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A noninvasive survey method for detecting wolverine

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A NONINVASIVE SURVEY METHOD FOR DETECTING WOLVERINE

by

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B.S. The University of Montana, USA, 1999

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Master of Science

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Approved by:

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Date
Abstract:

The wolverine (Gulo gulo) is one of the least understood mammals of North America. The species range underwent a major contraction in the early 1900’s due to fur trapping and human expansion. Wolverine appear to have recolonized some parts of their former range, yet, they are believed to be declining or remain absent in other areas, resulting in concern from the conservation community. While many survey techniques have been developed for wolverine, they all have design limitations or biases that prevent their application for broad scale distribution surveys across differing habitats. During 2003 and 2004, I modified and tested a historically popular management tool, snow track surveys, to provide managers with a more reliable and scientifically defensible method for detecting the presence of wolverine.

My first objective was to modify and test a sampling framework developed by Squires et al. (In Press) for lynx (Lynx canadensis) to determine it’s effectiveness at detecting wolverine in 4 mountain ranges in southwestern Montana. I investigated detection rates and the extent to which the method was limited by topography or administrative restrictions. I detected 64 wolverine tracks in 3 of the 4 mountain ranges during 1550 km of surveys. I used computer simulations to model the probability of detecting wolverine. Both my simulations and field surveys identified the importance of visiting each survey unit 3 or more times during the same winter to ensure high detection probabilities.

My second objective was to develop and test a genetic technique for providing reliable species identification and individual identification from noninvasive genetic samples collected along putative wolverine snow tracks. I completed 54 backtracks of putative wolverine and collected 169 hairs and 58 scats. Amplification rates of mitochondrial DNA (mtDNA) were 74% and 24% for scats and hairs, respectively, and genetic analysis confirmed 35 snow tracks (64%) as wolverine. Amplification rates of nuclear DNA (nDNA) from scats and hairs were 52% and 16%, respectively, and produced individual genotypes for 23 of the 54 snow tracks (43%).

The ability of this method to provide species identification for 63% of tracks and individual identifications for 43% of tracks holds promise for the collection of more reliable distribution data and population monitoring of wolverines.
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Finally, I wish to thank the wolverine of southwestern Montana. Snow tracking such a rare and amazing animal was a privilege (if not a bit exhausting). Learning the distances they travel, the topography they navigate, and the manner in which they eke out a living was nothing less than astonishing. Unfortunately, there is often a cost associated with such privilege. The increased awareness of wolverine in southwest Montana, due in part to my research, resulted in many of these study animals paying the ultimate price.
Chapter 1: Introduction

BACKGROUND

The wolverine (*Gulo gulo*) is a rare and elusive species that entered the American consciousness as the topic of incredible folklore and trapper’s tales. Rigorous research attempts to shed scientific light on wolverine ecology have been confounded by the fact that the species naturally occurs at low densities, inhabits often remote tundra and boreal forest habitats, and has huge spatial requirements. Despite major research efforts in Alaska, Canada, and the contiguous United States, the wolverine continues to be one of the least understood mammals in North America (Banci 1994). We do know that healthy wolverine populations have low reproductive rates and occur at low absolute densities compared to similar-sized carnivores (Banci 1994), traits that affect their ability to recover from population declines. Uncertainty regarding the effects of winter recreation on wolverine reproduction, the effect of human-caused mortality on population connectivity and persistence, habitat alteration, and the adequacy of landscapes capable of sustaining wolverine has resulted in concern over the conservation of the species.

Petitions to list the wolverine for protection under the Endangered Species Act were filed in 1994 and 2000 based on concerns over loss of habitat, over-utilization, and a contraction in distribution since the 1970’s (Biodiversity Legal Foundation 2000). The United States Fish and Wildlife Service (USFWS) determined protection was unwarranted in 1995 (Federal Register Vol. 60., No. 75, 1995) and declined to consider the species for protection under the Endangered Species Act in 2003 (Federal Register, Vol. 68, No. 203, 2003), citing the lack of understanding of the species distribution in the contiguous United States as one primary reason. Historical observations, anecdotal
reports, and trapper harvest records vary in reliability and do not account for effort (McKelvey et al. 2000, Aubry and Lewis 2003), making them, alone, inadequate for determining wolverine distribution. In addition, formal attempts by state and federal agencies to monitor trends in wolverine occurrence on a more regional basis have either not been formally conducted or have been hindered by conflicting detection results (Forkan et al. 1999, John Squires, unpublished data), an inability to complete all planned surveys over multiple years (B. Giddings., Montana Fish, Wildlife, and Parks furbearer coordinator, personal communication), and unequal sampling effort (McKelvey et al. In Press). The resulting uncertainty regarding wolverine distribution hinders our ability to manage and conserve this species within the contiguous United States.

Winter snow track surveys have historically been a popular management tool for state and federal agencies because they were relatively easy to conduct, believed to have high detection rates, and were well suited to the high daily movement rates and wide-ranging habits of many carnivores (McKelvey et al. In Press, Squires et al. In Press). Unfortunately, they have been hindered by non-representative designs and unreliable results. The political and management ramifications of making incorrect or equivocal track identifications (“false positives”) regarding rare or protected species demand that data are reliable and defensible (Halfpenny et al. 1995). In this thesis, I attempt to build upon the concept of winter track surveys by incorporating noninvasive genetic techniques and a more intensive sampling plan to improve the method's ability to provide high detection probabilities and unequivocal results.
OBJECTIVES

My overall objective was to improve our understanding of wolverine distribution and ecology by providing a reliable track survey framework for detecting wolverine in the contiguous United States. My thesis is composed of 2 main chapters. In chapter 1, I modified and tested a winter snow track survey method that was designed to be spatially representative and was successfully applied to detecting lynx in the contiguous United States (Squires et al. In Press). I then tested the method in 4 mountain ranges in southwestern Montana to determine actual detection rates for wolverines, the logistical requirements of implementing the method, and the extent to which the method was limited by terrain or other factors. I used computer simulations to model survey effort relative to detection probability to optimize efficiency in surveying for wolverine.

In chapter 2, I developed and tested a noninvasive genetic technique to be used in combination with snow track surveys to provide definitive species identification through the genetic analysis of mitochondrial DNA (mtDNA). Collecting genetic samples from snow tracks is a relatively new method (Flagstad et al. 2004, Schwartz et al. 2004, McKelvey et al. In Press), and no results have been reported regarding the frequency that wolverine hair and scat samples can be found along a snow track. Therefore, we do not understand whether this technique has adequate sample collection rates to be a feasible method. I addressed this issue by designing and testing a snow tracking protocol for collecting genetic samples from putative wolverine tracks and reported results on sample collection rates, sample distribution, and genetic amplification rates. I also investigated whether the method could be used not only to detect the species, but to detect and identify actual individuals through analysis of nuclear DNA (nDNA). A method capable
of both species and individual identification would not only provide detection data for
distribution, but could be applied in the monitoring of wolverine populations.

The 2 chapters of this thesis are intended to provide a comprehensive survey
method that can be used to better conduct surveys for wolverine in the contiguous United
States. The format is directed at land and wildlife managers in that I present results
regarding logistic issues and effort calculations to address concerns regarding
applicability, expected results, and logistic effort. The survey sampling plan and the
noninvasive sample collection technique presented, when used in conjunction with one
another, are aimed at getting the most data possible from snow tracks, regardless of age
and condition, and are capable of meeting stringent data standards. It is my hope that this
method will help to improve our understanding of wolverine distribution and population
ecology, thereby, helping to inform management and conservation decisions regarding
this rare species.

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Chapter 2: Testing a Winter Survey Method for Detecting Wolverine

**ABSTRACT:** Concerns over the status of wolverine (*Gulo gulo*) populations in the contiguous United States have elevated the need for a more comprehensive understanding of wolverine distribution. We used snow tracking to test whether we could detect and confirm wolverine presence in 4 mountain ranges in southwest Montana. We completed 2 surveys of a grid consisting of 76 survey units, each unit measuring 8 km by 8 km, overlaid on potential wolverine habitat. Surveys were conducted by traversing 10 km within each survey unit in search of wolverine snow tracks. We detected 64 wolverine tracks in 3 of the 4 mountain ranges during 2 surveys covering a total of 1550 km. We completed 88% and 84% of survey units during surveys 1 and 2, respectively, while surveys of the remaining units were restricted due to a lack of road and trail access and low snow pack.

We used computer simulations to model the probability of detecting wolverine given 2 different survey unit sizes and differences in gender-based spatial requirements. Simulations were most effective at detecting wolverine during the period 4-10 days after a major snow or wind event. In the field, detection probabilities were highest 4-7 days after a major weather event, after which time detections decreased. Both our simulations and field surveys identified the importance of visiting each survey unit 3 or more times during the same winter to ensure high detection probabilities.

**INTRODUCTION**

The wolverine is a rare and elusive species that inhabits the boreal and tundra zones of Eurasia and similar areas north of the 38th parallel in North America. The historical distribution (pre-1900’s) of wolverine in North America stretched from Alaska
and Canada south through the Cascades and Sierra Nevada range to southern California (Grinnell et al. 1937); south along the Rocky Mountains to Arizona and New Mexico (Hash 1987); and may have included multiple Northern Plains and Great Lakes states (de Vos 1964). Wolverine distribution in the contiguous United States underwent a significant contraction in the late 1800’s and early 1900’s as a result of heavy harvest pressure and expanding human settlement (Hash 1987, Lyon et al. 1994). The wolverine is currently absent from the Great Lakes and Northern Plains states and its occurrence is uncertain in the western United States outside of Idaho, Montana, and Wyoming (Banci 1994). While wolverine are known to exist in these 3 states, an understanding of distribution and population connectivity is necessary for the conservation and management of the species.

Our existing understanding of wolverine distribution is inadequate to accurately predict areas that wolverine inhabit. Wolverine are often associated with remote areas where they are difficult to survey and study. Aside from a general association with remote areas, we have little information about the landscape characteristics that are associated with wolverine occurrence. The presence of large ungulate populations and areas undeveloped by humans have been cited as important requirements (Banci 1994), yet these factors alone are poor predictors of wolverine presence. Harvest results, unverified sightings, and a variety of small-scale surveys have not provided an understanding of wolverine distribution at the appropriate scale over the time period necessary to properly manage the species.

Cegelski et al. (2003) suggested that wolverine in Montana exhibited genetic structuring that is indicative of isolation and a lack of gene flow. Such findings,
combined with the naturally low density of wolverine, have raised concerns over wolverine persistence in the contiguous United States. The lack of ecological understanding and a perception of low population numbers have led to the filing of 2 petitions to list the wolverine for protection under the federal Endangered Species Act (ESA). The United States Fish and Wildlife Service (USFWS) responded to these petitions by citing the lack of distribution data as a major factor in its final decision not to consider the wolverine for protection (Federal Register, Vol. 60, No. 75, 1995; Federal Register, Vol. 68, No. 203, 2003), hence, the importance of our study.

Many survey methods have been developed for determining wolverine occurrence, including aerial transect surveys (Becker 1991), surveys of potential denning habitat using helicopters (Heinemeyer et al. 2001), ground-based snow-track surveys (Thompson et al. 1989, Stephenson and Karczmarczyk 1989, Halfpenny et al. 1995, Beier and Cunningham 1996, Becker et al. 1999), track counts at bait stations (Copeland 1993), remote camera stations (Copeland 1993), sooted track plates (Taylor and Raphael 1988, Zielinski and Kucera 1995), hair-collection devices (McDaniel et al. 2000), trapper questionnaires (Groves 1988), and harvest results (Johnson 1991). However, all of these methods exhibit limitations that have prevented their widespread application.

We employed winter snow track surveys to detect the presence of wolverine in 4 mountain ranges in SW Montana. Given the high vagility and relatively low density of this species, we believe traversing large tracts of habitat to detect snow tracks may be more effective for detecting wolverine than is luring individual animals into camera stations, hair collection devices, or bait stations. However, while snow track surveys may be efficient, they have been plagued by design, track identification, and sampling issues.
Recently, Squires et al. (In Press) presented a sampling framework for detecting Canada lynx that is spatially representative, has a high probability of detection, and has shown promising results. Here, we test this method, with slight modifications, to determine whether the method is more generalizeable to other rare species, in particular, the wolverine. Our specific objectives were to:

1) quantify the detection rates of this survey method for determining wolverine presence in a SW Montana;

2) determine the extent to which the method is limited by topography, administrative closure, access, or other logistical considerations;

3) use computer simulations to predict the probability of detecting wolverine given different survey unit sizes, # of survey iterations, and snow frequencies;

4) provide information on the effort required to implement the method.

STUDY AREA

The study area was composed of all Beaverhead-Deerlodge National Forest lands within the Pioneer, Beaverhead, Anaconda-Pintler, and Flint Creek mountain ranges in southwest Montana (Figure 1). The Pioneer Mountains are located east and south of the Big Hole River valley and are bounded to the east by Interstate 15 and State Highway 43 to the north and west. Elevations range from approximately 1,830 m to 3,350 m with the highest peaks located in the eastern portion of the range. The dominant forest cover for the Pioneer Mountains is lodgepole pine (*Pinus contorta*). Lodgepole pine gives way to Douglas-fir (*Pseudotsuga menziesii*) and sagebrush (*Artemisia* spp.) steppe at lower elevations and on south-facing slopes. Mixed Engelmann spruce (*Picea engelmanni*)/subalpine fir (*Abies lasiocarpa*) forests are found on wet aspects at higher elevations.
Whitebark pine (*Pinus albicaulis*) occurs at the highest elevations near timberline. Riparian communities are dominated by willows (*Salix* spp.) that often transition into sagebrush dominated meadows.

The Beaverhead range is located on the Idaho/Montana border on the west side of the Big Hole valley. The range is oriented in a north/south fashion and abuts the Anaconda-Pintler mountain range at Highway 43 near Lost Trail Pass. The Anaconda-Pintler range continues in a northeast direction to the town of Anaconda. Habitat types in these ranges are similar to those in the Pioneers, although sagebrush openings are less common due to more mesic conditions. Higher elevations are dominated by mixed subalpine forests changing to lodgepole pine at mid-elevations on most aspects. Douglas-fir is found on drier sites at lower elevations. The Flint Creek range is located north of the town of Anaconda and is bordered by Highway 1 to the south and west and I-90 to the north and east. Vegetation types are similar to the Anaconda-Pintler and Beaverhead range.

**METHODS**

**Snow track surveys**

Protocols for performing the surveys closely followed guidelines outlined by Squires et al. (In Press). We defined potential wolverine habitat as all forested areas and areas above tree line (Hornocker and Hash 1981), which primarily excluded low elevation sagebrush and agricultural land, and delineated this habitat using Beaverhead-Deerlodge National Forest GIS vegetation layers in ArcGIS 8.3. We overlaid this habitat with a survey grid consisting of 8 km x 8 km survey units and assigned each unit a unique number (Figure 1). Technicians randomly selected a unit to survey each day from the
population of units, and continued without replacement until all survey units had been completed. A unit was considered “surveyed” when 10 km had been traversed across the unit. We maximized detection rates by attempting to traverse across the entire unit, rather than completing 10 km within a small portion of the survey unit. Our survey was designed to be “representative” in that survey units were spatially distributed across all potential wolverine habitat (Squires et al. In Press); yet, surveys within each unit were conducted with preference given to the subalpine fir/whitebark pine habitats in each unit to maximize detection probabilities. Existing roads and trails were used for survey routes when possible, and we used skis or snowshoes to survey units that lacked the roads, trails, or tree spacing to allow snowmobile travel.

Data regarding survey routes and wolverine detections were collected with a data logging GPS unit (Trimble Geoexplorer 3). If time allowed, additional units adjacent to the selected unit were also surveyed each day in the same manner. We used this “cluster” sampling design because the cost and time required to travel to another randomly selected unit during the same day typically precluded completion of that unit. We completed surveys of all units twice during the 2004 winter.

A single detection is all that is needed to establish species presence within a survey area; yet, multiple detections are useful in providing more accurate spatial use information. We quantified the survey effort needed to first detect a wolverine by measuring the distance traveled in all survey units leading up to a detection. Because multiple detections in the same survey unit or in adjacent survey units on the same day may not be independent (i.e. they potentially belong to the same individual), we only estimated distance/detection to the first track found per day. We did not curtail
subsequent surveys after the initial day with a detection, but rather continued to census our survey units to increase our sample size of latency to first detection by measuring effort anew the day following each detection. We determined average effort required to detect wolverine, the proportion of survey units that could not be completed using the survey method, and the proportion that contained wolverine detections.

**Simulations**

The probability of detecting a target species must be as close to 1 as possible for a survey method to accurately provide presence/absence data. We addressed this issue by modifying a task-specific TurboPascal program (Squires et al. In Press) to model detection probabilities relative to our survey design. Because wolverine have large, gender-based differences in home range size and average daily distances traveled, we developed separate simulations for males and females. We tested the effect of survey unit size by using 2 different unit sizes, 8 km x 8 km (64 km²) and 12 km x 12 km (144 km²), which in the model were represented by an 8 km transect and a 12 km transect, respectively. The 64 km² survey unit was used by Squires et al. (In Press) for lynx; however, wolverine have a much larger average home range size. We tested the larger size survey unit to determine if we could decrease our survey effort without sacrificing a high probability of detection. The results were 4 unique simulations that quantified the probability of detecting a male or female wolverine using 64 km² and 144 km² sampling units.

We designed a simulation program that randomly placed a wolverine within a circular home range and randomly moved the animal according to tortuosity and average daily movement inputs, recording the number of days required until the animal crossed
the survey transect. Home range size was calculated as an average of published minimum convex polygon home ranges (Hornocker and Hash 1981, Magoun 1985) and our own telemetry data, resulting in home range sizes of 840 km$^2$ for males and 300 km$^2$ for females. We calculated a tortuosity metric (Benhamou 2004) of 1.7 (animal path distance/straight-line distance) from our unpublished snow tracking data and applied this metric to average daily distances of 4.7 km/day for females and 6.3 km/day for males (Magoun et al. 2004, Copeland, J., Personal Communication) to replicate realistic wolverine travel patterns. The simulation was repeated 1000 times for each of the 4 designs. We determined how the probability of detection changed based on the number of days since the last track-obscuring snow and the number of times a survey unit was visited within a single winter.

RESULTS

Snow track surveys

The first survey was conducted from 18 January through 2 March 2004 and required 26 “crew days” to complete 67 of the 76 survey units (88%); 3 of the remaining units provided limited snowmobile access (<6.5 km surveyed) and 6 were not completed due to a lack of road and trail access (i.e. no skiing was attempted during this survey). We completed a total of 799 km of transects during the first survey and detected 31 wolverine tracks. Wolverine were detected in 16 of the 70 units that were at least partially surveyed (23%). The average distance required to first detect a wolverine was 42 km.

The second survey was conducted between 3 March and 22 March 2004 and required 22 “crew days” to complete. We completed 64 of the 76 units (82%); 2 of the
remaining units were partially surveyed (< 6.5 km surveyed), 3 were not completed due to access, and 7 were not completed due to inadequate snow cover. We completed a total of 748 km of transects in the units surveyed and detected a total of 34 wolverine tracks. Wolverine were detected in 15 of the 66 units surveyed (23%). The average distance required to first detect a wolverine during the second survey was 61 km.

The combined surveys resulted in 96% of the survey units being completed at least once and 78% of survey units being completed twice. Three survey units could not be completed during either survey due to inadequate access, 3 units were completed a single time using skis, and 7 units could not be completed a second time due to a lack of snow coverage at the end of the second survey. We detected a total of 64 putative wolverine tracks in 23 of the 76 units (30%)(Table 1).

Detection rates were highest for surveys conducted 4 to 7 days after snow (8 detections/17 units; 47%). Fourteen percent (12 of 84 units) of survey units that were completed between 1 and 3 days after a snow event had detections, while the detection rates for surveys conducted during periods ≥ 7 days after snow was 24% (11 of 41 units).

**Simulations**

The probability of detection increased in all scenarios as the number of days since the last snowfall and the number of times a unit was surveyed during a single winter increased (Table 2). The smaller home ranges of female wolverine resulted in higher probabilities of detection using both the 64 km² and 144 km² survey units than for those of male wolverine. While the probability of detecting an animal in our simulations continued to increase with time, our ground effort suggested that the probability was at its maximum at approximately 7 days after a major snowfall, after which time track
deterioration and the tracks of multiple species confounded detection activities. Using this 7-day cut off, the maximum probability of detecting a female wolverine using a 64 km$^2$ unit ranged from 0.58 for a single survey to 0.93 for three surveys in a single winter. Using a 144 km$^2$ unit, these probabilities increased to 0.80 and 0.99, respectively. The maximum probability of detecting a male wolverine using a 64 km$^2$ unit size, again using the 7-day cut off, was 0.40 for a single survey and 0.79 for three surveys in a winter. These increased to 0.51 for a single survey and 0.88 for 3 surveys in a winter for a 144 km$^2$ survey unit size.

DISCUSSION

Detection rates

Our survey protocol revealed the presence of wolverine in 3 of the 4 mountain ranges surveyed in southwest Montana (Table 1). The limited detections in the Anaconda-Pintlers and no detection in the Flints were informative relative to major issues raised by Zielinski and Kucera (1995), namely: 1) the importance of a representative sampling design; and 2) the importance of conducting multiple surveys in a single winter.

Surveys in the Pioneer, Beaverhead, and Flint Creek ranges were spatially representative in that survey units were delineated and completed across the entire area of interest. Conversely, surveys in the Anaconda-Pintler range were restricted by the presence of a federal wilderness area, which we decided \textit{a priori} not to survey due to logistical considerations. While we were still able to detect wolverine by surveying around the periphery of the wilderness, we believe detection rates were lower as a function of wolverine inhabiting large areas not subject to our surveys. Our inability to survey the wilderness for logistic reasons should not be confused with an inability to
apply the method in the wilderness area. Not all geographic areas are conducive to snowmobile-based surveys because of restricted access resulting from a lack of roads and trails, administrative closures (i.e. federal wilderness), or topography; however, these restrictions can be overcome by conducting the surveys using skis and snowshoes. Although these methods of travel require more effort and time, they are no less effective at detecting wolverine. Therefore, when planning a survey effort, potential areas with limited snowmobile access should be identified and the necessary steps taken to ensure these units are surveyed to avoid the exclusion of large areas of wolverine habitat.

The lack of detections in the Flint Creek range provided evidence that multiple visits to each survey unit during a single winter are needed to improve detection probabilities. We know from our trap checking activities, which are part of a larger research effort, that at least one wolverine used this area, yet no tracks were detected during either survey. Increasing the number of times the units were visited, and, if possible, choosing a window of opportunity with the best probability of detection (4-7 days after snow), would have increased the chance that this animal(s) was detected.

Detection probabilities

The simulated probability of detecting wolverine varied according to the time since last snowfall, the size of the survey unit, and the gender of the animal. We investigated the probability of detecting wolverine given gender-based differences in movement rates and spatial arrangements to better understand the method’s effectiveness; however, the goal of a survey protocol is to find a wolverine regardless of these factors. From a management perspective, the probabilities of detection from the simulations serve as conservative guidelines. Given the design of the simulations, these probabilities of
detection are more representative of areas with small, non-overlapping populations (i.e. a single individual or multiple adult males); however, in areas where a male wolverine territory overlaps at least one female, as is typical in resident populations, the detection probabilities would be higher given the presence of multiple animals within the same survey unit.

The probability of detection also increased using the larger survey unit size. While smaller survey units, such as 4 mi x 4 mi or 8 km x 8 km, have been used for previous forest carnivore surveys (Zielinski and Kucera 1995, Squires et al. In Press), surveys focused solely on wolverine may improve efficiency by using the 12 km x 12 km, or larger, unit. The tradeoff is that at some point in increasing the unit size configuration, the length of the survey transect needed for larger units would become difficult to complete in a single day. Conversely, when surveying for multiple species of interest, we recommend using a unit size based on the species with the smallest home range size to prevent individuals from inhabiting a portion of a survey unit and potentially being missed by survey efforts.

Simulated detection probabilities increased as the period after a snowstorm increased, which allowed more time for tracks to accumulate; however, this pattern held true up to approximately 7 days during our actual surveys, at which point a variety of factors made detection probabilities decline. Snow tracking is typically relegated to a relatively small time period when there is adequate snow accumulation; therefore, time and logistical constraints may dictate that one can not wait for the period of 4-10 days after snow to conduct a survey. Rather than planning a survey to occur during a certain time period after snowfall (e.g. >72 hours after snow) to ensure high detection
probabilities, it may be more feasible to increase detection probabilities by revisiting each survey unit multiple times during the same winter. Based on our simulations, we estimated that 3 surveys conducted during optimal times (4-7 days after snowfall) in the same winter, using the 8 km x 8 km survey unit, should provide detection probabilities no lower than 0.79. However, if surveys are conducted at less optimal times or if a higher probability is required, we suggest increasing the number of survey visits to more than 3 in a winter, or conducting multiple years of surveys.

 Costs and logistics

The first step in preparing for a survey is to delineate the geographic area of interest and to prepare the survey grid and maps, which can be completed within a day by an experienced GIS user. The second step is to determine the number of survey crews that will be needed based on the size of the area to be surveyed. Due to safety concerns, each crew should consist of 2 trained personnel. We found no economies of scale, relative to expenditures, by having more than a single crew. Rather, the deciding factor should be the number of crews necessary to survey the area of the size selected within the window of reasonable tracking conditions specific to the area under consideration. While we reported that 22 and 26 “crew days” were required to complete the first and second surveys in our 4,864 km² study area, the number of survey units that can be completed within a day, and under reasonable tracking conditions, will depend on local weather conditions, access, and the skill of observers. Managers should consider the number of units in the survey area, the number of times each unit will be surveyed, and the expected number of units that can be completed in a day, in determining the appropriate number of crews. We recommend erring on the conservative side and having too many crews finish
earlier than expected than in having too few crews unable to complete the surveys as designed. The downside to this approach is that more funding is necessary to outfit the additional crews.

Each crew will typically require 1 4-wheel drive vehicle, 1 trailer, 2 snowmobiles, housing, and salaries for all or portions of a 3 month period. Size and accessibility of the survey area will dictate fuel costs for both the vehicle and snowmobiles, but this expense should not be underestimated. In addition, each crew member will require safety and field training and equipment including, at a minimum, an avalanche beacon, two-way radio, GPS unit, winter clothing, backpack, helmet, and snowshoes or skis. We recommend each crew have at least 1 satellite phone for areas with poor radio coverage and in case of emergencies. These costs will vary depending on the group performing the survey (e.g. non-government organizations, state agencies, and federal agencies) and whether existing equipment can be used.

MANAGEMENT IMPLICATIONS

The survey method we tested was successful at detecting wolverine in fairly typical habitats of Montana. The method’s flexibility regarding the mode of transportation allows each survey to be custom tailored to the accessibility of each specific site. The presence of a winter snow pack is the only prerequisite for employing the method.

This track detection method, when combined with a noninvasive genetic sampling technique to provide definitive track identification (Flagstad et al. 2004, 2005, Squires et al. In Press, McKelvey et al. In Press, Chapter 2), provides a survey framework for attempting to better delineate and understand wolverine distribution in the contiguous
United States. Improved occurrence and distribution data provided by such a framework is necessary to address wolverine conservation concerns regarding population status and connectivity, impacts of harvest and winter recreation, and general land management.

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Figure 1: Our study area and location of survey units in southwest Montana during 2004.
Table 1: Wolverine detection results from 2 surveys conducted in 4 mountain ranges in southwestern Montana in 2004.

<table>
<thead>
<tr>
<th>Location</th>
<th># of survey days</th>
<th># of survey days with detections</th>
<th>% of survey days with detections</th>
<th># of units with detections</th>
<th>Total track detections</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Survey 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pioneers</td>
<td>12</td>
<td>5</td>
<td>42%</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Beaverheads</td>
<td>3</td>
<td>3</td>
<td>100%</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Pintlers</td>
<td>6</td>
<td>3</td>
<td>50%</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Flints</td>
<td>5</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>26</td>
<td>11</td>
<td>42%</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td><strong>Survey 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pioneers</td>
<td>12</td>
<td>7</td>
<td>58%</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Beaverheads</td>
<td>2</td>
<td>1</td>
<td>50%</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Pintlers</td>
<td>5</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flints</td>
<td>3</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>22</td>
<td>8</td>
<td>36%</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>48</td>
<td>19</td>
<td>40%</td>
<td>31</td>
<td>64</td>
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</tbody>
</table>
Table 2: Simulated probabilities of detecting wolverine tracks based on the number of days after snow fall during which the survey is conducted and the number of times a unit is surveyed during a single winter. Separate simulations conducted for each gender and survey unit size.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Survey Unit Size</th>
<th>1 survey per winter</th>
<th>2 surveys per winter</th>
<th>3 surveys per winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 day</td>
<td>4 day</td>
<td>7 day</td>
</tr>
<tr>
<td>Female</td>
<td>8 km</td>
<td>0.19</td>
<td>0.44</td>
<td>0.58</td>
</tr>
<tr>
<td>Female</td>
<td>12 km</td>
<td>0.25</td>
<td>0.61</td>
<td>0.80</td>
</tr>
<tr>
<td>Male</td>
<td>8 km</td>
<td>0.10</td>
<td>0.29</td>
<td>0.40</td>
</tr>
<tr>
<td>Male</td>
<td>12 km</td>
<td>0.14</td>
<td>0.38</td>
<td>0.51</td>
</tr>
</tbody>
</table>
Chapter 3: The Efficacy of Using Snow Tracks in Providing Genetic Data from Wolverine

**ABSTRACT:** Collecting noninvasive genetic samples from putative wolverine (*Gulo gulo*) snow tracks is an effective method for providing definitive species identification for use in presence/absence surveys. We completed 54 backtracks of approximately 1.4 km each and collected 169 hairs and 58 scats. Amplification rates of mitochondrial DNA (mtDNA), used for species identification, were 74% and 24% for scats and hairs, respectively. The average distance to collect a sample containing high quality mtDNA for species identification was 1330 m. Genetic analysis confirmed 35 snow tracks (64%) as wolverine. The remaining 19 snow tracks consisted of 8 that did not provide samples and 11 that contained non-amplifiable samples. Collection of both hairs and scats provided 28% more track verifications than would have occurred using only one type of sample.

Collecting noninvasive samples from snow tracks can also provide individual wolverine identification that may provide a basis for more complex monitoring such as minimum population estimates, tests of relatedness, or mark re-capture population estimates if sample sizes are large enough. We analyzed nuclear DNA (nDNA) from the same samples to produce individual genotypes. Amplification rates of nDNA from scats and hairs were 52% and 16%, respectively, and produced individual genotypes for 23 of the 54 snow tracks (43%).

**INTRODUCTION**

The development and refinement of molecular genetic techniques have provided new opportunities to study rare and elusive species through noninvasive means (Taberlet et al. 1997, Sloane et al. 2000, Palomares et al. 2002). We addressed the use of applying
noninvasive genetic sampling to winter snow track surveys to improve our ability to provide reliable wolverine detection and population data. Snow track surveys are commonly used to determine species presence and/or distribution. Properly designed surveys are an efficient method for searching large tracts of habitat for rare and highly vagile species. However, the ability to implement winter snow track surveys to delineate species occurrence and distribution requires accurate species identification. Evidence to substantiate species identification has historically been limited to a suite of track measurements, photos, and track casts (Halfpenny et al. 1995), none of which are completely reliable. This is confounded by the fact that field biologists are often faced with making track identifications under a broad array of snow, track, and weather conditions and in areas where the presence of sympatric species of similar size is possible. These sources of error are difficult to control, may produce ambiguous results, and ultimately, call into question the results of such surveys. Halfpenny et al. (1995) stressed that survey methods must provide unequivocal evidence that will hold up to the scrutiny of both the professional community and the court system because of the economic and management ramifications associated with the reporting of rare species presence.

We believe that noninvasive genetic techniques may provide an opportunity for improving snow track methods to meet these more stringent standards. Many of the problems with analyzing low quality, low quantity DNA have been identified (Kohn and Wayne 1997, Frantzen et al. 1998, Taberlet et al. 1999, Mills et al. 2000) and mitigated through the development of new laboratory and statistical techniques (Navidi et al. 1992, Taberlet et al. 1996, Taberlet et al. 1999, Alpers et al. 2003, McKelvey and Schwartz
Analysis of mtDNA from noninvasive hair and scat samples has been used to determine species identification (Foran et al. 1997a,b; Kohn and Wayne 1997, Reed et al. 1997, Ernest et al. 2000, Mowat and Strobeck 2000, Lucchini et al. 2002, Riddle et al. 2003). Noninvasive sampling has also been used to identify individuals within a species, through the analysis of nDNA, allowing for population estimates and population genetic analysis (Taberlet et al. 1997, Woods et al. 1999, Sloane et al. 2000, Palomares et al. 2002, and Hedmark et al. 2004). While analysis of mtDNA and nDNA can be completed on the same genetic samples, microsatellite analysis is more difficult because nDNA is present in far lower copy numbers per cell than mtDNA (Taberlet et al. 1996). Field methods associated with non-invasive sampling still need refinement given that DNA quality, which dictates the success of noninvasive techniques, varies by species, sample type (i.e. hair, scat, feather, urine, etc.), and by the method used to collect the sample.

While many methods, such as hair corrals (Woods et al. 1999, Mowat and Strobeck 2000), glue patches (Sloane et al 2000, Mowat and Paetkau 2002), and scats from tracks and trails (Ernest et al. 2000, Palomares et al. 2002), have been employed for the noninvasive collection of genetic samples, attempting to collect both hair and scat samples directly from the snow pack is a relatively new idea. Collecting genetic samples from snow tracks to determine species identifications of forest carnivores was first performed by Squires et al. (In Press) on two lynx tracks in Wyoming, and later refined on lynx by McKelvey et al. (In Press) and Schwartz et al. (2004). Flagstad et al. (2004) and Hedmark et al. (2004) used scat samples collected along wolverine snow tracks in Scandinavia to determine individual genotypes and reported that nDNA quality (i.e. genotype success rate) was higher than that reported for other species. However, this is
the extent of our knowledge regarding noninvasive sampling of wolverine using snow tracks as a sampling medium. We do not have an understanding of the distribution of hairs and scats along wolverine tracks (i.e. frequency and location), the ability of field technicians to detect such samples when they are present, nor the relative quality of the DNA between hairs and scats. Such an understanding is necessary before we can consider implementation of the method for monitoring and management purposes.

Our goal was to test the feasibility (i.e. capabilities and limitations) of using noninvasive genetic sampling based on snow tracks to document the presence of wolverine and to provide genetic-based ecological data. Our specific objectives were to: 1) determine if hair and scat samples can be consistently collected from backtracks; 2) quantify the backtracking distance required to do so; 3) determine amplification rates for mtDNA from these samples to assign reliable species identifications to putative wolverine snow tracks; 4) determine amplification rates for nDNA to determine if samples from backtracks can be used to address more complex questions; and 5) review the logistic requirements for the application of this noninvasive method.

STUDY AREA

The study area was composed of all Beaverhead-Deerlodge National Forest lands within the Pioneer, Beaverhead, Anaconda-Pintler, and Flint Creek mountain ranges in southwest Montana (Figure 1). The Pioneer Mountains are located east and south of the Big Hole River valley and are bounded to the east by Interstate 15 and State Highway 43 to the north and west. Elevations range from approximately 1,830 m to 3,350 m with the highest peaks located in the eastern portion of the range. The dominant forest cover for the Pioneer Mountains is lodgepole pine (*Pinus contorta*). Lodgepole pine gives way to
Douglas-fir (*Pseudotsuga menziesii*) and sagebrush (*Artemisia* spp.) steppe at lower elevations and on south-facing slopes. Mixed Engelmann spruce (*Picea engelmannii*)/subalpine fir (*Abies lasiocarpa*) forests are found on wet aspects at higher elevations. Whitebark pine (*Pinus albicaulis*) occurs at the highest elevations near timberline. Riparian communities are dominated by willow (*Salix* spp.) that often transition into sagebrush dominated meadows.

The Beaverhead range is located on the Idaho/Montana border on the west side of the Big Hole valley. The range is oriented in a north/south fashion and abuts the Anaconda-Pintler mountain range at Highway 43 near Lost Trail Pass. The Anaconda-Pintler range continues in a northeast direction to the town of Anaconda. Habitat types in these ranges are similar to those in the Pioneers, although sagebrush openings are less common due to more mesic conditions. Higher elevations are dominated by mixed subalpine forests changing to lodgepole pine at mid-elevations on most aspects. Douglas-fir is found on drier sites at lower elevations. The Flint Creek range is located north of the town of Anaconda and is bordered by Highway 1 to the south and west and I-90 to the north and east. Vegetation types are similar to the Anaconda-Pintler and Beaverhead ranges.

**METHODS**

**Backtracking and sample collection**

As part of a larger wolverine study, we completed a survey to detect wolverine snow tracks during the winters of 2003 and 2004 (Chapter 2). We followed all snow tracks approximately 2 km using snowshoes or backcountry skis depending on snow conditions, topography, and vegetation types. We collected genetic samples (hairs and
scats) from footprints, daybeds, foraging areas, tree boles, and from coarse woody debris along the path of the animal. Hair and scat samples were stored in 50 ml vials (Fisherbrand) filled with silica gel desiccant (Reagent A.C.S. 10-18 mesh, Fisherbrand). All hairs from a single location were considered a single sample; therefore, a “hair sample” may have consisted of a single hair or many hairs. We used the vial itself to scoop up the sample and the snow surrounding it to prevent sample contamination. This technique prevented follicles on hairs that were frozen into the snow from being stripped off during collection, as well as the loss of the sample during transfer to the vial.

Vials containing hair samples were stored in a dark area at room temperature until delivered to the lab. In 2003, we dried all scat samples in a paper bag over a lamp for 4-6 hours and stored them in fresh desiccant vials. In 2004, we preserved scats by soaking them in a 95% ethanol bath for 24 hours, draining, and then leaving the cap vented until evaporation was complete. We delivered all samples to the USFS Rocky Mountain Research Station’s genetics laboratory in the Wildlife Ecology Unit within 3 months of initial collection.

**Per-unit-effort analysis**

To quantify the distance required to collect genetic samples, we recorded the path of the animal and the distance between samples using a GPS unit (Trimble Geoexplorer 3). GPS points were collected every 7 seconds to create a line feature delineating the animal’s track. Point features were created for sample locations, daybeds, and foraging areas by averaging at least 20 GPS locations. Because each backtrack route contains hundreds or thousands of points, the GPS “error” associated with each point due to differences in habitat types overstated distances traveled (D’Eon et al. 2002, Frair et al.)
2004, DeCesare et al. In Press) and had to be corrected. We performed a series of post-processing steps to remove this sampling bias without losing the true tortuosity of the track (Decesare et al. In Press). We used the “Surface Length” extension (Jenness 2004) for ArcGIS 8.3 to calculate both the 2 dimensional and 3 dimensional track distances to reflect the influence of slope on the distance traveled in mountainous terrain.

We determined how far a person would have to walk on a putative wolverine track to collect a hair or scat sample. We calculated the number of backtracks that produced samples and the average distance between samples for each backtrack. However, not all samples collected contain adequate mtDNA for species identification; therefore, we factored in amplification rates to determine the frequency of samples that are capable of providing species identification. We used the distribution of these amplified samples among all backtracks to determine the proportion of backtracks that achieved our goal of providing a positive wolverine species identification.

We investigated how other factors influence backtracking success by comparing the frequency that scats and hairs were collected, the location along the track that they were collected, and their respective amplification rates to determine if one sample type was better suited to the technique or whether both sample types should be collected. We used logistic regression to investigate how amplification rates for single hairs with and without follicles compare to multiple hairs with and without follicles (Gagneux et al. 1997, Goossens et al. 2004). We used this understanding of the relationship between the number of hairs and presence of follicles relative to amplification rates to inform sample collection protocols.

We hypothesized that the condition of the track (i.e. the amount of debris in the
print, the depth of the track in the snow, the condition of the snow pack, and the amount of snow in the track) could affect the observer’s ability to detect a sample that is present, as well as actual deposition rates of hair by the wolverine. We tested this hypothesis by categorizing each track into 1 of 3 qualitative “track condition” groups (Good, Fair, and Poor) based on track age, depth of snow in the track, and the amount of debris in the track. We used Multivariate Response Permutation Procedures (MRPP) (Mielke et al. 1981, Zimmerman et al. 1985) to test relationships between track condition and the distance required to collect a hair sample.

Mitochondrial DNA analysis

Genomic DNA was extracted from hair samples using the Dneasy tissue kit (Qiagen) with modifications for hair samples (Mills et al. 2000). DNA was extracted from scat samples using the QiAmp DNA Stool Mini kit (Qiagen). Species identification was determined using restriction digest of a 442 bp segment of the cytochrome b region that is diagnostic for wolverine (Riddle et al. 2003). All samples not producing wolverine identification using restriction digest were subsequently sequenced. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). Both strands were sequenced and analyzed on a Li-Cor 4300 DNA imager using standard protocols. Subsequent sequences were compared to reference samples collected by the lab or to sequences located in the NIH Genbank. We used the genetic results to determine the specific samples collected on backtracks that provided species identification.

Microsatellite analysis

We screened genetic samples for microsatellite analysis by using only those samples that had adequate mtDNA for species ID. Given the higher copy numbers per
cell in mtDNA, this screening procedure (Taberlet et al. 1999) reduced the cost and effort associated with analyzing poor quality samples. Twenty-nine microsatellite markers were obtained from various mustelids and other species (Table 1) and used to analyze tissue samples collected from 15 wolverine captured in the study area. We calculated the probability of identification of siblings (Waits et al. 2001) for each marker and then ordered all markers from highest to lowest. Samples were analyzed at 7 loci to ensure that genotyping error was insignificant (p<0.004) (Figure 2) using a multi-tube approach (Navidi et al. 1992, Taberlet et al. 1996) with 3 repeats. Gels were scored and per locus genotypes were screened for consistency (Mowat and Paetkau 2002). Any samples that amplified at <4 loci were discarded. For samples with scoreable products at 3-6 loci, lab personnel re-amplified any missing or inconsistent loci 3 additional times. Samples were then re-screened, and any samples not containing consistent scores at 6 or more loci were discarded. Per locus genotypes were accepted when analysis produced 3 consistent homozygote or 2 consistent heterozygote scores (Flagstad et al. 2004).

**Logistic requirements**

We provided estimates of equipment, time, and personnel requirements necessary for completing our specific snow tracking surveys. These estimates are intended to provide guidelines for decision makers, since the intensity and duration of survey efforts will vary by site and project. We did not provide any cost estimates because they vary by agency or group, the ability to borrow equipment, and they are quickly outdated.

**RESULTS**

**Sample collection and distribution along snow tracks**

We completed 54 backtracks that were 77 km in total length and averaged 1430 m
Variation in backtrack length was due to the amount of time available to backtrack, the inability to follow a track due to track condition or topography, and technician effort. We collected 169 hair samples and 58 scat samples from snow tracks and found that 46 of 54 tracks (85%) contained at least 1 genetic sample. Hair samples were found in footprints (n=112; 66%), daybeds (n=23; 14%), on tree boles or other woody debris (n=18; 11%), and in the snow at foraging sites (n=16; 9%). Scat samples were collected from footprints (n=48; 83%), foraging sites (n=8; 14%), and daybeds (n=2; 3%).

The average distance between hair samples was 490 m (SE=70), while the average distance between scat samples was 1435 m (SE=341). When combined, the average distance between all samples was 370 m (SE=45). We removed all non-amplified samples from the analysis to determine the distribution of samples capable of providing species identification, and found the average distance between amplified hairs was 2025 m (SE=454), while amplified scats were 3990 m (SE=981) apart. By collecting both types of samples, the average distance between any amplified sample decreased to 1330 m (SE=212).

**Species identification**

The overall amplification rate of mtDNA for all scat samples, regardless of species, was 74% (n=43/58). Of the 43 scats that amplified, 22 belonged to wolverine (50%), while the others belonged to red fox (*Vulpes vulpes*) (n=13), coyote (*Canis latrans*) (n=5), ungulates (n=2), and marten (*Martes americana*) (n=1). The overall amplification rate of mtDNA for hair samples, regardless of species, was 28% (n=47/169). Of the 47 that amplified, 40 (85%) produced wolverine species
identifications, with ungulates (n=3), coyotes (n=2), and squirrel (n=2) making up the difference. Samples containing multiple hairs had a higher amplification rate (p=0.001) than single hair samples. Hair samples with at least one follicle had significantly higher amplification rates (35% amplification rate, p=0.021) than hair samples without follicles (15% amplification rate).

Amplification rates of hair samples also varied between track conditions. We found weak support that the average distance between all hair samples (meter/sample) was smaller for good and fair tracks than for poor tracks (p=0.059), and strong support that the average distance between amplified hairs was smaller for good and fair tracks compared to poor tracks (P=0.0022). Lastly, amplification rates of hair sample mtDNA varied by the location along the track in which they were collected (Table 2).

Specific amplification rates for wolverine scats and hairs were not reported because our sample was confounded by genetic samples from other species; hence, we did not know the proportion of non-amplified samples that belonged to wolverine. We approximated amplification rates by removing samples collected from snow tracks where multiple species tracks were documented; thereby removing all potential non-wolverine samples (n=77). From this subset, we estimated the average amplification rates of mtDNA for wolverine was 65% for scat (n=15/23) and 22% (27/125) for hair samples.

We verified that 35 of the 54 tracks (65% of all tracks, 76% of tracks with at least 1 sample) could be assigned to wolverine. The remaining tracks either did not have samples (n=8) or did not have samples with adequate mtDNA to provide species identification (n=11). Eighteen tracks with multiple samples produced multiple species identifications, with all but 1 containing at least 1 wolverine sample. Other species
represented were red fox (n= 8 tracks), ungulates (n= 5 tracks), coyote (n= 4 tracks), and marten (n= 1 track).

**Microsatellite analysis**

Sixty two samples were analyzed and reliable genotypes were produced for 14 scats (64%) and 21 hairs (53%) (Table 2). Calculating nDNA amplification rates for all wolverine samples was again confounded by an unknown proportion of samples from other species. Thirty eight percent (n=14/37) of the 37 potential wolverine scats (58 total less 21 identified as other species) had nDNA that amplified. Ten of these non-amplified scats could be non-wolverine because they were collected from tracks with multiple species, so if we remove these scats from the sample, the best possible nDNA amplification rate was 52% (n=14/27). We performed the same analysis on hair for an overall amplification rate for nDNA from wolverine hair samples of 16% (n=27/167). Amplifiable samples were well distributed among backtracks, allowing us to produce a reliable genotype for 23 of the 54 backtracks (43%). We identified a minimum population of 11 unique individuals in the study area using samples collected from backtracks.

**Logistic requirements**

The primary costs in backtracking putative wolverine are the salaries for at least 2 field technicians for the duration of the survey, the vehicles and gas needed to deliver them to the track (often a 4x4 pickup and 2 snowmobiles), and the genetic analysis costs for all samples collected. Safety gear (helmets, avalanche transceivers, two-way radios, GPS units) and collection equipment (snowshoes, DNA sample vials, and desiccant) are also expenses that should be considered.
The complete survey and backtracking effort for our 4,864 km² study area required 102 “crew” days, which included 2 complete surveys of the study area. Forty-eight “crew days” were spent surveying for putative wolverine tracks and 54 “crew days” were spent backtracking. More specifically, technicians spent a total of 135 hours completing 54 backtracks of varying length (mean = 2.5 hours/backtrack), which is the time spent following the track in one direction and does not include the return trip. In general, we expect a single backtrack to require at least a half a day, and sometimes an entire day, depending on the distance traveled.

**DISCUSSION**

**Using backtracks to determine species identification**

Collecting genetic samples from wolverine snow tracks provided reliable species identification from the majority of snow tracks. A 100% success rate was neither expected nor necessary when attempting to determine species occurrence using genetic data. Our ability to verify 65% of all tracks resulted in a high probability of confirming wolverine as long as multiple tracks are encountered over the course of the larger survey effort. While tested on a snowmobile/snowshoe/ki based survey method, this verification technique could be applied to snow tracks detected using any survey method, whether ground- or aerially-based. Highly degraded tracks that would otherwise be difficult to identify can now be included in surveys. McKelvey et al. (In Press, pg.6) recognized the value of this method by stating “if DNA samples are collected, the snow track is no longer used for species identification; instead, it is used as a DNA-collection device. This would enable biologists to obtain species identifications of tracks that would otherwise be ambiguous and greatly expand the conditions under which snow-tracking
surveys could be conducted, increasing both their efficiency and representativeness”.

The average distance to collect an amplifiable sample (1.3 km) was comparable to the rate of 1.21 km reported by McKelvey et al. (In Press) for lynx snow tracks. Amplification rates for mtDNA using our method were much higher for scats (65%) than hairs (22%) when collected from the snow pack. However, hairs were encountered 3 times more frequently than scats and thereby required significantly less effort to collect. Although many researchers (Gagneux et al. 1997; Foran et al. 1997a,b; Goosens et al. 1998) decided a priori to use only scats or multiple hairs with follicles to ensure quality samples, we believe the failure to examine the quality of all available sample types may result in missed opportunities. We believe our overall mtDNA amplification rates for hair from this method were lower than results from hair collection methods for other species (Mills et al. 2000, Sloane et al. 2000, Mowat and Paetkau 2002) because we collected all hair samples (i.e. fragments, single hairs, multiple hairs) rather than only multiple hairs with follicles. More selective collection of hairs from daybeds and foraging sites from subsequent tracking efforts in 2005 increased our amplification rates for hair to 88% (J. Squires, unpublished data), which is consistent with other published results. Despite lower amplification rates, the hair samples that did amplify were well distributed across backtracks and greatly improved our overall track verification results. While we could have improved amplification rates for hairs by collecting only multiple hairs or hairs with follicles, we note that single hairs and hairs without follicles still produced species identifications on occasion. We believe the benefit (i.e. the verification of additional tracks) of collecting and analyzing all genetic samples outweighs the relatively small additional costs of the analysis; hence, we recommend collecting all hair
samples regardless of number and follicle presence.

We supported this line of reasoning by viewing our results from the perspective of collecting only a single type of sample versus collecting both types of samples. We found that had we collected only scat, we would have provided species identification for 20 of the 54 tracks (37%). If we collected only hair, then 22 of the tracks (40%) would have produced wolverine species identifications. Yet, by collecting both sample types, a total of 35 (65%) tracks produced species ID, which is a 25-28% increase. These results are surprising in that they reveal the importance of considering not only amplification rates for samples, but also the relative effort of collecting each type of sample. In other words, hair samples may have lower DNA quality than scat, but if we can collect three hair samples for every one scat sample, in the end the two sample types perform almost identically (37% vs. 40%) with regards to the number of wolverine backtracks they confirm. By collecting both types of samples, we not only confirm a higher percentage of overall tracks, but we reduce the average distance required to find a high quality sample from a high of 4 km (scat) to 1.3 km (combined).

Although scats are highly visible, hair samples in snow tracks are difficult to observe. Two previous sampling efforts collected hair samples in high probability locations, such as daybeds or foraging sites, while ignoring potential samples within the footprints of animals (McKelvey et al. In Press), or ignored hairs altogether (Flagstad et al. 2004). While observing and collecting hair samples from footprints requires greater attention from the observer in scanning all footprints, we found this to be well worth the effort as footprints provided 62% of all hair samples. Hairs from footprints accounted for 53% of hair samples providing mtDNA species identification and 52% of hair samples...
providing nDNA-based individual identifications. These results support our assertion that the scanning of footprints for hair samples is included in any snow track protocol given the substantial improvement in collection and amplification rates.

Good and fair quality tracks produce more amplifiable samples per km than poor quality tracks; yet, we do not suggest that poor tracks be overlooked. The ultimate goal of this method is to detect wolverine in areas where their occurrence is uncertain, so we would expect that tracks are infrequently encountered in these areas. A small sample size of putative wolverine snow tracks limits the opportunity to collect genetic samples; therefore, we suggest that all putative wolverine tracks be followed regardless of track quality. Time constraints may not allow for technicians to wait for better tracking conditions, and the fact that poor tracks still produced at least one sample 61% of the time they were encountered should not be discounted. While samples in footprints and foraging sites may be obscured by snow and debris, samples in daybeds and on tree boles are more persistent and can remain visible even on the worst of tracks. Backtracking putative wolverine tracks to determine species or individual identification boils down to the laws of probability. The further the distance traveled, the higher the likelihood of collecting quality samples, and therefore the higher the probability of producing identification. The majority of the financial cost is incurred in finding and transporting people to a putative wolverine track, so we recommend taking full advantage of every opportunity by following the track as far as time and energy allow.

The presence of a single track that was verified as a species other than wolverine raises an important issue with this method. The backtracking file documented the presence of multiple canid tracks along with the putative wolverine track.
distance was short and only 3 hair samples were collected. We do not consider this a misidentification of the track, but rather a function of small sample size and probability. We suggest keeping detailed field notes regarding the presence of multiple species on a track and collecting as many genetic samples as possible to control for this problem. Another approach would be to follow the putative wolverine track until other species are no longer present and to collect multiple samples on that portion of the snow track.

Individual genotypes

The ability to produce genotypes from 43% of our snow tracks suggests that this method is capable of providing the necessary data for more complex analysis regarding minimum population estimates, individual relatedness, and mark-recapture population estimates (Flagstad et al. 2004, Bellemain et al. 2005), which ultimately may provide a framework for better monitoring and managing wolverine. The historical use of snow track surveys as a relative index of wolverine population numbers and trends is unreliable because of the high daily movement rates and large spatial use by wolverine. Hornocker and Hash (1981) first reported that the wide-ranging movement of a single wolverine over short time periods produces a false impression of abundance. When used in management decision making, such false impressions could have drastic effects on wolverine populations. However, the use of snow track surveys in which the individual identity of the animal can be determined through the use of noninvasive sampling provides a more accurate method for monitoring population trends, especially when conducted over large areas.

While no published data exist for the nDNA amplification rates for wolverine hair, our rate of 38%-64% for scat is slightly lower than the 70% reported by Flagstad et
al. (2004). We cannot explain this difference from a laboratory analysis perspective. Samples were fresh and collected from snow, inhibitor-binding substances were used during DNA extraction, and we used the same Hotstar Taq Polymerase (Qiagen) as Flagstad et al. (2004). We did note a difference in the methods used to store scat samples prior to analysis; however, Frantzen et al. (1998) reported that ethanol, freezing, and drying storage methods all produced similar amplification rates for mtDNA and nDNA.

We noted an interesting result regarding genotyping rates of scats and hairs. Scats had higher amplification rates of both mtDNA and nDNA than hairs. Yet, only 8 of the 17 scats that provided mtDNA also produced nDNA (47%), while 14 of the 16 hairs that provided mtDNA also produced nDNA (82%). Given the small sample size, we could not determine if this was a function of chance or if scats have a higher variability between the quality of n(DNA) and mt(DNA) within a given sample than hair.

MANAGEMENT IMPLICATIONS

Snow track surveys are an important tool for studying and monitoring wildlife; however, track misidentification is a major shortcoming. We addressed this limitation by presenting a backtracking method that can be applied to any snow track survey method in an attempt to provide more definitive identification using genetic analysis. Using snow tracks as a collection device and genetic analysis as a reliable identifier removes observer error and allows for application of snow track surveys over a much broader range of track conditions than is possible with the traditional method.

The ability to collect individual identifications from this method may provide the basis for a noninvasive monitoring tool for providing data on species population structure, connectivity, and size. Such data would be crucial for informing ESA and other
management decision making regarding rare and secretive species. While we present this method using wolverine as the focal species, we speculate that the basic concept of collecting noninvasive samples from snow tracks applies to many species that are active in snowy habitats.

LITERATURE CITED


detections. United States Forest Service, Pacific Southwest Research Station, Albany, California. 163 pages.


Figure 1: Our study area and location of survey units in southwest Montana during 2004.
Table 1: Microsatellite markers used for nDNA analysis of genotypes for wolverine in southwestern Montana during 2003 and 2004.

<table>
<thead>
<tr>
<th>Markers screened:</th>
<th>Markers selected:</th>
<th>Repeat:</th>
<th>Species:</th>
<th>Reference:</th>
</tr>
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<tr>
<td>Mvis 075</td>
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</tr>
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<td>CA</td>
<td>mink</td>
<td>O'Connel et al. 1996</td>
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</tr>
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<td>CA</td>
<td>mink</td>
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<td></td>
</tr>
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<td>Gg14</td>
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<td>wolverine</td>
<td>Davis &amp; Strobeck 1998</td>
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<td>Ggu 216</td>
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<tr>
<td>Ggu 234</td>
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<td>Duffy et al. 1998</td>
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<tr>
<td>Lut 604</td>
<td>CA</td>
<td>eurasian otter</td>
<td>Dallas &amp; Piertney 1998</td>
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<tr>
<td>Ma 1</td>
<td>(TG)TA(TG)</td>
<td>marten</td>
<td>Davis &amp; Strobeck 1998</td>
<td></td>
</tr>
<tr>
<td>Ma 2</td>
<td>TG</td>
<td>marten</td>
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<tr>
<td>Ma 3</td>
<td>(TG)c(TG)</td>
<td>marten</td>
<td>Davis &amp; Strobeck 1998</td>
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</tr>
<tr>
<td>Ma 8</td>
<td>TG</td>
<td>marten</td>
<td>Davis &amp; Strobeck 1998</td>
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<td>T(TG)</td>
<td>marten</td>
<td>Davis &amp; Strobeck 1998</td>
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<td>Ma 19</td>
<td>TG</td>
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<td>(CA)</td>
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<td>Duffy et al. 1998</td>
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<tr>
<td>Ma 9</td>
<td>(T)14(TG)4</td>
<td>marten</td>
<td>Davis &amp; Strobeck 1998</td>
<td></td>
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</table>
Figure 2: Probability of selecting 2 individual wolverine with identical genotypes from a population in SW Montana during the winters of 2003 and 2004 as a function of the number of microsatellite markers used in analysis.
Table 2: The frequency and percentages of hair samples collected during 2003 and 2004 in SW Montana in each of 4 snow track location categories and the associated amplification rates of wolverine mtDNA and nDNA for these samples.

<table>
<thead>
<tr>
<th>Location</th>
<th>Total # of hairs</th>
<th>% of total hair samples</th>
<th># of hairs with gulo mtDNA</th>
<th>% hairs with gulo mtDNA</th>
<th># of hairs with gulo nDNA</th>
<th>% of hairs with gulo nDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Footprints</td>
<td>112</td>
<td>66%</td>
<td>21</td>
<td>19%</td>
<td>11</td>
<td>10%</td>
</tr>
<tr>
<td>Daybeds</td>
<td>23</td>
<td>14%</td>
<td>9</td>
<td>39%</td>
<td>7</td>
<td>30%</td>
</tr>
<tr>
<td>Tree Boles</td>
<td>18</td>
<td>11%</td>
<td>8</td>
<td>44%</td>
<td>2</td>
<td>11%</td>
</tr>
<tr>
<td>Foraging Sites</td>
<td>16</td>
<td>9%</td>
<td>2</td>
<td>13%</td>
<td>1</td>
<td>6%</td>
</tr>
</tbody>
</table>
Chapter 4: Conclusion

Effective management and conservation of the wolverine requires an improved understanding of all aspects of wolverine ecology. An important and necessary first step is a better understanding of the current distribution of the species along the periphery of its range, namely the contiguous United States. By understanding which areas support or do not support persistent wolverine populations, we may be able to infer the biotic and/or abiotic factors affecting wolverine population dynamics.

In this thesis, I have taken a popular survey technique, snow track surveys, and provided a sampling framework to improve probabilities of detection and to provide more reliable occurrence data. I developed a noninvasive genetic sampling protocol for use in combination with snow track surveys to provide highly reliable and defensible species identification. I tested this combined method in 4 mountain ranges in southwestern Montana to provide potential users with empirical results regarding the method's utility and feasibility. Results from these tests were promising in that I had high detection rates of putative wolverine tracks and was able to verify the majority of these tracks as wolverine using genetic analysis. By presenting a comprehensive analysis of this winter survey protocol, I have attempted to provide wildlife and land managers with a complete picture of the capabilities and limitations of this method for detecting wolverine. It is my hope that this method raises the bar regarding data standards for species surveys, assists in addressing one of the fundamental data needs relative to wolverine conservation, and ultimately, helps ensure the persistence of this amazing animal.