Research Article

Comparison of noninvasive genetics and camera trapping for estimating population density of ocelots (*Leopardus pardalis*) on Barro Colorado Island, Panama

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Abstract
Estimates of population density are essential for the effective conservation and management of any threatened species. Accurately estimating density of elusive carnivores can be a challenge, however. One approach to this challenge is integration of DNA collected noninvasively from feces with capture-recapture modeling. To date, the bias and precision of this technique have seldom been evaluated in the field. We compared density estimates of ocelots (*Leopardus pardalis*) derived from fecal noninvasive genetic techniques to density estimates from camera trapping in the same population, during the same study period. Density estimates from the two techniques were comparable, especially when using spatially explicit capture-recapture models. Population density estimated using the program DENSITY was 1.74/km^2 (SE = 0.584) from noninvasive genetics and 1.59/km^2 (SE = 0.464) from camera trapping. These estimates also represent the highest reported ocelot population density within the species range.

Keywords: Elusive species conservation, ocelot, spatially-explicit capture-recapture.

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Introduction

Population density is a parameter of vital importance for species conservation. For rare or elusive species, however, acquiring reliable density estimates based on visual observation or capture can be difficult or impossible [*1*]. As a result, two noninvasive approaches are now commonly used to estimate abundance or density of elusive carnivores in conjunction with capture-recapture (CR) models: camera trapping [*2, 3*] and noninvasive genetics [*4, 5*]. To obtain CR density estimates from camera trapping, individual animals are identified from photographs based on morphological differences such as unique pelage patterns [*3, 6*], allowing researchers to construct capture histories for each individual. For the many elusive species without unique individual markings, however, one realistic option for estimating population density using CR methods is noninvasive genetics, in which individuals are identified from remotely collected DNA using genetic markers [*7*].

Since its first application by Karanth in 1995 [*2*], camera trapping has been widely used, reviewed, and evaluated. To date >100 published studies have used camera trapping in conjunction with CR techniques to estimate abundance or density, including >50 since 2010 [*8*]. Additionally, the accuracy of abundance and density estimates from camera trapping has been evaluated through comparisons with populations of known size [*9*], comparisons with estimates from telemetry [*9-13*], and via simulation [*14, 15*]. As a result camera trapping is considered a reliable method for estimating abundance and density in rare or elusive species [*16, 17*].

Use of noninvasive genetics to estimate abundance has recently become more common, but few studies have compared estimates derived from noninvasive genetics with estimates from other sources to evaluate the accuracy or potential bias of the technique [but see *18-21*]. In addition, most noninvasive genetics studies have only estimated abundance and not population density [*1, 19, 20*]. Abundance by itself is a less valuable parameter for conservation, as it makes comparisons with other studies and other populations difficult [*1*]. Estimating abundance with CR models is relatively straightforward. Estimating density can be more difficult, however, because it requires accurate estimation of the effective survey area (ESA) [*22*], which is a challenge in geographically open populations and in areas with patchy habitat [*23*]. In the past, most camera trap and noninvasive genetics studies that estimated density have used derivations of the mean maximum distance moved (MMDM) between captures to estimate ESA, but the validity of this method has been debated [*10, 11, 15, 24*]. More recently, sophisticated maximum-likelihood and Bayesian spatially-explicit capture-recapture (SECR) models have been developed that estimate density directly by modeling ESA explicitly, using the geographic locations of captures [*25-27*].

To our knowledge, only one study [*28*] has directly compared estimates of population density from noninvasive genetics with estimates from camera trapping in the same population during the same time. In the previous study, however, the number of recaptures from noninvasive genetics was far below the minimum number required for the SECR analysis used [*29*], and thus was invalid. Our primary objective was to directly compare density estimates from noninvasive genetics with estimates from camera trapping, using sample sizes large enough for valid SECR analyses. As a secondary objective, we compared traditional CR models with a newer SECR model. We did this in a population of ocelots (*Leopardus pardalis*), a medium-sized felid, on Barro Colorado Island (BCI) in Panama. Ocelots are considered of least conservation concern in the core of their range, but many populations are threatened by habitat loss, and some northern populations are critically endangered [*30*]. Ocelots are a suitable organism for such a study because individuals can be identified for CR analyses based both on unique spot patterns [*6*] and unique DNA genotypes collected noninvasively from fecal samples. Our island study site also is a suitable setting

for such a study because it is relatively closed geographically, yet is large enough to maintain a stable breeding population. For our density estimates from camera trapping, we did not face the same problems with estimating ESA inherent in other studies, as we were simply able to use island area as our ESA. We think our camera-trapping results reflect a relatively unbiased estimate of population density, making them an excellent standard for comparing estimates from fecal noninvasive genetic techniques.

Methods

Study Area

Field work was conducted on BCI, a 15.43-km^2 island in the Panama Canal waterway, at a research station operated by the Smithsonian Tropical Research Institute (Fig. 1). BCI (9°10'N, 79°51'W) sits within Gatun Lake, an artificial body of water created in 1912 by the damming of the Chagres River to create the Panama Canal, and is part of the protected 54-km^2 Barro Colorado Nature Monument. Vegetation is tropical moist forest, and topography is dominated by hills that reach a maximum elevation of 165 m above sea level. Mean temperature is 27 °C, and average annual precipitation is 2,600 mm, with 90% of rainfall occurring from May through November [*31*]. Forest cover on the island is a mix of old growth forest and mature secondary forest >80 years old, all of which is suitable habitat for ocelots.

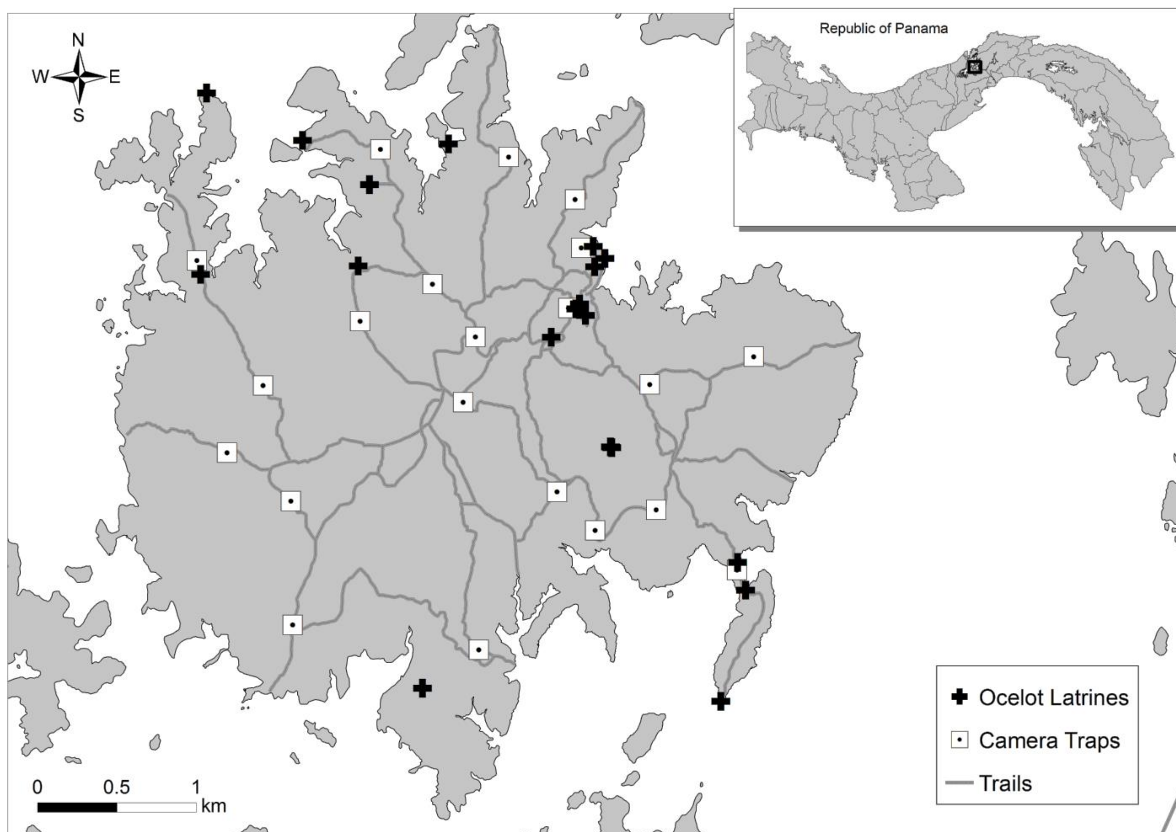

Fig. 1: Map of Barro Colorado Island in Panama, our study area for estimating population density of ocelots (*Leopardus pardalis*) from camera trapping and noninvasive genetics.

Scat surveys

Ocelots commonly defecate at latrine sites that are used by multiple individuals of both sexes [*32, 33*]. These latrines are typically located in large cavities or overhanging buttresses of large trees, underneath buttresses of fallen trees, or underneath human structures (Fig. 2).The primary objective of our surveys was to locate as many ocelot latrines as possible. All scats used in this study were found at such latrines. Seven latrines were located during a pilot study in 2011, five of which were still in use by ocelots in 2012. To find additional latrines in 2012, we walked all 39.5 km of trails on BCI (Fig. 1) a minimum of 3 times (once every 33 days). In addition, we walked >390 km of random, off-trail transects throughout the island in search of latrines. Once a latrine was located, all scats were removed, and it was revisited every 4-7 days for the remainder of the study to collect additional scats. Scat collection was conducted over 99 days from 29-January to 6-May 2012. The study length was chosen as a balance between being long enough to obtain sufficient data to estimate density, while still being short enough to satisfy assumptions of demographic closure [*15*]. Scats decompose quickly in the warm and tropical environment of Panama, so all collected scats were deposited by animals during the study period or shortly beforehand (within several days).

Fig. 2. An ocelot (*Leopardus pardalis*) defecating at a latrine under the buttress of a large tree on Barro Colorado Island, Panama.

Camera trapping

We conducted camera trapping using an array of 21 Reconyx PC900 and RC55 trail cameras (Reconyx Inc., Holmen, Wisconsin). Cameras were distributed evenly throughout the entire island so that gaps between cameras that could contain an ocelot home range were unlikely (Fig. 1), ensuring all individuals on the island had a capture probability > 0 [*2*]. The total grid size of camera traps (10.18 km^2) was several times larger than an ocelot home range (3.5 km^2 for males, 1.5 km^2 for females [*34*]) as suggested for unbiased density estimates [*15, 35*]. We placed all cameras along trails to maximize capture probability. Eighteen cameras were part of an ongoing, multi-year camera trap survey of the BCI mammal community; three additional cameras were placed to fill gaps. Each camera station consisted of one camera and could therefore photograph only one side of an animal at a time. For all individuals, however, right and left sides were observed in the same sequence of photographs at least once either before or during our study period, allowing profiles to be paired together for individual identification. Cameras were active for the same 99-day period that scat surveys were conducted. Photo sequences were considered independent if they were >30 minutes apart.

DNA extraction and species identification

We extracted DNA from scats using the Qiagen QAIamp DNA stool mini kit (Qiagen, Valencia, California) following the manufacturer's recommendations. For species identification, we amplified a 126-bp fragment of the mitochondrial gene ATP6 by polymerase chain reaction (PCR) using primers ATP6-DF3 and ATP6-DR1 following conditions from Chaves et al. [*36*]. PCR products were sequenced on an Applied Biosystems 3730xl DNA analyzer, and resulting sequences were compared to reference sequences using the online tool DNA Surveillance Carnivora [*36*].

Individual identification

Scat samples were genotyped at 4 microsatellite loci (FCA075, FCA077, FCA088, and FCA132) originally developed for the domestic cat (*Felis catus*) [*37*]. We initially screened 22 loci previously found to be variable in ocelots [*38*]. The 4 loci used for individual identification were selected based on degree of variability, success of amplification, and ease and clarity of allele scoring. As DNA from noninvasively collected fecal samples is often low quality and prone to genotyping errors such as allelic dropout and false alleles [*39, 40*], we used a multiple tubes approach [*41*] whereby each sample was genotyped three to nine times until reliable consensus genotypes were obtained. Genotypes were only accepted as reliable if a minimum of three identical heterozygote profiles, or five identical homozygote profiles, were observed (see Appendix 1 for PCR conditions). Checks for departure from Hardy-Weinberg equilibrium and probability of individual identity [*42*] were calculated using the program GENALEX [*43*].

Sex identification

For sex identification, we used felid-specific primers that amplify a 200-bp segment of the AMELY gene [*44*], which is only present on the Y-chromosome of males (See Appendix 1 for PCR conditions). PCR amplifications were performed in triplicate along with male and female positive controls and a negative control, and PCR products were visualized on agarose gel. Samples were identified as male if they showed amplification of the Y-linked marker for all three replicates, and were identified as female if no amplification was observed.

Density estimation

We used two CR models to estimate density from both our noninvasive genetic and camera trap data to provide a comparison of common approaches. First, we ran traditional closed CR analyses in the program CAPTURE [*45*] to estimate abundance. CAPTURE was implemented within the program MARK [*46*]. To

convert abundance estimates to density from our genetic data, we buffered sample locations with MMDM as well as ½-MMDM [*3, 47*], which can be conceptualized as a proxy for home range radius. We then used the sum of the resulting buffers to estimate ESA [*3, 19*]. For our camera trapping data, both the MMDM and the ½-MMDM buffer areas were larger than the area of the entire island, so we instead used island area (15.432 km^2) as our ESA for estimating density. MMDM and ESA estimates were calculated in the program DENSITY [*29*]. Density estimates were then derived by dividing abundance estimates by ESA.

We also estimated density directly using the SECR model in the program DENSITY [*29*], which estimates density using a maximum-likelihood approach [*25, 26, 48*]. For our genetic data, we considered ocelot latrines as one might consider camera traps or hair snares, as discrete geographic locations where animals could be passively detected. DENSITY allows detectors to be considered as active or inactive during each sampling occasion. Thus, latrines known before commencement of the study were considered as active during the entire study period, and latrines discovered in the interim of the study were considered as inactive prior to discovery and active thereafter. Sampling occasions were three days, giving a total of 33 occasions during the study. All models were run under full likelihood with a Poisson distribution and the half-normal detection function. We ran multiple model combinations including various levels of individual heterogeneity, and we ran all model combinations both with and without sex as a covariate. The best-fit model was chosen using the Akaike Information Criterion adjusted for small sample size (AIC_c) [*26, 48*].

Results

Density from noninvasive genetic sampling

We collected 63 scats from 19 ocelot latrines on BCI, and we genetically confirmed 55 of these (87%) as being from ocelots. From these 55 scats, we obtained complete 4-locus genotypes from 43 samples (78%), which consisted of 12 unique genotypes and 31 recaptures from 16 latrines. Six individuals were identified as male, and six as female. All four loci were in Hardy-Weinberg equilibrium, and the number of alleles per locus was 3-6 (Appendix 2). Based on allele frequencies within the sampled population, probabilities of individual identity among unrelated individuals $P_{(ID)}$ was 0.00031, and among siblings $P_{(ID)sibs}$ was 0.038 [*42*].

The discriminant function model selection algorithm in CAPTURE selected model M_0 (the null model) as the most appropriate model (selection score of 1), with model $M_{h\text{-jacknife}}$ a close second (selection score of 0.94). As model M_0 is highly sensitive to violations of the assumption of homogeneous capture probability [*45*], and as some heterogeneity in capture probability at ocelot latrines is probable, we report results from both model M_0 and M_h (Table 1). Model M_0 produced an abundance estimate of 12 (SE = 0.918) and model M_h produced an abundance estimate of 15 (SE = 2.433). Capture probability was 0.189 for model M_0 and 0.152 for model M_h. MMDM from noninvasive genetic sampling was 632 m (SE = 117), resulting in ESA buffers of 9.760 km^2 (MMDM) and 3.114 km^2 (½-MMDM). The program DENSITY selected the null model g0(.)σ(.), without sex as a covariate, as the best-fitting model (Appendix 3), resulting in a density estimate of 1.742/km^2 (SE = 0.584).

Density from camera trapping

Twenty-one cameras were active during the 99-day sampling period resulting in 1,824 camera days. Seven cameras failed for short durations of the study, and this censoring was accounted for in the analyses [*16*]. This effort resulted in 398 independent photo sequences and 370 recaptures of 28 individual ocelots. Of these, four individuals were kittens photographed with their mother, which were excluded from further

density analyses. Mean number of captures per individual was 15.3 (SE = 2.71). Mean number of captures per camera was 17.5 (SE = 3.45). Of the 24 photographed adult ocelots, nine were males and 15 were females.

The discriminant function model selection algorithm in CAPTURE selected model $M_{h\text{-jackknife}}$ as the best-fitting model of those available. Model M_h is also the most commonly used model in camera trapping studies as it accounts for heterogeneity in capture probability [*49*]. Model M_h resulted in an abundance estimate of 28 (SE = 2.12) ocelots (Table 1), with a capture probability of 0.262. MMDM from camera trapping was 1,509 m (SE = 289). DENSITY selected the null model g0(.)σ(.), with sex included as a covariate, as the best-fitting model (Appendix 4) resulting in a density estimate of 1.587/km^2 (SE = 0.464).

Table 1 Population density estimates of ocelots (*Leopardus pardalis*) from Barro Colorado Island, Panama. CAPTURE and DENSITY are software programs designed to estimate abundance or density using capture-recapture models. MMDM is a method of estimating effective survey area based on the mean of the maximum distance moved between captures.

Method and Model	Abundance (N)	Density		
		N/KM^2	SE	95% CI
Camera trapping				
CAPTURE $M_{h(jackknife)}$	28	1.815	0.163	1.686 – 2.464
DENSITY	-----	1.587	0.464	0.908 - 2.786
Noninvasive genetics				
CAPTURE M_0- MMDM	12	1.229	0.094	1.229 - 1.844
CAPTURE M_0 - ½ MMDM	12	3.854	0.294	3.854 - 5.780
CAPTURE $M_{h(jackknife)}$ MMDM	15	1.537	0.249	1.332 - 2.459
CAPTURE $M_{h(jackknife)}$ ½ MMDM	15	4.817	0.781	4.174 - 7.707
DENSITY	-----	1.742	0.585	0.917 - 3.302

Discussion

Our estimates of ocelot population density derived from noninvasive genetics and camera trapping were highly comparable. Point estimates were similar, and 95% confidence intervals from all models overlapped, except those that used ½-MMDM to estimate ESA. Estimates from camera trapping had greater precision than those from noninvasive genetics, but these differences were not substantial (Table 1). Because we obtained density estimates from noninvasive genetics that were comparable, despite a relatively small sample size, to estimates from camera trapping, from which we had a large sample with many recaptures, our results support the validity of noninvasive genetic techniques for estimating density of elusive carnivores.

Density estimates from noninvasive genetics that used ½-MMDM to calculate ESA were substantially higher than other estimates, in some cases by more than a factor of three. Thus, we agree with other researchers that use of ½-MMDM can greatly overestimate density [*10-12, 15, 19*]. Our density estimates that used full MMDM to estimate ESA were more in line with our other estimates, but this result is likely

coincidental rather than biologically meaningful. Although MMDM methods have been used to estimate density in many studies, we agree with recent criticisms that use of MMDM methods has little theoretical or biological justification [*15, 24*]. This shortcoming is evident in our study, as MMDM distances calculated from camera trapping (1,509 m) and noninvasive genetics (632 m) were substantially different even though they were derived from the same population at the same time. The underestimation of ocelot movements from noninvasive genetics can likely be attributed to two factors. First, our camera array covered the entire island, whereas ocelot latrines had a more clumped distribution (Fig. 1). Second, our sample size from camera trapping was larger, and contained a far greater number of recaptures. It is logical that a large number of recaptures will be needed to effectively estimate movement distances [*15*], an issue widely acknowledged for estimation of home range size from telemetry data [*50, 51*]. Another problem with using MMDM to estimate survey area is that area buffers may contain both suitable and non-suitable habitat. For example, when we used MMDM and ½-MMDM to estimate ESA from camera trapping, both were larger than the area of the entire island and thus contained large portions of water, which is obviously not suitable ocelot habitat.

For our camera trapping dataset, DENSITY selected models that included sex as a covariate to be preferable to those that did not include sex (Appendix 4). This suggests, not surprisingly, that there is heterogeneity between the sexes in terms of movement and capture probability. Including sex as a covariate in the model, however, did not have a large effect on estimates of density. For our noninvasive genetic dataset, models that included sex as a covariate did not outperform those without sex (Appendix 3). This is likely due to our much smaller sample size from noninvasive genetics. Large datasets are typically necessary to benefit from inclusion of covariates, as covariates increase the number of model parameters [*15*].

For all of the reasons above, recently developed SECR models such as DENSITY [*29*] are clearly preferable for estimating population density in noninvasive studies. This is especially true for studies with small sample sizes, small survey areas [*52*], or in study locations with heterogeneous habitat. A key advantage is that SECR models do not require contentious post-hoc estimates of survey area to convert estimates of abundance to density [*25, 53*]. Furthermore, SECR models allow the user to specify suitable and non-suitable habitat for inclusion in the model, and they allow for inclusion of covariates such as sex [*15*]. Additionally, SECR models allow users to designate which detectors were operational during each sampling occasion, avoiding bias introduced by occasional camera trap failure common in traditional CR models [*16*].

Despite our efforts to obtain scat samples from throughout BCI, the spatial extent of our effective sampling area was unequal between camera trapping and noninvasive genetics, due to the difficulty of finding ocelot latrines. This difference reflects the reality that most researchers will face when conducting studies that rely on collection of scats. With camera trapping, researchers pre-define the sampling area by choosing the placement of camera traps. With fecal noninvasive genetic sampling, effective sampling area is instead defined by where study animals defecate, and by the ability of researchers to recover scat samples [*1*]. Fortunately, SECR models take differences in sampling area into account when estimating density, making it possible to compare density estimates from different spatial extents. We think that our ability to estimate similar densities from noninvasive genetics and camera trapping, despite differences in spatial extent, lends further support to noninvasive genetics as a viable method.

Our estimated ocelot densities were higher than estimates reported from anywhere else in the species range [*54, 55*]. Even our density estimates based on minimum number known alive (1.55/km^2; a

conservative lower bound estimate) are 1.6 times greater than the densest ocelot population previously reported from the northwestern Amazon of Peru (0.947/km^2) [*55*]. Our unusually high densities could be the result of numerous factors, including high prey availability [*56*], frustrated dispersal due to the high cost of dispersing from island to mainland across open water, or the vigilant protection BCI receives from poaching of both ocelots and their prey. High densities could also be the result of mesopredator release, as jaguars (*Panthera onca*) and pumas (*Puma concolor*) were resident in the BCI area in the early 20th century, but are now only infrequent visitors to the island [*57, 58*].

Implications for conservation

Our study provides evidence that noninvasive genetic techniques can generate accurate estimates of population density, especially when used in conjunction with SECR models. We do caution, however, that further studies should be conducted on a variety of species in diverse environments to further verify the accuracy of these techniques. Our study was conducted in a high-density population of a species that defecates at latrine sites, and thus we were able to find scats relatively easily. For low density populations, it may be necessary to use aids such as scat detection dogs, which although expensive can greatly increase detection rates [*22*]. In sum, we think that fecal noninvasive genetics provides a promising tool for the estimation of density in elusive animals, especially for species in which individuals cannot be identified from camera trapping. These techniques have great potential to aid in the conservation of the many elusive species worldwide for which population density and conservation status are poorly known.

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Appendix 1. PCR Conditions

PCR conditions for species identification can be found in Chaves et al. (2012). PCR conditions for microsatellite amplification and sex ID were as follows. Reactions included 1 µl 10X Buffer, 0.8 µl of MgCl, 0.2 µl of 10 mM DNTPs, 0.2 µl of 20 mM fluorescently-labeled forward primer, 0.2 µl of 20 mM reverse primer, 0.04 µl of AmpliTaq Gold® 360 DNA Polymerase (Life Technologies; Grand Island, NY, USA), 0.1µl of BSA, 0.2 µl of GC-enhancer, 5.76 µl of water, and 1.5 µl of template DNA for a total reaction volume of 10 µl. Thermocycling conditions were as follows: initial denaturation at 95ºC/10’, followed by 50 cycles of 95ºC/5”, 55ºC/15”, 72ºC/45”, and a final extension of 72ºC/10’. Microsatellite forward primers were fluorescently labeled with one of four dyes (FAM, NED, PET, or VIC). Each microsatellite was amplified individually and then the loci were pooled together and genotyped on an Applied Biosystems 3730xl DNA analyzer.

Appendix 2. Summary of 4 microsatellite loci used for individual identification. H_o is observed heterozygosity. H_e is expected heterozygosity under Hardy-Weinberg equilibrium. All loci were in Hardy-Weinberg equilibrium (HWE probability > 0.05).

Locus	Size	# alleles	H_o	H_e	HWE probability
FCA075	110-128	5	0.769	0.782	0.826
FCA077	133-137	3	0.615	0.492	0.463
FCA088	92-110	5	0.846	0.809	0.564
FCA132	167-179	6	0.769	0.788	0.559
Mean	na	4.75	0.762	0.7226	0.5872

Appendix 3. AIC table for density estimates from noninvasive genetic sampling from the program DENSITY.

Model	Sex as covariate	# Parameters	ML Log likelihood	AICc	ΔAICc	Density	SE	95% CI
g0[.]s[.]	No	3	-186.49	381.97	--	1.7423	0.5846	0.919-3.305
g0[h2]s[.]	No	5	-186.00	392.00	10.03	1.7417	0.5851	0.918-3.306
g0[.]s[h2]	No	5	-186.45	392.90	10.93	1.7410	0.5828	0.919-3.298
g0[h2]s[h2]	No	6	-185.86	400.52	18.55	1.7456	0.5874	0.919-3.317
g0[.]s[.]	Yes	6	-191.58	411.95	29.98	1.8074	0.9057	0.717-4.577
g0[h2]s[.]	Yes	8	-191.11	446.21	64.24	1.8186	0.9176	0.717-4.629
g0[.]s[h2]	Yes	8	-191.57	447.15	65.18	1.8057	0.9046	0.716-4.570
g0[h2]s[h2]	Yes	9	-191.58	491.15	109.1	1.8053	0.9085	0.713-4.587

Appendix 4. AIC table for density estimates from camera trapping from the program DENSITY.

Model	Sex as covariate	# Parameters	ML Log likelihood	AICc	ΔAIC_c	Density	SE	95% CI
g0[.]s[.]	Yes	6	-1072.52	2161.98	--	1.5867	0.463	0.908-2.786
g0[.]s[h2]	Yes	8	-1071.33	2168.26	6.28	1.5835	0.463	0.906-2.782
g0[h2]s[.]	Yes	8	-1071.43	2168.46	6.48	1.5842	0.463	0.906-2.783
g0[h2]s[h2]	Yes	9	-1071.33	2173.51	11.53	1.5826	0.463	0.905-2.781
g0[.]s[.]	No	3	-1115.23	2237.65	75.67	1.5617	0.322	1.105-2.330
g0[.]s[h2]	No	5	-1113.69	2240.71	78.73	1.5575	0.321	1.104-2.325
g0[h2]s[.]	No	5	-1114.12	2241.57	79.59	1.5613	0.322	1.105-2.330
g0[h2]s[h2]	No	6	-1113.92	2244.77	82.79	1.5551	0.321	1.104-2.321