Monitoring jaguar populations *Panthera onca* with non-invasive genetics: a pilot study in Brazilian ecosystems

S. ROQUES, M. FURTADO, A. T. A. JÁCOMO, L. SILVEIRA, R. SOLLMANN, N. M. TÔRRES, J. A. GODOY and F. PALOMARES

SUPPLEMENTARY MATERIAL 1

DNA extraction for species and sex identification

Primer pairs were redesigned to exclude human gene amplification and were optimized for a range of temperatures (53–60°C) and template DNA quantities (10–50 ng). This was to evaluate the robustness of the amplification from low-quality DNA and the likelihood of false females as a result of failed amplification of the shorter male-specific product. Amplifications were performed four times, with one male positive control DNA, one female positive control DNA and one negative control. PCR involved 4 µl of DNA extract in a final volume of 20 µl, containing 67 mM Tris-HCl, pH 8, 16 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.25 mM deoxynucleotide triphosphates, 0.8 mg/ml bovine serum albumin, 0.6 µM of each primer and 0.4 U Taq polymerase (Bioline). Denaturation at 94ºC for 2 minutes was followed by 40 cycles of denaturation at 92ºC, annealing at 58ºC and extension at 72ºC, each step lasting 30 seconds, and a final extension step of 5 minutes at 72ºC. The PCR products were run on 2% agarose gels. Females were recorded whenever the upper band was seen at least three times with no amplification of the lower male-specific band, whereas for males only two independent amplifications of the male-diagnostic band were required.

Optimization of microsatellite markers for individual identification

To optimize both data quality and yield all faecal DNA extracts were first evaluated for quality by direct amplification of the Fca82b locus. This locus was selected for its high amplification robustness; the samples that failed to amplify this locus would probably not amplify the remaining 10 loci. The faecal samples showing positive amplification at this locus were selected for further genotyping. For the genotyping of faecal samples we followed a multiple-tube approach in which each DNA sample was genotyped four times (Taberlet et al., 1996, 1999; Taberlet & Luikart, 1999; Goossens et al., 2000). Microsatellite amplification was carried out through two sequential amplifications: a multiplex PCR that included the whole set of 11 primer pairs and a reduced number of cycles (Bellemain & Taberlet 2004; Piggott et al., 2004) was followed by the amplification of each locus separately, using the PCR products as templates. The PCR was carried out using 14 µl of DNA extract in a final volume of 60 µl and 0.02 µM of each primer, bovine serum albumin at 0.8 µg/µl and 25 cycles. Each marker was amplified separately using 4 µl of PCR product in a final volume of 20 µl, using the annealing temperatures listed in Supplementary Table S1. For Amelogenin amplifications we used the same conditions and cycling, except we used 0.2 µM labelled forward primer and 0.2 µM unlabelled reverse primer. Alleles were scored using *GeneMapper v. 4.0* (Life Technologies, Carlsbad, USA). We obtained a unique consensus genotype for each faecal sample from the four PCR replicates following Taberlet et al. (1996) and calculated a quality index value as described by Miquel et al. (2006).

Individual identification can be hampered by two potential problems. Firstly, if too few loci are examined, multilocus genotypes in a population may not be unique and individuals with the same genotype will be indistinguishable (e.g. Taberlet & Luikart 1999). Secondly, genotyping errors may produce different genotypes for the same individual, falsely suggesting a new individual. To overcome these problems we grouped all identical and complete consensus genotypes together

manually or using *GIMLET* (Valière, 2002). Because we had incomplete profiles, only consensus genotypes with seven or more successfully genotyped loci and a quality index ≥0.5 were considered for further analysis. We then estimated the distribution of the expected number of mismatches between different genotypes for three different kinship classes (Kalinowski et al., 2006; ×Fig. 2). The expected distributions of mismatches among consensus genotypes calculated for the Pantanal indicated that any two full siblings would differ by at least two loci (P mismatch=0.0015). Similarly for all consensus genotypes in the Caatinga, the probability of two siblings sharing the same genotype at all but one or two loci is low (P=0.0018 and P=0.0128, respectively). Thus we considered that any two samples with genotypes differing by one or two loci belonged to the same individual. Samples with incomplete genotypes were assigned to an individual when the matching probability of the matching genotype was lower than 1%. The match probability of each incomplete genotype observed (Woods et al., 1999) was calculated using *GIMLET* (Valière, 2002). We used sex identification as an additional criterion to assign a given consensus genotype to the same or different individuals.

SUPPLEMENTARY TABLE S1 Measures of diversity (primer sequences) at 11 microsatellite loci in the Pantanal and Caatinga regions of Brazil (Fig. 1), with annealing temperature, PCR amplification success, error rates (allelic dropout and false alleles), allelic richness, allelic range, expected and observed heterozygosity, probabilities of identity (for siblings and for unrelated individuals).

Locus	Primer sequences (5´-3´)	Annealing temperature	PCR success (%)	Allelic dropout*	False alleles*	Allelic richness	Allelic range (bps)	Expected heterozygosity	Observed heterozygosity	Probability of identification (siblings)	Probability of identification (unrelated)
FCA115	F: Fam-CTCACACAAGTAACTCTTTG R:CCTTCCAGATTAAGATGAGA	57				10.86	189–219	0.79	0.77	3.73×10^{-1}	3.34×10^{-2}
Fca547b	F:Vic-GGTGACAAAACAAAACAAAGCA R:GGAGCCTGCATAGGATTCAC	60	96	0.071	0	7.00	226–235	0.72	0.77	4.19×10^{-1}	8.17×10^{-2}
Fca26	F:Fam-GGAGCCCTTAGAGTCATGCA TGTACACGCACCAAAAACAA	60	96	0	0	4.00	129–151	0.46	0.45	6.03×10^{-1}	2.78×10^{-1}
FcaA176	F: Pet-GGAAACTTGGAAAGCAAAACC R:TCCACAGTTGGAGTTCTTAAGG	57				6.91	211–225	0.73	0.91	4.15×10^{-1}	9.58×10^{-2}
Fca90a	F:Fam-ATCAAAAGTCTTGAAGAGCATGG R:TGTTAGCTCATGTTCATGTGTCC	60	96	0	0	5.91	106–114	0.73	0.77	4.19×10^{-1}	1.07×10^{-1}
Fca566b	F:Ned-TGCTCAAACAGATAAGGCTGAA R:CCCACTCATGCTGTCTCTCA	57	100	0	0	7.95	162–175	0.80	0.91	3.69×10^{-1}	4.62×10^{-2}
Fca24	F:Pet- CCCAGCTTTGTCTCTTACTGTG R:CATCCTCCCCTAATGCCC	60	89	0	0	7.82	220–230	0.75	0.77	4×10^{-1}	8.37×10^{-2}
Fca82b	F:Vic-TCACCGCTTAAGAAGAGGCTA R:GTGAAGCTTCCGAAATGAGG	57	89	0	0	5.95	192–208	0.66	0.73	4.59×10^{-1}	1.20×10^{-1}
Fca126	F:Pet- GCCCCTGATACCCTGAATG R:CTATCCTTGCTGGCTGAAGG	57	96	0	0	6.00	143–163	0.76	0.91	3.94×10^{-1}	6.58×10^{-2}
Fca77	F:Ned-GGCACCTATAACTACCAGTGTGA R:ATCTCTGGGGAAATAAATTTTGG	57	96	0	0	5.96	135–155	0.67	0.59	4.54×10^{-1}	1.11×10^{-1}
Fca43a	F:Ned-GAGCCACCCTAGCACATATACC R:AGACGGGATTGCATGAAAAG	60	100	0	0.083	3.96	113–121	0.66	0.50	4.68×10^{-1}	1.64×10^{-1}
Overall			95	0.0008	0.0092	6.57		0.70	0.74	4.30×10^{-5}	4.19×10^{-12}

*Calculated for the faecal samples from captive jaguars

SUPPLEMENTARY TABLE S2 Measures of genetic diversity at 11 microsatellite loci in the Pantanal (Caiman Ecological Refuge; N=34) and the Caatinga (Serra da Capivara National Park; N=17) regions, with the polymerase chain reaction (PCR) amplification success, error rates (allelic dropout and false alleles), mean number of alleles per locus, allelic richness based on 12 individuals, allelic range, expected heterozygosity, observed heterozygosity, and total number of private alleles in each site.

Locus	Sampling location	PCR success (%)	Allelic dropout	False allele	Mean no. of alleles per locus	Allelic richness	Allelic range (bps)	Expected heterozygosity	Observed heterozygosity	Total no. of private alleles
Fca115	Caatinga	0.84	0.102	0.000	7	6.28	189–218	0.81	0.82	2
	Pantanal	0.79	0.088	0.000	16	12.54	189–219	0.87	0.79	11
Fca547b	Caatinga	0.62	0.000	0.000	6	6.00	223–233	0.78	0.53	0
	Pantanal	0.75	0.162	0.000	8	6.47	223–235	0.70	0.79	2
Fca26	Caatinga	0.98	0.029	0.000	5	4.95	131–151	0.65	0.82	2
	Pantanal	0.99	0.045	0.000	6	5.10	127–151	0.70	0.79	3
Fca176	Caatinga	1	0.258	0.114	7	7.17	209–231	0.78	0.88	5
	Pantanal	0.74	0.167	0.000	7	5.08	211–225	0.74	0.88	3
Fca90a	Caatinga	0.92	0.060	0.125	7	6.48	106–120	0.78	0.88	3
	Pantanal	1	0.042	0.000	4	3.99	106–114	0.71	0.71	0
Fca566b	Caatinga	0.98	0.012	0.000	6	5.63	163–173	0.69	0.65	1
	Pantanal	0.95	0.038	0.111	8	7.11	162–175	0.81	0.91	3
Fca24	Caatinga	0.86	0.068	0.000	4	4.95	224–230	0.31	0.47	0
	Pantanal	0.65	0.015	0.000	6	5.10	220–230	0.52	0.50	2
Fca82b	Caatinga	0.96	0.018	0.018	5	4.84	194–216	0.65	0.65	1
	Pantanal	0.95	0.042	0.000	7	5.68	192–216	0.62	0.65	3
Fca126	Caatinga	0.99	0.000	0.000	4	4.00	143–159	0.68	0.94	0
	Pantanal	1	0.036	0.000	6	5.43	143–163	0.74	0.91	2
Fca77	Caatinga	0.98	0.013	0.000	4	3.82	145–155	0.56	0.82	1
	Pantanal	0.91	0.078	0.000	6	5.06	135–155	0.66	0.65	3
Fca43a	Caatinga	0.70	0.010	0.000	6	4.66	113–121	0.67	0.88	1
	Pantanal	0.97	0.167	0.000	4	3.83	113–123	0.66	0.47	0
Overall	Caatinga	0.89	0.052	0.023	6	5.20		0.67	0.76	16
	Pantanal	0.88	0.080	0.010	7	5.94		0.70	0.73	32