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Effect of the TNR Program on Genetic Diversity of the Feral Cat Population at Abilene Christian University

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Effect of the TNR Program on Genetic Diversity of the Feral Cat Population at Abilene
Christian University

An Honors College Project Thesis

Presented to

The Department of Agriculture and Environmental Sciences

Abilene Christian University

In Partial Fulfillment

of the Requirements for

Honors Scholar

by

Kimery Lin Hankins

December 2017

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This Project Thesis, directed and approved by the candidate's committee,
has been accepted by the Honors College of Abilene Christian University
in partial fulfillment of the requirements for the distinction

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ABSTRACT

The feral cat population at Abilene Christian University is of primary concern to the community in terms of a wildlife management perspective. Due to the Trap-Neuter-Return program (TNR), initiated to maintain the cat colony, reduced genetic variability is highly probable. As a result, the suspected narrowed gene pool could create immunocompromised animals susceptible to certain diseases affecting the population. Some of these diseases could also have zoonotic implications for society. The effectiveness of TNR was assessed by comparing the DNA sequence obtained from the mitochondrial DNA control region between 17 cats of the feral population and 20 independently collected cats from Taylor Jones Humane Society, the Abilene Animal Shelter, and a handful of housecats. Blood samples of 1 mL were drawn from each cat. DNA was extracted from whole blood samples and amplified utilizing PCR techniques. Haplotype networks of the genetic material created a baseline for comparison of non-related cats to the colony on campus.

Effect of the TNR Program on Genetic Diversity of the Feral Cat Population at Abilene Christian University



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INTRODUCTION

Trap-Neuter-Return (TNR) programs are widely utilized and accepted within society as a means to control free-roaming feral cat colonies. Many institutions and local communities have implemented this type of program in order to maintain cat populations in their area (Longcore et al., 2009). The consequences of this system are very significant from a public health and wildlife management point of view because they directly affect the overall health of the cats along with the environment in which they inhabit. Questions that determine the effectiveness of such an initiative would be those that analyze the impact the program has upon the gene pool of the cats, how the spread of infectious and possibly zoonotic diseases are controlled by this type of management, how other wildlife in the area is affected by the invasive feline species, and how other locally developed organizations like animal shelters are impacted by TNR methods.

Studies have been done from various regions that have demonstrated that for the most part, TNR programs are an efficient and effective means in controlling feral cat populations over time. Not only do they help reduce the number of cats that free-roam in the community (Gale et al., 2003), they also aid in managing the prevalence of communicable diseases between other cats such as feline leukemia and feline immunodeficiency virus (Crawford et al., 2002). TNR initiatives have also proven to help control zoonotic outbreaks by enforcing regimented health check and vaccination policies on the cats that are admitted to the program (AVMA, 2004). However, from a wildlife conservationist outlook, the effects of TNR are more negative than beneficial (AVMA, 2004). The hosting of an invasive species in a particular location can be seen as

detrimental to the health of the natural ecology that would have existed in the absence of the cat population. This unequal distribution of wildlife potentially does more harm to the environment than good by eradicating native species (Longcore et al., 2009).

Nevertheless, the overall benefit of TNR programs cannot be negated especially when considering the significance they have for urban societies on maintenance of the feral colonies. Research has shown that TNR efforts have a positive impact upon the number of animals impounded by local shelters, albeit a very small effect within the larger scheme (Isaza et al., 2014). This effect in conjunction with the aforementioned advantages of TNR programs gives reason as to how implementation of such work is useful for communities worldwide. But only through a more conscientious effort by humans can all the facets of an effective TNR strategy be determined.

The goal of the research being conducted at Abilene Christian University with the population of feral cats on campus is to analyze how effective the TNR program has been on the gene pool of the cats collected. This is the initial step in determining how other areas like wildlife and disease spread are impacted by this type of management. Once this baseline for relatedness is drawn, other aspects can then be examined to discuss whether the TNR approach is beneficial to the overall community. These conclusions can be drawn by discerning whether more closely related cats are the main contributors or carriers of infectious diseases as opposed to cats that are not related. Research determining relatedness can be accomplished by compiling data from DNA sequences extracted from the mitochondria in samples of whole blood. Studies utilizing blood samples have been applied to humans in cases where extracting and sequencing the DNA

comes both from the mitochondria and nucleus simultaneously (Ahmad et al., 2007). Some research has also analyzed mitochondrial DNA from samples of blood from cheetahs in order to compare the results to other feline species. This allows scientists to draw conclusions about the evolution of the wild cats from one another in the population as well as divergence from other mammalian groups (Bradley et al., 2000). Similar studies have been accomplished with feral European cats as compared to domesticated cats within the area. Blood and tissue samples collected allowed the extraction of DNA in order for researchers to analyze how each population has affected the other genetically over time (Beaumont et al. 2001).

In summary, TNR programs have the potential for great benefits for society in terms of managing feral cat colonies. However, the effectiveness of the system must be analyzed before relying too heavily on this type of method. By beginning with first determining the relatedness of the cats within the population as compared to randomly selected cats from various locations, extracted DNA from mitochondria permits analysts to ascertain the specific regions of genetic material that are identical in differing cats. This information can then be linked to recurring characteristics in the clowder of cats that may potentially lead to more detrimental effects for both the cat population itself as well as the ecology surrounding it.

MATERIALS AND METHODS

The feral cats were caught using humane cages placed around campus by ACU landscape management. Under supervision of a veterinarian, the breed and sex were determined and 1 mL of blood was drawn for analysis. Samples for the random

population were taken from cats in separate households as well as the local animal shelters. DNA was extracted according to OMEGA bio-tek EZNA Tissue DNA Kit protocol. PCR was performed with the following reagents: 15.25 μ l of sterile water, 2.0 μ l of 10X Standard Taq Reaction Buffer (New England BioLabs Inc.), 0.25 μ l of 10 mM dNTPs, 1.0 μ l of PCR primer JHmtF3 -gatagtgcttaatcgtgc and 1.0 μ l of primer JHmtR3-gtcctgtggaacaatagg (Tarditi et. al. 2011), 0.2 μ l of NEB Taq DNA Polymerase (New England BioLabs Inc.). The thermocycling conditions were as follows: initial denaturation: 95°C for 5 minutes, 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, followed by a final extension of 72°C for 5 minutes. The products were visualized through gel electrophoresis and sent to DNA Analysis Facility on Science Hill at Yale University (New Haven, CT) for sequencing. A haplotype network was constructed using TCS version 1.21 (Clement et al. 2000) under the criterion of a parsimony network in which connections have probability of at least 95%.

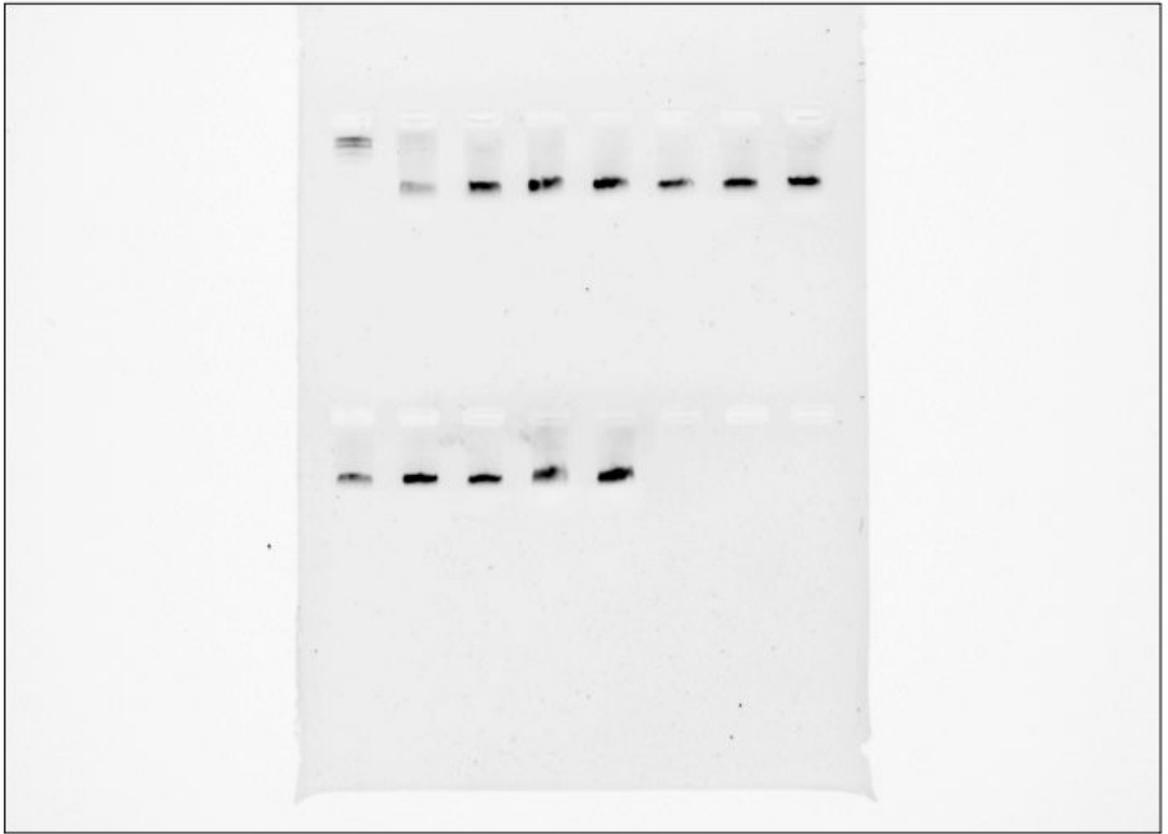


Figure 1: Gel electrophoresis of DNA. After the DNA was amplified using PCR, it was run through 1% agarose gel using SybrGreen dye to detect the presence of the DNA.

Table 1: PCR recipe table. The formulations allow for the calculation of the amounts of solutions used to prepare samples for PCR cycling conditions. This represents one set of feline blood prepared.

PCR - Feline spp.						
Date	20-Mar-17	Recipe:	Conc.		Amount (µl)	Master Mix (µl)
# of Reaction	14	sterile water			15.25	213.50
PCR Profile:	Cat2011	10X PCR Buff	10.0	X	2.00	28.00
94°C	1 min	dNTP mix	10.0	mM	0.25	3.50
94°C*	1 min	primer 1	5.0	µM	1.00	14.00
56°C	1 min	primer 2	5.0	µM	1.00	14.00
72°C	1 min	Taq	5.00	U/µl	0.20	2.80
72°C	10 min	undiluted DNA*			0.30	
4°C	Hold				20.00	
*Replicates	35					
Samples:						
1	<i>Feline</i>	Tabby M*				
2	<i>Feline</i>	Torti F*				
3	<i>Feline</i>	Blk M*				
4	<i>Feline</i>	Cat 14 F				
5	<i>Feline</i>	Cat 16 M				
6	<i>Feline</i>	Cat 21 M				
7	<i>Feline</i>	Cat 13 F				
8	<i>Feline</i>	Cat 7 M				
9	<i>Feline</i>	Cat 4 F				
10	<i>Feline</i>	Cricket SF				
11	<i>Feline</i>	Marty NM				
12	<i>Feline</i>	Kitty SF				
	<i>Negative</i>					
		* means feral				

RESULTS

The results of the DNA sequencing revealed that there are 5 different haplotypes of the specified mitochondrial DNA present in the random cat sample. Of the 5 haplotypes present, the feral cat population exhibited 2 of the same haplotype groups, but the most common haplotype in the ACU population was not detected from the random sample.

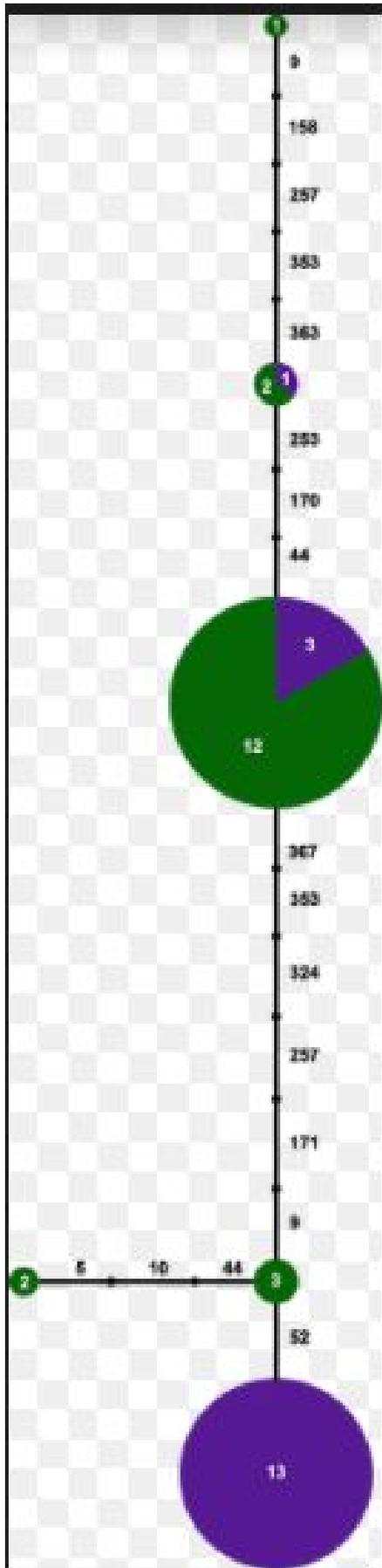


Figure 2: Haplotype network. Each colored group represents a different haplotype. The purple refers to the feral cat population and the green refers to the random cat population. Each black dot between the group represents a mutation that occurred between the different groups, with the number indicating the location of the mutation along the strand of DNA.

DISCUSSION

From the data it appears as though the feral cat population has substantially fewer haplotypes than the random cat population, and the most common haplotype at ACU is not common in the random population. Though the populations under question are small, it can be inferred that the feral cat population has a reduced gene pool. While there are 12 cats from our random sample that share a common haplotype, it does not necessarily mean they are related because populations often contain a most common haplotype. So rather than analyze the amount of cats within each haplotype, we focused on the spread of each population and number of haplotypes. It is possible that because we obtained smaller sample populations that there are more haplotypes that exist within Abilene and even within the feral cat population.

CONCLUSION

The feral cat population at ACU has a seemingly substantial decrease in the number of haplotypes when compared to a random sample of unrelated cats, and it appears that an uncommon haplotype not found in the random sample has become dominant in the ACU population. Further research needs to be done in order to determine several key factors affecting gene flow in the feral cat population; research targeting the paternal lineage of

the species, immigration rates to determine if any cats are moving into the area, and a larger sample size for an increase in accuracy of information would help further the results of not only TNR but also many other population characteristics.

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