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An Assessment of the Tobacco Root Mountain Range in Southwestern Montana as a Linkage Zone for Grizzly Bears

Abstract

We studied the Tobacco Root mountain range in southwestern Montana for the presence of grizzly bears and to determine if grizzly bears used this area as a travel corridor connecting to other source populations. Due to the proximity of the Tobacco Root mountain range to other grizzly bear occupied mountain ranges (i.e. Madison, Gallatin, and Gravelly Ranges) we speculated that dispersal of grizzly bears into the Tobacco Roots would be probable. We used non-invasive hair snagging methods, passive infrared cameras, fecal collection, and track identification from the spring of 1999 to the autumn of 2000. We collected and analyzed 255 hair and scat samples and obtained 480 photos. We detected no grizzly bears using the Tobacco Root mountain range as either a travel corridor or a linkage zone. However, the presence of a reproducing black bear population indicated the area might provide suitable grizzly bear habitat. As grizzly bear populations increase and the availability of suitable habitats within the Greater Yellowstone Ecosystem decrease, the Tobacco Root Range is likely to have high potential for grizzly bear occupancy.

Introduction

Grizzly bears (*Ursus arctos*) have been a topic of heated debate since they were listed as threatened under the Endangered Species Act in 1975. Space and solitude are essential if grizzly bear populations are to remain at sustainable levels (Craighead et al. 1982). The Greater Yellowstone Ecosystem (GYE) has the potential to sustain a large genetically viable grizzly bear population, but only if the population that is in place has the ability to exchange genetic material with outside populations (Craighead et al. 1999). Thus, management of linkage zones to maintain and enhance movement opportunities is a critical part of the successful application of metapopulation theory to grizzly bear conservation (Servheen et al. 2001).

Providing linkages for movement between populations is a central theme of metapopulation theory (Servheen et al. 2001). Servheen et al. (2001) defined the differences between linkage zones and corridor systems. They define a corridor as an area used merely for travel between ecosystems;

linkage zones are areas that will support low-density carnivore populations often as seasonal residents. Determining if an area that lies between established populations is suitable habitat for grizzly bears is the first step in identifying a corridor or linkage zone.

The Tobacco Root mountain range is unique because it is situated in an area northwest of the GYE adjacent to areas occupied by grizzly bears. Its close proximity to mountain ranges that are known to be inhabited by grizzly bears (Madison, Gallatin, and Gravelly ranges) make the Tobacco Roots attractive when looking into the possibility of dispersal of grizzly bears from these occupied habitats. Additionally, the range has been identified as suitable habitat and a potential corridor for grizzly bears (Craighead Environmental Research Institute, personal communication), possibly contributing to the linkage of the GYE with the Northern Continental Divide Ecosystem (Figure 1).

Our primary objective was to determine if grizzly bears were present in the Tobacco Root Range. We hypothesized that if present, grizzly bears were using the Tobacco Root Range to disperse from source populations because of its proximity to the GYE, orientation of the range, and the vegetative food sources available.

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Study Area

The 629.1 km² study area covered nearly the entire Tobacco Root Range (Figure 1). The Tobacco Root Range, located 60 km northwest of Yellowstone National Park, is composed of steep mountain slopes, with many peaks > 3,200 m. The cover types of the range consist of Douglas-fir (*Pseudotsuga menziesii*), engelmann spruce (*Picea engelmannii*), lodgepole pine (*Pinus contorta*), and whitebark pine (*Pinus albicaulis*). Fire, timber harvest, cattle grazing, recreational activities, and mining have all had an impact on the landscape.

The Tobacco Root range is accessible for motorized travel and is frequented for recreational activities such as camping, horseback riding, fishing, hunting, and off road motorized travel. Private landownership is generally restricted to the low elevation areas surrounding the range with a few in holdings deeper within the range itself.

We documented many of the known foods utilized by grizzly bears (Craighead et al. 1995) within our study area. Such foods include but are not limited to yampah (*Perideridia gairdneri*), cow parsnip (*Heracleum maximum*), whortleberry

(*Vaccinium scoparium*), yellow glacier lily (*Erythronium grandiflorum*), whitebark pine, elk (*Cervus elaphus*) and other mammal species.

Methods

There are many different ways in which to detect the presence of a species in an ecosystem. Obtaining DNA is a definitive method by which to confirm species, sex, genetic population structure and genealogies (Woods et al. 1999). Our primary method of obtaining DNA was hair collection using existing methods to remove hair samples from bears in a non-invasive manner. The roots of mammalian hair may contain sufficient DNA for analysis when genetic material at specific loci is amplified using the polymerase chain reaction (PCR) (Higuchi et al. 1988).

A 27-cell grid system, each cell 5 km² in size, was placed over the study area to distribute sampling efforts (Figure 1). Due to the relatively small size of the study area, we chose not to use the standard cell size of 8 km² (Mowat and Strobeck 1999). Grid lines were based on section lines established by the USGS. This format enabled us to exclude areas of land that were in private ownership.

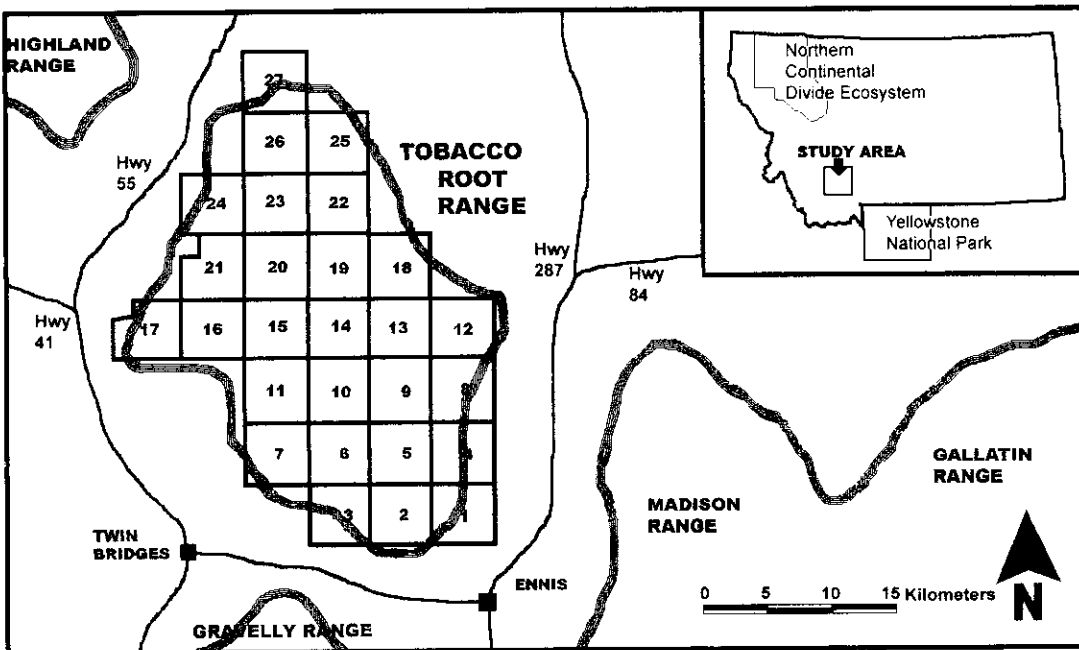


Figure 1. Study Area grid pattern and the proximity of the Tobacco Root Range to the Greater Yellowstone Ecosystem and the Northern Continental Divide Ecosystem.

One baited hair-snagging site was placed in each of the 27 cells. To ensure the greatest chances of grizzly bear detection, hair-snagging sites were placed in suitable habitat within the cell as determined by adequate cover or food. All baited hair-snagging sites were located ≥ 0.8 km from any road or trail to help avoid possible human/ bear conflicts and human tampering. To maximize capture variability, all hair-snagging sites were located ≥ 1 km from any other hair-snagging location (Mowat and Strobeck 1999). Due to snow pack in June and early July, hair-snagging sites were mostly located $< 2,100$ m in elevation. As the snow pack melted, hair-snagging sites were progressively moved throughout the study area to sample different elevations and plant communities.

Each hair-snagging site was operated between 15 June and 15 September in both the 1999 and 2000 seasons. Hair-samples were recovered and bait replenished at each site after 14-day intervals. This method resulted in a total of 4550 hair-snagging nights over the study period.

Hair-snagging Methods

A perimeter fence was built by fastening a single strand of barbed wire (2-strand wire, 4-point barbs, 17 cm spacing between barbs) around a grouping of trees, 20-30 m in circumference. The barbed wire was fastened to the outside of each perimeter tree with 3.8-cm fencing staples about knee height from the ground. The wire was first hand-tensioned then extra fence staples were added to increase the tautness. Irregularities in the terrain were filled with debris to ensure a uniform wire height (Woods et al 1999). Cable clamps and fence staples attached a 0.9-cm steel cable to two trees so that a bait container hung 3-4 m above the ground, 3-4 m from each cable-supporting tree, and in such a way that the bait container was situated near the center of the perimeter fence. The bait consisted of 1 kg of fresh animal or fish parts and ~ 1 L of water. The bait container was made from 3.8-L plastic jugs. Four 3x5 cm holes were cut at the top of each jug. The holes were then securely covered with nylon screen to prevent insect contamination. Fabric wicks were attached to the lid of the container to help loft the scent.

Hair-snagging sites were checked on 14-day intervals, at which time each individual 4-point barb was carefully examined. Hair samples from each barb were considered an individual sample to simplify collection methods and to avoid con-

tamination of individual hairs. Each sample was placed in a paper envelope, labeled, and stored at room temperature in an airtight, moisture free container. To ensure a moisture free environment, humidity sponges were added. All hair samples were handled with latex gloves to prevent contamination with human DNA. Fecal sampling was also incorporated into the study. Feces were collected opportunistically throughout the hair sampling periods and only scat that was thought to be less than one week old was collected to ensure that DNA was not degraded. As scat was found 2 ml of sample was collected using latex gloves and placed in a vial filled with a 90% ethanol solution (Lisette Waits, University of Idaho, Moscow, personal communication). Samples were then stored in a freezer until time of analysis.

In April 1999 we conducted an aerial survey of the entire range to locate den emergent bears or tracks that we could mark with a GPS unit and revisit these sites during the hair-snagging period. On this flight we located only one set of tracks on a north-facing slope and were unable to determine if they were grizzly bear or black bear (*Ursus americanus*).

During the 2000 season we deployed six 35-mm fully automatic cameras with flash. The cameras were equipped with passive infrared sensors that differentiated between animal heat and ambient temperature. These cameras were systematically distributed to different cells on bi-weekly intervals, enabling photo sessions at each of the hair-snagging sites for a total of 27 14-day photo sessions.

DNA Analysis Methods

We extracted DNA using standard phenol/chloroform procedure (Sambrook et al. 1989). EtOH precipitated samples were reconstituted in 20-50 μL dH_2O . We rejected using chelex extraction because this method results in a larger volume of final extract, and consequently lower DNA concentration. Six markers developed for bear (G1A, G10B, G10C, G1D, G10L and G10X) (Paetkau et al. 1995) were run in 10 μL hexaplex PCRs. We used 1.39 μL of PE buffer I, 1 μL of 10mM Dntp, 0.2 μL of each 10 mM primer (0.2 forward, 0.2 reverse), 0.3 μL Amplitag Gold (5U/ μL), 0.1 μL of 2.5 mM BSA, and 4.81 μL of DNA extract. PCR conditions were 12 min at 95°C, 20 cycles of 30 s at 95°C, 30 s at 58°C (decreasing 0.1°C per

cycle to 56°C), 1 min at 72°C, 15 cycles of 30s at 95°C, 30s at 56°C, 1 min at 72°C, final extension of 30 min at 72°C, followed by a 4°C hold.

Markers were labeled with fluorescent dyes HEX, TET, and FAM, and run with TAMRA 500 internal size standard on a PE 377 Genetic Analyzer for 2 hr. Alleles were scored by Genotyper. We calculated error rates on a per allele basis from duplicate runs for heterozygote loci, where three or four (out of four) alleles amplified. Individual assignment analyses were performed in identity (Amos and Harwood, 1998).

Results

Two field seasons consisting of 4550 hair-snagging nights and analysis of 255 hair samples from the Tobacco Root Range resulted in no detection of grizzly bear DNA. One hair sample indicated possible grizzly bear DNA. This sample returned with both black bear and grizzly bear DNA, which suggested to us that a grizzly bear either consumed a black bear or there was a lab error. We suggest that this sample is not definitive enough to conclude grizzly bears were present.

We obtained consensus genotypes for black bear samples at up to six microsatellite loci (G1A, G10B, G10C, G1D, G10L, G10X) for 121 hair samples that we genotyped two or more times. Genotypes were obtained for all 6 loci for all but 8 samples. For these samples, raw error rates were 0.16 dropouts per allele tested and 0.05 misprints per allele tested, similar to other studies using noninvasive samples collected in the field as a source of DNA, although higher than average for hair samples (Creel et al 2003). These 121 samples yielded 106 distinct genotypes, which could be considered an estimate of minimum black bear population size (Kohn et al 1999, Wilson et al 2003). Based on microsatellite data from a wolf (*Canis lupus*) population of known size with similar error rates, we suspect our estimate of black bear population is too high, perhaps by a factor of three to four (Creel et al. 2003). With the rates of dropout and misprinting we obtained, resampled individuals are likely to contribute more than one genotype to the data set, causing an overestimation bias. Thus, we do not believe that the microsatellite data provide a reliable estimate of the size of the black bear population. However, even adjusting conservatively for overestimation bias, these data suggest that more than 25 black bears were sampled in the study area.

Analysis of 25 fecal samples also resulted in no detection of grizzly bear DNA. Our analysis of microsatellite genotypes for black bear feces supported that our genetic sampling detected many individuals.

Over the study period, 378 nights of infrared camera detection gave no evidence of grizzly bear activity at any of the 27 hair removal sites. Photographs revealed a variation in age structure of black bears, capturing single adults, females with cubs, pairs of adults, and sub-adults at different times and at different sites.

Discussion

From spring 1999 to fall 2000, our data suggest that no grizzly bears were detected using the Tobacco Root Range as either a travel corridor or linkage zone. Our results corroborate what others have suspected, that no grizzly bears currently occupy the Tobacco Root Range (M. Patroni, R. Wiseman, Inter-Agency Grizzly Bear Study Team, personal communication). Further research into past mortality/trapping records indicated the same findings (Craighead et al. 1998).

The methodology used in this study had greater potential for detection due to the smaller cell size used, as compared to similar studies, which utilize a cell size of 8 km² (Mowat and Strobeck, 1999). The bait attractant was efficient at luring black bears into the hair removal sites; this was evident from the analysis of hair and fecal samples, camera detection, and personal observation.

Our analysis of microsatellite genotypes for black bear hair and feces supported two important points. First, our genetic sampling detected many individuals. Thus, if grizzly bears were present, even at a density far lower than the black bear population, it is reasonable to expect that the sampling program would have detected them at least once. Second, the study area sustains a substantial black bear population, which use many of the same foods and habitats as grizzly bears. Therefore, the area could be expected to be used by grizzly bears.

CERI has identified the Tobacco Root Range as suitable habitat for grizzly bears and we documented many of the food sources and habitat requirements of grizzly bears within our study area. As grizzly bear populations increase and desired bear habitat decreases, it is conceivable that at some point in time grizzly bears will colonize

the Tobacco Root mountain range. To maintain genetic diversity the grizzly bear population in the GYE needs to exchange genetic material with outside populations. Thus, management of the Tobacco Root Range as a potential linkage zone to maintain and enhance flow of genetic material is a critical part of the successful application of metapopulation theory to grizzly bear conservation (Servheen et al. 2001).

We conclude that grizzly bears probably do not have an ecologically significant presence in the Tobacco Root Mountains, despite substantial use by black bears. If the Tobacco Roots serve as a linkage between Yellowstone National Park and other areas for the movement of grizzlies, then such movements are apparently not common. Despite detecting 280 genetic samples, 480 photos, and numerous tracks, no clear evidence of grizzly bear presence was obtained. However, our data do not allow the conclusion that the area is completely unused by grizzly bears.

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