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TECHNICAL NOTE

Tiger (*Panthera tigris*) scent DNA: a valuable conservation tool for individual identification and population monitoring

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Abstract Genetic monitoring of tiger source populations is a conservation priority, yet due to low sample sizes and poor DNA quality, scat DNA has failed to produce the powerful studies needed to inform management decisions in humid, tropical landscapes. Here, we report the first successful extraction of DNA from tiger scent marks, a hitherto neglected genetic resource. We show that tiger scent DNA quality is equal or superior to scat DNA, and as scent marks are encountered 2–8 times more frequently in the wild than scats, they constitute an important genetic resource for monitoring populations and individuals.

Keywords *Panthera tigris* · Non-invasive sampling · Scent sprays · Individual identification

Fewer than 3500 tigers (*Panthera tigris*) remain in the wild, occupying <7 % of their historical range despite intense conservation efforts. Seventy percent of these tigers occur within 42 source populations covering <0.5 % of their historic range (Walston et al. 2010). Evaluating the success of conservation interventions requires rigorous monitoring of tiger densities and connectivity among populations. DNA analysis from scats has traditionally been used for genetic monitoring of wild tigers, but scat detection rates are generally low, particularly in humid, tropical environments, which limits their utility as a genetic resource (Smith 2012; A. Johnson, pers. comm.). Scent marks by contrast, constitute an untapped genetic resource, as they are more frequently deposited and have much higher detection rates than scats (Smith et al. 1989; Yudakov and Nikolaev 2012). Surveying over 664.4 km in the Russian Far East revealed scent spray to scat deposition ratios of 319:38 for males, and 109:46 for females (Yudakov and Nikolaev 2012). In Chitwan National Park, a subtropical forest in Nepal, the detection ratio was 612:28 (Smith et al. 1989) and in Tambling Wildlife Nature Conservation, a dense lowland tropical forest reserve in southern Sumatra, the detection ratio was 53:15 over 9 months of patrol activity (unpublished data). Tigers spray on trees and overhanging leaves along territory boundaries as a means of olfactory communication (Fig. 1). To boost the effectiveness of genetic monitoring of tigers, we examined the potential for DNA amplification from tiger scent marks for individual identification and gender determination.

Scent DNA samples were collected from three captive tigers (2 males, 1 female) in southern Ontario in November 2013 and June 2014. Two to four samples were collected per individual and estimated time between marking and sampling varied from 10 min to 39 h. Scent marks were swabbed using sterile cotton buds, the swabs placed in a vial containing 500 µL Buffer ASL (Qiagen) and stored at room temperature. The cotton tip was excised and placed in a sterile 2.0 mL tube with the initial Buffer ASL aliquot

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Fig. 1 Scent marks are typically 10–30 cm in diameter, 42 cm–1.7 m above ground, and sprayed on trees (*left*, Sanjay Gubbi/NCF/Panthera) or underneath overhanging leaves such as wild ginger (*right*, Rob Pickles/Panthera). Fresh sprays are commonly detected in the wild due to their unique aroma

Table 1 Percentage of successful independent PCR, and allelic dropout and false allele rates using DNA from captive tiger scent sprays

Locus	% Positive PCR	Allelic dropout	False allele
FCA100	83	0.136	0.000
FCA124	83	0.209	0.000
FCA126	83	0.000	0.056
FCA212	83	0.333	0.000
FCA229	83	0.000	0.064
Mean	83	0.136	0.024

Allelic dropout and false allele rates are calculated as mean values over total number of successful PCRs

and 15 μL Proteinase K (Qiagen). Samples were incubated at 65 °C for 2 h, after which an additional 15 μL of Proteinase K was added prior to incubation at 37 °C for 12 h. 500 μL Buffer AL (Qiagen) was added and the sample incubated at 65 °C for 10 min, after which 500 μL of cold 100 % ethanol was added and the sample incubated at 4 °C for 1 h. The remainder of the extraction process followed the suggested QIAGEN DNeasy Blood & Tissue Kit protocol, except DNA was eluted using 50 μL Buffer AE heated to 70 °C and left to incubate on the spin column membrane for 30 min prior to centrifugation. DNA was stored at −20 °C until analyzed.

Species identification was tested by amplifying a 110 bp fragment of the *cytochrome oxidase b* mitochondrial gene using primers H15149 (Kocher et al. 1989) and Farrel-R (Farrell et al. 2000). Sequencing followed Caragiulo et al. (2014). Five microsatellite loci in two multiplex groups (Menotti-Raymond et al. 1999) were used for individual identification (Table S1). Gender was determined using fluorescently labeled primers for the amelogenin region of the sex chromosomes (Pilgrim et al. 2005). Each gender typing reaction consisted of 3.50 μL of QIAGEN MasterMix, 0.70 μL of Q-solution, 0.20 μL of 10 μM amelogenin primers (Pilgrim et al. 2005), 0.20 μL of DNase-free water, and 2.0 μL of DNA template. Both microsatellite and gender-typing PCRs were done in triplicate using the multiple tubes approach (Taberlet et al. 1996). All PCRs were prepared and analyzed as per Caragiulo et al. (2015). Genotyping error rates were estimated using GIMLET version 1.3.2 (Valière 2002).

All *cytochrome oxidase b* sequences were successfully identified as tiger. All samples yielded reliable consensus genotypes, except DNA from a single swab that failed completely in all three replicates. Gender was correctly confirmed for all individuals. The PCR success rate (Table 1) and genotyping error rates (Table 2) are comparable to tiger genetic studies using scat and fall below the thresholds described by Smith and Wang (2014) for effective estimation of genetic variation and population subdivision. Comparable error rates are expected in tiger habitat with similar temperature ranges to those experienced during collection. Although error rates in the tropics may be higher, overall collection of scent DNA samples in tiger genetic studies in addition to scats, would significantly increase overall sample sizes, facilitating resolving individual genotypes and enabling more powerful genetic studies to take place. Since genetic connectivity is key to the long-term viability of the remaining 42 source populations, increased and high quality monitoring, using novel techniques such as the one presented here, are critical for the effective conservation management of wild tigers.

Table 2 Comparison of PCR success and error rates (mean across loci) for tiger sprays versus tiger scats

Study	Location	Sample Size	Sample type	Collection environment	% Positive PCR	Allelic dropout	False allele
This study	–	14	Spray	Captive	83	0.136	0.024
Unpublished data	Lao PDR	21	Scat	Mixed forest	69	0.285	0.004
Smith (2012)	Sumatra	27	Scat	Tropical rainforest	54	0.340	0.050
Mondol et al. (2009)	Northern India	50	Scat	Dry deciduous	90	0.0067	0.000
Reddy et al. (2012)	Northern India	103	Scat	Tropical dry forest	92	0.037	–
Gour et al. (2013)	Central India	75	Scat	Mixed forest	82.5	0.047	–
Sharma et al. (2013)	Central India	463	Scat	Mixed forest	–	0.011	0.006
Bhagavatula and Singh (2006)	Southern India	28	Scat	Unknown	60	0.3765	0.0235

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