PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL

DISSERTAÇÃO DE MESTRADO

GENÉTICA DE POPULAÇÕES DE ONÇA-PINTADA (*PANTHERA ONCA*) EM BIOMAS BRASILEIROS

FERNANDA PEDONE VALDEZ
ORIENTADOR: EDUARDO EIZIRIK

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Sumário

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Resumo

Atualmente, a redução do tamanho populacional constitui um problema real para muitas populações selvagens e cativas, e muitos estudos têm sido realizados nesse contexto com o intuito de identificar áreas de manejo e proteção de espécies ameaçadas. O manejo de populações naturais tem como objetivo assegurar hábitats adequados para manter populações em tamanhos viáveis evitando assim que os efeitos do endocruzamento se pronunciem, além de manter corredores para facilitar a dispersão dos organismos e consequentemente o fluxo gênico entre populações. Os grandes carnívoros, por necessitarem de grandes áreas de vida e serem usualmente perseguidos por caçadores, são bastante vulneráveis à fragmentação de hábitats Devido ao fato de serem predadores-topo, sua extinção local geralmente leva à alteração de todo o ecossistema. Desta forma, sua conservação afeta, de maneira direta e indireta, muitos outros organismos. Os carnívoros estão entre os organismos que causam maiores desafios e preocupações às autoridades referentes à conservação. Áreas consideravelmente grandes são necessárias para englobar a área de vida de um único individuo, e territórios ainda maiores são necessários para abranger uma comunidade inteira deste grupo. A onça-pintada, o maior felídeo das Américas, encontra-se atualmente em menos de 50% da sua distribuição original e muitas áreas remanescentes não apresentam tamanho e disponibilidade suficiente de presas para manter uma população saudável em longo prazo. No Brasil, a Bacia Amazônica e o Pantanal são as maiores áreas de distribuição da espécie onde ainda se encontra populações grandes o suficiente para uma viabilidade por um longo período de tempo. No entanto, pouco se sabe sobre a dinâmica das populações de onça-pintada nesses biomas, havendo assim uma extrema necessidade de análises em nível genético-populacional da espécie. O presente trabalho teve como objetivo analisar 52 indivíduos provenientes do Pantanal brasileiro amostrados no período de 2001 a 2008, e fazer inferências genéticas sobre esta população, bem como compará-la com uma área previamente estudada na Mata Atlântica, onde altos níveis de estruturação foram encontrados. Foram analisados índices de diversidade genética intra-populacional e calculados índices de estruturação (F_{st} e R_{st}) assim como análise Bayesiana de estruturação no programa STRUCTURE. Os níveis de variabilidade foram bastante altos e comparáveis com aqueles encontrados para a espécie quando analisada de uma forma mais ampla (em escala filogeográfica). Quando as amostras do Pantanal foram analisadas em relação à sua estruturação, os dados indicaram a presença de apenas uma população, sugerindo que a região amostrada (Pantanal sul) consiste de uma só unidade genética. No entanto, quando as populações da Mata Atlântica foram incluídas na análise, as amostras do Pantanal foram alocadas em duas unidades genéticas incompletamente diferenciadas, devido provavelmente à influência de parentesco entre alguns dos indivíduos desta região, em combinação à provável miscigenação histórica com áreas transicionais entre os dois biomas. Este parece ter sido o caso da população amostrada na área de influência da UHE Porto Primavera (MS/SP), situada no limite interior do bioma Mata Atlântica e que atualmente encontra-se extinta por ação humana. Os resultados obtidos apóiam a inferência de que, no passado, havia conectividade genética entre populações do Pantanal e da Mata Atlântica de Interior, embasando o delineamento de possíveis ações de manejo a fim de retomar a conexão entre estes dois biomas. Além disso, os dados do Pantanal representam a primeira amostragem de uma população geneticamente saudável de onças-pintadas, podendo servir como base para a avaliação e monitoramento da variabilidade observada nesta espécie em regiões fragmentadas.

Abstract

Population size reduction currently constitutes a real problem for several natural populations, and many studies have focused on the identification and management of adequate protected areas for threatened species. The conservation of natural areas aims to guarantee good-quality habitats to keep populations at a viable size, which includes an avoidance of potentially deleterious effects of inbreeding. Additionally, conservation plans aim to keep dispersal corridors and consequently to maintain gene flow among populations that were originally connected. Large carnivores, due to their need for large home ranges and frequent persecution by hunters, are extremely vulnerable to habitat fragmentation. Since they are top-predators, their local elimination may cause the alteration of the whole ecosystem, while their protection might impact the persistence and demography of several co-occurring species. Carnivores usually cause major challenges to conservation managers, since considerably large areas are necessary to encompass the home range of a single individual and even larger regions are required to comprise an entire population. The jaguar is the largest predator in the Americas, and currently persists in less than 50% of its original distribution. Also, many remnant areas do not contain sufficient habitat and prey base to hold viable populations in the long term. In Brazil, the Amazon Basin and the Pantanal are the largest areas of the current distribution of the species, and where populations with high probability of long-term survival are still found. However, little is known about the dynamics of jaguar populations in these biomes, and thus population-genetic analyses are extremely necessary. The present study aimed to analyze 52 individuals sampled in the southern Pantanal from 2001 and 2008 to make genetic inferences on jaguar populations, as well as to compare them to previously investigated fragments in the Atlantic Forest biome, where high levels of structuring had been found. We estimated genetic differentiation among populations using an AMOVA approach to generate F_{st} and R_{st} indices. Population structuring analyses were also performed with the Bayesian approach implemented in the software STRUCTURE. Variability indices were quite high, and comparable to those found for the species when analyzed under on a phylogeographic scale. When Pantanal populations were assessed separately, a single genetic cluster was inferred, supporting the expectation of demographic connectivity throughout this area. However, when Atlantic Forest jaguars were also included in the analysis, the Pantanal population was divided into two groups, probably due to relatedness among some of the sampled individuals, combined with complex historical admixture with respect to transitional areas between the two biomes. One such area was likely the region adjacent to Porto Primavera dam, on the bank of the upper Paraná river, whose population has already been extirpated due to human activities. Results obtained in the present study support the idea that, in the past, there was genetic connectivity between populations from the Pantanal and the Upper Paraná region, which helps provide baseline data to aid in the design of adequate management actions that may reconnect these biomes. Moreover, the data shown here represent the first jaguar sampling of a genetically healthy local population, and may be used as a comparative reference for the evaluation and monitoring of the observed variability in fragmented regions.

Apresentação

Esta dissertação é um dos requisitos exigidos para obtenção do título de mestre pelo Programa de Pós-Graduação em Zoologia, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul.

Os resultados aqui apresentados foram gerados ao longo dos meus últimos dois anos de estudo, realizados no Laboratório de Biologia Genômica e Molecular, vinculado a esta universidade, sob orientação do Prof. Dr. Eduardo Eizirik.

O trabalho está sendo apresentado sob a forma de um artigo científico a ser submetido para a revista *Conservation Genetics*, respeitando as regras da mesma. As normas de submissão para os autores estão disponíveis no endereço eletrônico: "http://www.springer.com/life+sci/ecology/journal/10592". Acompanha o artigo um resumo estendido (versão em português e inglês, ver acima). Tabelas e figuras são apresentadas após o corpo do texto.

Introdução

A conservação a longo prazo de carnívoros de grande porte apresenta-se como um grande desafio (Noss et al. 1996) devido a sua baixa densidade populacional e sua necessidade de territórios grandes e saudáveis com alta disponibilidade de presas (Marinho-Filho & Machado 2006). Além disso, o conhecimento acerca dessas espécies é bastante escasso, fato que dificulta o planejamento de estratégias de conservação efetivas (Karanth & Chellam 2009). Os grandes carnívoros são vulneráveis à perda e à fragmentação de hábitat, além de sofrerem inúmeras vezes perseguição direta e indireta por humanos devido aos seus requerimentos ecológicos conflitarem com aqueles da população local (Woodroffe & Ginsberg 1998; Inskip & Zimmermann 2008). Devido a estas características, estes animais estão entre os mamíferos mais ameaçados (Schipper et al. 2008). Devido ao fato de serem predadores de topo em muitos biomas, a sua eliminação pode causar alterações significativas no ecossistema inteiro (Noss et al. 1996, Woodroffe & Ginsberg 1998, Crooks & Soulé 1999, Crooks 2002), ao mesmo tempo, sua proteção pode impactar a persistência e a demografia de muitas espécies simpátricas (Caro 2003, Seddon & Leech 2008, Grigione et al. 2009). Por esta razão, eles são comumente conhecidos como "espécie guarda-chuva" (ou seja, mantendo estas espécies na paisagem, outras serão também protegidas – Caro 2003), e usualmente empregadas como espécies bandeira para a implementação de parques nacionais e reservas.

Desenvolvimento urbano e conversão de terras são os maiores perigos para grandes carnívoros, assim áreas saudáveis e corredores de dispersão devem ser mantidos para garantir a viabilidade a longo prazo destas populações (Grigione et al. 2009). Uma vez que os números populacionais estão diretamente ligados à disponibilidade de espaço (Marinho-Filho & Machado 2006), unidades de conservação de tamanho grande o suficiente para manter populações saudáveis são extremamente importantes para a proteção dos grandes carnívoros. Este é um problema crítico, uma vez que tem se notado que muitas áreas designadas para a conservação não são grandes o suficiente para manter uma população viável destas espécies (e.g. Sollman et al. 2008).

A onça-pintada (Panthera onca) é o maior predador das Américas e sua distribuição e persistência é estritamente dependente de ambientes saudáveis com presa abundante (Silveira 2004). Sua distribuição original estendia-se desde o sudeste da América do Norte, passando pela América Central e do Sul até o sul da Argentina. Atualmente esta espécie ocupa menos de 50% da sua área original, sendo que a maioria das populações remanescentes sofreu níveis severos de redução demográfica e fragmentação. A Bacia Amazônica e o Pantanal ainda mantêm as maiores populações estimadas da espécie, as quais exibem as mais altas probabilidades de sobrevivência a longo prazo (Sanderson et al. 2002).

O objetivo principal do presente trabalho foi investigar as populações de onça-pintada na porção sul do Pantanal, no estado do Mato Grosso do Sul, para testar se há continuidade genética ou se alguma subdivisão espacial pode ter ocorrido em uma escala regional neste bioma. Nós também comparamos as amostras do presente estudo com populações remanescentes do bioma Mata Atlântica. Para isto analisamos dados gerados em um estudo prévio (Haag 2009) que havia indicado a existência de diferenciação populacional devido à deriva genética gerada em um curto período de tempo (30-40 anos) causada pela fragmentação do hábitat. A comparação entre os dados genéticos dos biomas Pantanal e Mata Atlântica permitiram uma inferência sobre a conectividade histórica entre estes dois ecossistemas, e resultaram ainda na geração de informação básica que pode ser útil no futuro para investigar e monitorar populações que venham a sofrer alguma redução ou fragmentação.

Artigo Científico

“Population genetics of jaguars in the Brazilian Pantanal: molecular evidence for broad population connectivity on a regional scale”

Fernanda Pedone Valdez, Taiana Haag Fernando Azevedo, Leandro Silveira, Sandra Cavalcanti and Eduardo Eizirik

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Original Article

Population genetics of jaguars in the Brazilian Pantanal: molecular evidence for broad population connectivity on a regional scale

Fernanda Pedone Valdez [1], Taiana Haag[1,2], Fernando Azevedo[3], Leandro Silveira[4], Sandra Cavalcanti[3] and Eduardo Eizirik [1,3]

[1]Faculdade de Biociências PUCRS. Av. Ipiranga 6681, prédio 12. Porto Alegre, RS 90619-900, Brazil

[2] Programa de Pós-Graduação em Genética e Biologia Molecular, Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, CP 15053, Porto Alegre, RS 91501-970, Brazil,

[3] Instituto Pró-Carnívoros. Av. Horácio Neto,1030, Casa 10, Parque Edmundo Zanoni, Atibaia, SP 12940-970, Brazil

[4] Jaguar Conservation Fund/Instituto Onça-pintada, Mineiros, GO 75830-000, Brazil

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Corresponding Author:

Eduardo Eizirik, Faculdade de Biociências PUCRS. Av. Ipiranga 6681, prédio 12. Porto Alegre, RS 90619-900, Brazil. Fax: 55-51-33203612. Email: eduardo.eizirik@pucrs.br

Running title: Jaguar population structure in the Pantanal region

Abstract

Habitat loss and human-caused mortality are the main threats to the conservation of carnivores worldwide since they lead to reduction and isolation of natural populations. Reduced populations suffer the action of genetic drift and inbreeding, having as a likely consequence the decline of genetic variability and adaptive fitness. Jaguar populations are extremely affected by human disturbances and have already been extirpated from half of their original geographic range. However, there are a few regions in the Americas that still maintain rather large jaguar populations, with moderate to high probability of long-term survival. In Brazil, the Amazon basin and the Pantanal are the best examples of such areas and may serve as a basis to understand jaguar biology in a setting that is less impacted by human disturbances than in most other regions. The current study aimed to analyze Pantanal jaguar populations sampled in the southern portion of this biome. We employed 12 microsatellite loci to characterize genetic diversity and population structure in this area on the basis of 52 samples collected in four different ranches. Although some indication of internal structure was observed (possibly due to the putative presence of related animals in the sample), our overall results supported the hypothesis that this area comprises a single population unit with high levels of genetic diversity. Comparisons with data previously collected from fragmented remnants of the inner Atlantic Forest revealed significant but rather low levels of genetic differentiation with respect to the Pantanal, and indicated the occurrence of recent connectivity between these biomes. Evidence for admixture between the Pantanal and a population located on the boundary of the Atlantic Forest biome corroborates the transitional nature of this area, whose jaguar population has now been locally extirpated. These data can serve as a basis for understanding the natural population dynamics of jaguars on a regional scale, and for supporting the design of conservation strategies that maintain and restore connectivity among currently isolated areas across the species' range.

Introduction

The long-term conservation of large carnivores presents considerable challenges (Noss *et al*. 1996) due to their low population densities and the need for large and healthy habitats with high prey availability (Marinho-Filho and Machado 2006). Furthermore, the knowledge on most of these species is still very scarce, further hampering the planning of effective conservation strategies on their behalf (Karanth and Chellam 2009). Large carnivores are highly vulnerable to habitat loss and fragmentation, and often suffer direct persecution from humans because their ecological requirements conflict with those of local people (Woodroffe and Ginsberg 1998; Inskip and Zimmermann 2008). Due to these characteristics, they are amongst the most threatened of all mammals (Schipper *et al.* 2008). Since they are top predators in many biomes, their local elimination may cause major alterations in the whole ecosystem (Noss *et al.* 1996, Woodroffe and Ginsberg 1998, Crooks & Soulé 1999, Crooks 2002), while their protection might impact the persistence and demography of several co-occurring species (Caro 2003, Seddon and Leech 2008, Grigione *et al.* 2009). For this reason, they are usually known as "umbrella species" (*i.e.* by maintaining these species in the landscape, others will also be protected - Caro 2003), and often employed as a flagship for the establishment of national parks and reserves.

Land development and conversion are major threats to large carnivore species, thus areas with healthy habitats and dispersal corridors should be kept to ensure long-term viability of such populations (Grigione *et al.* 2009). Since population numbers are directly linked to the availability of space (Marinho-Filho and Machado 2006), conservation units of sufficient size are extremely important for the protection of large carnivores. This is a critical issue, as it has been shown that many areas designated for conservation are not large enough to hold viable populations of these species (e.g. Sollman *et al.* 2008).

The jaguar (*Panthera onca*) is the largest predator in the Americas, and its distribution and persistence are strictly dependent upon healthy habitats and an abundant prey base (Silveira

2004). Its original distribution extended from southeastern North America, Central America, and South America to southern Argentina. The species currently occupies less than 50% of its original range, with most remaining populations suffering severe levels of demographic reduction and fragmentation. Overall, the Amazon Basin and the Pantanal hold the largest estimated populations of jaguars, which therefore exhibit the highest probability of long-term survival (Sanderson *et al.* 2002). In Brazil, most conservation units expected to play a role in jaguar conservation are insufficient to hold the 650 individuals estimated to be required for long-term viability in this species (Eizirik *et al.* 2002, Sollmann *et al.* 2008). It could therefore be argued that they should be connected to each other to allow for demographic connectivity across broad areas and to increase the probability of long-term persistence of jaguar populations (Cullen *et al.* 2005). To assess whether this strategy is adequate to maintain or restore original levels of population connectivity, it is important to investigate the genetic structure and demographic dynamics of remaining jaguar populations, and to infer their original patterns of gene exchange. Although some initial studies have described historical patterns of population subdivision and connectivity on broad geographic scales (Eizirik *et al.* 2001, Ruiz-Garcia *et al.* 2006), little is known with respect to the structure and dynamics of jaguar populations in a local or regional context.

The Pantanal is the largest natural floodplain in the world, covering approximately 160,000 km^2 within Brazil, Paraguay, and Bolivia. This ecosystem is influenced by four biomes: Amazon rainforest, Cerrado, Chaco, and Atlantic Forest (Adámoli 1982). The large variety of vegetation and soil types makes the Pantanal one of the richest biomes in the Neotropics with respect to its biodiversity (Nunes da Cunha 2006). Despite the alteration of the original habitat and severe retaliation pressure because of cattle losses, this ecosystem still harbors one of the largest jaguar populations on Earth, likely due to the climatic characteristics of this biome, that makes it difficult to be accessed (Sanderson 2002, Soisalo and Cavalcanti 2006), especially during the wet season, when most of the area is flooded.

Although many studies have been performed on jaguar ecology in the Brazilian Pantanal (e.g. Schaller and Crawshaw 1980, Crawshaw and Quigley 1991, Quigley and Crawshaw 1992, Dalponte 2002, Silveira 2004, Soisalo and Cavalcanti 2006, Azevedo and Murray 2007, Cavalcanti and Gese 2009), only one preliminary assessment of genetic diversity for this species in the region has been published (Eizirik *et al.* 2008). That study indicated that Pantanal jaguars maintain considerably high levels of genetic variability, but did not investigate the spatial distribution of this diversity, nor its historical connection to adjacent biomes such as the Atlantic Forest.

The goal of the present study was to investigate jaguar populations in the southern portion of the Pantanal, in the Brazilian state of Mato Grosso do Sul, to test whether they are genetically continuous or if spatially-oriented subdivision may occur on a regional scale in this biome. We also aimed to compare the sampled areas from this biome with remaining population fragments from the inner Atlantic Forest. For that purpose, we reanalyzed a data set generated in a previous study (Haag 2009) which had indicated that habitat fragmentation likely induced drift-mediated differentiation among small forest fragments in a very short period of time (30-40 years). The comparisons between Pantanal and Atlantic Forest genetic data allowed the inference of historical connectivity between the two biomes, and the generation of baseline information that may serve in the future to investigate and monitor additional population fragments that become reduced and isolated due to human intervention.

Materials and Methods

Sample collection and laboratory procedures

Sampling was performed in the southern Pantanal region between 2001 and 2008 in the context of field ecology and behavioral studies (e.g. Silveira 2004, Azevedo and Murray 2007, Cavalcanti and Gese 2009). Samples from each ranch were analyzed separately, and referred to

initially as four different local populations. A total of 53 blood samples were collected from wild-caught animals considering all four ranches: a) San Francisco Ranch (n =11) – 2003 and 2004; b) Sete Ranch (n =10) – from 2001 to 2003; c) Ecological Refuge Caiman (n =22) – 2003, 2005, and 2006; and São Bento Ranch (n =10) – 2008 (Table 1). In order to compare these Pantanal populations with four fragments previously sampled in the inner Atlantic Forest (data generated by Haag 2009) we analyzed a composite data set comprising 102 individuals from both biomes.

Blood samples were preserved with EDTA and in some cases also mixed with an equal volume of a salt-saturated solution (100mM Tris, 100mM EDTA, 2% SDS) to facilitate the conservation of good-quality DNA content in a field setting. All samples were stored at -20°C prior to DNA extraction. Genomic DNA was extracted from blood samples using a phenol-chloroform protocol (Sambrook *et al.* 1989) with slight modifications. Each sample was amplified for 12 microsatellite loci by the Polymerase Chain Reaction (PCR, Saiki *et al.* 1985): one locus containing a dinucleotide repeat (FCA742), two loci with trinucleotide repeats (F146 and F98), and nine loci with tetranucleotide repeats (FCA740, FCA723, FCA453, FCA441, FCA391, F124, F85, F53 and F42). These primers were originally developed for the domestic cat (*Felis silvestris catus*) (Menotti-Raymond *et al.* 1999, 2005) and have been optimized and standardized for jaguars (Eizirik *et al.* 2001, 2008; Haag 2009) and other wild cats (Trigo *et al.* 2008). Every forward primer was 5'-tailed with an M13 sequence (Boutin-Ganache *et al.* 2001) and used in combination with an M13 primer that had the same sequence but was dye-labeled (with FAM, HEX or NED) on its 5' end. PCR reactions were performed in a 10 ul volume containing approximately 10ng of genomic DNA, 0.25U of Platinum Taq DNA polymerase (Invitrogen), 1x PCR Buffer (Invitrogen), 2.0 mM $MgCl_2$, 200 uM of each dNTP, 0.2 uM of the reverse primer, 0.0133 uM of the M13-tailed forward primer, and 0.16 uM of the M13-fluorescent primer (FAM, NED or HEX). The reaction profile started with a denaturing step at 94°C for 3 min, followed by 10 touchdown cycles of denaturing at 94°C for 45s, annealing at 60-51°C for 45s, and extension at 72°C for 1.5 min. Thirty cycles of the same profile were run

afterwards with fixed annealing temperature of 50°C, followed by a final extension of 72°C for 30 min. PCR reactions were carried out for each locus separately, and products from 2 to 4 loci were diluted and pooled together based on yield, size range, and fluorescent dye (Table 3).

PCR products were then run on a MegaBACE 1000 automated sequencer with the ET-ROX 550 internal size ladder (GE Healthcare) and analyzed with the GENETIC PROFILER 2.2 software to determine fragment length. Negative controls, *i.e.* PCR reactions without genomic DNA, were run for each batch of reactions and genotyped to monitor the presence of any exogenous DNA.

Data analysis

The estimated frequency of null alleles was calculated with the software CERVUS 3.0.3 (Marshall *et al.* 1998), with large positive values potentially indicating the presence of non-amplified alleles that occur most often when the microsatellite locus is cloned for one species and amplified in others, due to mutations in the primer binding sites. Likewise, the software MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) was used to identify possible genotypic errors that might occur during data recording due to stutter peaks, as well as to assess the existence of null alleles and large-allele dropout. These genotyping mistakes, when present, might lead to erroneous estimates of population genetic parameters.

Only individuals that had at least 70% of the loci genotyped (*i.e.* 8 out of 12) were included in the analysis. The final set of genotypes was tested for any evidence of deviation from Hardy-Weinberg equilibrium (HWE), employing the exact test of Guo & Thompson (1992) with 10,000 dememorization steps (Excoffier *et al.* 2005) and for linkage disequilibrium (LD) across pairs of loci using the software packages ARLEQUIN 3.11 (Excoffier *et al.* 2006) and FSTAT 2.9.3.2 (Goudet 2002). Significance levels ($\alpha = 0.05$) for departures from HWE or inferred LD were adjusted for multiple simultaneous comparisons with the sequential Bonferroni approach (Rice 1989).

Genetic diversity indices were measured as the observed heterozygosity (H_O), expected heterozygosity (H_E), and number of alleles per locus (A) employing ARLEQUIN and FSTAT. The software FSTAT was also used to identify private alleles and to estimate allelic richness (AR) for each population. The latter is a measure of the observed number of alleles per population that is normalized to account for differences in sample size among them (Petit *et al.* 1998).

Population differentiation and structure analysis

A set of statistical tests was performed to evaluate the existence of population genetic structure caused by long-term or recent isolation, such as due to historical geographic barriers or human-induced habitat fragmentation. F_{st} and R_{st} indices were calculated among pairs of populations using the AMOVA approach implemented in ARLEQUIN. Each test was run with 10,000 permutations to evaluate the statistical significance of the calculated value (α=0.05). Pairwise genetic distances were calculated for the Pantanal data set (i.e. containing the four local populations sampled in this biome) as well as for the composite data set (Pantanal + Atlantic Forest populations).

In addition, we assessed the existence of potential population subdivision using the Bayesian clustering method implemented in the program STRUCTURE 2.3.1 (Pritchard *et al.* 2000; Pritchard and Wen 2004). We initially conducted five independent runs for each value of K (number of clusters), ranging between 1 and 8 when analyzing the Pantanal samples by themselves, and between 1 and 12 when also including the Atlantic Forest populations. All analyses used no prior population information, along with the admixture model with correlated allele frequencies (which is considered to be the best configuration in the case of subtle population structure - Falush *et al.* 2003). For this initial set of analyses, an MCMC procedure of 1,000,000 generations was performed following a burn-in of 500,000 steps. An additional set of analysis was performed for the composite data set using 1,000,000 steps of burn-in and 2,000,000 steps of sampling. This was conducted to establish a chain length that was sufficient

to achieve convergence, which was assessed by comparing the likelihoods among different replicate runs, as well as by plotting the likelihood scores along the sampled portion of each chain.

On the basis of these initial results (in which some variation was still observed among replicates with the same K, especially in the case of the composite data set), we performed a final set of analyses that included a longer MCMC procedure (2,000,000 generations for burn-in and another 2,000,000 for sampling). Given the ranges of probabilities for different K values observed in the initial analyses, this final set included five runs for each K between 1 and 4 for the Pantanal alone, and between 3 and 8 for the composite data set.

Results

Data quality control

Given the established cutoff that samples with less than 70% of the loci reliably genotyped would be excluded from the analyses, we removed bPon156 from the final data set. Therefore, our analyses were performed on the basis of a total sample of 52 jaguar specimens from the Pantanal. We initially assessed the occurrence of deviations from HWE and linkage equilibrium assuming that all individuals from the Pantanal constituted a single population, and in both tests found no significant evidence of disequilibrium. We therefore accepted the null hypothesis that all four local populations constituted a single genetic unit, as inferred by lack of disequilibrium that could have been caused by underlying subdivision, and therefore did not test for disequilibrium within each local area.

The estimated frequency of null alleles for all loci calculated in the software CERVUS was either negative or very low (≤ 0.13) (Table 3). Furthermore, the software MICROCHECKER found no evidence for null alleles, large-allele dropout, or stutter peaks

influencing the data set. These results allowed the use of the entire twelve-locus panel to make population-level inferences.

Genetic Diversity

All 12 loci were polymorphic and showed high levels of diversity. The number of alleles per locus ranged from 3 (F146 and F98) to 13 (FCA742) with a mean of seven. For each local population, the expected heterozygosity ranged from 0.66 (Caiman) to 0.71 (São Francisco). When calculated jointly for all local populations, the mean observed (H_O) and expected (H_E) heterozygosity were identical (both 0.71). These indices of genetic diversity per population (observed and expected heterozygosity, allelic richness, and number of alleles) are presented in Table 2, while additional information on the analyzed loci is shown in Table 3. The entire genotype data is available upon request.

Population structure and differentiation

F_{st} values among the four pairs of populations within the Pantanal region were all considerably low (0.03 - 0.053) even though statistically significant (Table 4). The highest value was estimated for Caiman *vs.* San Francisco (0.053; P = 0.000), while the other indices were lower than 0.05. On the other hand, R_{st} values were mostly non-significant (see Table 4), except for Caiman *vs.* San Francisco (0.07; P = 0.0048) and San Francisco *vs.* São Bento (0.10; P = 0.0016).

Assignment tests performed with the program STRUCTURE showed similar results among different sets of analysis (see Materials and Methods), although the variance among replicate runs was lowest when the longest MCMC procedure was performed; therefore, only results from the latter are presented here. This Bayesian approach indicated that the Pantanal data set was best explained with K =1 (LnP(D) = -1.793,98), *i.e.* by considering all four local populations as a single genetic unit (Table 5).

255 The STRUCTURE analyses of the composite data set (Pantanal + Atlantic Forest)
256 resulted in a best likelihood of six populations (LnP(D) = -3.478,86; see Table 6). In this case,
257 three of the four populations from the Atlantic Forest region (Iguaçu, Morro do Diabo, and
258 Ivinhema) tended to cluster separately from all others, although they presented some migrant or
259 admixed individuals (Figure 2). The fourth genetic cluster mostly contained animals from the
260 Porto Primavera population in the Atlantic Forest, but also appeared to be involved in admixture
261 with other areas. The Porto Primavera sample contained several individuals presenting genetic
262 compositions indicative of admixture with the other Atlantic Forest fragments, along with some
263 samples that displayed similar profiles to those from the Pantanal (see Figure 2). This pattern
264 suggested that the Porto Primavera population might contain admixed ancestry from both the
265 Atlantic Forest and Pantanal biomes. An intriguing observation was that, under this subdivision
266 scenario, the Pantanal population was split into two main genetic clusters, along with evidence
267 of admixture with Porto Primavera (see Figure 2). There was no obvious biological or spatial
268 pattern to the subdivision in the Pantanal, except that some individuals from Caiman ranch
269 tended to cluster more strongly into one of the clusters. One possibility to explain this pattern
270 (not seen when the Pantanal populations were analyzed by themselves) was that this Bayesian
271 approach overestimated the number of clusters in this case, due to a combination of relatedness
272 among individuals in one or more local areas (Pritchard and Wen 2004) and the complex
273 influence of admixture from the Atlantic Forest populations, especially Porto Primavera (see
274 Figure 2).
275 F_{st} and R_{st} indices comparing the Pantanal to the Atlantic Forest populations revealed
276 contrasting patterns of genetic structuring in these two biomes (Table 7). F_{st} measures of
277 differentiation were moderate to high (0.06 to 0.20) and statistically significant ($P < 0.001$) for
278 all comparisons. However, R_{st} values were only significant for Pantanal *vs.* each one of the
279 Atlantic Forest populations, which would be consistent with a longer period of separation
280 between these two ecosystems relative to within-biome comparisons, since the R_{st} index takes
281 into account allele size, incorporating a stepwise mutation model (Slatkin 1995).

Discussion

Genetic diversity indices found in the present analysis corroborate the hypothesis that Pantanal jaguars maintain levels of variability expected for large healthy populations (Frankham *et al.* 2002). Even though loss of habitat and severe retaliation because of cattle losses have been major threats in that region, population dynamics have not been considerably affected, probably due to the high prey availability and difficulty to human access in some areas (Soisalo and Cavalcanti 2006).

Levels of variability were moderate to high: expected heterozygosity (H_E) ranged from 0.66 to 0.71 (Table 2) and the mean number of alleles per locus was seven. These values were similar to those found in a previous study that analyzed 29 microsatellite loci throughout the species' geographic distribution (H_E =0.74 and A = 8.3; Eizirik *et al.* 2001) and were quite lower than those found in jaguar populations from Colombia and other South American countries analyzed with 12 microsatellite loci (H_E =0.84 and A = 11.3; Ruiz-Garcia *et al.* 2006). Additionally, Uphyrkina *et al.* (2001) investigated leopard populations (recognized as subspecies) across the distribution of the species and found different levels of diversity among them; the mean expected heterozygosity (H_E = 0.79) was slightly higher than that found in the current study (H_E = 0.71). Nevertheless, any comparison between results obtained in these studies is very difficult to draw, since only 3 out of 12 loci analyzed here (FCA441, FCA391 and FCA453) were used in the latter work, 2 out of 12 (FCA441 and FCA453) were used by Eizirik *et al.* (2001), and only 1 out of 12 (FCA391) was employed by Ruiz-Garcia *et al.* (2006). Therefore, marker overlap with respect to those studies was too low to allow any meaningful comparison. Moreover, the present study aimed to perform population-level analyses, while the studies mentioned above sampled across a wider distribution and pursued a more phylogeographic focus.

One previous study investigating jaguar genetics in Atlantic Forest fragments (Haag 2009) employed almost the same panel used in the present investigation, plus locus FCA741 that was monomorphic (*i.e.* bearing no variation) in the Pantanal samples and thus excluded

from the present analysis. The similarity between the sampling scheme and the geographic scope in the current study and in Haag (2009) made it possible to compare them and their results might lead to better conservation plans yet to be designed (see Materials and Methods for detailed information). Genetic diversity in that study was high (H_E = 0.73) even though spatially divided into four groups. The presence of private alleles in all populations but Morro do Diabo supported the interpretation that very little, if any, gene flow is occurring among the habitat remnants in that area. The region has suffered enormous pressure from fragmentation since large tracts of land in the region were converted to farming and ranching activities. Moreover, in 1982 the Itaipu Binacional hydroelectric power plant was constructed, flooding thousands of hectares of riverine habitat and likely eliminating altogether the gene flow between the northern and southern parts of the Upper Parana region (Haag 2009). Our idea in this study was to evaluate, on the same spatial scale, a set of local populations that occur in a still rather continuous environment, and likely maintain levels of connectivity resembling the original population dynamics of the species.

Pantanal populations

Among the sampled populations within the Pantanal region, genetic diversity values did not change considerably. Allelic richness, calculated using a minimum sample size of eight diploid individuals, varied from 4.3 to 4.7, and observed heterozygosity ranged from 0.69-0.73 (indices of genetic diversity per locus and per population are presented in Table 2). A lower percentage of private alleles (16%; see Table 8) than that found in the Atlantic Forest populations (25%; Haag 2009) indicates that those areas in the southern Pantanal are currently more connected and present some level of gene flow among themselves.

Our Bayesian analysis performed in the program STRUCTURE showed that the four ranches investigated in the region comprise a single population from a genetic standpoint (Table 5 and Figure 2). Estimates of differentiation were low, although statistically significant (F_{st} values ranged from 2.9% - 5.3%, $P < 0.01$; see Table 4). The estimated values were consistent

335 with the hypothesis of a panmictic population in the region, although implying some degree of
336 local differentiation, perhaps caused by the sampling of some related individuals in one or more
337 ranches, thus driving the fixation indices to significant (albeit low) values. These results might
338 be explained by some ecological and behavioral traits of the species. Jaguars exhibit territorial
339 behavior, which means they present very low degree of spatial overlap with conspecifics
340 (Azevedo and Murray 2007), juveniles tend to disperse to establish their new territory, whereas
341 adults establish large home ranges, with males setting larger areas and dispersing longer
342 distances than females (Oliveira 1994, Cavalcanti and Gese 2009). An ecological investigation
343 conducted in the Pantanal region (Cavalcanti and Gese 2009) estimated an area of 57km^2 during
344 the wet season and 69km^2 during the dry season for female jaguars. For males, size of home
345 ranges was 152km^2 and 170km^2 in the wet and dry seasons, respectively. These estimates
346 indicate that jaguars are expected to roam widely in these areas, thus likely maintaining
347 demographic and genetic connectivity across this still continuous landscape.
348 Although little is still known on jaguar dispersal patterns, recent evidence has supported
349 the notion that corridors with good-quality habitat are required for their movement, since habitat
350 fragmentation is a strict barrier for these animals (Cullen *et al*. 2005). The fact that the primary
351 activity in the Pantanal is the cattle ranching (Harris *et al.* 2005) and that the jaguar does not
352 avoid it as much as agriculture (Oliveira 1992, Hoogesteijn and Mondolfi 1992) supports the
353 hypothesis that this region still encompasses one of the largest populations of the species with
354 high probability of long-term survival (Sanderson *et al* 2002).
355 Taking into account that the four ranches sampled in the Pantanal region may be
356 considered a single population, we compared them to four fragment sites in the Atlantic Forest
357 previously analyzed by Haag (2009). Despite the fact that the sampling scheme of both studies
358 was very similar, results found in each one of them were considerably different. That study
359 found moderate to high levels of population structuring between the four sites (Table 7) and
360 related that to strong, recent genetic drift affecting these fragments due to their small effective
361 size (N_E) (this was especially the case of two sites: Ivinhema State Park, N_E = 12.3 and Morro

362 do Diabo State Park N_E = 4.6; Haag 2009). In contrast to that pattern, the Pantanal samples
363 showed lower levels of differentiation (Table 4) and comprised a single genetic cluster (Table 5
364 and Figure 2). However, when analyzed jointly with the Atlantic Forest samples, the Pantanal
365 was divided into two groups, with no apparent biological or geographic pattern to this
366 subdivision. Our interpretation is that the presence of related individuals (as mentioned above)
367 might have led to this overestimated K, as we have observed in our previous study (Haag 2009).
368 Interestingly, this pattern of Pantanal subdivision only emerges when these samples are
369 analyzed jointly with those from the Atlantic Forest, suggesting that a mixture of two
370 phenomena is responsible for this observation. These would be local relatedness along with
371 some degree of historical admixture between the Pantanal and Atlantic Forest populations,
372 which would highlight subtle different in allele frequencies among individuals.

373 Distances among areas are very similar in both regions: within the Pantanal region
374 pairwise distances ranged from 15km (Caiman and Sete) to 80km (Sete and São Bento) and
375 between areas in the Atlantic Forest distances ranged from 80km (Morro do Diabo and Porto
376 Primavera) to 380km (Porto Primavera and Iguaçu); however, the Atlantic Forest fragments
377 have been disconnected in the few decades due to fragmentation and construction of
378 hydroelectric dams (D.A. Sana pers. obs.), leading to population size reduction and
379 consequently strong action of genetic drift (Page and Holmes 1998). The Pantanal region is still
380 rather well-preserved, allowing individuals to wander across the landscape during their lifetimes
381 following a dynamics that is likely more similar to the species' original life history. Therefore,
382 this population might serve as a model to understand jaguar genetic structure and demographic
383 dynamics, and provide valuable baseline information against which fragmented populations
384 may be compared to assess any effect of loss of diversity, inbreeding and correlated deleterious
385 processes driven by small population size and isolation due to human activities.

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Table 1: Jaguar samples analyzed in the present study, including the geographic location of each ranch, individual ID, sex of each individual, and contact information.

Population name	Geographic Coordinates	Individual	Sex	Contact
Caiman Ecological Refuge	19°48’00’’S	bPon333	M	Jaguar Conservation Fund
	56°16’12’’W	bPon339	M	
		bPon340	F	
		bPon341	M	
		bPon342	F	
		bPon343	F	
		bPon344	F	
		bPon345	F	
		bPon349	M	
		bPon350	M	
		bPon351	F	
		bPon352	F	
		bPon155	F	
		bPon156	F	
		bPon157	F	
		bPon158	F	
		bPon159	F	
		bPon160	F	
		bPon161	M	
		bPon162	F	
		bPon163	M	
San Francisco Ranch	20°04’60’’S	bPon67	M	CENAP/ICMBio; I. Pró-
	56°36’00’’W	bPon68	F	Carnívoros
		bPon69	F	
		bPon70	M	
		bPon71	F	
		bPon72	F	
		bPon73	M	
		bPon74	F	
		bPon75	M	
		bPon76	M	
		bPon77	M	
São Bento Ranch	19°28’05’’S	bPon144	F	CENAP/ICMBio; I. Pró-
	56°59’26’’W	bPon145	M	Carnívoros
		bPon146	F	
		bPon147	F	
		bPon148	F	
		bPon149	F	
		bPon150	M	
		bPon151	M	
		bPon152	F	
		bPon153	F	
Sete Ranch	19°56’60’’S	bPon360	M	CENAP/ICMBio; I. Pró-
	56°24’60’’W	bPon361	F	Carnívoros

	bPon362	F	

Table 1 : Continued.

Sete Ranch	bPon363	M	CENAP/ICMBio; I. Pró-Carnívoros
	bPon364	M	
	bPon379	M	
	bPon384	M	
	bPon385	F	
	bPon386	M	
	bPon388	M	

Table 2: Measures of diversity at 12 loci in the four populations analyzed in this study. Sample size (N), observed number of alleles (A), observed (Ho) and expected (He) heterozigosities were calculated with the program ARLEQUIN, while allelic richness (AR) was calculated with the software package FSTAT.

	Caiman Ecological Refuge (*n*=22)					San Francisco Ranch (*n*=11)					São Bento Ranch (*n*=10)					Sete Ranch (*n*=10)				
Locus	N	A	AR[a]	Ho	He	N	A	AR[a]	Ho	He	N	A	AR[a]	Ho	He	N	A	AR[a]	Ho	He
FCA742	20	9	6.46	0.95	0.81	11	7	6.78	0.82	0.87	10	9	8.30	0.80	0.90	9	7	6.88	1.00	0.89
FCA723	18	6	4.31	0.78	0.69	11	33	2.93	0.64	0.54	10	5	4.76	0.70	0.71	10	4	3.60	0.40	0.54
FCA740	20	5	3.45	0.55	0.60	11	5	4.92	0.73	0.80	10	4	3.80	0.70	0.73	10	3	3.00	0.30	0.65
FCA441	20	5	3.67	0.35	0.49	11	3	3.00	0.73	0.63	9	5	4.89	0.67	0.80	8	3	3.00	0.50	0.54
FCA391	21	5	4.07	0.76	0.62	11	6	5.91	1.00	0.86	10	5	4.93	0.90	0.77	9	5	4.88	0.89	0.80
F98	21	3	3.00	0.67	0.66	11	3	3.00	0.55	0.68	10	3	2.99	0.60	0.49	9	3	3.00	0.78	0.58
F53	18	5	4.12	0.67	0.67	11	4	3.93	0.73	0.74	10	6	5.73	0.90	0.81	10	5	4.96	1.00	0.81
F124	21	6	4.52	0.71	0.74	11	5	4.70	0.91	0.75	10	5	4.57	0.70	0.68	10	6	5.56	0.70	0.78
F146	20	3	2.19	0.20	0.19	11	2	2.00	0.27	0.45	9	2	1.99	0.22	0.21	9	3	2.88	0.22	0.38
F85	20	8	6.62	0.80	0.85	11	6	5.59	0.82	0.81	10	4	3.80	0.80	0.68	9	6	5.66	0.89	0.74
F42	19	7	6.15	0.89	0.84	11	6	5.37	0.82	0.72	10	6	5.95	0.90	0.85	8	7	7.00	0.75	0.83
F453	16	6	5.48	0.94	0.79	11	4	3.92	0.73	0.65	10	6	5.54	0.70	0.75	10	5	4.76	0.80	0.71
Overall		5.7	4.50	0.69	0.66		4.5	4.34	0.73	0.71		5	4.77	0.71	0.70		4.7	4.60	0.69	0.69

[a]Allelic Richness calculated for the minimum sample size of eight diploid individuals.

Table 3: Fluorescent label and multiplex panel employed for each locus within the optimized four-panel multiplex used in this study. Observed heterozygosity (Ho) and expected heterozygosity (He) was calculated with the software FSTAT, while the number of alleles (A), allelic size range in base pairs, and estimated frequency of null alleles (F(Null)) were calculated with CERVUS for each locus. The overall mean across loci is shown at the bottom.

Locus	**Label**	**Panel**	**A**	**Range (bp)**	**Ho**	**He**	**F(Null)**
FCA740	NED	1	5	300-316	0.57	0.70	0.094
FCA453	HEX	1	6	192-216	0.81	0.76	-0.032
F85	NED	1	9	139-183	0.82	0.80	-0.024
F124	FAM	1	8	207-231	0.75	0.75	-0.006
FCA441	NED	2	7	149-177	0.52	0.62	0.059
FCA391	NED	2	6	215-243	0.86	0.76	-0.070
F98	FAM	2	3	189-195	0.65	0.67	0.014
FCA723	FAM	3	7	200-244	0.65	0.63	-0.028
FCA742	NED	3	13	142-180	0.90	0.87	-0.023
F146	FAM	3	3	173-182	0.22	0.30	0.128
F42	NED	4	7	243-275	0.85	0.85	-0.007
F53	FAM	4	6	164-196	0.80	0.78	-0.016
Mean	-	-	7	-	0.71	0.71	-

Table 4: Pairwise Fst (below the diagonal) and Rst values (above the diagonal) for the four populations analyzed in the Pantanal region.

	Caiman	San Francisco	São Bento	Sete
Caiman	–	0.074*	0.031	-0.010
San Francisco	0.053*	–	0.103*	0.033
São Bento	0.042*	0.039*	–	-0.027
Sete	0.029*	0.039*	0.046*	–

Significant values: *P<0,01

Table 5: Mean values of likelihood and likelihood variance across 5 runs for each assumed number of populations (K) calculated with the program STRUCTURE using a MCMC procedure of 2,000,000 generations following a burn-in of 2,000,000 steps for the Pantanal populations.

K	LnP(D)	Var [LnP(D)]
1	-1.793,98	31,84
2	-1.903,36	268,74
3	-2.388,00	1.300,96
4	-2283,14	1.176,36

Table 6: Mean values of likelihood and likelihood variance across 5 runs for each assumed number of populations (K) calculated with the program STRUCTURE using a MCMC procedure of 2,000,000 generations following a burn-in of 2,000,000 steps for the composite data set (Pantanal + Atlantic Forest).

K	LnP(D)	Var [LnP(D)]
3	-3609,18	258,92
4	-3597,24	370,08
5	-3560,52	442,58
6	-3478,86	424,44
7	-3527,3	577,9
8	-3612,4	783,32

Table 7: Pairwise Fst (below the diagonal) and Rst values (above the diagonal) between the whole Pantanal population and the four Atlantic Forest populations.

	Pantanal	Iguaçu	Morro do Diabo	Ivinhema	Porto Primavera
Pantanal	–	0.137*	0.207*	0.106*	0.062*
Iguaçu	0.081*	–	0.004	0.057	0.053
Morro do Diabo	0.163*	0.203*	–	0.027	0.046
Ivinhema	0.113*	0.128*	0.122*	–	0.045
Porto Primavera	0.079*	0.062*	0.079*	0.067*	–

Significant values: *P<0.01

Table 8: Observed allele frequencies at each locus for each local population sampled in the Brazilian Pantanal. The number of genotyped individuals is shown in parentheses. Private alleles are double underlined.

		Caiman	San Francisco	São Bento	Sete
Locus	**Allele**	**(20)**	**(11)**	**(10)**	**(9)**
FCA742	**142**	0.025	-	-	-
	150	0.050	-	-	-
	152	0.275	0.091	0.200	0.111
	154	0.025	0.136	0.050	-
	156	-	0.091	0.050	-
	158	0.125	0.182	0.200	0.167
	160	-	0.091	-	0.222
	162	0.075	0.136	0.100	0.167
	164	0.100	-	0.150	0.167
	170	0.300	0.273	0.100	0.111
	174	-	-	0.100	0.056
	178	0.025	-	-	-
	180	-	-	0.050	-
Locus	**Allele**	**(18)**	**(11)**	**(10)**	**(10)**
FCA723	**200**	0.028	-	-	-
	208	0.028	-	-	-
	220	-	-	0.050	-
	232	0.306	0.273	0.100	0.250
	236	0.167	0.091	0.200	0.050
	240	0.444	0.636	0.500	0.650
	244	0.028	-	0.150	0.050
Locus	**Allele**	**(20)**	**(11)**	**(10)**	**(10)**
FCA740	**300**	0.050	0.091	-	-
	304	0.400	0.273	0.350	0.300
	308	0.500	0.182	0.350	0.500
	312	0.025	0.318	0.250	0.200
	316	0.025	0.136	0.050	-
Locus	**Allele**	**(20)**	**(11)**	**(9)**	**(8)**
FCA441	**149**	0.025	-	-	-
	157	-	-	0.333	-
	161	-	-	0.167	-
	165	0.125	0.227	0.167	0.313
	169	0.700	0.545	0.278	0.625
	173	0.025	-	-	-
	177	0.125	0.227	0.056	0.063
Locus	**Allele**	**(21)**	**(11)**	**(10)**	**(9)**
FCA391	**215**	0.071	0.091	0.150	0.167
	223	0.571	0.227	0.400	0.222
	231	0.119	0.136	0.100	0.056
	235	0.024	0.182	0.250	0.222

239	0.214	0.227	-	0.333

Table 8: Continued.

	243	-	0.136	0.100	-
Locus	**Allele**	**(21)**	**(11)**	**(10)**	**(9)**
F98	**189**	0.286	0.227	0.150	0.611
	192	0.262	0.364	0.700	0.167
	195	0.452	0.409	0.150	0.222
	F53				
Locus	**Allele**	**(18)**	**(11)**	**(10)**	**(10)**
F53	**164**	0.028	0.364	0.100	0.150
	180	-	-	0.050	-
	184	0.167	0.273	0.100	0.200
	188	0.500	-	0.350	0.300
	192	0.056	0.091	0.150	0.100
	196	0.250	0.273	0.250	0.250
Locus	**Allele**	**(21)**	**(11)**	**(10)**	**(10)**
F124	**203**	0.024	-	-	0.050
	207	0.333	0.136	0.300	0.400
	211	0.357	0.409	0.500	0.250
	215	0.190	0.136	-	0.150
	219	0.071	0.045	0.100	-
	223	0.024	-	0.050	0.100
	227	-	-	0.050	-
	231	-	0.273	-	0.050
Locus	**Allele**	**(20)**	**(11)**	**(9)**	**(9)**
F146	**173**	0.900	0.682	0.889	0.778
	176	0.075	0.318	0.111	0.167
	182	0.025	-	-	0.056
Locus	**Allele**	**(20)**	**(11)**	**(10)**	**(9)**
F85	**139**	0.150	0.091	0.050	-
	143	-	-	-	0.111
	147	0.250	0.273	0.450	0.444
	151	0.225	0.182	0.350	0.278
	155	0.025	-	-	0.056
	159	0.125	0.318	0.150	0.056
	163	0.050	0.045	-	-
	179	0.075	0.091	-	0.056
	183	0.100	-	-	-
Locus	**Allele**	**(19)**	**(11)**	**(10)**	**(8)**
F42	**251**	0.105	0.500	0.150	0.125
	255	0.184	0.045	-	0.188
	259	0.289	0.182	0.100	0.063
	263	0.026	0.091	0.300	0.063
	267	0.132	0.136	0.150	0.375
	271	0.132	0.045	0.150	0.125
	275	0.132	-	0.150	0.063

Table 8: Continued

Locus	Allele	(16)	(11)	(10)	(10)
FCA453	**192**	0.344	0.545	0.450	0.150
	196	0.094	0.091	0.050	0.500
	200	0.094	-	0.100	-
	208	0.125	-	0.050	0.100
	212	0.063	0.136	0.100	0.050
	216	0.281	0.227	0.250	0.200

Figure 1: Map of sampled Pantanal populations and Atlantic Forest sites. The inset shows the geographic location of the Pantanal and the Atlantic Forest biome within the Brazilian territory, while the main map depicts the sampling locales within each ecosystem. The largest distance between the Pantanal ranches is 80km, whereas the distance between Pantanal and Porto Primavera (the closest Atlantic Forest fragment) is approximately 450km.

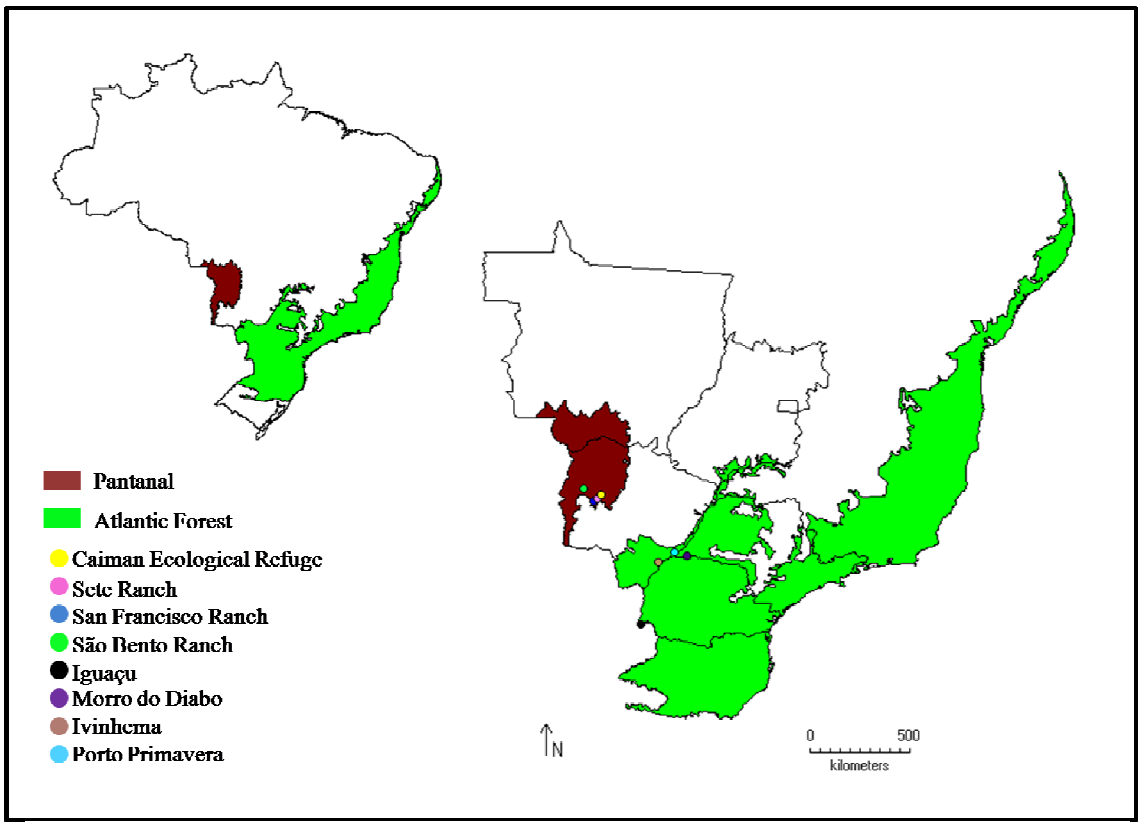
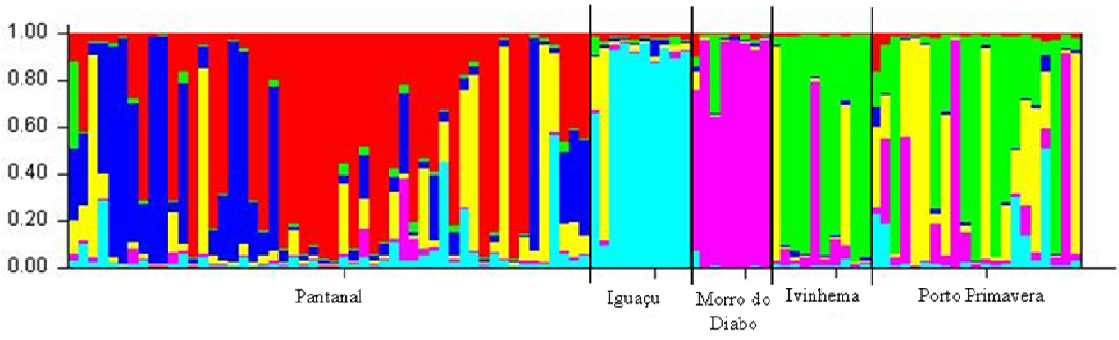


Figure 2: Proportional membership of each jaguar sample in the composite data set (Pantanal as a single population and the four Atlantic Forest fragments) inferred by STRUCTURE. Each individual is represented by a vertical bar.



Considerações finais

Os índices de diversidade genética encontrados nas populações do Pantanal corroboram a hipótese de que a as onças-pintadas presentes neste bioma ainda mantêm os níveis de variabilidade esperados para uma população grande e saudável (Frankham et al. 2002). Ainda que a perda de hábitat e a retaliação devido ao ataque ao rebanho tenham sido perigos constantes nesta região, a dinâmica populacional ainda não foi consideravelmente afetada, provavelmente devido à alta disponibilidade de presas e à dificuldade de acesso a muitas áreas pelos humanos (Soisalo and Cavalcanti 2006).

Os níveis de variabilidade foram de moderados a altos: heterozigosidade esperada variou entre 0.66 e 0.71 e o número médio de alelos por loco foi de sete. Estes valores são bastante similares aos encontrados em um estudo que analisou 29 locos de microssatélites ao longo da distribuição geográfica da espécie (heterozigosidade 0.74 e número médio de alelos por loco de 8,3; Eizirik et al. 2001). A análise Bayesiana realizada no programa STRUCTURE indicou a existência de apenas uma população e os índices de diferenciação foram extremamente baixos, ainda que significativos. Estes valores são consistentes com a hipótese de uma população panmítica na região, havendo, no entanto, algum nível de diferenciação local, que pode ter sido causado por amostragem ou parentesco de alguns indivíduos capturados nas áreas vizinhas.

A partir dos resultados gerados pelo programa STRUCTURE e pelos índices de diferenciação genética, comparamos as amostras do Pantanal como uma única população com os fragmentos remanescentes da Mata Atlântica. A amostragem dos dois biomas foi relativamente semelhante embora os resultados tenham sido bastante divergentes. No bioma Mata Atlântica os fragmentos indicaram níveis de diferenciação genética de moderado a alto, enquanto que no Pantanal esses valores foram bastante baixos e a análise populacional resultou em apenas uma população para toda a área. Desse modo, analisamos a população Pantaneira com a da Mata Atlântica no programa STRUCTURE e o resultado foi a subdivisão do Pantanal em duas populações e as quatro populações da Mata Atlântica se mantiveram. Porém nenhuma explicação biológica foi encontrada para este fenômeno de subdivisão das amostras do Pantanal e isto só pode ser visto quando mais amostras são incluídas na análise, o que pode indicar a presença de indivíduos mais relacionados entre si que leva o programa a superestimar o número de populações.

Conclui-se então que as onças-pintadas presentes no bioma Pantanal ainda encontram-se dispostas em uma população grande e saudável, provavelmente devido ao bom estado de preservação que este bioma se encontra, o que permite que os animais dispersem por

longas distâncias, mantendo assim o fluxo gênico entre eles. Assim, esta população deve servir como modelo para entendermos a estruturação genética e a dinâmica demográfica da espécie, gerando informação básica de extrema importância para o estudo da perda de diversidade, do endocruzamento e dos processos decorrentes do pequeno tamanho populacional e do isolamento devido à ação humana.