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Testing Kinship via Mitochondrial DNA on Colony vs. Non-Colony Cats

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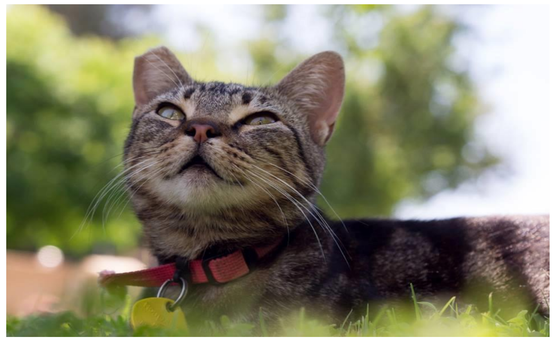
Introduction

This study focuses on mitochondrial DNA (mtDNA), specifically among feral cat (*Felis catus*) populations. Feral cats were once domesticated and have reverted to their wild state. There is an emerging importance to studying feline mtDNA related to kinship in the feral cat populations to understand their social structure. mtDNA is inherited in all offspring through the mother and will all be the same, barring mutations. Past research shows the importance of mtDNA in examining matrilines. The mammalian mtDNA is passed down maternally and is the case for all life forms, however, paternity for cats is highly variable. In each litter, there can be more than one father involved. This phenomenon is due to the mating process in cats. The degree of relatedness will be compared between the two populations of cats. The expected hypothesis of this study is that there will be some difference in degree of relationship in the colony versus the non-colony feral cats in question. Colony versus non-colony, in a sense, refers to the social structure in which they originated.

As forensics advance, more technologies are applied to wildlife forensics. This discipline aids in connecting wildlife crimes. This study impacts wildlife forensics by examining social structure of feral cats. Feral cats are often "demonized" and humans may commit crimes against them for this reason. They prey on native fauna, otherwise categorized as rodents, birds, and other small domesticates in the community. For example, an article by A.S. Glen shows a feral cat responsible for the killing of a native rodent species. Other than wildlife forensics, mtDNA is studied in forensic casework and is used for mitochondrial disease studies, to see what can be passed down through maternal DNA. There are also studies found stating that humans kill feral cats because they are a problem to their properties and domesticated pets, which is a crime. These studies present a connection to the Macdonald Triad. Prior research shows that one of the three main traits of a violence-prone human is animal cruelty, with the other two being bed-wetting and fire-setting. Also, feral cat hair has also been used to connect crime scenes to suspects and victims by the Locard Exchange Principle.

For this research, the mtDNA of feral cats from ear tip tissue was studied to compare colony versus non-colony cats for degree of relationship. These ear tips were provided by Dr. Morrow through TNR, from her veterinarian clinic, Frankie's Friends. TNR is an acronym for a process of trapping, neutering, and returning, and technique that aids in controlling feral cat populations. Feral cats are taken from their environment by humane traps, then spayed or neutered by a veterinarian, and returned to their environment. A feral cat's left ear tip is cut off if it has been spayed or neutered, which is a signal that the animal has been sterilized. TNR has been supported for population management in several published studies.



Materials and Methods

Collection
- TNR samples via Frankie's Friends
- 40 colony cats (4 colonies), 40 non-colony cats

Optimal Extraction Method
- Qiagen QIAamp DNA Mini Tissue Kit

Quantification
- NanoDrop Lite Spectrophotometer by Thermo Scientific

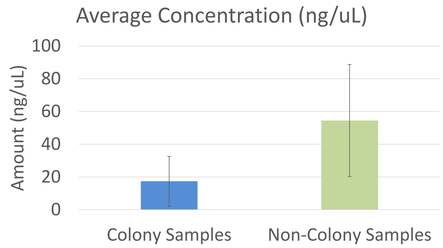
Amplification
- **Primers**: Lf15926 (5' ATATAAAATACTTTGGTCTTGTAAACC 3') and Hf3 (5' GGGTGTATAATACCCCCTGGGGTGAGTTG 3') **PCR profile**: cycled at 96°C for 5 min., followed by 25 cycles of: 94°C for 1 min. 30 sec., 55°C for 1 min. 15 sec., and 72°C for 1 min. 30 sec., then: 72°C for 2 min. and held at 4°C
- **ABI Big Dye Kit PCR profile**: cycled for 25 cycles at: 96°C for 10 sec., 50°C for 5 sec., and 60°C for 4 min., then: samples held at 4°C.

Sequencing
- 3130 ABI Genetic Analyzer

Analysis
- Chromas Software

Results

A representation of each population set of NanoDrop results are shown in the sections below. The tables are from the first extraction set of each population, showing the success of the extraction method. The average measured concentration from NanoDrop values between the two populations with standard deviation bars included is shown below.



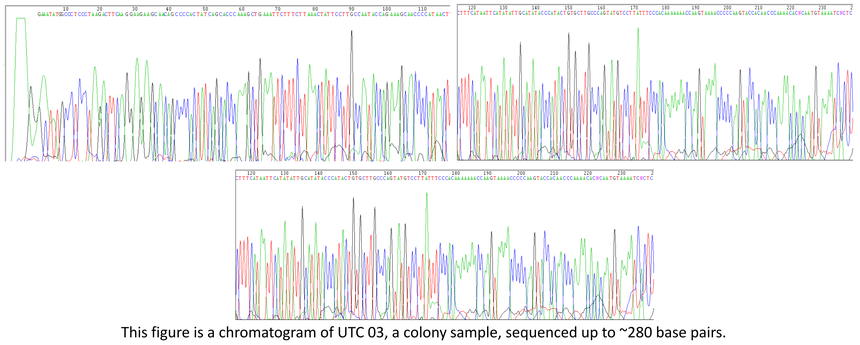
The IDT Blast results stated that the GC content was 55.2% for primer Hf3 and 25.9% Lf15926. Although the GC content was low for primer Lf15926, the amplification was successful for HV1 of the samples.

All samples were sequenced up to ~300 base pairs. An example of the two populations' sequences are shown in the sections below.

Colony

Sample	Purity (260/280)	Amount [ng/uL]
JCF 1	1.50	50.6
JCF 2	1.65	26.3
JCF 3	1.69	18.1
JCF 4	1.48	61.8
UTC 1	1.32	23.1
UTC 2	1.45	4.9
UTC 3	1.34	34.3
+	1.58	4.3
-	1.62	4.9

This table is the first set of colony cat samples, containing two colonies and a positive and negative control.

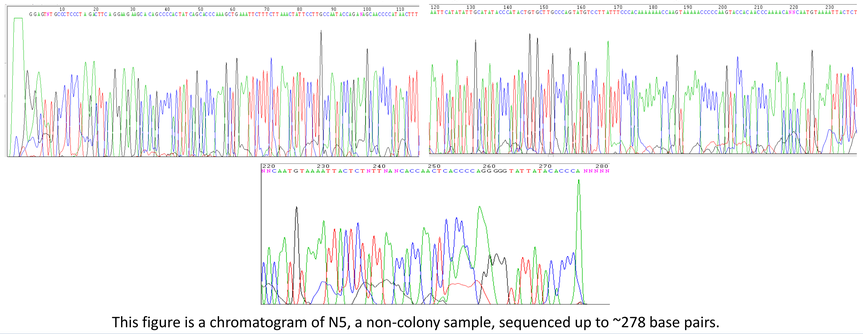


This figure is a chromatogram of UTC 03, a colony sample, sequenced up to ~280 base pairs.

Non-Colony

Sample	Purity (260/280)	Amount [ng/uL]
N1	1.74	18.7
N2	1.35	141.9
N3	1.86	83.0
N4	1.82	61.4
N5	1.82	81.6
N+	1.91	2.4
N-	1.92	2.4

This table is the first set of non-colony cat samples, containing 5 samples and a positive and negative control.



This figure is a chromatogram of N5, a non-colony sample, sequenced up to ~278 base pairs.

Discussion

There is a lack of research in the wildlife forensics discipline, specifically in the social structure of feral cats and crimes against them. This study was based on the foundation of the TNR feral cats and the degree of relatedness between them. Feral cats draw public attention because of their associated, destructive behaviors. This is largely why TNR was introduced in multiple areas of the world where feral cat populations are troublesome. TNR controls the population as well as halts specific groups from reproducing.

Feral cats are also demonized in wildlife forensics for preying on native fauna. The Songbird Study done in the UK debunked this stigma of feral cats and stated that after studying the fecal material and tissue samples of feral cats, they could not be held responsible for killing the local songbirds. However, it is reported in the US that millions songbirds are killed every year by feral cats. That statistic is based on a computer model, but not based on fieldwork, which is important to state because there is no way to know feral cats are fully responsible for this without studying the situation in real time.

Humans can be responsible for crimes against feral cats as well, which is a trait linked to the Macdonald Triad. It is often true that humans are the predator, poisoning or killing off feral cats to stop them from reproducing, instead of participating in TNR. Many clinics, similar to Frankie's Friends, will allow cats to be dropped off, with a small fee, in order to be neutered or spayed and then released or adopted.

Cats can also be a key piece of evidence linking a human to a crime. There are many instances where cat hair is found on a human from a crime scene or on a weapon or accessory to a murder or break in. Cat hair can link the crime to the suspect via mtDNA from the root of the hair or even trace evidence analysis. Cat DNA is also a linking piece of evidence. This is very important in cases of violent crimes that occur in a home with pets or crimes against wildlife.

The Qiagen QIAamp DNA Mini Tissue Extraction Kit was the best method for this study. The overnight incubation allowed for efficiency and it proved successful for the remainder of the study.

NanoDrop values were consistently 1.5 - 1.9 for the purity and the size of the substrate depended on the amount [ng/uL]. The non-colony ear tips were much larger than the colony ear tips which is completely subjective.

The primer set used (Lf15926 and Hf3) was optimal for sequencing was successful in sequencing up to approximately 280 base pairs. The longest sequence was up to 310 base pairs while the shortest sequence was 255 base pairs.

Future Directions

Moving forward, statistical analysis will be completed in order to accept or reject the hypothesis. The sequences within each colony will be compared before the colonies themselves are compared. The two individual populations (colony vs. non-colony) will then be compared against each other to evaluate degree of relationship of feral cats.

Should this study prove a significant difference within the feral cat community, the database started by this study can be expanded by future students. This may create larger statistical differences and variations. It would also be important to the study for reproducibility and further hypothesis support. To continue this study, or branch off of this study, genotyping could be done with the samples used here or along with an expanded database of samples.

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Thank You