Using Fecal DNA and Spatial Capture-Recapture to Characterize a Recent Coyote Colonization

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Abstract - The arrival of a novel predator in an ecosystem necessitates many wildlife-management decisions that should be based on sound demographic data. *Canis latrans* (Coyote) has experienced a dramatic range expansion across North America since the early 19th century, completing its colonization of the continental US in the mid-Atlantic region over the past 20 years. Their arrival in the suburbs of Washington, DC, has generated much public attention, and demonstrated a need for demographic information about this species. To address the challenges of surveying an elusive animal, we used fecal DNA to describe the population genetics and demographics of a newly colonized Coyote population at Marine Corps Base Quantico (MCBQ) in northern Virginia. We collected 331 scats over a period of 2 years at MCBQ, resulting in identification of 23 unique individual Coyotes and 41 total Coyote captures that were analyzed using spatial capture–recapture models. We found evidence of colonization by multiple genetic lineages and a low population density of 0.047 individuals/km^2. Importantly, this study incorporates a new class of models on individual animals identified by genotype data derived from fecal DNA and demonstrates the utility of these models in surveying elusive animals.

Introduction

When a new species colonizes an area, estimating demographic parameters is often the first step in designing an effective wildlife-management strategy. *Canis latrans* Say (Coyote) has experienced a dramatic range expansion in eastern North America over the past 100 years (Parker 1995). East of the Mississippi River, they have colonized eastward along 2 general routes: a northern route moving across the Great Lakes region and into the northeastern US and a southern route moving through the southeastern US. Based on reports of Coyote presence from both primary literature and state and federal wildlife agencies (Parker 1995, Mastro 2011), these 2 fronts appear to converge along the Appalachian Mountains and in the mid-Atlantic region (Delaware, the District of Columbia, Maryland, and Virginia). The mid-Atlantic region is the last area to have been colonized by Coyotes in the continental US; the animals were first detected in the area as late as 1993 (Parker

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1995). Northern Virginia is at the heart of this region; it is loosely defined and consists of several counties and independent cities radiating to the south and west of Washington, DC. This area has a dense human population and has experienced massive human development in the past decade (GMUCRA 2010). Public opinion regarding Coyote presence in Northern Virginia has been charged and polarized, with residents noting the need for more information on the new colonists (Battiata 2006). There have been many genetic, ecological, behavioral, and management studies on Coyotes throughout much of North America (e.g., Gehrt and Prange 2007, Hailer and Leonard 2008, Kamler and Gipson 2000, Kays et al. 2008, Prugh et al. 2005, Riley et al. 2006, Sacks et al. 2008, Way et al. 2010, Wheeldon et al. 2010), but very limited research has been conducted in the mid-Atlantic States (but see Bozarth et al. 2011 and Mastro 2011), despite its location as a convergence zone between two waves of colonization.

Non-invasive molecular methods provide a fast and reliable way to characterize a population of an elusive species. Fecal DNA analysis has been used to examine population demographics of several carnivore species, including *Lynx rufus* (Schreber) (Bobcat; Ruell et al. 2009), *Ursus arctos* L. (Brown Bear; Bellemain et al. 2005), Coyote (Kohn et al. 1999, Prugh et al. 2005), *Canis lupus* L. (Gray Wolf; Lucchini et al. 2002, Valière et al. 2003), *Vulpes macrotis* Merriam (Kit Fox; Smith et al. 2006), *Puma concolor* L. (Puma; Miotto et al. 2007), and *Gulo gulo* (Wolverine; Flagstad et al. 2004). Demographic parameters such as relatedness, population size, and genetic structure can be obtained without the need to capture animals, thus eliminating potential stress for the animals and modification of their natural behavior (Archie et al. 2007, 2008; Spiering et al. 2010). Coyotes are ideal candidates for non-invasive study because they can be difficult to trap but tend to defecate on roads and trails which makes their feces (scat) easy to detect (Macdonald 1980).

The detection of large carnivores for estimating population demographic parameters can be hampered by 2 major issues: the violation of assumption of population closure due to their large home ranges, and sparse data sets due to their elusiveness. Estimates of home range for Coyotes with established territories vary from an average of 4.1 km^2 in northeastern Kansas (Kamler and Gipson 2000) to 46.4 km^2 in eastern Maine (Harrison et al. 1989). Home-range sizes for transient Coyotes range from an average of only 12.4 km^2 in southern Texas (Windberg and Knowlton 1988) to 219.7 km^2 in southern British Columbia (Atkinson and Shackleton 1991). In populations with large home-ranges, individual animals may move into and out of the study site, thus influencing estimates of detection probability and abundance (Efford 2004, Gardner et al. 2009, Royle and Young 2008). In addition to potential violations of population-closure assumptions, large carnivores can be difficult to detect, resulting in small and incomplete data sets that may bias demographic estimates (Smallwood and Schonewald 1998). Population models that are not spatially explicit rely on a variety of ad hoc methods to buffer the study area to account for individual movements outside of the area instead of incorporating spatial data in the capture–recapture model (Boulanger and McLellan 2001, Boulanger et al. 2004, Dice 1938, Karanth and Nichols 1998). These models also do not formally account

for the detection of heterogeneity that results from the lower likelihood of detecting animals that live on the edge of the study site.

To address these concerns, we used a spatially explicit capture–recapture model that incorporated detection-location information with detection history to eliminate the need for post hoc buffering, and used a Bayesian framework to effectively deal with a small sample size. This class of models has been successfully applied to genotypes derived from fecal DNA data (e.g., Moore and Vigilant 2014), as well as genotypes derived from hair samples (e.g., Gardner et al. 2010b, Kéry et al. 2011, Russell et al. 2012), data collected from camera traps (e.g., Gardner et al. 2010a, Reppucci et al. 2011, Royle et al. 2011), and a variety of other sources. The objective of this study was to describe demographic parameters for a newly colonized population of Coyotes and to use this new class of models to provide the first estimates of Coyote density in the mid-Atlantic region.

Field-site Description

We conducted this study at Marine Corps Base Quantico (MCBQ), located 35 miles south of Washington, DC, in Virginia, and spanning 243 km^2 over 3 counties (Fauquier, Prince William, and Stafford). The base was established in 1917 and was expanded during World War II to its current size. Though MCBQ was an active military reservation, less than 30% of its holdings were used for strictly military operations. The majority of the land was used for a suite of purposes including forest and wildlife management, potable water production, waste disposal, and outdoor recreation (Marine Corps Base Quantico 1996). In the face of burgeoning suburban development that now completely surrounds the base, MCBQ has become a de facto wildlife preserve, hosting a wide variety of plant and animal species (C.A. Bozarth, pers. observ.). The study site was composed of second-growth deciduous forest, open fields, lakes, streams, and human development. A 4-lane highway (I-95) divides the base into western and eastern sides, with a secondary road connecting the sides and running underneath the highway. Coyotes were first observed on MCQB in 1997 (T. Stamps, MCBQ Natural Resources and Environmental Affairs, pers. comm.). Coyotes are known to adjust their behavior and habitat use to avoid human activity (Gehrt et al. 2009, Riley et al. 2003, Tigas et al. 2002), and thus, the Coyote population at MCBQ should be more insular than in a landscape with a patchy matrix of developed and non-developed areas.

Methods

Sample collection

We collected 331 carnivore scats at MCBQ during November 2006–October 2008. We systematically removed scat each month along the same eight 500-m transects on roads dispersed throughout the base and then collected scat 6–10 days later to ensure the freshness of the scat samples for molecular analyses. We marked the location of each scat collection on a GIS map using Google Earth v5.0 (Keyhole, Inc. 2009). Although transects were non-random due to the restrictions of

ongoing military training at the site, randomly generated locations would have been ineffective because carnivores often deposit scats on roadways (Macdonald 1980), and humans are inefficient at locating scat in vegetation (Smith et al. 2001, 2003). We also obtained tissue samples from 7 Coyotes killed by hunters on the base. We included these samples as amplification controls and to measure the power of our methods to distinguish individual animals when screening microsatellite loci (see below), but did not use them in the spatially explicit population-abundance model because we did not have precise locality data for them. We stored collected scats and tissues in zip-locked plastic bags in the field and within hours of collection moved them to freezers set at -4 °C.

Genetic data collection

We extracted DNA from scats using QIAamp DNA Stool Mini Kits and from tissues using DNeasy DNA Extraction Kits (Qiagen Inc., Valencia, CA). To mini-mize the risk of contaminating stock DNA with post-PCR products, we conducted DNA isolation in a dedicated lab with a separate air-handling system from the area where PCR amplifications were conducted. We also used aerosol-barrier tips for all pre-PCR procedures to eliminate sample-to-sample contamination and used negative controls in the DNA extraction and PCR amplification steps to control for contamination.

Scat of sympatric carnivore species may not be readily identifiable by visual examination (Davison et al. 2002); thus,we identified species by amplifying a short section of the control region that is variable in length for each of the potential canid species (Bozarth et al. 2010). We reliably differentiated scat deposited by Coyote, *Urocyon cinereoargenteus* (Schreber) (Gray Fox), and *Vulpes vulpes* L. (Red Fox). We removed samples that did not amplify from the data set.

We verified individual identities of animals detected from scat using up to 6 highly variable tetranucleotide microsatellite-loci (not all 6 loci amplified for all samples) and used tissue samples as "known individual" controls. We used prim-ers FH2001, FH2096, FH2137, FH2140, FH2159, and FH2235 obtained from the canine genome map (Francisco et al. 1996) and previously adapted for use in population genetic studies of Coyotes by Prugh et al. (2005). We performed PCR amplification in a 25-µL-reaction volume containing: 5 µL of template DNA (di-rectly from kit extraction); 0.5 µM of each FAM, HEX, or TET-labeled forward and unlabeled reverse primer; 1x PCR Buffer II (Applied Biosystems, Foster City, CA); 2 mM $MgCl_2$; 1 unit of Amplitaq Gold™ polymerase (Applied Biosystems); 0.008 mg/mL bovine serum albumin (BSA, New England Biolabs, Ipswich, MA); and 0.2 mM deoxynucleoside triphosphate. We employed negative and positive controls for each PCR reaction. We amplified DNA in PTC-100 and PTC-200 thermocyclers (MJ Research, Waltham, MA) using the following program: initial denaturation at 95 °C for 10 min; 45 cycles of 1 min at 95 °C, 1 min at 58 °C, and 1 min at 72 °C; and final extension at 72 °C for 10 min. We separated and detected PCR products by capillary electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems) and an ABI 3100 Genetic Analyzer (Applied Biosystems). We analyzed gels with

GeneMapper Analysis Software version 4.0 (Applied Biosystems) and typed all 7 Coyote tissue samples at these same loci.

We assigned sex to individuals using amplification and restriction-enzyme digestion of sex-chromosomal zinc-finger genes, following protocols designed for Kit Foxes and other canids in Ortega et al. (2004). Using this method, we amplified a short (195-bp) fragment of the zinc-finger protein genes (*Zfx* and *Zfy*) that contains a *Taq*I digestion site unique to the *Zfy* gene. When the *Taq*I enzyme digests these fragments, products show a single band for females and a double band for males. We separated and detected PCR products by capillary electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems). We analyzed gels with GeneMapper Analysis Software version 4.0 (Applied Biosystems).

Analysis of microsatellite DNA

We subjected each DNA extract to independent PCR amplifications for each locus at least 3 times for heterozygotes and 5 times for homozygotes as recommended by Taberlet et al. (1996) for non-invasive samples. After each sample was successfully typed at all 6 loci, we determined the reliability of each observed multilocus score using the program Reliotype, which assesses the reliability of an observed multilocus genotype using a maximum-likelihood approach for minimizing genotyping errors (Miller et al. 2002). Due to the low-quality DNA often present in scat samples (Taberlet et al. 1996), not all sample extracts amplified at all 6 loci. For each of the samples with incomplete data, we compared known loci (verified by at least 3 independent amplifications) to complete genotypes by eye to conservatively assign new genotypes to these samples when possible. To estimate the probability that individuals can be differentiated by these 6 loci, we calculated the unbiased probability of observing identical multilocus genotyopes between 2 individuals sampled from the population for unrelated individuals ($P_{ID-UNBIAS}$) and for siblings ($P_{ID-SIBS}$) using the program GIMLET (Valière 2002). We also confirmed that the genotypes were unique at all 6 loci for our 7 Coyote tissue samples.

We used GENEPOP version 4.0 (Raymond and Rousset 1995) to calculate allelic diversity and to test for departure from Hardy-Weinberg and genotypic linkage equilibrium. To control for false positives that may arise in multiple comparisons, we employed a sequential Bonferroni correction (Holm 1979). We calculated observed heterozygosity (H_O) and expected heterozygosity (H_E) for each locus and for the population using CERVUS version 3.0 (Marshall et al. 1998).

To investigate the colonization history of the Coyote population, we evaluated the relatedness of sampled individuals. A high level of relatedness between individuals may indicate colonization by a single group of related individuals, as opposed to colonization by multiple unrelated individuals. To estimate pairwise relatedness (R), we used KINSHIP version 1.3.1 (Goodnight and Queller 1999) and the method described by Queller and Goodnight (1989). We also used KINSHIP to test the likelihood that individuals were related at the level of full-siblings. The program calculates the likelihood of the primary hypothesis ($R = 0.5$) and the null hypothesis ($R = 0$) for each pair of individuals. To calculate significance, KINSHIP

runs a simulation that generates pairs of individuals using the primary hypothesis and allele frequencies of the data set to determine the ratio needed to reject the null hypothesis at various significance levels.

Due to their great dispersal ability, Coyote populations have been shown to exhibit low levels of genetic structure (Lehman and Wayne 1991, Roy et al. 1994). However, because this population was recently colonized and the habitat in the area is heavily fragmented and separated by roads and a 6-lane highway with heavy traffic 24 hrs/day, there was a potential for restricted gene flow within the study area. To test for signatures of population genetic structure, we used the Bayesian model-based approach implemented in STRUCTURE v2.3.2 (Pritchard et al. 2000). STRUCTURE uses discontinuities in multilocus allele profiles detected in terms of Hardy-Weinberg equilibrium and linkage disequilibrium to assign each genotype to a genetic cluster. Because there are no a priori assumptions about population assignments, STRUCTURE can provide an unbiased indicator of geographical patterns of population structure. Because we expected high levels of gene flow within the study site, we used the population admixture model, which assigns genotypes probabilistically to clusters and thereby allows individuals to have mixed ancestry from different clusters. We ran 2 sets of simulations: 1 with alleles independent and 1 with alleles correlated as recommended by Pritchard et al. (2010). For each of these 2 trials, the user specifies the number of clusters (K). Because we assumed high levels of gene flow, we expected to find the best support for $K = 1$ (panmixia). We ran each simulation (for $K = 1 … 6$) 5 times (100,000 iterations burn-in and then 100,000 iterations MCMC). We used the program Structure Harvester v0.56.4 (Earl 2009) to identify the most likely K. We used the program CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007) to visualize the mean of multiple STRUCTURE runs for the optimal K.

Spatially explicit model

To estimate the density of Coyotes and the population size at the study site using fecal DNA, we applied a spatial capture–recapture model (Borchers and Efford 2008, Gardner et al. 2009, Royle et al. 2009). The model addresses the movement of individuals by assuming that each individual has an activity center and that the probability of capture is a monotonic function of the distance from the activity center to a capture location (see Royle et al. 2014 for more details). We recorded the latitude and longitude of the centroid of each transect and used this as the "trap" location. For this model, we then summed the encounters across the repeat detections for each of the 2 years of study. We combined the data for both years to estimate the baseline encounter rate and σ (the scaling parameter for the distance function), but we estimated population size for each sampling period separately (November 2006–October 2007 and November 2007–October 2008) to account for possible population-size fluctuations.

The model can be formulated as a generalized linear mixed model. To begin, we assumed that each individual i in the population had an activity center at a fixed point, $\mathbf{s}_i = (s_{1i}, s_{2i})$ for $i = 1, 2, … n$ individuals and these activity centers were uniformly distributed across some region S. We denoted the transect locations as

$x_j = (x_{1j}, x_{2j})$ for $j = 1, 2, \ldots$ J transects. Next, we defined the encounter history y_{ijkt}, i.e., the number of times an individual i was captured in transect j during a sampling occasion k in year t, as a Poisson random variable with mean λ_{ij} such that:

$$y_{ijkt} \sim \text{Poisson}(\lambda_{ij}).$$

We used a Poisson distribution to allow for the potential of multiple recaptures of an individual at a sampling location during any sampling occasion. To model the relationship between an individual i's activity center and the transect j, we used the half-normal detection function. This yields a log-linear model of the form used by Royle et al. (2009) and results in the following log-link formulation:

$$\log(\lambda_{ij}) = \log(\lambda_0) + (1/2\sigma^2)d_{ij}^2,$$

where $d_{ij}^2 = \|\mathbf{s}_i \ x_j\|^2$ is the squared distance from individual i's activity center to transect j, s is the scaling parameter for the distance function, and λ_0 is the baseline encounter rate, defined as the expected number of captures at a sampling location during a sampling occasion given that an individual's activity center is located precisely at that sampling location.

The activity centers, $\mathbf{s}_i$, are not observed in this formulation of the model, and thus, d_{ij}^2 is a latent random effect. To carry out inference on N, the number of activity centers ($\mathbf{s}_i$) in the state-space S, we assumed that the $\mathbf{s}_i$'s are distributed uniformly over some prescribed region S, an arbitrarily large polygon containing the sampling locations. Because N is unknown, we used a technique called data augmentation (Royle et al. 2007), which effectively creates a zero-inflated version of the data set. Based on this formulation of the model, density is a derived parameter estimated as $N/|S|$, where N is the estimated number of activity centers ($\mathbf{s}_i$) in the state-space S $|S|$ is the area of S. The state-space and data augmentation are statistical tools to estimate abundance; however, caution should be taken in making inferences outside the surveyed area if conditions (e.g., habitat) in the state-space are not consistent with those observed in the surveyed area. For this analysis, we specified S as a rectangle centered on the study site with an area of 1452 km². We augmented the data by adding 97 more potential individuals with all zero encounter histories. Ro more information on choice of state-space and data augmentation, see Chapter 5 of Royle et al. (2014). We used a Bayesian framework for analysis of the model. We checked the output to ensure the chains converged by visual inspection and by checking that all R̂ values were less than 1.1 (Gelman and Hill 2007). We implemented the model in the freely available software package WinBUGS (Gilks et al. 1994). Posterior parameter estimates are based on 3 chains run for 15,000 iterations after a burn-in period of 5000 and iterations thinned by 3. In this model, transient individuals are assumed to occur randomly in the study.

We also ran program GIMLET (Valière 2002) with both 1000 and 10,000 iterations using the Kohn et al. (1999), Chessel (Valière 2002), and Eggert et al. (2003) methods to verify that there were not enough recaptures to estimate population size using rarefaction.

Results

We successfully identified 77 Coyotes, 62 Gray Fox, and 59 Red Fox out of the 331 scats collected. We did not identify scat deposited by either *Canus lupus familiaris* L. (Domestic Dog) or Gray Wolf. Coyotes in Northern Virginia are known to have hybridized with *Canis lycaon* Say (Eastern Wolf; Bozarth et al. 2011); the hybrids are known as Coywolves. Our method of species identification does not differentiate between Coyote and Eastern Wolf, ensuring that all Coywolves are appropriately included in the analysis. Of the Coyote samples, 41 met our criteria for identifying microsatellite alleles and could confidently be assigned a genotype (53% success rate). We found 30 unique genotypes, 23 of which were detected from scat samples and 7 from tissue samples from the individuals killed by hunters. Two genotypes, represented by only 1 scat sample each, were unique based on 4 and 5 loci, respectively, and we confirmed each locus with multiple successful amplifications. All other individual identifications were based on the full 6 loci. All 6 microsatellite loci were polymorphic in this population, with an average of 8 alleles/locus (range = 6–11 alleles/locus). We found locus FH2137 to be significantly out of Hardy-Weinberg equilibrium after sequential Bonferroni correction and locus FH2159 approaching significance ($P = 0.0115$) after Bonferroni. Observed and expected heterozygosity for these 2 loci ranged from 0.533–0.933 and 0.684–0.854, respectively, with locus FH2159, but not locus FH2137, showing significant homozygote excess after Bonferroni correction ($P = 0.003$ and 0.049, respectively). We found departures from linkage equilibrium at 5 of 15 comparisons (4 of which included locus FH2159). Average H_O was 0.756 and H_E was 0.798. We found an estimated overall $P_{ID-UNBIAS}$ of 5.217e-08 and an estimated overall $P_{ID-SIBS}$ of 3.197e-03. None of the genotypes recovered from scat samples matched the genotypes from the 7 tissue samples, and none of the 7 tissues known to come from different animals had identical genotypes, corroborating that we had high power to distinguish individual animals using our loci. We found 18 males, 11 females, and 1 genotype for which sex could not be determined, despite multiple replicates for each PCR.

The estimated relatedness (R) between all pairs of individuals ranged from -0.470 to 0.912, with the average relatedness for the entire population at 0.001. When tested against the primary hypothesis of $R = 0.50$ (relatedness coefficient for full siblings), only 31 out of 435 pairwise comparisons were significant, indicating that most sampled individuals were not closely related.

For the STRUCTURE model with alleles correlated, alpha converged but with high variability, and the plot of delta K did not peak (as expected for resolved data sets). For the model with alleles independent, alpha converged, and the results were more discernable. The plot of mean log likelihood indicated $K = 3$ as the best fit for the data. The plot of delta K produced a clear peak at $K = 3$. In examining the Q-plots for each of the 5 replicate runs of $K = 3$, we found that 4 of the 5 clearly divided individuals into 3 clusters and 1 did not. We used the program CLUMPP to find the mean of the $K = 3$ outputs (including the replicate of $K = 3$ that did not divide individuals as clearly into 3 clusters; excluding it produced

similar results) and could distinguish the 3 clusters (Fig. 1). To visualize popula-
tion structure at the study site, we mapped the proportion of membership in each
of the $K = 3$ clusters for each of the 8 transects and then for the west and east
sections of the base, as divided by I-95. Division by transects did not produce
any discernible pattern, but division by I-95 indicated some structure, though we
collected few samples east of I-95. Two of the genotypes (19 and 17) that were
assigned to cluster 2 (0.827 and 0.845 membership respectively) were only de-
tected east of I-95. A third genotype (30) only detected east of I-95 had 0.433
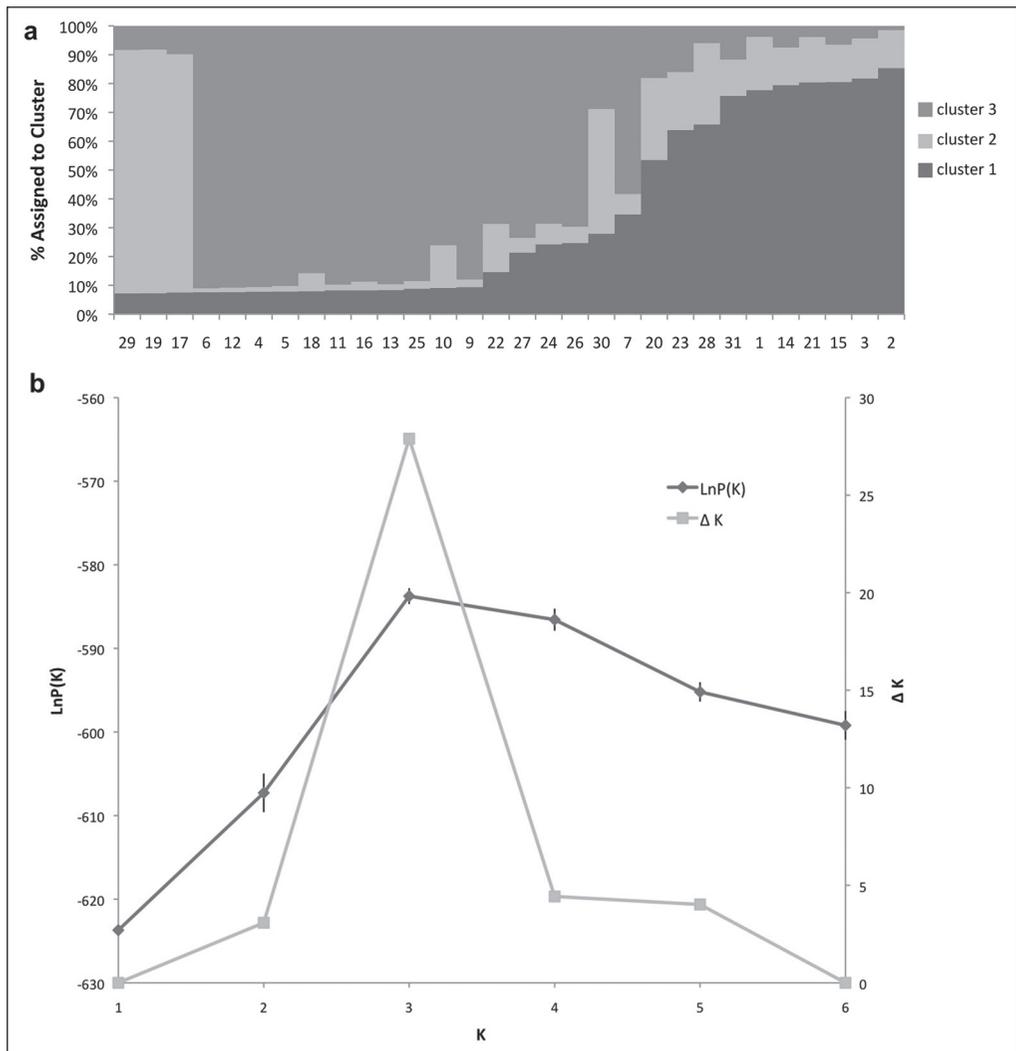

Figure 1. (a) Mean of 5 replicates of genetic assignment results for Coyotes at MCBQ for
$K = 3$ from the program STRUCTURE as averaged by the program CLUMPP. Genetic popu-
lation clusters are coded with different shades of gray. The fraction of each color for each
individual represents the probability of assignment to that cluster. (b) Magnitude of ΔK (rate
of change in the log probability) and $\ln P(K)$ (posterior probability of the data) as a function
of K (populations) detected 3 genetic clusters in the sampled population.

membership in cluster 2. The only other individual that had high assignment to cluster 2 (genotype 29) was found west of I-95. More intensive sampling east of I-95 is needed to strengthen these tentative results.

Genetic results showed that the 23 Coyote individuals made 41 visits to the 8 transects during the 2-year survey. During the survey, encounters at each transect varied from 1 to 12, with 12 individuals detected only once. For the two different study periods, we estimated the posterior mean number of activity centers ($\hat{N}$) as 58.81 and 75.88, respectively, in the state-space ($S = 1452$ km^2) and the density ($\hat{D}$) at 0.04 (95%BCI: 0.02, 0.07) and 0.05 (95%BCI: 0.03, 0.08) individuals/km^2 in the two periods, respectively. We used the area of the base (243 km^2) to estimate the number of individuals with activity centers on the base at 9.96 individuals in the first year and 12.69 individuals the second year (mean = 11.3 individuals). The posterior mean estimate for σ, the scaling parameter for the distance function, was 2.60 with a 95% posterior interval of (1.92, 3.58). Based on the posterior mean estimate of σ, we estimated the mean home-range radius to be 6.33 km by assuming a bivariate normal model for movement. The posterior mean for λ was estimated at 0.11 with a 95% posterior interval of (0.05, 0.21). λ is the baseline encounter rate under the Poisson encounter model used here, which resulted in an estimated detection probability of 0.10. The combination of low detection and larger σ suggests that Coyotes utilizing MCBQ have activity centers located not just within the base but also in the surrounding areas. This pattern is also seen in the posterior densities of activity centers for captured and uncaptured individuals, which indicates spatial heterogeneity in Coyote density (Fig. 2). The posterior summaries of model parameters are summarized in Table 1.

None of the rarefaction methods in program GIMLET produced a curve with an asymptote.

Discussion

We found high levels of polymorphism and heterozygosity in this newly colonized population of Coyotes. Given the precautions we took for multiple replicate PCR reactions, the departure from Hardy-Weinberg equilibrium for locus FH2137 as well as the departure from linkage equilibrium and homozygote excess for locus FH2159 were most likely due to small sample size and not allelic dropout.

Table 1. Posterior summaries of model parameters for this study of Coyotes at Marine Corps Base Quantico, where 23 individual Coyotes were observed during 2 years. We define N_1 and N_2 as the number of estimated activity centers in the state-space S (rectangular area of 1452 km^2 around the study area used for analyses) during years 1 and 2 respectively. The baseline encounter rate under the Poisson encounter model is λ_0 and the scaling parameter for the distance function is σ.

	Mean	SD	2.5%	Median	97.5%
λ_0	0.11	0.04	0.05	0.11	0.21
σ	2.60	0.43	1.92	2.55	3.58
N_1	58.81	17.832	29.00	47.00	99.00
N_2	75.88	19.12	42.00	62.00	114.00

Additionally, all 6 loci have previously been used in a population genetic study on Coyotes and were found to have insignificant error rates ($P < 0.01$), taking into account both allelic dropout and false allele rates (Prugh et al. 2005). The average H_O of 0.756 is similar to values reported for Coyote populations in other regions (0.69–0.74; Prugh et al. 2005, Riley et al. 2006, Sacks et al. 2005). The male to female sex ratio was slightly skewed at 1.6:1. This finding could be the result of the small sample size (30 individuals over a 2-year period) or differences in the detection of the sexes; our skewed sex ratio is probably not due to the detection of transient individuals because males and females are equally common dispersers (Bekoff 1977).

Pairwise relatedness was low between most pairs of individuals ($R = 0.001$). Because the program KINSHIP bases relatedness values on allele frequencies
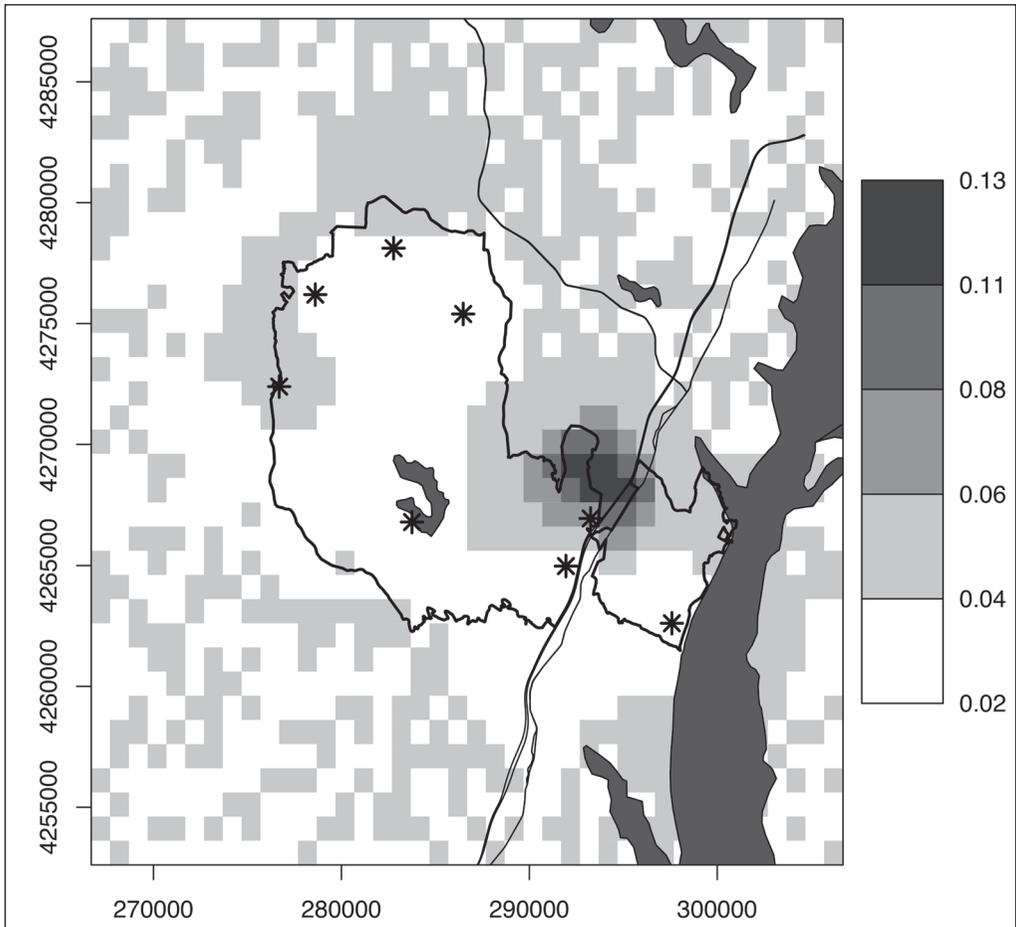

Figure 2. Map of the posterior mean density of activity centers for Coyotes across the state space for year 1 of sampling, specifically, $E[N_1(b)|data]$, where $N_1(b)$ is the estimated number of activity centers located in pixel *b*. MCBQ is outlined in black, black asterisks represent the location of the center of the sampled transects, black lines show Interstate 95 and other highways, and dark gray polygons represent major waterways. The map is displayed in universal transverse mercator (UTM) Zone 18 north coordinates with 1 km x 1 km cells.

given in the data instead of a population of random individuals, relatedness values may be biased low. Thus, we can conservatively state that the sampled population was composed of unrelated individuals, which may indicate multiple colonization events from diverse geographical regions.

The STRUCTURE assignment tests determined that 3 population clusters best fit the data. We expected to find panmixia ($K = 1$) because of the high gene flow and previously reported weak genetic structure in Coyote populations (Lehman and Wayne 1991) and in other canid species (Gray Wolf: Vilà et al. 1999; *Vulpes lagopus* (L.) [Arctic Fox]: Dalen et al. 2005). The detection of 3 distinct population clusters may support the fact that this newly colonized population of Coyotes was the result of multiple sources of colonization from several family groups, as opposed to a single colonization event by members of 1 closely related group of individuals. In addition, a previous genetic study using mitochondrial DNA from the same individuals in this population also detected 5 different matrilines, all of which have been previously reported in diverse surrounding geographic localities, and is further evidence of multiple sources of colonization from unrelated individuals (Bozarth et al. 2011). The apparent genetic structure caused by the division of the base by I-95 could be due to our small sample size east of the highway, although previous studies have found that a highway can be a genetic (but not physical) barrier to Coyote movement in southern California (Riley et al. 2006). In addition, results from the mtDNA analysis in Bozarth et al. (2011) show the presence of 5 haplotypes at MCBQ, with all occurring west of the highway, but 1 haplotype occurring only east of the highway. More extensive sampling on both sides of the highway may strengthen the preliminary evidence of structure found here.

The population-density estimates at MCBQ (mean = $0.047/km^2$) are comparable to estimates on urbanized Cape Cod, MA ($0.06–0.15$ individuals/km^2; Way et al. 2002). Our slightly lower estimate may be due to lower availability of food, the recentness of the colonization, or violation of model assumptions. Coyote density is strongly correlated with the abundance of potential prey (e.g., Clark 1972, Mills and Knowlton 1991, O'Donoghue et al. 1998, Prugh et al. 2005, Rose and Polis 1998). At the study site, however, the density of small mammals, anthropogenic food sources (including road kill), and other prey items appear to be abundant (Robinson 2005). In addition, Coyotes, as generalist predators, make use of a variety of food sources on the base, including *Odocoileus virginianus* Zimmermann (White-Tailed Deer), fruits, insects (including crickets and beetles), and birds (Robinson 2005). Because the density estimate is lower than, but still similar to, estimates on Cape Cod, the effect of food availability may not be strong enough to tease apart the slight differences in density estimates. Other examples of low Coyote density estimates occur during prewhelping, when densities are expected to be biased low because of reduced detectability (0.01 individuals/km^2; Springer and Wenger 1981) and during winter surveys in harsh climates where Coyote densities are expected to be genuinely low due to mortality (0.05 individuals/km^2; Brock 1992). A slightly low population-density estimate may also be due to the recentness of the Coyote colonization (see Parker [1995:81] for a discussion of fluctuating population

densities in newly colonized populations). Though density estimates at our site were consistent over a 2-year period, density was slightly higher the second year. Coyotes have only been recorded at MCBQ since 1997, making this a very recent colonization. The high number of individuals detected by genotyping from scats and the high haplotype diversity (Bozarth et al. 2011) of this population also indicate a population still in flux. Finally, our density estimate may be biased due to the small number of sampling transects and the wide spacing, which resulted in a low number of captures and recaptures. Of all individuals identified, only 3 were detected on more than one transect, none were detected at more than 2 transects, and 13 were detected only once. If transects had been located closer to one another, we would have expected more recaptures, and this is an important study-design issue for future studies to consider when placing transects or trap locations (see Sollmann et al. 2012, Sun et al. 2014). In order to fit the model to this small amount of data, we had to assume a demographically closed population over the duration of each sampling year. This assumption was most likely violated and may also have caused bias in our estimates of density.

The overall picture of Coyote population density in the literature is one of incredible variation, due to a variety of factors. Estimates of density range from 2.0 individuals/km^2 in southern Texas (Windberg and Knowlton 1988) to the smallest density estimates discussed above. Kays et al. (2008) conducted a large-scale scat survey of Coyotes in the Adirondacks region of northern New York and found that the number of scats captured was directly proportional to the number of individuals detected. There is much variation in density estimates, which suggests that scat surveys can be a useful tool for providing unbiased density estimates.

With the half-normal detection function, we can convert the scale parameter (σ) to a home-range radius (6.33 km) of a circle. While it is understood that home ranges are never exactly circular in nature (Samuel and Garton 1985, Smith 1983), this fact does not imply that using a circular detection function is inadequate or unrealistic. This assumption is necessary for the model, and recent studies show that selective space usage, which would violate this assumption of home-range shape, does not bias estimates of population size in SCR models (Efford 2014). Converting the home-range radius (6.33 km) to the area of the circle using the formula area = π (radius)^2 yields a rather large estimate of 125.82 km^2. Home-range sizes for Coyotes are highly variable, ranging from 4.1 km^2 in northeastern Kansas (Kamler and Gipson 2000) to 219.7 km^2 for transient Coyotes in Southern British Columbia (Atkinson and Shackleton 1991), though most reported home-range sizes are between 12 and 50 km^2 (e.g., Gese et al. 1988, Grinder and Krausman 2001, Harrison et al. 1989, Holzman et al. 1992, Major and Sherburne 1987, Way et al. 2002, Windberg et al. 1997). In our study, the paucity of recaptures (41 total captures for 23 individuals), the low number of captures, resultant scarcity of data, and the potential for transients to be included may have inflated this high home-range size estimate (hence the wide confidence bounds on σ; Table 1).

The map of the posterior density of activity centers (Fig. 2) highlights the need for dealing with spatial heterogeneity in a capture-recapture model. Coyote activity

is centered on one area of the study site, which coincides with the presence of an old landfill site. The landfill is covered and now exists as an open area dominated by tall grass, with planted *Pinus* (pine) trees and natural second-growth forest surrounding it, making this area an ideal habitat for Coyotes. The presence of a perennial pond may also attract Coyotes as well as potential prey species. The spatially explicit model we used directly addresses this spatial heterogeneity by using the location of individuals in relation to the captures. These hotspots of activity can be used to better understand Coyote habitat preferences in a heterogeneous landscape and inform population management.

Using noninvasive molecular techniques, we characterized the genetic variability, population relatedness, and population structure of a newly colonized Coyote population. We predict that population density and genetic structuring across the highway will increase in the future as more Coyotes exploit the abundant food sources and establish territories. Additionally, we have used a new class of spatially explicit capture–recapture models that effectively deal with violations of closure and small sample size to estimate the population density, population size, home-range radius, and detection probability of these Coyotes. By sharing some parameters across years, we were able to make inferences despite the small sample sizes in each year. This population genetic study of Coyotes in the mid-Atlantic region focused on combining this new class of spatial capture–recapture models with DNA data collected from scat. Molecular studies using fecal DNA and spatially explicit capture–recapture models could also be used to estimate these key demographic parameters for other elusive animals.

Acknowledgments

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