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

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MARÇO DE 2017

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Tese apresentada ao Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para a obtenção do título de Doutor em Ecologia e Recursos Naturais.

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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).

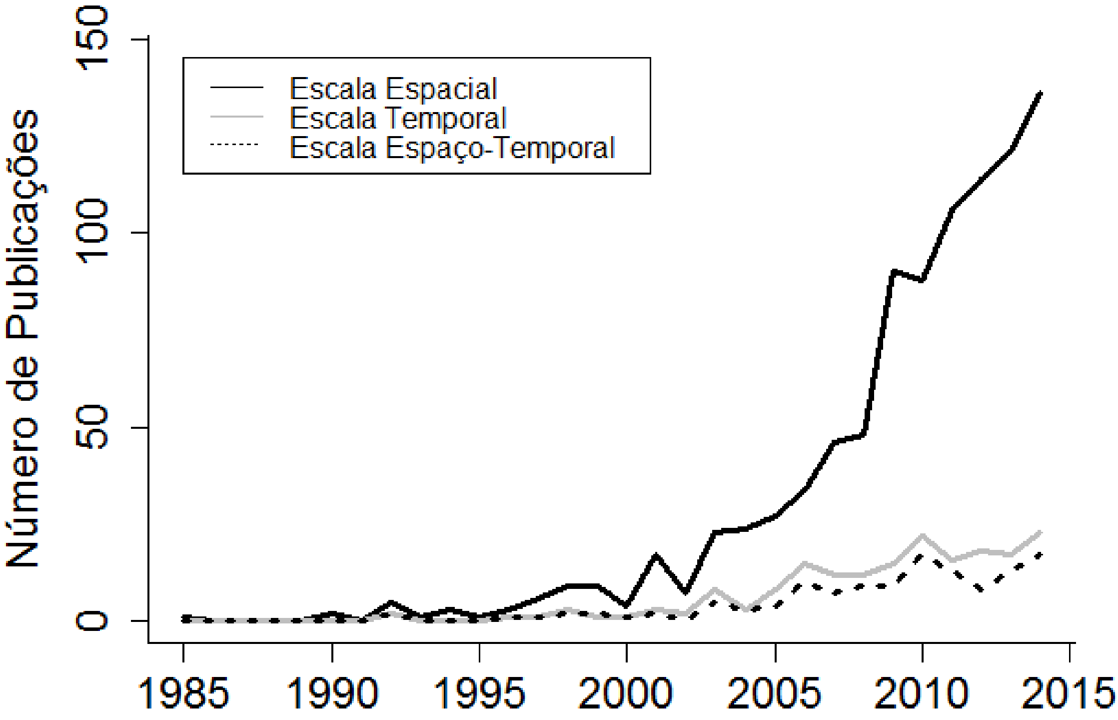

Figura a – Número de publicações encontradas no banco de dados do SCOPUS contendo no título, resumo ou palavras-chaves os termos: (1) *landscape genetic* + *population* + *conservation*, (2) *temporal scale* + *conservation genetic*, e (3) *spatial scale* + *temporal scale* + *genetic conservation*.

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).

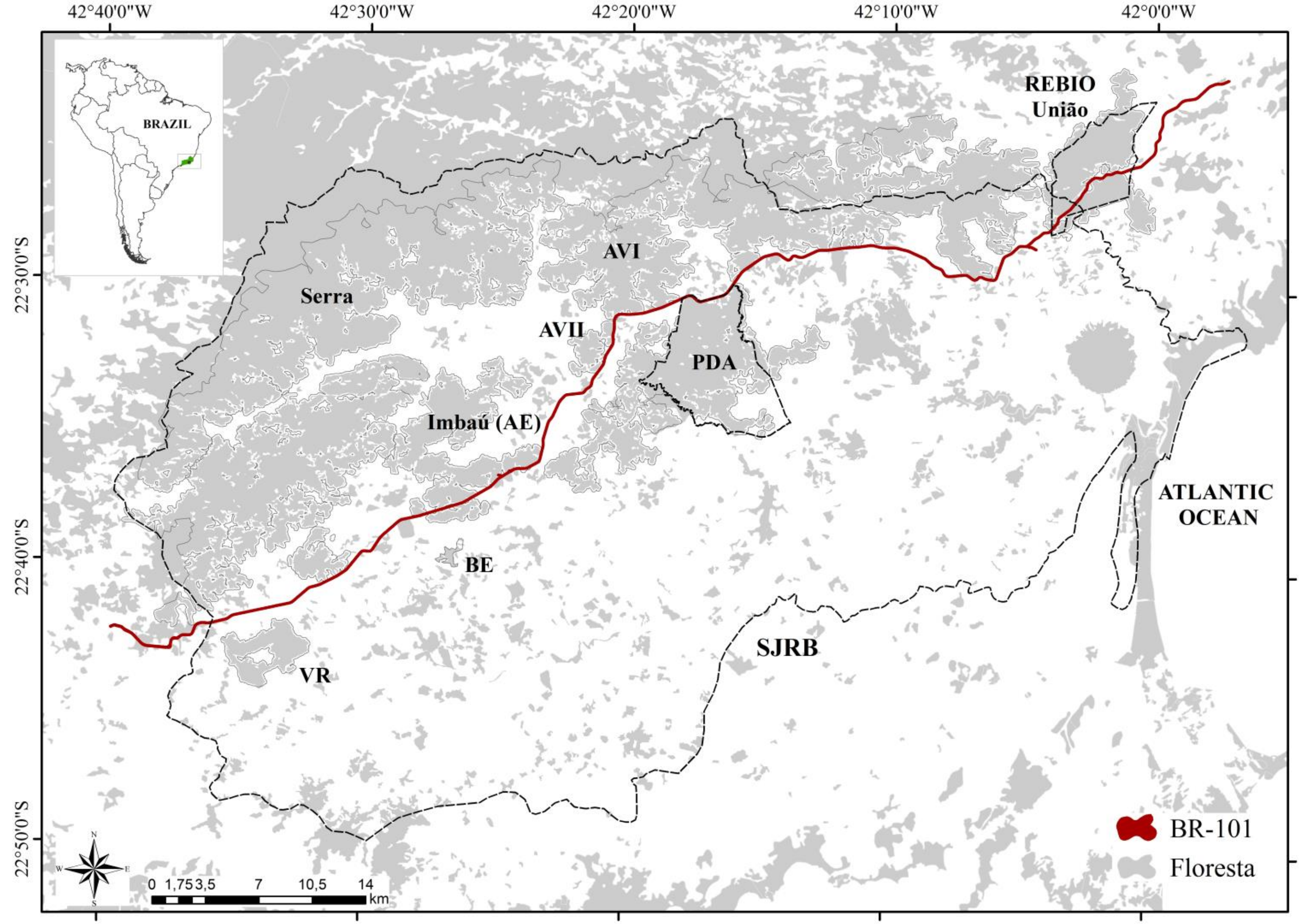

Figura b – Área de estudo e principal área de ocorrência do *L. rosalia* na Bacia do Rio do São João (SJRB) e REBIO União, Rio de Janeiro, Brasil. As siglas indicam os fragmentos florestais com ocorrência conhecida da espécie e amostrados nesse estudo, incluindo a Reserva Biológica de Poço das Antas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

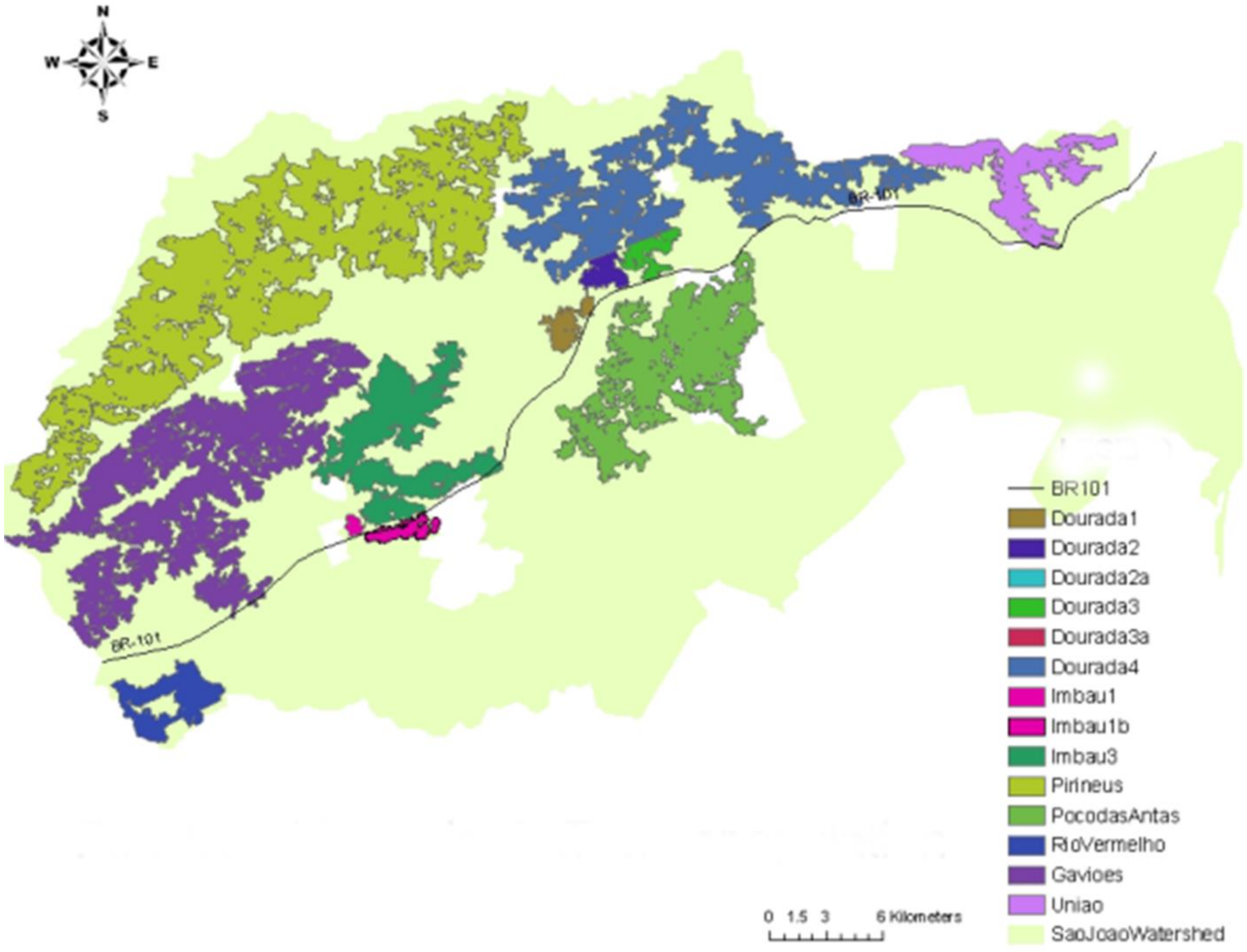

Figura c – Onze unidades funcionais do *L. rosalia* na Bacia do Rio São João delimitadas por Mickleberg (2011) usando dados de marcação e recaptura (Fonte: Mickelberg 2011).

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 1

FACTORS AFFECTING MOVEMENT AND DISPERSAL OF NEOTROPICAL PRIMATES ACROSS FRAGMENTED LANDSCAPE: A REVIEW[1]

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Author Contributions

All authors contributed to discuss the results and to review the manuscript; and AMM wrote the manuscript.

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[1] Manuscrito em preparação para submissão em periódico internacional.

Abstract

Arboreal primates are sensitive to deforestation because it has immediate effects on their individual dispersal. This study is a review of articles published from 1995–2015 that directly or indirectly assessed the movement patterns of Neotropical primates (Platyrrhini) within landscapes modified by habitat loss and fragmentation. The objectives of the review were to assess which species are most vulnerable to the anthropogenic effects, which aspects of the landscapes most affect primates, which conservation actions are most recommended, and to identify significant gaps in knowledge. We found a strong taxonomic bias in the literature, with some genera not addressed, and a lack of systematic studies of dispersal capacity. These gaps limit the kinds of generalizations that can be drawn to make conservation recommendations. Exogenous factors such as patch size and isolation, landscape permeability, resource availability, and physical barriers are the most cited to influence the movement of primates within human-modified landscapes. Although the endogenous factors that influence primate movement are still not completely understood, we found that some taxa were better able to use and survive in altered landscapes – e.g., *Alouatta* – than others – e.g., *Ateles*. Matrices structurally similar to the original habitat, such as riparian forest and agroforestry, were frequently used by primates for feeding, resting and travel. They are important strategies for maintaining or increasing connectivity among primate populations. We suggest the implementation of the conservation strategies that utilize vegetation in the matrix similar to that of the original primate habitat, and that can be economically viable. We suggest that exogenous and endogenous factors that influence Neotropical primate movement should be studied in detail, as they are essential for the effective landscape restoration projects that aim to conserve primates in the long-term.

Keywords: arboreal primates; fragmentation; landscape connectivity; movement ecology.

Introduction

Most Neotropical primates threatened by extinction occur in human-modified landscapes with intense habitat loss and fragmentation – e.g., *Leontopithecus* spp., *Brachyteles* spp., *Ateles* spp., *Sapajus* spp., *Chiropotes* spp. and others (IUCN 2016). These two processes of landscape change, often mistakenly lumped into the term fragmentation, affect different aspects of the primate´s response to habitat (Arroyo-Rodríguez & Mandujano 2009). Habitat loss refers to the reduction of the amount of habitat in the landscape, whereas habitat fragmentation is the breaking apart of the remnants in smaller habitat patches (Fahrig 2003). Our major interest is in understanding how both these processes of landscape changing interfere with two key aspects of primate behavioral ecology: movement and dispersal (definitions in Table 1.1). These two are of utmost importance to conservation, because they are essential for the maintenance of the dynamics and viability of primate populations (Nathan 2008; Henriques-Silva *et al.* 2015).

Recent studies that habitat loss and fragmentation may affect the ecology and behavior of arboreal Neotropical primates (Arroyo-Rodríguez & Mandujano 2009; Arroyo-Rodríguez & Dias 2010). Some endogenous characteristics that increase primate sensitivity to habitat degradation include canopy feeding strategies (Garber 1987), travel routes (e.g., Vié *et al.* 2001; Cervera *et al.* 2015), reliance on trees for protected sleeping sights (e.g., Aquino & Encarnacion 1986; Dietz *et al.* 1997; Di Bitetti *et al.* 2000; Vié *et al.* 2001; Raboy *et al.* 2004) and large home ranges (Milton & May 1976). Because of species differences in biology and behavior, the response to habitat loss and fragmentation, as well as the time frame of their respond, vary among primate species (Arroyo-Rodríguez & Dias 2010). The negative effects of landscape changes on population demography are immediate in some species, whereas others exhibit considerable time lag in their response to reduction in forest area and landscape connectivity (Tilman *et al.* 1994).

Reduction of the primates dispersal is the main response to habitat loss and fragmentation (Arroyo-Rodríguez & Mandujano 2009). Arboreal primates depend on forest continuity to disperse and, consequently, to avoid the negative concerns of inbreeding. So, landscape fragmentation, and the consequential reduction of forest connectivity, directly affects gene flow between arboreal primate populations (e.g., Xue *et al.* 2011) and interferes in population dynamics and viability (e.g., Zeigler *et al.* 2013). In the context of this study, we define forest connectivity as the degree of continuity of forest patches, which can be guaranteed by structural connections between forest patches, allowing primates movement without traveling on the ground.

Landscape connectivity results from a combination of environmental characteristics and disperser behavior (Tischendorf & Fahrig 2000; Bonnell *et al.* 2013; Henriques-Silva *et al.* 2015; Vasudev & Fletcher 2015). Environmental characteristics are determined by landscape structure (composition plus configuration; Fahrig 2003) which includes matrix permeability, patches size and structural connections (e.g., Anzures-Dadda & Manson 2007; Raboy *et al.* 2010; Silva *et al.* 2015). On the other hand, dispersal is determined by forces that promote it, such as competition for mates (Henriques-Silva *et al.* 2015) and food (Anderson *et al.* 2007), and by contrary forces such as increased predation risk (Anderson *et al.* 2007; Martinez & Wallace 2011; Anzures-Dadda *et al.* 2011; Henriques-Silva *et al.* 2015) and cost of survival (Henriques-Silva *et al.* 2015) associated with dispersal. Thus, connectivity is a function of the individual capacity to move through the different elements that compose the landscape, given the inherent costs and benefits of movement and dispersal (Mandujano *et al.* 2004; Vasudev & Fletcher 2015).

Factors affecting primate's movement and dispersal

The decision to use or move through the matrix of fragmented landscapes depends on the interaction between exogenous and endogenous factors (Fig. 1.1, based on Bowler & Benton 2005; Fischer & Lindenmayer 2007; Arroyo-Rodríguez & Dias 2010). Exogenous factors, i.e. the variables related to the landscape structure, have been shown to be key factors affecting population structure and persistence in Neotropical primates (Carretero-Pinzón *et al.* 2016). In addition, endogenous factors, which include the biology and behavior of primates also, have an effect on population dynamics and viability through their influence on movement and dispersal capacity.

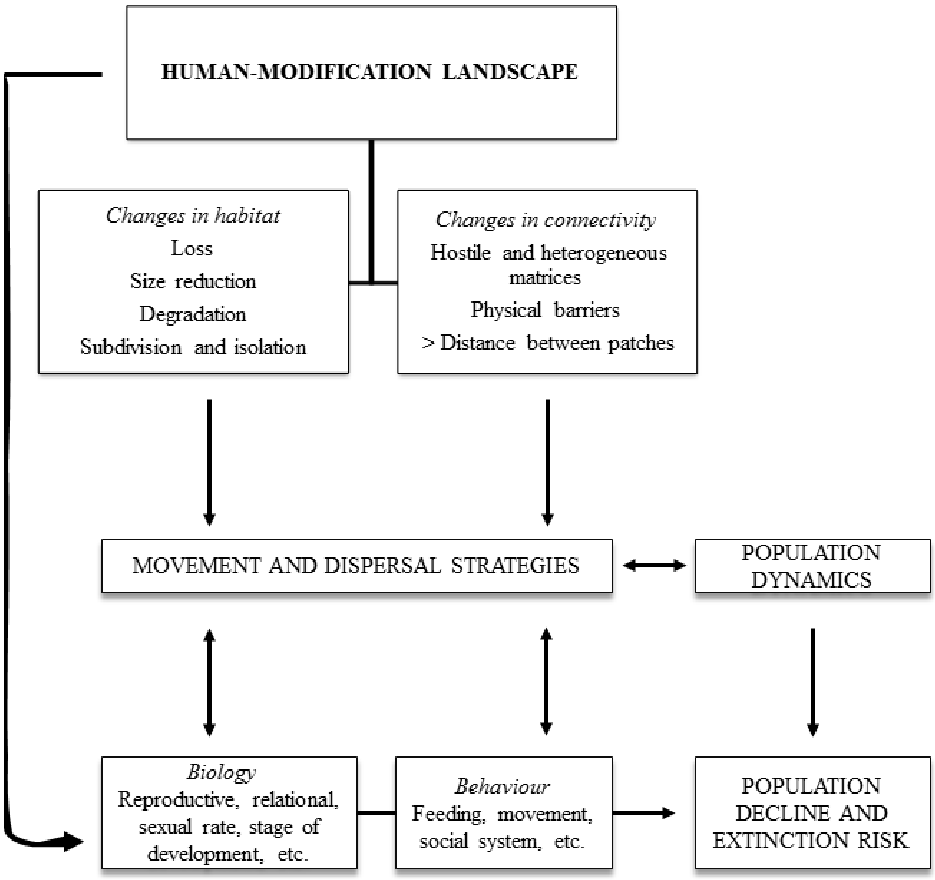

Figure 1.1– Association between exogenous and endogenous factors that affect movement ecology and dispersal behavior of Neotropical primates and consequently their population viability (modified from Bowler & Benton 2005; Fischer & Lindenmayer 2007; Arroyo-Rodríguez & Dias 2010)

Habitat patch size influences dispersal rate in many vertebrates (Bowler & Benton 2005). The early source-sink model assumed that large patches serve as the source of colonists for small patches which act as sinks (Pulliam 1988). However, some habitat patches are too small to sustain a local population or even an individual territory (Fahrig 2003). As a consequence, the emigration events probably occur most commonly from small fragments that cannot sustain a resident population, because they not reach at least the minimum territory size or do not contain preferential foods and other resources. In this sense, we can also expect that immigration will be greater in larger habitat patches that offer at least the minimum resources required to support the primate populations. Moreover, the habitat isolation, mainly due to the distance among habitat patches, is the factor that most affects the colonization of new areas (Macarthur & Wilson 1967). The probability of animals dispersal diminishes as the distance between habitats increases, due to the costs of traveling through the matrix, by the increased predation risk and the energy expended by the animal (Bowler & Benton 2005; Martinez & Wallace 2011).

The events of emigration and immigration between habitat patches also depend upon the primate´s ability to cross non-habitat portion of the landscape – the matrix. The individual will be more likely to emigrate when the environment is less hostile or resistant to its movement, and some species are more likely than others to move throughout open areas (Silva *et al.* 2015). Neotropical primates are less likely to travel through open areas since they are generally arboreal and dependent of trees for movement (Arroyo-Rodríguez & Mandujano 2009; Silva *et al.* 2015). The more open the matrix, the less permeable it will be to the movement of arboreal primates (Anderson *et al.* 2007). Agriculture is another type of matrix that may cause the isolation of natural habitats and populations, because it tends to reduce canopy cover and vegetation complexity within the matrix (Mader 1984). Depending on the crop type, it may represent an environment devoid of food resources and negatively influence primate’s decision to move or disperse (Bowler & Benton 2005). If the matrix prevents movement, primate populations will be confined to small patches, reducing the overall population size and the probability of persistence at local and regional levels (Fahrig 2003).

Other exogenous factors that may alter animal behavior and affect movement and reproductive success are human-built linear structures such as roads, channels, railways and power lines (Mader 1984). Roads (Mader 1984; Trombulak *et al.* 2000), rivers channels (Harcourt & Wood 2012) and hydroelectric dams (Cosson *et al.* 1999; Benchimol & Venticinque 2014) can act as physical barriers, isolating habitat patches and preventing gene flow between populations. Matrix structure is therefore a key variable to be considered in studies of dispersal capacity.

The degree in which habitat loss and fragmentation affect the persistence of primate populations also depends on the species inherent capacity to tolerate biotic and abiotic changes in the environment (Mader 1984; Trombulak *et al.* 2000; Anderson *et al.* 2007; Harcourt & Wood 2012). We refer to these biological traits as endogenous factors, including – but not limited to – age, sex (Jack & Fedigan 2004a; b; Boinski 2005; Boinski *et al.* 2005a; b), body size, diet (Bowler & Benton

2005) and locomotion type (Fleage 1999). The ability to make predictions based on endogenous factors can provide a means of rapidly assessing potential impacts of habitat modification and provide the basis for preliminary conservation actions, rather than waiting for the outcomes of long-term monitoring of primate response to landscape fragmentation (Carretero-Pinzón *et al.* 2016).

Discerning the role of these factors (exogenous and endogenous) separately can be difficult. For example, *Alouatta seniculus* persisted in abundance in forest islands resulting from a hydroelectric dam presumably due to its diet and larger body size in comparison to other primates (Cosson *et al.* 1999). Conversely, another large primate, *Ateles hybridus* was reported as severely affected by habitat loss and fragmentation, because of its body size and frugivorous diet, requiring large areas for subsistence. Long reproductive cycles and the rarity with which members of this species moved between adjacent forest fragments further increased the sensitivity of *Ateles hybridus* to habitat loss and fragmentation (Link *et al.* 2010). Body size and diet were important for both species, but contradictory results emphasize the interaction of exogenous and endogenous factors in determining primate persistence. Similarly, another study suggested that small fragments (1-10 ha) may sustain groups of highly folivorous primates – *Alouatta seniculus*, *Pithecia pithecia* and *Saguinus midas* – but may not sustain the large frugivorous species – *Ateles panicus* and *Chiropotes santans* (Gilbert 2003). Together, these studies demonstrate the importance of diet on the ability of primate species to adapt to and their ability to persist in human-modified landscapes.

Therefore, in this review we aim to present the results of a bibliographic analysis identifying trends and gaps in the research pertaining to movement and dispersal of Neotropical primates living fragmented landscapes. Published studies were examined to determine which exogenous factors (e.g., patch size, distance between patches, landscape structure and configuration, resource availability, physical barriers and predation) and endogenous factors (e.g., body size, type of locomotion, diet, and gender) affect movement and dispersal of Neotropical primates in fragmented landscapes (Fig. 1.1). The following questions are addressed: Which Neotropical primates taxa have their movement or dispersal most affected by fragmentation? Which anthropogenic landscape structures most affect the movement of primates through the matrix? Which actions are recommended for conservation of Neotropical primates within fragmented landscapes? We discuss the implications of our findings for conservation. Finally, we discuss directions for future research in applied movement ecology for the conservation of primates in light of the current knowledge and existing gaps in information.

Material and methods

Input

We conducted a search of original research articles published between 1995 and 2015 using the SCOPUS database (http://www.scopus.com) and Google Scholar (https://scholar.google.com.br/). We searched these databases for relevant articles using various combinations of the following search terms within the title, abstract and/or keywords: fragmentation, habitat, primate, dispersal, emigration, immigration, barriers to dispersal, connectivity, landscape, movement and/or movement ecology. A summary of the search strategy used is presented in Figure 1.2. We defined the terminology relevant to movement and landscape ecology in Table 1.1.

Processing – excluded papers

We limited the results to Neotropical primates belonging to the families Atelidae, Cebidae, Pithecidae and Aotidae. We selected the studies that provided direct or indirect information about the landscape elements that may facilitate or impede the movement of primates in a scenario of human-modification and landscape fragmentation. Many of the resulting articles used the keywords primates and dispersal to refer to the dispersal of seeds by primates, while others used the keywords primates and barriers to dispersal to refer to phylogeography and biogeography studies. Since we are interested in the contemporaneous processes that impinge on population dynamics, we discarded these kinds of studies from our review.

Output papers

Of around 500 articles found in the first phase of the bibliographic search, only 64 (around 13%) met the criteria of our research (Fig. 1.2). We maintained the studies that examined the occurrence, richness and abundance of primates in terms of landscape cover and configuration as well as those that investigated the use of space according to taxon. We considered these works as relevant because they provided indirect information about primate movement capacity in different patches and matrices. We also considered studies that investigated the consequences of restricted dispersal for the conservation of populations as well as dispersal behavior in continuous landscapes. Academic theses and dissertations providing information relevant to understanding primate movement in altered landscapes were also included opportunistically, though they did not appear in our literature search. After the filtering process, a total of 64 articles were selected. From each of these chosen publications, we collected basic information about the research conducted including taxon studied, data and general conclusions provided relevant to our topic (see Appendices A).

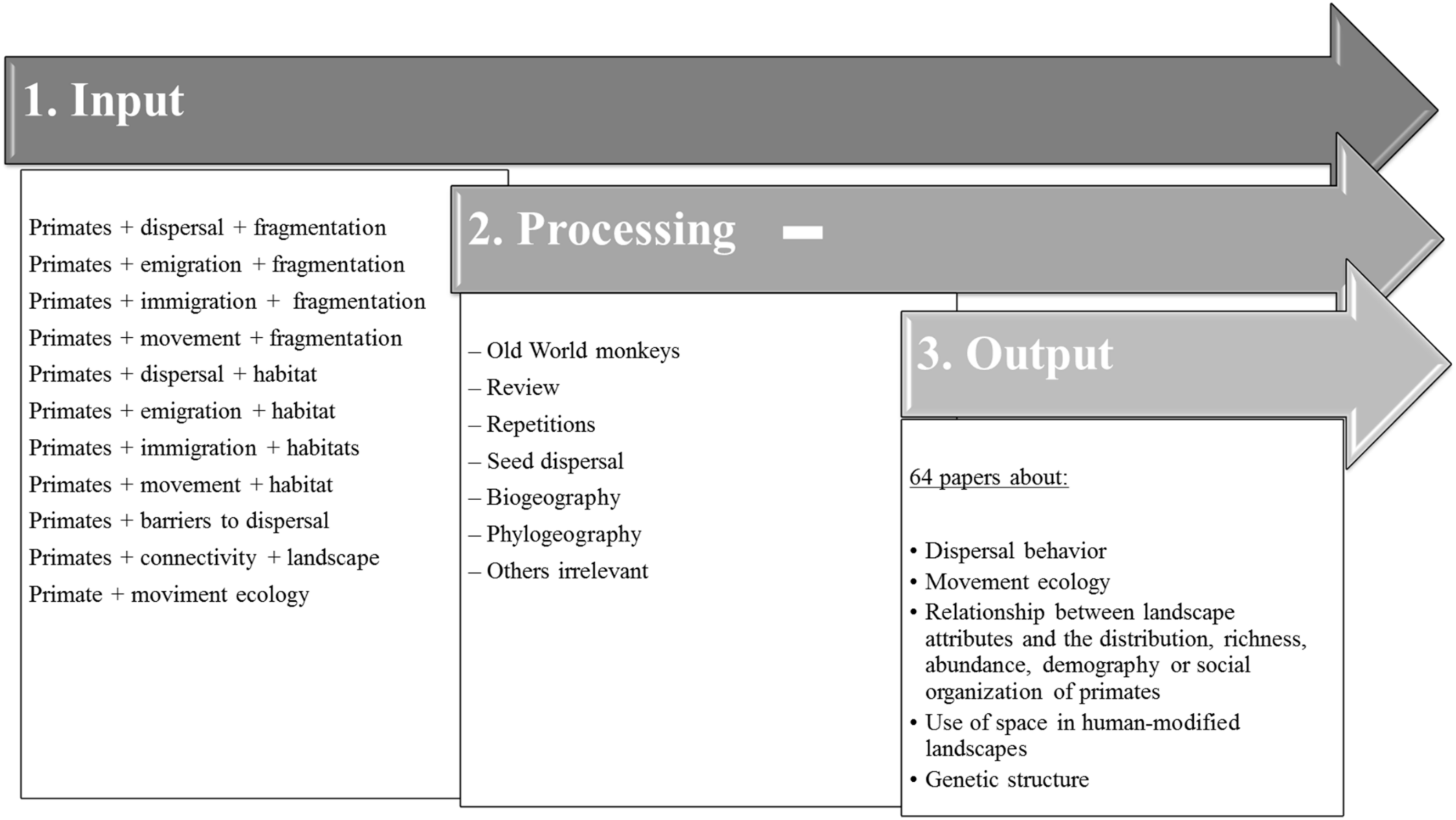

Figure 1.2 – Search strategies used during the bibliographic review. Symbol equal (-) represent the articles excluded in the processing

Table 1.1 – Terminology relevant to movement and landscape ecology

Term	**Definition**
Movement	Individual’s or group’s movement related to their biological processes (Burel & Baudry 2005).
Dispersal	Individual’s or small group’s movement from their home range to another, composed of three steps: emigration, movement through the landscape, and immigration (Bowler & Benton 2005).
Natal dispersal	Dispersal that occurs when an individual leaves their natal group at the first time (Raboy 2002).
Parallel dispersal	Joint dispersal (emigration and immigration) of two residents or related individuals (Jack & Fedigan 2004b)
Emigration	Dispersal stage where an individual decides to leave the current group or territory (Bowler & Benton 2005).
Immigration	Dispersal stage where an individual decides to enter and remain in the new group or territory (Bowler & Benton 2005).
Philopatry	Individuals of one sex dispersing more often than those of the opposite sex, the opposite sex tending to remain in the natal group (Greenwood 1980).
Gene flow	Movement of alleles between populations.
Barriers	Natural or anthropogenic landscape elements that impede animal crossing or drastically reduce dispersal capacity (Mader 1984).
Connectivity	Degree in which the landscape facilitates or impedes the movement of the animal between habitat patches. Connectivity may be (1) structural, related to landscape elements and configuration; and (2) functional, related to the individual’s behavioral responses to landscape variables (Tischendorf & Fahrig 2000).
Corridor	A linear strip of vegetation with phytophysiognomy that differs from the surrounding matrix and connects two non-continuous habitat patches.
Isolation	Fragment-scale attribute that impedes movement. It generally used to characterize the isolation by distance between two habitat fragments (Fischer & Lindenmayer 2007; Arroyo-Rodríguez & Mandujano 2009).
Landscape	Spatial unit where at least one factor is heterogeneous. Space delimited by criteria in accordance with the study object (Burel & Baudry 2005).
Fragmented landscape	Landscape with breaking apart of native vegetation into smaller patches surrounded by a human-modified matrix, independent of habitat loss (Fahrig, 2003)
Modified landscape	Landscape modified by human that includes native and non-native vegetation, and that may or may not be characterized by habitat loss and fragmentation.
Habitat	Place where the species live and that has all the resources they use (Burel & Baudry 2005).
Patch/fragment	Homogeneous forest element in the landscape, defined by its size, form and type of vegetation (restricted and nonlinear) and isolated by a matrix of inadequate environment (Burel & Baudry 2005; Arroyo-Rodríguez & Mandujano 2009).
Matrix	Dominant element in the non-habitat landscape. Always human-modified. May or may not be impermeable (Burel & Baudry 2005).
Landscape resistance	Related to the impermeability of patches or matrices, *i.e.* the relative cost of different types of surfaces that permit or impede the movement. (Burel & Baudry 2005).
Stepping stones	Small patches that are spread across the landscape and facilitate inter-habitat movement (Fischer & Lindenmayer 2007; Arroyo-Rodríguez & Mandujano 2009)

Results and discussion

Movement and dispersal of Neotropical primates in human-modified landscapes

The number of publications documenting primate responses to habitat fragmentation has increased rapidly over the last 10 years; however, the numbers of publications that deal specifically with primate dispersal or movement in fragmented landscapes have lagged behind (Fig. 1.3). In our review of 64 articles specifically targeting research on primate movement through fragmented landscapes, 23 studies (35%) identified direct movement through or use of the matrix, and 22 papers (34%) measured rate of dispersal or gene flow (Appendices A). These last 22 articles, only eight observed successful dispersal through the matrix, as defined by the establishment of a new territory in another fragment by the dispersing individual or primate group. Combined, these results reflect the increasing importance placed on understanding the consequences of habitat loss and fragmentation for primate ecology and conservation, while also demonstrating the need for increasing efforts to understand the limits on primate movement and dispersal as well as the exogenous and endogenous factors that define these limits for each species.

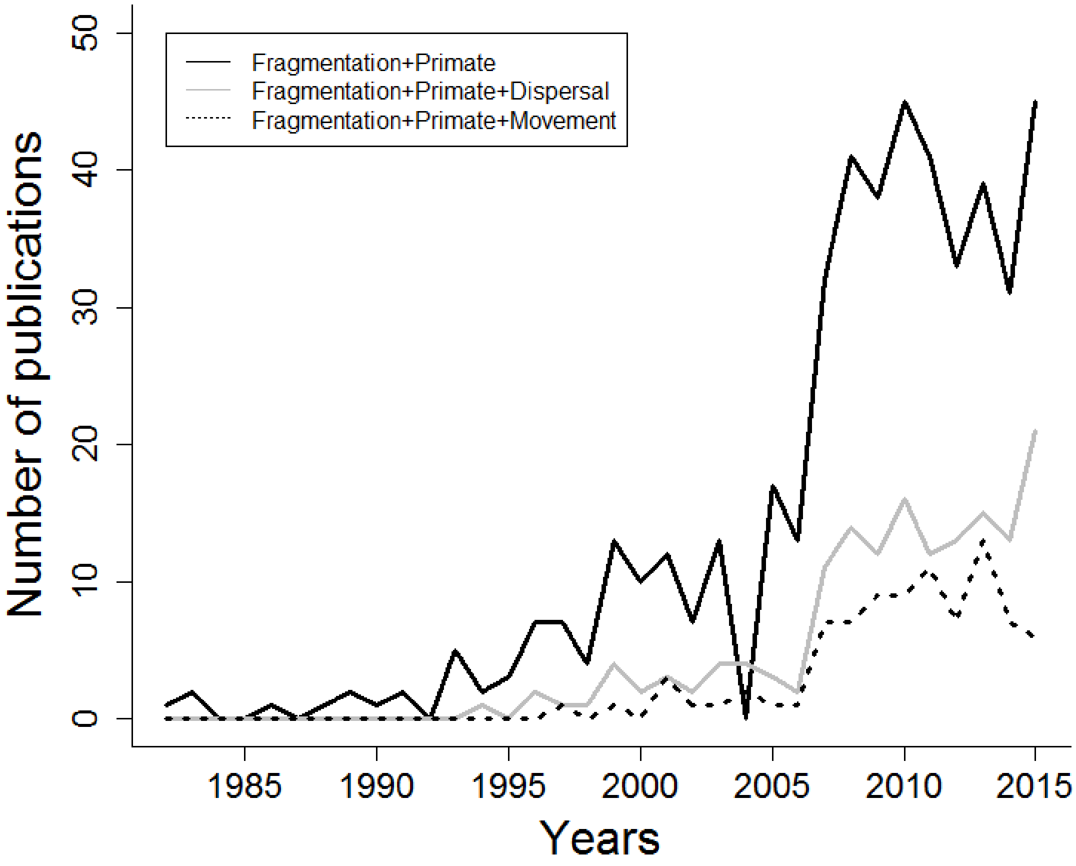

Figure 1.3 – Publication numbers present in the SCOPUS database from 1985 to 2015 that contain the following terms in their title, abstract or keywords: fragmentation plus primates (solid black line); fragmentation plus primates and dispersal (solid gray line); and fragmentation plus primates and movement (dashed line)

Our review also reveals a taxonomic bias in the study of primate movement in fragmented landscapes (Fig. 1.4). The majority of studies presented in this review focused on primates from the families Atelidae and Cebidae – 75% and 67% of the studies, respectively. Pithecidae and Aotidae were less represented – with 25% and 2%, respectively. The genus *Alouatta* (Atelidae) was the most studied, while *Aotus* (Aotidae), *Lagothrix* (Atelidade), and *Callithrix* (Cebidae) were those with the fewest studies. None of these studies reported *Aotus*, *Ateles, Callithrix* or *Lagothrix* moving through the matrix (Table 1.2). According to IUCN (2016), *Ateles* and *Lagothrix* are the Neotropical primate genera most threatened and their species are included in categories ranging from ‘vulnerable’ to ‘critically endangered’. *Aotus spp.* are categorized as being of ‘least concern’, ‘vulnerable’ or ‘data deficient’ (IUCN 2016). Among the species of *Callithrix*, *C. flaviceps* is ‘endangered’ by extinction and *C. aurita* is ‘vulnerable’ (IUCN 2016). The future of these primates, particularly *Ateles* and *Lagothrix,* may be compromised, and future research should be aimed at understanding their dispersal strategies in the current scenario of landscape modification and fragmentation. In addition, knowing the movement patterns of non-threatened species such as *Callithrix jacchus* and *Callithrix penicillata* is also important. These two species are often introduced outside their ranges as the result of the illegal wildlife trade and can act as invasive species posing a threat to biodiversity where they were introduced (Morais Jr 2010; Oliveira & Grelle 2012). Observations of primate movements through the matrix or their absence may reflect the capacity or inability of primates to adapt to new environmental conditions. Alternatively, our results may also reflect the lack of research on this aspect of primate movement ecology or the difficulties inherent in studying particular primate species (Pinto *et al.* 2013).

The genera *Brachyteles* (Atelidae), *Cacajao* (Pithecidae), *Callimico* and *Cebuella* (Callitrichidae) were not included in any of the studies considered in this review, reflecting a lack of information regarding the movement and persistence of these taxa within modified and fragmented landscapes. We conducted a new bibliographic search through the SCOPUS database, using the keywords mentioned above, but substituting the term *primate* with the names of the four genera not previously found. We found one additional article that used theoretical rates of dispersal to model the future of a *Brachyteles* population (Eduardo & Brito 2012)*,* and another article about the use of habitat by *Callimico* (Porter *et al.* 2007). However, none of these genera were the subject of any study that quantified movement or dispersal in a modified and fragmented landscape. The lack of information about functional connectivity between populations can seriously diminish the effectiveness of conservation actions (Vasudev & Fletcher 2015). *Brachyteles*, for example, are primates highly threatened by extinction that require large areas of forest (Mendes *et al.* 2008). Empirical studies about species dispersal to understand species-specific thresholds of connectivity are urgently need. Only with this kind of data we can understand how landscape affects persistence, thus refine population viability models to more accurately reflect current primate status in a human-modified landscape (Eduardo & Brito 2012).

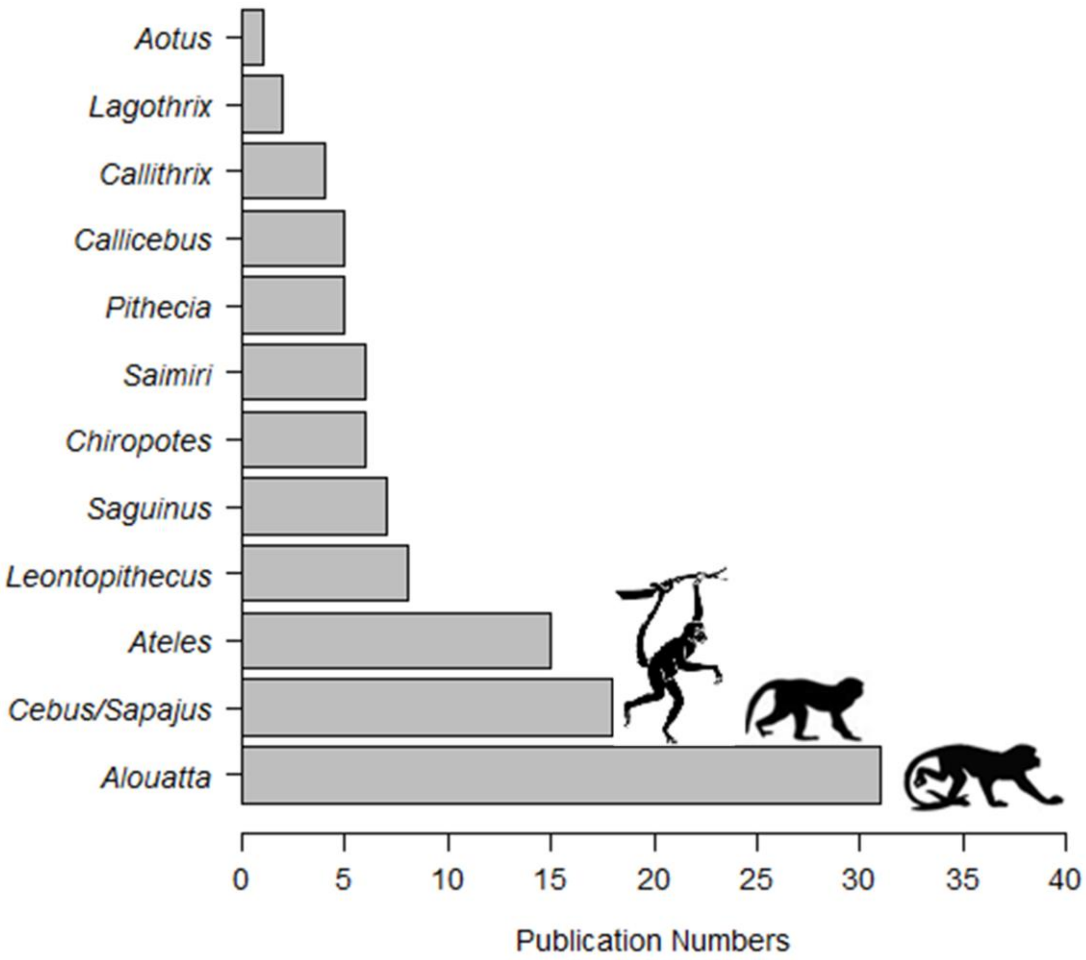

Figure 1.4 – Publication numbers assessed in this review for each taxonomic group of Neotropical primates

Howler monkeys (*Alouatta*) were reported as able to withstand habitat changes and as capable of surviving in and moving through disturbed and highly human-modified patches (Estrada *et al.* 1999a; Cosson *et al.* 1999). It was most cited genus in relation to events of movement through the matrix or through non-native forests (Table 1.2). After habitat loss and isolation caused by flooding due to a hydroelectric dam, *Alouatta seniculus* persisted in fragments of up to 1 ha (Cosson *et al.* 1999). Other studies show that howler monkeys can cross open areas (Oklander & Corach 2013) and also use crown to crown canopy bridges made of rope suspended above narrow roads to move between patches with a high percentage of urbanized landscape (Teixeira *et al.* 2013). However, this information and its application to the conservation of the taxon should be used with caution, because other studies indicated that howler monkey populations have also been reported be negatively affected by the fragmentation process and habitat disturbance (Anzures-Dadda & Manson 2007; Candelero-Rueda & Pozo-Montuy 2010).

Table 1.2 – Summary of studies that examined movement of primates through the matrix and/or forest fragments and the maximum (or probable) distance traveled

Species	**Maximum distance (km)**		**Reference numbers supporting**	
	Matrix	*Forest*	*Matrix*	*Forest*
Alouatta guariba clamitans	Unknown	Unknown	[17]	[17]
Alouatta palliata	0.65	0.77	[8, 13, 31, 43, 48]	[51, 61]
Alouatta pigra	0.09 and 0.31[A]	5.30[C]	[6, 44]	[6, 24, 44, 57, 60]
Alouatta seniculus	1.30	Unknown	[19, 62]	[19, 62]
Aotus azarae	–	1.22	–	[47]
Ateles geoffroyi	–	34.8[C]	–	[24]
Callicebus spp.	Unknown	Unknown	[36, 62]	[36, 62]
Callicebus olallae	0.04	Unknown	[22]	[22]
Cebus capucinus	Unknown	Unknown	[39]	[39, 40, 42, 49
Chiropotes chiropotes	0.03 – 0.15[B]	0.4–3[D]	[7]	[29, 55]
Leontopithecus caissara	–	4.12	–	[50]
Leontopithecus chrysomelas	Unknown	4.00	[4]	[54]
Leontopithecus rosalia	Unknown	6.60	[34, 35]	[35]
Pithecia pithecia	0.03 – 0.15[B]	11.5	[7]	[23]
Saimiri spp.	Unknown	Unknown	[62]	[25, 58]
Saguinus sp.	0.02 – 1.4[B]	Unknown	[19]	[59]
Sapajus paella	0.03 – 0.15[B]	Unknown	[7, 62]	[62]

[A] Respectively, mean distance that the group moved through pastureland, and straight-line distance from the edge of the habitat fragment to the farthest point to use scattered trees beyond the mango plantation.
[B] Shortest and longest distances between fragments, and probably traveled by primates.
[C] Distances traveled in around 175 h of sampling.
[D] Distances traveled in a small 1-ha fragment and in the forest, respectively.
Unknown: Primates travel through spatial element, but the study did not report the distance.

Exogenous factors that influence movement and dispersal

Landscape structure and composition – that refers to the landscape permeability (including quality habitat and/or permeability matrix) to primate movement according to vegetation structure (basal area, canopy complexity, etc.) – was the factor most cited (24) as influencing the distribution, movement and dispersal of Neotropical primates. Other cited factors creating resistance to movement included distance between patches, patch size, availability of food resources (particularly fruit), physical barriers, and predation by domestic animals or carnivores (as summarized in Fig. 1.5 and Table 1.3). The degree of habitat perturbation, temperature, fragment aggregation and edge effect were also mentioned (14 articles).

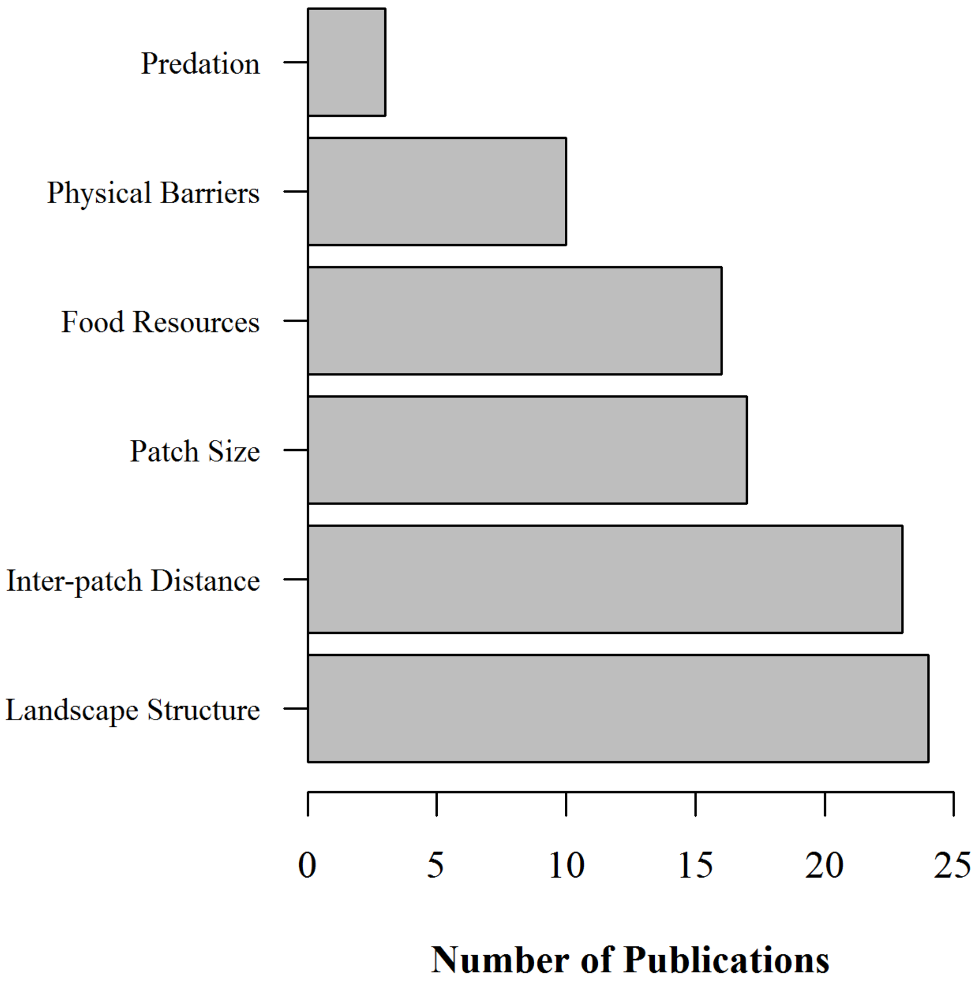

Figure 1.5 – Exogenous factors that influence primate movements and the publication numbers that cited them. Landscape structure = referred to vegetation structure (basal area, canopy type, perturbation degree, etc.) of the habitat or matrix that facilitated or limited the primate movement; inter-patch distance; patch size = size of habitat fragment; food resource = availability of food resources (especially fruits); physical barriers = rivers and floods; and predation

Table 1.3– Exogenous and endogenous factors that influence the primate's dispersal or movement through a human-modified and fragmented landscape and summary of 50 recent studies that supported the expected results.

Factors	Expected results	Taxon	Supporting references
Landscape structure	1. The lower the structural complexity of the matrix (presence of arboreal species), the fewer the number of events of primates moving in the matrix.	*Alouatta* spp., *Ateles* spp., *Callicebus* spp. and *Cebus* spp.	[31, 36, 32, 39, 44, 45]
Inter-patch distance	1. The greater the inter-habitat distance, the fewer the number of events of individuals crossing the matrix.	*Alouatta palliata*, *Callicebus olallae* and *Leontopithecus rosalia*	[8, 13, 22, 31, 32, 35]
	2. The greater the distance, the greater the genetic differentiation between populations.	*Saimiri oerstedii* and tamarins	[18, 33]
Patch size	1. The smaller the patch size, the less distance travelled and the fewer the number of movements.	*Ateles geoffroyi*, *C. santanas chiropotes* and *Leontopithecus rosalia*	[21, 29, 35, 55]
	2. The smaller the patch size, the greater the number of emigration events	–	–
Food resources	1. Matrices are used for feeding activities.	*Alouatta* spp., *Leontopithecus chrysomelas*, and *Cebus capucinus*	[28, 39, 44, 62]
	2. Food resources determine the pattern of movement.	*Alouatta* spp., *Ateles geoffroyi* and *Saguinus geoffroyi*	[24, 31, 44, 51, 56, 57, 59]
Physical barriers	1. Roads, rivers and water bodies impede movement.	*Alouatta* spp., *Ateles* spp., *Cebus* spp., *Chiropotes sagulatus*, *Lagothrix cana*, *Saguinus* spp., *Saimiri* spp. and *Pithecia* spp.	[19, 30, 41, 46]
Predation	1. Primate predation events are observed in the matrix.	*Sapajus apella* (three predation)	[62]

ENDOGENOUS	Body size	1. Primate species with larger body size are seen more often in the matrix than small primates.	*Callicebus olallae*	[22]
	Locomotion type	1. Species with quadruped locomotion engage more in movements through open areas than brachiating species.	–	–
	Diet	1. Species with a specialist diet, such as frugivorous species, require moving over large distances and look in more hospitable areas its preferential food.	–	–
	Gender	1. Females engage least movement through the matrix than males.	*Alouatta caraya*	[14]

The literature suggests that some primates are not capable or willing to cross open fields, and those matrices with phytophysiognomies similar to that of the surrounding forest were used more often (Table 1.4). For the majority of cases in which structural connectivity (or landscape resistance) was considered an important factor restricting primate movement (16 articles), the matrix was composed primarily of pasture (12 of 16 studies). Various studies showed that movement frequency and matrix permeability increased with vegetation height and crown cover (Estrada & Coates-Estrada 1996; Estrada *et al.* 1999a; Williams & Vaughan 2001; Anzures-Dadda & Manson 2007; Hopkins 2011; Pozo-Montuy *et al.* 2011; Plante *et al.* 2014). In the absence of matrix elements similar to the original habitat, primates demonstrate some flexibility in their choice of alternative substrates for locomotion, including canopy bridges made of rope (Teixeira *et al.* 2013) and wire fencing (Martinez & Wallace 2011). Species that were not observed crossing a non-forested matrix such as *Ateles* spp. (Michalski & Peres 2005; Lees & Peres 2008; Link *et al.* 2010; Boyle & Smith 2010b), *Aotus brumbacki* (Carretero-Pinzón 2013) and *Lagothrix* spp. are probably among the Neotropical primate taxa most threatened and sensitive to the effects of fragmentation.

Primate populations separated by large distances of structurally connected forest may not be nearly as isolated from one another as those populations separated by short distances of relatively impermeable matrix. The primate movement seems to be inversely proportional to distance between fragments (Estrada & Coates-Estrada 1996; Mandujano 2005; Martinez & Wallace 2011; Mickelberg 2011). The biology and behavior of each primate species interfere in their capacity for movement and dispersion through of different scenarios of human-modified landscapes (Fig. 1.1). Di Fiore and Valencia (2014) suggest the existence of a biological limit on the distance that tamarins species are capable of traveling under favorable conditions. For this reason, empirical research measuring the distance that primates are capable of travelling are important, particularly for those species with fragmented populations separated large distances by hostile matrices.

Table 1.4 – Summary of matrix elements used by primates in their biological activities.

Species	**Matrix elements**	**Supporting references**
Alouatta clamitans	Rope pathways from crown to crown	[17]
Alouatta palliata	Agroforestry, mixed plantation, living fences, annual crops and pasture	[8, 31, 32, 43]
Alouatta pigra	Shrubs, living fences, isolated and scattered trees, eucalyptus and orchards as mango plantations	[6, 44]
Alouatta seniculus	Shrubs, living fences, *Ficus* trees and pastures	[19, 62]
Ateles geoffroyi	Agroforestry and mixed plantations	[32]
Callicebus ornatos	Living fences	[62]
Callicebus olallae	Wire fence isolated and scattered trees and pasture	[22]
Cebus capucinus	Riparian forest, living fences, plantation of mango and palm	[39]
Leontopithecus chrysomelas	Agroforestry and pasture	[28, 53]
Leontopithecus rosalia	Pasture	[34, 35]
Saguinus midas	Leafless trees	[19]
Saimiri sciureus	Shrubs, living fences and pasture	[62]
Sapajus apella	Riparian forest, shrubs, living fences and pasture	[62]

Many studies inferred that isolation by distance is the main factor that influences primate abundance, distribution (Anzures-Dadda & Manson 2007; Boyle & Smith 2010b; Pozo-Montuy *et al.* 2011; Arroyo-Rodríguez *et al.* 2013) and primate movement (Estrada & Coates-Estrada 1996; Williams & Vaughan 2001; Mandujano *et al.* 2004; Boyle *et al.* 2009; Mickelberg 2011; Zeigler *et al.* 2011) (Fig. 1.5). On the other hand, our review revealed more direct observations of primate movement within the forest than within the matrix. Furthermore, few studies investigated the distance that primates are capable of crossing the matrix. The maximum distance traveled by primates in the matrix varied from 0.02 to 1.4 km, while within the forest the traveled distance varied from 0.4 to 34.8 km (Table 1.2). These results showed that primates traveled greater and more frequent travel distances through forest as compared to matrix, indicating that structural connectivity affects movement strategies and that the degree of isolation is not merely distance dependent.

Patch size was the third most cited of the exogenous factors responsible for the limitation of the primate movements (Fig. 1.5). The theoretical expectation is that small patches would lead to more emigration, albeit population density is a significant intervening variable (Bowler & Benton 2005). Studies with Neotropical primates have documented that time travelling was lesser in fragments than continuous forest (*Ateles geoffroyi*, Chaves *et al.* 2011) and that traveled distance within a fragment was directly proportional to the size of the fragment traveled (*Chiropotes santanas chiropotes*, Boyle *et al.* 2009). Five studies correlated the distribution and abundance of primates with patch size (Estrada & Coates-Estrada 1996; Estrada *et al.* 1999a; Anzures-Dadda & Manson 2007; Boyle & Smith 2010b; Silva *et al.* 2015), but no studies related the inter-patch distance traveled or the frequency of emigration events to the size of the fragments. Consequently, we cannot assert if patch size influences emigration rate in Neotropical primates.

The availability food resources within the matrix was also another frequently cited factor (Fig. 1.5) that influenced: (a) patterns of primate habitat use and movement (Estrada *et al.* 1999b; Boyle & Smith 2010a; Madden *et al.* 2010; Hopkins 2011; Pozo-Montuy *et al.* 2011; Bonnell *et al.* 2013; Fernández *et al.* 2013; Plante *et al.* 2014); (b) distribution and (c) abundance of primates (Boyle & Smith 2010b; Pozo-Montuy *et al.* 2011). The matrix was used by primates for (a) movement (Cosson *et al.* 1999; Williams & Vaughan 2001; Mandujano *et al.* 2004; Raboy *et al.* 2004; Anzures-Dadda & Manson 2007; Boyle & Smith 2010b; Mickelberg 2011; Pozo-Montuy *et al.* 2011, 2013; Paula 2013; Teixeira *et al.* 2013; Silva *et al.* 2015), (b) feeding activities (Williams & Vaughan 2001; Raboy *et al.* 2004; Pozo-Montuy *et al.* 2011, 2013), (c) rest (Williams & Vaughan 2001; Pozo-Montuy *et al.* 2011, 2013) and (d) as sleeping sites (Carretero-Pínzón 2013; Raboy *et al.* 2004). Some frugivore-folivore species use the matrix if it has food resources in it and is close proximity to the forest (Anderson *et al.* 2007; Wieczkowski 2010). Agriculture is often considered less permeable to the movement of arboreal primates (e.g., Estrada & Coates-Estrada 1996; Estrada *et al.* 1999a; Pozo-Montuy *et al.* 2011; Blair & Melnick 2012). However, the lack of food resources in degraded forest patches, and the presence of alternative foods in the matrix, such as cultivated fruits, may encourage primates to the use the matrix (e.g., Hopkins 2011; Pozo-Montuy *et al.* 2011).

Some studies also cited rivers (Chaves & Stoner 2010; Palminteri *et al.* 2011; Rabelo *et al.* 2014), floods caused by hydroelectric dams (Cosson *et al.* 1999; Benchimol & Venticinque 2014), roads (Vié *et al.* 2001; Williams & Vaughan 2001; Teixeira *et al.* 2013) and palm plantations (Blair & Melnick 2012), as barriers hindering movement, dispersal or negatively influencing gene flow (Fig. 1.5). For rivers, the distance between banks and the presence of trees on both banks seem to be significant factors to primate movement (Chaves & Stoner 2010). Roads are potential physical barriers, and are present on all landscapes where Neotropical primates occur; therefore assessing their impact is critical to conservation efforts. None of the aforementioned studies considered roads as possible barriers, nor have studies quantitatively evaluated the influence of roads on the movement of Neotropical primates. Studies report casual observations that some species (e.g., bearded sakis) are reluctant to cross roads (dirt roads) (Vié *et al.* 2001). Teixeira *et al.* (2013) also reported that *Alouatta clamitans* was suffering high mortality rate due to road kills in a small rural road (width not reported) and that rope canopy-canopy pathways placed above roads were used by individuals. Given the ubiquitous presence of roads, including major highways, on the where landscapes of a great number of Neotropical primate species occur, quantitative studies are critical to evaluate how these elements affect dispersal capacity.

Predation was the final exogenous factors assessed in this review. We found only three studies reported the influence of predation events in primate's movement (Boinski *et al.* 2005b; Martinez & Wallace 2011; Carretero-Pinzón, 2013) We found no studies evaluating the effects of predation pressure in the matrix by domestic or wild animals on primate movement. Given that a large number of primate species occur within urban or agricultural landscapes in which domestic dogs and cats (feral or owned) as well as raptors are commonplace, the effects of predation on primate dispersal capacity warrants more systematic study.

Endogenous factors that influence movement and dispersal

Body size is an endogenous factor that plays an important role in the decision making process of dispersal. The perceived scale of and costs involved in crossing landscape barriers are dependent upon body size. For a small arboreal primate, the energy expenditure of crossing a pasture using quadruped bounding may limit the distance that can be traversed (Mech & Zollner 2002). In addition, predation risk may be higher for small primates (Martinez & Wallace 2011). Martínez and Wallace (2011) observed *Callicebus* traversing distances of less than 50 meters of matrix using quadruped bounding when presented with open ground, but choosing their predominant mode of quadruped walking over wire cattle fences where available. The predominant locomotion style of a species, which can be somewhat correlated with body size, may be a key variable influencing the likelihood of a primate to move over open spaces (pastures, crops, barren ground), in as much as, non-predominant locomotion is more energetically costly than the predominant locomotion style. To date there is insufficient data for a consensus on the role of body size or locomotion style on the dispersal capacity of Neotropical monkeys.

On the other hand, large body size, the ability to cross open areas (Oklander & Corach, 2013) and use canopy bridges made of rope suspended above narrow roads to move between patches with a high percentage of urbanized landscape (Teixeira *et al.* 2013), combined with a broad diet have been found to be important characteristics allowing the persistence of *Alouatta seniculus* in fragmented landscapes (Cosson *et al.* 1999; Gilbert 2003). Thus, the species dietary breadth or ecological flexibility may be also affect dispersal capacity by both perception of available resources within fragment (patch quality) and the attractiveness of food resources in the matrix. Species with a generalist diet (e.g., frugivore-folivore) may find resources more easily than those with more restrictive diets (e.g., some frugivore species). Generalists may be more likely to find food within their fragment, but may also be attracted to items in the matrix (in a hedgerow, orchard or corridor), thus enhancing movement capacity. Narrow diet breadth species may be required to move over greater distances (Bonnell *et al.* 2013) or to less hospitable areas to find food, particularly in the dry season. Despite insufficient data are available to prove this relationship between diet and movement, the most cited factor as a strong motivator for primates to move was the search for food resources (e.g., Estrada *et al.* 1999b; Fedigan & Jack 2009; Fernandez-Duque 2009; Boyle & Smith 2010a; b; Hopkins 2011; Pozo-Montuy *et al.* 2011; Zeigler *et al.* 2011; Bonnell *et al.* 2013).

Gender may be another important variable. If there are sex-differences in dispersal, then the sex that disperses will be more affected by fragmentation. Males may be more prone than females to expose themselves to risk, due to their generally reduced parental investment and the role of competition for reproduction (Dreber & Hoffman 2009). Genetic analyses of *Alouatta caraya* showed also that in continuous forests, dispersal rate did not differ between the sexes, whereas in matrix areas the dispersal of females was more limited (Oklander *et al.* 2010). Furthermore, movement of solitary males through the matrix has been extensively reported in *Alouatta palliata* (Mandujano *et al.* 2004)*, Saimiri sciureus* and *Sapajus apella* (Carretero-Pizón 2013), but not one event of solitary females moving through the matrix has been reported.

There are different costs associated to different social groupings during dispersal, and the inter-patch movements of through the matrix of complete or partial social groups have been documented – four of *Alouatta palliata* (Estrada *et al.* 1999a; Mandujano *et al.* 2004; Anzures-Dadda & Manson 2007; Hopkins 2011), and one of *Saimiri sciureus* (Carretero-Pizón 2013). Parallel dispersal is common in *Cebus capucinus* and *Leontopithecus rosalia* (Jack & Fedigan 2004a; b; Fedigan & Jack 2009; Paula 2013). Parallel dispersal increases the benefits of indirect fitness and the chance of survival for reproductively immature dispersers (Jack & Fedigan 2004a; b). Dispersal in pairs or small groups of individuals may also increase the protection against predators and optimize the discovery and use of resources during travel and in the new territory, particularly for the youngest individuals. These advantages may motivate pairs or small groups of individuals to move together through fragmented landscape.

The socio-ecological context within the fragments may also influence individual decision and consequently dispersal rates. The type of social system and intra-specific competition or population density (Fedigan & Jack 2009; Spehar *et al.* 2009; Zeigler *et al.* 2011) will determine the number of reproductive vacancies, the presence of extra-group partners (Lazaro-Perea *et al.* 2000; Paula 2013), the likelihood of infanticide (Fedigan & Jack 2009) and the probability of inbreeding (Boinski *et al.* 2005b). These conditions interact with exogenous factors such as inter-habitat distance, matrix structure and predation risk shaping the individuals decision making strategy.

Notwithstanding, we found no studies that systematically associate the persistence or dispersal capacity of primates in human-modified landscapes with intrinsic biological characteristics. There is a lack of data on proximate factors such as perceptual range, the relevant sensory stimuli related to habitat cues and ecological traps or the role of conspecific attraction; all of which are variables shown to be important for terrestrial vertebrates (Bowler & Benton 2005). There is not enough information available to make inferences about the relative role of endogenous traits on primate movements in human-modified landscapes. This information is important to assess the capacity of primates to adapt to large scale habitat changes and should be better evaluated in future studies.

Implications for conservation

Models have suggested that, in the future, local extirpation of primates will occur due to the reduction of their dispersal potential – e.g., *L. chrysomelas* (Zeigler *et al.* 2013). Studies must evaluate the effect of potential barriers or methods of connectivity in a way that provides accurate information for conservation planning. It is worth considering how scientific data enters into the conservation decision making process. Modeling is useful and commonplace tool to construct scenarios of population and habitat viability over time (Brook *et al.* 2002). The results of these models are incorporated into Population and Habitat Viability Assessments or other group based decision processes (e.g., National Action Plans (PANs). However, for reliable inferences, to be used in conservation actions decisions, these models have to be built upon reliable and accurate data, which is still scarce. Thus, the lack of knowledge about a species dispersal capacity can result in unwarranted assumptions and misguided modeling. If the assumptions underestimate dispersal capacity, then resources can be wasted establishing landscape connections where none are needed. If dispersal capacity is overestimated, then gene flow may be below the necessary threshold and population viability will be compromised.

One recommendation is that if the rate of dispersal by primates is lower than the rate of deforestation, an solution at the regional level would be habitat restoration through the creation of corridors, stepping stones or enrichment of the matrix (Mandujano *et al.* 2004; Anderson *et al.* 2007; Boyle & Smith 2010b). However, these actions need to be guided by the ecological information and behaviors of each species (Anderson *et al.* 2007). Mandujano *et al.* (2004) highlight the relevance of stepping stones, among other strategies, to increase the capacity of dispersal in brown howler monkeys. Isolated trees, living fences, riparian forests, and habitat patches that are substantially smaller than the home range of the primate also may function as stepping stones. In Brazil, the country with the highest primate diversity, the National Action Plans (PAN) recognize that fragmentation and agricultural crops are a problem and recommend an overall increase in connectivity by 20%, mentioning corridors specifically as indicators (Escarlate-Tavares *et al.* 2016). However, are there enough data published to make generalizations on whether forest corridors are necessary for Neotropical primates, or if hedgerows are sufficient or is it preferable to strive for a permeable matrix solution such as, agro-forestry or commercial orchards or tree plantations?

The enrichment of the matrix in agricultural areas and pastures, so that the matrix resembles the structure of the original habitat, enriched with tree species (Pozo-Montuy *et al.* 2011) that provide food and safe passage to primates (Williams & Vaughan 2001) seems to be the best conservation strategy. Agro-forest systems can be implemented to reestablish or maintain landscape connectivity, while serving as alternative habitat for some Neotropical primate species (Raboy & Dietz 2004; Zeigler *et al.* 2011). However, the only well-studied example is the cacao agro-forests used by the *L. chrysomelas* in southern Bahia, Brazil (Oliveira *et al.* 2011). Furthermore, matrices enriched with orchards, native fruit species or agro-forest systems, are an example of an economically viable conservation measures for rural communities.

Currently there are few data to support a decision of which of these conservation actions would be best suited in general. We expect that the greater the complexity of the vegetation in the matrix, the lower the crossing cost and greater the inter-habitat distance that a primate will be able to travel. Neotropical primates are primarily arboreal, but there are large interspecific differences in the propensity of using the ground for travelling. There are studies reporting that Neotropical primates can travel short distances (<100 m) across the ground in open areas – e.g., *Leontopithecus rosalia* (Holst *et al.* 2006) and *Alouatta palliata* (Mandujano 2005), and some move over ground with relative frequency – e.g., *Sapajus flavius* (Bezerra *et al.* 2014) and *Alouatta pigra* (Pozo-Montuy *et al.* 2013). Furthermore, it is also clear that some species are able to use the elements of modified landscapes more readily than others – e.g., *Alouatta* spp. (Gilbert 2003; Pozo-Montuy *et al.* 2013) versus *Ateles hybridus* (Link *et al.* 2010). A key variable is how much non-habitat individuals are willing to routinely traverse on the ground.

One important conservation issue is the effect of roads on movement of primates. Long-term monitoring studies suggest that roads are a significant barrier to primate movement (Vié *et al.* 2001; Williams & Vaughan 2001; Teixeira *et al.* 2013). Canopy to canopy bridges have been suggested as a solution for the passage of primates over these barriers (Teixeira *et al.* 2013). However, roads vary greatly in width, materials and frequency of transit. Are canopy-canopy bridges a solution for highways that are over 50 meters in width, with heavy traffic of large vehicles at high speed? There is no data relevant to that question, but intuition and the precautionary principle tells us it the null hypothesis is that they would not work. Data are needed to evaluate the best designs and management of these bridges in order to guarantee that are efficiency for primates crossing.

Suggestions for future research

There is no enough data published to assess the efficacy of different connectivity structures to primate movement, and determine which primates are most vulnerable to the dispersal limitations in human-modified landscapes. One type of data needed is how the primates move, which structures of the landscape are used. The research on corridors and other connectivity structures needs to be more precise providing be information on rate of successful dispersal and if it achieves the necessary gene flow within one generation. Do corridors increase dispersal or just direct movement (see Haddad *et al.* 2003)? Are roads barriers? How to transpose roads? Which size needs a structure? Should it follow the same design criteria as corridors? Often, the information regarding how often primates move, why they move, and what type of matrix they cross are based on a few exploratory or sporadic observations. These data have underpinned species survival modeling over the long term (e.g., Eduardo & Brito 2012; Zeigler *et al.* 2013). More research is necessary in all of those aspects to better understand dispersal in a species-habitat conservation context and confer greater reliability upon population viability models and, consequently, to conservation programs (Bowler & Benton, 2005).

Two limitations to the development of studies that provide quantitative information about the factors affecting dispersal capacity and movement appear to be the lack of studies focused on dispersal and the lack of long term studies evaluating its success. To study dispersers requires a large scale monitoring (several social units over several years) and either telemetry equipment to follow movements or capture-mark-recapture studies to document successful dispersal in relation to exogenous variables. Population genetic studies can also provide data on population structure and gene flow and help to understand the dispersal processes in a smaller time scale. If these studies are designed with landscape attributes then inferences could be made about the role of exogenous factors on gene flow. Most of the studies reviewed emphasized the behavior of groups of animals within fragments. These studies to the fragment level are not able to respond the question about the changes in broad landscape level and should be considered with caution when trying to understand how changes in the landscape affect the persistence of populations (e.g., Anzures-Dadda & Manson 2007; Raboy *et al.* 2010; Silva *et al.* 2015).

Perceptual distance is an important variable for movement between patches and it may be related to body size (Mech & Zollner 2002). However, we unknown if there are species differences in perceptual distance and do these correlate with primates' body size. Furthermore, researches on how costly it is to traverse pastures or any other substrate that requires an unusual or non-typical locomotion style for the primates are also need. These data can be used, for example, to design (length, width and plant species composition) a corridor or a road crossing structure. Thus, these kinds of studies can allow us to define patterns based upon endogenous factors can be used to predict the response of individual species to landscape changes, and as such, obtain responses quickly about conservation actions to be implemented in order to save those species at risk of extinction (Carretero-Pinzón *et al.* 2016).

Final considerations

We summarized some of the factors that may affect the movement, dispersal and persistence of the primate populations in fragmented landscapes. The main conclusions are:

- Little information exists regarding the movement patterns of some endangered primate species. Studies of these taxa are urgently needed to understand the responses of these animals to the current threat of human landscape modification.

- Endogenous factors may be hypothesized to influence dispersal capacity in a human-modified landscape. However, there is a lack of systematic studies of the role of these factors.
- The main exogenous factors that influence the movement of primates are the landscape structure, the inter-patch distance, the patch size, the availability of food resources, the physical barriers, and the risk of predation. However, it is not yet clear how all these factors jointly affect movement decisions, and studies at the broad landscape scale must be done.
- The influence of physical barriers to movement (such as roads), the relationship between the type of matrix and the distance traveled are largely unknown.
- Elements in the matrix that resemble surrounding forest structure make it more permeable to the movement of primates than open areas.

CAPÍTULO 2

TEMPORAL GENETIC DYNAMICS OF REINTRODUCED AND TRANSLOCATED POPULATIONS OF THE ENDANGERED GOLDEN LION TAMARIN (*Leontopithecus rosalia*)[2]

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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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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.

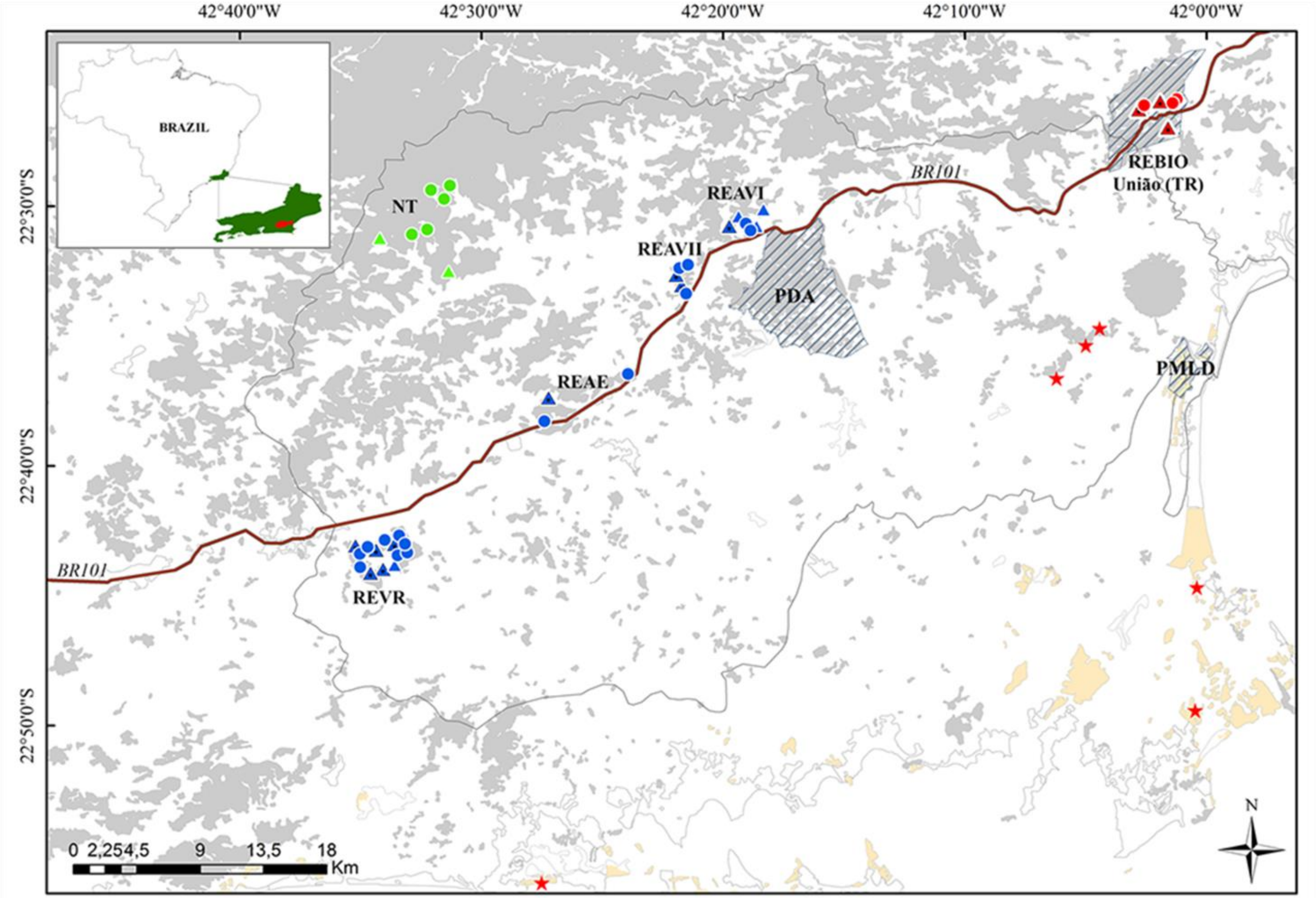


Figure 2.1 – Current geographic distribution of *Leontopithecus rosalia* in Rio de Janeiro state, Brazil. The largest area enclosed the São João River Basin. The dashed polygons enclosed the reserves of full protection: REBIO União, PDA and PMLD. In gray are lowland and gallery forest and beige are restinga 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 – $\chi^2 = 5.93$, d.f. = 1, *P-value* = 0.02) and that of the reintroduced individuals (Appendices B5, only in *set 2* – $\chi^2 = 8.92$, 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 ($\chi^2 = 5.94$, d.f. = 1, *P-value* = 0.015) and reintroduced ($\chi^2 = 9.77$, *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.

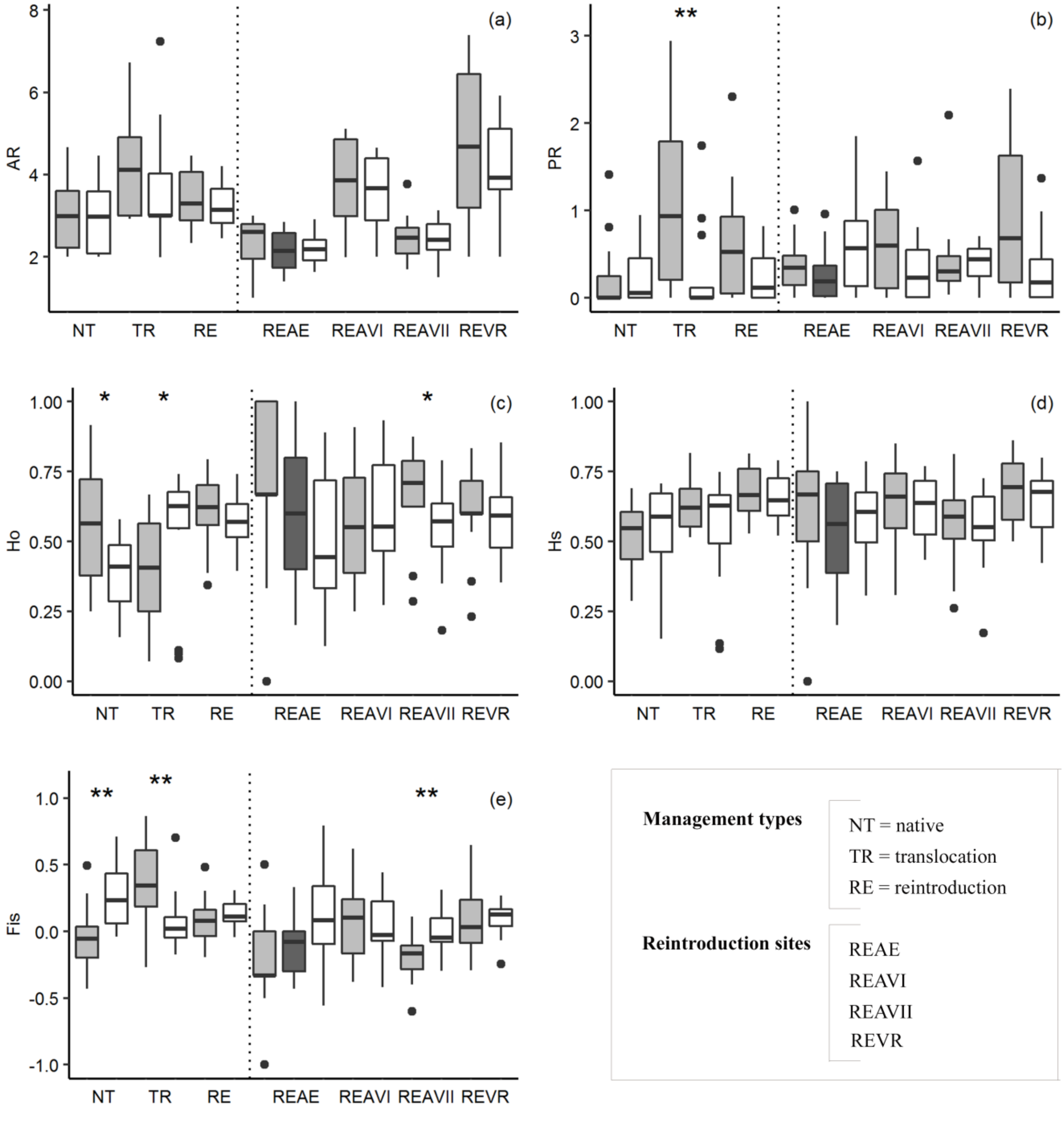


Figure 2.2 – Temporal changes in genetic diversity of *Leontopithecus rosalia* for sampling sites attributes based in history and types of management – NT, TR and RE – and for sampling sites based in spatial distribution– REAE, REAVI, REAVII and REVR. Indices of genetic diversity were estimated through (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.

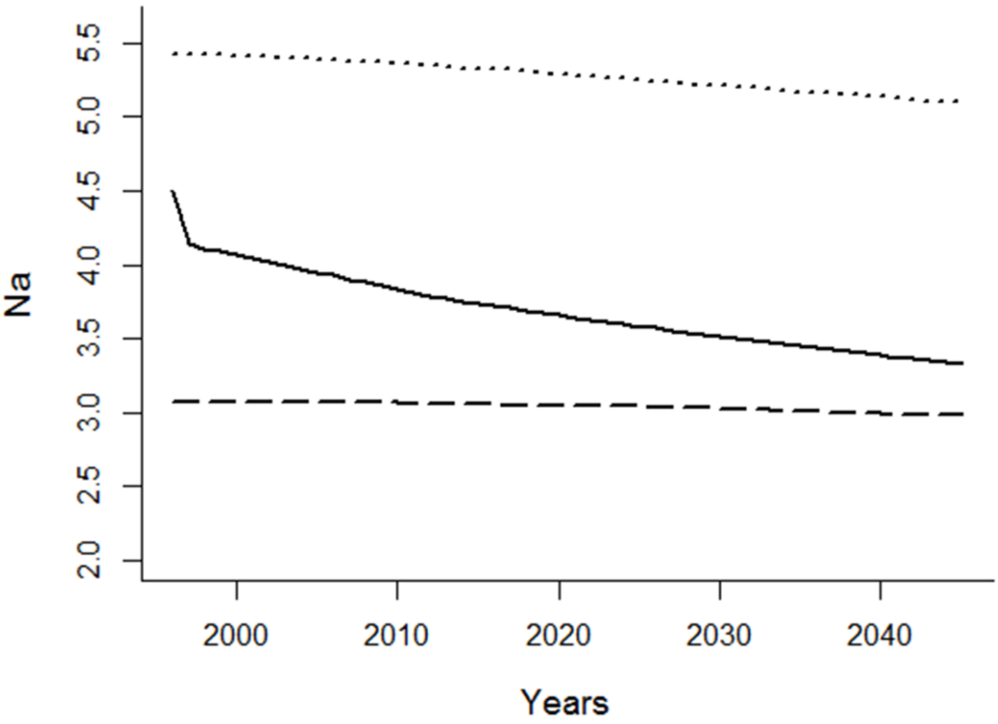


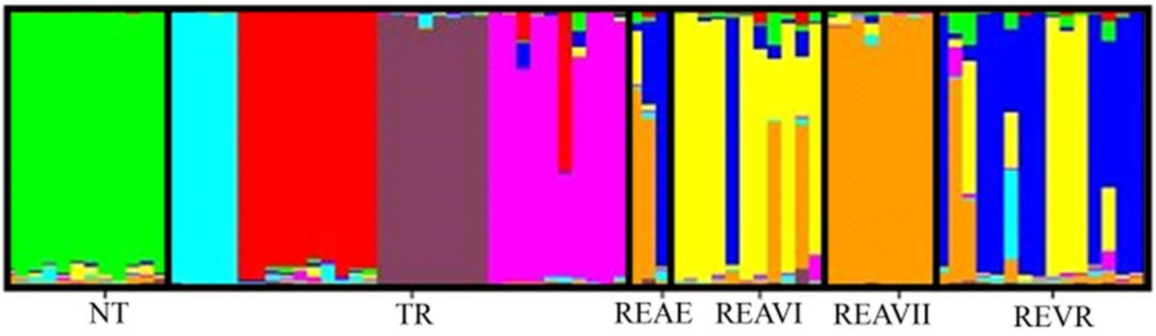
Figure 2.3 – Simulated loss of mean number of alleles per locus (*Na*) over 50 years (1996-2045) in native (dashed line), reintroduced (dotted line) and translocated (continuous line) populations of *Leontopithecus rosalia* assuming constant size through time.

Temporal genetic structure

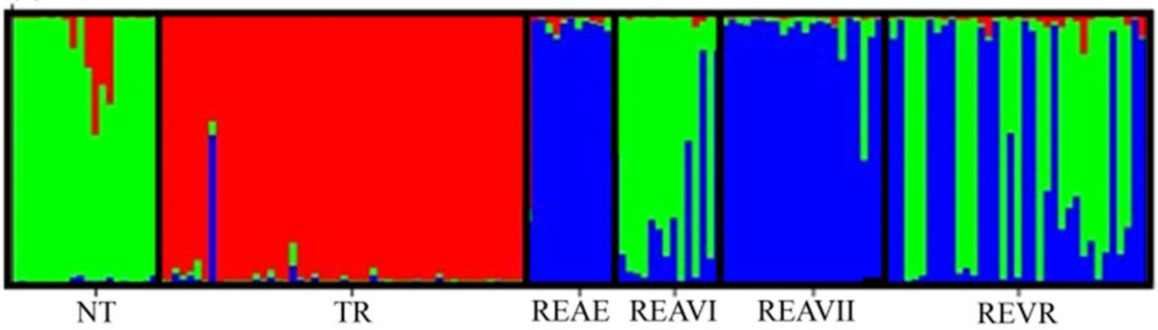
The Bayesian cluster analyses in both the historic and the recent periods showed the highest values of K statistics when K = 2. In both the historic and the recent periods, when K = 2, the translocated populations comprised a single distinct cluster of all the other sites (Appendices B7). In the sequence, 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.

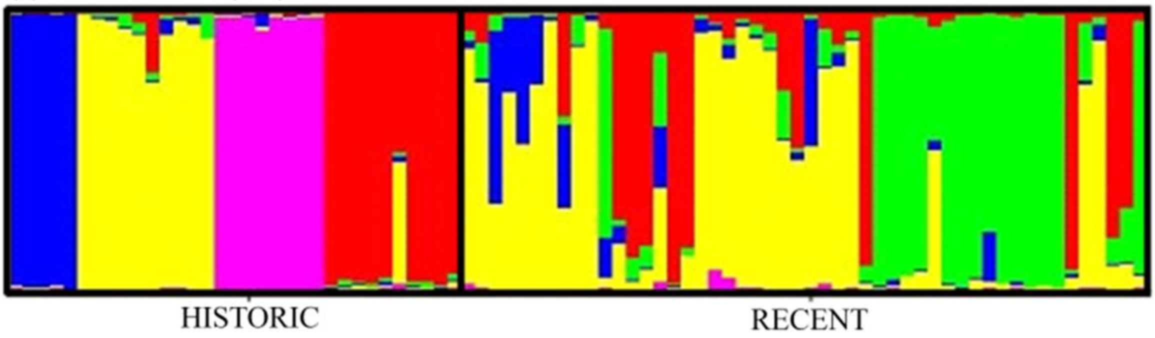
(a) Historic



(b) Recent



(c) Translocated (TR)



(d) Reintroduced (RE)

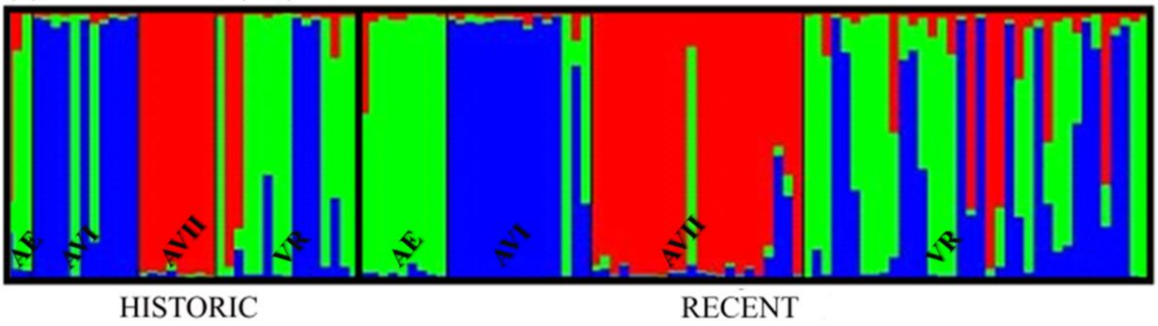


Figure 2.4 – Structure results for *Leontopithecus rosalia* from forest remnants in Brazil's Atlantic Forest showing suboptimal values of K between sampling sites in each study periods – (a) historical and (b) recent period – and within sampling sites through two generations times – (c) translocated (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.

CAPÍTULO 3

LANDSCAPE CONNECTIVITY INFLUENCES GENE FLOW OF ENDANGERED GOLDEN LION TAMARIN (*Leontopithecus rosalia*) FROM ATLANTIC FOREST[3]

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Author Contributions

AMM, CRRM, PMGJr and MCR designed the research; ADG and PMGJr provided logistical support for molecular analysis; PMGJr and MCR provided analytical tools; MCR and PMGJr provided financial support; AMM conducted the molecular analysis and ANF assisted in the laboratory; CRRM and ADG contributed field database and general conceptualization of the study; AMM, MCR, BBN and RM analyzed data; AMM, BRA, JWR and BBSN conducted spatial analyses; CRRM, PMG Jr, BBSN, BRA, RLM and MCR contributed to review the manuscript; and AMM wrote the manuscript.

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[3] Manuscrito em preparação para submissão em periódico internacional.

Abstract

Most species of Neotropical primates live in human modified environments. Habitat loss and fragmentation in these environments threatens the viability of populations because these processes reduce landscape functional connectivity. To assess which landscape variables influence the gene flow in *Leontopithecus rosalia*, an endangered arboreal primate species living in a highly fragmented region, we genotyped 201 individuals using 14 microsatellite loci. We aimed to answer the following questions: (1) What is the dispersal distance of the *L. rosalia*? (2) Is dispersal sex-biased? (3) What are the relative contributions of management strategies, distance, roads and landscape resistance to genetic kinship? We hypothesized that (1) gene flow decreased between more distant sites, (2) males dispersed more than females, and (3) management and the landscape resistance were the variables that most influence genetic kinship. The pairwise distances between genetic kinship and the spatial distances were compared by correlation, regression and generalized linear models. We found positive spatial population-structure up to 8 km. The spatial structuring did not differ between females and males, suggesting that both sexes contribute equally to gene flow. Variation in pairwise genetic kinship was explained by the interaction between landscape resistance and management. These results indicated that different environments affect the dispersal of the *L. rosalia* at different degrees of landscape permeability. We recommend that the genetic management strategies not be implemented in isolation, and the expert opinion and the species dispersal distance must be considered during the planning of conservation measures to reconnect populations.

Keywords: expert knowledge; individual-based analyses; isolation by resistance; landscape genetics; pairwise genetic kinship; sex-biased dispersal.

Introduction

Dispersal is among the main factors influencing species evolution and persistence. It is directly affected by landscape changes, particularly habitat loss and fragmentation (Fischer & Lindenmayer 2007; Nathan 2008; Arroyo-Rodríguez & Dias 2010; Henriques-Silva *et al.* 2015). In addition to external factors – e.g., inter-habitat distance, matrix composition and the presence of barriers – the intrinsic characteristics of organisms such as its sex, and its capacity to move and navigate through various landscape elements affect the movement and dispersal strategies of species (Bowler & Benton 2005; Nathan *et al.* 2008). The interaction of these external and organismal factors makes the environment either less hostile or more resistant to the movement of individuals (Bowler & Benton 2005) and determines landscape connectivity (Tischendorf & Fahrig 2000; Bonnell *et al.* 2013; Henriques-Silva *et al.* 2015; Vasudev & Fletcher 2015). In this study, we understand landscape connectivity to be the capacity of landscape to promote or impede animal movements (Taylor *et al.* 1993), with direct and indirect consequences for gene flow. Genetic connectivity in species with high dispersal capacity is not significantly hindered when they occur in discontinuous landscapes – e.g., white-browed scrub wren (Shanahan *et al.* 2011), racoons (Dharmarajan *et al.* 2014) and mountain lions (Castilho *et al.* 2011). In contrast, species that rely on tree cover to move become genetically differentiated when they are distributed along of a heterogeneous landscapes – e.g., yellow-throated scrub wren (Shanahan *et al.* 2011); Eastern Fox snakes (Row *et al.* 2010) and golden lion tamarins (Grativol *et al.* 2001).

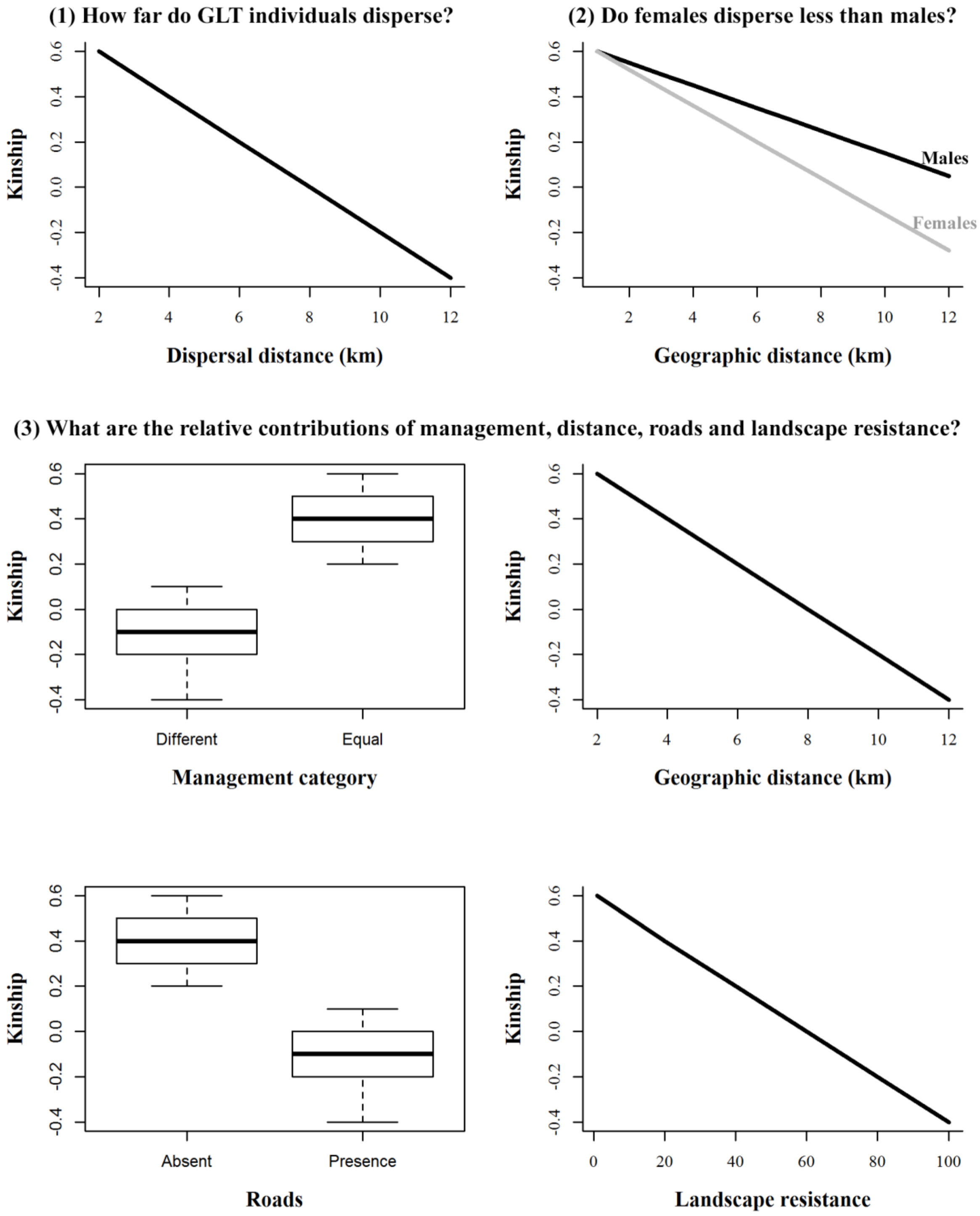
Arboreal primates are an example of animals whose dispersal is sensitive to forest connectivity (Arroyo-Rodríguez & Mandujano 2009; Arroyo-Rodríguez & Dias 2010). Arboreal primate species threatened with extinction occur in fragmented Neotropical landscapes (Estrada *et al.* 2017). However, few studies have investigated how the movement and dispersal of these primates are affected by distinct landscape attributes such as geographic distance (e.g., Mandujano *et al.* 2004; Mickelberg 2011), the road presences (e.g., Teixeira *et al.* 2013) and the structure of inter-habitat matrix (e.g., Estrada & Coates-Estrada 1996; Pozo-Montuy *et al.* 2011; Silva *et al.* 2015). Even fewer is the number of landscape genetics studies that investigated how human landscape modification affects the gene flow of arboreal primates under threat of extinction (e.g., Blair & Melnick 2012; Hagell *et al.* 2013). These studies are important because they enable the identification of landscape attributes that affect the behavior, survival and reproduction of species, and they may facilitate the planning of future conservation actions (Sork & Waits 2010).

The Golden Lion Tamarin (GLT), *Leontopithecus rosalia*, is an arboreal primate threatened mainly by the loss and fragmentation of its natural tropical forest habitat. GLT had been critically endangered, but its conservation status has improved due to conservation measures (Ruiz-Miranda *et al.* 2010; Kierulff *et al.* 2012), which include reintroduction of captive-born individuals, translocation of isolated social groups to a continuous forest, and managing the species as a metapopulation. One of the problems is that the region where GLT current inhabit and where animals were reintroduced and translocated is highly fragmented, and the low landscape connectivity and reduced gene flow may be the main threats to the maintenance of their populations (see chapter 2). Furthermore, observations of GLT individuals crossing open areas are rare (Dietz *et al.* 1997; Grativol *et al.* 2001; Coelho 2009), indicating that matrix composition may be a key factor restricting the primate's dispersal.

It is not yet clear how the GLT and other ecologically similar primates disperse through different landscape scenarios. Nor do we understand how the modification of their habitat interferes in their behavior at various levels, from the individual to the population level. For example, it is possible that females disperse less than males in a hostile landscape, since the costs of this action are greater than the benefits for the female (see chapter 1). The extensive research on lion tamarins has emphasized within-fragment ecology (e.g., Baker & Dietz 1996; Dietz *et al.* 1997; Raboy & Dietz 2004; Nascimento *et al.* 2011), and our knowledge is limited regarding the dispersal of these primates through non-forest and non-native landscapes. For the planning of future conservation measures, we need to understand how the different elements of landscape affect the dispersal and gene flow of the GLT populations in the wild.

Golden lion tamarin also offers an opportunity to evaluate the effects of different landscape elements on its dispersal, given the extensive monitoring of the species through collections of genetic samples at different spatial and temporal scales. Previous studies have made it possible to discern between the effects of different management actions and the effects of the landscape on genetic structure (see chapter 2). Because new populations were founded trough reintroduction and translocation (Kierulff *et al.* 2012), little change in the percentage of forest cover has been observed in their habitat (Seabra 2012), which also reduces the possible influence of historical landscapes on their recent gene flow (Hall & Beissinger 2014).

In this research, we assess which landscape attributes interfere in the dispersal of golden lion tamarins. We assess the genetic variability of different GLT populations distributed throughout their current range in the southeastern Brazil. We asked the following questions: (1) How far GLT individuals disperse? (2) Are female dispersers more affected by landscape changes than male dispersers? (3) What are the relative contributions of management, geographic distance, roads (physical barriers) and landscape resistance to the GLT genetic kinship? We hypothesized that (1) gene flow decrease between more distant sites, (2) males disperse more than females, (3) genetic kinship is negatively affected by geographic distance, landscape resistance and the road presence, and tend to be lower between individuals managed by different conservation strategies (native or unmanaged, reintroduced and translocated) (Fig. 3.1).



(1) How far do GLT individuals disperse?

(2) Do females disperse less than males?

(3) What are the relative contributions of management, distance, roads and landscape resistance?

Figure 3.1 – Expectations for our three questions related to genetic kinship of Golden lion tamarin within Brazilian Atlantic Forest: (1) gene flow decrease between more distant sites, (2) males disperse more than females, (3) genetic kinship is negatively affected by geographic distance, landscape resistance and the road presences, and tend to be lower between individuals managed by different conservation strategies (native or unmanaged, reintroduced and translocated)

Material and Methods

Study area and sampling

The study area is the São João River Basin (SJRB) and the União Biological Reserve (REBIO União), located in southeastern Brazil, within the Atlantic Forest, a highly fragmented biome (Ribeiro *et al.* 2009). To ensure that the extent of the study area was larger than the area occupied by the actual GLT populations (Anderson *et al.* 2010), we also analyzed a 5 km buffer beyond the boundaries of the SJRB (Fig. 3.2). Our study area has been subdivided into two parts by the federal highway BR-101. The northern portion is the most preserve, with higher amounts of forest cover and less number of isolated fragments (Procópio de Oliveira *et al.* 2008). In the southern portion the Poço das Antas Biological Reserve (PDA) and other large and isolated populations of GLT are located (Fig. 3.2).

Because in landscape genetics research, sampling is recommended to represent largely the landscape variables (Storfer *et al.* 2007), we selected of sampling sites simulating multi-patch ecological corridors using LSCorridors package (Ribeiro *et al.* 2017, see below in *Spatial analyses* section). We simulated 100 routes between 18 fragments (source and target) that contained genetic samples collected in the years 2007-2013 and calculated the average value of the corridor cost. After, we selected 12 fragments that corresponded to a minimum of ten sampled individuals and covered various ranges of corridor cost. Thus, we selected social groups from all of the managed units (MU) in the SJRB and REBIO União, which constitute the main areas of current GLT occurrence. According to the literature, the MUs are units established by the AMLD and collaborating researchers, whose goal is to support the management and conservation of the GLT (Holst *et al.* 2006; Mickelberg 2011). In this study, MU refers to a set of eight functionally connected forest fragments established on the basis of (1) field observations of individuals' movements (Mickelberg 2011) and (2) population genetic structuring (Freitas 2012; Moraes *et al.* 2017). The MUs were designated with the following names: PDA, REBIO União, AVI, AVII, Imbaú, Serra, BE and RV (Fig. 3.2).

PDA and REBIO União consist of public protected forests, while the MUs of AVI, AVII, Imbaú, Serra, BE and RV consisted of forest fragments inside private areas. Descendants of the genetically unmanaged population have remained distributed mainly in the fragments of PDA and Serra. Descendants of the animals born in captivity and reintroduced have remained distributed mainly in the forest fragments of the AVI, AVII, Imbaú, RV, and BE. REBIO União shelters the descendants of the six isolated social groups from different fragments in the coast of Rio de Janeiro that were translocated to this reserve.

We sampled between 2-15 GLT individuals of 48 social groups (mean = 4.0, SD = 2.7) distributed in the study region during the period 2007-2013 (hereafter referred to as ‘recent’). Of a total of 201 individuals sampled in the recent period, 152 were used from the databank of chapter 2. All genetic samples were collected by the Golden Lion Tamarin Association (AMLD; *Associação Mico-Leão-Dourado*) field team following capture procedures that are described in detail elsewhere (Dietz *et al.* 1994). For each sample, we selected individual identification, sex, and social group based on field records from 2009. Additionally, we also analyzed the genotypes of GLTs residing in the SJRB and REBIO União in the period 1996-1997 (hereafter referred to as ‘historic’) – 78 individual from 19 social groups (chapter 2) – to performed the dispersal distance analyses (see below). All social groups sampled are indicated in the Figure 3.2.

Genetic analysis

Detailed report on DNA extraction, amplification and genotyping procedures are provided in the chapter 2. We extracted DNA using DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA, USA) following the manufacturer’s protocol. Individual genotypes were amplified from fourteen microsatellite loci developed for *L. rosalia* (P2BH6 and P5BE6; Grativol *et al.* 2001), *L. chrysopygus* (Lchu 3, 4, 6, 7, 8 and 9; Perez-Sweeney *et al.* 2005) and *L. chrysomelas* (Leon 2, 3, 21, 27, 30 and 31; Galbusera & Gillemot 2008). The amplified protocol and PCR conditions are described in detail in the chapter 2.

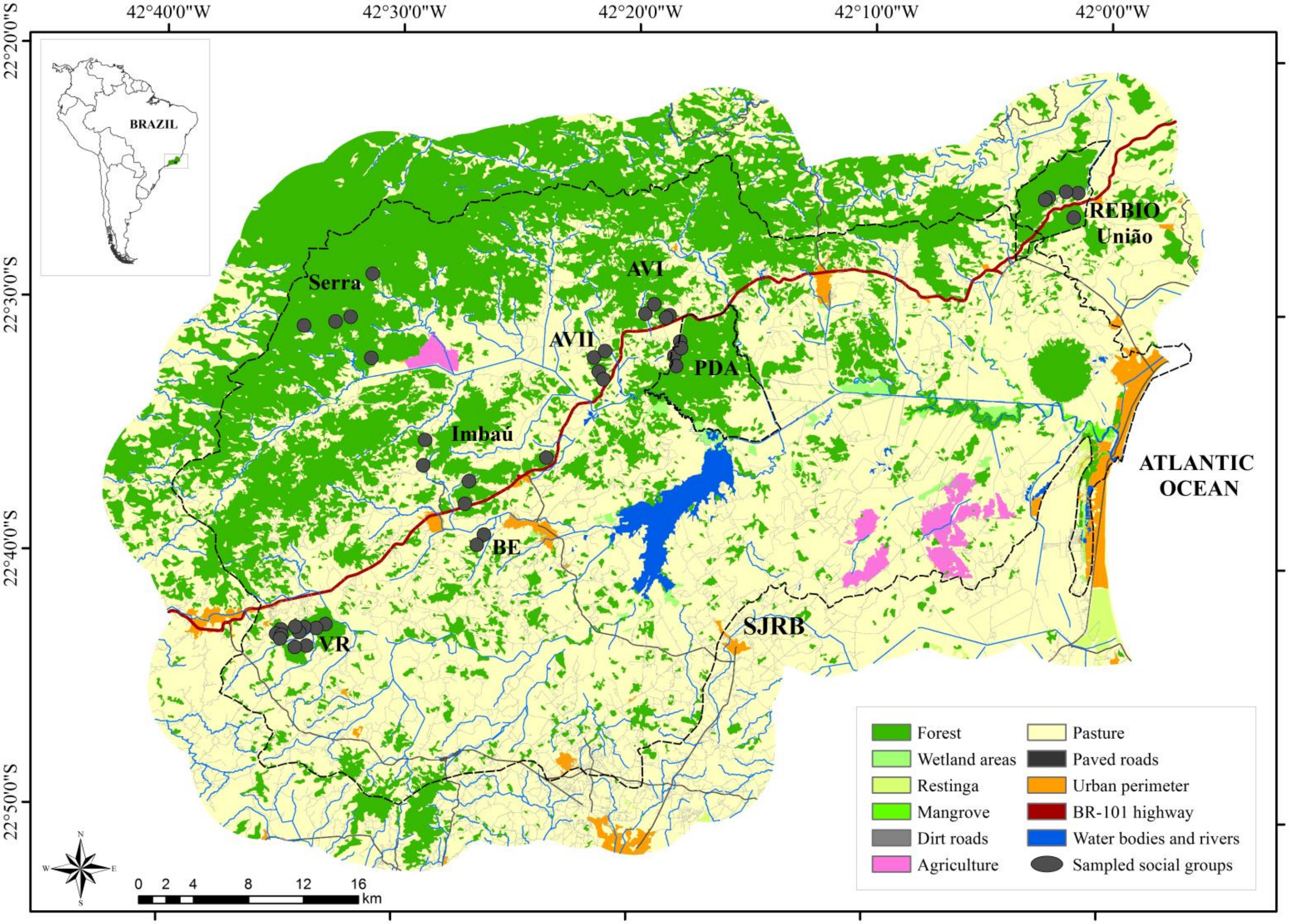


Figure 3.2 – Land cover map of the study area the current geographic distribution of the golden lion tamarin in the Rio de Janeiro State, Brazil. The dots represent the locations of the sampled social groups for which corridors were simulated.

For samples with missing loci, we amplified additional PCR replicates. When an individual sample was missing more than 30% of the loci, the individual was removed from the analysis. To estimate the genotyping error rate and confirm reliability, we re-amplified 20 (10%) randomly chosen samples from each locus. We estimated the error rate as the ratio between the observed number of allelic differences and the total number of allelic comparisons. Estimating genotyping errors is important because the errors may bias the final conclusions, especially for individual-based studies (Bonin *et al.* 2004). We also used MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) to detect and estimate null allele frequencies and to search for loci with short allele dominance and stuttering error. Next, we performed Hardy-Weinberg Equilibrium (HWE) tests in GENALEX version 6.5 (Peakall & Smouse 2006) and corrected significant deviations from the HWE using the Bonferroni confidence interval (Rice 1989).

We then estimated an individual-based relatedness parameter; this is recommended over assignment tests, which perform relatively poorly when genetic structure is weak (Jones & Wang 2012). We would have obtained a weak population genetics signal because of the genetic management of GLT that occurred on a short time scale (see chapter 2). Using the program SPAGeDi (Hardy & Vekemans 2002), we computed the kinship (or coancestry) coefficients between individuals, F_{ij}, as defined by Loiselle *et al.* (1995). We chose this particular kinship coefficient because it is indicated for non-equilibrium populations (independent of HWE) and for datasets with low frequency alleles. Furthermore, it is independent of mating systems (Vekemans & Hardy 2004).

To address the hypothesis that the GLT's dispersal patterns generally differ between sexes, we specified the sex of individuals in this analysis. We generated three matrices of pairwise inter-individual genetic distances: (1) all individuals (global analyses); (2) male samples only; and (3) female samples only. To further test sex-biased dispersal, we calculated the intra-individual inbreeding coefficient over 14 loci (as recomended by Goudet *et al.* 2002), using SPAGeDI. Following this, we calculated the average and standard deviation of the inbreeding coefficient of the sexed groups, and we test significant differences between them using a Kruskal-Wallis test performed in R 3.2.2 software (R Core Team software 2016).

Spatial analysis

Land use map for the study area was created through a mosaic of maps, which included: (1) the mapping of SJRB, made from LANDSAT 5/2010 satellite images (Seabra 2012); and (2) the 5 km area adjacent to SJRB, based on the *Atlas de Remanescentes Florestais da Mata Atlântica 2013–2014* (SOS Mata Atlântica & INPE 2015). Using GIS tools, we also made manual edits based on Google Earth images to guarantee that all forest fragments and connecting elements (strip-shaped riparian forest) were included on the final map. The land use map contained 11 land use classes: forest, wetland areas, *restinga*, mangrove, agriculture, pasture, urban areas, water bodies and rivers, small dirt and paved roads, and the BR-101 highway (Fig. 3.2).

We attributed resistance values to each pixel of this land use, using ARCGIS 10.3.1 (ESRI). Resistance was determined by researchers and field specialists who had developed studies with lion tamarins in the Atlantic Forest over a minimum of five years. Twelve specialists were selected and consulted through face-to-face interviews following Perera *et al.* (2012) protocol. In these interviews, the specialists attributed a value between 1 and 100 to each land cover class, representing a given environment's resistance to the movement of GLTs, with 1 indicating the most favorable and least resistance environment and 100 indicating the less favorable. The final resistance was calculated as the median of the values given to each of the land cover classes: forest (1); wetland areas (20); *restinga* (20); mangrove (20); dirt road (37.5); agriculture (40); pasture (55); paved roads (75); urban perimeter (92.5); paved highway with high traffic volume, i.e. BR-101 federal highway (100); and water bodies and rivers (100) (Fig. 3.2). The higher the resistance value the more difficult the dispersal of GLT within landscape. To generate the final resistance map we also included altitude, since GLT are rarely encountered in areas above 550 m (Kierulff & Rylands 2003). Each pixel of the resistance map was divided by the function $1/\{1 + \exp [0.02 * (\text{altitude} - 650)]\}$. Note that after this correction, due to landscape topology, the higher the latitude, the lower the probability that the GLT would pass through that location and therefore greater the resistance.

We used the location of the GLT social groups as source-target on our analysis. We defined a buffer of 500 m around each GLT social group location as source-target polygons. Each buffer of 500 m was rasterized with a different code – an integer value that refers to the identification of each social group. The size of this buffer was defined based on information about their most frequent movements (400–600 m, Mickelberg 2011) and the size of home ranges estimated for GLT (47–67 ha; hankerson & Dietz 2014). This was necessary because the grain must be less than the dispersal distance or the species' home range (Anderson *et al.* 2010). After generating both resistance and raster source-target maps, we calculated four landscape variables (see below) using 100 corridor replicates generated by the LSCorridor LSCorridor software (Ribeiro *et al.* 2017). LSCorridors simulates multiple ecological corridors between pairs of sources and targets based on the *least-cost path* (LCP) and *conditional minimum transit cost* (CMTC) algorithms (Adriaensen *et al.* 2003; Pinto & Keitt 2009) and simulated multiple paths between the pairs.

The following landscape variables were calculated using the simulated corridors: (1) *Euclidean distance (ED)* – straight-line distance (given in km) that separates two individuals; (2) *Resistance-based distance (RD)* – effective distance (given in km) between two individuals, assuming that GLTs prefer to move along the most permeable paths; (3) *Landscape resistance (LR)*, which is a measure of functional cost, calculated as the sum of the costs of crossing each pixel of the simulated corridors (Adriaensen *et al.* 2003; McRae 2006; Spear *et al.* 2010); (4) *Landscape connectivity (LC)*, which is calculated as the ratio between the average Euclidean distance and the average landscape resistance between pairs of individuals – LC = ED/LR (Santos 2014). ED, RD, and LR were calculated for each corridor and averaged over all corridors to generate a single value for each pair of individual locations. Finally, we created a binary matrix as a measurement of the presence or absence of paved roads (*roads*) on the route between two individuals, aiming on testing the effect of this variable on each pairwise genetic kinship. The *roads* variable was obtained based on the source *Base Cartográfica da Secretaria do Ambiente/IBGE* (1:25,000 scale).

Influence of environment on genetic variation

First, we assessed whether geographic distance influences dispersal potential of the GLT. The alleles obtained in the chapter *2* for the historical period (1996–1997) and obtained in this study for the recent period (2007–2013) were plotted on the land use map according to the geographic coordinates of the social groups. The minimum distance between locations where the same alleles were found in the historical and recent periods were used to estimate the Euclidean dispersal distance (Appendices C1). These analyses were performed in R 3.3.0 (R Development Core Team 2016) using the raster (Hijmans *et al.* 2016) and rgdal packages (Bivand *et al.* 2016).

To investigate the influence of sex on dispersal, we tested the relationship between the pairwise kinship coefficient – F_{ij} (Loiselle *et al.* 1995) –, ED and RD, for of all individuals, only males, and only females. Average kinship pairwise coefficients were calculated to 15 distance classes, which varied from zero to 66 km when using *ED*, and from 0 to 91 km when using *RD*. For individuals belonging to the same social group, we modified each of their geographic coordinates by 1 meter so that permutations were performed using the location of all individuals. The multilocus statistical averages were obtained using 9999 permutations and the jackknife estimator. We also performed a regression of the pairwise kinship of all individuals, females and males using the total intervals of both *ED* and *RD*. Then, we compared the coefficients of determination (r^2) obtained from 18 models (Table 3.1) to determine which intervals and geographic distances had the most influence on the genetic kinship between all individuals, only females and only males (as before, Soare *et al.* 2014). All these analyses were performed in the program SPAGeDi (Hardy & Vekemans 2002)

As recommended by the SPAGeDi software authors, tested for spatial autocorrelation using all the loci and then excluding separately each of the three loci with the highest percentage of missing data: Leon3, Lchu9 e Lchu7. This was done because the autocorrelation tests based on individuals did not consider the presence of missing data in the analyses (Hardy & Vekemans 2002). We observed differences in the significance tests only when we excluded Leon3. The number of pairwise comparisons and the proportion of individuals in each distance interval were represented as suggested by the software authors. The coefficient of variation (CV) of the number of times each individual was represented varied between 0.5 and 1.5.

To understand the effects of conservation management (shown to influence GLT genetic structuring; chapter 2) and landscape elements on genetic kinship, we used generalized linear models (GLM) with Gaussian distribution for the response variable, F_{ij}. We considered the following management categories for individuals: native or unmanaged, translocated, reintroduced, and origin unknown by researchers. For each pair of individuals, we attributed a numeric code that refers to the management category of both individuals, and expected that individuals in the same management category were more closely related than individuals of different categories.

Besides *management*, we also tested for the influence of the landscape variables (*ED*, *RD, LR, LC* and *roads*) on genetic kinship. Firstly, we applied Spearman's correlation to check if there were high correlations between the explanatory variables. A pair of variables was considered highly correlated when the correlation coefficient was greater than 0.7 (Appendices C2) (Zurr *et al.* 2009). To choose one of each pair of correlated variables, we performed variable selection using Akaike's information criterion (AIC; Akaike 1974), and we removed the variable with the highest AIC among the univariate linear models containing correlated predictors (Burnham & Anderson 2002). At the end, the explanatory variables used in the generalized linear models were *management*, ED, LR and *roads*.

We then adjusted univariate and multivariate GLMs. We assumed a Gaussian distribution of our response variable F_{ij} and adjusted the models using the "glm" function of R 3.3.0 software (R Development Core Team 2016). We then compared the explanatory power of each model using a competing model approach proposed by Burnham and Anderson (2002), which used the "AICtab" function in the "bbmle" package (Bolker & R Development Core Team 2016). First, we tested the power of the univariate models. We also included a null model that tested the absence of any effect (the intercept only model). Then, the most significant explanatory variable among the univariate models (*management*) was added to the effect of the other explanatory variables to adjust additive models. Again, the explanatory power of each univariate and multivariate model was tested by AIC model selection. The most plausible model was identified using the evidence weight (wAIC), representing the relative probability of a model in comparison to the other models in the set (Burnham & Anderson 2002).

Results

Quality of genetic data

In total, we compared 560 alleles. From these, 28 alleles (from two individuals) were excluded because they could not be typed for double amplification, and another 26 alleles were typed for only one of the two extracts. We found no allelic dropout, false alleles or contaminations among the 16 amplified and checked genotypes. In this study, only 4% of the 5628 amplified alleles were missing data. In total, 91 alleles across 201 individuals were amplified in 14 microsatellite loci. Microsatellite polymorphism ranged from three to nine alleles per locus.

Evaluation of the microsatellite dataset using MICRO-CHECKER revealed no evidence of genotyping errors due to stuttering or allele dropout, but indicated that null alleles might be present. However, the frequency of null alleles was decreased and inconsistent across the loci and MUs (Appendices C3). Only the Lchu9 locus had a frequency of null alleles greater than 0.1 in more than three situations. Nevertheless, Lchu9 was maintained in the analyses because previous calculation of the mean kinship coefficient showed no significant difference between the results of excluding or including it (as before in chapter 1). A second step involved analyzing genetics at the social group level; the situations with sufficient data for analysis showed no evidence of null alleles. Likewise, deviations from the HWE were low and inconsistent across loci and MUs (Appendices C4). At the social group level, deviation from the HWE occurred at only two loci in a single social group from REBIO União (on the south side of BR-101).

Effect of distance on genetic variation

Dispersal distance

The number of shared alleles was higher between nearby individuals and decreased with both ED and RD increased between individuals. Euclidean distance measured between points of allele presence over time showed a median allele dispersal of 1.70 km (10-90% percentiles = 0-16 km) with dispersal distance ranged principally up to 8 km (Fig. 3.3). Similarly, we found positive and significant values of pairwise kinship coefficients for three distances classes of *ED* and *RD* corresponding to values ≤ 8 km, and regression analysis showed a significant correlation between them (see below).

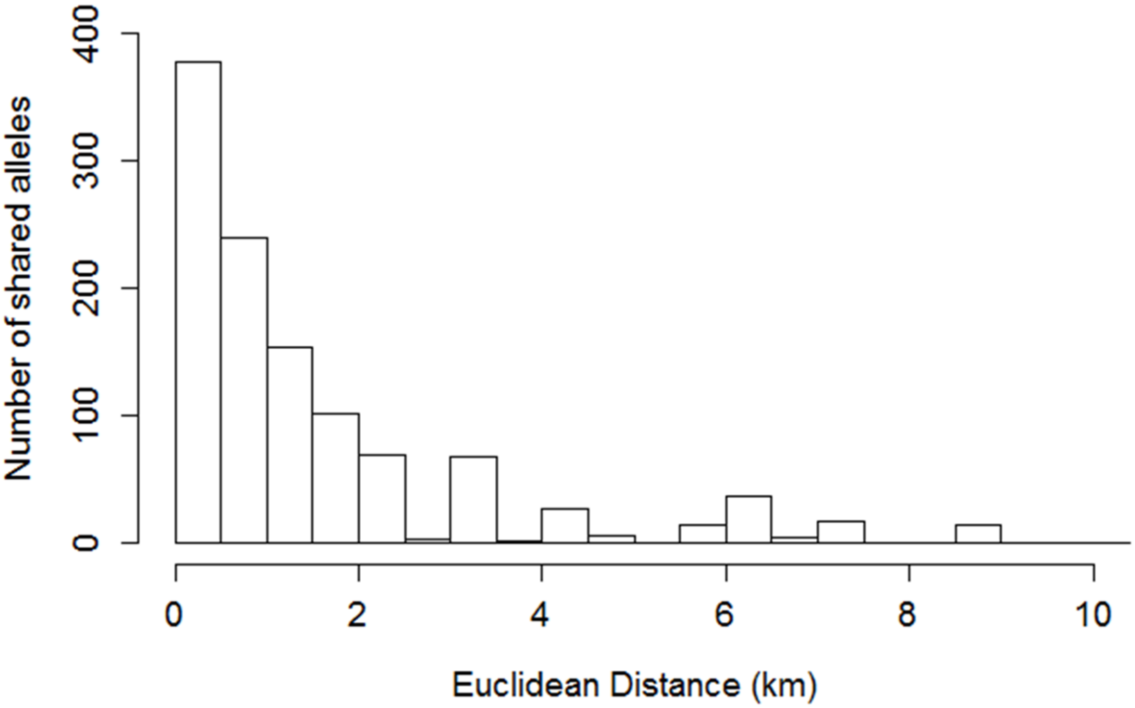


Figure 3.3 – Euclidean distance between recent locations (2009–2013) and nearest last locations (1996–1997) for each analyzed allele from golden lion tamarins distributed in the São João River Basin and REBIO União, Rio de Janeiro State, Brazil.

Test of sex-biased dispersal

The global autocorrelation analyses found positive and significantly higher than expected values of F_{ij} at greater *RD* distances (8 km) than *ED* distances (5 km). The F_{ij} values also varied between slightly positive and negative and were no significant in 11–21 km by *ED* and 17–31 km by *RD*. When excluding the loci with the greatest proportion of missing data (Leon3, 15%), the pattern of variation in F_{ij} remained the same, though significantly different than expected, in all distance classes except the fourth interval of *ED* (11 km). Thus, we found significantly lower values, especially after 24 km (more conservatively, after 15.5 km) according to *ED* and 31 km (more conservatively, after 17 km) according to *RD* (Fig. 3.4).

In general, the distance decay curves differed slightly between the *ED* and *RD* variables considered in the univariate autocorrelation models (Fig. 3.4). When analyzing males and females separately, we observed a similar pattern in the spatial genetic structuring of both sexes and of the global analysis (Fig. 3.4, Table 3.1). For females, we found positive and significant spatial structuring up to an average *ED* of 5.6 km and up to an average *RD* of 8.6 km (Figs. 3.4b and e). Males showed positive and significant spatial structuring up to an average *ED* of 9 km and up to an average RD of 8 km (Figs. 3.4c and f). Although the coefficient of determination between the pairwise kinship coefficient and *ED* was greater for males than for females (Table 3.1), there was no difference between the intra-individual inbreeding coefficient calculated using the 14 loci for all individuals (0.19±0.03), only females (0.20±0.03), and only males (0.19±0.03).

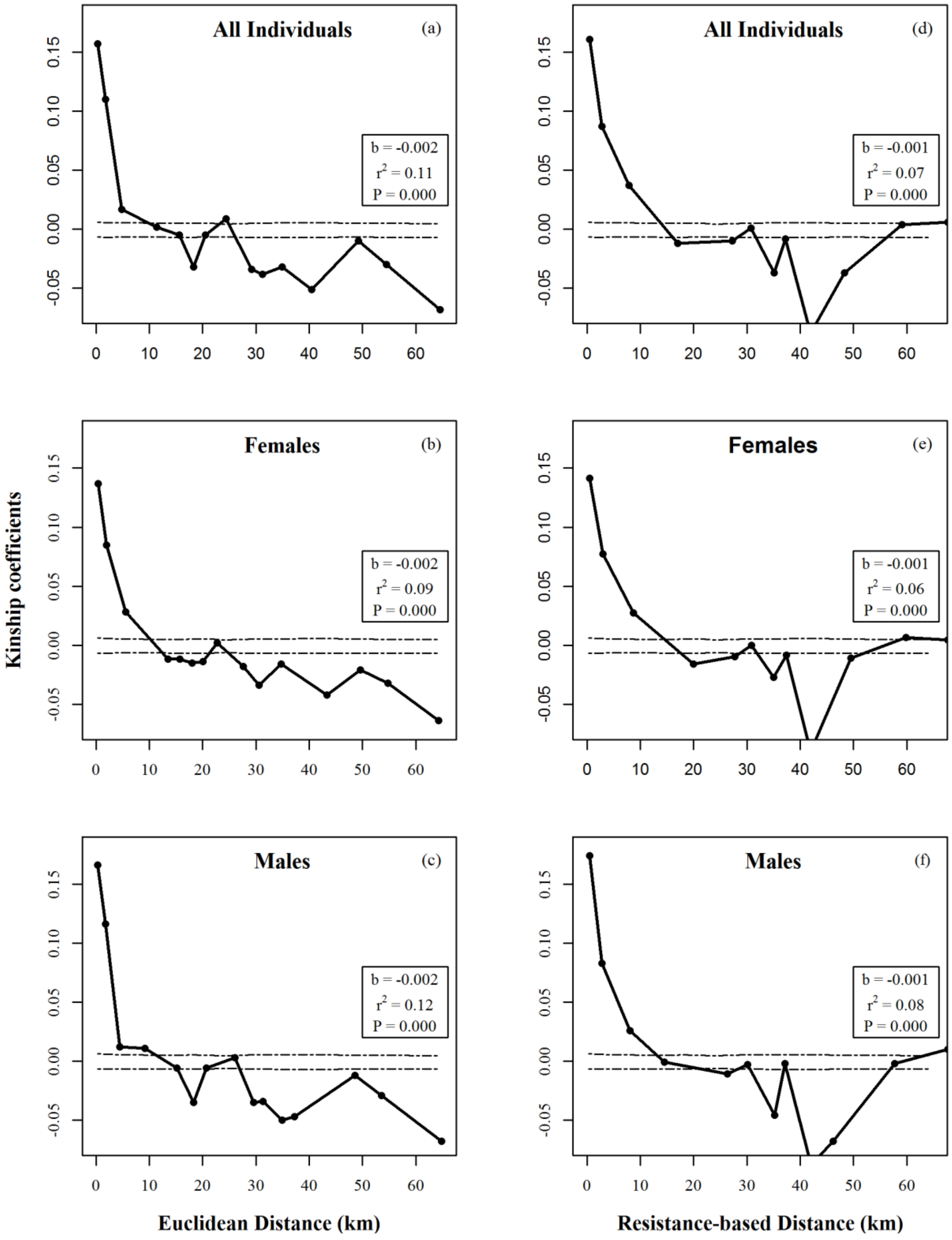


Figure 3.4 – Correlogram plots of kinship coefficients (F_{ij}) between 201 sexed individuals (first line), 91 female (second line), and 109 male (third line) golden lion tamarins, across 15 distance intervals in the São João River Basin and REBIO União, Rio de Janeiro State, Brazil. The upper and lower 95% confidence levels are given for each sexed group (dashed line)

Table 3.1 – Kinship pairwise coefficient (F_{ij}) within sexed groups of GLTs – all individuals, females and males – significantly predicted by Euclidean distance and Resistance-based Distance (both in km) over three class distances. Slope (*b*), coefficient of determination (r^2) and significance level (*P*, two-tailed).

		Euclidean Distance (ED)				**Resistance-based Distance (RD)**			
Group	**Extent of spatial interval (km)**	**N**	***b***	**r^2**	***P***	**N**	***b***	**r^2**	***P***
All individuals	0-10	4465	-0.02	0.16	0.00	4185	-0.02	0.16	0.00
	0-6	3675	-0.03	0.14	0.00	3089	-0.03	0.14	0.00
	0-2	2166	-0.03	0.03	0.00	1739	-0.06	0.08	0.00
Females	0-10	822	-0.02	0.12	0.00	760	-0.02	0.13	0.00
	0-6	700	-0.03	0.12	0.00	590	-0.03	0.15	0.00
	0-2	422	-0.03	0.02	0.00	341	-0.06	0.08	0.00
Males	0-10	1419	-0.02	0.19	0.00	1335	-0.02	0.21	0.00
	0-6	1123	-0.03	0.16	0.00	941	-0.03	0.15	0.00
	0-2	649	-0.04	0.04	0.00	527	-0.64	0.09	0.00

Landscape influences on genetic variation

As expected, the model *management*, which predicted that the kinship between GLT individuals is sensitive to the management type, was selected as the best univariate model for the pairwise kinship genetics (wAIC = 1, Table 3.2). When considering management and spatial variables, the most plausible model for explaining the variation in pairwise genetic kinship considered the influence of *managemen*t and the *LR* (wAIC = 1). Thus, the kinship between GLT individuals decreased as *LR* increased (Fig. 3.5). On the other hand, *ED* and *roads* were less important for explaining kinship – although the values of resistance of the roads were also incorporated in the *LR* – while the null model presented the lowest wAIC value (< 0.001, Table 3.2).

Table 3.2 – Rank of the univariate and multivariate models using model selection based on AIC. K is number of parameters of each model. The most plausible models are underlined

		AIC	**ΔAIC**	**K**	**wAIC**
UNIVARIATE	*Management*	**-31500.3**	**0.0**	**11**	**1**
	ED	-29179.1	2321.3	3	<0.001
	Roads	-28630.7	2869.6	3	<0.001
	LR	-27496.0	4004.4	3	<0.001
	Null	-26720.7	4779.6	2	<0.001
MULTIVARIATE	*LR + Management*	**-31719.2**	**0.0**	**12**	**1**
	Roads + Management	-31611.5	107.7	12	<0.001
	ED + Management	-31504.5	214.8	12	<0.001
	Management	-31500.3	218.9	11	<0.001
	ED	-29179.1	2540.1	3	<0.001
	Roads	-28630.7	3088.5	3	<0.001
	LR	-27496.0	4223.3	3	<0.001
	Null	-26720.7	4998.5	2	<0.001

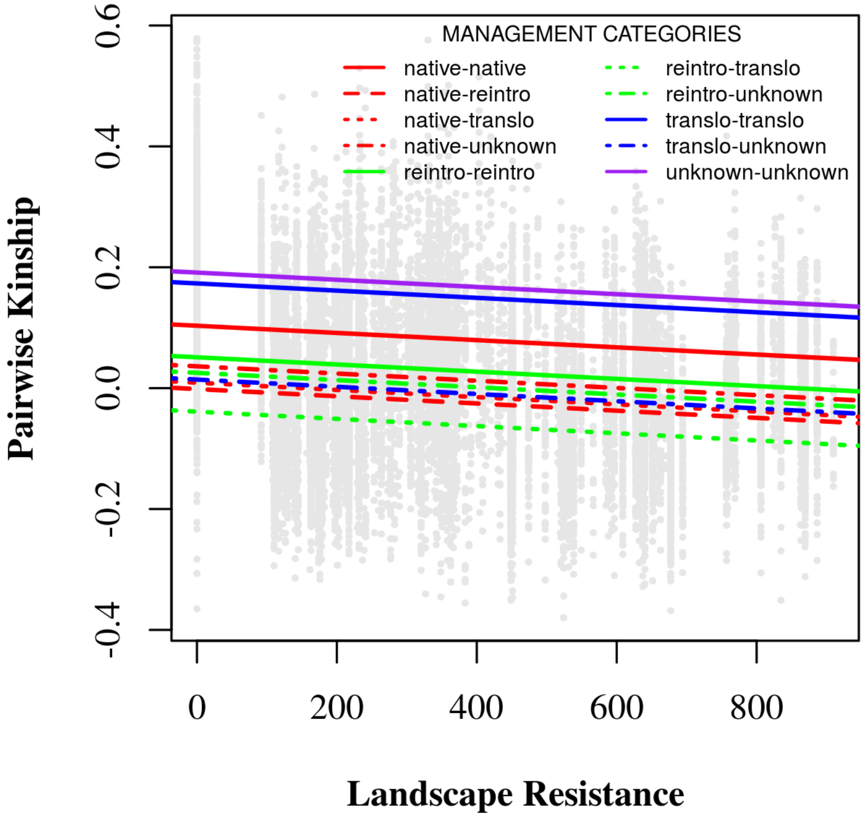


Figure 3.5 - Scatterplot of most plausible multivariate model (management + landscape resistance) for explaining the genetic kinship variation observed between individuals of *Leontopithecus rosalia* residing in the São João River Basin and REBIO União, Rio de Janeiro State, Brazil.

Discussion

Despite the effect of *management* on the genetic structure of the GLT, and although few generations have passed between the sampling period and the founding of some of the populations (see chapter 2); we were able to identify negative influence of forest discontinuity on GLT gene flow. The explanatory variables that best explained genetic variation between GLT individuals were *management* and *landscape resistance* showing that different environments – that make up the matrix – affect the dispersal of the *L. rosalia* at different degrees of landscape permeability. Even though geographic distance was not the most plausible predictor in our models, it was significantly correlated with the kinship between GLT individuals independently of their sexes. A previous genetic simulation study based on spatially explicit agents (Di Fiore & Valencia 2014), had shown that the social system of tamarins lead to a rapid genetic differentiation among social groups, while a pattern of isolation by distance was established over time. When a barrier (which fragmented the populations) was added into the simulations, isolation by distance prevailed, but the barrier caused greater and stronger genetic differentiation over time of the tamarins.

Population genetic tests

We considered the evidence of null alleles and of deviations from the HWE as consequences of the management implemented for the conservation of GLTs (see chapter 2) and of induced structuration, generally observed in monogamous species and/or species with familial organization (Anderson & Dunham 2008). GLT social system consists of closed familial groups that seldom accept dispersers, and the GLT possesses limited dispersal capacity (Baker *et al.* 1993; Dietz & Baker 1993; Baker & Dietz 1996). Furthermore, these results also reflect the expanding process of population structuring – i.e., the Whalund effect – that is indeed occurring in the SJRB and the REBIO União (see also Freitas 2012; and chapter 2).

Effect of distance on genetic variation

Dispersal distance

Analysis of the allele’s variation over time and autocorrelation showed a pattern of positive and weak spatial genetic correlation at around 8 km and strong spatial genetic structuring at around 2 km, which correspond to the first two distance classes in Figure 3.4. Similarly, field research based on the calculation of *ED* between capture and recapture points identified GLT movement most frequently up to 2 km and rarely beyond this distance, though GLTs may travel for up to 6.6 km (Mickelberg 2011). Together, these data suggest that GLTs disperse most frequently within the limits of their own– ≤ 67 ha (Hankerson & Dietz 2014)–and adjacent groups’ home ranges and rarely over distances longer than 8 km. Similar pattern was also observed in another primate species, *Propithecus tattersalli* (Quéméré *et al.* 2010). However, significant and positive autocorrelation of GLTs was also observed at greater distance intervals (around 8 km) than the 1.5 km observed for *P. tattersalli* (Quéméré *et al.*

2010). We believe that GLTs may travel these long distances of 6–8 km more frequently if the landscape becomes sufficiently permeable to its movement. These results showed the greater importance of connectivity in determining the dispersal pathways in the first distance intervals (up to 8 km) which GLTs probably move most frequently. Dispersal over long distances has a positive effect on the persistence of populations in a dynamic landscape; however, if the number of potential dispersers in low, it ceases to be an advantage (Johst *et al.* 2002).

Test of sex-biased dispersal

Although the field evidences indicate the existence of sex-biased dispersal among GLTs (Baker & Dietz 1996; Paula 2013), we found a similar spatial genetic structuring between males and females. A previous study on *L. chrysopygus*, based on genetic data, had already contradicted the notion that dispersal differs between males and females (Martins *et al.* 2014). However, field evidence showed that the proportion of male migrants is greater than female migrants (Paula 2013). Females tend to remain in their social group of origin and occupy a reproductive vacancy, while males tend to migrate (Baker & Dietz 1996).

Although we did not observe a statistical difference between the inbreeding coefficients of males and females, and the distance decay curves differed slightly between them, the geographic distances had greater explanatory power for autocorrelation of the males than of the females. Thus, our results indicate that the frequency of successful dispersal – i.e., dispersal followed by reproduction – must occur in a similar way for both sexes; however, the males may engage in more attempts of dispersal. We believe that male GLTs encounter more favorable conditions and have a greater propensity to disperse. However, male dispersers do not always become the reproducers in a new group. While females are accepted into a new group only when a reproductive vacancy arises, males may be accepted whether or not a space is available (Baker *et al.* 2002).

Landscape influences on GLT genetics

Our results showed that the reduction in landscape permeability is the principal factor limiting GLT dispersal and gene flow. The most important landscape variable for GLT dispersal was *LR*, which was present together with the variable *management* in the most plausible model. Moreover, *RD* appears to be a more realistic option than *ED* for understanding how the organism moves through space. When we performed the regression analysis considering the first three distance intervals, the *RD* had greater explanatory power than the *ED* for autocorrelation. Similar to the GLT, other primate species have been also threatened by forest discontinuity – e.g., *Alouatta pigra* (Pozo-Montuy *et al.* 2011), *Saimiri oerstedii* (Blair & Melnick 2012), and *Rhinopithecus bieti* (Liu *et al.* 2009). Due to the evidence found for various primate taxa, we recommend that the cost-distance relationship, which defines matrix permeability, be considered during the planning of management and conservation actions for arboreal primates.

Our results also indicated that pasture has a medium resistance to GLT dispersal. Coelho (2009) described an event of GLT dispersal across pasture areas and the use of stepping stones between source and target fragments. It is likely that small distances (≤ 100 m) over open areas are traversable by GLTs (Mickelberg 2011; AMLD unpublished data), and by other primate species as well – e.g., the *Alouatta palliata* (Mandujano 2005). Congruently, our results showed that although GLTs are sensitive to forest discontinuity, but pasture does not represent an impassable barrier. However, that statement does not apply to roads and highways, which are gaps of short distances, but with high crossing costs. We believe that for short distances of pasture and other open agricultural areas, the enrichment of the matrix with scattered trees or another spatial element – e.g., ecological corridors and stepping stones (Mandujano *et al.* 2004; Boyle & Smith 2010a) – makes the environment structurally similar to the original habitat and can facilitate inter-habitat movements.

For the federal highway BR-101 was assigned the highest resistance by specialists. Probably, BR-101 may be one of the main barriers to GLT dispersal. This highway will soon be widened, and its negative impacts on the populations will increase, becoming an impassable physical barrier. Roads are present across worldwide, and expansion projects provide an opportunity to better understand the response of fauna to environmental modification. Together, new management actions must be implemented to increase landscape connectivity and minimize the impact of roads on dispersal, using, for example, fauna passages or keeping intact high canopy-trees near roads (e.g., Teixeira *et al.* 2013).

Final considerations

Our results suggest that genetic management supported by animal reintroduction and translocation is not able to guarantee by itself the long-term viability of populations of the GLT – it is paramount to restore the landscape connectivity (see also chapter 2). Furthermore, our results also indicate that the effects of landscape discontinuity on the genetic structure of wild populations may be occurring at a much faster pace than can be fully quantified. Losses at the genetic level could occur rapidly and within a short time frame in arboreal species with closed social systems distributed in fragmented habitat (Di Fiore & Valencia 2014). The use of expert knowledge about the ecology and biology of the species may be help to minimize these effects of habitat fragmentation and in the reestablishment of functional connectivity. The scale and extent in which the conservation measures for the reestablishment of functional connectivity will be implemented also must be defined previous and based on the dispersal potential of the species.

Based on our results, we recommend for the conservation of endangered species occurring in fragmented landscapes that (1) landscape connectivity will be implemented associated to other management measures such as reintroduction and translocations; (2) expert opinions be used in the simulation of corridors intended to reestablish the connectivity of populations; (3) the distance that conservation action will be implement should be based on the dispersal potential of the species; and (4) an evaluation of the effect of roads on the dispersal potential, as well as empirical testing of measures that can minimize those effects. The use of endangered species with greater ecological restrictions (e.g., arboreal primates) to delimit conservation actions may be a good strategy, since it ensures that other less demanding species are also conserved over time (Lambeck 2007).

CAPÍTULO 4

ESTRUTURA E DISTRIBUIÇÃO ESPACIAL DAS POPULAÇÕES DE *Leontopithecus rosalia* E CARACTERIZAÇÃO DE SEU ESTADO DE CONSERVAÇÃO

Resumo

A persistência das espécies no atual cenário de fragmentação dos habitats depende da capacidade de cada uma delas funcionar como uma metapopulação. Os objetivos desse trabalho foram (1) delimitar espacialmente as populações de *Leontopithecus rosalia*, (2) investigar a existência de estrutura metapopulacional e (3) caracterizar as populações do ponto de vista espacial e genético e (4) indicar prioridades para a conservação da espécie. Para isso, usamos ferramentas de genética da paisagem para testar a hipótese de que existem pelo menos oito populações locais de *L. rosalia* na Bacia do Rio São João e REBIO União, Rio de Janeiro, Brasil. Cenários espaciais de estrutura populacional foram delimitados a partir do cálculo da distância baseada na resistência (ou distância efetiva), usando como base duas classes de distância de dispersão – 2,8 e 8 km – e os dados de ocorrência atual do *L. rosalia*. Os cenários delimitados usando a distância efetiva de 2,8 km corroboraram a estrutura genética populacional*.* Por isso, nós consideramos que esses cenários são os que melhores representam a dinâmica populacional do mico-leão-dourado: realísticos. Já os cenários que usaram 8 km de distância para delimitar populações regionais foram considerados como cenários ideais, pois poderão se tornar funcionais se a conectividade da paisagem for reestabelecida. A abordagem metodológica utilizada, com base em ferramentas de genética da paisagem, foi capaz de indicar regiões onde os *L. rosalia* podem estar se comportando como uma metapopulação e fragmentos onde as populações estão isoladas e podem perder diversidade genética se medidas de conservação não forem implementadas.

Palavras-chave: dispersão; estrutura espacial; estrutura genética; fragmentação; metapopulação.

Introdução

Atualmente, umas das principais preocupações de biologistas da conservação têm sido a destruição e fragmentação do habitat (Hanski 1998) e sua influência sobre a dinâmica populacional de várias espécies ameaçadas de extinção (Akçakaya *et al.* 2007; Hastings 2014). Associadamente, a teoria de metapopulações se tornou popular na biologia da conservação (Hanski & Simberloff 1997) com o objetivo de caracterizar populações espacialmente estruturadas e que mantém entre si movimentos ocasionais de indivíduos (Hanski 1998; Lopez & Pfister 2001; Akçakaya *et al.* 2007). Apesar de ser crescente o uso do termo metapopulações, os modelos metapopulacionais são difíceis de serem identificados em sistemas naturais. Por isso, é preciso conhecer bem a ecologia e biologia das espécies antes de caracterizá-las como uma metapopulação (Lamy *et al.* 2012).

A teoria de metapopulações avalia os processos de extinção, migração e colonização com o objetivo de contribuir com o manejo do espaço e a conservação das populações (Akçakaya *et al.* 2007). Os processos de extinção e recolonização são os principais fatores para se caracterizar uma metapopulação (Opdam 1991). Já o tamanho das manchas de habitat e sua distribuição no espaço são os principais fatores para a manutenção da dinâmica metapopulacional (Ovaskainen & Hanski 2001). Enquanto, os tamanhos dos fragmentos afetam diretamente a taxa de extinção local, o grau de isolamento entre eles interfere na taxa de recolonização. Dessa forma, a persistência de uma metapopulação em uma paisagem fragmentada depende tanto das características da paisagem como da capacidade da espécie de se mover e dispersar através dela. Como resultado da combinação desses dois fatores, a população se torna panmítica, relictual ou funcionar como uma metapopulação (Ovaskainen & Hanski 2001).

As análises de metapopulação geram informações sobre a dinâmica populacional em cada mancha de habitat com o objetivo de orientar programas de conservação em relação ao sistema como um todo (Hanski & Simberloff 1997; Dolrenry *et al.* 2014). Algumas medidas de conservação aplicadas em nível populacional são: proteção de habitat, redução da predação e remoção de espécies exóticas, restauração florestal, implementação de corredores de dispersão, translocações e reintroduções (Akçakaya *et al.* 2007). O mico-leão-dourado (GLT, na sigla em inglês), *Leontopithecus rosalia*, é um exemplo de primata ameaçado de extinção para o qual essas medidas de conservação têm sido aplicadas dentro de um contexto metapopulacional (Kierulff *et al.* 2012). A espécie é endêmica da Mata Atlântica, um ecossistema altamente degradado e fragmentado (Ribeiro *et al.* 2009). Provavelmente, ela apresenta-se organizada em metapopulações como uma resposta às modificações humanas da paisagem (Akçakaya *et al.* 2007). Na última Avaliação da População e Hábitat Viável (PHVA, na sigla em inglês), em 2005, os pesquisadores estabeleceram sete “Unidades de Manejo” (MUs, sigla em inglês) classificadas com base no grau de isolamento e na disponibilidade de habitat para o *L. rosalia* (Holst *et al.* 2006).

No entanto, o fato de estar distribuído em manchas, não torna o sistema populacional do *L. rosalia* necessariamente uma metapopulação (Harrison & Taylor 1997). Do mesmo modo, o fato de estar distribuído numa paisagem contínua, também não o torna uma população panmítica, pois ele exibe naturalmente um padrão de isolamento por distância (ver capítulo 3 e Di Fiore & Valencia 2014). Portanto, o conceito de metapopulação deve ser usado apenas se o *L. rosalia* estiver organizado em subpopulações conectadas por migrações e que exibem algum tipo de dinâmica metapopulacional (Elmhagen & Angerbjörn 2001). Devido à dificuldade de observar em campo eventos de extinção, consideramos que, se os indivíduos de *L. rosalia* são capazes de mover e dispersar através da paisagem e se existe habitat disponível, eventos de extinção e recolonização local podem ocorrer a todo o momento. Assim, os objetivos desse estudo foram: (1) delimitar espacialmente as populações de *L. rosalia*, (2) investigar se o seu sistema populacional apresenta-se como metapopulação, (3) caracterizar as populações em termos genéticos e espaciais, e (4) indicar prioridades para a sua conservação. Para isso, foram utilizados métodos de genética da paisagem com o intuito de testar a hipótese de que há pelo menos oito populações locais de *L. rosalia* na atual área de distribuição da espécie. O método genético é o mais indicado e informativo para esse tipo de estudo (Driscoll 2007), pois gera resultados mais robustos que o método direto, permitindo avaliar o padrão de dispersão da espécie (e não apenas sua mobilidade) em uma ampla escala de distribuição geográfica (Lampert *et al.* 2003).

Metodologia

A área de estudo esta localizada na Bacia do Rio São João (SJRB) e Reserva Biológica União (REBIO União), Rio de Janeiro, Brasil, que constitui a principal área de distribuição atual do *L. rosalia* (Procópio de Oliveira *et al.* 2008). Foram analisados os sítios descritos no capítulo 2 e 3 (Fig. 4.1), amostrados no período recente (2007-2013). Os sítios de VR, Imbaú/AE, AVI, AVII e BE são constituídos por descendentes de animais reintroduzidos e nascidos no cativeiro. Os sítios de Serra (NT no capítulo 2) e PDA são compostos por descendentes de animais nativos. Já a REBIO União (TR no capítulo 2) abriga descendentes das populações translocadas de fragmentos isolados no litoral do Rio de Janeiro para o contínuo de florestas nessa unidade de conservação (Kierulff *et al.* 2012).

Para delimitar espacialmente as populações de *L. rosalia* utilizamos uma abordagem de agregação espacial baseada em duas classes – 2,8 e 8 km – de distância baseada na resistência (*RD*, também conhecida como distância efetiva). Para cada classe de distância foi gerado um mapa no programa ARCGIS 10.3.1 (ESRI) através da ferramenta *cost distance*, utilizando (1) o mapa de resistência para o movimento de *L. rosalia* produzido com base na opinião de 12 especialistas (ver capítulo 3) e (2) os pontos de ocorrência da espécie (Morais, dado não publicado) (Fig. 4.1). Nós optamos pela distância baseada na resistência (*RD*), pois a resistência da paisagem – que é a relação entre o custo para o movimento e a distância (Adriaensen *et al.* 2003; McRae 2006; Spear *et al.* 2010) – foi a variável que melhor explicou a variação genética do *L. rosalia* na SJRB e REBIO União (capítulo 3). As classes de distâncias de 2,8 e 8 km também foram escolhidas com base nos resultados do capítulo 3, supondo que indivíduos *de L. rosalia* dispersem mais frequentemente dentro de 2,8 km (denominados populações locais) e ocasionalmente dentro de 8 km (denominados populações regionais) de *RD*. Na sequência, esses cenários de estrutura espacial foram confrontados com as análises de estrutura genética.

Para examinar padrões de estrutura genética recente, foram usados 14 microssatélites genotipados em 201 indivíduos de *L. rosalia* distribuídos na SJRB e REBIO União no período de 2007-2013 (Fig. 4.1). Maiores informações sobre as análises laboratoriais, erros de genotipagem e desvios do Equílibrio de Hardy Weinberg (HWE) podem ser consultados em maiores detalhes nos capítulos 2 e

3. As análises de estrutura genética foram realizadas sem informação espacial *a priori* no programa STRUCTURE versão 2.3 (Pritchard *et al.* 2000). De acordo com Sacks *et al.* (2016), consideramos o nível mais alto de K para identificar subpopulações fontes e escoadoras. Foram consideradas como subpopulações escoadoras aquelas com muitos indivíduos atribuídos a outras subpopulações, enquanto as subpopulações fontes são aquelas com a grande maioria dos indivíduos (ou todos) atribuídos ao *cluster* de origem (Sacks *et al.* 2016). As corridas foram realizadas assumindo *admixture model* e frequências alélicas correlacionadas. Para estimar a probabilidade *a posteriori* de K *clusters* – Pr (X/K), utilizamos 10 corridas independentes de K = 1-N, MCMC de 1.000.000 interações e *burn-in* de 200.000 *sets*. Para determinar o número de *clusters* genéticos para o conjunto de dados de 14 microssatélites utilizamos: (1) os valores ótimos das probabilidades *a posteriori* – dados como LnP(K) – e sua variância (Pritchard *et al.* 2000), e (2) o valor modal de ΔK (Evanno *et al.* 2005). Todos os resultados foram gerados usando o aplicativo HARVEST STRUCTURE (Earl & VonHoldt 2012).

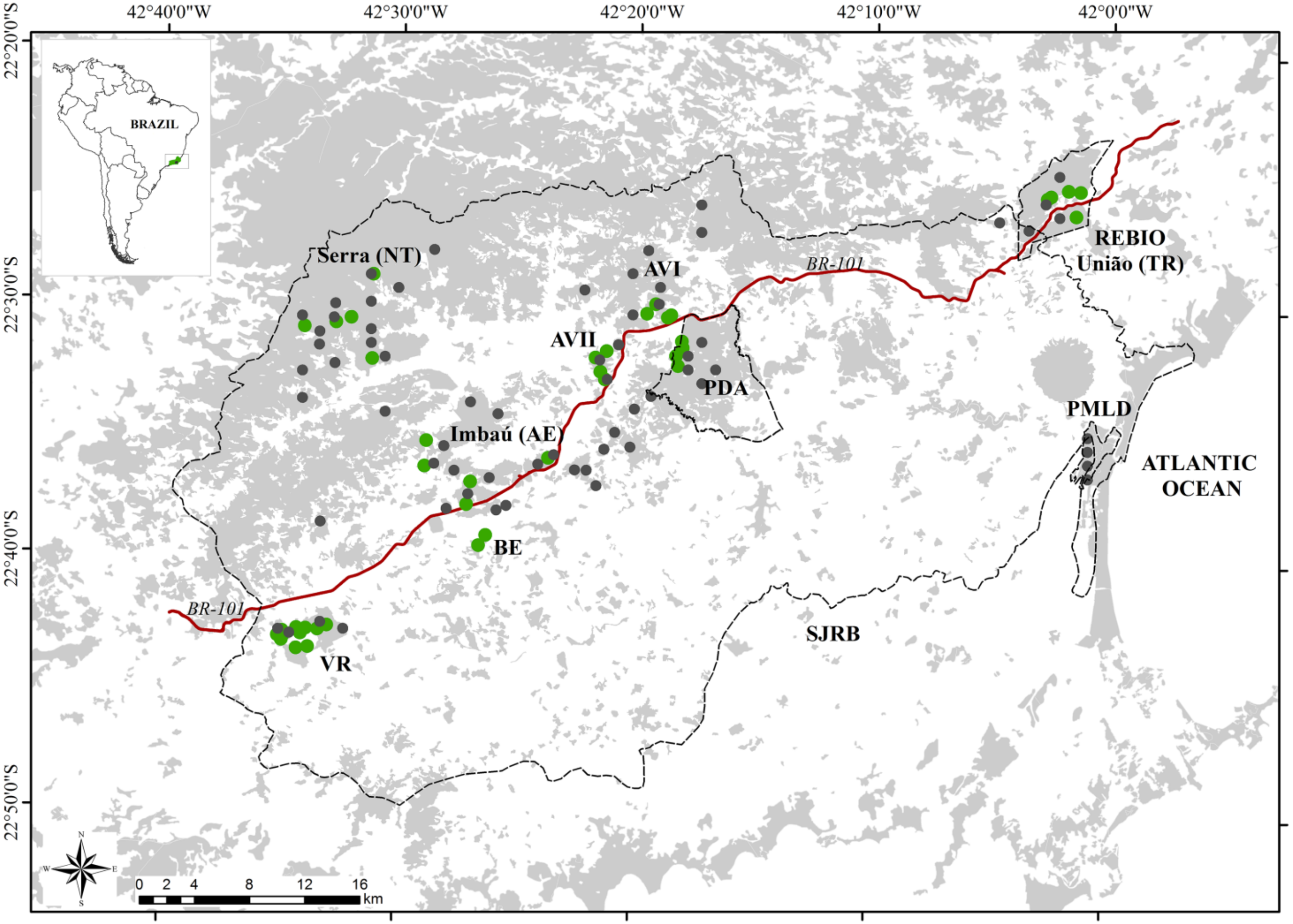


Figura 4.1 – Principal área de ocorrência atual do mico-leão-dourado e área de estudo. Pontos em verde mostram os pontos de amostragem genética e em preto os pontos de ocorrência conhecidos. As linhas pontilhadas delimitam as reservas de proteção pública. Blocos em cinza representam as áreas de floresta e em branco de não floresta.

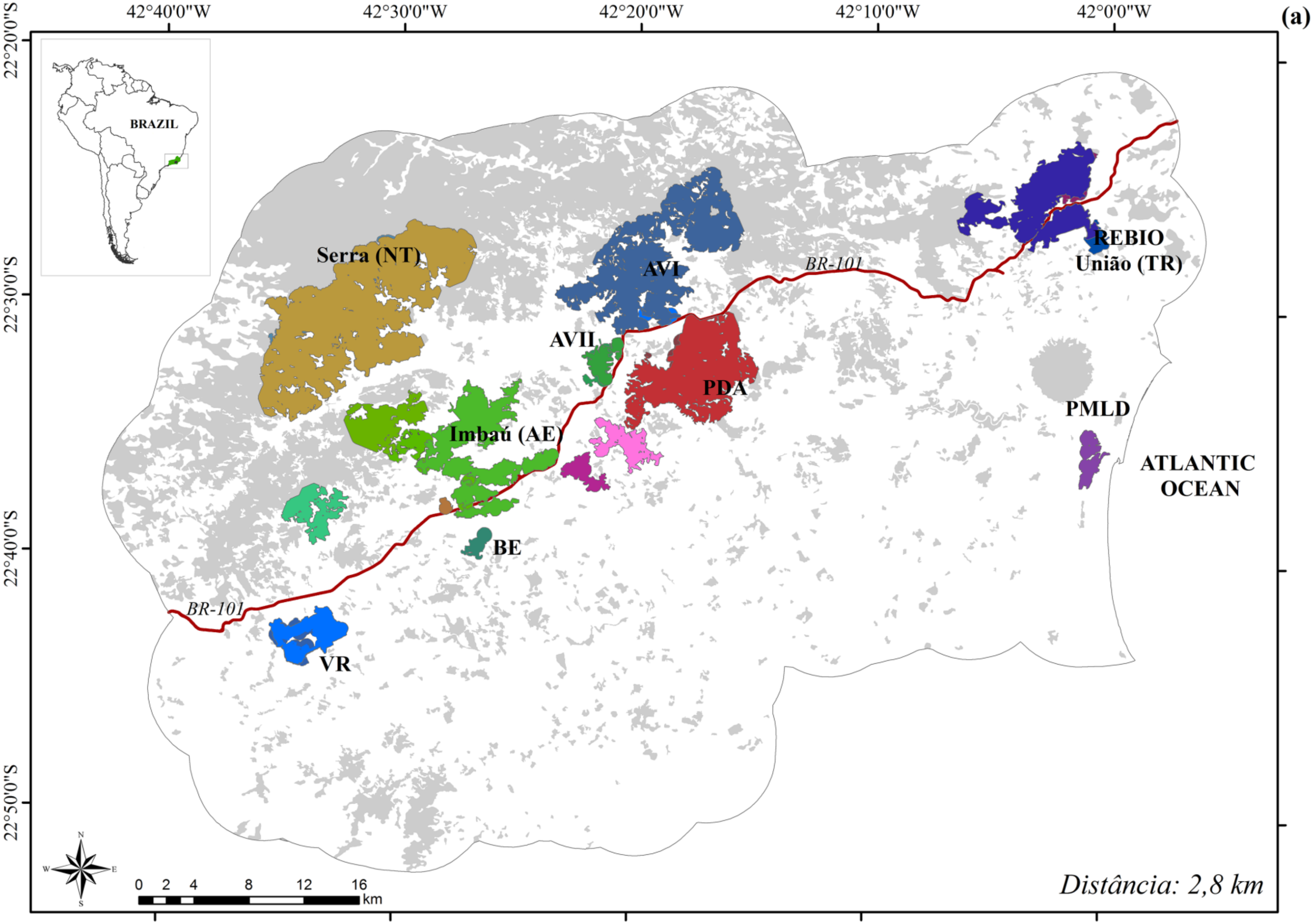
A análise de variância molecular (AMOVA) (Excoffier *et al.* 1992) também foi realizada no programa GENALEX versão 6 (Peakall & Smouse 2012) usando 9999 permutações, para testar a hipótese de que há oito *clusters* genéticos na área de estudo. O poder de inferência da AMOVA aumenta quando ela é calculada para uma hipótese de fronteira populacional estabelecida com base em informações biológicas. Além disso, é uma ferramenta poderosa quando a estruturação é fraca, dando maior confiabilidade aos resultados (Meirmans 2015). Por isso, ela é indicada para nosso conjunto de dados auxiliando no teste de nossa hipótese.

Depois de identificada as populações de *L. rosalia* na SJRB e REBIO União, foram calculados os índices de diversidade genética para cada uma delas, com o intuito de avaliar seu estado de conservação sob o ponto de vista genético. A heterozigose esperada padronizada (H_S) foi estimada através do programa GENEALEX e riqueza alélica (*AR*) e riqueza de alelos privados (*PR*) corrigidos pelo tamanho do menor sítio amostral foram estimadas no programa HP-RARE (Kalinowski 2005). Por último, reunida toda informação acerca da conservação do GLT, nós usamos as premissas que devem ser cumpridas por uma metapopulação clássica para discutir a aplicação do termo metapopulação ao sistema populacional do *L. rosalia* (Hanski *et al.* 1995).

Resultados

Os cenários de estrutura espacial mostraram que existem até 12 populações locais de *L. rosalia* na SJRB e REBIO União, quando usado 2,8 km de *RD*, e que o nível de estruturação espacial pode ser menor, se as populações forem funcionalmente conectadas por 8 km de *RD* (Fig. 4.2). As análises de estrutura genética sem informação *a priori* mostraram uma subestruturação em 5-8 *clusters* genéticos (Fig. 4.3), distribuídos de forma similar ao observado nos cenários de estrutura espacial. Os maiores valores de K, que corresponderam ao segundo melhor valor de LnP(K) e ΔK (Apêndices D), foram observados quando K = 5 e K = 8. Os resultados obtidos em cinco ou oito *clusters* foram similares entre si e mostraram uma subestrutura dentro da REBIO União. Ambos os *clusters* mostraram também, relativa mistura dentro das populações, mais evidenciada em Imbaú/AE (Fig. 4.3). De forma congruente ao encontrado nas análises do

STRUCTURE, a estatística *F* mostrou significativa estrutura genética através das oito populações hipotéticas (F_{ST} = 0.094, P = 0.000). Todas as comparações entre pares de populações foram significativas, exceto entre Serra e AVI (Tabela 4.1) que tiveram pouca ou nenhuma diferenciação. Também, similar ao STRUCTURE, maior variância molecular foi observada dentro das populações (91%) do que entre as unidades populacionais hipotéticas (9%).


(a)
42°40'0"W
42°30'0"W
42°20'0"W
42°10'0"W
42°0'0"W
22°20'0"S
22°30'0"S
22°40'0"S
22°50'0"S
BRAZIL
Serra (NT)
AVI
AVII
PDA
Imbaú (AE)
BE
VR
REBIO
União (TR)
PMLD
ATLANTIC
OCEAN
BR-101
BR-101
N
S
W
E
0
2
4
8
12
16
km
Distância: 2,8 km

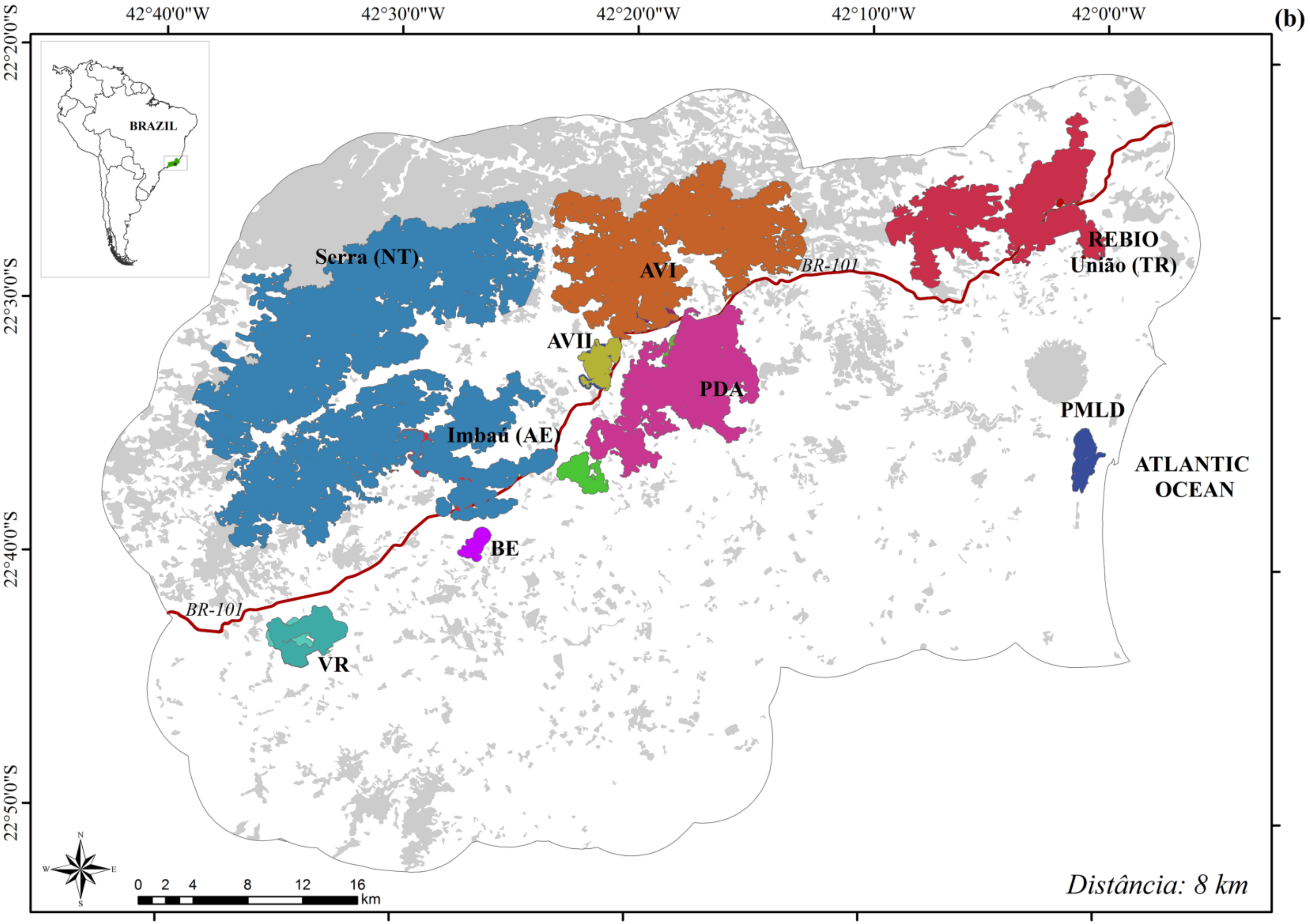


Figura 4.2– Cenários de estrutura espacial simulados para GLT na Bacia do Rio São João e REBIO União, Rio de Janeiro, Brasil, usando dois intervalos de distâncias baseada na resistência: (a) 2.8 km e (b) 8 km.

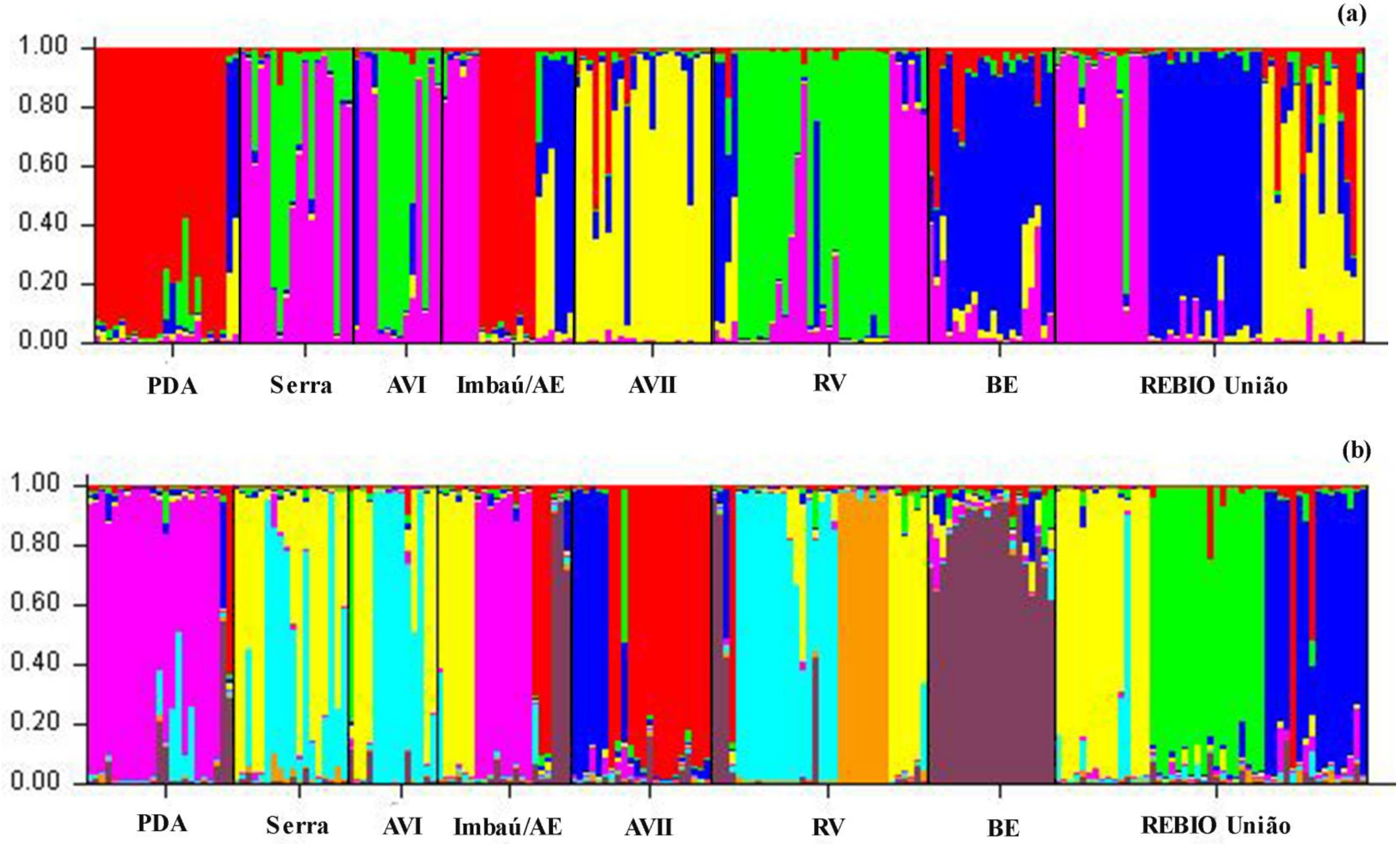


Figura 4.3 – Subestrutura genética recente (2009–2013) do GLT distribuído na Bacia do Rio São João e REBIO União, RJ, Brasil, sem usar informação espacial *a priori*. Em (a) K = 5 e em (b) K = 8.

Tabela 4.1 – Valores de F_{ST} par a par (abaixo da diagonal) e os valores de significância (acima da diagonal) baseados em 9999 permutações. A comparação par a par não significativa está destacada em negrito (*P* = 0.05).

	PDA	***Serra***	***AVI***	***AE***	***AVII***	***VR***	***BE***	***União***
PDA	-	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Serra	0,20	-	**0,05**	0,00	0,00	0,00	0,00	0,00
AVI	0,15	0,02	-	0,00	0,00	0,01	0,00	0,00
AE	0,04	0,11	0,06	-	0,00	0,00	0,00	0,00
AVII	0,14	0,23	0,16	0,09	-	0,00	0,00	0,00
VR	0,14	0,05	0,03	0,07	0,15	-	0,00	0,00
BE	0,13	0,16	0,10	0,06	0,11	0,10	-	0,00
União	0,10	0,11	0,06	0,03	0,06	0,06	0,07	-

Em sequência, nós comparamos nossos cenários de estrutura espacial com os cenários de estrutura genética sem informação *a priori*, e consideramos que os cenários que delimitaram as populações locais, baseados em 2,8 km de *RD*, foram os mais realísticos. Já os cenários que simularam as populações regionais, baseados em 8 km de *RD*, foram considerados cenários idealizados, no sentido que planos de manejo para reestabelecer a conectividade funcional na SJRB e REBIO União devem ser realizados. Assim, com base em ambos os cenários de estrutura (espacial e genético), consideramos como populações locais para as análises de diversidade genética: PDA; Serra, REBIO União; AVI; AVII; VR; BE e Imbaú/AE. REBIO União apresentou a maior diversidade genética, enquanto os sítios nativos – PDA e Serra – as menores. Entre os sítios reintroduzidos, BE foi a população com menor diversidade genética (Tabela 4.2).

Tabela 4.2 – Índices de diversidade genética calculados para oito populações de mico-leão-dourado na Bacia do Rio São João e REBIO União. Riqueza alélica (*AR*), riqueza de alelos privados (*PR*) e heterozigose esperada padronizada (H_S) foram calculadas a partir de 201 indivíduos e 14 microssatélites.

	PDA			**Serra**			**AVI**			**AE**			**AVII**			**VR**			**BE**			**REBIO União**		
Primers	***AR***	***PR***	***Hs***	***AR***	***PR***	***Hs***	***AR***	***PR***	***Hs***	***AR***	***PR***	***Hs***	***AR***	***PR***	***Hs***	***AR***	***PR***	***Hs***	***AR***	***PR***	***Hs***	***AR***	***PR***	***Hs***
Leon30	3,2	0,0	0,6	2,0	0,0	0,1	3,7	0,2	0,4	3,7	0,0	0,6	4,1	0,6	0,6	4,3	1,3	0,6	3,0	0,0	0,6	4,5	0,7	0,7
Leon21	2,0	0,0	0,5	3,0	0,0	0,6	3,9	0,9	0,7	2,7	0,0	0,4	4,4	0,5	0,7	3,7	0,3	0,7	2,0	0,0	0,5	5,2	0,6	0,7
Lchu8	4,5	0,0	0,7	4,2	0,5	0,7	4,6	0,7	0,7	5,0	0,0	0,8	5,5	1,6	0,8	3,3	0,0	0,6	3,9	0,0	0,6	4,3	0,0	0,7
Lchu3	2,0	0,0	0,4	3,0	0,0	0,6	3,0	0,0	0,6	3,0	0,0	0,6	2,9	0,0	0,6	3,0	0,0	0,7	3,0	0,0	0,7	3,0	0,0	0,6
P2BH6	4,5	0,2	0,7	4,7	0,0	0,7	6,5	0,5	0,9	4,8	0,0	0,7	3,7	0,0	0,6	5,7	0,0	0,8	4,0	0,0	0,7	5,1	0,0	0,8
Lchu9	3,0	0,0	0,6	2,8	0,0	0,5	2,0	0,0	0,3	3,0	0,0	0,6	2,9	0,0	0,6	2,6	0,0	0,6	3,0	0,0	0,6	2,9	0,0	0,6
Leon27	3,0	0,0	0,7	2,8	0,0	0,5	3,0	0,0	0,5	3,0	0,0	0,6	3,4	0,9	0,6	2,7	0,0	0,5	3,0	0,0	0,7	3,0	0,0	0,6
Leon31	3,9	0,0	0,5	1,9	0,0	0,2	3,0	0,0	0,5	4,3	0,0	0,5	5,2	0,0	0,8	4,5	1,3	0,6	4,6	0,1	0,7	5,0	0,3	0,6
Lchu7	2,4	0,0	0,5	2,0	0,0	0,5	2,8	0,8	0,5	2,8	0,0	0,6	3,0	0,0	0,6	2,8	0,0	0,5	3,0	0,0	0,6	3,0	0,0	0,7
Lchu6	3,0	0,1	0,3	4,3	0,1	0,6	5,2	1,0	0,7	3,8	0,0	0,7	2,9	0,0	0,5	4,8	0,4	0,8	3,8	0,0	0,7	3,6	0,2	0,7
P5BE6	3,0	0,0	0,7	2,0	0,0	0,4	3,0	0,0	0,6	3,4	0,0	0,6	3,9	0,5	0,4	3,4	0,3	0,6	3,0	0,0	0,6	4,3	0,2	0,6
Lchu4	4,2	0,0	0,6	3,0	0,0	0,7	3,0	0,0	0,7	4,3	0,0	0,7	4,1	0,0	0,7	4,1	0,0	0,7	2,9	0,0	0,6	4,3	0,0	0,7
Leon2	3,9	0,0	0,7	3,4	0,0	0,6	3,6	0,6	0,5	5,2	0,4	0,8	3,1	0,0	0,5	3,5	0,0	0,6	4,9	0,1	0,8	4,7	0,1	0,7
Leon3	2,5	0,0	0,5	2,9	0,0	0,5	3,0	0,0	0,5	4,5	0,3	0,7	3,7	0,0	0,7	3,7	0,1	0,5	3,8	0,0	0,6	4,1	0,2	0,7
Média	3,2	0,0	0,6	3,0	0,0	0,5	3,6	0,3	0,6	3,8	0,1	0,6	3,8	0,3	0,6	3,7	0,3	0,6	3,4	0,0	0,6	4,1	0,2	0,7
DP	0,8	0,1	0,1	0,9	0,1	0,2	1,1	0,4	0,1	0,8	0,1	0,1	0,8	0,5	0,1	0,9	0,4	0,1	0,7	0,0	0,1	0,8	0,2	0,1

Discussão

Cenário atual das populações do Mico-leão-dourado

Nossos resultados indicaram que populações locais de *L. rosalia* encontram-se delimitadas por 2,8 km de distância baseada na resistência. As análises de estruturação genética e de variância molecular foram congruentes entre si e com os cenários de estrutura espacial delimitados usando a distância de dispersão de 2,8 km. Corroborando, o coeficiente médio de relacionamento obtido entre indivíduos de GLT separados por essa classe de distância (0–2.8 km) foi 0,09 (capítulo 3). Segundo Loiselle *et al.* (1995) irmãos-completos tem um coeficiente de relacionamento igual à 0,25 e meios-irmãos à 0,125. Juntos, esses resultados indicam que, provavelmente, até 2,8 km de distância baseada na resistência, a dispersão é suficientemente alta para manter uma população conectada geneticamente. Quando a distância baseada na resistência que separava dois indivíduos somava em torno dos 8 km, o coeficiente médio de relacionamento, apesar de positivo e significativamente correlacionado, foi próximo de zero (0.04) (ver capítulo 3 para maiores detalhes). Além dos movimentos diários que mantêm os indivíduos dentro de uma população geneticamente conectada, muitas espécies necessitam de movimentos menos frequentes e por longas distâncias para persistirem ao longo do tempo (Lindenmayer & Fischer 2006). Caso a conectividade funcional na SJRB e REBIO União seja restaurada, movimentos menos frequentes de *L. rosalia* podem ocorrer em distâncias de até 8 km que mantenham populações geneticamente conectadas.

Diferentes cenários de K explicam diferentes processos biológicos (Meirmans 2015). Para avaliar o padrão de estrutura genética na SJRB e REBIO União sem informação *a priori*, nós usamos o segundo melhor e maior valor de K, porque o GLT possui um sinal fraco de estruturação, como observado no capítulo 2. Além disso, a interpretação dos maiores valores de K permitem indicar possíveis populações fontes e escoadouros em uma dinâmica metapopulacional (Sacks *et al.* 2016). As análises de estrutura genética mostraram que Serra e AVI constituem uma única população. Provavelmente, esse resultado é uma consequência do manejo na fundação de AVI (capítulo 2). Contudo, apesar de improvável como mostrado nos cenários de estrutura espacial, o fluxo gênico entre essas duas populações não pode ser descartado, ou, pode ainda, ser resultado da combinação desses dois fatores: manejo e resistência da paisagem (capítulo 3). Já as semelhanças observadas entre Serra e Imbaú/AE, provavelmente, são resultado da permeabilidade da paisagem (ver Figura 4.2b), que favoreceu o fluxo gênico entre elas (Freitas 2012). As demais semelhanças observadas entre pares de populações foram consideradas efeitos da fundação (capítulo 2).

Delimitadas as populações de *L. rosalia*, avaliamos sua diversidade genética e seu grau de ameaça sob o ponto de vista genético. Apesar da população na REBIO União estar perdendo diversidade alélica ao longo do tempo, como observado no capítulo 2, ela apresentou a maior diversidade genética e o mais distinto padrão de estruturação. Esse resultado mostra a importância dessa unidade para a conservação da espécie e reforça a necessidade de medidas de conservação para reconectá-la às demais populações da SJRB. Além disso, nossos resultados de estrutura populacional genética mostraram que existe uma subestruturação na REBIO União. Parte dessa subestrutura corresponde aos lados sul e norte da rodovia federal BR-101. A BR-101 está sendo duplicada e, provavelmente, se tornará uma barreira intransponível entre o lado sul e norte da REBIO União e toda a SJRB (maiores informações no capítulo 3). Além da divisão da população na REBIO União, outras duas importantes populações, VR e PDA, se tornarão totalmente isoladas com a duplicação da BR-101 e a conservação de ambas deve ser tornar ainda mais difícil. Se passarelas funcionais não forem implantadas, as populações ao sul, que já são geneticamente distintas, podem perder ainda mais diversidade genética ao longo do tempo (capítulo 2).

Em contraposição à REBIO-União, Serra, PDA e BE tiveram a menor diversidade genética. BE é pequena e encontra-se isolada desde sua fundação. Como consequência, apresentou baixa riqueza alélica e de alelos privados. Sua baixa diversidade genética, em comparação com as demais populações reintroduzidas, mostra a baixa prioridade de BE sob o ponto de vista da genética da conservação. Serra, assim como PDA, abrigam os descendentes das populações nativas, não manejadas, que sofreram intensa redução populacional até os anos de 1980 (Coimbra-Filho & Mittermeier 1973; Rylands *et al.* 2002). A redução e o isolamento histórico dessas duas populações devem ter contribuído para a redução de sua diversidade genética. Apesar disso, Serra pode estar funcionando como fonte para Imbaú/AE, contribuindo com o fluxo gênico e aumentando da diversidade genética nessa região (ver também Freitas 2012; Holst *et al.* 2006).

O Mico-leão-dourado funciona como uma metapopulação?

O novo mapeamento florestal da SJRB mostrou que existem grandes áreas de florestas (Seabra 2012). Associadamente, o último levantamento populacional *do L. rosalia* na SJRB identificou manchas de habitat vazias (MORAIS, dado não publicado). Juntas, essas duas pesquisas mostraram que existem áreas de florestas na SJRB que não estão colonizadas por indivíduos de *L. rosalia*, o que indica que a espécie não consegue ter acesso a todo o espaço habitável. Também quando comparamos o último censo populacional (Morais, dado não publicado) com o anteriormente realizado (Kierulff & Rylands 2003) e com o cenário até então proposto para a distribuição das populações na SJRB e REBIO União (Holst *et al.* 2006), observamos que as populações de *L. rosalia* podem estar em expansão. Corroborando com essa hipótese, estudos moleculares identificaram um grupo social entre a Serra e Imbaú/AE que possui relação genética com ambas as vizinhança (Freitas 2012), reforçando a teoria de que essas populações estão em expansão e que elas possuem alguma conectividade entre si.

Usando como base os resultados dessa pesquisa, conseguimos definir e delimitar populações isoladas e, outras, que atuam possivelmente como uma metapopulação. A provável distribuição das populações delimitadas por 2,8 km de distância baseada na resistência foi similar as UMMs delimitadas com base no habitat disponível e na conectividade da paisagem (Holst *et al.* 2006) e congruente com observações de movimento de indivíduos (Coelho 2009; Mickelberg 2011; Freitas 2012). Foram identificadas populações isoladas na porção sul e norte da SJRB devido à passagem da rodovia BR-101 e à resistência da paisagem (ver também capítulo 3). Na porção norte da SJRB há populações potencialmente isoladas pela resistência da paisagem (ex. AVII) e outras pela distância geográfica (ex. REBIO União). Recomendamos que medidas para restabelecer a conectividade estrutural e funcional entre AVII e AVI, como a implementação de corredores florestais, sejam realizados. Já na REBIO União, provavelmente, a resistência da paisagem e a distância geográfica atuam conjuntamente para seu isolamento. Esperamos que o novo corredor florestal implementado entre a REBIO União e o fragmento florestal vizinho (Dourada) (AMLD, dado não publicado) minimizem os efeitos do isolamento por resistência. Porém, ainda será necessário resolver o isolamento por distância e a resistência causada pela passagem da BR-101 em seu interior. Uma maneira de aumentar o fluxo gênico pode ser a translocação de populações isoladas para a vizinhança da REBIO União e a construção de passagens de faunas sobre a BR-101 unindo as duas porções da unidade.

Finalmente, nossos resultados indicaram que Imbaú/AE e Serra podem estar funcionando como um sistema metapopulacional, assim como anteriormente observado (Holst *et al.* 2006; Freitas 2012). Segundo Holst *et al.* (2006) Serra funciona como uma fonte para AVI. Porém, de acordo com os resultados desse estudo, não podemos descartar os efeitos da fundação das populações reintroduzidas na estrutura observada (capítulo 2). Medidas para aumentar e restaurar a conectividade funcional entre essas populações também devem ser implementadas, com o intuito de manter o fluxo gênico entre elas por longo prazo.

Além dos processos de migração e colonização, a teoria de metapopulações também avalia o processo de extinção (Akçakaya *et al.* 2007). Não existem relatos de eventos de extinção de populações de *L. rosalia* observados em campo. Entretanto, análises de viabilidade populacional identificaram probabilidades variáveis de extinções entre populações de *L. rosalia*. Algumas delas tiveram probabilidade igual à zero de se extinguirem em 100 anos (Holst *et al.* 2006; Mickelberg 2011; Mickelberg, não publicado), o que reforça a provável existência de um modelo continente-ilha (Harrison & Hastings 1996). Além da probabilidade de extinção, as populações de *L. rosalia* têm diferentes tamanhos populacionais (Kierulff & Rylands 2003; Holst *et al.* 2006; Mickelberg 2011; Morais dado não publicado) e diferentes conectividades estruturais (Procópio de Oliveira *et al.* 2008; Seabra 2012) e funcionais da paisagem em seus habitats (ver capítulo 2), indicando que elas possuem uma dinâmica assíncrona através da paisagem.

Com base nas informações reunidas consideramos que:

(1) a SJRB possui habitats capazes de sustentar populações locais de *L. rosalia* e que elas não ocupam todo o espaço habitável (Morais, dado não publicado);

(2) algumas populações são grandes o suficiente para sobreviver e persistir em 100 anos (Holst *et al.* 2006; Mickelberg 2011; Mickelberg, dado não publicado) mostrando que o *L. rosalia* pode funcionar como uma metapopulação do modelo continente-ilha;

(3) existem manchas de habitat não colonizadas e que são estruturalmente (Procópio de Oliveira *et al.* 2008; Seabra 2012) e funcionalmente conectadas, considerando que o *L. rosalia* seja capaz de atravessar curtas distâncias de matriz (capítulo 3) (Holst *et al.* 2006; Mickelberg 2011), o que permitem a recolonização;

(4) as populações possuem dinâmica assíncrona e independente, sendo a extinção simultânea de todas elas improvável.

Recomendamos que o manejo florestal para a restauração da conectividade funcional seja realizado dentro de um limiar de 8 km de distância baseada na resistência. Nós esperamos que, se a paisagem for funcionalmente conectada dentro desse limiar de distância, pelo menos quatro grandes metapopulações em equilíbrio poderão ser mantidas na SJRB e REBIO União (como indicado na Figura 4.2b). A fragmentação dos habitats torna populações que antes eram contínuas em esparsas (Driscoll 2007) e sua conservação nessa paisagem vai depender de sua capacidade de funcionar como uma metapopulação. Se os indivíduos não forem capazes de se mover entre as manchas e recolonizar novos habitats, a espécie poderá acumular eventos de extinções populacionais locais e regionais e alcançar a extinção globalizada (Hanski 1998).

Considerações finais

Nós usamos ferramentas de genética da paisagem e informações da literatura para identificar as populações de *L. rosalia* reprodutivamente isoladas, bem como aquelas que podem ter entre si movimentos ocasionais. Não conseguimos encontrar informações diretas sobre processos de extinções locais, mas supomos que, considerando um possível processo de expansão populacional (baseado nos registros de novos pontos de ocorrência; Morais, dado não publicado) e que as populações tem uma dinâmica assíncrona (Holst *et al.* 2006; Mickelberg 2011; Mickelberg, dado não publicado), extinções locais podem estar ocorrendo a qualquer momento através da SJRB e REBIO União.

A dinâmica metapopulacional depende dos eventos de dispersão, extinção e recolonização de fragmentos variadamente distribuídos numa matriz menos adequada. Essa dinâmica de extinção e recolonização tem um efeito de fundação que pode afetar a estrutura genética das populações (Hanski 1998). Cosentino *et al.* (2012) mostrou que o efeito fundador influenciava a estrutura genética metapopulacional de uma espécie de salamandra, *Ambystoma tigrinum*, mas não mais que outros fatores como a localização e a conectividade da paisagem. Similarmente, tanto o efeito fundador como a descontinuidade da floresta afetaram a estrutura genética do *L. rosalia* (capítulo 3). Esses resultados mostram que, estudos que avaliam a dinâmica populacional devem, portanto, considerar o efeito dos diferentes processos ecológicos e biológicos sobre a estrutura genética populacional (Meirmans 2015).

Nossos resultados indicam também que, se a conectividade funcional da paisagem for reestabelecida, a distância de dispersão do *L. rosalia* pode ser maior que o observada por métodos diretos (Mickelberg 2011). Os modelos metapopulacionais ajudam a entender a dinâmica das populações em paisagens fragmentadas e a delimitar estratégias de conservação. Eles podem ser usados para caracterizar diferentes grupos faunísticos, porém, é preciso ter cautela. Dependendo da espécie de estudo, amplas escalas temporais e espaciais devem ser consideradas. Conhecer a ecologia da espécie também é importante (Smith & Green 2005; Lamy *et al.* 2012). Além disso, conclusões baseadas apenas em dados de marcação e recaptura podem gerar resultados superficiais se a escala de amostragem não for adequada, ou, se as conclusões basearem-se apenas na mobilidade do animal e não na dispersão efetiva (e.g., Marsh *et al.* 1999; 2000; Lampert *et al.* 2003). Nesse sentido, nossa abordagem espacializada baseada em métodos de genética da paisagem se mostrou eficiente na definição das populações locais e regionais de *L. rosalia* na SJRB e REBIO União, podendo contribuir com as estratégias de conservação da espécie ou de outras ecologicamente similares.

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Consequences of Reintroductions: an Example From Oregon Pronghorn Antelope (*Antilocapra americana*). *Journal of Wildlife Management*, **69**, 1463–1474.

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Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: Where are we now? *Molecular Ecology*, **19**, 3496–3514.

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Teixeira FZ, Printes RC, Godoy Fagundes JC *et al.* (2013) Canopy bridges as road overpasses for wildlife in urban fragmented landscapes. *Biota Neotropica*, **13**, 117–123.

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Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: A largely untapped resource for applied conservation and evolution. *Evolutionary Applications*, **3**, 244–262.

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Zeigler SL, Neel MC, Oliveira L, Raboy BE, Fagan WF (2011) Conspecific and heterospecific attraction in assessments of functional connectivity. *Biodiversity and Conservation*, **20**, 2779–2796.

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APÊNDICES

Apêndice A

A1 – References list used for the quantitative analyses in the review paper "Factors affecting movement and dispersal of Neotropical primates across landscape fragmentation: a review"

REFERENCES	SPECIES STUDIED	SUMMARY	REFERENCE NUMBER
Abondano LA, Link A. 2012. The Social Behavior of Brown Spider Monkeys (*Ateles hybridus*) in a Fragmented Forest in Colombia. International Journal of Primatology 33:769–783.	*Ateles hybridus*	It evaluated the behavioral flexibility of endangered primates as a response to ecological challenges, e.g., anthropogenic habitat disturbance	27
Anzures-Dadda A, Manson RH. 2007. Patch- and landscape-scale effects on howler monkey distribution and abundance in rainforest fragments. Animal Conservation 10:69–76.	*Alouatta palliata*	It examined the distribution and abundance of *Alouatta palliata* as a function of both patch quality and landscape connectivity. Primate distribution was correlated with fragment area and abundance, whereas primate abundance was correlated with corridor abundance, canopy height and fragment area.	48
Arroyo-Rodríguez V, González-Perez IM, Garmendia A, Solà M, Estrada A. 2013. The relative impact of forest patch and landscape attributes on black howler monkey populations in the fragmented Lacandona rainforest, Mexico. Landscape Ecology 28:1717–1727.	*Alouatta pigra*	It assessed the effect of three metrics at the fragment scale (size, shape and isolation) and five metrics at the landscape scale (forest cover, edge density, distance between patches, and matrix permeability) on the composition and structure of primate population	37
Beaudrot LH, Marshall AJ. 2011. Primate communities are structured more by dispersal limitation than by niches. Journal of Animal	*Cebus paella*	It tested whether geographic distance was more important than similarity to determine resemblances among communities of South American primates	38

Ecology 80:332–341.			
Benchimol M, Venticinque EM. 2014. Responses of primates to landscape change in Amazonian land-bridge islands-a multi-scale analysis. Biotropica 46:470–478.	*Alouatta macconnell, Ateles paniscus, Chiropotes sagulatus, Pithecia chrysocephala, Saguinus midas, Saimiri sciureus* and *Sapajus apella*	It investigated how the primate community in the Brazilian Amazon responded to changes in landscape structure and the formation of terrestrial islands in 21 years of isolation due to flooding by a hydroelectric dam	46
Blair ME, Melnick DJ. 2012. Scale-dependent effects of a heterogeneous landscape on genetic differentiation in the Central American squirrel monkey (*Saimiri oerstedii*). PloS one 7:e43027.	*Saimiri oerstedii*	It examined gene flow and performed a landscape genetic analysis.	18
Boinski S, Ehmke E, Kauffman L, Schet S, Vreedzaam A. 2005a. Dispersal patterns among three species of squirrel monkeys (*Saimiri oerstedii*, *S. boliviensis* and *S. sciureus*): II. Within-species and local variation. Behaviour 142:633–677.	*Saimiri boliviensis, S. sciureus* and *S. oerstedii*	It reported and described the pattern of dispersal.	58
Bonnell TR, Dutilleul P, Chapman CA, *et al.* 2013. Analysing small-scale aggregation in animal visits in space and time: The ST-BBD method. Animal Behaviour 85:483–492.	*Alouatta pigra* and *Ateles geoffroyi*	It evaluated the efficiency of the method for estimating habitat aggregation and the intensity with which the animal uses the home range, considering its diet	24
Boinski S, Kauffman L, Ehmke E, Schet S, Vreedzaam A. 2005b. Dispersal patterns among three species of squirrel monkeys (*Saimiri oerstedii*, *S. boliviensis* and *S. sciureus*): I. Divergent costs and benefits. Behaviour 142:525–632.	*Saimiri boliviensis, S. sciureus* and *S. oerstedii*	It reported the dispersal of a forest population of the Neotropical primate *Saimiri sciureus* compared to other two species of *Saimiri*.	25
Boyle SA, Lourenço WC, da Silva LLR, *et al.* 2009. Travel and spatial patterns change when	*Chiropotes satanas chiropotes*	It examined how primates moved and the spatial patterns in three fragments of 1 ha,	29

Chiropotes satanas chiropotes inhabit forest fragments. International Journal of Primatology 30:515–531.		10 ha and 100 ha versus a continuous area	
Boyle SA, Smith AT. 2010a. Can landscape and species characteristics predict primate presence in forest fragments in the Brazilian Amazon? Biological Conservation 143:1134–1143.	*Alouatta macconnelli, Ateles paniscus, Cebus apella, Chiropotes satanas, Pithecia pithecia* and *Saguinus midas*	It measured the distribution and persistence of six primates in different isolated fragments.	7
Boyle SA, Smith AT. 2010b. Behavioral modifications in northern bearded saki monkeys (*Chiropotes satanas chiropotes*) in forest fragments of central Amazonia. Primates 51:43–51.	*Chiropotes satanas chiropotes*	It investigated the behavioral differences of five groups in forest fragments and two in continuous areas	55
Campos FA, Bergstrom ML, Childers A, *et al.* 2014. Drivers of home range characteristics across spatiotemporal scales in a Neotropical primate, *Cebus capucinus*. Animal Behaviour 91:93–109.	*Cebus capucinus*	It estimated home range and movement through four temporal and three spatial scales	40
Carretero-Pinzón X. 2013. An Eight-Year Life History of a Primate Community in the Colombian Llanos. In: Marsh LK, Chapman CA, editors. Primates in Fragments: Complexity and Resilience. New York: Springer Science+Business Media. p 159–182.	*Alouatta seniculus, Saimiri sciureus, Callicebus ornatus, Aotus brumbacki* and *Sapajus paella*	It tested the effects of forest fragment size on primate population densities. Variation in population densities were attributed to group composition, vegetation and fragment size. All species studied utilized fencerows to traverse the matrix between fragments, though observations of *Callicebus* and *Aotus* were rare.	62
Cervera L, Lizcano DJ, Tirira DG, Donati G. 2015. Surveying Two Endangered Primate Species (*Alouatta palliata aequatorialis* and *Cebus aequatorialis*) in the Pacoche Marine and	*Alouatta palliata aequatorialis* and *Cebus aequatorialis*	It evaluated the effects of anthropogenic disturbance and habitat characteristics on population densities	12

Coastal Wildlife Refuge, West Ecuador. International Journal of Primatology 36:933–947.			
Chaves ÓM, Stoner KE, Arroyo-Rodríguez V, Estrada A. 2011. Seasonal Differences in Activity Patterns of Geoffroyi's Spider Monkeys (*Ateles geoffroyi*) Living in Continuous and Fragmented Forests in Southern Mexico. International Journal of Primatology 32:960–973.	*Ateles geoffroyi*	It tested the effects of forest type (continuous and fragmented), season (dry and rainy), and their interaction on spider monkey activity patterns	21
Chaves ÓM, Stoner KE. 2010. River crossings by *Ateles geoffroyi* and *Alouatta pigra* in southern Mexico: A preliminary report. Revista Chilena de Historia Natural 83:435–442.	*Alouatta pigra* and *Ateles geoffroyi*	It assessed the ability of primates to cross a river, and if this behavior is related to deforestation or human population size on the disturbed riverbank	16
Clarke MR, Collins DA, Zucker EL. 2002. Responses to deforestation in a group of mantled howlers (*Alouatta palliata*) in Costa Rica. International Journal of Primatology 23:365–381.	*Alouatta palliata*	To evaluate the effects of partial deforestation of the home range of a group of free-ranging howlers. It compared the daily travel lengths and migration before, during, and after habitat destruction.	2
Cosson JFF, Ringuet S, Claessens O, *et al.* 1999. Ecological changes in recent land-bridge islands in French Guiana, with emphasis on vertebrate communities. Biological Conservation 91:213–222.	*Alouatta seniculus, Ateles panicus, Cebus apela* and *Saguinus midas*	It evaluated the movement of vertebrates in a scenario of landscape fragmentation on land islands due to the construction of a hydroelectric dam. It observed *Saguinus* using leafless tress to cross channels between the islands.	19
Di Fiore A, Valencia LM. 2014. The Interplay of Landscape Features and Social System on the Genetic Structure of a Primate Population: An Agent-Based Simulation Study Using "Tamarins." International Journal of Primatology	*Callithrix, Leontopithecus* and *Saguinus*	It used spatial simulation tools to explore the potential effects of social structure and the landscape on the gene flow of tamarins.	33

35:226–257.			
Dias PAD, Coyohua-Fuentes A, Canales-Espinosa D, Rangel-Negrín A. 2015. Group Structure and Dynamics in Black Howlers (*Alouatta pigra*): A 7-year Perspective. International Journal of Primatology 36:311–331.	*Alouatta pigra*	Which habitat and landscape-scale attributes best predicted variation among groups in structure and dynamics.	9
Estrada A, Anzures A, Coates-Estrada R. 1999a. Tropical rain forest fragmentation, howler monkeys (*Alouatta palliata*), and dung beetles at Los Tuxtlas, Mexico. American journal of primatology 48:253–262.	*Alouatta palliata*	It observed the presence of *Allouatta* and the beetle that composes its diet in 38 isolated fragments and 15 agricultural habitats composed of cacao, coffee, citrus, pepper and mixed crop plantations	31
Estrada A, Coates-Estrada R. 1996. Tropical rain forest fragmentation and wild populations of primates at Los Tuxtlas, Mexico. International Journal of Primatology 17:759–783.	*Alouatta palliata* and *Ateles geoffroyi*	It conducted a survey of the occurrence of the study species in 26 forest fragments and 44 agricultural sites	32
Estrada A, Juan-Solano S, Ortíz Martínez T, Coates-Estrada R. 1999b. Feeding and general activity patterns of a howler monkey (*Alouatta palliata*) troop living in a forest fragment at Los Tuxtlas, Mexico. American journal of primatology 48:167–83.	*Alouatta palliata*	It observed the pattern of activities in a small fragment	61
Fedigan L, Jack K. 2009. Female dispersal in a female-philopatric species, *Cebus capucinus*. Behaviour 146:471–497.	*Cebus capucinus*	It reported and assessed the motives driving the dispersal of philopatric females.	42
Fernández VA, Kowalewski M, Zunino GE. 2013. Who is coordinating collective movements in black and gold howler monkeys? Primates	*Alouatta caraya*	It examined how factors such as sex, age, reproductive status and dominance affect the coordination of movement for food or rest	56

54:191–199.			
Fernandez-Duque. 2009. Natal dispersal in monogamous owl monkeys (*Aotus azarai*) of the Argentinean Chaco. Behaviour 146:583–606.	*Aotus azarai*	It reported and described the pattern of natal dispersal.	47
Fortes VB, Bicca-Marques JC. 2008. Abnormal Pelage Color in an Isolated Population of *Alouatta guariba clamitans* Cabrera, 1940 in South Brazil. International Journal of Primatology 29:717–722.	*Alouatta guariba clamitans*	It reports the occurrence of individuals with abnormal pelage color in an isolated population in southern Brazil	1
Gilbert KA. 2003. Primates and Fragmentation of the Amazon Forest. In: Marsh LK, editor. Primates in fragments: Ecology and conservation. Hammond, Louisiana, USA: Kluwer Academic/Plenum Publishers. p 145–157.	*Allouatta seniculus*, *Ateles paniscus*, *Cebus apella, Chiropotes santanas*, *Pithecia pithecia* and *Saguinus midas*	It evaluated of primate ecology and behavior in isolated reserves and a continuous forest	15
Hopkins ME. 2011. Mantled Howler (*Alouatta palliata*) Arboreal Pathway Networks: Relative Impacts of Resource Availability and Forest Structure. International Journal of Primatology 32:238–258.	*Alouatta palliata*	It tested the influence of crown connectivity and resource distribution on the quantity and selection of pathways	51
Howard AM, Nibbelink NP, Madden M, *et al.* 2015. Landscape influences on the natural and artificially manipulated movements of bearded capuchin monkeys. Animal Behaviour 106:59–70.	*Sapajus libidinosus*	It evaluated how goal-directed travel is impacted by landscape variables	10
Jack KM, Fedigan L. 2004a. Male dispersal patterns in white-faced capuchins, *Cebus capucinus*. Part 1: Patterns and causes of natal emigration.	*Cebus capucinus*	It reported and examined the dispersal pattern of males.	49

Animal Behaviour 67:761–769.			
Jack KM, Fedigan L. 2004b. Male dispersal patterns in white-faced capuchins, *Cebus capucinus*. Part 2: Patterns and causes of secondary dispersal. Animal Behaviour 67:771–782.	*Cebus capucinus*	It reported and examined the patterns of male secondary dispersal derived from 16 years (1985-2000) of behavioral and demographic data collected on multiple groups.	64
Lazaro-Perea C, Castro CSS, Harrison R, *et al.* 2000. Behavioral and demographic changes following the loss of the breeding female in cooperatively breeding marmosets. Behavioral Ecology and Sociobiology 48:137–146.	*Callithrix jacchus*	It described the behavioral and demographic changes that follow the formation of reproductive vacancies in three groups of marmosets.	52
Lees AC, Peres CA. 2008. Conservation value of remnant riparian forest corridors of varying quality for Amazonian birds and mammals. Conservation Biology 22:439–449.	*Ateles* sp., *Callicebus moloch*, *Cebus apella*	It investigated the effect of width and the degree of degradation of runners formed due to riparian forest restoration in Brazil on the species richness	45
Link A, de Luna A, Alfonso F, Giraldo-Beltran P, Ramirez F. 2010. Initial effects of fragmentation on the density of three Neotropical primate species in two lowland forests of Colombia. Endangered Species Research 13:41–50.	*Alouatta seniculus, Ateles hybridus, Cebus albifrons*	It compared the population density of monkeys between continuous forest and recently fragmented forest	20
Madden D, Garber PA, Madden SL, Snyder CA. 2010. Rain-forest canopy-connectivity and habitat selection by a small Neotropical primate, Geoffroy's tamarin (*Saguinus geoffroyi*). Journal of Tropical Ecology 26:637–644.	*Saguinus geoffroyi*	It identified different canopy habitats used by this tamarin	59
Mandujano S. 2005. A metapopulation approach to conserving the howler monkey in a highly	*Alouatta palliata*	Its objective was answering the question: if our goal is to increase the viability of the	13

fragmented landscape in Los Tuxtlas, Mexico. In: Alejandro Estrada, Garber PA, Pavelka MSM, Luecke L, editors. New perspectives in the Study of Mesoamerican Primates: Distribution, Ecology, Behavior, and Conservation. New York: Springer. p 513–538.		howler monkey metapopulation in a highly altered landscape, what must we increase, the area of remaining habitat fragments, the connections among them, or both?	
Mandujano S, Escobedo-Morales LA, Palacios-Silva R. 2004. Movements of *Alouatta palliata* among forest fragments in Los Tuxtlas, Mexico. Neotropical Primates 12:126–131.	*Alouatta palliata*	It presents data on the movements in a highly modified landscape and a preliminary model of interchange between fragments.	8
Martinez J, Wallace RB. 2011. First observations of terrestrial travel for olalla's titi monkey (*Callicebus olallae*). Neotropical Primates 18:49–52.	*Callicebus olallae*	It presented a series of observations concerning terrestrial movements of the monkeys.	22
Martins MM, Nascimento ATA, Nali C, *et al.* 2014. A test for sex-biased dispersal in the black-faced lion tamarin (*Leontopithecus caissara*): inferences from microsatellite markers. Studies on Neotropical Fauna and Environment 50:14–20.	*Leontopithecus caissara*	It assessed the dispersal pattern and the system of cooperation during reproduction using molecular data.	50
Michalski F, Peres CA. 2005. Anthropogenic determinants of primate and carnivore local extinctions in a fragmented forest landscape of southern Amazonia. Biological Conservation 124:383–396.	*Alouatta seniculus, A. belzebul*, *Ateles marginatus*, *Callicebus moloch*, *Callithrix argentata*, *C. emiliae* and *Cebus apela*	It assessed the influence of fragmentation–landscape measures and perturbation degree –on the local extinction of five primates	3
Mickelberg JL. 2011. Understanding and managing isolation in a fragmented population of golden lion tamarins, *Leontopithecus rosalia*.	*Leontopithecus rosalia*	It identified the factors that affect the movement in a fragmented landscape, such as distance, fragment and population sizes and the structural connectivity.	35

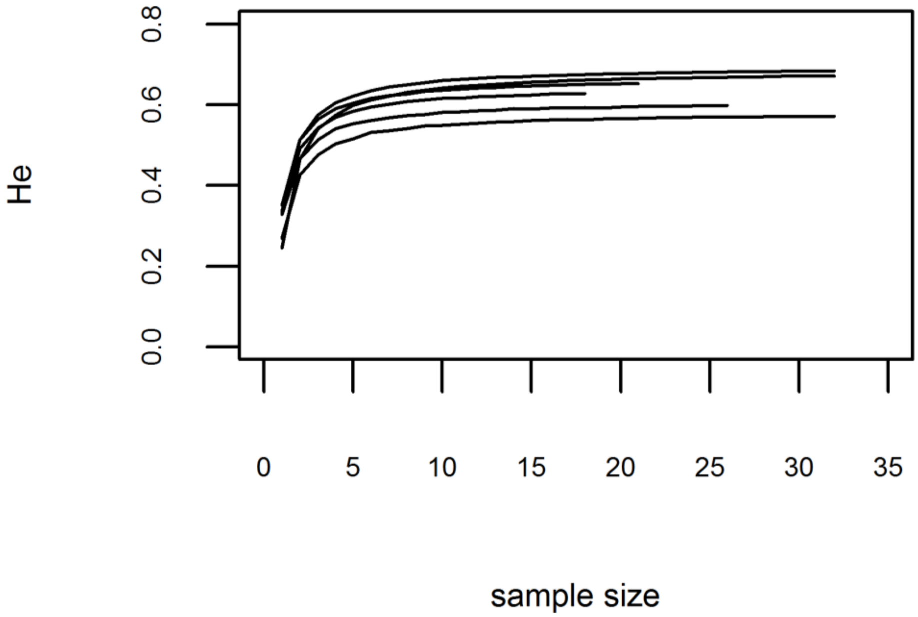
Munõz D, Estrada A, Naranjo E, Ochoa S. 2006. Foraging ecology of howler monkeys in a cacao (*Theobroma cacao*) plantation in Comalcalco, Mexico. American Journal of Primatology 68:127–142.	*Alouatta palliata*	It assessed the food ecology of a primate in a cacao agroforest	43
Oklander L, Corach D. 2013. Kinship and Dispersal Patterns in *Alouatta caraya* Inhabiting Continuous and Fragmented Habitats of Argentina. In: Marsh LK, Chapman CA, editors. Primates in Fragments: Complexity and Resilience. New York: Springer Science+Business Media. p 399–412.	*Alouatta caraya*	It assessed how habitat reduction and fragmentation affect the gene flow of howler monkey populations.	63
Oklander LI, Kowalewski MM, Corach D. 2010. Genetic Consequences of Habitat Fragmentation in Black-and-Gold Howler (*Alouatta caraya*) Populations from Northern Argentina. International Journal of Primatology 31:813–832.	*Alouatta caraya*	It compared the genetic structure of two populations to understand how habitat reduction and fragmentation affect gene flow.	14
Palminteri S, Powell G, Endo W, *et al.* 2011. Usefulness of species range polygons for predicting local primate occurrences in southeastern Peru. American Journal of Primatology 73:53–61.	*Alouatta sara*, *Ateles chamek*, *Cebus albifrons*, *C. apela, Callicebus brunneus*, *Lagothrix cana*, *Saguinus fuscicollis, S. imperator*, *Saimiri boliviensis* and *Pithecia irrorata*	It examined the accuracy of species range maps for conservation planning at the landscape scale in the Peruvian Amazon	30
Paula VR De. 2013. Comportamento de dispersão dos micos-leões-dourados (*Leontopithecus rosalia*, Linnaeus, 1766) reintroduzidos e seus descendentes nascidos em vida livre.	*Leontopithecus rosalia*	It assessed the behavioral bases of dispersal of the golden lion tamarin testing the influence of three proximal factors on the behavior of natal emigration.	34
Plante S, Colchero F, Calmé S. 2014. Foraging strategy of a Neotropical primate: How intrinsic	*Alouatta pigra*	It identified foraging decision rules of howler monkeys, according to food preferences,	57

and extrinsic factors influence destination and residence time. Journal of Animal Ecology 83:116–125.		the location of high-quality patches and trees with food, and the phenology of food resources.	
Pozo-Montuy G, Serio-Silva JC, Bonilla-Sánchez YM. 2011. Influence of the landscape matrix on the abundance of arboreal primates in fragmented landscapes. Primates 52:139–147.	*Alouatta pigra*	It assessed the effects of the matrix on the abundance of primates	44
Pozo-Montuy G, Serio-Silva JC, Chapman CA, Bonilla-Sánchez YM. 2013. Resource Use in a Landscape Matrix by an Arboreal Primate: Evidence of Supplementation in Black howlers (*Alouatta pigra*). International Journal of Primatology 34:714–731.	*Alouatta pigra*	Investigation about how arboreal primates exploit resources when residing in a smaller fragment as opposed to larger ones. Description which environments within the matrix they prefer if they supplement their diet using resources from it	6
Pozo-Montuy G, Serio-Silva JC. 2007. Movement and resource use by a group of Alouatta pigra in a forest fragment in Balancán, México. Primates 48:102–107.	*Alouatta pigra*	It observed the activity pattern of groups	60
Rabelo RM, Silva FE, Vieira T, *et al.* 2014. Extension of the geographic range of *Ateles chamek* (Primates, Atelidae): Evidence of river-barrier crossing by an amazonian primate. Primates 55:167–171.	*Ateles chamek*	It confirmed the occurrence of *Ateles* in different locations on an island	41
Raboy B. 2002. The Ecology and Behavior of Wild Golden-Headed Lion Tamarins (*Leontopithecus chrysomelas*).Thesis. Departament of Biology.	*Leontopithecus chrysomelas*	It described the pattern of dispersal and reproduction.	54
Raboy BE, Christman MC, Dietz JM. 2004. The use of degraded and shade cocoa forests by endangered Golden-headed lion tamarins	*Leontopithecus chrysomelas*	It investigated how *L. chrysomelas* uses flooded areas, cabrucas and secondary and mature forests	28

Leontopithecus chrysomelas. Oryx 38:75–83			
Silva LG da, Ribeiro MC, Hasui E, Da Costa CA, Cunha RGT da. 2015. Patch size, functional isolation, visibility and matrix permeability influences Neotropical primate occurrence within highly fragmented landscapes. PLoS ONE 10:1–20.	*Callicebus nigrifrons, Callithrix aurita* and *Sapajus nigritus*	It assessed whether the size and isolation of the fragment and the visibility and permeability of the matrix are important to explain the occurrence of three species of Neotropical primates	36
Silva SSB, Ferrari SF. 2009. Behavior patterns of southern bearded sakis (*Chiropotes satanas*) in the fragmented landscape of eastern Brazilian Amazonia. American Journal of Primatology 71:1–7.	*Chiropotes satanas*	It monitored the behavior of two groups: a large group (34) in a continuous area and a small group (7) on an island	5
Spehar S, Di Fiore A, Schmitt C, Link A. 2009. Dispersal patterns in sympatric woolly and spider monkeys: integrating molecular and observational data. Behaviour 146:437–470.	*Ateles belzebuth, Lagothrix poeppigii*	It used molecular data and opportunistic observation of dispersal events to determine the dispersal patterns of two primate species.	26
Teixeira FZ, Printes RC, Godoy Fagundes JC, *et al.* 2013. Canopy bridges as road overpasses for wildlife in urban fragmented landscapes. Biota Neotropica 13:117–123.	*Alouatta guariba clamitans*	It monitored the use of rope bridges as passages by howler monkeys	17
Valero A, Byrne RW. 2007. Spider monkey ranging patterns in Mexican subtropical forest: Do travel routes reflect planning? Animal Cognition 10:305–315.	*Ateles geoffroyi yucatanensis*	It described how spider monkeys moved between feeding sites.	11
Vié J-C, Richard-Hansen C, Fournier-Chambrillon C. 2001. Abundance, use of space, and activity patterns of white-faced sakis (*Pithecia pithecia*) in French Guiana. American Journal of	*Pithecia pithecia*	The aim of this study was to collect ecological data from translocated animals and their possibly association resident populations.	23

Primatology 55:203–221.			
Williams H, Vaughan C. 2001. White-faced monkey (*Cebus capucinus*) ecology and management in Neotropical agriculture landscape during dry season. Rev. Biol. Trop. 49:1199–1206.	*Cebus capucinus*	Habitat use was studied in an agricultural landscape during late dry season	39
Zeigler SL, Neel MC, Oliveira L, Raboy BE, Fagan WF. 2011. Conspecific and heterospecific attraction in assessments of functional connectivity. Biodiversity and Conservation 20:2779–2796.	*Leontopithecus chrysomelas*	It assessed the influence of the social regime and the conspecific and heterospecific attraction on functional connectivity. Group movements across the matrix and dispersal of individuals within forest habitat were observed.	53
Zeigler SL, Vleeschouwer KM De, Raboy BE, De Vleeschouwer KM, Raboy BE. 2013. Assessing Extinction Risk in Small Metapopulations of Golden-headed Lion Tamarins (*Leontopithecus chrysomelas*) in Bahia State, Brazil. Biotropica 45:528–535.	*Leontopithecus chrysomelas*	It assessed the population viability and the vulnerability of 10 metapopulations due to habitat loss and small population size	4

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.

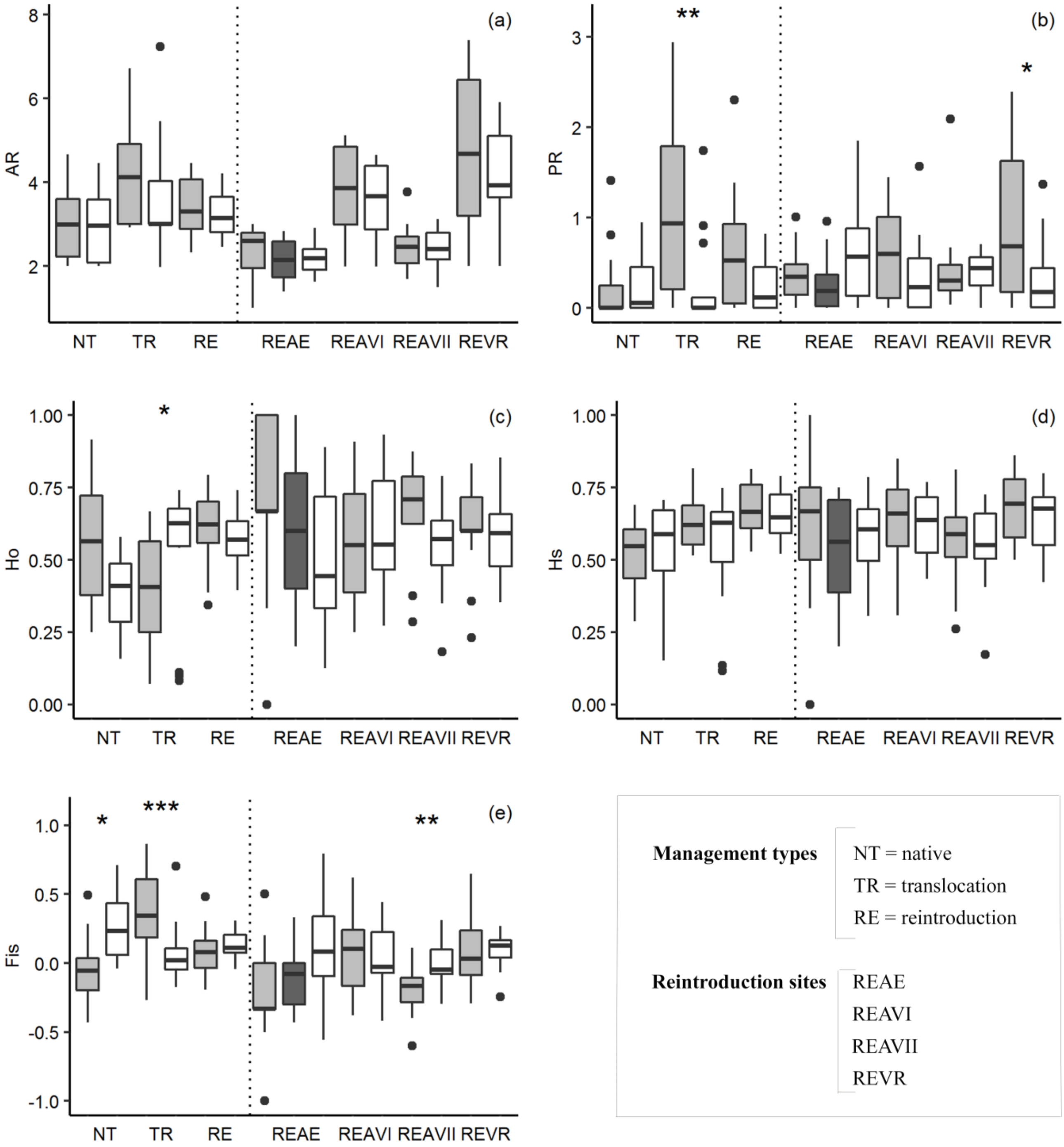
	Native		**Translocated**		**Reintroduced(RE)**						
					AE		**AVI**		**AVII**	**VR**	
	1996	**2007**	**1996**	**2009**	**2003**	**2009**	**1997**	**2009**	**2009**	**1997**	**2009**
Leon30	-0.2	0.1	**0.3**	0.1	-0.1	-0.1	-0.2	0.1	0.1	0.0	0.1
Leon21	0.1	**0.3**	**0.3**	0.0	-0.6	-0.3	**0.2**	0.1	0.0	0.1	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 < α < 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

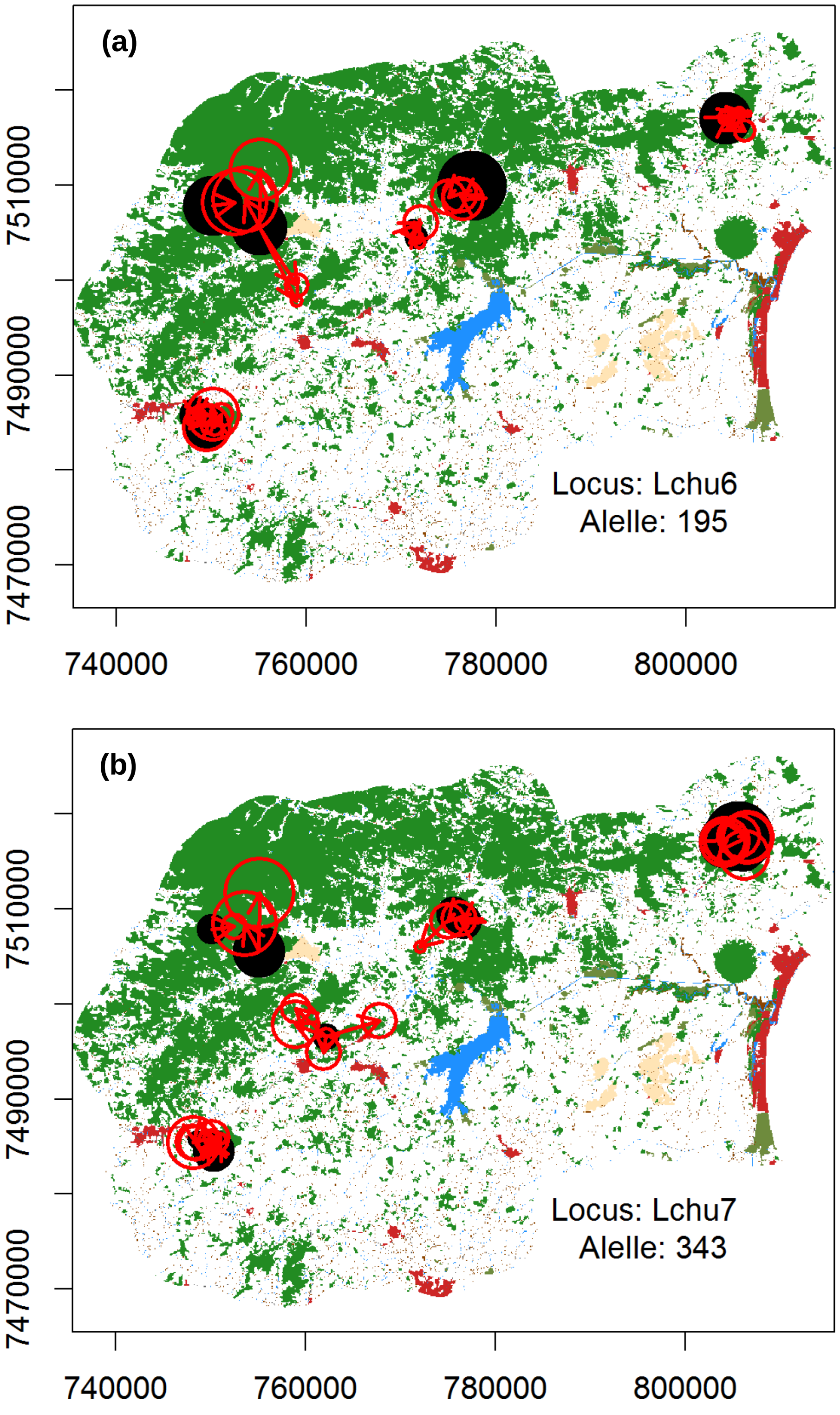
	229	0.05	0.10	-	-	-	-	-	0.05	-	-	-	-	-
Leon3	**309**	-	-	-	-	-	-	-	-	-	-	-	0.03	0.01
	311	-	-	-	-	-	-	**0.06**	-	-	-	-	-	-
	315	0.32	0.54	0.21	0.26	0.17	0.25	0.11	0.14	0.23	0.43	0.32	0.03	0.11
	317	-	-	0.15	0.24	-	-	-	0.05	0.10	-	**0.08**	0.03	0.01
	319	0.68	0.46	0.65	0.50	0.33	0.25	-	0.45	0.33	0.36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

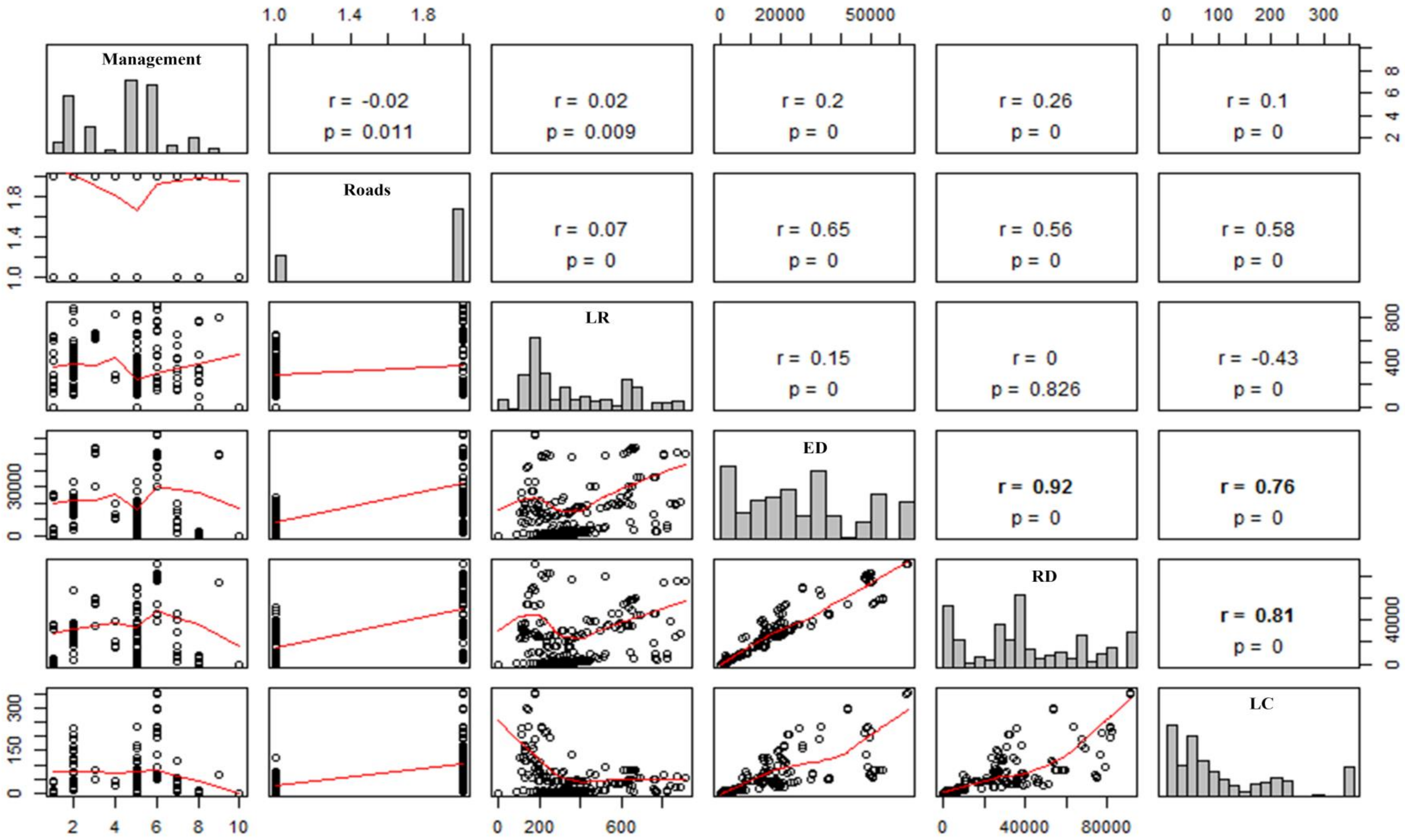
	K	**Rep**	**Mean LnP(K)**		**Stdev LnP(K)**		**ΔK**	
			SET 1	*SET 2*	*SET 1*	*SET 2*	*SET 1*	*SET 2*
Historic	1	10	-3,023,920,000	-2,305,680,000	0.813497	0.500666	—	—
	2	10	-2,758,660,000	-2,109,510,000	0.525357	0.835929	207,763,474	144,856,750
	3	10	-2,602,550,000	-2,034,430,000	16,337,772	7,801,574	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	—	—

Apêndices C



C1 – Illustration showing how was calculated the Euclidean distance between two time periods for each estimated allele using 14 microsatellite loci and 178 samples of golden lion tamarins distributed in the São João River Basin, Rio de Janeiro State, Brazil. The circles represent the presences and amplitude represents the frequencies of the since allele in 1996–1997 (black) and 2009–2013 (yellow) periods. (a) allele 195, Lchu6 locus, and (b) allele 343, Lchu7 locus.



C2 – Scatterplots of response variables. The lower panels show scatterplots with a smoother added to visualize the patterns, and the upper panels contain the Spearman’s correlation coefficients. Response variables: management, paved roads (roads), landscape resistance (LR), Euclidean distance (ED), resistance based-distance (RD), and landscape connectivity (LC)

C3 – Null allele estimate for *Leontopithecus rosalia* in each of fourteen microsatellites across each Management Unity (MU) from São Joao River Basin. Estimates > 0.1 are highlighted in boldface.

Loci/ MU	**PDA**	**AVI**	**AVII**	**União**	**Imbaú**	**RV**	**BE**	**Serra**
Leon30	-0.1	**0.2**	0.1	**0.2**	0.0	0.1	0.0	-0.1
Leon21	**0.2**	0.1	0.0	0.0	-0.2	**0.3**	-0.3	0.0
Lchu8	0.1	0.1	0.0	0.0	0.0	0.0	-0.1	0.0
Lchu3	0.1	-0.1	0.0	**0.2**	0.0	**0.3**	-0.2	0.1
P2BH6	**0.2**	0.0	0.0	0.0	0.1	0.1	-0.1	0.0
Lchu9	**0.3**	**0.3**	0.1	**0.3**	0.1	**0.3**	0.1	**0.3**
Leon27	0.1	0.0	0.1	0.2	-0.1	0.1	-0.1	-0.3
Leon31	0.1	**0.3**	0.0	0.1	0.1	0.1	0.0	0.1
Lchu7	0.0	-0.2	-0.1	0.0	0.1	0.0	0.0	-0.1
Lchu6	0.1	0.0	0.0	0.0	0.0	0.1	-0.2	0.0
P5BE6	0.0	0.1	0.1	0.1	-0.2	-0.1	**0.2**	0.0
Lchu4	0.1	**0.3**	0.0	0.0	0.1	0.1	-0.4	-0.1
Leon2	0.1	-0.1	0.0	0.0	0.1	0.1	0.0	-0.1
Leon3	0.1	0.0	-0.1	-0.1	0.0	0.1	-0.2	0.0

C4 – *P* values of Hardy Weinberg Test of *Leontopithecus rosalia* in each fourteen microsatellites across each Management Unity (MU) from São Joao River Basin. Significant p values after Bonferroni correction are highlighted in bold type. *P* interval after Bonferroni correction: 0.0036 < α < 0.0500.

Loci/ MU	**PDA**	**AVI**	**AVII**	**União**	**Imbaú**	**RV**	**BE**	**Serra**
Leon30	0.77	**0.00**	0.01	**0.00**	0.84	**0.00**	**0.00**	1.00
Leon21	0.09	0.38	**0.00**	**0.00**	0.69	0.06	**0.00**	0.18
Lchu8	0.04	0.77	0.12	0.02	0.69	0.69	0.79	0.80
Lchu3	0.39	0.63	0.73	0.01	0.09	0.07	**0.00**	0.62
P2BH6	0.01	0.27	0.63	0.63	0.33	0.10	**0.00**	0.71
Lchu9	**0.00**	0.01	0.06	**0.00**	0.03	**0.00**	**0.00**	0.01
Leon27	0.34	0.81	0.14	**0.00**	0.42	0.31	0.54	0.43
Leon31	**0.00**	**0.00**	0.00	0.13	0.01	**0.00**	0.01	0.06
Lchu7	0.84	0.24	0.22	0.01	0.23	0.03	0.21	0.62
Lchu6	0.02	0.01	0.54	0.01	0.03	0.20	0.08	0.20
P5BE6	0.37	0.21	**0.00**	**0.00**	0.17	**0.00**	0.25	0.88
Lchu4	0.17	0.02	0.35	0.94	0.30	**0.00**	0.35	0.52
Leon2	0.03	0.24	**0.00**	0.57	0.11	0.18	0.26	0.08
Leon3	0.51	0.80	0.81	0.06	**0.00**	0.58	0.01	0.80

Apêndices D

D1 – Resultados da estrutura genética do *Leontopithecus rosalia* em remanescentes de Floresta Atlântica, Rio de Janeiro, Brasil. O LnP (K) foi usado de acordo com Pritchard *et al.* (2000) e ∆K com Evanno *et al.* (2005). Em negrito, valores subótimos de K interpretados nesse estudo com objetivo de indicar populações fontes e sumidouros num sistema metapopulacional.

K	Mean LnP(K)	SD LnP(K)	Delta K
1	-7068	0,2	-
2	-6487	11,4	31,6
3	-6266	34,0	2,9
4	-6144	489,7	0,3
5	-5860	14,4	**10,9**
6	-5734	31,5	1,4
7	-5651	124,8	0,5
8	-5504	7,4	**10,8**
9	-5437	8,5	2,8
10	-5394	34,5	0,3
11	-5341	29,0	-