Non-Invasive Indexing of Red and Gray Fox Populations at Brookhaven National Laboratory

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Gray fox – field camera – Brookhaven National Laboratory

Red fox (photo – Jennifer Higbie) – Brookhaven National Laboratory

ABSTRACT

The red fox (*Vulpes vulpes*) and the gray fox (*Urocyon cinereoargenteus*) have sympatrically inhabited the greater Long Island area over the last several hundred years. In recent years, speculation has grown regarding the population size of each species. While the red fox has historically been known to adapt well to ecological disturbances, including those of an anthropogenic nature, and is largely considered to have a thriving population in the Long Island area, recent studies of the last thirty years suggest the gray fox populations have struggled with such anthropogenic disturbances of the last century. A previous Brookhaven National Laboratory (BNL) study in 2006 confirmed the presence of gray fox on BNL property using non-invasive fecal DNA analysis via mitochondria DNA markers and automated camera documentation. This project further studied the extent of the gray fox presence at BNL for the 2007 season by using the non-invasive techniques of fecal DNA extraction and automated field cameras. Gray fox presence was confirmed through both methods over the course of the study. While apparently much less common than the red fox, the gray fox species appears to be present and established at BNL and, presumably, in similar habitats throughout the Long Island area.

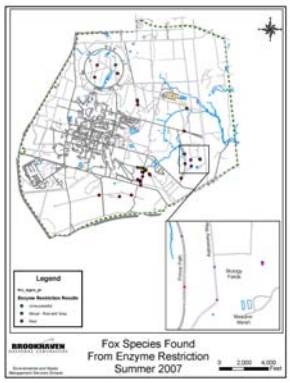

Figure 1: Scat samples with successful enzyme restriction from summer 2007 collection – labeled by species (refer to legend)

INTRODUCTION

Throughout many parts of North America, the gray fox (*Urocyon cinereoargenteus*) and the red fox (*Vulpes vulpes*) coexist sympatrically. While in many parts of the country, gray fox populations have grown in the past 100 years due to abandonment of farmland and subsequent woodlands growth [1], it is likely that gray fox populations of the Long Island area have followed the opposite trend [2]. Data on fox populations in the area is scarce, and most of the available information is outdated and may not account for recent changes in habitat due to anthropogenic disturbances [2].

Gray foxes typically prefer a habitat of mixed hardwood/pine with fairly dense undergrowth [3]. While the diet of the red fox is comprised of mostly small mammals and insects with a mix of some plant material, the gray fox is considered more omnivorous, with over half of its diet (with some seasonal variation) coming from plant materials such as berries [4]. With a balanced omnivorous diet, it seems to follow that gray fox species would tend to reside in dense forest habitats with high availability of both small game and vegetation. Anthropogenic disturbances affecting these preferred habitats of gray foxes would create a somewhat transient lifestyle in which the species would likely have a much lower survival rate [5]. In disturbed habitats, red fox tend to have a much higher survival rate than gray and have been found increasingly more often in suburban and urban settings over the last century [6].

A study at BNL in the summer of 2006 examined the presence of the gray fox species on laboratory property [7] by using techniques and strategies largely based on a 1997 Smithsonian Institution zoological study [8]. Based on these and other studies, this project relied on several key points: a) DNA can be successfully extracted from fecal samples due to the shedding of epithelial cells from the digestive tract b) these fecal samples can be effectively preserved to undergo DNA extraction c) samples can easily be obtained by walking forest paths and roads, as canids tend to follow these established routes [9].

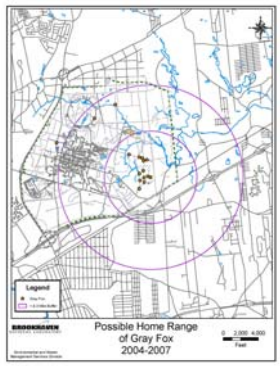

Figure 2: Buffer around known activity of gray foxes gathered from 2004-2007 -- smaller circle represents 1 mile radius range, larger circle represents 2 mile radius range. Foxes typically maintain a home range with a radius of about 2 miles.

MATERIALS AND METHODS

The Laboratory was first divided into walking transects from a vegetation map to be walked regularly. The GPS location was recorded for each sample prior to collection using a Thales handheld GPS/GIS device. The scat samples were collected in resealable bags, preserved with silica gel in the ratio of 4g of silica gel to 1g of sample and then stored in a freezer in preparation for DNA extraction.

For the mitochondrial DNA (mtDNA) extraction, the protocol from the Qiagen QIAamp DNA Stool Mini Kit was followed. After DNA extraction, a portion of the DNA was then run through a Polymerase Chain Reaction (PCR) following standard protocols of a Taq PCR kit. A portion of each PCR product was run on a 0.8% agarose gel to test the success of the PCR. Successful PCR products then underwent enzyme restriction using AluI and HinfI enzymes following standard protocol. Enzyme restriction products were then run on a 2% agarose gel to determine species of sample.

In addition to scat samples, a digital field camera was used to supplement the results of the scat species identification. Camera sites were chosen based on a variety of factors, including: area of scat collection success, reported sighting locations, and likely habitats.

RESULTS

Overall, 51 scat samples were collected from the field. All of these samples plus one additional sample from 2006 underwent DNA extraction and PCR. Of the 52 samples, 40 had a successful PCR (77% success rate). Of the 40 successful PCR products run through enzyme restriction, 28 returned a positive red fox result, 9 samples returned positive bands for both red and gray fox, and 3 samples returned no result. In the case of the double species positive results, the result could not be classified as exclusively gray fox or red fox. A result with two positive readings was therefore classified as a "mixed" positive sample for this project. In these "mixed" results, intensities of the bands varied, but distinct bands were observed for each species. These 9 mixed samples underwent an additional PCR and enzyme restriction and were run through an acrylamide gel to confirm the initial results. From the reading of the enzyme restriction products on the acrylamide gel, 8 samples came back exclusively gray positive, with one sample yielding no result.

In approximately four weeks of use, the Reconyx automated field camera also returned positive results for the gray fox. In one camera location, the camera captured the gray fox on film on at least five distinct occasions. Additionally, in one set of pictures during a brief time period, two gray foxes appeared together in one picture. Furthermore, in the same camera location, a gray fox and a red fox appeared within seconds of one another.

Two gray foxes (at night) – field camera– Brookhaven National Laboratory

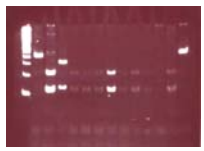

Figure 3: 7.5 % acrylamide gel – enzyme restriction products -- Lane 1: ladder – Lane 2: red fox positive control – Lane 3: gray fox positive control – Lane 4: domestic dog positive control – Lanes 5-13: scat samples – Lane 14: control (no enzyme)

DISCUSSION AND CONCLUSION

While foxes are known to be transient in their juvenile life stages as they establish their territory and disperse from their natal dens, it appears that the gray foxes identified through this study are permanent residents of the study site. Since two adult gray foxes were captured on camera in the same photo, it is very unlikely that both foxes were transient individuals just passing through the area; instead, it is more likely that these individuals have an established home range that encompasses a part or parts of BNL.

Additionally, it appears that red and gray fox populations have direct habitat overlap with one another. With appearances by both species with such a small temporal gap on the camera, it is clear that the species have some level of habitat overlap, albeit to an unknown degree. Moreover, the scat samples that returned mixed species positive results were obtained in an isolated geographic area of approximately 0.25 square miles. In the first round of laboratory testing, the only positive gray fox results were coupled with a red fox positive result was well. This mixed positive result was possibly the result of territorial marking by one or both of the species upon the other's feces. It would therefore be unclear which species produced the actual fecal sample, but the mixed positive result would confirm the species presence of the gray fox nonetheless. It is also possible that some degree of contamination between samples occurred. To test the validity of the first set of results for these mixed samples, a second round of tests was run. The second run returned results of exclusively gray for 8 of 9 of these previously "mixed" samples (with the other sample returning no result). Although the second round of testing showed the exclusive gray fox species identification result, it is unclear whether the first round of testing actually showed trace red fox DNA within the sample or if it was simply a result of contamination. To make this matter more clear, further tests on the sample would have to be run to retest for this trace red positive result.

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