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Population Genetics of Jaguars (*Panthera onca*) in the Brazilian Pantanal: Molecular Evidence for Demographic Connectivity on a Regional Scale

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Abstract

Habitat loss and fragmentation are important threats to carnivores worldwide, and can be especially intense for large predators. Jaguars have already been extirpated from over half of their original area of distribution, and few regions still maintain large populations. For these, detailed understanding is crucial for setting appropriate recovery targets in impacted areas. The Pantanal is among the best examples of a region with a large jaguar population in a healthy environment. Here, we analyzed 12 microsatellite loci to characterize genetic diversity and population structure of 52 jaguars sampled in 4 localities of the southern Pantanal, and compared them with prior studies of heavily fragmented populations of the Atlantic Forest. Although we observed some internal structure among the Pantanal localities, our results indicated that this area comprises a single population with high genetic variability. Moreover, our comparative analyses supported the hypothesis that the strong population structure observed in the Atlantic Forest derives from recent, anthropogenic fragmentation. We also observed significant but low levels of genetic differentiation between the Pantanal and Atlantic Forest populations, indicating recent connectivity between jaguars occurring in these biomes. Evidence for admixture between the Pantanal and a population on the western boundary of the Atlantic Forest corroborates the transitional nature of the latter area, where the jaguar population has already been extirpated. Our results can be used to understand jaguar population dynamics in a region that is less disturbed than the Atlantic forest, and to support the design of conservation strategies that maintain and restore natural connectivity among currently isolated areas.

Resumen

La pérdida y fragmentación de hábitat constituyen importantes amenazas para los carnívoros a nivel mundial, las cuales pueden ser particularmente intensas para los grandes depredadores. Los jaguares ya han sido extirpados en más de la mitad de su área de distribución original y pocas regiones todavía

sostienen poblaciones grandes. Por ello, un conocimiento detallado es crucial para establecer metas de recuperación adecuadas en las áreas impactadas. El Pantanal brasileño se encuentra entre los mejores ejemplos de una región con una población grande de jaguares ocupando un ambiente saludable. En este trabajo analizamos 12 loci microsatélites para caracterizar la diversidad genética y la estructura poblacional de 52 jaguares muestreados en cuatro localidades del sur del Pantanal y las comparamos con estudios previos realizados en poblaciones fuertemente fragmentadas del Bosque Atlántico. Aunque observamos cierta estructura interna entre las localidades del Pantanal, nuestros resultados indicaron que esta área comprende una única población con una alta variabilidad genética. Además, nuestros análisis comparativos sustentaron la hipótesis de que la marcada estructura poblacional observada en el Bosque Atlántico es derivada de un proceso de fragmentación antropogénica reciente. También observamos niveles bajos pero significativos de diferenciación genética entre las poblaciones del Pantanal y del Bosque Atlántico, lo cual indica una conectividad reciente entre los jaguares que ocupan estos biomas. La evidencia de mezcla entre el Pantanal y una población ubicada en el límite occidental del Bosque Atlántico corrobora la naturaleza transicional de ésta última región, donde la población de jaguares ya ha sido extirpada. Nuestros resultados pueden utilizarse para comprender la dinámica poblacional del jaguar en regiones menos perturbadas que el Bosque Atlántico, así como para sustentar el diseño de estrategias de conservación que mantengan y restauren la conectividad natural entre áreas actualmente aisladas.

Subject areas: Conservation genetics and biodiversity; Population structure and phylogeography
Key words: Carnivora, conservation genetics, Felidae, gene flow, Neotropics

Conservation of large carnivores presents considerable challenges since they require large areas and good-quality habitat (Noss et al. 1996). At the same time, knowledge of most carnivore species is still scant, hampering the planning of effective conservation strategies on their behalf (Karanth and Chellam 2009). Because its distribution and persistence are strictly dependent upon good-quality habitats and an abundant prey base, the jaguar (*Panthera onca*) is a good example of such issues (Miller and Rabinowitz 2002). Currently, the species occupies approximately half of its original distribution (which extended from southeastern North America to south-central Argentina), with most remaining populations suffering severe levels of demographic reduction and fragmentation (Sanderson et al. 2002; Zeller 2007; Galetti et al. 2013; Zeller et al. 2013). Overall, the Amazon Basin and the Pantanal region are considered strongholds, harboring the largest estimated populations of jaguars, which therefore have the highest probability of long-term survival (Sanderson et al. 2002).

Although jaguar ecology has been studied extensively 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, 2010; Azevedo and Verdade 2012), only 2 preliminary assessments of genetic diversity for this species in the region have been published (Eizirik et al. 2008; Roques et al. 2014). These studies indicated that Pantanal jaguars maintain considerable 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.

Previous genetic analyses focusing on inland Atlantic Forest jaguars have shown that populations inhabiting remaining fragments have very small effective sizes, with evidence of drift-induced genetic differentiation, likely due to anthropogenic demographic reduction and isolation in the last 30–40 years (Haag et al. 2010). To test the hypothesis that this regional pattern of differentiation is indeed caused by recent fragmentation, it is important to analyze samples encompassing a similar spatial scale, but collected in a region that has not undergone such intense habitat loss and alteration. Such a comparison is possible with the Pantanal, which harbors some of the nearest jaguar populations relative to the inland Atlantic Forest, and still contains a relatively continuous landscape, less affected by fragmentation (Nunes da Cunha et al. 2006; Cavalcanti et al. 2012).

The goal of the present study was therefore to investigate jaguars from the southern portion of the Pantanal, in the Brazilian state of Mato Grosso do Sul, to test whether samples collected from 4 nearby locations are genetically continuous or if spatially oriented subdivision may occur on a regional scale in this biome. We also aimed to genetically compare jaguars from the southern Pantanal with remaining population fragments from the inland Atlantic Forest, around 500 km away toward the southeast (Figure 1), so as to assess the existence of historical connectivity between these biomes. In addition to testing the hypothesis of anthropogenic differentiation among Atlantic Forest fragments, we also were interested in Pantanal data as a baseline against which to compare and monitor additional jaguar populations elsewhere that may become reduced and isolated in the future due to human disturbance.

Materials and Methods

Study Area and Sample Collection

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 4 other South American biomes: Amazon rainforest, Cerrado, Chaco, and Atlantic Forest (Adámoli 1982). The large variety of vegetation and soil types makes the Pantanal one of the most biodiverse biomes in the Neotropics (Nunes da Cunha et al. 2006). Despite the persecution in retaliation to cattle predation, as well as alteration of the original habitat, this ecosystem still harbors one of the largest jaguar populations on Earth (Sanderson et al. 2002; Soisalo and Cavalcanti 2006). However, the species has recently been assessed as "near threatened" in the Pantanal biome, mainly due to these factors (Cavalcanti et al. 2012).

Sampling was performed 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, 2010). A total of 53

blood samples were collected from capture and release of wild jaguars inhabiting 4 different ranches (i.e., study areas, Figure 1): San Francisco Ranch (n = 11), sampled from 2003 to 2004; Sete Ranch (n = 10), sampled from 2001 to 2003; Caiman Ecological Refuge (n = 22), sampled in 2003, 2005, and 2006; and São Bento Ranch (n = 10), sampled in 2008 (Supplementary Table S1). Samples from each ranch were analyzed separately, and initially treated as 4 different local populations named after each ranch. They were preserved with EDTA and in some cases also mixed with an equal volume of a salt-saturated solution (100 mM Tris, 100 mM EDTA, 2% SDS) and stored at –20 °C prior to DNA extraction.

Laboratory Procedures

Genomic DNA was extracted from blood samples using a phenol-chloroform protocol (Sambrook et al. 1989) with slight modifications. Each sample was amplified by the polymerase chain reaction (PCR, Saiki et al. 1985) for 12 microsatellite loci originally developed for the domestic cat (*Felis silvestris catus*; Menotti-Raymond et al. 1999, 2005) and optimized and standardized for jaguars (Eizirik et al. 2001, 2008; Haag et al. 2010): F42, F53, F85, F98, F124, F146, FCA391, FCA441, FCA453, FCA723, FCA740, and FCA742 following conditions described in Haag et al. (2010). PCR products were then run on a MegaBACE 1000 automated sequencer with the ET-ROX 550 internal size ladder (GE Healthcare, Amersham, UK) and analyzed with the GENETIC PROFILER 2.2 software to determine fragment length. Negative controls were run for each batch of reactions, and genotyped with the same procedure to monitor the presence of any exogenous DNA.

To compare these Pantanal populations with 4 fragments previously sampled in the inland Atlantic Forest (Green Corridor, Ivinhema, "Morro do Diabo" and "Porto Primavera"), we analyzed a composite data set comprising individuals from both biomes, including the data set reported by Haag et al. (2010) and the one generated in this study. Both data sets were generated using the same laboratory procedures and genotyping equipment, and multiple controls were run to assure that genotypes from both studies were fully comparable. Given the results reported by Haag et al. (2010) showing that the 4 Atlantic Forest fragments were genetically distinct amongst themselves, they were treated as separate populations throughout the comparative analyses described below. Although the average distance among sampling locations was smaller for the Pantanal than the Atlantic forest, several pairs of locations within both ecosystems were separated by similar distances (Figure 1), which allowed a comparison of the genetic structure observed in these 2 regions.

Data Analysis—Genetic Diversity

Only individuals genotyped for at least 70% of loci (i.e., 8 out of 12 loci) were included in the analyses. We used the software MICRO-CHECKER 2.2.3, which employs Monte Carlo simulation of expected allele-size differences (Van Oosterhout et al. 2004), to identify possible genotyping 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.

The final set of genotypes was tested for evidence of deviation from Hardy–Weinberg equilibrium (HWE), employing the exact test of Guo and 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 (α = 0.05) for departures from HWE or inferred

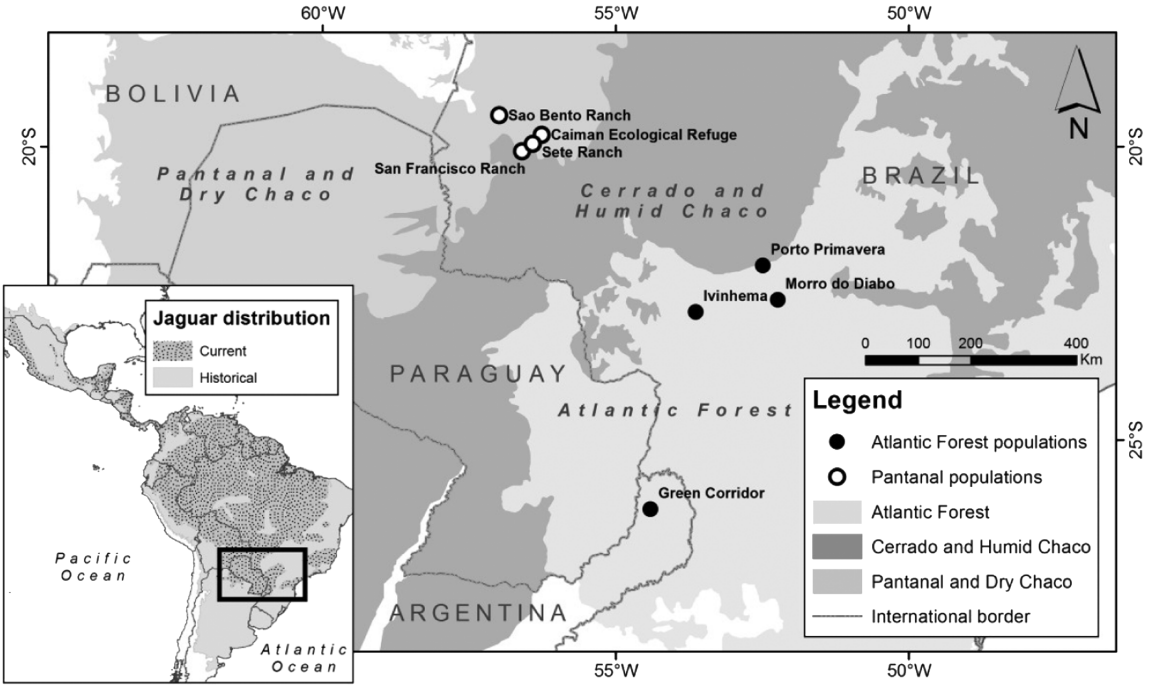

Figure 1. Locations of jaguars sampled in the southern Pantanal, as well as analyzed Atlantic Forest populations. The inset shows the geographic location of the Pantanal and the Atlantic Forest biomes, while the main map depicts the sampling locales within each ecosystem. Only midpoint locations of each site are shown [see Figure 1 in Haag et al. (2010) for a detailed depiction of the Atlantic Forest sites, including information on the severe fragmentation of native ecosystems in that area]. The distance between the sampling locations in the Pantanal and Porto Primavera (the closest Atlantic Forest fragment) is approximately 500 km. Within each ecosystem, a similar spatial scale is assessed by comparisons between São Bento and the other 3 ranches in the Pantanal (distance of ca. 80 km in all 3 cases), and comparisons among the 3 northern Atlantic Forest sites (smallest pairwise distance among sampled sites: 42 km for Porto Primavera vs. Morro do Diabo; 70 km for Porto Primavera vs. Ivinhema; 120 km for Ivinhema vs. Morro do Diabo).

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 for local populations separately, as well as for samples from all Pantanal populations pooled together. We also used FSTAT 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). To test whether sample sizes influenced the number of alleles detected in a population, AR and the number of private alleles were also computed for the entire data set (comprising the Pantanal as one population and the 4 Atlantic Forest populations mentioned above), following a rarefaction method that compensates for uneven sample sizes, as implemented in the software HP-Rare (Kalinowski 2004, 2005).

Data Analysis—Population Structure

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 anthropogenic habitat fragmentation. Global and pairwise F_{ST} and R_{ST} indices, as implemented in ARLEQUIN, were calculated for the Pantanal data set (i.e., containing the 4 ranches sampled in this ecosystem) as well as for the composite data set (Pantanal + Atlantic Forest fragments). Each test was run with 10 000 permutations to evaluate the statistical significance of the calculated value ($\alpha = 0.05$). We also used D_{EST}, a recently developed index of population differentiation, which we calculated as the arithmetic mean across loci using the software SMOGD 1.2.5 (Crawford 2010). According to Jost (2008), this measure is more reliable than the others, since it is not biased by within-population heterozygosity.

In addition, we assessed the existence of potential population subdivision using the Bayesian clustering method implemented in the program STRUCTURE 2.3.3 (Pritchard et al. 2000; Falush et al. 2003; Pritchard and Wen 2004). We initially conducted 10 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 and correlated allele frequencies. For this initial set of analyses, we performed an MCMC procedure of 1 000 000 generations, following a burn-in of 500 000 steps. An additional set of analyses was performed for the composite data set (Pantanal + Atlantic Forest) 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 10 runs for each K between 1 and 4 for the Pantanal alone, and between 3 and 8 for the composite data set. Results of the final STRUCTURE analyses were analyzed using STRUCTURE HARVESTER (Earl and vonHoldt 2012), including an assessment of the most likely number of distinct genetic clusters employing the method proposed by Evanno et al. (2005).

In addition to the STRUCTURE analyses, we also assessed the genetic structure of our composite data set using Bayesian approach implemented in BAPS 6.0 (Corander et al. 2006, 2008). We ran 10 replicates of individual clustering for each value of K (K = 1–12), as well as multiple replicates in which K was allowed to vary up to $K = 20$.

Data Archiving

The final set of genotypes generated in this study was deposited in the Dryad electronic repository (http://datadryad.org/), in accordance with the policy of the journal.

Results

Data Quality Control

Given our established cutoff of a minimum of 70% reliably genotyped loci, we removed 1 individual, bPon156, from the final data set. Therefore, our analyses were performed on a total sample of 52 jaguars from the Pantanal. We found no evidence of null alleles, large-allele dropout, or stutter peaks influencing the data set. These results allowed us to use the entire 12-locus panel to make population-level inferences.

We initially assessed the occurrence of deviation 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. This result indicated that it was not necessary test for disequilibrium within each local area, and that we could assume for subsequent analyses that all Pantanal samples represent a single population unit.

Genetic Diversity

All 12 loci were polymorphic and had high levels of diversity. When samples from all Pantanal populations were pooled together, the mean expected (H_E) and observed (H_O) heterozygosities were 0.69 and 0.71, respectively; the mean number of alleles per locus was 7, ranging from 3 (F146 and F98) to 13 (FCA742). When each local population (i.e., each ranch) was analyzed separately, we observed H_E values ranging from 0.66 (Caiman) to 0.71 (San Francisco), while H_O ranged from 0.69 (Caiman and Sete) to 0.73 (San Francisco; Table 1). AR calculated in FSTAT for the complete Pantanal sample (i.e., pooling the 4 ranches) was 6.63, ranging from 4.34 (San Francisco ranch) to 4.77 (São Bento ranch) when local populations were assessed separately (Table 1).

AR calculated with HP-Rare for the composite data set was similar between the Pantanal (treated as a single population) and some of the Atlantic Forest populations. However, richness was higher in the Pantanal (4.81) than in Morro do Diabo (3.33), which is the smallest population sampled in our Atlantic Forest data set. The 2 largest populations had the most private alleles (Pantanal and Green Corridor; 0.50 and 0.89, respectively) while the smallest population had the fewest (Morro do Diabo = 0.07).

Population Structure and Differentiation

Although we found no deviation from HWE or linkage equilibrium suggestive of population structure in the Pantanal, F_{ST} and R_{ST} values indicated some degree of genetic differentiation among the local populations sampled in this biome (0.045 [$P = 0.000$] and 0.047 [$P = 0.004$], respectively). Pairwise F_{ST} values were all low (0.03–0.053) but statistically significant (Table 2). The highest value was estimated for Caiman versus San Francisco (0.053; $P = 0.000$), while other estimates were lower than 0.05. On the other hand, R_{ST} values were mostly nonsignificant (Table 2), except for Caiman versus San

Table 1. Measures of diversity at 12 loci in the 4 populations of jaguars analyzed in this study

	Caiman Ecological Refuge (n = 22)					San Francisco Ranch (n = 11)					São Bento Ranch (n = 10)					Sete Ranch (n = 10)				
Locus	N	A	AR[a]	H_O	H_E	N	A	AR[a]	H_O	H_E	N	A	AR[a]	H_O	H_E	N	A	AR[a]	H_O	H_E
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	3	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

Sample size (N), observed number of alleles (A), observed (H_O), and expected (H_E) heterozygosities were calculated with ARLEQUIN, while AR was calculated with FSTAT.
[a]AR calculated for the minimum sample size of 8 diploid individuals.

Francisco (0.07; P = 0.0066) and San Francisco versus São Bento (0.10; P = 0.0012). The alternative index D_{EST} also showed a low degree of differentiation (Table 2) for all pairwise comparisons, with the highest value (0.051) again being between the Caiman and San Francisco populations.

To provide an exact comparison, we estimated the same pairwise differentiation indices for the 4 Atlantic Forest populations previously reported by Haag et al. (2010), but restricting the data set to the same 12 loci analyzed here. The results were very different from those observed in the Pantanal, with several pairwise combinations indicating high differentiation between fragments (Table 3). Of all comparisons, those involving the "Morro do Diabo" and "Green Corridor" fragments tended to yield the highest values of differentiation indices. The difference in patterns of differentiation between the Pantanal and Atlantic Forest was particularly visible when the respective F_{ST} and D_{EST} indices were compared, especially considering the pairs of locations at similar geographic distances (Tables 2 and 3).

Results from the Bayesian approach implemented in the program STRUCTURE were consistent across the different sets of analysis (Materials and Methods section). Since the variance among replicate runs was lowest when the longest MCMC procedure was performed (2×10^6 steps of sampling after 2×10^6 steps of burn-in), only these results are presented. This analysis indicated that the Pantanal data set was best explained with K = 1 [mean Ln P (D) = −1793.98; Pr(X|K) = 1], that is, by considering all 4 local populations as a single genetic unit (Supplementary Table S2).

The STRUCTURE analyses of the composite data set (52 individuals from the Pantanal and 59 from the Atlantic Forest) achieved the highest

Table 2. Pairwise F_{ST} (below the diagonal), R_{ST} (left number above the diagonal), and D_{EST} (right number above the diagonal) for the 4 jaguar sampling localities (ranches) analyzed in the Pantanal region

	Caiman	San Francisco	São Bento	Sete
Caiman	—	0.053[+]/0.051	**0.016/0.029**	−0.01/0.038
San Francisco	0.053***	—	**0.077[+]/0.044**	0.020/0.046
São Bento	**0.043****	**0.039***	—	**−0.041/0.023**
Sete	0.031*	0.039*	**0.048****	—

Values in bold types represent pairwise comparisons among the farthest sampling sites (ca. 80 km from each other).
[+]P < 0.05; *P < 0.01; **P < 0.001; ***P < 0.0001.

Table 3. Pairwise F_{ST} (below the diagonal), R_{ST} (left number above the diagonal), and D_{EST} (right number above the diagonal) for the 4 populations of jaguars analyzed in the Atlantic Forest region

	Green Corridor	Morro do Diabo	Ivinhema	Porto Primavera
Green Corridor	—	-0.050/0.336	0.011/0.224	0.052/0.118
Morro do Diabo	0.205***	—	**0.034/0.114**	**0.050/0.133**
Ivinhema	0.131***	**0.122****	—	**0.023/0.063**
Porto Primavera	0.051***	**0.081****	**0.063*****	—

Values in bold types represent pairwise comparisons among the northern sampling sites, whose reciprocal distances overlapped with those of the Pantanal locations highlighted in Table 2 (see Figure 1 for more details on the spatial relationships among sites).
**P < 0.001; ***P < 0.0001.

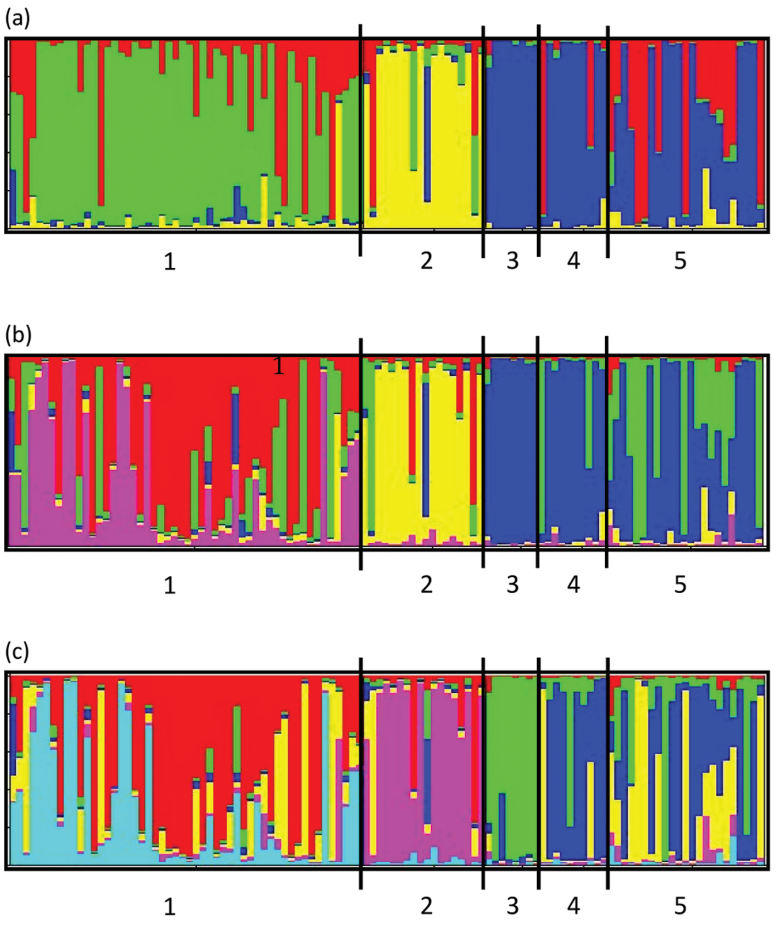

Figure 2. Proportional membership (q) of each jaguar sample inferred with STRUCTURE using the composite data set (Pantanal and Atlantic Forest) with $K = 4$ (a), $K = 5$ (b), and $K = 6$ (c), without any use of prior population information. Additional analyses, with $K = 3$, $K = 7$, and $K = 8$ are shown in Supplementary Figure S1. Each color represents a genetically defined population cluster. Each individual is represented by a vertical bar, with the length of each color per bar indicating the probability of membership in each genetic cluster. The locality of origin is indicated by numbers below the graph: (1) Pantanal, (2) Green Corridor, (3) Morro do Diabo, (4) Ivinhema, (5) Porto Primavera.

Table 4. Pairwise fixation indices (F_{ST}, R_{ST}, and D_{EST}) between the Pantanal (treated as a single population) and the 4 sampled Atlantic Forest populations of jaguars (see text for details)

	F_{ST}	R_{ST}	D_{EST}
Green Corridor	0.068***	0.133***	0.18
Morro do Diabo	0.165***	0.162**	0.29
Ivinhema	0.114***	0.099**	0.21
Porto Primavera	0.076***	0.039*	0.16

Significant values for F_{ST} and R_{ST} only: $^{*}P < 0.01$; $^{**}P < 0.001$; $^{***}P < 0.0001$.

likelihood score when 6 genetic populations were assumed [mean Ln P (D) = −3796.79; Pr(X|K) = 0.999; Supplementary Table S4]. In this scenario, 3 of the 4 Atlantic Forest populations (Green Corridor, Morro do Diabo, and Ivinhema) tended to cluster separately from all others (i.e., to have their own predominant genetic cluster), although they contained some migrant or admixed individuals (Figure 2). The Pantanal individuals were mostly allocated into 2 genetic clusters not shared with the other populations (red and light blue in Figure 2c), but also displayed substantial assignment to the sixth cluster (yellow in Figure 2c), which was also present in Atlantic Forest populations. Interestingly, the Porto Primavera sample contained mostly individuals with genetic compositions indicative of admixture with the other Atlantic Forest fragments and with the Pantanal (Figure 2).

When we assessed the STRUCTURE results using the method proposed by Evanno et al. (2005), there was strong support for $K = 4$ as the best fitting scenario of population structure with the composite data set (Supplementary Figure S2). Under this scenario (Figure 2a), some patterns were similar to those observed with $K = 6$, while a few interesting differences emerged. In particular, the subdivision of the Pantanal sample into 2 mostly endemic clusters was not apparent, while its connectivity to Porto Primavera became even more visible. The "Morro do Diabo" population showed the least connectivity to all others, while the remaining areas harbored migrant or admixed individuals indicating shared ancestry with other sites. This striking distinctiveness of the "Morro do Diabo" population was also visible in the BAPS analyses, even though this approach tended to over-split the composite data set (the best scenario was $K = 8$; Supplementary Tables S3 and S5).

Estimates of pairwise fixation indices between the Pantanal and Atlantic Forest fragments revealed interesting patterns (Table 4). F_{ST} values ranged from around 0.07–0.16, while R_{ST} varied between 0.04 and 0.16. In both cases, all comparisons were statistically significant ($P < 0.001$). D_{EST} values were consistently higher (0.16–0.29) than both traditional indices, showing a very similar trend across populations relative to that observed with F_{ST}. Overall, comparisons with

the Porto Primavera population consistently yielded the lowest values, while those with Morro do Diabo were consistently the highest. Interestingly, F_{ST} and D_{EST} values were very similar for comparisons involving Porto Primavera and Green Corridor, but a different trend was observed with R_{ST}, which indicated more substantial differentiation between Green Corridor and the Pantanal (Table 4).

Discussion

Genetic diversity indices found in the present study corroborate the hypothesis that Pantanal jaguars maintain considerably high levels of variability, as expected for large and healthy populations (Frankham et al. 2002). A comparison with diversity estimates for 3 other outbred felid species (*Leopardus geoffroyi*, *L. guttulus*, and *L. colocolo*) that have been previously genotyped for 7 of the same microsatellite markers and sampled on broader spatial scales (Trigo et al. 2008) reveals comparable levels of variation at most loci (Table 1), supporting this conclusion. Even though hunting and habitat degradation have been important threats to jaguars in this region, their population dynamics may not have been substantially affected so far, probably due to high prey availability and difficulty of human access in some areas (Soisalo and Cavalcanti 2006).

Levels of variability observed in this study were similar to those reported previously in an analysis of 29 microsatellite loci assessed throughout the species' geographic distribution (H_E = 0.74 and A = 8.3; Eizirik et al. 2001), but 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). However, direct comparisons with results from these studies should be avoided, since only 2 out of the 12 loci analyzed here (FCA441 and FCA453) were used by Eizirik et al. (2001), and only one (FCA391) was employed by Ruiz-Garcia et al. (2006). Moreover, the present study had a population-level scope, while those 2 others encompassed a broader geographic scale. One of the Pantanal populations analyzed here (Caiman Ecological Refuge) was also investigated in an independent study based on noninvasive fecal samples (Roques et al. 2014), which revealed a similar level of genetic diversity to that reported here. However, in this case none of the 11 microsatellite loci employed in that study overlapped with our panel, again precluding a more detailed comparison across data sets.

A more direct comparison with respect to our Pantanal data set is possible with our previous study that investigated jaguar genetics in remnant Atlantic Forest fragments (Haag et al. 2010), employing almost the same marker panel used here. The overall genetic diversity observed in that study was also high (e.g., H_E = 0.73), but strongly structured into 4 distinct spatial clusters (see below).

Among the local populations sampled within the Pantanal, genetic diversity values did not vary considerably (Table 1). We found a lower percentage of private alleles (16%; Supplementary Table S3) than that we found in the Atlantic Forest populations (25%; Haag et al. 2010), indicating that these areas in the southern Pantanal are currently more connected, likely presenting gene flow among them. Furthermore, the Bayesian clustering performed with the program STRUCTURE indicated that the 4 ranches investigated in the region comprised a single population (Supplementary Table S2). Estimates of differentiation were low, though statistically significant (Table 2). Estimated values were consistent with the hypothesis of a panmictic population in the region, although implying some degree of local differentiation, perhaps caused by the sampling of some related individuals in one or more ranches, thus driving fixation indices to significant (albeit low) values.

A detailed comparison of our Pantanal data set with that reported by Haag et al. (2010) for 4 Atlantic Forest fragments revealed very different patterns for the 2 biomes. Haag et al. (2010) found moderate to high levels of population structure among these 4 sites, and inferred that they were caused by strong, recent, genetic drift affecting these fragments, which harbored very small effective population sizes (especially in the case of the Morro do Diabo and Ivinhema fragments). Here, we repeated the analyses of the Atlantic Forest fragments using the exact same marker set we used for the Pantanal (i.e., 12 instead of 13 loci from the original study), and found the same pattern of strong regional structure (Table 3). In contrast, the Pantanal samples had much lower levels of differentiation (Table 2) and comprised a single genetic cluster when assessed with STRUCTURE (Supplementary Table S2).

To assess whether differences in the spatial scale of sampling for the 2 biomes could have influenced the observed contrast in genetic structure, we focused particular attention on pairwise comparisons between sites located at similar linear distances in the 2 regions (Figure 1 and Tables 2 and 3). In the Pantanal, the comparisons between São Bento and the other 3 sites spanned a linear distance of around 80 km, while in the Atlantic Forest, comparisons among the 3 northern sites spanned a similar spatial scale (ca. 40–120 km). It might therefore be hypothesized that these pairwise comparisons within each biome would display similar levels of genetic structuring. The observed patterns differ strongly from this expectation, as the level of population structuring was substantially more pronounced in the Atlantic Forest. Of all the populations assessed on this scale, "Morro do Diabo" was the most strongly differentiated from all others (Table 3 and Figure 2), which is consistent with its smaller effective size [as reported by Haag et al. (2010)] and more severe isolation due to recent habitat fragmentation (see below).

These results can be interpreted in the light of the current understanding of ecological and behavioral traits of this species, as well as differences in habitat connectivity between the 2 biomes. Jaguars are territorial, with moderate degrees of spatial overlap with conspecifics (Azevedo and Murray 2007). Juveniles tend to disperse to establish their new territory, whereas adults establish large home ranges, with males occupying larger areas and dispersing longer distances than females (Cavalcanti and Gese 2009). An ecological investigation conducted in the Pantanal estimated a home range of 57 km^2 during the wet season and 69 km^2 during the dry season for female jaguars. For males, the size of home ranges was 152 km^2 and 170 km^2 in the wet and dry seasons, respectively (Cavalcanti and Gese 2009). These estimates indicate that jaguars are expected to roam widely in these areas, thus likely maintaining demographic and genetic connectivity across this rather continuous landscape.

Equivalent estimates of jaguar home range sizes in 2 Atlantic Forest sites overlapped with those of the Pantanal [Iguaçu/Iguazu National Parks: 16–138 km^2 for males and 9–70 km^2 for females—Crawshaw et al. (2004); Morro do Diabo State Park: 88–177 km^2 for males and 44–132 km^2 for females—Cullen et al. (2005)]. The difference between the 2 biomes therefore does not lie in the home range size or individual dispersal capabilities of local jaguars, but rather in the present continuity of native cover in each of these regions. Recent evidence supports the notion that corridors with good-quality habitat are required for jaguar movement, and that in this surveyed region of the inland Atlantic Forest (and specifically in the "Morro do Diabo" population) they strongly avoid the heavily deforested matrix and tend to stay within their local fragment (Cullen et al. 2005).

Overall, these observations corroborate the interpretation that the genetic differentiation detected in the Atlantic Forest populations

has been induced by recent, anthropogenic fragmentation (Haag et al. 2010), as opposed to natural structuring on a regional scale. The comparison between the patterns observed in the 2 biomes illustrates the value of characterizing the genetic structure of populations that have not yet been severely modified by human activities. The Pantanal region is still reasonably preserved, allowing individuals to wander across the landscape following a dynamics that is likely more similar to the species' original life history. Therefore, this population might serve as a model to understand the natural demography and genetic structure of jaguars, and to provide valuable baseline information against which fragmented populations may be compared. Such a comparison should be useful to assess the effects of loss of diversity, inbreeding and correlated deleterious processes driven by small size and anthropogenic isolation among population fragments remaining in other biomes across the species' range.

Finally, our study strongly suggests recent genetic connectivity between the Pantanal and Atlantic Forest biomes (Figure 2), supporting the view that jaguar populations have maintained gene flow over broad geographic scales (Eizirik et al. 2001). In particular, our results indicate that the Porto Primavera population, located in a swampy, transitional habitat on the western boundary of the Atlantic Forest (Figure 1), showed strong evidence of recent genetic connectivity with the Pantanal. The recent extirpation of this transitional population due to the flooding of a hydroelectric dam (Haag et al. 2010; D. Sana, personal communication) has further contributed to the severing of the historical genetic connections between these biomes. Restoring such connections and maintaining those that still persist are critical challenges for ongoing jaguar conservation efforts.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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