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TECHNICAL NOTE

Molecular tracking of jaguar melanism using faecal DNA

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Abstract Major evolutionary questions remain elusive due to persistent difficulties in directly studying the genetics of variable phenotypes in natural populations. Many phenotypic variants may be of adaptive relevance, and thus important to consider in the context of conservation genetics. However, since the dynamics of these traits is usually poorly understood in the wild, their incorporation in conservation strategies is difficult to accomplish. For animals which exhibit intriguing phenotypic variation but are difficult to track in the wild, innovative approaches are required to investigate such issues. Here we demonstrate that non-invasive DNA sampling can be used to study the genetics and ecology of melanism in the jaguar, by directly genotyping the molecular polymorphism underlying this coloration trait. These results open new prospects for the in-depth investigation of this polymorphism, and highlight the broader potential of non-invasive DNA-based phenotype tracking for wildlife in general.

Keywords Phenotypic polymorphism · *Panthera onca* · *MC1R* · Non-invasive sampling

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Introduction

The use of non-invasive sampling from free-ranging animals has revolutionized the study of ecological, behavioral, genetic and epidemiological aspects of many taxa (Smith and Wayne 1996; Beebee and Rowe 2008). In the last two decades, the fields of conservation genetics and molecular ecology have grown dramatically, largely due to the increased ability to analyze natural populations using molecular markers applied to samples such as scats. Many research groups routinely employ faecal DNA to identify field-collected samples at the species or individual level, as well as to determine gender, kinship, genetic diversity, or diet (e.g. Hedmark et al. 2004; Livia et al. 2007; Deagle and Tollit 2007). However, up to now we are unaware of any study that has applied faecal DNA to identify a morphological phenotype. This is largely due to the fact that the molecular basis of such traits remains unknown for most naturally occurring polymorphisms, precluding the development of assays to survey the dynamics of

morphological variants and their underlying alleles. If this impediment is overcome, the population genetics of loci influencing natural phenotypic polymorphisms could be studied directly using non-invasive sampling, thus opening up tremendous prospects for the investigation of their evolutionary and ecological significance.

The jaguar (*Panthera onca*) is the largest felid in the Americas and has been the focus of considerable scientific and conservationist attention in the last decades (e.g. Medellín et al. 2002). Melanism (dark background coloration) has been long known to occur in this species, representing a dramatically visible coat color polymorphism whose ecological impact and adaptive significance remain a mystery. Although melanistic jaguars are common in zoos and often seen in the wild, no study has so far addressed the ecological role of this variant, and even its frequency in natural populations has not been surveyed systematically. Therefore, the conservation relevance of this phenotype cannot currently be assessed, and may be an issue in the context of dwindling natural populations.

Studying this trait in the wild using conventional methods poses major challenges given low densities and high costs of capture-based operations, especially when compared to the effectiveness of scat collection in the field. We have previously identified a 15-bp deletion in the jaguar *MC1R* gene (the "*MC1R*-Δ15" allele) that was perfectly associated with melanism (Eizirik et al. 2003). These analyses were based on blood or tissue samples,

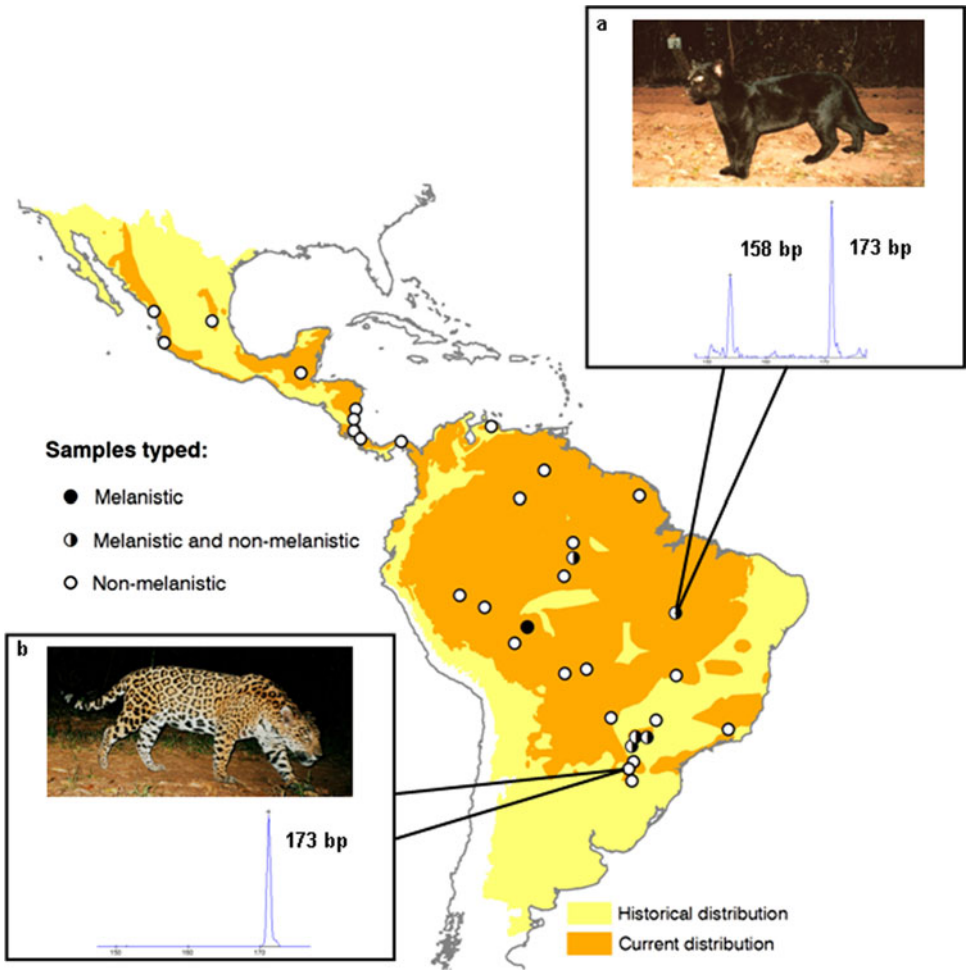

Fig. 1 Range map of the jaguar in the Americas, depicting its historical and current distribution—Modified from Sanderson et al. (2002) and Zeller (2007). *Circles* indicate sample collection locales for our association study between melanism and *MC1R* variation (*white*: one or more non-melanistic samples from a given site; *black*: one or more melanistic samples from a site). *Insets*: **a** Heterozygous *MC1R* genotype (containing the melanism-related *MC1R*-Δ15 allele that yields a 158-bp PCR product, and the 173-bp wild-type allele) obtained from a scat sample collected at an Amazonian field site where camera traps also recorded the presence of melanistic animals (camera-trap photo of melanistic individual taken in Morro do Diabo State Park, SP, Brazil; credit: Instituto de Pesquisas Ecológicas); **b** Homozygous wild-type *MC1R* genotype (containing only the 173-bp wild-type allele) obtained in six independent PCR trials from a scat sample collected at an Argentinean field site where only non-melanistic animals were detected by camera-trapping (camera-trap photo of wild-type jaguar from Iguazú National Park, Argentina; credit: A. Paviolo, C. De Angelo and M. Di Bitetti—Proyecto Yaguareté, Argentina)

Table 1 Genotyping results for the melanism-related *MC1R* polymorphism in field-collected jaguar scats

Collection site	N[e]	Genotypes identified[f]			Predicted phenotype
		158/158	158/173	173/173	
Ivinhema State Park[a]	2	–	–	6	Wild-type
Iguaçu National Park[b]	2	–	–	6	Wild-type
Misiones Province[c]	5	–	–	6	Wild-type
Cantão State Park[d]	14	–	–	6	Wild-type
Cantão State Park[d]	1	3	1	4	Melanistic

Genotypes are given as all possible combinations of alleles 158 (*MC1R*-Δ15) and 173 (wild-type allele)

[a] Mato Grosso do Sul state, southwestern Brazil

[b] Paraná state, southern Brazil

[c] Multiple Atlantic Forest sites in northeastern Argentina (adjacent to Iguaçu National Park)

[d] Tocantins state, southern Amazonian region, northern Brazil

[e] Only samples that could be reliably and reproducibly genotyped are included. Five additional samples from Cantão State Park yielded *MC1R* genotypes, but our replication threshold for phenotype prediction could not be reached after 12 genotyping attempts: two of them yielded four replicates each of the 173/173 genotype; two others yielded a single replicate each of 173/173; and one sample was typed once as 158/158 and three times as 173/173

[f] Values indicate the number of times each *MC1R* genotype was independently observed for each faecal sample

prompting the question of whether this variant could be genotyped in natural populations using scats. To pursue this possibility, it is important to ascertain that the association between genotype and phenotype is affirmed by additional sampling, and to test whether this molecular variant can be effectively and reliably typed in faecal samples.

In the present study we addressed these issues by performing three sequential analytical steps: (1) an expanded association study testing the ability of our molecular assay to predict the melanistic phenotype; (2) a blind test using faecal samples from known animals to assess whether scat DNA-based phenotype prediction is reliable and reproducible; and (3) field tests assessing whether the quality of samples found in wild settings is sufficient to interrogate this nuclear genetic locus. Details of materials and methods are provided as online Supplementary Information.

Results and discussion

The association study included 95 broadly sampled jaguar individuals (Fig. 1 and Supplementary Information) whose color was known, and resulted in 100% correspondence between the melanistic phenotype and presence of the *MC1R*-Δ15 allele, thus strongly implicating the latter in this morphological variant. The blind test was conducted with 34 faecal samples collected from captive jaguars whose identity and coloration were hidden from the geneticist performing the molecular assay, and only revealed after reaching a pre-established threshold of reliable genotypes (three for heterozygotes [or at least two independent scores for each allele], and six for homozygotes). In 32 out of 34 samples this threshold could be reached, in every case correctly predicting the individual's phenotype (see Supplementary Information). In the two additional samples the phenotype was also predicted correctly, with four and five homozygote genotypes, respectively. This experiment demonstrated that it is possible to reliably predict a jaguar's coat color on the basis of scat DNA.

Finally, we applied this approach to survey field-collected scats identified as having been deposited by jaguars based on mtDNA sequencing (Haag et al. 2009). Twenty-three jaguar scats from the southern Amazon region (P.E. Cantão, TO, Brazil) and ten from the Atlantic Forest biome (Argentina and southwestern Brazil) were analyzed; 15 among the former and 9 among the latter could be reproducibly genotyped by reaching our threshold (Table 1). No evidence of melanism was detected in the Argentinean Atlantic Forest sites or in adjacent Iguaçu Park (Brazil) in agreement with current camera-trapping data (Fig. 1; Table 1). In contrast, at least one melanistic animal was detected by scat-DNA genotyping of the *MC1R* polymorphism in the Amazonian site, where this phenotypic variant has been observed. These results demonstrate that it is possible to survey the presence and frequency of melanism in natural jaguar populations using non-invasive DNA, and create an opportunity for in-depth investigation of the evolutionary forces driving the dynamics of this polymorphism in the wild.

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References

Beebee T, Rowe G (2008) An introduction to molecular ecology. Oxford University Press, Oxford

Deagle BE, Tollit DJ (2007) Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition? Conserv Genet 8:743–747. doi:10.1007/s10592-006-9197-7

Eizirik E, Yuhki N, Johnson WE et al (2003) Molecular genetics e evolution of melanism in the cat family. Curr Biol 13:448–453. doi:10.1016/S0960-9822(03)00128-3

Haag T, Santos AS, De Angelo C et al (2009) Development and testing of an optimized method for DNA-based identification of jaguar (*Panthera onca*) and puma (*Puma concolor*) faecal samples for use in ecological and genetic studies. Genetica. doi:10.1007/s10709-008-9347-6 (in press)

Hedmark E, Flagstad Ø, Segerström P et al (2004) DNA-based individual and sex identification from wolverine (*Gulo gulo*) faeces and urine. Conserv Genet 5:405–410. doi:10.1023/B:COGE.0000031224.88778.f5

Livia L, Francesca V, Antonella P et al (2007) A PCR-RFLP method on faecal samples to distinguish *Martes martes, Martes foina, Mustela putorius* and *Vulpes vulpes*. Conserv Genet 8:757–759. doi:10.1007/s10592-006-9203-0

Medellín RA, Chetkiewicz C, Rabinowitz A, Redford KH, Robinson JG, Sanderson E, Taber A (eds) (2002) El jaguar en el nuevo milenio: una evaluación de su condición actual, historia natural y prioridades para su conservación. Prensa de la Universidad Nacional Autônoma de México/Wildlife Conservation Society, Mexico

Sanderson EW, Redford KH, Chetkiewicz CB, Medellin RA et al (2002) Planning to save a species: the Jaguar as a model. Conserv Biol 16:58–72. doi:10.1046/j.1523-1739.2002.00352.x

Smith TB, Wayne RK (1996) Molecular Genetic approaches in conservation. Oxford University Press, New York, p 483

Zeller K (2007) "Jaguars in the New Millennium Data Set Update: The State of the Jaguar in 2006". Report to the Wildlife Conservation Society's Jaguar Conservation Program. Wildlife Conservation Society. Takoma Park, MD, USA