# UNDERSTANDING THE INFLUENCE OF BANDED MONGOOSE (*Mungos mungo*) SOCIAL STRUCTURING ON DISEASE TRANSMISSION USING MOLECULAR TOOLS

By

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# Understanding the Influence of Banded Mongoose (*Mungos mungo*) Social Structuring on Disease Transmission Using Molecular Tools

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## Abstract

Understanding the disease transmission dynamics in wildlife species can be difficult and can prove more complicated if the population structure of a socially living species is shaped by territoriality. Understanding the connections and movements of individuals between groups is vital to documenting how a disease may be spread. The presence of a heterogeneous landscape can further complicate attempts to describe transmission of an infectious disease. Here, I sought to understand how dispersal patterns of individual banded mongooses (*Mungos mungo*) could potentially influence disease transmission. Banded mongooses are small fossorial mammals that live in social groups ranging from 5 to 75 individuals and defend their territories against rival troops. The focal population of mongooses for this study lives across a complex environment in the Chobe district of northern Botswana and is faced with a novel strain of tuberculosis, *Mycobacterium mungi*. To infer genetic structure and individual movements between troops, I utilized microsatellite genetic markers and population genetic analyses.

I found moderately strong genetic structuring ($F_{ST}$ = 0.086) among 12 troops of banded mongooses in the study area in 2017-18. The best supported number of genetic clusters was $K$ = 7, with a considerable amount of admixture between troops in urban areas. Compared to the average pairwise differentiation values of troops residing in natural habitats ($F_{ST}$ = 0.102), urban troops had a lower level of differentiation ($F_{ST}$ = 0.081), which suggests more gene flow between these troops. Among 168 mongooses genotyped, 20 were identified as being likely dispersers, with the majority moving across anthropogenic environments, suggesting that dispersal is heightened in urbanized areas.

To assess whether temporal variation had an effect on genetic structure and gene flow between troops, I compared population genetic results from 5 troops in 2008 to those from the same 5 troops in 2017. Genetic differentiation was lower between troops living in urban environments than in natural environments for both 2008 and 2017. This result suggests higher gene flow across the anthropogenic landscapes at both times steps. The overall genetic structuring of the troops persisted over almost a decade, with the exception of observing more mixture and admixture in 2017 than in 2008. The effective population sizes ($N_e$) of troops were larger in 2008, which would indicate that genetic variability declined as time progressed. For 11 individuals confirmed to have *M. mungi*, an assignment test suggested that 3 mongooses were likely dispersers. This finding would contradict that of previous work, which suggested that sick banded mongooses refrained from dispersing. Sequencing of the *M. mungi* strains would be

needed to determine whether these dispersers moved while sick or became infected after entering their new troop.

These findings suggest that emphasis should be placed on closely monitoring banded mongoose troops in areas with heavy human influence. Here we see lower pairwise differentiation, higher gene flow estimates, and more frequent dispersal events. Heightened dispersal potentially can result in elevated disease transmission between troops in urban habitats. With disease transmission being the result of complex interactions between environment, host, pathogen, and time, results from this study contribute to understanding of disease transmission dynamics.

# Understanding the Influence of Banded Mongoose (*Mungos mungo*) Social Structuring on Disease Transmission Using Molecular Tools

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Abstract (General Public)

Understanding how groups of the same species are connected is important for assessing how wildlife diseases spread across a landscape. For social species, connections are established by the movements of individuals between different groups; however, these can prove difficult to observe. Further complicating our ability to infer connections and movements, groups often live under different environmental conditions, which can influence movement rates.

I studied banded mongooses (*Mungos mungo*) living in northern Botswana to assess the role of individual movement on the potential for disease transmission. Banded mongooses are small ground-dwelling mammals that live in troops of 5-75 individuals and defend group home-ranges. In Botswana, some troops are infected with a species of tuberculosis (TB, caused by the bacterium *Mycobacterium mungi*) that is unique to banded mongooses. Using molecular genetic tools, I estimated how genetically similar troops were to one other and estimated the rates of movement of individuals between troops. I found that troops living in urban environments tended to be more genetically similar to one other compared to troops living in natural environments within nearby Chobe National Park. I also detected more cases of individuals moving between troops in urban settings, with little evidence of movement between troops living in natural areas. These results suggest that there is more genetic exchange and a higher degree of connection between troops living in areas heavily influenced by people. With more connections between town-dwelling troops, we would expect to see higher rates of disease transmission between these urban troops, and hence should monitor their movement and health status closely.

I also assessed how genetic structure and connections between banded mongoose troops changed over time by comparing results for collections of samples made in 2008 and 2017. Although more movement was detected in 2017, the overall pattern of genetic connections remained similar over the ten-year period. In particular, there was greater genetic similarity between troops in town compared to troops in natural environments in both years. Additionally, I genetically assigned TB-positive individual mongooses to their troop of origin to determine whether sick individuals moved out of their original troops. I identified three sick individuals as probable dispersers, although it is difficult with the information available to know whether they moved while infected or became ill after joining a new troop.

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## Table of Contents

## List of Tables

## List of Figures

## Chapter 1

## Introduction

The emergence and spread of novel pathogens is of increasing concern, affecting both human and animal health (1–3). The characterization of pathogen transmission dynamics is fundamental to the development of intervention and mitigation approaches. Host, pathogen, and environmental attributes interact to determine pathogen persistence and distribution across landscapes (4,5). These interactions can be strongly influenced by social structuring, movement, and contact between infected and susceptible hosts (6). Territoriality can exert even finer-scale influence on transmission dynamics for directly transmitted pathogens, influencing disease spread. For directly transmitted pathogens in the absence of a reservoir (7), species immigration and emigration between groups and populations can dramatically influence pathogen spread from local to global scales (8). Models play a critical role in understanding and forecasting disease outbreaks (7). Critical to model accuracy is the estimation of dispersal (7), a key behavior that will influence the spatiotemporal dynamics of infectious disease spread across complex and changing landscapes (9).

Unlike estimates of birth and deaths, characterizing and estimating dispersal in social species continues to present challenges as it is difficult to observe and are influenced by decisions that are made at both the individual and group level. Here, molecular tools can be usefully applied to identify dispersal dynamics in a population where these behaviors might otherwise remain unknown. Across systems, genetic analytical techniques are being applied to understand population structure and movement of hosts (10,11), as well as their invading pathogens (12,13). For example, molecular genetic tools were used to investigate the influence of individual dispersal on the spread of chronic wasting disease (CWD) in white-tailed deer (*Odocoileus virginianus*). CWD prevalence was found to be higher in herds with greater genetic differentiation, suggesting lower levels of dispersal had important influence on CWD spread (14).

In order to evaluate the influence of dispersal on infectious disease, we use a model host-pathogen system in Chobe District, northern Botswana. Banded mongooses (*Mungos mungo*) are members of Family Herpestidae that live in socially structured troops ranging from 5-75 individuals. These small, diurnal mammals are territorial, although home ranges often overlap and particular den sites are often used by multiple troops at different times. Troops live in relatively egalitarian social systems and have less reproductive skew compared to other group living carnivores. As a result, individuals tend to stay within their natal troop to help raise offspring and breed. A previous genetic study for the species (15) has suggested that dispersal between troops is rare, however, this study site was in a protected area with little human impact. Our study site consists of natural and urban environments which can influence wildlife behavior and movements. The close aggregation of troops in such a complex landscape makes the system in Chobe District ripe for exploring host dispersal and disease transmission potential.

In our study population, banded mongooses are infected with a novel and emerging tuberculosis (TB) pathogen in the *Mycobacterium tuberculosis* complex, *Mycobacterium mungi* (16,17). This

particular pathogen has a unique pathway of environmental transmission (16) where the pathogen is environmentally transmitted through social communication behaviors that expose mongoose to infected anal gland secretions and urine. Spread of this pathogen across the landscape appears to occur through two primary processes, 1) troop overlap and investigation of infected scent marks placed in overlapping boundary areas, and 2) movement of infected individuals into a new group or area with pathogen transmission occurring through intensive exposure to infected scent marks via various social behaviors. This system was used to evaluate dispersal behavior and infectious disease transmission potential in banded mongoose using molecular tools and genetic analytical approaches. Information obtained from these analyses will provide critical insight into the potential role of dispersal in disease spread and identify dispersal estimates that will be used in spatially explicit disease models.

In Chapter 2, I determined the population structure and genetic diversity of banded mongoose troops living in the Chobe District of northern Botswana. The dataset was primarily composed of non-invasively collected fecal samples, ensuring a high proportion of individuals from most troops were represented. Levels of differentiation between troops and assignment tests using multilocus genotypes helped to identify dispersal estimates across this population of banded mongooses.

In Chapter 3, I assessed the temporal shifts in the population structure, genetic diversity, and dispersal estimates between 2 sampling periods in the same population, with a 9-year time step. The genotypes of individuals infected with *M. mungi* were also used in assignment tests to determine if infected individuals potentially dispersed.

Finally, in Chapter 4, I provide a summary analysis of my work, identifying future areas of research that might contribute to advancing our understanding of this disease system and disease ecology in general.

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## Chapter 2

## Assessing Disease Transmission Potential of Banded Mongooses (*Mungos mungo*) Using Molecular Estimates of Dispersal


### Abstract

Many factors contribute to the characterization of disease transmission dynamics in wildlife populations. For socially living species, the degree of connectivity between individuals and groups can play an integral role in infectious disease transmission across populations and landscapes. Assessing movement and dispersal patterns for a particular species and population allows an improved understanding of the factors that shape and alter social networks. Banded mongoose (*Mungos mungo*) troops in northern Botswana provide an important opportunity to exam these dynamics in a population that spans both natural and urban landscapes. This group-living species is infected with a novel tuberculosis pathogen, *Mycobacterium mungi*. Using microsatellite DNA derived from fecal and tissue samples (2017-18, $n$=168), we evaluated genetic structure, gene flow, and dispersal dynamics for the population. Genetic structure was detectable and moderately strong ($F_{ST}$= 0.086), with $K$=7 being the most probable number of genetic clusters. Considerable indications of admixture in certain troops suggest formation of new groups through recent fusion events. Differentiation was higher for troops inhabiting natural ($F_{ST}$= 0.102) areas compared to troops in urban areas ($F_{ST}$= 0.081), suggesting increased levels of gene flow between urban dwelling troops. Twenty probable dispersers were identified across sampled troops, with the majority of these movements occurring in the urban landscapes. Molecular data suggest dispersal and admixture are considerably elevated in our study system compared to mongoose populations living in natural areas in Uganda. Our findings suggest that anthropogenic landscapes may increase dispersal behaviors. As urban landscapes grow, it is increasingly important to understanding how urban landscapes might influence wildlife population movements and potential for infectious disease transmission.



### Keywords

Dispersal, Infectious Disease, Urban Landscapes, Population Structure, *Mungos mungo*, Non-invasive Sampling


### Introduction

Our capacity to predict infectious disease dynamics remains limited, particularly in free-ranging wildlife populations. One of the most important challenges is the characterization of pathogen transmission dynamics, processes strongly shaped by interactions and attributes of the host, pathogen, and the environment. Sociality and social structuring in the host can have a critical influence on pathogen dynamics, establishing the degree of connectivity within and between groups which can influence the potential for pathogen spread (1–5). Landscape type and structure interacts with species behavior in interdependent ways to influence these processes.

Dispersal behavior can be a critical factor influencing the topography of group connections within and between populations that determines the potential for disease transmission (7). Here, we define dispersal as an individual or group of individuals permanently leaving their natal site to establish themselves in another area or patch in a landscape (6)) Our understanding of these behaviors within and across species remains limited given the often difficult process of in observing these movements.

Together with the social ecology of a species, dispersal dynamics are also influenced by landscape structure and environmental characteristics (8,9). For example, anthropogenic landscapes can have a profound effect on the dispersal potential of a species possibly hindering individual travel in certain circumstances (10,11) or, conversely, increasing movement and species distribution (12). Increased density and connectivity of raccoons living in urban landscapes in Illinois appears linked to increased transmission of rabies in the area (13). Another example can be seen with banded mongooses (*Mungos mungo*) in the Northern Botswana. This population lives across a mixed land use area ranging from natural environments to urbanized areas, with movement behavior of mongooses varying according to land type and proximity to humans (14). Evidence from previous studies suggests (15) that mongoose dispersal behavior in this region diverges significantly from populations living in a protected area in Uganda, where dispersal from the natal troop is limited (16,17). Increased dispersal of infected individuals in a population will have a clear influence on disease transmission potential across social groups.

While dispersal behavior can influence disease transmission dynamics, infectious disease itself can modify dispersal behaviors, influences that can occur at both the individual and group level (18). Banded mongooses living in Northern Botswana are infected with an emerging tuberculosis pathogen in the Mycobacterium tuberculosis complex, *M. mungi*. This novel tuberculosis (TB) pathogen is transmitted primarily through infected scent marks and olfactory communication behaviors between individuals within and between groups (19). Evidence suggest that clinically ill mongoose disperse less frequently than healthy mongooses (20). While more data is needed, there is also evidence to suggest that healthy individuals residing in troops with more infected mongooses may be more likely to disperse compared to individuals living with a higher proportion of healthy troop mates (20). Bidirectional interactions between dispersal and disease may have critical implications for infectious disease dynamics. As dispersal behavior and connectivity can vary so importantly across a species range, it is becoming clearer that a single estimate of dispersal may not accurately reflect true dispersal patterns, compromising the accuracy of model predictions (21).

In northern Botswana, the banded mongoose population provides an important opportunity to study the manner in which dispersal behavior may vary across social groups and influence pathogen transmission potential. Using microsatellite DNA markers, we characterize genetic variation, differentiation, and population structure across multiple troops. We use this information to evaluate dispersal dynamics in this population and implications to TB transmission.

## Methods

*Study Area and Species*

Our long-term study site is located in the northern part of Botswana and includes the northeastern portion of the Chobe National Park, a northern portion of the Kasane Forest Reserve, and the towns of Kasane and Kazungula (Figure 1). Since the identification of *M. mungi* in 2000, banded mongoose troops in this region have been monitored intensively (Alexander, 2010). Banded mongoose are small fossorial carnivores that live in social groups that can number from 5 to 75 individuals (22). This territorial species has a predominantly egalitarian social system, with low or left reproductive skew (23). Mongoose troops in our study area occurred across protected and unprotected landscapes including the growing urban center of Kasane (Figure 1). Denning sites tended to be distributed along the Chobe River [Fig. 1] (22). Radio collars (VHF) were fitted to 1 or 2 mongoose in each study troop previously described (14).

*Sample collection*

We extracted host DNA from tissue and fecal samples form banded mongoose. Banded mongooses use latrine sites for defecation, providing a localized area to collect fecal samples from a majority of troop members. The evening prior to a sampling event, a troop of interest was selected and tracked to their denning site using radio telemetry. Most of the preexisting fecal boluses were removed from the latrine site so that fresh samples could be collected the following morning with a reduced risk of cross-contamination. To obtain samples that were fresh and with the least host DNA degradation, troops were observed as they emerged from the den shortly after sunrise. Mongooses that exited the den were counted and monitored to see where they moved and for signs of defecation (squatting, lifted tail, etc.). The latrine site was approached for sample collection at least 10 minutes after the last individual left to ensure that most members had the opportunity to defecate without disturbance. Even after leaving the communal latrine site, the troop of interest was followed in case individuals defecated in an alternate location. Distinguishing fresh fecal samples from older samples was based on color, firmness, and moisture of the stool [30]. Using a sterilized surgical blade, the outer surface of each fecal bolus was carefully removed, avoiding surfaces in contact with the soil, which can act as a PCR inhibitor (25,26). The fecal matter was transferred into a sterilized 1.5mL microcentrifuge tube and placed in a portable cooler with ice packs.

Blood and tissue samples were obtained from banded mongooses during live-capture activities and animals opportunistically obtained (hit by car, dog attacks, human persecution) or collected in association with other management activities. Each sample was assigned an animal identification number along with demographic data such as the sex of the animal, life-stage (juvenile, sub-adult, adult), date of capture, and troop with which the animal was affiliated at the time of capture or carcass discovery.

Fecal DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio®, Carlsbad, CA). DNA samples were then were stored in a -20°C freezer. Extractions were done within 24-48 hours in an attempt to obtain the highest quality and yield of the limited host DNA available in this sample type (36,37). For DNA originating from blood or tissue, Quick-DNA Plus Kits (Zymo®, Irvine, CA) were used for the extraction process. Both kits were used according to the manufacturer's specifications.

*Genotyping*

Genotyping was performed at 20 microsatellite loci (27,28) derived from previous studies of banded mongoose and other members of the Family Herpestidae. In the Ugandan study (27) species specific microsatellites were identified as polymorphic with no indication of segregation of null alleles among troops.

Three multiplexed polymerase chain reactions (PCR) were used for genotyping individual samples. To ensure strong amplification at each locus without allelic dropout or other molecular artifacts, amplification conditions for each multiplex were tested and visualized. This was done by assessing the fluorescence of amplicons subjected to electrophoresis in an ethidium bromide-stained 3.5% agarose gel with a 100 base-pair molecular weight ladder. Based on these gel images, adjustments to reagent concentrations were subsequently applied to optimize the assay. Each reaction was carried out in a 10-µl reaction which consisted of 5µl of Qiagen Mulitplex PCR Kit buffer, 1µl of a 10x primer mix, 1µl of bovine serum albumin (BSA), 2µl of water, and 1µl of 5ng DNA template. The conditions for the PCR protocol were as follow: 1 cycle of an initial activation phase of 15 minutes at 95°C; followed by 34-cycles of denaturation for 30 seconds at 95°C, annealing for 45 seconds at 57°C, and extension for 30 seconds at 72°C; and a final extension for 10 minutes at 72°C. Forward primers were fluorescently labeled with 6-FAM, NED, PET, or VIC dyes, ensuring that different primers with similar product lengths (within 10 base-pairs) would exhibit different labeling. The PCR products were sent to the Cornell University Biotechnology Resource Center (Ithaca, NY) for fragment analysis using an ABI 3730 Genetic Analyzer.

Given the small amounts of host DNA in fecal samples (29,30), a multiple-amplification approach (31) was implemented to ensure accurate genotyping of mongoose fecal DNA. For the initial PCR, if an individual was scored as heterozygous at a given locus, it was recorded as heterozygous. Results for loci that were scored as homozygous were compared to a second PCR for the same individual. If the locus was heterozygous in the second PCR, then the individual was recorded as heterozygous at that locus. Individuals for whom both PCRs indicated homozygosity for the same allele at the same locus were considered homozygous. If an individual was homozygous at one allele for a locus for the initial PCR and homozygous at a different allele at the same locus for the additional PCR, the individual was recorded as heterozygous for the observed alleles at that locus. Results that showed three or more alleles at one locus across the two combined PCRs we assumed to have been cross-contaminated with another sample and were removed from the study.

*Genetic Analysis*

Amplicon sizes were visualized and scored using GeneMarker ver 2.6.2 (32). Each individual's genotyping file was manually uploaded, and peaks were scored automatically using the default software settings and a GS-500 size standard (Applied Biosystems). Fluorescence peaks were then manually scored on three separate occasions to ensure consistent allele calling. Since fecal samples were collected without knowing the individual donor, we screened for any duplicate samples using Microsatellite Toolkit (33). If fewer than four alleles differentiated individuals, then they were considered duplicates. Any duplicated multilocus genotypes were removed from the dataset.

MICROCHECKER (34) was used to assess the possibility of genotyping errors attributed to null alleles, large allele dropout, and the accidental scoring of stutter peaks (34). MICRO-CHECKER also estimated frequencies of any null alleles, which may allow data for the locus to be utilized in further population genetic analyses (34), for example, in distinguishing the effects of inbreeding and loss of heterozygosity due to Wahlund effect(34).

Exact tests of deviation from Hardy-Weinberg and linkage equilibria, as well as observed and expected heterozygosities were estimated using Arlequin ver 3.5(35). Microsatellite Toolkit(33) and Arlequin provided allelic size ranges, and Arlequin calculated Garza-Williams *m* indices(35) per locus for each troop sampled in the study. Estimates of fixation indices, genetic distances between populations, and analysis of molecular variance (AMOVA) were also calculated using Arlequin.

Assignment of individuals into given numbers of multilocus microsatellite clusters was accomplished through the use of program STRUCTURE (36). This software program was applied to assess population structuring across the study area and to assign individual mongooses to subpopulations using Bayesian clustering approaches (36). GENECLASS2 (37) was used for similar and complementary purposes to estimate inferred population of individual origin and identification of apparent migrants (37). This program utilizes Bayesian and distance-based criteria to assess demographic structure of populations and uses Monte-Carlo resampling for probability computations. Both STUCTURE and GENCLASS2 detect migrant individuals within each population cluster, with GENECLASS2 proving to be especially useful with the detection of first-generation migrants using likelihood computations (37).

Rather than solely relying on the genotypic data to create clusters of genetically similar individuals, the LOCIPRIOR model (38), which is within the admixture model of STRUCTURE, was also employed in identical runs. This model uses prior information regarding the location, or troop, from which each sample was derived to better inform the clustering algorithm. With the LOCIPRIOR model, STRUCTURE takes into consideration that the sampling location may be the true location of origin for each individual, as long as genetic data also provides support for this claim. This means individuals will not automatically assign to a troop based off location alone, but the model has the potential to help the program's confidence when clustering. The LOCIPRIOR model is ideal for populations with detectable structuring that may be weak when

relatively few individuals are sampled per group or location. This was an important aspect to consider particularly for the scale of our study site.

Effective population sizes ($N_e$) were estimated to assess genetic variability and rate of genetic change driven by genetic drift within the population (39). Both single and two-sample approaches were applied. The single-sample method of Waples and Do (40) uses a random linkage disequilibrium (LD) approach which has been shown to have high precision for microsatellite data.

GROUPRELATE(41) was used to assess the relatedness of individuals within and across troops. This Excel macro algorithm performs 1000 randomizations to test for average relatedness values by generating new genotypes from the presented allele frequency distributions. Within-group relatedness was estimated for each troop as well as pairwise values of between-troop relatedness. Values of $r>0.25$ are indicative of potential first- and second-degree relationships, while conversely, values of $r<-0.25$ suggest no relation (41). GROUPRELATE is particularly useful for identifying patterns of relatedness between and within sexes; however, sex assignment was not available for the majority of individuals because the samples were collected at communal latrine sites.

*Study permissions*

Methods for this study were conducted adhering to the procedures of the Virginia Tech Institutional Care and Use Committee (IACUC 16-217-FIW) and under a permit issued by the Botswana Ministry of Environment, Natural Resources Conservation, and Tourism (EWT 8/36/4 XXXVIII (23)).

## Results

*Genotyping and Loci Metrics*

From the 167 individual fecal samples that were collected, 77% were successfully amplified at a minimum of 13 loci. DNA from 49 additional blood or tissue samples were also genotyped and included in the dataset. After the identification and removal of 9 duplicate samples, multilocus data from 168 individuals across 12 troops were used in for analyses.

Due to a lack of polymorphism or unreliable PCR amplification, five of the original 20 microsatellite loci (*MmAAC5, Mm18-1, Mm7-5, Mon-67,* and *Mm19*) were omitted from the study, leaving data from 15 loci for analysis [Table 3]. MICROCHECKER detected the presence of null alleles at loci *Mm10-7* (*n* = 1 troop), *Mon-16* (*n* = 1 troop), *Mm2-10* (*n* = 2 troops), *Mon-38* (n = 1 troop), and *Ss13-8* (*n* = 3 troops). Because the null alleles were not detected consistently across the majority of troops, data from these loci were retained in the analysis. Eleven loci showed deviations from Hardy-Weinberg equilibrium (HWE) in at least one troop, but none deviated from HWE in more than half the troops. Some departures from equilibrium would be expected within a group-living social system in violation of underlying Hardy-

Weinberg assumptions (e.g., small numbers of breeders within troops, mixing among demes, generational overlap, etc.), so data from each locus were retained in the analysis. Tests for linkage disequilibrium after a Bonferroni correction showed no significant outcomes of apparent linkage heritability between loci across the troops.

*Population Structure and Differentiation*

Structure was able to provide an estimate for the appropriate number of genetic clusters for the banded mongoose population in our study area. Posterior support for various numbers of clusters ($K$) from $K$= 1 to 15 were evaluated, with support for each $K$ tested using 10 iterations. Each iteration was tested using 50,000 burn-ins and 500,000 Markov chain Monte Carlo (MCMC) repetitions. Based on the highest mean estimate of the log probability of the data (Ln$P(D)$), STRUCTURE identified $K$= 7 as the most likely number of clusters. Using the Evanno et al. (42) ad-hoc $\Delta K$ statistic, $K$ = 2 was the best-supported number of clusters.

While varying levels of admixture were observed across troops [Fig. 2], genetic signatures unique to particular troops could be seen. For example, troops CGL, CCH, CSL, MOGO, and KUBU-KWA were comprised predominantly of their own genetic cluster. However, there were individuals within some of these troops that exhibited high probabilities of belonging to an alternate genetic cluster than the troop from which they were sampled. Alternatively, troops - such as WDL, LIB, and SEF - appeared to be the result of several distinct genetic clusters merging [Fig. 2].

$F_{ST}$ *and* $R_{ST}$ metrics of genetic differentiation [Table 3] identify considerable genetic structuring among the troops. Mean $F_{ST}$ and $R_{ST}$ values for pairwise comparisons between troops were 0.086 and 0.076, respectively. The mean $F_{ST}$ of troops residing in the urbanized area of Kasane (CSL, WDL, FOR, LIB, MOGO, PLAT, WA-WP, MOW, and SEF) was 0.081. This is compared to the average $F_{ST}$ values for troops living in the natural landscape of the Chobe National Park (CGL and CCH, average= 0.108) and the mixed-use area of Kazangula (KUBU-KWA; average= 0.086). Within Kasane, the highest $F_{ST}$ average for a troop belonged to WA-WP (0.103) with lowest belonging to LIB (0.063). The largest $F_{ST}$ for the population was for CGL with a value of 0.126. Results from the global AMOVA [Table 1] indicated that ~8% of genetic variation was among troops, ~5% among individuals within troops, and 87% within individual mongooses across troops.

*Group Relatedness*

Results from troop relatedness showed considerably higher values of relatedness [Table 4] within troops (mean = 0.127) than between troops (mean = -0.018, Table 5). Although some troops such as CSL and SEF had intragroup relatedness values relatively close to 0 [Table 4], all within-troop values were considerable larger than troop pairwise comparisons.

*Effective Population Sizes*

Estimates of $N_e$ [Table 7] ranged from 3 to 29, with estimates for two troops being unbounded. For certain groups, such as CCH and MOGO, $N_e$ estimates were approximately the number of individuals sampled. Although their sample size was the third lowest in the population ($n$=8), WDL by far had the largest effective population size ($N_e$= 28.2). Other troops, such as CGL and KUBU-KWA, had considerably lower effective population sizes compared to their actual sample size [Table 7]. Important to note, CGL and KUBU-KWA also had had very distinct genetic signatures indicated by the STRUCTURE Q plot [Fig. 2] and high pairwise differentiation values relative to other troops ($F_{ST}$= 0.125 and 0.086, respectively).

*Dispersal and Detection of First-Generation Migrants*

Individual assignment and detection of first-generation migrants using GeneClass2 led to the assignment of 148 individuals (88%) to the troop from which they were sampled. Five of the 12 twelve troops in the study area included individuals that apparently originated from other troops [Table 6], with CSL contributing the most immigrants ($n$= 8). There was no indication that troops with adjacent or overlapping home ranges exchanged more migrants than spatially distant troops. In fact, two troops on the eastern edge of the study area, SEF and KUBU-KWA (Figure 1), produced the highest numbers of detectable migrants, 9 and 5, respectively [Table 6]. The test for first-generation migrants identified 11 individuals likely to have emigrated away from their parental troops [Table 9]. Each detection of first-generation migrants corresponded with individuals identified from the initial assignment test [Table 6]. SEF had the largest number of first-generation immigrants with 4, while KUBU-KWA had 3.

## Discussion

Infectious disease transmission dynamics are closely tied to contact rates within and between groups, particularly in social species like banded mongooses. Our results indicate that banded mongooses in Northern Botswana appear to have higher levels of dispersal compared to another banded mongoose study population in Uganda (17), highlighting the potential for species behavior to vary significantly across a species range. Landscape heterogeneity and the presence of an endemic disease may have influence on dispersal behaviors in the Botswana population.

*Troop Structure, Movements, and Relatedness*

Data suggest that there are 7 genetic clusters in the population over the 12 troops sampled. Certain troops had prominent signals of admixture, such that they appeared to be amalgamations of two or more genetically distinct groups. This type of troop composition could be the result of individuals or groups from different troops, either voluntarily leaving or forcibly evicted, coming together to form a new troop in an available territory and interbreeding. For example, individuals from CGL may have joined individuals from LIB or other troops to establish troop WDL [Fig.

2]. Similarly, this pattern of cohort fusion leading to troop formation is also observed in previous banded mongoose studies in Uganda (17).

Data suggest direct troop mixture in the population such as that seen within CSL [Fig. 2], where individuals originating from KUBU-KWA, MOGO, and PLAT appear to have assimilated. Troops within urban environments were more genetically similar than troops in the mixed and protected land areas, suggesting higher levels of dispersal and gene flow in these anthropogenic landscapes.

Overall, twenty-one apparent migrants or dispersing mongooses were detected among the 168 individuals genotyped (12.5%) [Table 6]. From this pool of dispersing individuals, 11 were recognized as first-generation migrants having dispersed from their natal troop in the recent past [Table 6]. There was no indication that troops with adjacent or overlapping home ranges exchanged more migrants that spatially distant troops. This population appears to mimic an Island Model in which individuals have more of an equal opportunity to immigrate to most troops in the study area. For some individuals it may also be more advantageous to disperse further away rather than stay close if inbreeding is more of a concern. Coupling this detection of immigration with the observation of admixture, genetic exchange is more frequent in this system than in other systems previously investigated for the species (28).

Dispersal events appear to be more frequent in the urban troops suggesting that this environment may positively influence the amount of intertroop movement. For a species having the ability to adapt and thrive in anthropogenic habitats, the abundance of denning and food resources can facilitate elevated levels of congregation (43–46). Laver and Alexander (2018) found that mongoose troops living in these anthropogenic landscapes tended to have smaller home ranges in the dry season and concentrated around buildings and human refuse. Troops in the urban land areas have also been documented using the same den sites on different occasions, behaviors that would increase exposure to environmental pathogens and possibly more troop-to-troop contact (Nichols and Alexander). Banded mongoose troops living in urbanized areas with abundant resources from human garbage (larger and more calorie-dense than insects) may be more receptive to immigrants than troops living in natural ecosystems where denning and food resources are more constrained (i.e. insects and small vertebrates). Foreign individuals may also be readily integrated into a troop as a source of genetic variation.

Anthropogenic habitats can influence gene flow both indirectly and directly. As a non-conventional mode of dispersal, human-mediated movement of wildlife can have important impacts on gene flow and pathogen movement (47–49). Here, banded mongooses can be moved incidental to human activities, distances that normal biological and environmental constraints would infrequently allow. An example of this is the case of an orphaned mongoose pup. He was brought to us in December of 2017 by local fisherman, but efforts to introduce the pup to the nearby KUBU-KWA troop in Kazangula failed. Genotyping and multiple assignment tests using this dataset revealed strong evidence that the pup was most likely a member of MOGO troop and a native of Kasane. The journey from Kasane to Kazungula (~8km) would have been a significant and unrealistic geographic distance for a lone pup to travel without human-mediated transport. In this particular case, the troop receiving the potential immigrant aggressively rejected

it. However, a more receptive troop might have absorbed such a dispersing individual, thus initiating or enhancing gene flow.

Even with evidence of inter-troop movements, levels of genetic differentiation between troops were reasonably high considering the limited size of our study area. Hartl and Clark (50) classified $F_{ST}$ values ranging from 0.05 to .15 as indicators of moderate genetic differentiation. With troops living in close proximity, some with overlapping home ranges (32), an average $F_{ST}$ of 0.086 is larger than might be expected. $F_{ST}$ values for mongoose population in Uganda were, however, higher than those estimated in this study (average $F_{ST}$ = 0.129) (28). This population, however, occurred exclusively in protected areas. Troops in our study area living in the town Kasane and the mixed-use area of Kazungula had lower $F_{ST}$ values on average (average $F_{ST}$ s= 0.081 and 0.086 respectively) than troops living in natural environments (average $F_{ST}$ = 0.108).

Previous studies of banded mongoose behavior have shown that members of the species are typically philopatric and often mate within their natal group (16,17). Findings from our troop relatedness analysis suggests that banded mongoose in our study site practice similar behaviors. Although the detection of mixture [Fig. 2] and dispersal events [Table 6] suggests some familial relationships most likely exist outside of troops, relatedness values within troops were considerably high compared to between-troop values [Table 4]. This supports the notion that strong familial relationships within troops persist and a high proportion of mating occurs between troopmates, even with gene flow and detectable genetic migrants present. As a result of intragroup breeding, smaller effective population sizes would be expected for well-established and older troops in the study system, as seen for CGL ($N_e$ = 3.0) and KUBU-KWA ($N_e$ = 8.6). These troops would most likely experience drift-mediated changes in allele frequencies (mean $F_{ST}$ for CGL = 0.125, for KUBU-KWA mean $F_{ST}$ = 0.086), rather than have frequency changes induced by genetic migrants, as multiple generations of individuals stay within the natal troop and the inflow of novel alleles is reduced.

The opposite is inferred for troops, such as WDL and LIB, that have few members but substantially higher estimates of $N_e$ (28.8 and 8.5, respectively). These smaller troops with higher genetic variability and admixture likely formed recently through the joining of cohorts from genetically distinct natal troops (17), possibly explaining their large effective population sizes. As a new troop progressively interbreeds over time, drift would lead $N_e$ to decline as generations of newly recruited pups remain philopatric. This may have important implications to pathogen movement and genetic structuring in the population. This will be influenced by the nature of the pathogen transmission. *M. mungi* is transmitted through an environmental route and lack of immigration may not isolate troops from exposure.

Important to note is the existence of troops in the study area where no individuals were sampled. It is likely that at least 2 or more troops reside between CGL and CCH within the park, however, their extremely cryptic nature made it difficult to collar and track them. Having un-sampled or "ghost" populations (51) can influence estimates of gene flow and allele frequency. Estimating the immigration and emigration of individuals from these ghost populations components can be a very difficult task (52). However, it is suggested that estimates of genetic migration are

marginally impacted if there is little to no immigration from these unaccounted populations or groups (51).

*Infectious Disease and Land Use*

Where Nichols et al (17) emphasized the critical role of relatedness in a social species to the overall population structure, our data suggest that infectious disease and land use may have the greatest influence on population structure in the Botswana system. These results are important and suggest that host-pathogen-environment interactions can have complex influences not only on infection dynamics, but on the ecology of the species. As mentioned before, a previous study suggested that *M. mungi* changes the behavior of its host, with clinically ill individuals unlikely to disperse in contrast to healthy mongooses (20). Conversely, Fairbanks et al. (53) assessed how altered behaviors within urban dwelling troops can affect disease transmission dynamics. Increased bouts of aggression observed at trash sites, compared to other foraging habitats, lead to more external injuries and a higher probability of the pathogen invading the host (53). When we couple behaviors altering transmission with the heightened dispersal we observed across the urban landscape for this study, we begin to see how anthropogenic environments can truly shape outbreaks and potentially pathogen movements.

Our findings suggest that accurate the prediction of disease spread may be difficult without inclusion of feedbacks and interactions between landscape and infectious disease and their influence on host movement behavior and disease transmission. These complex bidirectional dynamics may be difficult to characterize but may be central to the outcome of disease invasions in a population.

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**Table 2.1**

| *Locus* | *Species Origin* | *Source* | $H_O$ | $H_E$ | *A* | *Range* | *% Variation* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *Among Troops* | *Among Indiv.* | *Within Indiv.* |
| *Mon-16* | *Mungos mungo* | *Sanderson et al. 2013* | 0.584 | 0.618 | 3.67 | 6.17 | 11.2 | 5.3 | 83.5 |
| *Mon-19* | *Mungos mungo* | *Sanderson et al. 2013* | 0.753 | 0.715 | 5.00 | 13.33 | 10.7 | -4.4 | 93.7 |
| *Mon-25* | *Mungos mungo* | *Sanderson et al. 2013* | 0.723 | 0.783 | 5.75 | 11.50 | 5.6 | 8.2 | 86.2 |
| *Mon-32* | *Mungos mungo* | *Sanderson et al. 2013* | 0.466 | 0.568 | 3.08 | 4.16 | 0.1 | 13.1 | 86.8 |
| *Mon-38* | *Mungos mungo* | *Sanderson et al. 2013* | 0.713 | 0.786 | 5.17 | 10.00 | 2.0 | 8.2 | 89.8 |
| *Mon-41* | *Mungos mungo* | *Sanderson et al. 2013* | 0.323 | 0.398 | 2.91 | 5.82 | 7.7 | 12.4 | 79.9 |
| *Mon-65* | *Mungos mungo* | *Sanderson et al. 2013* | 0.339 | 0.304 | 2.56 | 7.56 | 15.1 | -11.6 | 96.5 |
| *Mon-66* | *Mungos mungo* | *Sanderson et al. 2013* | 0.759 | 0.690 | 4.55 | 12.91 | 11.5 | -9.0 | 97.5 |
| *Mon-68* | *Mungos mungo* | *Sanderson et al. 2013* | 0.702 | 0.695 | 4.42 | 19.83 | 7.5 | -0.3 | 92.8 |
| *Mon-69* | *Mungos mungo* | *Sanderson et al. 2013* | 0.539 | 0.484 | 3.17 | 28.67 | 14.3 | -8.6 | 94.3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Mon-70* | *Mungos mungo* | *Sanderson et al. 2013* | 0.751 | 0.789 | 6.42 | 33.00 | 7.263 | 2.255 | 90.5 |
| *Mm2-10* | *Mungos mungo* | *Waldick et al. 2003* | 0.631 | 0.729 | 4.75 | 20.17 | 4.6 | 16.0 | 79.4 |
| *Mm10-7* | *Mungos mungo* | *Waldick et al. 2003* | 0.423 | 0.532 | 3.83 | 6.67 | 13.9 | 15.7 | 70.5 |
| *Ss11-12* | *Suricata suricatta* | *Griffin et al. 2001* | 0.580 | 0.525 | 4.00 | 57.09 | 7.6 | -5.1 | 97.5 |
| *Ss13-8* | *Suricata suricatta* | *Griffin et al. 2001* | 0.657 | 0.791 | 6.92 | 24.17 | 9.9 | 16.1 | 74.0 |
| | | | | | | Mean | 8.2 | 4.6 | 87.2 |

**Table 1.** Descriptive information and genetic variability metrics for polymorphic loci used for the genetic analyses of banded mongooses in the Chobe district of northern Botswana. Average observed heterozygosities ($H_O$) and expected heterozygosities ($H_E$) are presented for each locus, along with the average number of alleles (*A*), and average allelic size range. Results of partitioning of genetic variation using AMOVA are displayed as *% variation* among troops, among individuals, and within individuals.

**Table 2.2**

| *Troop* | $H_O$ | $H_E$ | *A* | *Range* | *M-ratio* | $F_{IS}$ |
|---|---|---|---|---|---|---|
| *CGL* | 0.636 | 0.660 | 4.13 | 13.73 | 0.301 | 0.038 |
| *CCH* | 0.646 | 0.651 | 4.87 | 17.73 | 0.275 | 0.008 |
| *CSL* | 0.570 | 0.656 | 5.40 | 19.73 | 0.274 | 0.131 |
| *WDL* | 0.676 | 0.661 | 3.93 | 19.29 | 0.203 | -0.044 |
| *FOR* | 0.578 | 0.576 | 3.93 | 16.80 | 0.234 | -0.004 |
| *LIB* | 0.619 | 0.674 | 4.23 | 15.23 | 0.277 | 0.064 |
| *MOGO* | 0.665 | 0.651 | 5.00 | 19.47 | 0.257 | -0.037 |
| *PLAT* | 0.536 | 0.571 | 4.13 | 16.40 | 0.252 | 0.050 |
| *WA-WP* | 0.452 | 0.585 | 2.93 | 15.00 | 0.195 | 0.250 |
| *MOW* | 0.579 | 0.648 | 4.86 | 17.57 | 0.277 | 0.104 |
| *SEF* | 0.674 | 0.704 | 5.00 | 19.29 | 0.259 | 0.040 |
| *KUBU-KWA* | 0.586 | 0.584 | 4.93 | 18.80 | 0.262 | -0.004 |
| ***Mean*** | 0.601 | 0.635 | 4.45 | 17.42 | 0.256 | 0.050 |

**Table 2.** Similar genetic diversity metrics as presented in Table 1, but specific for the 12 individual banded mongoose troops. Average inbreeding coefficients ($F_{IS}$) within the troops are also presented. The mean of each values from across the troops have been calculated to produce an average value representing the study area.

**Table 2.3**

| | *CGL* | *CCH* | *CSL* | *WDL* | *FOR* | *LIB* | *MOGO* | *PLAT* | *WA-WP* | *MOW* | *SEF* | *KUBU-KWA* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *CGL* | - | **0.180** | **0.188** | **0.119** | **0.067** | **0.065** | **0.194** | **0.184** | **0.308** | **0.310** | **0.147** | **0.077** |
| *CCH* | **0.118** | - | 0.014 | 0.049 | **0.067** | -0.002 | **0.123** | **0.058** | -0.078 | 0.022 | 0.014 | **0.088** |
| *CSL* | **0.104** | **0.066** | - | 0.064 | **0.090** | 0.034 | **0.112** | **0.072** | -0.041 | 0.002 | -0.003 | **0.096** |
| *WDL* | **0.098** | **0.075** | **0.050** | - | **0.047** | -0.051 | **0.094** | -0.002 | 0.052 | **0.101** | 0.017 | 0.017 |
| *FOR* | **0.126** | **0.132** | **0.098** | **0.075** | - | -0.039 | **0.122** | **0.080** | **0.110** | **0.166** | 0.038 | 0.008 |
| *LIB* | **0.109** | **0.089** | **0.044** | 0.035 | **0.055** | - | **0.075** | -0.010 | 0.082 | **0.095** | 0.005 | -0.038 |
| *MOGO* | **0.130** | **0.062** | **0.046** | **0.076** | **0.144** | **0.069** | - | **0.046** | **0.126** | **0.171** | 0.041 | **0.136** |
| *PLAT* | **0.146** | **0.097** | **0.068** | **0.050** | **0.078** | 0.023 | **0.078** | - | -0.004 | **0.084** | 0.030 | **0.097** |
| *WA-WP* | **0.148** | **0.122** | 0.033 | **0.110** | **0.104** | **0.094** | **0.116** | **0.083** | - | -0.080 | 0.017 | **0.117** |
| *MOW* | **0.167** | **0.097** | **0.032** | **0.107** | **0.177** | **0.067** | **0.046** | **0.097** | **0.100** | - | 0.043 | **0.159** |
| *SEF* | **0.118** | **0.050** | 0.015 | **0.068** | **0.111** | **0.062** | **0.034** | **0.085** | **0.089** | **0.053** | - | 0.033 |
| *KUBU-KWA* | **0.120** | **0.091** | **0.062** | **0.056** | **0.085** | **0.052** | **0.100** | **0.082** | **0.130** | **0.107** | **0.064** | - |

**Table 3.** Pairwise $F_{ST}$ (below the diagonal) and $R_{ST}$ values (above the diagonal) compared between the 12 mongoose troops. Bold font indicates values that are statistically significant ($\alpha$ = 0.05) after tests of 10100 permutations.

**Table 2.4**

| *Troop* | *Average Relatedness within Troops* |
|---|---|
| *CGL* | 0.156 |
| *CCH* | 0.109 |
| *CSL* | 0.034 |
| *WDL* | 0.130 |
| *FOR* | 0.129 |
| *LIB* | 0.131 |
| *MOGO* | 0.090 |
| *PLAT* | 0.186 |
| *WA-WP* | 0.181 |
| *MOW* | 0.150 |
| *SEF* | 0.050 |
| *KUBU-KWA* | 0.180 |
| *Mean* | 0.127 |

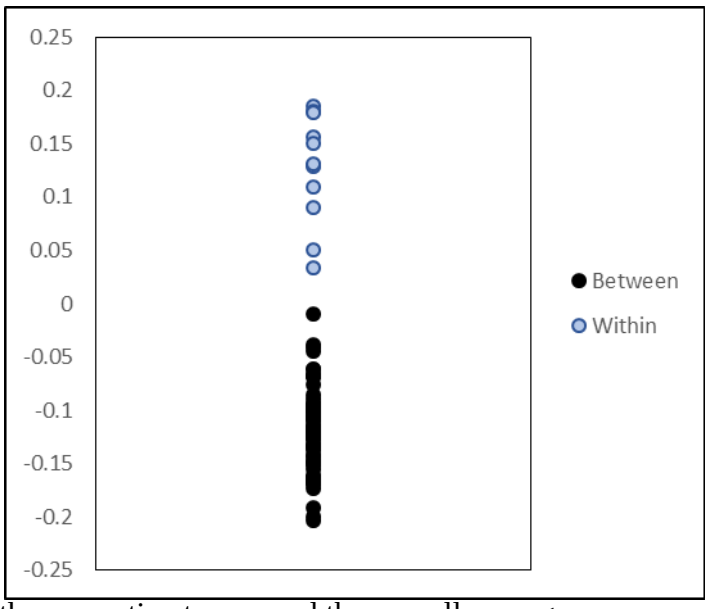


**Table 4.** Average relatedness values within the respective troops and the overall average relatedness value between all troops in the study area. The graph depicts relatedness values within each troop (blue data points) against the pairwise relatedness values between all the troops (black data points). All values are statistically significant at $\alpha = 0.05$ after 1000 repetitions.

**Table 2.5**

| *Troop* | *CGL* | *CCH* | *CSL* | *WDL* | *FOR* | *LIB* | *MOGO* | *PLAT* | *WA-WP* | *MOW* | *SEF* | *KUBU-KWA* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *CGL* | - | | | | | | | | | | | |
| *CCH* | -0.155 | - | | | | | | | | | | |
| *CSL* | -0.123 | -0.085 | - | | | | | | | | | |
| *WDL* | -0.147 | -0.118 | -0.068 | - | | | | | | | | |
| *FOR* | -0.170 | -0.162 | -0.085 | -0.137 | - | | | | | | | |
| *LIB* | -0.150 | -0.132 | -0.045 | -0.103 | -0.100 | - | | | | | | |
| *MOGO* | -0.167 | -0.096 | -0.062 | -0.118 | -0.173 | -0.095 | - | | | | | |
| *PLAT* | -0.191 | -0.144 | -0.076 | -0.101 | -0.098 | -0.061 | -0.110 | - | | | | |
| *WA-WP* | -0.167 | -0.127 | -0.010 | -0.168 | -0.134 | -0.152 | -0.105 | -0.097 | - | | | |
| *MOW* | -0.200 | -0.126 | -0.039 | -0.156 | -0.204 | -0.106 | -0.068 | -0.125 | -0.118 | - | | |
| *SEF* | -0.164 | -0.093 | -0.041 | -0.134 | -0.163 | -0.117 | -0.069 | -0.142 | -0.123 | -0.097 | - | |
| *KUBU-KWA* | -0.164 | -0.125 | -0.068 | -0.114 | -0.107 | -0.089 | -0.131 | -0.108 | -0.149 | -0.135 | -0.119 | - |

**Table 5.** Pairwise between-group relatedness values.

**Table 2.6**

| | | $N_e$ Values | |
|---|---|---|---|
| *Troop* | *n* | 0.05 $P_{crit}$ | 95% CI |
| *CGL* | 11 | 3.0 | 2.3-6.1 |
| *CCH* | 15 | 16.3 | 10.7-27.8 |
| *CSL* | 27 | 13.1 | 10.4-16.8 |
| *WDL* | 8 | 28.8 | 7.2- *Infinite* |
| *FOR* | 15 | 10.7 | 6.4-19.2 |
| *LIB* | 7 | 8.5 | 2.7-65.3 |
| *MOGO* | 17 | 14.1 | 9.5-22.7 |
| *PLAT* | 22 | 7.9 | 5.4-11.3 |
| *WA-WP* | 4 | *Infinite* | 2.9- *Infinite* |
| *MOW* | 13 | 7.5 | 4.2-12.5 |
| *SEF* | 9 | *Infinite* | 35.6- *Infinite* |
| *KUBU-KWA* | 19 | 8.6 | 6.1-12.2 |

**Table 7.** Samples sizes (*n*) and estimated effective population size ($N_e \pm$ 95% confidence interval) for each of the 12 troops. The $N_e$ estimated accounted for allele frequencies as low as 0.05 ($P_{crit}$= 0.05).

**Table 2.7**

| *Troop of Assignment* | *Troop Where Individuals Were Sampled* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *CGL* | *CCH* | *CSL* | *WDL* | *FOR* | *LIB* | *MOGO* | *PLAT* | *WA-WP* | *MOW* | *SEF* | *KUBU-KWA* |
| *CGL* | **11** | - | - | - | - | - | - | - | - | - | - | - |
| *CCH* | - | **15** | - | - | - | - | - | - | - | - | - | - |
| *CSL* | - | - | **27** | - | *1* | - | - | *1*$^{m=1}$ | - | - | - | - |
| *WDL* | - | - | - | **8** | - | - | - | - | - | - | - | - |
| *FOR* | - | - | *1*$^{m=}$ | - | **15** | - | - | *1*$^{m=1}$ | - | - | - | - |
| *LIB* | - | - | - | - | - | **7** | - | - | - | - | - | *1* |
| *MOGO* | - | - | *1* | - | - | - | **17** | - | - | - | - | - |
| *PLAT* | - | - | - | - | - | - | *1*$^{m=1}$ | **22** | - | - | - | - |
| *WA-WP* | - | - | - | - | - | - | - | - | **4** | - | - | - |
| *MOW* | - | - | - | - | - | - | - | - | - | **13** | - | - |
| *SEF* | - | *1*$^{m=1}$ | *2* | - | - | - | - | *2* | - | 1$^{m=1}$ | **9** | *3*$^{m=2}$ |
| *KUBU-KWA* | - | - | *4*$^{m=2}$ | - | *1*$^{m=1}$ | - | - | - | - | - | - | **19** |

**Table 6.** Results from the assignment and first-generation detection tests showing where individuals were sampled (*x*-axis) compared to where individuals were assigned (*y*-axis). The superscript ($m=i$) denotes how many of the individuals assigned to alternate troops were inferred first-generation migrants.

**Figure 2.1**

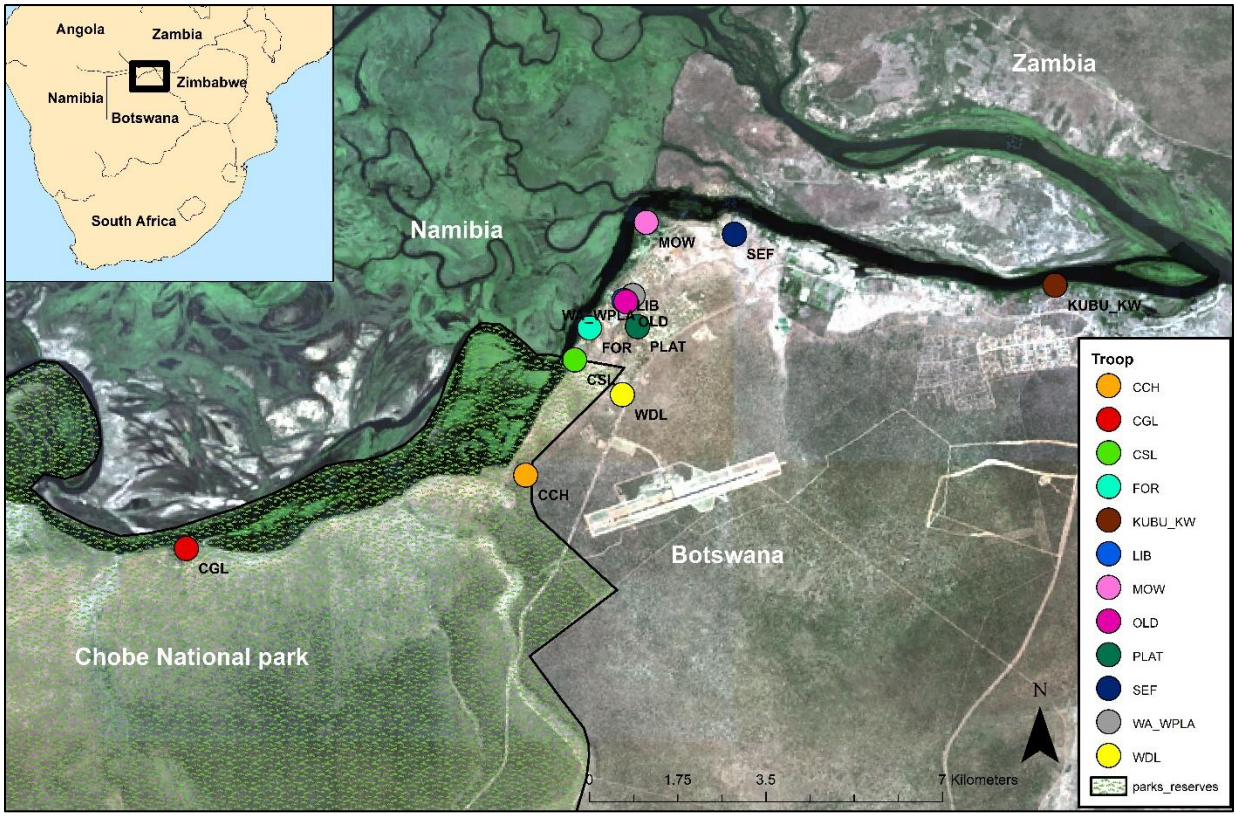


**Figure 1.** Spatial organization of denning sites belonging to 12 troops of mongooses along the Chobe River in northern Botswana.

**Figure 2.3**

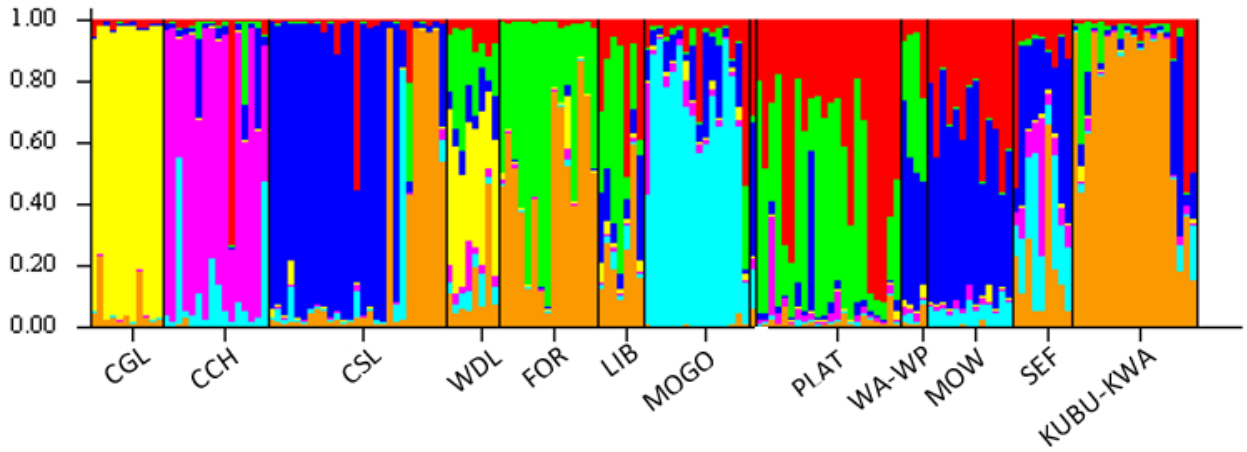


**Figure 2.** Genetic structure of banded mongoose troops in northern Botswana inferred using program STRUCTURE. Troops are arranged in sequential order based on geographic location from east to west. The graph shows the probability coefficients (*q*) for each individual (represented by a colored histogram bar) showing individual assignment to 7 inferred genetic clusters (*K*) using the LOCPRIOR model. Important to note is that WA merged with WP and KUBU had joined with a nearby troop, KWA, at the time of sampling.

## Chapter 3

## Temporal dynamics of banded mongoose troop structure along the Chobe River, Botswana


### Abstract

With time, populations of wildlife can experience changes in spatial distribution and gene flow between groups of conspecifics. Temporal variation is especially important for social and territorial species since networks of interaction and group connectivity are dynamic characteristics. Further, factors such as the presence of an infectious disease and the complexity of the landscape may affect how a population is distributed and interacts over time. The banded mongoose (*Mungos mungo*) is a cooperatively, group-living species in Family Herpestidae that defends territorial home ranges against rival troops. In northern Botswana, banded mongoose troops are affected by a novel strain of tuberculosis, *Mycobacterium mungi*, with troops living in an array of environmental conditions. Genetic samples collected from select troops in 2008 and 2017 were screened in a temporal comparison to assess population genetic structure and gene flow over the sample period. Overall, the genetic structuring of troops was similar from 2008 to 2017, although more admixture and mixture was detected in 2017. However, effective population sizes decreased over time, suggesting a reduction of gene flow as troops aged. For both time periods, pairwise $F_{ST}$ values were smaller for troops living in urban areas compared to troops in periurban/rural villages or natural landscapes. This observation suggest that urban environments may facilitate more movement and gene flow between troops over other landscapes. Multilocus genotypes from 11 individuals that tested positive for *M. mungi* were subjected to assignment tests using the 2008 dataset. Three individuals were identified as likely dispersers, however, it is unclear if animals moved with the infection or were subsequently infected after dispersal. Research results suggest that urban landscapes may exert important influences on wildlife population genetics, an issue with important implications to wildlife conservation.



### Keywords

Temporal Variation, banded mongoose, dispersal, genetic structure, *Mycobacterium mungi*


### Introduction

In wildlife populations, spatial distribution and genetic variability are often subject to change as time progresses (1,2). Behavioral interactions and genetic connectivity between groups are not static and change with time (3). This is particularly important for species that are highly social and territorial, as groups may experience changes in demographic structure, home range, and levels of gene flow. Thus, it is key to sample and analyze data from various time periods in order to accurately characterize within- and between-group gene flow and structure (4). Although

difficult to identify a priori, various ecological factors may drive the temporal changes of group structure that we see in these group living, territorial species.

One potentially modifying factor is the presence of infectious disease. Over time, a pathogen can greatly influence social interactions and even the inter-group movement of individual hosts (5). Pathogen invasion either can suppress the level of host movement (6) or enhance the emigration of healthy individuals potentially avoiding infection within a disease infected troop (7). Landscape heterogeneity is another ecological factor that influences the connectivity between groups (8), especially as certain areas in a landscape change over time and resource availability fluctuates.

Banded mongooses (*Mungos mungo*) offer an important system for the study of group dynamics through time. These cooperatively living members of Family Herpestidae live in highly social troops and defend their territory against opposing groups (9). Typically philopatric, members tend to stay within their natal troop (9,10), meaning that individuals are highly related within rather than between troops. New troops form when small cohorts of same-sex individuals are evicted or voluntarily disperse (10,11). If they meet with a same-sex cohort of the opposite sex from another troop, theses troops may merge and form a new troop in an available territory.

In Northern Botswana, banded mongooses are infected with a novel *Mycobacterium* pathogen, *Mycobacterium mungi* (12,13). This tuberculosis pathogen is shed though anal gland secretions and urine, which the mongoose host uses for olfactory communication. *M. mungi* invades through lacerations in the nasal planum or skin in association with the olfactory inspection of secretions or the marking of conspecifics. This infected population lives across the urban wildlife continuum. Results of previous work (Chapter 2) identify less between-group genetic differentiation and increased rates of gene flow in urban troops compared to those in natural landscapes. The urban landscape also appears to influence disease transmission where mongooses using human waste exhibit higher levels of intergroup aggression, injury and disease transmission (7). Infection also appears to influence dispersal movement, with sick mongooses less likely to disperse and there being no evidence of eviction by group members (6). Here, we comparatively evaluate genetic structuring among focal troops at 2 time steps (2008 and 2017) using microsatellite markers. We also evaluate disease status among identified immigrant mongoose to test whether clinically ill mongooses dispersed or remained philopatric.

## Methods

### *Study Site*

The study population occurs across various land use types in Northern Botswana and has been the subject of study since the identification of *M. mungi* infection in 2000 (12). Five troops were selected for evaluation based on the samples available and the time steps selected (2008 and 2017). Mongooses in this study den along the Chobe River in a small northeastern portion of Chobe National Park and the towns and Kasane and Kazungula. Detailed information on the ecology of these troops have previously been reported (7,14).

*Sample Collection*

Fecal samples were collected from July to August of 2017 (Fig. 1). Banded mongooses defecate at latrine sites each morning right after exiting the den, allowing non-invasive collection of a fecal sample from a majority of troop members. DNA was extracted from feces on the same day as collection using the PowerSoil DNA Isolation kit (MoBio, Carlsbad, CA) according to the manufacturer's protocol. Blood and tissue samples were also collected across all troops through live captures and opportunistic necropsies of individuals found dead. Zymo Quick-DNA Plus Kits (Zymo, Irvine, CA) was used to extract DNA from tissue samples.

Archived DNA samples from 2008 were used to provide a temporal comparison. These samples had been extracted from blood and tissue of mongooses across the same 5 troops in the study area. Individual mongooses were captured using 81.3 cm x 25.4 cm x 30.5 cm Tomahawk live traps (Tomahawk Inc., Hazelhurst, Wisconsin, USA) and chemically immobilized to obtain genetic samples and morphological measurements (14). Along with the 2008 samples, extracted DNA from captured and necropsied banded mongooses infected with *M. mungi* (2009-2011) was used in an effort to assign sick individuals to their troop of origin. The methods for infection status determination are described in a previous study (13).

*Genotyping*

Using polymorphic microsatellite markers previously described (Chapter 2), 15 loci were amplified for each individual. Using Polymerase chain reactions (PCR), analyses were conducting using 3 separately optimized multiplexes and fluorescently labeled forward primers for each locus. Reactions were set up using 5µl of Qiagen Multiplex PCR Kit Buffer, 1µl of bovine serum albumin, 2µl of water, 1µl of a 10x forward and reverse primer mix, and 1µl of DNA template, for a total volume of 10µl. The amplification protocol included: 1 cycle of activation for 15 minutes at 95°C; 34-cycles of denaturation for 30 seconds at 95°C, annealing for 45 seconds at 57°C, and extension for 30 seconds at 72°C; and a final extension for 10 minutes at 72°C. PCR products were sent to the Cornell University Biotechnology Resource Center (Ithaca, NY) for amplification fragment-size analysis using an ABI 3730 Genetic Analyzer.

DNA samples extracted from feces were subjected to a multiple-amplification process (16) to ensure that the most probable genotype was reliably scored. Individuals that were heterozygous at a locus were recorded as heterozygotes for those alleles. For homozygous genotypes, an additional PCR was run to confirm homozygosity. If the second run also revealed a homozygous genotype, then it was considered homozygous. However, if the genotype was scored as heterozygous after the second PCR, then the locus was recorded as heterozygous for that

individual. Instances where more than two alleles were amplified were regarded as having contamination among multiple individuals, resulting in removal of the sample from the study.

*Statistical Analyses*

Raw data produced from fragment-size profiles were analyzed using GeneMarker ver 2.6.2 (17). Fluorescence peaks representing the fragment lengths were manually scored using the software's default settings against a GS-500 molecular-weight size standard (Applied Biosystems). Individuals were manually genotyped 3 times on separate occasions to ensure precise scoring. The datasets were checked for redundant sampling of particular individuals; multilocus genotypes were considered redundant if fewer than 4 alleles distinguished individuals; duplicated entries were removed from the dataset. After the datasets were finalized, MICROCHECKER (18) was used to check for the presence of null alleles, allelic dropouts, and the possible scoring of stutter peaks within each troop.

Arlequin (19) was used to estimate observed and expected heterozygosities across all the troops and test for deviations from Hardy-Weinberg equilibrium (HWE). The program was also used to test all loci for significance in linkage disequilibrium and to estimate the M-Ratio for each troop. Certain fixation indices ($F_{ST}$ and $F_{IS}$) were also estimated using Arlequin, and an analysis of molecular variance (AMOVA) was also conducted to estimate proportions of genetic variation.

STRUCTURE was used to assign individuals to a given number of genetic clusters ($K$) and determine the appropriate number of clusters in the population supported by the multilocus data. Using Bayesian clustering algorithms, the program was used to infer the best fit of genetic structuring for both timeframes of interest (2008 and 2017). Separate runs of Structure were conducted for the 2008 and 2017 time periods initially using the admixture model at 50,000 burn-ins, 500,000 repetitions, and 10 iterations for each value of $K$, the set number of clusters, from 1 to 10. Additional STRUCTURE analyses were run using the LOCIPRIOR model incorporated within the admixture model, allowing sampling location to inform the assignment algorithm.

GENECLASS2 (22), which utilizes Bayesian and genetic distance-based criteria to assess demographic structure of populations and Monte-Carlo resampling for probability computations, was used to assign individual multilocus genotypes from subsequent years to candidate troops of origin in 2008 and 2017. This program was also used to assign individuals known to be infected with TB to their troop of origin to test whether sick individuals stay philopatric.

To assess the rate of change and genetic variability between the 5 focal troops in 2008 and 2017, effective population sizes ($N_e$) were estimated using $N_E$ESTIMATOR v2 (23). This software program utilizes various methods to estimated effective population sizes, however, we focused on using the random linkage disequilibrium method of Waples and Do (24) for this study. This approach has been demonstrated to perform at a high precision when using microsatellite markers.

*Study Permissions*

This study was conducted following the guidelines set out by the Virginia Tech Institutional Care and Use Committee (IACUC 16-217-FIW) and a permit from the Botswana Ministry of Environment, Natural Resources Conservation, and Tourism (EWT 8/36/4 XXXVIII (23)).

## Results

Fifty-one individual mongooses from the 2008 field season and 86 from the 2017 field season were successfully genotyped at a minimum of 13 microsatellite loci. MICROCHECKER showed no evidence of null alleles, which supported the use of all loci for further analyses. Deviations of genotype frequencies from Hardy-Weinberg expectations were seen at some loci, but none had departure across a majority of troops, and data from these loci were thus retained. Considering the small scale of the study site and the social structure of the species (overlapping generations, non-random mating), we would expect to see deviations from HWE at some loci in some troops. There were few departures from linkage disequilibrium in either sampling year, especially after applying a Bonferroni correction to the tests.

*Genetic Variability*

Mean observed and expected heterozygosities declined somewhat from 2008 to 2017 (Table 1). The average allelic size range decreased from 18.21 to 17.51, while the average number of alleles rose from 3.97 to 4.83. The mean $F_{IS}$ values increased with time.

*Genetic Structure*

Results from program STRUCTURE and the Evanno et al. (25) method suggested that the best-supported number of clusters was $K = 3$ for 2008 and $K = 4$ for 2017(Fig. 2). At $K = 3$ (Fig. 2a), troop CGL has a unique and uniform genetic signature in 2008 and 2017. In both time periods, individuals in troop CCH show signs of admixture from troops CGL and CSL. A noticeable difference can be seen in the genetic composition of troop CSL among the two years (Fig. 2a); in 2008, CSL was almost exclusively blue and uniform, while in 2017, there was an apparent contribution of genetic migrants from KUBU-KWA. Troop MOW show signs of shared ancestry from CSL and KUBU in 2008, while in 2017, MOW appears almost solid blue like CSL. Troop KUBU was rather distinct in 2008, but signs of direct mixture from CSL and MOW could be seen in 2017.

At $K = 4$ (Figure 2b), CGL still exhibits a unique and solid genetic signal for both sampling periods. CCH and CSL share a similar signature in 2008. However, in 2017, CCH appears to have its own, more distinctive genetic cluster separating it from all the other troops. CSL and KUBU maintain their own unique genetic signatures in both periods, but they also show signs of

exchanging genetic migrants. With 4 inferred clusters MOW exhibits a unique blue genetic signature in 2008, separating it from the other troops. However, MOW is less distinct and appears very similar to CSL in 2017.

*Effective Population Sizes*

Effective population sizes and their respective confidence intervals (Table 2) decreased for all 5 troops from 2008 to 2017(Table 2), although it is important to note that CCH and MOW had undefined values in 2008. Sample sizes in 2008 were smaller than sample sizes from 2017, with the exception of CGL ($n$ = 16 and $n$ = 11 respectively; Table 2). Also worth noting; the number of samples was more representative of the actual troop size in 2017 compared to 2008 (expect for CSL in 2008).

*Troop Differentiation*

Pairwise $F_{ST}$ values (Table 3) show indications of moderate genetic structuring between troops in both 2008 and 2017. However, the values estimated for 2008 (mean = 0.05) were considerably lower than those almost a decade later (average = 0.096). Higher mean $F_{ST}$ values were seen for troop CGL, with them located at the extreme western end of the study site (2008 mean = 0.050, 2017 mean = 0.127). Similar patterns can be seen for troop MOW to the east (2008 average = 0.055, 2017-18 average = 0.101) and KUBU at the very eastern edge of the study area (2008 mean = 0.074, 2017-18 mean = 0.095). $F_{ST}$ values for troop CCH were extremely low in 2008 (mean = 0.015), but increased considerably in 2017(mean = 0.093).

*Assignment Tests of Sick Individuals*

An attempt to use GeneClass2 to assign individuals from troops in 2017 using the dataset from 2008 proved largely unsuccessful. Exact assignment probabilities were generally very low, resulting in most individuals being assigned to multiple troops. This is an indication of the algorithm's inability to make clear assignments. However, the algorithm was able to assign 11 TB-positive individuals (sampled from 2009 to2011) to their most likely troop of origin using allele frequencies from the 2008 dataset (Fig. 1). Troop CCH had the largest proportion of sick individuals assigned, with 4 that were located in the troop and 3 found to have dispersed out to another troop. The only detectable immigrants ($n$ = 3) were in CSL, which also had 2 TB-infected native individuals. The 2 individuals sampled from CGL were confidently assigned back to CGL.

## Discussion

The spatial structure and distribution for individuals of a social species like the banded mongoose fluctuates with progression in time, especially when faced with landscape

complexities and a pathogen. Molecular insight such assessing genetic structuring and gene flow from different sampling periods can help uncover some of these changing patterns. Our results suggest that the overall genetic structuring remained similar from 2008 to 2017 in spite of there being some differences in gene flow and genetic variability in the population. Genetic differentiation appears to be lower between troops residing across environments with heavy anthropogenic influences. There was also evidence indicating the dispersal of 3 individuals confirmed to be infected with *M. mungi*.

There was an apparent change in allele frequency over time as we observe a decrease in the average allelic size range and an increase in the average number of alleles from 2008 to 2017. After 9 years and multiple generations of younger mongooses replacing older generations, this result is not unexpected. The changes in allelic size range and number of alleles were such that *M*-ratios also increased since 2008 (Table 1). M ratios provide an indication of a reduction the in population size. Since smaller *M*-ratios are observed earlier in 2008, the increase could be interpreted as troops becoming larger and more genetically diverse between the 2 time periods. This finding, however, may have been influenced by factors other than temporal variation, notably dissimilar sample sizes. For 4 of the 5 troops, sample sizes were larger in 2017 than in 2008 (Table 2), which could have significantly contributed to the change in allele frequency and the differences in *M*-ratio values.

Overall, the clustering of multilocus genotypes produced by STRUCTURE were reasonably consistent from 2008 to 2017 (Fig. 1). At $K = 3$, STRUCTURE output clearly distinguished the park troop CGL from the other troops sampled in both time periods (Fig. 2a). As we assess the clustering patterns of troops in town, we see more signs of admixture between troops. This is a possible indication that gene flow between troops is more prevalent in troops living in urban environments compared to naturally living troops, perhaps as a result of increased intertroop movement.

Although the overall genetic structure for the population appears to be similar across time, there are some differences in gene flow and potentially genetic migration to mention. The other troop located in Chobe National Park, CCH, appears to have some CGL ancestry, but results showed heavy genetic influence from a town troop, CSL, in the graph for both time periods (Fig. 2a). At K=4, the distinction of CCH becomes less apparent in 2008, but greatly intensified in 2017 to the point where CCH is clearly differentiated from other troops (Fig. 2b). This development of genetic uniqueness in 2017 appears to be the result of restricted gene flow and establishment of a distinct genetic signature over a 9-year interval. That is, as CCH matured in its home range, fewer individuals may have dispersed out and immigration may have been suppressed by highly philopatric and territorial group members. Alternatively, the detection of a unique genetic cluster for CCH between the 2 time periods also may also be explained by the relatively small number of samples analyzed from 2008 ($n = 3$) compared to 2017 ($n = 15$). With only 3 members of CCH genotyped, the STRUCTURE algorithm may have had difficulty assigning these individuals to their own unique cluster. Even if CCH is was truly significantly differentiated from all other troops in 2008, this distinction may not have been captured by the insufficient sampling.

Direct mixing of individuals between KUBU-KWA and CSL from the 2017 dataset was inferred at both *K* equals 3 and 4 (Fig. 2). In the 2008 results output, there were indications of admixture suggesting shared ancestry between troops, but no direct mixture comparable to that from 2017. MOW seemed to be considerably less differentiated to CSL in 2017, which would suggest increased rates of gene flow between the troops across the study area. These patterns suggest that movement of individuals became more fluid for certain troops as time elapsed. Demographic changes, landscape changes, the establishment of migration corridors, food availability, or other influencing factors may have helped facilitate the increased dispersal (26,27). However, because sample sizes from 2017 ($n$ = 85) were considerably larger than in 2008 ($n$ = 47), it is less likely that migrant individuals were detected in the earlier dataset, even if they were existent in the study area at the time.

Toward the western and eastern borders of the study area, pairwise $F_{ST}$ appears to be higher compared to troops located in the middle of the study area for both 2008 and 2017 (Table 3). Troops living on the edges have greater spatial separation from the central, town-dwelling troops, which would contribute to the lack of gene flow inferred. For the troops living in urban environments, the bounty of readily available human resources may foster the aggregation of different troops into certain areas. This proximity would allow individuals to interact more than they would in a more resource-poor natural environment (28). Taking into consideration the lower overall $F_{ST}$ values in 2008 (mean = 0.05) than in 2017 (mean = 0.096), the issue of sample-size differences must be recognized. Drastic variation in sample sizes for particular troops may cause allele frequencies to appear more similar than was accurate. Hence, the importance of capturing more genetic variability within groups is stressed for population genetic studies.

Estimated effective population sizes declined from 2008 to 2017 (Table 2). As troops of banded mongooses progress through time, breeding tends to become more exclusive within the troop and individuals are less likely to leave (11). Apparent inbreeding (indicated by the mean $F_{IS}$ value increasing by 0.036 from 2008 to 2017) and generational overlap may have contributed to the reduction of effective population sizes over time. Although differences in sample size may have influenced the variation in results for other temporal analyses in this study, the smaller samples sizes of 2008 yielded larger, though poorly bounded, effective population sizes in contrast to 2017 (Table 2). This supports that notion that genetic variability was higher in the past and is decreasing within troops as time passes.

The GeneClass2 algorithm struggled assigning individuals from 2017 using allele frequencies from 2008 with high probability. If multilocus genotype frequencies changed significantly over multiple generations, this would be sufficient to drastically reduce the accuracy of assignments. To help solve this issue we would need develop a more rigorous and continual sampling scheme for this population of mongooses. I would recommend sampling each troop at least once a generation in order accurately capture to capture gene flow and shifts in genetic structuring. Having multiple datasets would tremendously help the power of future assignment tests.

GeneClass2 was able to help assign TB-positive individuals to a troop of origin, with 8 of 11 being assigned to their natal troop. Identifying infected dispersers was somewhat surprising as

our previous observational studies (6) had suggested that clinically ill individuals were less likely to disperse from their natal troop. It is possible that the 3 individuals immigrated into CSL from CCH before becoming symptomatic from infection by *M. mungi*. Individual mongooses may be able to transfer the disease from troop to troop as "Trojan Horses" (29), only to develop symptoms after assimilating into a new group. Another possibility is that these individuals became infected after dispersing into CSL. To answer this question, future research might be directed at sequencing variable regions of the *M. mungi* strains found in CCH and CSL and conducting an assignment test using the strains from the dispersing CCH members. The results from a pathogen genetic structure analysis could yield insights, identifying sources of sick migrants and routes of TB transmission.

Population structure appears to have remained relatively stable over an interval of almost a decade. Through both years, we can see that most gene flow and movement patterns are within urban areas as opposed to troops living in Chobe National Park. However, smaller sample sizes (especially for troops such as CCH and MOW) may have limited the analysis of the 2008 data.

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**Table 3.1**

| *Year* | *Troop* | $H_O$ | $H_E$ | *A* | *Range* | *M-Ratio* | $F_{IS}$ |
|---|---|---|---|---|---|---|---|
| *2008* | | | | | | | |
| | *CGL* | 0.702 | 0.660 | 4.67 | 19.27 | 0.242 | -0.082 |
| | *CCH* | 0.762 | 0.748 | 3.33 | 16.57 | 0.201 | 0.036 |
| | *CSL* | 0.586 | 0.609 | 4.67 | 19.33 | 0.242 | 0.027 |
| | *MOW* | 0.571 | 0.676 | 2.93 | 14.71 | 0.199 | 0.160 |
| | *KUBU* | 0.612 | 0.638 | 4.23 | 21.14 | 0.200 | -0.048 |
| | **Mean** | 0.647 | 0.667 | 3.97 | 18.21 | 0.217 | 0.019 |
| *2017-18* | | | | | | | |
| | *CGL* | 0.636 | 0.660 | 4.13 | 13.73 | 0.301 | 0.038 |
| | *CCH* | 0.646 | 0.651 | 4.87 | 17.73 | 0.275 | 0.008 |
| | *CSL* | 0.570 | 0.656 | 5.40 | 19.73 | 0.274 | 0.131 |
| | *MOW* | 0.579 | 0.648 | 4.86 | 17.57 | 0.277 | 0.104 |
| | *KUBU-KWA* | 0.586 | 0.584 | 4.93 | 18.80 | 0.262 | -0.004 |
| | **Mean** | 0.603 | 0.640 | 4.83 | 17.51 | 0.278 | 0.055 |

**Table 1.** Key genetic diversity metrics for 5 banded mongoose troops sampled in 2008 and 2017 and characterized for variation at 13 microsatellite loci. Metrics: average observed ($H_O$) and expected ($H_E$) heterozygosities, average number of alleles per locus (*A*), average allelic size range in base-pairs, Garza and Williamson *M* ratio, and $F_{IS}$, the within-troop departure of genotype frequencies from Hardy-Weinberg equilibrium, also known as the inbreeding coefficient.

**Table 3.2**

| | *2008* | | | | *2017* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *$N_e$ values* | | | | *$N_e$ values* | |
| *Troop* | $n_{Troop}$* | $n_{Sample}$ | *0.05 $P_{crit}$* | *95% CI* | $n_{Troop}$ | $n_{Sample}$ | 0.05 $P_{crit}$ | 95% CI |
| *CGL* | 34 | 16 | 23.8 | 13.4-60.8 | 16 | 11 | 3.0 | 2.3-6.1 |
| *CCH* | 23 | 3 | Undefined | 0-Inf | 28 | 15 | 16.3 | 10.7-27.8 |
| *CSL* | 18 | 14 | 16.2 | 9.8-32.6 | 35 | 27 | 13.1 | 10.4-16.8 |
| *MOW* | 8 | 3 | Undefined | 2.0-Inf | 23 | 13 | 7.5 | 4.2-12.5 |
| *KUBU* | 21 | 11 | 18.8 | 8.3-140.4 | 22 | 19 | 8.6 | 6.1-12.2 |

**Table 2.** Troop-level sample sizes ($n$) and estimated effective population sizes ($N_e$) for 2008 and 2017. Allele frequencies as low as 0.05 ($P_{crit}$) were accounted for and 95% confidence intervals (CI) are presented. The columns $n_{Troop}$ indicate the number of individuals in the troop at the time of sampling. Columns $n_{Samples}$ indicate the number of samples collected and successfully genotyped for the analyses.

*Troop sizes were recorded at the end of the dry season.

**Table 3.3**

| | *CGL* | *CCH* | *CSL* | *MOW* | *KUBU* |
|---|---|---|---|---|---|
| *CGL* | - | 0.005 | **0.073** | **0.054** | **0.067** |
| *CCH* | **0.118** | - | -0.013 | -0.003 | **0.070** |
| *CSL* | **0.104** | **0.066** | - | **0.092** | **0.079** |
| *MOW* | **0.167** | **0.097** | **0.032** | - | **0.078** |
| *KUBU* | **0.120** | **0.091** | **0.062** | **0.107** | - |

**Table 3.** Pairwise $F_{ST}$ values between troops in 2008 (above the diagonal and highlighted in blue) and 2017 (below the diagonal). Bold font indicates values that were statistically significant ($\alpha = 0.05$) after a randomization test with 10,100 permutations.

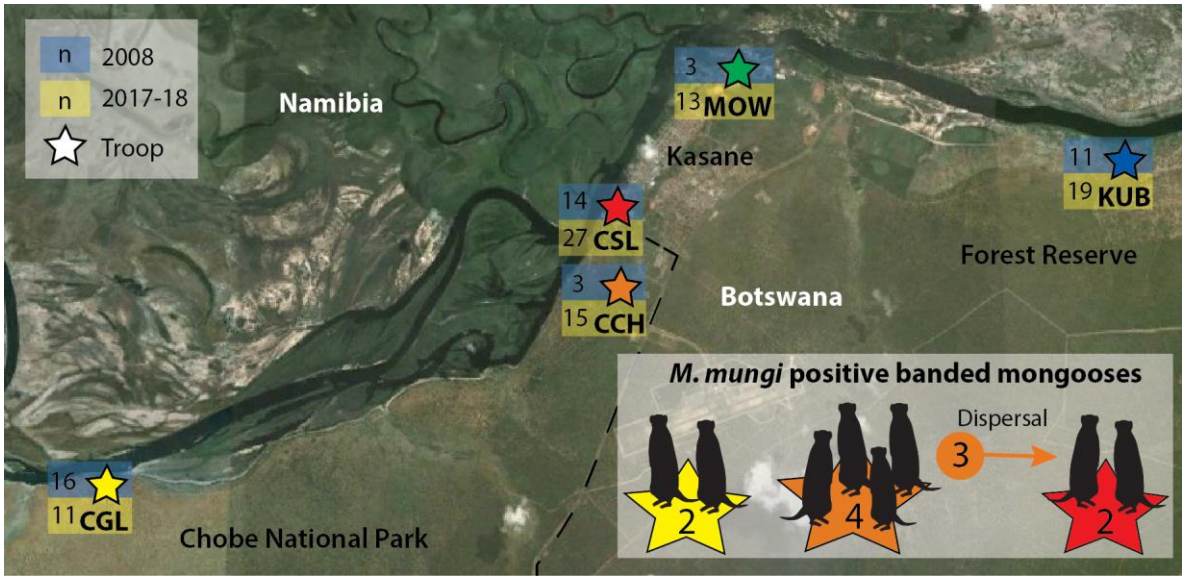


**Figure 3.1.** Five troops were selected for this (geographic positions are represented with colored stars). Sample size is noted for each time step (blue box = 2008, yellow box = 2017). A depiction of the distribution of TB positive individuals is provided in the inset in lower right-hand corner. GeneClass2-based assignments of TB-positive mongooses were performed using the 2008 dataset. The stars in this inset figure are colored to correspond with the stars on the map, and numbers indicate how many sick individuals were sampled from the troops. Circles with numbers represent TB-positive individuals believed to have dispersed from their natal troop (the color of the circle indicates the probable troop of origin). The arrow indicates to what troop dispersers moved and were sampled.

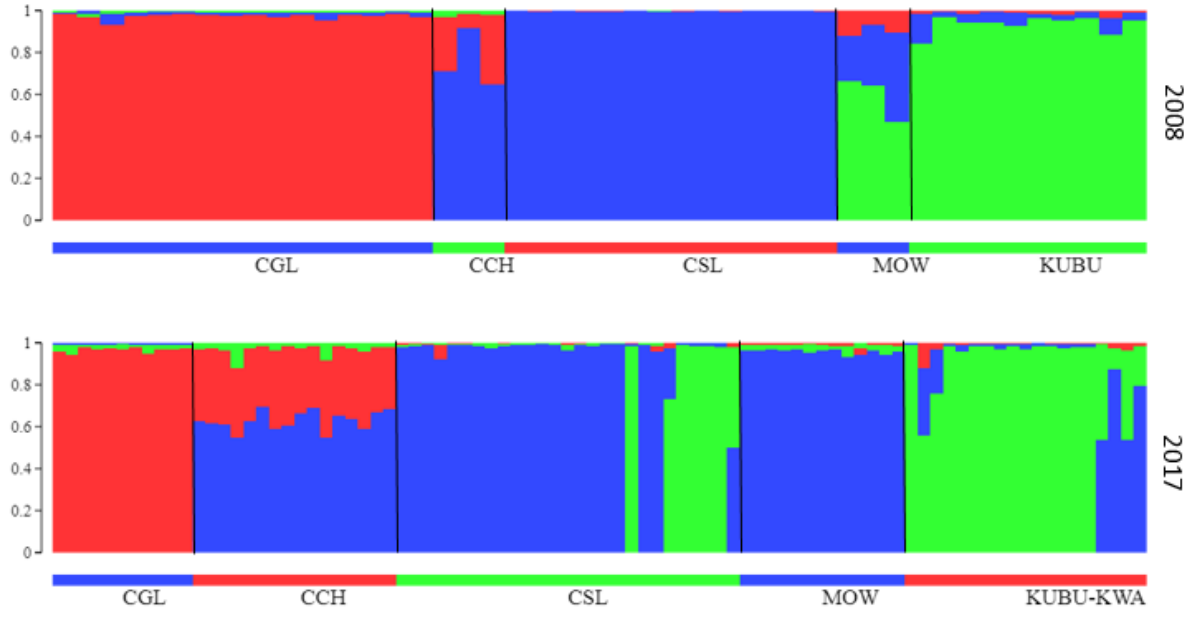


**Figure 2a**

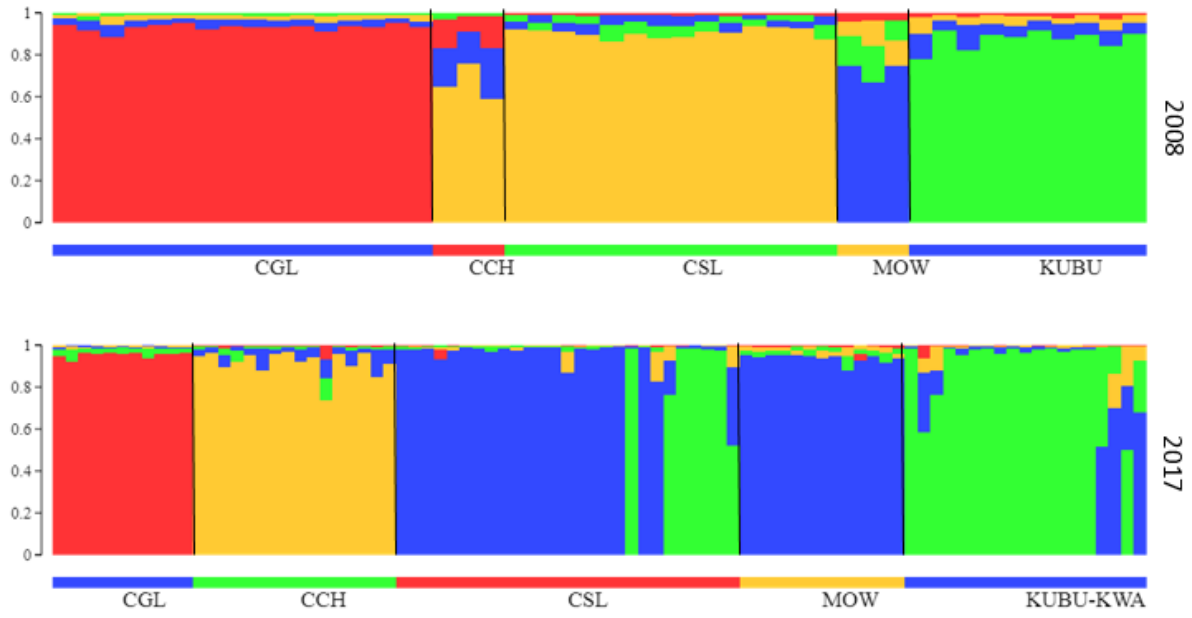


**Figure 2b**

**Figure 3.2.** *Q* plots showing the probability of assignment of individuals to set numbers *K* of multilocus genetic clusters inferred by STRUCTURE. Samples from 2008 and 2017 are presented together for temporal comparisons for $K = 3$ (Fig. 2a) and $K = 4$ (Fig. 2b). Runs were performed using the LOCIPRIOR function under the admixture model.

## Chapter 4

## General discussion

Many factors contribute to the dynamics of disease transmission across a landscape. Among those, host movements can a profound effect on the on the potential for a pathogen to spread and persist in an environment (1–4). Social behaviors of a species influences how individuals disperse through a given area, especially for species that are highly territorial (5,6). That is, territorial behavior may be expected to inhibit pathogen spread if dispersing individuals are met agonistically in enemy home ranges. The complexity of the landscape is a powerful characteristic that can alter behaviors and movement patterns of a host. This is particularly true for wildlife that inhabit and thrive in urbanized environments (7–9). Here human behaviors and practices, such as storage and disposal of waste, can affect how far individuals and groups will travel in search of resources and how they will congregate if the resource is abundant.

Banded mongooses living in northern Botswana provide an excellent opportunity to study host dispersal and the possible spread of a pathogen in the face of infectious disease and environmental complexities. This a cooperatively breeding mammal living in troops from 5 to 75 individuals that guards their territory against opposing troops (10). Previous studies (11) have suggesting that members of a troop tend to stay within their natal troop rather than disperse. However, this research was conducted on a population living in a natural environment (11,12).

In our study area, I examined 12 troops living in environments ranging from natural areas in the Chobe National Park, mixed-use areas like Kazungula, and the urbanized town of Kasane. These mongoose troops are faced with a novel strain of tuberculosis called *Mycobacterium mungi* (13). This particular mycobacterium is unique because it is shed through anal gland secretions, used for scent marking and olfactory communication in mongooses, and invades the host through lesions on the body. Since the discovery of *M. mungi* in 2000 (14), mongoose troops in the study area have been carefully observed and screened for the disease to understand its transmission. Home ranges were estimated using radio telemetry (15,16), scent marking behavior was studied using photos and video from camera traps (Nichols and Alexander), and foraging behavior was observed as troops visited human garbage sties (15,17). Dispersal of healthy and sick individuals was also estimated using observational data. Fairbanks et al. (18) found that individuals with clinical signs of *M. mungi* refrained from dispersing from the troop while healthy mongooses did disperse. Although observational studies can provide insight into host movements, this approach is limited in its scope for detecting rare dispersal events, especially with a species that has no distinguishing markings between individuals. For this particular study, I used genetic markers to determine the structuring and estimate rates of gene flow between the troops in our study system in 2017. Assignment tests also were conducted to detect the number of dispersers of present and immediate past generations. Additionally, comparisons were made to a dataset from 2008 to assess temporal variation in population structuring and dispersal.

## Key Findings

Results from the Bayesian clustering and assignment algorithms of STRUCTURE (19) show that genetic structuring is sufficiently strong to be detectable, with $K = 7$ as the most likely number of

inferred clusters present. Troops residing in more natural environments and on extreme ends of the study area (CGL, CCH, and KUBU-KWA) tend to have a distinct and uniform genetic signature. However, in the central and urbanized areas, mixture and dispersal between troops appears to be sufficiently frequent to preclude genetic differentiation from arising. This interpretation is supported by the pattern of pairwise $F_{ST}$ values among troops, indicating lower differentiation between town troops than between troops living in natural habitat. For the most part, a persistent pattern of genetic structuring was seen for 5 troops of focal interest (CGL, CCH, CSL, MOW, and KUBU) when analyzing data for temporal structural consistencies among sampling periods in 2008 and 2017. These findings suggest that urbanization may positively influence movement of individuals between troops and across the landscape. Previous studies (15,16) showed that the core home range and denning sites of banded mongooses inhabiting the town of Kasane are close to from human refuse disposal sites. Mongooses congregating at sites with human waste and may be less hostile and more accepting of immigrants because food resources are not a constraining factor.

Results from GENECLASS2 (20) suggest that individuals confirmed positive for being infected with *M. mungi* typically assign back to the troop from which they were sampled. This finding supports those from previous work (18) that clinically infected banded mongooses tend not to disperse as frequently as healthy individuals. However, there were 3 individuals (originating from CCH) that had a higher assignment probability to a neighboring troop (CSL). Plausible explanations for this observation are that (1) these mongooses became infected after immigrating into CSL, or (2) they could have traveled while the disease was latent and later displayed clinical signs of infection.

**Future Directions**

Future research should be aimed at carefully monitoring the genetic structure and dispersal patterns of the host. This is especially true for urban areas where I inferred the highest levels of gene flow, which could correspond with disease transmission potential. Nichols et al. (11) conducted a similar study in which they assessed the population genetics of banded mongooses in Queen Elizabeth National Park, Uganda over a long temporal scale (from 2000 to 2009) with intensive sampling. They were able to gain deep insight into the population structuring and relatedness between and within troops to better understand troop dynamics over almost a decade.

Although the comparison between 2008 and 2017 datasets provided an interesting contrast for my purpose of assessing temporal variation of genetic structure among troops, more intensive sampling in 2008 would have strengthened these comparisons. That is, a more complete set of samples for 2008 would have strengthened comparisons of genetic structuring, levels of differentiation, and higher detection probability for possible dispersers. Going forward, I propose an intensive sampling scheme initially over a 4-year period to accurately capture shifts in genetic structure and gene flow across mongoose troops in the Chobe district. Part of the sampling strategy would include collecting DNA in both the wet and dry season to help determine if seasonality has an effect on detectable dispersal. Characterizing these seasonal dispersal patterns can also help us understand if these movements primarily occur just before (late dry season) or after (wet season) breeding has commenced. I would predict dispersal happens right before the

ovulation of females in a group since females may be more receptive to the mating attempts of less related male. This idea is supported by a previous study (21) which suggested that female banded mongooses tend to select against mating with closely related males as a strategy for avoiding inbreeding.

Another recommendation would be to incorporate additional microsatellite loci. The more polymorphic genetic markers used, the more power and potentially more insight it would provide for future analyses. This is particularly true for a study area of such small scale that we have in northern Botswana. Using more loci would likely strengthen the signal of genetic structuring and further distinguish troops with some shared ancestry. Previous research from Sanderson et al. (21) provide additional microsatellite markers derived from banded mongooses and other wildlife proven to work for the species. It would be appropriate to test each newly incorporated marker on a subset of samples to assess the presence of null alleles, deviations from Hardy-Weinberg equilibrium, linkage disequilibrium, and the level of polymorphism across the population.

Efforts also should be aimed at sequencing the genomes of *M. mungi* isolates from individuals infected in each troop. This would be useful in determining whether the pathogen is evolving within particular troops and assessing whether sequences from the respective isolates correspond with mongooses within or dispersing from certain areas. If the genetic variability of the pathogen is sufficiently high among troops for precise assignment tests to be conducted, this also could strengthen inference of host and pathogen movement patterns. Such methods are being utilized to better understand epidemiology in the context of population genetics. For example, in a study of lions and the feline immunodeficiency virus, Kerr et al. (22) tracked host movements by analyzing the molecular signatures, differentiation, and structuring of the pathogen in Kruger National Park, South Africa. In our particular context, such information would be useful in determining whether the infected individuals from CCH that dispersed into CSL acquired *M. mungi* after joining the troop, or whether they had emigrated carrying a different strain from their natal troop. The genotypes from the host and the sequences from the pathogen also could be used simultaneously to better understand pathogen transmission, especially across landscapes larger than our study area in northern Botswana. On a phylogeographic scale, haplotypes from mongooses across the entire species range and their respective TB strains (where existent) could also be gathered to determine evolutionary overlap, and possibly coevolution of the host and pathogen in this system.

Additionally, it would be beneficial to track genetic variation of individual immunology as it relates to presence and spread of *M. mungi* across the landscape. Capturing the characteristics and variation of the major histocompatibility complex (MHC) would be vital to understanding the potential presence of disease susceptibility in certain mongooses. Ideally, we would have MHC representation from both infected and uninfected animals to help determine allelic diversity across the study site and begin to understand whether some closely related troops harbor innate immunity or resistance to the disease. Examining the adaptive variation of the transcriptome also may yield information as to how *M. mungi* is potentially altering the gene expression of mongooses infected. For tissues where we tend to see high concentrations of the

pathogen within the host (nose, spleen, liver), there may be up- or down-regulation of expressed genes occurring in cells in response to presence of *M. mungi*. It may also be the case that environmental factors in natural versus urban landscapes contribute to differences in transcriptomes which could affect susceptibility to illness.

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