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Centro de Ciências Biológicas e da Saúde

Programa de Pós-Graduação em Genética Evolutiva e Biologia Molecular

PAOLA ANDREA AYALA-BURBANO

Genômica populacional do Mico-Leão-Preto (*Leontopithecus chrysopygus*, Mikan 1823): uma espécie ameaçada de extinção

São Carlos

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TESE DE DOUTORADO

Genômica populacional do Mico-Leão-Preto (*Leontopithecus chrysopygus*, Mikan 1823): uma espécie ameaçada de extinção

Paola Andrea Ayala-Burbano

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"Não acredite em ninguém que diga que, já que a natureza se baseia na luta pela vida, nós precisamos viver assim também. Muitos animais sobrevivem não eliminando uns aos outros ou mantendo tudo para si, mas cooperando e compartilhando"

Frans de Waal

"Em que termos devemos pensar nesses seres, não humanos, ainda possuindo muitas características semelhantes a humanos? Como devemos tratá-los?
Certamente devemos tratá-los com a mesma consideração e gentileza que mostramos a outros humanos;
E como reconhecemos os direitos humanos, também devemos reconhecer os direitos dos grandes macacos?
Sim"

Jane Goodall

"No se nace en vano al pie de un volcán"

(Mariano Melgar)

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Genomic analyses reveal loci associated to local adaptation in the endangered black lion tamarin (*Leontopithecus chrysopygus*, Mikan,1823)

I. RESUMO

A espécie *Leontopithecus chrysopygus*, endêmica da Mata Atlântica do estado de São Paulo, é considerada ameaçada de extinção pela IUCN e pelo IBAMA. Estima-se que atualmente a população de vida livre desta espécie não ultrapasse 1500 animais. Em cativeiro, existem pouco mais de 50 indivíduos, os quais apresentam dificuldade em se reproduzir, fato que tem contribuído para o declínio populacional e aumento nos níveis de endogamia. Ações prioritárias para a conservação desta espécie conhecida como mico-leão-perto (MLP) têm ressaltado a importância de estudos que ampliem o conhecimento sobre a diversidade genética em fragmentos florestais com distintos graus de alteração. Este conhecimento é especialmente relevante para auxiliar o monitoramento e manejo das populações de vida livre, e também dos grupos de cativeiro, os quais se constituem numa fonte importante para programas de conservação, mas necessitam de renovação de seus planteis. Com o objetivo de contribuir com o Programa de Conservação do Mico-Leão-Preto e com o Plano de Ação Nacional para a Conservação (PAN) dos Primatas da Mata Atlântica, este trabalho realizou, incialmente, análises de pedigree e microssatélites integradas para inferir parâmetros demográficos e de diversidade genética e avaliar a estrutura genética dos grupos de cativeiro da espécie. Posteriormente, foi feita uma revisão sistemática na literatura para levantar informações acerca de aspectos sobre ecologia, comportamento, genética, e conservação de populações do mico-leão-preto em diferentes paisagens. Finalmente, nós examinamos a diversidade genética neutral e não-neutral e investigamos sinais putativos de seleção diferencial em populações silvestres do mico-leão-preto utilizando polimorfismos de nucleotídeo único (SNPs) prospectados através do emprego de genotipagem por sequenciamento (GBS) de dados nex-gen. Além disso, determinamos o grau de parentesco e caracterizamos a estrutura genética das populações com base nos SNPs identificados. A metodologia utilizada e os principais resultados referentes às abordagens empregadas e suas implicações estão apresentados e discutidos nesta tese em três capítulos organizados na forma de artigos científicos.

Palavras chave: mico-leão-preto, Studbook, Ecology, Behavior, *Genotyping By Sequencing*, SNPs, genômica da conservação, diversidade genética, adaptação, seleção.

II. ABSTRACT

The species *Leontopithecus chrysopygus*, endemic to the Atlantic Forest of the state of São Paulo, is considered endangered by the IUCN and IBAMA. It is estimated that currently the free-living population of this species does not exceed 1500 animals. In captivity, there are just over 50 individuals, which have difficulty reproducing, a fact that has contributed to the population decline and increase in inbreeding levels. Priority actions for the conservation of this species known as black lion tamarin (MLP) have highlighted the importance of studies that expand the knowledge about genetic diversity in forest fragments with different degrees of alteration. This knowledge is especially relevant to assist the monitoring and management of wild populations, as well as captive groups, which constitute an important source for conservation programs, but need to renew their stocks. Overall, with the objective of contributing to the Black Lion Tamarin Conservation Program and the National Action Plan for the Conservation (PAN) of Primates of the Atlantic Forest, first we carried out integrated pedigree and microsatellites analyzes to infer demographic and genetic diversity parameters, and assess the genetic structure of captive groups of the species. Next, a systematic literature review was carried out to gather and raise relevant information about ecology, behavior, genetics, and conservation of black lion tamarin populations in different landscapes. Finally, we examined neutral and non-neutral genetic diversity and investigated putative signals of differential selection in wild populations using single nucleotide polymorphisms (SNPs) prospected by sequencing genotyping (GBS) of next generation data. In addition, we determine the degree of relatedness and characterize the genetic structure of populations based on the identified SNPs. The methodology used and the main results regarding the approaches used and their implications are presented and discussed in this thesis in three chapters organized as scientific articles.

Keywords: black lion tamarin, Studbook, ecology, behavior, Genotyping by Sequencing, SNPs, conservation genomics, genetic diversity, adaptation, selection.

III. INTRODUÇÃO DA PROBLEMÁTICA

A perda e alteração dos habitats naturais são processos considerados ameaças graves à biodiversidade global (FISCHER; LINDENMAYER, 2007). Enquanto que a perda está relacionada a redução na quantidade de habitats, a alteração se relaciona a processos como a fragmentação de áreas contínuas que ocasiona perda de conectividade da paisagem (FAHRIG, 2003b). Alterações nas paisagens, gerando fragmentos com vários tamanhos e formas, estão comumente associadas a mudanças no microclima pela maior incidência de radiação solar, precipitação (efeito de borda nos fragmentos). Estes eventos modificam o ambiente físico e biológico, alterando processos naturais das comunidades vegetais e animais (ARISTIZABAL et al., 2018; BRODIE; POST; LAURANCE, 2012; HENZI et al., 2017). Além das modificações na estrutura e funcionamento da dinâmica dos ecossistemas, a perda e alteração dos habitats leva ao isolamento de áreas remanescentes (EWERS; DIDHAM, 2006; FISCHER; LINDENMAYER, 2007). Este fenômeno pode afetar negativamente processos diversos em espécies animais dependentes de florestas, como, por exemplo, a dispersão de juvenis e a movimentação diária de indivíduos em busca, de recursos alimentares ou de sítios para descanso (COOPER; WALTERS, 2002; SAUNDERS, 1980). Tais alterações, podem reduzir ou eliminar o fluxo gênico entre populações que antes da fragmentação mantinham conexão, contribuindo com o aumento dos riscos de declínio populacional (FAHRIG, 2003ª).

Dentro deste contexto inclui-se a Mata Atlântica, uma floresta extremamente fragmentada que atualmente possui apenas 11.4% de sua área original. Cerca de 80% de seus fragmentos possui menos de 50 hectares de extensão, com exceção de seis áreas florestais contínuas, localizadas especialmente no estado de São Paulo (RIBEIRO et al., 2009). Ainda assim, este bioma abriga uma alta diversidade de espécies vegetais e animais incluindo espécies dependentes de floresta, como os primatas, para qual são relatadas 24 espécies, com 80% delas endêmicas e 62% consideradas ameaçadas (RYLANDS; MITTERMEIER; RODRÍGUEZ-LUNA, 1997; RYLANDS et al., 1996). Este alto índice de ameaça aos primatas da Mata Atlântica está relacionado ao preocupante desmatamento ocorrido neste bioma, estimado em uma taxa anual (2019-2020) de aproximadamente 13.053 ha/ano (FUNDAÇÃO SOS MATA ATLÂNTICA E INPE, 2021)

Neste cenário , as espécies de primatas que habitam a Mata Atlântica, têm sobrevivido em pequenos remanescentes de floresta por vezes isolados e extremamente degradados (RIBEIRO et al., 2009). Embora algumas espécies de primatas generalistas costumam ser mais resilientes pela sua capacidade de utilizar uma maior variedade de recursos em paisagens

alteradas, e diferentes tipos de vegetação (BICCA-MARQUES; CALEGARO-MARQUES, 1994; TUTIN; WHITE; MACKANGA-MISSANDZOU, 1996), as alterações decorrentes da perda e fragmentação do habitat podem impactar negativamente suas populações de diversas formas, tanto em aspectos demográficos (tamanho do grupo ou a densidade) e comportamentais (estratégias para obter recursos), quanto em aspectos genéticos (fluxo gênico, endogamia, deriva) (FRANKHAM; BRADSHAW; BROOK, 2014; HOFFMANN; SGRÒ; KRISTENSEN, 2017; MARSH; CHIARELLO; MARSH, 2003). No entanto, a extensão em que a modificação da paisagem resulta em isolamento do habitat costuma afetar diretamente os níveis de variabilidade genética e o seu padrão de distribuição nas populações, modificando sua estrutura e interferindo no processo de manutenção e sobrevivência de uma espécie, fatores estes intimamente relacionados com a propensão de ameaça à extinção (LANDE, 1988).

Especificamente, *Leontopithecus chrysopygus*, Mikan 1823, espécie endêmica da Mata Atlântica do estado de São Paulo, conhecida popularmente como mico-leão-preto (MLP) e foco deste estudo, se destaca exatamente por sua capacidade de persistir em habitats perturbados onde outros primatas não conseguiriam sobreviver, incluindo paisagens fragmentadas e reduzidas a pequenas manchas florestais, muitas vezes alteradas (COSTA, 1997; CULOT et al., 2015; VALLADARES-PADUA, 1993). Atualmente a literatura aponta para existência de populações de MLP distribuídas em três principais regiões do estado: Alto, Médio e Baixo Paranapanema (GARBINO; REZENDE; VALLADARES-PADUA, 2016). Esta espécie que faz parte da família Callitrichidae, já foi considerada extinta e é provavelmente o mico-leão que enfrenta o maior grau de isolamento quando comparado aos outros três micos-leões que compõem o gênero, todos endêmicos da Mata Atlântica e considerados também ameaçados, a saber: o mico-leão-dourado (*L. rosalia*); o mico-leão-de-cara-dourada (*L. chrysomelas*), e o mico-leão-de-cara-preta (*L. caissara*) (MEYER; PIE; PASSOS, 2014).

Mudanças ligadas à modificação histórica e atual da paisagem têm ameaçado efetivamente a viabilidade do MLP; o que se reflete em declínios populacionais e extinções locais na última década (GARBINO; REZENDE; VALLADARES-PADUA, 2016), e eventualmente em mudanças comportamentais e ecológicas em fragmentos com diferentes características e graus de modificação (CULOT , comunicação pessoal). A ocorrência do MLP em fragmentos relativamente pequenos e isolados, e ocasionalmente próximos a cidades, levanta questões sobre como a espécie lida com as altercações ambientais que podem ter um efeito prejudicial na sua persistência, tornando suas populações mais vulneráveis a processos estocásticos, como epidemias e catástrofes ecológicas e genéticas (MARSH, 2003). Estudos

preditivos já mostraram, que os aumentos de temperatura latitudinais, previstos para ocorrer nos próximos 30 a 60 anos, farão com que o MLP perca a maior parte do habitat considerado adequado à sua sobrevivência (MEYER; PIE; PASSOS, 2014), estimado em apenas 2% (92.239 km2) da distribuição original da espécie (REZENDE; SOBRAL-SOUZA; CULOT, 2020).

A sobrevivência de uma espécie em fragmentos que diferem em sua fisionomia e outros atributos ecológicos pode estar relacionada a processos de seleção diferencial de regiões específicas do genoma, associados a potenciais adaptativos diversificados, os quais podem promover diferenciação genética não casuística (pelo efeito de deriva) entre as populações (STORZ, 2005). A compreensão de mecanismos genômicos subjacentes à adaptação diferencial aos distintos ambientes de ocorrência do MLP é um passo crucial para uma melhor compreensão dos processos evolutivos que moldam o padrão de distribuição da diversidade e estrutura genética não-neutral de suas populações. Assim, compreender os processos que resultaram na atual diversificação das populações desta espécie pode auxiliar nas decisões de conservação que buscam manter a viabilidade populacional em longo prazo por meio da conexão entre fragmentos e translocações de indivíduos entre fragmentos. Além disso, a manutenção de populações representativas e autossustentáveis em condições *ex-situ* pode auxiliar também sua conservação pelo desenvolvimento de programas de manejo integrado, como vistas às reintroduções na natureza, caso estas sejam necessárias (Programa de Conservação Mico-Leão-Preto, 2021). Neste sentido, as análises dos registros genealógicos da espécie, as quais direcionam a formação de casais para realização de acasalamentos dirigidos e manutenção da espécie em condições *ex-situ*, devem incluir estratégias complementares que permitam inferir sobre os níveis de diversidade genética dos grupos cativos, com vistas a evitar os efeitos deletérios da redução da variabilidade genética e da depressão por endocruzamento. Complementarmente a este primeiro passo, estudos que investiguem os níveis de diversidade genética adaptativa em populações *in-situ* devem ser posteriormente implementados também nas populações *ex-situ*, visando manter representatividade da diversidade neutral e não-neutral da espécie, também em cativeiro.

Considerando a problemática aqui apresentada, este trabalho teve como principais objetivos: (i) Avaliar os dados de diversidade genética das populações *ex-situ* do MLP através de análises moleculares e de *Studbook*, visando comparar as estimativas de diversidade genética e fornecer dados adicionais para direcionar a escolha de reprodutores; (ii) Analisar dados ecológicos, genéticos e comportamentais disponíveis para o MLP na literatura, visando encontrar padrões para aspectos relevantes à sobrevivência da espécie e à diversificação de suas

populações naturais; e (iii) Caracterizar os níveis de diversidade genética, para locos neutrais e que se comportam como *outliers*, em populações de vida-livre do MLP que habitam fragmentos com características paisagísticas e atributos ecológicos diferenciados, visando inferir sobre a existência de assinaturas genômicas potencialmente associadas aos ambientes estudados.

Para alcançar as metas pretendidas, e dada a importância do programa de conservação dos grupos *ex-situ* para o manejo integrado e conservação da espécie, inicialmente análises utilizando dados de Studbook e dados de marcadores de microssatélites foram realizadas nas populações de cativeiro. Em seguida. compilamos os dados disponíveis na literatura para o MLP e os analisamos de forma integrativa com o fim de entender como o comportamento, a ecologia e a genética da espécie variam entre as regiões e fragmentos onde a mesma encontra-se atualmente distribuída. Finalmente, realizamos estudos genômico-populacionais para medir variáveis genéticas de diversidade neutra e adaptativa e identificar sinais associadas de adaptação local em distintas populações *in-situ* do MLP. Os principais achados e suas implicações estão apresentados e discutidos nesta tese, juntamente com a metodologia empregada, em três capítulos organizados na forma de artigos científicos, como descrito a seguir:

Capítulo 1*"Studbook and molecular analyses for the endangered black-lion- tamarin; an integrative approach for assessing genetic diversity and driving management in captivity".* Este capítulo foi publicado na revista *Scientific Reports* (https://doi.org/10.1038/s41598-020-63542-2) e aborda análises tradicionais baseadas no Studbook e moleculares baseadas em microssatélites com o objetivo de avaliar a estrutura e diversidade genética e inferir parâmetros demográficos nos grupos de MLP mantidos em cativeiro. Através da análise integrativa desses dados estimamos a diversidade da população fundadora inicial e propomos o uso de um índice, baseado em dados moleculares, como um parâmetro complementar para avaliar pares de acasalamento e para auxiliar na tomada de decisão de manejo para a formação dos casais reprodutores em condições *ex-situ.*

Capítulo 2 *"Ecological, behavioral, and genetic aspects of black-lion-tamarin (Callitrichidae: Leontopithecus chrysopygus, Mikan 1823): a review"*. Este capítulo está em processo de submissão para publicação na revista *Mammals Review* e analisa um conjunto expressivo de dados levantados da literatura, revelando informações relevantes sobre ecologia, comportamento e genética do MLP. Uma síntese sobre dados de demografia e recursos chave

para a sobrevivência da espécie foi realizada com o objetivo de identificar variações em aspectos ecológicos e comportamentais e apontar direções para lacunas e futuros estudos no MLP. Além disso fizemos uma análise preditiva de mudanças nos parâmetros de diversidade genética ao longo de 100 anos, com a finalidade de avaliar a viabilidade de uma população de vida livre com o maior valor de diversidade genética observado para a espécie até o momento.

Capítulo 3 "*Genomic analyses reveal loci associated to local adaptation in the endangered black-lion- tamarin (Leontopithecus chrysopygus, Mikan 1823)"*. Este capítulo está em fase de preparação de um manuscrito que tem como foco inferir sobre a diversidade e estrutura genética não-neutral da espécie, e sobre a existência de locos potencialmente associados aos ambientes estudados, usando dados genômicos produzidos através de genotipagem por sequenciamento GBS (*Genotyping By Sequencing*). Esta informação é especialmente significativa devido a capacidade do MLP para sobreviver em um ambiente em constante mudança, mas também no contexto das rápidas alterações climáticas globais. Baseados na premissa da capacidade do MLP de persistir tanto em habitats conservados quanto perturbados e nos eventos atuantes da seleção natural, considerando que estes podem moldar padrões de adaptação local uma vez que, dependendo das condições ambientais locais, populações distintas podem responder a diferentes pressões seletivas, testamos a hipótese da existência de sinais de seleção diferencial nesta espécie potencialmente associados aos ambientes em que ela ocorre. Tal conhecimento é extremamente relevante para uma espécie rara e em extinção, uma vez que permitir compreender os mecanismos desta espécie para sua adaptação a habitats diversificados e para seu uso futuro em planos de manejo *in-situ*, e também *ex-situ/in-situ* integrado.

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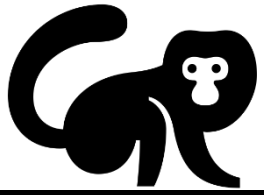

Chapter 1

Studbook and molecular analyses for the endangered black-lion-tamarin; an integrative approach for assessing genetic diversity and driving management in captivity

ABSTRACT

Breeding strategies based on molecular markers have been adopted by ex-situ conservation programs to assess alternative parameters for the genetic diversity estimates. In this work we evaluated molecular and studbook data for captive populations of black-lion-tamarin (BLT), an endangered primate endemic to Brazil's Atlantic Forest. Pedigree analyses were performed using BLT studbook information collected from 1973 to 2018. We analyzed the whole captive population since its foundation; the current captive population (CCP); and all extant BLTs in the Brazilian captive population (BCP), separately. Microsatellite analyses were implemented on the BCP individuals from the eighth generation (BCP-F8) only to avoid generation overlap. The expected heterozygosity for BCP-F8, using molecular, data was 0.45, and the initial expected heterozygosity was 0.69. Kinship parameters showed high genetic relationships in both pedigree and molecular analyses. The genealogy-based endogamy evidenced a high inbreeding coefficient, while the molecular analyses suggested a non-inbreeding signature. The Mate Suitability Index showed detrimental values for the majority of potential pairs in the CCP. Nevertheless, some individuals evidenced high individual heterozygosity and allele representation, demonstrating good potential to be used as breeders. Thus, we propose the use of molecular data as a complementary parameter to evaluate mating-pairs and to aid management decision-making.

1.1 INTRODUCTION

Captive breeding programs have been recognized as a powerful alternative for rescuing endangered species and for biological conservation (FRANKHAM, 2010; RUDNICK; LACY, 2008). Often based on pedigree analyses, ex-situ management plans aim to maintain demographically stable populations, retaining genetic diversity, limiting inbreeding, and avoiding adaptation to captivity (FOOSE; BALLOU, 1988; HEDRICK; MILLER, 1992; IVY et al., 2009; LACY, 1995; RUDNICK; LACY, 2008). However, this is not an easy task, and consequently captive groups tend to present lower levels of genetic diversity and higher inbreeding rates than expected (FRANKHAM, 2010; IVY; LACY, 2012), challenging the success of these captive breeding programs. On the other hand, wild endangered species often present small and fragmented populations subjected to bottleneck effects and absence of gene flow, and low genetic diversity levels are commonly also observed in nature (AYALA-BURBANO et al., 2017; FRANKHAM, 2008). This is the case for the black-lion-tamarin (BLT), *Leontopithecus chrysopygus* (Callitrichdae, Platyrrhini), an endangered primate inhabiting exclusively the Atlantic Forest of São Paulo state in Southeast Brazil (AYALA-BURBANO et al., 2017; KIERULFF et al., 2008) .

The population size of *L. chrysopygus* in nature is small (BALLOU; VALLADARES-PÁDUA, 1997), currently estimated at a total of a thousand individuals living in a few small forest fragments (REZENDE, 2014). This species was assumed to be extinct about 65 years, when a small population was rediscovered in the Morro do Diabo State Park (SP, Brazil) (COIMBRA-FILHO, 1970). At that time, a population census estimated that only about 200 animals existed in nature. In 1973, the first seven wild individuals of two contiguous subgroups of BLT were brought into captivity, at the Biological Bank of Tijuca in Rio de Janeiro (Rio de Janeiro, Brazil) (COIMBRA-FILHO, 1976). In 1985, because of the construction of the Rosana Hydroelectric dam, invading about 3,000 ha of the protected Morro do Diabo State Park, eight wild groups were rescued. Of these animals, one group of six individuals was brought to the Rio de Janeiro Primatology Center (CPRJ; Guapimirim, RJ, Brazil), and the seven other groups, totaling 31 BLTs, were kept in a vivarium, and transferred to a nearby forest fragment later (CARVALHO; CARVALHO, 1989; VALLADARES-PÁDUA, C.B.; RYLANDS, 1986). However, due to the poor health condition of these transferred animals, only sixteen BLTs (six males, eight females and two animals with no gender information) survived and were relocated to the Zoological Park Foundation of São Paulo State (FPZSP; São Paulo, SP, Brazil), starting a new group in captivity in 1986 (KLEIMAN; RYLANDS, 2002).

In 1987, the International Committee for the Preservation and Management of BLTs was organized in order to contribute to the management of the captive groups of this species. From this initiative, the studbook for the black-lion-tamarin, describing genealogical records for the captive animals, was created in the same year(SIMON, 1988). The first captive group of BLT overseas emerged in 1990, when six individuals were transferred from CPRJ to the Jersey Wildlife Preservation Trust (Jersey Zoo, Jersey, Channel Islands), currently known as Durrell Wildlife Conservation Trust (DWCT). The animals kept at Jersey Zoo successfully produced offspring, and some *L. chrysopygus* were transferred to other institutions in Europe, North America and Australia. However, the majority of these individuals died (KLEIMAN; RYLANDS, 2002), and nowadays there are only extant captive BLT overseas in Jersey (AYALA-BURBANO et al., 2017).

Similarly, to most ex-situ breeding programs, the management of *L. chrysopygus* in captivity has been implemented based only on pedigree analyses (AYALA-BURBANO et al., 2017), aiming to minimize population average kinship and preserve representative genetic diversity (FERNÁNDEZ; TORO, 1999; LACY et al., 1995; SONESSON; MEUWISSEN, 2001). Although this strategy has been considered appropriate to avoid inbreeding (MARSHALL et al., 1999), even if a pedigree has been properly scored for a captive group since its foundation, founder relationships are generally unknown, and for management purposes it is commonly assumed that the founders are unrelated (BALLOU, 1983). Moreover, captive breeding programs often recruit few founders, in general from a single population, representing a small proportion of the total genetic diversity of a species (RUDNICK; LACY, 2008).

To compensate for the lack of knowledge about the initial genetic diversity and relationships between the founders, various institutions that manage endangered species have recently tried to combine molecular data with pedigree analyses (FERRIE et al., 2013; GAUTSCHI et al., 2003; HENKEL et al., 2012; IVY et al., 2009; MCGREEVY; DABEK; HUSBAND, 2011; OGDEN et al., 2007), although studies integrating both types of data are still scarce (ITO et al., 2017).

In the present work, we performed studbook and microsatellite analyses to assess population genetic structure and infer demographic and genetic diversity parameters in the captive groups of *L. chrysopygus*. We analyzed molecular and pedigree data and estimated genetic diversity for F0. The most common pedigree-based index used to choose mates in breeding programs was compared with the individual heterozygosity obtained by microsatellite

markers. Our findings suggest that although genealogical analysis has been beneficial, an integrated approach including molecular data might be useful for a better understanding of genetic diversity and the structure of the BLT population in captivity, and for proper metapopulation management.

1.2 METHODS

1.2.1 Ethical requirements and research permits

The present study was approved by the Ethics Committee on Animal Experimentation (Federal University of São Carlos, São Carlos, São Paulo, Brazil), under CEUA-UFSCAR number 9805200815; the Authorization System and Biodiversity Information of the Chico Mendes Institute for Biodiversity Conservation (Ministry of Environment, Federal Government, Brazil), under SISBIO-ICMBio numbers 50616-1; and the National System of Genetic Patrimony Management and Associated Traditional Knowledge (Ministry of Environment, Federal Government, Brazil), under SISGEN number A411359. The approved experimental protocols included the capture of live animals in captivity, and the anesthesia using direct inhalation equipment and blood collection procedures. The animals were handled by a veterinarian who released them safely after blood collection. These procedures followed all ethical and legal recommendations proposed by the institutional and licensing committee and the American Society of Primatologists for the Ethical Treatment of Non-Human Primates (https://www.asp.org/society/resolutions/EthicalTreatmentOfNonHumanPrimates.cfm).

1.2.2 Studbook data and pedigree analyses

We analyzed all records of *L. chrysopygus* registered in the International Studbook for the black-lion-tamarin (unpublished current version). We considered all BLTs kept in captivity from 1973 to 2018, including the founders, ancestors and their offspring, and we carried out analyses separately for three set of individuals: the whole captive population (WCP), including all living or dead captive BLTs; the current captive population (CCP), comprising all extant captive individuals maintained in Brazil and overseas until 2018; and the Brazilian captive population (BCP), including only living captive BLT adults in Brazil (AYALA-BURBANO et al., 2017).

For demographic inferences we implemented three different analyses for evaluating the consequences of the applied random mating system and its evolution over time by using the

Endog 4.8 software (GUTIERREZ; GOYACHE, 2004). First, we calculated the pedigree depth by considering the proportion of known ancestors per generation for each offspring, and then we added the interval of generations, defined as the mean age of parents when their progeny is selected to be parent, considering the relationships between mother-daughter, mother-son, father-daughter and father-son (JAMES, 1977). Finally, we estimated the equivalent complete generations based on the proportions of individuals with both known parents. This parameter is also known as the mean equivalent generation (*ge*) and it is calculated as the sum of all known ancestors $\left(\frac{1^n}{2}\right)$, where *n* is the number of the i^{th} generation separating an individual from each known ancestor (e.g. parents=1, grandparents=2, great-grandparents=3, ...) (MAIGNEL; BOICHARD; VERRIER, 1996). The complete pedigree was constructed using Pedigree Viewer version 6.5.2.0 (KINGHORN, 1994).

Fertility (*Mx*) was calculated considering the individual fertility or reproductive potential information for each age class. Mortality (*Qx*) was estimated as the proportion of individuals entering an age class versus animals that died before reaching the age class *x* + 1. Survival (*Lx*) was determined as the proportion of individuals surviving from birth to the beginning of the age group *x*. The proportional change in population size from one year to the next, based on life table calculations (expected lambda-λ), and the instantaneous rate of change of the population, averaged for males and females I, were also estimated. A lambda value greater than one indicates an increase in the population. A value of *r* greater than one also means that the population is increasing. All these estimators were calculated using PMx software(LACY; BALLOU; POLLAK, 2012).

For the pedigree-based genetic inferences, we determined genetic diversity by calculating the total effective number of founders (*fe*) (LACY, 1989) and total effective number of ancestors (*fa*)(BOICHARD, 1997), using the Endog 4.8 software(GUTIERREZ; GOYACHE, 2004), and founder genome equivalents (*fge*) (LACY, 1989) using PMx (LACY; BALLOU; POLLAK, 2012). The degree of remaining genetic diversity (i.e., expected heterozygosity originated by limited numbers of founders and its balanced contribution) was calculated based on the following expression: $\frac{He}{H0} = 1 - \left(\frac{1}{2fge}\right)$, in which *H0* = 1. The inbreeding coefficient (*F*) was estimated to illustrate the trend in mean inbreeding across years. Likewise, mean kinship (*Mk*) was also calculated as complementary information to that provided by the inbreeding coefficient (*F*). F_{ST} and mean coancestry (*fij*) were calculated following Caballero and Toro(CABALLERO; TORO, 2000, 2002), considering the genetic

divergence between each pair of zoos which hold the species based on the pedigree data. These latter parameters were calculated using PMx software (LACY; BALLOU; POLLAK, 2012).

The effective population size (*Ne*) was estimated based on two approaches (*NeI* and *Nec*) implemented in Endog version 4.8 (GUTIERREZ; GOYACHE, 2004). First, *Ne* was calculated to estimate the founder population size and to detect the existence of bottlenecks and possible consequences of the mating strategy, via the individual increase in inbreeding (*NeI*), as proposed by De la Rosa *et al.*(DE LA ROSA; CERVANTES; GUTIÉRREZ, 2016). To calculate *NeI*, the coefficient of individual increases in inbreeding (ΔFi), determined according to Falconer and Mackay (FALCONER; MACKAY, 1996) and modified by Gonzales-Recio *et al.*(GONZÁLEZ-RECIO; LÓPEZ DE MATURANA; GUTIÉRREZ, 2007) and Gutiérrez *et al.*(GUTIÉRREZ; CERVANTES; GOYACHE, 2009), was used. The modified method proposed by Gutiérrez *et al.*(GUTIÉRREZ; CERVANTES; GOYACHE, 2009) is considered the most appropriate to analyze permanently subdivided populations. *Ne* was also calculated using the increase in coancestry (*Nec*) proposed by Cervantes *et al.*(CERVANTES et al., 2011), which is suitable when mixing of populations becomes a usual practice. We also calculated the ratio of the effective population size (*NeI*) to the census size of living captive-born individuals (*NeI*/*N*). Mate Suitability Index (*MSI*) was determined for all potential pairs in the current captive population of BLTs using PMx (LACY; BALLOU; POLLAK, 2012).

1.2.3 Biological samples and molecular analyses

Biological samples of all BLTs from the Brazilian captive population were obtained by collecting about 0.5 mL of fresh blood from each individual, using *vacutainers* containing EDTA (3.6 mg). The animals were anesthetized by direct induction using inhalation equipment calibrated with isoflurane (2-5%) and oxygen (2 L/min), and were then released back into their respective enclosures. Blood samples were stored at -20°C for subsequent DNA extraction. Genomic DNA was obtained following the phenol protocol (SAMBROOK; FRITSCH; MANIATIS, 1989). The DNA integrity was confirmed using 1% agarose gels under constant voltage (100V for 45 min) (Supplementary Fig. S7A) and the quantification was performed using GE NanoVue Plus, GE Healdthcare Spectrophotometer.

Polymerase chain reactions (PCRs) for the microsatellite amplifications followed procedures proposed by Ayala-Burbano *et al.*(AYALA-BURBANO et al., 2017). We firstly tested a panel of 22 loci previously described for *Leontopithecus* species (GALBUSERA; GILLEMOT, 2008; GRATIVOL; BALLOU; FLEISCHER, 2001; PEREZ-SWEENEY et al.,

2005) and posteriorly selected 15 polymorphic loci. PCR-amplified products were visualized in 2% agarose gel (Supplementary Fig. S7B). Genotyping was performed in an ABI3730XL automatic sequencer (Applied Biosystems, Foster City, CA, USA), using GS 500 Liz size standard, and the alleles were scored in the software Geneious version 6.0.6 (https://www.geneious.com). Each sample genotyped as homozygous was confirmed by a minimum of three replications. We also performed multiple PCRs for random samples, in order to identify genetic inconsistencies, according to recommendations proposed by the ISFG (*International Society for Forensic Genetics*) for the area of non-human DNA typing (LINACRE et al., 2011). More details related to the technical procedures employed for DNA amplification and genotyping of the STR (Short Tandem Repeats) loci are available in Supplementary Information.

Before the statistical analyses, we estimated the occurrence of null alleles, allelic dropout and stuttering for all scored alleles using Micro-Checker (VAN OOSTERHOUT et al., 2004). Subsequently, lack of linkage disequilibrium (LD) between loci was verified in Genepop version 4.0.10 (ROUSSET, 2008). We used the linkage disequilibrium method to assess the effective population size. Genetic diversity parameters were inferred by calculating the number of alleles (Na), effective number of alleles (Nae), expected (He) and observed (Ho) heterozygosity using GenAlEx version 6.4 (PEAKALL; SMOUSE, 2006).

The proportion of remaining genetic diversity represented in the eighth generation of the BCP was calculated as $\frac{\mathrm{He}}{\mathrm{H0}} = (1 - \frac{1}{2\mathrm{Ne}})^{\mathrm{t}}$, in which H0 is the initial heterozygosity in the F0 generation, He is the expected heterozygosity calculated by molecular data, t is the number of generations, and Ne is the number of individuals that produced offspring in a specific generation. Ne was calculated by harmonic mean (FRANKHAM; BALLOU; BRISCOE, 2010), where $\mathrm{Ne} = \frac{1}{\frac{1}{\mathrm{Ne1}}+\frac{1}{\mathrm{Ne2}}+\frac{1}{\mathrm{Ne3}}\cdots\frac{\mathrm{N}}{\mathrm{Net-1}}}$. From the ratio between molecular heterozygosity expected for F8 (He) and for F0 (H0), we calculated the remaining genetic diversity (rGD), considering the effective population size, and then we estimated the genetic diversity for F0.

Allelic richness (Ra) and inbreeding coefficient (f) were calculated using Fstat version 2.9.3.2 (GOUDET, 2001). The mean relatedness (r_m) between individuals was estimated using Coancestry (WANG, 2011). This software calculates seven different relatedness estimators, and after testing all of them, we choose the estimator based on Triade likelihood (TrioML), which showed the smallest variance among all the estimators tested (WANG, 2011).

Individual heterozygosity, based on the internal relatedness index (IR) (AMOS; BALMFORD, 2001) was calculated for the individuals recently transferred from Brazil to England, using GENHET (COULON, 2010), in order to add a relevant molecular genetic diversity parameter to the *MSI* obtained from pedigree data.

1.3 RESULTS

1.3.1 Genealogical and demographic inferences based on pedigree data analyses

The whole captive population of BLTs consists of 517 animals (Supplementary Fig. S1), of which 466 have already died, including 35 wild founders and three individuals with unknown parents (Table 1.1). The Brazilian captive population of BLTs includes 37 adults recorded in the 2014 studbook (WORMELL, 2014). Of these, 17 individuals were maintained at the Primatology Center of Rio de Janeiro, 16 at the Zoological Park Foundation of São Paulo, and four at the São Carlos Ecological Park (PESC; São Carlos, SP). However, some of these animals were relocated among the zoos during the years 2015-2018, including five BLTs that were recently transferred from both CPRJ (two animals) and FPZSP (three animals) to Jersey Zoo. In addition, other BLTs were born and one wild individual from Pratânia municipality was brought to captivity. Currently, there are 55 living animals in the captive population. Eight of them are in Jersey Zoo, one in Magdeburg Zoo (German) and 46 are in Brazil (15 at CPRJ, 26 at the FPZSP, three at PESC, and two at Belo Horizonte Zoological, (BH Zoo; in Minas Gerais state) (Supplementary Table S1.1).

The pedigree graphical representation for the WCP revealed nine generations of BLTs in captivity up to 2018, with several non-breeding individuals and some others showing higher reproductive rates (Supplementary Fig. S1.1). Related to the pedigree depth, until the fifth generation back, the completeness level for the WCP was 92% for the parent generation, 71% for the grandparent generation and 45% for the great-grandparent generation. The Brazilian captive population showed an overlap of generations including animals from the 5th, 6^{th}, 7^{th}, and 8^{th} generations. Thus, for the integrative approach, we calculated genetic diversity estimators for the individuals comprising the eighth generation (BCP-F8), which included the descendants from the prior generations without the parents (Fig. 1.1).

The longest generation interval values were found for father-daughter for the WCP (6.32 years) and father-son for the CCP (11.04 years) populations. The average generation interval for WCP and CCP was 5.44 and 7.57 years, respectively. The generation intervals calculated

across all pathways are presented in Table 1.2. More details of demographic and genealogical results, including age structure, fertility (*Mx*), mortality (*Qx*), survival *(Lx*), expected lambda-λ, instantaneous rate of change of the population (*r*), and reproductive peaks in captivity are shown in Supplementary Information (Fig. S1.2-S1.6)

Table 1.1 - Founders' data registered from 1973 to 2018 in the International Studbook for the black-lion-tamarin (BLT), showing local of capture, transfer location, year of capture, number of individuals captured, and the current status of the fragments where BLTs still occurs.

Founders introduced from nature				
Local of capture	**Transfer location**	**Year of capture**	**Number of individuals**	**Fragments with BLTs**
Morro do Diabo State Park	CPRJ	1973	7	yes
Morro do Diabo State Park	CPRJ	1985	4	yes
Morro do Diabo State Park	FPZSP	1986	14	yes
Morro do Diabo State Park	FPZSP	1987	1	yes
Ribeirão Bonito Farm	FPZSP	1991	3	no
Wild (Missing location)	PEMQB*	1998	1	-
Wild (Missing location)	CPRJ	1999	1	-
Buri	Sorocaba	2003	1	no
Buri	FPZSP	2007	1	yes
Morro do Diabo State Park	FPZSP	2014	1	yes
Pratania	FPZSP	2017	1	yes

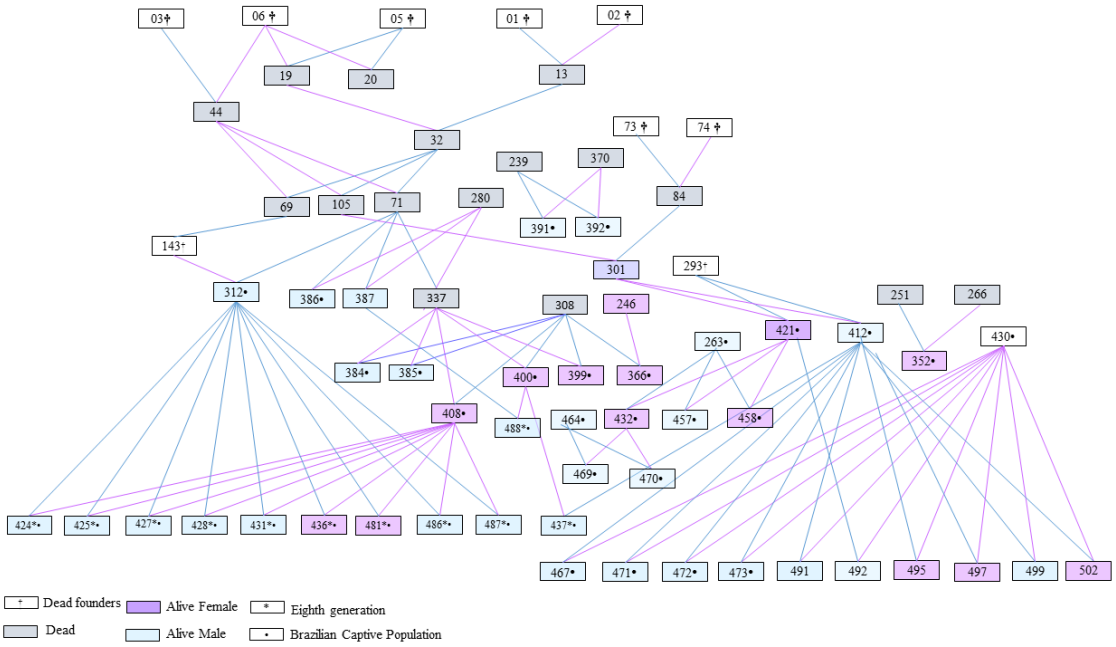

Figure 1.1 - Kinship tree depicting the 37 black-lion-tamarins in the Brazilian Captive Population (BCP) and the 11 individuals in the eighth generation (BCP-F8). All BCP individuals descend from 10 wild animals, of which only one (430) is still living. Note the current severe skew in breeding contribution. White squares: founders; grey squares: dead individuals; blue squares: alive males; and purple squares: alive females.

Table 1.2 - Mean of generation interval (in years), considering the four paths (father-son; father-daughter, mother-son and mother-daughter) in the whole (WCP) and the current captive (CCP) populations of black-lion-tamarin. Number of individuals (N), Standard deviation (SE).

	WCP			CCP		
Parents-offspring	**N**	**Years**	**SE**	**N**	**Years**	**SE**
Father-Son	53	5.76	3.35	7	11.04	4.24
Father-Daughter	58	6.32	2.68	5	8.81	3.36
Mother-Son	54	4.33	2.25	7	5.24	1.76
Mother-Daughter	58	5.29	2.29	5	4.71	1.87
Total	223	5.44	2.75	24	7.57	3.94

1.3.2 Measures of the probabilities of gene origin based on pedigree data

The effective number of ancestors (*fa*) calculated following Boichard *et al.* (BOICHARD, 1997) was 10 for WCP and eight for both CCP and BCP. The effective number of founders (*fe*) was equal to 10 for WCP, CCP and BCP (Table 1.3). These results show a markedly lower number of non-captive potentially contributing individuals than the total number of wild animals registered in the current version of the BLT Studbook (Table 1.1). For CCP, the proportion of remaining genetic diversity (*rGD*) based on the founder genome

equivalent (*fge*) was 87.2%. When we considered only BCP, *fge* was equal to 83.9% (Table 1.3).

Table 1.3 - Demographic and gene origin statistics for the whole (WCP), current (CCP) and Brazilian captive (BCP) populations of black-lion-tamarin.

	WCP	**CCP**	**BCP**
Number of individuals	517	55	37
Number of founders	35	13	13
Effective number of founders (*fe*)	10	10	10
Effective number of ancestors (*fa*)	10	8	8
Founder genome equivalent (*fge*)	27	3.93	3.12
Remaining genetic diversity (*rGD*) (%)	-	87.2	83.9

1.3.3 Inbreeding, mean kinship and effective population size based on pedigree data analyses

The inbreeding coefficient (*F*) was higher in BCP and CCP than in WCP (Table 1.4). The inbreeding values ranged from 0.0119 in 1984 to 0.1070 in 2018 (Supplementary Table S1.2), showing a curve fluctuating according to the number of inbred individuals in each year (Fig. 1.2).

Table 1.4 – Inbreeding statistics (*F*) for the whole (WCP), current (CCP) and Brazilian (BCP) captive populations of black-lion-tamarin. Unk (Unknown).

	WCP				**CCP**				**BCP**		
	Total	Male	Female	Unk Sex	Total	Male	Female	Unk Sex	Total	Male	Female
Nº of records	517	242	188	87	55	32	15	8	37	24	13
F	0.052	0.111	0.134	0.338	0.101	0.169	0.234	0.226	0.108	0.092	0.112
Minimum F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Maximum F	0.338	0.338	0.226	0.226	0.395	0.395	0.216	0.395	0.225	0.225	0.215

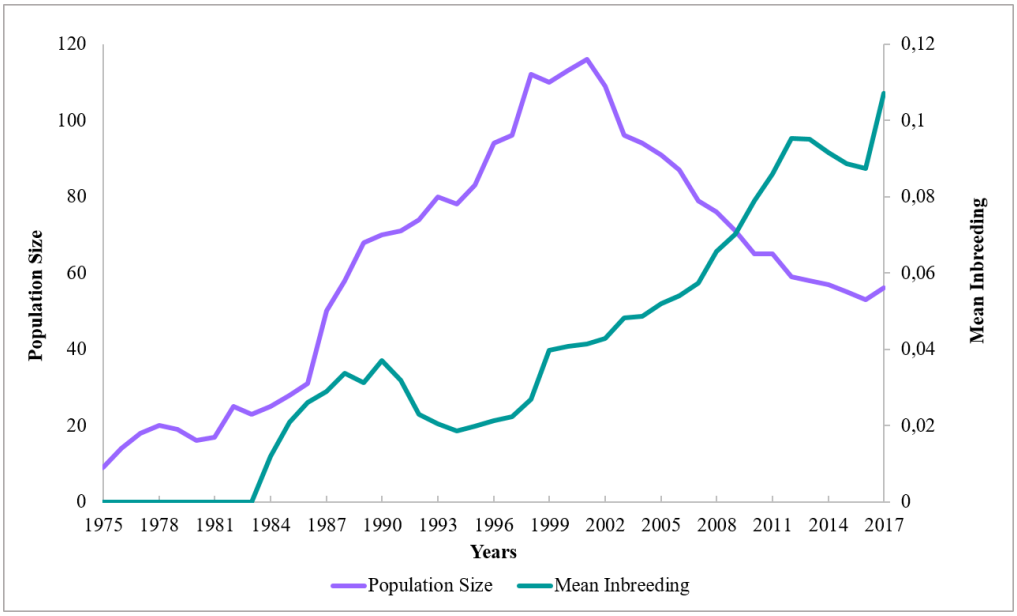

Figure 1.2 - Variation in mean inbreeding and population size in the whole captive population of black-lion-tamarin (BLT/WCP).

Mean kinship statistics showed slightly superior values in the CCP and BCP (Table 1.5). The realized effective population size (*NeI*) and the value of *Nec*, which assumes random mating occurring in the near future, are shown in table 1.5. The ratio *Nec*/*Ne* for CCP was equal to 0.30.

The genetic structure, based on Wright's *F*-statistics (F_{ST} between groups) and mean coancestry (*fij*) within and between zoos, is shown in table 1.6. The *F*-statistics evidenced that Jersey Zoo and CPRJ are the most genetically distant captive groups, whereas mean coancestry and F_{ST} values showed that PESC and CPRJ are the most related ones.

Table 1.5 - Effective population size and mean kinship for the whole (WCP), current (CCP) and Brazilian (BCP) captive populations of black-lion-tamarin.

	WCP	**CCP**	**BCP**
Effective population size (*NeI*)	-	16.85	15.35
Effective population size (*Nec*)	-	11.58	12.59
Mean equivalent generation (*ge*)	2.64	3.66	2.61
Mean kinship (*Mk*, %)	13.34	12.74	16.04

Table 1.6 - Genetic structure based on Wright's *F-statistics*. F_{ST} values below the diagonal, and mean kinship between zoological parks (*Mk*) above the diagonal. Mean coancestry (*fij*), within (diagonal) and between subpopulations (off diagonals), for the current population (CCP) of black-lion-tamarins.

	F_{ST} – Mk				**Mean Coancestry (*fij*)**				
Zoos	Jersey-Mag	CPRJ	FPZSP	PESC	Zoos	Jersey	CPRJ	FPZSP	PESC
Jersey-Mag		0.136	0.095	0.073	Jersey-Mag	16	12	9	8
CPRJ	0.106		0.070	0.139	CPRJ	12	31	8	13
FPZSP-BH	0.022	0.077		0.055	FPZSP-BH	9	8	12	6
PESC	0.044	0.008	0.021		PESC	7	13	6	23

1.3.4 Genetic diversity inferences based on molecular and pedigree data integrative analyses

For the integrative approach, we considered the eleven individuals from BCP in the eighth generation (BCP-F8), which were born between 2005 and 2012. Currently, 10 of these are alive and consequently are included in the CCP as well (Fig. 1.1). The set of 15 microsatellite loci was successfully amplified in these samples, with no indication of null alleles, stuttering, allelic dropout and significant LD ($p>0.05$). Despite literature report some issues for dinucleotide loci (ANDREASSEN et al., 2012; IYENGAR, 2014; LINACRE et al., 2011), no genetic inconsistencies were found for these loci, after following the technical procedures employed for DNA amplification and genotyping (see Supplementary Information). The obtained electropherograms (EPGs) evidenced specific allele patterns with proper quality (Supplementary Fig. S1.8). After sequencing and alignment of the amplicons, the expected motifs were searched, and the microsatellite sequences were confirmed for all heterologous loci.

In total, we computed 31 alleles, ranging from two to three per locus, with an average of 2.06 alleles per locus, and average allelic richness equal to 2.07. The equivalent genetic diversity estimators, based on both pedigree and molecular data, are shown in table 1.7. The effective population size (NeI) showed a higher value when calculated through pedigree analyses. The ratio of Ne/N was equal to 0.18 and 0.12 for genealogical and molecular data, respectively. The founder genome equivalent was 1.42, and the mean effective number of alleles was 1.84. The degree of kinship (*Mk*) based on pedigree data showed a high value concordant with that observed using the pedigree inbreeding index (F = 0.19). Kinship based on molecular data (r_m) confirmed a high degree of relatedness. The molecular inbreeding

coefficient was negative (f = -0.58), as a consequence of an excess of heterozygosis, the observed mean (Ho = 0.73) being higher than the expected heterozygosity (He = 0.45).

Table 1.7 - Pedigree and molecular genetic diversity indices for the black-lion-tamarins of the Brazilian captive population in the eight generation (BCP-F8).

Pedigree data	***N***	***Ne***	***Ne/N***	***Mk***	***F***	***fge***	***rGD***
BCP-F8	11	2.0	0.18	0.35	0.20	1.42	65%
Molecular data	**N**	**Ne**	**Ne/N**	**rm**	**f**	**Nae**	**rGD**
BCP-F8	11	1.4	0,12	0.38	-0.58	1.84	65%

The remaining genetic diversity measured by pedigree analyses was 65%. Remaining genetic diversity and heterozygosity for F0, calculated based on the integrative approach, were 65% and 0.69, respectively. The values of *MSI* estimated by PMx for all extant BLTs from CCP, including individuals from Jersey, resulted in a total of 480 simulated potential couples, in which 80% were considered at least as slightly detrimental (4, 5, 6, ~) (Fig. 1.3). In contrast, the individual heterozygosity, based on the IR Index, for the three individuals from FPZP (studbook numbers 471, 472, 497) and the two individuals from CPRJ (studbook number 436, 487), which were recently transferred from Brazil to Jersey, ranged from -0.654 to -0.088 (Table 1.8), indicating high heterozygosity.

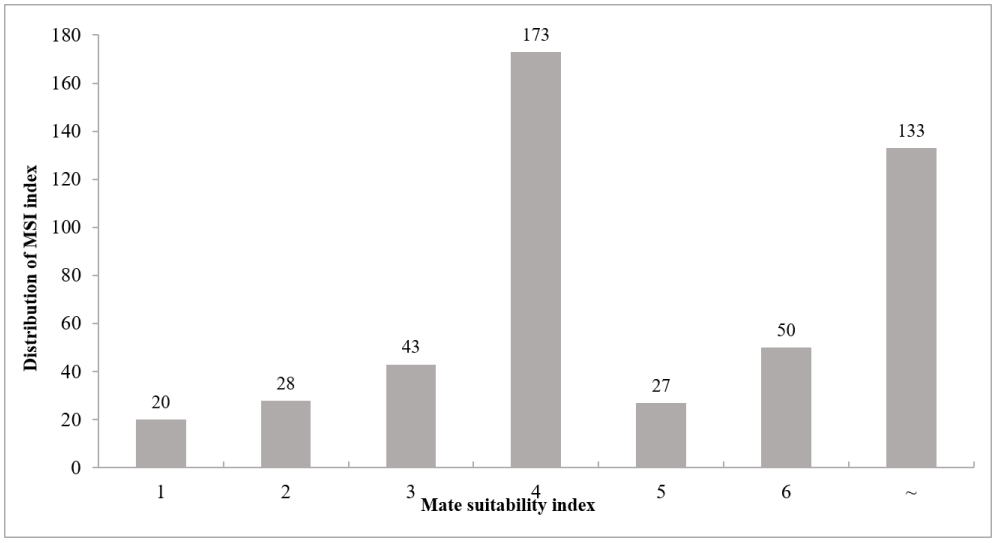

Figure 1.3 - Distribution of the mate suitability indices (MSI) for the 55 black-lion-tamarin pairings (480 simulations in total). Scores (1 to ~) indicating 20 mating pairs very beneficial (1); 28 moderately beneficial (2); 43 slightly beneficial (3); 173 slightly detrimental (4); 27 detrimental (5); 50 very detrimental (6); and 133 very highly detrimental (~). Values of IR ranging from -1 to +1. When the IR values are higher, the individual heterozygosity estimates are lower.

Table 1.8 – Information for the black-lion-tamarins transferred from the Zoological Park Foundation of São Paulo (FPZSP) and Primatology Center of Rio de Janeiro (CPRJ) in Brazil to Jersey Zoo.

Studbook number	Sex	Age (years)	Origen	Generation	Internal relatedness (IR)
436	Female	8	CPRJ	8	-0.410
487	Female	5	CPRJ	8	-0,589
497	Female	4	FPZSP	6	-0.590
471	Male	5	FPZSP	6	-0,088
472	Male	5	FPZSP	6	-0.654

1.4 DISCUSSION

Setting up an efficient captive breeding program requires a precise knowledge of the genetic diversity and genealogical data of the populations to be managed (GOYACHE et al., 2003). In this sense, pedigree analyses can provide relevant information for the management of species in captivity (FERRIE et al., 2013). However, the effectiveness of pedigree-based approaches depends on its completeness and depth, since deeper pedigrees usually generate more accurate and robust inferences (GOYACHE et al., 2003). According to the present study, 92% of ancestral relationships in the whole captive population of BLTs are well known. Taking into account such high pedigree depth value, the demographic and genetic inferences raised herein, based on the BLT studbook data, should be considered as reliable.

Overall, the captive BLT population is well established; nevertheless, its age structure is typical for a slowly growing population ($\lambda>1$), showing an explicit decline from the year 2001, in both sexes. The whole captive group reached a maximum population size of 114 (59 males and 55 females) in 2000, but in the subsequent years it suffered a drastic and continuous decline, though new births have contributed to the growth of the current captive population in Brazil. We also observed an increase in the average generation interval over the years. These results are quite probably due to management efforts aimed at minimizing inbreeding by the reuse of less related animals as reproducers (NAGY et al., 2010), that in this case are the oldest BLTs. In addition, we verified low rates of reproduction of founder and non-founder wild animals, and also a high number of unrelated captive animals that never reproduced at all. Thus, despite the huge efforts to avoid matings between closer relatives, or in recent years to decrease accumulation of inbreeding as much as possible, the BLT captive population has been showing an increase in inbreeding over time and high kinship values.

High rates of inbreeding and kinship can promote genetic diversity loss and inbreeding depression in future generations, compromising fitness-related features such as viability, birth weight and fecundity (TEMPLETON; READ, 1984). Unfortunately, some possible evidence of inbreeding depression, such as bone deformities, low copulation rates, low sperm motility, high rates of infertility and cleft lip that cause infant deaths during tooth changes, have already been observed in the Brazilian captive population of BLTs (PISSINATTI, A., personal communication). Additionally, the European population has experienced a high incidence of gallbladder problems (WORMELL, personal communication). In spite of this, the short-term goal of the BLT breeding program in captivity has been achieved by the high survival rates in the infant and juvenile age groups. However, the long-term goal for BLT management consists of maintaining genetic diversity levels and avoiding inbreeding depression (ESCARLATE-TAVARES; MAFRA; JERUSALINSKY, 2016).

Captive populations often have a very small number of founders, which are considered unrelated and consequently have inbreeding rates assumed as zero (BALLOU, 1983; RUDNICK; LACY, 2008). In our study, we know the origin of the founders, which came from two neighboring groups of the same population that lived in the Morro do Diabo State Park and might be genetically related. Consequently, the kinship and inbreeding values calculated by pedigree analyses were high, compromising the viability (ESCARLATE-TAVARES; MAFRA; JERUSALINSKY, 2016) of the captive population of BLTs in the long-term.

Fortunately, according to measures of probabilities of gene origin, our data showed a greater value of effective size when compared to the effective number of founders, ancestors and founder genome equivalent values. The relationship between the effective size and the population size (NeI/N) shows that the pedigree-based recommendations are being directed to the equalization of the families, and the sex ratio and the number of individuals throughout the generations, aspects considered very advantageous (FRANKHAM; BRISCOE; BALLOU, 2002). Moreover, molecular data have shown a higher observed heterozygosity than expected, which leads us to infer those zoos are managing the population appropriately. Nevertheless, the PMx analyses showed that the *MSI* values are at least detrimental (LACY; BALLOU; POLLAK, 2012) for the majority of potential couples of the CCP. From the total of simulated mate pairs, 174 and 30 showed values of *MSI* that were slightly detrimental and detrimental, respectively. The remaining ones were considered very detrimental and very highly detrimental. On the other hand, only 20% pairs had *MSI* values considered as beneficial for breeding

programs, whereas the BLTs recently transferred from Brazil to Jersey showed *MSI* values moderately or slightly beneficial when we simulated pairing with BLTs from Jersey Zoo.

The *MSI* is the most common parameter used to select mates in captive breeding programs, and considers differences in genetic diversity, kinship, inbreeding coefficient and unknown ancestry, all calculated only by pedigree data (BALLOU; EARNHARDT; THOMPSON, 2001; LACY; BALLOU; POLLAK, 2012; RALLS; BALLOU, 2004). Considering the *MSI* values found here, we estimated the Internal Relatedness index as a complementary parameter to the *MSI*, in order to gain some insights based on molecular data as well.

The IR index is a method for estimating individual heterozygosity and considers that rare alleles count more than common alleles. Negative IR values indicate higher heterozygosity, whilst positive values are attributed to more homozygous individuals (AMOS et al., 2001). In our study, despite the fact that the transferred BLTs have shown moderately or slightly beneficial *MSI* values, they all showed negative IR values, indicating that these individuals have high heterozygosity, besides allele representativeness, and consequently are valuable as breeders (APARICIO; ORTEGO; CORDERO, 2006). In fact, these animals have already mated and successfully produced offspring in Jersey (WORMELL, personal communication). Alternatively, if only very homozygous individuals are available for forming mate-pairs, genetic differentiation among the potential breeders and their allele representativeness (RALLS et al., 2018) must be considered in addition to *MSI* scores.

Management decisions must take into account the possibility of changes in genetic diversity by mating between genetically more divergent individuals (RALLS et al., 2018; RUDNICK; LACY, 2008). Previous molecular analysis, using the same set of microsatellites used here, showed private alleles in each captive group from Brazil and Europe, evidencing genetic structuring among them (AYALA-BURBANO et al., 2017). In addition, the pedigree analyses performed here pointed to greater genetic differentiation between the Jersey and CPRJ captive groups. It is noteworthy that the animals that successfully mated in Jersey are from CPRJ (487) and FPZSP (472), these latter being descended from the CPRJ group. In this case, the genetic diversity increment was beneficial to the metapopulation management of BLTs (VALLADARES-PÁDUA et al., 2001).

Notwithstanding this, changes in the genetic diversity of source and recipient populations, by movement of individuals, are not always beneficial to both populations. Such groups need to be carefully managed to maintain the maximum of allele richness, to avoid

inbreeding, but also potential outbreeding depression. Therefore, combining multiple genetic diversity measures, based on both molecular and studbook data, might produce a more robust data set (JOST et al., 2017; LÓPEZ-CORTEGANO; PÉREZ-FIGUEROA; CABALLERO, 2019; RALLS; BALLOU, 2004; ZHANG et al., 2018).

When the initial genetic diversity of the founding captive population is unknown, it is hypothetically considered equal to 1, as proposed by the PMx model commonly used for calculating pedigree parameters (LACY, 1995; LACY; BALLOU; POLLAK, 2012). However, our findings suggest that despite the remaining genetic diversity is about 65% in both pedigree and integrated analyses, the expected heterozygosity represented in the founder individuals, based on the integrative approach, would be about 0.69. Such results show a more coherent value of genetic diversity for F0, reinforcing the idea that genetic diversity inferences must be specific for each breeding program and cannot be extrapolated from hypothetical assumptions (IVY; LACY, 2012; JOST et al., 2017).

Molecular analyses are essential for populations with unknown genetic diversity and can be relevant to monitoring genetic diversity across generations in conservation actions (FIENIEG; GALBUSERA, 2013; JONES et al., 2002; MARSDEN et al., 2013; RUSSELLO; AMATO, 2004). According to recommendations of the ISFG (*International Society for Forensic Genetics*) for the area of non-human DNA typing (LINACRE et al., 2011), they have also potential to be used, by the community of forensic scientist, for investigations involving poaching, smuggling and illegal trade of protected species (ESTRADA; RABOY; OLIVEIRA, 2012; IYENGAR, 2014; OKLANDER et al., 2019)

DNA-based studies can still simulate, estimate and compare genetic diversity levels in breeding programs (WANG et al., 2004). Genetic management of threatened species has experienced an increase in the last few years (CARROLL et al., 2014; ITO et al., 2017; IVY et al., 2009; SMITH et al., 2014), and more recently has been improved by a combination of pedigree and molecular information (GRUEBER; WATERS; JAMIESON, 2011). For the BLT captive breeding program, we highlight that an integrative approach could be of benefit in terms of allele representativeness and also for considering a more plausible genetic diversity estimate for the founding population.

Overall, to promote the long-term success of the BLT conservation program, we recommend including genetic diversity parameters based on molecular data, in addition to the pedigree analyses and *MSI* scores. Microsatellite-based values of expected heterozygosity, individual heterozygosity, allele richness, private alleles, population structure, inbreeding and

kinship could be monitored over generations, helping to evaluate gains and losses of genetic diversity more effectively, and identifying individuals potentially better suited for reproduction and for relocation in captivity (ITO et al., 2017; LÓPEZ-CORTEGANO; PÉREZ-FIGUEROA; CABALLERO, 2019; RALLS; BALLOU, 2004). Finally, we must take into account that an integrated *in situ* and *ex situ* approach is strongly indicated for the metapopulation management of BLTs and to help shield this species from its imminent risk of extinction, since in nature *L. chrysopygus* has a small population size and a very low genetic diversity level.

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1.6 SUPPLEMENTARY MATERIAL 1

1.6.1 Population dynamics

The complete pedigree of BLT from 1973 to 2018 consists of 517 animals and nine generations (Fig. S1.1). The proportional change in population size (ƛ) from a year to the next, based on the life table calculations, and the instantaneous rate of population change (*r*), measured for males and females, was ƛ =1.016 and *r* = 0.016 for the Whole Captive Population (WCP); ƛ = 1.158 and *r* = 0.072 for the Current Captive Population (CCP); and ƛ = 1.075 and *r* = 0.147 for the Brazilian Captive Population (BCP). The total life expectancy observed for BCP (7.3 years) and CCP (7.4 years) was similar, although differences between males and females within BCP (10.1 years and 4.4 years, respectively) and within CCP (9.9 years and 5 years, respectively) were found. It was clearly observed that the captive population of BLT is aging and showing a male bias, with a lower number of females (Fig. S1.2A). The current captive population shows a non-stable age structure, since a female deficit was observed in the 0-3 years age class (Fig. S1.2B). Demographically, the whole captive population of BLT began to decline, especially in the number of females from the year 2000 (Fig. S1.3). Nowadays, the age structure of the current population is relatively old, since many individuals are above 7 years old and there is no male-female balance. Thus, the structure of the captive population of BLT is typical for a slowly growing population (λ>1).

The mortality (Qx) and fertility (Mx) analyses for WCP showed a clear difference between sexes when ages were considered, gradually increasing throughout life in females (Figs. S1.4A and S1.4B). Despite such differences, the life expectancy was similar in both males and females (~7.5 years) in CCP. Annual mortality rates by age-class showed high infant mortality and similar fluctuations in both sexes up to the age 13 years (Figure S1.4A). In general, we observed, in a way that only 50% of males and 58% of females survived to the age of first reproduction (18 months approximately) (Fig. S1.4C). We observed an increase in mortality rates in some age classes for both females and males, when we considered the year 2014 (BCP) and 2018 (CCP) (Fig. S1.5). When we compared this year, the mortality rates showed significant differences between the sexes, according to the Mann-Whitney test (Female, U=106.5, *P=0.00*: Males, U=118, *P=0.00*). Age-specific fertility rates showed an onset of fertility at age of 1 year for females and 2 years for males. We also observed a differential relation between reproductive peaks, considering males and females, in which fertility increases at 3 and 2 years old for the males and females, respectively (Fig. S1.4B). The reproduction for BLT has preferably been occurring in captivity from September to October, with the major birth

peak occurring in October, and smaller peaks in May, June and July (Fig. S1.6). Litters with one, two or three infants were commonly observed, with a larger number of birth twins (162) than single (88) and triplets (21); and higher mortality rates for triplets (54.5%) and twins (40.7%).

1.6.2 Technical procedures for amplification and genotyping of the STR loci

We used 15 microsatellite loci previously described for *Leontopithecus* species, from which eight were for *Leontopithecus chrysopygus,* three for *Leontopithecus rosalia,* and four for *Leontopithecus chrysomelas* (Table S1.3). Polymerase Chain Reactions (PCRs) were first performed using the annealing temperatures (Ta) described initially for each species, in order to test the amplification patterns and determine the expected fragment sizes for each locus. Then, annealing temperatures were adjusted for improving the amplification patterns of some loci (Table S3). PCRs were performed in 10 µl of reaction volume, containing template 1ul of DNA (50 ng), and forward primer (0.12 pmol), reverse primer (0.46 pmol), M13 primer (0.46 pmol), $MgCl_2$ (0.63mM), BSA (0.25mg/ml), and 1X GoTaq Master Mix (Promega, Madison, WI). We used an Eppendorf Mastercycler Gradient® Thermal Cycler (Eppendorf AG, Hamburg, Germany) equipment under the following programming: 5 minutes at 94 °, followed for 35 cycles of 30 s at 94 ° C, 45 s at the primer-specific annealing temperature, 45 s at 72 °C. Finally, 10 cycles of 30 seconds at 94 °C, 45 s at 53 °C (annealing temperature of M13 tail), and 45 s at 72 °C. Each locus was standardized with a specific fluorophore. M13 primers were labeled with FAM, PET, NED or VIC, following the methodology proposed by Schuelke (2000) (Table S3). Amplification patterns were confirmed by electrophoresis using 2% agarose gels, under constant voltage (100V for 35 min). After obtaining amplicons into the expected sizes and without spurious bands, PCR-amplified products were purified using the PEG (Polyethylene glycol 20%) protocol, according to Lis & Schleif (1975); and then sequenced in an automatic sequencer ABI3730XL (Applied Biosystems, Foster City, CA, USA), aiming to confirm the microsatellite motifs for the heterologous loci. The obtained sequences were aligned and compared to the reference microsatellite sequences available in Genbank (https://www.ncbi.nlm.nih.gov/genbank/), using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). After confirming the motifs, random PCRs were performed, and the labeled products were genotyped.

1.6.3 Supplementary Figures

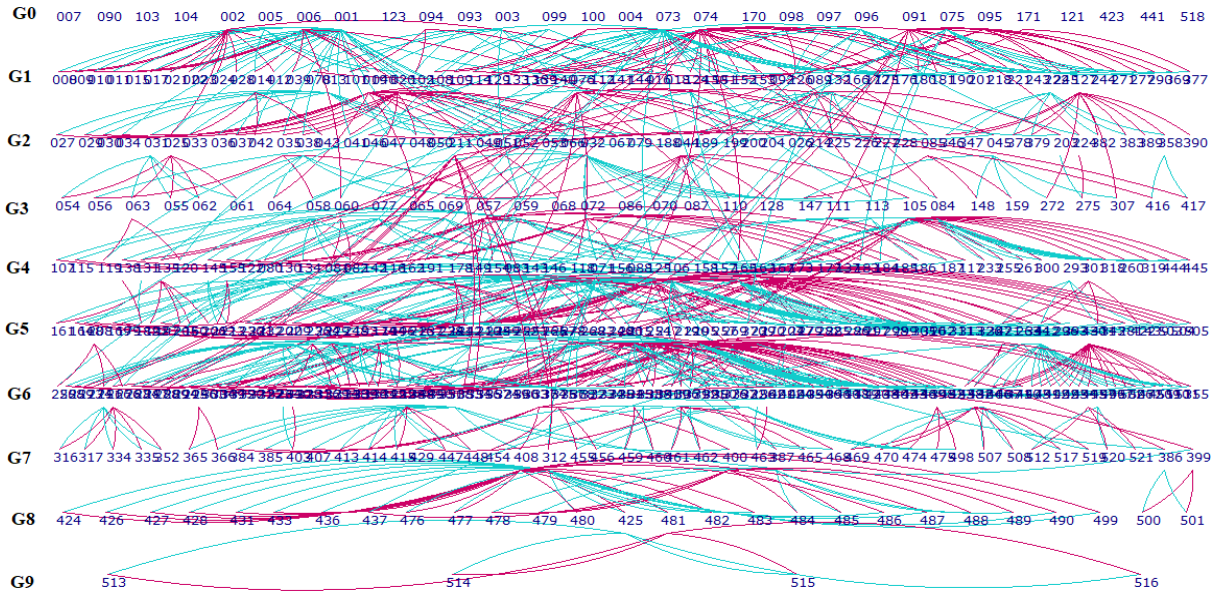


Figure S1.1 - Pedigree graphic of the whole captive population of the black-lion-tamarin according to data from Studbook. G0 represents the wild founders. G1-G9 represent the descendants. Pink lines indicate females. Blue lines indicate males.

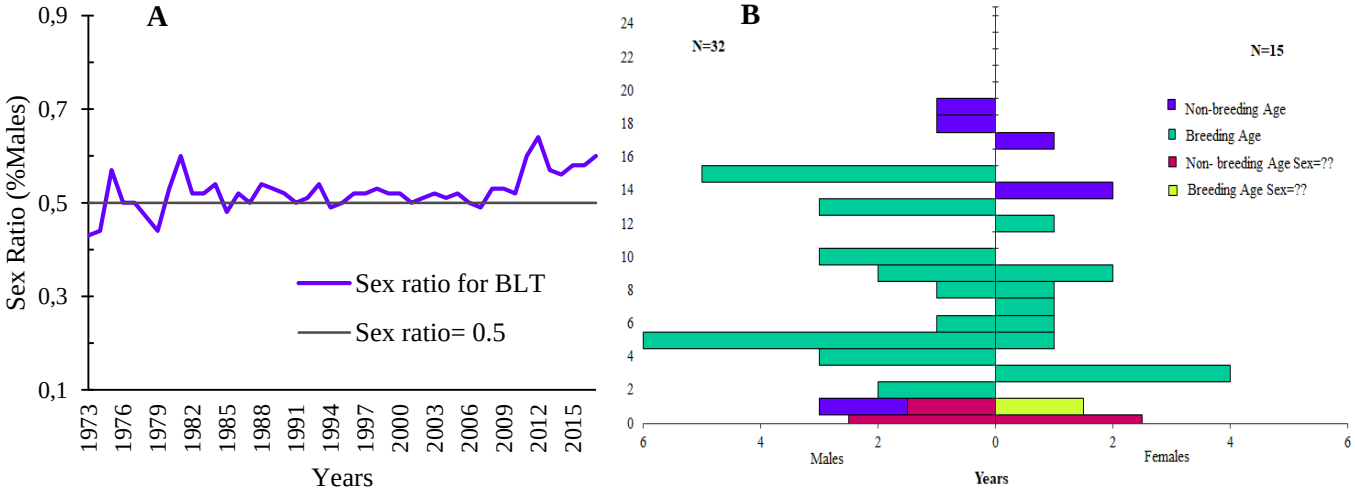


Figure S1.2 - Representation of population dynamics of black-lion- tamarin in captivity. (A) Sex ratio across years showing a male bias. (B) Age pyramid for the Current Captive Population (CCP) at the end of 2018. Number of males and females (N).

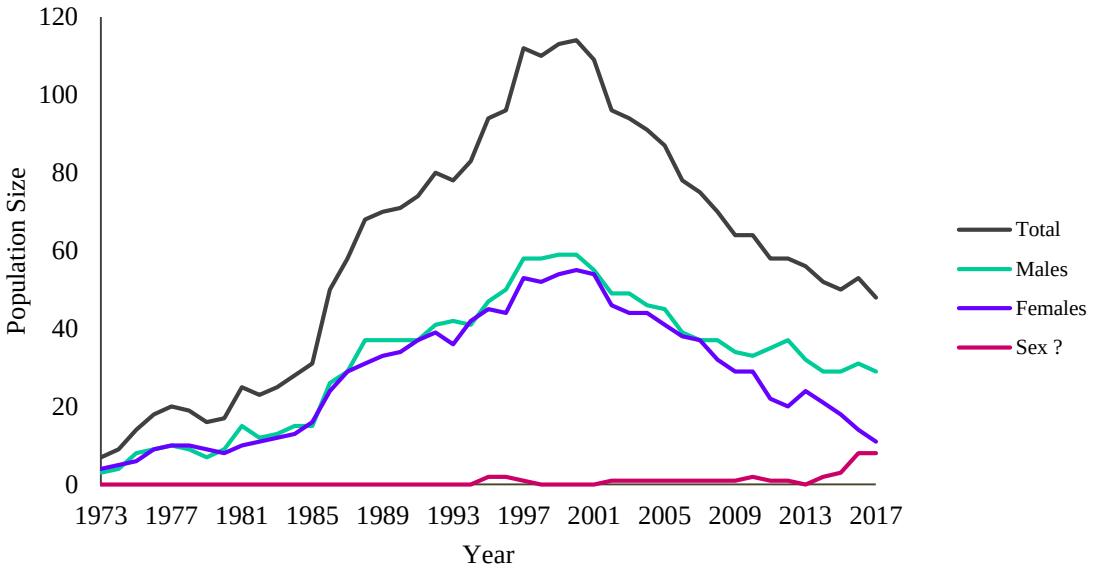


Figure S1.3 - Population Size Fluctuation: number of black-lion-tamarins in captivity from 1973 to 2017.

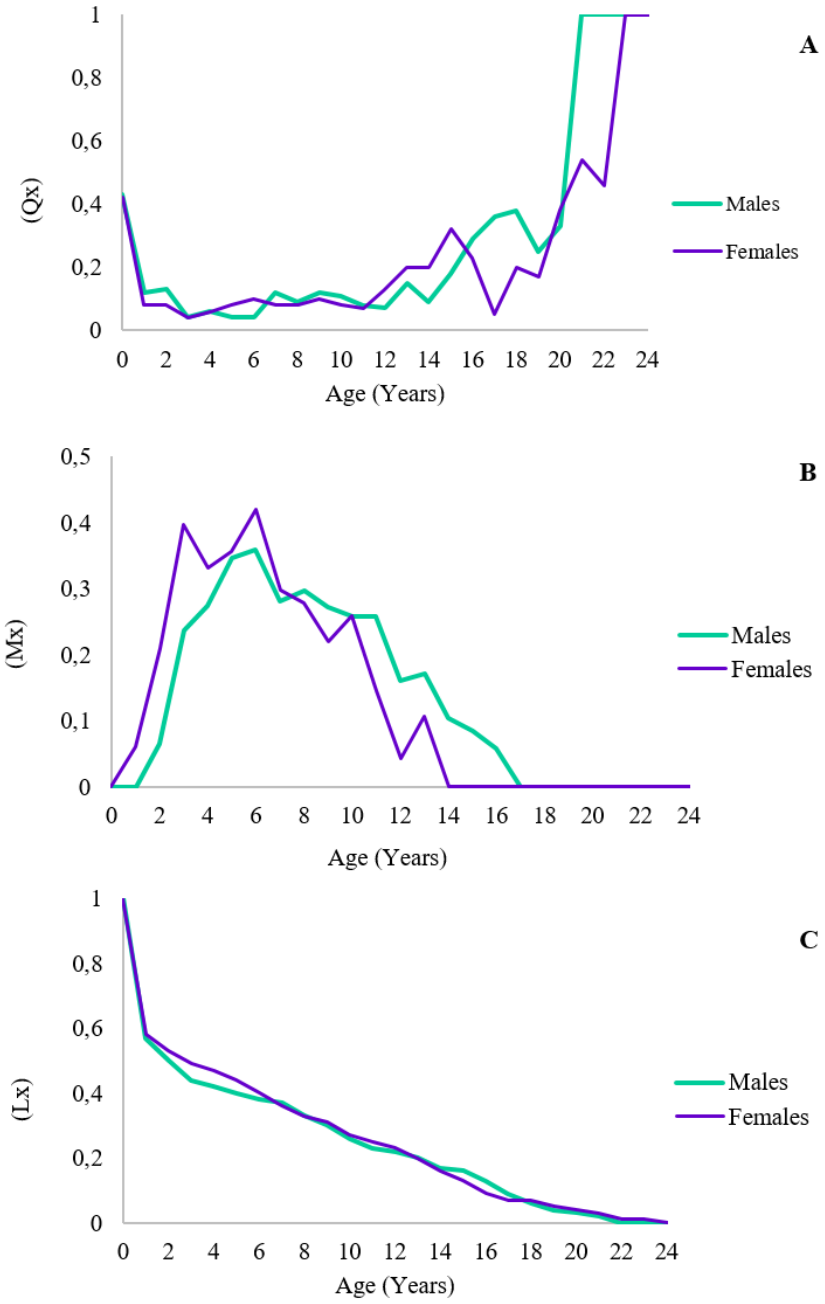


Figure S1.4 - Representation of population dynamics for the whole captive population of black-lion-tamarins. (A) Mortality rate (Qx): proportion of died individuals within an age group (B). Fecundity (Mx): the average number of same-sex young born to individuals in that age class. (C) Survivorship (Lx): proportion of individuals that survive from birth to the beginning of a given age class.

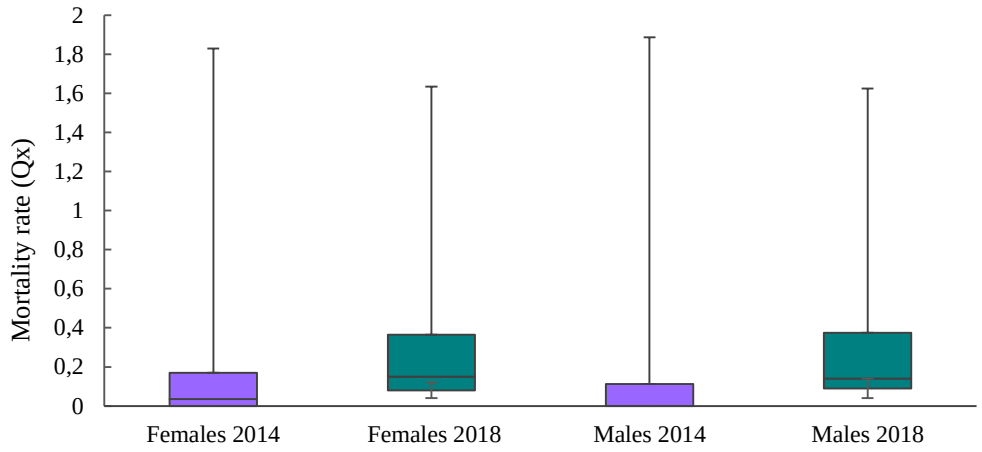


Figure S1.5 - Box plot for the male and female black-lion-tamarins in Brazilian Captive Population (BCP) and Current Captive Population (CCP). Note the increase in the mortality rate between 2014 and 2018 for both males and females.

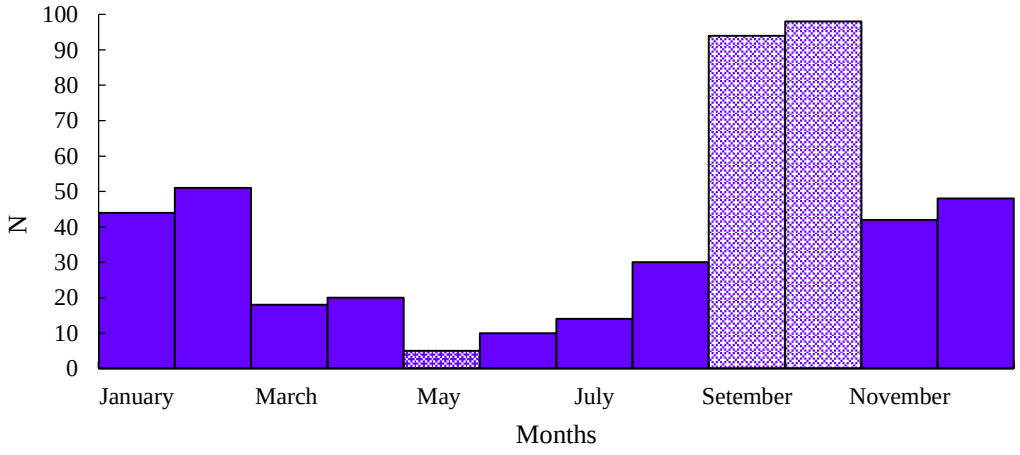


Figure S1.6 - Histogram of number of births (N) per month for the Whole Captive Population of black-lion-tamarins.

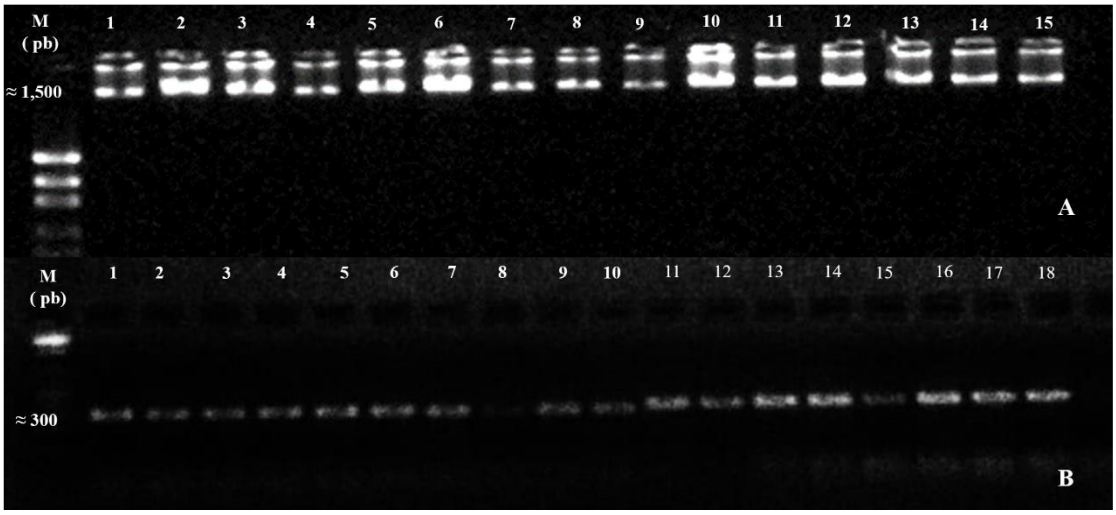


Figure S1.7 - (A) Agarose gel (1%) evidencing DNA profiles from blood samples of black-lion-tamarins. (B) Agarose gel (2%) showing amplicon profiles obtained for two loci, Leon 30c73 (samples 1-10) and Leon 11c72 (samples 11-18), amplified in different samples of black-lion-tamarins. M: molecular weight marker (Low DNA Mass Ladder above and 1Kb plus below).

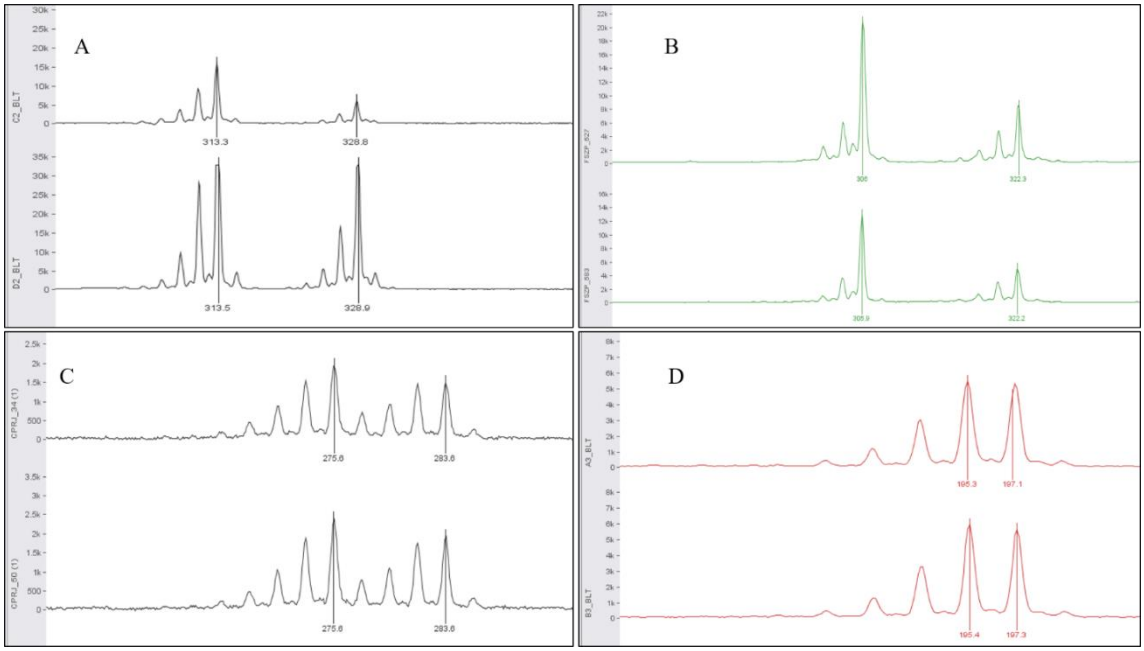


Figure S1.8 - Electropherograms (EPGs) showing heterozygous patterns evidencing two alleles amplified in DNA samples of black-lion-tamarins for the loci: (A) Leon 3c20 labeled with NED; (B) 11c72 labeled with VIC; (C) Leon30c72 labeled with NED; and (D) Lchu06 labeled with PET.

1.6.4 Supplementary Tables

Table S.1.1 - Summary for the captive populations of the black-lion-tamarins in 2014 and 2018. *São Carlos Ecological Park (PESC, SP); Zoological Park Foundation of São Paulo State (FPZSP, SP); Primatology Center of Rio de Janeiro (CPRJ, RJ); Jersey Zoo, Belo Horizonte Zoo (BH); Magdeburg Zoo (Mag).

Studbook number	Sex	Birth date	Death date	Zoo in 2014	Transfer to	Population 2014	Population 2018
391	M	12/01/2002		PESC		BCP-2014	CCP-2018
500	M	09/10/2010	06/03/2013	PESC		BCP-2014	
352	F	30/09/1999		PESC		BCP-2014	CCP-2018
501	M	11/03/2013		PESC		BCP-2014	CCP-2018
386	M	31/10/2001		PESC	FPZSP	BCP-2014	CCP-2018
399	F	19/09/2002		PESC	FPZSP	BCP-2014	CCP-2018
385	M	31/10/2001		FPZSP	BH	BCP-2014	CCP-2018
421	F	04/10/2004	02/12/2015	FPZSP		BCP-2014	
422	F	04/10/2004		FPZSP		BCP-2014	CCP-2018
458	F	09/01/2008		FPZSP		BCP-2014	CCP-2018
457	M	09/01/2008		FPZSP		BCP-2014	CCP-2018
392	M	12/01/2002		FPZSP		BCP-2014	CCP-2018
263	M	30/08/1995	28/08/2016	FPZSP		BCP-2014	
464	M	Wild		FPZSP		BCP-2014	CCP-2018
432	F	11/04/2007		FPZSP		BCP-2014	CCP-2018
469	M	13/08/2011	19/09/2012	FPZSP		BCP-2014	
470	M	13/08/2011		FPZSP		BCP-2014	CCP-2018
412	M	17/092003		FPZSP		BCP-2014	CCP-2018
467	F	17/09/2010		FPZSP		BCP-2014	CCP-2018
473	M	31/01/2012		FPZSP		BCP-2014	CCP-2018
430	F	Wild		FPZSP		BCP-2014	CCP-2018
471	M	20/09/2011		FPZSP	Jersey	BCP-2014	CCP-2018
472	M	30/01/2012		FPZSP	Jersey	BCP-2014	CCP-2018
491	M	10/09/2012		FPZSP			CCP-2018
492	M	10/09/2012		FPZSP			CCP-2018
495	F	13/08/2013		FPZSP			CCP-2018
496	F	26/12/2013		FPZSP			CCP-2018
TE64	M	03/03/2015		FPZSP			CCP-2018
TE65	U	31/08/2015		FPZSP			CCP-2018
TE66	U	31/08/2015		FPZSP			CCP-2018
TE67	U	11/11/2015		FPZSP			CCP-2018
TE72	M	05/02/2016		FPZSP			CCP-2018
TE73	M	05/11/2014		FPZSP			CCP-2018
TE74	U	23/09/2016		FPZSP			CCP-2018
TE75	U	27/10/2016		FPZSP			CCP-2018
TE76	U	27/10/2016		FPZSP			CCP-2018
502	F	26/12/2013		FPZSP	BH		CCP-2018
312	M	11/10/1997		CPRJ		BCP-2014	CCP-2018
408	F	18/02/2003		CPRJ		BCP-2014	CCP-2018
486	M	02/10/2011		CPRJ		BCP-2014	CCP-2018
487	M	02/10/2011		CPRJ	Jersey	BCP-2014	CCP-2018
427	M	13/02/2007		CPRJ		BCP-2014	CCP-2018
366	F	08/11/2000	13/06/2016	CPRJ		BCP-2014	
384	M	31/10/2001	09/04/2015	CPRJ		BCP-2014	
436	F	29/09/2008		CPRJ	Jersey	BCP-2014	CCP-2018
387	M	31/10/2001		CPRJ		BCP-2014	CCP-2018
400	F	19/09/2002	16/08/2015	CPRJ		BCP-2014	
488	M	02/10/2011		CPRJ		BCP-2014	CCP-2018

437	M	27/10/2008		CPRJ	BCP-2014	CCP-2018
481	F	01/01/2010		CPRJ	BCP-2014	CCP-2018
431	M	26/10/2007		CPRJ	BCP-2014	CCP-2018
424	M	14/12/2005	22/07/2014	CPRJ	BCP-2014	
425	M	27/12/2006		CPRJ	BCP-2014	CCP-2018
428	M	13/02/2007		CPRJ	BCP-2014	CCP-2018
TE68	M	06/01/2016		CPRJ		CCP-2018
TE69	M	06/01/2016		CPRJ		CCP-2018
TE70	U	05/12/2016		CPRJ		CCP-2018
TE71	U	05/12/2016		CPRJ		CCP-2018
410	M	06/08/2003		Jersey		CCP-2018
419	M	10/02/2004		Jersey		CCP-2018
468	M	22/03/2011		Jersey		CCP-2018
497	F	23/12/2013		Jersey		CCP-2018
333	M	15/11/1998		Mag		CCP-2018

Table S1.2 - Inbreeding coefficient values over the years for the whole captive population of black-lion-tamarins.

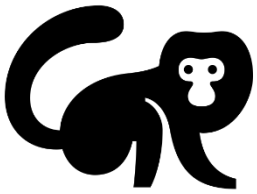
Year	**1974**	**1975**	**1976**	**1977**	**1978**	**1979**	**1980**	**1981**	**1982**
Inbreeding	0	0	0	0	0	0	0	0	0
Year	**1983**	**1984**	**1985**	**1986**	**1987**	**1988**	**1989**	**1990**	**1991**
Inbreeding	0	0,0119	0,0208	0,026	0,029	0,0337	0,0313	0,037	0,0318
Year	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**
Inbreeding	0,0229	0,0204	0,0185	0,0199	0,0212	0,0223	0,0268	0,0397	0,0408
Year	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**
Inbreeding	0,0414	0,0428	0,0483	0,0486	0,0519	0,0541	0,0574	0,0656	0,0702
Year	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**
Inbreeding	0,0788	0,086	0,0952	0,095	0,0916	0,0887	0,0874	0,107	0,107

Table S1.3 - Summary information on the homologous and heterologous microsatellite loci validated for the black-lion-tamarin.

Locus	Primer Sequence (5`-3`)	Repetitive unit	Annealing Temperature (°C)	PCR product size range	ABI Label	GenBan Acess	Source
***Leon2**[a]	F: CTGCTTCTTGTTCCACTTCTTCTC R: GTTTGGGTGGTTGCCAAG	$(CA)_{18}(CG)(CA)_3$	56	219-223	FAM	AY706915	Perez-Sweeney et al. 2005
***Leon15c85**[a]	F: CTGCTTCTTGTTCCACTTCTTCTC R: GTTTGGGTGGTTGCCAAG	$(GA)_{17}$	60	284-296	FAM	AY706920	Perez-Sweeney et al. 2005
***Leon3c20**[a]	F: CTGTATGTGATCGCTTTTACCTG R: AAGGCAATCTAACTAATCAACACTC	$(GT)_{22}$	60	312-318	NED	AY706916	Perez-Sweeney et al. 2005
***Leon21c75**[a]	F: CAGTTGAGGGAACAGGAATTA R: CACTGCACTGACAGAGCAAG	$(GT)_{19}(NA)1(GT)_5$	60	294-298	FAM	AY706922	Perez-Sweeney et al. 2005
***Leon30c73**[a]	F: GGACCTGATTGAAGCAGTC R: TTCCCTGAGAATCTAATGGAG	$(TC)_{25}(AA)(TC)(TG)_{16}$	60	274-284	NED	AY706927	Perez-Sweeney et al. 2005
***Leon31c97**[a]	F: TGGTCCAGAGAAATGATGTC R: GTAATTCCTTGGATTTATGCC	$(GA)_2(CA)_2(GA)_{19}(TT)(GA)(CA)_4$	55	328-340	PET	AY706928	Perez-Sweeney et al. 2005
***Leon11c72**[a]	F: AGGATTACAGGTGCCCAC R: TTGCATATTGTGTTCAACTTC	$(GT)_{21}$	60	307-323	VIC	AY706921	Perez-Sweeney et al. 2005
***Leon35c42**[a]	F: GTGGAAAGGTTTCAGAATATC R: TGCAGTTGTCCACACTTTA	$(CT)_{16}(CA)_9(T)(AC)_3$	58/60	219-223	FAM	AY706929	Perez-Sweeney et al. 2005
Leon26c10[a]	F: TTCATCTCAATGACACGAAAC R: CATCGAGTGTCCTGCTGT	$(TG)_{17}(AG)_{15}(GT)_3(CT)(GT)$	58	266	VIC	AY706924	Perez-Sweeney et al. 2005
Leon27c13[a]	F: AAGCGCAGATTTATTGATAGG R: TGCAGGTAAATGATGGTAATG	$(CA)_{11}$	60	213	PET	AY706925	Perez-Sweeney et al. 2005
***Lchu01**[b]	F: GCTCAGGTGTTATTTATGTCCAAA R: GTTTCTTGCAACTATCTTGCATGTTCTGC	$(TTTA)_8$	58	213-225	FAM	DQ979343	Galbusera and Gillemot 2007
Lchu02[b]	F: AGATTCTGCCTCAAGAAATTCAGT R: GTTTCTTTCTAGATCCAGGTCGGCAAT	$(A)_3\ T(AGAA)_2$	60	314	FAM	DQ979344	Galbusera and Gillemot 2007
Lchu03[b]	F: AAGGCATGATGTATCTTGTTCTCA R:GTTTCTTATCTTTCTGTATGTGTCTCCCTGTCT	$(GATA)_{13}$	58	336	VIC	DQ979345	Galbusera and Gillemot 2007
Lchu04[b]	F: TGACCAAAGAAAATGCAAAA R: GTTTCTTGCACAGGGTATTTAGCAGGA	$(AGAT)_{14}$	58	396-400	VIC	DQ979346	Galbusera and Gillemot 2007
Lchu05[b]	F: TGATGCTAAAACAGAAGCATTT R: GTTTCTTGTCCTGATGTTCACAAAACCT	(GAAG)11	58	256	VIC	DQ979348	Galbusera and Gillemot 2007

***Lchu06** [b]	F: GCCTTAATTAGCACCAGAACC R: GTTTCTTACCACTCCAAGCCTTCAGTA	(CA)8	55	193-195	PET	DQ979349	Galbusera and Gillemot 2007
***Lchu07** [b]	F: TCTCATTTCTTCTCATGGACTC R: GTTTCTTCTTGACTCACAGCATGACCT	$(TG)_{16}$	55	342-348	FAM	DQ979350	Galbusera and Gillemot 2007
***Lchu08** [b]	F: CACGGCAATGTGGGAATAA R:GTTTCTTTTCAGTAGTTGGGACTGGGATAA	$(TG)_{23}$	58	224-236	VIC	EF583690	Galbusera and Gillemot 2007
Lchu09 [b]	F: TTCATTGTAGCATTGTTGGTCAT R: GTTTCTTTTGCCTCCTCATAGTTCCTCAT	$(CA)_{19}$	58	418-422	FAM	EF583691	Galbusera and Gillemot 2007
***Lr. P2BH6** [c]	F: TCTGTTTGAATCCCCAGTCC R: GCAGTCCCTCAAGGTTTTCT	$(CA)_{19}$	58	132-136	PET	AF320577	Grativol et al. 2001
***Lr. P5BE6** [c]	F: TGTGCATGCTTGCCTGTGTC R: ATCTCACTGGACCCACCT	$(CA)_{27}$	58	120-130	PET	AF320580	Grativol et al. 2001
***Lr. P3AF1** [c]	F: CCATCCTGGCCAACATAGGT R: GTAGCTGGGATTACAGGCAC	$(CA)_{23}$	62	126-130	FAM	AF320581	Grativol et al. 2001

Specific primers developed for [a] *Leontopithecus chrysopygus*; and heterologous primers developed for [b] *Leontopithecus chrysomelas*, and [c] *Leontopithecus rosalia*.
*Polymorphic loci selected to be used for the subsequent analyses of genetic diversity in the black-lion-tamarins from captivity.



Chapter 2

Ecological, behavioral, and genetic aspects of black lion tamarin (Callitrichidae: *Leontopithecus chrysopygus*, Mikan 1823): a review

ABSTRACT

The black-lion-tamarin (BLT), *Leontopithecus chrysopygus* is an endangered primate inhabiting exclusively in the remaining Atlantic Forest of São Paulo state in Brazil. Changes linked to the historical and current modification of the landscape have threatened its viability reflecting in population declines and local extinction within the last decade. Despite its important role in the ecosystem balance, due to the seed dispersion there is a knowledge gap in relation in relation to this species. Here, we reviewed the studies published between 1970 and 2021, about ecological, behavior, and genetic aspects in order to understand how BLTs' populations vary across different regions and types of habitats where they occur. Overall, the studies about BLTs were relatively low throughout the years (mean 2.2 ± SD 1.69), and were distributed unequally, being ecology the most studied subject (66.66%), followed by genetics (20.37%) and behavior 12.37%. Currently, there are 14 populations of BLT registered after the rediscovery of the species in the wild in the 1970s. Morro do Diabo and Carlos Botelho State Park areas are considered the unique large remnants with more than 10,000 hectares of continuous forest, where the species has been registered. The rest of the areas where the BLT is found correspond to middle and small-size fragments not exceeding 5,000 hectares; and with some of them close to water bodies characterizing riparian forests. Our study provides a complete overview a perspective for future research. We highlight relevant patterns related to the type of fragment (continuous, fragment or riparian) or region (Lower, Middle and Upper Paranapanema) where the BLT occurs and population densities, sex-ratio bias, home range sizes, rest and move time. In addition, we compared the genetic diversity parameters and performed simulations analyses of changes in genetic diversity for a wild population that showed the highest heterozygosity in order to predict genetic diversity declines under a single bottleneck event. Our review allowed us to have a more comprehensive view of the BLT's resilience and genetic status pointing to the main information gaps in the current knowledge about the ecology, behavior, and genetic aspects. These topics are of special interest for the conservation of the BLT populations given the expansion of changes in the physiognomy of the landscape and anthropogenic climate change that can affect its viability.

2.1 INTRODUCTION

The *Leontopithecus chrysopygus* (Callitrichidae) - known as black lion tamarin (BLT) - is a small arboreal primate that occurs only in the remaining Atlantic Forest of São Paulo State, in Brazil (HERSHKOVITZ, 1977; MITTERMEIER, 2013). Compared to the other three lion tamarins (*Leontopithecus rosalia, Leontopithecus chrysomelas* and *Leontopithecus caissara*), *L. chrysopygus* is the widest and most longitudinally distributed species (MEYER; PIE; PASSOS, 2014). Its current distribution is within three hydrographic basins, limited in the west by the Paraná River, in the north by the Tietê River, and along both margins of the Paranapanema River (COIMBRA-FILHO; MITTERMEIER, 1973). However, deforestation, wood extraction, and fires have restricted their extant populations into few remnants and forest patches (MEYER, 2017).

For centuries, habitat destruction processes have reduced BLT populations until the species has been declared extinct in 1905, maintaining this status for about 65 years (COIMBRA-FILHO, 1970). Fortunately, during the 1970s, few individuals were found in the Pontal do Paranapanema region (COIMBRA-FILHO, 1970). Nowadays, there are 15 BLT populations registered in 14 municipalities located in three main regions of São Paulo state: Upper, Middle and Lower Paranapanema. The BLT occurrence in relatively small and isolated fragments, and occasionally near cities, raises questions about how primate species deal with environmental disturbances, which may have a detrimental effect on their persistence, making their populations more vulnerable to stochastic processes, such as epidemic, demographic, ecological and genetic catastrophes (MARSH; CHAPMAN; ARROYO-RODRÍGUEZ, 2013).

Despite the increasing literature addressing the impacts of habitat loss and fragmentation on primates (MARSH, 2003), further studies considering different scenarios are still missing for most taxa (GALÁN-ACEDO et al., 2019), including for threatened species, as BLT (IUCN, 2021). In this sense, gathering available data for a species and analyzing them in an integrative manner may help to understand how the species behavior, ecology, and genetic vary across the regions of its distributional range, but also according to the environmental context, i.e., whether it occurs in a fragment, continuous or riparian forest.

In attempt to raise relevant information about ecology, behavior and genetics of BLT, we carried out a literature review in published and unpublished studies for the species, and performed statistical analyses when sufficient data were available. We evaluated the variation of demography, home range, diet, sleeping behavior, activity budget, use of strata and

substrates, and seed dispersal according to the region and the type of forest remnant where the species were found. We also summarized the genetic data available for BLTs, and performed a simulation analysis using microsatellite genotypes previously produced by Ayala-Burbano et al. (2017) for a wild population, which enables to predict changes in the genetic diversity parameters across 100 years. Through these approaches we could sum up important behavioral, ecological and genetic characteristics of BLT, highlight some interesting variation between regions and environmental context, but also identify relevant issues and localities needing further studies. In addition, we highlighted the importance of these data for the ongoing conservation programs and provided some insights for future surveys that would allow establishing more accurate decisions for the management of the species.

2.2 METHODS

The review of the literature was carried out through three main steps: (i) data searching and compilation; (ii) data mining and extraction; and (iii) data analysis, as described below.

2.2.1 Data searching and compilation

We used two major sources to raise studies on behavioral, ecological or genetic aspects of the BLT. First, we searched the Web of Science and Google Scholar libraries using the following keywords and combinations: "*Leontopithecus*" "*Leontopithecus chrysopygus*" "black lion tamarin", "Atlantic Forest", "ecology", "behavior", "genetics", "demography", "home range", "activity budget", "seed dispersal", "diet", "foraging behavior". We did not restrict the year of publication nor the language. Next, we downloaded articles and 'grey literature', such as reports from symposiums and congresses, theses, technical reports and management plans. Furthermore, some papers were obtained directly from the authors, since they were not available on the web. We also included data archives from previous studies conducted by the Primatology Laboratory of University of São Paulo State (LaP, UNESP) and by the Institute for Ecological Research (IPÊ), and compiled by Forero-Sánchez (2021).

2.2.2 Data mining and extraction

The full data sets were manually examined to identify those reporting aspects related to the keywords and their combinations. Studies that did not mention the three main subjects (ecology, behavior and genetics) were removed from the subsequent steps. We quantified the

research effort (number of publications) and research coverage (diversity of subjects), and organized the data set according to eight target topics: (1) demography; (2) home range; (3) sleeping sites; (4) activity budget; (5) diet; (6) seed dispersal; (7) use of strata and substrates; and (8) genetic diversity. Then, we extracted specific information that we judged relevant to characterize each of the eight-target topic, as described in table 2.1. Topics or information of interest with insufficient data for statistical approaches (e.g., from a single BLT group) were analyzed descriptively.

Table 2.1 - Data about behavior, ecology, and genetics of black lion tamarin extracted from the literature review.

Target topics	***Specific information of interest***
Demography	Number of individuals, group composition (male, female, infant, juvenile), births and / or deaths and immigration or emigration of individuals.
Home range	Study period, distributions of days of sampling effort, data sampling method, home range estimation method.
Sleeping sites	Family and species of plants used as shelters, number of individuals used, tree height, hollow height entrance, diameter at breast height, type of sleeping site (trees, hole trees or lianas), tree shelter position (branch, canopy or trunk).
Activity budget	Study period, sampling effort, sampling method, percentage spent on each activity: moving, resting, foraging, feeding and social interactions.
Diet	Family and species of plant consumed, percentage contribution of the most consumed items, type of items consumed (fruits or gum), sampling effort, study period.
Seed dispersal	Family and species of plant retrieved from feces, fruit size, seed size, percentage of germination of defecated seeds and control treatments.
Use of stratum and substrates	Study period, sampling method, stratum in meters, stratum in category (understory, middle layers, canopy), total percentage of time spent in each stratum, activity in each stratum and substrate.
Genetic Diversity	Number of individuals analyzed, type of molecular marker used, number of loci used, expected and observed heterozygosity, haplotypic diversity, number of alleles per locus, nucleotide diversity, inbreeding coefficient, genetic structure.

2.2.3 Data analyses

To characterize the variation in BLT behavioral and ecological characteristics, we compared the available data among the different studied regions (Lower, Middle and Upper Paranapanema) and also among the three different remnant types where the species were found: continuous (>10,000 ha), fragments (<5,000 ha) and into riparian forest. Next, we performed statistical analysis to compare regions and remnants.

Initially, we used unpaired t-student or one-way ANOVA tests for comparing data; however, when the data did not fit the requirements of these both parametric tests (normality and homogeneity of variance) we performed Mann-Withney or Kruskall-Wallis tests, respectively. For pairwise post-hoc comparations we performed Tukey or Wilcoxon tests in case of parametric or non-parametric data, respectively. For activity budget data, in which the proportion of behavior types is dependent of each other (e.g., CASELLI; SETZ, 2011), we used a principal component analysis (PCA) based on the correlation matrix of the five behavioral categories (moving, resting, foraging, feeding, and social interaction) in order to reduce the dimension of our data set to two orthogonal axes. Then, we chose the component that best explained the variation of activity pattern and performed an unpaired t-test and one-way ANOVAs for comparing regions and remnant types, respectively. We also used a chi-square test to compare the absolute number of adult males and females among group in the overall data. All analyses were performed in R software version 4.0.5 (R Development Core Team, 2020).

Noteworthy, we standardized sampling effort of the different studies in days to enable easier comparisons. Thus, for studies with sampling effort in hours, we considered an average observation of ten hours per day and then divided the total number of hours by ten. For studies with sampling effort in months we considered an average of five observation days per month and then multiplied this number by the number of months of each study. As an exception, for demographic data, we established the sampling effort in months, since most of data collection was carried out once a month or biweekly.

For simulate changes in genetic diversity, under a single bottleneck event, we chose the wild population that showed the higher values of heterozygosity and downloaded genotype data, available in the BLT database (https://www.bltdatabase.ufscar.br/), from a Capão Bonito population previously analyzed (AYALA-BURBANO et al., 2017). We performed a prediction analyses using the BOTTLESIM 2.6 software (KUO; JANZEN, 2003), and assessed the chance of retaining 90% of observed genetic variation, as suggested by Frankham et al. (2002) to predict population viability.

The allele frequencies and census population size (N) were used to predict the genetic diversity in the future, considering only genetic drift as an evolutionary force. Mutation, migration, and selection were not assumed in the model. We estimated the observed heterozygosity (HO) and observed number of alleles (OA) for the next 100 years, considering 100%, 75% 50% and 25% of the census population size retained. The simulated scenarios were

ran following 100% of completely overlapping generations; a dioecious reproductive system; 16 years for expected longevity of the species (FORERO-SÁNCHEZ, 2020; MORAES et al., 2017); 2 years age for reproductive maturation; census population size of 74 individuals (Forero-Sánchez, 2020; sex ratio = 1male:1female), and 1,000 iterations.

2.3 RESULTS AND DISCUSSION

2.3.1 Data source

The whole data set included 75 studies performed from 1970 to 2021, since the rediscovery of BLT. After filtering the data, we retained 55 published studies, in which 34 were papers, 20 were theses (four undergraduate, 12 master, and four doctoral theses), and one was a report. Among the unpublished data, there were 16 spreadsheets obtained by Institute for Ecological Research (IPÊ), that describing the employed methods and their respective results for 14 BLT groups studied from 1993 to 2006. Such information was compiled and organized in a single document available in Supplementary Material 2 (see Tables S2.2–S2.8). Subsequently, the data will then be added to a black lion tamarin database, available at www.bltdatabase.ufscar.br.The list of the selected bibliography references and the records raised for the eight target topics are provided in Supplementary Material 2 (Table S2.1).

Publications were distributed unequally across the three main subjects (ecology, behavior and genetics) and the topics (demography, home range, sleeping sites, activity budget, diet, seed dispersal, use of strata and substrates, and genetic diversity). Ecology was the most studied subject (66.7%), followed by genetics (20.4%) and behavior 12.4%). In relation to topics, studies relating to demography (22.38%), diet (19.40%), and genetic (16.42%) were the most reported followed by studies on activity budget (13.43%) and home range (11.94%). Studies about sleeping sites (5.97%), use of strata and substrates (5.97%), and seed dispersal (4.74%) were less assessed. Overall, the number of studies about BLTs was relatively low throughout the years (mean 2.2 ± SD 1.7) with a little peak between 1997 and 2003 (Figure 2.1).

Most of the studies were published in international journals (45.8%), covering all target topics searched here. These international publications comprise older papers (from the 1970s, 1980s, and 1990s) mainly on ecology and behavior, as well as more recent papers (2000s, 2010s, and 2020s) on genetics and ecology, combining climate, landscape models and conservation matters. Studies from Brazilian journals (23.7%) were published in Portuguese between 1970 and 2004, and focused mainly on behavioral and ecological issues, with special

interest in diet and seed dispersal. Theses comprised researches performed from 1976 to 2021 and included almost all target topics published in both English and Portuguese languages.

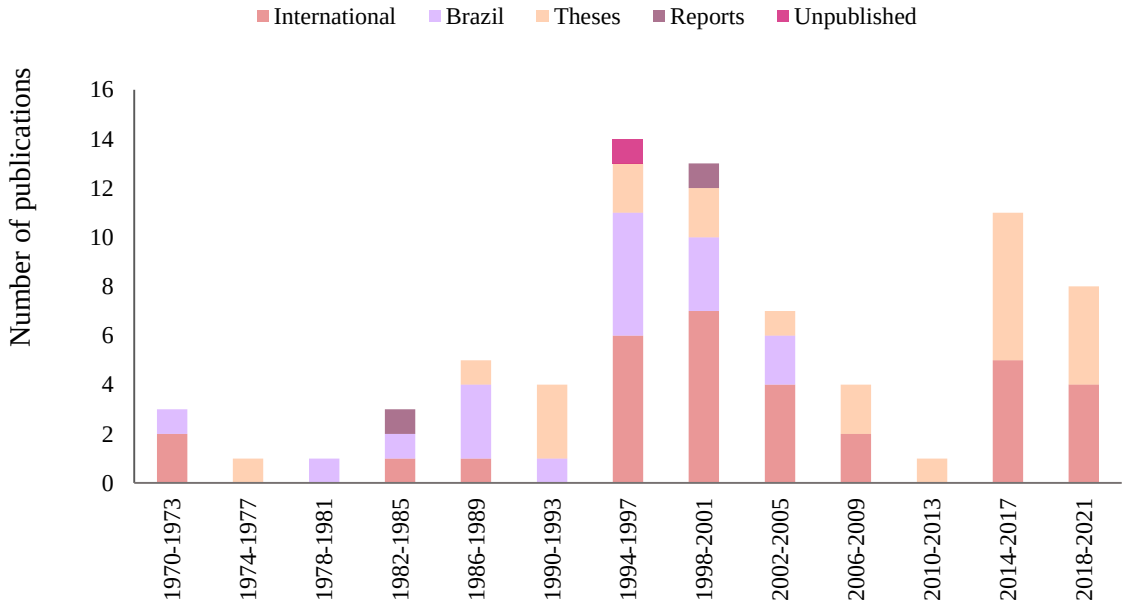


Figure 2.1 - Total number of studies performed from 1970 to 2021 for the black lion tamarin, comprising publications in international and Brazilian journals (International and Brazil respectively) theses, reports and one unpublished work, which was raised after a literature review on the searched topics.

2.3.2 Contemporary population occurrence sites

Similarly to other species of the genus (*L. chrysomelas*, Rylands, 1989; *L. rosalia*, Dietz et al., 1997), BLT seems to have a strong ability to survive in both preserved and disturbed environments. This ecological and behavioral flexibility has been of vital importance for the survival of this species, which is under constant environmental changes and pressures (VALLADARES-PADUA, 1993).

Currently, there are 14 populations of BLT registered after the rediscovery of the species in the wild in the 1970s; two of them are in the continuous areas of Morro do Diabo State Park (Lower Paranapanema), and Carlos Botelho State Park-Paranapiacaba (Upper Paranapanema). Six populations are in fragmented remnants with some degree of protection located in Lower, Middle and Upper Paranapanema regions: Black Lion Tamarin Ecological Station-Ponte Branca, Mosquito Farm (translocated population), Caetetus Ecological Station, Angatuba

Ecological Station, Paranapanema Ecological Station, and Capão Bonito National Forest. The other six populations are also located in the three regions of the Paranapanema, but in private rural properties: Santa Maria Farm, Santa Monica Farm, Rio Claro-Turvinho Farms-Borebi, Taquarivaí and Apiai-Guaçu rivers, Buri-Itapeva, and Guareí-Areia Branca rivers (Figure 2.2; Table 2.2).

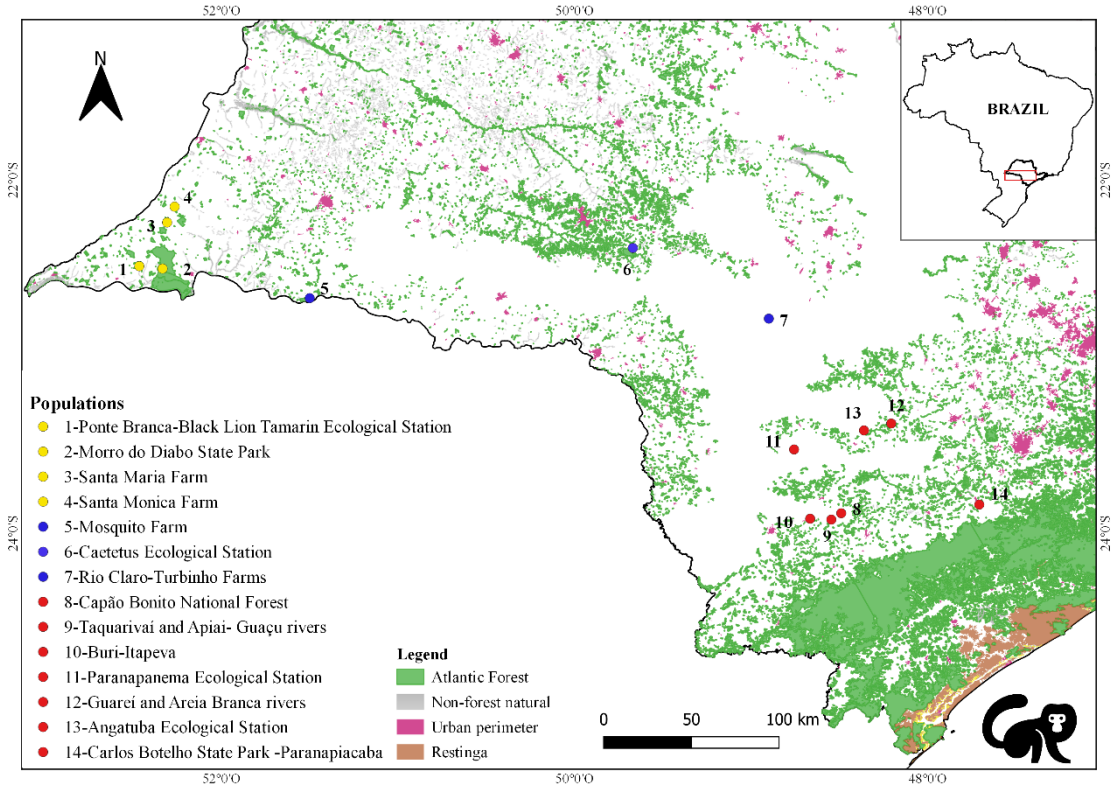


Figure 2.2 - Populations of black lion tamarin in the remnants of the Atlantic Forest of the Lower (yellow dots), Middle (blue dots), and Upper (red Paranapanema. Source: S.O.S. Mata Atlântica. Black lion tamarin icon was drawn by Gabriel Figueiredo from the Noun Project.

Table 2.2 - Summary of the current populations registered for the black lion tamarins (BLT) from 1970 to 2021, describing municipality, region of Paranapanema, remnant types, area in hectares (ha), estimated number of individuals (N), density of individuals per hectare, method used to estimate the population size and the source of the data. RPPNS: Private Natural Patrimony Reserve.

AREAS OF INTEGRAL PROTECTION								
Population	**Municipality**	**Region**	**Remnant**	**Area (ha)**	**N**	**Density**	**Method**	**Source***
Morro do Diabo State Park	Teodoro Sampaio	Lower	Continuous	35.000	1.142	0.035	Linear transect	Paranhos, 2006
Carlos Botelho State Park-Paranapiacaba	São Miguel Arcanjo	Upper	Continuous	32.000	320	0.010	Extrapolation	Forero-Sánchez, 2020
Caetetus Ecological Station	Gália	Middle	Fragment	2.254	35	0.016	Linear transect	Passos,1997
Angatuba Ecological Station	Angatuba	Upper	Fragment	1.394	46	0.033	Linear transect-Play Back	Culot, et al.,2018
FEDERAL CONSERVATION UNITS								
Population	**Municipality**	**Region**	**Remnant**	**Area (ha)**	**N**	**Density**	**Method**	**Source**
BLT Ecological Station-Ponte Branca	Euclides da Cunha Paulista	Lower	Fragment	1.306	46	0.035	Extrapolation	Forero-Sánchez,2020
Paranapanema Ecological Station	Paranapanema	Upper	Fragment	635	6	0.010	Extrapolation	Forero-Sánchez,2020
Capão Bonito National Forest	Buri	Upper	Riparian	357	35	0.098	Survey	Caldano et al, 2016
PRIVATE RESERVE OF NATURAL HERITAGE RPPNS								
Population	**Municipality**	**Region**	**Remnant**	**Area (ha)**	**N**	**Density**	**Method**	**Source**
Mosquito Farm	Narandiba	Middle	Fragment	1.534	14	0.008	Survey	Medici, 2001 Culot, et al., 2018
Rio Claro and Turvinho Farms	Lençóis Paulista-Borebi	Middle	Riparian	1.799	83	0.046	Extrapolation	Forero-Sánchez, 2020
PRIVATE RURAL PROPERTIES								
Population	**Municipality**	**Region**	**Remnant**	**Area (ha)**	**N**	**Density**	**Method**	**Source**
Buri-Itapeva	Buri, Itapeva	Upper	Riparian	4.933	163	0.033	Extrapolation	Forero-Sánchez, 2020
Taquarivaí and Apiai-Guaçu rivers	Taquarivaí	Upper	Riparian	2.831	277	0.097	Extrapolation	Forero-Sánchez, 2020
Santa Maria Farm	Presidente Epitacio	Lower	Fragment	515	18	0.035	Extrapolation	Forero-Sánchez, 2020
Santa Monica Farm	Presidente Epitacio	Lower	Fragment	484	3	0.007	Survey	Culot, et al., 2018
Guareí-Areia Branca rivers	Guareí	Upper	Fragment-Riparian	284	40	0.140	Extrapolation	Forero-Sánchez, 2020

* Extrapolation: values determined based on those from close fragments with similar characteristics or for fragments with no ecological studies on the area, the lowest population density recorded for the species was adopted. **Except for the work of Paranhos (2006), which estimated population density, all studies related to population size (N) estimates.

2.3.3 Population size and density

Accurate estimates of BLT population sizes are essential to better understand the specific needs of the species according to the region and the type of remnant, and hence guide the conservation programs that are in progress (CULLEN; RUDRAN, 2003; PARANHOS, 2006). In our review, we found relative density estimations, reported by individuals per hectare (ha) from groups with known population sizes, only for one population from the Lower (Morro do Diabo State Park), two from the Middle (Mosquito Farm and Caetetus Ecological Station), and one from the Upper (Capão Bonito National Forest) Paranapanema. For the other ten populations, the estimated number of individuals and densities were based on recent observations (see Table 2.2). In such cases, population size and density estimates were extrapolated through the observations as follows: (i) if a fragment did not have a prior population density estimate, the value was determined based on those from close fragments with similar characteristics (e.g., size, successional state, vegetation type, and possible connectivity); (ii) for fragments with no ecological studies on the area, the lowest population density recorded for the species was adopted to estimate population size (FORERO-SÁNCHEZ, 2020) .

The estimated densities for the total 14 registered populations of BLT ranged from 0.007 to 0.140 individuals per ha (mean 0.047 ±0.042). We observed that BLT densities in riparian forests (mean: 0.093 ±0.038; $F_{(2,11)} = 12.53$, $p=0.001$) were higher than in continuous (mean: 0.022 ±0.017; Tukey test, $p=0.018$) and fragment remnants (mean: 0.020 ±0.013; Tukey test, $p=0.001$). At the region level, the densities (Kruskal-Wallis $X^2=2.62$, df=2, $p=0.26$) showed no variations (Figure 2.3).

High densities in riparian forests could be an initial response of BLT to fragmentation, by crowding in the remnant habitat, before other threats such as hunting or pathogens can reduce its population (LINK et al., 2010). Moreover, it can also be the result of four non-exclusive factors: higher food productivity at riparian forest edges, competitive release when potential competitors are depleted or extirpated, absence of top predator, and the tolerance of BLT to habitat reduction and fragmentation (GONZÁLES-SOLIS et al., 2001; PERES; DOLMAN, 2000). However, high densities may have a negative effect on animals' fitness due to the higher susceptibility to parasites and diseases (ALLAN; KEESING; OSTFELD, 2003; BOYLE; SMITH, 2010; GILBERT, 1994) than those living in lower densities. We should point out that variations in BLT densities can also be attributed to sampling method inaccuracies (MENDES; KOPROWSKI; GALETTI, 2019; PARANHOS, 2006).The small number of sightings

documented in the studies and the relatively large sampling effort resulted in a high variation and a confidence interval that is close to double the average estimated.

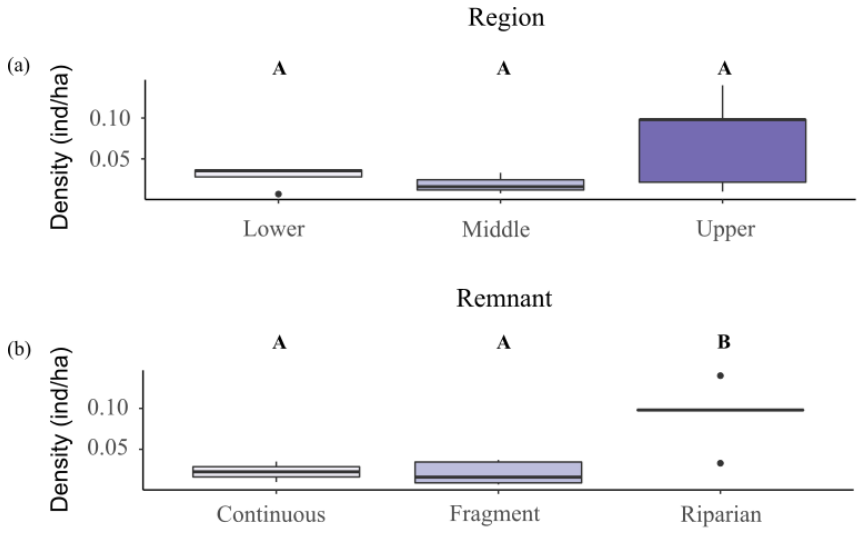


Figure 2.3 - Black lion tamarin densities observed in (a) the regions of Lower, Middle and Upper Paranapanema; and in (b) the different types of remnants (Continuous, Fragment and Riparian Forest). The horizontal lines show the medians; boxes limits indicate the 25th and 75th percentiles. Black dots represent outliers. Different letters represent significant different values ($p<0.05$).

2.3.4 Sampling effort

When we compared the sampling effort per region, we found that the Middle Paranapanema was the most studied one, registering 1.388 days with nine BLT groups analyzed. The Lower and Upper Paranapanema regions showed sampling efforts equal to 431 days on 7 groups, and 177 days on 5 groups, respectively. Regarding the types of remnants, the continuous forest represented by Morro do Diabo State Park showed a relatively low sampling effort, with a total of 329 days on 6 groups. The forest fragments were the most studied remnant type (1.024 days on 7 groups) with the Mosquito Farm and Caetetus Ecological Station as the areas presenting the largest sampling effort. Riparian forest totalized 644 days on 7 groups, with the Rio Claro and Turvinho Farms as the most studied site (Figure 2.4). These results indicate that populations from the Lower and Upper Paranapanema regions, and from the continuous forest remnants require greater sampling effort.

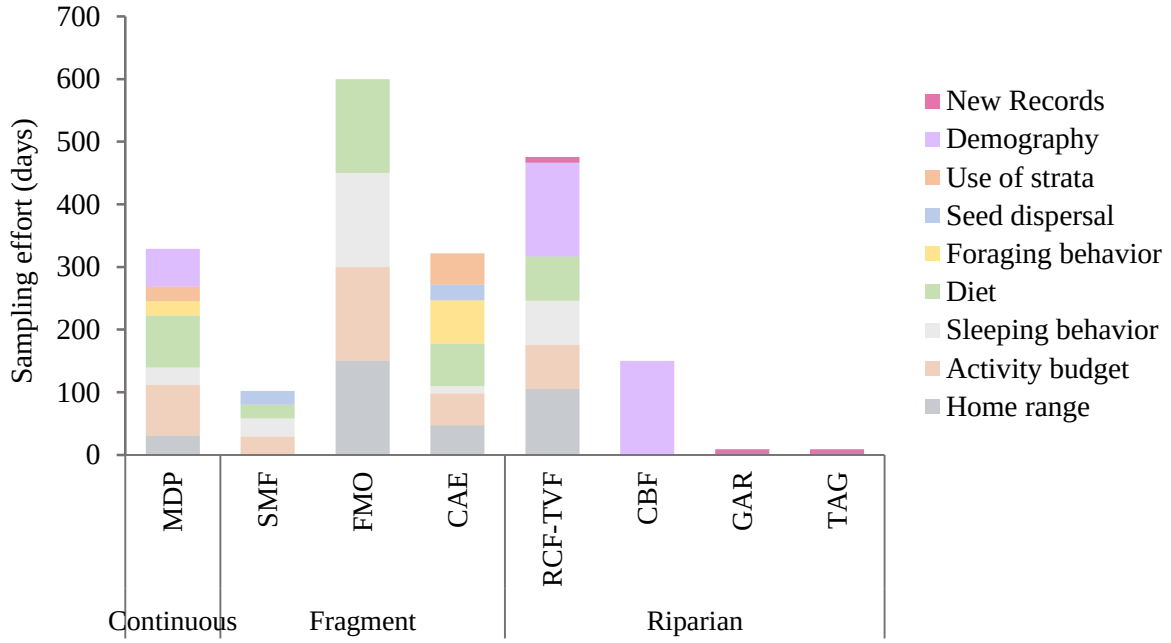


Figure 2.4 - Number of days sampled for each study site in relation to the areas assessed (Continuous, fragments and riparian forest). MDP: Morro do Diabo State Park; SMF: Santa Maria Farm; FMO: Mosquito Farm; CAE: Caetetus Ecological Station; RCF-TVF: Rio Claro and Turvinho Farms; CBF: Capão Bonito National Forest; GAR: Guareí-Areia Branca rivers; TAG: Taquarivaí-Apiai-Guaçu rivers;

2.3.5 Demography

For demographic inferences, we compiled and analyzed data from 23 BLT groups collected in 10 remnants from 1985 to 2015. The group sizes ranged from two to nine individuals, and the mean group size was 5.4 individuals ± 1.9. The mean composition of these groups was 2.4 ± 1.2 adult males (range 0–4; N=34), 1.4 ± 0.7 adult females (range 1–3; N=34), 0.8 ± 1.0 juvenile (range 0–3; N=34), and 1.1 ± 1.3 infants (range 0-4; N=34). Eleven groups had more than one adult female, 18 had more than one adult male, nine had no juvenile, and five groups had no infants.

According to evaluated regions, the group size in the Lower Paranapanema was smaller than in the Upper Paranapanema region ($F_{(2,34)}$ =4.18, p=0.023, Tukey test, p=0.030). Additionally, BLT groups in continuous forest had a lower number of individuals than in riparian forest ($F_{(2,34}$ =6.79, p=0.003, Tukey test, p=0.002). The proportion of adults and non-adults per group was similar ($F_{(2,31)}$ =1.11, p=0.34). The non-adult (juvenile and infants) proportion did not differ among regions (Kruskal–Wallis K^2=2.07, df=2, p=0.35) or remnants (Kruskal–Wallis K^2=0.97, df=2, p=0.61) (Figure 2.5).

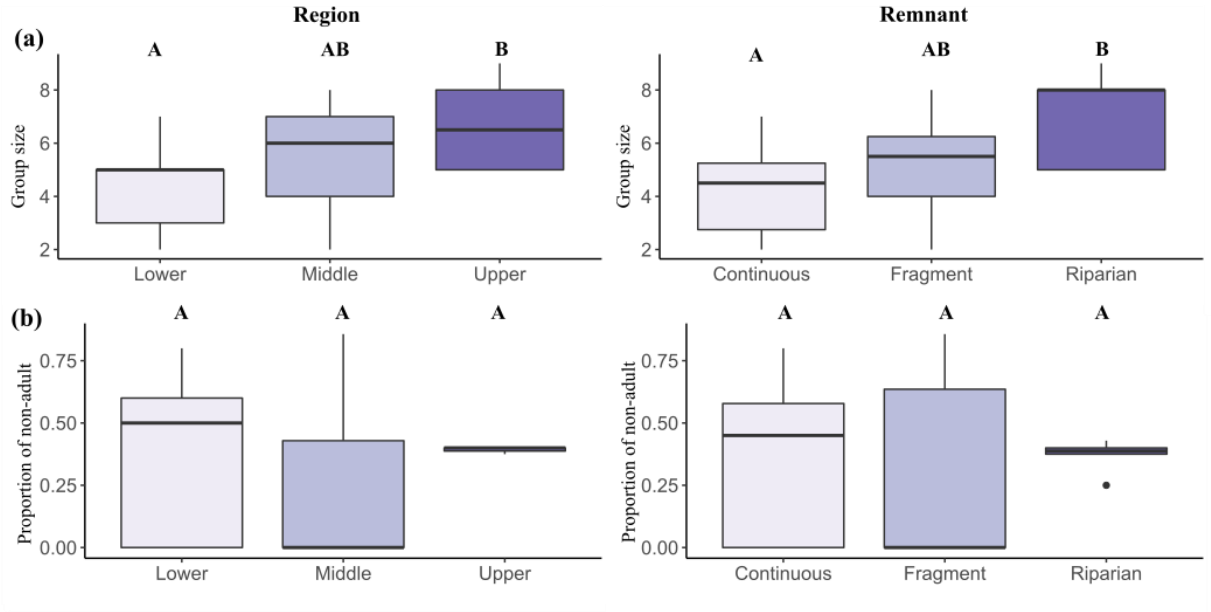


Figure 2.5 - Box plots showing (a) the variation of group sizes; and (b) the number of non-adult individuals according to the region (Lower, Middle, Upper Paranapanema) and the type of remnants (Continuous, Fragment, Riparian Forest). The horizontal lines show the medians; boxes limits indicate the 25th and 75th percentiles. Black dots represent outliers. Different letters represent significant different values ($p<0.05$).

Overall, the number of adult males did not differ from the number of adult females (X^2=11.36, d.f. =33, p=0.99) in a group. However, we found a sex-ratio more biased for males in the Middle and Upper Paranapanema (mean sex-ratio = 2.2 males: 1 female and 2.5 males: 1 female, respectively) when compared with Lower Paranapanema (mean sex-ratio = 1.2 males: 1 female), although the difference was significant only between the Middle and Lower Paranapanema (Kruskal–Wallis X^2=10.46, df=2, p<0.001; Wilcox p=0.005), possibly because the sample size in the Upper region was small (N=3).Similarly, considering vegetation types, the sex-ratio was also biased for males (Kruskal–Wallis X^2=11.95, df=2, p=0.003) in fragments (mean sex-ratio = 2.3 males: 1 female; Wilcox p=0.006) and riparian forests (mean sex-ratio = 2.2 males: 1 female; Wilcox p=0.01) when compared with continuous remnants (mean sex-ratio = 1.1 male: 1 female).

The differences in group size and adults' sex ratio in continuous (Lower Paranapanema) and fragmented sites (riparian and fragments) may be explained by the social behavior, which may vary according to the habitat type. In general, group size is thought to be a trade-off between the costs of reduced foraging efficiency and the benefits of lower predation risk (CHAPMAN; WRANGHAM; CHAPMAN, 1995; TERBORGH; JANSON, 1986). As a result,

when increasing the size of groups, the individuals' foraging efficiency is reduced, requiring them to spend more time moving in order to satisfy their nutritional needs (ONDERDONK; CHAPMAN, 2000). Consequently, in fragments or riparian forests, where it was observed a greater group size for BLT, increasing the area of foraging outside the boundaries of its home range can be harmful, because it would require traveling to open areas in search of resources, making them exposed to predators. On the other hand, increased demographic stochasticity, when populations decline, might imply variance in the number of births and deaths, causing adult sex ratios to be biased (UMAPATHY; KUMAR, 2003).

Also, adult male migration maybe reflects inbreeding avoidance (PUSEY; WOLF, 1996; STOW et al., 2001) due to higher levels of within-group relatedness. In addition, in cooperatively breeding primate species (e.g. callitrichids), when helpers are absents, the sex ratio appears to skew towards the helping sex, whereas when helpers are present, the sex ratio appears to be equal (BOULTON; FLETCHER, 2015; GOWATY; LENNARTZ, 1985; PEN; WEISSING, 2000; WEST; SHELDON, 2002). This sexual bias towards helpers is expected for males due to their increased investment in baby carrying and food provision (DUNBAR, 1995), which would lead them to disperse later by providing long-term assistance in the natal setting (MCGREW; MCLUCKIE, 1986) . Thus, our findings suggest that BLT groups seem to be more limited in their dispersal opportunities in fragmented and riparian forests, resulting in larger group size in these areas, a higher number of adult males per group, and male sexual bias.

Although the dispersion in tamarin species are common (GARBER et al., 1993), the sex-related dispersal strategies appear differ in both fragmented and continuous habitats| (e.g. *Leontopithecus rosalia*; PAULA, 2013). In 14 BLT groups, migration cases of individuals were reported in continuous, fragments and riparian forests. Thirteen cases of group input with mean of 0.67 ± 0.61 individuals per group, and 21 cases of group output with a mean of 0.94 ±1.10 individuals per group. The data also prevent us from estimating specific migration rates or determining the effects of mortality on group stability, although our analysis revealed that the continuous forest (N=7) had more cases of migrations-input than the fragments (N=4) or riparian forest (N=2). This appears to support our findings about greater dispersion (probably for males) in the continuous forest, which would keep the sex ratio in this remnant at 1:1 (Table 2.3).

According to the annual reproduction pattern, we observed that it was mostly seasonal. The births occurred from August to March, with a peak between November and December (70.6%), the warmest and wettest period of the year. There were only two reports of

reproductions in July, one in the Fazenda Mosquito in 2002 and another in Morro do Diabo in 2004. The number of births per group and per year varied from one to four with a mean of 1.68 ± 0.76. The number of births per female in each year was one, generally twins (Table 2.3), as commonly reported in callitrichids (HAIG,1999). Nevertheless, although breeding by secondary females is infrequent, we observed two cases in which two females reproduced in the same group during the same period in Morro do Diabo State Park and Mosquito Farm (GARCIA et al., 2020). Other BLT groups in the Angatuba region showed the same pattern, with four and three infants observed inside groups at the same time region (GARCIA et al., 2020).

While reproductive repression of subordinate females has been recorded in captive callitrichid studies (ABBOTT; HODGES; GEORGE, 1988; SALTZMAN; SCHULTZ-DARKEN; ABBOTT, 1996), secondary wild females also have been observed reproducing (*Callithrix jacchus*, Scanlon et al., 1988; *Saguinus mystax*, CULOT et al., 2011; GARBER et al., 1993); *L. rosalia*, (Dietz & Baker, 1993); *Saguinus fuscicollis*, GOLDIZEN et al., 1996; HERRERA; KNOGGE; HEYMANN, 2000). The change in mating system reported in lion tamarins might be related to isolation caused by habitat fragmentation and saturation of their habitat, resulting in a decrease in dispersal success and limitation of reproduction outside the natal group (BAKER; DIETZ; KLEIMAN, 1993). In addition, the quality of the habitat appears to be another factor that can lead to the abandonment of a monogamous mating system, because resources are more abundant and distribution patterns are more abundant in secondary and edge forests than in mature forests (RYLANDS; DE FARIA, 1993).

Table 2.3 - Number and mean of birth, number of infants per year and total number of deaths and migrations in relation to the study site and study period. Migration *in*, refers to the entry of individuals to a certain group. Migration out, refers to the output of individuals in a group.

Population	Region	Remnant	Period	Number of groups per site evaluated	Number of Infants per group	Total Births in the period	Ratio number of infants per birth	Total Death in the period	Total Migration *in*	Total Migration *out*
Morro do Diabo	Lower	Continuous	1986,2002 and 2004	3	4/4/2	5	2	-	7	2
Morro do Diabo	Lower	Continuous	2017 and 2019		-	3	1.3	-	-	-
Mosquito Farm	Middle	Fragment	1995-2006	4	14/6/6/6	19	1.68	30	4	6
Ponte Branca	Lower	Fragment	2005-2007	1		1	2	-	-	2
Caetetus	Middle	Fragment	1986 and 1990	1		-	-	-	1	1
Rio Claro Farm	Middle	Riparian	1993-1994	2	2/2	4	1	4	2	10
Rio Claro Farm	Middle	Riparian	2018 and 2019			1	2	-	-	-
Capão Bonito	Upper	Riparian	2012	2	2	1	2	-	-	-
Guareí	Upper	Riparian	2018 and 2020		-	2	2	-	-	-

The body mass data were collected for 55 individuals of 12 BLT groups from four populations: Morro do Diabo State Park, Ponte Branca, Mosquito Farm and Buri-Itapeva. The mean body mass varied from 300 g (infant; N=5) to 592 g (adult; N=17) in females, and from 325 g (infant; N=6) to 619 g (adult; N=21) in males (Table 2.4). There was no difference between body mass of adult males and adult females (Kruskal–Wallis K^2= 2.76, df=1, p=0.096). Also, body mass of adults did not differ among remnant types or regions (Kruskal-Wallis K^2=4.64, df=2, p=0.097; in this case all groups from continuous areas were in the Lower Paranapanema, from fragments in the Middle, and from riparian in the Upper). However, adult males were 5% and 3% larger than females in the riparian forest and in the fragments, respectively, when compared with continuous forest.

Table 2.4 - Mean and standard deviation of body mass 55 individuals of 12 BLT groups of Morro do Diabo State Park, Ponte Branca fragment, Mosquito Farm and Buri according to age and sex classes.

Age	Sex	Sample size	Weights (Mean ± SD)
Infant (<1 month)	Male	6	324.7 ± 84.53
	Female	1	300.0 ± 0
Juvenile (3-6 month)	Male	5	494.0 ± 61.9
	Female	5	440.0 ± 65.2
Adult (12+month)	Male	21	618.9 ± 70.04
	Female	17	591.9 ± 74.3

2.3.6 Home range

We gathered home range data from 13 BLT groups from the Lower and Middle Paranapanema (no data was found for groups in the Upper Paranapanema). The home range estimates were carried out using five methods with distinct sampling efforts, and, in some cases, for the same social group. Most studies used grid cell count as sampling method to home range estimates (N=6). However, minimum convex polygon (MCP), and Kernel methods have also been used for home range estimation (Supplementary Material, Table S2.2).

Considering all available data, home range of BLT groups ranged from 64 ha to 199 ha (mean: 114.35 ±42; N=8) in the continuous forest and from 54 ha to 118 ha (mean: 72.74±27, N=9) in the fragments. In the riparian forest there was no variation (mean: 40, N= 2). For comparison between regions and remnant types, we selected only the values estimated by MCP and Grid cell count. Also, for groups with more than one home range estimate, we used that value estimated with the greatest sampling effort. The home range size in the Lower Paranapanema was greater than in the Middle Paranapanema region (Mann-Whitney: U=24,

p=0.013). Regarding to remnant type, the home range size was greater in the continuous forest than in the fragments (Mann-Whitney: U=15, *p*=0.034) (Figure 2.6).

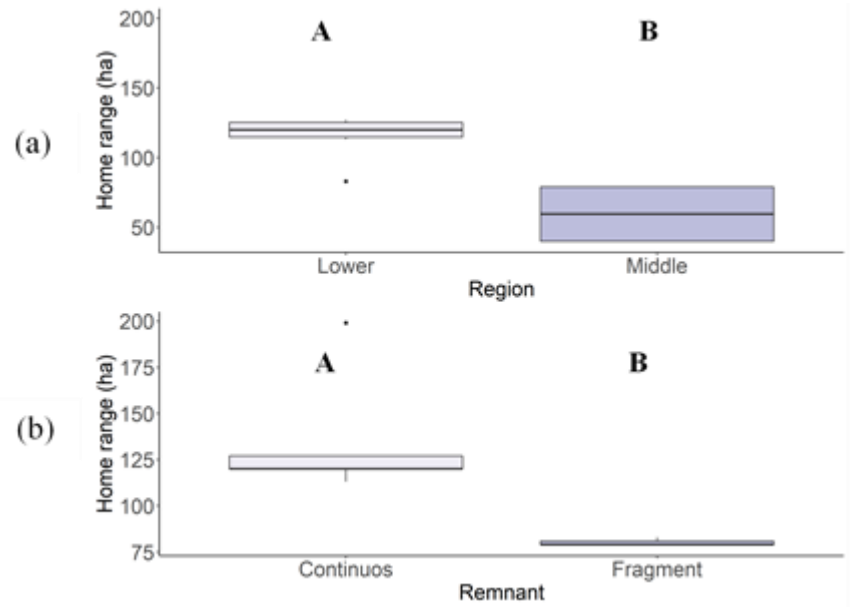


Figure 2.6 - Box plots showing the variation of home range size in populations of black lion tamarins considering (a) regions and (b) remnants types. The horizontal lines show the medians; boxes limits indicate the 25th and 75th percentiles. Black dots represent outliers. Different letters represent statistical different values ($p<0.05$).

The ability of primates to adapt home range size to habitat availability has been linked to their ability to survive in fragments (CRISTÓBAL-AZKARATE; ARROYO-RODRÍGUEZ, 2007). Similarly, the observed retraction of the home range in both fragments and riparian forest for BLT, may be a response to the reduced fragment size, the higher population density in these areas, and also, to patterns of resources availability. Furthermore, an increase in neighboring groups in the area, may increase territoriality and agonistic interactions, leading to a reduction in the home range area (LUDWIG; AGUIAR; ROCHA, 2005; NACIMENTO, 2008; PASSOS, 1997a). On the other hand, as long as agonistic encounters are avoided, groups should be able to expand their home range and maximize their chances of finding resources (PASSAMANI; RYLANDS, 2000). Thus, it is possible that in the continuous forest, the BLT has implemented a group strategy that allows them to spend more time and energy looking for resources in different locations, minimizing encounters with other groups and expand the home range, as observed in other callitrichids (PASSAMANI; RYLANDS, 2000).

2.3.7 Sleeping sites

We found four publications, from six social groups, that reported the plant species used as sleeping sites by the BLT (Supplementary Material, Table X). Groups of BLT can occupy a greater or lesser number of sleeping sites depending on the environment in which they occur. In the Lower Paranapanema, for example, the two groups observed were found using 25 and 14 hollow-type shelters, respectively (Carvalho et al., 1989). In the Middle Paranapanema, 36 (Caetetus Ecological Station; PASSOS, 1992), 57 (Mosquito Farm; MEDICI, 2001) and 32 (Rio Claro Farm; COSTA, 1997; MEDICI, 2001) hollows-type shelters have been identified in three distinct groups.

Although tree hollows are prevalent night shelter sites for BLTs, other types of sleeping sites were identified: seven lianas, and two palm tree canopy inserts (*Syagrus romanzoffiana*) for one group (Rio Claro Farm; COSTA, 1997) and, four lianas, and two palm tree canopy inserts (*Syagrus romanzoffiana*) for the other (Rio Claro Farm; MEDICI, 2001), suggesting that the use of hollows does not seem to be an ecological restriction for BLT groups living in secondary forests. Thus, the type and quality of the environment seem to be defining the sleeping sites of the groups. In addition, the smaller size of the home range area and the physiognomy of the riparian forests also seems to result in a smaller number of hollows used. This is due to the fact that relatively larger trees capable of forming hollows have been removed through time, leaving them with almost no hollows. Based on the whole records, 62 plant species were reported being used as sleeping sites by BLTs, with 16 species described in the Lower Paranapanema (continuous forest) and 46 species in the Middle Paranapanema (29 species in the fragments, and 17 species in the riparian forest). The most used species as sleeping site were *Tabebuia avellanedae* (Bignoniaceae, Morro do Diabo State Park) in the Lower, and *Endlicheria paniculata* (Lauraceae, Rio Claro Farm), *Aspidosperma polyneurun* (Apocynaceae, Caetetus Ecological Station), *Boungainvillea glabra* (Nyctaginaceae, Mosquito Farm) in the Middle Paranapanema. The species *Diatenopteryx sorbifolia* was commonly reported in the three remnants, *Terminalia* sp. was common in both continuous and riparian forest, *Aspidosmerma polyneuron* in continuous and fragment, and *Nectandra megapotamica* and *Alchornea triplinervia* in fragments and riparian forest (see the complete list in Supplementary Material 2, Table S2.3).

The mean or range height of the hollows was recorded only in two of the studies (MEDICI, 2001; PASSOS, 1992). At the Mosquito Farm (Middle and fragment), for example, a minimum height of 1 m and a maximum height of 15 m (mean=6.59 m; N=57) of hollow

entrance were observed (MEDICI, 2001). The minimum height was 0.94 m and the maximum were 14 m (mean=3.20 m; N=22) in Rio Claro Farm (Middle and riparian forest), and the height ranged from 3 to 30 m in Caetetus Ecological Station (Middle and fragment) (COSTA, 1997; PASSOS, 1992). For lianas the minimum and maximum height were 17.8 m to 42.3 m Rio Claro Farm (COSTA, 1997).

Considering all available data on hollow entrance height (CARVALHO et al., 1989; COSTA, 1997), we observed that the mean height of the entrances of hollows (up to 25 meters in continuous forest and 14 meters in riparian) were not different between remnant types or regions (Mann–Whitney: U=268, p=0.053) (all continuous data were from Lower and all riparian data were from Middle Paranapanema). The amount of information available about sleeping sites is scarce, and future studies enhancing our knowledge about sleeping site choice could make clearer the differences among remnant types.

Sleeping sites are a key resource for primate survival and they must select them according to strict criteria. Predators are the main determinants of the choice of a sleeping place, because sleeping sites tend to provide concealment and/or inaccessibility (ANDERSON, 1998). However, other variables, such as thermoregulation (ANDERSON; MCGREW, 1984; DAWSON, 1979), parasites (ANDERSON, 2000), territoriality and availability of resources competition (ANDERSON; MCGREW, 1984), comfort and hygiene (ANDERSON, 1998), foraging, proximity to water / food (CHAPMAN, 1989; COSTA, 1997; PASSOS, 1992) ranging patterns (TILSON; TENAZA, 1982), protection against rain (HEYMANN, 1995) are also referred to as important variables that affect their choices.

In general, most observations have shown that BLTs rarely return to the same tree they used the night before. However, in continuous forest (Morro do Diabo State Park, CARVALHO et al., 1989), fragments (Caetetus Ecological Station, PASSOS 1992) and riparian forests (Rio Claro farm, COSTA, 1997) it has been recorded that some sites have been used in two, four and 12 consecutive nights respectively. This pattern may be related to the shelter's position in relation to the availability of food in a given area what would reduce the distances travelled to food places and vice versa (COSTA, 1997; PASSOS, 1992).

2.3.8 Activity budget

We gathered activity data from 13 BLT groups collected in five forest fragments. Overall, the BLT groups dedicated 34 % of the time resting (N=15), 20% feeding (N=15), 19% moving (N=14), 12% foraging (N=15), and 2.3% in other activities such as social interactions

(N=9) (Supplementary Material 2, Table S.2.4). The time dedicated to rest was always the highest whatever the type of remnant (continuous: 36.2%, fragment: 24.78% and riparian: 52.1%) or the region (Lower: 35.5%; Middle: 31.8%).

The principal component analysis (PCA) of activity budget data revealed that the first two axes of the PCA explained 81% of the variability in activity budget (Table 2.5). While moving behavior was better explained by PC2, the other behavioral categories were better explained by PC1 (Table 2.5), thus we choose these two components to evaluate the activity pattern of BLTs among vegetation types and regions. There was not difference in PC1 (t=-1.10, df=12, p=0.293) or PC2 (t=-1.71, df=12, p=0.112) between Lower and Middle Paranapanema. Additionally, there was not difference in PC1 values ($F_{(2,12)} = 1.76$, $p = 0.213$), but significant differences in PC2 ($F_{(2,12)} = 6.36$, $p = 0.013$) among remnant types. PC2 values were higher in fragment than continuous forest remnants (Tukey test p=0.013), but not other comparisons (Tukey test p>0.05). These results indicate that although the pattern of activities of BLT groups are similar between regions, BLT groups from fragments spent more time moving than those from continuous vegetation type. The time spent travelling can be a good indicator of distances covered and the associated energy expenditure (ASENSIO et al., 2007) since they must change trees more often in order to satisfy daily feeding or resting requirements (MARTÍNEZ-MOTA et al., 2007) adjusting their behavior in fragmented forests.

Table 2.5 - Principal component analysis (PCA) results for the activity budgets in black lion tamarin groups. * Proportion represents the variation in activity budget explained by each PCA axis.

Components	Eigenvalue	Proportion*	Factor loadings				
			Moving	**Resting**	**Foraging**	**Feeding**	**Social interactions**
PC1	2.82	56.4	0.416	**-0.786**	**0.89**	**0.787**	**0.787**
PC2	1.23	24.6	**0.839**	0.204	-0.254	-0.435	0.483
PC3	0.64	12.9	0.288	0.54	0.253	0.367	-0.266

Behavioral studies evaluating other species of the genus *Leontopithecus* show similar patterns such as a greater proportion of time moving in relation to feeding, rest and foraging (*L. chrysomelas*, RYLANDS; DE FARIA, 1993) ; *L. rosalia*, PERES, 1986); *L. caissara*, PRADO, 1999). The presence of other resident groups in both the fragments and riparian forests could increase competition and defense for the territory, affecting the activity budget during these events (MEDICI, 2001; PERES, 1986). Moreover, possibly some fragments have

characteristics of successive forests and the fact that food resources are spread more widely will result in groups spending more time traveling than investing time in any other activity (MEDICI, 2001). Our results allow us to emphasize that time budget patterns cannot be generalized to BLT because they seem to be closely related to the spatial distribution of food resources in each habitat.

2.3.9 Use of strata and substrates

Five studies reported strata and substrates used by BLT during activities, all of them in the Morro do Diabo State Park and Caetetus Ecological Station and Rio Claro Farm. All studies used the scan sampling method for data collection, with different sampling efforts reported. BLT used from forest floor to canopy stratum, but they were registered mainly in the understory and middle strata (Supplementary Material 2, Table S2.5). In general, the studies reported that the forest floor was used to play and to search for insects. The middle strata were used to feed, move, forage and rest, while in the upper stratum, only to feed on fruits and gums (ALBERNAZ, 1997; PASSOS, 1994; PASSOS; ALHO, 2001).

Regarding the substrates, BLT was registered using lianas, branches, foliage, soil and trunk (COSTA, 1997; PASSOS; ALHO, 2001). In Caetetus Ecological Station, the branches and trunk were the most used substrates by BLTs to forage mainly on prey insects (45.52% and 22.39 % respectively) and to move and rest (36.49% and 32.76%) (PASSOS; ALHO, 2001). Foliage and trunks also are important substrates for BLT, because they were observed eating plant items and foraging (PASSOS; ALHO, 2001) . The foraging for prey was reported on palm fronds, in the sheaths of fronds, flowers, epiphytes, fallen dried seed pods and among clusters of fruits. Foraging species include *Syagrus romanzoffiana*, *Syagrus oleracea*, *Euterpe edulis*, and *Cariniana estrellensi*(PASSOS; KEUROGHLIAN, 1999). Vine entanglements, bamboo thickets, and materials in the process of decomposition, such as rotting logs, were among the other prey foraging microhabitats.

Observations in Caetetus Ecological Station show differences in the capture success of prey in relation to the season (dry vs. rainy; PASSOS; ALHO, 2001). During the dry season, the BLT spends more time looking for *Cariniana estrellensi* species dry fruits, spider web, and sprouting foliage. However, substrates as hollows, palm, bamboo, lianas, bark and epiphytes showed no seasonal variation in prey capture (PASSOS; ALHO, 2001). In this sense, prey foraging also seems to be highly adaptable, as evidenced by the use of a wide variety of microhabitats and forest types (PASSOS; KEUROGHLIAN, 1999; VALLADARES-PADUA,

1993), and seems to be adapted to patterns of prey availability (daily, weekly, and seasonally) (KEUROGHLIAN; PASSOS, 2001). This reinforces the idea that this species is more flexible than other members of the genus, and evidence of its plasticity in response to environmental and seasonal factors in the BLT distribution area.

2.3.10 Diet

We gathered a total of 187 food records for 14 social groups from 10 publications, in which 62.56% (N=117) corresponded to fruit consumption (ripe and unripe pulp), 27.27% to gum (N=51) (polysaccharides of natural origin, which is found in the woody parts of plants or in seed coatings), 7.48% to invertebrates (N=14) and 1.60% other items, such as flowers, nectar and vegetative parts (N=3) (Supplementary Material 2, Table S2.6). Insects of the Coleoptera and Blattaria orders were the most consumed animals, in addition to insects from the Phasmida, Hemiptera, Orthoptera and Lepidoptera orders. Among the rare registers, anurans were reported twice.

In sum, the diet of black lion tamarin includes at least 98 fruit species and 39 gum species, belonging to 35 and 22 families respectively. Myrtaceae (27 species), Moraceae (10 species), Fabaceae (five species) and Cactaceae (six species) provided the largest numbers of fruit species in the BLT diet. With regard to gum, the most frequently reported families were Fabaceae (seven species) and Rutaceae (four species). BLTs also eat flowers, twigs and nectar from the Cactaceae, Bignoniaceae and Euphorbiaceae families, but these items were registered only in the diet studies developed in the Caetetus Ecological Station.

From total plant species registered, only 11 were consumed by groups from both Lower and Middle Paranapanema. Also, we observed relatively low overlap in the plant species consumed by BLT according to remnant type, with more species shared between continuous and riparian forests (Figure 2.7). Only two species were common in the diet of three areas: *Syagrus romanzoffiana* and *Enterolobium contortisiliquum*.

Considering the total richness of plant species registered in BLTs' diet per group (mean: 18.1 ± 13.5 SD, range: 1-53, N=13), group from fragments in the Middle Paranapanema region showed the most diverse diet (Figure 2.7), although few data is available in both regions, preventing complete list from being obtained. However, no differences were observed when comparing the diversity of plant species consumed standardized by number of days of sampling effort between regions (t =-1.54, df=4.33, p=0.191) or among remnants ($F_{(2,10)}$=3.73, p=0.061).

Our findings suggest that in places fruit scarcity, BLT appears to temporarily adjust the diet to available resources by consuming a more diversified diet (MEDICI, 2001; SUSSMAN; KINZEY, 1984; VALLADARES-PADUA, 1993) , which is the most common response to food scarcity in primates (HEMINGWAY; BYNUM, 2005). Behavioral flexibility, such as the ability to switch diets and increase consumption of other items, may be a key adaptive response to seasonal environments, reducing the effects of seasonal fruit scarcity(NORCONK, 2011). As a result, in areas where high quality food, such as fruit, are scarce, foraging efficiency must be maximized by including low-quality foods (items that taking more time and energy to digest, e. g. plant parts) in the diet (CASELLI; SETZ, 2011; SUSSMAN; GARBER, 1987).

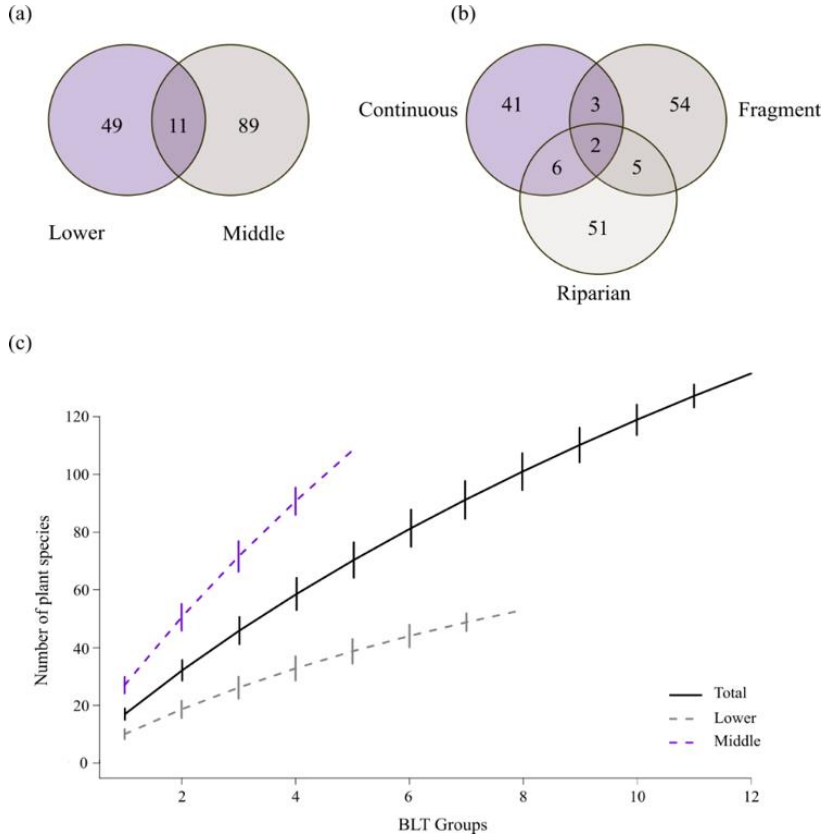


Figure 2.7 - Number plant species consumed (fruits, gum, vegetative parts) by black lion tamarins showing the overlap in the consume of species per (a) region; (b) type of remnant; and (c) the rarefaction number of plant species consumed by BLT groups in the Lower and Middle Paranapanema.

The percentage of total time spent feeding has only been documented for 6 social groups. Fruits were always the most consumed item, regardless of region (Lower Paranapanema mean: 75.10% ± 9.43 SD; N=4); Middle Paranapanema mean: 72.79% ± 5.43 SD; N=2) or remnant type (Continuous mean: 75.10% ± 9.43 SD; N=4; Fragment: 68.95%; N=1; Riparian:

76.64%; N=1) as observed with other species of family Callitrichidae (CARDOSO et al., 2011; EGLER, 1992; LAPENTA; PROCÓPIO DE OLIVEIRA; NOGUEIRA-NETO, 2007; PERES, 1993; RYLANDS, 1989). Interestingly, only one to six fruit species per studied group accounted for 50% of the overall feeding representation, resulting in a total of 27 species (Table 2.6).

Table 2.6 - Percentage contribution of the most consumed (sum > 50%) plant species per item ingested (fruit and gum) by each studied group of black lion tamarins. MD= Morro do Diabo State Park; CAE = Caetetus Ecological Station; Fmos =Mosquito Farm; FRC= Rio Claro Farm.

Food item	Region & Remnant	Group ID	Family	Species	Records of consumption (%)	Sampling Effort (days)	Study period	References
Fruit	Lower Continuous	MD16	Myrtaceae	*Myrceugenia ovata*	15.5	60	Jul 1989-Jun 1990	Valladares-Pádua, 1993
			Arecaceae	*Syagrus romanzoffiana*	10.2			
			Myrtaceae	*Campomanesia* sp.	8.9			
			Rutaceae	*Helietta longifoliata*	7.6			
			Meliaceae	*Cabralea canjerana*	5.9			
			Myrtaceae	*Myrcia* sp.	4.8			
		MD17	Myrtaceae	*Eugenia uvalha*	10.9	60	Jul 1989-Jun 1990	Valladares-Pádua, 1993
			Arecaceae	*Syagrus romanzoffiana*	10.9			
			Annonaceae	*Xylopia brasiliensis*	10.7			
			Myrtaceae	*Myrcia* sp.	10.3			
			Myrtaceae	*Eugenia* sp.	5.8			
			Moraceae	*Ficus enormis*	5.3			
		PMD18	Myrtaceae	*Eugenia* sp.	22.3	60	Jul 1989-Jun 1990	Valladares-Pádua, 1993
			Arecaceae	*Syagrus romanzoffiana*	19.4			
			Myrtaceae	*Myrcia* sp.	14.4			
		PMD19	Myrtaceae	*Myrcia* sp.	30.0	60	Jul 1989-Jun 1990	Valladares-Pádua, 1993
			Myrtaceae	*Psidium* sp.	13.9			
			Arecaceae	*Syagrus romanzoffiana*	10.4			
	Middle Fragment	CAE5	Arecaceae	*Syagrus romanzoffiana*	43.45	53	Jan-Jun 1989; May 1990-Jan 1991	Passos,1999
			Cannabaceae	*Celtis iguanaea*	10.31			
		FMos7	Anacardiaceae	*Tapirira guianensis*	19.59	150	Mai-Jun 1993;	Medici,2001
			Annonaceae	*Rollinia sylvatica*	16.97		Oct-1993;	
			Cactaceae	*Pereskia aculeata*	16.10		Jan 1996- Feb 1998	
	Middle Riparian	FCR4	Arecaceae	*Syagrus romanzoffiana*	48.15	36	Nov 1992-Oct 1993	Mamede Costa,1997
			Moraceae	*Ficus* sp.	7.05			
		FCR5	Arecaceae	*Syagrus romanzoffiana*	47.94	105	Jan 1993-Dec 1994	Mamede Costa,1997
			Moraceae	*Ficus insipida*	10.11			
Gum	Middle Fragment	CAE5	Rutaceae	*Pilocarpus pauciflorus*	70.11	53	Jan-Jun 1989; May 1990; Jan 1991	Passos,1999
		FMos7	Fabaceae	*Enterolobium contortisiliqu*	44.16	150	Mai-Jun 1993;	Medici,2001
			Fabaceae	*Inga uruguensis*	6.81		Oct-1993; Jan 1996- Feb 1998	
	Middle Riparian	FRC4	Combretaceae	*Terminalia* sp.	24.24	36	Nov 1992-Oct 1993	Mamede Costa,1997
			Euphorbiaceae	*Croton urucurana*	17.41			
			Myrsinaceae	*Rapanea umbellota*	14.27			
		FRC5	Combretaceae	*Terminalia triflora*	26.03	105	Jan 1993-Dec 1994	Mamede Costa,1997
			Euphorbiaceae	*Croton urucurana*	25.41			

Comparing regions, more fruit plants species was included among the most consumed species in the Lower Paranapanema than in the Middle (Wilcoxon, W=15, *p*=0.047), indicating the BLT's needs for seeking for more diverse fruit resources to achieve their minimal requirements in fragment or riparian remnants. In the Lower Paranapanema and Continuous Forest for example, the most consumed families were Myrtaceae and Arecaceae. For the Middle Paranapanema, Arecaceae and Moraceae were the most important families in the diet (Table 2.6).

When fruit availability is lower, tamarins enrich their diet by eating insects and gum, as a complementary source of their diet (FERRARI; LOPES, 1989; RYLANDS, 1982; VALLADARES-PADUA, 1993). However, only in the middle of Paranapanema the percentage of gum consumption was reported, and the most consumed families were Fabaceae, Combretaceae and Euphorbiaceae. There is no reported diet data for Upper Paranapanema so far. Due to few available data, we cannot compare BLT diet among remnant types. However, in Continuous forest, it was observed an opportunistic consumption of exudates that increased during fruit shortage (VALLADARES-PADUA, 1993). Also, in some fragments (Mosquito Farm) or riparian forests (Rio Claro Farm), the gum is not only used as an alternative source in the dry season, but in varying proportions throughout the year (COSTA, 1997; MEDICI, 2001). It has also been reported notable differences in gum consumption by BLTs in comparison with other species of the genus (RYLANDS, 1996). Animal prey, on the other hand, represents an important item in the diet of BLT which actively manipulate forest floor to search for insects. Unlike other lion tamarins (*L. rosalia*, RYLANDS, 1993; *L. chrysomelas*, FERRARI, 1993), which depend on bromeliads to capture their prey, BLTs search for and capture prey on a number of substrates (as shown previously in the topic use of stratum and substrate). In this sense, despite the fact that lion tamarins evolved to explore mature forests, fragmentation caused them to occupy forests at different levels of succession (RYLANDS, 1993) and have adapted their diet to factors like: habitat quality, plant-species composition, abundance, temporal availability, spatial distribution as well as the overall seasonality of ripe fruit production (PERES, 1993; RABOY; CANALE; DIETZ, 2008) that are important factors that may contribute to the interspecific variation in diet of BLT.

2.3.11 Seed dispersal

We found two studies for two fragments: Santa Maria Farm (ALCOLEA, 2016) and Caetetus Ecological Station (PASSOS, 1997b). In both studies, samples of feces were collected

in all events of defecation by the individuals, and then seeds were retrieved for germination monitoring. Seed sizes were reported only for the group of Santa Maria Farm, as ranging from 1 to 24.5 mm long, and from 1 to 11.5 mm wide (N=20) (ALCOLEA, 2016) (Supplementary Material, Table S2.7). There were 188 defecation events reported for this fragment, with 93 % (N=175) having seeds in their feces. Small seeds (<3.0 mm) were deposited in greater numbers by BLT than medium (approximately 3 to 12.0 mm) or large (>12.0 mm) seeds. This relationship was also reported to other Callitrichidae species, such as *C. jacchu*s, *Saguinus niger* (CASTRO et al., 2003), *L. rosalia* and *L. chrysomelas*(CARDOSO et al., 2011), which evidenced the smallest seed being eaten and the biggest one being discarded .

The number of seeds contained in the feces ranged from one to 1500, with one to four different plant species per feces. In the feces collected during the two studies, seeds from 16 plant species belonging to 12 families were identified, with seven of these species (43.75 %) in the Santa Maria fragment and nine in the Caetetus ecological station (56.25 %). The Cannabaceae, Moraceae Myrtaceae families, with three and two species respectively, were the most representative.

BLT dispersed seeds of a large proportion of the fruit species they handled. From 63.63% (ALCOLEA, 2016) to 100 % (PASSOS, 1997b) of ingested fruits species were detected in their feces (Supplementary Material 2, Table S2.7). *Syagrus romanzoffiana* was the only species in the Santa Maria Farm that was not found in feces, owing to the largest dimensions (36.1 mm long and 1 to 23.4 mm wide; ALCOLEA, 2016). Also, it was reported that the distance of deposition varied from 4 to 828 meters with a mean 343.8 m ± 225.8 (N=37), considering the mother tree that produced the defecated seeds(ALCOLEA, 2016). However, 70% of the seeds deposited until 450 m from the mother tree. Regarding the homogeneity of the deposit places, the greatest number of deposited seeds occurred near the upper edge of the home range, in comparison to the central region or near the lower edge (ALCOLEA, 2016). Also, the deposition of feces in the home range was not at random, but rather depended on the location of food trees (up to approximately 210 m) and sleeping/resting trees (up to approximately 100 m) (Santa Maria Farm; ALCOLEA, 2016). This pattern seems to be common in some groups of lion tamarins (KIERULFF; PROCÓPIO-OLIVEIRA; BECK, 2002). The concentration of seeds around the feeding and resting/sleeping places may indicate a high probability of defecation after the periods of resting (ALCOLEA, 2016). Also, diurnal resting sites are more likely to be found in areas with a high density of fruiting trees, and, therefore, this location can vary seasonally (MUÑOZ et al., 2011). Additionally, the daily range

may change the spatial pattern of seed distribution in relation to the original plant distribution, when groups travel to the borders of their ranges during territorial encounters as reported to some groups of lion tamarins (KIERULFF; PROCÓPIO-OLIVEIRA; BECK, 2002). Consequently, primates' presence in fragmented habitats will benefit regeneration, particularly when they cross habitat borders and distribute seeds to habitats other than the one in which they were ingested (CHAPMAN; RUSSO, 2005).

In Caetetus, the germination experiments were carried out by separating the seeds contained in the feces and allowing them to germinate on filter paper, kept damp and under natural light and temperature conditions. As a control, seeds from the same fruit species were collected and germinated under the same conditions (PASSOS, 1997b). In the Santa Maria fragment, germination was also examined under natural light and temperature, but in a vivarium. The authors evaluated three treatments: seeds from fruits with pulp, seeds from fruit without pulp and seeds retrieved from the feces (ALCOLEA, 2016). From both evaluated BLT groups, less than 50% percentage of plant species had seed germination rate higher in feces than in control treatments. At Caetetus Ecological Station four from nine species (PASSOS, 1997b), and at Santa Maria three from nine species (ALCOLEA, 2016), showed a higher seed germination rate after passing through the digestive tract. No cases in which seeds from fruits had a higher rate of germination were reported.

Primates that include a significant fraction of insects in the diet (frugivore-insectivores and insectivore-frugivores), appear provide low dispersal quality (FUZESSY et al., 2015). According to Fuzessy et al. (2015), the germination percentage is not significantly affected as the seed passes through the frugivore-insectivore primate tract guts and an increase of 18% in the average germination time was observed. However, the BLT pattern seems to be contrary because the germination rate in each fragment was very high. Also, interestingly, 70% of the feces that contained seeds were deposited at a relatively far distance from the mother tree (until 450 meters) contrary to what was observed for other *Leontopithecus* spp. (e.g., *L. rosalia*, mean=107.5 ± 97.4 m, Lapenta, 2006). This is important because seeds planted near parents have a lower chance of germination and seedling establishment than seeds planted farther out (BALCOMB; CHAPMAN, 2003). In this context, despite the fact that only two studies were evaluated, we must emphasize BLT's ability as a disperser of many plant species in its home range. Therefore, we may suggest that the absence or decline of BLT populations, as with other dispersers, contributes to a reduction in plant populations growth (DALLING et al., 2002), a gene flow disruption between plant populations (BACLES; LOWE; ENNOS, 2006), and an

increased likelihood of extinction of plant species that depend on them (CORDEIRO; HOWE, 2003; GALETTI et al., 2006; TRAVESET; GONZÁLEZ-VARO; VALIDO, 2012).

2.3.12 Genetics

We found 10 studies addressing genetic diversity issues in BLTs to date; of which five are scientific articles and five are theses (Supplementary Material 2, Table S2.8). The first genetic studies were performed in the 1980s, and analyzed BLTs from captive and wild individuals using allozymes (FORMAN et al., 1986; VALLADARES-PADUA, 1987). After a hiatus of about two decades, Perez-Sweeney et al. (2005) described for the first-time microsatellite loci for the species, generating genetic diversity information for few individuals from Lower Paranapanema. About ten years later, new genetic data were produced for a wild population from Upper Paranapanema (CALDANO, 2014). However, population genetic approaches in BLTs have intensified only from 2015 onwards, when three theses (AYALA-BURBANO, 2015; JAVAROTTI, 2021; VELTRONI, 2018) and two scientific articles (AYALA-BURBANO et al., 2017, 2020) were published in a broader number of captive and wild populations.

The scarce amount of genetic data for the species is possibly due to the difficulty to capture of obtaining biological samples. In captivity, population sizes are very reduced as well (10-33), and reproduction does not occur regularly, thus ex-situ groups have been managed to retain genetic diversity and avoid inbreeding depression (AYALA-BURBANO et al., 2017, 2020). Translocations among the institutions that keep the species under human care have been occurring since the first captive group was founded in 1973. Despite that, the captive groups present high inbreed rates, because they have a common origin based on the founder population that was raised from just seven wild individuals from Morro do Diabo State Park, although few wild individuals from other regions (e.g., Ribeirao Bonito, Sorocaba, Buri, and Angatuba) have been brought to captivity throughout the years (AYALA-BURBANO et al., 2017, 2020).

Genetic diversity estimators based on microsatellite markers for both ex-situ and in-situ populations are low (Supplementary Material 2, Table S2.8), but comparable to those from the other *Leontopithecus* species (GALBUSERA; GILLEMOT, 2007; GRATIVOL; BALLOU; FLEISCHER, 2001; MARTINS et al., 2011; MARTINS; GALETTI, 2011; MORAES et al., 2017; PEREZ-SWEENEY et al., 2005). When we compared genetic diversity estimated by allozyme markers, BLT populations evidenced very lower levels than to those estimated by microsatellites, showing mean expected heterozygosity (He) values ranging from 0 to 0.003,

using, respectively, 25 and 47 loci, for 16 individuals from captivity, and seven individuals from Morro do Diabo and Caetetus Ecological Station (FORMAN et al., 1986; VALLADARES-PADUA, 1987).

Diversity genetic data can depend on the nature of the type of molecular markers, in which allozymes are considered less polymorphic than microsatellite ones (TURLURE; VANDEWOESTIJNE; BAGUETTE, 2014), but also may reflect the life history of the species (SPITZE, 1993), that in such case has been suffered the negative impacts of the habitat loss and fragmentation, and, consequently, population size reductions and stochastic genetic events, in which endogamy, bottleneck and drift effects are enhanced. Therefore, even when genetic diversity parameters are estimated by more polymorphic markers, low levels of genetic variation can be observed (FRANKHAM, 2005; HARTL; CLARK, 2007; SPIELMAN; BROOK; FRANKHAM, 2004).

When 15 neutral homologous microsatellite markers were used in BLT (Perez-Sweeney el al., 2005), the results showed that microsatellites are much more polymorphic than allozymes indeed, revealing a mean expected heterozygosity of 0.295 for 14 individuals from Morro do Diabo (N=9) and from a central region of Sao Paulo state (N=5). However, only nine loci evidenced polymorphism. Posteriorly, 10 individuals from Capão Bonito National Forest were analyzed by Caldano (2014) using 10 of these homologous microsatellites, eight loci described for L. chrysomelas (GALBUSERA; GILLEMOT, 2008), and two for L. rosalia (GRATIVOL; BALLOU; FLEISCHER, 2001). From the total of 20 loci tested, 14 showed polymorphisms, evidencing higher heterozygosity level than those observed by Perez-Sweeney et al. (2005).

In 2015, new heterologous microsatellites were prospected through in silico mining in genomic data available for *C. jacchus* (PARDO, 2015). Sixty loci showed satisfactory amplification patterns, however, after genotyping only three markers were polymorphic (PARDO, 2015). Two years later, Ayala-Burbano et al. (2017) tested a set of 22 loci and choose 15 makers evidencing the most suitable Polymorphism Information Content (PIC) values to be employed for genetic diversity and population structure analyses in captive and wild populations of BLTs. Thus, the Brazilian and European captive groups were compared with the same wild population previously evaluated by Caldano (2014), using now a standardized panel of microsatellites: eight homologous (PEREZ-SWEENEY et al., 2005), and seven heterologous, three developed for *L. rosalia* (GRATIVOL; BALLOU; FLEISCHER, 2001) and four for *L. chrysomelas* (GALBUSERA; GILLEMOT, 2007).The results revealed expected

heterozygosity values ranging from 0.410 to 0.462 for captive and wild groups, with no significant differences among both.

Genetic structure analyses evidenced three main clusters suggesting that the Brazilian and European captive groups are markedly differentiated among them and from the wild population of Capão Bonito National Forest (AYALA-BURBANO et al., 2017). From then on, other studies have been used this same set of informative microsatellites for genetic approaches in BLTs, although some loci have evidencing absence of polymorphism or unsuccessfully amplification patterns mainly for wild individuals and feces/hair samples, respectively (Freitas PD, personal communication).

In 2020 an integrative analysis was carried out in captive groups for assessing genetic diversity and driving management through molecular and studbook data (AYALA-BURBANO et al., 2020). The authors were able to infer the initial diversity of the founder population (F0) by integrating the remaining genetic diversity assessed by pedigree and microsatellite data for the extant generation (F8). In addition, it was also suggested to evaluate mating-pairs using a molecular index (Individual heterozygosity-IR), as a complementary parameter to the Mate Suitability Index (MSI), which is based only on genealogical data. The IR index indicated that some individuals with low values of MSI had high heterozygosity, besides allele representativeness, and consequently were valuable as breeders.

In nature, Javarotti (2021) has assessed genetic diversity estimators for new sampled wild populations in addition to monitoring the captive groups throughout generations. The mean values of expected and observed heterozygosity, and inbreeding coefficients did not differ across captive generations. However, alleles have been lost, resulting in a decline in allelic richness. On the other hand, rare alleles that were unique to the Brazilian captive population are now shared with the European group, due to the recent translocations among institutions (AYALA-BURBANO et al., 2020). Regarding the wild populations, Santa Maria Farm and Guareí and Areia Branca rivers showed expected and observed heterozygosity, inbreeding, and allelic richness values different from those reported for Capão Bonito National Forest, evidencing genetic structure according to the population differentiation analysis (JAVAROTTI, 2021).

Interestingly Capão Bonito (He=0.436) and Guareí (He=0.215), both riparian forests from Upper Paranapanema, evidenced respectively higher and similar values of expected heterozygosity than those found in Morro do Diabo (He=0.295), a continuous forest remnant. Such data suggest that riparian forests may also play an important role for the BLT groups,

since the structural characteristics of riparian forests allow for long-distance genetic flow, resulting in higher genetic diversity in these habitats than in other vegetation formations (PUTH; WILSON, 2001).

Another interesting approach reported by Javarotti (2021) combined microsatellite and mitochondrial data to infer about social and reproductive behavior in nature. Matrilineal genetic structure through mitochondrial markers has previously been performed using D-loop markers in both captive and wild groups (VELTRONI,2018) . This study revealed haplotype networks and historical genetic diversity patterns, that combined to kinship relations established by microsatellite provided insights into the BLT cooperative breeding system and historical connectivity. As brief examples, the authors found haplotypes sharing among populations from the Lower and the Upper Paranapanama, suggesting the existence of ancestral haplotype in distant areas currently unconnected which possibly might have kept gene flow in the past (JAVAROTTI, 2021; VELTRONI, 2018). In addition, it was observed that infants and/or juveniles from a same group had different haplotypes, indicating the existence of polygyny in BLT, system that has already been reported for *L. rosalia* (BAKER; BALES; DIETZ, 2002; SALES, 2009), and more recently also suggested for *L. chrysopygus* (GARCIA et al., 2020), a species previously considered as monogamic (RYLANDS, 1996).

Other genetic studies in *Leontopithecus* species have been also used for addressing ecology (MORAES et al., 2018), ethology (MARTINS et al., 2015), and evolutionary issues (FREITAS et al., 2018), besides conservation and management (MORAES et al., 2017). Genetic data can offer insights into dispersion and parentage, aiding to better comprehension of relevant aspects related to social interactions and reproduction (DOLOTOVSKAYA; ROOS; HEYMANN, 2020). In addition, genetic diversity estimates can be useful to predict the viability of populations across generations (Martins et al., 2011), providing relevant data to simulate how populations may respond to a bottleneck event in terms of retaining genetic diversity and evolutionary potential to adapt to environmental changes (BISHOP et al., 2009; CORTI et al., 2011; TAYLOR; JAMIESON, 2007).

When we simulated the trend of genetic diversity in terms of number of alleles and observed heterozygosity for the population of Capão Bonito National Forest, the wild population that evidenced the higher values of heterozygosity (see Supplementary Material, Table S2.8), we predicted loss in genetic diversity over the next 100 years (Figure 2.8). We observed that the simulated values for observed heterozygosity (HO) decrease faster than the simulated values for the allele diversity (OA). In all simulated bottleneck scenarios, HO was

projected to drop below the 90% threshold in 40 years, but not OA. As a consequence, even if the population size is constant over the next 100 years, the levels of genetic diversity will decline, putting the population's variability in risk. These results could reflect the low allelic richness and/or the low frequency of rare alleles observed in the Capão Bonito population. It is expected that in the presence of a high frequency of rare alleles, these will be rapidly lost while intermediate- and high-frequency alleles will be preserved (FUERST; MARUYAMA, 1986). Consequently, the heterozygosity will decline more slowly than allele number. However, in our analysis we found opposite results, but similar to other studies reported for endangered species (CORTI et al., 2011).

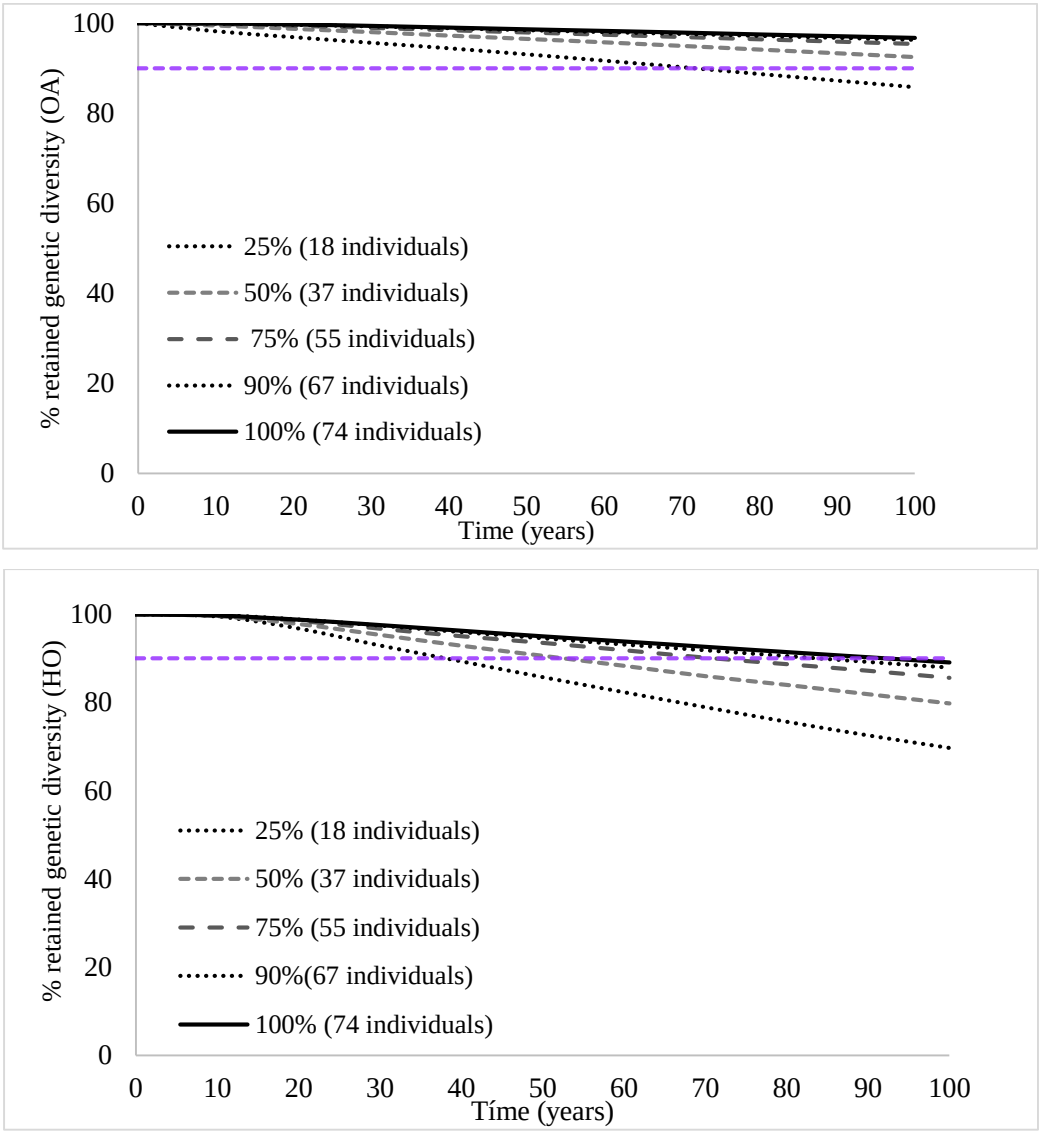


Figure 2.8 - Predicted genetic diversity for the black lion tamarin population from Capão Bonito National Forest over the next 100 years using BOTTLESIM software. The observed number of alleles (OA) and the observed heterozygosity (HO) were projected to decline (sex ratio: 1:1).

2.4 FINAL CONSIDERATIONS

Few areas of the Atlantic forest still retained original characteristics, and therefore lion tamarins reside in habitats that have been drastically changed by human activity, with ecological conditions that are significantly different from those in which the species has raised (GUIDORIZZI, 2008). Understanding how these changes impact BTL populations is critical to the species surviving in nature for longer. The available data on ecology, behavior, and genetic diversity of BLT is limited and strongly biased towards few remnants. Despite that, our study was able to reveal relevant issues for the species. Differences in some ecological and behavioral aspects between continuous and fragmented areas, particularly in relation to social organization, feeding, and use of time and space were found. Besides, our analyzes show that this species stands out for its ability to persist in conserved and disturbed habitats, including fragmented landscapes and small fragments of forests where other primates would not be able to survive (ESTRADA et al., 2017).

The high resilience of BLT has allowed the species to survive in highly impacted habitats (COIMBRA-FILHO, 1976; VALLADARES-PADUA, 1993). However, habitat fragmentation may offer three distinct negative consequences (OFFERMAN et al., 1995) on BLT populations. First, although the diversity of plants consumed in the fragments is greater, remnants may not provide sufficient resources (food, shelter, and breeding sites) to keep a viable population in the long-term. We observed that BLTs spend more time moving around in fragmented areas, possibly to obtain the necessary resources. Second, groups tend to be more limited in their dispersion in fragmented and riparian remnants than in contiguous ones.

The genetic data showed that wild populations are structured. However, the existence of ancestral haplotype among distant fragmented areas, currently genetically differentiated, suggests ancient connectivity. The isolated and saturated nature of some fragments are factors that may influence on their reproductive strategies, since reproduction outside of the natal group seems to be limited. In addition, populations may be vulnerable to the negative effects of reduced gene flow (COUVET, 2001).

Finally, changes in the microclimate are common in the fragments and riparian forests, increasing depredation rates and competition for resources (OFFERMAN et al., 1995) as a consequence of the increase in density. Thus, though populations from riparian forest had evidenced similar or even higher levels of heterozygosity from continuous areas, stochastic evolutionary events are more conspicuous in small populations, affecting in theory, primarily genetic diversity by allele loss, and then heterozygosity and fitness reductions, essential

components for adaptability and persistence of populations (FUERST; MARUYAMA, 1986). However, we observed a contrary pattern for Capão Bonito population that could reflect the low allelic richness and/or low frequency of rare alleles observed. Overall, both estimators are relevant to avoid local extinctions and guarantee population viability and evolutionary potential in long-term (FRANKHAM; BRISCOE; BALLOU, 2002).

Given the importance of these fragments as genetic reservoirs and also for restoration actions, this study emphasizes the importance of conserving large but also small and medium sized forest fragments and increasing efforts in connecting remnants. The future of BLT has long been assumed to be largely dependent on the successful conservation of Morro do Diabo State Park, which contains the species' largest population (HOLST et al., 2006; VALLADARES-PADUA et al., 2002). However, the Middle and Upper Paranapanema populations, despite their medium or small population size, are significant in terms of genetic and evolutionary aspects. Within the context of the Black Lion Tamarin Conservation Program (BLTCP), some strategies aimed at restoring and connecting small fragments in the region of the Lower Paranapanema have already been implemented, such as the creation of a 20 km corridor with about 2.5 million trees planted (Instituto de Pesquisas Ecológicas, 2021). This corridor connects the southern portion of Morro do Diabo State Park, with the BLT Ecological Station, making this area the largest forest corridor of Brazil (REZENDE, 2013).

New corridors, in addition to the current ones, are expected to be established between the next 10 years to connect populations from the Lower Paranapanema, covering approximately 45 thousand hectares. In this scenario, extant populations genetically structured should be able to disperse and carry out effective gene flow through areas currently unconnected, reducing the deleterious effects of increased inbreeding, loss of genetic diversity and population structuring.

Conservation strategies aimed at restoring degraded areas and/or connecting habitat fragments where BLT populations are isolated should be guided by knowledge of the species biology, ecology, behavior, and genetics within its range of distribution. Long-term studies with annual monitoring are also recommended for monitoring population patterns and assessing the impact of management decisions in any conservation initiative (PARANHOS, 2006).

Currently, some populations of the Lower Paranapanema (Morro do Diabo State Park, Santa Maria, Santa Monica, Ponte Branca), Middle Paranapanema (Rio Claro Farm) and the Upper Paranapanema (Guareí) are being monitored by ecological and genetics studies. However, there is an urgent need to focus on efforts that allow knowledge of the real status of

BLT populations across the species distribution. Besides, we would like to stress the need for current studies relating the floristic characteristics of the forest fragments and ecology, food choice, seed dispersal, use of the stratum, foraging behavior, and genetic diversity and structure.

The inclusion of genetic analysis becomes essential given that one of the objectives of the BLTCP is managing small and isolated populations through the formation of new groups and translocations to protected forests. Therefore, genetics studies that evaluate inbreeding, effective gene flow, and genetic diversity and structure are imperious. As BLT populations are mostly small, isolated, and with reduced gene flow, they tend to suffer from the deleterious effects of inbreeding, which would compromise the fitness of the species in the long term (COUVET, 2001). The isolated populations tend to diverge genetically across generations, regardless of their common origin (LA HAYE; NEUMANN; KOELEWIJN, 2012). Therefore, estimating the degree of historical and contemporaneous genetic diversity and characterizing the genetic structure will enable the identification of priority areas for conservation and population translocations. On the other hand, demographic information such as effective population sizes, along with genetic parameters, allowing to determine the amount of genetic variation maintained over time (ALLENDORF; HARRIS; METZGAR, 1991), and consequently the potential of persistence and viability for particular populations. Finally, genetic relatedness and kinship may help with the study of aspects related to the cooperative system observed in nature (HUCK et al., 2005), but also to monitoring captive populations, since they represent an insurance for future reintroductions in nature. Therefore, captive populations may retain genetic diversity representativeness from the wild to be used in further ex-situ and in-situ integrated conservation plans. Studies of non-neutral markers to search signals of differential selection and loci associated to the diverse environments and conditions where the species currently occurs must be urgently performed to give insights into adaptive process as well.

Overall, this review allowed us for a more comprehensive holistic view of the BLT's resilience and genetic status, pointing out gaps in topics of special interest for the conservation of the species, which will be important for studies of threat prediction, given the expansion of changes in the physiognomy of the landscape and genetic structure of the populations.

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2.6 SUPPLEMENTARY INFORMATION 2

2.6.1 Supplementary tables

Table S2.1 - Reference list used for the quantitative analyses in the review paper ―" ecological, behavioral, and genetic aspects of black lion tamarin (Callitrichidae: *Leontopithecus chrysopygus*, mikan 1823): a review"

Autor	Year	Title	Journal/Type	Volume	Issue	Pages
Coimbra-Filho, A.	1970	Acerca da redescoberta de *Leontideus chrysopygus* (Mikan, 1823) e apontamentos sobre sua ecologia.	Primates	30	2	249-268
Coimbra-Filho, A., Mittermeier, R.A.	1973	Distribution and Ecology of the Genus *Leontopithecus* LESSON, 1840 in Brazil	Primates	14	1	47-66
Mittermeier, R.A., Valladares-Padua, C., Valle, C., Coimbra-Filho, A.F.	1985	Major program underway to save the black lion tamarin in São Paulo, Brazil	Primate Conservation	6		19-21
Forman, L., Kleiman, D., Mitchell, R., Dietz, J., Ballou, J., Phillips, L., Coimbra-Filho, A.F., O´Brien, J.	1986	Genetic variation within and among lion tamarins	American Journal of Physical Anthropology	71		1-11
Valladares-Padua, C. B.	1987	Black Lion tamarin *Leontopithecus chrysopygus*: status and conservation.	Master thesis			
Carvalho, C.T., Albernaz, A.L., Lucca, C.A.	1989	Aspectos da bionomia do mico-leão-preto (*Leontopithecus chrysopygus* Mikan) (Mammalia, Callithricidae).	Ver. Instituto da Floresta	1	1	67-83
Carvalho, C.T., Carvalho, C.F.	1989	A organização social dos sauís-pretos (*Leontopithecus chrysopygus* Mikan), na reserva em Teodoro Sampaio, São Paulo (Primates, Callithricidae).	Ver. Brasil. Zool.	6	4	707-717
Keuroghlian, A.	1990	Observations on the behavioral ecology of the black lion tamarin (*Leontopithecus chrysopygus*) at Caetetus Reserve, São Paulo, Brazil.	Master thesis-West Virginia University			
Passos, F.C.	1992	Hábito alimentar de mico-leão-preto *Leontopithecus chrysopygus* (Mikan, 1823) (Callitrichidae, Primates) na Estação Ecológica dos Caetetus, município de Gália, SP.	Master thesis-Campinas State University			99
Valladares-Padua, C.B.	1993	Ecology, behavior and conservation of black lion tamarins (*Leontopithecus chrysopygus* Mikan, 1823)	Doctoral dissertation-University of Florida			208
Passos, F.C.	1994	Behavior of the Black Lion tamarin, *Leontopithecus chrysopygus*, in different forest levels in the Caetetus Ecological Station, São Paulo, Brazil.	Neotropical Primates	2		40-41
Valladares-Padua, C.B., Padua, S.M., Cullent, L.	1994	The conservation biology of the Black Lion tamarin, *Leontopithecus chrysopygus*: first ten years' report.	Neotropical primates	2		36-39
Valladares-Padua, C.B., Cullent, L.	1994	Distribution, abundance and minimum viable metapopulation of the Black Lion tamarin (*Leontopithecus chrysopygus*).	Dodo, Journal of the Wildlife Preservation Trusts	30		80-88

Albernaz, A. L.	1997	Home Range Size and Habitat Use in the Black Lion Tamarin (*Leontopithecus chrysopygus*).	International Journal of Primatology	18	6	877-887
Mamede-Costa, A.C.	1997	Ecologia de um grupo de micos-leões-pretos (*Leontopithecus chrysopygus* Mikan, 1823) na mata ciliar da Fazenda Rio Claro, Lençóis Paulista, SP.	Master thesis-Paulista State University			109
Passos, F.C.	1997	Seed dispersal by Black Lion tamarin *Leontopithecus chrysopygus* (Primates, Callitrichidae) in southeastern Brazil.	Mammalia	61	1	109-111
Passos, F.C.	1997	Padrão de atividades, dieta e uso do espaço em um grupo de mico-leão-preto (*Leontopithecus chrysopygus*) na Estação Ecológica dos Caetetus, SP.	Doctoral dissertation-Federal University of São Carlos			144
Valladares-Padua, C.B.	1997	Habitat analysis for the metapopulation conservation of Black Lion tamarins (*Leontopithecus chrysopygus*, Mikan, 1823).	A primatologia no Brasil	6		13-26
Mamede-Costa, A.C., Gobbi, N.	1998	The black lion tamarin Leontopithecus chrysopygus-its conservation and management	ORYX	32	4	295-300
Mamede-Costa, A.C., Godoi, S.	1998	Consumption of *Syagrus romanzoffiana* (Arecaceae) fruits, by black lion tamarins (*Leontopithecus chrysopygus*) in south-eastern Brazil.	Mammalia	64	2	310-313
Valladares-Padua, C.B., Ballou, J.D.	1998	*Leontopithecus chrysopygus* metapopulation management action plan.	Report			
Passos, F.C.	1999	Dieta de um grupo de mico-leão-preto *Leontopithecus chrysopygus* (Mikan) (Mammalia, Callitrichidae) na Estação Ecológica do Caetetus, São Paulo.	Resvista Brasilera de Zoologia	16	1	269-278
Passos, F.C., Keuroghlian, A.	1999	Foraging behavior and microhabitats used by black Lion tamarins, *Leontopithecus chrysopygus* (Mikan) (Primates, Callitrichidae).	Revista Brasileira de Zoologia	16	2	219-222
Passos, F.C., Kim, A.C.	1999	Nectar feeding on *Mabea fistulifera* Mart. (Euphorbiaceae) by Black Lion tamarins, *Leontopithecus chrysopygus* Mikan, 1823 (Callitrichidae), during the dry season in southeastern Brazil.	Mammalia	63	4	519-521
Peres-Sweeney B.	2000	The molecular systematics of Leontopithecus, population genetics of *L. chrysopygus*, and the contribution of these two sub-fields to the conservation of *L. chrysopygus*.	Doctoral dissertation			
Keuroghlian, A., Passos, F.C.	2001	Prey foraging behavior, seasonality and time-budgets in black lion tamarins, *Leontopithecus chrysopygus* (MIKAN 1823) (MAMMALIA, CALLITRICHIDAE).	Brazilian Journal of Biology	61	3	455-459
Medici, E.P.	2001	Translocação e Manejo Metapopulacional de Mico-leão-preto, *Leontopithecus chrysopygus* Mikan, 1823 (Callithricidae – Primates).	Master thesis-Federal University of Minas Gerais			115
Passos, F.C., Alho, C.J.	2001	Importância de diferentes micro-habitats no comportamento de forrageio por presas do mico-leão-preto, *Leontopithecus chrysopygus* (Mikan) (Mammalia, Callitrichidae).	Revista Brasileira de Zoologia	18	1	335-342
Valladares-Padua, C.B., Martins, C.S., Wormell, D., Setz, E.Z.	2001	Preliminary evaluation of the reintroduction of a mixed wild-captive group of Black Lion tamarins *Leontopithecus chrysopygus*.	Dodo	36		30-38
Lima, F.S., Silva, I.C., Martins, C.S., Valladares-Padua, C.	2003	On the occurrence of the Black lion tamarin (in Buri, São Paulo, Brazil)	Neotropical Primates	11	2	76-77

Martins, C. S	2003	Conservação do mico-leão-preto (*Leontopithecus chrysopygus*):três tipos de manejo avaliados através da ecologia e comportamento.	Doctoral dissertation-Campinas State University			171
Medici, E.P., Valladares-Padua, C.P., Rylands, A.B., Martins. C-S.	2003	Translocation as a Metapopulation Management Tool for the Black Lion Tamarin, *Leontopithecus chrysopygus.*	Primate Conservation	19		23-31
Röhe, F., Antunes, A.P., Tófili, C.F.	2003	The discovery of a new populations of black lion tamarin in the Serra de Paranapiacaba, São Paulo, Brazil	Neotropical Primates	11	2	75-76
Perez-Sweeney, B.M., Valladares-Padua, C., Burrell, A.S., Di Fiore, A., Satkoski, J., Groot, P.J., Boag, P.T., Melnick, D.J.	2005	Dinucleotide microsatellite primers designed for a critically endangered primate, the black lion tamarin (*Leontopithecus chrysopygus*).	Molecular Ecology Notes	5	2	198-201
Paranhos, K.M.	2006	Estimativas populacionais para espécies raras, o mico leão preto como modelo	Master thesis-Federal University of Paraná			62
Perez-Sweeney, B.M., Valladares-Padua, C., Martins, C.S., Morales, J.C., Melnick, D.J.	2008	Examination of the Taxonomy and Diversification of Leontopithecus using the Mitochondrial Control Region	International Journal of Primatology	29	1	245-263
Rezende, G.	2013	Sucesso em Programas de Conservação de Espécies da Fauna Ameaçada: A história do Programa de Conservação do Mico-Leão-Preto.	Master thesis--Ecological Research Institute (IPÊ)			129
Caldano, L.	2014	Censo populacional e avaliação da variabilidade genética das populações de mico-leão-preto (Leontopithecus chrysopygus Mikan, 1823) na Floresta Nacional de Capão Bonito- SP	Master thesis-Federal University of São Carlos			63
Rodrigues, S., Gagetti, B., Piratelli, A.	2014	First record of *Leontopithecus chrysopygus* in Carlos Botelho state park, São Miguel Arcanjo, São Paulo, Brazil	Mammalia	80	1	1--4
Ayala-Burbano, P.A.	2015	Variabilidade genética e verificação de paternidade da colônia cativa do mico-leão-preto (Leontopithecus chrysopygus) (Primates, Callithricidae) utilizando marcadores microssatélites	Master thesis-Federal University of São Carlos			98
Culot, L., Griese, J., Knogge, C., Tonini, M., Mulato, M., Genaro, C., Pinto, B., Mantovani, B., Bertanha, A., Soares, B., de Oliveira, F., Batista, R., Port-Carvalho, M.	2015	New records, reconfirmed sites and proposals for the conservation of black lion tamarin (*L. chrysopygus*) in the middle and upper Paranapanema	Neotropical Primates	22	1	32-39
Alcolea, M.	2016	Dispersão de sementes pelo mico leão preto, *Leontopithecus chrysopygus* (Primates, Callitrichidae) em um fragmento de Mata Atlântica	Bachelor thesis-Paulista State University			43
Caldano, L., Monticelli, C., Galetti, P.	2016	Demography of the black lion tamarin (Leontopithecus chrysopygus, Mikan) in Capão Bonito national forest (state of São Paulo)	Neotropical Primates	23	1	40-41
Garbino, G., Rezende, G., Valladares-Padua, C.	2016	Pelage Variation and Distribution of the Black Lion Tamarin, Leontopithecus chrysopygus	Folia primatologica	87	4	244-261
Lopes, B.	2016	Influência da paisagem me das características locais na ocorrência do mico-leão preto (*Leontopithecus-chrysopygus*, Callitrichidae)	Master thesis-Paulista State University			109
Winter, M.	2016	Aspectos comportamentais e ecológicos do mico-leão-preto (Callitrichidae: *Leontopithecus chrysopygus*): uma revisão bibliográfica	Bachelor thesis-Paulista State University			40
Ayala-Burbano, P.A., Caldano, L., Galetti, P.M., Pissinatti, A., Marques, M., Wormell, D., Freitas, P.	2017	Genetic assessment for the endangered black lion tamarin *Leontopithecus chrysopygus* (Mikan, 1823), Callitrichidae, Primates	American Journal of primatology	79	12	1-13

Freitas, P., Mendez, F., Chavez-Congrains, K., Galetti, P.M., Coutinho, L., Pissinatti, A., Bustamante, C.	2018	Next-Generation Sequencing of the Complete Mitochondrial Genome of the Endangered Species Black Lion Tamarin Leontopithecus chrysopygus (Primates) and Mitogenomic Phylogeny Focusing on the Callitrichidae Family	Genes, Genomes, Genetics	8	6	1985-1991
Culot, et al.	2018	ATLANTIC-PRIMATES: a dataset of communities and occurrences of primates in the Atlantic Forests of South America.	Ecology	100	1	
Pardo, P.	2018	Identificação, caracterização e validação de sequências microssatélites no genoma do mico-leão-preto (Leontopithecus chrysopygus)	Master thesis-Federal University of São Carlos			47
Veltroni, Y.	2018	Estrutura genética matrilinear e relações de parentesco no mico leão preto, Leontopithecus chrysopygus (Primatas) uma espécie ameaçada que apresenta sistema de reprodução cooperativa	Bachelor thesis-Federal University of São Carlos			60
Ayala-Burbano, P.A., Galetti, P.M., Wormell, D., Pissinatti, A., Marques, M., Freitas, P.	2020	Studbook and molecular analyses for the endangered black-lion tamarin; an integrative approach for assessing genetic diversity and driving management in captivity	Scientific reports	10	6781	1--11
Garbino, G., da Silva, L., Amaral, R., Rezende, G., Pereira, V., Culot, L.	2020	Predation of treefrogs (Anura: Hylidae) with toxic skin secretions by the black lion tamarin (*Leontopithecus chrysopygus*, Callitrichidae).	Primates	61	4	567-572
Rezende, G., Sobral-Souza, T., Culot, L.	2020	Integrating climate and landscape models to prioritize areas and conservation strategies for an endangered arboreal and conservation strategies for an endangered arboreal primate	American Journal of Primatology	82	12	1-9
Forero-Sánchez Francy	2020	Análises de Viabilidade Populacional no Planejamento para a Conservação do Mico-Leão-Preto	Master thesis- Instituto de Pesquisas Ecológicas			152
Javarotti, N.	2021	Estrutura genética e relações de parentesco no mico leão preto (Leontopithecus chrysopygus) inferidas por marcadores de microssatélites	Bachelor thesis-Federal University of São Carlos			61

Table S2.2 - Home range estimates from 13 BLT groups collected in the Pontal and Middle Paranapanema.

Region-Remnant	Forest fragment complex	ID group	Study period	Distribution of days of sampling effort	Sampling effort (days)	Area (ha)	Method	Source
Lower-Continuous	Morro do Diabo State Park	PMD1	Out/1986 - Jan/1987	Out (8) -Nov (4) Dec (6) – Jan (5)	23	106	Composite	Albernaz,1997
		PMD1	Out/1986 - Jan/1987	-	23	127	Convex polygon	
		PMD1	Out/1986 - Jan/1987	-	23	64	Grid cell count	
		PMD2/PMD3	1985	-	60	65.83	Radiolemetry	Carvalho et al.,1989
		PMD16	1988	-	40	113	Grid cell count	
		PMD17	1988	-	40	120	Grid cell count	
		PMD18	1988	-	40	199	Grid cell count	
		PMD19	1988	-	40	120	Grid cell count	
Lower-Fragment	Santa Maria Farm	SMa	Feb/2015-May/2015	-	29	81.1	Convex polygon 1 point every 5 min	Culot & Messaoudi unpublish data
		SMa	Feb/2016-May/2016	-	29	85.7	Kernel 75%, 1 point every 5 min	
		SMa	Feb/2017-May/2017	-	29	20.6	Kernel 50%, 1 point every 5 min	
		SMa	Mar-Sep /2015	Mar (2) -May (4) Jun (4)- Aug (5) Sep (7)	22	82.9	Convex polygon 1 point every 5 min	Alcolea,2016
		CAE9	Jan-Jun/1989	Jan (6) – Feb (5) Mar (5)-Apr (6) May (5)-Jun (3)	30	79	Grid cell count	
		CAE9	Mar/1990-Mar1991	Apr (5) – May (3) Jun (3)- Jul (4) Sep (3)-Out (3)	23	54	Grid cell count	
	Mosquito Farm	FMos	1995/1998	-	150	79	Grid cell count	Medici,2001
Middle-Riparian	Rio Claro Farm	FRC4	1992-1995	-	36	40	Grid cell count	Medici,2001
		FRC5	1993-1195	-	105	40	Grid cell count	

Table S2.3 -Species of plants used as sleeping sites by five groups of BLTs, collected in the Morro do Diabo State Park (PMD), Rio Claro Farm (FRC4 and FRC5), Caetetus Ecological Station (CAE) and Mosquito Farm (FMos). Diameter at Breast Height (DBH), type of shelter (T=tree; H=Hollow; L=Liana) and tree shelter position (B=branch; C= canopy; T=trunk) are also listed.

Region-Remnant	Group ID	Family	Specie	Number of individuals	Tree height (m)	Hollow height entrance (m)	DBH (m)	Type	Tree shelter position	Source
Lower Continuous	PMD3	Anacardiaceae	*Astronium graveolens*	-	-	-	-	-	-	Carvalho et al.,1989
		Apocynaceae	*Aspidosmerma polyneuron*	3	-	17,6,7	1.20,1.10,0.60	T, O, L	-	
		Bignoniaceae	*Tabebuia avellanedae*	5	-	12,17 18,25,25	1.50,1.30 0.60	T	-	
		Burseraceae	*Protium heptaphyllum*	2	-	3.5,5.0	0.50,0.60	T, H		
		Combretaceae	*Terminalia sp.*	1	-	4	0.45	T	-	
			Lonchocarpus leucanthus	1	-	8	0.60	T	-	
			Holocalyx balansae	-	-	-	-	-	-	
		Fabaceae	*Poecilanthe parviflora*	-	-	-	-	-	-	
			Peltophorum dubium	1	-	7	0.55	T	-	
		Meliaceae	*Trichilia pallida*	1	-	15	0.70	T	-	
			Cabralea canjerana	-	-	-	-		-	
		Moraceae	*Ficus enormis*	3	-	1,5	1.40	T	-	
		Myrtaceae	*Campomanesia xanthocarpa*	2	-	6,12	0.70	T	-	
		Nyctaginaceae	*Bougainvillea arborea*	1	-	3	0.38	T, H	-	
		Phytolaccaceae	*Gallesia integrifolia*	2	-	6,12	0.75,2.0	T, H	-	
		Sapindaceae	*Diatenopteryx sorbifolia*	1	-	8	0.50	T	-	
Middle Riparian	FRC4	Arecaceae	*Syagrus romanzoffiana*	2	8,12	8,8	20.1,25.8	I	C	Costa,1997
		Combretaceae	*Terminalia sp.*	1	11	10	17.8	L	B	

	Euphorbiaceae	*Alchornea urikana*	1	10	7	45.5	H	T	
	Fabaceae	*Copaifera langsdorffii*	1	-	-		H	T	
		Erythrina sp.	1	10	10	29	L	C	
	Lauraceae	*Endlicheria paniculata*	3	12,15,20	7,8,9	64.3,63.6, 37.6	H, L	B, T, T	
		Nectandra sp.	1	14	14	46.2	H	B	
		Persea pyrifolia	1	20	13	86.6	H	B	
	Lythraceae	*Lafoensia sp.*	1	1	0.94	18.5	H	T	
	Malvaceae	*Luehea divaricata*	1	14	10	42.3	L	T	
	Meliaceae	*Cedrela fissilis*	1	8	8	21.3	L	B	
		Cedrela odorata	1	14	14	35.5	L	C	
	Phytolaccaceae	*Gallesia integrifolia*	1	15	12	29	H	B	
	Rutaceae	*Balfourodendron riedelianum*	1	16	9	41.1	H	T	
	Sapindaceae	*Diatenopteryx sorbifolia*	1	20	9	52.2	H	T	
FRC5	Arecaceae	*Syagrus romanzoffiana*	4	-	-	-	-	-	Medici, 2001
	Combretaceae	*Terminalia inflora*	-	-	-	-	-	-	
	Euphorbiaceae	*Alchornea triplinervia*		-	-	-	-	-	
	Fabaceae	*Copaifera langsdorffii*	5	-	-	-	-	-	
		Erythrina sp.	-	-	-	-	-	-	
	Lauraceae	*Endlicheria paniculata*	8	-	-	-	-	-	
		Nectandra megapotamica	6	-	-	-	-	-	
		Persea pyrifolia	-	-	-	-	-	-	
	Lythraceae	*Lafoensia pacari*	4	-	-	-	-	-	
	Malvaceae	*Luehea candicans*	-	-	-	-	-	-	
	Meliaceae	*Cedrela fissilis*	-	-	-	-	-	-	
		Cedrela odorata	-	-	-	-	-	-	
		Guarea kunthiana	-	-	-	-	-	-	
	Rutaceae	*Galipea jasminiflora*	-	-	-	-	-	-	

			Balfourodendron nedelianumii	-	-	-	-	-	-	
		Sapindaceae	*Diatenopteryx sorbifolia*	-	-	-	-	-	-	
Middle Fragments	CAE	Anacardiaceae	*Astronium graveolens*	1	-	-	-	-	-	Passos,1992
		Apocynaceae	*Aspidosmerma polyneuron*	7	-	-	-	-	-	
		Burseraceae	*Protium widgrenii*	-	-	-	-	-	-	
		Elaeocarpaceae	*Sloanea sp*	-	-	-	-	-	-	
		Euphorbiaceae	*Alchornea triplinervia*	3	-	-	-	-	-	
			Croton floribundus	1	-	-	-	-	-	
		Fabaceae	*Inga sp.*	-	-	-	-	-	-	
			Piptadenia gonoacantha	-	-	-	-	-	-	
			Pterodon pubescens	-	-	-	-	-	-	
		Meliaceae	*Cabralea canjerana*	-	-	-	-	-	-	
		Moraceae	*Chlorophora tinctoria*	1	-	-	-	-	-	
		Opiliaceae	*Agonandra excelsa*	3	-	-	-	-	-	
		Phyllanthaceae	*Savia dictyocarpa*	-	-	-	-	-	-	
		Rutaceae	*Zanthoxylum hyemale*	-	-	-	-	-	-	
		Sapindaceae	*Diatenopteryx sorbifolia*	2	-	-	-	-	-	
Middle Fragments	FMos	Boraginaceae	*Patagonula americana*	2	-	-	-	-	-	Medici,2001
		Euphorbiaceae	*Pachystroma longifolium*	4	-	-	-	-	-	
		Fabaceae	*Anadenanthera macrocarpa*	2	-	-	-	-	-	
		Lauraceae	*Nectandra megapotamica*	3	-	-	-	-	-	
		Lecythidaceae	*Craniana estrellensis*	-	-	-	-	-	-	
		Meliaceae	*Cabralea canjerana*	-	-	-	-	-	-	

		Guarea macrophylla	-	-	-	-	-	-
	Myrtaceae	*Campomanesia xanthocarpa*	11	-	-	-	-	-
		Eugenia uniflora	-	-	-	-	-	-
		Myrciaria sp.	-	-	-	-	-	-
	Nyctaginaceae	*Boungainvillea glabra*	21	-	-	-	-	-
	Phytolaccaceae	*Gallesia integrifolia*	3	-	-	-	-	-
	Sapindaceae	*Diatenopteryx sorbifolia*		-	-	-	-	-
	Sapotaceae	*Chrysophyllum gonocarpum*	-	-	-	-	-	-

Table S2.4 - Activity Budget of black-lion tamarins reported fin 15 studies from 1986 to 2015.

Forest fragment complex	Group ID	Study period	Sampling effort (days)	Sampling Method	Activity Budget (%)					Source
					Moving	Resting	Foraging	Feeding	Social interaction	
Morro do Diabo State Park	PMD	Out/1986-Jan/1987	23	Scan Sampling	6.9	11.8	26.8	54.0	NA	Albernaz,1997
Morro do Diabo State Park	PMD	Out/1986-Jan/1987	23	Scan Sampling	3.5	6.3	20.4	41.9	NA	Albernaz,1997
Morro do Diabo State Park	PMD	Out/1986-Jan/1987	23	Scan Sampling	1.5	0.4	1.9	4.4	NA	Albernaz,1997
Morro do Diabo State Park	PMD16	1988	96	Radiotelemetry	10	70	5	8.0	0.55	Valladares-Pádua, 1993
Morro do Diabo State Park	PMD17	1988	96	Radiotelemetry	15	52	8	9.5	0.5	Valladares-Pádua, 1993
Morro do Diabo State Park	PMD18	1988	96	Radiotelemetry	11	60	6	8.0	0.5	Valladares-Pádua, 1993
Morro do Diabo State Park	PMD19	1988	96	Radiotelemetry	16	53	6	10.0	0.	Valladares-Pádua, 1993
Santa Maria	SMa1	May-Sept 2015	20	Instantaneous scan	29.7	30.2	10.2	25.6	4.3	Alcolea, 2016
Caetetus	CAE1	Nov/1988-Jun/1989-Apr/1990-Dec/1990	83	Scan Sampling	NA	17.0	16.3	6.55	NA	Keuroghlian, Passos, 2001
Caetetus	CAE2	Dec/1993-Mar/1994	12	Radiotelemetry-Scan sampling	33.0	14.4	16.8	35.8	NA	Passos,1994
Caetetus	CAE9	1989-1991	53	Radiotelemetry - Direct observation	35.6	16.2	18.3	23.3	6.6	Passos,1999

Caetetus	CAE5	Jan-Jun/1989 - Apr-oct/1990 - Jan/1991	53	Radiotelemetry - Scan sampling	35.6	16.2	18.3	23.3	6.6	Passos,1992
Fazenda Mosquito	Fmos7	1995-1998	150	Focal	23.0	54.7	4.68	13.8	0.41	Medici,2001
Fazenda Rio Claro	FRC4	1992-1993	36	Radiotelemetry - Focal	18.4	53.0	11.4	17.2	NA	Mamede Costa,1997
Fazenda Rio Claro	FCR5	1993-1994	105	Focal	21.5	51.3	11.5	17.0	0.50	Medici,2001

Table S2.5 - Use of different strata for BLT and percentage foraging activity and prey consumption related to height classes where behaviors developed.

Forest fragment complex	Group ID	Study period	Sampling effort (days)	Sampling Method	Strata (meters)	Category	Total time (%)	Foraging time (%)	Prey time (%)	Source
Morro do Diabo State Park	PMD16, PMD17, PMD18, PMD19	Nov/1988-Jul/1989	40	Scan sampling	7-8.5	Middle layers	-	-	-	Valladares-Pádua, 1993
Morro do Diabo State Park	PMD1	Out/1986 - Jan/1987	23	Scan sampling	0-4	Forest floor	18	-	-	Albernaz,1997
					4-6	Understory	20	-	-	
					>10	Middle layers	20	-	-	
Caetetus Ecological Station	CAE2	Dec/1993-Mar/1994	12	Scan sampling	0-8	Floor and Understory	-	29.9	-	Passos,1994
					8-16	Middle layers	-	57	-	
					>16	Canopy	-	13.1	-	
Caetetus Ecological Station	CAE6	Dec/1993-Feb/1995	55	Scan sampling	0	Forest floor	-	0.58	0.75	Pasos & Alho, 2001
					1-10	Understory	-	54.91	71.64	
					10-20	Middle layers	-	35.55	21.64	
					>20	Canopy	-	8.96	5.97	

Table S2.6 - Total food records for black lion tamarins according to region and vegetation type.

FAMILY	SPECIES	ITEM CONSUMED				STUDY REGION			VEGETATION TYPE			REFERENCE
		Fruit	Gum	Flower Nectar	Others	Lower	Middle	Upper	Continuous	Fragment	Riparian	
Acanthaceae	*Mendocia velloziana*	x					x			x		Passos, 1992,199
Anacardiaceae	*Lithraeae molleoides*		x				x				x	Mamede Costa, 1997
	Myracrodruon urundeuva	x					x				x	Medici, 2001
Annonaceae	*Tapirira guianensis*	x	x							x	x	Passos,1992, 1999; Medici, 2001
	Duguetia lanceolata	x					x			x	x	Passos,1992, 1999; Medici, 2001
	Rollinia sylvatica	x					x			x	x	Medici, 2001
	Xylopia sp	x				x			x		x	Mamede Costa, 1997
	Xylopia brasiliensis	x				x			x			Valladares-Padua, 1993
Arecaceae	*Euterpe edulis*		x				x			x		Passos, 1992,1999
	Syagrus romanzoffiana	x	x			x	x		x	x	x	Carvalho & Albernaz, 1989; Passos, 1992; Valladares-Pádua, 1993; Passos, 1999; Mamede Costa, 1997 Medici, 2001; Alcolea, 2016
Araceae	*Philodendron sp*	x					x				x	Mamede Costa, 1997
Asteraceae	*Gochnatia polymorpha*		x				x				x	Medici, 2001
Bignoniaceae	*Stizophyllum perforatum*				x		x			x		Passos, 1992,1999;
Bombacaceae	*Chorisia speciosa*		x				x			x		Medici, 2001
Boraginaceae	*Cordia eucalyculata*	x				x	x		x	x		Passos, 1992,1999
	Cordia superba	x					x			x		Passos, 1992,1999
	Cordia sellowiana	x					x				x	Mamede Costa, 1997
Burseraceae	*Protium spruceanum*	x					x			x		Passos,1999
	Protium widgrenii	x					x			x		Passos, 1992
Cactaceae	*Cereus hildmannianus*	x				x				x		Alcolea, 2016
	Cereus sp.	x				x			x			Valladares-Padua, 1993
	Epiphyllum phyllanthus			x			x			x		Passos, 1992,1999
	Pereskia aculeata	x				x	x		x	x		Carvalho & Albernaz,1989, Passos, 1992,1999, Medici, 2001
	Pereskia sp	x					x				x	Mamede Costa, 1997
	Rhipsalis sp.	x				x	x		x	x	x	Carvalho & Albernaz;1989; Mamede Costa, 1997; Medici, 2001
	Zygocactus sp.	x				x			x			Carvalho & Albernaz,1989; Valladares-Padua, 1993
Cannabaceae	*Celtis fluminensis*	x				x				x		Alcolea, 2016
	Celtis iguanaea	x					x			x		Passos, 1992,1999
	Celtis pubescens	x					x			x		Passos, 1992,1999
	Celtis spinosa	x					x				x	Mamede Costa, 1997; Valladares-Padua,1993
Celastraceae	*Maytenus cestrifolia*	x	x				x				x	Mamede Costa, 1997

Combrataceae	*Terminalia sp.*	x	x		x	x	x	x	x	Carvalho & Albernaz, 1989; Valladares-Padua, 1993; Mamede Costa, 1997, Albernaz, 1997, Passos,1992, 1999
	Terminalia triflora	x	x			x			x	Medici, 2001
Ebenaceae	*Diospinus inconstants*	x				x			x	Mamede Costa, 1997
Euphorbiaceae	*Croton floribundus*		x			x		x		Passos, 1992,1999
	Croton urucurana	x	x			x			x	Medici, 2001
	Mabea fistulifera			x		x		x		Passos & Kim,1999
	Sebastiana serrata	x			x		x			Valladares-Padua, 1993
Fabaceae	*Anadenanthera falcata*	x	x		x		x		x	Mamede Costa, 1997; Valladares-Padua,1993
	Anadenanthera macrocarpa		x			x		x		Medici, 2001
	Acacia polyphylla		x			x		x	x	Mamede Costa, 1997; Medici,2001
	Centrolobium tomentosum		x			x			x	Medici, 2001
	Copaifera langsdorffii	*x*	x			x			x	Medici, 2001
	Enterolobium contortisiliquum	x	x		x	x	x	x	x	Carvalho, Carvalho 1989; Carvalho, Albernaz, 1999; Mamede Costa,1997, Medici, 2001
	Inga marginata		x			x		x		Passos, 1992,1999
	Inga striata	x				x		x		Passos,1992,1999
	Inga uruguensis	x	x			x		x	x	Medici, 2001
	Inga sp.	x	x		x	x	x		x	Carvalho, Albernaz, 1989; Mamede Costa, 1997
	Lonchocarpus guilleminianus		x			x		x		Medici, 2001
	Myroxylon peruiferum		x			x		x	x	Medici, 2001
	Pithecelobium edwallii	x			x		x			Carvalho, Albernaz,1989
	Piptadenia gonoacantha		x			x			x	Mamede Costa, 1997
Lauraceae	*Endlicheria paniculata*		x			x			x	Mamede Costa, 1997
	Nectandra megapotamica		x			x		x		Medici, 2001
	Ocotea velutina	x	x			x		x	x	Medici, 2001
Loranthaceae	*Struthanthus vulgaris*	x				x		x		Passos, 1992,1999
Lythraceae	*Lafoensia pacari*	x				x			x	Medici, 2001
	Lafoensia sp	x				x			x	Mamede Costa, 1997
Malvaceae	*Luehea candicans*	x	x			x			x	Medici, 2001
	Luehea divaricata		x			x			x	Mamede Costa,1997
Melastomataceae	*Leandra sp*	x				x			x	Mamede Costa, 1997
Meliaceae	*Cabralea canjerana*	x			x		x			Valladares-Padua, 1993
	Cedrela fissilis		x			x		x		Medici, 2001

	Cedrela odorata		x			x				x	Mamede Costa,1997; Medici, 2001
	Trichilia catigua	x			x				x		Passos, 1992,1999; Alcolea, 2016
	Trichilia pallida	x			x			x			Valladares-Pádua, 1993
Menispermaceae	*Abuta sp.*	x									Passos, 1999, Mamede Costa, 1997
Moraceae	*Chlorophora tinctoria*	x			x			x			Carvalho, Albernaz,1989
	Ficus enormis	x			x			x			Valladares-Padua, 1993
	Ficus guaranitica	x				x			x		Passos,1992,1999
	Ficus insipida	x				x			x	x	Medici, 2001
	Ficus luschnathiana	x			x				x		Alcolea, 2016
	Ficus organensis	x				x			x		Passos, 1992,1999
	Ficus tomentella	x				x			x		Passos, 1992,1999
	Ficus trigona	x				x			x		Passos, 1992,1999
	Ficus sp	x				x				x	Mamede Costa, 1997
	Soroceae bemplardii	x				x				x	Mamede Costa,1997
Myrsinaceae	*Rapanea umbellota*	x	x			x				x	Mamede Costa,1997
Myrtaceae	Campomanesia sp.	x			x			x			Valladares-Pádua, 1993
	Campomanesia xanthocarpa	x				x			x		Medici, 2001
	Eugenia blastantha (Berg) Legr.	x				x			x		Medici, 2001
	Eugenia cf. hiemalis Cambess.	x			x				x		Alcolea, 2016
	Eugenia melanogyna	x			x			x			Carvalho, Albernaz,1989
	Eugenia sulcata	x				x			x		Passos, 1992,1999
	Eugenia uniflora	x				x			x		Medici, 2001
	Eugenia aff. ramboi D. Legrand	x			x				x		Alcolea, 2016
	Eugenia uvalha	x			x			x			Valladares-Pádua, 1993
	Eugenia sp	x			x	x		x	x		Carvalho, Albernaz, 1989; Alcolea, 2016, Medici, 2001
	*Eugenia sp*1	x				x				x	Mamede Costa, 1997
	*Eugenia sp*2	x				x				x	Mamede Costa, 1997
	Eugenia sp. no.2	x			x			x			Valladares-Pádua, 1993
	Myrcia splendens	x			x				x		Alcolea, 2016
	Myrcia sp		x			x			x	x	Mamede Costa, 1997
	Myrcia sp no.1	x			x			x			Valladares-Padua, 1993
	Myrcia sp.no.2	x			x			x			Valladares-Pádua, 1993
	Myrcia guianensis	x				x				x	Medici, 2001
	Myrciaria ciliolata (DC.) Berg	x				x				x	Medici, 2001
	*Myrciaria*sp. no.1	x			x			x			Valladares-Padua, 1993
	Myrciaria sp.	x			x	x		x	x		Carvalho, Albernaz,1989, Passos, 1992,1999, Medici, 2001
	Myrceugenia ovata	x			x			x			Carvalho, Albernaz,1989; Valladares-Padua, 1993
	Neomithrantes obscura	x				x				x	Mamede Costa, 1997

	Plinia rivulans(Cambess.) Rotman.	x				x			x	Medici, 2001
	Psidium sp. no.1	x			x		x			Valladares-Padua, 1993
	Psidiumsp. no.2	x			x		x			Valladares-Padua, 1993
	Psidium sp	x			x		x			Carvalho, Albernaz,1989; Medici, 200
Nyctaginaceae	*Pisonia aculeata*		x		x	x	x	x		Carvalho, Albernaz,1989; Medici, 2001
Peraceae	*Pera obovata*	x	x			x			x	Mamede Costa, 1997; Medici, 2001
Polygonaceae	*Coccoloba sp*	x				x			x	Mamede Costa, 1997
	Ruprechtia laxiflora Meissm.		x		x	x	x		x	Carvalho, Albernaz,1989; Mamede Costa, 1997
Rhamnaceae	Colubrina glanulolosa	x				x		x	x	Mamede Costa, 1997; Medici, 2001
	Rhamnidium elaeocarpum	x				x		x		Passos,1992,1999
Rosaceae	*Prunus selowii*		x			x		x		Passos, 1992,1999
Rubiaceae	*Chomelia pohliana*	x				x		x	x	Medici, 2001
	Ixora gardneriana	x				x			x	Medici, 2001
	Randia armata	x			x			x		Alcolea, 2016
Rutaceae	*Citrus aurantium*		x			x		x		Medici, 2001
	Balfourodendron riedelianum	x			x		x			Carvalho, Carvalho,1989
	Galípea jaminiflora		x			x			x	Mamede Costa, 1997
	Helietta longifoliata	x			x		x			Valladares-Padua, 1993
	Metrodorea nigra		x			x		x		Medici, 2001
	Pilocarpus pauciflorus		x			x		x		Passos, 1992,1999
Salantaceae	*Phoradendron rubrum*	x				x			x	Mamede Costa,1997
Salicaceae	*Casearia decandra*	x	x			x		x	x	Mamede Costa, 1997; Medici, 2001
	Casearia sp		x			x			x	Mamede Costa, 1997
Sapindaceae	*Allophylus edulis*	x				x		x		Medici, 2001
	Cupanis vernalis		x			x			x	Medici, 2001
	Matayba elaeagnoides	x	x			x			x	Medici, 2001
	Matayba guianensis	x	x			x			x	Mamede Costa, 1997; Medici, 2001
Sapotaceae	*Chrysopyllum gonocarpum*	x			x	x	x	x		Carvalho, Albernaz,1989; Passos, 1992,1999
Urticaceae	*Cecropia cinerea*	x			x		x			Carvalho, Albernaz,1989
	Cecropia pachystachya	x				x		x		Medici, 2001
	Cecropia sp.	x			x		x			Carvalho, Carvalho,1989
Viscaceae	*Phoradendron quadrangulare*	x			x			x		Alcolea, 2016
Vochysiaceae	*Vochysia tucanorum*	x	x		x	x	x	x		Valladares-Padua, 1993; Mamede Costa, 1997; Medici, 2001

Table S2.7 - Fruit species consumed by the BLT and dimensions (mean and SD) of seeds and fruit in the Santa Maria and Caetetus Ecological Station fragment. SMa1= Santa Maria Farm; CAE3= Caetetus Ecological Station.

							Mean fruit size		Mean seed size		
Region-Remnant	**Group ID**	**Study period**	**Sampling effort (days)**	**Sampling Method**	**Family**	**Specie**	**Length (mm)**	**Width (mm)**	**Length (mm)**	**Width (mm)**	**Source**
Lower Fragment	SMa1	Mar-Sep/2015	22	Collect of feces	Arecaceae	*Syagrus romanzoffiana*	36.1±4.8	23.4±2.5	24.5±1	11.5±1.7	Alcolea, 2016
					Cactaceae	*Cereus hildmannianus*	105±20.3	64.6±6	1±0	1±0	
					Cannabaceae	*Celtis fluminensis*	11.7 ± 1.1	11.2 ± 1.5	10.1 ± 0.8	8.8 ± 0.8	
					Meliaceae	*Trichilia catigua*	9.9 ± 0.1	7.1 ± 0.1	8.97±0	6.1±0	
					Moraceae	*Ficus luschnathiana*	11 ± 0.8	10 ±0.8	1 ± 0	1 ± 0	
					Myrtaceae	*Eugenia aff. ramboi*	6.7 ±0.7	6.2 ± 0.6	5.7 ± 0.7	3.9 ± 0.5	
						Eugenia cf. hiemalis	12.7 ± 2.8	8.1 ± 0.9	8.8 ± 0.6	6.68 ± 1	
						Eugenia sp.	12.1 ± 1.4	11.9 ± 1.5	9.5	8.9	
						Myrcia splendens	9 ± 0.1	4.84 ± 0.3	6.9 ± 0.01	3.1 ± 0.1	
					Rubiaceae	*Randia armata*	28.6 ± 3.1	18.7 ± 2.7	7.2 ± 0.8	6.2 ±0.5	
					Viscaceae	*Phoradendron quadrangulare*	4.84 ± 0.2	4.1 ± 0.5	3.71 ±0.3	2.68 ± 0.1	
Middle Fragment	CAE3	Jan-May/1989	25	Collect of feces	Acanthaceae	*Mendoncia velloziana*	-	-	-	-	Passos, 1997
					Boraginaceae	*Cordia ecalyculata*	-	-	-	-	
					Burseraceae	*Protium widgrenu*	-	-	-	-	
					Cannabaceae	*Celtis iguanaea*	-	-	-	-	
						Celtis pubescens	-	-	-	-	
					Fabaceae	*Inga striata*					
					Moraceae	*Ficus organensis*		-	-	-	
						Ficus tomentella		-	-	-	
					Rhamnaceae	*Rhamnidium elaeocarpum*		-	-	-	

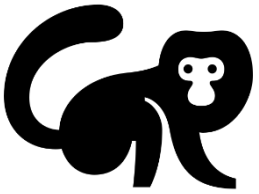
Table S2.7 - Percentage of germination of defecated seeds, seeds with pulp, and without pulp from fruits eaten by BLT at the Santa Maria fragment and Caetetus Ecological Station. The number in parentheses represents the amount planted in the experiment. The asterisk shows the species whose treatments there was significant difference in germination. SMa1= Santa Maria Farm; CAE3= Caetetus Ecological Station.

Region-Remnant	Group ID	Study period	Sampling effort (days)	Sampling Method	Family	Specie	Germination Fruits (%)	Germination seeds (%)	Germination feces (%)	Source
Lower Fragment	SMa1	Mar-Sep/2015	22	Collect of feces	Cannabaceae	*Celtis fluminensis*	0 (30)	0 (30)	33.33	Alcolea, 2016
					Cactaceae	*Cereus hildmannianus**	0	16.6 (30)	66.6 (30)	
					Meliaceae	*Trichilia catigua*	0	0 (2)	0 (1)	
					Myrtaceae	*Eugenia aff. ramboi*	50 (30)	40 (30)	70 (30)	
						Eugenia cf. hiemalis	36.6 (30)	46.6 (30)	53.3 (30)	
						Eugenia sp.	0(8)	(0)	0(2)	
					Moraceae	*Ficus luschnathiana**	56.5 (46)	53.5 (56)	76.7 (56)	
					Rubiaceae	*Randia armata*	(0)	0 (15)	0 (9)	
					Viscaceae	*Phoradendron quadrangulare*	(0)	0 (30)	0 (30)	
Middle Fragment	CAE3	Jan-May/1989	25	Collect of feces	Acanthaceae	*Mendoncia velloziana **	-	0 (33)	16.7 (30)	Passos, 1997
					Boraginaceae	*Cordia ecalyculata**	-	0 (11)	4.5 (22)	
					Burseraceae	*Protium widgrenu**	-	12.5 (12)	80.3 (76)	
					Cannabaceae	*Celtis iguanaea*	-	46.7 (15)	55.5 (18)	
						*Celtis pubescens**	-	16.5 (170)	31.8(170)	
					Fabaceae	*Inga striata*	-	76.6 (47)	100 (13)	
					Moraceae	*Ficus organensis*	-	71.4 (21)	71.4 (21)	
						Ficus tomentella	-	86 (50)	90 (50)	

	Rhamnaceae	*Rhamnidium elaeocarpum*	-	91.8 (12)	93.5 (31)

Table S2.8 - Summary of the nuclear and mitochondrial genetic diversity of Black lion tamarin groups based on microsatellite and D-loop markers. The studies are organized by the marker type. N= samples size analyzed in wild (W) or captive (C); He= expected heterozygosity; Hd= haplotypic diversity; a= mean number of alleles per locus; π= nucleotide diversity; *Fis*= inbreeding coefficient.

Region-Remnant	Forest fragment complex	N (Wild or Captive)	Marker	N loci	He or Hd	Na or π	*Fis*	Source
	Captive	-	Allozyme	47	0.003	-	-	Forman et al., 1986
Lower-Middle Contínuos-Fragment	Morro do Diabo/Caetetus and cative	16 (C) /7 (W)	Allozyme	25	0.000	-	-	Valladares-Pádua 1987
Middle - Fragment	Central region of São Paulo	14 (W)	Microsatellites	11	0.295	-	-	Perez-Sweeney et al., (2005)
Upper-Riparian	National Forest of Capão Bonito	10 (W)	Microsatellites	14	0.450	2.42	-0.363	Caldano,2015
Upper Riparian	National Forest of Capão Bonito	10 (W)	Microsatellites	15	0.403	2.00	-0.639	Ayala-Burbano et al., 2017
	FPZSP/CPRJ/Durrell	20/17/16 (C)	Microsatellites	15	0.462/0.461 0.410	2.26/2.33/2.00	-0.488/ -0.624/-0.72	Ayala-Burbano et al., 2017
	BCP-F8	11 (C)	Microsatellites	15	0.450	2.06		Ayala-Burbano et al., 2020
Lower Fragment	Santa Maria	9 (W)	Microsatellites	12	0.267	1.667	-0.413	Javarotti,2021
Upper Riparian	Guareí	8 (W)	Microsatellites	12	0.215	1.750	-0.167	Javarotti,2021
Upper Riparian	National Forest of Capão Bonito	10 (W)	Microsatellites	12	0.436	2.083	−0.58	Javarotti,2021
	FPZSP/CPRJ/Durrell	27/14/15 (C)	Microsatellites	12	0.4428/ 0.466/ 0.412	2.08/2.16/2.08	-0.376/ -0.457/-0.475	Javarotti,2021
	FPZSP/CPRJ/Durrell	24/19/1 (C)	D-loop	-	0.66	0,00650	-	Veltroni,2018
Lower Fragment	Santa Maria	11 (W)	D-loop	-	0.69	0.00657	-	Veltroni,2018
Upper Riparian	National Forest of Capão Bonito	5 (W)	D-loop	-	0.491	0.00558	-	Veltroni,2018



Chapter 3

Genomic analyses reveal loci associated to local adaptation in the endangered black lion tamarin (*Leontopithecus chrysopygus*, Mikan, 1823)

ABSTRACT

Improve our understanding of genomic mechanisms influencing local adaptation to different environments is essential in the context of rapid global climate and environmental changes. Here, we examine neutral and non-neutral genetic diversity and investigate putative signals of differential selection in wild populations of the endangered black lion tamarin (BLT, *Leontopithecus chrysopygus*, Mikan, 1823), a primate species endemic to the Brazilian Atlantic Forest restricted to the Brazilian state of São Paulo. In addition, we determine kinship and genetic structure using approximately 3.000 bi-allelic single nucleotide polymorphisms (SNPs). We found evidences for strong population structuring into five genetic clusters across the sampled distribution range. Such high differentiation suggests that populations of BLT have been isolated for a considerable period of time. A significant isolation by distance (IBD) pattern was revealed suggesting that geographic isolation is one of the main factors influencing the restricted gene flow among the BLT populations. The genetic diversity levels were low and similar between populations. Kinship analyses showed close relationships within each population. Despite this, none population showed significant inbreeding coefficients, instead exhibited heterozygote excess. In this sense, some individuals from a same population had no relationships, evidencing moving among populations possibly for avoiding mating between relatives. Loci under differential selection were associated with temperature and precipitation seasonality and fragment size, indicating these environmental parameters could be drivers for local adaptation. Functional annotation showed these outlier SNPs are within genes involved in relevant physiological processes, such as lipid metabolism, host-virus interaction, immunity, regulation of blood pressure and fertilization. Our findings are relevant in the context of rapid global environmental and climate changes. Complementary studies should further investigate the potential adaptive role of the outliers identified here to guide effective conservation strategies, aiming at for adapting to a constantly changing environment by restoring degraded areas and/or connecting habitat fragments where BLT populations occur.

3.1 INTRODUCTION

In the era of anthropogenic environmental and climate change, improving our understanding of genomic mechanisms underlying local adaptation is a crucial step towards better understanding evolutionary processes that shape populations of several taxa including endangered species (ANDREWS et al., 2021). In general, small, isolated or declining populations, as those of the black lion tamarin (*Leontopithecus chrysopygus*; BLT), tend to exhibit reductions in genetic diversity and consequently in their adaptive and evolutionary potential (FRANKHAM, 2008, 2010; O'GRADY et al., 2006). This arboreal primate is an endemic species to the Brazilian Atlantic Forest of São Paulo state, and is considered threatened by extinction mainly due to the environmental disturbances caused by habitat loss (IUCN, 2021). As a result, the remaining fragments are no longer structurally intact (SCHWITZER et al., 2011), and processes such as edge effects may alter your dynamics, influencing the forest microclimate (by an increase in temperature, light, or decrease in humidity for example), the hydrological regimes, and/or the vegetation structure (KAPOS, 1989; LAURANCE et al., 2011; MALCOLM, 1998; SCHWITZER et al., 2011).

The BLT is probably the lion tamarin that faces the greatest degree of population isolation (MEYER; PIE; PASSOS, 2014). Nowadays, it is estimated the existence of approximately 1,500 wild individuals only, distributed in few remnants of forest displaying distinct landscapes and ecological resources (CULOT, personal communication). Historical and current modification of the habitat have threatened the BLT viability, reflecting indeed in population declines and local extirpations within the last decade (GARBINO; REZENDE; VALLADARES-PADUA, 2016). Behavioral and ecological changes in fragments with different characteristics and degrees of modification have also been reported for the species (CULOT personal communication).

According to recent studies on climatic changes, the tendency of latitudinal temperature increases, which is expected to occur over the next 30–60 years, might promote the loss of most of the currently suitable habitat for BLT within its distribution range (MEYER; PIE; PASSOS, 2014).. Actually, climate- and landscape-based niche modeling showed that only 2% of the original geographic distribution of BLT (92,239 km^2) can be considered suitable for the species (REZENDE; SOBRAL-SOUZA; CULOT, 2020) . Thus, despite of the ability of this species to survive in disturbed environments, understanding genomic processes involved in the current

diversification of the BLT populations become a relevant matter to raise evolutionary and adaptive issues useful to help with conservation decision-making (ANDREWS et al., 2021; BEKKEVOLD et al., 2020; HARRISSON et al., 2017). In this sense, genomic studies using next-generation sequencing (NGS) technologies, as Genotyping by Sequencing (GBS), are allowing to measure genetic variables of neutral and adaptive diversity (e.g. levels of heterozygosity and inbreeding), and social structure (e.g. kinship, effective size), as well as to identify loci under differential selection associated with environment traits (ALLENDORF; HOHENLOHE; LUIKART, 2010; STEINER et al., 2013).

Genome-environment associations analyses (GEA) have become a powerful tool to explore signals of local adaptation in heterogeneous landscapes (FRICHOT et al., 2013; HANCOCK et al., 2008; JOOST et al., 2007; PONCET et al., 2010). Methods for identifying polymorphisms with high correlation with environmental variables could be based on univariate approaches, as Latent Factor Mixed Models (LFMM), or multivariate ones, as Redundancy Analysis (RDA). When taking into account population structure, univariate association approaches are strong tools for accurately detecting even weak adaptation signatures, since they test for single-locus–single-predictor correlation (FRICHOT et al., 2013; GÜNTHER; COOP, 2013; LOTTERHOS; WHITLOCK, 2015; RELLSTAB et al., 2015). However, the limitation of these approaches is that they assess each locus independently, limiting polygenic signals from being detected (FORESTER et al., 2018). Alternatively, multivariate analyses, incorporating the effects of multiple loci and predictors, can be implemented to solve this constraint (CAPBLANCQ et al., 2018; FORESTER et al., 2018; RELLSTAB et al., 2015). Therefore, combining such approaches to search outliers may improve the chances of detecting complex selection patterns given the advantages of both univariate and multivariate GEA methods (RELLSTAB et al., 2015).

Overall, in the present study we analyzed SNPs derived from GBS data and investigated local adaptation signatures in black lion tamarins from five wild populations presenting distinct landscapes and ecological attributes. We examined neutral and non-neutral genetic diversity and inferred on population structure and kinship into each sampled population. We employed a combination of outlier searching and GEA methods to identify genome-wide signatures of selection. In addition to applying univariate (LFMM) genome–environment association methods to identify individual SNPs showing evidence of divergent selection in response to climate variation, we also used a multivariate method (RDA) to identify subtler signatures of polygenic climatic adaptation. Finally, we annotated the candidate genes exhibiting genomic

signatures of differential selection, and explored their biological functions and implications for fitness and potential adaptive in BLT.

3.2 MATERIALS AND METHODS

3.2.1 Ethical requirements and research permits

The present study was approved by the Ethics Committee on Animal Experimentation (Federal University of São Carlos, São Carlos, São Paulo, Brazil), under CEUA- UFSCAR number 9805200815; the Authorization System and Biodiversity Information of the Chico Mendes Institute for Biodiversity Conservation (Ministry of Environment, Federal Government, Brazil), under SISBIO-ICMBio numbers 50616-1; and the National System of Genetic Patrimony Management and Associated Traditional Knowledge (Ministry of Environment, Federal Government, Brazil), under SISGEN number A411359. The biological sampling from the alive individuals were conducted under anesthesia using direct inhalation devices, using inhalation anesthesia equipment calibrated with Isoflurane (2– 5%) and Oxygen (2 L/min). The animals were handled by a veterinarian who released them safely after the blood collections. These procedures followed all ethical and legal recommendations proposed by the American Society of Primatologists for the Ethical Treatment of Non-Human Primates.

3.2.2 Sample collection and DNA extraction

We sampled a total of 21 black lion tamarins from three fragments located in the Lower Paranapanema (westernmost portions of São Paulo state) and two fragments in the Upper Paranapanema (easternmost portions of São Paulo state), both areas where the species is currently distributed (Figure 3.1). The total sampling corresponds to eight different groups: two groups from Morro do Diabo State Park (PMD, N=5), one from Santa Maria Farm (SM, N=4), one from Ponte Branca (PB, N=4), two from Capão Bonito National Forest (CB, N=6) and two from a Riparian Forest located in Guareí (GU, N=2). These samples areas present differences in terms of matrix type, fragment sizes, elevation (see Table S3.1) and consequently key resources.

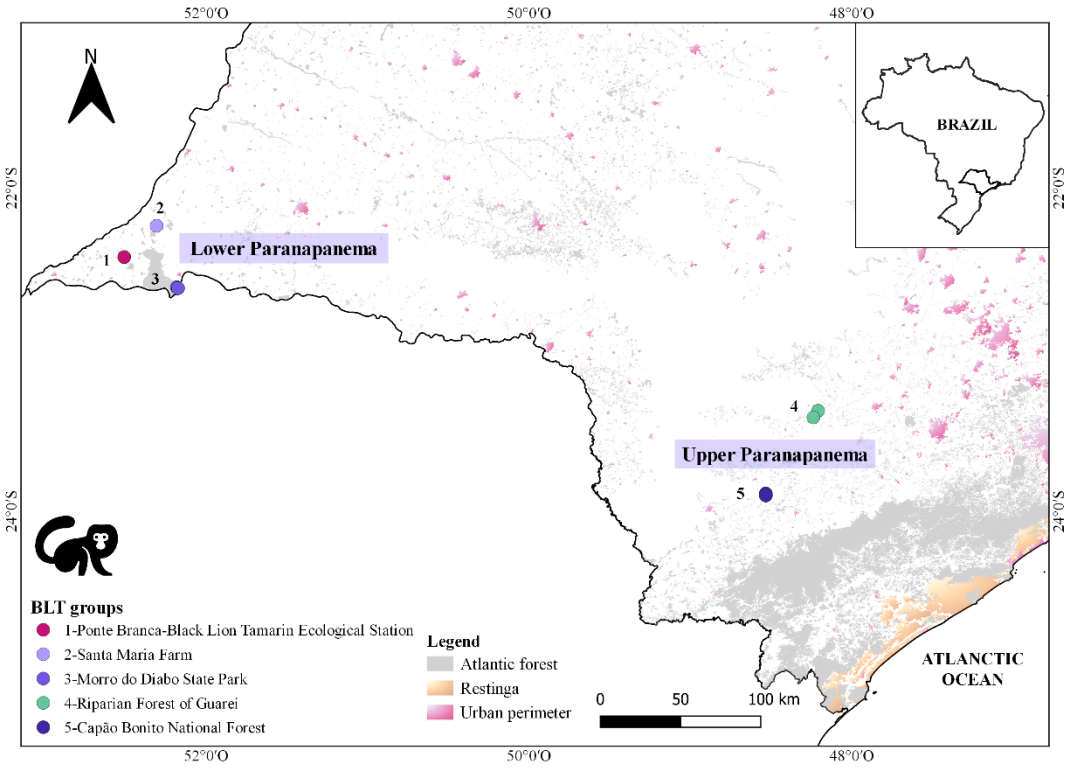


Figure 3.1 - Sampled collection sites for Black Lion Tamarin groups. Three sites were located within Lower Paranapanema: Ponte Branca (pink dots), Santa Maria (violet dots), Morro do Diabo State Park (purple dots), and two within Upper Paranapanema: Riparian Forest of Guareí (green dots) and Capão Bonito National Forest (blue dark dots).

Overall, we collected about 0.3 mL of fresh blood from each individual was collected using vacutainers containing EDTA (3.6 mg). Blood samples were preserved at 20 °C for subsequent DNA extractions. DNA were extracted using a phenol protocol (SAMBROOK; FRITSCH; MANIATIS, 1989) , and then quantified on a Qubit 2.0 (Life Technologies, Carlsbad, California, EUA). The quality of the DNA samples was evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, EUA). Samples with insufficient DNA concentration were concentrated with a SpeedVac (Thermo Scientific, Waltham, Massachusetts, EUA), resuspended in a final volume of 15 µl of water, and requantified.

3.2.3 Genotype-by-sequencing analysis

Library preparations for Genotype-by-sequencing followed the protocol proposed by Elshire et al. (2011), using the *Pst*I restriction enzyme. All samples were vacuum-lyophilized

after digestion, and then adapters with indexing sequences (barcodes) were ligated on each DNA sample. After restriction and ligation reactions, libraries were size-selected for fragment sizes between 200 bp and 450 bp. Pooled samples were sequenced quantified using real-time PCR with the KAPA Biosystems Quantification Kit (Illumina, San Diego, California,). Subsequently, the four pools were mixed in equimolar proportions in a single sample. The pooled libraries were evaluated on a BioAnalyzer Agilent 2100 equipment, using a high sensitivity DNA kit (Thermo Fisher Scientific, Waltham, Massachusetts, EUA), and then quantified on a real-time PCR equipment with a KAPA Biosystems Quantification kit (Illumina, San Diego, California,) diluted to 2pM. The next-gen sequencing was performed on a HiSeq 2500 Illumina Platform (Illumina, Inc, San Diego, California) and a single lane.

Raw sequencing reads were demultiplexed, quality filtered, and clustered using the API version of the seven-step computational pipeline iPyRad v.0.7.24 (EATON; OVERCAST, 2020) on computational cluster of the Laboratorio de Bioinformática y Genómica de la Biodiversidad, Universidad Nacional Mayor de San Marcos, Peru. Demultiplexing step used sample-specific barcode sequences, allowing one mismatch in the barcode sequence. Base calls with a Phred quality score under 20 were converted to Ns, and reads containing more than five low quality bases in a read were discarded. Subsequently, reads were clustered using a threshold of 90% sequence similarity. Sequences were then aligned to the black lion tamarin reference genome (unpublished data) to improve the clustering. Reads were clustered using a 90% sequence similarity threshold both within a single individual and between samples to genotype polymorphisms. Orthologous loci were identifyed with a minimum coverage of 6× per locus. The final filtering and quality control for the genotype data were realized in the R package r2vcftools (https://github.com/nspope/r2vcftools), a wrapper for VCFtools (DANECEK et al., 2011). The data were filtered for excess heterozygosity based on deviations from Hardy–Weinberg equilibrium (HWE, $p < 0.0001$), linkage disequilibrium (LD, $r2 < 0.8$), and an overall minor allele frequency (MAF) of 0.05 %, retaining only GBS loci found at least in one individual from all groups.

3.2.4 Detecting SNPs putatively under selection and defining data sets

For identifying SNPs putatively neutral or under selection we first implemented a Principal Component Analysis (PCA) using a Mahalanobis distance-based approach with PCAdapt v.4.3.3 (LUU; BAZIN; BLUM, 2017) in R (R Development Core Team 2020). The Mahalanobis distance was computed for each bi-allelic SNP and the scores that did not follow

the distribution of the bulk of distance points were considered outliers (LUU; BAZIN; BLUM, 2017). Thus, PCAdapt assumes that markers excessively related to population structure are candidates for differential selection. Individuals are not classified into predefined populations in the PCAdapt; instead, population structure is determined using PCA, and loci under putative selection are identified as those that are excessively correlated with population structure. To assess the best supported genetic clustering among the sampled individuals, 10 principal components (PC) were used, and the optimal number of PC was determined using the Cattell's graphical rule, following Luu et al. (2017). Outliers were identified based on a false discovery rate (FDR) of 0.05, using the R package *qvalue* (STOREY; TIBSHIRANI, 2003). Loci with global minor allele frequency (MAF <0.05) were excluded. PCAdapt outliers were scored based on q-value corrected p-values, with K set to 5. The results obtained from the PCAdapt were used to define neutral and non-neutral SNP datasets, and then used for genetic diversity and population structure assessments, and kinship analyses.

3.2.5 Population structure and genetic diversity

Based on the PCAdapt results, we used three different datasets for the genetic diversity and population structure analyses as follows: neutral, outlier, and combined neutral and non-neutral SNPs. First, we carried two tests to determine the population structure, and then we performed the genetic diversity estimates.

Initially we employed a multivariate approach based on a Discriminant Analysis of Principal Components (DAPC), using the package Adegenet (JOMBART, 2008), implemented in R (R Development Core Team 2020), without the sampling areas as priors for populations. The optimal number of PCs to be retained was selected using an alpha score optimization with the *optim.a.score* function in the Adegenet package, considering PCs accounting for 80% of the variance. To determine the appropriate number of discriminant functions to represent the datasets, the Bayesian Information Criterion (BIC) was adopted. Next, we used the function *snmf* of the R package LEA (FRICHOT; FRANÇOIS, 2015) to estimate individual ancestry and population clustering by utilizing an non-negative matrix factorization (sNMF) and least-square optimization procedure of ancestry coefficients. This approach is capable of efficiently analyzing big bi-allelic datasets without loss of accuracy when compared with Bayesian clustering programs, like Structure (PRITCHARD; STEPHENS; DONNELLY, 2000) and Admixture (ALEXANDER; NOVEMBRE; LANGE, 2009). This analysis has the advantage of being substantially more computationally efficient, robust to many of Hardy-Weinberg's

assumptions, and more appropriate for dealing with inbred lineages. The *snmf* function was used with K = 1-10, with 100 repetitions per K value. The cross-entropy criterion was adopted to determine the value of K that best explained the results.

Considering the genetic clusters assigned by *snmf* and DAPC analyses, we then estimated genetic diversity also for the three datasets, using the R package HIERFSTAT (GOUDET, 2005). Observed (Ho) and expected (He) heterozygosity, overall FST as well FIS were calculated using *basic.stats function*. The *boot.ppfis* function was used to calculate the confidence intervals for populations-specific FIS with 1.000 bootstrap replicates. Pairwise FST, following Weir and Cockerham (1984), was calculated using the *genet.dist* function with R package HIERFSTAT. Significance values were calculated using 1,000 bootstrap replications with *pwfst* function of the dartR package (GRUBER et al., 2018). Effective population sizes (Ne) employing the heterozygosity excess method and the lowest allele frequency value of 0.05 were estimated using NEESTIMATOR 2.1 (Do et al., 2014). Finally, to test isolation by distance, mantel correlation between geographic and genetic distances was assessed using the *mantel.randtest* function with R package ad4 (DRAY; DUFOUR, 2007). Geographic distance matrices were generated using the *dist* function of R based on Euclidean distance. Nei's genetic distance matrix (Nei, 1978) was calculated using the *dist.genpop* function with the R package Adegenet v.1.3-1 (JOMBART; AHMED, 2011). The significance values were assessed using 999 permutations and 95% confidence intervals.

3.2.6 Analysis of pairwise Relatedness

The neutral SNPs-based pairwise relatedness coefficients were estimated within each familiar group using the R package r2vcftools (https://github.com/nspope/r2vcftools), a wrapper for VCFtools (DANECEK et al., 2011), through the function *relatedness*, and based on the method described by Manichaikul et al. (2010). This method uses a robust algorithm to perform pairwise relationship inferences independently of sample composition or population structure (MANICHAIKUL et al., 2010). The criteria listed in table 3.1 were used to reliably infer on the relationship using kinship coefficients.

Table 3.1 - Relationship inference criteria based on estimating relatedness coefficients (φ) to SNPs data.

Relationship	Examples	Φ^*	Genomic inference criteria*	Inference criteria
1st degree	Monozygotic twin	$1/2$	$> \frac{1}{2^{3/2}}$	> 0.354
1st degree	Parent-offspring	$1/4$	$\left(\frac{1}{2^{5/2}}, \frac{1}{2^{3/2}}\right)$	[0.177,0.354]
1st degree	Full-sibs	$1/4$	$\left(\frac{1}{2^{5/2}}, \frac{1}{2^{3/2}}\right)$	[0.177,0.354]
2nd degree	Half-sibs	$1/8$	$\left(\frac{1}{2^{7/2}}, \frac{1}{2^{5/2}}\right)$	[0.084,0.177]
3rd degree	First cousins	$1/16$	$\left(\frac{1}{2^{9/2}}, \frac{1}{2^{7/2}}\right)$	[0.0442,0.0884]
Unrelated	-	0	$< \frac{1}{2^{9/2}}$	< 0.0442

* See Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, et al. (2010)

3.2.7 Genotype Environment Association (GEA)

To identify genomic signatures potentially linked to local adaptations associated with environmental variable we employed, respectively, the univariate- and multivariate-GEA methods: latent factor mixed models (LFMM) and redundancy analysis (RDA). We considered a total of 22 environmental variables, 19 obtained from the WorldClim v.2.1 bioclimatic database (FICK; HIJMANS, 2017) which were transformed into a raster layer with a spatial resolution of 30 seconds (1 km2) and three variables additional: minimum and maximum elevations, the area sizes (Table S3.1). Correlations between environmental variables were evaluated using a function for the variance inflation factor (VIF), implemented in the *usdm* R package (NAIMI et al., 2014). Values of VIF < 5 indicate that multicollinearity among the predictors should not be a problem for the model, so the variables must be retained (Table S3.3). Once the predictor variables were determined, we evaluate more subtle genomic signs of adaptation, by detecting atypical loci as candidates for functional importance using both approaches described below.

A global RDA was employed using the *rda* function in the VEGAN 2.4-5 (OKSANEN et al., 2017) package in R (R Development Core Team 2020). This analysis has shown high power to detect potential signals of multilocus adaptation because it allows finding the ideal combination of many loci and environmental predictors simultaneously (FORESTER et al., 2018; RELLSTAB et al., 2015). An ANOVA with 1,.000 permutations was implemented to

determine the significance (alpha 0.05) of the global RDA and the significance of each RDA axis. Candidate SNPs were identified based on the loading of each locus in ordination space that were ± 2.5 standard deviation from the mean loading like cutoff of each axis to identify candidate SNPs. Finally, the Pearson correlation coefficient was used to analyses the association between each candidate SNP and the environmental variables.

The univariate latent factor mixed model (FRICHOT et al., 2013) was implemented with LFMM package (CAYE et al., 2019). This method employs latent component mixed models that take into consideration neutral population structure, when evaluating associations between gene variation and candidate environmental variables (BEKKEVOLD et al., 2020). Thus, environmental variables are considered as fixed effects in the model, and population structure is modeled using latent factors. We used population clustering results from sNMF and DAPC to guide the choice of k latent factors. Instead of using all raw predictor variables we employed the variables with VIF< 5, also used for RDA. We ran LFMM using the previously identified number of genetic clusters (k = 5) as latent factors, to account for the underlying neutral genetic structure. We then calculated the genomic inflation factor (α) and modified it until a calibrated distribution of adjusted p-values was found, and re-adjusted the p-values for FDR using the q-value of 0.05, based on the Benjamin–Hochberg algorithm (CAYE et al., 2019) . Thus, we investigated genomic signatures related to local adaptation, accounting for the underlying population structure. The scrip used for LFMM are available in https://github.com/jdalapicolla/LanGen_pipeline/blob/master/07-LFMM2.R.

After identifying the outliers, the resulting data obtained were used to construct a Venn diagram and evaluate the congruence of outlier SNPs identified across each of these tests.

3.2.8 Gene prediction and functional annotation

The outlier SNPs common in at least two of the methods used were used as queries in nucleotide searches with BLASTX with E-value 1E-3 against the nr database at the National Center for Biotechnology Information (NCBI), using Blast2GO (CONESA et al., 2005). Later, the functional annotation data were obtained based on the Gene Ontology (GO) for homologous sequences with E-value < 1E-6, annotation cut-off > 55, and a GO weight > 5. The output GO annotation was then classified in multilevel biological processes, molecular functions and cellular components. Then, the annotation with Blas2Go was compared to the annotation results obtained for the BLT's reference genome using the Funannotate pipeline (https://zenodo.org/record/4054262#.YWqsK2ZKjEY).

3.3 RESULTS

3.3.1 Genotyping by Sequencing analyses

The GBS sequencing of libraries resulted in a total of 3.026.810 raw reads, which were aligned with the *Leontopithecus chrysopygus* genome. Following the removal of samples with low-quality data, 29 ones were maintained for further analysis. The quality pre-filtering procedure retained a total of 217.851 SNPs. After the subsequent filtering, based on observed heterozygosity, global minor allele frequency (MAF <0.05) and LD (R2 > 0.8), 3.017 SNPs were retained for a total of 21 individuals from the five sampled populations.

3.3.2 Neutral and putatively non neutral SNPs identification

PCAdapt, following the Cattell's graphical, showed that most of the variation was accounted for at K=5, with the main proportion of explained variance for the two first PCs (Figure S3.1). The distribution of the p-values was visualized with a Manhattan plot and a QQ-plot, and then these values were used to compute the q-values (Figure S3.2). After FDR adjustment at 0.05, 345 SNPs were identified as outliers (Figure S3.1). Thus, we considered three different datasets for the subsequent genetic diversity and structure analyses: the combined dataset (3.017 SNPs), the neutral dataset (including 2.672 SNPs), and the outlier dataset (345 SNPs).

3.3.3 Genetic diversity and population structure estimates

The sNMF and DAPC analyses using only outilier SNPs evidenced five genetic clusters. The sNMF analysis provided the lowest cross-entropy for a K=5, with less support for alternative K values (6-10; Figure S3.3). The best K-value reported for this dataset using this method is considered robust as it exhibited the lowest cross-entropy criterion value across 100 replicate runs of all K values tested. Similarly, the DAPC multivariate analysis returned the most likely BIC as K=5 (Figure S3.4), and retained 18 PCs and two discriminant functions that explained 100% of the total variance (DA1=76.43% and DA2=23.57%), revealing strong separation among five discrete clusters: Guareí, Ponte Branca, Morro do Diabo, Santa Maria and Capão Bonito (Figure 3.2). All individuals fell within their respective population-specific of the sampling sites tested, reinforcing the findings of a five-population pattern.

On the other hand, the sNMF analysis using the combined and neutral datasets evidenced three genetic clusters when the lowest cross-entropy value was considered. Four or five clusters were evidenced when these values begin to plateau (Figure S3.3), and K=5 was

taken into account because they produced the same clusters for outlier's dataset and also that have the same geographic signal (Figure 3.2). DAPC analysis also revealed strong structuring in five clusters for both datasets (Figures S3.4), being retained 18 PCs and two discriminant functions that explained 100% of the total variance (DA1=95.8% and DA2=4.2% for the neutral dataset; DA1=66.31% and DA2=33.69% for the combined dataset).

The global FST value calculated to outliers' loci (FST = 0.6769) was almost two times greater than the values found for the neutral (FST = 0.3404) and combined (FST = 0.3438) datasets. The pairwise FST values confirmed the relatively high levels of genetic differentiation across BLT populations for all datasets, indicating large genetic differences among the wild populations ($p < 0.001$) (Figure 3.3). The highest genetic differentiation was found to Santa Maria and Capão Bonito populations, followed by Ponte Branca and Capão Bonito ones for neutral and combined datasets. For outlier's dataset, the highest differentiation was observed to Santa Maria e Guareí. Although the lowest value of differentiation was observed between Santa Maria and Morro do Diabo populations, their FST indexes were also significantly distinct from zero. The Mantel test showed a significant positive correlation between genetic distance and geographic distances for all datasets: neutral ($r = 0.70$, $P = 0.016$), outliers ($r = 0.517$, $P = 0.046$), and combined ($r = 0.65$, $P = 0.017$) SNPs (Figure 3.4).

Regarding the genetic diversity estimates, the outliers evidenced the lowest values of observed (Ho=0.1883) and expected (He= 0.1189) heterozygosity than the neutral (Ho = 0.2492; He=0.1988) and combined (Ho = 0.2423; He=0.1897) datasets. In addition, we observed that He values among populations were similar within each dataset. For outliers, the higher expected heterozygosity value was observed in Morro do Diabo, while for neutral and combined datasets was observed in Capão Bonito (Table 3.2). The average FIS for all datasets did not deviate significantly from random mating ($p = 0.001$). However, we observed mean negative values for all populations (Fis outliers= -0.5757; Fis neutral= -0.2536; Fis combined= -0.2772) and for all datasets (Table 3.2), indicating the excess of heterozygosis. The effective population size was higher in Guareí for all datasets and showed the lowest values of inbreeding coefficient for neutral and combined datasets. On the other hand, Santa Maria showed the lower effective population size and the higher inbreeding coefficient for all datasets (Table 3.2).

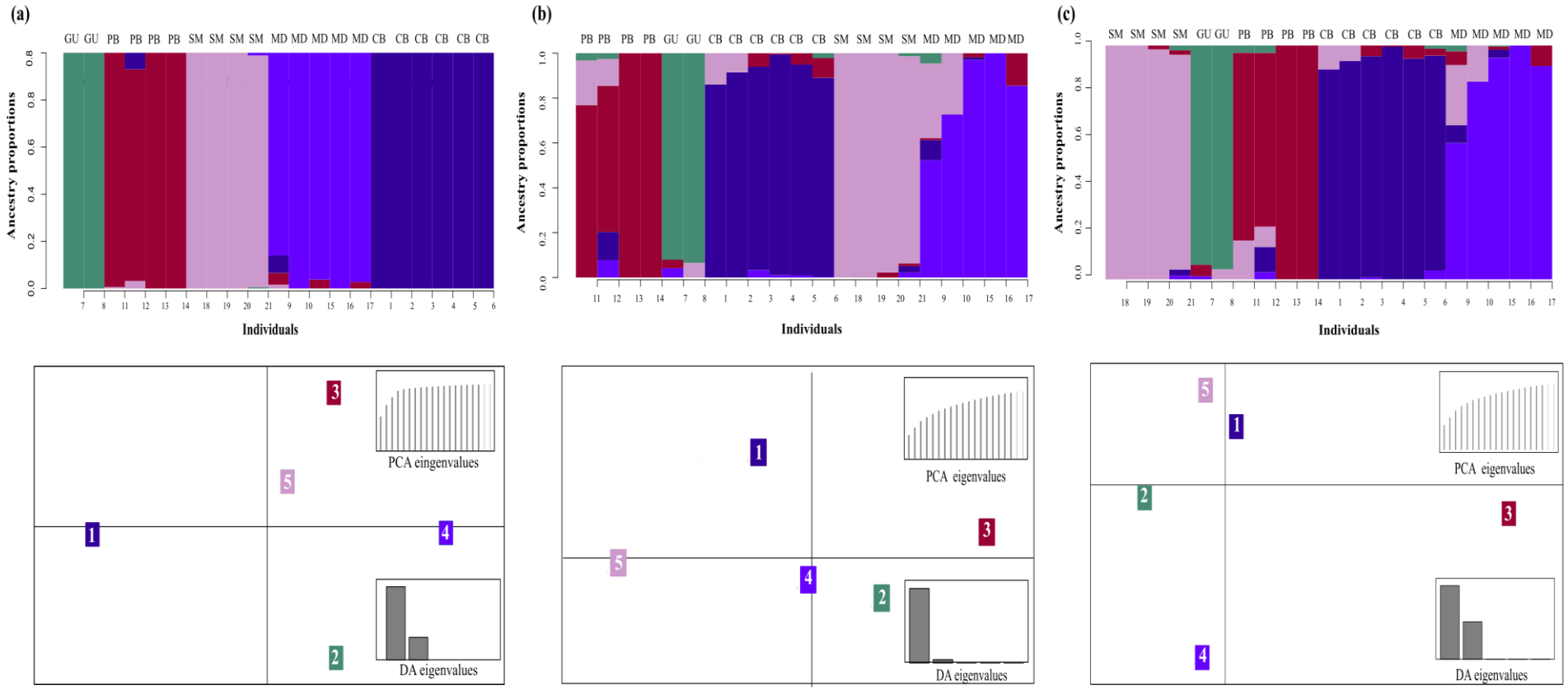


Figure 3.2 - Genetic structure of black lion tamarin populations assessed by two approaches based on outliers' (345 loci), neutral (2.672) and combined (3.017) dataset. Estimates of admixture proportions inferred with sNMF for the with the best supported number of ancestral populations (K = 5). (b) Discriminant analysis of principal components (DAPC) showing the scatterplot of the first two principal components and percent of DA for each axis. Colors are representative of the admixture proportions of individuals estimated with sNMF. GU=Guareí; PB=Ponte Branca; SM=Santa Maria Farm; MD=Morro do Diabo State Park; CB=Capão Bonito National Forest.

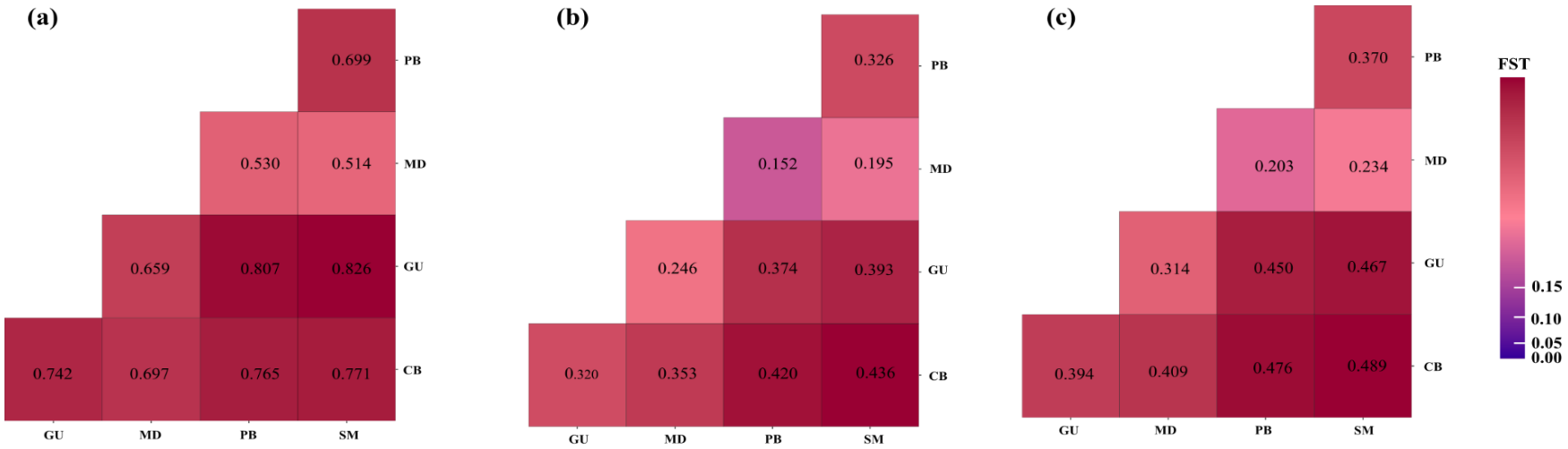


Figure 3.3 - Heatmap pairwise F_{ST} (Weir & Cockerham, 1984).) values estimated for outliers (a), neutral (b) and combined datasets between the wild populations. All pairwise comparisons of FST were significant p<0.001 with 1000 replicates. GU=Riparian Forest of Guareí; PB=Ponte Branca; SM=Santa Maria Farm; MD=Morro do Diabo State Park; CB=Capão Bonito National Forest.

Table 3.2 - Summary genomic diversity statistics for the outliers (345), neutral (2.672) and combined (3.017) datasets for the BLT populations. N: Number of samples genotyped; He: expected heterozygosity averaged across loci; Ho: observed heterozygosity averaged across loci; pairwise FIS: average of inbreeding coefficient; Ne: effective population size.

		Outliers				Neutral				Combined			
Population	**N**	**He**	**Ho**	**Fis**	**Ne**	**He**	**Ho**	**Fis**	**Ne**	**He**	**Ho**	**Fis**	**Ne**
Capão Bonito	6	0.1522	0.2406	-0.4377	1.7	0.2241	0.2971	-0.1939	3.5	0.2159	0.2906	-0.2113	3.2
Guareí	2	0.0891	0.1512	-0.5312	2.3	0.1562	0.2234	-0.1690	8.9	0.1486	0.2151	-0.1919	7.4
Morro do Diabo	5	0.1699	0.2865	-0.5310	1.6	0.2066	0.2839	-0.2129	3.4	0.2024	0.2842	-0.2395	3.1
Ponte Branca	4	0.0785	0.1362	-0.5919	1.5	0.1451	0.2123	-0.2690	3.0	0.1375	0.2040	-0.2874	2.8
Santa Maria	4	0.0703	0.1258	-0.6448	1.4	0.1518	0.2285	-0.3228	2.5	0.1425	0.2168	-0.3389	2.4

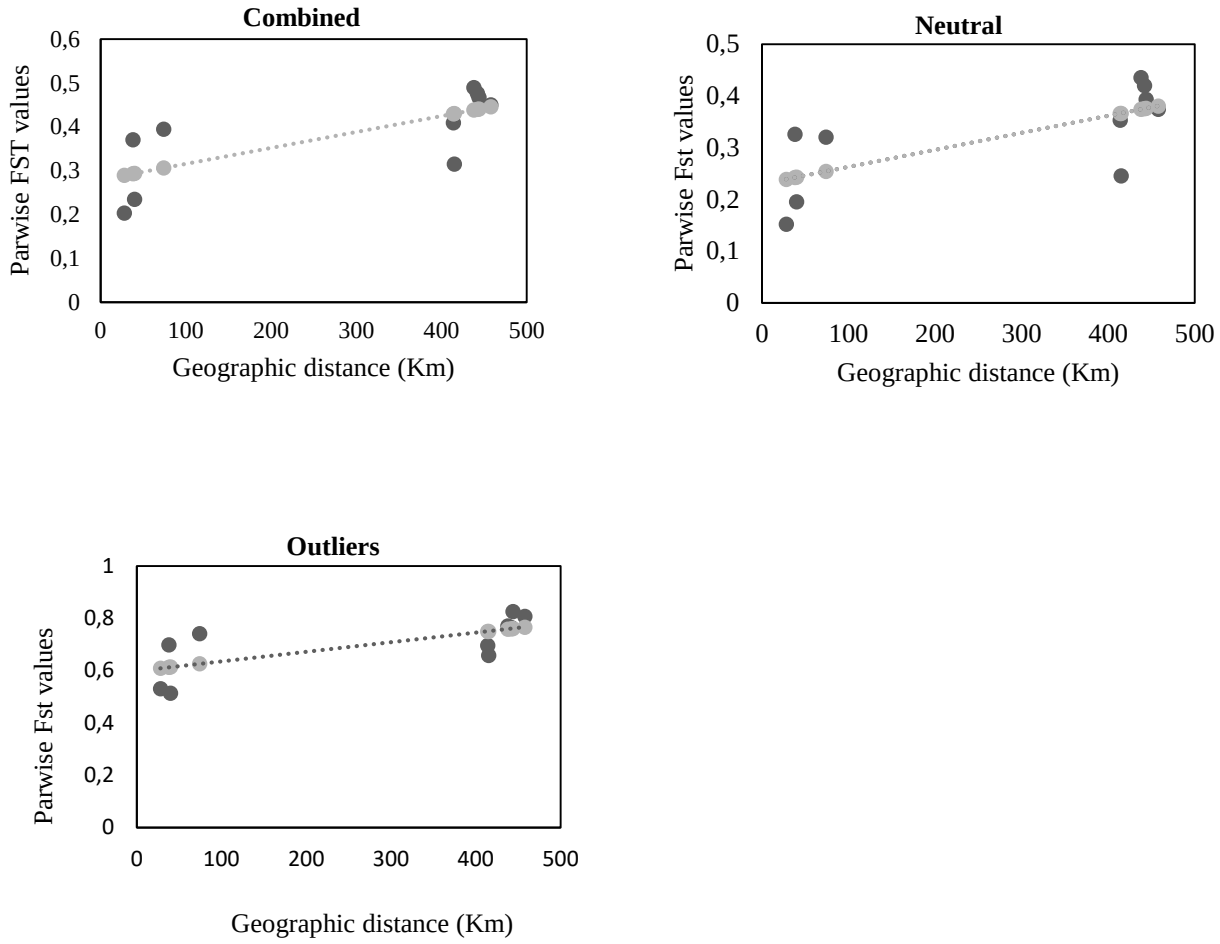


Figure 3.4 - Mantel test for isolation by distance (IBD) using pairwise linearized FST and geographic distance (km) among five populations of BLT for combined single nucleotide polymorphism (SNP) loci (3.017); only neutral SNP loci (2.672); and only SNP loci identified as outliers (345).

3.3.4 Relationship inference based on genomic data

Kinship coefficients were mostly positive, showing close genetic relatedness within the populations in general. We inferred 16 pairs as full-sibs (1st degree relatives) and nine as half-sibs (2nd degree relatives). Such analyses pointed twins in Ponte Branca (N=1), and Capão Bonito (N=1). On the other hand, unrelated BLTs were evidenced in Morro do Diabo (N=2), Ponte Branca (N=1) and Guareí (N=1). Kinship values found according to the relationship inference criteria based on the kinship coefficient (Φ) are shown in Supplementary Table S3.3.

3.3.5 Genotype Environment Association (GEA) analyses

The RDA model was significant (F3,17 = 3.83, p=0.001) and identified 131 SNPs that showed strong association with the environment variables. The first three axes also showed significance (F1,17 = 7.382, p=0.001; F1,7 = 2.567, p =0.001; F1,7 = 1.547, p=0.030; Table S3.3). The first (64%) and second (22%) axis explained most of the adaptive genetic variance among the populations. The first RDA axis was strongly correlated to temperature seasonality (-0.9282545) and the RDA2 and RDA3 axes were correlated with precipitation seasonality (0.8049704) and fragment size (-0.7769677), respectively. We found 66 outlier SNPs on the second RDA axis. Of these, 59 were most strongly correlated with precipitation seasonality (BIO15), five with temperature seasonality (BIO4), and two with fragment size (AREA). The third axis evidenced 46 outliers SNPs most strongly correlated with fragment size, seven with temperature seasonality and 12 with precipitation seasonality, totaling 65 outliers (Figure 3.5; Table S3.4). Many of these outliers SNPs revealed a high correlation with more than one environmental variable, suggesting they are likely interacting with the multivariate environment. The first and second RDA axis were also correlated strongly with the five populations The genetic variation in Capão Bonito was correlated with a temperature seasonality. Meanwhile, genotype differences between Morro do Diabo, Ponte Branca and Santa Maria, all from the Pontal of Paranapanema region, were correlated with fragment size and precipitation seasonality. Guareí was correlated with precipitation seasonality only.

The LFMM indicated a total of 371 loci with strong correlations to environmental variables and K=5. Of these, 153 were correlated predominantly with temperature seasonality (PC2). On the other hand, 124 SNPs were correlated with fragment size (PC1) and 94 predominantly with precipitation seasonality (PC3) (Table S3.5). Histograms of p-values from the LFMM results suggest that the false-positive rate was well controlled (Figure S3.5).

Comparations among LFMM, RDA and PCAdapt methods showed nine common loci. When we considered PCAdapt and RDA, PCAdapt and LFMM, and LFMM and RDA comparisons, we found, respectively, 64, 36 and four common SNPs. The overlap among methods for detecting loci potential associated with local adaptation is represented by a Venn diagram (Figure 3.6).

3.3.6 Gene prediction and functional annotation

The annotation of the 113 outliers evidenced after comparations among methods showed 18 SNPs into genes with coding products already known (E-value 1E-3). The functional categorization revealed proteins involved in relevant physiological process, such as binding, structural, transport and catalytic activities, evidencing important roles to fitness related to immunity, responses to stimulus, and species interactions, for example (Figure 3.7). The main molecular and biological functionalities for the annotated loci are listed according to the loci localization in the scaffold of the BLT's reference genome, considering the position of the outlier SNPs derived from GBS-loci analyses, gene products, GO categories, change of base in SNP, and environmental predictors (Table 3.3). The loci candidates to local adaptation were plotted in a graphical considering the genotypes frequencies, the populations and the environment variable associated (Figure 3.8).

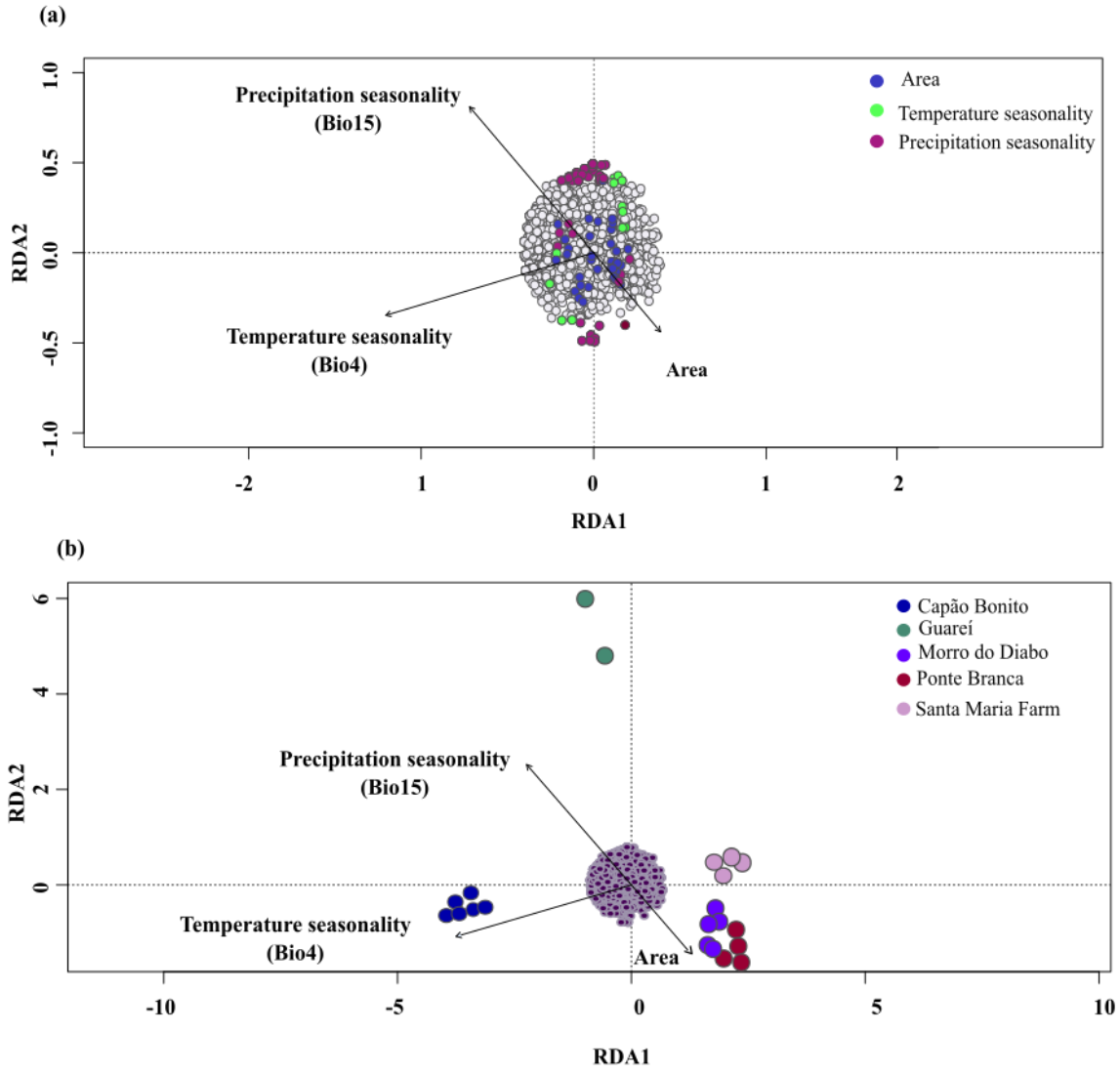


Figure 3.5 - Biplots summarizing results of global redundancy analysis (RDA). The axes 1 and 2 were plotted with symmetrical scaling using 3017 SNPs. Environmental predictors are represented as black vectors, where length reflects the amount of variance in SNP genotypes explained by that variable and angles of arrows represent the correlation between variables. (a) Candidate SNPs from RDA axis 1 are highlighted in colors based on the environmental predictor with the strongest correlation, while all other SNPs are the white dots. (b) Groups of BLT are highlighted in colors, and SNPs are grey

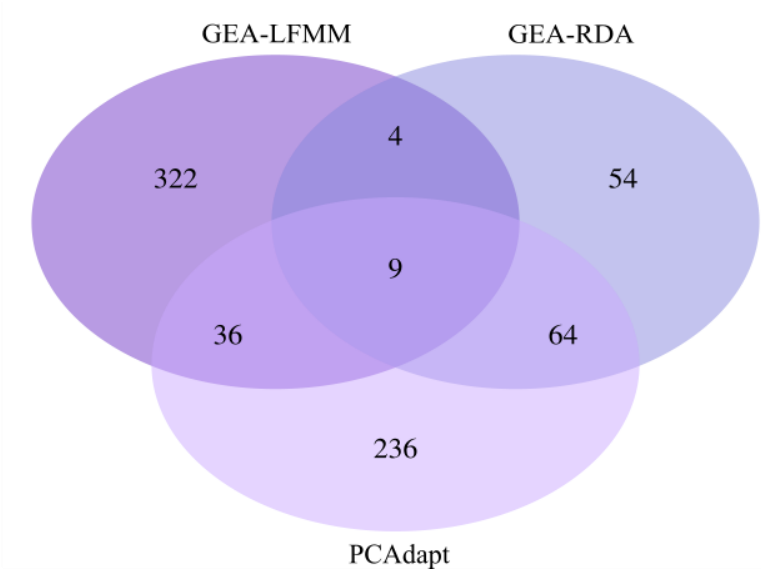


Figure 3.6 - Venn diagram showing the intersection of candidate SNPs for Black lion tamarin identified by PCAdapt, GEA-LFMM and GEA-RDA. Putative adaptive loci identified using environmental association tests, employed mean temperature seasonality, precipitation seasonality and fragment size (area).

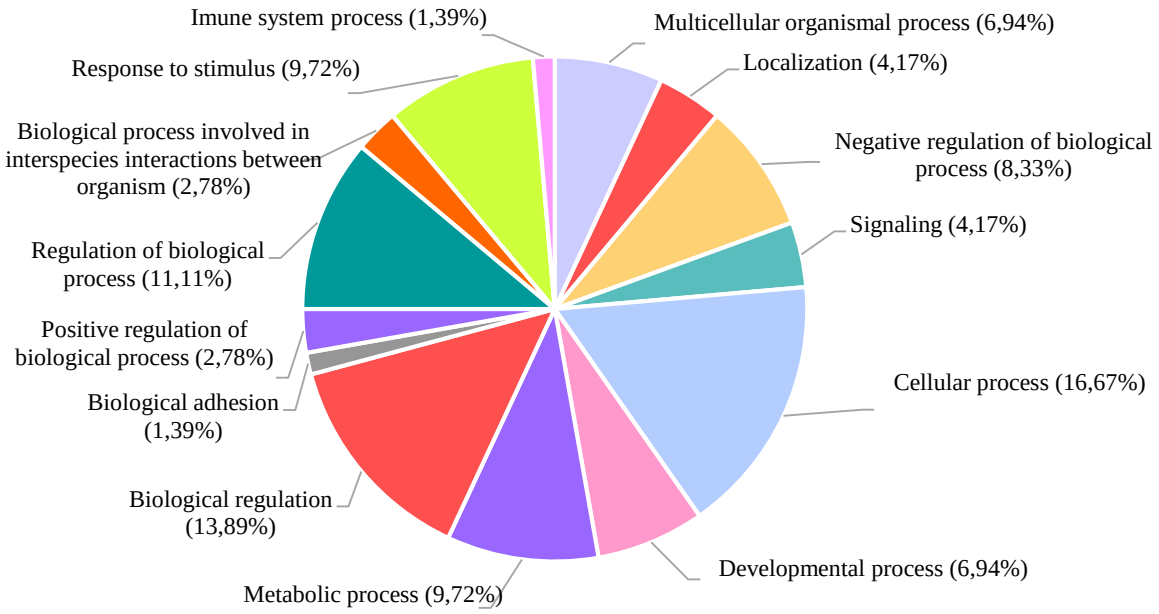


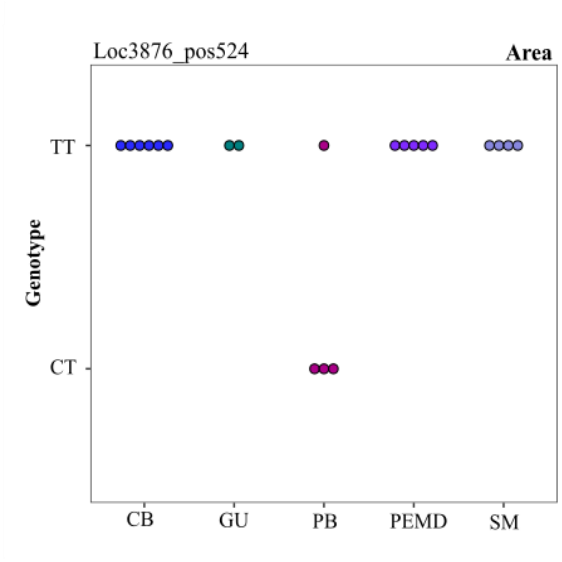
Figure 3.7 - Blast2GO annotation whit the putative functional category, and distribution of 18 significant hits.

Table 3.3 - BLASTx, gene ontology (GO) annotation, change of base in SNP, and environmental predictors (Bio4= temperature seasonality; Bio15= precipitation seasonality) for 18 outlier loci with positive BLAST hits. P: biological process F: molecular function, C: cellular component.

Chromosome and Position	Locus ID	REF-ALT*	Gene description	Gene Ontology	Method	Predictor
Scaffold2554 132502	loc3876_pos524	C-T	Mitotic spindle assembly checkpoint protein MAD1	P: regulation of mitotic cell cycle phase transition; C: nuclear envelope; C: mitotic spindle	RDA/PCAdapt	Area
Scaffold7327 100705	loc10262_pos109	A-G	Alcohol dehydrogenase 1C	F: oxidoreductase P: oxidation: cytosol	RDA/PCAdapt	Area
Scaffold10279 2168949	loc13502_pos175	A-T	BC-1514 protein-like	Uncharacterized protein	RDA/LFMM/PCAdapt	Area
Scaffold15452 1978711	loc19183_pos142	C-G	DNA-direct polymerase, eta, isoform CRA_c	F: DNA-binding P: DNA repair, DNA replication	RDA/PCAdapt	Bio15
Scaffold20674 5157433	loc27865_198	C-T	Myotubularin-related protein 3	F: Hydrolase; F: Protein phosphatase P: Lipid metabolism	RDA/PCAdapt	Area
Scaffold20900 1230062	loc28324_pos245	C-T	AT-hook-containing transcription factor isoform X1	P: regulation of inflammatory response; C: cytosol	RDA/PCAdapt	Area
Scaffold31990 3756773	loc38201_pos186	C-T	Methyl-CpG-binding domain protein 1	F: DNA-binding P: Transcription regulation	RDA/PCAdapt	Bio15
Scaffold34081 5980105	loc40115_pos96	T-C	Keratin, type I cytoskeletal 18	P: response to fructose; F: scaffold protein binding; C: nucleolus; C: cytosol	RDA/PCAdapt	Bio15
Scaffold41527 3129977	loc44388_pos95	G-A	Protein GVQW1-like	P: regulation of transcription, DNA-templated; C: nucleus	RDA/LFMM/PCAdapt	Bio4/Bio15
Scaffold45170 1138165	loc46599_pos117	G-A	PDZ domain-containing protein 7 isoform X4	C: membrane; C: integral component of membrane	RDA/PCAdapt	Area
Scaffold45321 1399108	loc46834_pos38	C-T	DNA (cytosine-5)-methyltransferase 1	F: Chromatin regulator, DNA-binding C: Transcription regulation	RDA/PCAdapt	Area
Scaffold55363 367245	loc52126_pos121	G-A	Peptidoglycan recognition protein 3	F: Antibiotic, Antimicrobial; C: Immunity, Innate immunity	RDA/PCAdapt	Area

Scaffold98159 2363422	loc71664_pos38	T-C	Leucine-rich repeat-containing protein 30	P: signal transduction; F: protein serine/threonine phosphatase activity; C: cytoplasm	RDA/PCAdapt	Bio4
Scaffold100088 1981659	loc72744_pos162	G-A	Hypothetical protein EGM_11598, partial	Uncharacterized protein	RDA/LFMM/PCAdapt	Bio4/Bio15
Scaffold220687 1832993	loc97376_pos84	G-A	Keratin, type II cytoskeletal 8	P: Host-virus interaction; P: hepatocyte apoptotic process; C: nucleus; C: cytosol	RDA/PCAdapt	Bio15
Scaffold336004 678677	loc107293_pos112	T-A	Nitric oxide synthase, brain	P: response to hypoxia; P: response to heat; P: negative regulation of blood pressure; F: scaffold protein binding; C: cytosol; C: cytoskeleton	RDA/PCAdapt	Bio15
Scaffold480300 602625	loc113194_pos148	G-A	ERBB2 isoform 18	P: carbohydrate metabolic process F: racemase and epimerase activity, acting on carbohydrates and derivatives; C: membrane	RDA/PCAdapt	Bio15
Scaffold517928 5984	loc114058_pos143	C-T	Nucleoplasmin-2 isoform X9	F: chromatin binding P: single fertilization P: oocyte differentiation	RDA/PCAdapt	Area

*Change of base in SNP. REF= reference allele; ALT=alternative allele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Genotype
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CT
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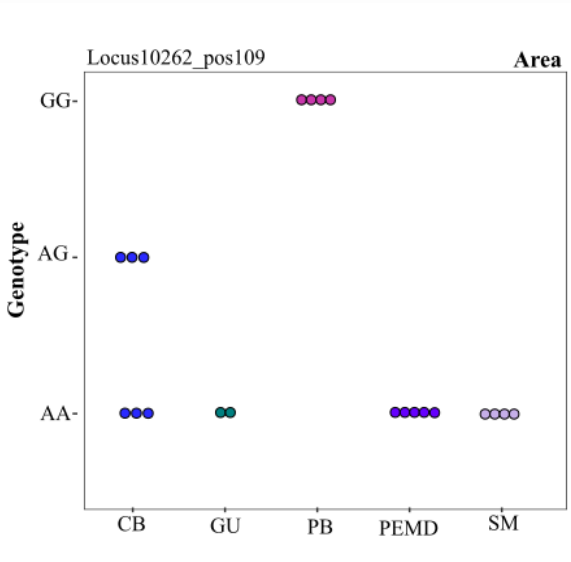
PEMD
SM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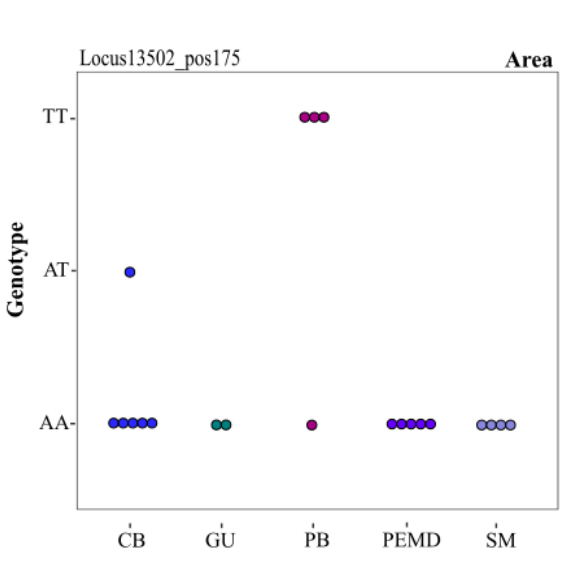
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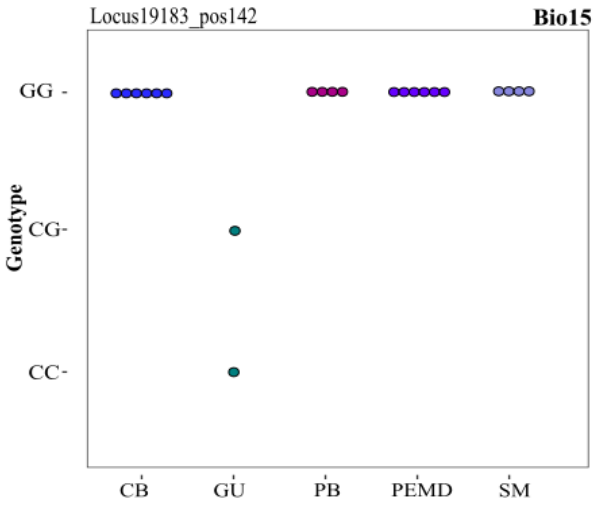
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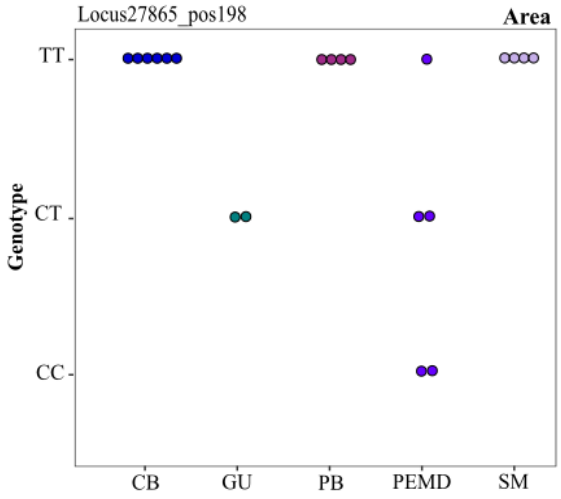
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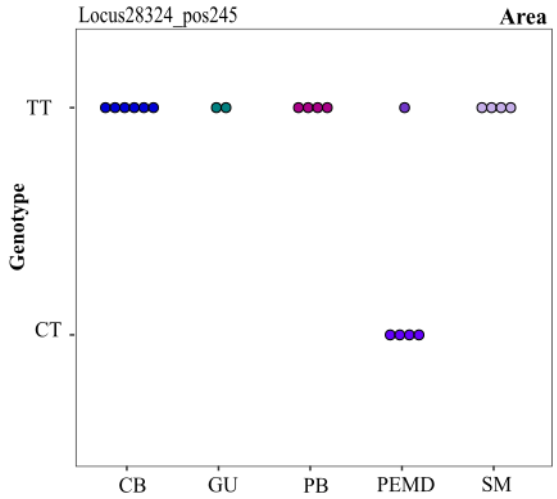
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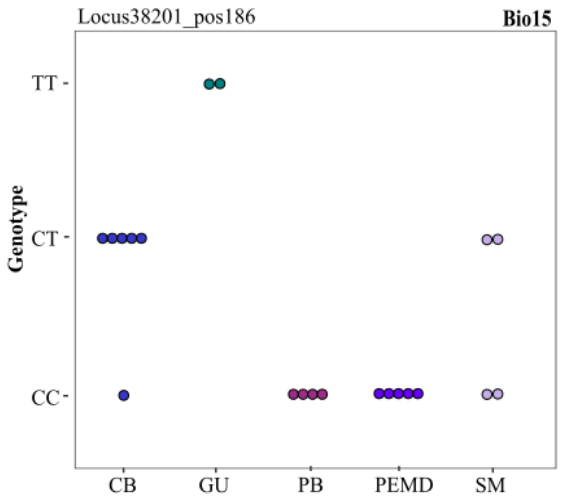
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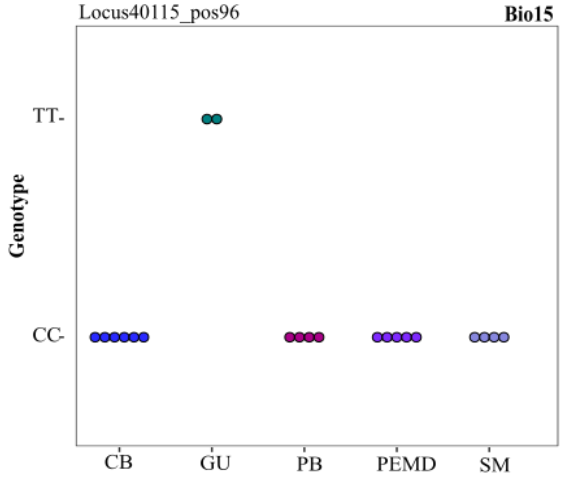
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Genotype
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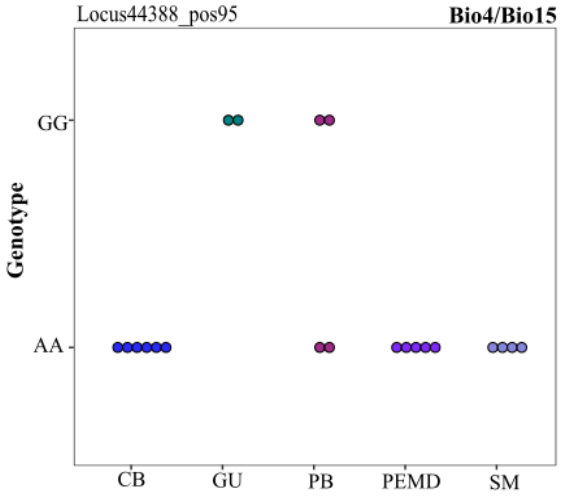
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Bio4/Bio15
Genotype
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PB
PEMD
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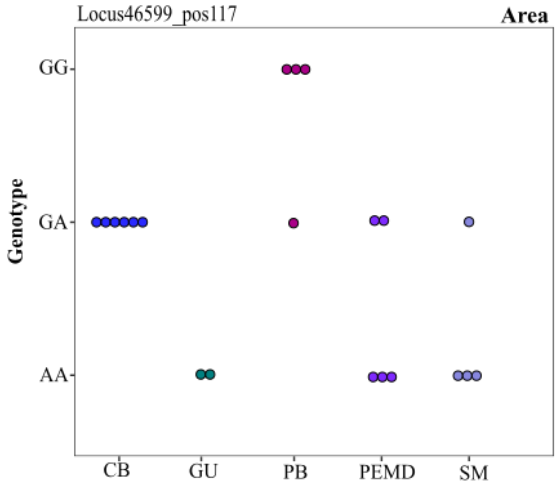
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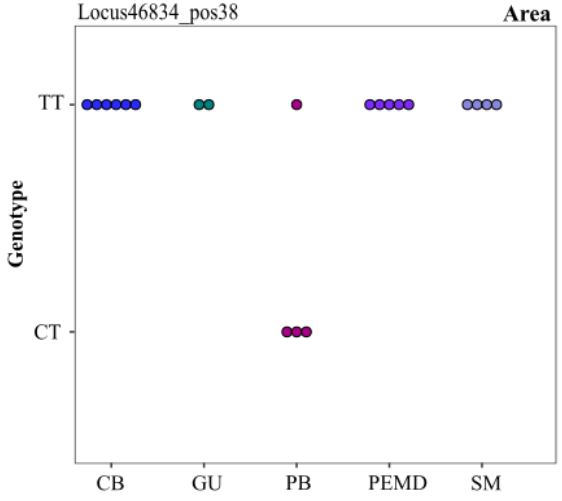
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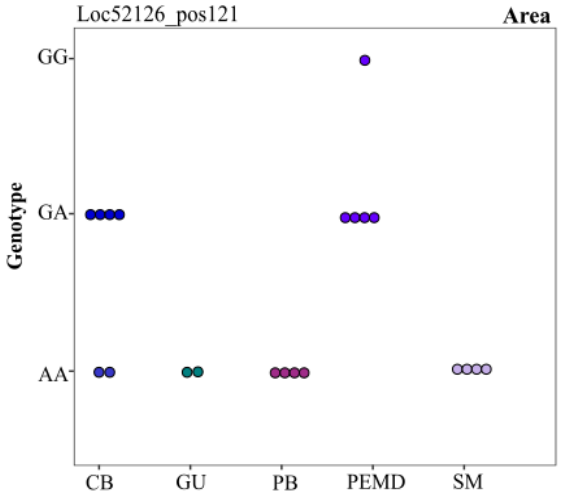
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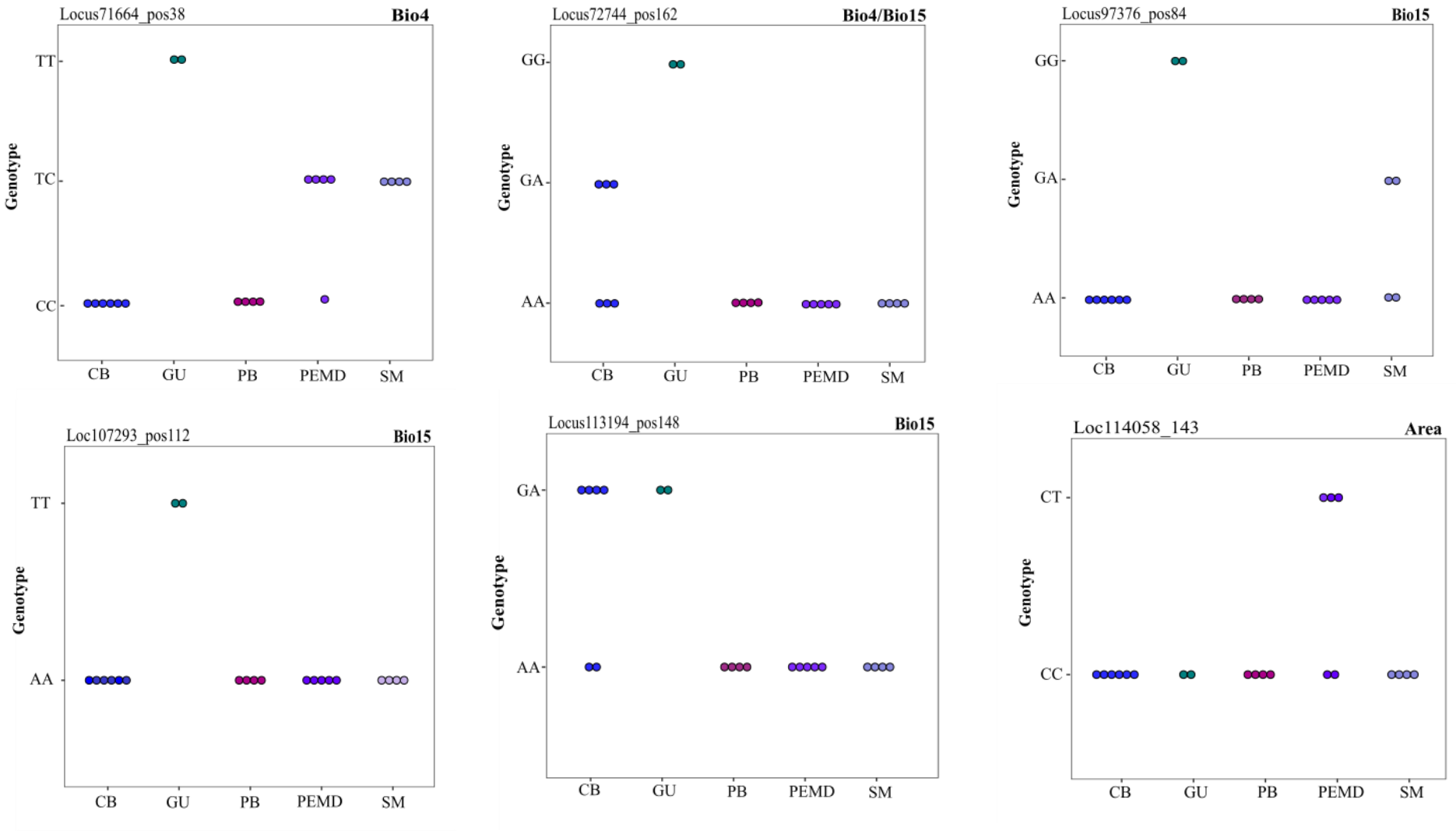

Figure 3.8. Genotypes frequencies of loci candidates to local adaptation and the environment variable associated for the five populations of BLT.

3.4 DISCUSSION

3.4.1 Populations divergency, genetic diversity and kinship analysis

We found evidence of strong population differentiation for the neutral, outlier, and combined SNP datasets, with sampling locations structured into five distinct groups according to the sNMF and DAPC analyses. Pairwise FST values for the outliers were high and significant, evidencing that Guareí and Santa Maria as the most diverged populations (Figure 3.3). When we considered only neutral SNPs, the pairwise FST comparisons between groups were also significant, indicating that such genetic divergences in BLT wild populations are driven by both non-neutral and neutral SNPs. In addition, we found a significant IBD pattern (Figure 3.4), indicating that geographic isolation is one of the main factors influencing the restricted gene flow among the populations and implying that gene flow is more likely to occur between nearby areas if there was connectivity between the fragments. The population structure pattern found based on large SNP datasets is consistent with that one previously detected using microsatellite markers, indicating that BLT populations have been isolated for a considerable period of time (JAVAROTTI, et al., in prep). On the other hand, studies based on mitochondrial data showed sharing of ancestral haplotypes between currently disconnected fragments from Lower and Upper Paranapanema, suggesting that these regions-maintained population gene flow in the past (MODENA, et al 2021).

Among the four lion tamarins, the BLT is probably the species that faces the greatest degree of isolation, given its extant populations are sparsely distributed throughout the state of São Paulo (KIERULFF et al., 2008; MEYER; PIE; PASSOS, 2014). This current scenario for the BLT's distribution range is due to Anthropocene historic and contemporary events related to the deforestation and environmental associated disturbances in the Atlantic rainforest. According to recent studies, the species currently occupies less than 1% of its original area that was estimated as 92,239 km^2 (REZENDE; SOBRAL-SOUZA; CULOT, 2020). The annual rate of deforestation for Atlantic Forest in the period 2019 to 2020 was 13,053 hectares. For the same period, the annual rate of deforestation in Sao Paulo state was 218 hectares, showing a 402 %rise over the previous period (2018-2019) (FUNDAÇÃO SOS MATA ATLÂNTICA E INPE, 2021). As result, the majority of current BLT populations are isolated in small fragments (with exception of Morro do Diabo and Carlos Botelho State Park-Paranapiacaba) ranging from 284 ha to 5.000 ha, some of which are close to riparian forests and have limited dispersal, resulting in low gene flow. In such a context, it is expected that populations exhibit low levels of genetic diversity due to genetic drift and inbreeding effects.

Indeed, we found low heterozygosity values for all populations analyzed and for all datasets (Table 3.2). However, we observed higher values of heterozygosity in Capão Bonito using neutral loci and, in the Morro do Diabo using non-neutral loci, despite no significant differences ($p>0.05$). When we combined both type of loci, the values showed the pattern observed for the non-neutral, probably because the higher number of non-neutral loci (2.672), when compared to the outliers (345). The highest heterozygosity found in Capão Bonito and Morro do Diabo could be the result of larger effective population size for these populations in relation to Santa Maria and Ponte Branca. In addition, the sNMF results indicated that the genetic diversity observed in Morro do Diabo and Capão Bonito could be the results of admixture from un-sampled individuals. Interestingly, Guareí showed the largest effective population size and the lowest inbreeding values.

Some authors have claimed that the low levels of genetic diversity observed in lion tamarins is probably a common feature shared among species from the Callitrichidae family (MARTINS et al., 2011; POPE, 1996) due to social structure, which corresponds to family groups composed of 3–6 probably related animals (LIMA et al., 2003). Our kinship results confirmed such family structure, showing close relationships within each group analyzed. Despite this, none of the populations analyzed here showed significant inbreeding coefficients. Conversely, they exhibited an excess of heterozygotes, suggesting a tendency of escaping from inbreeding (outbreeding). These data may be associated with the behavior of no sex-biased dispersal found in tamarins (GARBER et al., 2016; LÖTTKER; HUCK; HEYMANN, 2004; MORAES et al., 2018) that could promote the introduction of new gene pools, and then the effective reproduction from mate pairs formed by individuals from different populations or familiar groups (FAULKES; ARRUDA; MONTEIRO DA CRUZ, 2003; HUCK; ROOS; HEYMANN, 2007).

The fact of finding unrelated individuals within some of the populations, in addition to the negative inbreeding values may be indicating dispersion of individuals from different populations, and the possibility of crossing between non-relatives. Studies that combined microsatellite and mitochondrial data also showed parental individuals from a same familiar group exhibiting different matrilineal structure, in addition the existence of some infants and/or juveniles with different haplotypes (JAVAROTTI, 2021). Dispersal of one or both sexes should work like a mechanism to maintain genetic variability and avoid inbreeding (FIEL; GUATELLI-STEINBERG, 2003; PUSEY; WOLF, 1996). Some primate species have adopted this mechanism and it has been well documented that both sexes often leave their natal groups

when they reach sexual maturity (e.g. *Alouatta seniculos*: CLUTTON-BROCK, 1989; *Callithrix jacchus*: FAULKES; ARRUDA; MONTEIRO DA CRUZ, 2003; *Saguinus mystax* HUCK; ROOS; HEYMANN, 2007; *Alouatta caraya*: RUMIZ et al., 1986). Nevertheless, it is it's worth noting that deforestation, and hence the continuous habitat fragmentation and loss have negative consequences in the dispersion and gene flow, limiting the increasing of genetic diversity. Consequently, in long-term, isolated populations tend to present higher crossing rates between relatives (CHIARELLO; GALETTI, 1994; OKLANDER; KOWALEWSKI; CORACH, 2010). Thus, although these populations are showing values of observed heterozygosity higher than those expected, the mean heterozygosity for the species is still low.

Studies using neutral markers, such as microsatellites, indeed have been revealed low levels of genetic variation in BLT (AYALA-BURBANO et al., 2017; PEREZ-SWEENEY et al., 2005) and other Leontopithecus species (GALBUSERA; GILLEMOT, 2008; GRATIVOL; BALLOU; FLEISCHER, 2001; MARTINS et al., 2015; MARTINS; GALETTI, 2011; MORAES et al., 2017), in addition to the excess of heterozygous observed in some populations of *L. chrysopygus* (AYALA-BURBANO et al., 2017) ; *L. caissara* (MARTINS; GALETTI, 2011); *L. chrysomelas* (GALBUSERA; GILLEMOT, 2007). On the other hand, when we compare the values of genetic diversity estimated by microsatellite loci and by SNPs derived from GBS, the values of heterozygosity are quite lower for large SNP datasets, considering both non-neutral and neutral. For the BLT, we found values of neutral heterozygosity ranging from 0.145 in Ponte Branca to 0.224 in Capão Bonito, while for 15 microsatellite markers the values ranged from 0 to 0.50 for Capão Bonito population (AYALA-BURBANO et al., 2017). For other species of Leontopithecus, the studies using microsatellites show similar values (MARTINS; GALETTI, 2011; MORAES et al., 2017, 2018b). However, when we compared the estimated values of heterozygosity using both neutral and non-neutral SNP datasets, we did not find significant differences ($p>0.05$). Therefore, regardless the nature of the molecular marker, the overall genetic diversity for BLT populations is low indeed.

3.4.2 Evidence for local adaptation

The use of combined univariate and multivariate methods to detect outliers' loci, allowed us to enhance the identification of single-locus and multi-locus adaptive signals, raising 113 outliers that were common in at least two methods (Figure 3.6). When environmental variations associated with adaptation are correlated to population structure, genome scans based on genetic differentiation, like those used in PCAdapt, detect true positives. (CAPBLANCQ et

al., 2018). For the BLT species, a significant genetic differentiation was observed, thus, PCAdapt was able to orient genetic variation in the habitat's direction. Even so, RDA has a larger statistical power than PCAdapt to detect SNPs outliers by taking advantage of information from environmental local conditions (FORESTER et al., 2018). Besides, the outlier's loci identified by RDA analysis would be indicating that most adaptations to local environmental conditions are polygenic (FORESTER et al., 2018). We also found that these environmental factors were both strongly associated with sampled populations, with the significant RDA axis separating the populations into five groups based on habitat differences (Figure 3.5). On the other hand, the advantages of a multivariate GEA-RDA compared to univariate GEA-LFMM analysis is that multivariate methods take all environmental variation into account at the same time, and it can simultaneously detect associations between different sets of loci and different sets of environmental variables (FORESTER et al., 2018).

Despite the diverse statistical methodologies employed by each test, the overlap between methods shows that the multiple outlier tests are likely detecting some of the same biological processes. The GEA analyses showed that temperature seasonality, precipitation seasonality, and fragment size, should be drivers for local adaptation in BLT populations. Indeed, such environmental variables could be recognized as important determinants of BLT distribution and its persistence under eminent climate change. The seasonality of temperature and precipitation, for example, is relevant because they are strongly related to plants phenology (MARQUES; ROPER; BAGGIO SALVALAGGIO, 2004; MORELLATO et al., 2000), and also key resources for BLT, providing sleeping sites, and substrates for foraging and feed. Really, some studies that quantify the effects of large-scale climate for frugivorous primate, reveal that climatic variability and El Nino events have a significant impact on potential primate resource levels (WIEDERHOLT; POST, 2010). On the other hand, the result of modification and the lack of connectivity between the remanent fragments may have resulted in changes in dynamics of the fragment, affecting microclimate through an increase in solar radiation, temperature, and wind as well as a decrease in humidity promoting mortality of the overall flora and fauna´s species that reside there (ARISTIZABAL et al., 2018; BRODIE; POST; LAURANCE, 2012). These modifications could have taken individuals to experience ecology and behavioral changes depending on the availability of resources, especially because different fragments display different resources (AYALA-BURBANO et al, in prep).

Despite of large variations in the seasonality of temperature and precipitation are not yet documented for the Atlantic forest, it was suggested that tropical region precipitation extremes

would be intensified by ~10% per warming degree (HUANG et al., 2019). In this scenario, primates are likely to experience more temperature change than the global mean, as well as precipitation changes that might vary from large increases in some areas to large decreases in others (GRAHAM; MATTHEWS; TURNER, 2016).

Variations in temperature and/ or precipitation, which were strongly associated with GEA-RDA and GEA-LFMM outliers, can influence physiological and behavioral processes (MCFARLAND et al., 2020; RASHAMOL et al., 2018) and then lead populations to local adaptation through divergent selective pressures. Changes in temperature, for example, can drive primates to employ a range of physiological mechanisms to cope with environmental challenges and maintain homeostasis (MCFARLAND et al., 2020). Thus, autonomic processes, such as the activation of pathways in the preoptic area of the hypothalamus, and that regulate the balance of heat production and loss (including altering blood flow to the skin through peripheral vasoconstriction and vasodilation) can be activated (HENZI et al., 2017; MCFARLAND et al., 2020; MORRISON; NAKAMURA, 2019). Besides, changes in microhabitat temperature could affect the ability of primates to thermoregulate themselves, and may force them to alter their activity patterns for selecting appropriate microclimates (HENZI et al., 2017).

To deal with high temperatures and/or low humidity, many primates tend to increase rest time as well as reduce displacement to feed (ARISTIZABAL et al., 2018). For BLT this pattern has already been observed, showing that the mean proportion of time dedicated to rest was higher than other behaviors between fragments with different characteristics (AYALA-BURBANO et al., in prep; COSTA, 1997; VALLADARES-PADUA, 1993). It is also known, that animals living in highly fragmented habitats likely suffer from high levels of physiological stress, and under the negative impact of additional short-term may exert rather strong pressures that force tropical species, for instance, to adjust their reproductive pattern (GRAHAM; MATTHEWS; TURNER, 2016). Among the main short-term stressors are reductions in the resource availability of high seasonality; intra- and intergroup competitions, extremes in temperature; sex-age dependent nutritional demands; changes in sex-ratios; and increases in parasitic infections, per example (BARRETT et al., 2013; PAVÉ; KOWALEWSKI, 2012; WIEDERHOLT; POST, 2010).

Changes in some of the short-term stressors have already been observed for BLT population from fragmented and riparian forests remnants, including, larger densities, higher number of adult males per group, male sexual bias, and home range retractions (AYALA-

BURBANO et al, in prep). Under these conditions, the BLT's ability to persist in an ever-changing environment will ultimately be determined by patterns of local adaptation, as environmental conditions can process different selective pressures and consequently lead populations to respond differently (FORESTER et al., 2018; MAHONY et al., 2019; TALBOT et al., 2017). In this way, some outliers detected herein provide insights about relevant physiological processes that may have experienced diverse selection pressures in response to climate and other environmental variations in the habitats where the wild populations of BLT were sampled.

As for highlights we found SNPs within genes related to important roles in fertilization, immunity, lipid metabolism, cell cycle, host-virus interaction, transcription regulation, synaptic plasticity and regulation of blood pressure (Table 3.3), such as the peptidoglycan recognition protein 3, the neucloplamin-2 protein, and the nitric oxide synthase. The peptidoglycan recognition protein 3 (PGLYRP3) is an antibacterial and anti-inflammatory innate immunity protein encoded by the PGLYRP3 gene and is found in epithelial cells, body secretions, and polymorphonuclear leukocytes (LIU et al., 2001; LU et al., 2006). The neucloplamin-2 protein is a core chaperone histones involved in chromatin reprogramming, especially during fertilization and early embryonic development, and probably is involved in sperm DNA decondensation during fertilization (BURNS et al., 2003; INOUE; AOKI, 2010). On the other hand, the nitric oxide synthase, brain protein, produces nitric oxide (NO) which is a messenger molecule with diverse functions throughout the body such as synaptic plasticity in the central nervous system , central regulation of blood pressure, smooth muscle relaxation, and vasodilatation via peripheral nitrergic nerves (FORSTERMANN; SESSA, 2012), that play a particular role in the relaxation of corpus cavernosum and penile erection (FORSTERMANN; SESSA, 2012; KIM et al., 1991) .

Similar approaches in plants (YODER et al., 2014), lobsters(BENESTAN et al., 2016) , fishs (DENNENMOSER et al., 2017), frogs (GUO et al., 2016), lizards (PRATES et al., 2018), rodents (FISCHER et al., 2011) and whistling hares (WATERHOUSE et al., 2018), have been reporting variations in genes that also underlying relevant y physiological processes, in response to differing environmental stressors. In our study we raise 18 genes linked to ecologically relevant physiological processes associated with specific environmental variables, that revealed variation genotype frequencies depending on the population. This information is especially significant in the context of rapid global climate change, as it aids our understanding of mechanisms possibly used by BLTs for adapting to a constantly changing environment. In

addition, this research raises relevant information on neutral and adaptive genetic variation that can be exploited to provide evidence-based recommendations, aiding management decision-makings, aiming at retaining adaptive diversity and guiding translocations or even reintroductions in areas of prior occurrence of the species, taking into account genetic-based conservation strategies.

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3.6 SUPPLEMENTARY INFORMATION 3

3.6.1 Supplementary Figures

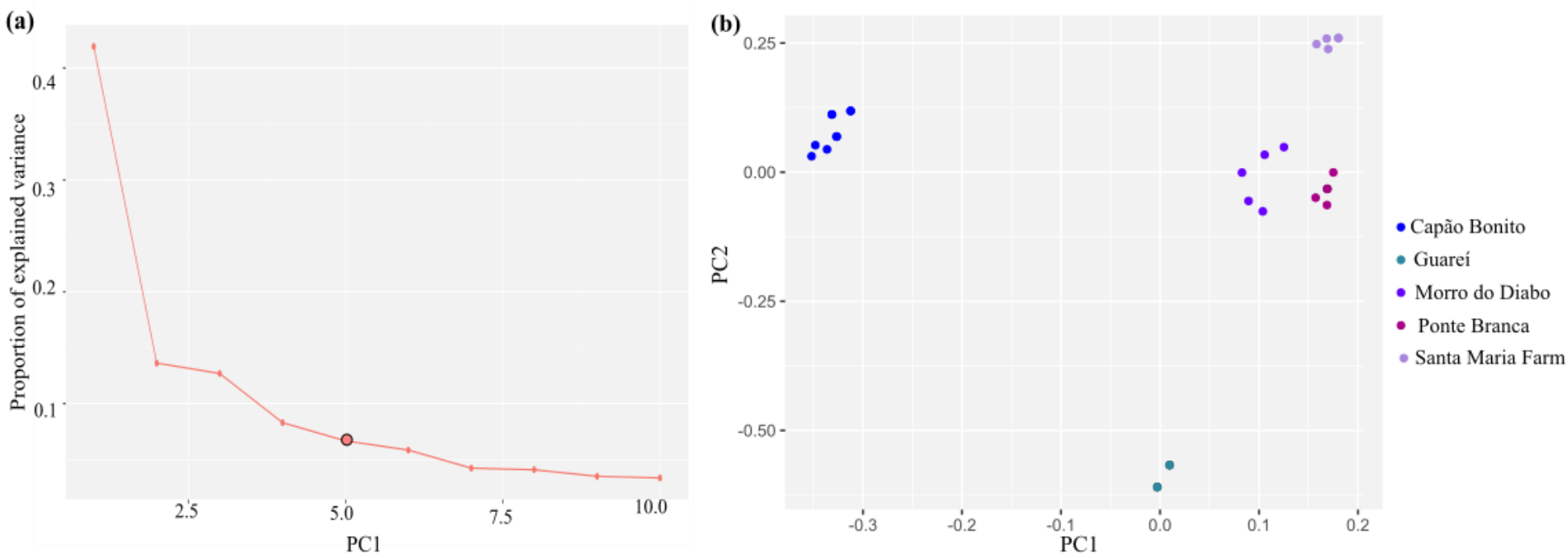

Figure S3.1 - (a) Scree plot produced in PCAdapt showing the percentage of explained variance for each PC and the selection of K=5 populations of Black lion tamarin. (b) Principal coordinate analysis (PCA) showing the scores on the first and second principal coordinates for the all-genomic dataset. Population structure confirms the results of the scree plot K=5.

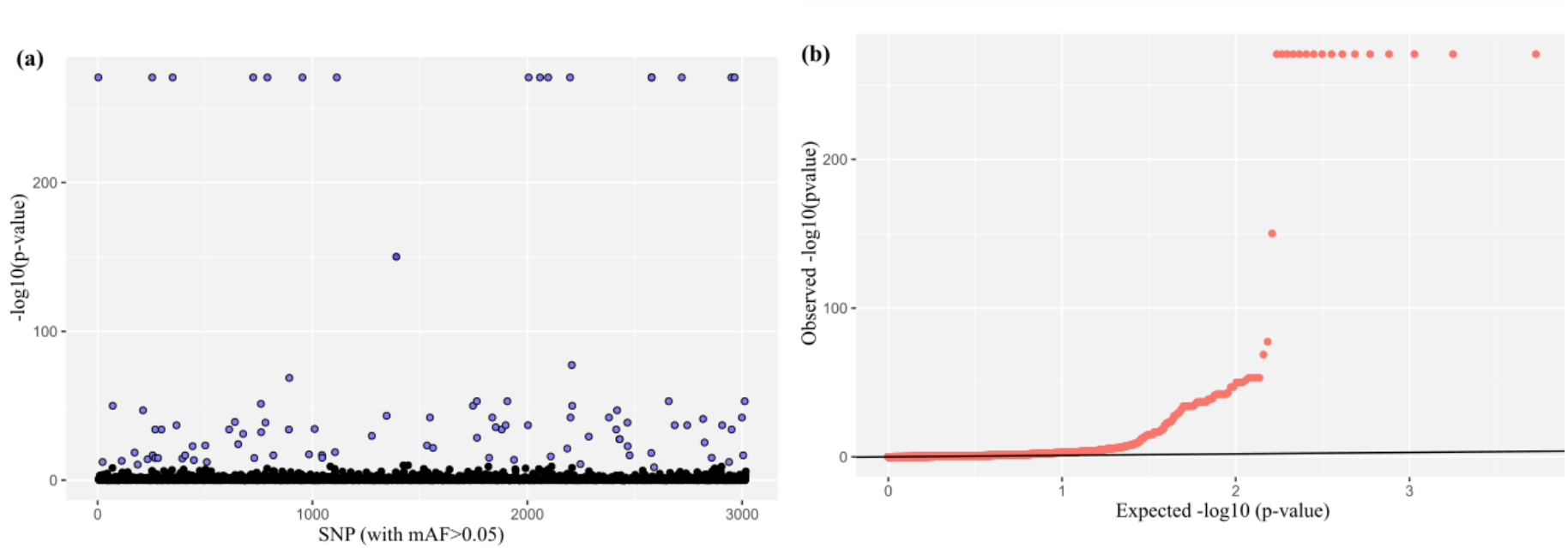

Figure S3.2 - (a) Distribution of the empirical p-values obtained by PCAdapt visualized through a Manhattan plot (up). Loci identified by the analysis as candidate loci with a signal of local adaptation are highlighted in blue. (b) QQ-plot (below) showing the cut off of 0.05%.

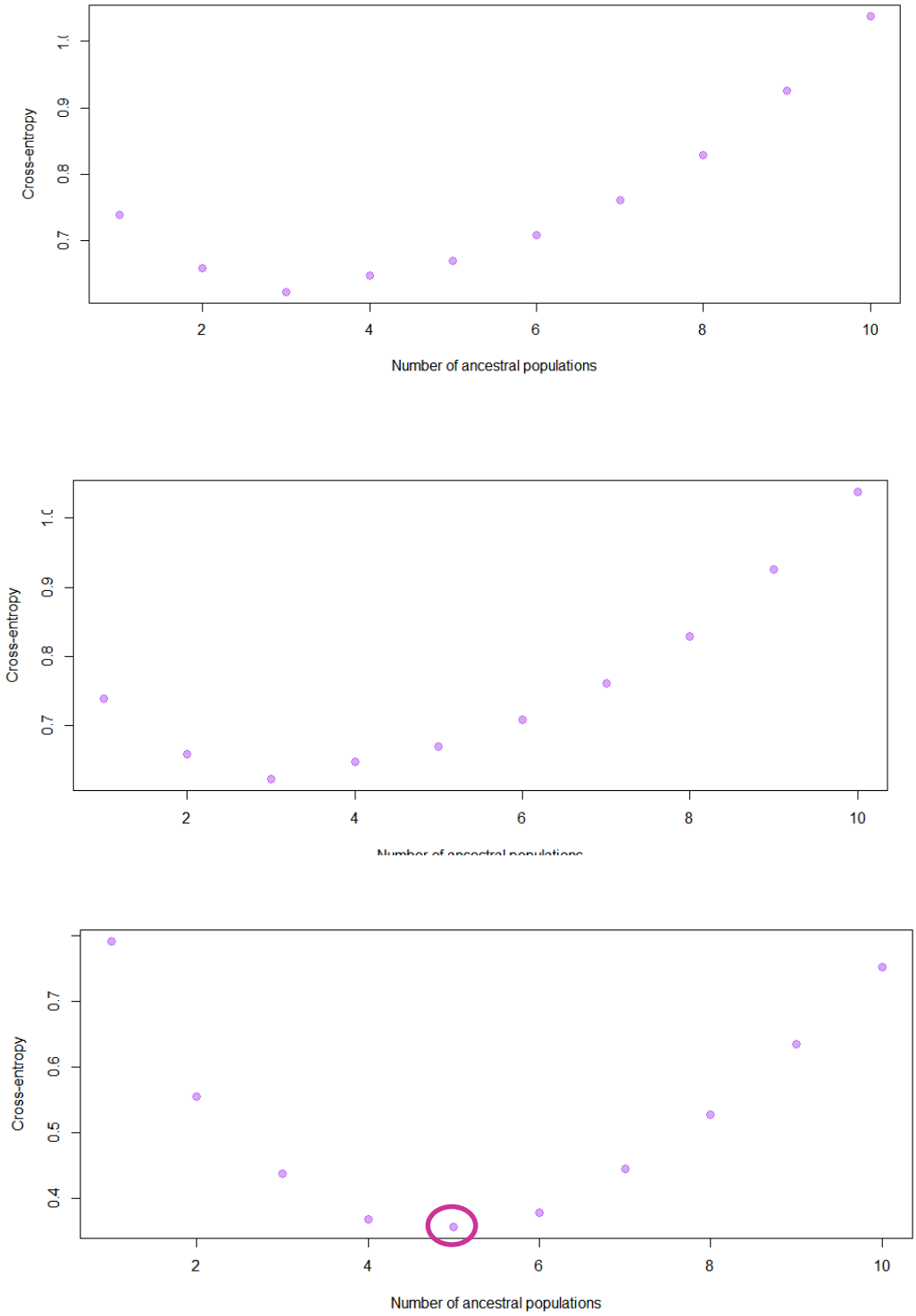

Figure S3.3 - Minimal cross-entropy for each number of ancestral populations (K) from 1 to 10 for combined (a), neutral (b) and outliers'(c) datasets. Red circle indicates the value of K that best represents the population history for outliers. For combined and neutral datasets, we chose five because the values of cross-entropy begin to plateau.

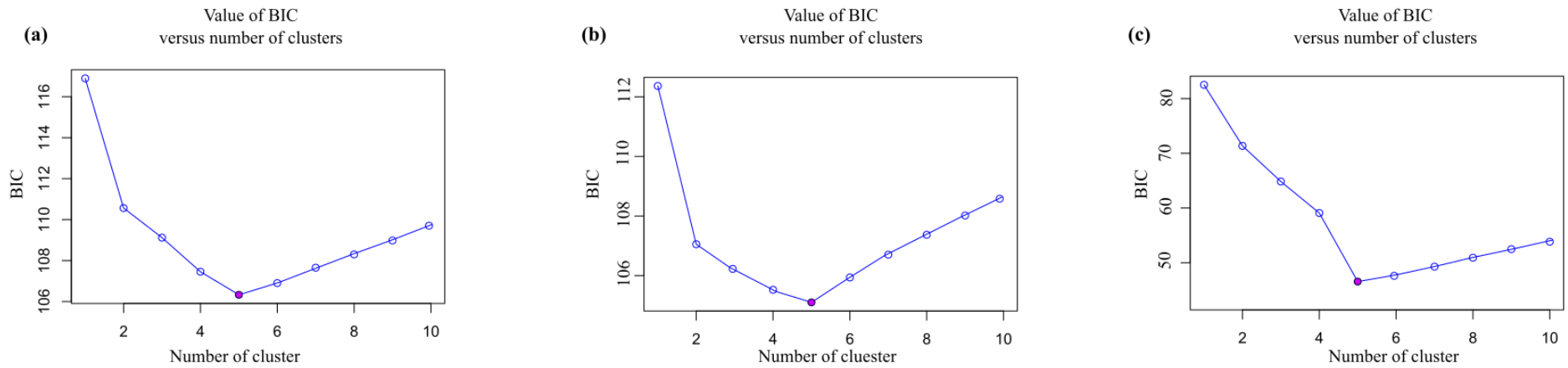

Figure S3.4 - Bayesian Information Criteria (BIC) used to obtain the optimal number of PCs and discriminant functions to retain in the DAPC analysis of the (a) Combined (b) Neutral and (c) outliers' datasets. Pink circles indicate the optimal number of PCs and discriminant functions retained for each dataset.

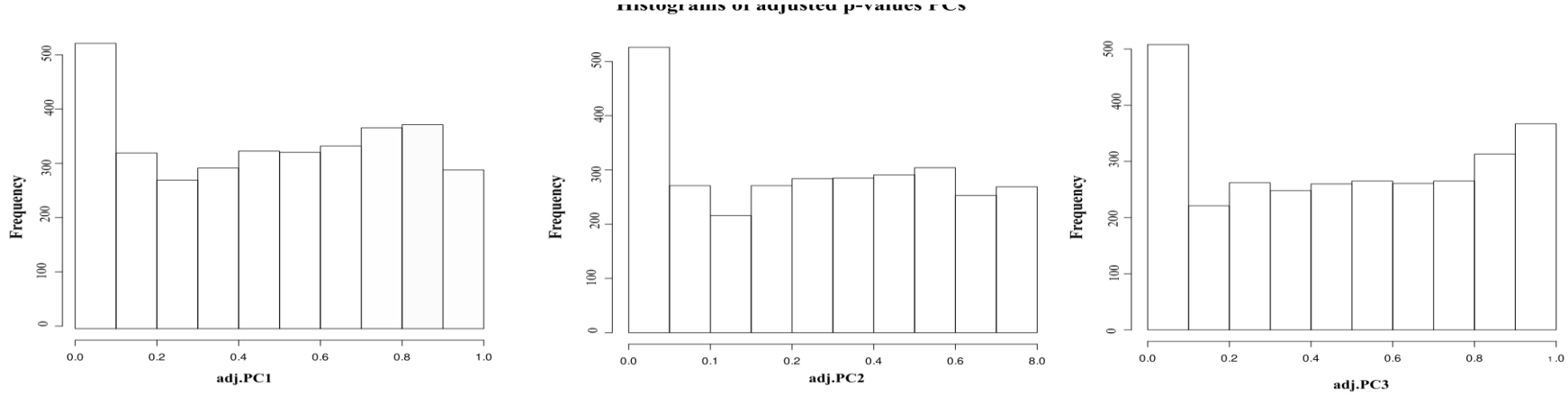

Figure S3.5 - Histograms of *P*-values from LFMM suggesting that the false-positive rate is well controlled in analyses

3.6.2 Supplementary tables

Table S3.1 - Total climatic variables considered initially to identify genomic signatures potentially linked to local adaptations.

Environmental variables	
Bio1 = Annual Mean Temperature	Bio12 = Annual Precipitation
Bio2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))	Bio13 = Precipitation of Wettest Month
Bio3 = Isothermality (BIO2/BIO7) (×100)	Bio14 = Precipitation of Driest Month
Bio4 = Temperature Seasonality (standard deviation ×100)	Bio15 = Precipitation Seasonality (Coefficient of Variation)
Bio5 = Max Temperature of Warmest Month	Bio16 = Precipitation of Wettest Quarter
Bio6 = Min Temperature of Coldest Month	Bio17 = Precipitation of Driest Quarter
Bio7 = Temperature Annual Range (BIO5-BIO6)	Bio18 = Precipitation of Warmest Quarter
Bio8 = Mean Temperature of Wettest Quarter	Bio19 = Precipitation of Coldest Quarter
Bio9 = Mean Temperature of Driest Quarter	Elevation (m)
Bio10 = Mean Temperature of Warmest Quarter	Fragment size (area)
Bio11 = Mean Temperature of Coldest Quarter	

Table S3.2 - Collection sites and identification of the 21 individuals analyzed. For each individual, the values of Temperature seasonality (BIO4), Precipitation seasonality (BIO15), and fragment size employed in GEA analyses are shown.

Sample ID	Forest fragment complex	Sex	Group	Longitude	Latitude	Area (ha)	Matrix type	Bio4	Bio15
CB1_02	Capão Bonito National Forest	Male	CB1	-48.532	-23.888	357		28.248	50.948
CB1_03	Capão Bonito National Forest	Male	CB1	-48.532	-23.888	357		28.248	50.948
CB2_07	Capão Bonito National Forest	Female	CB2	-48.532	-23.901	357	Agriculture, silviculture, roads	28.349	50.326
CB2_08	Capão Bonito National Forest	Male	CB2	-48.532	-23.901	357		28.349	50.326
CB2_09	Capão Bonito National Forest	Male	CB2	-48.532	-23.901	357		28.349	50.326
CB2_10	Capão Bonito National Forest	Male	CB2	-48.532	-23.901	357		28.349	50.326
GB1_01	Riparian forests of Guareí	Male	GB3	-48.208	-23.376	100	Sugar cane, pasture, silviculture	24.916	61.153
GC1_07	Riparian forests of Guareí	Male	GC1	-48.237	-23.417	100		24.913	58.953
SM2_02	Santa Maria Farm	Female	SM2	-52.308	-22.231	515		25.129	46.237
SM2_03	Santa Maria Farm	Female	SM2	-52.308	-22.231	515	Sugar cane, pasture, roads	25.129	46.237
SM2_05	Santa Maria Farm	Female	SM2	-52.308	-22.231	515		25.129	46.237
SM2_07	Santa Maria Farm	Male	SM2	-52.308	-22.231	515		25.129	46.237
PB1_01	Ponte Branca	Male	PB1	-52,508	-22,425	1.303		25.443	43.765
PB1_02	Ponte Branca	Female	PB1	-52,508	-22,425	1.303	Sugar cane, pasture	25.443	43.765
PB1_04	Ponte Branca	Male	PB1	-52,508	-22,425	1.303		25.443	43.765
PB1_05	Ponte Branca	Female	PB1	-52,508	-22,425	1.303		25.443	43.765
PEMD_04	Morro do Diabo State Park	Female	PEMD1	-52,182	-22,612	34.000		25.911	42.214
PEMD_05	Morro do Diabo State Park	Female	PEMD1	-52,174	-22,617	34.000	Sugar cane, pasture	25.911	42.214
PEMD_06	Morro do Diabo State Park	Male	PEMD2	-52,174	-22,617	34.000		25.880	42.214
PEMD_07	Morro do Diabo State Park	Male	PEMD2	-52,174	-22,617	34.000		25.880	42.214
PEMD_08	Morro do Diabo State Park	Male	PEMD2	-52,174	-22,617	34.000		28.880	42.214

Table S3.3 - Results of relationship inference based on pairwise kinship coefficient (Φ) by genomic data for groups of black lion tamarin using neutral dataset. *Individuals who have a twin relationship.

Morro do Diabo	**PEMD_04**	**PEMD_05**	**PEMD6F**	**PEMD7B**	**PEMD8B**
PEMD_04		0.1922770	0.0225179	0.1107300	0.0185512
PEMD_05	0.1922770		0.1673550	0.2780490	0.1686370
PEMD6F	0.0225179	0.1673550		0.2365360	0.2460850
PEMD7B	0.1107300	0.2780490	0.2365360		0.2249840
PEMD8B	0.0185512	0.1686370	0.2460850	0.2249840	
Santa Maria	**SM2_02**	**SM2_03**	**SM2_05**	**SM2_07**	
SM2_02		0.290792	0.286441	0.247672	
SM2_03	0.290792		0.299470	0.192982	
SM2_05	0.286441	0.299470		0.148760	
SM2_07	0.247672	0.192982	0.148760		
Ponte Branca	**PB_01**	**PB_02**	**PB_04**	**PB_05**	
PB_01		-0.0476804	0.2226180	0.2099550	
PB_02	-0.0476804		0.1696110	0.1672680	
PB_04	0.2226180	0.1696110		0.3701190*	
PB_05	0.2099550	0.1672680	0.3701190*		
Capão Bonito	**CB2_07**	**CB2_08**	**CB2_09**	**CB1_10**	
CB2_07		0.348907*	0.221146	0.211803	
CB2_08	0.348907*		0.205832	0.201102	
CB2_09	0.221146	0.205832		0.190616	
CB2_10	0.211803	0.201102	0.190616		
Capão Bonito	**CB1_02**	**CB3**			
CB1_02		0.179034			
CB1_03	0.179034				
Guareí	**GB1_01**	**GC1_07**			
GB1_01		0.101045			
G07	0.101045				

Table S3.4 - RDA loading on constrained axis from the RDA analysis of genome-wide SNPs against 3 environmental variables. The Variance Inflation Factors (VIF) are likewise revealed to be less than 5, indicating that multicollinearity among these predictors should not be an issue for the model.

Variable	RDA1	RDA2	RDA3	VIF
Area	0.2955	-0.4356	-0.7769	1.202
BIO4 (Temperature seasonality)	-0.9282	-0.34464	0.1090	1.074
BIO15(Precipitation seasonality)	-0.5447	0.8049	-0.1536	1.196

Table S3.5 - Redundancy analysis (RDA) with area, temperature and precipitation seasonality, identified 131 outlier loci-environment associations. We found outlier loci associated with area (n=48), temperature seasonality -Bio04 (n=12), and precipitation seasonality-Bio15 (n=71). Values below each environmental variable represent correlations. The environmental variable with the highest correlation is listed under the "Pred" column along with its corresponding correlation with each given single nucleotide polymorphism (SNP) locus.

Axis	Chromosome	Position	SPN_ID	Loading	Area	Temperature seasonality (Bio4)	Precipitation seasonality (Bio15)	Pred	R2
2	Scaffold2	1709205	loc65_pos79	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold493	1843727	loc396_pos91	-0,221	0,439	0,180	-0,653	Bio15	0,653
2	Scaffold1437	2336012	loc2382_pos85	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold3411	254426	loc4925_pos119	0,260	-0,299	-0,309	0,764	Bio15	0,764
2	Scaffold5203	3408961	loc8363_pos83	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold6272	4574089	loc9338_pos125	0,249	-0,289	-0,309	0,721	Bio15	0,721
2	Scaffold15452	1978711	loc19183_pos142	0,260	-0,299	-0,309	0,764	Bio15	0,764
2	Scaffold15452	4639880	loc19319_pos79	0,220	-0,331	-0,004	0,802	Bio15	0,802
2	Scaffold15650	2920492	loc19703_pos50	0,228	-0,365	0,043	0,855	Bio15	0,855
2	Scaffold15808	3233811	loc20184_pos76	0,244	-0,346	-0,080	0,840	Bio15	0,840
2	Scaffold15889	1467257	loc20435_pos14	0,231	-0,365	0,038	0,863	Bio15	0,863
2	Scaffold16391	4620256	loc22128_pos68	0,228	-0,365	0,043	0,855	Bio15	0,855

2	Scaffold18303	1962661	loc25390_pos83	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold19429	405580	loc26346_pos108	0,234	-0,451	-0,347	0,586	Bio15	0,586
2	Scaffold19429	413592	loc26349_pos140	0,234	-0,451	-0,347	0,586	Bio15	0,586
2	Scaffold19429	413592	loc26349_pos153	0,234	-0,451	-0,347	0,586	Bio15	0,586
2	Scaffold20674	5515401	loc27871_pos58	0,220	-0,244	-0,349	0,586	Bio15	0,586
2	Scaffold21386	2004915	loc28603_pos230	0,219	-0,258	-0,357	0,575	Bio15	0,575
2	Scaffold21386	2004915	loc28779_pos20	0,260	-0,299	-0,309	0,764	Bio15	0,764
2	Scaffold21709	292678	loc29439_pos76	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold23490	1948367	loc30727_pos106	0,225	-0,365	0,049	0,846	Bio15	0,846
2	Scaffold24157	18802	loc32050_pos67	0,220	-0,519	-0,360	0,500	Area	0,519
2	Scaffold31990	3756773	loc38201_pos186	-0,220	0,407	-0,145	-0,879	Bio15	0,879
2	Scaffold33455	2801980	loc39172_pos78	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold34081	5980105	loc40115_pos96	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold34839	7705501	loc40971_pos84	-0,212	0,337	0,421	-0,479	Bio15	0,479
2	Scaffold35326	6158844	loc41568_pos43	0,219	0,080	-0,604	0,504	Bio4	0,604
2	Scaffold35916	210294	loc41819_pos65	-0,251	0,382	0,337	-0,680	Bio15	0,680
2	Scaffold41527	1977333	loc44359_pos4	0,255	-0,328	-0,199	0,811	Bio15	0,811
2	Scaffold41527	3129977	loc44388_pos95	0,224	-0,038	-0,399	0,632	Bio15	0,632
2	Scaffold42681	858953	loc45757_pos92	0,240	-0,346	-0,074	0,831	Bio15	0,831
2	Scaffold47001	4464540	loc47915_pos62	0,218	-0,435	0,018	0,778	Bio15	0,778
2	Scaffold47817	110151	loc48255_pos48	0,267	-0,289	-0,487	0,672	Bio15	0,672
2	Scaffold53860	11025773	loc51423_pos138	0,220	-0,519	-0,359	0,500	Area	0,500
2	Scaffold56840	610499	loc52859_pos173	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold70464	3447	loc60205_pos38	-0,270	0,312	0,327	-0,787	Bio15	0,787
2	Scaffold76381	1867313	loc62770_pos123	0,255	-0,328	-0,199	0,811	Bio15	0,811
2	Scaffold79554	90707	loc64232_pos161	0,234	-0,314	-0,139	0,768	Bio15	0,768
2	Scaffold89030	400302	loc67430_pos71	-0,251	0,382	0,337	-0,680	Bio15	0,680
2	Scaffold90198	6346763	loc68038_pos84	0,227	-0,148	-0,557	0,498	Bio4	0,557
2	Scaffold90451	909627	loc68109_pos167	0,240	-0,346	-0,074	0,831	Bio15	0,831
2	Scaffold91070	46108	loc68162_pos56	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold98159	2363422	loc71664_pos38	0,219	-0,390	-0,616	0,354	Bio4	0,616
2	Scaffold99689	595774	loc72559_pos17	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold100088	1981659	loc72744_pos162	0,231	-0,365	0,038	0,863	Bio15	0,863
2	Scaffold106952	3805016	loc73948_pos58	0,228	-0,365	0,043	0,855	Bio15	0,855

2	Scaffold107288	1438952	loc74368_pos70	0,231	-0,192	-0,234	0,727	Bio15	0,727
2	Scaffold109121	2426221	loc74814_pos39	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold110946	428107	loc76352_pos167	-0,260	0,299	0,309	-0,764	Bio15	-0,764
2	Scaffold112682	3237997	loc77218_pos51	-0,270	0,312	0,327	-0,787	Bio15	-0,787
2	Scaffold117383	782749	loc79189_pos29	-0,267	0,302	0,381	-0,741	Bio15	-0,741
2	Scaffold131853	2216896	loc82538_pos37	0,244	-0,346	-0,080	0,840	Bio15	0,840
2	Scaffold151429	979936	loc85921_pos3	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold191264	18594	loc93325_pos77	0,227	-0,330	-0,400	0,554	Bio15	0,554
2	Scaffold192316	667418	loc93437_pos114	0,237	-0,346	-0,069	0,822	Bio15	0,822
2	Scaffold195688	110842	loc93940_pos57	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold220687	1832993	loc97376_pos84	0,266	-0,294	-0,434	0,703	Bio15	0,703
2	Scaffold239423	166	loc99235_pos47	-0,204	0,430	0,503	-0,361	Bio4	0,503
2	Scaffold277778	3094909	loc103270_pos136	0,238	-0,314	-0,146	0,779	Bio15	0,779
2	Scaffold286391	1540007	loc104311_pos69	-0,205	0,334	0,648	-0,294	Bio4	0,648
2	Scaffold310599	532205	loc105384_pos68	0,219	-0,310	-0,072	0,754	Bio15	0,754
2	Scaffold330900	291613	loc107141_pos11	-0,267	0,289	0,487	-0,672	Bio15	0,672
2	Scaffold333066	74601	loc107199_pos26	0,219	-0,310	-0,072	0,754	Bio15	0,754
2	Scaffold336004	678677	loc107293_pos112	0,270	-0,312	-0,327	0,787	Bio15	0,787
2	Scaffold440724	722561	loc111714_pos8	0,219	-0,310	-0,072	0,754	Bio15	0,754
2	Scaffold480300	602625	loc113194_pos148	0,220	-0,385	0,151	0,891	Bio15	0,891
3	Scaffold737	1297682	loc1636_pos94	0,200	-0,429	-0,110	-0,401	Area	-0,429
3	Scaffold1912	10717443	loc3177_pos110	-0,164	0,579	0,320	-0,087	Area	0,579
3	Scaffold2554	132502	loc3876_pos524	-0,164	0,682	-0,250	-0,279	Area	0,682
3	Scaffold2863	71430	loc3879_pos67	-0,210	0,431	0,367	0,462	Bio15	0,462
3	Scaffold5147	5993328	loc7895_pos168	0,237	-0,510	-0,132	-0,478	Area	
3	Scaffold5450	220639	loc8611_pos113	0,190	-0,339	-0,204	-0,513	Bio15	-0,513
3	Scaffold7179	420964	loc9960_pos64	0,168	-0,524	-0,250	-0,036	Area	-0,524
3	Scaffold7272	818758	loc10248_pos49	0,190	-0,840	0,447	0,419	Area	-0,840
3	Scaffold7327	100705	loc10262_pos109	0,191	-0,737	0,017	0,218	Area	-0,737
3	Scaffold7899	890971	loc11033_pos195	-0,164	0,682	-0,250	-0,279	Area	0,682
3	Scaffold8743	2301326	loc11536_pos10	-0,166	0,602	-0,272	-0,121	Area	0,602
3	Scaffold9226	487748	loc12198_pos288	0,201	-0,533	0,356	-0,213	Area	-0,533
3	Scaffold10279	2168949	loc13502_pos175	0,209	-0,589	0,145	-0,163	Area	-0,589
3	Scaffold10279	2553691	loc13513_pos19	-0,182	0,668	0,119	-0,148	Area	0,668

3	Scaffold14521	16314741	loc17815_pos23	-0,164	0,498	-0,583	0,058	Bio4	-0,583
3	Scaffold15808	7362071	loc20331_pos134	-0,164	0,682	-0,250	-0,279	Area	0,682
3	Scaffold16080	536994	loc21140_pos161	-0,164	0,682	-0,250	-0,279	Area	0,682
3	Scaffold17602	2029221	loc23356_pos85	-0,173	0,379	0,246	0,335	Area	0,379
3	Scaffold18241	2263809	loc24569_pos106	0,210	-0,431	-0,367	-0,462	Bio15	-0,462
3	Scaffold20674	5157433	loc27865_pos198	0,188	-0,607	-0,285	-0,006	Area	-0,607
3	Scaffold20900	1230062	loc28324_pos245	0,237	-0,510	-0,132	-0,478	Area	-0,510
3	Scaffold21514	678221	loc29015_pos53	-0,177	0,426	-0,083	0,274	Area	0,426
3	Scaffold21709	1344027	loc29507_pos39	-0,193	0,570	-0,042	0,102	Area	0,570
3	Scaffold31399	1536135	loc37406_pos35	-0,190	0,645	-0,386	-0,058	Area	0,645
3	Scaffold33490	1929613	loc39208_pos153	-0,160	0,288	0,145	0,423	Bio15	0,423
3	Scaffold39265	527520	loc43397_pos155	-0,193	0,471	-0,628	0,283	Bio4	-0,628
3	Scaffold39265	1759231	loc43435_pos84	-0,178	0,444	-0,619	0,244	Bio4	-0,619
3	Scaffold41724	2323995	loc44656_pos87	-0,169	0,642	0,294	-0,173	Area	0,642
3	Scaffold42264	605507	loc45535_pos183	0,210	-0,431	-0,367	-0,462	Bio15	-0,462
3	Scaffold45170	1138165	loc46599_pos117	-0,180	0,707	0,246	-0,231	Area	0,707
3	Scaffold45321	1395052	loc46833_pos61	-0,164	0,682	-0,250	-0,279	Area	0,682
3	Scaffold45321	1399108	loc46834_pos38	-0,164	0,682	-0,250	-0,279	Area	0,682
3	Scaffold49090	48930	loc48867_pos106	-0,165	0,326	0,424	0,383	Bio4	0,424
3	Scaffold53109	1324629	loc50420_pos4	0,180	-0,283	-0,154	-0,551	Bio15	-0,551
3	Scaffold54316	452330	loc51607_pos64	-0,166	0,659	-0,384	-0,228	Area	0,659
3	Scaffold55363	367245	loc52126_pos121	0,172	-0,407	0,362	-0,275	Area	-0,407
3	Scaffold59442	679602	loc54476_pos82	0,169	-0,463	-0,192	-0,157	Area	-0,463
3	Scaffold59821	234352	loc54644_pos105	0,200	-0,429	-0,110	-0,401	Area	-0,429
3	Scaffold64461	4034992	loc56405_pos114	-0,164	0,741	-0,310	-0,388	Area	0,741
3	Scaffold69677	3009436	loc59819_pos209	0,177	-0,426	0,083	-0,274	Area	-0,426
3	Scaffold71932	610183	loc60848_pos12	-0,173	0,380	-0,243	0,333	Area	0,380
3	Scaffold76162	5508058	loc62280_pos88	0,183	-0,385	-0,201	-0,383	Area	-0,385
3	Scaffold80359	29999	loc64580_pos198	-0,170	0,260	0,310	0,540	Bio15	0,540
3	Scaffold92548	530300	loc69246_pos27	0,180	-0,283	-0,154	-0,551	Bio15	-0,551
3	Scaffold97034	193968	loc71263_pos21	0,186	-0,401	-0,102	-0,375	Area	-0,401
3	Scaffold106952	2267629	loc73898_pos101	-0,165	0,490	-0,401	0,080	Bio4	-0,401
3	Scaffold107288	1430797	loc74366_pos46	0,180	-0,283	-0,154	-0,551	Bio15	-0,551
3	Scaffold123530	124399	loc80779_pos106	-0,164	0,682	-0,250	-0,279	Area	0,682

3	Scaffold125807	1824373	loc81460_pos74	0,200	-0,429	-0,113	-0,403	Area	-0,429
3	Scaffold162906	3590331	loc88606_pos17	-0,180	0,283	0,154	0,551	Bio15	0,551
3	Scaffold198389	1214765	loc94502_pos49	0,192	-0,606	0,776	-0,027	Bio4	0,776
3	Scaffold198389	1214776	loc94502_pos60	0,192	-0,606	0,776	-0,027	Bio4	0,776
3	Scaffold199639	4989222	loc94844_pos89	0,200	-0,429	-0,113	-0,403	Area	-0,429
3	Scaffold200986	483991	loc95140_pos112	-0,176	0,645	-0,542	-0,140	Area	0,645
3	Scaffold204279	253059	loc95438_pos71	0,212	-0,803	0,418	0,218	Area	-0,803
3	Scaffold244690	1591929	loc99805_pos44	0,179	-0,650	0,600	0,131	Area	-0,650
3	Scaffold249217	268766	loc100498_pos217	-0,183	0,760	-0,278	-0,311	Area	0,760
3	Scaffold259411	1634314	loc101508_pos59	0,180	-0,283	-0,154	-0,551	Bio15	-0,551
3	Scaffold281752	205607	loc103704_pos95	-0,164	0,682	-0,250	-0,279	Area	0,682
3	Scaffold294880	332040	loc104696_pos102	0,185	-0,617	-0,297	0,035	Area	-0,617
3	Scaffold326135	864226	loc106349_pos79	0,177	-0,623	0,430	0,096	Area	-0,623
3	Scaffold352948	390711	loc108416_pos31	-0,182	0,756	-0,277	-0,309	Area	0,756
3	Scaffold497055	136618	loc113519_pos136	0,183	-0,385	-0,204	-0,384	Area	-0,385
3	Scaffold512254	549924	loc113974_pos122	0,180	-0,283	-0,154	-0,551	Bio15	-0,551
3	Scaffold517928	5984	loc114058_pos143	0,200	-0,429	-0,113	-0,403	Area	-0,429

Table S3.6- Latent factor mixed-model analyze (LFMM) identified 371 outlier loci-environment associations. We found outlier loci associated with area (n=124), temperature seasonality -Bio04 (n=153), and precipitation seasonality-Bio15 (n=94). The environmental variable and the component which was associated is listed under the "Associated Varible" and “Pred” column corresponding with each single nucleotide polymorphism (SNP) locus.

Chromosome	Position	SNP_ID	Associated Variable	Pred
Scaffold2	1709205	loc65_pos79	PC1	Area
Scaffold260	351235	loc104_pos183	PC1	Area
Scaffold690	10470806	loc1586_pos138	PC1	Area
Scaffold882	5955234	loc1862_pos99	PC1	Area
Scaffold1437	1495889	loc2337_pos33	PC1	Area
Scaffold1912	16475581	loc3285_pos37	PC1	Area
Scaffold3704	334578	loc5359_pos74	PC1	Area
Scaffold4469	4723482	loc6470_pos52	PC1	Area
Scaffold4921	77041	loc7051_pos111	PC1	Area
Scaffold4985	2716709	loc7279_pos56	PC1	Area
Scaffold5076	2454757	loc7587_pos131	PC1	Area
Scaffold5217	700778	loc8440_pos215	PC1	Area
Scaffold5867	730361	loc9037_pos46	PC1	Area
Scaffold6522	2855884	loc9726_pos23	PC1	Area
Scaffold9218	6946901	loc12030_pos87	PC1	Area
Scaffold9736	458116	loc12843_pos241	PC1	Area
Scaffold10148	2390179	loc13156_pos197	PC1	Area
Scaffold10279	2168949	loc13502_pos175	PC1	Area
Scaffold10463	751431	loc13750_pos178	PC1	Area
Scaffold15650	4865174	loc19744_pos159	PC1	Area
Scaffold16106	7026386	loc21822_pos56	PC1	Area
Scaffold16106	8467096	loc21870_pos63	PC1	Area
Scaffold16391	329432	loc22064_pos126	PC1	Area
Scaffold18300	51887	loc25175_pos145	PC1	Area
Scaffold18303	80333	loc25302_pos96	PC1	Area
Scaffold18303	4519993	loc25452_pos43	PC1	Area
Scaffold18809	5144001	loc25870_pos225	PC1	Area
Scaffold18809	6191264	loc25899_pos128	PC1	Area

Scaffold20674	2772088	loc27814_pos78	PC1	Area
Scaffold20811	8238630	loc28141_pos14	PC1	Area
Scaffold21738	5038038	loc30060_pos35	PC1	Area
Scaffold23873	6288363	loc31338_pos308	PC1	Area
Scaffold24130	1531493	loc31859_pos41	PC1	Area
Scaffold31399	256560	loc37332_pos13	PC1	Area
Scaffold33649	2001559	loc39781_pos83	PC1	Area
Scaffold34396	738497	loc40187_pos80	PC1	Area
Scaffold34396	738498	loc40187_pos81	PC1	Area
Scaffold34451	407782	loc40241_pos76	PC1	Area
Scaffold37184	1869084	loc42478_pos153	PC1	Area
Scaffold37184	2659994	loc42509_pos81	PC1	Area
Scaffold41248	1637624	loc43959_pos58	PC1	Area
Scaffold45669	863503	loc46917_pos18	PC1	Area
Scaffold49059	907481	loc48608_pos87	PC1	Area
Scaffold49158	2625262	loc49093_pos90	PC1	Area
Scaffold53860	5474544	loc51221_pos112	PC1	Area
Scaffold56931	565772	loc53222_pos199	PC1	Area
Scaffold57284	3198815	loc53415_pos90	PC1	Area
Scaffold64968	434846	loc57152_pos47	PC1	Area
Scaffold65451	709440	loc57667_pos67	PC1	Area
Scaffold65945	2636106	loc58164_pos63	PC1	Area
Scaffold67127	3795145	loc58903_pos28	PC1	Area
Scaffold67872	2096732	loc59155_pos120	PC1	Area
Scaffold70407	1684360	loc60187_pos92	PC1	Area
Scaffold71249	3512915	loc60506_pos15	PC1	Area
Scaffold71612	484459	loc60549_pos13	PC1	Area
Scaffold71851	28377	loc60657_pos9	PC1	Area
Scaffold71851	450362	loc60686_pos54	PC1	Area
Scaffold76162	11851451	loc62487_pos77	PC1	Area
Scaffold79234	5162282	loc64196_pos45	PC1	Area
Scaffold84183	1524627	loc66198_pos45	PC1	Area
Scaffold84183	1524659	loc66198_pos77	PC1	Area

Scaffold87480	4163924	loc66986_pos89	PC1	Area
Scaffold88271	363033	loc67268_pos15	PC1	Area
Scaffold89447	43499	loc67493_pos128	PC1	Area
Scaffold90091	641056	loc67650_pos56	PC1	Area
Scaffold90091	1125908	loc67790_pos147	PC1	Area
Scaffold90198	6369744	loc68040_pos11	PC1	Area
Scaffold93753	1845039	loc70034_pos76	PC1	Area
Scaffold94742	463447	loc70265_pos49	PC1	Area
Scaffold94742	518548	loc70269_pos94	PC1	Area
Scaffold94742	1906167	loc70412_pos124	PC1	Area
Scaffold97474	321255	loc71320_pos18	PC1	Area
Scaffold98630	5317471	loc72320_pos23	PC1	Area
Scaffold99689	1848287	loc72605_pos143	PC1	Area
Scaffold99689	1848335	loc72605_pos191	PC1	Area
Scaffold104853	733826	loc73294_pos10	PC1	Area
Scaffold105255	569690	loc73389_pos62	PC1	Area
Scaffold107288	1430797	loc74366_pos46	PC1	Area
Scaffold109121	4727324	loc74931_pos193	PC1	Area
Scaffold110236	2048017	loc75897_pos273	PC1	Area
Scaffold111768	691791	loc76774_pos259	PC1	Area
Scaffold112178	1457572	loc77063_pos73	PC1	Area
Scaffold113004	288773	loc77236_pos231	PC1	Area
Scaffold115855	53062	loc78668_pos30	PC1	Area
Scaffold117018	449149	loc79104_pos67	PC1	Area
Scaffold119067	88324	loc79508_pos87	PC1	Area
Scaffold119326	1315258	loc79753_pos95	PC1	Area
Scaffold120396	127522	loc79964_pos31	PC1	Area
Scaffold120445	94633	loc79990_pos112	PC1	Area
Scaffold120929	1328900	loc80194_pos82	PC1	Area
Scaffold122613	445816	loc80629_pos265	PC1	Area
Scaffold132471	583589	loc82727_pos249	PC1	Area
Scaffold132648	114648	loc82853_pos76	PC1	Area
Scaffold140819	175031	loc83943_pos37	PC1	Area
Scaffold141095	246889	loc84053_pos83	PC1	Area

Scaffold144388	53808	loc84438_pos20	PC1	Area
Scaffold151686	1236276	loc85945_pos75	PC1	Area
Scaffold158856	689744	loc87144_pos74	PC1	Area
Scaffold161964	147616	loc87703_pos97	PC1	Area
Scaffold174788	433180	loc90407_pos141	PC1	Area
Scaffold195688	110842	loc93940_pos57	PC1	Area
Scaffold196341	1250748	loc94128_pos13	PC1	Area
Scaffold217206	8655	loc96838_pos128	PC1	Area
Scaffold220427	31510	loc97333_pos182	PC1	Area
Scaffold220427	31556	loc97333_pos228	PC1	Area
Scaffold248964	563304	loc100364_pos113	PC1	Area
Scaffold249217	1020953	loc100562_pos3	PC1	Area
Scaffold255374	100897	loc101118_pos36	PC1	Area
Scaffold269237	374244	loc102529_pos83	PC1	Area
Scaffold274905	677043	loc103044_pos92	PC1	Area
Scaffold275226	497783	loc103086_pos46	PC1	Area
Scaffold277181	1300822	loc103201_pos172	PC1	Area
Scaffold281752	356033	loc103723_pos27	PC1	Area
Scaffold294880	332758	loc104697_pos123	PC1	Area
Scaffold295847	180494	loc104728_pos33	PC1	Area
Scaffold311901	76796	loc105551_pos80	PC1	Area
Scaffold335784	285	loc107248_pos46	PC1	Area
Scaffold354038	637998	loc108503_pos20	PC1	Area
Scaffold401891	873162	loc110355_pos176	PC1	Area
Scaffold416299	496	loc110655_pos9	PC1	Area
Scaffold422645	132293	loc110816_pos89	PC1	Area
Scaffold435221	840115	loc111481_pos147	PC1	Area
Scaffold458743	418823	loc112368_pos49	PC1	Area
Scaffold462060	333	loc112421_pos52	PC1	Area
Scaffold737	3176442	loc1666_pos46	PC2	Bio4
Scaffold1912	2707724	loc2958_pos119	PC2	Bio4
Scaffold1912	13676800	loc3228_pos90	PC2	Bio4
Scaffold1935	375387	loc3351_pos183	PC2	Bio4

Scaffold2359	3620803	loc3675_pos180	PC2	Bio4
Scaffold2911	806116	loc4032_pos41	PC2	Bio4
Scaffold2911	1043449	loc4042_pos17	PC2	Bio4
Scaffold3704	789358	loc5374_pos82	PC2	Bio4
Scaffold3985	1313805	loc5919_pos212	PC2	Bio4
Scaffold4441	71684	loc6261_pos80	PC2	Bio4
Scaffold4441	290978	loc6287_pos207	PC2	Bio4
Scaffold5148	3572561	loc8119_pos84	PC2	Bio4
Scaffold6522	2855949	loc9726_pos88	PC2	Bio4
Scaffold7271	2968988	loc10180_pos96	PC2	Bio4
Scaffold7272	592526	loc10233_pos65	PC2	Bio4
Scaffold7899	1033167	loc11041_pos131	PC2	Bio4
Scaffold8157	59998	loc11120_pos65	PC2	Bio4
Scaffold9078	2633583	loc11760_pos100	PC2	Bio4
Scaffold9218	1860521	loc11926_pos9	PC2	Bio4
Scaffold9374	2795061	loc12265_pos99	PC2	Bio4
Scaffold10148	4336653	loc13181_pos218	PC2	Bio4
Scaffold10463	533848	loc13712_pos49	PC2	Bio4
Scaffold10463	967778	loc13781_pos76	PC2	Bio4
Scaffold11088	133204	loc14472_pos104	PC2	Bio4
Scaffold11460	4013422	loc14718_pos52	PC2	Bio4
Scaffold12622	53207	loc15828_pos74	PC2	Bio4
Scaffold12873	303721	loc16042_pos92	PC2	Bio4
Scaffold13099	1509903	loc16446_pos96	PC2	Bio4
Scaffold13893	1601097	loc16993_pos74	PC2	Bio4
Scaffold14521	2711739	loc17424_pos113	PC2	Bio4
Scaffold14521	12049963	loc17709_pos103	PC2	Bio4
Scaffold14521	18443592	loc17891_pos84	PC2	Bio4
Scaffold15241	2868909	loc18853_pos77	PC2	Bio4
Scaffold16106	7578079	loc21841_pos231	PC2	Bio4
Scaffold17554	2350989	loc23150_pos23	PC2	Bio4
Scaffold17627	831002	loc23395_pos3	PC2	Bio4
Scaffold17663	1271768	loc23542_pos134	PC2	Bio4

Scaffold18059	3120624	loc24020_pos97	PC2	Bio4
Scaffold18303	106991	loc25304_pos28	PC2	Bio4
Scaffold18809	6989930	loc25915_pos115	PC2	Bio4
Scaffold19028	69458	loc25927_pos177	PC2	Bio4
Scaffold19834	67423	loc27037_pos32	PC2	Bio4
Scaffold23266	223707	loc30637_pos210	PC2	Bio4
Scaffold23873	4129728	loc31287_pos68	PC2	Bio4
Scaffold24130	2362676	loc31882_pos163	PC2	Bio4
Scaffold24130	6099317	loc32027_pos168	PC2	Bio4
Scaffold24404	5457699	loc32322_pos61	PC2	Bio4
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