

DNA Analysis of Hair and Scat Collected Along Snow Tracks to Document the Presence of Canada Lynx

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Abstract

Snow tracking is often used to inventory carnivore communities, but species identification using this method can produce ambiguous and misleading results. DNA can be extracted from hair and scat samples collected from tracks made in snow. Using DNA analysis could allow positive track identification across a broad range of snow conditions, thus increasing survey accuracy and efficiency. We investigated the efficacy of DNA identification using hairs and scats collected during the winter along putative Canada lynx (*Lynx canadensis*) snow tracks and compared our findings to those obtained using hair-snaring techniques during the summer. We were able to positively identify 81% and 98% of the hair and scat samples, respectively, that were collected in or near snow tracks. Samples containing amplifiable lynx DNA were collected at rates of 1.2–1.3 per km of lynx tracks followed. These amplification rates and encounter frequencies validate the collection and use of DNA samples from snow tracks as a feasible technique for identifying Canada lynx and possibly other rare carnivores. We recommend that biologists include the collection of hairs and scats for DNA analysis as part of snow-tracking surveys whenever species identification is a high priority. (WILDLIFE SOCIETY BULLETIN 34(2):451–455; 2006)

Key words

DNA, hair snares, lynx, *Lynx canadensis*, noninvasive sampling, scats, snow tracking.

Snow tracking has been widely used to assess the presence and relative abundance of mammalian species (Thompson et al. 1989, Becker et al. 1998). Animals prone to wide-ranging movements provide extensive track signatures. For example, Canada lynx (*Lynx canadensis*) move about 5–9 km per day (Mowat et al. 2000). Because of the abundance of such signs, many carnivore biologists believe that snow tracking may be the most efficient method for detecting the presence of rare and wide-ranging species where conditions permit (Squires et al. 2004). However, snow tracking has several limitations: obtaining representative surveys is both labor-intensive and expensive—especially in areas with limited access—and track identification is prone to error (see Aubry and Lewis 2003).

The likelihood of track misidentification increases as snow conditions deteriorate. Thus, tracking is constrained to very specific and sometimes atypical snow conditions. Because snow conditions and access vary spatially and temporally, using snow tracks to monitor organisms at regional or national scales is particularly problematic due to difficulties achieving equal sampling effort across locations (Squires et al. 2004).

Reliability of track identification can be improved with photographs or track casts for other researchers to evaluate. These methods have the added benefit of documenting the observation (Halfpenny et al. 1995). However, snow-track identification remains highly dependent on the judgments of field personnel

who vary in their abilities to accurately identify tracks. Thus, the observer bias that has confounded breeding-bird surveys (Sauer et al. 1994, Link and Sauer 1998, Lloyd and Plaganyi 2002) is problematic for snow-tracking surveys as well.

Methods have recently been developed for snaring hairs from Canada lynx (McDaniel et al. 2000) and analyzing DNA from resulting samples to document species presence (Mills et al. 2000). We used these methods to evaluate lynx distributions in 45 national forests and 5 national parks with potential lynx habitat in the western mountains and northern tier states in the contiguous United States. Although primary detection relied on hair snares, we conducted follow-up snow-tracking surveys in areas where positive lynx detections occurred. Additionally, Squires et al. (2004) used snow tracking to delineate local distributions of lynx in Montana and Wyoming, and stressed the importance of using snow tracks as DNA “collection devices” to facilitate positive species identification. Thus, determining the feasibility of incorporating noninvasive genetic sampling is an important step towards reducing the ambiguity and observer bias associated with using snow tracking to monitor forest carnivores.

If DNA could be routinely collected by following snow tracks, and if a large proportion of samples could provide species identifications, it would offer many potential advantages. First, multiple detection methods are always advantageous, especially if strengths and weaknesses of available methods differ. Second, the method could be added to existing snow-tracking survey protocols, greatly enhancing their reliability. Third, if DNA is

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collected, a clear snow track is no longer required for reliable species identification. This would enable species identifications of tracks that would otherwise be ambiguous, and would greatly expand conditions under which snow-tracking surveys could be conducted, increasing efficiency and relevance. Last, this approach could produce physical evidence of species presence that could be verified independently by other researchers. If a lynx track is suspected, the protocol would be to follow it and attempt to obtain a DNA sample. The DNA sample then becomes the track identifier, rather than the judgment of a field observer.

Our objective was to evaluate the efficacy of this approach by 1) estimating the rates that hairs and scats are encountered along Canada lynx snow tracks; 2) estimating the proportion of samples that could be identified to species, and the proportion that were lynx; and 3) comparing results obtained from snow tracks with those obtained from hair snares in the same area. We then used our findings to provide recommendations for collecting lynx DNA samples along snow tracks.

Study Areas

This study was conducted in 4 study areas located in 3 of the northern tier states: the Meadows and Black Pine Basin study areas in north-central Washington, the Clearwater study area in west-central Montana, and the North Shore study area in northeast Minnesota (Fig. 1). The Meadows study area (200 km²) was 25 km northwest of Conconully, Washington, in the northeastern Cascade Range on the Okanogan National Forest and Loomis State Forest. The Black Pine Basin study area (210 km²) was 15 km southwest of the Meadows area on the Okanogan National Forest, about 3 km northwest of Mazama, Washington. The Clearwater study area (1,800 km²) was located in the Clearwater drainage, approximately 48 km northeast of Missoula, Montana. The North Shore study area (2,000 km²) on the Superior National Forest was located 7–25 km inland along the Lake Superior coast and centered approximately 150 km northeast of Duluth, Minnesota.

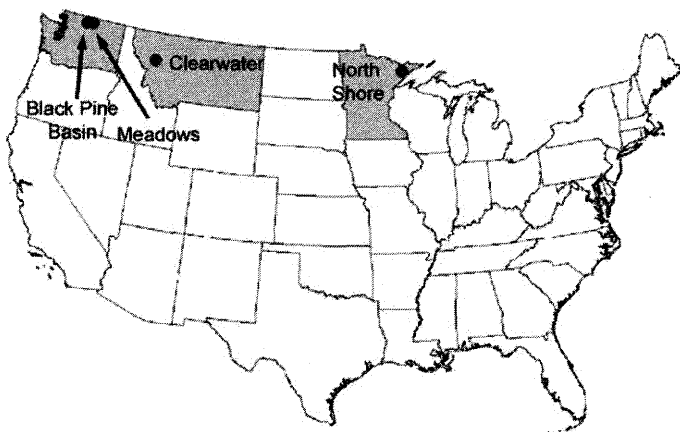


Figure 1. A map showing the locations of the study areas. DNA samples were collected in the Meadows, Black Pine Basin, and North Shore study areas. Daybed frequencies were recorded in the Meadows, Black Pine Basin, and Clearwater study areas. Scat frequencies were recorded in the Meadows and Black Pine Basin study areas.

Methods

We estimated frequency of occurrence, DNA amplification rates, and species composition of hairs and scats collected by following putative lynx snow tracks in the Meadows, Black Pine Basin, and North Shore study areas. Because daybeds (described below) were particularly good locations for collecting hairs, we also estimated the frequency of daybed encounters along lynx snow tracks in the Meadows, Black Pine Basin, and Clearwater study areas. Last, we compared results obtained along snow tracks with those obtained using hair snares during summer in the Meadows study area. Contingency tables were used for all comparisons; reported χ^2 values are Yates corrected (Zar 1999).

If genetic samples are randomly distributed along snow tracks, then the number of samples per kilometer will be Poisson distributed. In this case, the probability of detecting at least 1 genetic sample having traveled X km is $1 - P^X$, where P is the probability of finding 0 samples in a 1-km segment, and is derived from the Poisson distribution. Making these assumptions, and to provide general guidance for designing a snow-tracking study for lynx, we investigated this relationship using data from the Meadows and Black Pine Basin study areas in Washington.

Field Methods

Snow tracking in Washington.—We divided each study area into 6 zones that averaged 34 km² in size. We randomly selected and searched a zone at the beginning of each field season, then searched the next sequential zone each working day after that. Tracks were located by traversing all accessible roads and trails within the zone by snowmobile. If we failed to locate putative lynx tracks in the zone, we searched the next sequential zone until a track was located or we ran out of fuel or time. We followed the freshest track if we found more than one lynx track in a zone. We attempted to follow each track for at least 2 km. We collected spatially referenced data on snow tracks and the locations of beds, other hair-collection sites, and scats with a differentially corrected Global Positioning System (GPS). We estimated distances along lynx tracks using Geographic Information System software. (Arc GIS, ESRI, Redlands, California)

While snow tracking, we searched for scats deposited along the trail and collected hairs in daybeds and ambush beds. Daybeds were sites where animals had curled up and rested, and were characterized by oval-shaped depressions in the snow where a crust had formed. Ambush beds were sites where animals had been sitting down in the snow with their front legs stretched out in front, presumably waiting for potential prey. Generally, ambush beds did not have ice crusts. In the Black Pine Basin, we also searched for hair in lookout beds, which are sites where animals sat upright on their haunches.

Once we located a bed, an observer would kneel or lie down in the snow with eyes approximately 30 cm from the surface of the bed. Typically, only a few hairs had been left at the site, and they were often difficult to see. Therefore, we scanned each bed from 2 or 3 different angles looking for hairs. We also collected hairs found opportunistically along the track. In the Meadows, we collected hairs and scats during 2 consecutive winters of snow tracking (2000–2001 and 2001–2002), but recorded associated substrates (daybeds, ambush beds, etc.) only during the second winter. All

relevant data were collected in Black Pine Basin during 2 consecutive winters of snow tracking (2002–2003 and 2003–2004). **Hair snaring in Washington.**—Hair-snaring surveys were conducted in the Meadows during summer 2001. We placed 25 sampling transects in areas where lynx had been detected during snow-tracking studies the previous winter (McDaniel et al. 2000). Each transect was placed 50–100 m parallel to a road or trail and consisted of 5 hair-snare stations spaced 50–100 m apart depending on visibility within each stand. We spaced stations such that adjacent stations were not visible to observers. Each station consisted of a 15 × 15 cm baited carpet pad imbedded with nails to snare hair, and an aluminum pie plate hung as a visual attractant. Using the protocol from McDaniel et al. (2000), carpet pads were baited with a combination of beaver castor, catnip oil, and catnip. Pads were left in the woods for 1 month and checked every 2 weeks. We re-baited pads during the first check.

Snow tracking in Montana.—We snow-tracked 15 (6 females, 9 males) radiocollared lynx in the Clearwater study area during the winters of 2000 through 2002. We used radiotelemetry to locate lynx sequentially to distribute the sampling effort among individuals. Once an animal was located, we followed its tracks for 2–3 km depending on track condition. Snow tracks and the locations of daybeds were spatially referenced using differentially corrected GPS (DeCesare et al. 2005).

Snow tracking in Minnesota.—Observations of lynx or their tracks were solicited from selected natural resource agency employees or screened from in-house reports. Fourteen target locations were selected, 8 of which were solicited from an individual who claimed to have regularly encountered lynx tracks for about 30 years. All target locations were based on observations that occurred from November 2001 through March 2002. From 22 February to 8 April 2002, we attempted to investigate all target locations. While searching for sign near a target location, lynx tracks were occasionally spotted from access roads but, more often, lynx sign was located by searching suitable forest cover types within a few hundred meters perpendicular to an access road. Once we located putative lynx tracks, we snow tracked for irregular distances and collected all hairs and scats encountered.

DNA Analyses

We placed hair samples in vials filled with 10–18 mesh silica desiccant to inhibit enzyme activity that degrades DNA. Scats were air-dried and placed in paper bags or vials containing silica desiccant. In the laboratory, we used the QIAGEN DNEASY™

Tissue Kit (Qiagen Inc., Valencia, California) and manufacturer protocols to extract DNA from hair and the QIAMP™ DNA Stool Minikit (Qiagen Inc.), and manufacturer protocols to extract DNA from scat. These protocols used 1–10 hairs with follicles or up to 220 mg of scat from each sample. Once DNA was extracted, we used 16S rRNA species identification methods detailed in Mills et al. (2000).

Results

Washington

Meadows study area.—We collected 48 hair samples and 47 scats during the study. Of these 95 samples, 84 were identified as lynx, 6 as nontarget species, and 5 could not be identified (Table 1). We followed lynx tracks a total of 104 km averaging 1.2 km of trail per lynx detection. We followed 61 km of lynx tracks during winter 2001–2002 and encountered 33 daybeds (1 per 1.8 km). We collected hairs from 88% (29/33) of encountered daybeds; 4 samples did not contain quality DNA and the rest were identified as lynx. All scats had quality DNA, and all were identified as lynx. Five samples were collected from 20 ambush beds (25%); 4 were lynx, and 1 was a canid. We collected 3 additional hair samples from various substrates, of which 2 were lynx. Hair-snaring surveys resulted in 37 samples during the summer of 2001, and 84% (31/37) contained quality DNA. Six samples were lynx and 25 were nontarget species. Amplification rates for hair samples collected along winter snow tracks vs. summer hair snares were similar ($\chi^2_1 = 0.22$, $P = 0.643$), but lynx detection rates were higher for snow tracking ($\chi^2_1 = 60.71$, $P < 0.001$).

Black Pine Basin study area.—We followed lynx tracks for 155 km and collected 84 hair samples and 64 scats. Hairs and scats were found at rates of 1 per 1.8 km and 1 per 2.4 km, respectively. We obtained quality DNA from 79% of hair samples (66/84) and 97% of scat samples (62/64). All 128 DNA samples were identified as lynx (Table 1). Snow tracking resulted in 1 lynx detection per 1.2 km of lynx trail followed. We obtained hairs from 74% of daybeds (58/78), 22% of ambush beds (23/103), and 1% of lookout sites (3/321). We encountered daybeds at a rate of 1 per 2.0 km of lynx tracks followed.

Montana

We followed 240 sets of lynx tracks (184 were >2.0 km) associated with 15 radiocollared lynx in the Clearwater study area. We snow tracked lynx for 582 km, and encountered 198 daybeds at a rate of 1 per 2.9 km of lynx tracks followed.

Table 1. Numbers of samples and results of DNA analyses of hairs and scats obtained along snow tracks in the Meadows and Black Pine Basin study areas in Wash., USA, and the North Shore study area in Minn., USA. A summer hair-snaring survey occurred concurrently in the Meadows study area.

Site	Sample type	Samples	Lynx	Other felids	Canids	Other ^a	Failed	Amplification success (%)
Meadows	Scats	47	47	0	0	0	0	100.0
	Hairs (snow tracking)	48	37	0	2	4	5	89.6
	Hairs (hair snaring)	37	6	6	10	9	6	83.8
Black Pine Basin	Scats	64	62	0	0	0	2	96.9
	Hairs	84	66	0	0	0	18	78.6
North Shore	Scats	17	16	0	0	0	1	94.1
	Hairs	20	13 ^b	0	0	1	6	70.0
All areas	All samples	317	247	6	12	14	38	88.0

^a Other includes all other species.

^b One of the samples identified as lynx subsequently was determined to be a lynx/bobcat hybrid (Schwartz et al. 2004).

Minnesota

We collected 20 hair samples and 17 scats in the North Shore study area. We obtained quality DNA from 70% (14/20) of the hair samples and 94% (16/17) of the scats. Of the 30 samples with quality DNA, 29 (97%) were identified as lynx (Table 1). One hair sample identified as lynx subsequently was determined to be a lynx–bobcat hybrid (Schwartz et al. 2004).

Overall, scats proved to be a better source of DNA than hairs ($\chi^2_1 = 17.61$, $P = <0.001$). Of the 128 scats collected in the Meadows, Black Pine Basin, and North Shore study areas, only 3 failed to amplify. Based on the average encounter rate of identifiable lynx samples from the Washington studies (1 sample per 1.2 km), and random distribution of samples along the tracks, there would be a 55.4% chance of obtaining at least 1 lynx DNA sample for each kilometer of lynx track followed. Snow tracking 4 km would result in a >95% chance of documenting lynx occurrence with DNA (Fig. 2).

Discussion

DNA amplification rates for hairs were variable. Rates from the Meadows study area were the highest (89.6%) and the North Shore the lowest (70.0%). In contrast, almost all scats collected contained useful DNA. DNA-amplification rates associated with scats are higher than might be expected based on previously published studies (Frantzen et al. 1998, Creel et al. 2003), possibly because scats were fresh and cold temperatures helped preserve DNA. In Washington, because of the encounter rates for daybeds and scats and the high probability of DNA amplification, following lynx tracks for 2–4 km would result in a high probability of obtaining a usable DNA sample.

The effort required to collect hairs or scats along lynx tracks was virtually identical between the Meadows and Black Pine Basin study areas (1 per 1.3 km and 1 per 1.2 km, respectively), and daybed encounter rates were also similar (1 per 1.8 and 1 per 2.0 km, respectively). Although DNA samples were not collected in Montana, daybeds were encountered at somewhat lower frequencies, 1 per 2.9 km. The range in DNA-amplification and daybed-

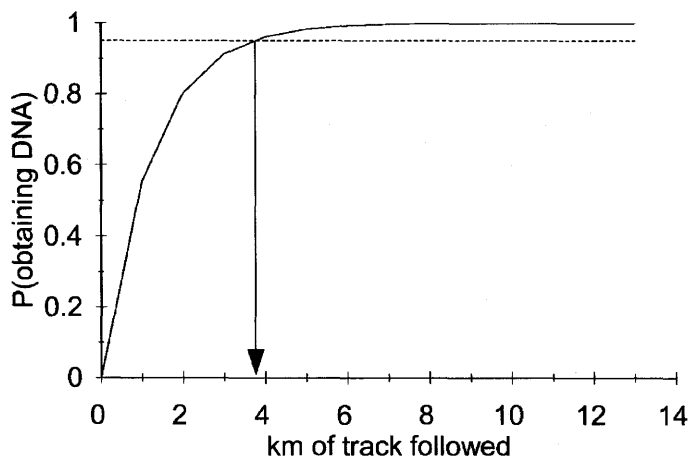


Figure 2. The probability of obtaining DNA based on random distribution of DNA samples along tracks. The sample collection rate is the average from the Meadows and Black Pine Basin study areas in Wash. State. Under these assumptions, a 4 km backtrack will produce useable DNA more than 95% of the time.

encounter rates indicate that opportunities to obtain DNA samples along snow tracks will vary by location. We therefore suggest that initial decisions concerning how far to follow snow tracks be made conservatively. If encounter rates are higher than expected, tracking distances can be reduced.

A detection method based on following snow tracks must be sufficiently intense and representative to have a high probability of crossing tracks if the target species is present in an area (Squires et al. 2004). The more days passed since snowfall, the greater the probability of finding tracks, assuming tracks continue to be identifiable. If species determinations are based on DNA, identification of tracks becomes less critical, and snow-tracking surveys can be conducted across a much wider range of snow conditions. Mistakes represent additional labor but do not produce spurious data. This enables researchers to conduct snow-tracking surveys in areas that could not be sampled with standard techniques due to inadequate or irregular snowfall. It also increases the number of days after snowfall that surveys can be conducted (Squires et al. 2004). However, even with increased sampling flexibility, implementing representative and consistent sampling efforts based on snow tracking may not be possible in areas where access is restricted or snow conditions are highly variable.

In the Meadows study area, DNA identification with samples collected by snow tracking (winter) and hair snaring (summer) documented the presence of lynx; however, samples obtained from snow tracks were much more likely to be lynx (88% vs. 16%). Summer methods are passive, and lynx are preferentially detected only to the extent that the lure is species-specific. However, snow-track identification serves as an effective screening mechanism that minimizes DNA analysis of samples from nontarget species. DNA amplification rates from hairs collected along snow tracks and on hair snares in the Meadows area were both relatively high (89.6 and 83.8%, respectively), but DNA from scats collected along snow tracks almost always amplified (100% in Meadows, 97.7% overall).

Snow tracking lynx in certain areas can be highly effective for obtaining DNA samples and thus result in rapid verification of their presence in an area. The relative efficacy of snow tracking versus hair snaring will, however, vary. Each method has strengths and weaknesses, and the efficacy of each technique will vary with snowfall patterns, topography, ease of access, lynx densities, and many other factors.

Encounter rates of daybeds, scats, and other sources of DNA along snow tracks have only been estimated for lynx. However, we believe this method would also be effective for other species. Among the mammalian carnivores that inhabit boreal forests with winter snowpacks, lynx have relatively large home ranges and travel long distances. Thus, the distances between beds and scats of many other carnivores will likely be shorter than for lynx. If so, then searching for hairs and scats while following the snow tracks of other species should also provide usable DNA samples, and may require shorter tracking distances.

In areas where a given species is of conservation concern, verifiable evidence of its presence and distribution is an essential first step for developing conservation plans or habitat management strategies. For rare and wide-ranging carnivores such as lynx and wolverine (*Gulo gulo*), putative presence in an area is often based on sighting reports or descriptions of tracks. Anecdotal observa-

tions of forest carnivores, however, are inherently unreliable (Aubry and Lewis 2003). In situations where anecdotal reports can be linked to particular snow tracks, the time and resources required to follow snow tracks to collect DNA samples for species identification will likely be worth the investment. Species-level identification uses simple assays and is much less time-consuming and expensive than individual identification. Using restriction enzyme digests (Mills et al. 2000), species-level identification from hair or scat samples takes 2 days. Per sample, we allocate a half-hour of technician time, and materials cost approximately \$5.00. We agree with Squires et al. (2004) that augmenting snow-tracking surveys with collection of hairs and scats for DNA analysis is recommended whenever reliable species identification is a high priority.

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