Petit RJ. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. Theor Appl Genet. 92: 832-839.

Gaieiro P, Mazzella C, Agostini G, Bertolazzi S, Rossato M. 2011. Genetic diversity among endangered Uruguayan populations of *Butia* Becc. species based on ISSR. Plant Syst Evol. 292:105-116.

Glassman SF. 1972. A revision of B.E. Dahlgren's index of American palms. Lehre: J. Cramer.

Glassman SF. 1987. Revisions of the palm genus *Syagrus* Mart. and other selected genera in the *Cocos* alliance. University of Illinois Press.

Gonzáles-Pérez MA, Caujapé-Castells J, Sosa PA. 2004. Allozyme variation and structure of the Canarian endemic palm tree *Phoenix canariensis* (Arecaceae): implications for conservation. Heredity. 93:307-315.

Goudet J. 2002. FSTAT: a program to estimate and test gene diversities and fixation indices. Version 2.9.3.2. [cited 2012 Dec 11]. Available from: http://www2.unil.ch/popgen/softwares/fstat.htm.

Grove RH. 1995. Green imperialism: colonial expansion, tropical island edens and the origin of enviromentalism. Cambridge University Press.

Hardy OJ, Vekemans X. 2002. SPAGeDi: a versatile computer program to

analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes. 2:618–620.

Honnay O, Jacquemy H, Bossuyt B, Hermy M. 2005. Forest fragmentation effects on patch occupancy and population viability of herbaceous plant species. New Phytol. 166:723-736.

Honnay O, Jacquemyn H. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. Conserv Biol. 21:823-831.

IUCN World Conservation Union. 2001. IUCN Red List Categories and Criteria Version 3.1. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland.

IUCN World Conservation Union. 2012. Red List of Threatened Species. Version 2010.1. [cited 2012 Dec 15]. Available from: http://www.iucnredlist.org.

James A, Maidman K. 2003. Palms of Dominica. Palms. 47:151-157.

Jamieson IG, Allendorf FW. 2012. How does the 50/500 rule apply to MVPs? Trends Ecol Evol. 27:578-584.

Jian S, Ban J, Ren H, Yan H. 2010. Low genetic variation detected within the widespread mangrove species *Nypa fruticans* (Palmae) from Southeast Asia. Aquatic Botany. 92:23-27.

Johnson D. 1996. Palms: their conservation and sustained utilization. Status survey and conservation action plan, Switzerland. IUCN The World Conservation Union.

Lande R. 1999. Anthropogenic, ecological and genetic factors in extinction. In: Conservation in a Changing World. Mace GM, Balmford A, Ginsberg J, editors. Cambridge, Cambridge University Press. p. 29-51.

Laurance WF, Useche DC, Rendeiro J et al. 2012. Averting biodiversity collapse in tropical forest protected areas. Nature. 489:290-294.

Liddle DT, Brook B, Matthews J, Taylor SM, Caley P. 2006. Threat and response: A decade of decline in a regionally endangered rainforest palm affected by fire and introduced animals. Biol Conserv. 132:362-375.

Loiselle BA, Sork VL, Nason J, Graham C. 1995. Spatial genetic structure of a

tropical understory shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot. 82:1420-1425.

Lorence DH, Sussman RW. 1986. Exotic species invasion into Mauritius wet forest remnants. J Trop Ecol. 2:147-162.

Malthus TR. [1798(1993)] An Essay on the Principle of Population. Oxford University Press.

Manohara TN, Linto EL, Renuka C. 2010. Diversity and conservation of palms in Andaman & Nicobar archipelago. Biodivers Conserv. 19:3655-3666.

Matos DMS, Bovi MLA. 2002. Understanding the threats to biological diversity in southeastern Brazil. Biodivers Conserv. 11:1747-1758.

Minardi BD, Voytena APL, Randi AM, Zaffari GR. 2011. Cultivo in vitro de embriões zigóticos de *Butia eriospatha* (Mart. Ex Drude) Becc.. INSULA Rev Bot. 40:70-81.

MMA (Ministério do Meio Ambiente). 2008. In: Lista oficial de espécies da Flora brasileira ameaçada de extinção. Diário Oficial da União de 24 de setembro de 2008, nº 185. Seção 1, p. 75-83. [cited 2012 Nov 8]. Available from:http://www.mma.gov.br/estruturas/ascom_boletins/_arquivos/83_19092008034949.pdf.

Moore HE. 1973. The major groups of palms and their distribution. Gentes Herb. 11:27-140.

Moore H.E 1979. Endangerment at the specific and generic level in palms. Principes. 23:47-64.

Morici C. 1997. *Coccothrinax barbadensis* in Antigua. Principes. 41:84-86.

Nazareno AG, Zucchi MI, Reis MS. 2011. Microsatellite markers for *Butia eriospatha* (Arecaceae), a vulnerable palm species from the Atlantic Rainforest of Brazil. Am J Bot. 98:e198–e200.

Nazareno AG, Reis MS. 2012. Linking phenology to mating system: exploring the reproductive biology of the threatened palm species *Butia eriospatha*. J Heredity. 103:842-852.

Nei M, Maruyama T, Chakraborty R. 1975. The bottleneck effect and genetic variability in populations. Evolution. 29:1-10.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590

Noblick LR. 2010. *Butia*. In: Lorenzi H, Noblick LR, Kahn F, Ferreira E, editors. Flora brasileira Lorenzi: Arecaceae (palmeiras). Instituto Plantarum, Nova Odessa, Brazil.

North SG, Bullock DJ, Dulloo ME. 1994. Changes in the vegetation and reptile populations on Round Island, Mauritius, following the eradication of rabbits. Biol Conserv. 67:21-28.

R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [cited 2012 Oct 12]. Available from: http://www.R-project.org.

Redford KH. 1992. The empty forest. BioScience. 42:412-422.

Reis MS, Mantovani A, Silva JZ, Mariot A, Bittencourt R, Nazareno AG, Ferreira SK, Steiner F, Montagna T, Silva AALS, Fernandes CD, Altrak G, Figueredo LG. 2012. Distribuição da diversidade genética e conservação de espécies arbóreas em remanescentes florestais de Santa Catarina. In: Vibrans AC, Sevegnani L, Gasper AL, Lingner DV, editors. Inventário florístico florestal de Santa Catarina. v. 1 Blumenau: Edifurb.

Reis MS, Ladio AH, Peroni N (*in press*) Landscapes with Araucarias in South America: evidence for a cultural dimension. Ecol Societ.

Reitz R. 1974. Palmeiras – Parte 1. Flora ilustrada catarinense, Herbário Barbosa Rodrigues, Brazil.

Rice WR. 1989. Analyzing tables of statistical tests. Evolution. 43:223-225.

Saunders DA, Hobbs HJ, Margules CR. 1991. Biological consequences of ecosystem fragmentation: a review. Conserv Biol. 5:18-27.

Schatz GE. 2009. Plants on the IUCN Red List: setting priorities to inform conservation. Trends Plant Sci. 14:638-642.

Shapcott A. 1998. The genetics of *Ptychosperma bleeseri* a rare palm from the Northern Territory, Australia. Biol Conserv. 85:203-209.

Shapcott A, Dowe JL, Ford H. 2009. Low genetic diversity and recovery implications of the vulnerable Bankoualé Palm *Livistona carinensis* (Arecaceae), from North-eastern Africa and Southern Arabian Peninsula.

Conserv Genet. 10:317-327.

Shapcott A, Quinn J, Rakotoarinivo M, Dransfield J. 2012. Contrasting patterns of genetic diversity between two endangered palms with overlapping distributions, *Voanioala gerardii* (Arecoideae) and *Lemurophoenix halleuxii* (Arecoideae), from North-east Madagascar. Conserv Genet. 13:1393-1408.

Smith SA, Donoghue MJ. 2009. Rates of molecular evolution are linked to life history in flowering plants. Science. 322:86-89.

Thebaud C, Strasberg D. 1997. Plant dispersal in fragmented landscapes: a field study of woody colonization in rain forest remnants of the Mascarenie Archipelago. In: Tropical Forest Remnants. Laurance WF, Bierregaard RO, editors. University of Chicago Press, Chicago, IL. p. 331-332.

Turner IM. 1996. Species loss in fragments of tropical rain forest: a review of the evidence. J Appl Ecol. 33:200-209.

Turchin P. 2000. Does population ecology have general laws? Oikos 94:17–26.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 4:535–538.

Weir BS, Cockerham CC. 1984. Estimating F-Statistics for the analysis of population structure. Evolution. 38:1358-1370.

Wilkie DS, Bennett EL, Peres CA, Cunningham AA. 2011. The empty forest revisited. Ann NY Acad Sci. 1223:120-128.

Wissel C, Stocker S. 1991. Extinction of populations by random influences. Theor Pop Biol. 39:315-328.

Wright S. 1943. Isolation by distance. Genetics. 28:114-138.

Zambrana NYP, Byg A, Svenning J-C, Moraes M, Grandez C, Balslev H. 2007. Diversity of palm uses in the western Amazon. Biodivers Conserv. 16:2771-2787.

CAPÍTULO 5

Este manuscrito segue as normas de formatação do período *Biodiversity and Conservation*

Where did they come from? Genetic diversity and forensic investigation of the threatened palm species *Butia eriospatha*

Núcleo de Pesquisas em Florestas Tropicais, Federal University of Santa Catarina, CP 476, 88040-900, Florianópolis, Santa Catarina, Brazil.

Address correspondence to Alison G. Nazareno at the above address, or e-mail: alison_nazareno@yahoo.com.br

Abstract

Few studies have assessed the genetic diversity that exists in individuals that were illegally traded. In this paper, we evaluate the genetic consequences of illegal trade of the palm species *Butia eriospatha*. Although it is protected by Brazilian environmental law, information about the genetic consequences of illegal trading which can be used to support conservation planning is still needed. The two main questions approached were: a) do illegally-traded individuals have higher levels of genetic diversity than those found in wild populations; and b) where did the illegally-traded individuals come from? To answer these questions, we used nine microsatellite loci to quantify the genetic diversity in eight wild populations (n=390) and one group of individuals (n=50) planted in an urban area of Southern Brazil. For the forensic investigation, an assignment exclusion-test was performed. Remarkably, the illegally-traded *B. eriospatha* individuals had more genetic variation than all of the studied wild *B. eriospatha* populations, suggesting that there is no single target population used by poachers. Accordingly, the multilocus assignment test indicated that the urban *B. eriospatha* individuals came from a variety of different populations, with 46% coming from populations not surveyed in this study. In light of these results, we discuss the very real problem of illegal trading of *B. eriospatha* that must be quickly addressed. Our results provide information that can be used to combat illicit trade and help support *B. eriospatha* conservation.

Keywords: Atlantic Forest, Assignment test, Bayesian model, Conservation genetics, Illegal trade, Microsatellites

Introduction

Hundreds of millions of plants and animals species around the world have been hunted and caught for food, leather, and medicine (Kate and Laird 1999; Arroyo-Quiroz et al. 2007; Larsen and Olsen 2007) and the majority are sold to private collectors (Alves and Filho 2007; Rosa et al. 2011; Natusch and Lyons 2012). While some of this trade is legal and does not harm wild populations, an alarmingly large proportion is illegal (Redford 1992; Destro et al. 2012), putting many wild plant and animal species on the verge of extinction (Redford 1992; Wilkie et al. 2011).

Some examples of illegal and unsustainable wildlife trade are well documented, such as poaching of elephants for ivory (Wasser et al. 2004; Wasser et al. 2010), bears for their skin, claws and canines (Shepherd and Nijman 2008), rhinos for their horn (Graham-Rowe 2011), and felines for their skin and bones (Kenney et al. 1995; Check 2006). A long-term study in India showed that at least four leopards (*Panthera pardus)* have been poached every week for the past decade (Mutterback 2012). Another problematic example comes from Brazil where due to illegal trafficking, the bird spix's macaw (*Cyanopsitta spixii*, *ararinha-azul*) is now extinct in the wild, with only 79 individuals left in the world (e.g. Qatar, Spain, Germany and Brazil) all being raised in captivity (Foldenauer et al. 2007). But the exploitation of species is not a new phenomenon. During the colonial period, the Brazilian tree 'Pau Brasil' (*Caesalpinia echinata*) was harvested and sent to Portugal in such large quantities that the species almost became extinct (Bueno 2006). Likewise, at the beginning of the 20th century in southern Brazil, the population of the Brazilian pine, *Araucaria angustifolia*, was almost completely decimated (Carvalho 2006).

Around the word, the illegal trade of species and their products is a lucrative business, providing high returns with relatively little risk (Destro et al. 2012). In Brazil, nearly 40 million animal specimens are captured from the wild annually, representing a total retail value of approximately US$2.5 billion a year (RENCTAS 2011). However, this amount is an underestimation; it does not consider the illegal trade of plants as data on plant poaching is rare. The ornamental plants of some botanical families (e.g. Orchidaceae, Cactaceae, Bromeliaceae and Cyatheaceae) and timber tree species (e.g. *Swietenia macrophylla*) are the most traded plants in Brazil. According to the database of CITES (Convention on International Trade in Endangered Species of Wild

Fauna and Flora that enforces regulations on international trade of species) during the period from 2006-2010, trade of *S. macrophylla* alone likely reached an estimated value of US$168 million (CITES 2010).

Even though some species are protected by environmental laws and by International agreements, we need to address trafficking of species from a multi-stakeholder approach in order to inform, facilitate and support conservation plans and to reduce this serious threat facing biological diversity. As pointed out recently (US Department of State, November 2012) by US Secretary of State, Hillary Clinton, "we need governments, civil society, businesses, scientists and activists to educate people about wildlife trafficking." Furthermore, identifying and protecting species that are jeopardized by illegal trade, such as the vulnerable palm species *Butia eriospatha*, can act as an insurance policy to preserve not only the future of the species, but also the futures of the species' ecological communities.

Trafficking of *B. eriospatha,* a threatened palm species endemic of Brazil, along with other threats imposed on the species, have contributed significantly to the species becoming at risk of local extinction. The remaining populations of the vulnerable palm species *B. ertiospatha* (IUCN 2012) are mainly consist of mature individuals aged one hundred years or older (Nazareno et al. 2011). Populations of this slow-growing, self-compatible, monoecious, and out-crossing palm species (Nazareno and Reis 2012) may be genetically impoverished due to the ongoing decrease in the number of reproductive individuals because of illegal trade. In Brazil, adult individuals of *B. eriospatha* have a high ornamental value, approximately US$3000, an amount which is more than 100 percent of the price that poachers pay to landowners. In Europe and North America, where this species is also sold, its price varies depending on the stem size (e.g. http://www.exoticplantsonline.co.uk). Interestingly, in a forum from one US website (http://forums.gardenweb.com) we found the following dialogue: Person 1: "We were attracted to the eriospathas because they're a real feather palm and we want them to be at the front of our building -- along with bananas, hibiscus, etc. -- to set the tropical tone. But at this point, the nearest I've found any sizable trees is Holland ..... or Brazil"; Person 2: "There's one place in California that might can help you, it's Brother Earth Nursery. I hope they can help you." This exchange highlights the susceptibility of this species to illegal trade.

Furthermore, the habitat in which this vulnerable palm occurs (highlands or *campos de altitude*, a subtype of the Atlantic Forest Domain; Reitz 1974) is not adequately protected by conservation policies (Overbeck et al. 2007). Even more concerning is the fact that the Atlantic Forest (with scattered, discontinuous grassland areas, especially on the plateaus in the southern region) has been reduced to about 7% of its original area (Morellato and Haddad 2000). Despite the significant fragmentation of the biome, researchers estimate that there are at least 20,000 plant species occurring in the biome (Myers et al. 2000), many of which are also at severe risk of extinction.

Although *B. eriospatha* is protected by Brazilian law (Instrução Normativa 06, MMA 2008), information about the genetic consequences of illegal harvesting are still needed in order to effectively support conservation programs. From this point of view, the goal of this study was to quantify the genetic diversity in wild *B. eriospatha* populations as well as the genetic diversity of one group of illegally-traded individuals that are now planted in luxurious homes, malls and public gardens in Southern Brazil. Considering that molecular forensic methods have enabled researchers to identify the originating population of individuals (Roman and Bowen 2000; Manel et al. 2002; Degen et al. 2013) using polymorphic molecular markers (e.g. microsatellites) and statistical approaches (e.g. assignment tests as proposed by: Rannala and Mountain 1997; Cornuet et al. 1999; Pritchard et al. 2000), we also investigated the originating population of the poached *B. eriospatha* individuals. Our results provide important information for decision-makers to help combat *B. eriospatha* trafficking in Brazil and abroad and thereby help support conservation strategies for this threatened palm species.

Materials and Methods

Sampling and study area

We sampled eight natural populations of *B. eriospatha* located in Santa Catarina State, Western Plateau, Southern Brazil (Figure 1). We do not provide the exact locations of natural populations in this study in order to reduce the risk of poaching. In addition, a group of 50 *B. eriospatha* individuals were sampled from a non-native, urban area (Figure 1). These plants were illegally traded and planted in malls, and public and private gardens in the city of Florianópolis, Santa Catarina, Southern

Brazil. The population size and the number of individuals sampled, and the distances between natural populations are shown in Tables 1 and 2, respectively. All of the natural populations included in the study have been negatively impacted by anthropogenic activities such as cattle farming, deforestation and the introduction of exotic species (e.g. *Pinus*) that are cultivated in large homogeneous stands**.**

Data analysis

The microsatellite data analyses followed two approaches. Our primary interest was in verifying the level of genetic diversity in wild populations as compared to a group of illegally-traded *B. eriospatha* individuals. Secondly, in order to identify the originating population of the poached *B. eriospatha* individuals, we checked the genetic homogeneity of each wild population using a Bayesian model. For forensic investigations, we conducted one exclusion-simulation method of assignment, based on multilocus genotype data, in order to determine the likely origin of the poached *B. eriospatha* individuals.

Genotyping and genetic analyses

Genomic DNA extraction from leaves was conducted using the NucleoSpin® kit (MACHEREY-NAGEL GmbH & Co. KG), according to the manufacturer's instructions. The PCR and profile used to amplify the nine microsatellite loci are described in Nazareno et al. (2011). PCR products were denatured and separated with 10% denaturing polyacrylamide gels stained with silver nitrate. Allele sizes were estimated by comparison with a 10 base pair DNA ladder standard (Invitrogen, Carlsbad, California, USA).

Figure 1. Highlands (dark gray areas) in the Atlantic Rainforest (IBGE 2004) where *Butia eriospatha* (Martius ex Drude) Beccari can occur in Southern Brazil (States of Paraná, Santa Catarina and Rio Grande do Sul). The black circles indicate the eight natural populations (A to H) and one urban area (X) from which genetic samples were obtained in Santa Catarina State, Southern Brazil.

Deviation from the Hardy-Weinberg equilibrium and linkage disequilibrium were tested for each *B. eriospatha* population. The significant values for linkage disequilibrium were corrected for multiple comparisons by Bonferroni correction (Rice 1989). Allele frequencies and the following parameters were then calculated: allelic richness (A), number of private (A_P) and rare alleles (R; defined as those with a frequency of less than 5%), observed heterozygosity (H_O), and expected heterozygosity (H_E, Nei 1978). Rarefaction approach was used to standardize A to the smallest sample size in each comparison. All of these analyses were run using the program FSTAT 2.9.3.2 (Goudet 2002). The inbreeding index (F_{IS}) was estimated and its significance (determined by 10,000 permutations over loci) tested using the program SPAGeDi (Hardy and Vekemans 2002). The genetic differentiation was estimated using an unbiased estimator of F_{ST} (Weir and Cockerham 1984) with FSTAT 2.9.3 (Goudet 2002). Null allele frequencies were assessed for all populations using the Microchecker software V 2.2.0 (van Oosterhout et al. 2004). If significant homozygosity was detected at a given locus, it was dropped and a modified average F_{IS} over loci was calculated. Significance was calculated from jackknife over loci. Likewise, estimates of genetic differentiation between populations was calculated using the ENA method (10,000 permutations) implemented in FreeNA (Chapius and Estoup 2007), which corrects for the presence of null alleles. Furthermore, F_{ST} values calculated with FSTAT 2.9.3 and FreeNA were used to investigate isolation by distance pattern. The relationship between the matrix of the logarithm of geographical distances and the matrix of pairwise genetic distance [$F_{ST}/(1 - F_{ST})$, Rousset 1997] was analysed via a Mantel's test (Mantel 1967) with 30,000 randomizations using the program IBDWS 3.23 (Jensen et al. 2005).

Identification of genetic units and forensic analysis

In order to test whether *B. eriospatha* populations were genetically differentiated without *a priori* classification of individuals, a Bayesian model was executed in a Markov Chain Monte Carlo (MCMC), as implemented in the STRUCTURE program, version 2.3.4 (Hubisz et al. 2009). In this model, the number of populations, K, is treated as a parameter processed by the MCMC scheme without any approximation providing a better estimation of K. Based on the spatial configuration and distribution of the sampled *B. eriospatha* populations and high

allozyme variation between *B. eriospatha* populations (F_{ST}=0.36, Reis et al. 2012), we performed our analysis under the assumption that the allele frequencies in different populations are not correlated with one another and that alleles carried at a particular locus by a particular individual originated in some known population (no admixture model). The *K* was set from 2 to 8 with each *K* estimate replicated 15 times with 100,000 burn-in iterations and 500,000 data iterations. In order to estimate the appropriate number of populations after distribution, we followed the protocol described by Evanno et al. (2005).

A Bayesian model-based assignment test (Rannala and Mountain 1997), implemented in the GeneClass 2.0 (Piry et al. 2004), was used in order to identify a possible source population. In this method, the assumption that the true population of origin has been sampled is not required. The exclusion simulation method was calculated based on the resampling algorithm described in Paetkau et al. (2004). In the GeneClass 2.0 program, the allele frequencies from a sampled population is used to compute the likelihood of a genotype occurring in the population; it compares the likelihood of the specific genotype to a distribution of the likelihoods of simulated genotypes for each investigated population. In our analysis, the genotypes were generated by MCMC simulations of 10,000 individuals for each of the sampled *B. eriospatha* populations. In order to exclude an individual from all but the true population of origin, one strict criterion was chosen (*p* value of 0.001; i.e. if a specific genotype is observed less than once in 1000 randomly simulated genotypes, the population will be excluded as the origin). Although null alleles can cause bias in assignment tests, no additional analysis to correct null alleles was performed since the microsatellite loci affected by null alleles did not alter the overall outcome of this test (Carlsson 2008).

Results

Genetic diversity

A total of 410 individuals from the wild populations and the urban area were surveyed (Table 1), in which 57 alleles were identified at nine microsatellite loci. As expected, the allelic richness for the illegally-traded *B. eriospatha* individuals planted in the urban area was higher than the sampled wild populations (Table 1). Of the 13 private alleles observed, 9 or 70% were found in the urban area. We also observed a

greater number of rare alleles in this group of individuals. As the expected heterozygosity (H_E) value is influenced by rare alleles, our results indicated that *B. eriospatha* plants that occur in the urban area of Florianópolis have a genetic potential (i.e., H_E) slightly higher than the *B. eriospatha* individuals in wild populations. The average observed heterozygosity (H_O) within wild populations was 0.36, ranging from 0.22 to 0.47 (Table 1). These values are considerably lower than the expected heterozygosity (H_E) assuming the Hardy-Weinberg equilibrium, which averaged 0.48.

For the eight wild *B. eriospatha* populations, the test for Hardy-Weinberg equilibrium found that of 144 locus-population combinations, 46, 34 and 20, or 32.0%, 23.6% and 13.9%, showed significant deviation at p=0.05, 0.01 and 0.001, respectively. The test for the genotypic disequilibrium in all wild population samples found that 79 of 288 locus combinations or 27.4% showed significant deviation at the p=0.05; however, none of the locus pairs were found to be in significant genotypic disequilibrium after the Bonferroni correction ($p < 0.001$).

The average F_{IS} values were 0.25 (ranging from 0.04–0.46) for all studied wild *B. eriospatha* populations. As the F_{IS} was positive and significantly different from zero for all but one population (Table 1), the pattern of excess homozygosity can be related to the presence of null alleles (Table S1). When loci with significant null alleles were omitted, the F_{IS} values remained positive and significantly different from zero for six of the seven populations (Table 1), indicating that most populations lose allelic richness through genetic drift. Contrary to expected for small populations, the population D seems to be less susceptible to implications of the genetic drift (Table 1).

TABLE 1 Population genetics estimates for eight *Butia eriospatha* (Martius ex Drude) Beccari populations sampled in Santa Catarina State,

Southern Brazil. Estimates for a group of 50 *B. eriospatha* individuals sampled in an urban area, in Florianópolis, Santa Catarina, are also presented. *N*, estimate of population size; *n*, sample size; *K*, number of alleles; A_R, allelic richness by rarefaction based on the minimum sample size of 29 individuals; A_P, number of private alleles; *R*, number of rare alleles (here defined as alleles with a frequency of less than 5%); H_E and H_O, expected and observed heterozygosity, respectively; F_{IS}, inbreeding index; F_{IS}^1 inbreeding index excluding the loci segregating for null alleles.

Samples	*N*	*n*	*K*	A_R	A_P/R	H_E/H_O	F_{IS}	F_{IS}^1
A (Curitibanos)	490	50	26	2.89	0/3	0.49/0.43	0.13*	0.08*
B (Marombas)	610	50	30	3.33	0/5	0.47/0.42	0.12*	0.08*
C (Sta Cecilia)	41	41	23	2.56	0/1	0.40/0.22	0.46*	0.12*
D (Matos Costa)	29	29	28	3.11	0/4	0.49/0.47	0.04	0.00
E (Irani1)	120	50	29	3.22	1/2	0.52/0.35	0.32*	0.13*
F (Irani2)	150	50	26	2.88	0/4	0.49/0.29	0.41*	0.12*
G (Fraiburgo)	40	40	25	2.67	0/2	0.47/0.29	0.38*	0.13*
H (Bela Vista)	100	50	33	3.67	2/10	0.50/0.43	0.15*	-0.06
X (Florianópolis)	200	50	44	5.11	9/12	0.62/0.40	nc	nc

* Significant at $P > 0.05$. nc, not calculated.

The overall estimate of genetic differentiation (Weir and Cockerham 1984) was significant among *B. eriospatha* populations (F_{ST}=0.23, p<0.05). This value was similar to the overall estimate of F_{ST} obtained after the correction for null alleles (F_{ST}=0.17, p<0.05). However, the geographic distance among *B. eriospatha* populations did not explain the pattern of genetic differentiation observed (i.e., lack of isolation by distance, Z=15.73, r=0.05, p=0.63; Figure 2A), suggesting

that genetic drift rather than gene flow contributed to the genetic divergence observed among these populations.

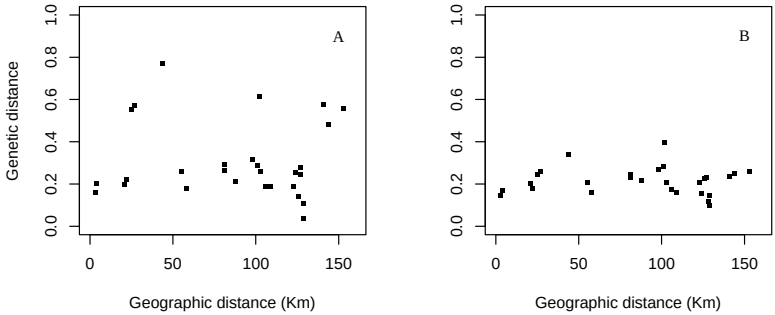


FIGURE 2 Scatter plots of pairwise genetic distance [$F_{ST}/(1- F_{ST})$] versus geographical distance (Km) for eight *Butia eriospatha* (Martius ex Drude) populations sampled in Santa Catarina, Southern Brazil. The geographic distance among populations did not explain the pattern of genetic differentiation quantified by the presence (A) and absence of null alleles (B).

Our results also indicated that null alleles inflated the estimates of genetic distance (Figure 2). However, even after the correction for null alleles in the F_{ST} pairwise estimates, no isolation by distance was observed for *B. eriospatha* populations (Z=10.79, r=0.09, p=0.70; Figure 2B). The matrix of geographic distance and the pairwise F_{ST} values quantifying genetic differentiation among *B. eriospatha* populations are presented in Table 2.

TABLE 2 Matrix of the geographic distances (above diagonal) and the genetic differentiation (below diagonal) between eight *Butia eriospatha* (Martius ex Drude) Beccari populations from Santa Catarina, Southern Brazil, based on nine microsatellite loci. Bold indicates the pairwise F_{ST} values using the ENA correction method as proposed by Chapius and Estoup (2007). Asterisks denote values that are significant at 0.05.

Populations	A	B	C	D	E	F	G	H
A	--	3.2	26.7	100.7	127.5	123.8	21.5	128.9
B	0.039	--	25.1	97.8	126.1	122.5	20.8	129.1
C	0.355*	0.364*	--	101.7	144.1	140.8	44.2	152.7
D	0.240*	0.223*	0.381*	--	80.8	80.5	87.8	126.7
E	0.123*	0.196*	0.325*	0.208*	--	4.4	106.2	58.3
F	0.159*	0.202*	0.367*	0.227*	0.168*	--	102.5	55.3
G	0.167*	0.181*	0.435*	0.181*	0.159*	0.206*	--	108.6
H	0.099*	0.035	0.358*	0.218*	0.150*	0.206*	0.157*	--
B	**0.027***	--						
C	**0.205***	**0.196***	--					
D	**0.220***	**0.211***	**0.284***	--				
E	**0.107***	**0.184***	**0.200***	**0.189***	--			
F	**0.133***	**0.171***	**0.191***	**0.197***	**0.144***	--		
G	**0.152***	**0.170***	**0.254***	**0.178***	**0.150***	**0.171***	--	
H	**0.091***	**0.040**	**0.206***	**0.188***	**0.137***	**0.172***	**0.137***	--

Bayesian analyses

Bayesian clustering without prior information about the geographical origins of populations showed that the highest likelihood value (ΔK) occurred at K=6 (Figure 3; Figure 1S at Supplementary Material), where the number of clusters (K) was similar to the number of wild populations sampled in this study (n=8). Although we expected a K equal to six due to the spatial clustering of populations (e.g., clustering of populations A and B, and E and F), the K value was not the result of population clusters. The difference between the number of clusters and the number of sampled populations was due to the grouping of three populations (A, B, and H) into only one unit. While it makes biological sense for

populations A and B to be grouped as they are located in close proximity to each other (less than 4.0 km), this result is noteworthy because the H population is separated from populations A and B by a distance of 129 km (Figure 1). However, this result strengthens our previous observations of lack of isolation by distance among *B. eriospatha* populations. Bayesian clustering with and without prior information about geographical origins of populations, considering both the allele frequencies in different populations are correlated with one another and the admixture model (data not show), also indicated that highest likelihood value (ΔK) occurred at K=6.

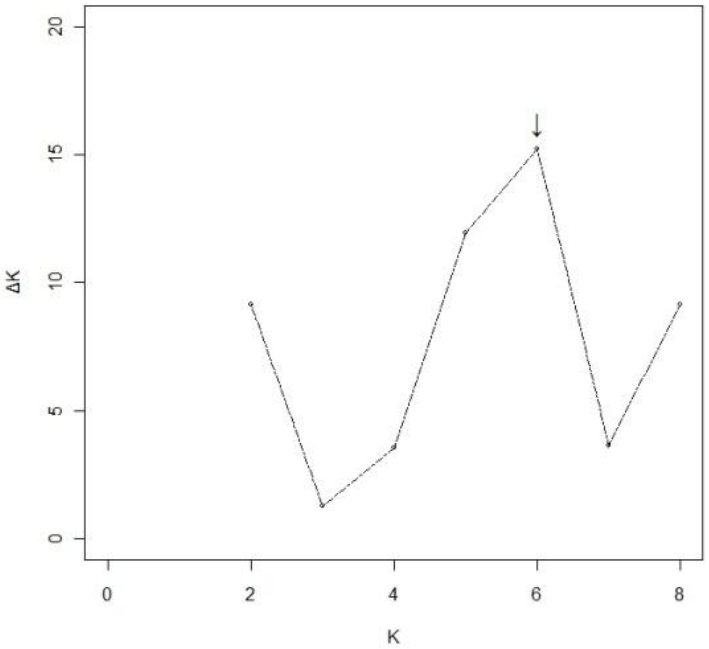


FIGURE 3 Magnitude of ΔK as a function of number of clusters, K. ΔK is an ad hoc statistic applied over the posterior probability data from the STRUCTURE program and it was calculated as the mean of the second order rate of likelihood L(K), L´´(K), divided by the standard deviation of L(K).The most appropriate number of K is indicated with an arrow.

The forensic analysis using the exclusion-simulation significance test found that 24 of the *B. eriospatha* individuals (48%) sampled in the urban area have an unknown origin ($p < 0.001$). For just three individuals (6%), we excluded all but one population as the probable population of origin. Two individuals were assigned to the set of populations A, B, and H. The other *B. eriospatha* individual was assigned to the D population. On the other hand, 23 out of the 50 *B. eriospatha* individuals (46%) may have come from several of the six populations identified *a posteriori* (K=6). For instance, for one individual we excluded only one of the six populations as not being its probable population of origin.

Discussion

Defined as any act that intentionally contravenes the laws and regulations established to protect biological resources, poaching (or illegal trade, Muth and Bowe 1998) is considered one of the most significant threats to biological diversity (Redford 1992; Alacs et al. 2010; Wilkie et al. 2011; Destro et al. 2012). However, few studies have assessed the genetic diversity that can occur in individuals that were illegally poached (Bodkin et al. 1999; Larson et al. 2002; Degen et al. 2013). In our study, we evaluated the genetic consequences of illegal trade of the vulnerable palm species *B. eriospatha*. One of the main issues assessed was whether *B. eriospatha* individuals that were illegally traded and planted outside their natural area have higher levels of genetic diversity than the ones found in wild populations.

Interestingly, the analysis of microsatellite allelic data revealed that the *B. eriospatha* individuals that were illegally traded had more genetic variation (i.e. allelic richness, expected heterozygosity) than all the studied wild *B. eriospatha* populations, suggesting that there is no preferred target source population for poachers. Private and rare alleles were also observed in greater numbers in the urban population than the wild populations. However, as sample size in population genetics approaches are *per se* a significant problem (Nazareno and Jump 2012), we must consider that the private alleles found in *B. eriospatha* individuals (56% of them being rare) from the urban area may be so rare in the wild that they were not present in our sample. Nevertheless, it is equally important to point out that based on the set of microsatellite markers used here (a total of 46 alleles in the wild populations), we

believe that our sample was adequate to detect low-frequency alleles. To be sure, there is no specific sample size required for such analysis; however, it is crucial for the sample to be representative of the wider population and thus it should be based on the degree of polymorphism of the genetic markers used.

While we found moderate to low genetic diversity in wild *B. eriospatha* populations, similar as the genetic diversity reported for other palm species (Dowe et al. 1997; Shapcott 1998; Perera et al. 2000; Gonzáles-Pérez et al. 2004; Shapcott et al. 2009; Jian et al. 2010), the continual decrease of *B. eriospatha* population sizes due to illegal trade and other deterministic factors (e.g. deforestation, habitat degradation, cattle grazing) can jeopardize the genetic variation that remains. Cornuet and Luikart (1996) pointed out that allelic richness is highly affected by population reduction due to the rapid elimination of rare alleles. In the studied wild *B. eriospatha* populations, the genetic consequences of human activities may have led to a loss of alleles (e.g. there is just one allele on locus *But18* in the G population; Table S2) and may contribute to the loss of other alleles in the near future (e.g. while allele 149 of locus *But11* could be lost in the B population, in C population this allele could be fixed; Table S2). Likewise, stochastic forces such as genetic drift can contribute to the loss and fixation of alleles, mainly if the *B. eriospatha* populations shrink in size and become spatially isolated. The loss of genetic diversity due to the threats facing this palm species is also reflected in the levels of inbreeding (i.e. fixation index) as observed in almost all of the studied wild *B. eriospatha* populations.

Similar to the results from the analysis of genetic diversity, the multilocus assignment exclusion-test also indicated that urban *B. eriospatha* individuals had varying origins. Even though we believe that our sample sizes were adequate, this result should be viewed with caution because we examined a modest number of individuals with nine microsatellite loci (H_E=0.49). For some species, Manel et al. (2002) reported a roughly consistent result of assignment test using eight microsatellite loci (H_E=0.60) with 30 to 50 individuals sampled per population. Thus, the set of microsatellites used in our analysis may have contributed to the number of individuals (n=23) that had more than one population assigned as the origin. Even though we were able to identify the origin of the majority of the *B. eriospatha* individuals (n=27 or 54%), we emphasize that our analysis could be improved if more polymorphic loci are added. Furthermore, the forensic analysis for this

palm species can be better clarified if other kinds of molecular markers (e.g. mitochondrial DNA) are used alongside nuclear markers (e.g. Nazareno et al. 2011; Nazareno and Reis 2011) to develop specific DNA profiles. DNA markers such as those suggested above have been validated in forensic analyses producing reliable results in identifying the geographic origin of a specimen (Avise et al. 1987; Campbell et al. 2003; DeYoung et al. 2003; Genton et al. 2005; Schwenke et al. 2006; Gomez-Diaz and Gonzalez-Solis 2007; Velo-Anton et al. 2007; Sanders et al. 2008; Degen et al. 2013).

The assignment test method used herein is appropriate when populations are significantly differentiated ($F_{ST} > 0.1–0.2$; Cornuet et al. 1999; Manel et al. 2002; Guinand et al. 2004). The moderately high inter-population differentiation over all populations ($F_{ST} = 0.17$) and the F_{ST} pairwise estimates were particularly notable for *B. eriospatha* populations, thus conforming to the conditions required for the exclusion-simulation significance test (Manel et al. 2002; Guinand et al. 2004). In this context, if a poacher claims to have obtained one *B. eriospatha* individual from the C population, but we believe that the individual came from the D population, an assignment test between the two populations can be easily undertaken (F_{ST} between C and D population=0.284, Table 2). However, it can be difficult to conduct such an analysis if this individual came from the A, B or H population ($F_{ST} = 0.03$-0.09, Table 2) which were grouped as one unit by the cluster analysis. While the structure analysis using the Bayesian algorithm allowed us to identify this group, the low pairwise F_{ST} values also supported this result. However, for the other *B. eriospatha* populations these estimates indicated that local adaptation and genetic drift are much stronger than gene flow. Furthermore, the lack of correlation between genetic differentiation and geographic distance suggest that an island model (Wright 1931, Maruyama 1970), rather than isolation by distance model, may best describe the population structure of this palm species. In fact, the island model is in line with the species' distribution pattern (e.g. *B. eriospatha* populations cover small areas with individuals in a clustered distribution) and with their specificity by habitat (highlands). However, even though the island model is biologically plausible for this palm species, the number of populations likely plays a role in shaping the population structure. As such, this issue can be better explored when samples from other populations become available.

Conservation perspectives

From a conservation perspective, the genetic diversity that exists in both wild *B. eriospatha* populations and illegally-traded individuals can be preserved. Although criminal charges and fines may be appropriate to control or decrease the illegal trade of this palm species, effective conservation strategies may be more feasible if compensatory mitigation (e.g. seed collection for genetic restoration) is targeted at the purchasers of illegally-traded *B. eriospatha* plants. Furthermore, even though *B. eriospatha* can adapt to varying local environments, its perpetuation in introduced habitats, like the urban area of Florianópolis, can be difficult since each individual is isolated and surrounded by buildings, homes, or motorways. As this palm species is able to reproduce by selfing (Nazareno and Reis 2012), genetic diversity can be lost in only a few generations due to inbreeding. In addition, as previously pointed out (Clegg et al. 2002; Estoup and Clegg 2003; Kolbe et al. 2004; Frankham 2005), colonization following introduction into an area can lead to genetic bottlenecks that would further reduce genetic variation. In light of this, we emphasize that conservation activities should be undertaken for this palm species. Otherwise, even though there is significant genetic variation in the urban *B. eriospatha* populations, this variation will become static in a few years because these individuals will become non-reproductive.

Whilst Brazil has the primary responsibility of protecting its own biological diversity, international agreements can provide additional assistance by cooperating in the protection of species (e.g. CITES; The Convention of Biological Diversity, CBD; International Union for Conservation of Nature, IUCN), their habitats (e.g. Biosphere Reserve Program; Ramsar Convention on Wetlands; World Heritage Convention), and ecosystem processes (e.g. United Nations Framework Convention on Climate Change, UNFCCC). We argue that it is time to update the conservation status of *B. eriospatha* in the IUCN from vulnerable to critically endangered due to the diverse threats facing it (e.g. cattle grazing, deforestation, illegal trade), as well as place this palm species in the Appendix I of the CITES. However, we expect that in next few years this species may be removed from protection acts. Scientific data about *B. eriospatha* (discussed herein and Nazareno and Reis 2012) can be used to stop the ongoing population decline and to prevent (local) extinction. However, Briggs (2009) pointed out that the difficulty of implementing effective conservation plans for many species

is often not due to a lack of scientific information but, rather, it is dependent on political, administrative and financial support (see Martín-Lópes et al. 2009 for a contraditory view). While we agree with this, Laurance et al. (2012) also warned that many of the published conservation studies have little relevance to real-world conservation activities. They importantly point out that this issue can be mainly explained by a lack of dialogue between conservation scientists and practitioners and by limited access to scientific information (i.e. few conservation managers and policy makers read the scientific literature).

In this context, our aim is to extend our research beyond academic borders. In order to facilitate this, we are forwarding our results to Brazil's Environmental Agency to ensure that *B. eriospatha* is included in the 2013 Agenda at the 16th meeting of the Conference of the Parties (COP 16), CITES in Thailand. We are also sending a report to Brazil's Public Ministry – an agency that monitors environmental protection activities and forwards complaints on to the Federal Court which is qualified to generate a Public Action – in order to inform the ministry about the criminal threats facing this palm species. In this latter document, the content will go beyond the results shown here and include a parallel analysis (AGN and MSR, unpublished data), where we show that almost all wild *B. eriospatha* populations (i) have a bimodal age structure made up of adult plants and seedlings, (ii) are highly threatened by cattle grazing, and (iii) would be at an extremely high risk of local extinction, with a greater than 50% reduction in the effective population size in only a few decades.

According with our findings, several conservation strategies are feasible for this species. For instance, sale of adult *B. eriospatha* individuals that are no longer reproductive may be permitted; however, for this to be implemented, long-term monitoring of wild populations and strict control by Brazil's Environmental Agency are required. Another possible conservation strategy for this species is related to cattle herding (AGN and MSR, unpublished data). Placing enclosures around some reproductive plants could encourage seedling survival, can thus prevent population decline. Since the fruits of *B. eriospatha* are appreciated and overexploited by local peoples, the develop of mechanisms that incorporate both use and conservation is a need also required in order to safeguard this genetic resource.

Finally, in this paper we demonstrate that the illegal trade of *B. eriospatha* is a real problem that must be quickly addressed. The use of

the molecular tools such those employed herein may be applied in future criminal investigations. However, a database containing information about allele frequencies from many populations and more informative molecular markers are necessary in order to obtain reliable results from forensic investigations. Investing in forensic databases and population genetics research are a necessary step in the conservation of this threatened palm species, particularly since public awareness is limited.

Acknowledgments

This study is part of the Doctoral Thesis (Plant Genetic Resources Program-Federal University of Santa Catarina, UFSC) of the first author. We are grateful to the Núcleo de Pesquisas em Florestas Tropicais (Rainforest Research Department) of the UFSC for assistance during field work. We would also like to thank the Physiology of Development and Plant Genetics Laboratory at UFSC for providing the infrastructure required for microsatellite analysis. The authors thank Dr. Evelyn R. Nimmo for editing the English of the manuscript and Renata Duzzioni for helping with Figure 1. AGN was supported by a doctoral fellowship from CNPq (National Council of Technological and Scientific Development) and MSR had financial support from CNPq (304724/210-6). The authors are grateful to FAPESC (Santa Catarina State Research Council, Brazil) for the financial support.

Supplementary Material

FIGURE 1S Ancestry of each individual to any of the six groups (each group is identified by one color), using all nine microsatellite markers analyzed, yielded by the Structure software. Bar length is proportional to the inferred ancestry values into each group for each individual.

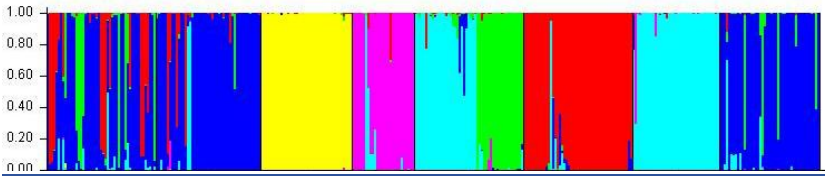


TABLE S1 Null allele frequency for eight *Butia eriospatha* (Martius ex Drude) Beccari populations sampled in Santa Catarina, Southern Brazil. * Presence of null allele based on Oosterhout's method.

	Populations							
Locus	A	B	C	D	E	F	G	H
But06	0.2153*	0.0462	0.2545*	0.1415*	0.2305*	0.3103*	0.0363	0.3007*
But07	-0.0370	-0.0331	0.1987*	0.1159	0.0969	0.1504*	0.2311*	-0.0795
But08	0.0716	0.0271	0.1956*	-0.4008	0.2192	0.2000*	0.2674*	-0.0590
But09	0.2409*	0.2247*	0.2204*	0.1089	0.0992	0.1998*	0.0462	0.3579*
But11	0.0784	-0.0547	0.1189	-0.2327	0.1426*	-0.2513	-0.1835	0.1766*
But16	0.1109	0.1145	0.2291*	0.1065	0.0000	0.2431*	0.2804*	0.1031
But17	0.1218	0.0889	0.2988*	-0.0318	0.2741*	0.2367*	0.2594*	-0.3099
But18	0.0131	0.1262	0.3389*	0.0345	0.2189	0.1941*	0.0000	0.0668
But23	-0.5725	-0.2095	-0.1760	-0.1025	0.0306	0.1241	0.2397*	-0.2424

TABLE S2 Allele frequency for eight *Butia eriospatha* (Martius ex Drude) Beccari populations sampled in Santa Catarina, Southern Brazil.

		Butia eriospatha populations							
Locus	Allele	A	B	C	D	E	F	G	H
But06	177		0.076		0.040	0.071		0.038	0.033
	179		0.304	0.703			0.278		0.033
	181	0.020	0.011	0.149	0.120		0.111		
	183	0.388		0.081	0.180	0.092	0.611	0.103	
	185	0.551	0.043	0.068	0.040	0.327		0.615	0.267
	187	0.041	0.565		0.480	0.510		0.244	0.667
	189				0.140				
But07	270				0.018			0.026	
	272							0.385	
	274				0.911			0.423	
	276		0.048		0.071	0.041	0.245		
	278	0.709	0.250	0.205		0.816	0.735	0.167	0.487
	280	0.291	0.702	0.795		0.112	0.020		0.513
	282					0.031			
But08	190					0.104			
	192					0.083		0.092	
	194	0.223	0.244		0.654	0.354	0.378	0.553	0.232
	196	0.032		0.146	0.077	0.229	0.051	0.250	0.024
	198	0.213	0.183	0.598		0.104	0.010	0.105	0.293
	200	0.117	0.061	0.171	0.269	0.125	0.551		0.024
	202	0.117	0.183	0.085			0.010		0.110
	204	0.191	0.159						0.146
	206	0.106	0.146						0.049
	208		0.024						0.122
But09	155								0.044
	159			0.192					0.044
	161		0.053	0.808	0.250	0.112	0.224		0.044
	163	0.422	0.574		0.286	0.214	0.367	0.538	0.633
	165	0.578	0.309		0.214	0.520	0.296	0.192	0.200
	167		0.064		0.143	0.153	0.112	0.269	
	169				0.036				0.033
	171				0.071				
But11	147					0.061	0.092		
	149	0.704	0.947	0.049	0.589	0.337	0.765	0.833	0.800
	151	0.296	0.053	0.951	0.411	0.602	0.143	0.167	0.200
But16	135								0.209
	137								0.012

	139	0.878	0.711	0.603	0.750	0.571	0.316	0.526	0.651
	141	0.122	0.289	0.397	0.250	0.429	0.684	0.474	0.128
But17	202	0.638	0.772	0.392	0.786	0.439	0.735	0.538	0.738
	204	0.362	0.228	0.671	0.214	0.561	0.265	0.462	0.262
But18	246	0.289	0.209	0.805	0.518	0.184	0.327		0.220
	250	0.711	0.791	0.195	0.482	0.816	0.673	1.00	0.780
But23	164	0.217	0.138	0.056					0.167
	168	0.511	0.500	0.625	0.346	0.333	0.564	0.526	0.578
	172	0.272	0.362	0.319	0.654	0.667	0.436	0.474	0.256

References

Alacs EA, Georges A, FitzSimmons NN (2010) DNA detective: a review of molecular approaches to wildlife forensics. Forensic Sci Med Pathol 6:180-194

Alves RRN, Filho GAP (2007) Commercialization and use of snakes in North and Northeastern Brazil: implications for conservation and management. Biodivers Conserv 16:969-985

Arroyo-Quiroz I, Pérez-Gil R, Leader-Williams (2007) Mexico in the international reptile skin trade: a case study. Biodivers Conserv 16:931-952

Avise JC, Arnold J, Martin Ball R, Bermingham E, Lamb T, Neigel JE et al (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann Rev Ecol Syst 18:489-522

Bodkin JL, Ballachey BE, Cronin MA, Scribner KT (1999) Population demographics and genetic diversity in remnant and translocated populations of sea otters. Conserv Biol 13:1378-1385

Briggs SV (2009) Priorities and paradigms: directions in threatened species recovery. Conserv Letters 2:101-108

Bueno E (1998) Náufragos, traficantes e degredados: as primeiras expedições ao Brasil. Editora Objetiva.

Campbell D, Duchesne P, Bernatchez L (2003) AFLP utility for population assignment studies: analytical investigation and empirical comparison with microsatellites. MOl Ecol 12:1979-1991

Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. J Heredity 99:616-613

Carvalho MMX (2006) O desmatamento das florestas de araucária e o Médio Vale do Iguaçu: uma história de riqueza madeireira e colonizações. Dissertation, Universidade Federal de Santa Catarina, Brazil

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621-631

Check E (2006) The tiger's retreat. Nature 441:927–930

CITES – Convention on International Trade in Endangered Species (2010) CITES Trade: a snapshot. http://www.cites.org/common/docs/CITES-trade-

snapshot-eng.pdf

Clegg SM, Degnan SM, Kikkawa J, Moritz C, Estoup A, Owens IPF (2002) Genetic consequences of sequential founder events by an island-colonizing bird. Proc Nat Acad Sci USA 99:8127–8132

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for inferring recent population bottlenecks from allele frequency data. Genetics 144:2001–2014

Cournuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989-2000

Degen B, Ward S, Lemes MR, Navarro C, Cavers S, Sebbenn AM (2013) Verifying the geographic origin of mahogany (*Swietenia macrophylla* King) with DNA-fingerprints. Forensic Sci International: Genetics 7:55-62

Destro GFG, Pimentel TL, Sabaini RM, Borges RC, Barreto R (2012) Efforts to combat wild animals trafficking in Brazil. In: Gbolagade Akeem Lameed (Org.). Biodiversity Enrichment in a Diverse World. InTech pp 421-436

DeYoung RW, Demarais S, Honeycutt RL, Gonzales RA, Gee KL, Anderson JD (2003) Evaluation of a DNA microsatellite panel useful for genetic exclusion studies in white-tailed deer. Wildlife Soc Bull 31:220–32

Dowe JL, Benzie J, Ballment E (1997) Ecology and genetics of *Carpoxylon macrospermum* H. Wendl. & Drude (Arecaceae), an endangered palm from Vanuatu. Biol Conserv 79:205-219

Estoup A, Clegg SM (2003) Bayesian inferences on the recent island colonization history by the bird *Zosterops lateralis lateralis*. Mol Ecol 12:657–674

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol Ecol 14:2611–2620

Foldenauer U, Borjal RJ, Deb A, Arif A, Taha AS, Watson RW, Steinmetz H, Bürkle M, Hammer S (2007) Hematologic and plasma biochemical values of Spix's Macaws (*Cyanopsitta spixii*). J Avian Med Surgery 21:275-282

Frankham R (2005) Resolving the genetic paradox in invasive species. Heredity 94:385

Genton BJ, Shykoff A, Giraud T (2005) High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. Mol Ecol 14:4275-4285

Gomez-Diaz E, Gonzalez-Solis J (2007) Geographic assignment of seabirds to their origin: combining morphologic, genetic, and biogeochemical analyses. Ecol Appl 17:1484–98

Gonzáles-Pérez MA, Caujapé-Castells J, Sosa PA (2004) Allozyme variation and structure of the Canarian endemic palm tree *Phoenix canariensis* (Arecaceae): implications for conservation. Heredity 93:307-315

Goudet J (2002) FSTAT: a program to estimate and test gene diversities and fixation indices. Version 2.9.3.2. http://www2.unil.ch/popgen/softwares/fstat.htm. Accessed 15 December 2012

Graham-Rowe D (2011) Endangered and in demand. Nature 480:S101-S103

Guinand B, Scribner KT, Topchy A, Page KS, Punch W, Burnham-Curtis MK (2004) Sampling issues affecting accuracy of likelihood-based classification using genetical data. Environmental Biology of Fishes 69:245–259

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

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resources 9:1322-1332

IBGE – Instituto Brasileiro de Geografia e Estatística (2004) Mapa da vegetação do Brasil e mapa de biomas do Brasil. http://www.ibge.gov.br

IUCN World Conservation Union (2012) Red List of Threatened Species. Version 2010.1. [cited 2012 Dec 15]. Available from: http://www.iucnredlist.org.

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. http://ibdws.sdsu.edu/. Accessed 12 December 2012

Jian S, Ban J, Ren H, Yan H (2010) Low genetic variation detected within the widespread mangrove species *Nypa fruticans* (Palmae) from Southeast Asia. Aquatic Botany 92:23-27

Kate Kt, Laird SA (1999) The commercial use of biodiversity. Earthscan Publications Ltd., London

Kenney JS, Smith JLD, Starfield AM, McDougal CW (1995) The long-term effects of tiger poaching on population viability. Conserv Biol 9:1127–1133

Kolbe JJ, Glor RE, Rodriguez-Schettino L, Chamizo-Lara A, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–181

Larsen HO, Olsen CS (2007) Unsustainable collection and unfair trade? Uncovering and assessing assumptions regarding Central Himalayan medicinal plant conservation. Biodivers Conserv 16:1679-1697

Larson S, Jameson R, Etnier M, Fleming M, Bentzen P (2002) Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. Mol Ecol 11:1899-1903

Laurance WF, Koster H, Grooten M, Anderson AB, Zuidema PA et al (2012) Making conservation research more relevant for conservation practitioners. Biol Conserv 153:164-168

Manel S, Berthier P, Luikart G (2002) Detecting wildlife poaching: Identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. Conserv Biol 16:650-658

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220

Martín-López B, Montes C, Ramírez L, Benayas J (2009) What drives policy decision-making related to species conservation? Biol Conserv 142:1370-1380

Maruyama T (1970) Effective number of alleles in a subdivided population. Theoret Popul Biol 1:273-306

MMA (Ministério do Meio Ambiente). 2008. In: Lista oficial de espécies da Flora brasileira ameaçada de extinção. Diário Oficial da União de 24 de setembro de 2008, nº 185. Seção 1, p. 75-83. http://www.mma.gov.br/estruturas/ascom_boletins/_arquivos/83_19092008034949.pdf. Accessed 8 November 2012

Morellato LPC, Haddad CFB (2000) Introduction: The Brazilian Atlantic Forest. Biotropica 32:786-792

Muth R, Bowe J (1998) Illegal harvest of renewable resources in North Amercia: towards a typology of the motivations for poaching. Society and Natural Resources 11:9-24

Mutterback M (2012) Leopard poaching is a bigger problem in India than previously believed. http://news.mongabay.com/2012/1030-mutterback-leopard-poaching.html

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853-858

Natusch DJD, Lyons JA (2012) Exploited for pets: the harvest and trade of amphibians and reptiles from Indonesian New Guinea. Biodivers Conserv 21:2899-2911

Nazareno AG, Jump AS (2012) Species-genetic diversity correlations in habitat fragmentation can be biased by small sample sizes. Mol Ecol 21:2847-2849

Nazareno AG, Reis MS (2011) The same bus different: monomorphic microsatellite markers as a new tool for genetic analysis. Am J Bot e265–e267

Nazareno AG, Reis MS (2012) Linking phenology to mating system: exploring the reproductive biology of the threatened palm species *Butia eriospatha*. J Heredity 103:842-852

Nazareno AG, Zucchi MI, Reis MS (2011) Microsatellite markers for *Butia eriospatha* (Arecaceae), a vulnerable palm species from the Atlantic Rainforest of Brazil. Am J Bot 98:e198–e200

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590

Overbeck GE, Muller SC, Fidelis A, Pfadenhauer J, Pillar VD, Blanco CC, Boldrini II, Both R, Forneck ED (2007) Brazil's neglected biome: The South Brazilian Campos. Perspectives in Plant Ecology, Evolution and Systematics 9:101-116

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol 13:55-65

Perera L, Russel JR, Provan J, Powell W (2000) Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (*Cocos nucifera* L.). Genome 43:15-21

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536-539

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Rannala B, Mountain J (1997) Detecting immigration by using multilocus genotypes. Proc Nat Acad Sci USA 94:9197-9201

Redford KH (1992) The empty forest. BioScience 42:412-422

Reis MS, Mantovani A, Silva JZ, Mariot A, Bittencourt R, Nazareno AG, Ferreira SK, Steiner F, Montagna T, Silva AALS, Fernandes CD, Altrak G, Figueredo LG. 2012. Distribuição da diversidade genética e conservação de espécies arbóreas em remanescentes florestais de Santa Catarina. In: Vibrans AC, Sevegnani L, Gasper AL, Lingner DV, editors. Inventário florístico florestal de Santa Catarina. v. 1 Blumenau: Edifurb.

Reitz R (1974) Palmeiras – Parte 1. Flora ilustrada catarinense, Herbário Barbosa Rodrigues, Brazil.

RENCTAS (2011) Rede Nacional de Combate ao Tráfico de Animais Silvestres. 1º Relatório Nacional sobre o Tráfico de Fauna Silvestre. http://www.renctas.org.br/. Accessed 14 December 2012

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223-225

Roman J, Bowen BW (2000) The mock turtle syndrome: genetic identification of turtle meat purchased in the south-eastern United States of America. Animal Conservation Biology 3:61-65

Rosa IL, Oliveira TPR, Osório FM, Moraes LE, Castro ALC, Barros GML, Alvez RRN (2011) Fisheries and trade of seahorses in Brazil: historical perspective, current trends, and future directions. Biodivers Conserv 20:1951-1971

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under

Sanders JG, Cribbs JE, Fienberg HG, Hulburd GC, Katz LS, Palumbi SR (2008) The tip of the tail: molecular identification of seahorses for sale in apothecary shops and curio stores in California. Conserv Genet 9:65-71

Schwenke PL, Rhydderch JG, Ford MJ, Marshall AR, Park LK (2006) Forensic identification of endangered Chinook Salmon (*Oncorhynchus tshawytscha*) using a multilocus SNP assay. Conserv Genet 7:989-989

Shapcott A (1998) The genetics of *Ptychosperma bleeseri* a rare palm from the Northern Territory, Australia. Biol Conserv 85:203-209

Shapcott A, Dowe JL, Ford H (2009) Low genetic diversity and recovery implications of the vulnerable Bankoualé Palm *Livistona carinensis* (Arecaceae), from North-eastern Africa and Southern Arabian Peninsula. Conserv Genet 10:317-327

Shepherd CR, Nijman V (2008) The trade in bear parts from Myanmar: an illustration of the ineffectiveness of enforcement of international wildlife trade regulations. Biodivers Conserv 17:35-42

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Velo-Anton G, Godinho R, Ayres C, Ferrand N, Rivera AC (2007) Assignment tests applied to relocate individuals of unknown origin in a threatened species, the European pond turtle (Emys orbicularis). Amphibia-Reptilia 28:475-484

Wasser S, Poole J, Lee P, Lindsay K (2010) Elephants, ivory, and trade. Science 327:1331-1332

Wasser SK, Shedlock AM, Comstock K et al (2004) Assigning African elephant DNA to geographic region of origin: applications to the ivory trade. Proc Nat Acad Sci USA 101:14847–14852

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the analysis of population structure. Evolution 38:1358-1370

Wilkie DS, Bennett EL, Peres CA, Cunningham AA (2011) The empty forest revisited. Ann NY Acad Sci 1223:120-128

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97:159

CONCLUSÕES

Com base em indicadores genéticos e ecológicos, este estudo contribuiu para o preenchecimento da lacuna de conhecimento que impedia uma avaliação adequada do *status* de conservação de *Butia eriospatha*. Nesse contexto, os resultados obtidos indicaram que *B. eriospatha* encontra-se em risco extremamente alto de extinção local em decorrência de atividades antrópicas relacionadas ao uso da terra e da exploração de seus recursos. Enquanto pressões antrópicas, como a venda ilegal de indivíduos de *B. eriospatha* e a fragmentação de habitats têm implicações diretas para a diminuição de seus tamanhos populacionais e redução de diversidade genética, a presença do gado – componente que vem alterando a regeneração natural da espécie – tem contribuído, indiretamente, para a diminuição de suas populações. Os resultados indicaram que planos de conservação são necessários para a espécie, principalmente para populações de *B. eriospatha* que apresentam tamanhos populacionais reduzidos em decorrência destas estarem mais susceptíveis às estocasticidades demográfica e genética. Em vista dos níveis e da distribuição da diversidade genética presentes nas populações naturais, atesta-se a importância de se estabelecer unidades de conservação que contemplem populações da espécie. Em decorrência dos níveis de diversidade genética observados nos indivíduos ilegalmente comercializados, a criação de bancos de germosplama torna-se também imprescíndivel para conservar a variação genética que ainda resta. Haja vista as ameaças e a ausência de planos de conservação para a espécie, com base nos resultados obtidos neste estudo, recomendações para a conservação de *B. eriospatha* foram indicadas, destacando-se: mapear e indicar populações com alta prioridade de conservação (i.e., populações com tamanhos populacionais reduzidos); monitorar as populações remanescentes; combater o tráfico ilegal; impedir o comércio internacional da espécie, inserindo-a no Apêndice I da CITES (Convenção que regula o comércio internacional de espécies ameaçadas da flora e da fauna); informar e alertar as pessoas que comercializam a espécie sobre a importância de conservá-la e o ônus de não fazê-lo; criar áreas protegidas e proporcionar alternativas econômicas aos agricultores em locais de ocorrência da espécie e impedir a herbivoria do gado sobre o componente regenerante, cercando alguns indivíduos reprodutivos. Enquanto as recomendações apresentadas neste estudo não esgotam todas as ações que podem beneficiar a conservação de *B. eriospatha*, elas devem ser consideradas

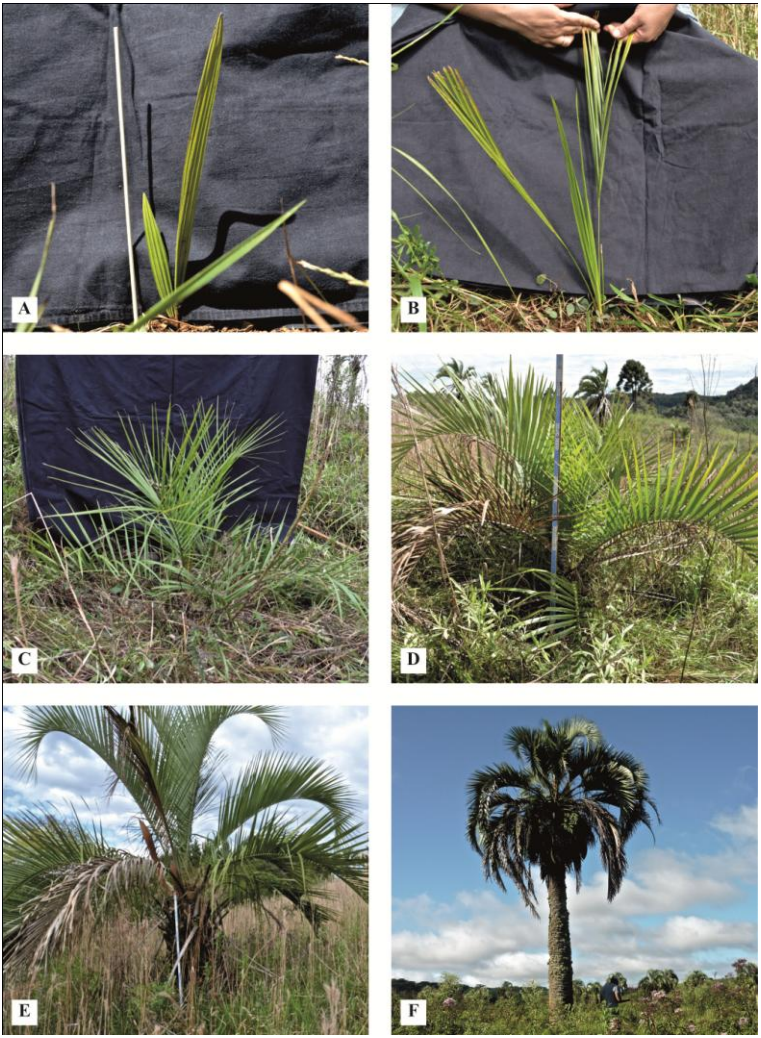
com alta prioridade no estabelecimento de estratégias efetivas para a sua conservação.

ENCAMINHAMENTOS FUTUROS

O objetivo desta tese vai além das informações biológicas obtidas para *Butia eriospatha*. Dois documentos estão sendo preparados e serão encaminhados para o IBAMA e para o Ministério Público Federal. O intuito é que a espécie possa constar no Apêndice I da CITES (Convenção que regula o comércio internacional de espécies ameaçadas da flora e da fauna). Além disso, com teor de denúncia, é esperado que o documento encaminhado ao Ministério Público e ao IBAMA possa dar subsídio para que medidas efetivas para conservar a espécie sejam realizadas. Enquanto estas ações possam ser efetivas, recomenda-se que *B. eriospatha* seja contemplado com estudos adicionais aos apresentados nesta tese. Estudos sobre o fluxo gênico contemporâneo, o entendimento da estrutura genética em fina escala, da biologia reprodutiva, da dinâmica demográfica em populações sem a presença do gado e o conhecimento dos níveis de diversidade genética em toda a área de ocorrência da espécie são necessários para a conservação deste recurso genético.

APÊNDICES

APÊNDICE 1. Estádios ontogenéticos (A-F) de *Butia eriospatha* (Martius ex Drude) Beccari obsevados em uma população no município de Matos Costas, Santa Catarina, Brasil. A descrição dos estádios ontogenéticos encontra-se no Capítulo 4.



APÊNDICE 2. Indicativo do comércio ilegal de *Butia eriospatha* (Martius ex Drude) Beccari na cidade de Florianópolis, Santa Catarina. Os orçamentos foram obtidos nas floriculturas Verde & Cia Garden Center e Primavera Garden Center, em Novembro de 2012.

verde&cia garden center

Ac/ Sr. Alison

Tel : 9994 - 8882

alisongn@hotmail.com

Florianópolis, 13 de Novembro 2012.

Orçamento de Material

Qtd.	Unid.	Especificação	Tam.	Preço unit.	Preço total
2	md	*Butia tronco (altura total + - 0,00m)*	6,00	R$ 4.500,00	R$ 9.000,00
1	pct	*Forthy plantio*	25 Kg	R$ 85,00	R$ 85,00
1	um	*Hormonio enraizador*	1 Lts	R$ 45,00	R$ 45,00
6	um	*Estaca*	1,50	R$ 15,00	R$ 90,00
6	m!	*Substrato para plantio*	m!	R$ 155,00	R$ 930,00
40	m	*Corda*	m	R$ 5,00	R$ 200,00
		SERVIÇOS TERCEIRO			
1	um	*Frete carreta (para transporte das plantas)*		R$ 1.450,00	R$ 1.450,00
1	um	*Serviço de guincho (plantio das plantas)*		R$ 780,00	R$ 780,00
2	um	*Container*		R$ 120,00	R$ 240,00

Total das mercadorias	R$	12.820,00
Mão-de-obra	R$	1.400,00
Total	R$	14.220,00

Obs. as covas estão a 3,00m da estrada conforme cliente

Condições:
Validade da proposta: *20 dias.*
De entrega: imediata / conforme disponibilidade de estoque .
De pagamento: *a negociar.*

André Bovee

Sem mais, atenciosamente .
Aceite do cliente:_____ Data: ___ / ___ / 2012.

Florianópolis 48 3234 0000 - Biguaçu 48 3285 0000
sac@verdecia.com.br

Florianopolis, 12 de novembro 2012

Rod. SC 401 km 04 Saco Grande Florianopolis- SC Fone(48)3238-1156
CNPJ:81.521.668/0001-86

Segue abaixo orçamento conforme solicitação :

Descrição	Porte (m)	Qtde	Valor Uni.	Valor Total
Butiá	5mt tronco	2	4.750,00	9.500,00
			Total	9.500,00

Vendedor: Lucas

Formas de pagamento: 50% do valor no pedido e 50% na entrega.
Entreganos no local,porém não fazemos plantio,lembrando que vais precisar de um guindaste para efetuar o plantio .

APÊNDICE 3. Demonstrativo do comércio ilegal de *Butia eriospatha* (Martius ex Drude) Beccari: o modo como a espécie é retirada em campo está ilustrado em A e B; as figuras C a F ilustram alguns dos locais em Florianópolis, SC, onde a espécie está plantada.