Carnivore Conservation and Search Dogs: The Value of a Novel, Non-invasive Technique in the Greater Yellowstone Ecosystem

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Abstract

In the Greater Yellowstone Ecosystem (GYE), habitat connectivity is a concern because large carnivores have difficulty dispersing successfully between protected areas. One area of high conservation value is the Centennial Mountains and surrounding valleys (1,500 km^2) along the Idaho–Montana border. They have been deemed important to connecting central Idaho with the GYE, and the range anchors the southern Yellowstone-to-Yukon system. The Centennials have also been identified as a peripheral sink area within the GYE. Despite the geographical appeal of the Centennials as a linkage zone, empirical investigation of their importance for large carnivores has received scant attention. This is due in part to the complex arrangement of public lands within the range, steep topography, and the difficulties associated with conducting research on large carnivores in the region. The aim of this project was to utilize a novel, non-invasive DNA sampling technique to examine the relative abundance of a suite of large carnivores in the Centennials and surrounding valleys. Search dogs specifically trained to locate the scat of four target species (black bears, grizzly bears, cougars, and gray wolves) were used to sample the study area. From DNA extractions, I can identify the samples not only to species, but also to gender and to individual animals, and can estimate sex ratios, densities, and (possibly) home ranges. I will discuss how search dogs are being used to examine various carnivore species' use of the landscape with respect to habitat parameters, public land management, and changes in land use patterns over time to examine human impacts on species distributions and movements. Finally, I will discuss the merits and limitations of this novel, non-invasive method for carnivore conservation research inside the GYE using preliminary data from this study.

Introduction

As the human population continues to boom in the Intermountain West, new subdivisions and increasing human density are occurring at accelerated rates. For example, areas adjacent to public lands are being sold and subdivided across western North America (Knight and Mitchell 1997). In fact, the fastest-growing region in the U.S. is the Intermountain West, with growth rates rivaling those of several African nations and exceeding that of Mexico (Knight and Mitchell 1997). Concentrated growth in limited areas raises serious conflicts among traditional agriculture, unprecedented urban expansion, and wildlife conservation. Of particular concern are wide-ranging carnivores, especially at the interface of wildland and urban or suburban areas. Little is known about how carnivores live in and move through these interface areas (Beckmann and Berger 2003b). Such gaps in knowledge impede prudent management, a situation that will likely be exacerbated in the future. Currently, the potential for loss of livestock, consumption of pets, property damage, and even injury or death to humans due to free-ranging large carnivores exists or has been documented (Herrero 1985; Beier 1991; Herrero and Higgins 1999). For these reasons, large carnivores both capture the public's imagination and inspire calls for carnivore control, protection, and translocation.

Knight and Mitchell (1997) point out that as population-driven, landscape-level changes occur, there are other associated changes, such as an increase in the number of pets, more vehicles and road-killed wildlife, and increasing human–carnivore interactions, leading the public to categorize those carnivores as "nuisance wildlife" (Knight and Mitchell 1997; Beckmann and Berger 2003a). Although our understanding of the impacts of these changes is limited, Knight and Mitchell (1997) state that several studies suggest that these types of changes result in an accumulation of human-adapted species (e.g., raccoons [*Procyon lotor*]) and a decline of species sensitive to human activities, such as large carnivores (Tyser and Worley 1992; Knight and Mitchell 1997; Beier 1995). In addition, these changes contribute to habitat fragmentation and species isolation. In the

Greater Yellowstone Ecosystem (GYE), isolation is of particular concern for the large carnivore species that currently occur inside both the Yellowstone National Park and the Grand Teton National Park core areas. Such core areas are of fundamental importance because they not only harbor populations of rare and sensitive species, but also could become completely isolated from other northern Rockies systems due to human activities in peripheral lands over the next few decades.

One area of high conservation value is the Centennial Mountains along the Idaho–Montana border west of Yellowstone National Park (Fig. 1). They have been deemed important to connecting central Idaho with the GYE, and the range anchors the southern Yellowstone-to-Yukon (Y2Y) system. The Centennials have also been identified as a peripheral sink area within the GYE (Noss et al. 2002). Lower levels of connectivity, higher road densities, and fewer refugia in the southern Y2Y region make this link particularly important. North–south connections through the Canadian and U.S. Rocky Mountains have received a fair amount of attention, but east–west corridors, such as the Centennial area, have not been intensively examined. The idea of connecting the GYE not only to the Yukon, but also to the Cascade Range and other areas to the west, has placed a new emphasis on these corridors. Noss et al. (2002) point out that for most "mega" species (e.g., grizzly bears [*Ursus arctos*], wolves [*Canis lupus*], and wolverines [*Gulo gulo*]) in the GYE, modeling predicts that core areas will remain strong sources of individuals over the next 25 years. However, because of human perturbations, namely road construction, these core areas may no longer be able to support populations of these "mega" species in the peripheral distribution (surrounding sink habitat) in the next 25 years (Noss et al. 2002). Because the Centennials have been not only delineated as an area of possible linkage within GYE, but also identified as a possible peripheral sink area, it is critical to examine whether the range is currently functioning as a linkage zone for large carnivores. This is particularly true given that more than half of the Centennial range falls outside the grizzly bear recovery zone, where mortality rates are often higher for bears than in more protected regions (M. Haroldson, U.S. Geological Survey, Interagency Grizzly Bear Study Team, pers. comm.). As Servheen et al. (2001) point out, linkage zones are different than corridors in that they are areas that could support carnivores at low densities over time, rather than areas that are strictly used just as travel lanes.

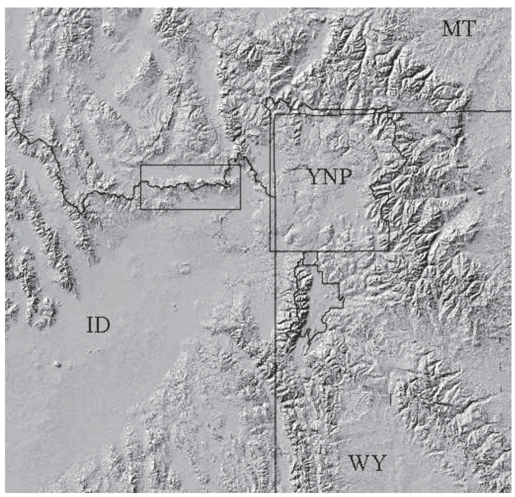

Figure 1. Location of the Centennial Mountains study area (black box) in the Greater Yellowstone Ecosystem (GYE). The Centennials form the Continental Divide between Idaho and Montana directly west of Yellowstone National Park (YNP). The Centennials have been identified as a potential linkage area for large carnivore populations in the GYE with central Idaho wilderness areas.

The primary species driving the interest in the Centennials are the federally protected grizzly bear and gray wolf. However, to a lesser degree there is also interest in more common species of large carnivores, such as black bears (*U. americanus*) and cougars (*Puma concolor*), as relatively no data are available concerning these two species in the Centennials despite the fact that both are hunted in the range. Increasing the significance of the issue of connectivity is the fact that portions of both U.S. Interstate 15 and U.S. Highway 20 bisect the area. In addition to potentially increasing the mortality risk of carnivores, human-altered landscapes may increase habitat fragmentation. The primary causes of habitat fragmentation, especially for bears, are human activities, including road building (Servheen et al. 2001). Habitat fragmentation isolates populations, potentially leading to losses of genetic diversity as well as population decline, and may result in the eventual extinction of a species or local population. Maintaining linkage opportunities between bear populations in the GYE and Salmon–Selway area could enhance grizzly bear recovery in the United States (Servheen et al. 2001). Therefore, if the Centennials are a significant impediment for grizzly bear dispersal from the GYE into the wilderness areas of central Idaho, there could be serious impacts on the population viability of grizzly bears within this ecosystem. Conversely, movement across the area is essential to prevent further fragmentation and isolation of bear populations inside the GYE.

A major concern associated with focusing issues of connectivity on a single, "mega" species, such as grizzly bears, is that successfully documenting whether a species is able to use an area as a linkage zone may only be tenable at longer temporal scales (e.g., several decades). If ecologists and conservationists want to understand the importance of the Centennial Mountains to connectivity within the Y2Y, then research should also focus on species that may generate data useful for determining the effectiveness of the range for connectivity at shorter time intervals. Thus, this project has taken a suite approach to examining connectivity for large carnivores in the Centennial Mountains. This suite approach allows examination of the region for species that use the landscape differently and thus have different habitat requirements for linking isolated populations, such as those found inside the GYE. By examining species such as black bears and cougars, rather than focusing solely on grizzly bears, it may be possible to generate data useful for determining the effectiveness of the range in connecting populations at shorter time intervals.

Effective management and conservation of carnivores requires that ecologists have reliable and detailed demographic information. However, for most species of carnivores, especially threatened and endangered (T&E) species, such data are often difficult to obtain. The causes of difficulty in data acquisition include low population densities, wide home ranges for individuals, and, in some cases, issues associated with trapping and marking rare species (Smith et al. 2003). Although the Centennial Mountains and two surrounding valleys only cover approximately 1,500 km^2, they are a microcosm of the entire GYE in that they contain BLM wilderness study areas, a U.S. Fish and Wildlife Service refuge, two national forests, and the U.S. Department of Agriculture Sheep Experiment Station, as well as Bureau of Reclamation lands, Idaho and Montana state lands, and private lands. This complex arrangement of public and private lands creates challenges when researchers attempt to address landscape-scale questions or conservation concerns such as long-distance migrations (LDMs) of ungulates, or connectivity issues for large carnivores. It can often be difficult to align all entities in order to conduct invasive (capture/handle) scientific studies. Acquiring permits to capture and handle animals from each agency can be challenging and, in many instances, logistical and monetary challenges can arise, making invasive studies difficult. In this research, for instance, using a suite approach to addressing connectivity issues for several large carnivore species would have made a capture study extremely expensive and impractical. Finally, when T&E species are involved, and because large carnivores can often be controversial with the general public, a non-invasive approach may often be a better option for certain research questions. Most methods of live capture and marking individuals to obtain demographic data have biases associated with them. For example, data can be biased by behaviors of target species (e.g., sex-biased trapping; see Smith et al. 2003). Capturing and marking also have associated risks of injury to both the study animal and researchers. Thus, as Smith et al. (2003) point out, non-invasive techniques to gather population demographic data have found recent favor among ecologists. For these reasons, a new, alternative method to sampling in a complex political landscape with rugged terrain, at a huge scale, and for a suite of relatively rare species of carnivores, was needed for this study.

Recent advances in molecular genetics have made fecal DNA technology a promising, viable option for researchers working on species that are difficult to capture and mark due to biological and political factors; for detecting species presence or absence; for identifying the sex of each individual; and for determining the identity of each individual (Smith et al. 2003). Fecal analyses have been used in the past to examine food habits, determine relative abundance of animals, infer habitat use, and estimate home range size, as well as in parasitology studies (Smith et al. 2003). DNA technology has advanced such that a well-designed study can use fecal DNA sampling to determine species, sex ratio, home range, paternity, and kinship, and even to produce population estimates for carnivores (Smith et al. 2003; Boulanger et al. 2004; McKelvey and Schwartz 2004a and 2004b; Paetkau 2004).

Acquiring data from feces (scats) of rare carnivores requires sampling across a large area on the landscape. Obtaining samples for populations with low densities and/or cryptic scats, in addition to human error in identifying the scat, may influence the reliability of demographic data (Smith et al. 2003). Because human limitations also prevent locating sufficient scats for such species, a more effective method of scat recovery was needed for this project (Smith et al. 2003). We employed a novel DNA sampling technique that has only been used intensively in the last several years (see Smith et al. 2003; Wasser et al. 2004). We used dogs specifically trained to locate the scat of four target species (black bears, grizzly bears, cougars, and gray wolves) to sample the Centennial range and surrounding valleys. This method was used in conjunction with fecal DNA analyses on black bear and grizzly bear populations as early as 1998 (Wasser et al. 2004). Dogs have also been used to locate scat of species such as San Joaquin kit foxes (*Vulpes macrotis mutica*), black-footed ferrets (*Mustela nigripes*), coyotes (*Canis latrans*), and lynx (*Lynx canadensis*) (Smith et al. 2003). In fact, in one study, dogs were used to detect the presence or absence of a target species, kit fox, with 100% accuracy despite the presence of sympatric striped skunks (*Mephitis mephitis*) and American badgers (*Taxidea taxus*) (Smith et al. 2001; Smith et al. 2003). In this paper, I describe this novel, non-invasive sampling technique using preliminary data from the Centennial Mountains study area as an example of its utility inside the GYE, and I discuss the merits and limitations of this technique.

Sampling with dogs

Four dogs (two Labrador retrievers, two German shepherds) were trained to detect scat of black bears, grizzly bears, cougars, and gray wolves using the techniques described in Smith et al. (2003). In order to sample the Centennials in a block design, a 5 × 5-km grid was overlaid onto the 1,500 km^2 study area, resulting in 60 grid cells (25 km^2 each). Using this grid size enabled us to detect the smallest home range of the four target species (i.e., that of female black bears) using data reported in the literature for similar systems (e.g., see Nagy and Haroldson 1990; Beier 1995; Logan and Sweanor 2001; Beckmann and Berger 2003a). The 60 cells were then individually numbered and stratified into five blocks of 12 cells each. Employing a random number generator, we then selected four grids per block, resulting in 20 cells' being sampled using transects in 2004. In 2005, we sampled the remaining 40 grid cells. Because grid cells were eliminated after they were searched, each of the 60 cells has been sampled with a transect once at this point in the study. Each triangle-shaped transect was six kilometers long, meaning that a total of 360 kilometers have been searched by dogs and their two-person handling teams (one handler and one orienter). These random-direction transects were triangular so that the dogs could return to the starting point without ever having to retrace their route, as occurs in straight-line transects. This avoided unnecessary energy expenditure by the dogs and kept them fresh for successive days during this intensive search work.

Each transect was recorded with a GPS unit on both the dog and the human handler, and the resulting transects were mapped using GIS software (ArcView 3.2, ArcMap). Each dog carried a GPS unit in order to map the distance covered off of the human-walked transect, to accurately estimate the total area sampled, and to estimate densities based on scat hit rates. Scats were collected in 95% ethanol in the field for transport to the DNA lab. For each scat collected, we recorded distance off the transect, altitude, slope aspect, quality of sample (degree of freshness), habitat, land management agency responsible for the site, fire history, logging history, ATV/snowmobile use level, presence of livestock (type and number), and distance to road, trail, building, fence, or any other anthropogenic structure. These covariates will later be included in spatial analyses models using multiple logistic regression and hierarchical partitioning analyses to examine the impacts of human activities in the Centennials on their ability to function as a linkage zone for carnivores.

DNA analyses

Species identification

The DNA isolation procedure involved freeze-drying the samples and then pulverizing them in order to uniformly distribute DNA in the samples. DNA was extracted from every sample using a QIAGEN Dneasy DNA extraction kit following the manufacturer's protocol. Extractions were carried out in a separate room under quasi-clean conditions to prevent contamination. Each sample was isolated a minimum of two times and tested. Negative controls (no scat added to extraction) were used with each set of extractions to test for contamination. After DNA was extracted, polymerase chain reaction (PCR) amplification and restriction enzyme analyses were performed. Scat samples that failed to produce PCR amplification after the second extraction were removed from the analyses.

Two methods were used for species identification, both involving mitochondrial (mt) DNA analysis. The first used a size difference between black bears and grizzly bears (Woods et al. 1999). A region of mtDNA was amplified via PCR, using primers that targeted a region of the cytochrome B gene of the mt genome. Black bears yielded a fragment approximately 15 bases larger than grizzly bears. One primer was fluorescently labeled, allowing visualization on an automated DNA sequencer for precise size comparisons. Positive and negative controls, in addition to the DNA isolation blanks, were included for each amplification. The second method of species identification used sequence analysis of a region of the cytochrome B gene. Samples were amplified using primers that target this region (Farrell et al. 2000), and sequence analysis was then performed (using Big Dye terminator chemistry on an ABI Prism 377 automated DNA sequencer). DNA sequences were edited and aligned using Sequencer (Genecodes). Sample sequences were compared with known sequences and with entries in GenBank using the megaBLAST program (National Center for Biotechnology Information) to identify species that possess sequences of high similarity. Sequence analysis was attempted on all samples that failed using the size-based method.

Individual and sex identification

Microsatellite genotyping was used to determine individual genotypes for the samples. Samples were genotyped in quadruplicate at seven microsatellite loci (G10A, G10B, G10C, G10J, G10L, CXX.20, and G10D) using 2–3 locus multiplexes, with one primer of each pair fluorescently labeled. Positive and negative controls were included in each amplification. Genotypes were determined following electrophoresis on an ABI Prism 377 using Genescan and Gentoyper software (ABI) and an internal size standard. Alleles were scored if they were detected at least twice across the four replicate amplifications. Multilocus genotypes were determined for the samples that met the scoring criteria at four or more loci.

Sex was determined using Sry and ZFX/ZFY coamplifications, with one primer of each pair fluorescently labeled. Electrophoresis and scoring of fragments was as described for the microsatellite amplifications, although gender amplifications were performed in duplicate. Six known bears (3 males, 3 females) were included, as was an amplification blank, to assist gender determinations for bears.

Preliminary results

To date, 60 transects have been sampled in the Centennial study area. Humans have walked 365.5 kilometers, and dogs have sampled 767.7 kilometers, covering covered 2.1 times the total distance of humans. The number of scats/km varied between dogs ($\overline{X}_{\text{scats/km}}$ = 0.376, range 0.242–0.746). A total of 289 scats have been located, of which only four are non-target species based on both field identification (2005 samples) and DNA analyses (2004 samples). To date, dogs have been 98.6% accurate in identifying only target species of carnivores. The four non-target scats identified by DNA analyses were all from red fox (*Vulpes vulpes*). Because some DNA analyses are still pending for samples collected in 2005, a preliminary breakdown of scat samples based only on field identification reveals that all taxa (ursids, canids, and felids) have been sampled using this technique: bears (n = 269), cougars (n = 11), and wolves (n = 5). In addition to data collected from scat, dogs have located den sites for carnivores, potential rendezvous sites, and kill sites in the Centennials. High-quality DNA samples (hair) were collected from carnivores at bed sites and kill sites.

Discussion

Dogs have been used by humans for millennia for a multitude of purposes including hunting, serving, rescuing, herding, protecting, leading, capturing and tracking wildlife, and even aversive conditioning of “nuisance” carnivores (e.g., Beckmann et al. 2004). More recently, dogs have been used as a conservation tool, as search dogs have been trained specifically to locate scat of target species of interest in order to obtain DNA samples. This novel sampling technique has merit as a useful tool for ecologists addressing landscape-scale conservation issues such as connectivity for populations of large carnivores via linkage zones. Yet, as with any technique, limitations exist. One limitation of the technique is that costs can be prohibitive at some level, as few people are expert at handling dogs for this type of work. However, for studies such as the one described here—examining a complex, landscape-scale phenomenon such as long distance movements for several wide-ranging species simultaneously—an invasive capture study would most likely be many orders of magnitude higher in cost than the use of search dogs.

One of the largest drawbacks of this sampling technique is that the dogs often outwork the DNA lab, finding many scat samples that are too old and degraded to be suitable for DNA amplification. This was especially true during the first year of this study as the lab attempted to obtain individual DNA fingerprints. Because of cost constraints, it was difficult to use dogs to walk transects prior to actual sampling bouts to clear old scats; thus, dogs located some very degraded scats during sampling. In many cases, dogs located scats that were little more than crumbs or were old enough to be covered by mold, both of which contribute to the breakdown and/or contamination of DNA (Wasser et al. 2004). In addition, because bears are only out of their dens for a relatively short period of time in the Centennials, clearing transects in spring or early summer after the snow was gone would not give animals much time to deposit new samples prior to the first snows of the following winter. Another limitation to this sampling technique is that because DNA from scat is considered low-quality DNA, lab expenses are generally higher than they are for higher-quality sources of DNA (e.g., tissue, blood, or hair).

In addition to these limitations, the technique is so new that relatively little is known about several of its methodological aspects. For example, little is known about differences in detection rates as environmental variables change. Wind speed, relative humidity, topography, age of sample, and temperature all influence the scent cones left by scat samples (Wasser et al. 2004), yet models do not currently exist for predicting the shape and size that scent cones take under various environmental conditions. Thus, accurately predicting the likelihood of detection by dogs under various conditions is currently impossible. Other methodological questions still to be worked out include: What happens to detection rates as more species are added to a dog's repertoire? Do dogs begin to lose the ability to detect the first species added to their scent search as more species are added? Do they start to generalize to all carnivores on the landscape at some point in time? Do different breeds of dogs and different individuals within a breed have various success rates at locating scats? All of these puzzles currently remain unanswered and are limitations to the usefulness of this technique, because ultimately, these variables affect the validity of using search dogs as a carnivore research tool.

There are several benefits to using dogs as a research technique for addressing certain conservation questions. Because search dogs are capable of covering extremely large areas on the landscape, they are useful for addressing landscape-scale questions. Dogs also have the ability to sample for multiple species simultaneously, as demonstrated in this project (although as acknowledged above, the effects of adding multiple species to a dog's search repertoire are unknown at this time). Because dogs have been shown to be up to four times more effective at locating scats than trained human observers, they are useful in sampling for rare or low-density species, such as most large carnivores (Smith et al. 2003; A. Whitelaw, Working Dogs for Conservation, pers. comm.). As with any non-invasive technique, search dogs present no risk to the carnivore species being studied, which is a large benefit when addressing research or conservation issues for T&E species. Non-invasive methods of data collection also eliminate potential trap biases that occur in capture studies, especially the sex-biased trapping often found in carnivore studies (see Smith et al. 2003). Search dogs are also useful in extremely rough terrain, such as that of the Centennial Mountains, where capturing species such as grizzly bears in leg snares would be a big challenge and would present risks to both bears and researchers. Finally, search dogs may be helpful during attempts to locate areas with higher densities of target species before an invasive capture study is begun. This may reduce costs associated with low trap success by increasing the probability of successful trap sets.

As demonstrated here, search dogs are a useful technique for sampling complex matrices of public lands for carnivores. Not only can they reduce the difficulty in acquiring permits and decrease some costs, they can also help sample multiple, rare species simultaneously to address certain conservation questions. However, many questions remain. Most notably, the effect on detection rates as more species are added to a dog's repertoire, whether different breeds of dogs and different individuals within a breed have various success rates at locating scats, and how environmental factors affect scent cones and detection abilities of dogs. Future research should address all of these questions to increase our understanding of the effectiveness of using search dogs as a carnivore conservation tool.

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