Use of scented hair snares to detect ocelots

John L. Weaver, Peggy Wood, David Paetkau, and Linda L. Laack

Abstract Biologists need a variety of tools to determine the population and genetic status of the ocelot (*Leopardus pardalis*), an elusive Neotropical cat that favors dense habitats. We developed and tested a technique that entices ocelots to rub on scented hair snares and uses DNA analysis of the hair to determine species, gender, and individual identity. Twenty-seven (84%) of 32 captive ocelots rubbed against the scented pads. In field tests at Laguna Atascosa National Wildlife Refuge in south Texas, we detected a minimum of 6 ocelots, including at least 3 of 4 radiocollared animals. Using a 6-locus microsatellite analysis, we made individual identification for 10 of 20 samples. Scented hair snares can provide useful information on the population and genetic status of ocelots and identification of key areas and connecting linkages. We suggest that surveys for ocelots deploy 1 station per 25–50 ha and check them every 1–2 weeks.

Key words carnivore detection, DNA, genetics, hair snare, *Leopardus pardalis*, ocelot

Ocelots (Leopardus pardalis) range from northern Argentina northward to south Texas (Sunquist and Sunquist 2002). The only United States population (estimated at <100) occurs in south Texas, where ocelots use scattered patches of dense thornscrub habitat (Tewes 1986, Laack 1991). Listed as an endangered species in the United States (United States Fish and Wildlife Service 1982), the ocelot is jeopardized by loss and fragmentation of habitat and vehicular collisions. To develop and implement an effective conservation strategy for ocelots, resource managers need a diverse set of information regarding occurrence, habitat use, population density and trends, dispersal, and genetic diversity. Biologists need a variety of tools to obtain such vital information about this secretive cat.

Traditional methods of physically capturing and radiocollaring carnivores can provide useful and reliable data but are expensive, time-consuming, and invasive. Recently, biologists have deployed automatic cameras to detect elusive carnivores and estimate population size using capture-recapture statistical modeling (Karanth 1995, Karanth and

Nichols 1998, Heilbrun et al. 2003), including ocelots (Trolle and Kéry 2003). Camera "capture" can provide useful data, particularly for species like spotted cats where variation in natural markings permits visual identification of individuals. Nonetheless, use of cameras brings up considerations such as initial cost, risk of theft, installation effort and maintenance, operational reliability, scale of deployment, and lack of data on some conservation concerns (e.g., genetics).

Biologists also have pursued another line of noninvasive techniques to obtain hair samples from carnivores for subsequent DNA analysis (e.g., Woods et al. 1999). Molecular-based methods have their own set of promises and pitfalls (Mills et al. 2000) but are becoming more cost-effective and reliable under careful and rigorous laboratory protocols (Paetkau 2003).

To expand the toolbox of techniques for ocelot conservation, we conducted a pilot study to 1) evaluate the rubbing response of captive ocelots to scented hair snares, and 2) determine the efficacy of scented hair snares for detecting wild ocelots

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Figure 1. Radiocollared ocelot rubbing on scented hair snare placed at Laguna Atascosa National Wildlife Refuge in south Texas during 2000, and (inset) ocelot hairs on snare pad.

and obtaining information on their status.

Methods and study area

Scented bair snares

Our method capitalizes on the natural cheek-rubbing behavior exhibited by many (but not all) species of small felids (Reiger 1979, Mellen 1993). The hair snare was a 10-cm \times 10-cm square of shortnapped carpet with a rubber backing. We used a power nailer to shoot 8-10 roofing nails (1.6 cm long) through the carpet pad from the backside in a circular arrangement (see inset, Figure 1). Small wires (8 mm) already attached to the nails facilitated snagging of hair. We rubbed 10 ml (0.3 oz) of a scent lure called Weaver's Cat Call (WCC; contact John Weaver, St. Ignatius, Mont.) into carpet fibers at the center of the pad and crumbled dried catnip (Nepeta cataria) on top of the scent paste. The rubbing and rolling response of cats to catnip is well known (Todd 1962, Palen and Goddard 1966). In previous trials with captive lynx (Lynx canadensis) and bobcats (L. rufus), significantly more animals responded to the WCC lure than to catnip

alone or various musk oils (J. L. Weaver, Wildlife Conservation Society, unpublished report 1996). Others have tested this original approach to scented hair snares for lynx (McDaniel et al. 2000).

We established stations by nailing a scented hair snare to a tree trunk at a height of 0.3 m above the ground. At sites where the tree trunk was more than 5 m from a desired location along a wildlife trail, we drove a wooden post into the ground immediately adjacent to the trail and mounted the pad on a wooden placard nailed to the top of the wood post (Figure 1). For a visual attractant, we tied an aluminum pie plate to a 5-cm leader of monofilament with a swivel at the loose end and clipped it onto a length of 19-gauge wire twisted around a branch above the carpet pad. The pie plate hung approximately 1 m above the ground and fluttered in the breeze.

Testing of captive ocelots

We tested 32 ocelots at 9 zoological facilities in March 1999 for their rubbing response to scented hair snares. We presented the scented pad by nailing it on a log within the ocelot's exhibit area or by affixing it on a block of wood that we wired to the side of the animal's holding cage at a height of 0.3 m. We provided an ocelot access to the pad for up to 1 hour and recorded its rubbing response.

Detection of wild ocelots

We tested the efficacy of scented hair snares to detect wild ocelots at Laguna Atascosa National Wildlife Refuge (LANWR), located along the Gulf coast of south Texas 48 km northeast of Brownsville. The refuge supported 35-40 ocelots and provided the largest protected area (18,294 ha) of natural habitat remaining in south Texas. The LANWR has been the center of ecological research on ocelots since 1982 (Tewes 1986, Laack 1991), and refuge biologists continue to monitor a number of radiocollared ocelots there. We selected a 600-ha area (the Island Fields Unit) where 4 radiocollared ocelots occurred.

During the first 5-night session in April 1999, we established scent stations along gravel and unimproved roads around the periphery of patches of suitable habitat at a density of 1 per 50 ha. Prior studies had noted that ocelots traveled these roads (Laack 1991). We placed hair-snare stations at sites where the visual attractant was visible >25 m in >1 direction (e.g., intersections of roads and trails, sharp bends in the road). During the second 5night session, we doubled the density of scent stations to 1 per 25 ha and placed the additional stations along wildlife trails that penetrated the dense thornscrub brush of the patch interior. We relocated at least half of the initial stations to new sites along the periphery of the unit. For this pilot investigation, we checked stations daily. We repeated the survey in May 2000.

To determine occurrence of the radiocollared ocelots on the study area during the test period, we located them from the ground by triangulation from designated receiving sites twice a day: once during their inactive period (0700–1800) and once during the first half of their active period (1800–2400). Two azimuths were taken, usually within 15 minutes and <500 m from the animal across level terrain; previous radiotracking here using a similar protocol had established an average triangulation error of 75 m (Laack 1991).

DNA analyses

We extracted DNA using QIAGEN's DNeasy kits (QIAGEN, Venlo, The Netherlands and Mississauga, Ont., Canada). To guard against possible contami-

nation, we kept DNA that had been amplified using polymerase chain reaction in an isolated facility apart from hair samples and genomic DNA extracts. We monitored for contamination by running blank samples (no hair added) with each set of samples that was extracted or amplified.

We determined the felid species of hair samples using a partial (A-only) sequencing of the 16S rRNA mitochondrial gene (D. Paetkau and J. Weldon, Wildlife Genetics International, unpublished report). Ocelot (Genbank AF006416) differed from bobcat (Genbank AF006417) at 9 diagnostic nucleotide positions (in bold) in the sequence GTA-GAGATTTGTTGTTCCTGTCGGTTAATTAGTAGG (Johnson and O'Brien 1997).

We screened 11 microsatellite markers based on published reports for other felid species (Menotti-Raymond and O'Brien 1995, Carmichael et al. 2000, Culver et al. 2000, Schwartz et al. 2000) and tested them with hair samples from captive ocelots. We used 6 markers (Fca43, Fca45, Fca90, Fca117, Fca559, and Lc118) because they exhibited considerable variability and produced clear, strong alleles for most samples from the captive ocelots.

We used a step-wise protocol of exclusion to ensure rigorous and conservative determination of identity (Paetkau 2003). We made 2 attempts to genotype ocelot hair samples for individual identity. We excluded samples that did not produce strong results for at least 4 loci because such samples can be prone to amplification errors (Taberlet et al. 1996) or the "shadow effect" (described by Mills et al. 2000). We initially scored samples using GENOTYPER software (Applied Biosystems, Foster City, Calif.). Two technicians independently confirmed the scoring of all complete genotypes that comprised the final declaration of individual ocelots. We scrutinized all pairs of genotypes that differed at only 2 loci for possible error and reanalyzed them if identification of an individual was based on only one available sample. All genotypes declared as individual ocelots differed at ≥2 of the 6 loci, thereby minimizing the potential errors of misidentification.

We determined gender based on presence or absence of the SRY gene (Y-chromosome), along with co-amplification of the ZFX/ZFY gene (both X-and Y-chromosomes) as an internal control. We followed the approach outlined in Aasen and Medrano (1990) but modified primers and reaction conditions to produce strong amplifications in felids. Known male and female samples included as

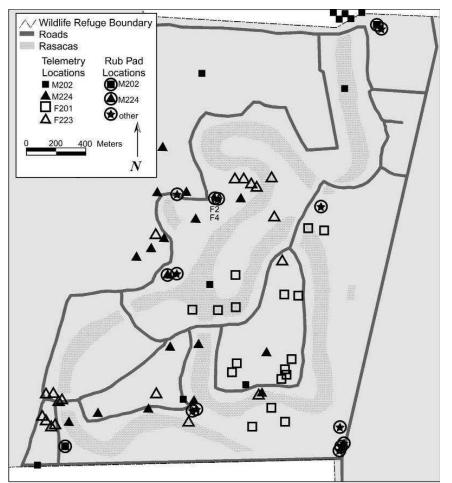


Figure 2. Locations of radiocollared ocelots and snares with ocelot hair in the Island Fields survey unit at Laguna Atascosa National Wildlife Refuge in south Texas, 6–15 April, 1999.

controls produced unambiguous and accurate results, whereas negative controls (water in place of sample DNA) failed.

Results

A high percentage (84%) of the 32 captive ocelots rubbed against the scented hair snares. There was no significant difference between male and females ($G_{\rm adj}$ =0.015, 1df, P>0.90). Some individuals approached the testing setup more timidly than others but rubbed within 10-15 minutes. Ocelots rubbed their cheeks, chin, and side of the throat for upward of 20 minutes.

Using scented hair snares at Laguna Atascosa NWR and DNA analysis, we detected a minimum of 6 ocelots, including at least 3 of the 4 radiocollared animals (Table 1). In 1999 at least 4 different ocelots made 5 rubs at 4 separate stations during

the first 5-night session (1 ocelot/15 station-nights or 1 rub/12 station-nights) (Table 1, Figure 2). Three individuals (M202, M224, and F4) visited stations on the second night of the survey. During the sec-5-night session, ocelots rubbed on 7 occasions at 5 stations (1 rub/17 station-nights) (Table 1). Only 1 of these samples, however, yielded adequate DNA for individual identification. Ocelot M202 was "recaptured" on night 10.

In 2000 at least 3 ocelots (F201, M202, and F5) made 5 rubs at 4 stations during the first 5-night session (1 ocelot/20 station-nights or 1 rub/12 station-nights) (Table 1, Figure 3). Two ocelots visited separate stations during the second session (1 ocelot/60 station-nights or 1 rub/60 station-nights). We detected all 3 of the identified ocelots (F201, M202, and F5) dur-

ing the first session and recaptured 2 during the second session.

Discussion

Efficacy of scented bair snares

Trials with captive occlots verified that this species has the rubbing response in its behavioral repertoire. A high percentage of both male and female occlots responded to the same scent (WCC) that we have found attractive to other felids. Mellen (1993) noted that males and females of several felid species, including occlots, rubbed at similar rates. Not all cats are expected to respond, however, because the capacity of an individual to detect and respond to catnip is governed by a dominant autosomal gene (Todd 1962).

One approach to assessing the probability of detection is to use the known sample of radiocol-

Table 1. Detection of ocelots rubbing on hair snares, Island Fields Unit - Laguna Atascosa National Wildlife Refuge, Texas, 6–25 April 1999 and 4–13 May 2000. Sample size (*n*) refers to number of snare stations.

Year: Session	n	Night	Station	Ocelot
1999: I	12	2	3	M202 ^a
	2	7	M224	
	2	9	F4	
	3	8	Op	
	4	8	NAC	
	4	9	F2	
1999: II	24	6	6	О
	8	1	O	
	8	14	Ο	
	9	6	Ο	
	9	12	O	
	10	14	M202	
	10	20^{2}	О	
2000: I	10	1	8	F5
	2	6	M202	
	3	2	О	
	3	9	O	
	4	8	F201	
2000: II	20	6	17	M202
	8	11	F201	

- a Gender and individual identity ·
- b O = identification to species only.
- ^c NA = no amplification of DNA.

lared animals. In 1999 we confirmed detection of the 2 male ocelots on the Island Fields unit, where they were located 55% and 80% of the 20 monitoring occasions (Figure 2). We could not confirm detection of F201 that year but did obtain several ocelot hair samples near her telemetry locations (85% on study unit) that, unfortunately, did not yield an individual identification. We did confirm her detection, however, at 2 stations the following year that were 100-300 m from a station with ocelot hair in 1999 (Figure 3). We could not confirm F223 because we did not have DNA reference material from her physical capture. Based upon the proximity of her radiolocations (95% on study unit), it is possible that F223 may have been 1 of 2 female ocelots (F2, F4) that rubbed at one station in 1999 (Figure 2).

The time to first detection was quite short in most instances. In both years, we detected most of the individual occlots during the first 5-night session when stations were placed along the periphery of the unit at a density of 1 station per 50 ha. We recaptured known occlots 4, 4, and 8 days after

initial detection.

Overall, we made individual identification for only 10 (50%) of 20 ocelot hair samples, which hampered the assessment. Some hair samples lacked roots, which contain the nuclear DNA needed for microsatellite analysis; in other samples with roots, the amount of nuclear DNA in the small, thin hairs was meager. In another study using similar hair snares, individual identification was achieved on 47% of 45 samples of lynx hair (J. L. Weaver, Wildlife Conservation Society, unpublished data). Samples of felid hair obtained from these hair snares yielded a lower rate of individual identification than did samples of bear hair collected from barbed-wire enclosures (>75%: Paetkau 2003). Perhaps this style of hair snare collected more hairs that were already loose or shed rather than plucking hairs with roots (Gagneux et al. 1997, Goosens et al. 1998).

Trolle and Kéry (2003) made identification of individual ocelots in 95% of 55 photographs and rephotographed 5 of 9 individuals. When we initiated our study in 1999, we were unsure of what effect camera flash might have on the rubbing response of ocelots. We deployed 2 cameras ad hoc and found that ocelots did rub on the pad at each station. Elsewhere in south Texas, United States Fish and Wildlife Service biologists have conducted ocelot surveys using both scented (WCC) hair snares and cameras at the same sites. Shinn (2002) documented 8 rubs by ocelots (3 by females, 5 by males) at 2 tracts of land on the Lower Rio Grande Valley National Wildlife Refuge. He recorded ocelot hair on snares at 5 of 6 stations where automatic cameras photographed ocelots. More recently, refuge biologists have videographed some of these same ocelots rubbing for 5-7 minutes on scented snares at these sites, which facilitated photographic identification (M. Sternberg and A. Chapa, United States Fish and Wildlife Service, unpublished

We did not detect any bobcats rubbing on the hair snares at Laguna Atascosa NWR, where ocelots are much more common (Laack 1991). Elsewhere in south Texas, however, biologists recorded bobcat hair on snares on 29 occasions at 16 different tracts but none at the 2 tracts where they detected ocelots (Shinn 2002).

Management implications

Like cameras, scented hair snares and DNA analy-

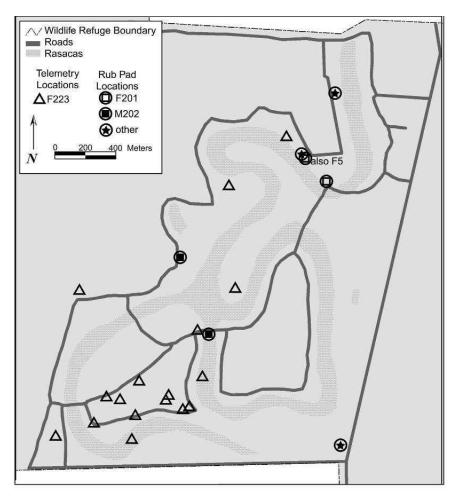


Figure 3. Locations of radiocollared ocelots and snares with ocelot hair in the Island Fields survey unit at Laguna Atascosa National Wildlife Refuge in south Texas, 4–13 May, 2000.

sis offer a non-invasive means of gathering data on ocelot distribution and density, population trends, and association with key habitat and landscape conditions. Scented hair snares, moreover, can provide unique and vital information on genetics that cannot be obtained with cameras. For example, our survey documented an extremely low level of genetic variation in the ocelot population at LANWR (mean number of alleles per locus=2.0), which corroborated the findings of Walker (1997) that ocelots in south Texas appear isolated from the nearest large population of ocelots 320 km (200 miles) south in northern Mexico.

With ocelots occurring in discrete sites and populations across this trans-border landscape due to habitat fragmentation, clearer understanding of the role of dispersal and colonization could enhance conservation of ocelots considerably. Indeed, dis-

persal remains one of the most enigmatic parameters in population and conservation biology for many species (Waser and Strobeck 1998). Recently, researchers in Australia used microsatellite DNA data and Bayesian statistics to assign dispersing animals to their source population as well as the population where they were subsequently "captured" (Eldridge et al. 2001). Hair snares offer a non-invasive means of gathering a larger number of strategic samples for tracking dispersal and ocelots charting regional connectivity.

We suggest that surveys for ocelots deploy a minimum of 1 station per 25-50 ha, with stations located both along the periphery and within patches of suitable thornscrub habitat. Our experience with other species elsewhere indicates that checking stations at 1-2-week intervals will yield

samples with adequate DNA. Surveys of 4-week duration would provide data suitable for most management purposes. Automatic cameras could be deployed simultaneously and selectively with hair snares to enhance data collection.

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