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Research Article

Hair-snares: A non-invasive method for monitoring felid populations in the Selva Lacandona, Mexico

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Abstract

Non-invasive techniques such as hair snares have been used in conjunction with molecular methods to study species that occur at low densities and have elusive behavior, as an alternative to invasive methods such as trapping and hunting. This study was designed to evaluate the use of hair snares as a non-invasive method for the collection of felid and other mammalian samples in the tropical rainforest of the Selva Lacandona, Chiapas, Mexico. Hair snares were placed along transects in Montes Azules Biosphere Reserve for four months a year in 2005 and 2006. Hairs were selected based on morphological characteristics and identification of species was done based on a diagnostic portion of mtDNA cytochrome *b* region. A total of 389 hits on 888 hair-snare checks were recorded, representing a capture rate of 43%. The species identified included margay (*Leopardus wiedii,* n=2), ocelot (*Leopardus pardalis,* n=1), jaguarundi (*Puma yagouaroundi,* n=1), gray fox (*Urocyon cinereoargenteus,* n=1), tayra (*Eira barbara,* n=3), coati (*Nasua narica,* n=1), four-eyed opossum (*Metachirus nudicaudatus,* n=6), and common opossum (*Didelphis marsupialis,* n=16). The present study is the first to report the successful collection of hair samples from jaguarundi and margay in the wild and hair samples from ocelots in tropical areas. The deficit of information on carnivore populations in tropical rainforests is due mainly to the lack of appropriate methodologies that are reliable and cost-effective. This study supports the assumption that hair-snaring is viable and cost-effective in ecosystems such as the Selva Lacandona, particularly when monitoring carnivore populations that have wide geographic distributions and low densities.

Key words: Felids; Hair-snares; *Leopardus pardalis; Leopardus wiedii;* Selva Lacandona; Mexico

Resumen

Las técnicas no invasivas tales como las trampas de pelo han sido utilizadas junto con métodos moleculares para estudiar especies de mamíferos que ocurren en bajas densidades o que presentan un comportamiento elusivo, tratando de encontrar una alternativa a métodos invasivos como la colecta. Este estudio evalúa el uso de las trampas de pelo para la obtención de muestras de felinos y otros mamíferos en bosques tropicales como la Selva Lacandona de Chiapas, México. Las trampas de pelo se colocaron en transectos dentro de la Reserva de la Biosfera Montes Azules durante cuatro meses en 2005 y 2006. Las trampas fueron reemplazadas cada mes y las muestras obtenidas se seleccionaron en base a las características morfológicas del pelo. La identificación de las especies se realizó con base en una porción del gen mitocondrial citocromo *b*. Un total de 389 muestras fueron colectadas en 888 revisiones de las trampas. Las especies reportadas incluyen: margay (*Leopardus wiedii,* n=2), ocelote (*Leopardus pardalis,* n=1), jaguarundi (*Puma yagouaroundi,* n=1), zorra gris (*Urocyon cinereoargenteus,* n=1), tayra (*Eira barbara,* n=3), coatí (*Nasua narica,* n=1), tlacuache cuatro ojos (*Metachirus nudicaudatus*, n=6) y tlacuache común (*Didelphis marsupialis*, n=16). El presente estudio es el primero en reportar la colecta de muestras mediante trampas de pelo de jaguarundi y margay en vida libre, y muestras de pelo de ocelotes en zonas tropicales. La falta de información de poblaciones de carnívoros en bosques tropicales se debe en gran medida a la carencia de un método confiable y de bajo costo. Este estudio demuestra que el trampeo de pelo es viable, de bajo costo y efectivo en ecosistemas como el del área de estudio, especialmente para monitorear poblaciones de carnívoros que presentan bajas densidades y grandes áreas de actividad.

Palabras clave: Felinos; trampas de pelo; *Leopardus pardalis; Leopardus wiedii;* Selva Lacandona; México

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Introduction

Distribution and abundance are key attributes to understanding the population ecology of a given species. However, gathering field data on these attributes is often difficult for mammals such as felids and other carnivores [1-3]. Non-invasive techniques have been used to study species that occur at low densities and have elusive behavior, providing an alternative to invasive methods such as trapping and hunting. Non-invasive techniques include tracking, automated camera systems, and feces and hair sample collection. Each of these techniques has intrinsic pros and cons and each has been used in various studies where results varied from the detection of a species to the identification of individuals [2, 4-6, 7, 8].

Due to the elusive behavior of felids, their large home ranges, and their frequently remote distribution areas, information on the ecology of wild populations is difficult to obtain with invasive methods. Combining molecular techniques with non-invasive sample collection has been shown to be successful in generating information such as population status that is required to develop viable management strategies in temperate areas.

Hair snares constitute a non-invasive technique to obtain hair samples in topical areas. This method can be an alternative to live trapping that is often logistically difficult, expensive, and invasive. In addition, hair snares have been more successful in obtaining samples in tropical ecosystems than scat collection due to the high decomposition rates of feces in the tropical rainforest [9]. This technique uses rub pads sprayed with specific scents to encourage individual animals to rub and leave hairs. Hair-snare sampling coupled with DNA identification has allowed researchers to assess aspects of carnivore communities such as occurrence and distribution, relative abundance, habitat fragmentation, and human disturbance [9, 10]. This approach has been previously undertaken in temperate areas for a variety of carnivore species [10], including lynx (*Lynx canadensis*) [11-13], bobcat (*Lynx rufus*) [9], puma (*Puma concolor*) [9, 12], and ocelot (*Leopardus pardalis*) [14]. Nevertheless, some researchers targeting puma and margay (*Leopardus wiedii*) using this technique were unsuccessful in obtaining results [15].

This paper aims to evaluate the usefulness of hair snares to collect mammalian samples, with particular interest in feline species in the Selva Lacandona, a tropical rainforest of southern Mexico. Five felid species occur in this area: jaguar (*Panthera onca*), puma, ocelot, jaguarundi (*Puma yagouaroundi*) and margay. Based on the usefulness shown by this technique in temperate areas and the urgent need of information on carnivores such as felids in tropical areas, the primary goals of this study were: (1) to test the use of hair snares to report mammalian presence in both disturbed and pristine sites of the Selva Lacandona, Chiapas, Mexico; (2) to test the use of hair snares combined with molecular techniques to obtain data as an alternative to conventional methods

used in tropical areas to study felid species; and (3) to report species of mammals present in the Selva Lacandona that could be targeted by using hair snares combined with molecular identification.

Methods

Study Area

The Selva Lacandona is located in the southeast portion of Chiapas (16º 05'-17º15' N, 90º30'-91º 30' W), limiting with Guatemala on the east, north and south, and with the Chiapas Highlands on the west [16]. Montes Azules Biosphere Reserve (MABR; 331,200 hectares) is the largest protected area of the Selva Lacadona region. It was established in 1978 and has been recognized internationally as part of UNESCO's Man and Biosphere Program (MAB-UNESCO) since 1979 [17]. The average annual temperature ranges from 24º to 26º C, with maximum and minimum values in May (28º) and January (18º), respectively. Mean annual rainfall is 2,500 to 3,500 mm, with 80% of rains falling between June and November [17].

Hair-snare surveys

Hair snares were made from 25 x 15 cm pieces of carpet with 2 velcro strips and carpet nails. Nails were placed through the carpet in a circular arrangement with 2 velcro strips on each side. Each trap was nailed to the base of a tree approximately 30 cm from the ground, and flagging tape was placed 2 m above each trap. To facilitate rubbing by mammals, we cleared the lowest branches of the tree where the hair-snare was nailed. Each trap was sprayed with a mixture of liquid catnip (*Napeta cataria*), that has been shown to produce rubbing behavior by felines. In addition, commercial carnivore bait for felids "Wildcat Lure # 2" (Hawbaker's Wildcat Lures, Mansfield, Louisiana) was used. Each trap location was identified using a Garmin GPS unit (Model # 12 XL), and locations were downloaded onto a digital mapping system using ArcView (Fig. 1). Prior to the study, hair snares and commercial baits (Wildcat Lure # 2 and catnip) were tested on captive felid species at the Miguel Alvarez del Toro Zoo in Tuxtla Gutierrez, Chiapas. This test on captive animals induced rubbing behavior and resulted in clusters of hairs being left on the hair snare (Fig. 2).

We evaluated the usefulness of hair snares to collect mammalian samples, with particular interest in feline species in the study area, using hair-snare stations. We conducted surveys for four months each during the dry seasons of 2005 (March-June) and 2006 (February-May). One hundred and eleven stations were set up each month at 150-m intervals along 12 line transects that were 1-4 km long. To test for the usefulness of hair snares under different habitat disturbance conditions, we placed hair snares along 8 transects inside and 4 transects outside MABR. Each transect contained 9 to 10 stations depending on accessibility. The predominant habitat type within MABR was pristine rainforest, while 1-3 km^2 secondary forest fragments surrounded by croplands (corn, beans, cacao, and bananas) were present outside the conservation area.

Hair snares were removed from transects each month in order to carefully extract all hair samples from them. On removal, hair snares were placed individually in plastic bags and labeled with the appropriate station number and date. Hairs were subsequently collected from each hair snare using a magnifying glass and stored in envelopes at room temperature with silica gel desiccant until scale patterns were analyzed and identified. Guard hair has a distinctive microstructure based on the scale pattern and has proved to be helpful for the identification of groups of mammals in the wild. Hence, scale patterns were developed from each sample obtained in this study using a imprinting technique and analyzed using a stereoscopic microscope and compared with hair catalogues [18-20]. Comparisons also were made with a reference collection of slides containing hair samples prepared from museum specimens of mammal collections at Mexico's National Autonomous University (UNAM) in Mexico City, El Colegio de la Frontera Sur (ECOSUR) in San Cristobal de Las Casas, and Instituto de Historia Natural (IHN) in Tuxtla Gutierrez. Only hairs with scale patterns similar to felids were selected for DNA analysis.

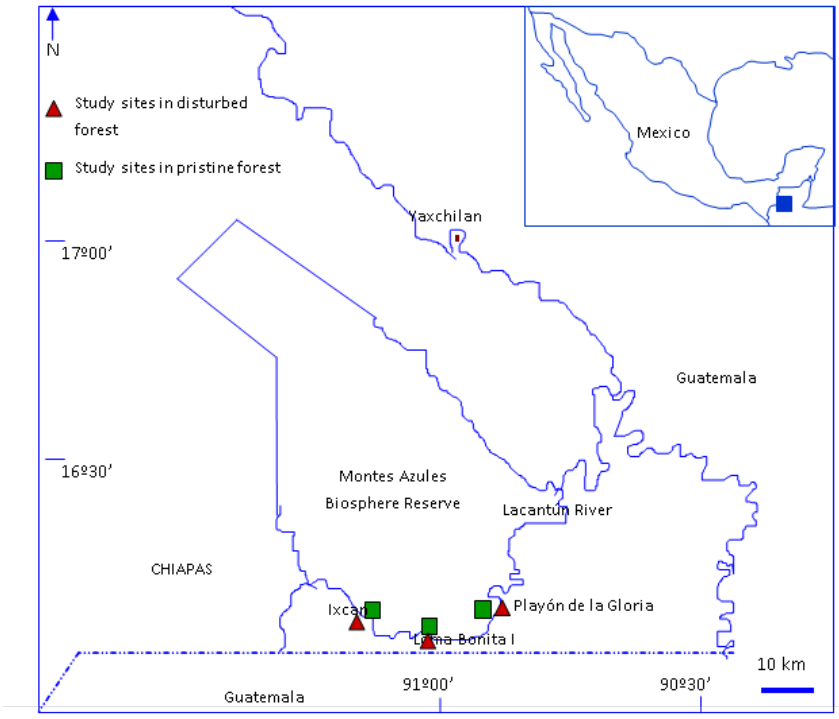

Fig. 1. Location of hair-snare transects in Montes Azules Biosphere Reserve and surrounding sites in the Selva Lacandona, Chiapas, Mexico.

Laboratory analyses

All hair samples were analyzed at the Natural Resources DNA Profiling and Forensic Centre, Trent University, Peterborough, Ontario, Canada. All DNA extractions were carried out using a Qiagen kit for tissue following a standard extraction protocol. Identification to the species level was done based on a diagnostic portion of mtDNA cytochrome *b* region obtained with primers specifically developed by one of the authors (NGA) placed in GeneBank (accession numbers FJ490205-FJ490209) for the five felid species present in the study area. Reference sequences for the five species were obtained from the blood of zoo specimens native to the Chiapas region. Wild species identification was based on analysis and comparison to these reference samples and sequences from GeneBank using the computer program Mega 3.1.

Results

We recorded 389 hits of mammals on 888 hair-snare stations over the two years of study, which represents a total hit rate of 43.8 percent. A total of 270 hits over 560 hair-snare stations (48.2%) was obtained inside MABR, while 119 hits over 328 hair-snare stations (36.3%) were registered in disturbed areas outside the protected area. When analyzing the utility of hair snares by the hits reporting mammalian presence and having 111 stations each day over a total period of 240 days, our results indicated a total rate of 14.6 hits/1000 trap-days ([389 total hits x 1000]/[111 hair-snare stations x 240 days]). In addition, information on the use of hair snares to assess the guild of felid species resulted in 0.15 hits/1000 trap-days over the entire study, having all of them inside the conservation area. The numbers of hits/1000 trap-days for each felid species were: margay (0.075); ocelot (0.07); and jaguarundi (0.07).

Based on scale patterns obtained by the previous examination, only 138 hair samples were selected for DNA analysis. Hairs were selected based on presence of scale patterns approximating those of felids and that had complete hair follicles at their roots. From the 138 samples a total of 41 produced DNA, while the rest failed to yield adequate amounts of non-degraded DNA. From these hair samples, we identified margay (n=2), ocelot

(n=1), and jaguarundi (n=1). Other species identified using the hair snares were gray fox (*Urocyon cinereoargenteus,* n=1), tayra (*Eira barbara,* n=3), coati (*Nasua narica,* n=1), four-eyed opossum (*Metachirus nudicaudatus,* n=6), and common opossum (*Didelphis marsupialis,* n=16). Eight samples were of unknown identity and two samples contained evidence of Mustelids. However, these were excluded due to the low confidence in identification.

Fig. 2. (a) A jaguar (*Panthera onca*) rubbing against the hairsnare baited with lures at the Tuxtla Gutierrez Zoo in Chiapas; (b) a hair sample obtained from the same jaguar.

Discussion

Our hair sample protocol proved useful to assess the presence of a variety of mammalian species in the Selva Lacandona. The percentages of hits recorded was similar to other studies that have reported 46-49% success [9, 15]. Initially, hair snares were designed to target carnivores and in this study to collect felid samples, but it is clear that this method may be used to attract other mammalian species as well. Sometimes this "bycatch" can provide useful information. The fact of knowing that a given species is in the area is often of interest, and this is especially useful in areas such as the tropics where inventories and information are needed. This method has potential for estimating diversity and population abundance, and it could be used in more detailed surveys of non-carnivore species.

A

B

C

D

E

F

Fig. 3. A, B: Views of Montes Azules Biosphere Reserve in Chiapas, Mexico. C, D: Jaguar and ocelot photographed by one of the authors (EN) in the study area. E, F: One of the authors (NG) placing hair snares on trees in the Selva Lacandona.

The hair-snare protocol used in this study allowed for identification of three out of five target felid species, but failed to detect high numbers of individuals of a particular species. Attraction to hair snares by ocelot, jaguarondi, and margay has been previously reported for captive animals [21, 22]. However, this study is the first to report a successful collection of hair samples from jaguarundi and margay in the wild, and hair samples from ocelots in tropical areas. Hair samples from ocelots were low in our study sites, although other researchers have obtained high numbers of hair samples when studying a population of ocelots using hair snares in the drier grasslands of Texas [14, 23]. Differences in the number of hair samples collected for this species may be due to lower population densities typical of tropical areas [24, 25]. The other two species targeted in this study were jaguar and puma. Collection of jaguar hair samples using hair snares in the wild has not been reported to date, while data on puma have produced low returns or non-occurrence [23, 9, 15].

Hair snares are likely to collect a variety of species, particularly in an area with such high diversity of mammals as the Selva Lacandona. To reduce total numbers of mixed samples one can shorten the intervals between checks. Kendall & Mckelvey [10] have suggested a modified box trap in which the door is prevented from locking, allowing captured animals to push the door open to escape but preventing any other animals from entering. Targeting felid species as seen in this and other studies using hair snares, often results in more samples from other mammalian species and this could be due to the nature of felid hairs, which are very short and fine compared to the coarser hair found in canids, ursids, and mustelids [10, 26-28]. In addition, based on the nature of felid hair, specific molecular techniques for low-yield DNA should be applied to increase success [29, 30].

Implications for Felid Conservation

The deficit of information on carnivore populations and specifically felids in tropical ecosystems is partially due to the lack of reliable cost-effective methodologies allowing managers to obtain data that will eventually lead to the development of appropriate management strategies. The hair-snare method and data of mammal species considered in this study indicate the potential that this technique has for obtaining samples from mammal populations in tropical ecosystems. However, refinements in the materials to make hair traps, in the baits used, and in techniques to extract hair samples from traps are needed to increase the utility of this methodology to study felid populations in rainforest ecosystems such as the Selva Lacandona (Fig. 3).

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