Non-Invasive Species Confirmation of Fox Populations at Brookhaven National Laboratory

Wendy Finn

Office of Science, Science Undergraduate Laboratory Internship (SULI)

University of Rhode Island

Brookhaven National Laboratory

Upton, New York

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Participant: _____
Signature

Research Advisor: _____
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ABSTRACT

Non-Invasive Species Confirmation of Fox Populations at Brookhaven National Laboratory. WENDY FINN (University of Rhode Island, Kingston, RI 02881) JENNIFER HIGBIE (Brookhaven National Laboratory, Environmental Services and Waste Management Division, Upton, NY 11973) TIM GREEN (Brookhaven National Laboratory, Environmental Services and Waste Management Division, Upton, NY 11973)

Information regarding the present day status of Fox populations on Long Island, NY is essential for an understanding of species diversity. Historically, Red Fox (*Vulpes vulpes*) and Gray Fox (*Urocyon cinereoargenteus*) occurred sympatrically on Long Island, NY. Although current population size estimates have not been established for either species it is speculated that the Red Fox has adapted to anthropogenic disturbances better than the Gray Fox. After the discovery of a deceased Gray Fox in the Relativistic Heavy Ion Collider at Brookhaven National Laboratory (BNL) in October of 2004, questions arose concerning the presence of this species in the area. To determine if the Gray Fox is utilizing areas of BNL as a home range, this study focused on observing mitochondrial DNA markers in feaces, which enable us to distinguish between the two species. A camera trap was implemented to determine areas of high fox activity. A positive scat sample and camera trap shot have confirmed the presence of gray fox at BNL.

INTRODUCTION

In October of 2004, a deceased juvenile Gray Fox *(Urocyon cinereoargenteus)* was discovered on the Relativistic Heavy Ion Collider (RHIC) road on Brookhaven National Laboratory (BNL) property. This discovery led to many questions concerning the abundance of this species at BNL. Information regarding Red Fox *(Vulpes vuples)* and Gray Fox populations on Long Island, New York is scarce and outdated with no current studies in progress on the subject. This preliminary study focuses on the method of analyzing mitochondrial DNA, which is extracted from suspected fox feces to distinguish between the two species. This non-invasive method of species identification is very useful in field studies as it imposes no stress on the animal in question and therefore does not alter the species usual movements and habits. The samples for this type of study are easy to obtain as canids tend to follow well-traveled game trails and roads for defecation and boundary marking [1].

Past literature states that although red and gray fox occurred sympatrically on Long Island, New York, the gray fox was the predominantly abundant species [2]. The gray fox was a more aggressive competitor when in its preferred habitat of undisturbed mature pine or hardwood combined with brushy undergrowth [3]. Since the dominant habitat on BNL is mixed oak-pine with a heavy understory of blueberry and huckleberry it falls into the preferred habitat type for gray foxes. With development limiting habitat, it is speculated that the red fox has adjusted to anthropogenic impacts more successfully than the gray fox, enabling it to become the abundant species [4].

The differences between the two species are mainly in pelage coloring with the gray fox having a black tipped tail and the red fox having a white tipped tail. The pelage of the gray fox is mostly gray but does include reddish marks along its neck. The red fox has black tipped ears and black legs that also help to distinguish it from the gray fox. Both species are crepuscular and nocturnal and share the same foraging techniques in their search for prey. They both are generally opportunistic feeders subsisting mainly on small mammals, insects, carrion and whatever berries may be in season except the gray fox is more inclined to subsist on insects and vegetation than the red fox [5]. Another distinguishing is that the gray fox is the only North American canid that has the ability to climb trees enabling it to escape from most terrestrial predators.

MATERIALS AND METHODS

Transects were walked on a daily basis with randomly chosen locations in the search for scat collection. Transects focused on the perimeter of BNL property in the more undeveloped sections. Sample collection was relatively easy as the foxes utilized roads on many occasions. All collected samples (n=58) were recorded with a gps point location. A Thales gps/gis hand held unit was taken into the field for data collection of fox signs such as scat (Figure One), tracks, kill sites and possible dens. Collected samples of feaces were placed in plastic Ziploc bags and later preserved with 4 grams of silica per one gram of scat and then frozen until mtDNA extraction could be performed.

A gray fox control was obtained from a skull provided by a taxidermist in Rhode Island. Once the tissue was removed from the skull it was preserved in ethanol and refrigerated until mtDNA extraction could be performed. A red fox sample was taken from a deceased kit found on the Long Island Expressway on the southern boundary of

the lab. A cheek swab obtained from a domestic dog was run to ensure no samples originated from wild or domestic dogs roaming on BNL property. Protocols from the Qiagen DNeasy Tissue kit were followed for mtDNA extraction of the controls.

Fecal extraction for mtDNA was performed on (n = 39) samples following protocols from the Qiagen QIAamp DNA Stool Mini Kit. A small portion of DNA from each sample was run on an .8% agarose gel to ensure successful extraction. Polymerase Chain Reaction (PCR) was conducted on all resulting mtDNA samples using a Taq PCR kit following standard protocols. A red fox tissue control accompanied all PCR rounds to ensure the primers successfully annealed to and replicated the desired segment of the cytochrome b gene. The red fox control tissue was also sequenced using Sequencher software to ensure a correct identification of the species. Following standard protocol, enzyme restriction was performed on successful pcr products using AluI and HinfI enzymes.

A Reconyx camera trap was used to locate areas of suspected fox activity. Camera locations were recorded as points with the Thales gps/gis unit (Figure Two). Once the camera confirmed presence of fox in the area transects would then be focused on that location in the search for sample collection. The camera card was reviewed and changed >3 times/week. If it was suspected that red foxes were identified on the camera then the camera would be relocated to a new area with the intent of locating a gray fox, assuming that the two species did not overlap home ranges or utilize the same areas of the laboratory.

RESULTS

The camera trap provided a positive result for a gray fox identification on the east portion of the laboratory. The fox was initially caught on the time set images on 07/21/06 at 04:28 hours and 07/22//06 at 02:50 hours. On 8/2/06 canned dog food was deposited in the line of the camera trap in the hopes of gaining clearer motion set images. An individual did return on 07/29/06 and was caught on the motion images where the specific pelage distinctions between red and gray foxes could be observed. The individual did not have the black legs and ear tips normally associated with the red fox but did have a darker pelage, muzzle and the black tip tail associated with the gray fox species.

Although 90% of DNA extraction performed on stools (n = 39) yielded that DNA was present in samples, PCR proved successful in (n = 14) or 26% of scat samples(Figure 3). Two samples produced unexpected PCR product. PCR was successful in yielding the desired 412bp segment.

Enzyme restriction of the control sample that was run on tissue from an assumed gray fox yielded bands that matched the expected patterns of a red fox. PCR was conducted again on the tissue and results were sequenced on Sequencher software. The resulting chain of nucleotides was compared to known sequences in the genbank database and the sample was returned back as *Vulpes vulpes*.

Enzyme restriction yielded 13 positive red fox samples(Figure 4). A sample found on the eastern portion of the lab was positive for gray fox. Two unknown pcr products yielded no bands during enzyme restriction.

DISCUSSION AND CONCLUSION

With known presence of red fox on the northern and southern areas of BNL it was interesting to find evidence of gray fox on the eastern portion of the property. It is unknown if this individual is related to the individual found deceased in the RHIC in October 2004. Although initially assumed to be a transient juvenile dispersing to establish a territory, it is now speculated that this incident may be the result of a permanent gray fox sub population on lab property. The RHIC area and East Fifth Avenue, where the gray fox was captured on camera, are about 1 mile apart from each other, a distance that could easily be encompassed in a gray fox home range size. It is possible these two individuals originated from the same natal range, but more information regarding the DNA sequences of each individual would be needed in order to determine their relatedness. If it was discovered that the RHIC area was encompassed within the gray foxes home range this would lead to more questions concerning red and gray fox interactions due to the well-documented red fox den located in the center of the RHIC.

Due to heavy precipitation sample collection was limited and quality of samples was compromised. Many samples had been exposed to sun, rain and other weather occurrences for unknown lengths of time effecting sample quality for DNA extraction. DNA of unknown species may have been extracted from the samples where DNA was

present in the initial gel but had no PCR success. The method of storing samples at –80° C in DET buffer may improve DNA extraction for future results [2].

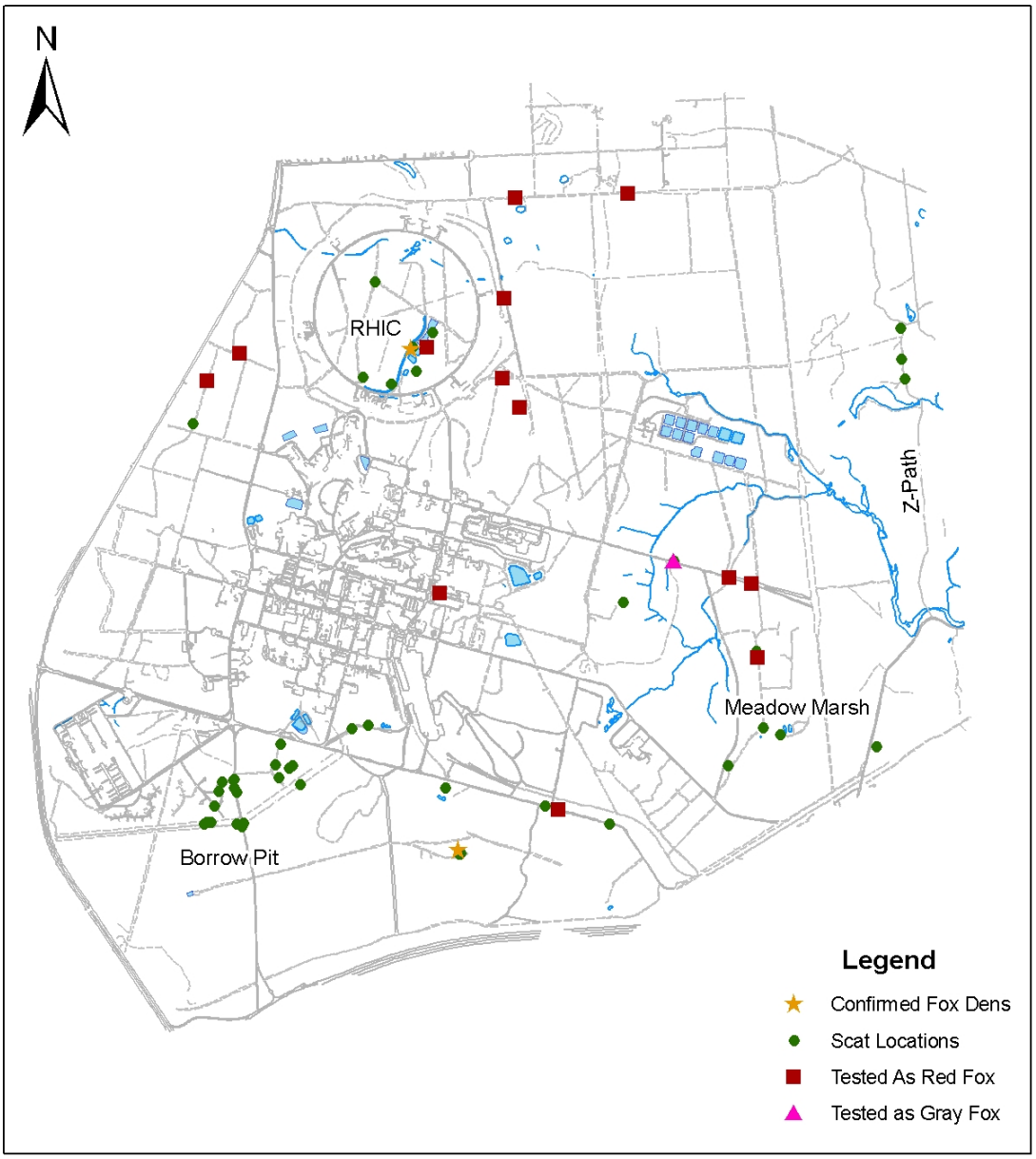
During the study, PCR was unreliable. There were several possible explanations for the low success rate. Primarily the magnesium contained in the PCR kit may have been mislabeled or contained a magnesium concentration that fell to low to produce successful results. Once magnesium levels were raised, the PCR yielded consistent results for the red fox tissue sample. Another reason for initial PCR failure was possibly due to mechanical complications or human error. One particular PCR machine did not yield results for PCR during the study. Once the machine was identified as a possible problem, it was removed from the study and all samples were repeated on an alternate unit. Lack of PCR product from the scat samples can be due to lack of fox DNA present in samples. It is unknown if the origin of DNA that resulted from extraction was from fox species or from prey and vegetation consumed by the defecating individuals. It is assumed that a lack of PCR product means the original DNA did not originate from a fox species.

An interesting note in the study came from the suspected gray fox control that turned out to in fact be of the species *Vulpes vulpes*. When the nucleotide sequence of this individual was run through genbank database it was discovered that this individual showed some regional mutations and contained a unique nucleotide sequence that was different from other published sequences.

The future of this project will be focused on locating more evidence of gray foxes utilizing habitat on BNL. The staff of the project also hope to begin identifying individuals of fox species through DNA sequencing in order to construct home range

sizes, determine survivorship and learn more about the interactions between red and gray fox species at BNL.

FIGURES

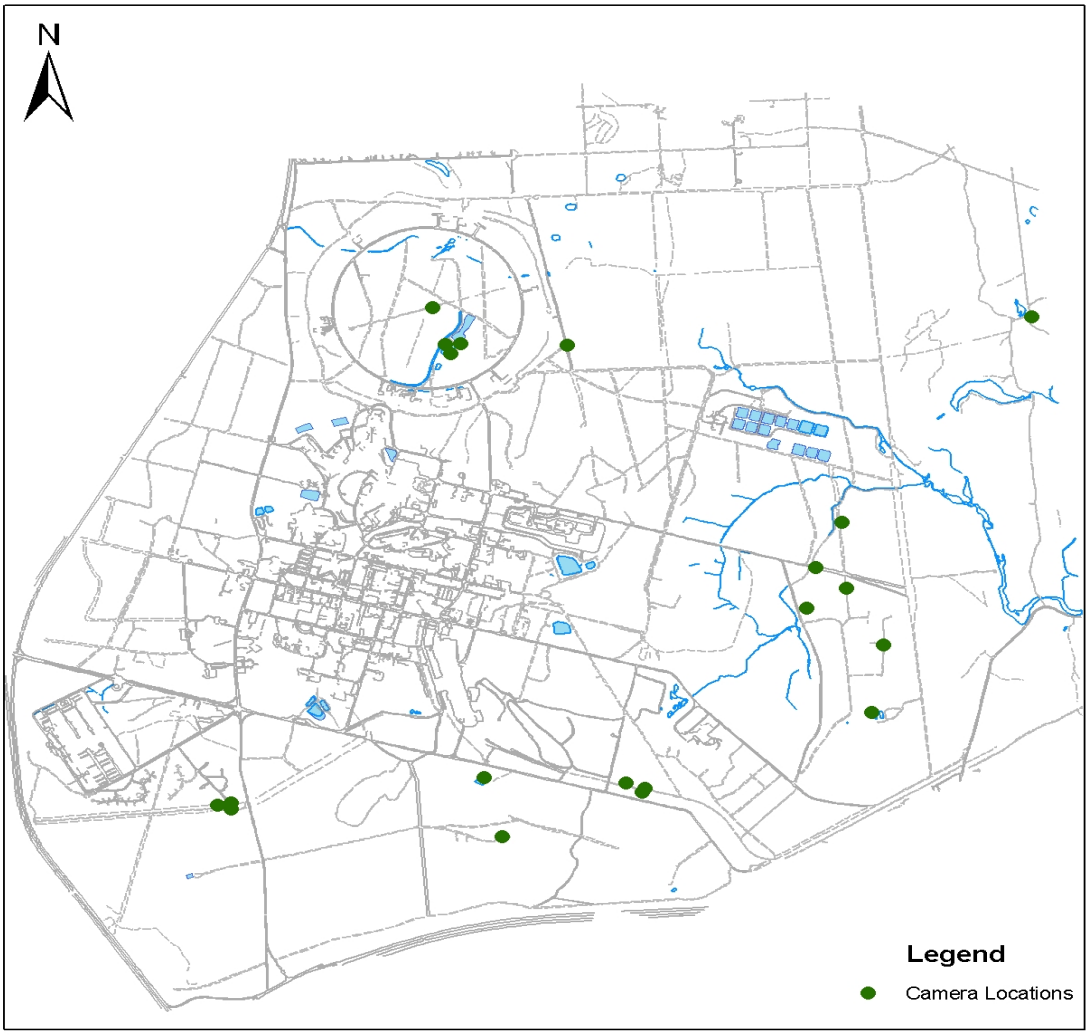


BROOKHAVEN NATIONAL LABORATORY

Figure 1

0 2,000 4,000
Feet

Location of Scat Collected
at Brookhaven National Lab in 2006



BROOKHAVEN NATIONAL LABORATORY

Figure 2

0 2,000 4,000
Feet

Camera Locations during 2006
at Brookhaven National Lab

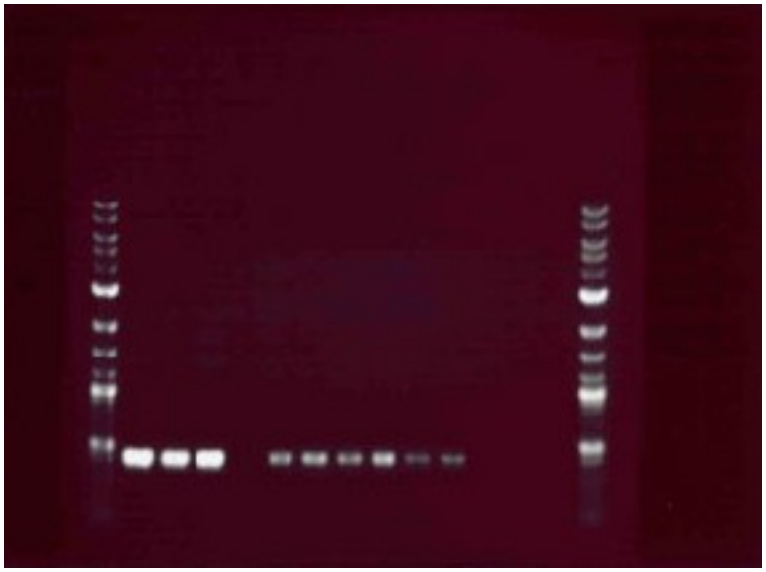


Figure 3
PCR Product Results from Scat are shown as 412bp Lines on a 0.8% Agarose gel

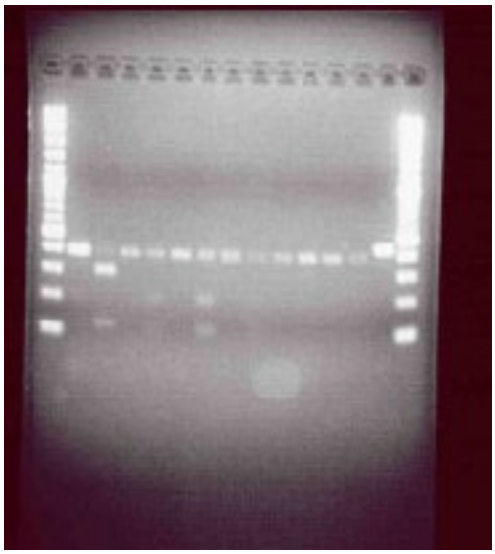


Figure 4
Results of Enzyme Restriction

References

1. Mech, L. David, The Wolf: The Ecology and Behavior of an Endangered Species. Pg.153
2. Connor, Paul F., The Mammals of Long Island, New York.
3. Chamerlain, Micheal J. and Leopold, Bruce D. Spatial Use Patterns, Seasonal Habitat Selection, and Interactions Among Adult Gray Foxes in Mississippi.
4. Macdonald, D.W. and Reynolds, J.C. www.canid.org
5. Hockman, J. Gregory, and Chapman, Joseph A., Comparative Feeding Habits of Red Foxes (Vulpes vulpes) and Gray Foxes (Urocyon cinereoargenteus) In Maryland.
6. Prugh, L.R., Ritlands, C.E., Arthur, S.M., and Krebs, C.J. Monitoring Coyote Population Dynamics by Genotyping Faeces.

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