**Cat Project of the Month – April 2010**

The IUCN/SSC Cat Specialist Group’s website (www.catsg.org) presents each month a different cat conservation project. Members of the Cat Specialist Group are encouraged to submit a short description of interesting projects.

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**Minimum number and diet assessment of recently documented pumas at Kofa National Wildlife Refuge in Arizona to address predation management for desert bighorn sheep conservation**

With the recent decline in desert bighorn sheep numbers on Kofa National Wildlife Refuge (Kofa NWR) in Arizona, predation by pumas has become an increasing concern and there is a critical need for continued information on the abundance and food habits of pumas on the refuge.

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**Background**

Predation by mountain lions on declining desert bighorn sheep populations has become a major concern to non-government organizations and state and federal wildlife managers in southwestern Arizona, especially on the Kofa National Wildlife Refuge (Kofa NWR). The only record of a mountain lion on Kofa NWR, prior to 2003, was of a hunter-killed mountain lion in the Kofa Mountains in 1944, after which mountain lions were thought to be largely transient in southwestern Arizona. However, between 2003 and 2007 direct sightings, detection of tracks and an extensive camera-trap survey revealed the presence of 5 different mountain lions (3 adults and 2 kittens) occupying the Kofa Mountains. This documentation, along with a declining population of desert bighorn sheep, led the Arizona Game and Fish Department (AGFD) to implement in 2007 an adaptive, site-specific predator management plan directed at removing any mountain lion that preys on more than 1 bighorn sheep killed during any 6-month period. GPS data from 4 radio-collared mountain lions also indicates that Kofa NWR and 4 nearby mountains ranges are providing habitat to mountain lions with exceptionally large movement ranges. These preliminary findings, and the concern over declining numbers of desert bighorn sheep, prompted further investigation into determining the abundance and diet of mountain lions on the Kofa NWR.

Kofa NWR has made significant progress to date in assessing its own mountain lion populations, but little is known about how they are related to mountain lions outside the refuge boundary. This information is necessary to investigate patterns of movement of mountain lions between the Kofa NWR and surrounding mountain complexes in the southwest Arizona region. The results from current studies involving camera trap detection, GPS-collar radio-tracking and scat DNA analysis, have demonstrated the need for expansion of this work to the surrounding areas. There is a critical need for continued information on the abundance of mountain lions and their food habits on the refuge and movements into the refuge to make decisions on mountain lion management within a regional context, since predator management actions have consequences that extend beyond the boundaries of the refuge.

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**Map showing the study region, samples obtained for genetic analyses and future sampling locations for pumas to estimate and assess connectivity and movement patterns.**
The results from the ongoing study on Kofa have shown promise for being applied region- and state-wide as a non-invasive genetic method to survey and manage a notoriously elusive species and to provide concrete data unobtainable by traditional survey methods. Information obtained from this study will be essential for the new Comprehensive Conservation Plan, scheduled for 2011. This study also contributes to objectives in the 2007 Investigative Report and Recommendations for the Kofa Bighorn Sheep Herd, which are to identify causes and extent of predation on bighorn sheep.

Agencies will use information from this study to draft and implement management action plans focused towards the benefit of the highly vulnerable desert bighorn sheep and mule deer population on Kofa NWR as well as sheep and mule deer populations in surrounding areas.

The results from this project will provide the AGFD and the U.S. Fish and Wildlife Service (USFWS) with:
1. Estimates of minimum population size of mountain lions in the southwest region of Arizona
2. Information on the source population(s) for mountain lions recently identified on the Kofa NWR and potential mountain lion movement/dispersal corridors
3. Estimates of relatedness of individual mountain lions recently identified on the Kofa NWR to the larger regional mountain lion population(s).
4. Mountain lion diet and estimates of food habits by individual mountain lions identified in the southwest Arizona region mountain complexes.
5. Information on effects of management actions involving removal of mountain lions from project area.
Methods
During Phase-I of this project, reference biological (DNA) samples were collected from representative locations in Arizona and are currently in possession by the Conservation Genetics Laboratory in the School of Natural Resources at the University of Arizona, Tucson. These samples were collected in locations that include mountain lion habitat within an approximate 300 mile radius from Kofa NWR. Scat samples from mountain lions have been collected extensively throughout Kofa NWR mountain complexes since Fall 2008.

Phase-II: Scat collection has been opportunistic but will be supplemented by a randomized sampling design based on prey reproductive cycles that fall within the planned study period: 2010-2012. Mountain lion scat samples will also be collected from new areas from which we do not have reference DNA samples. These areas include mountain ranges in southern California and to the north and south of the refuge – e.g., Black Mountains, Cabeza Prieta NWR, Gila Bend Mountains and Pinacate Biosphere Reserve (Mexico). AGFD wildlife managers and interns have contributed scats from these areas and will continue to do so in support of this project. To maximize our final yield of usable DNA from scat samples and obtain a relatively large sample size, collection efforts will be continuous (year-round).

Field Data Collection
All scats found suspected to be of mountain lion origin based on morphology will be collected based on a standard field collection protocol for further DNA analysis (see attached). Sampled locations will be recorded using handheld GPS units along with time, date and habitat type details for mapping purposes after DNA analysis. Scat samples collected will be desiccated and frozen as soon as possible, to prevent any further DNA degradation, and transported to the laboratory.

Laboratory Analysis
DNA will be extracted from epithelial cells on the surface of the scat samples. PCR amplification will be done using mitochondrial DNA markers to confirm species identity. Once identified, DNA from mountain lion scat samples will be PCR amplified using microsatellite DNA markers to generate individual genotypes. Using software packages (mentioned below), all genotypes obtained will be analyzed for relatedness, structure, isolation by distance and migration. At the end of the analysis, we hope to map a best estimate of gene flow across the compared sub-populations. We will also perform a rarefaction analysis to obtain a minimum population estimate during each of the three years. DNA will also be extracted from prey remains, such as tissue and bone fragments, found inside the scat samples, for identification of prey species. Scat components identified will be compared to a database divided into biological year periods to estimate any seasonal variation in prey selection by mountain lions.

Project location
Mountain complexes in the Kofa NWR and surrounding mountain complexes in southwest Arizona and southern California, including the Plomosas, Harquahalas, Black Mountains, Cabeza Prieta NWR, Gila Bend Mountains, and Pinacate Biosphere Reserve (Mexico).

Results
We collected 105 scat samples suspected to be mountain lion in origin between 2007 and 2009 from the Kofa and Castle Dome Mountains in Kofa NWR. We extracted and PCR-amplified DNA from the surface of the samples and, using a species-identification PCR assay, identified 56 scat samples to be from mountain lion, 12 from bobcat (Lynx rufus) and 3 from coyote (Canis latrans).
The remaining 37 scat samples failed to yield PCR-amplifiable DNA. We then analyzed the mountain lion scats (along with 4 male lion tissue reference samples collected during radio-collaring efforts on the Kofa NWR) in a genotyping assay employing 3 microsatellite loci chosen from the domestic cat genetic linkage map. We successfully genotyped 4 tissue reference samples from previously captured mountain lions (all males) and 21 of 56 mountain lion scat samples. Among the 25 obtained genotypes, we identified 11 unique genotypes (individuals). Then, using a PCR-based assay with the 4 tissue samples as male controls and 1 other female mountain lion control sample (collected by AGFD near Sabino Canyon, Tucson, Arizona), we identified an additional 2 female and 2 male mountain lions. The assay failed to yield PCR products for the remaining 3 unknown individuals.

We concluded that a minimum of 11 individual mountain lions used the Kofa NWR between 2007 and 2009, including at least 2 females and 5 males identified from scat samples (see map) and 1 male identified only from the reference tissue samples. We surmise that success in identifying, genotyping and sexing mountain lion scats can be limited by DNA degradation caused by exposure to sunlight and moisture prior to collection.

Diet analysis

We isolated prey remains (i.e., two morphologically dissimilar bone or connective tissue fragments) from each of the 56 mountain lion scats, pulverized each fragment, and extracted DNA from the powder. We successfully identified prey species from all bone fragments using the species-identification PCR assay described above. We determined that a majority (~50%) of mountain lion diet was composed of mule deer. We also documented 4 other different prey species (see graph). Our high success in identification of prey remains is possibly due to the preservation of DNA in bones complemented with the protection offered by scat material against direct exposure to sunlight and moisture.

For wildlife agencies plagued by a lack of robust information on mountain lion populations, genetic techniques provide an effective, non-invasive and potentially less expensive way to sample populations over large areas. Our analysis of mountain lion scats provided data, in conjunction with on-going camera-trap surveys and radio-collaring efforts, on the minimum number and sex of mountain lions. Wildlife managers may exploit such genetic analyses to monitor wild populations of cryptic species with questions not just limited to population parameters, but also individual relatedness and movements at the landscape level. We foresee non-invasive genetics will become increasingly useful to wildlife managers as management agencies are called upon to obtain baseline information in support of management actions.

Taking into consideration the low success rate on PCR amplifiable DNA recovery from feces, we wish to extend our fecal sample collection to obtain a large enough sample size that will allow for drawing conclusions on a robust minimum number estimate and construction of a diet profile for the pumas on the Kofa NWR.
Future plan
In southwestern Arizona, mountain lion management decisions in the future will require information on how mountain lions in Kofa NWR fit within the larger regional mountain lion population. To address this, we are continuing this project to the next phase and plan to use both microsatellite and single-nucleotide polymorphism (SNP) markers to analyze mountain lion DNA samples collected at a regional scale. Based on relatedness estimates from this analysis, we plan to map out a best estimate of gene flow across the compared populations or sub-populations. To initiate this phase of the project, we plan to obtain mountain lion DNA samples from the surrounding mountain ranges in southern California and to the north and south of Kofa NWR, including the Pinacate Biosphere Reserve in Mexico. Our ability to achieve the project’s goals would benefit from any collaboration with other researchers via contribution of representative mountain lion DNA samples from areas surrounding Kofa NWR. Anyone who has or can obtain scat or tissue samples from mountain lions should contact the primary author if interested in contributing samples to this project.

Project Information
Duration: Phase I: June 2008 – November 2009, Phase II: Jan 2010 - 2012
Location (see map): Kofa National Wildlife Refuge, Arizona
Sponsor(s): United States Fish and Wildlife Service (USFWS)
Project address 9300 E 28th St., Yuma, Arizona 85365, USA
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Protocol IN-FIELD SCAT SAMPLE HANDLING, COLLECTION AND STORAGE PROTOCOL PDF