Using Non-invasive DNA analysis to assess Territory of Red and Gray Fox Populations at Brookhaven National Laboratory

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Red Fox (photo- Jennifer Higbie) Brookhaven National Laboratory


Gray Fox (photo- Jennifer Higbie) Brookhaven National Laboratory

ABSTRACT

The red fox (Vulpes vulpes) and the gray fox (Urocyon cinereoargenteus) have coexisted on Long Island for hundreds of years. Due to historic ecological disturbances, the presence of these populations on Long Island became questionable. Many people speculated that the gray fox was extinct from the island by 1927 (10). However, research conducted at Brookhaven National Laboratory (BNL) in 2006 and 2007 confirmed the presence of both red and gray foxes on the property. Past studies have speculated that the red fox has adapted better to the ecological disturbances throughout Long Island than the gray fox. This study investigated the presence of red and gray fox territories for the 2008 season. Population size and home range sizes were the primary interests in this study. Non-invasive fecal DNA extraction and automated field cameras enabled identification of each fox species and their respective locations at BNL. The red fox species was confirmed throughout the property using both methods, however, the gray foxes have apparently abandoned their previous home range at BNL. The absence of the gray fox is apparent as there have not been recent sightings or positive scat results.

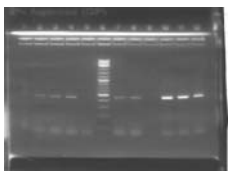
Figure 4. Polymerase Chain Reaction (PCR)-- Lane 6--Ladder, Lane 10, 11, 12--Red fox, Gray fox, Dog controls, Lane 1,2,3,4,5,7,8--positive PCR, Lane 9--Distilled Water

INTRODUCTION

Background

Red foxes (Vulpes vulpes) and gray foxes (Urocyon cinereoargenteus) are members of the family *Canidae.* They are similar in size, weighing between 7 and 15 pounds, yet they are fairly distant relatives (11). Both species are nocturnal as well as omnivorous, feeding on insects, small mammals, and berries, depending on availability (4,5). The red fox can be differentiated by its white-tipped tail and stark black legs, whereas the gray fox has a black-tipped tail and a coarse gray-silver coat (4,5). As the gray fox is the only canine that has the ability to climb, it often relies on a dense woodland habitat. Contrastingly, the red fox is much more adaptable and has been known to flourish in many areas, from dense forests and woodlands to suburban and urban environments (9). The gray fox tends to be the more aggressive of the two species and is thought to dominate during inter-species encounters (1). However, contrasting abilities to adapt to different vegetation and ecological disturbances in the last 100 years may have lead to varying population sizes in the surrounding BNL area (3). Consequently, there may be new forms of inter-specific competition between the two fox species.

Study Site

Historically, populations of red foxes and gray Foxes have coexisted throughout North America (10). However, little information is available regarding fox inter-specific competition in the Long Island area as the gray fox was thought to be extinct from the area by 1927 (10). Amazingly, the presence of both the gray and red fox species has been confirmed at Brookhaven National Laboratory in studies over the last two years (8). A study conducted in 2007 to non-invasively assess the fox populations in the Brookhaven area found a mixed fecal sample of red and gray fox DNA (3). This sample illuminated the possibility of fecal marking for territory as a form of inter-specific competition on the BNL property. This prompted the question of whether or not home ranges of red and gray foxes overlap onsite.

Purpose and Techniques

This study focused on finding overlays of red and gray fox territory for the 2008 season. Using an automated field camera and non-invasive fecal DNA techniques the study assessed the different areas inhabited by each species in comparison with previous years. This process was completed under the assumption that (1) red and gray fox scat can be obtained from routine walking of forest paths, (2) fecal matter and DNA can be preserved, and (3) DNA can be obtained and extracted from epithelial cells caught in feces.

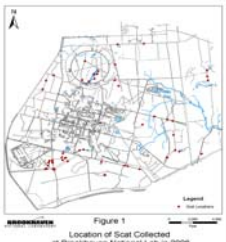

Figure 1. Scat samples collected without enzyme restriction from summer 2006

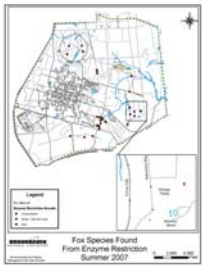

Figure 2. Scat samples with successful enzyme restriction from summer 2007 collection – labeled by species

Figure 3. Scat samples with and without successful enzyme restriction from summer 2008 collection – labeled by species or other


Figure 5. Automated Reconyx Field Camera

METHODS

Roads and forest paths on BNL property were walked on a daily basis with the goal of fresh scat collection. Fox scat normally entails the general home range and/or territorial boundaries for foxes. The location of each sample was recorded on a handheld GPS along with the collection date. The sample was then preserved with 4g silica per 1g scat and stored in a freezer until the extraction.

The fecal DNA extractions (n=81) were conducted using the protocols from Qiagen QIAamp DNA Stool Mini Kit. Polymerase Chain Reaction (PCR) was run for all of the extracted samples using a Taq PCR kit and following standard protocol. Each PCR round was accompanied by control DNA (red fox, gray fox, or dog) to ensure that primers were successfully annealed and that they amplified the selected strands. Small portions of the resulting PCR products were then run on a 2.0% agarose E-gel® to determine the successfulness of the extraction and PCR procedures.

The positive PCR results (n=19) underwent standard enzyme restriction using Hinf and Taq enzymes. Again, the results were run on a 2.0% E-gel® with a red and gray fox control to determine the species of each sample.

In addition, automated field cameras were set up around BNL to support the locations of fox activity. Locations for these cameras were chosen based on likely habitats and reported sightings.

RESULTS

Overall, 56 samples were collected from BNL and extracted. The extraction and PCR produced positive results (18% success). Enzyme restriction produced 4 results. Out of 120 enzyme restrictions 24 attempts were positive (20% success). The results were 2 red fox scat samples, 0 gray fox, and 2 incomplete digestions. These can be found in figure 3 at their respective locations.

25 different samples were taken from Brookhaven State Park (36% extraction success) and were restricted to reveal 8 red foxes.

The automated field camera was placed in 5 different locations and 2 different habitats over a course of 10 weeks. A red fox was caught on camera on 3 distinct occasions, however, no gray fox was ever witnessed.


Figure 6. Taq I Enzyme Restriction-- Lane 6--Ladder, Lane 7, 8, 9--Red Fox, Lane 10--Red Fox control, Lane 11--Gray Fox control. Restriction results often showed different band intensities.

CONCLUSION AND DISCUSSION

While the number of scat samples collected was relatively high, the return rate on the DNA extraction was lower than expected. Weather conditions affect the quality of DNA in the samples and high amounts of precipitation often compromised the yield. Additionally, positive PCR results often turned up negative after enzyme restriction. This may have resulted from unsuccessful repeated PCR. The fact that the success rate of Brookhaven State Park scat was double that of BNL may be attributed to relative freshness of the scat or a simple coincidence.

Red foxes seem to be well established throughout the Brookhaven Lab property. Scat results revealed that they are present and abundant as in years past. However, the gray fox population did not even appear to be subsiding on the property. Even though the success rate of the samples was low, the lack of gray fox sightings and scat results may suggest that the animals no longer rely on BNL as a natal home range. That is not to say that the gray fox has disappeared from BNL all together, yet the continued use of the area as a base for the species is doubtful. The 2007 fox studies at BNL documented a pair of gray fox adults with a natal den and an established home range. It is somewhat odd that permanent resident foxes would seemingly vacate their established territory.

The apparent absence of the gray fox population from BNL could have been caused by a variety of factors. An expansion of red fox territory, lack of resources, or other encroachments of habitat may have prompted a movement to other surrounding areas. Also, offsite winter trapping may have simply exhausted the area of gray fox. Further studies should be performed to monitor for gray fox and possible home range reestablishment. Also, future research should evaluate whether red and gray fox interactions occur in this island habitat and if they play a role in territorial movement.

REFERENCES

(1) Ohio Division of Wildlife Service. *Notes on the Gray Fox.* 2006. July 24, 2008. http://www.dnr.state.oh.us/Portals/9/pdf/pub112.pdf.

(2) Blankenship, Terry L. *Coyote Interactions with other carnivores. Coyotes in the Southwest: A Compendium of our Knowledge, Symposium Proceedings.* Dec. 13, 1995. August 5, 2008. http://texnat.tamu.edu/symposia/coyote/p6.htm.

(3) Mallin, P. *Non-Invasive Indexing of Red and Gray Foxes at Brookhaven National Laboratory.* 2007.

(4) Association of Fish and Wildlife Agencies. *Basic Management Practices for Red Foxes* 2005.

(5) Association of Fish and Wildlife Agencies. *Basic Management Practices for Gray Foxes* 2005.

(6) Homer, Winslow. *Red Fox Life and Habitat.* Aug. 12, 2008. http://www.terrierman.com/lifehabitatRedFox.htm.

(7) Beals,M., L. Gross, S. Harrell. *Inter-specific competition: Lotka-Volterra.* 1999. July 15, 2008. http://www.tiem.utk.edu/bioed/bealsmodules/competition.html.

(8) Green, Timothy. *Mammals.* Environmental and Wasted Management Services Division, Brookhaven National Laboratory. Feb. 1, 2008. July 21, 2008. http://www.bnl.gov/esd/wildlife/Mammals.asp.

(9) Fox, David L. *Animal Diversity Web: Foxes.* University of Michigan. 2008. July 21, 2008. http://animaldiversity.ummz.umich.edu/site/accounts/information/Vulpes_vulpes.html.

(10) Connor, Paul F. The Mammals of Long Island. New York. pp.48-51. 1971.

(11)NBII. *Genetic Diversity Chart.* http//www.nbii.gov/portal/server.pt.

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