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DINÂMICA TEMPORAL E ESPACIAL DA VARIAÇÃO GENÉTICA DE UM PRIMATA DA MATA ATLÂNTICA AMEAÇADO DE EXTINÇÃO, *Leontopithecus rosalia* (Linnaeus, 1766)

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UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE
DARCY RIBEIRO – UENF
CAMPOS DOS GOYTACAZES
MARÇO DE 2017

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Aprovada em 09 de março de 2017

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Dedico este trabalho aos pais agricultores – representados nos meus pais – que trabalharam de sol a sol para formar filhos doutores e, ainda assim, são comprometidos com a preservação do meio ambiente. A todas essas famílias que depositam na educação sua esperança e, mesmo na simplicidade, constroem um Brasil melhor. Dedico também àqueles que, muitas das vezes no anonimato, trabalham pela conservação dos recursos naturais e pelo direito de todo ser humano ter acesso a eles. Espero que o meu trabalho não seja em vão e que ele contribua com a conservação do meio ambiente para o favorecimento de todos.

AGRADECIMENTOS

Agradeço primeiramente Deus por toda ajuda e consolo em todos os momentos da minha vida. Muito obrigada.

Ao meu orientador Carlos Rámon Ruiz-Miranda e coorientadores Milton Cézar Ribeiro e Pedro Manoel Galetti Jr pela confiança depositada, pelos ensinamentos durante a minha formação acadêmica, pelas oportunidades de crescimento pessoal e profissional e por toda a ajuda, amizade e compreensão nos momentos adversos de dificuldade.

Aos laboratórios LabMC/UFSCar e LEEC/UNESP pelo apoio financeiro e suporte logístico.

À Associação Mico-leão-dourado pelo suporte e pela troca de ideias. Obrigada a todos, especialmente, à Andreia Martins e equipe de campo pelo fornecimento de dados e coleta de amostras. Sem vocês esse trabalho não seria possível. Ele vem de vocês e é para vocês.

Às professoras Adriana Grativol, Patrícia Freitas e Lucia Lorini por me receberem nos seus laboratórios, pelo apoio logístico e pelas contribuições.

À Peter Galbusera e Kristel De Vleeschouwer pelo suporte financeiro e contribuições na minha formação.

À UENF e a CAPES pelo apoio financeiro e concessão de bolsa de estudo.

À coordenação do Programa de Pós Graduação em Ecologia e Recursos Naturais, UENF, pelas contribuições que viabilizaram a realização desse trabalho. Em particular, agradeço a secretaria do curso, Elizabete Costa, sempre pronta a nos ajudar.

À Dra. Maria Lúcia Lorini, pela revisão desta tese.

À M. King, nativo na língua inglesa, pela tradução do texto.

Aos membros da banca examinadora. Por terem aceitado o convite de participar da avaliação deste trabalho e por suas contribuições.

Aos amigos e colegas de trabalho, essenciais nesse processo. Pelas contribuições na minha formação acadêmica e pelo apoio psicológico. Obrigada, especialmente:

(i) À dupla, Brenda Alexandre (LabVert) e Bernardo Niebuhr (LEEC) que me acompanharam desde o começo e em todas as etapas. Pela amizade que se fortaleceu e por todas as contribuições: “tamojuntos”;

(ii) À todos membros do LabMC/UFSCar, minha família são-carlense. Pela amizade, acolhida e parceira dentro e fora do laboratório. Agradeço especialmente à Mariana Correa, Rosane Santos e Carmem Ruíz pela hospedagem e apoio; Paola Ayala e Carla Gestich pelas discussões do trabalho; à Gisele Oréfice, Ueslei Lopes, Regina Yabe, Bruno Saranholi, Camilla Alves, Alline Braga e Andiara Silos pelas contribuições e por serem meu alento em momentos de dificuldade;

(iii) Ao Arystene Nicodemo e Paola Ayala por me auxiliarem nos trabalhos de bancada, principalmente, quando me ausentei para a qualificação;

(iv) À todos os membros do LEEC/UNESP. Pela amizade, acolhida e parceria. Agradeço especialmente à John Ribeiro, Carolina Carvalho, Renata Muylaert, Paola Tokumoto e Márcio Morais pelas revisões, sugestões e ensinamentos. E, à Irene Mendoza pela acolhida e por me receber na sua casa e sua família;

(v) À Adriana Grativol, Malinda Henry, Talitha Mayume, Marianna Louro, Eliliane Vasconcelos e Marcelita Marques pelas revisões e sugestões, hospedagem e por me representarem na UENF nos momentos em que estive afastada;

À minha família amada: minha mãe Joana, meu pai Paulo, minha irmã Andreza, meu cunhado Rogy e meu companheiro Alisson. Pela ajuda, apoio, compreensão e “puxões de orelha” durante essa caminhada. Com vocês, as dificuldades se tornaram sorrisos.

À todos que, com boa intenção, colaboraram para a realização e finalização desse trabalho.

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RESUMO

O mico-leão-dourado é um exemplo de primata arborícola ameaçado de extinção devido à fragmentação e perda de seu habitat original. Atualmente ele encontra-se distribuído na Bacia do Rio São João, estado do Rio de Janeiro, Brasil. Ele já esteve criticamente ameaçado, mas melhorou sua categoria de ameaça devido à realização de programas de conservação que incluíram, dentre outras medidas: (1) a reintrodução em vida livre de animais nascidos no cativeiro; e, (2) a translocação de grupos sociais isolados em fragmentos florestais no litoral do estado do Rio de Janeiro para um contínuo de florestas na Reserva Biológica União. Depois que essas medidas foram aplicadas, a população cresceu na natureza. Porém, até o presente momento, nenhum outro estudo havia avaliado em nível molecular as consequências dessas medidas de manejo para a diversidade genética. Nós amplificamos 14 loci de microssatélites em 239 indivíduos de mico-leão-dourado capturados em dois períodos de tempo: histórico (1996-1997) e recente (2007-2009). O tamanho efetivo populacional da população translocada aumentou através do tempo, enquanto que na população reintroduzida tendeu a diminuir. Já a diversidade genética variou caso a caso, de acordo com o número de fundadores das novas populações (translocada e reintroduzida) e sua taxa de sobrevivência e dispersão. Provavelmente, devido ao pouco ou nenhum fluxo gênico após a fundação das novas populações, as populações translocada e reintroduzida também passaram por um processo de estruturação genética ao longo do tempo, constituindo grupos genéticos correspondentes aos locais de soltura de seus fundadores. Contudo, faltava-nos saber quais elementos da paisagem estão causando essa estruturação. Essa informação torna-se fundamental para o planejamento de medidas de restauração da conectividade funcional e para evitar que as populações voltem a perder a diversidade genética. Por isso, correlacionamos o relacionamento genético obtido entre 201 indivíduos residentes na Bacia do Rio São João no período recente com (1) o manejo realizado, (2) a distância, (3) a presença de estradas e (4) a resistência de diferentes superfícies da paisagem ao movimento do mico-leão-dourado atribuída por 12 especialistas que trabalham na conservação

da espécie. O manejo e a resistência da paisagem foram as variações que melhor explicaram o relacionamento genético entre indivíduos de mico-leão-dourado, mostrando que a heterogeneidade da paisagem influência a dispersão do primata. Portanto, é importante que a relação distância-custo seja levada em consideração durante o delineamento de estratégias de restauração da conectividade funcional. Algumas populações de mico-leão-dourado encontram-se recentemente isoladas e podem perder diversdidade genética ao longo do tempo. Outras, porém, comportam-se como uma metapopulação, mantendo maior diversidade genética. É preciso que medidas de conservação sejam realizadas para resgatar as populações isoladas e para aumentar a conectividade da paisagem na porção norte da Bacia do Rio São João. Nós esperamos que, se a conectividade funcional for restaurada, indivíduos de micos-leões-dourados poderão se dispersar por até 8 km de distância e metapopulações em equilíbrio sejam mantidas nesse limiar.

Palavras-chaves: dispersão; genética da conservação; genética da paisagem; manejo; primatas neotropicais.

ABSTRACT

The golden lion tamarin is an example of arboreal primate endangered due to fragmentation and loss of the original habitat. It is currently distributed in the São João River Basin, state of Rio de Janeiro, Brazil. It has already been critically endangered but has improved its threat category due the implementation of conservation programs that included, among other measures: (1) reintroduction of captive animals into wild life, and (2) translocation of isolated social groups into forest fragments on the coast of the state of Rio de Janeiro to a continuous forest in the União Biological Reserve. After these measures were applied the wild population grown. However, to date, no other study has evaluated at the molecular level the consequences of these management strategies for genetic diversity. We amplified 14 microsatellite loci in 239 golden lion tamarins captured in two periods: historical (1996-1997) and recent (2007-2009). The effective population size of the translocated population increased over time, while in the reintroduced population it tended to decrease. Genetic diversity varied on a case-by-case basis, according to the number of founders in the new populations (translocated and reintroduced) and their survival rate and dispersal. Probably, due the little or absence of gene flow after the founding of new populations, the translocated and reintroduced populations also underwent into a genetic structuring process over time, constituting genetic groups corresponding to the release sites of their founders. However, we lacked to know which elements of the landscape are causing this structure pattern. This information is essential for future approaches aiming to restore functional connectivity and to prevent the reduction of genetic diversity on these populations again. Therefore, we correlated the genetic kinship obtained among 201 individuals living in the São João River Basin in the recent period, with the (1) type of management performed, (2) distance, (3) presence of roads, and (4) landscape resistance to the movement of golden lion tamarins, attributed by 12 specialists working on the conservation of this species. The management and the landscape resistance were the variables that best explained the genetic kinship between golden lion tamarin individuals, showing that the heterogeneity of the landscape

influences the dispersal of this primate. Therefore, the distance-cost relationship must be taken into account during the design of functional connectivity restoration strategies. For example, some populations of golden lion tamarins are recently isolated and may lose genetic diversity over time. Others, however, behave as a metapopulation, maintaining greater genetic diversity. Further, conservation measures need to be undertaken to rescue isolated populations and to increase the connectivity of the landscape in the northern portion of the São João River Basin. We hope that the restoration of the functional connectivity will make the individuals of the golden lion tamarins disperse to up 8 km of distance and that a metapopulation equilibrium can be maintained at this threshold.

Keywords: dispersal; conservation genetic; landscape genetic; management; Neotropical primates.

INTRODUÇÃO GERAL

Considerações iniciais

Atualmente, os processos antropogênicos têm sido os principais responsáveis pela extinção das espécies (Mace *et al.* 2008). Eles são considerados fatores de ameaças quando, de alguma forma, causam o declínio populacional (Mace & Lande 1991). Dentre eles, a perda e a alteração dos habitats têm sido os processos com maior destaque e maior preocupação dentro da biologia da conservação (Fahrig 2003; Akçakaya *et al.* 2007; Fischer & Lindenmayer 2007). A perda dos habitat consiste na redução da quantidade de habitat disponível na paisagem, enquanto que alteração inclui processos como a fragmentação, que se caracteriza pelo rompimento da continuidade do habitat (Fahrig 2003). Juntos a perda, a degradação e a subdivisão dos habitat alteram a biologia e o comportamento das espécies e podem, eventualmente, contribuir com seu declínio populacional (Fischer & Lindenmayer 2007). A perda do habitat afeta negativamente toda a biodiversidade. Já a fragmentação, pode afetar tanto negativa como positivamente as relações ecológicas, dependendo das características biológicas de cada espécie (Fahrig 2003). Algumas espécies de morcegos, por exemplo, podem aumentar sua abundância em paisagens fragmentadas, devido maior acesso a sítios de forrageio e abrigos (Ethier & Fahrig 2011).

Dessa forma, a conservação de uma dada espécie em uma paisagem antropogênica vai depender da interação entre os processos de ameaça que estão ocorrendo e as características biológicas daquela espécie (Ovaskainen & Hanski 2001; Mace *et al.* 2008). O tamanho populacional, a abrangência da distribuição geográfica, a estocasticidade demográfica e a probabilidade de extinção dentro de um dado tempo, vão definir o grau de ameaça de cada espécie nesse novo cenário da paisagem (Mace *et al.* 2008). Provavelmente, as espécies mais generalistas sofrem menos impactos negativos, devido à sua dieta generalista, a alta abundância em habitat degradados e uma ampla distribuição geográfica – ex. *Alouatta seniculus* (Cosson *et al.* 1999). Em contraposição, as espécies mais especialistas, como o *Ateles hybridus*, são mais afetadas pelo processo de perda e fragmentação dos habitats, porque elas geralmente possuem

uma dieta restritiva, requerem grandes áreas para sua subsistência e raramente se movem entre manchas de florestas (Link *et al.* 2010).

Os primatas arborícolas são um bom exemplo de táxon animal ameaçado pelo processo de fragmentação dos habitats, devido às suas restrições ecológicas que incluem, entre outras características, a dependência da continuidade da floresta para realizar movimentos (Arroyo-Rodríguez & Mandujano 2009). Espécies mais sensíveis a uma dada ameaça, como os primatas, são consideradas espécies guarda-chuva e são usadas para definir o nível mínimo aceitável em que a ameaça pode ocorrer. Assim, os requisitos de movimento exigidos pela maioria das espécies de primatas são usados para definir as estratégias de conectividade da vegetação, porque abrangem os requisitos de movimento exigidos por outros táxons (Lambeck 2007).

A restrição do movimento é a principal resposta das espécies animais ao processo de modificação da paisagem (Lindenmayer & Fischer 2006; Fischer & Lindenmayer 2007). A perda e, principalmente, a fragmentação dos habitats restringem as estratégias do movimento animal em diferentes escalas, tais como o movimento diário realizado dentro da área de vida e a dispersão para outro território ou outra subpopulação dentro de uma metapopulação (Lindenmayer & Fischer 2006). Se o movimento é interrompido por causa do isolamento dos habitats, a população pode se tornar estruturada e perder diversidade genética ao longo do tempo devido à falta de fluxo gênico (Ortego *et al.* 2008; Habel *et al.* 2015). Por isso é importante que estudos que procuram entender a dinâmica e a persistência das populações em paisagens fragmentadas usem medidas de dispersão efetiva, que não envolvam apenas o movimento do indivíduo, mas também, o sucesso reprodutivo na nova população. É importante também, avaliar se ambos os sexos respondem da mesma forma às modificações da paisagem, porque o isolamento pode resultar em manchas de habitat com indivíduos de um único sexo (Lindenmayer & Fischer 2006).

Dessa forma, a dispersão é um dos principais processos para conservação das espécies (Nathan 2008; Henriques-Silva *et al.* 2015). Ela também é fundamental para a manutenção da dinâmica de recolonização metapopulacional (Hanski 1994). A conservação de uma dada espécie vai depender da sua capacidade de se adaptar ao novo cenário da paisagem e de funcionar como uma metapopulação (Hanski 1998). Porém, sabemos pouco sobre

o processo de dispersão efetiva através de paisagens modificadas. Isso acontece, porque dados de dispersão efetiva são muito difíceis de obter por meio de técnicas de campo (Lindenmayer & Fischer 2006; Driscoll 2007). Uma maneira eficiente de se obter informações acerca da dispersão entre manchas de habitat e através dos diferentes elementos que compõem uma matriz é usando ferramentas de genética da paisagem (e.g., Taylor *et al.* 2011; Munguia-Vega *et al.* 2013; Schwalm *et al.* 2014; Mullins *et al.* 2015).

Tradicionalmente estudos de genética de populações se restringiam a testes de isolamento por distância (Sork & Waits 2010). No entanto, graças ao aumento do número de marcadores moleculares disponíveis (Segelbacher *et al.* 2010) e da facilidade de acesso aos recursos de sistemas de informação geográfica (Habel *et al.* 2015), foi possível incorporar outras variáveis espaciais aos estudos de genética de populações. Dessa maneira, se tornou possível também, inferir sobre como as mudanças da paisagem estão afetando o comportamento, a sobrevivência e a reprodução das espécies (Sork & Waits 2010). Porém, além das múltiplas escalas espaciais, também as escalas temporais afetam os diferentes processos biológicos das espécies, como a dispersão e o fluxo gênico (Anderson *et al.* 2010; Segelbacher *et al.* 2010; Keis *et al.* 2013; Davis *et al.* 2014). Assim, um dos maiores desafios metodológicos dentro da biologia da conservação, atualmente, é interpretar como os padrões genéticos espaciais são o resultado de múltiplos processos bióticos e abióticos que operam em diferentes escalas de tempo e espaço (Sork & Waits 2010).

Influência dos processos temporais e espaciais sobre a variação genética

Devido sua importância para a biologia da conservação, a variação genética tem sido um crescente alvo de investigação (Segelbacher *et al.* 2010). Ela pode ser afetada por múltiplas escalas espaciais e temporais (Anderson *et al.* 2010; Keis *et al.* 2013; Davis *et al.* 2014; Habel *et al.* 2014). Desde 2003, o número de publicações científicas que tem usado variáveis espaciais para explicar a variação genética tem crescido substancialmente (Fig. a). A ciência que explica a influência das características da paisagem sobre a variação genética é chamada genética da paisagem. Ela foi citada pela primeira vez por volta dos anos de 1980, mas somente no ano 2003 foi proposta como uma ciência (Manel

et al. 2003). Manel *et al.* (2003) definiram a genética da paisagem como uma disciplina que integra as características espaciais com processos micro evolutivos, como fluxo gênico, deriva genética e seleção. Anos mais tarde, ela foi redefinida para integrar a genética de populações, a ecologia da paisagem e as estatísticas espaciais (Storfer *et al.* 2007).

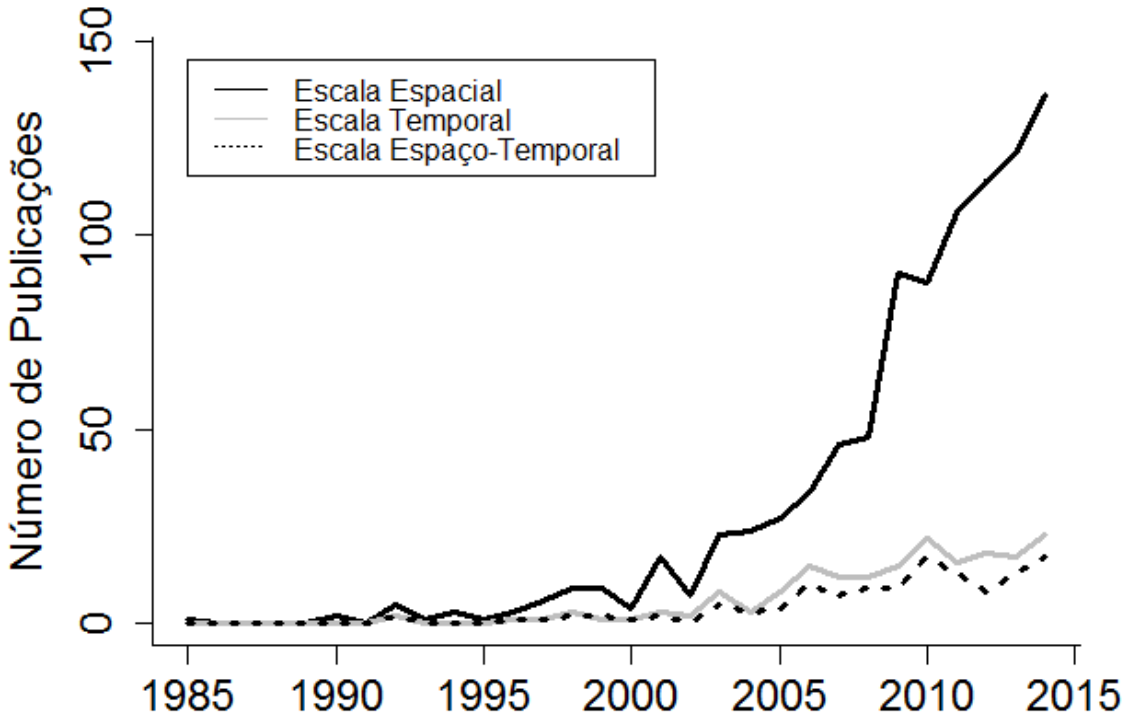

Figura a – Número de publicações encontradas no banco de dados do SCOPUS contendo no título, resumo ou palavras-chaves os termos: (1) *landscape genetic* + *population* + *conservation*, (2) *temporal scale* + *conservation genetic*, e (3) *spatial scale* + *temporal scale* + *genetic conservation*.

Os estudos de genética da paisagem permitem avaliar a contribuição da variação da paisagem, em diferentes escalas espaciais, para a estrutura genética dentro e entre as populações (Sork & Waits 2010). Já os modelos temporais ajudam a entender quais forças determinam a diferenciação das populações (Bezault *et al.* 2011; Guillemaud *et al.* 2011) e testam quais as causas da perda de diversidade genética ao longo do tempo (Ramakrishnan *et al.* 2005; Habel *et al.* 2014, 2015). As amostragens em múltiplas escalas temporais permitem também: (1) monitorar e avaliar os programas de conservação, tais como medidas

de reintroduções e translocações de indivíduos (Jason Kennington *et al.* 2012; Smyser *et al.* 2013); e (2) planejar novas medidas de conservação, uma vez que, conhecemos a causa primária de diferenciação genética (Habel *et al.* 2014). Enquanto as escalas espaciais são importantes para avaliarmos o efeito da paisagem sobre a variação genética (Storfer *et al.* 2007; 2010), as escalas temporais são importantes para investigarmos eventos demográficos e declínios populacionais (Ortego *et al.* 2011; Bristol *et al.* 2013; White *et al.* 2014).

Conjuntamente, as amostragens em múltiplas escalas espaciais e temporais podem ajudar a identificar eventos passados e atuais que governam a diferenciação das populações (Habel *et al.* 2014). Por ser a dinâmica e estruturação populacional resultado de múltiplos processos temporais, espaciais e biológicos; amostragens genéticas espaço-temporais tendem a aumentar o poder das análises e geram informações mais precisas sobre mecanismos de diferenciação das populações e sobre seu *status* de ameaça (Habel *et al.* 2015). Entretanto, apesar das vantagens listadas, o número de publicações que incorporam dados temporais é muito menor que aqueles que incorporam amostragens espaciais. Menor ainda tem sido o número de pesquisas que incorporaram ambas as escalas de espaço e tempo (Fig. a). Possivelmente, isso ocorre por causa da dificuldade de se obter amostras temporais e de analisá-las (Habel *et al.* 2014).

Se a heterogeneidade genética temporal não é considerada, a diferenciação populacional pode ser interpretada de forma equivocada, especialmente, em situações em que a estruturação é fraca (Storfer *et al.* 2007). Somente quando amostragens espaços-temporais múltiplas de uma população forem incluídas nas pesquisas de genética da conservação, seremos capazes de identificar empiricamente os efeitos recentes de processos de longo prazo (Habel *et al.* 2014), tais como a fragmentação (e.g., Reed *et al.* 2011) e declínios populacionais (e.g., Bristol *et al.* 2013; Yuan *et al.* 2014). Por isso, em condições ideais, estudos que buscam mensurar a resposta dos organismos às diferentes estruturas da paisagem, usando a variação genética, devem definir previamente sua escala espacial e temporal de análise (Anderson *et al.* 2010).

O Mico-leão-dourado como espécie modelo

Taxonomia e status de conservação

Leontopithecus rosalia (Linnaeus, 1766), conhecido como mico-leão-dourado (GLT, sigla em inglês), é uma espécie de primata do gênero *Leontopithecus*, da família Cebidae e infraordem Platirrini (Groves, 2001). O gênero *Leontopithecus* reúne as espécies mais exuberantes da família, todas ameaçadas de extinção: *L. rosalia* (Linnaeus, 1766) (mico-leão-dourado), *L. chrysomelas* (Kuhl, 1820) (mico-leão-da-cara-dourada), *L. chrysopygus* (Mikan, 1823) (mico-leão-preto) e *L. caissara* Lorini & Persson, 1990 (mico-leão-da-cara-preta). As espécies de micos-leões são preferencialmente frugívoras, adaptam-se bem a vegetações secundárias ou perturbadas e são territorialistas (Auricchio, 1995). Apesar do atual contexto de ameaças, o mico-leão-dourado representa um dos poucos mamíferos em todo mundo – e o único exemplo de primata – que melhorou seu *status* de conservação devido aos programas de manejo e conservação (Hoffmann *et al.* 2011). Antes do manejo ele era classificado pela "União Internacional para a Conservação da Natureza" (IUCN, sigla em inglês) na categoria "criticamente ameaçada", sendo que atualmente é considerado "em perigo", devido à redução de seu habitat e isolamento de suas populações (Kierulff *et al.* 2008).

Distribuição geográfica e área de estudo

O mico-leão-dourado (GLT) é endêmico da Floresta Atlântica do estado do Rio Janeiro. Originalmente, ele se distribuía por toda a baixada costeira do estado do Rio de Janeiro, em áreas abaixo dos 550 m de altitude (Kierulff & Rylands 2003). Devido à perda e isolamento de seus habitats, a porção principal de sua área de distribuição atual é a Bacia do Rio São João (SJRB, sigla em inglês), localizada entre 22º50'S, 42º40'W e 22º20'S, 42º00'W, na região centro-norte do estado do Rio de Janeiro, Brasil. Nossa área de estudo engloba a SJRB e a Reserva Biológica União (REBIO União), que juntas representam o principal conjunto de fragmentos de ocorrência atual do GLT (Fig. b).

A Bacia é originalmente composta por florestas de Mata Atlântica que inclui floresta ombrófila densa, montana, submontana e de terras baixas, mangues, brejos e restingas (Bidegain & Pereira 2005). As formações florestais

nas áreas planas são altamente fragmentadas e isoladas, principalmente, por pastagens. Além disso, a passagem da rodovia federal BR-101 no interior da SJRB subdivide-a em duas grandes porções. Na porção norte encontra-se a área de maior cobertura vegetal e conectividade florestal. Nela encontram-se também os maiores remanescentes florestais, situados nas áreas montanhosas da Bacia. Já a porção sul tem a menor cobertura florestal, ocorrendo predominantemente na matriz de pasto. Apesar de ser mais fragmentada, a porção sul detém também importantes fragmentos florestais sob o ponto de vista da conservação do mico-leão-dourado, como é o caso da Reserva Biológica de Poço das Antas (PDA) e do fragmento florestal de Rio Vermelho (REVR) (Procópio de Oliveira *et al.* 2008; Seabra 2012).

Entre meados da década de 70 e 80 houve uma expressiva construção de canais de drenagem de água e redução da cobertura vegetal. Já entre 1995 e 2010 houve um expressivo aumento das áreas urbanas. Em compensação, nesse mesmo período, houve também um acréscimo no número de áreas florestadas. Associadamente, nos anos para o quais foram relatados decréscimos da cobertura natural, houve também um aumento da classe de pastagem, agricultura e vegetação secundária e vice-versa. Entre o período de 1995 e 2010 em torno de 125 km^2 de coberturas naturais foram convertidas para agricultura, pastagem ou vegetação secundária, enquanto que 163 km^2 foram convertidas no sentido oposto (Seabra 2012).

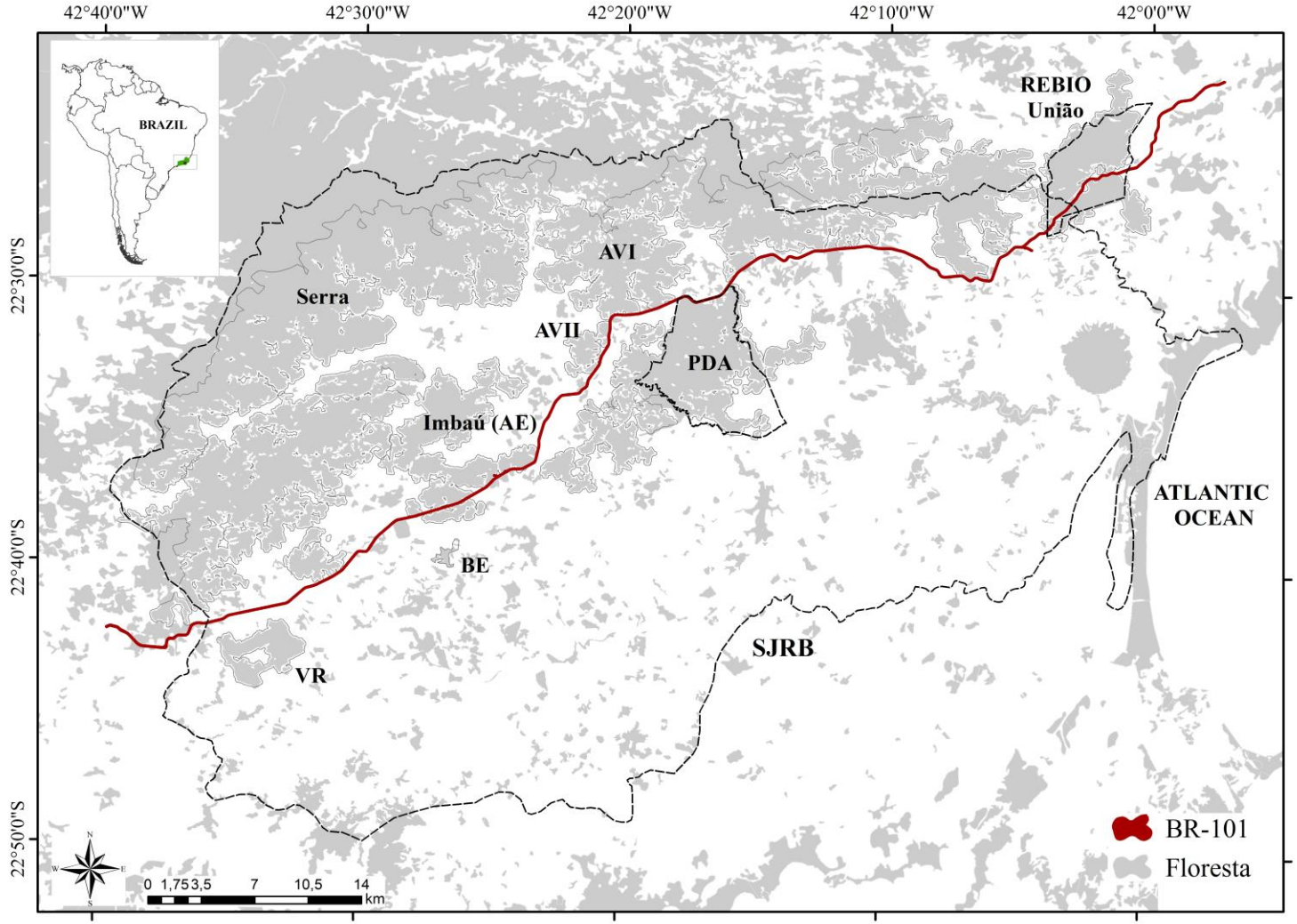

Figura b – Área de estudo e principal área de ocorrência do *L. rosalia* na Bacia do Rio do São João (SJRB) e REBIO União, Rio de Janeiro, Brasil. As siglas indicam os fragmentos florestais com ocorrência conhecida da espécie e amostrados nesse estudo, incluindo a Reserva Biológica de Poço das Antas (PDA) e remanescentes florestais distribuídos em propriedades particulares – Serra, VR, BE, AVI e AVII.

Ecologia

Quando se trata da conservação de uma espécie ameaçada, devido à redução e isolamento de seu hábitat, é preciso também considerar o seu comportamento e adaptação ao processo de modificação da paisagem. Os GLTs são altamente sociais, com sistemas fechados de difícil aceitação de dispersores (Baker & Dietz 1996). Esses comportamentos tornam a dispersão mais difícil, sendo a espécie dotada de uma estruturação natural causada pela alta sociabilidade e pela constituição de grupos familiares. Tais características acabam sendo intensificadas, quando as populações estão inseridas numa paisagem fragmentada, com o isolamento dos habitat (Di Fiore & Valencia 2014).

A dispersão dos micos-leões é realizada por ambos os sexos, porém casos de imigração de fêmeas são mais raros e relacionados com a substituição de uma fêmea reprodutora que sumiu do grupo. Os machos migram com mais facilidade e são admitidos num novo grupo com comportamentos de injúria advindos de indivíduos do mesmo sexo (Baker & Dietz 1996). A dispersão é realizada, geralmente, por duplas de machos migrantes aparentados com 2 a 4 anos de idade, que repõem ou se unem aos machos reprodutores pré-existentes no grupo. Já as fêmeas se tornam vagantes em sua maioria. Eles começam a reproduzir antes dos 4 anos, idade em que a taxa reprodutiva começa a se igualar aos machos reprodutivamente maduros (Holst *et al.* 2006).

Normalmente, os micos-leões são monogâmicos, sendo as filhas subordinadas às suas mães e com potencial reprodutivo inferior. Porém, quando em habitats saturados e com recursos intensamente explorados, as oportunidades de reprodução fora do grupo natal são reduzidas e a poliginia feminina estimulada (Baker & Dietz 1996). A gravidez das filhas ocorre mais comumente com a presença de machos migrantes sem parentesco. No entanto, não é claro se as cópulas das filhas são realizadas com machos extra grupais ou em relações incestuosas (Dietz & Baker 1993). No remanescente de Rio Vermelho, um fragmento isolado na porção sul da SJRB e onde foram reintroduzidos indivíduos de cativeiro, os grupos sociais aumentaram sua taxa de poliginia em 75%, diminuindo o potencial de reprodução fora do grupo natal (Coelho 2009).

O comportamento social, a concepção do que é um o habitat adequado e as características estruturais da paisagem podem estar limitando o movimento e a

conectividade funcional do habitat dos micos-leões (Zeigler *et al.* 2011; Di Fiore & Valencia 2014). Paralelamente, análises genéticas indicaram baixa conectividade e ineficiente dispersão de *L. chrysomelas*, mesmo em uma paisagem relativamente contínua (Moraes 2011). Apesar de alguns autores relatarem ter visualizado a dispersão de GLTs atravessando áreas abertas, os registros são muito raros, mostrando que esses ambientes representam barreiras potenciais para a dispersão da espécie (Dietz *et al.* 1997; Grativol *et al.* 2001; Coelho 2009). Além disso, a maioria das pesquisas com o táxon têm se restringido ao território dos grupos, dentro das florestas (Dietz *et al.* 1997; Raboy & Dietz 2004), sendo nosso conhecimento acerca da movimentação desses primatas através da matriz inter-habitat ainda muito limitado.

Segundo Mickelberg (2011) a distância média percorrida pelo GLT é 847 m. A pesquisadora, usando de dados de monitoramento da Associação Mico-Leão-Dourado (AMLD), observou que maioria dos animais realizava movimentos entre 400 e 600 m e poucos viajavam por mais de 2 km. Ainda, com base nesses dados de movimentação, Mickelberg (2011) delimitou onze unidades funcionais de manejo metapopulacional na SJRB (Fig. c). Até o presente momento nos faltava saber se o movimento observado por Mickelberg (2011) tem sido suficiente para manter o fluxo gênico e conservar a diversidade genética. Além disso, não sabíamos também quais elementos espaciais tornam a paisagem mais ou menos permeável aos movimentos do GLT.

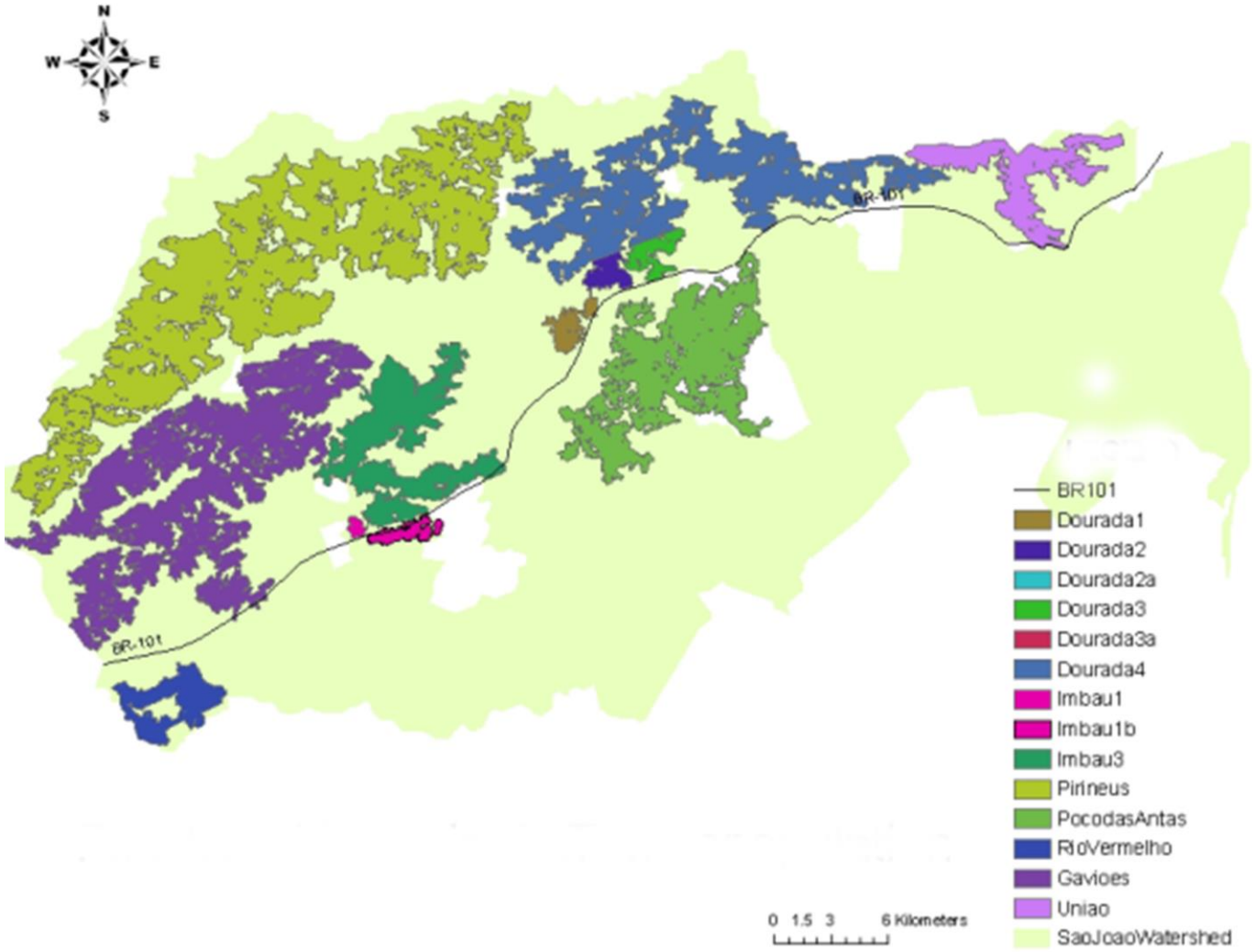

Figura c – Onze unidades funcionais do *L. rosalia* na Bacia do Rio São João delimitadas por Mickleberg (2011) usando dados de marcação e recaptura (Fonte: Mickelberg 2011).

História de conservação

Nos anos 1960 a 1970, Adelmar F. Coimbra-Filho chamou a atenção para a destruição do habitat do mico-leão-dourado na região da SJRB, estado do Rio de Janeiro. Nesse período, as áreas florestais mais importantes para a conservação dos GLTs localizavam-se nos município de Silva Jardim, Casimiro de Abreu e Cabo Frio, ao longo do Rio São João. Em 1964 o mico-leão-dourado foi incluído na lista de espécies ameaçadas de extinção. Em 1975 restavam apenas 10% de floresta densa e 30% de floresta degradada na sua área de distribuição. De 1969 a 1971, os esforços de conservação foram centralizados no estabelecimento de uma área protegida para a conservação do mico-leão-dourado e, em 1974, foi criada a Reserva Biológica de Poço das Antas (PDA) com 3000 ha, mais tarde ampliada para 5000 ha (Rylands *et al.* 2002; Kierulff *et al.* 2012).

Na década de 1980 se iniciaram os principais programas de conservação, incluindo a reintrodução na SJRB de animais nascidos em cativeiro. Isso foi feito com o intuito de recuperar as populações silvestres de GLT que estavam ameaçadas de extinção, devido à redução drástica de seu hábitat e seu tamanho populacional, relatado para o período de 1960 e 1970. No ano de 1981 começou o Programa de Conservação do Mico-leão-dourado e em 1992 foi criada a Associação Mico-Leão-Dourado (AMLD). Em 1991 se iniciou o levantamento completo das florestas dentro da área original de ocorrência do GLT no Rio de Janeiro e em 1994 grupos ameaçados foram translocados para 2400 ha de mata na Fazenda União (atual Reserva Biológica União), no município de Rio das Ostras, a 20 km de PDA (Kierulff *et al.* 2012).

Um dos objetivos do manejo foi conservar a diversidade genética das populações selvagens, através do aumento do tamanho populacional do GLT e do resgate de grupos ameaçados (Kierulff *et al.* 2002). Por isso, foram translocados para a antiga Fazenda União 42 micos-leões-dourados, oriundos de seis grupos sociais que estavam isolados em fragmentos de 20-200 ha situados no litoral do Rio de Janeiro. Em 1983, foram reintroduzidos em fazendas particulares na SJRB 146 micos-leões-dourados da geração F1 nascida em cativeiro (de 33 fundadores distribuídos em 30 zoológicos) e, no ano de 2000, 7 indivíduos nascidos em vida livre. Como resultado desses esforços, a população silvestre aumentou para mais de 1000 indivíduos, distribuídos na SJRB (Kierulff *et al.* 2012). Além disso,

análises de pedigree mostraram que a população reintroduzida reteve 96% da sua diversidade genética em relação à população original (Mickelberg 2011).

O mico-leão-dourado é um exemplo de primata que esteve criticamente ameaçado pela intensa redução e isolamento de seus habitat (Kierulff *et al.* 2008). Porém, graças aos diferentes esforços de conservação aplicados para salvar a espécie da extinção (Kierulff *et al.* 2012), seu *status* de conservação melhorou e suas populações voltaram a crescer na natureza. Atualmente, o programa de conservação do GLT é reconhecido internacionalmente, por ele ser o único exemplo de primata do mundo a melhorar sua categoria de ameaça devido às medidas de conservação (Procópio de Oliveira *et al.* 2008). Além disso, é um dos poucos exemplos de programa de conservação de longo prazo que inclui monitoramento populacional e a coleta de material genético por anos consecutivos. Por todas estas razões o GLT representa uma oportunidade ideal de avaliação de medidas de manejo aplicado para conservação de uma espécie de primata sensível à fragmentação e sua adaptação durante esse processo.

Objetivos

O mico-leão-dourado esteve em vias de ser extinto na natureza. Por causa disso, desde o início da década de 80, medidas de conservação vem sendo realizadas para salvar a espécie na natureza. Desde então e, principalmente, após a criação da AMLD, os animais residentes na SJRB e REBIO União foram periodicamente monitorados (Rylands *et al.* 2008; Kierulff *et al.* 2012). Durante esse período, amostras genéticas de gerações de animais reintroduzidos, translocados e nativos foram sendo coletadas. Associadamente, a dinâmica temporal do espaço na sua principal área de ocorrência foi analisada (Seabra 2012). Como resultado, Seabra (2012) observou pouca variação na cobertura florestal da SJRB entre os ano de 1995 a 2010. Devido a essas condições conjuntamente, o mico-leão-dourado oferece uma oportunidade ímpar de se avaliar a variação de processos ecológicos no espaço e no tempo, usando ferramentas genéticas que são as mais indicadas quando se pretende medir a dispersão efetiva (Lindenmayer & Fischer 2006; Driscoll 2007).

Apesar das pesquisas realizadas até o momento, pouco se sabe sobre o padrão de movimentação dos GLTs através dos elementos espaciais que compõem uma paisagem fragmentada. Pesquisas já mostraram que o sucesso e o engajamento na dispersão podem estar sendo influenciados pelo tamanho e proximidade de fragmentos e pela complexidade da matriz (Mickelberg 2011; Moraes 2011). Além disso, os GLTs são naturalmente estruturados, devido ao seu sistema social fechado com baixas oportunidades de dispersão e reprodução (Moraes 2011; Di Fiore & Valencia 2014). Nesse contexto, atualmente uma das prioridades para a conservação da espécie é estabelecer a conectividade funcional de modo a otimizar o impacto do movimento sobre sua demografia, genética e distribuição através da paisagem (Kierulff *et al.* 2012). Antes, porém, precisamos entender como as diferentes escalas de tempo e espaço afetam a estrutura genética populacional do GLT e, dessa forma, avaliar e indicar medidas de conservação.

Por isso, esse estudo teve como objetivo avaliar a dinâmica temporal e espacial da variação genética da(s) população(ões) de *Leontopithecus rosalia* inserida(s) numa paisagem fragmentada com o propósito de:

(1) comparar a dinâmica temporal genética de populações reintroduzidas e translocadas de um primata da Mata Atlântica ameaçado de extinção e inserido num habitat fragmentado com de uma população nativa;

(2) entender como os diferentes elementos espaciais presentes em uma paisagem fragmentada afetam a dispersão de um primata neotropical arborícola, e;

(3) propor cenários de estruturação populacional do mico-leão-dourado para, a partir deles, indicar prioridades para sua conservação.

Como nosso foco é a conservação de primatas arborícolas em paisagens fragmentadas e, sendo a dispersão o principal processo de conservação das espécies (Henriques-Silva *et al.* 2015); fizemos previamente uma revisão sobre a dispersão de primatas neotropicais arborícolas em paisagens modificadas pela perda e fragmentação dos habitat.

Estrutura da tese

O objetivo principal desse projeto de pesquisa foi entender como as interferências antropogênicas – particularmente a fragmentação – afetam a conservação de primatas neotropicais arborícolas. Durante o processo de fragmentação dos habitats, as mudanças nas estratégias de movimento e dispersão são as principais respostas dos primatas à redução da conectividade da paisagem. Por isso, no **capítulo 1,** fizemos uma revisão sobre o que sabemos e o que falta explorar acerca do movimento e dispersão de primatas neotropicais arborícolas em paisagens fragmentadas. Nos capítulos subsequentes, usamos o mico-leão-dourado como uma espécie modelo para testar empiricamente algumas hipóteses e gerar informações úteis para a conservação de diferentes espécies de primatas arborícolas, ou de espécies ameaçadas de extinção com restrições ecológicas similares.

O mico-leão-dourado é uma espécie para a qual medidas de conservação têm sido aplicadas numa curta escala de tempo. Por isso, no **capítulo 2**, usando ferramentas de genética da conservação, nós analisamos como diferentes medidas de manejo – particularmente os programas de reintrodução e translocação de animais – afetaram a estrutura genética do mico-leão-dourado através de duas gerações. Isso foi feito, porque diferentes processos, em diferentes escalas temporais e espaciais, afetam a dinâmica e estrutura das espécies.

Uma vez identificada a influência das medidas de manejo sobre a variação genética do mico-leão-dourado, no **capítulo 3**, nós investigamos como diferentes variáveis da paisagem – tais como distância, presença de estradas e resistência da paisagem – têm afetado a dispersão efetiva do mico-leão-dourado. Por fim, no **capítulo 4**, reunimos as informações acerca da dinâmica temporal e espacial do mico-leão-dourado para avaliar o atual cenário de sua conservação dentro de um contexto metapopulacional. A partir desse cenário, nós propomos direcionamentos para os futuros planos de manejo e conservação da espécie.

CAPÍTULO 2

TEMPORAL GENETIC DYNAMICS OF REINTRODUCED AND TRANSLOCATED POPULATIONS OF THE ENDANGERED GOLDEN LION TAMARIN (*Leontopithecus rosalia*)[1]

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Author Contributions

AMM, CRRM, MCR and PMGJr designed the research; ADG and PMGJr provided logistical support for molecular analysis; PMGJr provided analytical tools and advised data analysis; CRRM, MCR, ADG, PMGJr provided financial support; AMM conducted the molecular analysis; LAF contributed genotyping database; JMD and CMK contributed field database; AMM and CSC analyzed data; all authors contributed to review the manuscript; and AMM wrote the manuscript.

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[1] Manuscrito publicado na revista “Conservation Genetics” (DOI 10.1007/s10592-017-0948-4)

Abstract

Reintroductions – captive-born animals introduced into the species' original distribution area – and translocations – free-living animals transferred to another location within the historical distribution area – are important conservation strategies for endangered species. Genetic analyses of 240 individuals from unmanaged, translocated and reintroduced populations of *Leontopithecus rosalia* were performed using 14 microsatellites. These samples were collected during two periods: (a) 1996–97 (historic), when individuals were translocated and reintroduced into forest fragments in the lowland Atlantic Forest, and (b) 2007–09 (recent). We hypothesized that effective population size and genetic diversity would increase over time and that these management strategies would affect the resulting population genetic structure. We found trends indicating that the effective population size at the translocation site increased while that at the reintroduction sites diminished over time. The inbreeding coefficient of the translocated population diminished over time (from 0.38 to 0.03) and was much lower than that of the native (0.29) and reintroduced (0.13) recent populations. We observed a greater genetic admixture among the reintroduced sites on the historic sampling, as well as a strong genetic structure at the translocation site. In the recent sampling, the population structuring became more site-related suggesting low or inconsistent gene flow between sampling sites. This research highlights how conservation management decisions have an important influence in the genetic outcome of translocations and reintroductions. Future conservation planning should consider population genetic monitoring before and after management measures and maintain population connectivity thereafter to avoid the negative effects of a population size reduction.

Keywords: conservation genetic; endangered species; genetic management; microsatellite; temporal genetic sampling.

Introduction

Reintroductions (captive-born animals introduced into the species' original distribution area) and translocations (free-living animals transferred to another location within the historical distribution area) (Kleiman 1989) are strategies useful to reverse the decline of endangered species and avert extinction (Griffith *et al.* 1989; Kleiman 1989; Fischer & Lindenmayer 2000; Seddon *et al.* 2007; IUCN/SSC 2013). The objective of these conservation strategies is to establish viable populations over time by increasing population size of new or existing populations and by increasing or maintaining genetic diversity (Griffith *et al.* 1989; Kleiman 1989; Sigg *et al.* 2005). Since reintroductions emerged as a conservation option, the number of reintroduced and translocated animal species has increased (for review, see Fischer & Lindenmayer 2000; Seddon *et al.* 2007), the same occurring for the number of successful programs (e.g., Parker 2008; Michaelides *et al.* 2015; Mowry *et al.* 2015).

Two of the main goals of translocation and reintroduction programs are to increase genetic diversity and to counteract the effects of inbreeding depression in small populations (IUCN/SSC 2013). Due to these putative genetic outcomes, several studies have sought to understand the genetic effects of reintroduction or translocation on genetic diversity, for example *Notiomystis cincta* (Brekke *et al.* 2011), *Mustela nigripes* (Cain *et al.* 2011), *Vulpes velox* (Cullingham & Moehrenschlager 2013; Sasmal *et al.* 2013), and *Psittacula echo* (Tollington *et al.* 2013). Few of these studies have used more than one sampling period to characterize the genetic diversity of these managed species (e.g., Cullingham & Moehrenschlager 2013; Tollington *et al.* 2013), which makes it difficult to monitor the consequences of reintroduction and translocation over time. Genetic monitoring over time is especially important for the planning of management strategies that will guarantee persistence of wild animals, particularly when they were reintroduced or translocated to a fragmented habitat (De Barba *et al.* 2010). Nevertheless, these measurements are rarely available.

The golden lion tamarin, *Leontopithecus rosalia*, provides an example of successful reintroductions and translocations. It is the only primate whose threat status has improved (from "critically endangered" to "endangered", Kierulff *et al.* 2008) through conservation efforts supported by translocations and reintroductions

(Procópio de Oliveira *et al.* 2008). *L. rosalia* is considered a model for similar conservation programs, particularly those developed for threatened species and/or Neotropical arboreal primates. One of the goals of the translocation and reintroduction of *L. rosalia* was to increase and recover the genetic diversity of wild populations (Kierulff *et al.* 2002). Yet, the consequences of these interventions for genetic diversity have not been quantified at the molecular level. This species provides an opportunity to assess temporal and spatial genetic processes in a species reestablished via reintroductions and translocations into a fragmented landscape.

The aim of this research was to assess whether the conservation programs supported by reintroduction and translocation were effective in increasing and recovering genetic diversity and, consequently, in the conservation of this endangered species. To achieve this, we assessed the genetic variability within and between (a) unmanaged wild, (b) translocated, and (c) reintroduced *L. rosalia* populations sampled in fragments of the Atlantic Forest with varied degrees of habitat loss and fragmentation over two time periods. Although this is a fairly limited timeframe to observe changes in genetic diversity (≤ 2 generations), two study periods generating genetic indices are more robust to evaluate management strategies than a single sampling period (De Barba *et al.* 2010; Habel *et al.* 2014). We hypothesized that (i) the effective population size (N_e) would increase over time as field surveys indicated a population increase, (ii) the genetic diversity would increase in accordance with the expectation for the effective population size, and (iii) the translocated and reintroduced populations would undergo genetic structuring due to founder effects, and the subsequent population isolation caused by habitat fragmentation. On the other hand, we expected that the native population would remain as a single population over time and distinct from the other populations due to two factors: it was not genetically managed and it remained isolated from the other populations.

Methods

The golden lion tamarin as a model species

The golden lion tamarin is an endangered, small arboreal primate – average body mass 598-620 g (Dietz *et al.* 1994) – endemic to the Atlantic Forest,

Rio de Janeiro State, Brazil, and threatened with extinction (Kierulff *et al.* 2008). Their social system consists of small family groups (3–14 individuals) with cooperative breeding and a typically monogamous mating system with occasional polygamy (Baker *et al.* 1993; Dietz & Baker 1993; Baker *et al.* 2002). Tamarins reach sexual maturity between 18–24 months of age (Dietz *et al.* 1994; Baker *et al.* 2002), and both sexes disperse with bias toward male dispersal (Baker & Dietz 1996; Baker *et al.* 2002). Successful reproduction is usually achieved around four years of age; generation time varies between six to seven years (Holst *et al.* 2006).

In 1964, *L. rosalia* was included in Brazil's first list of threatened species. In 1975, estimates suggested less than 400 individuals remained in the wild (Coimbra-Filho & Mittermier 1977). At this time, *L. rosalia* occurred in only two regions along São João River Basin (SJRB), Rio de Janeiro state (RJ), Brazil: Poço das Antas Biological Reserve (PDA) and the private forest fragments in northwestern SJRB (Fig. 2.1). These small populations were considered incapable of sustaining themselves over time without demographic intervention and the restoration of forest habitat (Kleiman *et al.* 1986). Therefore, a long-term conservation program began in 1981 and included, among other measures, (i) the reintroduction of captive animals in SJRB, and (ii) the translocation of wild isolated social groups to a continuous forest of a private farm, currently protected as União Biological Reserve (REBIO União) (Kleiman *et al.* 1986; Beck *et al.* 1991; Kierulff 2000; Kierulff *et al.* 2002).

In 1984, the reintroduction program for *L. rosalia* started based on criteria including the number of individuals in captivity, habitat suitable for the species, and the necessity of increasing genetic diversity of the wild population. Between 1984 and 2000, 147 individuals born in captivity were reintroduced into the wild. The reintroduced population had a pedigree lineage distribution equivalent to a captive population (Ballou *et al.* 2002; Mickelberg 2011). The individuals were reintroduced in social groups in forest fragments uninhabited by and distant from other areas occupied by the species. Generally, one social group was reintroduced per forest fragment, except in large fragments (e.g., REVR in Fig. 2.1). By 1997, 95% of the individuals were reintroduced. However, only 14% of the captive-born reintroduced animals remained alive at this date. When the

reintroductions were concluded, the number of reintroduced *L. rosalia* and their offspring born in the wild reached 359 individuals (Kierulff *et al.* 2002).

Translocations began later than reintroductions. The decision to translocate wild individuals of *L. rosalia* was made after a regional-wide survey done between 1990–92 detected small populations living in small fragments (0.2 – 2 km^2), with high risk of total habitat loss (Kierulff & Rylands 2003). Between 1994-1997 all 42 tamarins from six social groups inhabiting four isolated coastal forest fragments were translocated to REBIO União (Fig. 2.1). This location was chosen due to its large size (around 2,500 ha) of preserved forest uninhabited by and distant from other areas occupied by the species (Kierulff & Procópio De Oliveira, 1996; Kierulff 2000). Even though the landscapes and timescales of the translocations and reintroductions were different, this study is a heuristic comparison of two different conservation actions on genetic diversity.

Study area and sample collection

The study area is located within the current *L. rosalia* distribution: SJRB and REBIO União (Fig. 2.1). SJRB is characterized by remnants of Atlantic Forest, a highly fragmented and threatened biome (Ribeiro *et al.* 2009). REBIO União is adjacent to SJRB and currently comprises 2,548 ha. The forests of both conservation units (SJRB and REBIO União) are divided in two sections by the BR 101 federal highway (ICMBio 2016). The vegetative cover is taller and the mean linear distance between fragments is less in the northern part than in the southern portion of BR 101 (Procópio de Oliveira *et al.* 2008). The descendant generations of the reintroduced captive-born animals as well as the unmanaged (native) *L. rosalia* are distributed throughout SJRB; the descendant generations of the translocated wild groups are distributed throughout REBIO União (Holst *et al.* 2006).

The geographic locations of the native, reintroduced, and translocated social groups sampled in this study in 1996–97 (hereafter referred to as ‘historic’), and in 2007 and 2009 (hereafter referred to as ‘recent’) are given in Figure 2.1. In the historic period we sampled the reintroduced individuals and their descendants, and the translocated individuals; while in the recent period only descendant individuals of both conservation strategies were sampled. We collected hair samples at six sampling sites. One site contained social groups of native

individuals (NT) inhabiting forests in the Serra, one site contained social groups of translocated individuals (TR) inhabiting in the REBIO União, and four sites contained social groups of reintroduced individuals (RE). Reintroduced sites were further classified according to their geographic locations: REAE (Andorinha-Estreito fragment in the region of Imbaú); REAVI (São Francisco and Igarapé fragments, Aldeia Velha region); REAVII (Vale do Cedro fragment, also Aldeia Velha region); and, REVR, known as Rio Vermelho, which is a large and isolated forest fragment (1,740 ha) containing approximately 20 social groups. Two fragments (PDA and *Parque Municipal do Mico-Leão-Dourado* – PMLD) both with known native animals were not sampled (Fig. 2.1).

We collected hair samples from 239 individuals from around 65 social groups; some of them were resampled between the study periods (Fig. 2.1). Eighty-two individuals were from the historic period, and 152 individuals were from the recent period. For REAE, additional samples (n = 5) were included from an intermediate period (2003). Animal capture followed the protocol described in Dietz *et al.* (1994). Hair samples of animals were collected by the Golden Lion Tamarin Association (*Associação Mico-Leão-Dourado* – AMLD) field team over consecutive years and stored in silica. We selected the individuals and sampling sites for this study with the assistance of AMLD field records, which identify the individual, sex, age or age category, date of birth, date of reintroduction and the social group of origin in a given period.

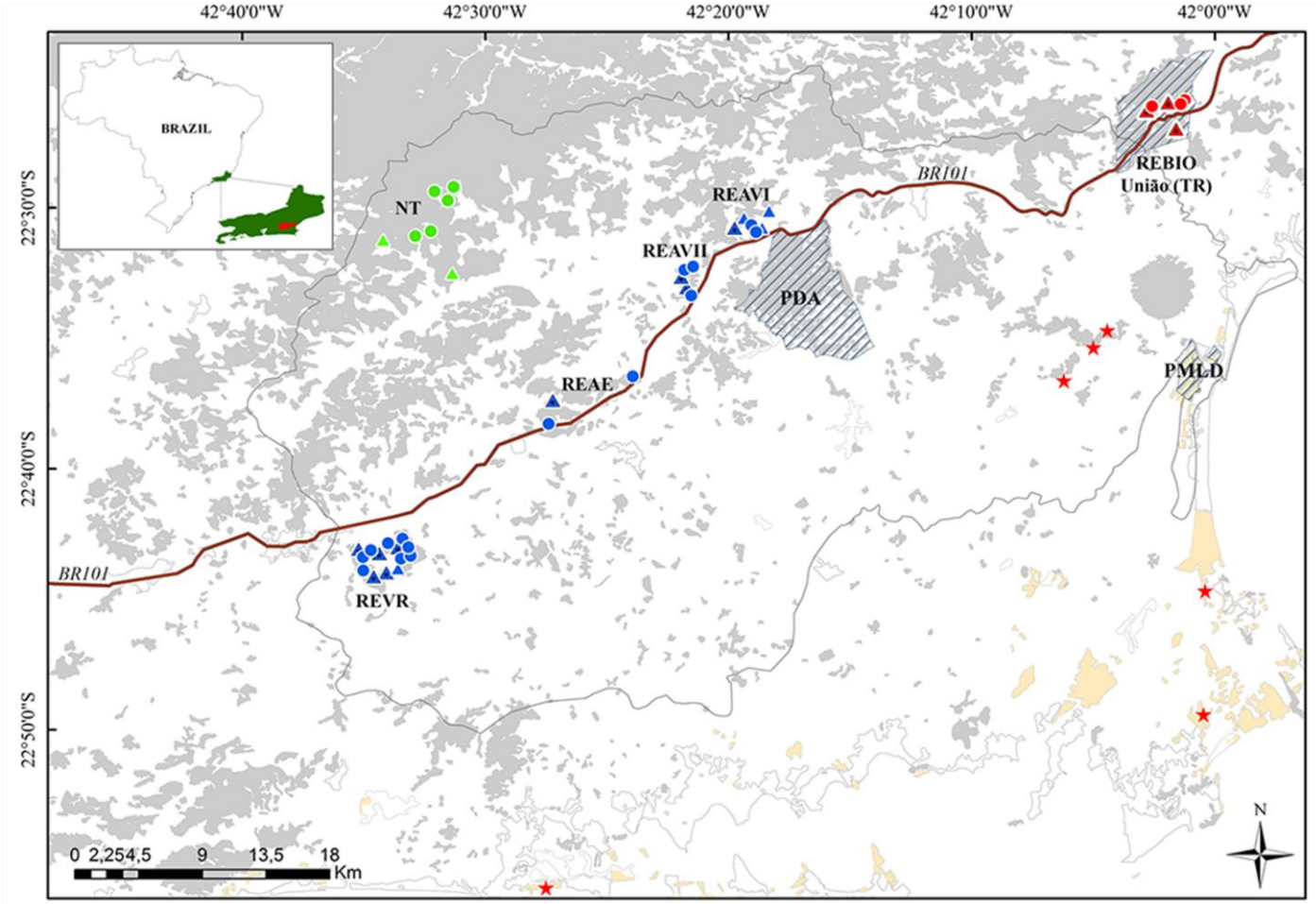

Figure 2.1 – Current geographic distribution of *Leontopithecus rosalia* in Rio de Janeiro state, Brazil. The largest area enclosed the São João River Basin. The dashed polygons enclosed the reserves of full protection: REBIO União, PDA and PMLD. In gray are lowland and gallery forest and beige are restinga forest. Symbols indicate the location of sampled social groups. Blue represents the reintroduced (RE), red the translocated (TR), and green the native (NT) social groups. Circles represent social groups sampled in the recent and triangles in the historic. The red stars represent the locations of translocated social groups before management. Source: S.O.S. Mata Atlântica.

A minimum number of sampled individuals were defined using accumulation curves based on the expected heterozygosity for all 14 microsatellite loci in each sampling site. Through this accumulation curves, we evaluate whether the sample size in each site would give a good estimate of genetic indices. We carried out accumulation curves by random sampling from one to the total number of sampled individuals in each site using 1,000 permutations. In each permutation for a given number of sampled individuals, we estimated the expected heterozygosity and thus built the accumulation curves. We observed equity in estimates of expected heterozygosity when we sampled five or more individuals per sample site (Appendices B1). This analysis was carried out in R 3.2.2 software (R code in Appendices).

DNA extraction and genotyping

We extracted DNA from hair samples using DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. We tested 17 microsatellites developed for *L. rosalia* (Grativol *et al.* 2001)*, L. chrysopygus* (Perez-Sweeney *et al.* 2005) and *L. chrysomelas* (Galbusera & Gillemot 2008). Three of them were monomorphics – Lchu1, 2 and 5 (Galbusera & Gillemot 2008) – and were excluded from the analyses. The primers for the remaining 14 useful microsatellites were constructed with M13 tails and used in combination with an M13-labeled primer, following the protocol established by Schuelke (2000).

PCR reactions (12 µL) contained 2 µL of DNA (about 10 ng), 0.46 pmol of each reverse and M13-fluorescent primers, 0.12 pmol of M13-tailed forward primer, 1x of the GoTaq Master Mix (Promega, Madison, WI, USA), and an addition of 0.63 mM of $MgCl_2$ and 0.25 mg/mL of BSA. We performed the DNA amplifications using a thermo Veriti® Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: 5 min at 94 °C, 35 cycles of 30 s denaturation at 94 °C, annealing for 45 s at 55–62 °C, extension for 45 s at 72 °C, and finally 10 cycles of 30 s denaturation at 94 °C, annealing for 45 s at 53 °C, extension for 45 s at 72 °C, followed by a final extension step of 10 min at 72 °C. PCR reactions were carried out for each locus separately, and products from one to eight loci were pooled together based on yield, size range and fluorescent dye for genotyping. The microsatellites' genotyping was performed in the ABI 3730XLs automatic sequencer (Applied Biosystems, Foster City, CA, USA) using GS 500

Liz size standard, and they were scored in Geneious R8 (Biomatters, Auckland, NZ). We amplified additional PCR replicates for samples with missing loci and 5% of the samples (12) were chosen randomly to estimate the genotyping error rate and to confirm reliability. We estimated the error rate as the ratio between observed number of allelic differences and total number of allelic comparisons (Bonin *et al.* 2004). The individual samples with more than 30% of missing loci even after additional amplification were removed from the analysis.

Data analyses

Sampling set and genetic tests

Because *L. rosalia* lives in family social groups (Baker *et al.* 2002) and therefore could have genetic structure reflecting this type of social organization, we tested the effects of the presence of family members on the population structure (Anderson & Dunham 2008). Three sample sets were tested:

- *set 1*, using the full set of samples (n = 239);
- *set 2,* excluding the young individuals (aged< 2 years) within the social groups in an attempt to reduce the influence of strongly related individuals on the results (n = 188);
- *set 3,* resampling one individual per social group through bootstrapping with 1,000 permutations, attempting to eliminate the bias of kinship (n = 69).

The Appendices B2 shows the individual numbers of *L. rosalia* in each study site and period used to test the different sampling sets. The accuracy of each sample set was evaluated through the accumulation curve of the expected heterozygosity (see *Study area and sample collection*, above). After resampling using *set 3*, at least seven sites retained a sample size below the minimum (< 5) (Appendices B2). Therefore, we considered *set 3* inadequate for the analyses, and used only *set 1* and *set 2*. Furthermore, we retained samples for REAE from the intermediate period, 2003, because of its small sample size in the historic period (n = 3).

We performed genetic analyses based on the management type (native, translocation, and reintroduction) and the spatial distribution of *L. rosalia* assigned as sampling sites (native, translocation, REAE, REAVI, REAVII, REVR). Thus, s*et 1* and *set 2* were analyzed through comparison of (1) the differences between the

management types, and (2) the changes that occurred within each sampling site over time. For each sample set we investigated the existence of possible errors in genotyping due to null alleles (frequency > 0.1), stuttering, or allele dropout in MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004). Null alleles occurring with low frequency (< 0.1) have no significant influence on the results (Carlsson 2008). We performed Hardy-Weinberg Equilibrium (HWE) tests in GENALEX version 6 (Peakall & Smouse 2006), and we corrected significant deviations from the HWE by using the Bonferroni confidence interval (Rice 1989).

Temporal changes in effective population size and genetic diversity

We estimated the effective population size (N_e) through the use of both single sample estimates and the temporal method, as implemented in NeEstimator version 2.01 (Do *et al.* 2014). The single point-in-time estimates the inbreeding effective size (N_{ei}) for all sites and times were generated considering random system and using the linkage disequilibrium method (LDNe) with bias correction. The LDNe method uses the correlation among alleles at unlinked loci and corrected for downward bias due to small sample sizes (Waples & Do 2008, 2010). The temporal method was applied to estimate the variance effective size (N_{ev}) overall and for all sites. This method is related to allele frequency changes due genetic drift. It was based on the unbiased estimator F_s that generally performs better than other temporal methods if allele frequency is skewed, a common feature in microsatellite data (Jorde & Ryman 2007). Generation time was set to 6 year, consistent with the generation time of *L. rosalia* (HOLST *et al.* 2006), and estimates were generated for all possible combinations of years within sites using Plan I (Waples 2005; Jorde & Ryman 2007). We assumed census population sizes (N_c) for all SJRB and REBIO União (1,500 individuals) and for each sampling site according to Holst *et al.* (2006) – see Table 2.1.

For both single point-in-time and temporal methods, we obtained 95% confidence intervals (CI) using a jackknife procedure (non-parametric data) and excluded allele frequencies lower than 0.02 (DO *et al.* 2014), because alleles that occur with low frequency may bias the results (Waples 2006). Due to the temporal methods having better precision than the moment methods (Wang 2005), and our research deals with populations in not in equilibrium, our N_{ei} results were considered only as tendencies over time (after Brekke *et al.* 2011). We calculated

the N_e to N_c ratio to evaluate if N_e based on the genetic data differed from that based on the population census (after Kamath *et al.* 2015).

Hierfstat package (Goudet 2005) was used in R 3.2.2 software (R Development Core Team 2015) to estimate allele frequencies and inbreeding coefficient (F_{is}), observed (H_o) and unbiased (H_s) heterozygosity, and allelic richness (*AR*) corrected by the sample size per sampling site across time. We calculated the private allele richness (*PR*) using HP-Rare (Kalinowski 2005), which was also corrected by the sample size per sampling site across time. We tested the data normality using Shapiro´s test. Since most data differ from normal distribution, we used Kruskal Wallis test (χ^2 and *P-value* reported) to test the existence of significant differences in temporal genetic variation at the sampling site level and at the types of management level. We also tested the significant differences between management types in the recent period. All these analyses were performed in R 3.2.2 software (R Development Core Team 2015).

To quantify the genetic consequences of reintroduction and translocation, we simulated the loss of genetic diversity over 50 years, measured by the mean number of alleles per microsatellite locus (N_a). We used BottleSim version 2.6 (Kuo & Janzen 2003) to simulate post-bottleneck population growth, using 1,000 iterations and the following parameters: life-span = 16 years; age at maturity = 4 years; completely overlapping generations; random mating; dioecious reproduction; same sex ratio of females and males; and constant size population. We used the historic dataset to validate the simulations and the chosen parameters up to the recent period, and we proceeded with the analysis until 50 years were reached using the same historic dataset. Following this, we calculated the annual average and standard deviation of loss of alleles over 50 years in the native, translocated and reintroduced populations.

Temporal genetic structure

The Bayesian clusters analyses were conducted to investigate the genetic structure changes within and between the study periods using the STRUCTURE 2.3 software (Pritchard *et al.* 2000). To estimate the posterior probability that the data fit the K clusters hypothesis (Pr (X/K)), we used 10 independent runs for K= 1 – N (number maximum of K estimated in each test), MCMC of 1,000,000 interactions, and a burn-in period of 200,000 sets. The analysis was done without

prior information from the origin population, using the models of admixture and correlated allele frequencies. We determined the number of genetic groups using the optimal value of the posterior probability (K (Pr (X/K)), given as LnP (K) (Pritchard *et al.* 2000), and the modal value of ΔK (Evanno *et al.* 2005). These results of K statistics were generated using HARVEST STRUCTURE (Earl & VonHoldt 2012).

Environmental, historical and demographic factors affect genetic structure. Therefore, we must evaluate different scenarios of K that explained different biological processes (Meirmans 2015). Other factors that influence the STRUCTURE results are the sample size (Kalinowski 2011), the type of sampling (Schwartz & McKelvey 2009) and the degree of kinship between individuals (Anderson & Dunham 2008). Hence, we considered the biological and historical aspects of *L. rosalia* in our interpretation of all the optimal and suboptimal values of the K statistic that were congruent between *set 1* and *set 2*. We investigated the temporal genetic structure through Bayesian clustering analyses comparing (1) the differences between sampling sites in each period and (2) the changes within each management type over the years.

Results

DNA quality and genetic tests

In total, 336 alleles were compared, except for 28 alleles (two individuals) that could not be typed for double amplification. Another 26 alleles were typed for only one extract, but not for the other. Allelic dropout, false alleles or contaminations were not found among the 282 alleles amplified and checked. In sequence, the frequency estimates of null alleles were inconsistent across the years and sites, and only the locus Lchu9 had a frequency of null alleles greater than 0.1 in more than three situations (Appendices B3a and B3b). Therefore, we performed analyses that both excluded and included the locus Lchu9. As there was no significant difference between the results generated with the use of all the loci and those generated while excluding the locus Lchu9, it was maintained in the analyses. Likewise, deviations from the HWE were inconsistent across the years and the sample sites, and they were observed mainly in the translocated samples in the historic and recent periods and in the reintroduced samples in the recent

period (Appendices B4a and B4b). Because results from *set 1* (using total individuals) and *set 2* (excluding the young individuals, probably related to adults in the social group) were similar, and *set 1* had fewer null alleles and preserved the variability of all the individuals in the sample set, we presented mainly the results of *set 1* (Appendices B3a and B3b). The results using *set 2* were also included in the Supplementary Material and when pertinent they were cited in the mainly text.

Temporal changes in effective population size and genetic diversity

The overall N_{ev} estimated for *L. rosalia* was 88 individuals (CI: 52–283). The estimates per sampling site by the single method showed that the N_{ei} of the translocated site increased over time. In contrast, a trend to decrease over time was observed in the N_{ei} of the reintroduced sites, although the confidence interval within each site in both study periods overlapped slightly, except in the REVR site (Table 2.1). All estimates concur that N_e was lower than N_c, except the N_{ev} estimated for REAVII site. As a consequence, low N_e / N_c ratios were observed in overall estimate (N_{ev} / N_c = 0.058) and most of the sampling sites in both study periods ($N_{ei} / N_c \leq 0.21$ and $N_{ev} / N_c \leq 0.66$ when excluding REAVII).

Table 2.1 – Effective population size estimation of the *L. rosalia* from São João River Basin and REBIO União using single (N_{ei}, Waples & Do 2008) and temporal (N_{ev}, Jorde & Ryman 2007) methods. Approximate size of sampling sites (Census size ≥) based on estimate of Holst *et al.* (2006). GT: generation time, CI: confidence interval.

Sampling site	Year	Sample Size	Census Size (≥)	GT	N_{ei}	95% CI for N_{ei}	N_{ev}	95% CI for N_{ev}
Native	1996	12	75	1.8	∞	52 – ∞	50	21 – ∞
Native	2007	20			3	2 – 6		
Translocated	1997	33	200	2	3	2 – 3	10	6 – 23
Translocated	2009	50			14	10 – 20		
REAE	1997	3	200	2	∞	∞ – ∞	13	5 – ∞
REAE	2009	9			7	3 – 16		
REAVI	1997	11	200	2	20	9 – 146	-47	-267 – ∞
REAVI	2009	15			10	6 – 18		
REAVII	1997	8	80	2	18	3 – ∞	104	12 – ∞
REAVII	2009	22			14	8 – 29		
REVR	1997	15	200	2	37	19 – 177	-123	82 – ∞
REVR	2009	36			15	11 – 20		

When comparing the management types in the recent period, the inbreeding coefficient of the translocated individuals was significantly lower than that of the native (Fig. 2.2 – χ^2 = 5.93, d.f. = 1, *P-value* = 0.02) and that of the reintroduced individuals (Appendices B5, only in *set 2* – χ^2 = 8.92, d.f. = 1, *P-value* = 0.003). The observed heterozygosity (H_o) of the native individuals was also significantly lower than that of the translocated (χ^2 = 5.94, d.f. = 1, *P-value* = 0.015) and reintroduced (χ^2 = 9.77, *P-value* ≤ 0.002) individuals (Fig. 2.2). Although we observed no significant temporal variation in the global genetic diversity of the reintroduced population, when the individuals were subdivided according to their spatial distribution, we recorded significant variations: inbreeding coefficient of the REAVII site increased (Fig. 2.2e and Appendices B5), while decreases were observed respectively in the H_o and *PR* of the REAVII (Fig. 2.2c) and REVR sites (only when considering *set* 2, Appendices B5). We also observed significant temporal differences in the translocated (Fig. 2.2b, c and e) and native sites (Fig. 2c: only in *set 1*, and Fig. 2.2e). H_o increased and F_{is} decreased in the translocated site, while H_o decreased and F_{is} increased in the native and in REAVII sites. On the other hand, *PR* (Fig. 2.2b) and allelic frequency (Appendices B6) showed the highest deficits of alleles over time in the translocated and REVR sites.

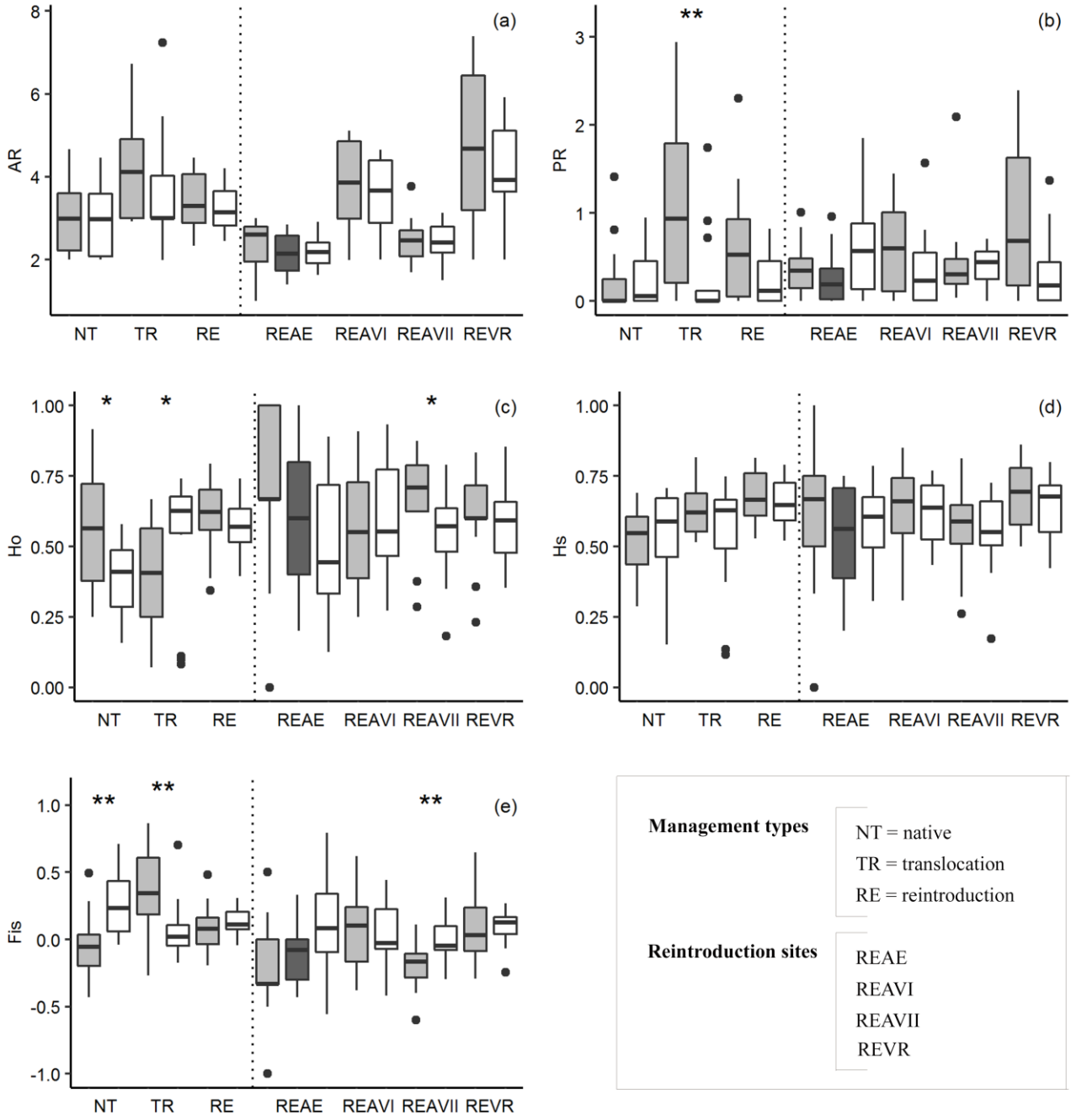

Figure 2.2 – Temporal changes in genetic diversity of *Leontopithecus rosalia* for sampling sites attributes based in history and types of management – NT, TR and RE – and for sampling sites based in spatial distribution– REAE, REAVI, REAVII and REVR. Indices of genetic diversity were estimated through (a) mean allelic richness – *AR*, (b) mean of allelic private richness – *PR*, (c) mean observed heterozygote – H_o, (d) mean unbiased expected heterozygosis – H_s , and (e) mean inbreeding coefficient – F_{is} Color periods: gray – historical; dark gray – intermediate (2003); and white – recent. Significant codes: *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05.

The simulation of the variation in average number of alleles until year 2045, using the dataset from the historic period and considering constant population sizes, also showed that in the first year after translocation the loss of alleles was more pronounced (rate of 0.36 alleles), and in the subsequent years the annual average loss of alleles was constant (0.02±0.005). In the reintroduced and native populations, the average losses of alleles over years were minor, around 0.01±0.002 and 0.002±0.001, respectively (Fig. 2.3). This simulation also produced the mean number of alleles expected to the recent period exactly the same as the results obtained in GENALEX using the dataset from the recent period, increasing our confidence in the sampling set used.

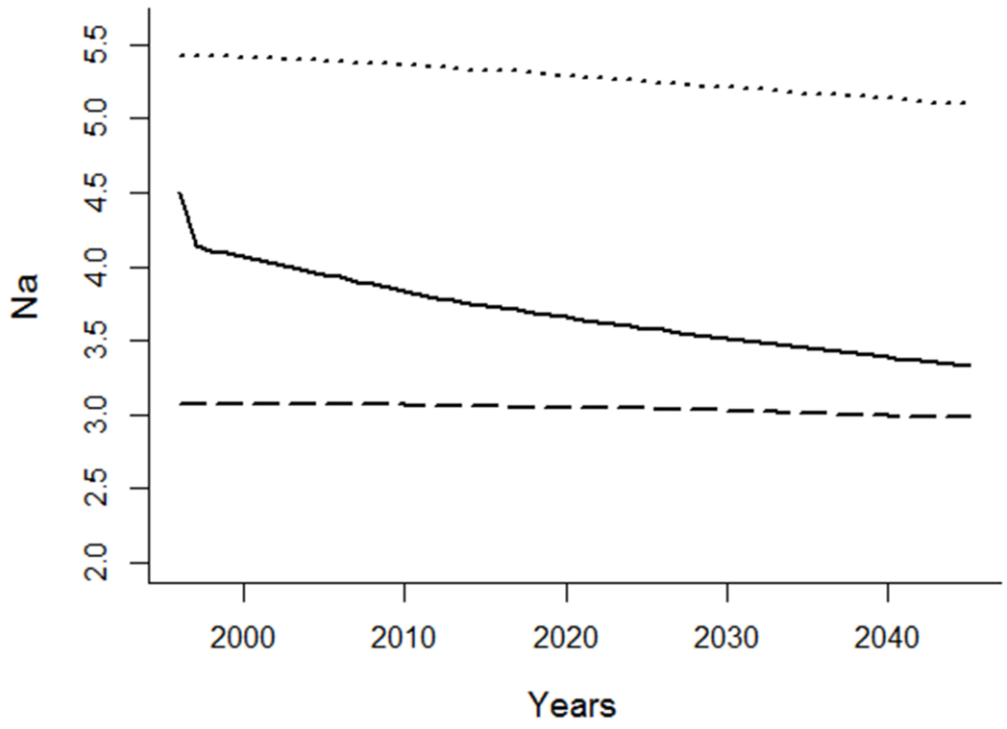

Figure 2.3 – Simulated loss of mean number of alleles per locus (*Na*) over 50 years (1996-2045) in native (dashed line), reintroduced (dotted line) and translocated (continuous line) populations of *Leontopithecus rosalia* assuming constant size through time.

Temporal genetic structure

The Bayesian cluster analyses in both the historic and the recent periods showed the highest values of K statistics when K = 2. In both the historic and the recent periods, when K = 2, the translocated populations comprised a single distinct cluster of all the other sites (Appendices B7). In the sequence, the sampling sites in the historic period presented the second highest biologically significant value when K = 8 (Fig. 2.4a). For K = 8, the translocated populations were sub-structured in four clusters assigned according to their original distribution prior to translocation. When the sampling sites of the recent period were analyzed together, the wild populations of *L. rosalia* was again strongly structured in two clusters and sub-structured in three clusters (Fig. 2.4b). In the recent period, the translocated individuals become more similar to each other and the reintroduced individuals become more similar to each other (Fig. 2.4).

When we investigated the Bayesian clustering within each management type and across the two generations, we did not observe a temporal genetic structure in the native population. In contrast, the translocated population were strongly structured in two clusters (historic and recent) and sub-structured in 3-5 clusters (Appendices B7) according their original distribution prior to the foundation of the new population (K = 5, Fig.2.4c). Neither the reintroduced nor the native populations presented temporal differentiation. The reintroduced, however, presented a strong structure when K = 3 (Fig. 2.4d) corresponding to the release site in the foundation.

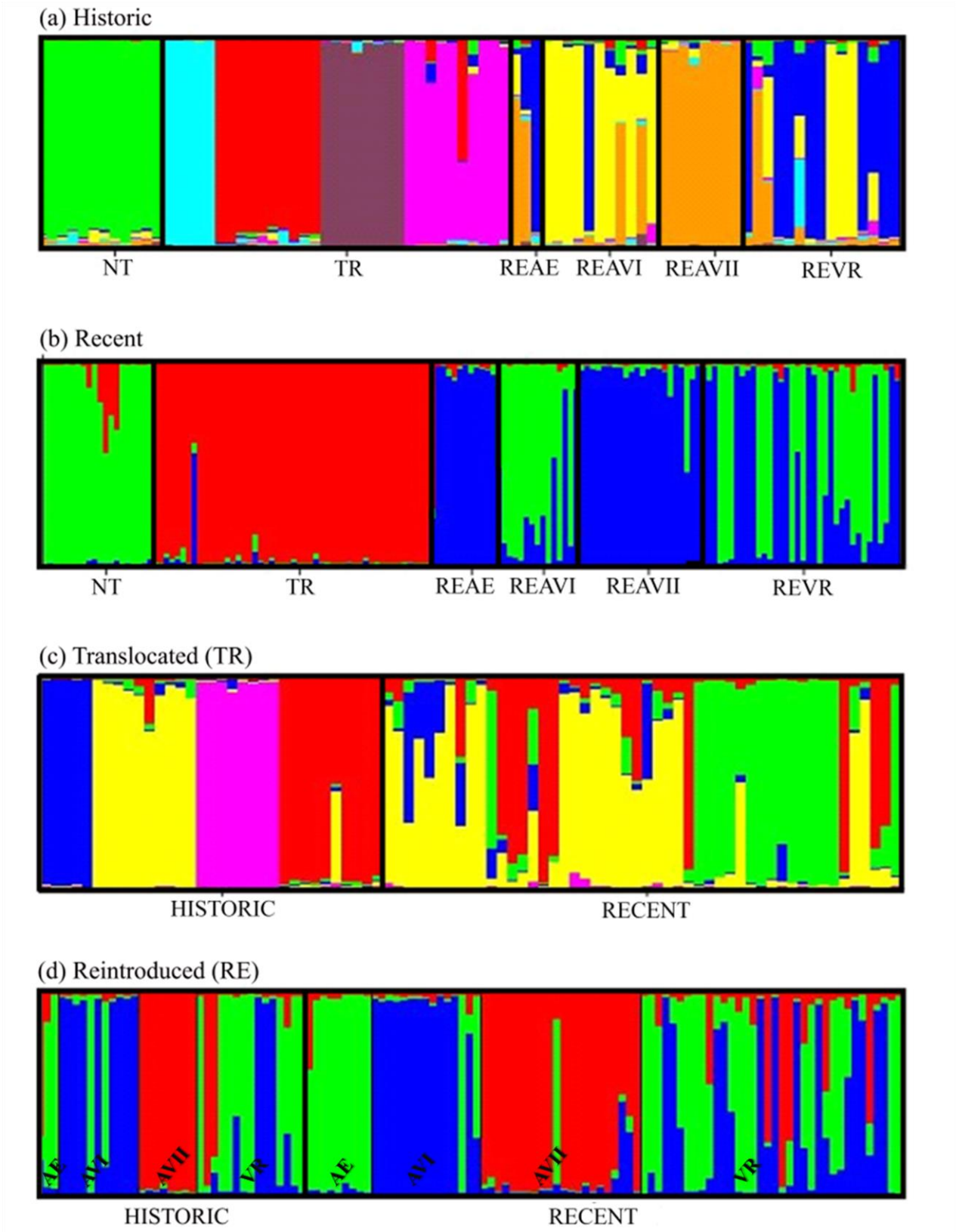

Figure 2.4 – Structure results for *Leontopithecus rosalia* from forest remnants in Brazil's Atlantic Forest showing suboptimal values of K between sampling sites in each study periods – (a) historical and (b) recent period – and within sampling sites through two generations times – (c) translocated (TR) and (d) reintroduced (RE). A single vertical line represents each individual and color segments represent partitioning into K clusters.

Discussion

Although the time between the two periods of study was ≤ 2 generations, our results show temporal genetic variation explained by the sampling sites and the management types. Despite the increase in size of the wild *L. rosalia* populations over time, the N_e increased only at the translocation site and tended to decrease in the reintroduction sites. The inbreeding coefficient of the translocated population diminished and the heterozygosity increased, whereas a tendency toward the opposite result was observed in the global evaluation of native and reintroduced populations. The average heterozygosity of a population is influenced not only by its founder size, but also, and more importantly, by the rate of population growth after its foundation (Maruyama & Fuerst 1985). On the other hand, the translocated *L. rosalia* showed a greater loss of alleles than the reintroduced individuals. When populations experience a bottleneck effect, the allelic diversity can be lost much more readily than the heterozygosity, through drift (Maruyama & Fuerst 1985; Allendorf 1986).

Genetic structuring was observed among all sites, although changes in its pattern were detected across the periods of time studied. A conspicuous within-site population structuring was observed in the translocated site in the founder period (historic), reflecting the mixing of isolated small populations. The population structuring became more site-related particularly in the recent period, suggesting admixture within the population as well as low or inconsistent gene flow between sampling sites after release. A similar result was observed for the swift fox, *Vulpes velox* (Cullingham & Moehrenschlager 2013). Probably, the temporal variation of genetic structuring resulted from a combination of the management type used during foundation of the populations, genetic drift due to the small effective population size, and the subsequent limitation of gene flow after individual translocations and reintroductions.

Temporal changes in effective population size and genetic diversity

Although the translocated and reintroduced populations of *L. rosalia* increased in size over time (Kierulff *et al.* 2002), their N_e was relatively low (based on Franklin & Frankham, 1998), while their genetic diversity varied on a case-by-case basis. The N_{ei} at the reintroduction sites had a tendency to decrease –

observed mainly in the REVR site – while the number of reproductive translocated adults increased over time. Congruently, researchers in the field reported that the number of reintroduced adults that survived after release was much lower than the cumulative number of individuals over time (see *Study species*, above). In contrast, the translocated animals had a high survival rate among adults post-release (annual average 82%) (Kierulff *et al.* 2002). Generally, translocations seem to be more successful for the establishment of a self-sustaining population than reintroduction measures (Griffith *et al.* 1989; Fischer & Lindenmayer 2000).

In addition to fluctuation in population size over generations, variance in family size and unequal sex ratio also affect the N_e of populations. N_e typically is much lower than the actual census population size, generally averaging about 10% of the actual population (Frankham 1995, 2010). Typically, *L. rosalia* lives in small family groups with monogamous and occasionally polygyny mating system (Baker *et al.* 1993; Dietz & Baker 1993). This may explain the low N_{ev} values observed in SJRB and REBIO União. Moreover, considering the overall population size estimate of 1,500 individuals for *L. rosalia* (Holst *et al.* 2006; Procópio de Oliveira *et al.* 2008), the expected N_e value is in agreement with the N_{ev} range obtained (52-283). This value is far less than the 500 individuals recommended to retain the evolutionary potential of the species (Franklin & Frankham 1998).

We also found a small N_{ei}/N_c ratio in all sampling sites. On the other hand, the N_{ev}/N_c ratio was high in the REAVII and native sites. We consider that the estimate of N_{ev} for REAVII site was inconsistent or the census population size for this area was underestimated. Nevertheless, considering that it is a small area and is extensively monitored by AMLD, we believe that the census value is likely a reasonable estimate. In contrast, we believe that the census population size of the native site is underestimated. In fact, current estimates show that the population size in this region is much higher than expected (Morais, pers. com.). In some sites where there was no evidence of variation in the genetic characteristic caused by a finite number of parents, the N_e estimates showed infinite and/or negative values, although sampling errors (Waples & Do 2010; Do *et al.* 2014) and bias due to the small sample size (Waples 2006; Jorde & Ryman 2007) cannot be discarded.

Genetic diversity is lost at a rate that depends on the effective population size, rather than the actual population size (Frankham 2010). It comprises both

allelic diversity and heterozygosity (Ballou & Foose, 2010). When we compared the recent observed heterozygosity among management types, our results showed that the translocated and reintroduced populations had higher heterozygosity rates than the native population. Similarly, when we evaluated the temporal changes in the allele frequencies in the reintroduced and translocated populations in comparison with the native population, our results showed that translocation and reintroduction achieved conservation goals: they maintained or recovered the depleted alleles. Furthermore, translocation recovered private alleles of small and isolated populations that were originally distributed in gallery forests of the São João River Basin and in *restinga* forests on the coast of Rio de Janeiro State (Kierulff 2000; Grativol *et al.* 2001; Kierulff *et al.* 2002). A similar conclusion was previously reached by Grativol *et al.* (2001), comparing a smaller sample size of translocated and native *L. rosalia*. In addition, Grativol (2003) reported private haplotypes in the translocated population. However, the possibility that such alleles were not yet sampled in the native population can be not excluded (Grativol *et al.* 2001).

When we evaluated the temporal changes within sites, the heterozygosity at the translocation site increased and the F_{is} decreased over time, while an inverse tendency was observed in the reintroduction and native sites. On the other hand, the greatest losses of alleles happened in the translocation population, particularly during the first years after translocation. Probably, the main factor responsible for the initial loss in alleles in the translocated population was the mixing of rare alleles in a large population during its establishment (Grativol *et al.* 2001). Alternatively, these lost alleles may be the result of unsampled descendants of founders in the recent sampling. Similarly, the initial increase in heterozygosity in the translocated population may result from mixing of genetically distinct social groups and may be a recent increase. Since the translocated population remains isolated, and without additional translocations, it is expected to experience drift and inbreeding in the near future (e.g., Kennington *et al.* 2012).

The second greatest loss of alleles over time was observed in the REVR reintroduced site. Both REVR and the translocated sites are isolated populations, whereas gene flow is probably facilitated by the structural connectivity of the landscape in the other remaining areas (Procópio de Oliveira *et al.* 2008). Even relatively large isolated populations can lose genetic diversity relative to their

sources (e.g., Mock *et al.* 2004). Additionally, the simulation of variation in allelic diversity over time also showed that losses may continue if the translocated population remains isolated over time. If migration is limited and inbreeding continues after founding, the genetic diversity may continue to decline over time (e.g., Kennington *et al.* 2012). Losses of alleles in the translocated population were greater initially and continued over time. In some cases, loss of alleles are greater initially, when the bottleneck effects are induced by reintroduction or translocation, but they cease over time (e.g., *Bristol et al.* 2013) if population connectivity is restored.

A similar pattern of losses of alleles was observed in the simulation with the reintroduced population, but was less intense than that observed in the translocated population. Similarly, pedigree analysis showed that the reintroduced *L. rosalia* population retained 96% of its genetic diversity relative to the source population (Mickelberg 2011). However, some reintroduced sites showed genetic diversity losses and an increase in the F_{is} over time. At the molecular level, the F_{is} of the REAVII site (located in Aldeia Velha) increased over time. Similarly at the pedigree level, Aldeia Velha had the largest F_i. According to pedigree analysis, the annual mean F_{is} of reintroduced *L. rosalia* increased most rapidly in the population from 1993 to 2000; thereafter inbreeding was steady, increasing only slightly at an average rate of 0.3% per year (Mickelberg 2011). Pair-wise comparisons also showed that the reintroduced population had a greater F_{is} than the translocated population. The native population also had lower heterozygosity and larger F_{is} than the translocated population, but it had the lowest loss of the average number of alleles over time (until 2045, see Figure 2.3). Moreover, the native as well as REAVII sites showed a decrease of in H_o over time only when considering the *set* 1 analysis, and can be therefore skewed by the family structure of the species.

Temporal genetic structure

Bayesian cluster analyses showed that population structuring of *L. rosalia* became more site-related over time; probably due to subsequent exposure of the founder populations to the process of genetic drift and isolation promoted by low forest patch connectivity. Reintroduction and translocation programs only have positive effects on demographics and genetic composition if the external factors that limit the population expansion are also controlled (Kleiman 1989). The ΔK

value was optimal when the wild *L. rosalia* population was strongly structured into two genetic clusters in both historic and recent periods. One cluster was composed of translocated individuals and the other cluster was a combination of native and reintroduced individuals. Differentiation of the translocated population may be the result of early separation from other populations of the species. The majority of the founders of the translocated population represent *L. rosalia* descendants of the coastal Atlantic Forest, which has a distinct physiognomy and is historically isolated by distance (Guedes-Bruni *et al.* 2006).

On the other hand, the suboptimal ΔK values showed the influence of management strategies and of spatial distribution on the population structure. In the historic period, there was a sub-structure in eight genetic clusters that revealed the genetic structure before the translocated population was established, and the mixing between and within reintroduced populations. Congruently, a high proportion of HWE deviations were observed particularly in the translocation site, and historic period, as a consequence of the mixing of different populations, i.e. the Whalund effect (Sinnock 1975). The translocated population was founded by isolated social groups, and the reintroduced populations were founded by the offspring of 33 founders distributed among 30 zoos and genetically managed using pedigree analysis (Kierulff *et al.* 2002; Kierulff *et al.* 2012). Lasting for two generations (11–12 years), this sub-structure was reduced to three genetic clusters – there was a greater homogenization within each of management type and distinction between them. These scenarios show the influence of management and the subsequent limitation of the gene flow, probably caused by physical barriers in the new habitat.

As we expected, we found no genetic evidence of isolation over time for the native population. Likewise, the reintroduced population showed no evidence of isolation over time; however, we observed a tendency toward genetic structuring that corresponds with the release sites. The representation of each site in the within-population structure of reintroduced groups is similar to those indicated by the pedigree analyses (see Mickelberg 2011). Conversely, a change in the genetic structure over time was observed in the translocated population. The greater representation of some translocated groups over others in the recent population brings a within-population genetic distinction over time (as see in Figure 2.4).

Nevertheless, these structuring scenarios could also be the result of the small sample size (Kalinowski 2011), specially for the historic period.

Final considerations

Genetic consequences of reintroductions and translocations depend on many local factors that need to be considered on a case-by-case basis. Differences in management between reintroduction and translocation can affect the genetic outcome of the conservation efforts and ultimately the viability measures of the population. For *L. rosalia*, the reintroduction used a captive population managed as one population through a studbook (Ballou & Cooper 1992; Ballou *et al.* 2002). Animals were also channeled to the wild via "Gateway Zoos" that provided key pre-release experience (Beck & Castro 1994; Stoinski *et al.*1997) and the in-situ reintroduction protocols were designed to keep the captive-born animals alive in the wild until reproduction (Beck *et al.* 2002). This management procedure likely reduced initial losses of allelic diversity. On the other hand, differential post-release mortality – which was high for the captive-born adult animals (44% survival to one-year) and low for their wild-born offspring (81% survival to one year of age; Beck *et al.* 2002) – and the fragmented structure of the landscape may have contributed to the low gene flow between populations, higher inbreeding coefficient than the translocated population and a trend of lower effective population size over time.

The translocated population was comprised of isolated social groups known to differ in genetic structure, to have low genetic diversity, inbreeding, and rare and private alleles (Grativol *et al.* 2001). When inbreeding is clearly a factor, the mixture of individuals from different populations is recommended (IUCN/SSC 2013). Because of these characteristics, managers decided to translocate entire social groups of *L. rosalia* to one protected area, as opposed to distributing the groups among existing populations. The groups of *L. rosalia* stayed as cohesive reproductive units after translocation, and reproduction in this population was successful (Kierulff 2000; Kierulff *et al.* 2002). As a consequence, translocated *L. rosalia* showed a relative decrease in inbreeding over time. Management notwithstanding, the translocated population showed a loss of allelic diversity.

Both populations (translocated and reintroduced) of *L. rosalia* showed an increase in population size, making a significant demographic contribution toward

a free-living metapopulation (Kierulff *et al.* 2002), albeit with some loss of genetic diversity. The genetic analyses of other reintroduced and translocated species, e.g., *Anticolocapra americana* (Stephen *et al.* 2005), and *Meleagris gallopavo merriami* (Mock *et al.* 2004), also showed that these populations grew rapidly but suffered a reduction in their genetic diversity. Future gene flow into these populations via natural dispersal could increase their genetic diversity (as observed by Ortego *et al.* 2011). We recommend that the success of reintroduction and translocation programs be measured both in terms of demographic and genetic monitoring outcomes.

Reintroductions and translocation should carefully consider and include landscape structure in long term planning. Our results indicated a low gene flow among *L. rosalia* populations after translocation and reintroductions actions. Thus, landscape connectivity influenced genetic diversity and population structure of *L. rosalia*. Furthermore, simulations suggest that if the translocated population of *L. rosalia* continues to be isolated, alleles will continue to be lost over time. Ongoing analyses of functional connectivity will be important to evaluate long-term population viability and to avoid the negative effects of small population size (as observed by Cullingham & Moehrenschlager 2013). We recommend future research investigating which landscape elements are responsible for the limitation of gene flow of *L. rosalia* populations and thus suggest appropriate measures for reconnection and restoration of their habitats. We suggest that habitat reconnections (Bouzat *et al.* 2009) and restocking (IUCN 1987) be done to promote the gene flow among populations of *L. rosalia*. In the case of REVR site, as habitat reconnection is impaired by the BR 101 road, we suggest its supplementation and/or translocation of some individuals to an unoccupied habitat in the northern of BR 101 road. The translocated site also has a geographically isolated population, although it is surrounded by unoccupied forest patches. We suggest that translocations to these forest patches might increase gene flow and improve the long-term persistence of these populations. The remaining population from the coastal region of Rio de Janeiro (PMLD, Fig. 2.1) is a possible candidate for animals to translocate. However, a previous genetic analysis of PMLD individuals will be needed to ensure that the genetic diversity in the founder population will be maximized.

The current long-term study indicates that a number of factors – such as the genetic composition and the number of founders, the genetic management of the populations, and the ability of each population to expand quickly and landscape connectivity – can influence the genetic diversity and structure of a population under conservation management. If under a small initial size in the foundation, low growth rate, and low migration rate, the genetic diversity of the established populations may become suppressed following a translocation or a reintroduction (Thrimawithana *et al.* 2013). Therefore, we recommend monitoring the population genetics before and after a translocation and/or reintroduction to help evaluate conservation programs and to have better conservation actions, and maintaining population connectivity thereafter to avoid the negative effects of a small population size. Translocation and reintroduction are useful conservation strategies, but they should be done in combination with other strategies (Kleiman 1989) such as habitat restoration, to guarantee a minimum population size and gene flow among the populations.

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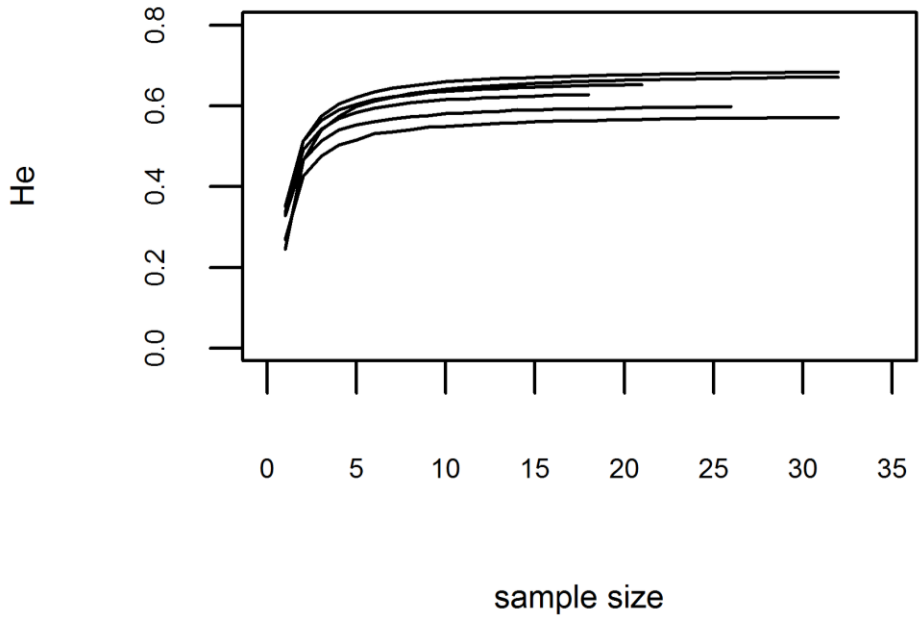
APÊNDICES

Apêndices B



B1 – Accumulation curve of expected heterozygosity for *Leontopithecus rosalia* from forest remnants in Brazil's Atlantic Forest using 14 microsatellites and 1000 permutations. Each line represents one sampling sites and the x-axis (sample size) refers to the size of the sample taken for estimate the genetic parameters (y-axis).

R code used to build the accumulation curves based on the expected heterozygosity for all 14 microsatellite loci in each sampling site

By Carolina da Silva Carvalho

How many individual I need to use?

```
 data <- read.table("genotypes.txt", head=T, sep="\t")

 head(data)
# pop ind Leon30 Leon30.1 Leon21 Leon21.1 Lchu8 Lchu8.1 Lchu3 Lchu3.1 P2BH6
P2BH6.1
# 1       S1   283     283    287     287   226    228   341    341   134    134
# 1       S2   279     283    287     297   228    232   337    341   134    134
# 1       S3   277     283    287     287   226    232   341    341   134    134
# 1       S4   279     283    287     287   230    232   341    341   132    132
# 1       S5   277     279    287     287   226    228   337    337   134    134
# 1       S6   279     283    287     297   228    232   341    341   134    134
```

Heterozigosity

```
       Simulacaohe <- function(data,nl,nsample,perm=1000){  # data=data; nl= loci
number, nsample= individuals number
         vetor.sim <- numeric(nsample)  #vector to store the He

         for (i in 1:nsample) {  ## calculation with 1 individual up to total sample number

           vetor.perm <- numeric(perm)  ## vector to save the result of each permutation

           for (j in 1:perm){

             sorteio <- dados[(sample(nsample,i)),] ## draw the data that will be used to
calculate He

               medialoco<-numeric(nl*2) ## vector to save the He for each locus

               for (k in seq(1,(2*nl),by=2) ){

                 n <-2*nrow(sorteio) ##  He = Sum of squared allele frequencies / 2x the
samples number

                   vetor <- c(sorteio[,k],sorteio[,(k+1)]) ## join the loci columns

                   alelos <- as.numeric(table(vetor))
```

```
            media.alelos <- alelos/n

            pi2 <- sum(media.alelos^2)

            resu <- 1-pi2 ## sum of square of allelic frequency

            medialoco[k] <- resu}  ## save the He by each locus

          He <- sum(medialoco)

          He.pop <- He/nl ## He of population = sum of He by locus / number of loci

            vetor.perm[j]<-He.pop
        }

        vetor.sim[i]<-mean(vetor.perm)
      }

      return(vetor.sim)
    }

## Building the accumulation curve

   plot(simulacaohe(data,nl,nsample,perm=1000 ),xlab="sample size",ylab="He",
ylim=c(0,0.8), xlim=c(0,35), cex.axis=0.5, cex.lab=0.6, type="n")
      lines(simulacaohe(data,nl,nsample,perm=1000 ),xlab="sample size",ylab="He")
```

B2 – Individual numbers of *Leontopithecus rosalia* in each study site and period used to test the different sampling sets – set 1, set 2 and set 3.

Sampling site	Period	SET 1	SET 2	SET 3
Native	1996	12	9	2
	2007	20	10	6
Translocated	1997	33	24	5
	2009	50	30	8
REAE	1997	3	3	1
	2003	5	4	1
	2009	9	9	5
REAVI	1997	11	9	6
	2009	15	15	3
REAVII	1997	8	5	3
	2009	22	22	4
REVR	1997	15	12	6
	2009	36	36	18

B3a – Null allele estimates for *Leontopithecus rosalia* in all 14 microsatellites across each sampling site-year using *set 1*. Estimates > 0.14 highlighted in bold type. In historic period, REAE and REAVII had insufficient data for null allele analysis.

	Native		**Translocated**		**Reintroduced (RE)**						
					AE		**AVI**		**AVII**	**VR**	
	1996	**2007**	**1996**	**2009**	**2003**	**2009**	**1997**	**2009**	**2009**	**1997**	**2009**
Leon30	-0.1	0.0	**0.3**	0.1	-0.1	0.0	0.1	0.1	0.1	0.1	0.1
Leon21	0.0	**0.2**	**0.3**	0.0	-0.6	-0.2	**0.2**	0.1	0.0	0.1	0.0
Lchu8	-0.1	0.1	0.0	0.1	0.0	-0.2	0.0	-0.2	-0.3	0.1	0.0
Lchu3	0.1	0.0	**0.2**	0.0	-0.1	0.1	-0.2	-0.1	-0.1	0.0	0.0
P2BH6	**0.2**	**0.2**	0.1	0.0	-0.3	-0.1	-0.2	0.0	0.1	0.0	0.0
Lchu9	0.0	**0.3**	**0.4**	**0.3**	-0.1	**0.3**	**0.3**	**0.2**	-0.1	**0.3**	0.1
Leon27	-0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.1
Leon31	-0.2	0.0	**0.3**	0.1	-0.3	0.1	**0.2**	-0.1	0.0	-0.1	-0.2
Lchu7	0.0	0.0	0.1	-0.1	-0.2	-0.1	0.0	0.0	0.0	0.1	0.1
Lchu6	-0.2	-0.1	0.1	0.0	-0.1	0.1	-0.1	-0.3	0.1	-0.2	-0.1
P5BE6	-0.2	0.1	**0.2**	0.0	-0.1	**0.3**	0.0	0.1	-0.1	-0.1	0.1
Lchu4	0.0	0.1	**0.2**	0.0	-0.3	0.1	-0.1	0.0	0.0	-0.1	0.0
Leon2	0.0	**0.2**	0.0	-0.1	-0.5	-0.1	0.1	0.1	-0.1	-0.1	0.0
Leon3	-0.4	**0.3**	-0.2	0.0	0.1	-0.2	-0.2	-0.2	-0.1	-0.1	0.1

B3b – Null allele estimates for *Leontopithecus rosalia* in all 14 microsatellites across each sampling site-year using *set 2*. Estimates > 0.14 highlighted in bold type. In historic period, REAE and AVII had insufficient data for null allele analysis.

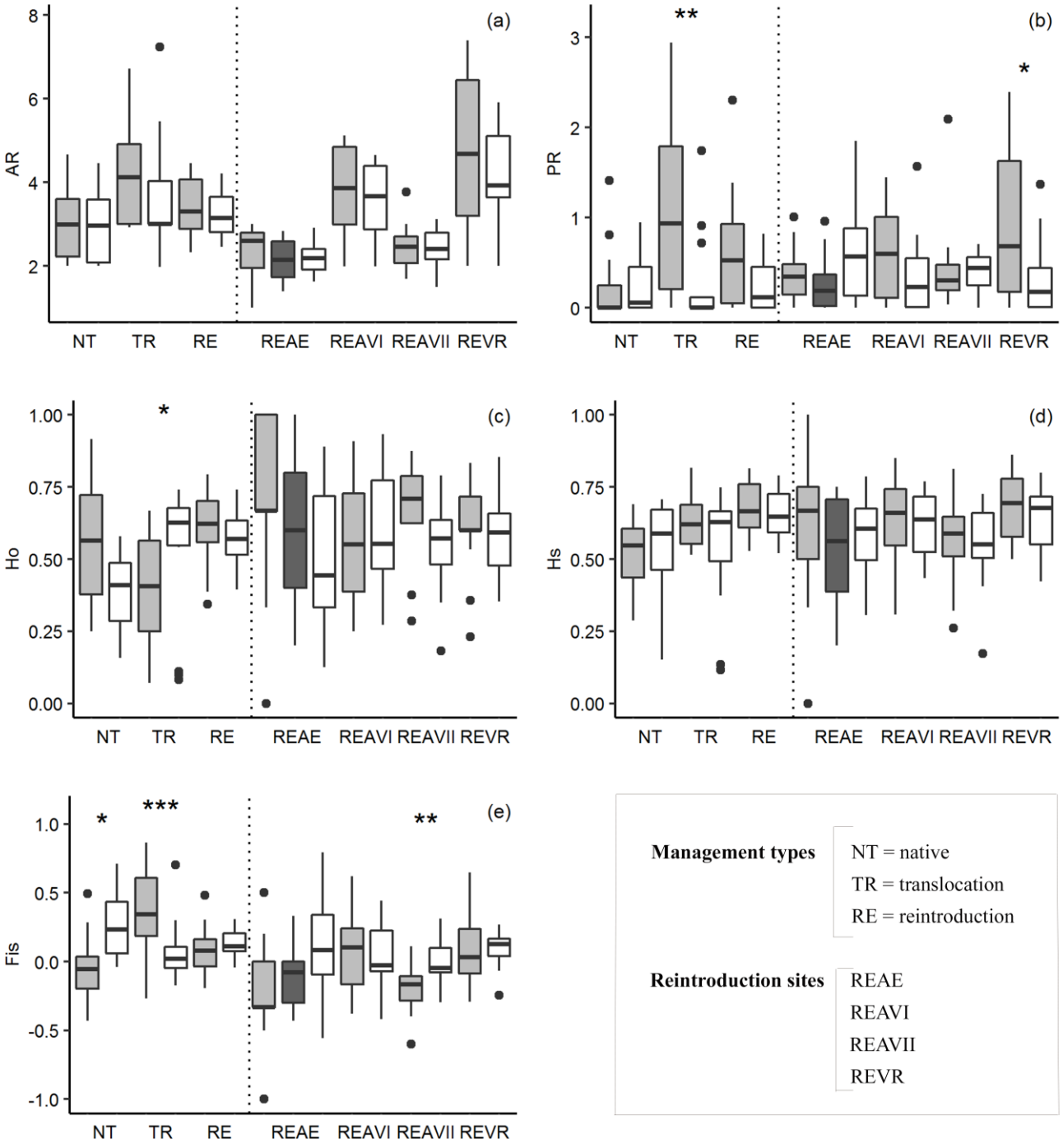
	Native		**Translocated**		**Reintroduced(RE)**						
					AE		**AVI**		**AVII**	**VR**	
	1996	**2007**	**1996**	**2009**	**2003**	**2009**	**1997**	**2009**	**2009**	**1997**	**2009**
Leon30	-0.2	0.1	**0.3**	0.1	-0.1	-0.1	-0.2	0.1	0.1	0.0	0.1
Leon21	0.1	**0.3**	**0.3**	0.0	-0.6	-0.3	**0.2**	0.1	0.0	0.1	0.0
Lchu8	-0.1	**0.2**	0.0	0.0	0.0	-0.5	0.0	-0.2	-0.3	0.1	0.0
Lchu3	**0.2**	0.0	**0.2**	-0.1	-0.1	0.0	-0.2	-0.1	-0.1	0.0	0.0
P2BH6	**0.2**	**0.2**	**0.2**	0.0	-0.3	-0.2	-0.2	0.0	0.1	0.0	0.0
Lchu9	0.0	**0.2**	**0.4**	**0.3**	-0.1	**0.2**	**0.3**	**0.2**	-0.1	**0.3**	0.1
Leon27	-0.2	0.1	0.1	-0.1	0.1	**0.2**	-0.2	0.1	0.0	0.1	0.1
Leon31	-0.2	0.0	**0.3**	0.1	-0.3	0.1	0.1	-0.1	0.0	-0.1	-0.2
Lchu7	-0.1	0.1	**0.2**	-0.1	-0.2	0.0	0.1	0.0	0.0	0.0	0.1
Lchu6	-0.2	-0.1	0.1	0.0	-0.1	-0.2	-0.3	-0.3	0.1	-0.2	-0.1
P5BE6	-0.2	-0.1	**0.2**	0.0	-0.1	**0.3**	-0.4	0.1	-0.1	0.0	0.1
Lchu4	0.0	**0.2**	**0.2**	0.0	-0.3	**0.2**	-0.1	0.0	0.0	-0.1	0.0
Leon2	-0.1	0.0	0.0	0.0	-0.5	0.0	0.1	0.1	-0.1	-0.1	0.0
Leon3	-0.3	**0.2**	-0.2	0.0	0.1	-0.2	-0.2	-0.2	-0.1	-0.3	0.1

B4a – *P* values of Hardy Weinberg Test of *Leontopithecus rosalia* in all 14 microsatellites across each sampling site-year using *set 1*. Significant p values after Bonferroni correction are highlighted in bold type. *P* interval after Bonferroni correction: 0.0036 < α < 0.0500. Missing data (-): monomorphic locus.

	Native		**Translocated**		**Reintroduced (RE)**								
					AE			**AVI**		**AVII**		**VR**	
	1996	**2007**	**1996**	**2009**	**1997**	**2003**	**2009**	**1997**	**2009**	**1997**	**2009**	**1997**	**2009**
Leon30	0.446	0.032	**0.000**	**0.000**	0.112	0.532	0.841	**0.001**	**0.000**	0.499	**0.000**	0.109	0.044
Leon21	0.977	0.041	**0.000**	0.432	0.809	0.809	0.260	0.005	**0.001**	0.513	**0.001**	0.323	**0.002**
Lchu8	0.898	0.009	0.253	0.928	0.861	0.392	0.090	0.921	0.302	0.042	0.188	0.022	**0.000**
Lchu3	0.402	0.831	0.009	0.510	0.112	0.729	0.764	0.461	0.667	0.353	0.514	0.425	0.943
P2BH6	0.054	0.003	**0.001**	0.058	0.809	0.392	0.494	0.869	0.650	0.337	0.990	0.463	0.010
Lchu9	0.135	**0.000**	**0.000**	**0.000**	0.729	0.729	0.079	0.037	0.338	0.199	0.120	0.029	0.120
Leon27	0.253	0.596	0.026	0.015	0.386	0.564	0.317	0.458	0.588	0.506	0.931	0.347	0.382
Leon31	0.909	0.317	**0.000**	0.042	0.682	0.861	0.414	0.210	0.160	0.362	0.971	0.912	0.788
Lchu7	0.838	0.911	0.536	0.202	0.392	0.729	0.266	0.868	0.973	0.136	0.834	0.703	0.294
Lchu6	0.488	0.987	0.000	0.060	-	0.083	0.999	0.748	0.291	0.362	0.950	0.494	0.489
P5BE6	0.254	0.780	**0.000**	0.166	0.386	0.729	0.044	0.556	0.066	0.659	0.974	0.617	0.471
Lchu4	0.396	0.316	**0.001**	0.935	0.262	0.392	0.307	0.221	0.297	0.624	0.549	0.889	0.588
Leon2	0.854	0.076	0.968	**0.000**	0.083	0.386	0.047	0.465	0.136	0.514	0.746	0.027	**0.000**
Leon3	0.122	0.033	0.310	0.127	0.392	0.343	0.948	0.430	0.531	0.150	0.408	0.997	0.864

B4b – *P* values of Hardy Weinberg Test of *Leontopithecus rosalia* in all 14 microsatellites across each sampling site-year using *set 2*. Significant p values after Bonferroni correction are highlighted in bold type. *P* interval after Bonferroni correction: $0.0036 < \alpha < 0.0500$. Missing data (-): monomorphic locus.

	Native		**Translocated**		**Reintroduced (RE)**								
						AE		**AVI**		**AVII**		**RV**	
	1996	**2007**	**1996**	**2009**	**1997**	**2003**	**2009**	**1997**	**2009**	**1997**	**2009**	**1997**	**2009**
Leon30	0.615	0.029	**0.000**	**0.000**	0.112	0.299	0.841	0.549	**0.000**	0.528	**0.000**	0.277	0.044
Leon21	0.612	0.022	**0.001**	0.516	0.809	0.677	0.260	0.090	**0.001**	0.136	**0.001**	0.324	**0.002**
Lchu8	0.814	0.009	0.842	0.872	0.861	0.789	0.090	0.724	0.302	0.020	0.188	0.064	**0.000**
Lchu3	0.072	0.764	0.047	0.143	0.112	0.775	0.764	0.549	0.667	0.708	0.514	0.647	0.943
P2BH6	0.160	0.322	0.007	0.282	0.809	0.710	0.494	0.863	0.650	0.528	0.990	0.841	0.010
Lchu9	0.022	0.115	**0.000**	**0.000**	0.729	0.775	0.079	0.051	0.338	0.576	0.120	0.114	0.120
Leon27	0.421	0.890	0.053	0.094	0.386	0.709	0.317	0.549	0.588	0.637	0.931	0.386	0.382
Leon31	0.865	0.601	**0.000**	0.089	0.682	0.821	0.414	0.202	0.160	0.576	0.971	0.758	0.788
Lchu7	0.606	0.719	0.168	0.258	0.392	0.576	0.266	0.862	0.973	0.386	0.834	0.778	0.294
Lchu6	0.549	0.686	**0.000**	0.327	-	0.655	0.999	0.260	0.291	0.427	0.709	0.605	0.489
P5BE6	0.294	0.733	0.008	0.261	0.386	0.804	0.044	0.959	0.066	0.505	0.974	0.788	0.471
Lchu4	0.801	0.528	0.017	0.888	0.261	0.427	0.307	0.429	0.297	0.821	0.549	0.881	0.588
Leon2	0.851	0.946	0.955	**0.000**	0.083	0.528	0.047	0.652	0.136	0.576	0.746	0.010	**0.000**
Leon3	0.249	0.241	0.353	0.663	0.392	0.172	0.948	0.599	0.531	0.290	0.408	0.996	0.864



B5 – Temporal changes in genetic diversity of *Leontopithecus rosalia* from forest remnants in Brazil's Atlantic Forest using *set2* and two different times in native (NT), tranlocated (TR) and REAVI, REAVII and REVR sites, and for three different times in REAE sampling site. Indices of genetic diversity were estimated through (a) mean allelic richness – *AR*, (b) mean of private allelic richness – *PR*, (c) mean observed heterozygote – H_o, (d) mean unbiased expected heterozygosis – H_s , and (e) mean inbreeding coefficient – F_{is} Color periods: gray – historical; dark gray – 2003; and white – recent. Significant codes: *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05.

B6 – Allele frequencies of *Leontopithecus rosalia* from forest remnants in Brazil's Atlantic Forest by sampling sites, period and loci. Alleles lost through time are in underlined and new alleles are in bold type. In N the mean through all fourteen microsatellite loci of sample sizes genotyped for each population and period.

						Reintroduced (RE)								
		Native		Translocated		AE			AVI		AVII		RV	
		1997	2009	1997	2009	1997	2003	2009	1997	2009	1997	2009	1997	2009
Locus	**Allele/N**	12	19	30	48	3	5	8	11	14	7	21	14	34
Leon30	**261**	-	-	0.09	0.04	-	-	**0.33**	0.14	0.27	0.14	0.19	0.10	0.11
	265	-	-	-	-	-	-	-	-	-	-	-	0.07	-
	269	-	-	0.24	-	-	-	-	-	-	-	-	-	-
	275	-	-	0.03	0.02	-	-	-	0.05	0.03	-	**0.05**	-	-
	277	0.25	0.08	0.03	0.01	-	0.20	0.06	-	-	-	-	0.07	0.15
	279	0.25	0.40	0.61	0.93	0.33	0.50	0.44	0.05	0.03	0.36	0.36	0.17	0.21
	281	-	-	-	-	0.33	0.10	0.06	-	**0.03**	-	-	0.27	0.18
	283	0.50	0.53	-	-	0.33	0.10	0.11	0.77	0.57	0.50	0.40	0.23	0.32
	285	-	-	-	-	-	0.10		-	**0.07**	-	-	0.10	0.03
Leon21	**283**	-	-	0.02	0.01	-	-	-	-	-	-	-	-	-
	285	-	-	0.02	-	-	-	-	-	-	-	-	-	-
	287	0.71	0.55	0.23	0.39	0.17	0.25	0.50	0.27	0.33	0.50	0.38	0.17	0.37
	289	-	-	-	-	0.17	0.13		-	-	-	-	0.03	0.06
	291	-	-	-	-	-	-	**0.39**	0.27	0.27	0.44	0.40	0.20	0.10
	293	-	-	-	-	-	-	-	0.09	0.07	-	-	0.03	0.03
	295	-	-	0.29	0.36	-	-	-	0.09	-	-	-	0.03	-
	297	0.29	0.45	0.42	0.24	0.50	0.13	-	0.27	0.27	0.06	0.17	0.23	0.36
	299	-	-	0.03	-	0.17	0.50	0.11	-	**0.07**	-	**0.05**	0.20	0.09
	301	-	-	-	-	-	-	-	-	-	-	-	0.10	-
Lchu8	**216**	-	-	-	-	-	-	-	-	-	0.19	-	-	**0.03**
	218	-	-	-	**0.01**	-	-	-	-	-	-	-	-	**0.01**
	222	-	-	-	**0.01**	-	-	-	-	-	-	-	-	-
	224	-	-	-	-	-	-	-	0.05	0.03	0.31	-	-	**0.01**
	226	0.13	0.03	0.15	0.26	0.67	0.40	0.63	0.41	0.43	0.25	0.68	0.27	0.31
	228	0.42	0.53	-	**0.02**	0.17	0.10	-	0.27	0.30	0.06	0.14	0.13	0.13
	230	0.04	0.15	0.38	0.29	0.17	0.50	0.38	0.05	0.10	-	-	0.27	0.21
	232	0.38	0.18	0.05	0.02	-	-	-	0.09	0.03	-	-	0.03	0.04
	234	0.04	0.13	0.42	0.40	-	-	-	0.14	0.10	0.19	0.18	0.30	0.26
Lchu3	**333**	-	-	0.23	0.38	0.33	0.88	0.44	-	-	0.25	0.27	0.23	0.06
	337	0.21	0.30	0.42	0.46	0.33	0.13	-	0.18	0.30	0.56	0.55	0.17	0.21
	341	0.79	0.70	0.35	0.16	0.33	-	0.56	0.82	0.70	0.19	0.18	0.60	0.73
P2BH6	**124**	-	-	0.02	0.03	-	-	-	-	-	-	-	-	-
	126	-	**0.08**	0.18	0.30	-	-	-	0.09	0.03	-	-	0.13	0.01
	128	-	**0.03**	0.15	0.12	0.17	0.20	0.11	-	**0.03**	-	-	0.17	0.19
	130	0.08	0.25	-	**0.01**	-			0.05	-	-	**0.02**	0.17	0.08
	132	0.17	0.08	0.11	0.05	0.50	0.30	0.56	0.32	0.23	0.31	0.16	0.10	0.07
	134	0.71	0.58	0.17	0.38	0.17	0.10	0.06	0.50	0.67	0.50	0.66	0.37	0.50
	136	0.04	-	0.32	0.04	0.17	0.40	0.28	0.05	0.03	-	**0.02**	0.03	-

	138	-	-	0.06	0.07	-	-	-	-	-	0.19	0.14	0.03	0.14
Lchu9	**420**	0.33	0.33	0.04	0.02	0.17	0.17	0.06	0.13	0.05	0.69	0.47	0.15	0.17
	422	0.13	0.28	0.39	0.21	0.83	-	0.38	0.50	0.68	0.31	0.42	0.38	0.41
	424	0.54	0.40	0.57	0.77	-	0.83	0.56	0.38	0.27	-	**0.11**	0.46	0.42
Leon27	**211**	0.42	0.44	0.34	0.34	-	-	-	0.77	0.65	0.50	0.35	0.39	0.29
	213	0.25	0.32	0.58	0.54	0.67	0.60	0.50	0.23	0.35	0.33	0.58	0.61	0.71
	215	0.33	0.24	0.08	0.12	0.33	0.40	0.50	-	-	0.17	0.08	-	-
Leon31	**328**	-	-	-	-	-	-	**0.06**	-	-	-	-	-	-
	330	-	-	0.14	0.06	-	-	**0.44**	0.23	0.37	-	**0.02**	0.04	0.04
	332	-	-	0.21	-	-	-	**0.06**	-	-	-	-	-	-
	334	0.09	0.10	-	-	-	-	**0.06**	0.18	0.10	0.06	0.02	0.07	-
	336	0.09	0.18	-	-	0.33	-	0.06	-	**0.03**	0.63	0.60	0.11	0.13
	338	-	-	0.09	-	0.33	0.20		0.18	0.03	0.31	0.33	0.14	0.21
	340	-	-	-	-	-	0.10	0.06	0.18	0.07	-	**0.02**	0.11	0.10
	342	0.82	0.73	0.53	0.94	0.33	0.70	0.25	0.23	0.40	-	-	0.50	0.50
	344	-	-	0.03	-	-	-	-	-	-	-	-	0.04	0.01
Lchu7	**341**	-	-	0.02	-	-	-	-	-	-	-	-	0.03	0.03
	343	0.55	0.68	0.58	0.65	0.33	0.20	0.44	0.28	0.29	-	**0.03**	0.33	0.26
	345	0.05	-	0.40	0.35	0.50	0.80	0.38	0.17	0.11	0.40	0.40	0.23	0.26
	347	0.41	0.32	-	-	0.17	-	0.19	0.56	0.61	0.60	0.58	0.40	0.45
Lchu6	**179**	-	**0.03**	0.02	-	-	-	-	-	-	-	-	-	-
	181	-	-	0.02	-	-	-	-	-	-	-	-	-	-
	183	-	-	-	-	-	-	**0.06**	-	-	-	-	-	-
	187	-	-	0.27	0.52	-	0.50	0.06	0.09	0.09	0.06	0.05	-	**0.06**
	193	-	-	0.08	0.01	-	-	-	-	-	-	-	-	-
	195	0.83	0.92	0.14	0.17	-	-	-	0.50	0.55	0.31	0.20	0.37	0.25
	197	0.17	0.05	0.30	0.19	1.00	0.50	0.83	0.41	0.36	0.63	0.75	0.60	0.63
	199	-	-	0.18	0.11	-		0.06	-	-	-	-	0.03	0.07
P5BE6	**123**	0.08	0.24	-	-	0.33	0.10	0.06	0.07	0.12	-	-	0.25	0.24
	129	-	-	0.03	0.11	-	-	-	-	-	0.14	0.07	0.08	0.13
	131	-	-	-	-	-	-	-	0.14	0.08	-	-	0.04	-
	133	0.46	0.37	0.32	0.41	0.67	0.90	0.56	0.57	0.58	0.86	0.91	0.38	0.40
	137	0.46	0.39	0.58	0.48	-	-	**0.38**	0.07	0.15	-	**0.02**	0.21	0.24
	139	-	-	0.08	-	-	-	-	0.14	0.08	-	-	0.04	-
Lchu4	**392**	0.04	-	0.08	-	0.25	0.30	-	0.10	0.32	0.56	0.40	0.07	0.09
	396	0.13	0.11	0.44	0.30	-	-	**0.25**	0.25	0.25	0.31	0.33	0.50	0.51
	400	0.67	0.58	0.15	0.35	0.50	0.40	0.42	0.35	0.32	0.13	0.24	0.18	0.20
	404	-	**0.06**	0.32	0.35	0.25	0.30	0.33	0.15	0.04	-	**0.02**	0.21	0.13
	408	0.17	0.25	0.02	-	-	-	-	0.15	0.07	-	-	0.04	0.06
	412	-	-	-	-	-	-	-	-	-	-	-	-	**0.01**
Leon2	**215**	-	-	0.02	-	-	-	-	-	-	-	-	-	-
	217	0.05	0.18	0.02	0.02	0.50	0.30	0.06	0.14	0.21	-	-	0.07	0.13
	219	-	-	-	**0.01**	-	-	**0.06**	-	-	-	-	0.03	0.03
	221	0.55	0.45	0.44	0.30	-	-	**0.11**	0.05	0.21	0.19	0.36	0.27	0.21
	223	-	-	0.02	0.20	-	-	**0.06**	-	-	-	-	-	**0.01**
	225	-	-	-	-	-	0.10		0.09	0.04	-	**0.02**	0.03	-
	227	0.35	0.28	0.52	0.47	0.50	0.60	0.72	0.68	0.54	0.81	0.62	0.60	0.63

	229	0.05	0.10	-	-	-	-	-	0.05	-	-	-	-	-
Leon3	**309**	-	-	-	-	-	-	-	-	-	-	-	0.03	0.01
	311	-	-	-	-	-	-	**0.06**	-	-	-	-	-	-
	315	0.32	0.54	0.21	0.26	0.17	0.25	0.11	0.14	0.23	0.43	0.32	0.03	0.11
	317	-	-	0.15	0.24	-	-	-	0.05	0.10	-	**0.08**	0.03	0.01
	319	0.68	0.46	0.65	0.50	0.33	0.25	-	0.45	0.33	0.36	0.34	0.27	0.23
	321	-	-	-	-	0.50	0.50	0.83	0.36	0.33	0.21	0.26	0.63	0.63

B7 – Genetic structure results of *Leontopithecus rosalia* from forest remnants in Brazil's Atlantic Forest using *set 1* and *set 2*. The LnP (K) was used according to Pritchard *et al.* (2000) and ΔK to Evanno *et al.* (2005). In gray highlighted, the optimal and suboptimal K statistic values that were congruent between *set 1* and *set 2* for historical and recent periods and for native (NT), translocated (TR) and reintroduced (RE) in both temporal periods. We interpreted all the optimal values and second highest biologically significant values of K that were concordant between *set 1* and 2, except for the changes within translocated site over the years. In the analyses of translocated site over time, we interpreted the particularly second highest value of K in the *set 1* and third highest value of K in the *set 2* (K = 5), because the geographical concordance of individual's ancestry probabilities in the historic period.

	K	Rep	Mean LnP(K)		Stdev LnP(K)		ΔK	
			SET 1	*SET 2*	*SET 1*	*SET 2*	*SET 1*	*SET 2*
Historic	1	10	-3,023,920,000	-2,305,680,000	0.813497	0.500666	—	—
	2	10	-2,758,660,000	-2,109,510,000	0.525357	0.835929	207,763,474	144,856,750
	3	10	-2,602,550,000	-2,034,430,000	16,337,772	7,801,574	3,310,733	0.208932
	4	10	-2,500,530,000	-1,957,720,000	11,009,092	4,950,825	0.674897	0.725132
	5	10	-2,405,940,000	-1,884,600,000	25,092,682	2,740,641	0.623289	10,906,209
	6	10	-2,326,990,000	-1,841,370,000	25,409,685	7,523,157	0.201498	0.739051
	7	10	-2,253,160,000	-1,803,700,000	30,174,426	12,793,401	0.114998	3,380,649
	8	10	-2,182,800,000	-1,757,660,000	1,421,267	4,817,607	72,062,461	22,650,251
	9	10	-2,214,860,000	-1,809,280,000	11,560,392	105,503,637	0.032871	0.542161
	10	10	-2,246,540,000	-1,815,160,000	14,656,072	11,300,069	—	—
Recent	1	10	-5,442,740,000	-4,269,110,000	0.107497	0.508702	—	—
	2	10	-4,946,590,000	-3,926,570,000	0.119722	0.133749	2,582,735,490	1,640,680,859
	3	10	-4,759,650,000	-3,803,470,000	0.538	0.537587	136,412,535	33,613,151
	4	10	-4,646,100,000	-3,698,440,000	9,361,624	3,986,143	0.314048	19,133,786
	5	10	-4,535,490,000	-3,669,680,000	13,582,215	12,530,301	6,726,444	2,216,228
	6	10	-4,516,240,000	-3,613,150,000	37,966,599	5,650,025	0.677964	2,651,316
	7	10	-4,471,250,000	-3,571,600,000	12,085,643	15,836,175	3,329,570	0.43508
	8	10	-4,386,020,000	-3,523,160,000	28,173,502	7,835,985	—	—

NT	1	10	-792,270,000	-482,280,000	0.226323	0.304777	—	—
	2	10	-749,620,000	-513,350,000	0.958935	22,928,839	40,096,582	0.657687
	3	10	-745,420,000	-529,340,000	3,520,038	43,827,546	7,548,214	0.841936
	4	10	-767,790,000	-508,430,000	11,404,819	36,467,826	—	—
TR	1	10	-2,427,610,000	-1,576,810,000	0.284605	0.363471	—	—
	2	10	-2,180,310,000	-1,432,150,000	0.144914	0.150923	715,874,011	408,751,251
	3	10	-2,036,750,000	-1,349,180,000	0.330824	0.325918	195,542,107	150,682,301
	4	10	-1,957,880,000	-1,315,320,000	5,726,896	21,936,514	0.244461	0.225651
	5	10	-1,880,410,000	-1,286,410,000	0.363471	0.993814	299,913,950	26,483,824
	6	10	-1,911,950,000	-1,283,820,000	31,023,441	11,975,234	0.268829	1,250,915
	7	10	-1,951,830,000	-1,296,210,000	10,552,625	4,479,447	7,786,688	8,016,615
	8	5	-1,909,540,000	-1,344,510,000	14,570,793	10,885,204	—	—
RE	1	10	-4,302,480,000	-3,828,130,000	0.468568	0.275076	—	—
	2	10	-4,094,720,000	-3,663,630,000	1,655,160	2,098,174	19,865,146	3,379,129
	3	10	-3,919,840,000	-3,506,220,000	1,964,801	2,156,025	52,427,691	50,036,522
	4	10	-3,847,970,000	-3,456,690,000	2,918,923	3,600,139	6,697,676	3,649,859
	5	10	-3,756,550,000	-3,394,020,000	0.556277	4,811,283	42,209,164	2,508,686
	6	10	-3,688,610,000	-3,343,420,000	1,050,344	7,355,089	26,981,640	4,565,546
	7	10	-3,649,010,000	-3,326,400,000	2,847,006	5,668,235	8,774,129	5,994,811
	8	10	-3,634,390,000	-3,343,360,000	10,163,491	27,815,591	1,642,152	1,719,539
	9	10	-3,603,080,000	-3,408,150,000	6,285,221	10,604,533	4,763,556	2,236,779
	10	10	-3,601,710,000	-3,449,220,000	41,452,957	38,074,307	—	—