## ORIGINAL ARTICLE

# Using detection dogs and genetic analyses of scat to expand knowledge and assist felid conservation in Misiones, Argentina

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## Abstract

Many carnivores require large ranges to meet their ecological and energetic needs; however, anthropogenic changes threaten species and their habitats. Camera traps have been used to effectively collect data on carnivores in a variety of habitat types; however, a single survey effort is typically limited to species that have similar body size, habitat use and movement patterns, and individual identification of animals is not always possible. We evaluated whether scat detection dogs could effectively survey for 4 wide-ranging felids that vary in these characteristics: jaguars (*Panthera onca*), pumas (*Puma concolor*), ocelots (*Leopardus pardalis*) and oncillas (*Leopardus tigrinus*). From June to October 2009 and May to August 2011, a detection dog-handler team detected 588 scats, from which 176 unique genotypes were detected. We assigned sex to 84.7% of the genotyped scats and identified 55 individuals multiple times. The effectiveness of these noninvasive techniques (detection dogs and genetic analyses of scat) not only opens the door for additional studies in areas that were previously difficult or impossible with standard survey techniques, but also provides conservationists with a set of tools that overcome some of the limitations associated with the use of camera traps alone.

**Key words:** conservation, detection dogs, felids, genetics, noninvasive techniques

## INTRODUCTION

Many carnivores require large ranges to meet ecological and energetic needs; however, anthropogenic changes put many species and their habitats under threat. Species that require expansive ranges are threatened as they navigate a heterogeneous landscape composed of protected areas, altered habitat, roads and human-occupied

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areas. Ensuring the long-term survival of wide-ranging carnivores in a fragmented ecosystem requires understanding the effects of forest fragmentation on their patterns of habitat use; however, many species are elusive, making studying and monitoring them extremely challenging.

One technique that has proven effective at surveying carnivores in a variety of habitats is camera traps (e.g. Silveira 2003; Gompper *et al.* 2006). While camera traps during a single survey effort are positioned in a particular habitat to target animals with a specific body size, they are able to capture other species with different body size but that overlap in habitat use (Kay & Slauson 2008). However, the identification of animals beyond the species-level is not always possible if individuals have no distinguishable characteristics and the sexes are monomorphic. In addition, the technique's dependence on attracting a species to a specific location, either through placement along an animal trail or in association with bait in an open area, may mean that it will fail to capture species that actively avoid these habitat types or avoid movements that overlap with potential predators or competitors. In addition, the use of this technique in areas with human activity may be difficult or impossible, due to an increased risk of equipment theft or destruction, resulting in gaps in data. We will show that 2 noninvasive techniques, genetic analyses of scat and detection dogs, can overcome these limitations, help to expand the data collected and fill in existing data gaps.

First, genetic analyses of scat switches the focus from attracting a species to a specific location to "capture" their presence, to locating evidence associated with the species' natural behavior and movement patterns, specifically scat. Scat is inevitably deposited during an individual's movements and has been used to define basic ecological parameters for many species; however, inaccurate visual species identification can result in incorrect conclusions (Reed *et al.* 2004). Genetic technique advances remove this error and allow this noninvasive information source to gain analysis power. DNA can be extracted from the scat and genetic analyses used to generate data that can confirm donor species, identify samples to the individual level, determine the sex of each individual, evaluate a species' distribution, evaluate the levels and distribution of genetic diversity, and estimate population sizes (e.g. Kohn *et al.* 1999; Palomares *et al.* 2002; Creel *et al.* 2003; Hedmark *et al.* 2004; Schwartz *et al.* 2004; Rodgers & Janečka 2013).

Second, detection dogs eliminate dependence on target species' visitation rate to a specific area and variability in locating scat. Detection dogs have proven to be effective at surveying wildlife species in a diverse array of habitat types (Smith *et al.* 2003; Wasser *et al.* 2004; Cablk & Heaton 2006; Long *et al.* 2007a,b; Vynne *et al.* 2011a). The olfactory search image of domestic dogs provides several advantages over the visual search image used by humans (Smith *et al.* 2003; Wasser *et al.* 2004; Cablk & Heaton 2006; Harrison 2006; Long *et al.* 2007a,b; DeMatteo *et al.* 2009; Vynne *et al.* 2011), including: (i) the visual appearance of samples does not affect the dog's ability to determine species identity; (ii) dogs can pinpoint the location of a target sample regardless of whether it is exposed or masked by the environment; (iii) dogs can locate multiple target species within a search area while ignoring non-target species; (iv) dogs can cover a larger geographic area faster, more efficiently and more completely than humans working alone; and (v) dogs are superior to humans in their ability to locate samples in areas with varying topography and dense vegetation. In addition, dogs' search image is not limited by the body size of the target species, the species' use or avoidance of trails or open areas, or the presence of people. These advantages provide researchers the ability to efficiently collect species-specific, large unbiased sample sizes across varying habitat so that meaningful habitat and population demographic analyses can be completed.

The province of Misiones, Argentina exemplifies an area facing ongoing anthropogenic effects where information on how wide-ranging carnivores use or avoid human modified habitats is largely unknown. Although camera traps have been successfully used in many surveys in the region (Kelly *et al.* 2008; Paviolo *et al.* 2008; Di Bitetti *et al.* 2010), data from outside of protected areas remains largely absent. Because detection dogs have been shown to be effective in the rough terrain and vegetation in Misiones (DeMatteo *et al.* 2009), we believe that when this technique is combined with genetic analyses of scat we will gain a tool that can be used to collect detailed population data for multiple species independent of body size and habitat use. Using detection dogs, we located samples from 4 felids (jaguar [*Panthera onca* Linnaeus, 1758], puma [*Puma concolor* Linnaeus, 1771], ocelot [*Leopardus pardalis* Linnaeus, 1758] and oncilla [*Leopardus tigrinus* Schreber, 1775]) that vary in their body mass, home range size and relative use of native *versus* altered habitat in Misiones. Using DNA extracted from the scats we conducted genetic analyses to confirm species identity, to distinguish individuals and to determine the sex of individuals. In addi-

tion, we conducted a preliminary analysis of how felid richness varied with the size and degree of isolation of the protected area.

## MATERIALS AND METHODS

### Study area

The Misiones Province, which is bordered by Brazil and Paraguay (Fig. 1), contains the largest remaining tract of the Upper Paraná Atlantic Forest ecoregion. Protected areas are located in a matrix that varies in degree of fragmentation or modification, with forest patch-es varying in size and connectivity (Fig. 1). While many tracts of native forest exist near and between protected areas, others are adjacent to small-scale agriculture, large monoculture stands of *Pinus* sp., *Eucalyptus*, native *Araucaria angustifolia*, areas of subsistence agriculture, pastures, bare ground or urban areas (Fig. 1). Protected areas, especially those along the eastern boundary of Misiones, are at risk of becoming isolated islands.

The region is characterized by a humid, subtropical climate with no distinct dry season (Crespo 1982). Average monthly rainfall typically exceeds 100 mm; however, in October and November rainfall is >200 mm.
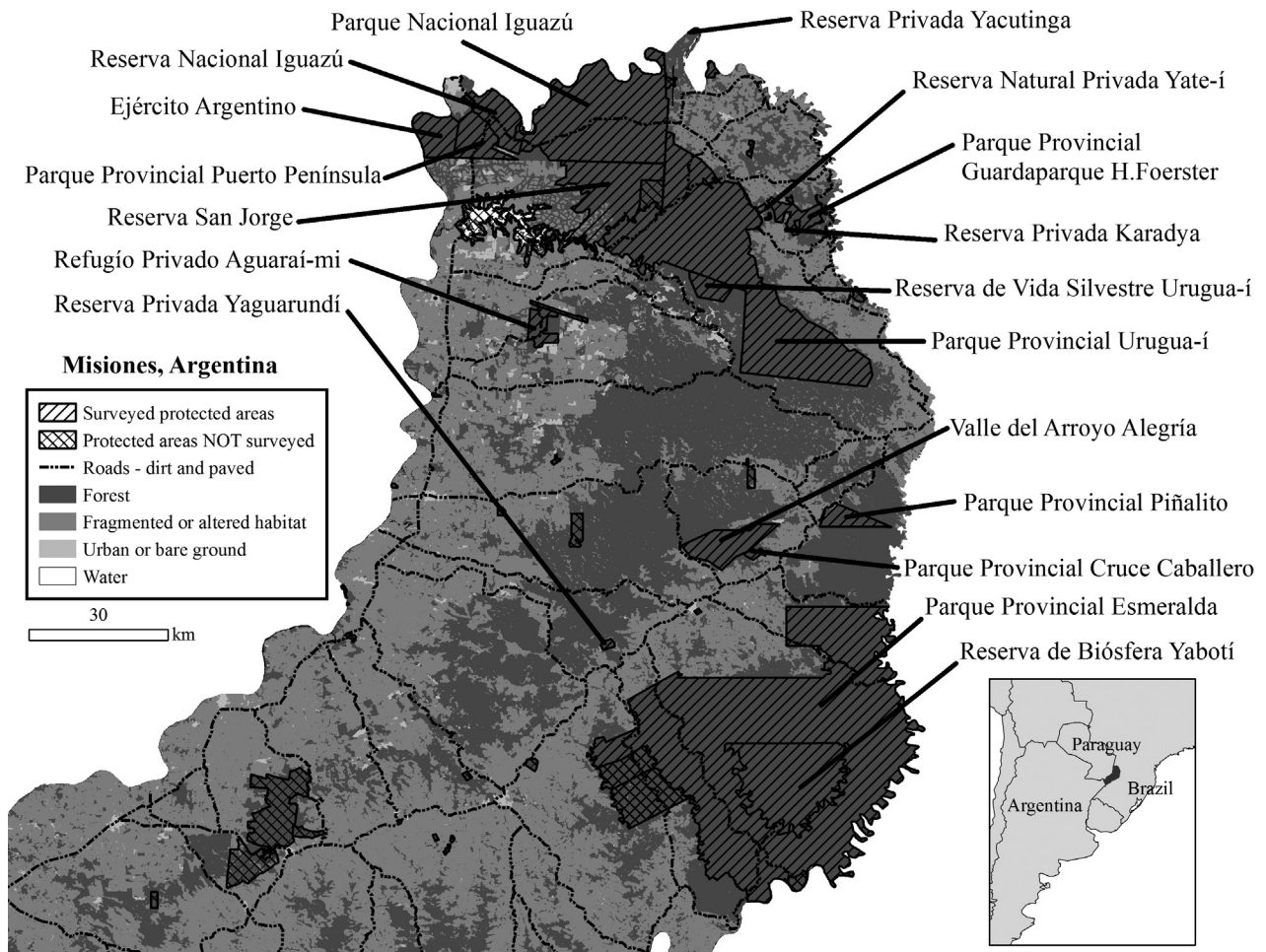


**Figure 1** Location of Misiones, Argentina, in South America (inset). Map of Misiones province showing 16 protected areas included in 2009/2011 surveys and the protected areas not included in the survey. These areas are shown in relation to the land-use pattern existing in Misiones in 2009: Forest, fragmented or altered areas, and urban or bare ground (Izquierdo *et al.* [2008] land cover map updated by A. Izquierdo for 2009 [pers. comm.]).

The hot season (late September to mid-April) is characterized by warm days (28–33 °C) and moderate nights (14–20 °C). In contrast, the cool season has moderate days (23–27 °C) and cool nights (9–12 °C).

Scats of jaguar, puma, ocelot and oncilla were collected from June to October 2009 (late fall to early spring) and May to August 2011 when lower daily temperatures were optimal for the detection dog. We surveyed a total of 16 protected areas (range: 15–236 313 ha; total area 470 815 ha; Fig. 1) and selected areas outside of protected areas including those adjacent to protected areas, private properties not connected to protected areas, monoculture plantations of pine, and dirt/paved roads, the majority of which are immersed in areas with subsistence farming, small villages, livestock and free-ranging domestic animals.

Survey routes consisted of 2-lane paved roads, 1 to 2-lane dirt roads, established trails through forested areas and existing machete cut trails through forest. A total of 149 routes (total = 861.55 km; range = 0.39–21.06 km; mean ± SD = 5.78 ± 3.50 km) were walked. Of this total, 79.7% (686.81 km) was within protected areas and 20.3% (174.74 km) was outside of protected areas. All survey tracks were walked a single time, with the exception of 1 dirt road that passed through an area with moderate human activity and feral domestic dogs. In this area, sections were walked 2 or 3 times in order to locate fresh target species scats that were not contaminated with domestic dog urine. Daily distance covered and time to cover this distance were dependent on sample processing time and how extreme the working conditions were for the dog (i.e. temperature, precipitation and presence/absence of shade). Based on previous studies, it is estimated that the dog walked an average distance of 4 to 6 times that walked by the human handler (Nussear *et al.* 2008; DeMatteo *et al.* 2009). During surveys, the dog worked off lead (except along major paved roads) and covered a distance of at least 15 m off the center of any track; however, the actual search areas may be much larger depending on wind, topography, sun, moisture, age of samples and vegetation.

### Detection dog and handler training

Although dog selection and training are key components of the success of a detection dog survey, training of the dog handler is at least as important because without proper training the handler is not able to interpret nonverbal cues from their dog and maximize the ability of their dog to find samples in varying field conditions. In addition, improper training of the handler can actual-

ly result in a negative effect on the dog's accuracy and field success. K. DeMatteo was the dog handler during both field surveys. In 2007, she became certified in the handling and training of conservation detection dogs through training provided by the PackLeader Dog Training facility (Gig Harbor, Washington, USA) and did her first field survey in Misiones (DeMatteo *et al.* 2009). Along with B. Davenport, K. DeMatteo worked to train the dog used in these surveys. K. DeMatteo is the person responsible for all ongoing dog maintenance when the dog is not in the field.

An adult male rescued Chesapeake Bay Retriever was used in both surveys (Fig. 2). Adopted at approximately 2 years of age, he was selected due to his strong ball drive and high energy level, 2 key factors that make a successful conservation detection dog (Smith *et al.* 2003). Prior to the start of the first survey in 2009, 3 weeks were spent in Misiones acclimatizing the dog to the area and working on basic training. The dog was trained using scat samples from both captive and wild animals, for which species identification had been genetically confirmed. DeMatteo *et al.* (2009) demonstrated that using scats from captive animals in training did not impede the dog's ability to recognize target species in the field. The dog was trained to indicate on 4 target species (jaguar, puma, ocelot and bush dog) and to ignore several nontarget species (maned wolf [*Chrysocyon brachyurus* Illiger, 1815], margay [*Leopardus wiedii* Schinz, 1821], jaguarundi [*Herpailurus yagouaroundi*



**Figure 2** Photo of the detection dog used in both field surveys. The use of an olfactory *versus* visual search image allows for the dog to effectively search large areas and locate samples independent of condition or visibility.

É. Geoffroy Saint-Hilaire, 1803], coyote [*Canis latrans* Say, 1823], bobcat [*Lynx rufus* Schreber, 1777], red fox [*Vulpes vulpes* Linnaeus, 1758] and various venomous snakes, including *Porthidium* sp. and *Bothriechis* sp.). While some nontarget species were not present in the area, they helped fine-tune the dog's search image. We did not formally include domestic cats (*Felis catus* Linnaeus, 1758) or domestic dogs (*Canis lupus familiaris* Linnaeus, 1758) as nontarget species because the dog had already demonstrated through natural exposure to these species that they were not confused with the felid or canid target species.

Incorporating scats from nontarget species in the detection dog training process is extremely important (B. Davenport, pers. observ.). Oncilla, which was inadvertently not included as a nontarget species during training in 2009, was indicated on by the dog during the first week of sample collection. Because the handler was not able to visually distinguish oncilla scat from ocelot scat, the detection dog quickly catalogued oncilla as a target species. While the oncilla was not originally a target species, gaining insight into its distribution and habitat use is important because it is one of the species that local land-owners blame for domestic poultry predations. In addition, the oncilla illustrates that even very small carnivores may be affected by habitat fragmentation when species home ranges are much larger than predicted by their size (Oliveira *et al.* 2010).

## Sample collection

In 2009, all scats that the dog indicated on were collected, independent of condition, which ranged from fresh with moist mucus layer to hard and dry, moldy, or brittle and crumbly. In 2011, scats where mold was evident were not collected, due to the lower extraction success with these samples in 2009. Each scat location was georeferenced using a GPS unit (Garmin eTrex®; Garmin, Schaffhausen, Switzerland) and condition/approximate age (Prugh *et al.* 2005; Smith *et al.* 2006; Michalski *et al.* 2011; Vynne *et al.* 2011b), composition/contents, and location relative to trail or road were recorded. Environmental factors such as rain, sun and insects can cause an inaccurate assessment of scat condition. Despite these factors, a best guess was made when classifying each scat as fresh (≤24 h), moderately old (between 24 h and 3 days) or old (>3 days).

The surface of the scat was swabbed with a cotton-tipped applicator soaked in phosphate buffered saline solution in order to collect cells sloughed from the digestive tract of the animal (Ball *et al.* 2007; Rutledge

*et al.* 2009). Each scat was swabbed in triplicate (2 for DNA extraction in the USA and 1 for storage in Argentina) and each swab was stored in a 1.7-mL polypropylene tube, labeled, and secured with parafilm. To maximize the quality of the DNA within each swab, each one was taken from different areas on the scat's exterior. Each scat was collected and stored in a labeled 18-oz Whirlpak® bag (Nasco, Fort Atkinson, WI) as a backup for DNA extraction and for future diet analyses. At the end of each field day, samples were placed in a −20 °C freezer.

## Genetic analyses

DNA was extracted from 2 independent swabs using a Qiagen (Venlo, Netherlands) DNeasy™ DNA extraction kit following a modified protocol by Vynne (2010). Extractions were carried out in a room separated from the one in which polymerase chain reaction (PCR) amplifications were performed to prevent cross-contamination of samples and PCRs. Negative controls (no scat material added to the extraction) accompanied each set of extractions and were used in species identification PCRs to test for contamination.

To identify species, a 110-bp (171-bp with primers) carnivore-specific region of mitochondrial cytochrome *b* gene (5′-AAACTGCAGCCCCTCAGAATGATAT-TTGTCCTCA-3′; 5′-TATTCTTTATCTGCCTATACA-TRCACG-3′ [Farrell *et al.* 2000]) was amplified with a modified version of the protocols and reagents of Farrell *et al.* (2000) and Miotto *et al.* (2007). Amplifications were performed on a MyCycler Thermal Cycler System (BioRad, Hercules, CA) in 25-μL volumes containing 2-μL DNA extract, 1× PCR Gold buffer [Applied Biosystems, Foster City, CA] 0.3-μM forward and reverse primer, 200 μM each dNTP, 5-mM $MgCl_2$, 150-μg/mL BSA (Ambion® - Life Technologies, Grand Island, NY) and 1-U Ampli*Taq* Gold DNA polymerase (Applied Biosystems). To minimize the potential for contamination in all reactions (including microsatellites and sexing), PCR set up was performed in a UV PCR Chamber (Plas Labs, Inc., Lansing, MI.) A negative control (no DNA added) was included in each PCR run to test for contamination. The PCR profile consisted of 10-min denaturation at 95 °C, followed by 40 cycles at 95 °C for 30 s, 49 °C for 45 s, 72 °C for 45 s, and a final 30 min extension at 72 °C. Purified PCR products were sequenced using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kits (ABI) and analyzed in an ABI 3100 Genetic Analyzer (ABI). Sequences were edited (Table 1) and aligned using Lasergene Se-

qman 8.1 (DNASTAR, Madison, WI) and compared with reference entries in GenBank using the Basic Local Alignment Search Tool (BLAST; Altschul *et al.* 1990) to identify sequences from Neotropical species that had high similarity and closely-matched sample sequences.

Scat samples identified as a jaguar, a puma, an ocelot or an oncilla were subsequently genotyped to distinguish individuals. We used 12 dinucleotide microsatellite loci that were originally developed for domestic cats (Menotti-Raymond & O'Brien 1995; Menotti-Raymond *et al.* 1999) and have successfully been used to screen blood, tissue, hair and scat samples from each of the target species (Carmichael *et al.* 2000; Ernest *et al.* 2000; Eizirik *et al.* 2001, Weaver *et al.* 2005; Grisolia *et al.* 2007; Miotto *et al.* 2007; Eizirik 2008; Janečka *et al.* 2008). An annealing temperature of 53°C was used with all reactions. Loci were amplified in 3 multiplex reactions: Group 1, FCA8-6FAM, FCA26-VIC, FCA43-NED, FCA78-PET; Group 2, FCA23-6FAM, FCA35-VIC, FCA45-NED, FCA96-PET; Group 3, FCA132-6FAM, FCA77-VIC, FCA90-NED, FCA126-PET. All amplifications were performed in 10 µL final volumes using a Qiagen Multiplex PCR Kit, following the manufacturer's instructions. A positive control or reference sample was included in all reactions to standardize allele calling. Fragment sizes were determined in an ABI 3100 Genetic Analyzer and scored against a GS600LIZ molecular size standard (Applied Biosystems) using GeneMapper 4.01 (Applied Biosystems).

For each locus, a genotype was confirmed in 4 identical homozygous profiles or 2 heterozygous profiles (Hedmark *et al.* 2004). We estimated the probability that 2 siblings or related individuals (parent-offspring) would have the same genotype (probability of identity for siblings, $P_{ID (sib)}$ [Waits *et al.* 2001]) in GenAlEx 6.4 (Peakall & Smouse 2006). Individual identities were assigned using GenAlEx 6.4 and confirmed by eye based on unique genotypes at a minimum of 10 microsatellite loci in jaguars ($P_{ID (sib)} = 0.0009$), pumas ($P_{ID (sib)} = 0.000023$) and ocelots ($P_{ID (sib)} = 0.000048$) and a minimum of 9 microsatellites in oncillas ($P_{ID (sib)} = 0.00026$), which is less than the threshold of <0.01 (Waits *et al.* 2001). For all species, tests for deviations from Hardy–Weinberg equilibrium at each locus, linkage disequilibrium and genic differentiation of alleles were performed with an alpha of 0.05 (with Bonferroni correction [Rice 1989]) using GenePop 4.0.10 (Raymond & Rousset 1995). Tests for presence of null alleles were performed using Micro-Checker 2.2.3 (Van Oosterhout *et al.* 2004) with the Brookfield 1 equation. Allelic richness was cal-

**Table 1** The 4 species-specific mitochondrial (mtDNA) sequences (110 bp) generated from the scats in this study. Sequences were compared with entries in GenBank to confirm species identity

| | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jaguar | T | G | T | A | G | G | A | C | A | T | A | T | C | C | T | A | T | A | A | A | A | G | A | A | G | C | T | A | G | C |
| Puma | – | Y | – | Y | R | R | – | – | – | – | – | R | – | – | – | – | – | T | – | – | – | – | R | A | R | G | – | T | G | – |
| Ocelot | – | T | – | T | G | A | T | A | – | G | G | – | – | – | – | – | – | C | – | – | – | – | G | G | T | G | – | T | G | – |
| Oncilla | – | T | – | T | G | G | G | T | – | G | – | – | – | – | – | – | – | T | – | – | – | – | G | A | G | G | – | – | G | – |

| | 31 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 60 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jaguar | T | A | G | C | T | A | G | T | G | A | A | C | A | T | A | A | C | G | A | T | G | R | C | G | A | T | T | A | A | T |
| Puma | – | – | R | – | G | Y | Y | – | A | – | – | – | – | – | – | R | G | T | T | – | R | – | – | – | – | – | – | – | G | – |
| Ocelot | – | – | G | – | C | C | T | – | R | – | – | – | C | – | T | T | C | G | T | – | G | – | – | – | – | G | G | G | G | – |
| Oncilla | – | – | A | – | T | T | A | – | T | – | – | – | T | – | A | A | T | A | T | – | A | – | – | – | – | A | G | G | G | – |

| | 61 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jaguar | A | T | T | C | A | G | G | T | T | G | A | G | A | A | G | T | G | G | T | A | G | G | A | C | T | T | G | G | C | C |
| Puma | – | Y | – | – | – | – | – | – | Y | – | – | A | – | G | A | – | – | – | – | G | A | – | G | – | – | – | – | – | – | – |
| Ocelot | – | T | – | – | – | T | – | – | T | – | – | G | C | G | G | G | – | – | – | G | G | – | G | – | – | – | – | – | – | – |
| Oncilla | – | T | – | – | – | T | – | – | T | – | – | G | C | G | G | G | – | – | – | G | G | – | G | – | – | – | – | – | – | – |

| | 91 |  |  |  |  |  |  |  |  |  |  | 110 |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jaguar | G | T | A | G | T | A | A | G | A | T | A | A | T | C | C | T | C | G | C | A |
| Puma | R | A | – | – | – | R | – | – | – | – | – | – | – | – | – | C | T | – | – | – |
| Ocelot | A | R | – | R | – | A | – | – | – | – | – | – | – | – | – | T | – | – | – | – |
| Oncilla | G | G | – | G | – | G | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

A '–' indicates positions where the identified base was the same for the four species.

culated with and without rarefaction correction (HP-RA-RE v. J-6-2006; Kalinowski 2005).

Scat samples genotyped to the individual level were subsequently sexed using a modified version of the protocol described in Pilgrim *et al.* (2005), which was successfully tested on a variety of felids. We used both the Amelogenin and the Zinc-finger loci to confirm the sex (Pilgrim *et al.* 2005). Amplifications for both loci were performed in 10 μL volumes containing 2 μL of DNA extract and followed the Pilgrim *et al.* (2005) protocol with the exception that fragment sizes were determined in an ABI 3100 Genetic Analyzer (Applied Biosystems) and scored against a GS600LIZ molecular size standard using GeneMapper. Known male and female samples were included in all reactions for comparison. A binomial test was used to test for a skew in the 50:50 proportions of males *versus* females.

### Assessment of genotyping errors

If a genotype that was determined to be heterozygous had 1 or more replicates in which only 1 of the 2 expected alleles was represented (homozygous), these replicates were interpreted to have allelic dropout. The rate of allelic dropout (number of heterozygous replicates with allelic dropout/total number of heterozygous replicates [with and without allelic dropout]) was calculated for each locus and each individual sample. If a homozygous profile had an allele in a single replicate that could not be reproduced in 4 additional replicates, we interpreted this as a false allele. The percentage of amplifications with false alleles was calculated for each species by dividing the number of false alleles by the total number of replicates.

### Use of trails *versus* roads

Using ArcGIS® 10.1 (ESRI®, Redlands, CA), all scats identified to the species level (jaguar = 57; puma = 42; ocelot = 104; oncilla = 244) were classified as either located on or near a road (dirt or paved) or human trail. To examine the fraction of all samples located relative to roads *versus* trails for all scats across species (male and female combined), the total distance surveyed on roads *versus* trails (2.5:1 roads:trails) was used to establish the expected proportion. For each species, a binomial test was used to test for a skew in the proportion of samples located on roads *versus* trails. In addition, independence likelihood ratio tests (Sokal & Rohlf 1995) were used to determine, for each species, if there was an association between sex and whether samples were located on roads and trails.

### Variation in felid richness

Protected areas vary in their size (Table 2), degree of isolation (Fig. 1) and degree of protection. The latter is determined by many factors, including presence of park guards, frequency of patrols, prevalence of poaching, and proportion of boundary that is adjacent to other protected areas *versus* human modified areas. As a general guideline, 5 categories of protection have been defined, with Parque Nacional Iguazú (P.N. Iguazú) considered the highest value, followed by other national parks and national reserves, provincial and state parks, private reserves and multiple use reserves (De Angelo *et al.* 2011). Using ArcGIS® 10.1 the variation in felid richness was evaluated with species detection or nondetection, total number of species present, species' relative abundance and qualities of the specific protected area.

## RESULTS

### Samples

The detection dog located a total of 588 scats in the 2 survey periods. Samples varied in their location relative to the center of the road and visibility to handler with no pattern was found among species. Of these, 447 (76.0%) were confirmed as 1 of the 4 felids (57 jaguar, 42 puma, 104 ocelot and 244 oncilla; Table 2) or canid (34 bush dog) targeted by the detection dog. The latter is not reported on in detail here. Twenty-two (3.7%) scats were identified as mixed species samples that contained various combinations of the 5 target species or a target species with the crab-eating fox (*Cerdocyon thous* Linnaeus, 1766) and likely represent a combination of DNA from the original scat and urine from another species. This mixed species identity was not identified through double peaks in the sequencing chromatograms but instead from the sequences of the 2 independent swabs taken from the exterior of the same scat. The majority (76.9%) of these mixed species scats were classified as older (>3 days). The remaining 85 scats either failed to amplify (*n* = 45 or 7.7%) or amplified only the DNA from prey items contained in the scat (*n* = 40 or 6.8%). A visual assessment of the latter scats confirmed the presence of hair from one of several genetically confirmed prey (South American field mouse [*Akodon montensis* Thomas, 1913], black-footed pygmy rice rat [*Oligoryzomys nigripes* Olfers, 1818], collared peccary [*Pecari tajacu* Linnaeus, 1758]), indicating that these were not detection dog failures.

Table 2 Summary of 447 felid scats (species) and 288 genotyped scats (individual) found in protected (number of hectares in parentheses) and unprotected areas in Misiones, Argentina. The number of scats identified to species-level and individual-level (no. scats species:individual), as well as the number of unique individuals (no. unique individuals) is reported. Sexes for the 176 unique individuals (male [M]:female [F]:unknown [U]) are shown. Sex was successfully assigned to 84.1% of the genotyped scat in the 4 felid species

| | Area (ha) | Jaguar | | Puma | | Ocelot | | Oncilla | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. scats species: individual | No. unique individuals (M:F:U) | No. scats species: individual | No. unique individuals (M:F:U) | No. scats species: individual | No. unique individuals (M:F:U) | No. scats species: individual | No. unique individuals (M:F:U) |
| Total | | 57:34 | 7:6:0 | 42:33 | 16:3:2 | 104:78 | 26:16:12 | 244:143 | 46:29:13 |
| Location | | | | | | | | | |
| Parque Nacional (P.N.) Iguazú | 54 380 | 17:11 | 2:3:0 | 13:13 | 5:0:1 | 35:25 | 8:6:6 | 4:1 | 1:0:0 |
| Parque Provincial (P.P.) Urugua-í | 84 000 | 9:1 | 1:0:0 | 9:4 | 3:1:0 | 17:14 | 6:3:1 | 37:17 | 5:1:2 |
| Reserva San Jorge | 21 163 | 2:2 | 1:1:0 | 3:2 | 1:0:0 | 6:4 | 2:1:1 | 22:18 | 9:7:2 |
| P.P. Puerto Península | 6900 | 9:1 | 0:1:0 | 4:4 | 3:0:0 | 3:2 | 1:0:1 | 52:29 | 7:3:1 |
| P.P. Esmeralda and Reserva de Biosfera Yabotí | 267 882 | 2:2 | 1:0:0 | 5:2 | 1:1:0 | 16:11 | 4:0:2 | 43:23 | 6:8:2 |
| Military Area – Ejército | 6951 | 2:1 | 0:1:0 | 2:2 | 0:1:0 | | | 6:5 | 3:1:0 |
| P.N. Iguazú, P.N. Urugua-í and Reserva San Jorge | 159 543 | 11:(5:4:2) | 1:0:0 | | | | | | |
| P.N. Iguazú, Reserva San Jorge, and outside protected areas | 75 543+ | 5:(2:2:1) | 1:0:0 | | | | | | |
| P.P. Cruce Caballero | 522 | | | 1:1 | 1:0:0 | 1:1 | 1:0:0 | 2:1 | 1:0:0 |
| Refugio Privado Aguará-mi | 3050 | | | 1:1 | 0:0:1 | | | 5:3 | 2:0:1 |
| Reserva de Vida Silvestre Urugua-í | 3149 | | | 2:2 | 1:0:0 | | | 1:0 | --- |
| P.P. Cruce Caballero and Valle del Arroyo Alegría | 8522 | | | 2:(1:1) | 1:0:0 | | | | |
| P.P. Guardaparque H. Foerster | 4309 | | | | | 8:6 | 2:1:0 | 11:8 | 2:2:0 |
| outside protected areas | | | | | | 5:2 | 0:2:0 | 21:15 | 5:3:1 |
| Reserva Yacutinga | 539 | | | | | 13:13 | 2:3:1 | | |
| Reserva Nacional Iguazú | 12 620 | | | | | | | 3:1 | 1:0:0 |
| Reserva Natural Privada Yate-í | 15 | | | | | | | 8:4 | 0:1:1 |
| Reserva Privada Karadya | 90 | | | | | | | 9:3 | 0:0:1 |
| Valle del Arroyo Alegría | 8000 | | | | | | | 7:5 | 1:1:0 |
| P.P. Piñalito | 3796 | | | | | | | 9:8 | 2:2:1 |
| Reserva Privada Yaguarundí | 400 | | | | | | | 4:2 | 1:0:1 |

Over half of the felid scats (*n* = 288 or 64.4%) successfully amplified at microsatellite loci, allowing us to identify 176 individuals (jaguar = 13, puma = 21, ocelot = 54 and oncilla = 88; Table 2). The amplification success was lower in 2009 when scats were collected independent of condition (47.9%) compared to 2011 when older scats were not collected (77.6%). Approximately one-third (*n* = 55) of the identified individuals (*n* = 176) were found multiple times (mean ± SD = 3.04 ± 1.8; range = 2–11; Table 3). While individual ocelots and oncillas had all samples located within a single protected, 2 jaguars and 1 puma had samples located in 2 or more adjacent protected areas. All 4 felids had at least 1 individual that was identified across both sampling periods.

### Assessment of genotyping errors

The degree of allelic dropout varied among loci (range = 3.6%–52.7%) and individual samples, with jaguars consistently having the lowest (mean ± SD = 18.8% ± 8.9%) compared to pumas (34.7% ± 8.3%), ocelots (30.8% ± 8.5%) and oncillas (30.0% ± 7.3%). In all species, the majority of the dropout could be attributed to a subset of samples that were classified as either moderately old or old, which follows findings by Vynne *et al.* (2011b) where DNA quality is associated with sample freshness. This effect is exemplified in the 2009 samples where all samples were collected independent of condition. For example, 5 (moderately old = 1 and old = 4) out of 12 puma samples accounted for 75% of the total allelic dropout across all loci. The percentage of total amplifications with false alleles for the 4 species was relatively low (range = 1.7%–3.4%), with a small range of variation seen for the number of false alleles across individual loci (range = 0–6.7%).

### Genetic analyses

No evidence of genotypic linkage disequilibrium was found. The average heterozygosity across all loci was similar for the 4 felids, with the jaguar being the lowest (0.623) compared to the puma (0.856), the ocelot (0.798) and the oncilla (0.749; Table 4). In jaguars all loci conformed to expectations under Hardy–Weinberg equilibrium; however, 2 loci in pumas (FCA8 and FCA26), 4 loci in ocelots (FCA8, FCA35, FCA96 and FCA132) and 4 loci in oncillas (FCA77, FCA78, FCA96 and FCA132) significantly deviated from Hardy–Weinberg equilibrium expectations (Table 4). Analysis in Microchecker suggested the presence of 1 or more null alleles in 5 of these loci (FCA26, FCA35, FCA77, FCA96 and FCA132; Table 4). The presence of null alleles would

make these loci less powerful in their ability to differentiate between individuals and could bias our estimate of heterozygosity and any estimates of effective population size. However, because these loci averaged a high number of amplifiable alleles (mean ± SD = 11.6 ± 2.2; range = 8–16) they were useful in differentiating individuals and any estimates of population size, while not conducted in this study, would be conservative. Although null alleles could bias our estimates of heterozygosity and any future calculations on effective population size, the latter would likely be a more conservative estimate and precautions could be taken by producing all estimates both with and without these loci (Eggert *et al.* 2003).

The number of loci that amplified was similar among jaguars (mean ± SD = 10.0 ± 0), pumas (10.5 ± 0.9), ocelots (10.4 ± 1.0) and oncillas (9.1 ± 0.9; Table 4). The allelic richness (number of alleles) for each locus varied with species with no pattern for low or high variability at any given locus. While jaguars had the lowest mean number of alleles across all loci (5.1 ± 1.1) when compared to pumas (11.1 ± 2.1), ocelots (10.5 ± 3.2) and oncillas (10.6 ± 2.7), no loci differed significantly among species (Table 4). When rarefaction was applied to account for differences in sample sizes among species, the average allelic richness across loci was similar with, jaguars being the lowest (4.71) compared to pumas (9.03), ocelots (7.08) and oncillas (6.31; Table 4). Sex was successfully assigned to 149 (84.7%) of the 176 individuals (Table 2) and the results were consistent across individuals that were identified multiple times. Using only confirmed sexes, the proportion of male *versus* female scats located (16 male:3 female) was significantly different for pumas (binomial test 1 male:1 female, *P* = 0.004). No significant differences were found for jaguars (7 male:6 female, *P* = 1.0), ocelots (26 male:16 female, *P* = 0.164) and oncillas (46 male:29 female, *P* = 0.064).

### Use of trails *versus* roads

Only the jaguar was detected more frequently on roads than on trails (binomial test, *P* = 0.039; Table 5). For jaguars, there was a tendency for females to be detected relatively more frequently on roads than males, although expected frequencies were low and this should be taken as an approximation (G test, $\chi^2 = 6.054$, *P* = 0.0139; Table 5). For pumas and oncillas there was no association between sex and the relative frequency of scats detected on roads *versus* trails (puma: $\chi^2 = 2.066$, *P* = 0.1507; oncilla: $\chi^2 = 0.343$, *P* = 0.5580; Table 5). In

**Table 3** Summary of the 55 individuals identified multiple times (jaguars [*n* = 6], pumas [*n* = 8], ocelot [*n* = 14] and oncilla [*n* = 27]) with sex (male [M]:female [F]:unknown [U]), location(s), year samples located and number of scats (no. scats) found are reported For each individual, the mean distance between scat (km) was calculated using ArcGIS® 10.1. If scats were found adjacent to each other the distance is equal to zero. If only 2 scats were found the SD is zero

| Species | Sex | Location | Sample year | No. scats | Mean distance ± SD (km) |
|---|---|---|---|---|---|
| Jaguar | M | Parque Nacional (P.N.) Iguazú | 2011 | 3 | 0 |
| | F | P.N. Iguazú | 2011 | 2 | 0 |
| | M | Parque Provincial (P.P.) Esmeralda – Reserva de Biosfera Yabotí | 2011 | 2 | 0.32 |
| | F | P.N. Iguazú | 2011 | 4 | 3.17 ± 3.01 |
| | M | P.N. Iguazú – Reserva San Jorge | 2011 | 5 | 10.1 ± 4.4 |
| | M | P.N. Iguazú – P.P. Urugua-í – Reserva San Jorge | 2009 & 2011 | 11 | 18.03 ± 11.1 |
| Puma | F | Military Area – Ejército | 2011 | 2 | 0.29 |
| | M | P.N. Iguazú | 2011 | 2 | 0.64 |
| | M | Reserva de Vida Silvestre Urugua-í | 2009 | 2 | 0.94 |
| | M | P.N. Iguazú | 2009 & 2011 | 5 | 1.34 ± 0.90 |
| | M | P.P Cruce Caballero – Valle del Arroyo Alegría | 2011 | 2 | 1.81 |
| | M | Reserva San Jorge | 2011 | 2 | 2.45 |
| | M | P.P. Puerto Península | 2011 | 2 | 2.64 |
| | M | P.N. Iguazú | 2009 & 2011 | 3 | 4.9 ± 2.67 |
| Ocelot | M | P.P. Urugua-í | 2009 | 2 | 0 |
| | M | P.N. Iguazú | 2011 | 2 | 0.17 |
| | M | P.N. Iguazú | 2011 | 3 | 0.30 ± 0.21 |
| | F | P.P. Guardaparque H. Foerster | 2011 | 3 | 0.33 ± 0.24 |
| | M | P.P. Urugua-í | 2009 & 2011 | 2 | 0.83 |
| | M | P.P. Guardaparque H. Foerster | 2011 | 2 | 0.92 |
| | F | P.N. Iguazú | 2011 | 2 | 1.04 |
| | U | P.P. Esmeralda – Reserva de Biosfera Yabotí | 2009 & 2011 | 2 | 1.16 |
| | M | Reserva Yacutinga | 2011 | 6 | 1.22 ± 0.96 |
| | F | Reserva Yacutinga | 2011 | 3 | 1.25 ± 0.57 |
| | F | P.P. Urugua-í | 2011 | 3 | 1.31 ± 0.43 |
| | M | P.P. Esmeralda – Reserva de Biosfera Yabotí | 2011 | 4 | 1.56 ± 0.90 |
| | M | P.P. Esmeralda – Reserva de Biosfera Yabotí | 2011 | 2 | 1.65 |
| | M | P.N. Iguazú | 2009 | 2 | 4.74 |
| Oncilla | F | outside protected areas | 2009 | 2 | 0 |
| | M | P.P. Guardaparque H. Foerster | 2011 | 2 | 0 |
| | M | Military Area - Ejército | 2011 | 2 | 0 |
| | M | P.P. Piñalito | 2011 | 2 | 0.01 |
| | F | P.P. Guardaparque H. Foerster | 2011 | 3 | 0.03 ± 0.02 |
| | M | outside protected areas | 2011 | 2 | 0.06 |
| | F | outside protected areas | 2011 | 2 | 0.09 |
| | U | Reserva Privada Karadya | 2009 | 3 | 0.11 ± 0.04 |
| | M | P.P. Urugua-í | 2011 | 6 | 0.12 ± 0.07 |
| | U | P.P. Urugua-í | 2009 | 2 | 0.15 |
| | F | P.P. Puerto Península | 2011 | 3 | 0.19 ± 0.07 |
| | M | P.P. Puerto Península | 2011 | 9 | 0.20 ± 0.16 |
| | M | P.P. Puerto Península | 2011 | 2 | 0.22 |
| | F | P.P. Puerto Península | 2009 | 7 | 0.25 ±0.14 |
| | F | P.P. Esmeralda – Reserva de Biosfera Yabotí | 2011 | 3 | 0.26 ± 0.18 |
| | F | Reserva Natural Privada Yate-í | 2009 | 3 | 0.30 ± 0.04 |
| | M | Valle del Arroyo Alegría | 2011 | 4 | 0.40 ± 0.22 |
| | F | P.P. Guardaparque H. Foerster | 2011 | 2 | 0.42 |
| | F | P.P. Piñalito | 2011 | 2 | 0.44 |
| | F | P.P. Piñalito | 2011 | 2 | 0.56 |
| | M | P.P. Urugua-í | 2011 | 3 | 0.57 ± 0.39 |
| | M | outside protected areas | 2009 | 4 | 0.61 ± 0.24 |
| | F | P.P. Esmeralda – Reserva de Biosfera Yabotí | 2009 & 2011 | 2 | 0.69 |
| | M | P.P. Esmeralda – Reserva de Biosfera Yabotí | 2009 & 2011 | 3 | 0.82 ± 0.34 |
| | M | P.P. Puerto Península | 2011 | 2 | 1.14 |
| | M | P.P. Esmeralda – Reserva de Biosfera Yabotí | 2009 | 3 | 1.30 ± 0.58 |
| | M | P.P. Urugua-í | 2009 | 2 | 1.68 |

**Table 4** Genetic diversity levels across 8 microsatellite loci (FCA#) for each of the 4 felids found in the north and central zones of Misiones, Argentina: †Locus did not amplify. $N_S$, number of scats; $N_I$, number of individuals detected; A, number of alleles (without rarefaction), $A_R$, richness with rarefaction; $H_o$, observed heterozygosity; $H_e$, expected heterozygosity. The null allele frequency ($N_a$: Brookfield 1) is reported for loci where null alleles may be present. Significant departures from Hardy–Weinberg equilibrium after Bonferroni correction (*$P < 0.05$ and **$P < 0.01$)

| | $N_S$ | $N_I$ | | FCA8 | FCA23 | FCA26 | FCA35 | FCA43 | FCA45 | FCA77 | FCA78 | FCA90 | FCA96 | FCA126 | FCA 132 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jaguar | 34 | 13 | A | 5 | † | 6 | § | 5 | 5 | 4 | 5 | 5 | 8 | 6 | 3 |
| | | | $A_R$ | 4.761 | † | 5.714 | § | 4.538 | 4.492 | 3.539 | 4.714 | 4.715 | 6.446 | 5.485 | 2.723 |
| | | | $H_o$ | 0.538 | — | 0.615 | — | 0.615 | 0.769 | 0.538 | 0.769 | 0.769 | 0.769 | 0.769 | 0.231 |
| | | | $H_e$ | 0.723 | — | 0.785 | — | 0.655 | 0.582 | 0.517 | 0.600 | 0.634 | 0.794 | 0.720 | 0.218 |
| | | | $N_a$ | — | — | — | — | — | — | — | — | — | — | — | — |
| Puma | 33 | 21 | A | 14 | 7 | 14 | 12 | 12 | 10 | 10 | § | 11 | 11 | 12 | 9 |
| | | | $A_R$ | 11.3414 | 5.90 | 11.848 | 9.159 | 9.706 | 7.671 | 8.350 | § | 8.922 | 9.159 | 9.483 | 7.827 |
| | | | $H_o$ | 0.905* | 0.810 | 0.611** | 0.650 | 0.857 | 0.619 | 0.810 | — | 0.857 | 1.000 | 0.667 | 0.632 |
| | | | $H_e$ | 0.923 | 0.783 | 0.933 | 0.854 | 0.892 | 0.749 | 0.843 | — | 0.873 | 0.841 | 0.866 | 0.856 |
| | | | $N_a$ | — | — | 0.156 | — | — | — | — | — | — | — | — | — |
| Ocelot | 78 | 54 | A | 8 | 7 | † | 15 | 8 | 11 | 11 | 14 | 7 | 16 | 9 | 9 |
| | | | $A_R$ | 6.389 | 4.316 | † | 8.201 | 6.988 | 6.489 | 8.700 | 9.617 | 5.382 | 9.187 | 6.015 | 6.552 |
| | | | $H_o$ | 0.704** | 0.679 | — | 0.569** | 0.926 | 0.635 | 0.830 | 0.870 | 0.830 | 0.553* | 0.792 | 0.580* |
| | | | $H_e$ | 0.814 | 0.627 | — | 0.782 | 0.846 | 0.785 | 0.885 | 0.899 | 0.740 | 0.835 | 0.784 | 0.780 |
| | | | $N_a$ | — | — | — | 0.116 | — | — | — | — | — | 0.154 | — | 0.108 |
| Oncilla | 143 | 88 | A | 10 | 10 | † | † | 11 | 11 | 12 | 15 | 5 | 13 | 11 | 8 |
| | | | $A_R$ | 5.830 | 4.835 | † | † | 7.292 | 7.186 | 7.154 | 8.153 | 2.723 | 7.763 | 6.582 | 5.556 |
| | | | $H_o$ | 0.727 | 0.568 | — | — | 0.736 | 0.718 | 0.655* | 0.731* | 0.244 | 0.351** | 0.727 | 0.464** |
| | | | $H_e$ | 0.796 | 0.686 | — | — | 0.854 | 0.839 | 0.802 | 0.867 | 0.278 | 0.741 | 0.825 | 0.806 |
| | | | $N_a$ | — | — | — | — | — | — | 0.079 | — | — | 0.219 | — | 0.187 |

**Table 5** Number of scat from male, female, and unknown jaguar, puma, ocelot and oncilla located on or near roads and human trails. Only those samples from scats identified as male or female were used in comparisons within species and sexes

|  | Jaguar | | Puma | | Ocelot | | Oncilla | |
|---|---|---|---|---|---|---|---|---|
|  | Roads | Trails | Roads | Trails | Roads | Trails | Roads | Trails |
| Male | 17 | 7 | 20 | 5 | 36 | 4 | 60 | 20 |
| Female | 11 | 0 | 3 | 3 | 14 | 9 | 33 | 14 |
| Unknown | 20 | 2 | 10 | 1 | 31 | 10 | 92 | 25 |

ocelots we found a very strong association of sex and path type, with female scats being positively associated with trails while male scats were found relatively more frequently on roads ($\chi^2 = 7.348$, $P = 0.0067$; Table 5).

### Variation in felid richness

Four adjacent protected areas (P.N. Iguazú, P.P. Urugua-í, Reserva San Jorge and P.P. Puerto Península) make up the largest contiguous area in northern Misiones (Fig. 1). While each of these areas has varying levels of protection (low/moderate to high) and poaching pressures, together they form only 1 of 2 areas where all 4 felids were found. The second area is the 267 882 ha represented by the P.P. Esmeralda and Reserva de Biosfera Yabotí in central Misiones (Fig. 1). Overall, as protected areas decreased in size and increased in degree of isolation from other protected areas, the number of species located declined (Table 2). The jaguar was unique in that it was exclusively located in or around these 2 large contiguous areas. While the puma overlapped with the jaguar's areas, it also showed variability in its association with small (Refugío Privado Aguaraí-mi: 3050 ha) and medium (P.P. Cruce Caballero and Valle del Arroyo Alegría: 8522 ha) areas where the protection levels are lower and potential for human–wildlife conflict (roads and local communities) is higher. Both ocelots and oncillas occupied the full range of possibilities and extended beyond those seen in jaguars and pumas by association with protected areas that have the highest poaching threat (e.g. P.P. Guardaparque H. Foerster and P.P. Piñalito) and highest degree of isolation (e.g. Reserva Yacutinga and Reserva Privada Yaguarundí).

### DISCUSSION

This study demonstrated that the use of 2 noninvasive techniques, a detection dog and genetic analyses of scats, has the ability to collect detailed population data for multiple species independent of the species' body size, physical appearance, habitat use and movement patterns. The detection dog was able to target multiple species as it worked across habitat types, including native forest, agriculture, monoculture plantations and pastures. Genetic analyses of each scat allowed us to expand our knowledge from knowing the habitat where the sample was located and suspected species identification to a confirmed species identity, as well as the individual and sex of the majority of scat. Amplification success was improved when older scats were not collected, which follows the findings of Vynne *et al.* (2011b).

A significant and unique male biased sex ratio was found in puma, which contrasts with a recent scat study conducted by Palomares *et al.* (2012), where a male biased sex ratio in scats was found for jaguars but not for pumas. While camera trap studies have shown a male sex bias in pumas (Negrões *et al.* 2010), data obtained from scat and camera trap studies are not always comparable because with the latter it is easier to confirm a male's identity than a female's (Palomares *et al.* 2012). Independence tests, however, suggests no association between sex and the type of path (road *versus* trail) in pumas. As no evidence exists that detector dogs preferentially search for 1 sex (Nussear *et al.* 2008), this difference could be explained by either the existence of a behavioral difference between sexes, with females avoiding roads and trails altogether, or by a real population sex bias.

Although we did not find an overall male biased sex ratio in jaguars, ocelots and oncillas, we did find behavioral differences between the sexes in their tendency to use roads and trails in both jaguars and ocelots. In jaguars, females seem to have a higher tendency than males to use roads compared to trails, contrary to the general pattern found by Palomares *et al.* (2012). In ocelots, contrary to jaguars, female scats were relatively more frequently found on trails than male scats, which were

relatively more frequently found on roads. No association between sex and road type was found in the oncilla. Although the results do not generate a definitive pattern and need to be interpreted with caution due to the number of samples with unknown sex, it is clearly evident that there is an overall sex bias in the sample of scats obtained from pumas and an association between sex and type of road in jaguars and ocelots. An explanation for these patterns remains elusive. While these sex biases should be further explored (Palomares *et al.* 2012), caution should be taken when interpreting them. Occupancy models (MacKenzie *et al.* 2005) can be used to ascertain whether sex has an effect on detectability and how detectability and occupancy vary in different situations (e.g. habitat and road type) according to the sex of the individuals. While a combination of methods may be necessary to understand these puzzling patterns, it is also clear that surveys for these 4 felids need to include both path types, roads and trails.

We were able to identify areas where all 4 target species, multiple individuals, and both sexes were detected *versus* those areas where some species are rare, ecologically extinct or absent due to isolation or habitat patch size. For example, while in the largest, most contiguous tracts of protected land all 4 target felids were detected, in the smallest, most isolated protected areas jaguars were not and pumas only occasionally. This finding supports previous studies in the ecoregion that found that jaguars had a much lower tolerance to human disturbance than pumas, suggesting that landscape heterogeneity may be a larger movement barrier for jaguars than pumas (De Angelo *et al.* 2011). In addition, in areas with high poaching levels, such as P.P. Guardaparque H. Foerster and P.P. Esmeralda/Reserva de Biosfera Yabotí, oncillas were relatively more abundant compared to areas with low poaching levels, such as P.N. Iguazú, where jaguars and pumas were relatively more abundant. Ocelots fell in between these 2 extremes, with high numbers in areas with low poaching levels but moderate levels in areas with high poaching levels. These patterns are similar to those found by Di Bitetti *et al.* (2010), who observed higher numbers of oncillas and margays in less protected areas where the larger species were found relatively less frequently. They suggest that this may reflect mesopredator release.

The ability of the detection dog to effectively search large geographic areas allowed us to locate multiple samples from 55 individuals. This ability to gain repeat samples allows for analyses of species' habitat use and movement patterns to be expanded. This expanded view allows for existing connectivity among protected areas to be evaluated and for locating areas of potential problems. For example, our results suggest that the largest areas in the northern portion of Misiones (P.P. Urugua-í, P.N. Iguazú and Reserva San Jorge) maintain connectivity, as evidenced by individual jaguars using more than 1 area. However, further evaluation is needed to evaluate potential negative effects from 2 high velocity paved roads (Ruta 12 and Ruta 19) that physically divide 2 provincial parks (P.P. Puerto Península and P.P. Urugua-í) and may disrupt connectivity within and among the areas in northern Misiones. Assessing these potential negative influences on the connectivity in the region is important given the increasing pressure by private entrepreneurs and the current Misiones Government to build paved roads within protected areas. Most protected areas would be crisscrossed by multiple roads with the inevitable increase in transit. This development will have definite negative impacts on carnivores due to the high risk of road kills (Haines *et al.* 2005, 2006). In fact, road kills of wild felids, including jaguars, pumas and ocelots, are becoming increasingly common and a big concern in northern Misiones, especially in P.N. Iguazú, P.P. Puerto Peninsula and P.P. Urugua-í.

While the north-central region of Misiones appears to have retained a relatively high degree of connectivity, the majority of native forest is unprotected (52.5% or 483 171.75 ha) and is located in a mosaic of monoculture plantations, small-scale agriculture, and small communities with subsistence agriculture and pastures (Fig. 1). The threat to the remaining forest is high given that this area is undergoing changes in land use, particularly from forests to grasslands for cattle grazing. Expanding detection dog surveys to include additional areas outside of protected areas will allow us to identify potential corridors that can support the movements of all 4 felids. Previously, expanding surveys into unprotected areas in Misiones has been hindered by the presence of people in the region resulting in high risk of camera trap theft. The use of detection dogs opens the door to documenting animal movement in the area so that conservation efforts to ensure biological corridors between the 2 zones can be evaluated.

Understanding animal movement between protected areas, efficacy of existing wildlife crossings and optimal locations for biological corridors (Ray *et al.* 2002; Nikolakaki 2004) will require increasing sample sizes, genotyping samples to investigate individual movements and estimate migration rates, broadening the range of protected areas surveyed, and increasing surveying ef-

forts in those areas surrounding and between protected areas, all of which are possible with the aid of detection dogs and advances in genetic techniques. The power of these analyses can be expanded by incorporating geographic information system (GIS) technology to evaluate habitat suitability. Areas where movement is most likely to occur can be identified by combining presence data (genetic data, camera trap photos, radio telemetry and road kills) in a weighted analysis with factors that affect animal movement (e.g. land use patterns, human population density, prey distribution, environmental factors, presence/absence of manmade *versus* natural structures and connectivity of protected areas) (Cuarón 2000; Clevenger & Waltho 2005; Dixon 2006; Cascelli de Azevedo & Murray 2007). The combination of these 3 techniques (detection dogs, genetic analyses of scat and GIS technology) not only opens the door for additional studies in Misiones, Argentina but also provides conservationists with a set of tools that overcome some of the limitations associated with the use of camera traps alone (Long *et al.* 2008).

The cost associated with the use of detection dogs for wildlife surveys can be extremely variable, depending on the specific circumstances surrounding the field survey, including the duration, location and objectives of the project, as well as field logistics. The majority of costs associated with working with a detection dog are related to selecting a dog, training both the dog and the handler, maintenance of the dog and genetic processing of collected scats. When acquiring a detection dog, there are several possible options. Each has been successfully used in the field but each has its own set of advantages and special considerations: (i) hiring a professional dog/handler team; (ii) purchasing a professionally selected and trained dog; and (iii) leasing a professionally trained dog and contract-associated handler training (MacKay *et al.* 2008). Which option is the best will depend on several factors, including the long-term goals of the project, the availability and interest of personnel, the funding, and the resources to care for a dog (Harrison 2006; Long *et al.* 2007b; MacKay *et al.* 2008). The costs associated with genetic analyses of collected scats are directly related to the number of samples to be processed, which analyses are desired (e.g. species-only identification *vs* species identification, individual identification and sex identification), whether there are personnel costs associated with sample processing, and equipment availability. Including a qualitative scale of DNA quality (Vynne *et al.* 2011b) can help to maximize success in the lab and minimize processing costs. In a comparative analysis of costs, both Long *et al.* (2007b) and Harrison (2006) determined that detection dogs had a higher cost compared to camera traps or hair snares; however, this cost was offset by the dog's high efficiency to detect species presence. In addition, they note that while the initial cost is higher with detection dogs, the long-term cost may be lower because only a single site visit is required.

## ACKNOWLEDGMENTS

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