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Evaluating Genetic Analysis of Scat as a Technique for Monitoring Species Richness in National Parks

Photo of mountain lion and cub, Saguaro National Park, Tucson Mountain District, taken by remote wildlife camera, January 2005

Lay Report

Western National Parks Association and Saguaro National Park
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Introduction

Conservation genetics is a fast-growing field that is continually discovering new ways to address ecological and conservation concerns with genetic tools. This Western National Parks Association funded research project was designed to investigate the potential use of non-invasive genetic techniques for determining the presence of mammal species in national parks. Knowledge of which species are present in a park or natural area is essential for measuring changes in biological communities over time. Surprisingly, for many groups of animals, it can be very difficult to determine which species are present. Although some mammals are common and highly visible, many others are nocturnal, rare, elusive, or difficult to identify. In addition, traditional methods of studying mammals, such as trapping, can be both stressful for the animals and very expensive.

Our focus was to determine the effectiveness of genetic analysis for estimating the number of terrestrial mammals (that is, all species of mammals except for bats) in the Tucson Mountain District of Saguaro National Park. Our study was based on DNA extracted from scat samples collected in the field. When mammals defecate, they release cells from the intestinal tract, which are deposited on a thin mucous membrane surrounding the scat. By taking scrapings from around the surface of scat samples we were able to recover DNA from those cells, which could then be used to identify the species that deposited them. In addition, to identify prey species we performed DNA extractions on bone material removed from predator scats. Results of our genetic analysis were then compared to results from remote wildlife photography, a more traditional technique for detecting presence of terrestrial mammals. To our knowledge, this was the first study to use DNA in an attempt to determine species richness (the number of species of mammals present) in a national park or other natural area.

Methods

Our study required development of several new sets of information. After collecting a large number of scat samples in the field on randomly located transects, our initial step in the lab was to develop primers. Primers are small, artificially-manufactured pieces of DNA that are designed to recognize a part of the entire genome of an animal and reproduce it millions of times in a process called polymerase chain reaction (PCR). We designed these primers to replicate a portion of the mitochondrial genome, because there are more than one thousand copies of the mitochondrial DNA genome (as opposed to the one copy of the nuclear genome) in each cell. We also designed our primers to replicate a relatively short section of DNA. Ultra violet light breaks down DNA, and because our scat samples had been exposed to the intense ultra violet light in the desert environment, we wanted to target a small section so that it would be more likely to be intact. In a final step, we tested our primers on a representative species from every family of terrestrial mammals that is known to occur in Saguaro National Park West so that we could be sure that if we recovered DNA, our primers would recognize it and be able to reproduce it into something useable for our analyses.

Once we had extracted DNA from our samples we entered the sequences into "BLAST", a computer program provided online by GenBank®, the genetic sequence database of the National Institute of Health. This program compares an unknown

genetic sequence to a vast database of sequences, then finds the closest match. To confirm these species identifications, we created a reference list of sequence data for all potential species of terrestrial mammals that could possibly be found in the park (including domestic dog and cat). We created this reference list using tissue samples from specimens collected in the Tucson area as well as existing published sequences on Genbank. Our reference list and positive samples were then entered into a phylogenetic tree-building program, which resolved them into a "family tree". Positive samples would "group" with the reference sequence that they were most closely related to. These trees provided us with statistical support for making positive species identifications.

Results

Of 141 total attempted DNA extractions from scat (103 samples) and the bones removed from scat (38 samples), 48 (34%) positively identified a mammalian species. From 103 scat sample extractions, 25 samples (24.3%) were positive, identifying nine species. From 38 bone sample extractions, 23 samples (60.5%) were positive, identifying five species. Many of 141 samples were from predators. Predator scat samples and the bone material extracted from them accounted for 116 samples (the remaining 25 samples were scat samples from non-predators, such as javelina and rabbits). Of the predator samples, 43 were positive (37.1%) and accounted for nine species. Whereas predator scat samples and bone samples did not account for a higher rate of new species accumulation, they did represent the greatest rate of positive species identification. In total, we identified 9 of 27 (33.3%) potential mammal species documented to occur in Saguaro National Park West: collared peccary, gray fox, hooded skunk, ringtail, mountain lion, bobcat, black-tailed jackrabbit, Bailey's pocket mouse, and white-throated woodrat. We also identified three bird species with the BLAST program, but did not do further analysis with the phylogenetic tree building program.

To compare our genetic techniques with other techniques for detecting mammal species, infrared-triggered wildlife cameras were placed in the park at random locations at upland and riparian sites for a total of 24 weeks. These cameras detected 17 species of mammals, 8 more than we detected using genetics.

Discussion

Whereas remote photography detected more species than genetic analysis of scat in our study, we gained very useful insights into using genetic techniques for estimating species richness. Most importantly, we demonstrated that genetic techniques work, and that there is great potential for using this method in other studies in national parks and other natural areas.

That we did not identify a larger number of mammal species in Saguaro National Park West through genetic techniques was primarily due to limitations of time and funding in this exploratory study. The majority of our time in the study was spent developing appropriate primers and the conditions under which they work most effectively. Now that this process has been completed, subsequent researchers who hope to use these techniques would not have to repeat it. However, because of this initial investment of time in our study, we were able to process a relatively small sample size (141 total extractions).

In addition, our results indicate sampling efficiency can be greatly increased by focusing extractions on predator scats and the bone material associated with them. Although we were able to identify herbivores from their scat using genetic techniques, we were able to identify herbivores more efficiently from bones within predator scat. In other words, focusing primarily on carnivores would have increased the percentage of positive samples, allowing us to process more samples and detect more species.

Finally, our results indicate that we could have increased our efficiency by repeating PCR replication for our negative samples. PCR is the chemical process by which DNA is multiplied exponentially to produce millions of copies of the DNA segment targeted by the primers. In very low concentration samples of DNA extract, it is sometimes necessary to repeat PCR for negative samples because the very few copies of DNA in the sample were not picked up and added to the reaction. This problem can produce a negative sample even though there is actually DNA in the extract, because it was simply missed. By repeating PCR one can increase the probability of detecting DNA in a sample of extremely low concentration, which is typical of scat samples.

We feel that this new technique has great potential application for monitoring mammal diversity in parks because it is relatively inexpensive, and is likely to become less expensive as DNA technology advances. Although a major goal of the National Park Service is to conserve natural resources for future generations, in many parks, including Saguaro, mammal species have gone extinct after park establishment. While long-term monitoring is essential for detecting trends and preventing future loss of species, monitoring has always been too time-consuming and expensive to implement in most parks. Despite disturbing downward trends for several species, Saguaro does not currently monitor populations of most mammals, nor is there a plan to do so.

Although genetic techniques cannot alone resolve this problem, we believe that use of mitochondrial DNA derived from scat does have the potential for being a more cost-effect approach to monitoring the entire community of mammals than other techniques. Although DNA from scat does not provide the kind of physical information derived from mammal trapping, nor the useful interpretive photographs derived from wildlife cameras, there are many other potential applications for this technique as well, ranging from understanding predator-prey dynamics to identifying individual animals for use in estimating population sizes.

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