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# **Population Genetics of Feral Cats in Harvey County, Kansas**

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### Abstract

Many cities across the country have thousands of feral cats roaming the streets, often causing problems for residents, and Newton, Kansas is no exception. Since 2013, Caring Hands Humane Society in Newton has used a Trap Neuter Release (TNR) program as a population control strategy. The effects of control strategies and a basic understanding of invasive species populations can be gathered through genetic analyses. In this study, tissue samples were gathered from 57 feral cats in Harvey County and the surrounding community. DNA was isolated from these samples and polymerase chain reactions (PCR) were run for each of seven tetranucleotide short tandem repeat (STR) loci in order to determine the genotype of each STR by capillary sequencing. Genotypes from each individual cat were used to obtain estimates of population structure, relatedness, gene flow, heterozygosity, genetic diversity, and allelic richness of the feral cat population in Harvey County. Estimates of population structure, relatedness, and gene flow enable the determination of necessary trapping locations and can help guide methods for future trapping efforts. Very little structure was found in the overall population, as indicated by the F statistics (Fst=0.011, p=0.050, Fis=0.243, p=0.001, Fit=0.251, p=0.001), relatedness (0.033), and the correlation coefficient of the Mantel test (Rxy=0.048, p=0.173). These findings also point to little relatedness among individuals within the population and long range dispersal ability of the cats. Estimates of heterozygosity, genetic diversity, and allelic richness, in addition to the other measures, help to establish a baseline understanding of the population genetics of feral cats in Newton, which future studies can use to monitor the effectiveness of population control strategies over time.

#### Keywords: Feral Cats, Control Methods, Population Genetics

## **1. Introduction**

Overpopulation of cats in the United States has led to a rise in feral cat populations. The definition and classification of a feral cat is largely based on the behavior and temperament of the animal. One way to define feral cats is as cats that cannot be handled by people and are unsuitable to be placed in a typical home<sup>1</sup>. Feral cats come from a variety of sources depending on the location, but the main sources are existing feral cats and lost and abandoned cats that have become unsocialized<sup>2, 3</sup>. Over 600 million domestic cats exist in the world today and are considered an exotic, non-native species wherever they occur<sup>4</sup>. This is in part due to their domestication, making them distinct from their wild ancestors, and in part because of their ability to overwhelm natural species and ecosystems<sup>4</sup>. One way to estimate feral cat populations is by their relative size to owned cat population. In the United States, the feral cat population is estimated to be one third to one half the number of owned cats<sup>1</sup>. Thus, the estimated feral cat population in the United States is around 60-100 million individuals<sup>5</sup>.

Feral cats in the United States have begun to create conservation issues in many urban areas. In addition, natural areas have continued to be converted to urban areas and this urbanization has placed increased importance on urban areas to act as wildlife habitat. Feral cat populations in these areas have caused concern about the interaction of feral cats with wildlife, especially birds and small mammals. The presence of feral cats causes many otherwise suitable

urban bird habitats to no longer be suitable, due to the cats' opportunistic predatory behavior and existence in high population sizes in relation to natural predators<sup>4</sup>. In one study, it was estimated that free-ranging cats kill 1.4-3.7 billion birds and 6.9-20.7 billion mammals annually<sup>6</sup>. In addition, cats have been implicated in the extinctions of 33 bird species<sup>4</sup>.

Feral cats also pose health risks to the human population. Toxoplasmosis is a common protozoal disease of cats that can be transmitted to humans, which can lead to serious illness in immunocompromised humans<sup>3</sup>. In addition to infecting human populations, feral cats are also responsible for transmitting diseases to other species as well as themselves. Disease and injury are much more prevalent among cats living in feral colonies than owned cats<sup>7</sup>. Two examples of these diseases are feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), both of which can have an adverse impact on feral cat health<sup>3</sup>.

Past methods for controlling feral cats have been to wait and see or do nothing, hoping that nature will take its course and the cats will be killed off or move away<sup>1</sup>. However, due to cats' great dispersal ability, natural barriers are ineffective at controlling populations and preventing recolonization<sup>8</sup>. Studies have shown that human perceptions of cats influence the selection methods used to control them<sup>1</sup>. Combined with the pressure on local authorities to act on the problem, doing nothing is no longer acceptable<sup>3</sup>. Therefore, multiple methods have been developed to control/reduce feral cat populations.

The following two methods aim to eliminate feral cat populations. The Kill on Site method involves using poisons or lethal chemicals to reduce cat populations. This method is generally not favored among the public but it is often used by local governments because it is perceived to be a permanent, relatively cheap solutions to feral cat problems<sup>1</sup>. However, poisons are not specific to cats and can pose risks to humans and other animal species, as well as causing a painful death for the cats. The Trap-Euthanize / Lethal Control (TE/LC) method involves setting traps to catch feral cats and subsequently administering lethal injections. According to one study<sup>7</sup>, this method is the optimal management decision because it balances the public interest in cats with the value that stakeholders place upon the conservation of the native wildlife. Other studies have shown that TE/LC is the most effective method when capture rates are greater than 97% of the total population<sup>9</sup>. It is also cheaper than other methods of control, with the estimated cost per cat at approximately \$71<sup>7</sup>.

Community members often feed feral cats; in one study it was found that 12% of the households in one county do  $so^{10}$ . It is therefore likely that many residents in these local communities are in favor of stabilization instead of elimination. Instead of decreasing the population, the goal of the following three methods is to stabilize the population. These approaches can accomplish population control while allowing a sensible number of cats to remain, which is often essential for pest control<sup>1, 3</sup>. The Trap-Vasectomy-Hysterectomy-Release (TVHR) method involves trapping, altering, and releasing the cats. In TVHR the alterations are vasectomies for males and hysterectomies for females. By performing a vasectomy, the male cat retains its dominance in the breeding hierarchy, preventing a "social vacuum" that draws in other cats. Some drawbacks include vasectomies being a more complicated surgical technique than castration and that this method allows negative male mating behaviors to exist, such as fighting, vocalization, and urine marking<sup>9</sup>. In the Trap-Test-Vaccinate-Alter-Return-Monitor (TTVARM) method, cats are trapped and tested for FeLV and FIV; if negative, they are vaccinated for a number of diseases including rabies and then altered by castration or ovariohysterectomy. In TTVARM programs, kittens and any other cats that are tame are fixed and vaccinated, then put up for adoption. Once cats are returned, the population continues to be monitored<sup>2</sup>. It is believed that TTVARM programs improve the health of feral cats and reduce the public health risks because cats are vaccinated at the time of neutering<sup>2</sup>. The-Trap-Neuter-Release (TNR) method involves trapping, altering by castration or ovariohysterectomy, and releasing cats back into the environment. Like the wait and see method, the hope is that natural attrition will eventually decrease numbers or at least maintain a stable number of cats<sup>1</sup>. The cost per cat is approximately \$158 per cat, cheaper than the Trap-Test-Vaccinate-Alter-Return-Monitor (TTVARM) method at \$2037.

Since February 2013, Caring Hands Humane Society in Newton, KS has adopted a TNR release program in an attempt to find a method of controlling the feral cat population that has public support. Before then, the policy had been euthanasia or trap and release to the surrounding country. This was done for about 15 years prior to the start of the TNR program but Kevin Stubbs, the director of Caring Hands, stated no decline in the population had been observed with this method<sup>11</sup>. However, Stubbs has determined that in the years since the TNR program was implemented, the program has shown signs of success<sup>12</sup>. With the TNR program, community members feed the feral cats and report when 20 or 30 cats have been observed in a particular area so that traps can be set by their food to capture them<sup>11</sup>. The humane society also vaccinates cats in addition to altering them, which makes the population safer and the cats less likely to carry infectious diseases once released<sup>12</sup>.

There are many studies debating the success of the TNR methods. One drawback is that dominant males within a TNR program become sexually inactive and are subsequently replaced in the breeding hierarchy by the next most dominant male<sup>9</sup>. In computer simulations<sup>9</sup>, TNR did not perform better than Lethal Control or Trap Vasectomy

Hysterectomy Release at any annual capture rate. In addition, TNR is not predicted to reduce or stabilize populations unless the program is supplemented with kitten removal, which can result in stabilization of small initial population sizes<sup>7</sup>. Furthermore, because many programs are small and local, it can be very hard to quantify the extent and success of TNR in most locations<sup>1</sup>.

More research is needed to understand feral cat populations and the effects of the various types of control efforts on these populations. A powerful method for gaining this kind of information is population genetics research, a valuable tool for analyzing wildlife species and one which has begun to be applied to invasive species. The goal of population genetics is to understand the forces that have an impact on levels of genetic variation<sup>13</sup>. The information gathered through population genetics research can contribute to the development of appropriate control strategies of invasive species<sup>9</sup>. Some of the information that population genetics can provide include estimates of genetic diversity, population structure, effective population size, gene flow and migration rate, and relatedness among members of a population units. An understanding of the specific population units of an invasive species is important for preventing rapid recolonization of the target population from an unidentified fraction or source population<sup>3</sup>. Knowledge of migration and immigration dynamics provide through this research is very valuable, as these dynamics can be difficult to study using only visual observations<sup>8</sup>.

Genetic testing has been available for cats since the 1960s and identification of individual cat identity, breed, and race can be done with short tandem repeat (STR) and single nucleotide polymorphism (SNP) panels<sup>17</sup>. The high variability in fragment size of STRs, in addition to their relative abundance in genomes, makes STRs very useful for population genetics<sup>18</sup>. In this study STRs are used to identify individual cats and gain a baseline understanding of population genetic of the feral cat population in Harvey County, Kansas.

#### 2. Methods

As part of their TNR program, Caring Hands Humane Society in Newton, KS divides the city into square mile areas and spends a month in each square mile setting up traps to catch feral cats. The residents who feed cats in that area stop feeding them a week beforehand so that the cats are hungrier and more likely to be caught. If possible, traps are set where residents and the humane society have seen the cats in the past. When cats are caught, they are transported to the humane society where they are spayed or neutered. The humane society then snips off the tip of one of each cats' ears, which allows them to identify which cats have already been spayed and neutered, and for the duration of this study the humane society froze these ear tips for use as tissue samples.

Ear tips were collected from the feral cats caught between February and May 2017. For each ear tip, the humane society provided the general location of where each cat was caught. A total of 57 ear tips were collected over this time period and these samples were kept frozen to prevent tissue decay. DNA extraction from the ear tip samples was performed using a Gentra Puregene Tissue Kit© following the protocol for 5-10 mg of frozen solid tissue outlined by the manufacturer.

Once DNA from every sample was extracted, polymerase chain reactions (PCR) were run and optimized for each of seven STRs<sup>8</sup>. Initial baseline conditions used were optimized following recommendations outlined by Menotti-Raymond and colleagues<sup>19</sup>. Either a forward or reverse fluorescent-tagged primer was used for each locus. The PCR products were diluted appropriately and each product was combined with the size standard LIZ. The fluorescently-tagged PCR products were then analyzed with an automated ABI3130© capillary sequencer.

Sizing of the raw STR data was performed with the software program *Geneious*  $11.0.5^{20}$ . Sizes for each of the STRs for each individual cat were then exported to Excel for further analyses. Using this STR data, basic population genetic parameters were obtained with several other software programs. Alleles per locus, observed heterozygosity, expected heterozygosity, deviation from Hardy-Weinberg equilibrium, and null allele probability were calculated with the software program *Cervus*  $3.0.7^{21}$ . Significant deviations from Hardy-Weinberg equilibrium were tested based on p-values and chi-squared values. Gene diversity, allelic richness, F statistics, and relatedness were calculated with the software program *Fstat*  $2.9.3.2^{22}$ . The relatedness of individuals in the population was estimated with the software program *Fstat* 2 using relatedness values created by Queller and Goodnight (1989)<sup>23</sup>. F statistics were analyzed for significance with 95% confidence intervals found after bootstrapping across all loci using the software program *Fstat*  $2^{22}$ .

In order to analyze the population structure, as well as the correlation between geographic and genetic distances, the approximate capture location for each cat was converted into geographic coordinates and the cats were sorted into subpopulations according to the address listed for each capture site. All cats were assigned to one of two subpopulations, one centered in Newton and the other one centered in Halstead, KS, based on proximity to the

subpopulation. The two subpopulations are identified as Pop1 and Pop2. The cats caught in and around Newton are listed as Pop1 and the cats caught in and around Halstead are listed as Pop2. The trapping locations of the cats and the designated subpopulations can be seen in Figure 1.

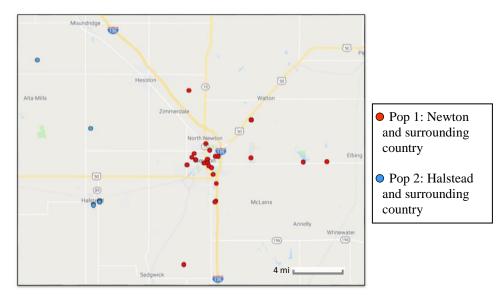


Figure 1. Map of the trapping location of each cat included in this study

Population structure was analyzed using the two designated subpopulations in the total population. Correlation between genetic distance and geographic distance was analyzed with a Mantel test, using the software program  $GenAlEx \odot 6.503^{24}$ . Further analysis on population structure was done with an AMOVA test and the accompanying F statistics that were also calculated in  $GenAlEx \odot 6.503^{24}$ . These F statistics were analyzed for significance with p-values.

### **3. Results**

After analysis of the raw STR data in *Geneious*<sup>©</sup> 11.0.5<sup>20</sup>, STR *FCA731* was excluded from further analyses because only 15 of the 56 total cats used in the study had usable data. The other six STRs were included in all further analyses.

For these remaining six STRs, the number of alleles observed per locus (k), the observed heterozygosities (HObs), and expected heterozygosities (HExp) for both subpopulations and the population overall can be seen in Table 1. For the population overall, significant deviation from Hardy-Weinberg equilibrium (HW), with levels of significance denoted by asterisks, and the probability of the presence of null alleles (F(Null)) are also listed in Table 1. In addition, the number (n) of individuals in each subpopulation and the overall population are given.

Table 1. Basic population parameters for each assigned subpopulation and for the population overall.

	Pop1 (n=41)			Pop2 (n=15)			Overall (n=56)				
Locus	k	HObs	HExp	k	HObs	HExp	k	HObs	HExp	HW	F(Null)
FCA441	4	0.585	0.746	4	0.667	0.674	4	0.607	0.735	NS	0.0889
F124	11	0.308	0.867	7	0.167	0.815	11	0.275	0.867	***	0.5201
FCA740	7	0.725	0.666	5	0.667	0.630	7	0.709	0.652	NS	-0.0564
FCA742	12	0.750	0.823	10	0.867	0.874	12	0.782	0.852	NS	0.0457
FCA723	12	0.512	0.874	8	0.467	0.717	13	0.500	0.853	**	0.2596
FCA733	13	0.829	0.866	9	0.867	0.830	13	0.839	0.855	NS	0.0024

With the exception of 2 loci, F124 and FCA723, these results are similar to the values found in previous studies<sup>8, 19</sup>, lending support to the accuracy of these results. F124 and FCA723 showed significant deviation from the Hardy-Weinberg principle, indicating either a lack of panmixia, lack of gene flow, genetic drift, or the presence of null alleles.

It is likely the presence of null alleles is a contributing cause to the significant deviation from the Hardy-Weinberg principle for alleles *F124* and *FCA723*, since the null allele probability seen in Table 1 is high.

More basic population parameters and the results of population structure analyses can be seen in the following tables and figures.

	A	llelic Richı	Gene Diversity		
Locus	Pop1	Pop2	Overall	Pop1	Pop2
FCA441	3.993	3.800	3.991	0.748	0.674
F124	8.681	7.000	8.596	0.874	0.845
FCA740	5.497	4.761	5.218	0.665	0.629
FCA742	8.499	9.456	8.671	0.824	0.874
FCA723	9.086	7.297	9.187	0.879	0.726
FCA733	9.370	8.321	9.236	0.866	0.829

Table 2. Allelic richness and gene diversity of the two designated subpopulations (and in the case of allelic richness, the total population) per locus.

Table 3. F statistics and relatedness values calculated for each locus, as well as for the loci overall.

Locus	Fst	Fis	Fit	Relat
FCA441	0.024	0.167	0.187	0.041
F124	0.018	0.683	0.689	0.021
FCA740	-0.014	-0.083	-0.098	-0.032
FCA742	0.042	0.067	0.106	0.076
FCA723	0.049	0.404	0.433	0.068
FCA733	-0.004	0.020	0.016	-0.008
Overall	0.021	0.224	0.240	0.033
95% CI	0.002-0.038	0.028-0.448	0.035-0.460	0.003-0.058
AMOVA Overall	0.011 (p=0.050)	0.243 (p=0.001)	0.251 (p=0.001)	

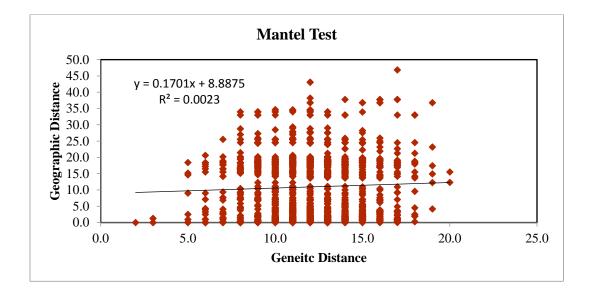


Figure 2. The results of the Mantel test showing the relationship between genetic and geographic distance. Rxy=0.048 (p=0.173)

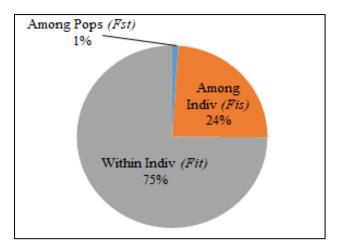


Figure 3. The percentages of molecular variance attributed to each of three different comparisons between individuals, subpopulations, and the total population

In Figure 3, among pops (Fst) refers to the molecular variance attributed to the subpopulations when compared to the total population. Among Indiv (Fis) refers to the molecular variance attributed to the individuals when compared to the subpopulations. Within Indiv (Fit) refers to the molecular variance attributed to the individuals when compared to the total population.

## 4. Discussion

The Mantel test found no significant correlation between geographic and genetic distances, as indicated by the correlation coefficient value of 0.048 (p=0.173) for the total population. This finding, in addition to the relatedness values generated for each locus and for the loci overall, as seen in Table 3, indicate there is little relatedness between individuals within subpopulations or among individuals within the total population. This strongly suggests individuals are equally likely to mate with an individual that is nearby as with an individual that is far away and are thereby exhibiting long range dispersal.

There are three categories from which molecular variance arises, as estimated by the F statistics: subpopulations to the total population (Fst), individuals to the subpopulations (Fis), and individuals to the total population (Fit). The AMOVA estimate of Fst (0.011, p=0.050) falls between the range of 0-0.05, which indicates little genetic differentiation when comparing subpopulations to the total population<sup>13</sup>. The estimates of Fis (0.243, p=0.001) and Fit (0.251, p=0.001) indicate the observed genetic differentiation is most attributable to individuals within the total population. These results, seen in Figure 3, suggest there is very little structure in the total population of feral cats and that the cats exhibit long-distance dispersal<sup>8</sup>. This is congruent with studies that have found similar levels of structure and levels of dispersal<sup>8</sup> and other studies showing that feral cats have large home ranges, varying from 118-262 ha<sup>25</sup>, to 178-2,486 ha<sup>26</sup>, to 42-840 ha<sup>27</sup>.

Relatedness values seen in Table 3 further support the finding that there is no significant population structure. The low relatedness values indicate low inbreeding, suggesting that cats are not preferentially seeking to mate with relatives and/or the feral cat population is sufficiently large and/or there is enough gene flow to prevent inbreeding. However, negative values in the relatedness column suggest that cats are preferentially selecting mates that are geographically farther away from the location in which they were captured than to other cats that were captured in more proximal locations. Other basic population genetic parameters explored were allelic richness and gene diversity at each locus. These values, seen in Table 2, indicate the diversity of each locus in this population and provide a basic understanding of each locus which can be useful for future studies. For instance, knowing the allelic richness for each of these loci would allow researchers to pick the minimum number of loci required to obtain unique genotypes for all of the individuals in the population.

These findings are important to keep in consideration when discussing the best control methods for feral cats in Harvey County. These low Fis, Fit, and Fst values, in addition to the likelihood of long-distance dispersal indicate that

control methods that do not take into account the total population of feral cats are not likely to succeed. Any control method that focuses on subpopulations or on small areas will likely face the challenge of quick recolonization. This is consistent with the observed success and failure rates of control methods in the literature, as TNR programs require a capture rate greater than 82% for complete elimination of the population in 4,000 days<sup>9</sup>. As our sample included cats from the countryside surrounding Newton and cats from another town, Halstead, this means that control methods need to include feral cats from the area surrounding Newton as part of the target population. Further studies are needed to obtain an estimate of effective population size and to monitor the same population over time, which would provide more insight as to the effectiveness of the control efforts.

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