Techniques and Technology Note

Evaluation of Noninvasive Genetic Sampling Methods for Felid and Canid Populations

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ABSTRACT Noninvasive sampling methods provide a means for studying species such as large mammalian carnivores that are difficult to survey using traditional techniques. Focusing on bobcat (*Lynx rufus*), we compared the effectiveness of noninvasive hair and scat genetic sampling in terms of field sample collection, species identification, and individual identification. We describe a novel hair-snare design and sampling protocol that successfully sampled 4 sympatric carnivore species, bobcat, mountain lion (*Felis concolor*), coyote (*Canis latrans*), and gray fox (*Urocyon cinereoargenteus*), in 3 habitat blocks in coastal southern California, USA. Scat surveys were also successful at sampling bobcats and other carnivores in the area. Hair and scat sampling methods had similar species identification success (81% and 87%, respectively) using mitochondrial DNA amplification and restriction enzyme digestion patterns. Therefore, for studies focused on the distribution and activity of a suite of carnivore species, we recommend a combination of noninvasive methodologies, for example, targeting hair and scat surveys toward species and sites where they are most effective. Because of a higher success rate for scat (85%) than for hair samples (10%) when using 4 microsatellite loci and a multiple-tubes approach to verify individual genotypes, we suggest scat sampling is a better choice for studies that require individual identification of bobcats. (JOURNAL OF WILDLIFE MANAGEMENT 71(5):1690–1694; 2007)

KEY WORDS bobcat, coyote, gray fox, hair snare, *Lynx rufus*, microsatellites, mitochondrial DNA, mountain lion, noninvasive sampling, scat.

Mammalian carnivores are inherently difficult to survey because of large home ranges, small populations, nocturnal activity, and wariness resulting from persecution by humans (Sargeant et al. 1998, Crooks 2002). Conventional techniques, such as live trapping for mark–recapture or radio-telemetry, are often logistically difficult. Indirect techniques, such as track and remotely triggered camera surveys, allow researchers to noninvasively monitor the distribution and activity of large mammals, but the resulting indices usually do not yield population estimates (Anderson et al. 2003) and provide no information regarding the genetic structure of populations. In contrast, noninvasive sampling of hair or scat and subsequent genetic analyses can be used to determine important behavioral and population characteristics of carnivores, such as density, dispersal, and genetic structure, which can be difficult to determine using other methods (Snow and Parker 1998).

Published hair snare methods for felids in North America primarily have focused on lynx (*Lynx rufus*) in northern forests (McDaniel et al. 2000, Mills et al. 2000). Our planning field tests using the lynx hair snare design did not collect hair samples from bobcats (*L. rufus*) or other carnivores in predominantly scrub habitat in the highly fragmented landscape of coastal southern California, USA. Therefore, we designed and tested a hair-snare device that allows noninvasive sampling of felids and other carnivores in this system, as well as in a variety of habitat types. Noninvasive scat sampling also has been used successfully to study a variety of carnivores (Kohn et al. 1999, Ernest et al. 2000, Creel et al. 2003), so we sampled scat concurrently with hair-snare surveys in order to compare their effectiveness in sample collection, species identification success, and

individual identification success. We focused on bobcats because they are sensitive to habitat fragmentation and are valuable indicators of landscape connectivity in the region (Crooks 2002).

STUDY AREA

We conducted surveys within 138 km^2 of the Nature Reserve of Orange County (NROC), a fragmented system of open space in coastal Orange County, California, south of Los Angeles. Vegetation consisted primarily of coastal sage scrub habitat interspersed with patches of chaparral, oak woodlands, nonnative grasslands, and riparian woodlands. This montage of habitat supported bobcat, mountain lion (*Felis concolor*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), and several other native and nonnative mesopredator species (Crooks 2002, George and Crooks 2006).

METHODS

We constructed hair snares using 5.1 × 15.2 × 61-cm pine boards as anchors. To create an initial visual attractant, we stapled a cluster of 3–5 white turkey feathers down by the feather shafts to the top of each board. We nailed a 10 × 10-cm square of stiff natural fiber carpet (unpainted welcome mat) to the top surface of each board. We baited carpet squares with approximately 2 mL of Russ Carman's Canine Call (Sterling Fur & Tool, Sterling, OH), a carnivore scent lure that has been successfully used to attract bobcats and other carnivore species to track and camera stations in southern California (Crooks and Soulé 1999, Crooks 2002).

In spring 2003, we placed 161 hair snares on the ground along dry creek beds, game trails, recreational trails, and roads, as these are habitual paths of movement for carnivores (Kohn et al. 1999, George and Crooks 2006). In coastal

southern California, female bobcats have smaller home ranges (1.55 ± 1.44 km^2) than males (Riley et al. 2003). Therefore, we placed approximately 1 snare/1 km^2 throughout study areas to ensure that every bobcat had a reasonable chance of being sampled. We sampled each snare for 4 consecutive sampling occasions, each lasting 3–4 days, for a total of 644 sampling occasions. We placed carpet squares with hairs in manila envelopes and stored them at room temperature away from heat and moisture. Within 3 months of their collection, we removed hairs from carpet squares, combined them as one sample, and stored them at $-20°$ C (Roon et al. 2003). While hair sampling, we also collected all fresh and intact mammalian carnivore scat samples encountered. We picked up scats using inverted labeled Ziploc bags (S. C. Johnson, Racine, WI) and added 1.905-cm silica gel beads (Sigma Aldrich, Inc., St. Louis, MO) to desiccate samples in an approximate 5(silica):1(scat) weight ratio (Wasser et al. 1997).

We conducted laboratory analyses using strict protocols and guidelines to cull poor-quality samples and to reduce genotyping errors (Paetkau 2003). We extracted DNA from all hair samples using the QIAamp® DNA Mini Kit standard tissue protocol (Qiagen, Inc., Valencia, CA). We pooled up to 15 hair follicles with shafts in each extraction because the benefit of increasing DNA concentrations outweighed the risk of combining DNA from multiple individuals (Gagneux et al. 1997). We extracted DNA from scat samples using the QIAamp DNA Stool Mini Kit protocol (Qiagen, Inc.). We extracted only potential felid scat samples, based on size, shape, and composition. We were conservative in culling samples to avoid missing atypical felid scats. To verify that we were not missing felid scats, we extracted 30 randomly selected culled scat samples that had been morphologically identified as non-felid and genetically identified them to species.

We identified hair and scat samples as bobcat, mountain lion, domestic cat, and non-felid species using the 16S rRNA protocol in Mills et al. (2000). We identified non-felid hair samples to canid species using the cytochrome *b* protocol in Paxinos et al. (1997). These mitochondrial DNA restriction digestion protocols allowed us to identify mixed species samples because they exhibited restriction patterns for >1 species. Species identification via sequencing of mixed-species samples would result in ambiguous sequences (Paxinos et al. 1997).

For individual identification, we tested 7 microsatellite loci (FCA023, FCA026, FCA045, FCA077, FCA090, FCA096, and FCA132; Menotti-Raymond et al. 1999) and identified a subset with the lowest genotyping error rates that differentiated individuals with confidence. We replicated genotypes 3 times for each locus using M13-tailed primers (Boutin-Ganache et al. 2001). We estimated genotyping error rates from matched hair and blood ($n = 31$) and scat and blood ($n = 25$) samples from bobcats trapped within the Santa Monica Mountains (SMM), California, north of Los Angeles (Riley et al. 2003, 2006). We estimated rates of allelic dropout (ADO) per replicate per locus from both heterozygous and all genotypes (Broquet and Petit 2004). We estimated rates of false alleles (FA) per replicate per locus from all genotypes (Broquet and Petit 2004).

To minimize genotyping error, we replicated genotypes using a multiple-tubes approach to obtain correct consensus genotypes (Taberlet et al. 1996). This approach assumed that errors occurred randomly among samples. If this assumption was violated, observed consensus genotype error rates could be different from expected (Gagneux et al. 1997). Therefore, we compared observed to expected consensus genotype error rates from the 3 replicates of SMM bobcat hair and scat genotypes at each locus. We estimated expected consensus genotype error rates per locus using the methods of Broquet and Petit (2004). Observed genotyping error rates were the number of incorrect consensus hair and scat genotypes, which we compared to matched blood sample genotypes, over the total number of genotypes for each locus. We scored consensus genotypes homozygous at a locus if only 1 allele appeared and scored heterozygous if 2 alleles appeared during any of the 3 replicates. We also performed a Cochran–Mantel–Haenszel (CMH) chi-square test, stratified by loci, using SAS PROC FREQ in SAS (SAS Institute, Cary, NC) to detect if there was a difference in rate of ADO between samples.

We used the probability of identity of siblings (P(ID)sib) and the observed probability of identity (P(ID)obs; Waits et al. 2001), calculated from 45 SMM bobcat genotypes (Riley et al. 2006), as upper and lower bounds in determining the most efficient and error-free set of loci that will differentiate individuals. The actual P(ID) was likely somewhere between these upper and lower bounds depending on the degree of relatedness of individuals in study areas (Waits et al. 2001).

Based on these analyses (see Results), we used 4 loci (FCA026, FCA045, FCA077, and FCA132) and an expanded multiple-tubes approach to genotype the hair and scat samples noninvasively collected in the NROC. The NROC samples were initially run for 3 independent replicates for each locus, with replicate number increased until we observed both alleles of heterozygous genotypes in 3 replicates and single alleles of homozygous genotypes in 6 replicates without any other allele. We matched final hair and scat genotypes using the Excel Microsatellite Toolkit (Park 2001).

RESULTS

Both hair-snare and scat surveys were successful in collecting mammalian carnivore samples (Table 1). Approximately 49% of sampling occasions collected hair samples and 91% of hair snares collected hair during ≥1 sampling occasion. Hair and scat samples readily identified to species (Table 1). The majority of hair samples (78%) were non-felid, primarily coyote, whereas a majority of the potential felid scat samples processed (56%) were bobcat. We verified that we were not missing felid samples using our culling method, as all 24 randomly chosen scat samples morphologically identified as non-felid that yielded successful

Table 1. Field collection, species identification, and individual identification success for hair and scat samples noninvasively collected from the Nature Reserve of Orange County, California, USA, 2003.

	Noninvasive sample type	
Sample type comparison	Hair	Scat
Field collection		
Carnivore samples collected	315	259
Species identification		
mtDNA restriction profile success[a]	81%	87%
% bobcat[b]	13%	56%
% mountain lion[b]	2%	1%
% non-felid[b]	78%	43%
% coyote[b]	47%	NA
% gray fox[b]	29%	NA
% domestic dog[b]	2%	NA
% mixed species[c]	7%	NA
Bobcat sample individual identification		
Genotyping success (4 loci)[d]	10%	87%
Unique genotypes	4	30

[a] Mitochondrial DNA (mtDNA) restriction profile success rates are from the samples extracted and identified to species using mtDNA restriction profiles ($n = 315$ for hair and $n = 111$ for scat).
[b] Species percentages are out of the samples that generated mtDNA restriction profiles ($n = 256$ for hair and $n = 97$ for scat). Hair samples species percentages do not include mixed-species samples containing these species. Non-felid scat samples were not differentiated to coyote, gray fox, and domestic dog, which are combined in the non-felid category. Only one domestic cat hair sample was identified and therefore not included in the table.
[c] Mixed-species sample concentration were bobcat and coyote, bobcat and gray fox, bobcat and domestic dog, mountain lion and gray fox, coyote and gray fox, and coyote and domestic dog. None of the hair samples appeared to consist of ≥ 3 species.
[d] Genotyping success rates are from the identified bobcat samples ($n = 39$ for hair and $n = 54$ for scat). Mixed-species hair samples containing bobcat DNA were included. None of the hair samples that genotyped exhibited 3 or more alleles at any locus, which would have indicated ≥ 1 bobcat in the sample. All 4 hair sample genotypes differed by >2 alleles.

mtDNA restriction profiles were genetically confirmed as non-felid.

The 5 loci with the lowest expected and observed genotyping error rates of ADO and FA estimated from 3 replicates of matched SMM were FCA026, FCA045, FCA077, FCA090, and FCA132 (Table 2). Observed consensus genotype error rates were sometimes different from expected, which was likely caused by a nonrandom distribution of errors among samples. We found evidence of different rates of ADO among samples (CMH $\chi^2_{25} = 72.8331$; $P < 0.001$). Although the use of 5 loci would have reduced P(ID)sib to 0.01 (Fig. 1) as recommended in Waits et al. (2001), error rates for 5 loci genotypes were undesirably higher than those for 4 loci for scat samples (Table 2). Four loci also had a small P(ID)sib and only 3 loci were necessary for P(ID)obs to fall to zero (Fig. 1). Therefore, we chose to genotype individuals with only the 4 most heterozygous and consistent loci (FCA026, FCA045, FCA077, and FCA132). For samples noninvasively collected in the NROC, the expected overall genotyping error rates when using the expanded multiple-tubes approach and summed across loci was estimated to be 0.0002 for hair samples and 4.294^{-6} for scat samples.

Complete genotyping of samples at all 4 loci was less successful for hair samples (10%) than for scat samples (87%; Table 1). The 4 successfully genotyped hair samples resulted in 4 unique bobcats, and the 47 successfully genotyped scat samples resulted in 30 unique individuals. Matching the 51 hair and scat samples yielded 33 individual bobcats.

DISCUSSION

Our hair sampling protocol performed well in the field to assess the distribution and activity of a variety of carnivore species. Both hair and scat sampling methods worked for bobcat, allowing collection of numerous samples from the field. The hair-snare protocol, however, was less useful for sampling just bobcat because processing numerous non-bobcat samples was both time-consuming and costly, and bobcat hair samples did not genotype well. In contrast, bobcat scat samples had high genotyping success, and conservatively ruling scat samples non-bobcat considerably

Table 2. Genotyping error rates for hair and scat samples obtained from trapped bobcats in the Santa Monica Mountains, California, USA, 1996–2000.

Sample type	Genotyping error	Locus[a] (%)						
		FCA026	FCA045	FCA077	FCA132	FCA090	4 loci[b]	5 loci[b]
Scat ($n = 25$)	ADO$_{het}$ per replicate[c]	1.9	9.3	10.7	14.3	10.9	9.1	9.4
	ADO$_{all}$ per replicate[c]	1.6	6.9	5.6	12.7	8.8	6.7	7.1
	FA$_{all}$ per replicate[c]	0.0	0.0	0.0	0.0	5.9	0.0	1.2
	Expected overall error rate[d]	0.0	0.5	0.3	1.6	6.7	2.4	9.1
	Observed overall error rate[d]	0.0	0.0	0.0	0.0	8.0	0.0	8.0
Hair ($n = 31$)	ADO$_{het}$ per replicate[c]	1.5	19.4	13.2	5.1	10.6	9.8	10.0
	ADO$_{all}$ per replicate[c]	1.1	15.3	8.0	4.3	5.7	7.2	6.9
	FA$_{all}$ per replicate[c]	0.0	3.5	4.5	3.2	0.0	2.8	2.3
	Expected overall error rate[d]	0.0	5.9	5.2	3.4	0.3	14.5	14.8
	Observed overall error rate[d]	0.0	6.9	6.7	6.5	0.0	9.7	9.7

[a] These 5 loci had the lowest genotyping error rates out of the 7 loci tested and are ordered by decreasing heterozygosity.
[b] Rates of allelic dropout (ADO) and false alleles (FA) per replicate were averaged across the first 4 loci and all 5 loci. Exp and obs overall error rates are summed across loci for the first 4 loci and all 5 loci.
[c] ADO$_{het}$ is the rate of ADO calculated from heterozygous genotypes. ADO$_{all}$ and FA$_{all}$ are calculated from all genotypes.
[d] Exp and obs overall error rates are the sum of both ADO$_{all}$ and FA$_{all}$ consensus error rates after 3 replicates.
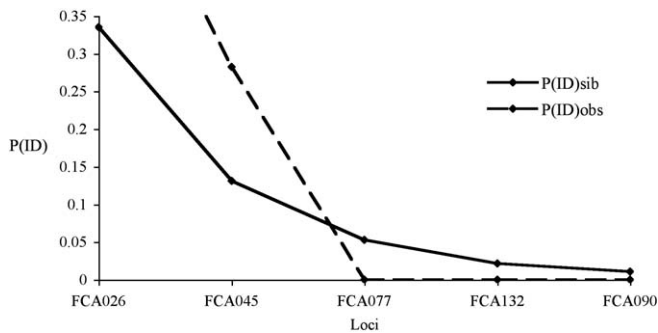
Figure 1. Probability of identity (P(ID)) statistics for 45 bobcats trapped in the Santa Monica Mountains, California, USA, 1996–2000. Loci are in order of greatest to least expected heterozygosity. The observed probability of identity (P(ID)obs) for each locus is the proportion of identical genotypes observed when including information from the previous loci. The probability of identity of siblings (P(ID)sib) for each locus is the product of the P(ID)sib for that locus and the previous loci.

reduced the proportion of nontarget species samples processed.

The much greater genotyping success of scat samples than hair samples was surprising. Our genotyping success for hair samples was much smaller than previously reported for bears (*Ursus americanus* and *U. arctos*; 90%, 6 loci, Woods et al. 1999; 81%, 6 loci, Mowat and Strobeck 2000) and martens (*Martes americana*; 77%, 6 loci; Mowat and Paetkau 2002). Conversely, our genotyping success for scat samples was greater than previously reported for badgers (*Meles meles*, 74%, 7 loci; Frantz et al. 2003), mountain lions (63%, 12 loci; Ernest et al. 2002), and coyotes (48%, 3 loci; Kohn et al. 1999). However, consistent with our results, Morin et al. (2001) found that chimpanzee (*Pantroglodytes verus*) feces contained more DNA than did hair, and recent noninvasive studies of lynx (*Lynx canadensis*) also found scat samples to have much greater genotyping success than hair samples (Lukacs 2005).

Our relatively low hair genotyping success may be due in part to the fine structure of felid hair, which may contain less DNA than that of species with coarser hair, or the occurrence of shed hairs, which contain much less DNA than plucked hairs (Gagneux et al. 1997). Storing hair samples at room temperature for up to 3 months before freezing also may have led to lower yields. However, Woods et al. (1999) and Mowat and Strobeck (2000) did not freeze hair samples for >1 month but still achieved high genotyping success. Our high scat genotyping success relative to other studies could be due to scat freshness (≤4 d old) at time of collection (e.g., compared to <17 weeks in Kohn et al. 1999 and unknown age in Ernest et al. 2002) and arid field conditions.

For studies in which individual identification of samples is necessary to address population genetic or demographic questions, researchers must take additional measures to verify that multi-locus genotypes have low rates of genotyping errors (Taberlet et al. 1996). Our rates of ADO and FA per replicate per locus for both hair and scat samples were relatively low and consistent with other studies (Broquet and Petit 2004). The use of the multiple-tubes

approach probably resulted in little to no sample misidentification, even with a nonrandom distribution of errors among samples.

MANAGEMENT IMPLICATIONS

Our hair sampling method, coupled with species identification via analyses of mitochondrial markers as described here, allows researchers to study and compare multiple species simultaneously for a systematic measure of carnivore distribution, relative activity, and community composition. This is of interest when studying how carnivore species may differ in their responses to anthropogenic disturbances, such as urbanization and habitat fragmentation (Crooks 2002; Riley et al. 2003, 2006). However, given the much greater genotyping success of scat compared to hair samples, we suggest scat sampling is a better choice for studies that require individual identification of bobcats, at least until laboratory methods for analyzing hair are improved. Individual identification of samples through microsatellite markers can then be used to estimate population sizes (Kohn et al. 1999) and assess genetic structure of populations (Snow and Parker 1998), which are largely unknown for bobcat and other carnivores in coastal southern California.

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