Translocation as a tool for the conservation of the jaguar *Panthera onca*: a case study in the Brazilian Atlantic Forest

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SUPPLEMENTARY MATERIAL 1 Genetics Analyses.

Methods

The microsatellite database developed in previous studies included samples from the Atlantic Forest (Haag *et al.* 2010; Srbek-Araujo *et al.* 2018), Pantanal (Valdez *et al.* 2015; Kantek *et al.* 2021) and Amazon (Lorenzana *et al.* 2020) ecoregions. In addition, we further complemented the database by genotyping these same markers in 13 additional jaguars (six from the Pantanal, one from the Atlantic Forest, five from the Cerrado and one skin sample (bPon-057) from Minas Gerais state, collected 300 km from Juiz' capture site.

To perform the genotyping, DNA from the samples was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN) following the manufacturer's instructions. DNA extracts were then genotyped for the following microsatellite loci, developed originally for the domestic cat (Menotti-Raymond *et al.* 1999, 2005): one dinucleotide repeat (FCA742), two trinucleotide repeats (F146 and F98) and nine tetranucleotide repeats (FCA740, FCA723, FCA453, FCA441, FCA391, F124, F85, F53, and F42). We scored the genotypes using the software Peak Scanner v1.0 (Applied Biosystems). To allow accurate genotype binning of the integrated dataset, we re-genotyped a subset of samples reported in the previous studies.

To asses assignment probabilities for different regional populations or ecoregions, we used the software STRUCTURE 2.3.4 (Pritchard *et al.* 2000) Given the initial results (see below) and the fact that some local populations present exacerbated differentiation due to genetic drift driven by anthropogenic habitat fragmentation (Haag *et al*. 2010; Srbek-Araujo *et al.* 2018), which might bias our estimates, we ran the analyses with the complete database (n = 251) and also with seven subsets: (i) excluding the Morro do Diabo population; (ii) excluding the Morro do Diabo, Porto Primavera and Ivinhema populations (the whole northern block of the Upper Parana Atlantic Forest); (iii) excluding the isolated Reserva Natural Vale (RNV) population (coastal Atlantic Forest); (iv) excluding the Pantanal samples; (v) excluding the Amazon samples; (vi) including only individuals from the Atlantic Forest and Cerrado ecoregions; and (vii) including only individuals from the Green Corridor (southern block of the Upper Parana Atlantic Forest), Cerrado and Amazon. For each subset, we performed five independent runs for a varying number of assumed clusters (K=1-10) using 1,000,000 MCMC iterations following a burn-in period of 500,000 steps, with no putative population information and under the admixed and correlated allele frequencies model. We subsequently performed a second batch of analyses using the complete dataset and the

USEPOPINFO model, predefining the population of origin of all individuals except for Juiz and bPon-057. Since we had only five individuals from the Cerrado and they did not form a clear-cut genetic cluster, in one of the analyses we assigned these individuals to the Amazon population (based on the previous results) and in the other we removed these individuals from the dataset.

Results

For most of the data sets, when the number of assumed genetic clusters was low (K=3-4), individuals of the Amazon, Cerrado and Green Corridor (Inner Atlantic Forest) formed one genetic cluster, in which Juiz was included. However, when K≥5, these individuals were divided into two or more clusters and Juiz usually clustered with samples from the Amazon and Cerrado. Interestingly, though, Juiz presented its highest membership coefficient (q=0.9664) when only individuals from the Atlantic Forest and Cerrado were considered in the analysis, clustering with individuals from the Green Corridor and Cerrado (Table S1). The individuals from the Cerrado ecoregion did not form a separate cluster, regardless of the subset analyzed and the number of clusters considered. Two of them (bPon-338 and bPon-354) usually presented high levels of membership probability in an 'Amazon' cluster, while bPon-334 usually presented higher similarity to individuals from the northern fragments of the Inner Atlantic Forest (Porto Primavera, Ivinhema and Morro do Diabo populations). The remaining ones presented high levels of admixture among all the ecoregions. In addition, bPon-057 also presented high levels of admixture, not being assigned to any population (q<0.6), except for the analysis of the subset containing only individuals from the Green Corridor, Cerrado and Amazon, in which it presented high membership probability to the cluster formed by individuals from the Green Corridor and Cerrado (considering K=2) (Table S1, Fig. S1).

Discussion
It is noteworthy that Cerrado individuals exhibited high levels of admixture, especially with the Atlantic Forest and the Amazon, and this admixture may reflect a historical role that the Cerrado played as a corridor for gene flow between these South American ecoregions. Given this result, and the currently small sample size for this ecoregion, the assessed molecular markers would not be able to reliably assign a sample to the Cerrado, due to its lack of diagnostic genetic features.

Moreover, although Juiz was most usually allocated within Amazon/Cerrado groups, we cannot exclude the possibility that it came from an Atlantic Forest (AF) population. Most remaining AF jaguar populations show high genetic differentiation, low diversity and low effective size (Haag *et al.* 2010; Srbek-Araujo *et al.* 2018). Small and isolated populations are more prone to inbreeding and loss of genetic diversity due to genetic drift (Gibbs 2001; Traill *et al.* 2010), making individuals from a population more similar to each other, but more different from other populations. Therefore, the high isolation and small size of AF populations could explain why Juiz presented low genetic similarity with individuals from the closest sampled population, RNV, and higher similarity with individuals from the farthest AF population, the Green Corridor. The Green Corridor comprehends the largest Atlantic Forest remnant, and its jaguar population probably represents to some degree the genetic composition of AF jaguars prior to the severe fragmentation of this ecoregion. In addition, we note that our current data base does not include some remnant jaguar populations from the coastal portion of the AF, including RDSP itself. Therefore, Juiz could have originated from a small, unsampled coastal AF population remnant (such as the ones mentioned above, none of which has been so far sampled for inclusion in our data base), which may also bear a unique genetic profile due to anthropogenic genetic drift. Interestingly, the other sample from Minas Gerais state, bPon-057, demonstrated high levels of admixture and could not be assign to any genetic cluster. This sample was collected within the Atlantic Forest ecoregion, ca. 300 km southwards of where

Juiz was captured, and may indicate that other unsampled jaguar populations from the costal Atlantic Forest present distinct genetic structure. However, as it is an old skin sample, it could suffer from allele drop-out and other genotyping errors, so that its results should be considered with caution.

Supplementary Table 1 Probability of assignment of jaguar individual "Juiz" to genetic clusters delimited with the Bayesian approach implemented in STRUCTURE, based on microsatellite genotypes (see text for details). For each data set (complete database or subsets including different sets of local populations), different values of K (number of assumed genetic clusters) were assessed. Labelled clusters indicate that they comprised a predominant membership from the respective ecoregions or local populations. Values underneath the labels are the probability of assignment to that cluster. Unlabeled genetic clusters imply that there was no predominant membership of a clear-cut set of populations (i.e. individuals were sampled at different sites and/or showed admixed ancestries from multiple sites). Assignments with probability ≥0.7 are highlighted in boldface types. See footnote for abbreviations referring to ecoregions and local populations.

	K	Genetic cluster							
Complete database	4	**Amaz/GC**	Pant	PP/MD/Iv	Pant/RNV				
		0.8906	0.013	0.0322	0.0642				
	5	Pant	GC/RNV	PP/MD/Iv	**Amaz**	Pant			
		0.0916	0.06	0.0312	**0.806**	0.0112			
	6	**Amaz**	PP/MD/Iv	Pant	Pant	RNV	Amaz/GC		
		0.8314	0.0278	0.0102	0.0692	0.034	0.0274		
	7	Pant/RNV	**Amaz**	Pant	Pant	Amaz	PP	PP/MD/Iv	
		0.0278	**0.8094**	0.009	0.0788	0.0222	0.0288	0.024	
	8	--	--	RNV	PP/MD/Iv	--	PP	Pant	Pant
		0.1188	0.2	0.0156	0.0238	0.5224	0.023	0.0884	0.008
Excluding MD	4	Pant	PP/Iv	Pant/RNV	**Amaz/GC**				
		0.014	0.0418	0.0704	**0.8738**				
	5	GC/RNV	Pant	**Amaz**	PP/Iv	Pant			
		0.0592	0.0974	**0.7942**	0.0382	0.011			
	6	RNV	Pant	Amaz	Pant	**Amaz**	PP/Iv		
		0.027	0.0712	0.0722	0.0098	**0.7901**	0.0298		
	7	--	PP/Iv	--	Pant	RNV	--	Pant	
		0.073	0.0302	0.5529	0.0714	0.015	0.2492	0.0082	
	8	PP/Iv	--	RNV	Pant	--	--	Pant	--
		0.033	0.4742	0.0166	0.0858	0.1313	0.0402	0.008	0.2109

	K	Genetic cluster							
Excluding MD, PP and Iv	4	GC/RNV 0.1182	Pant 0.0154	**Amaz 0.7608**	Pant 0.1056				
	5	Pant 0.013	**Amaz 0.821**	Amaz/GC 0.054	Pant 0.0722	RNV 0.0398			
	6	Pant 0.011	RNV 0.0212	-- 0.0606	-- 0.5958	-- 0.2301	Pant 0.0812		
	7	-- 0.0216	-- 0.0274	Pant 0.009	-- 0.4404	RNV 0.014	GC 0.3976	Pant 0.09	
	8	GC 0.4098	-- 0.0308	-- 0.0616	-- 0.016	-- 0.0316	RNV 0.0134	-- 0.4126	-- 0.0242
Excluding RNV	4	PP/MD/Iv 0.032	Pant 0.062	Pant 0.0136	**Amaz/GC 0.8924**				
	5	GC 0.0478	Pant 0.012	PP/MD/Iv 0.0294	**Amaz 0.8448**	Pant 0.066			
	6	-- 0.145	Pant 0.0808	-- 0.6362	Pant 0.01	PP/MD/Iv 0.027	0.1009		
	7	GC 0.1204	Pant 0.0906	PP/MD/Iv 0.0264	PP 0.0268	**Amaz 0.7055**	Pant 0.009	Amaz 0.0214	
	8	-- 0.5446	PP/MD/Iv 0.0256	-- 0.1235	Pant 0.008	-- 0.0496	PP 0.023	Pant 0.081	GC 0.1447
Excluding Pant	4	PP/MD/Iv 0.0274	**Amaz 0.8441**	GC/RNV 0.0943	Amaz 0.0342				
	5	Amaz 0.0294	RNV 0.0488	PP/MD/Iv 0.022	**Amaz 0.8331**	PP 0.0666			
	6	-- 0.0632	MD/Iv 0.0204	-- 0.2502	RNV 0.0246	PP 0.0622	-- 0.5794		
	7	-- 0.1402	RNV 0.0212	-- 0.0542	-- 0.4501	MD/Iv 0.018	PP 0.0214	GC 0.2949	

	K	Genetic cluster							
	8	--	MD/Iv	RNV	--	--	--	GC	PP
		0.1808	0.0154	0.0206	0.3008	0.0798	0.1614	0.2226	0.0186
Excluding Amaz	4	PP/MD/Iv	Pant	**GC/RNV**	Pant				
		0.0994	0.178	**0.7095**	0.013				
	5	--	Pant	PP/MD/Iv	RNV	Pant			
		0.5295	0.0108	0.0608	0.2775	0.1214			
	6	PP/MD/Iv	**GC**	Pant	RNV	PP	Pant		
		0.033	**0.8658**	0.0492	0.0134	0.0296	0.009		
	7	PP	**GC**	Pant	--	Pant	PP/MD/Iv	RNV	
		0.0402	**0.8212**	0.059	0.0212	0.0094	0.0358	0.0132	
	8	--	RNV	--	Caiman	PP/MD/Iv	--	--	PP
		0.0629	0.0114	0.1426	0.0078	0.0368	0.6221	0.083	0.0334
Including AF and Cer	3	PP/MD/Iv	RNV	**GC/Cer**					
		0.0162	0.0174	**0.9664**					
	4	RNV	PP	**GC**	MD/Iv				
		0.0176	0.0264	**0.9426**	0.0134				
	5	RNV	PP	--	--	MD/Iv			
		0.0138	0.0502	0.5363	0.3897	0.01			
	6	RNV	--	PP	MD	PP	--		
		0.0152	0.6237	0.014	0.01	0.0248	0.3123		
	7	RNV	--	PP	PP	--	MD	--	
		0.0156	0.4923	0.0186	0.0572	0.0674	0.009	0.3399	
	8	--	PP	PP	--	--	Iv	RNV	MD
		0.2412	0.0212	0.0158	0.3938	0.2958	0.009	0.0152	0.008
Including GC, Cer and Amaz	2	**Amaz**	GC/Cer						
		0.7782	0.2218						
	3	Amaz	Amaz	GC					
		0.605	0.037	0.358					

	K	Genetic cluster							
	4	Amaz 0.117	Amaz 0.0288	GC 0.3852	Amaz 0.469				
	5	-- 0.1726	GC 0.3172	-- 0.3142	-- 0.1248	-- 0.0712			
Using prior information on population of origin for the reference individuals (POPINFO) – Cerrado included in the Amazon Population	3	Pant/RNV 0.0224	**GC/Amaz/Cer 0.9326**	PP/MD/Iv 0.045					
	4	Pant 0.013	PP/MD/Iv 0.0316	Pant/RNV 0.0638	**Amaz/GC/Cer 0.8916**				
	5	PP/MD/Iv 0.0314	Pant 0.0958	GC/RNV 0.0616	**Amaz/Cer 0.8001**	Pant 0.0112			
	6	PP/MD/Iv 0.0278	Pant 0.01	Pant/RNV 0.0338	**Amaz/Cer 0.8307**	Amaz/GC 0.0288	Pant 0.0688		
	7	Pant 0.009	PP/GC 0.0284	PP/MD/Iv 0.0238	**Amaz/Cer 0.8106**	Pant/RNV 0.0276	Amaz/Cer 0.0216	Pant 0.079	
	8	-- 0.1918	RNV 0.015	Pant 0.008	PP/Iv/MD 0.0238	PP 0.0228	-- 0.4636	-- 0.1942	Pant 0.0808
Using prior information on population of origin for the reference individuals (POPINFO) – Cerrado excluded from the analysis	3	Pant/RNV 0.0198	**Amaz/GC 0.942**	PP/Iv/MD 0.0382					
	4	**Amaz/GC 0.9**	Pant/Amaz/RNV 0.0588	PP/Iv/MD 0.0292	Pant 0.012				
	5	Pant 0.0112	Pant 0.093	GC/RNV 0.0598	PP/Iv/MD 0.0304	**Amaz 0.8056**			
	6	Pant 0.01	PP/Iv/MD 0.027	-- 0.0448	RNV 0.0334	Pant 0.0703	**Amaz 0.8144**		
	7	**Amaz 0.759**	RNV 0.0266	Pant 0.009	PP/Iv/MD 0.025	Pant 0.0832	GC/PP 0.0286	Amaz 0.0686	
	8	GC 0.2865	PP 0.0208	Pant 0.008	-- 0.0888	-- 0.4682	RNV 0.015	PP/Iv/MD 0.0254	Pant 0.0872

Abbreviations: AF - Atlantic Forest; Amaz – Amazon; Cer – Cerrado; GC – Green Corridor; Iv – Ivinhema; MD – Morro do Diabo; Pant - Pantanal; PP – Porto Primavera; RNV – Reserva Natural Vale.
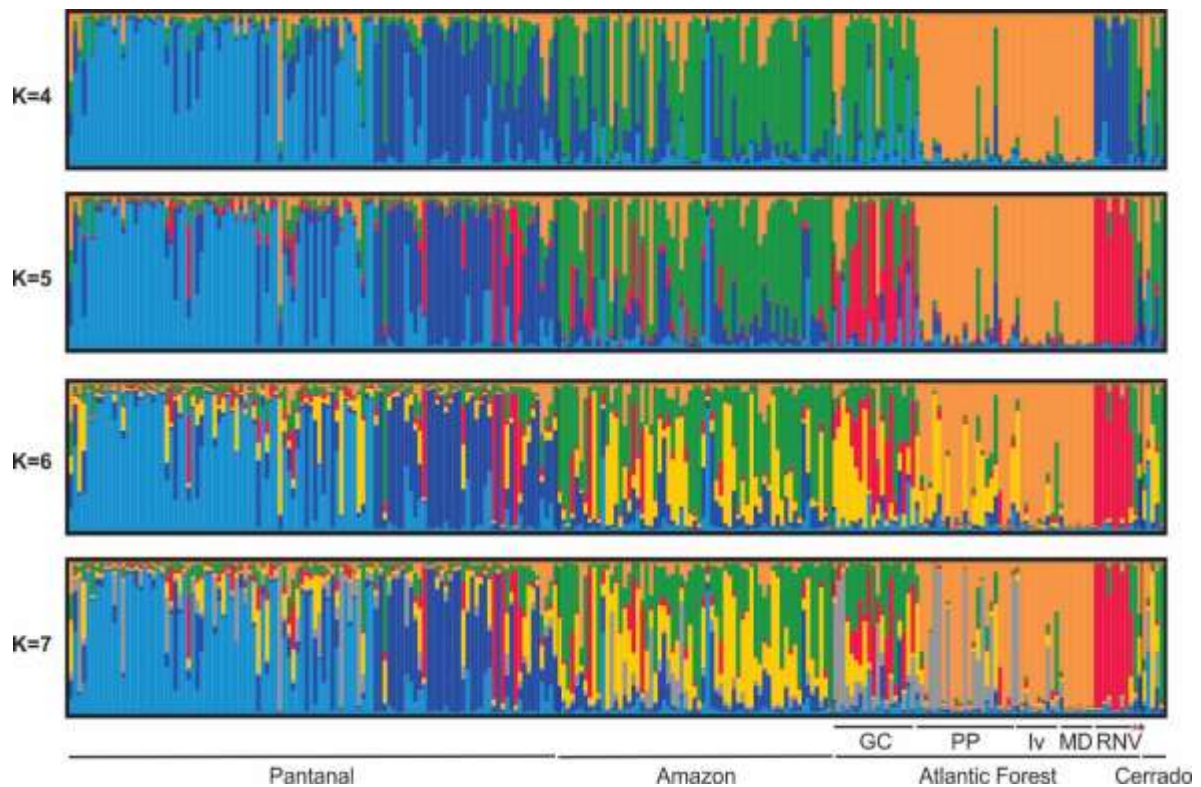
SUPPLEMENTARY FIG. 1. Genetic structure of jaguar populations analyzed in the present study with the program Structure. Vertical bars represent individuals; colors indicate their respective proportion of membership (q) in K distinct genetic clusters. Panels indicate the results for the complete data set with assumed values of K ranging from 4 to 7. GC = Green Corridor; PP = Porto Primavera; IV = Ivinhema; MD= Morro do Diabo; RNV = Reserva Natural da Vale.