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

Patrick Mallin
Office of Science, Science Undergraduate Laboratory Internship Program

College of William and Mary, Williamsburg VA

Brookhaven National Laboratory
Upton, New York

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Participant: _____
Signature

Research Advisor: _____
Signature

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ABSTRACT

A Non-Invasive Indexing of Red and Gray Fox Populations at Brookhaven National Laboratory. PATRICK MALLIN (College of William and Mary, Williamsburg, VA 23186) JENNIFER HIGBIE (Brookhaven National Laboratory, Upton, NY 11973).

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 on BNL property 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 on the BNL site and, presumably, in similar habitats throughout the Long Island area.

1. 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 Data on fox populations in the area is scarce, and most of the available information is outdated and may not account for recent changes in habitat due to anthropogenic disturbances [2].

While both species of fox are members of the taxonomic family *Canidae*, the two species are fairly distant relatives [1]. The two differ in size, with the gray fox being slightly smaller and lighter on average. Additionally, unlike the red fox, the gray fox is able to climb trees and partially retract its claws. The gray fox is considered to be the more aggressive of the two and would tend to dominate an interspecies encounter [1]. The two species can also be easily be visually identified by their characteristic markings on their pelage. The red fox has red-orange fur, a white underbelly, black colorations on its legs and ear tips, and a white-tipped tail. Conversely, the gray fox has coarse gray fur with black colorations near its eyes and on the tip of its tail. The gray fox does, however, have reddish-brown fur on its front and parts of its lower half, causing some identification confusion with the red fox.

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 BNL study 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].

2. MATERIALS AND METHODS

The Laboratory was first divided into walking transects on a site vegetation map. Since the goal of the transect walking was to collect fox scat samples, these transects focused primarily on the more heavily forested regions of the site which would be conducive to fox activity based on known fox habitat preferences. Transects were walked at random with an equal share of walking time each week. The GPS location was

recorded for each sample prior to collection using a Thales handheld GPS/GIS device. Additionally, other objects of interest such as fox tracks, dead animals, and other signs of possible fox activity were noted and recorded on the GPS device. The scat samples were collected in resealable bags and 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. Used as a common wildlife field camera, the Reconyx automated digital camera allowed for nighttime pictures, motion-triggered pictures, and daytime color pictures. The camera was used only during the last four weeks of the study due to availability but was placed in several locations on the BNL site, with the ultimate goal of catching a gray fox on camera. Camera sites were chosen based on a variety of factors, including: area scat collection success, reported sighting locations, and likely habitats.

3. 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.

4. 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 the BNL site.

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" result 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 test for this trace red positive result again.

Since the majority of the walking transects followed roads and paths of the site, it is possible that transects that were created did not accurately portray the proper ratio of fox populations of the site. Although red and gray fox species, and the *Canidae* family in general, are known to follow established forest paths and roads [8], the gray fox is known to be more reclusive, with a preferred habitat of mature pine or hardwood with shrubby undergrowth [3]. Walking the roads and paths for scat collection may not create the most representative sample of fox populations. The reclusive nature of the gray fox and its preference for a denser forest habitat may have skewed the scat collection data in which the percentage of red fox in the area was notably higher.

Although the scat samples collected were of varying quality, surprisingly there did not appear to be a strong correlation between scat quality and DNA Extraction and PCR success rate. Of the 52 samples obtained, 40 samples returned a positive PCR result, yielding an overall extraction success rate of 77%. This success rate was relatively high compared to a similar BNL study in 2006, and other fecal DNA extraction success rates, typically less than 40 % [10]. This high success rate can be attributed to effective preservation of the samples, effective potency of chemicals in extraction kit, along with other factors.

Since this study just briefly analyzed the interactions between red and gray fox, more research needs to be done on this species-species interaction in the future. Because

many top food-chain mammals such as black bears, wolves, coyotes, and medium-sized cats are absent in the Long Island area relative to comparable climates of North America, the fox is the top predator in the area with few or no predators besides man. This top predator status is fairly unique for the fox to have compared to other parts of the country, and behavioral research studying red fox and gray fox interactions along with fox and other predator interactions could offer some unique scientific insight.

In order to further study aspects of the fox populations on site such as behavior, home range, dispersion patterns, etc., a controlled trapping and radio-telemetry program would need to be implemented. Since gray fox are reclusive by nature and extremely difficult to visually track, a trapping and tracking program would provide invaluable information about the gray fox populations in the area with minimal animal stress.

5. ACKNOWLEDGEMENTS

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FIGURES

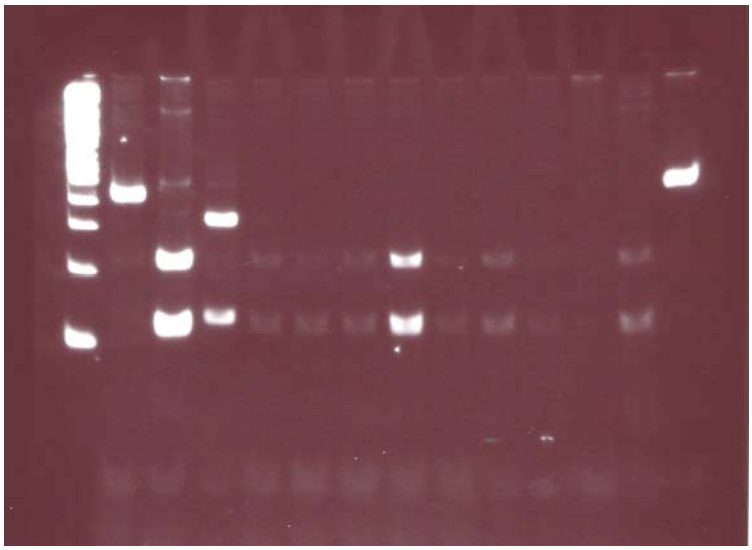

Figure 1: Acrylamide Gel – Enzyme Restriction Product
Well 2: Red Fox Positive Control
Well 3: Gray Fox Positive Control
Well 4: Dog Positive Control
Wells 5-13: Scat samples
Well 14: Control – No Enzyme

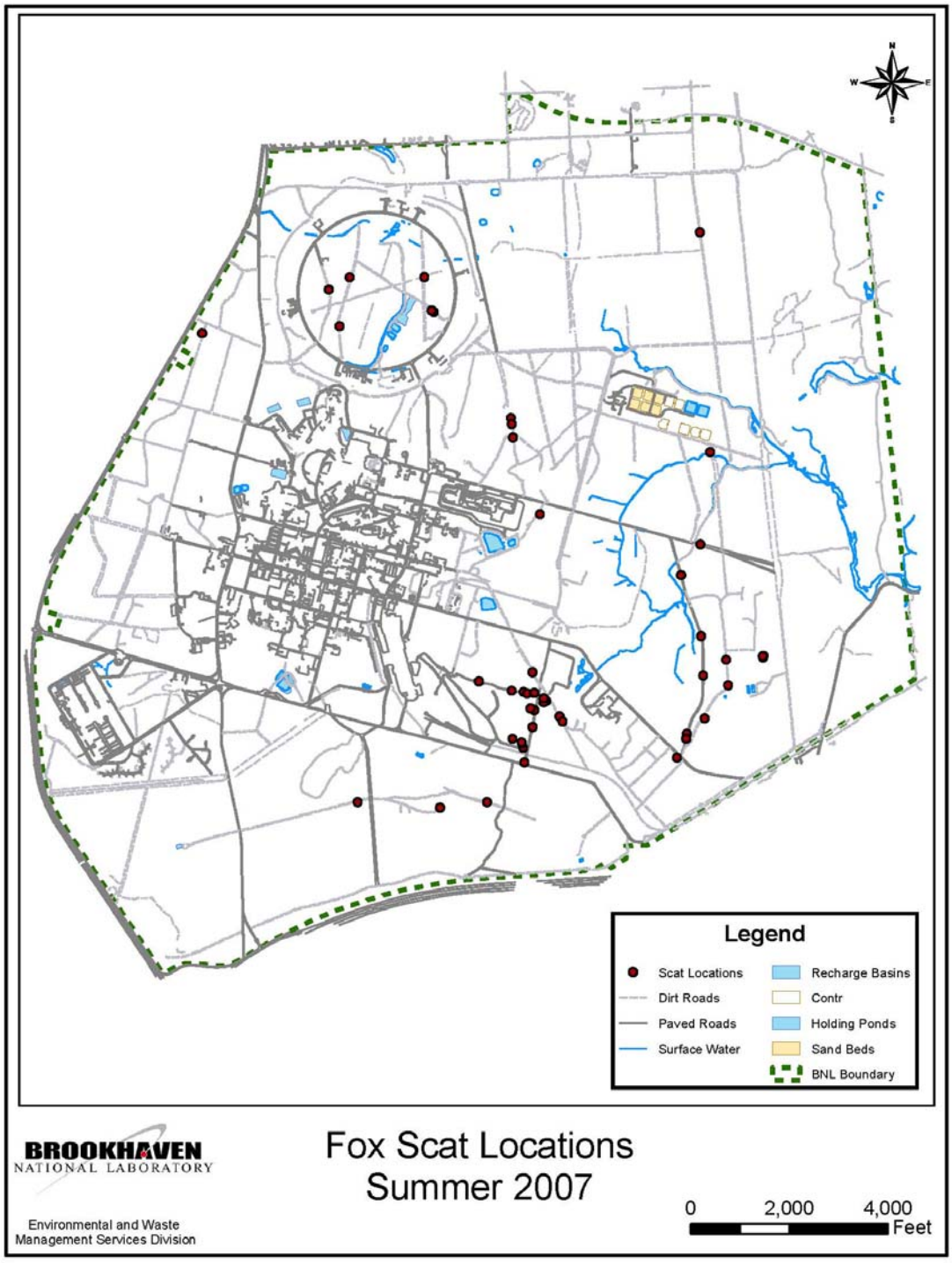

Figure 2: Scat Sample Distribution Summer 2007

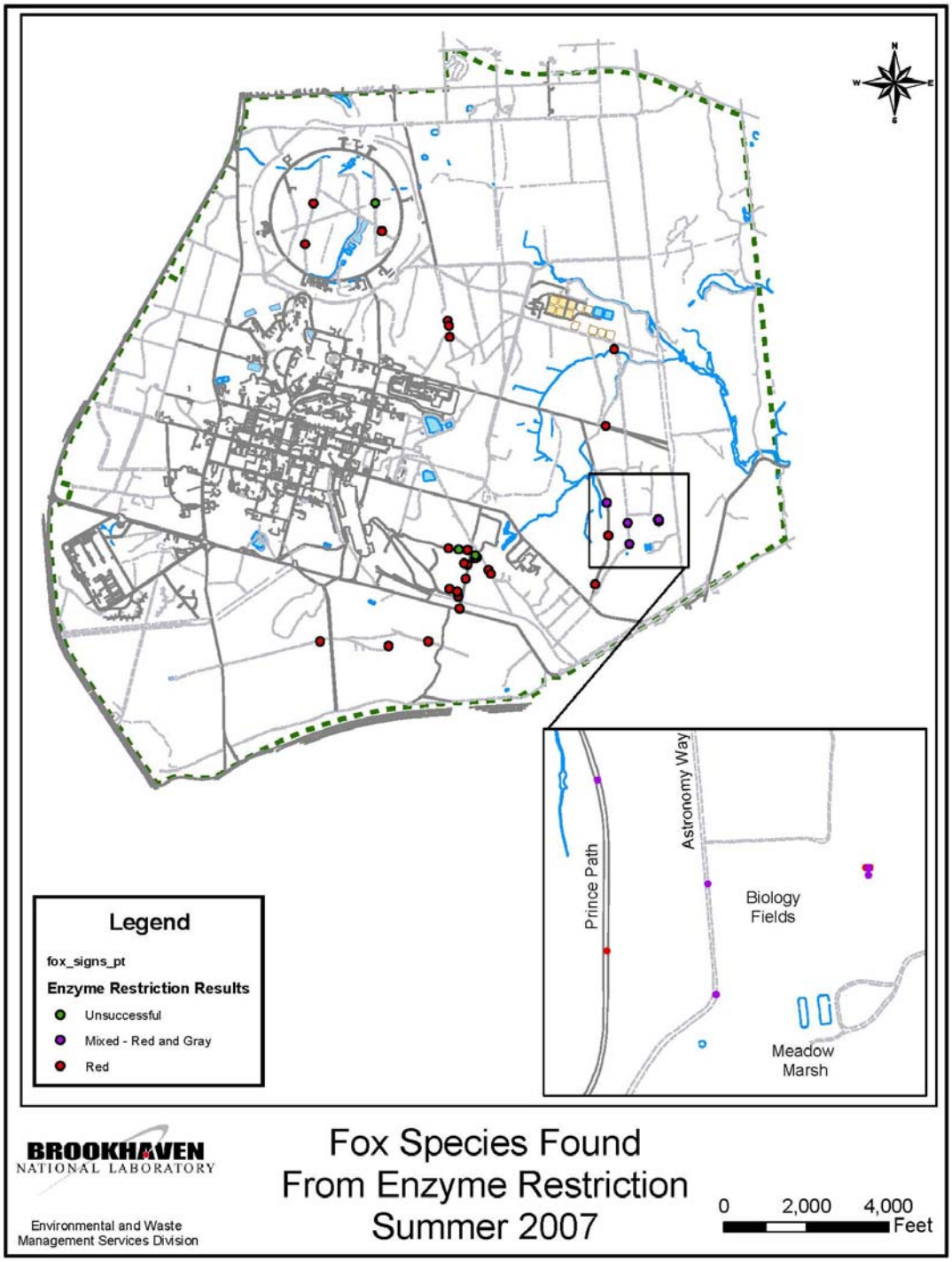

Figure 3: Summer 2007 Scat Distribution by Species