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GENETIC STRUCTURE AND DEMOGRAPHIC ANALYSIS OF KEY DEER (*ODOCOILEUS VIRGINIANUS CLAVIUM*)

by

VICKI LEE VILLANOVA
B.S. University of Florida, 2012

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Sciences
at the University of Central Florida
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Major Professor: Eric A. Hoffman

ABSTRACT

Recent improvements in genetic analyses have paved the way in using molecular data to answer questions regarding evolutionary history, genetic structure, and demography. Key deer are a federally endangered subspecies assumed to be genetically unique (based on one allozyme study), homogeneous, and have a female-biased population of approximately 900 deer. I used 985bp of the mitochondrial cytochrome *b* gene and 12 microsatellite loci to test two hypotheses: 1) if the Moser Channel is a barrier to gene flow, I should expect that Key deer are differentiated and have reduced diversity compared to mainland deer and (2) if isolation on islands leads to a higher probability of extinction, I should expect that Key deer exhibit a small population size and a high risk of extinction. My results indicate that Key deer are genetically isolated from mainland white-tailed deer and that there is a lack of genetic substructure between islands. While Key deer exhibit reduced levels of genetic diversity compared to their mainland counterparts, they contain enough diversity of which to uniquely identify individual deer. Based on genetic identification, I estimated a census size of around 1,000 individuals with a heavily skewed female-biased adult sex ratio. Furthermore, I combined genetic and contemporary demographic data to generate a species persistence model of the Key deer. Sensitivity tests within the population viability analysis brought to light the importance of fetal sex ratio and female survival as the primary factors at risk of driving the subspecies to extinction.

To my mom and dad for always encouraging me to follow my dreams.

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Thank you to those who have helped get me to where I am today. I grew up down the street from the Homosassa Springs Wildlife State Park and my parents took me there all the time, encouraging me to have a passion for wildlife and the environment. Thank you to my wildlife park "family" who taught me how to drain an otter pond and clean muck out of a waterway. It was here where I first learned what an amazing animal Key deer are and how to find their poop.

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LIST OF ABBREVIATIONS

BPK	Big Pine Key
cyt*b*	cytochrome *b* gene
HWE	Hardy-Weinberg Equilibrium
mtDNA	mitochondrial DNA
NNK	No Name Key
PCR	Polymerase Chain Reaction
P_{ID}	Probability of identity
P_{sib}	Probability of sibship
UAGC	University of Arizona Genetics Core

CHAPTER ONE: INTRODUCTION

In the past, scientists have utilized both demographics and genetics as a means to address the conservation of species of concern (e.g. Petit *et al.* 1998; Degner *et al.* 2007; Bristol *et al.* 2013; Grayson *et al.* 2014; Robert *et al.* 2015). Despite that both demographics and genetics have utility in devising plans for long-term conservation planning, researchers have argued about which data type provides the best evidence for short-term planning (Lande 1988; Caughley 1994; Hedrick *et al.* 1996). Demographically, when there are too few individuals in a population, random factors such as demographic stochasticity, environmental variation, and rare catastrophic events may drive a population to extinction (Gilpin & Soule 1986; van Noordwijk 1994). For example, in 1980 the endangered dusky seaside sparrow experienced a severe bottleneck, due to habitat alteration, in which the population declined to only six individuals. Consequently, those six individuals were all male, condemning the population to eventual extinction (Avise & Nelson 1989). On the other hand, genetic drift can also put populations at risk of extinction by loss of genetic diversity (e.g. Miller & Lambert 2004; Cheng *et al.* 2012) . For small populations genetic drift can increase the probability that deleterious alleles will increase in frequency and that rare alleles will be lost from the population (Soule 1973). In the 1990s, the Florida panther (*Puma concolor coryi*) was exhibiting kinked tails, cowlicks, and sperm and heart defects as a result of inbreeding depression (Roelke *et al.* 1993). To battle the possible extinction of the subspecies, management brought Texas cougars (*P. c. stanleyana*), the closest geographical population, to Florida (Pimm *et al.* 2006). The introduction of genetic diversity reduced the effects of inbreeding depression and encouraged the recovery of the Florida panther (Johnson *et al.* 2010). Collectively, demographic instability and reduced genetic diversity can increase the chance of

populations being caught in an extinction vortex (van Noordwijk 1994; Tanaka 2000), where small populations succumb to inbreeding depression and genetic drift leading to a further reduction in genetic diversity over time, and hence, reduces population size further. Ultimately, the understanding of both demographics and diversity are crucial in small populations.

Historically, dissimilarity in sample collection between genetics and demographics limited researchers to choose which type of data to use for the investigation of how best to conserve a species. Genetic data have proven to be powerful given their ability to assess loss of genetic diversity relative to non-endangered species (e.g. O'Leary *et al.* 2014), amount of gene-flow among populations (e.g. Robinson *et al.* 2012), and species delimitations (e.g. Brown *et al.* 2014). Moreover, genetic techniques have facilitated the evaluation of historical demography though the estimation of population expansion (e.g. Hoffman & Blouin 2004) and admixture (e.g. Zachos *et al.* 2008) via mismatch distribution analysis and historical effective population size via coalescent techniques such as Bayesian skyline plots (e.g. Grazziotin *et al.* 2006). Contemporary demographic techniques have addressed a separate but equally important set of questions with regard to conservation including age structure (e.g. Martins *et al.* 2006), survival (e.g. Pradel *et al.* 1997), and census size (e.g. Rice & Harder 1977; Cantor *et al.* 2012).

Recent advances in analyses have enabled genetic techniques to evaluate questions of contemporary demography, opening the door for studies that combine the investigation of genetic diversity, structure and evolutionary history with estimates of contemporary demographic parameters. In combination, these techniques can provide an improved view of conservation for a particular species. A major step enabling combined genetic/demographic analyses was the acquisition of noninvasive genetic material facilitating researchers to incorporate genetic data as a

means to estimates census size (e.g. Mowat & Paetkau 2002; Boersen *et al.* 2003; Coster *et al.* 2011; Morán-Luis *et al.* 2014) and sex ratio (e.g Lindsay & Belant 2008; Brinkman & Hundertmark 2009; Morán-Luis *et al.* 2014). However, few studies have used genetic data to simultaneously answer questions regarding genetic diversity, structure and evolutionary history as well as contemporary demographic questions (e.g. Sugimoto *et al.* 2014). Moreover, the combination of these genetic and demographic data can be combined in predictive models to evaluate the long-term survivability of the species of interest. These models [i.e. population viability analysis (PVAs)] incorporate genetic information, life history data and estimates of population parameters, alongside probabilistic functions of stochastic events, to determine the probability of persistence of a species.

In this study, I sought to evaluate how putative isolation of a wide-ranging species impacts genetic diversity, structure and evolutionary history as well as contemporary demography of Key deer (*Odocoileus virginianus clavium*), a subspecies of white-tailed deer (*O. virginianus*). Moreover, I used these data to generate a species persistence model (i.e. PVA) to evaluate the extinction probability of this island subspecies. Islands provide a model system in which to study the impact of small populations (Frankham 1998) and reduced genetic diversity (Frankham 1997), as well as the evolutionary history of how natural fragmentation impacts continental species under quasi-isolated conditions. Insular populations are typically founded by only a few individuals (Frankham 1998). Thus, the initial founding population must be large enough to avoid detrimental stochastic events and contain adequate genetic variation to adapt under fragmented conditions; if not, the population will be unable to persist. Furthermore, taxa that reside on islands can be seen as a replicate of mainland populations, demonstrating how different selection

pressures may influence the species. Given their unique evolutionary history, islands provide an ideal setting to apply a combination of phylogeographic and demographic tools in which to elucidate how evolutionary history, genetic diversity, and demography have influenced the contemporary composition of a species.

Florida contains a large density of near-shore islands which facilitate examining how insular systems impact population demography and genetic diversity. During the last glacial maximum, about 18,000 years ago, the landmass of Florida was much greater in area and extended beyond the Dry Tortugas (Lazell Jr. 1989). Eight thousand years later, with rising sea level associated with glacial retreat, the land south of modern day Florida became disjoined, with the intervening ocean establishing a geographical barrier between the mainland and the Florida Keys (Lazell Jr. 1989). The contemporary Florida Keys are categorized by three groups of islands: Upper, Middle, and Lower Keys. The Lower Keys are the farthest group of islands from the mainland and are separated from the Middle Keys by the 11-km wide Moser Channel. The Lower Keys contain numerous subspecies which were historically described based on geographic isolation and morphological distinction of mainland sister taxa. The largest of these taxa, Key deer, have been found to be genetically unique relative to their mainland sister taxa (Ellsworth *et al.* 1994). In addition to their genetic differentiation, Key deer have numerous physical (Hardin *et al.* 1976; Klimstra *et al.* 1991; Klimstra 1992) and behavioral characteristics (Hardin *et al.* 1976) which set them apart from their mainland counterparts. Additionally, anthropogenic influences have further impacted the natural history of Key deer. Most importantly, the subspecies was hunted to near extinction in the early 1950s and has been listed as federally endangered since 1967 (USFWS 1999).

In general, this study sought to evaluate how genetic data can be used to evaluate genetic and contemporary demographic questions regarding the conservation status of Key deer. Specifically, I used the mitochondrial cytochrome *b* gene and variation present in 12 microsatellite loci to assess two hypotheses. First, I hypothesized that if the Moser Channel is a barrier to gene flow, then Key deer should be differentiated and have reduced genetic diversity compared to mainland deer. This hypothesis is based on previous work conducted by Ellsworth *et al.* (1994), who identified a single, unique haplotype found in Key deer using restriction enzymes. This hypothesis is further supported by two sources of evidence: a) the geographic distance between the Keys and mainland Florida, which would suggest that Key deer are unable to disperse from the Keys to the mainland; and b) research of other insular species which has shown that island populations typically contain reduced levels of genetic diversity compared to their mainland counterparts (Frankham 1997). Second, I hypothesized that if isolation on islands leads to a higher probability of extinction, then Key deer should exhibit a small population size, a female-biased sex ratio, and a PVA analysis should indicate the fragility of the current population growth rate. This hypothesis is based on previous estimates of Key deer census size [even a consistent trend of 5% annual increase in Key deer census size (Lopez *et al.* 2004) starting with an estimate of 587 deer (Roberts 2005) would lead to a prediction of 900-1000 deer], sex ratio [studies on Key deer which have also shown a female-biased adult sex ratio (Lopez *et al.* 2003)], and studies that have shown that insular species exhibit higher risk of extinction, which are typically exacerbated by low genetic diversity, population size, and suboptimal habitat (Alcover *et al.* 1998; Frankham 1998; Manne *et al.* 1999; Ricklefs 2009).

CHAPTER TWO: METHODS

Sampling

In order to evaluate broad-scale differentiation of white-tailed deer throughout Florida, I collected tissue samples from 6 counties in Florida, USA (Fig. 1): Citrus (n=1), Santa Rosa (n=1), Collier (n=30), Palm Beach (n=8), Monroe (n=10), and Orange (n=30). Additionally, I collected samples from Ohio (n=2) and West Virginia (n=2) to compare with the Florida population. I also obtained 22 sequences from GenBank (Table 1) to determine where my samples fall into the greater New World deer phylogeny and to be used as outgroups. All white-tailed deer samples were donated by individuals as a result of legal hunting, road kill, or by Florida Fish and Wildlife Conservation Commission.

To compare genetic differentiation between mainland white-tailed deer and Key deer and to evaluate genetic structure and demography within Key deer, I additionally collected fecal (n= 350) and tissue (n=21) samples from Key deer originating from No Name (NNK) and Big Pine Key (BPK) during two sampling sessions (Fig. 2). These two islands represent the core of the Key deer population and contain approximately 75% of the global Key deer population (Lopez 2001). The initial sampling occurred from April 2013 through May 2013; the second session occurred from July 2013 until March 2014. To ensure that collections were sampled uniformly throughout NNK and BPK, I established 29 1-km grids covering these islands using ArcMap10. The size of grids was based on the monthly home range size of male Key deer (USFWS 1999) and the amount of effort needed to collect fecal samples across the two islands. Using a random number generator, I assigned a direction and distance along the edge of each grid to mark the starting point of each transect. I then walked each transect in an approximate straight line to a

point 1-km away on the opposite side of the grid. Along each transect, I continuously searched for piles of fecal pellets. Pellet groups which were scattered or contained an abnormally high amount of pellets were not collected in order to reduce the risk of a sample being from multiple individuals. Additionally, only pellet groups which appeared to be shiny with a mucus sheen were sampled to ensure the highest probability of successful DNA extraction (Brinkman *et al.* 2010). Moreover, I did not collect samples within 24 hours of rainfall to maximize collection of pellets with high DNA quality. Due to environmental conditions in the Keys, I could not estimate the number of days pellets were exposed to weather conditions. For each pellet group which met my criteria, I collected 6 pellets and georeferenced the sample site using a Garmin GPSmap 60CSx. I used fresh gloves for each pellet group and stored samples in Drierite desiccant (W. A. Hammond Drierite Co., Xenia, OH). Fecal samples were collected during the parturition season; however, fawn pellets are easily distinguished in size from adult and yearling pellets and were not collected in this study. In addition to the collection of fecal pellets, tissue samples were taken from Key deer using biopsy darts (PneuDart, Inc.) in grids with high human population density due to the difficulty in locating fecal pellets and the inability to walk a transect through private property.

For sex identification, I used the same fecal samples which I collected for the previous objective. Additionally, for methodological control of sex identification, I collected fresh fecal samples from three Key deer males and three Key deer females. One control sample male was collected from BPK, the remaining five control samples were donated by the Ellie Schiller Homosassa Springs Wildlife State Park, Florida (samples collected from their captive Key deer population). These six fecal samples were only used to validate the methodology for sex identification and were not used in any other analyses.

DNA Extraction

I extracted DNA from tissue using serapure beads following the protocol of Rohland & Reich (2012). Fecal DNA was extracted using two pellets following the QIamp Stool Kit (QIAGEN) manufacturer's instructions with two modifications. First, to account for absorption of the lysis buffer and maximize DNA yield, I added the minimum amount of lysis buffer to each sample to obtain a final amount of 1.4mL lysis buffer. The amount of lysis buffer varied by sample depending on how much of the liquid was absorbed by the pellets. Second, I used 100uL of water heated to 70° C for the elution step, following recommendations by Tursi *et al.* (2013).

DNA Amplification

For the broad scale phylogenetic analyses, I sequenced 985 base pairs of the mtDNA cytochrome *b* gene. Amplification of the cyt*b* gene was conducted in 40uL reactions using the following concentrations: 4uL of 10x PCR Buffer, 4uL of 25mM $MgCl_2$, 4uL 10mM dNTP, 0.4uL of DMSO, 1.8uL each of 10uM forward and reverse primer, 0.8uL of *Taq* polymerase, and 4uL of DNA (50ng/uL). Primers were developed based on published mitochondrial genomes of *O. virginianus* within GenBank (Forward 5'-GTCATTCAACTACAAGAACACYA-3'; Reverse 5'-TATTGAATGTACTACAAAGACTTA-3'). Amplification conditions were as follows: 5min at 95°C, 30 cycles of 1min at 95°C, 30sec at 54°C, 1min at 72°C, followed by a final extension for 15min at 72°C. Subsequent PCR product was sequenced at Eurofins Genomics and University of Arizona Genetics Core (UAGC).

To assess fine scale genetic structure and demographic parameters, I genotyped all samples using twelve previously published polymorphic microsatellite loci which had been

optimized for *O. virginianus* (Table 2). Microsatellite PCR products were genotyped at UAGC. PCRs for fecal DNA were conducted in 15uL reactions using the following concentrations 0.3uL of 40mM dNTP, 1.5uL of GeneAmp® 10X PCR Gold Buffer, 0.15uL of DMSO, 0.1875uL of 10uM forward primer, 0.75uL of 10uM 6-fam dye, 0.75uL of 10uM reverse primer, 0.15uL of AmpliTaq Gold® DNA Polymerase, and 1.5uL of DNA (specific concentration of $MgCl_2$ are shown in Table 2). Amplification conditions were based on the protocol of Anderson *et al.* (2002). The following modifications were made for fecal DNA: initial denaturation of 5 minutes, followed by 40 cycles of 30sec at 95°C, 30sec at T_A (specific annealing temperatures are shown in Table 2), and extension for 1 min at 95°C, followed by a final extension of 10 min at 72°C. For tissue DNA, the protocol was the same as for fecal DNA, but the initial denaturation conducted for 4min and the amplification was run for 35 cycles. All fecal samples were initially amplified across 12 loci under the optimal conditions. Samples which failed at greater than 50% of the loci were discarded from the study. The remaining samples were rerun under the same conditions if loci failed to amplify during the initial screening. Samples which failed to amplify a second time under the initial conditions were rerun at decreasing annealing temperature in 2 degree increments until an annealing temperature of 46°C was reached (Fig. 3). Samples which failed to display clear peaks went through the amplification temperature-cycle twice.

Sex identification was determined using intron 7 of the zinc-finger locus (Lindsay & Belant 2008). Amplification of intron 7 was conducted in 10uL reactions following the protocol of Lindsay & Belant (2008). The X-linked allele (displayed for males and females) is visualized as a smaller band on a 2% agarose gel, while the Y-linked allele (males only) is double the size of the X-linked allele caused by an insertion in the Y-linked allele of intron 7. The larger, Y-linked,

allele is at greater risk of allelic dropout in degraded samples such as feces. To monitor allelic dropout, each PCR reaction was run with two positive controls: one male and one female. As previously stated, these positive controls were fecal pellets collected from Key deer with known sex.

Analyses

To address the genetic isolation of Key deer in hypothesis one, I edited cyt*b* sequences in Sequencher v5.1 (Gene Codes Inc., Ann Arbor, MI, USA) and aligned the data in MEGA6 (Tamura *et al.* 2013) using ClustalW. I first created a TCS network (Clement *et al.* 2000) using popART (http://popart.otago.ac.nz) to find unique haplotypes. Next, I determined the highest likelihood models of evolution for my cyt*b* data and evaluated partitioning of the gene based on codon position using PartitionFinder v1.1.1 (Lanfear *et al.* 2012). I constructed a Bayesian phylogeny utilizing MrBayes v3.2.2 (Ronquist *et al.* 2012) and partitioned my data by first, second and third codon positions. Each partition was run under a separate model: HKY+G, K80+I, and HKY, respectively (see Results). I ran MrBayes with only unique haplotypes identified from TCS and with two independent runs of 5×10^6 generations and the first 10,000 trees were discarded as burn-in to generate the phylogeny. To evaluate mitochondrial diversity in terms of nucleotide and haplotype diversity, I used the program DnaSP v5.0 (Librado & Rozas 2009).

To assess nuclear genetic diversity and structure between the Keys and mainland and within the Keys, I first determined allele sizes using the program GENEMARKER (SoftGenetics, LLC) and used GenAlEx6 (Peakall & Smouse 2006) to assess if loci were in Hardy-Weinberg

Equilibrium (HWE) for each population. Next, I used FSTAT (Goudet 2001) to estimate levels of allelic richness and tested for significance between Key deer and mainland deer via Welch's t-test in R (R Core Team 2013). Finally, to test for structure within and between the mainland and the Keys I ran the program STRUCTURE (Pritchard *et al.* 2000) with 10 independent runs for each value of K (1-6), 100,000 burn-in, and 500,000 iterations. I used the Evanno method (Evanno *et al.* 2005) to estimate ΔK as implemented in the program STRUCTURE HARVESTER (Earl & VonHoldt 2011). To complement the findings in STRUCTURE, I estimated F_{ST} between the mainland and the Keys using the program GenePop v4.0 (Rousset 2008).

I calculated the probability of identity (P_{ID}) and probability of sibling identity (P_{sib}) using GenAlEx6 as a means to uniquely identify individuals. The ability to distinguish between individuals and siblings is crucial to calculating a census size and is based on the amount of genetic diversity within the population. I identified unique individuals and possible recaptures of the same individual utilizing two programs, which use different methods to correct for error. The first program, COLONY (Jones & Wang 2010), was used to determine full sibs under an assumption of a 20% error rate. The inclusion of an error rate corrects for known issues involving noninvasive genetic sampling such as allelic dropout and false alleles (Waits & Paetkau 2005). By including error into the analyses, I was able to account for inconsistencies in identifying recaptured individuals which may not be exact matches due to allelic dropout. I estimated genotyping error rates in the fecal samples by re-amplifying eleven loci across sixteen samples which had been shown to work successfully. The second program, CERVUS (Kalinowski *et al.* 2007), identified unique individuals under the conditions of 4 mismatching loci and 6 matching loci. Assigned matches from COLONY and CERVUS were further scrutinized by eye to confirm

matching individuals. Any single allele that was identified as different between samples disqualified the classification of samples as matches. However, I allowed four instances of allelic dropout between possible matches. In many cases, four cases of allelic dropout were not required to have a match between individuals. Additionally, the majority of possible matches had missing data for at least one locus (e.g. matched at 10 loci with the eleventh locus missing entirely). To account for missing data, I recalculated P_{ID} and P_{sib} for all matches such that the recalculated value only included loci in which data were present. If the P_{ID} and P_{sib} did not exceed the threshold values of 0.001 and 0.05 (Schwartz & Monfort 2008), respectively, they were recorded as the same individual.

To estimate census size, I used two methodologies: mark-recapture in the program MARK (White & Burnham 1999) and spatially-explicit capture recapture (SECR) in the package *secr* (Efford 2014) in R. In MARK, I used the standard closed capture model (Otis *et al.* 1978) which assumes that, for the duration of the experiment, the population does not change through immigration, emigration, births, or deaths. For the purpose of the study, I can assume that movement into and out of NNK and BPK are negligible given that the majority of the Key deer individuals inhabit these two islands (Barrett & Stiling 2006). I further evaluated the assumption of a closed system using the program CloseTest (Stanley & Burnham 1999). CloseTest tests for closure in time-specific data using the null hypothesis from Otis *et al.* (1978) against the Jolly-Seber open model (Stanley & Burnham 1999). Since CloseTest suggested I met the assumptions of a closed capture model, I tested two biologically plausible closed capture models based on Otis *et al.* (1978). The first model assumed that the probability of capture and recapture remained equal and constant between first and second captures. The second model allowed time (sampling

occasion) to remain constant within captures, but vary between first and second captures. I used Akaike's Information Criterion (AIC_c, adjusted for sample size) to determine which model best explained the data. The second method to estimate census size utilized SECR analysis. SECR differs from traditional mark-recapture in that it includes individuals found in the same sampling session ("occasion") and uses the coordinates of each sample to estimate the density of Key deer. I generated a mask area based on the ArcMap shapefile of BPK and NNK using the *maptools* package in R. For the SECR analysis, I tested two models, following the same models I used in MARK, and assessed the best model using AIC_c. From the calculation of density estimated in *secr*, I multiplied this value by the area of BPK and NNK to estimate the census size (N=DA). Additionally, I estimated effective population size using COLONY to compare the amount of diversity present in the Keys population to the estimated census and calculated a census size/effective size ratio.

To estimate the sex ratio within the Key deer population as part of the second hypothesis, I counted the number of males and females identified in the Key deer population using gel electrophoresis. The total number of males and females found in the Key deer population was then divided by the total number of samples which successfully amplified.

Finally, I combined the genetic and demographic data into a species persistence model using the program VORTEX v10.0.8.0. Life history traits and genetic data were based on results from this study, previously published literature for Key deer, or standard VORTEX values (Table 3). To determine aspects of the model that impacted species persistence, I ran 20 iterations for each model while changing individual model parameters, these included: catastrophes, mate monopolization, maximum age of male reproduction, levels of inbreeding depression, fecundity,

carrying capacity, fetal sex ratio, male survival, and female survival. Not all model parameters impacted probability of extinction (see Results); however, for those that did, I ran sensitivity analyses to determine how varying parameters related to persistence. Specifically, I varied percent males born (59%, 66%, and 74%) and female mortality in the sensitivity analyses. The values for fetal sex ratio represent the two published extremes (Hardin 1974; Folk & Klimstra 1991) and an intermediate value. Adult female mortality was modeled using two methods: constant mortality rate and a function to account for negative density-dependent survival:

$$=18*(EXP(N/K)/EXP(K/K))$$

with N=census size and K=carrying capacity. The constant in the equation for female mortality represents the published value for adult mortality (Lopez *et al.* 2003). In the sensitivity analyses, female mortality was evaluated under three different levels: decreased mortality (10% fawns, 10% adults), baseline mortality (28% fawns, 18% adults), and increased mortality (38% fawns, 28% adults). I ran the sensitivity analyses for 100 samples to model changes due to stochasticity. I simulated the data to look 50 years into the future and evaluate likelihood of persistence.

CHAPTER THREE: RESULTS

Sequencing

A total of 985bp were successfully amplified from 100 samples used for haplotype and phylogenetic analyses. From these samples, I identified 18 unique haplotypes (Fig. 4) which were used to create the phylogeny. The final tree was partitioned based on codon position and resulted in an average standard deviation of split frequencies of about 0.0029. Each codon was represented by a different model of DNA evolution: HKY+G, K80+I, and HKY (Kimura 1980; Hasegawa *et al.* 1985), first, second, and third codon positions, respectively.

Based on the haplotype network, the most distinct group (three haplotypes found in the Keys and Collier County) are separated by 23 base pairs from the next most closely related group. Within Ohio there are two haplotypes. One Ohio haplotype was shared between Ohio and Collier County and was more closely related to the samples from West Virginia than the Florida haplotypes. The second haplotype found in Ohio was more closely related to samples in Florida. Within the Keys, I found that all samples exhibit a single unique cyt*b* haplotype. The Keys haplotype differs from its closest related haplotype, found in Collier County, by one base pair.

The phylogeny produced interesting results with regards to Key deer, white-tailed deer, and placement of genera within New World deer. Based on the phylogenetic analysis, I found that Key deer, like mule deer (*O. hemionus*) are nested within white-tailed deer (Fig. 5). However, the genus *Odocoileus* itself forms a monophyletic clade, excluding *O. virginianus* 6 (Fig. 5). Additionally, two other genera within New World deer (*Pudu* and *Mazama*) formed a paraphyletic clade that includes the monophyletic *Odocoileus* clade (Fig. 5).

Population Genetics

I found all 12 microsatellite loci were polymorphic within and among the mainland deer samples, whereas only 11 of the loci were polymorphic within the Key deer population. In the mainland I tested for Hardy-Weinberg equilibrium (HWE) in Collier, Orange, Monroe, and Palm Beach counties. After conducting a sequential Bonferroni correction (Rice 1989), only three loci were out of HWE (loci R and IGF1 in Collier County and locus R in Orange County). I saw no patterns of loci or populations that were consistently out of HWE; therefore, all populations and loci were used in downstream analyses, despite the possibility for low frequency null alleles in some populations. Citrus and Santa Rosa counties, as well as Ohio and West Virginia, were not tested for HWE due to small sample sizes. However, samples from these populations were not evaluated for within-population levels of genetic diversity or among-population genetic differentiation. In contrast to the mainland populations tested, the Keys population deviated from HWE expected values in 11 out of 12 loci. However, this result was not surprising given known issues associated with noninvasive genetic sampling (Waits & Paetkau 2005). The average error rate across all loci was 8.52% (Table 2). The one locus that did not deviated from HWE was Locus BL25, the monomorphic locus in the Keys. Average allelic richness varied from 3.37 in the Keys to 5.51 in Orange County (Table 4). Key deer were found to contain significantly reduced levels of allelic richness compared to the mainland population (Welch's t-test; t=-2.771, df=20.501, P= 0.012). Based on the genetic diversity estimate, the P_{ID} (2.4×10^{-9}) and P_{sib} (2.4×10^{-4}) were calculated to be less than the threshold (Schwartz & Monfort 2008) which allowed for the genetic tagging of individuals.

Based on STRUCTURE, I identified K=2 clusters as the best fit for the data using the Evanno *et al.*(2005) method: the Keys and mainland Florida (Fig. 6). Although all pairwise F_{ST} values were significant except for the comparison between Collier and Monroe Counties, the numerical values were much greater between the Keys and the mainland (0.155 – 0.207) than among mainland populations (0.022 – 0.074; Table 5), further supporting that these populations fall into two clusters (i.e. mainland versus Keys). One caveat of the high F_{ST} value is that the Keys population is out of HWE leading to a possible inaccurate estimate of F_{ST}. However, Smith & Wang (2014) determined that when error rates are less than 20%, estimates of F_{ST} and genetic diversity are able to be evaluated. Only one locus exhibited an error rate above 20% (Table 2); hence, these data should reflect accurate estimates of differentiation between Keys and mainland populations.

Demographics

I was able to successfully genotype 164/350 samples collected (47% success rate). Combined with 21 tissue samples, I identified 173 unique deer to be used in downstream demographic analyses. Within sampling session one I found six matches (i.e. samples that were identical within the first sampling session), sampling session two had two matches. Comparisons between sampling sessions revealed eight recaptures of sampling session one deer in sampling session two. The model assumptions did not significantly (P=2.0) deviate from those of a closed population model based on the program CloseTest. In MARK and *secr*, the most supported model stated that the probability of capture remains constant within sampling sessions, but varies between sessions (Table 6). Both programs gave similar results: MARK estimated a census size of 986.69 (SE = 316.81) individuals and *secr* estimated 1,006.93 (SE = 242.30) individuals

(Table 6). These numbers are surprisingly higher than the genetic effective population size, which was estimated to be 11 individuals (95% CI: 6-28). Therefore, the ratio of effective/census population size is approximately 0.011. Finally, I was able to successfully amplify intron 7 of the zinc-finger locus in 70 samples. Out of the 70 samples, I was able to identify 65 females and five males showing a heavily female-biased adult population.

Population Viability Assessment

By altering individual parameters, I found that only two variables (female survival and fetal sex ratio) impacted long-term census size and species persistence of the Key deer. When all remaining variables were substituted with alternative values (Table 3), the model of species persistence was minimally impacted. In contrast, when female survival is increased or decreased beyond the value estimated from field data (Lopez *et al.* 2003), I found that female survival itself is the primary factor impacting persistence (Fig. 7). Whereas when female survival is at the value estimated from the field, persistence is dependent upon fetal sex ratio. Fetal sex ratio impacted the rate of extinction such that at higher male-biased fetal sex ratios (e.g. 74% males), extinction was reached more quickly (Fig. 7). Under all scenarios, when the species is extinction bound, density-dependent mortality slows the rate of extinction.

CHAPTER FOUR: DISCUSSION

This study highlights the utility in using modern genetic techniques to answer questions related to both genetics and contemporary demography. My results indicate that Key deer are genetically isolated from mainland white-tailed deer and that there is a lack of genetic substructure between BPK and NNK. Moreover, Key deer exhibit reduced levels of genetic diversity compared to their mainland counterparts; however, they contain enough diversity of which to uniquely identify individual deer. Based on genetic identification, I estimated a census size of around 1,000 individuals with a heavily skewed female-biased adult sex ratio. Moreover, I was able to combine genetic and contemporary demographic data to generate a species persistence model of the Key deer. Sensitivity tests within the PVA brought to light the importance of fetal sex ratio and female survival as the primary factors at risk of driving the subspecies to extinction. Below, I discuss the evolutionary history of Key deer, contemporary demographic estimates of Key deer and how each of these factors contributes to species persistence.

Evolutionary History

Overall, I sequenced and acquired samples from GenBank in which to evaluate the phylogenetic relationship of three genera of New World deer (*Odocoileus, Mazama* and *Pudu*) as a means to determine the placement of Key deer within this phylogeny. I found that all three genera are paraphyletic. *Pudu* and *Mazama* are paraphyletic with regard to each other, which parallels the findings of Duarte *et al.* (2008) and Hassanin *et al.*(2012). *Odocoileus* is paraphyletic owing to a single sample of *O. virginianus* that was sampled from the most southern

part of the contiguous species range (i.e. Colombia). Other than this one sample, *Odocoileus* forms a monophyletic clade, which contains white-tailed deer, mule deer and Key deer. However, white-tailed deer are not monophyletic as both mule deer and Key deer are nested within the white-tailed deer phylogeny. The mule deer phylogenetic placement may be largely impacted by introgression between mule deer and white-tailed deer (Cathey *et al.* 1998). Key deer, on the other hand, are likely nested within white-tailed deer owing to the recent isolation of the population (6-10 kya; Lazell Jr., 1989).

Focusing on *Odocoileus* found in Florida, I found that there is high haplotype diversity among all samples and even within populations. There are three possible causes to the high levels of diversity observed: translocation, historic polymorphism, and long distance dispersal. In the 1900s, southeastern white-tailed deer were overexploited and subsequently restocked with deer ranging throughout the United States (Blackard 1971). Although in recent years, translocations have been stopped owing to measures to decrease the spread of chronic wasting disease (Garrison & Gedir 2006), historic translocations from previously isolated regions could leave the observed pattern. Historic polymorphism could also explain the pattern of diversity. Under this scenario, historic abundance and large effective population sizes of white-tailed deer could enable the retention of historic diversity even as drift causes haplotypes to be lost over time. Lastly, white-tailed deer range throughout the Americas and have been shown to exhibit low structure between broad regions (Robinson *et al.* 2012), suggesting that long-distance dispersal can occur. However, males are the primary dispersers in white-tailed deer and thus are unlikely to impact mtDNA genetic structure.

Despite the haplotype diversity found within Florida, I did not identify high haplotype diversity in the Key deer. Indeed, Key deer all contained a single mtDNA haplotype. This haplotype was most closely related to two haplotypes from Collier County and these three haplotypes were quite distinct from the next closest haplotype (2.3% uncorrected sequence divergence). Key deer haplotype diversity indicates a lack of structure within Key deer. Although the lack of structure contrasts with studies of Key deer movement (Lopez 2001), it is paralleled by the nuclear markers, which supported a single panmictic population between BPK and NNK. In addition, I identified a reduced level of allelic richness relative to mainland deer, supporting the lack of gene flow between Key deer and mainland deer identified using mtDNA. To further provide evidence, previous to this study I screened an additional seven loci (BM415, K, BBJ11, eth152, O, D, BM848), which I chose not to genotype across all samples owing to monomorphism within the Key deer samples. The inclusion of these monomorphic loci would almost certainly have further decreased the genetic diversity estimates for the Keys. It is not surprising that Key deer exhibit such a reduction in genetic diversity relative to their mainland white-tailed deer ancestors. In addition to their insularization, Key deer experienced an extreme bottleneck due to overexploitation and the population plummeted to about 25 individuals in the early 1950s (USFWS 1999).

Contemporary Demography

Upon examination of the census estimates for both models in MARK and *secr*, I found that they were similar and ranged from about 987-1012 individuals. Other studies have evaluated the census size estimates between *secr* and other mark-recapture programs (e.g. CAPTURE and MARK) and found similar population estimates between programs (e.g Gray & Prum 2012;

Rayan *et al.* 2012). A census size estimate of around 1,000 individuals suggests that the Key deer population is continuing to increase from the estimated 25 individuals in the 1950s until present or that previous studies have underestimated census size. The last census count of Key deer on BPK and NNK estimated 555-619 deer in 2005 (Roberts 2005). In a previous study by Lopez *et al.* (2004), estimating a population size between 453-517 in 2001, they noted that the Key deer population on BPK and NNK is increasing at about 5% annually. Based on the 5% annual increase starting from the last census estimate in 2005, the Key deer population should consist of around 900 individuals, which is similar to my estimate, thus matching the prediction of Lopez *et al.* (2004) and has not yet stabilized.

The continuous increase of the population since the early 1950s may be due to 1) the population recovering and not reaching carrying capacity or 2) recent habitat changes have favored the Key deer. Historic population size has been estimated to be between 600-700 individuals (Seal *et al.* 1990) with previous estimates of carrying capacity to be around 607 (Harveson *et al.* 2006). However, my current estimate exceeds historical estimates and carrying capacity. Thus, my data suggest that carrying capacity has not been reached. Humans may have artificially increased their carrying capacity by the addition of fresh water and food (Peterson *et al.* 2005). In fact, Key deer have increased their use of urban developments from the times of 1970-2000 (Harveson *et al.* 2007). Even with few houses providing additional sustenance, the extra resources can significantly influence population dynamics (Peterson *et al.* 2005).

Previous work on sex ratio in Key deer focused on two separate life-history stages and found contrasting results. In two different studies (Hardin 1974; Folk & Klimstra 1991), the fetal sex ratio was observed to be skewed towards males. Contradictory to the fetal sex ratio, Lopez

(2001) reported an adult female-biased (approximately 3:1) sex ratio during an observational study. My results support a pattern similar to Lopez (2001); however, my results suggested a more striking 13:1 female: male adult sex ratio. Here, the explanation for the extreme female-biased sex ratio does not likely have natural causes. Rather, road mortality data likely play a role in skewing the sex ratio. Specifically, studies have revealed a greater number of male deaths each year caused by deer-vehicle collisions (DVCs; Lopez *et al.* 2003). Moreover, deer behavior is a likely culprit as to why DVCs favor male deer. Similar to mainland white-tailed deer, Key deer males are the primary dispersers (Lopez 2001), making them more likely to move across roads than female deer and collide with vehicles.

Persistence Modeling

I utilized both genetic and demographic data to evaluate the persistence of Key deer for the next 50 years. The genetic data informed the number of populations to be equal to one, consisting of all individuals found on BPK and NNK and provided the input of allele frequency data into the model to evaluate loss of genetic diversity. The demographic data informed the initial census size for all models and provided information with regard to the adult sex ratio. Surprisingly, only two variables (female survival and fetal sex ratio) were the main drivers of species persistence. The models illuminated the importance of females within the Key deer population. Variables that increased the number of females increased the likelihood of long-term persistence. On the other hand, the number of males does not influence time to extinction. Assuming that Key deer, similar to mainland white-tailed deer and other ungulates, are polygamous (Clutton-Brock 1989), I should expect that it would require few males to sustain the population. In other words the limiting factor, with regard to long-term persistence of Key deer, is

the number of females. When evaluating the combined role of fetal sex ratio and female survival, this study revealed that the tipping point for species persistence in the Key deer is near 66% fetal sex ratio, 28% female fawn survival, and 18% adult female density-dependent survival. Values less favorable lead to extinction while values more favorable lead to growth capped at carrying capacity.

In summary, Key deer provided a model system in which to use modern genetic techniques to evaluate questions related to traditional genetic data (e.g. genetic structure and diversity) and demography. I found that Key deer are genetically isolated from mainland white-tailed deer and contain reduced levels of genetic diversity. However, they contain enough genetic diversity to identify individual deer to estimate census size using genetic tagging. Through genetic mark-recapture, I found that the Key deer population is continuing to increase from their historic population size of around 25 individuals. To evaluate population stability in Key deer, management should continue to monitor the census size of the population. Moreover, because females are critical for Key deer survival, future studies should focus on obtaining more accurate estimates of fetal sex ratio and methods to reduce female mortality. Ultimately, I provide evidence that Key deer are recovering and under continued management practices, I expect their continued persistence into the next 50 years.

APPENDIX A: FIGURES

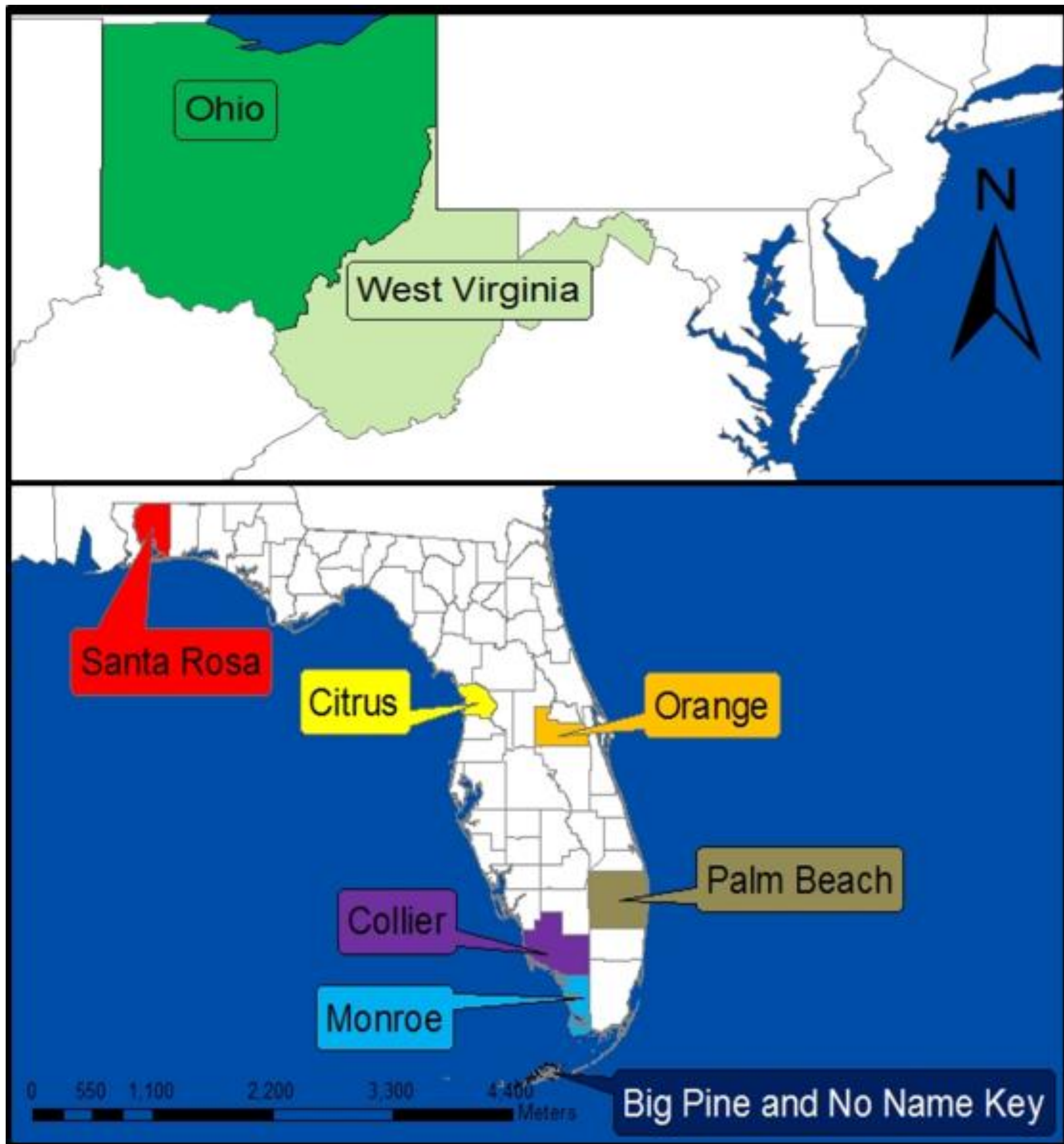

Figure 1. Map of sampling locations. Samples were collected from six counties within Florida to represent the Florida mainland population. Ohio and West Virginia samples were collected as outgroup samples.

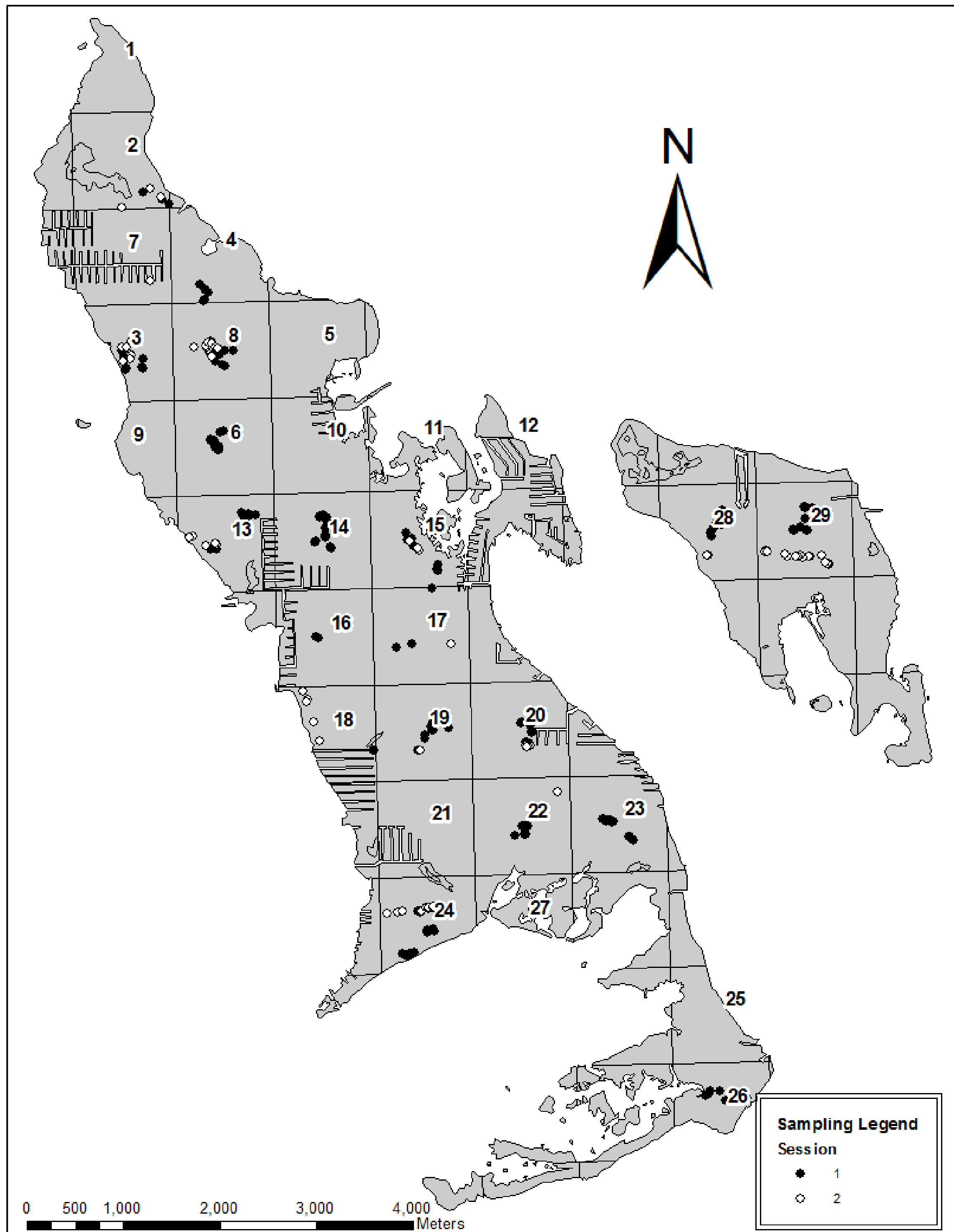

Figure 2. Sampling locations of Key deer. BPK and NNK are the easternmost islands in the Lower Keys of Florida. Sampling session one is denoted by pink dots; second session by green dots.

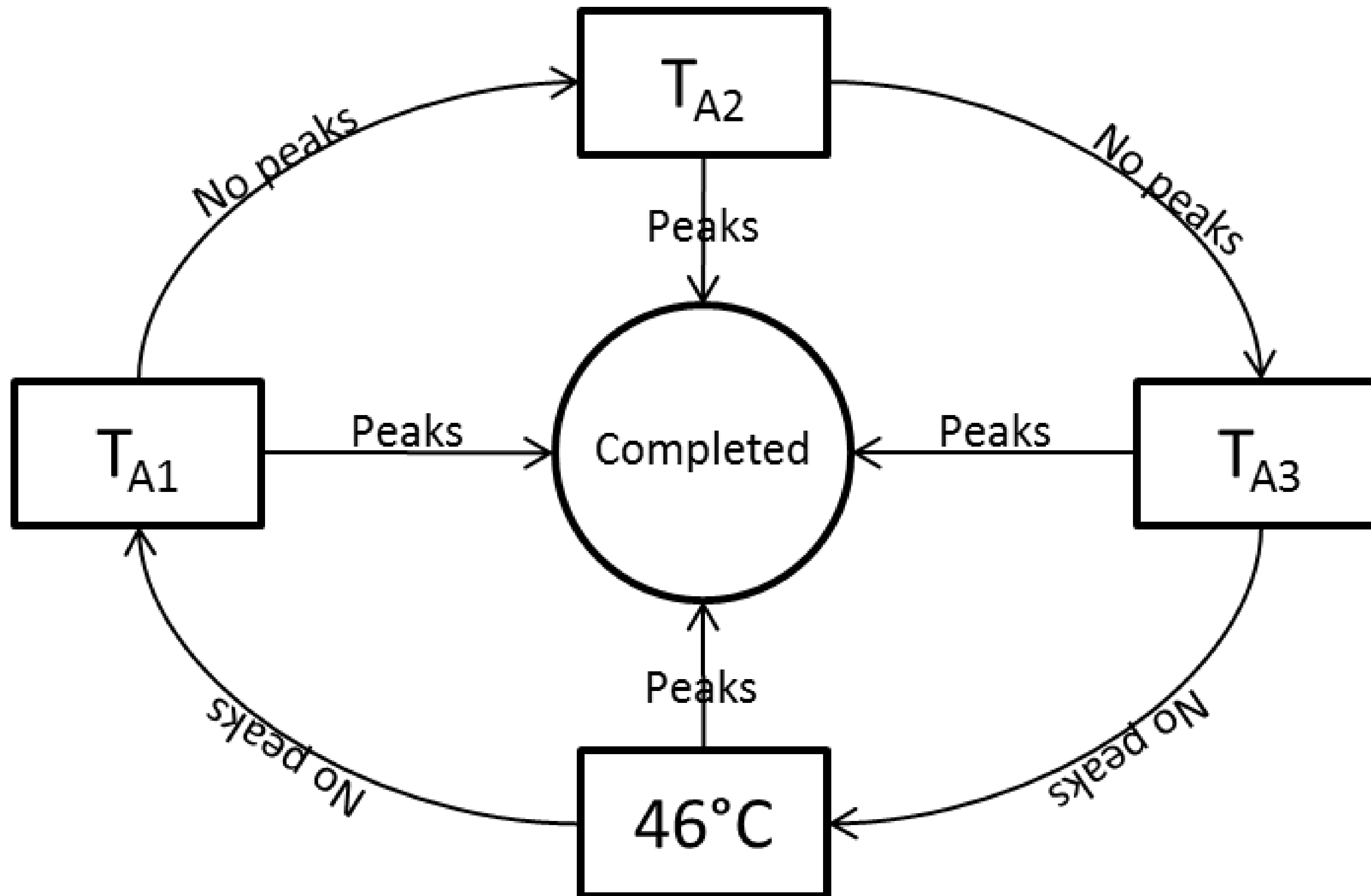

Figure 3. Amplification cycle of fecal samples. Samples were run repeatedly until a clear peak was distinguishable or until a sample went through the cycle two times per locus.

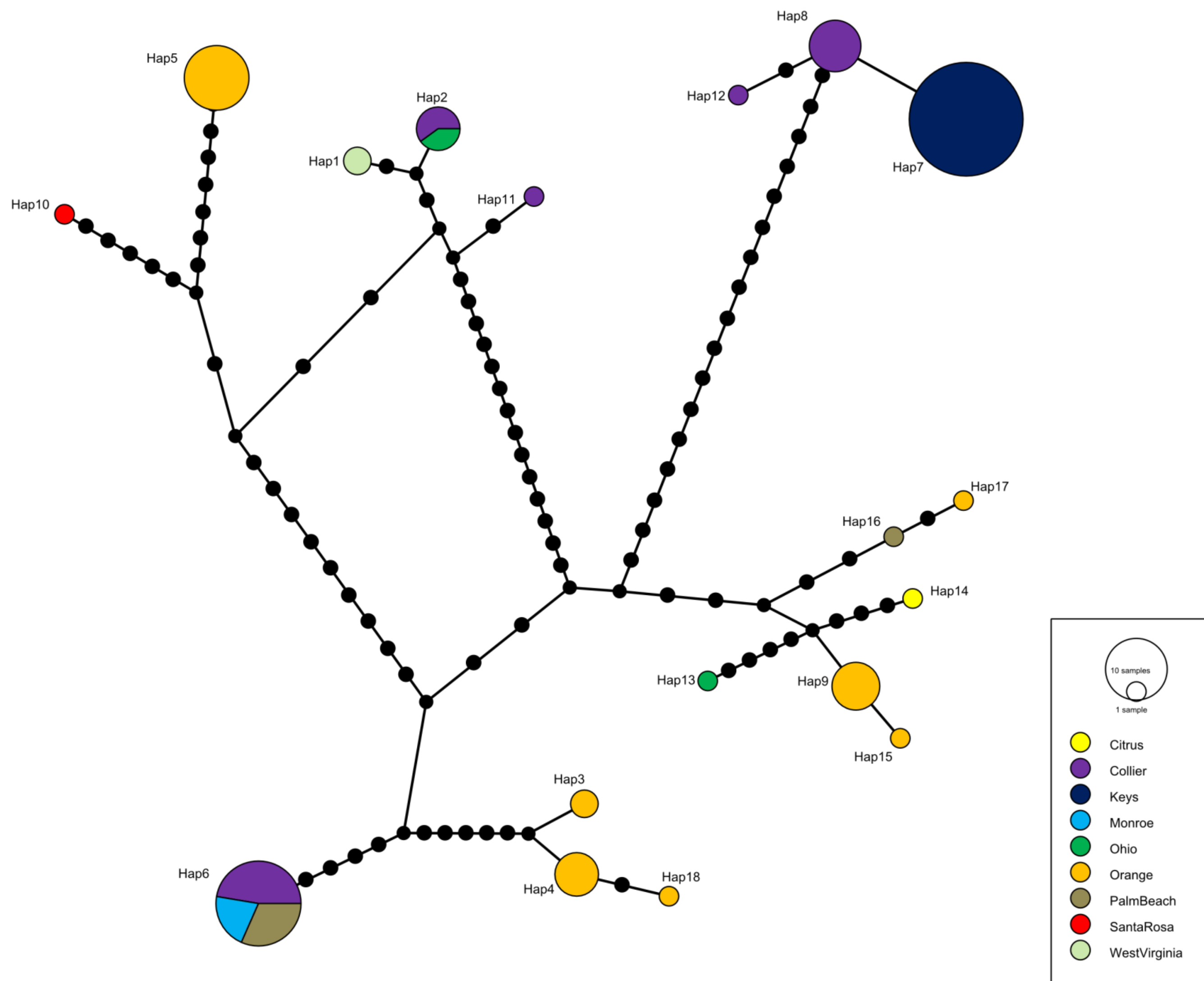

Figure 4. Haplotype network of samples collected in this study. As shown, the haplogroup containing the Keys and Collier County are several basepairs away from the next related haplotypes. Samples from Orange County are not shared with any of the other populations.

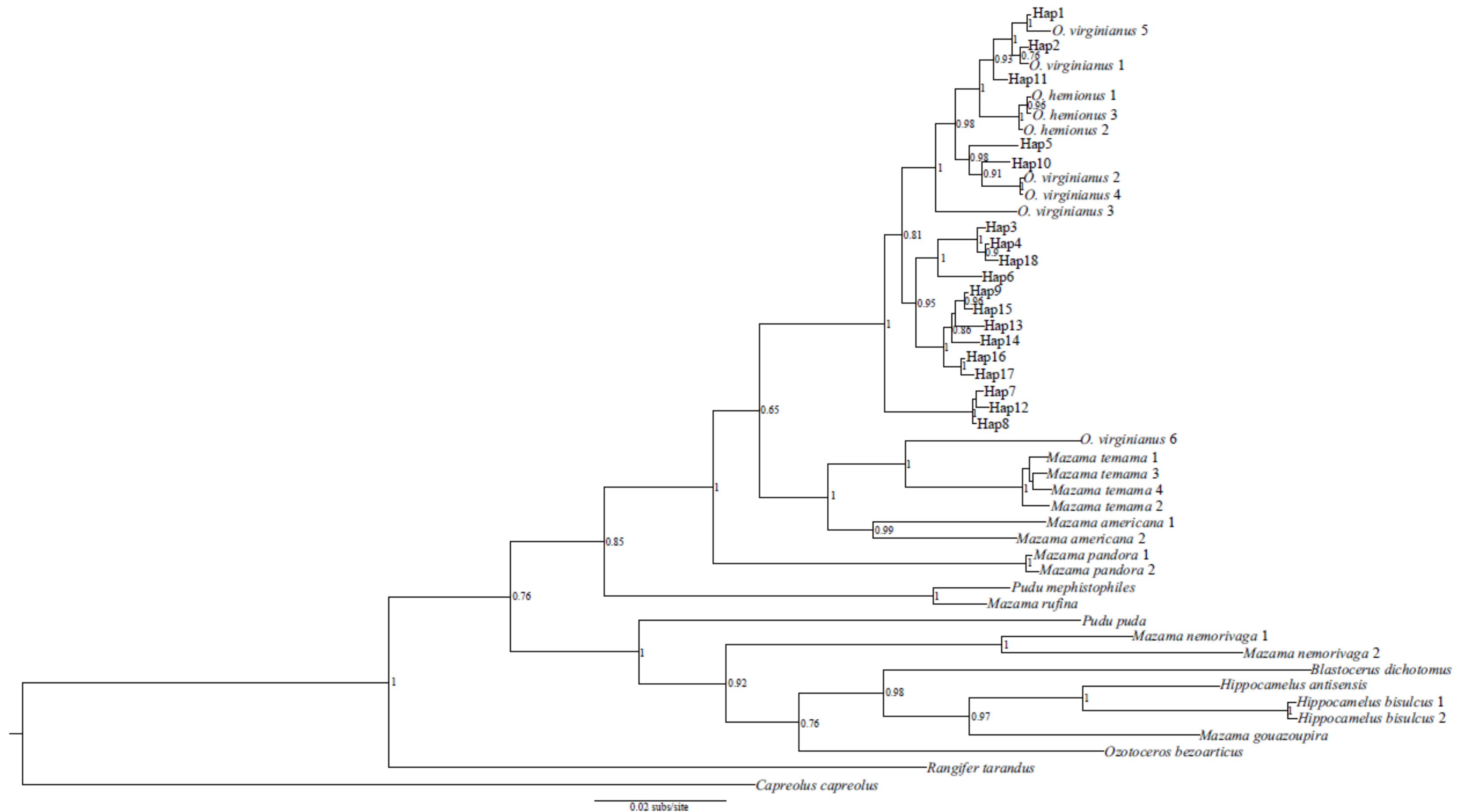

Figure 5. New World deer phylogeny based on the cyt*b* gene. Posterior probabilities, greater than 0.5, are indicated at their respective nodes. Samples used in this study are named according to their haplotype number. The remaining samples found in the phylogeny were obtained from GenBank. Numbers next to taxon name correspond to the GenBank accession numbers in Table 1. Outgroups: *Rangifer tarandus* and *Capreolus capreolus*.

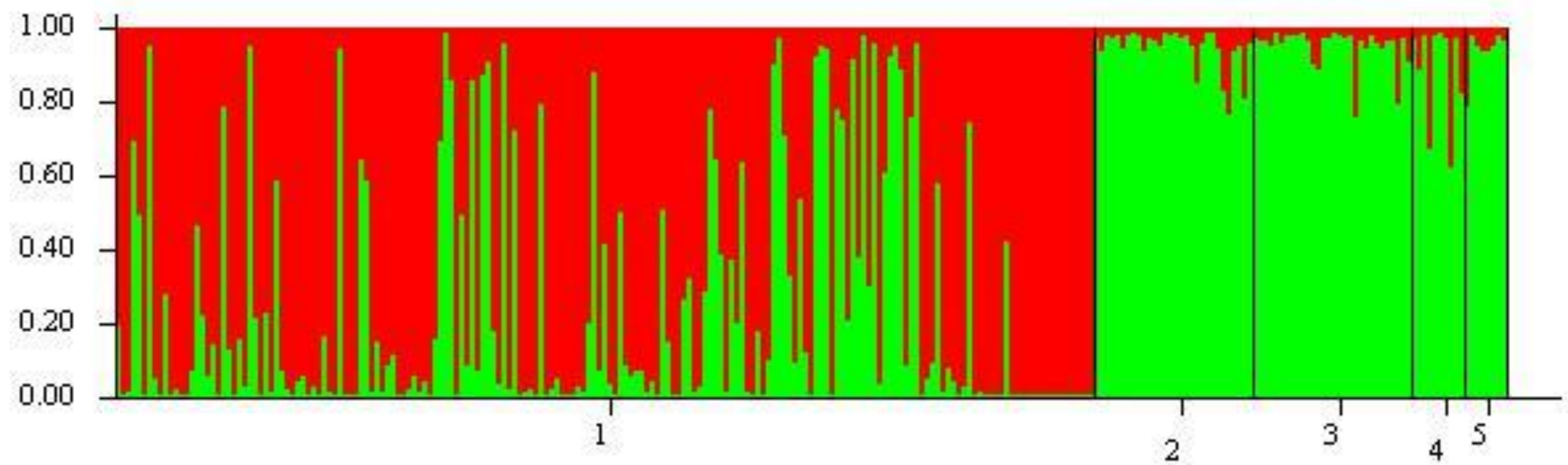

Figure 6. Output of the STRUCTURE analysis when K=2. The output shows structure between the Keys (Keys=1) and mainland (Collier=2, Orange=3, Monroe=4, Palm Beach=5) with no structure within the Keys or mainland.

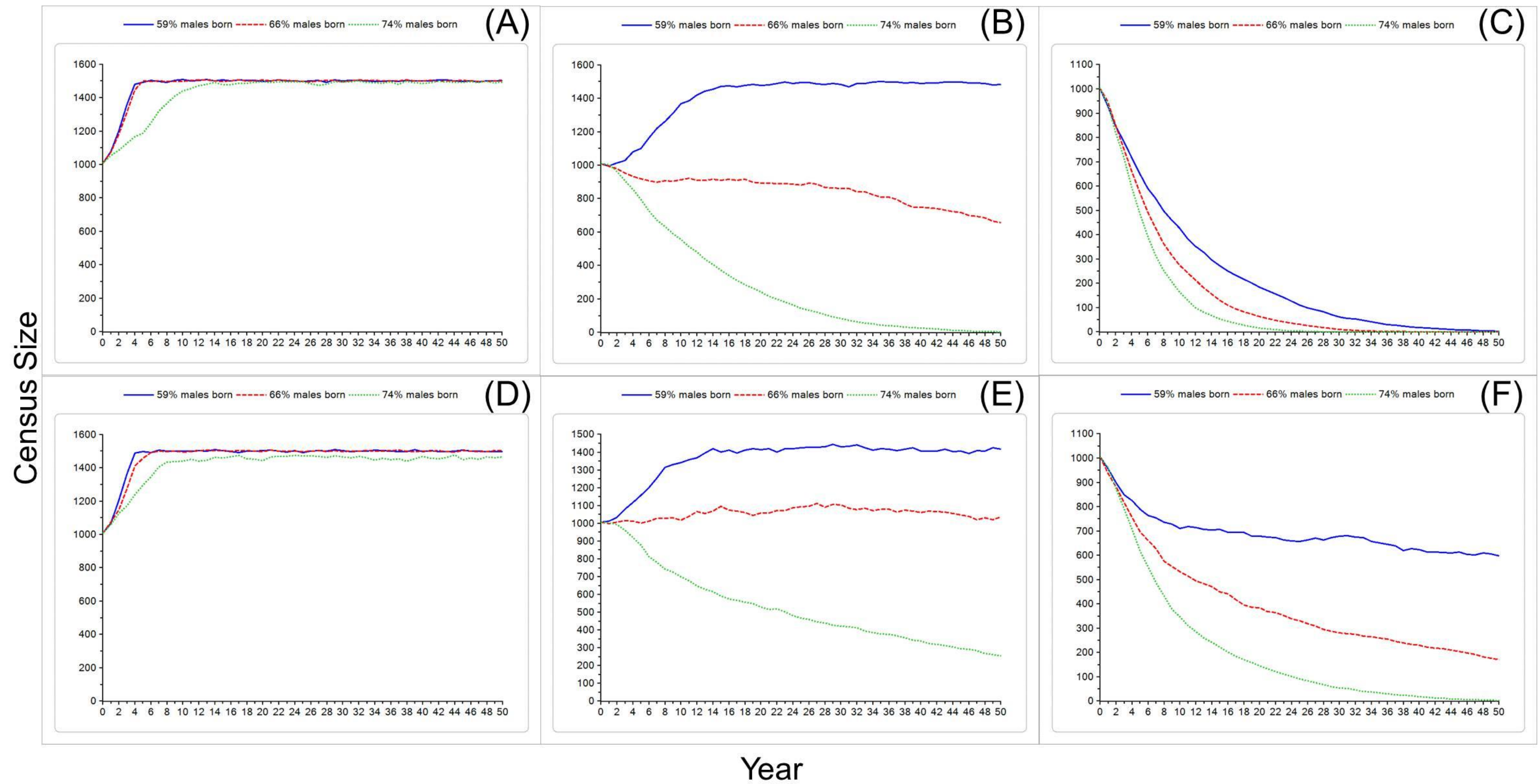

Figure 7. VORTEX simulations for persistence of Key deer. All six graphs are shown with changing fetal sex ratios, but each graph varies according to female mortality. (A) Decreased and constant mortality, (B) baseline and constant mortality, (C) increased and constant mortality, (D) decreased density-dependent mortality, (E) baseline and density-dependent mortality, and (F) increased and density-dependent mortality.

APPENDIX B: TABLES

Table 1. GenBank samples used in phylogeny. Sample names given correlate to taxa naming. Citations correlate to studies which generated the sequence and accession number refers to GenBank.

Name in Phylogeny	Citation	Accession Number
O. virginianus 1	Pitra *et al.* 2004	AY607035
O. virginianus 2	Gilbert *et al.* 2006	DQ379370
O. virginianus 3	Hassanin *et al.* 2012	JN632671
O. virginianus 4	Hassanin *et al.* 2012	JN632672
O. virginianus 5	Cronin *et al.* 2006	DQ673136
O. virginianus 6	Hassanin *et al.* 2012	JN632673
O. hemionus 1	Hassanin & Douzery 1999	AF091630
O. hemionus 2	Naidu *et al.* 2012	HM222707
O. hemionus 3	Hassanin *et al.* 2012	JN632670
Mazama temama 1	Unpublished	KC146956
Mazama temama 2	Unpublished	KC146957
Mazama temama 3	Unpublished	KC146958
Mazama temama 4	Unpublished	KC146959
Mazama americana 1	Hassanin *et al.* 2012	JN632657
Mazama americana 2	Hassanin *et al.* 2012	JN632656
Mazama pandora 1	Unpublished	KC146954
Mazama pandora 2	Unpublished	KC146955
Pudu mephistophiles	Hassanin *et al.* 2012	JN632691
Mazama rufina	Hassanin *et al.* 2012	JN632661
Pudu puda	Hassanin *et al.* 2012	JN632692

Name in Phylogeny	Citation	Accession Number
Mazama nemorivaga 1	Hassanin *et al.* 2012	JN632659
Mazama nemorivaga 2	Hassanin *et al.* 2012	JN632660
Blastocerus dichotomus	Hassanin *et al.* 2012	JN632603
Hippocamelus antisensis	Hassanin *et al.* 2012	JN632646
Hippocameuls bisulcus 1	Duarte *et al.* 2008	DQ789177
Hippocameuls bisulcus 2	Duarte *et al.* 2008	DQ789178
Mazama gouazoupira	Hassanin *et al.* 2012	JN632658
Ozotocerus bezoarticus	Hassanin *et al.* 2012	JN632681
Rangifer tarandus	Unpublished	NC_007703
Capreolus capreolus	Hassanin *et al.* 2012	JN632610

Table 2. Primers and PCR conditions for microsatellite data generated for Key deer. All PCR reactions were run using a standard protocol (see Methods). Concentration of $MgCl_2$ and primer annealing temperatures varied by locus (TA). Error rates within noninvasive samples are calculated from the re-amplification of 16 samples.

Locus	Citation	$[MgCl_2]$	T_A	Error rate
BL25	Bishop *et al.* 1994	2.0mM	52	N/A
ILSTS011	Brezinsky *et al.* 1993	2.0mM	52	6.25%
OarFCB193	Talbot *et al.* 1996	2.0mM	52	0%
INRA011	Vaiman *et al.* 1992	2.0mM	52	0%
Cervid1	DeWoody *et al.* 1995	2.5mM	52	25%
P	Jones *et al.* 2000	2.0mM	52	0%
R	Jones *et al.* 2000	3.5mM	52	12.5%
IGF1	Kirkpatrick 1992	3.0mM	52	6.25%
N	Jones *et al.* 2000	2.0mM	52	18.75%
Rt9	Wilson *et al.* 1997	3.0mM	54	12.5%
BM4107	Talbot *et al.* 1996	2.5mM	52	12.5%
Q	Jones *et al.* 2000	2.0mM	52	0%

Table 3. Final parameters input into program VORTEX. All data input into the persistence model were based on results from this study, previously published Key deer literature, or standard VORTEX values. During model examination, parameters in bold were evaluated for impacts on species persistence. The (†) illustrates values used in the standard model when multiple values were evaluated, yet variation did not influence likelihood of extinction. Parameters italicized were used in sensitivity analyses due to their importance in species persistence.

Parameter	Value	Source
Inbreeding depression	**Inbreeding depression = 0, 3, 6.29*†, 12**	*Standard value given by VORTEX
	Percent due to recessive lethal alleles = 25, 50*†	*Standard value given by VORTEX
Reproductive system	Polygynous*	*Based on white-tailed deer (Clutton-Brock 1989)
	Age of first offspring females = 1*	*(USFWS 1999)
	Age of first offspring males = 2*	*(USFWS 1999)
	Maximum lifespan = 7*	*(Lopez *et al.* 2003)
	Maximum number of broods per year = 1*	*(USFWS 1999)
	Maximum number of progeny per brood = 3*	*(USFWS 1999)
	Sex ratio at birth -- in % males = 59, 66, 74	See Methods
	Maximum age of female reproduction = 7*	*(Lopez *et al.* 2003)
	Maximum age of male reproduction = 3*†, 7	*(Klimstra 1992)
Reproductive rates	% adult females breeding = 82*	*(Folk & Klimstra 1991)
	SD in % breeding due to EV = 10*	*Standard value given by VORTEX
	Distribution of number of offspring per female pe brood (4 combinations tested: 1, 2, 3 offspring respectively):	*(Folk & Klimstra 1991; USFWS 1999)
	Combination 1 = 83, 17, 0	
	Combination 2 = 60, 40, 0	
	Combination 3 = 20, 50, 30	
	Combination 4*† = 82, 17, 1	
Mortality rates	***Females = See Methods***	(Lopez *et al.* 2003)
	SD due to EV = 0.1*	*Standard value given by VORTEX
	Males – Age 0-1 = 28, 32*†	*(Lopez *et al.* 2003)
	Males – Age 1-2 = 18, 39*†, 50	*(Lopez *et al.* 2003)
	Males – Age after age 2 = 18, 39*†, 50	*(Lopez *et al.* 2003)
	SD due to EV = 0.1*	*Standard value given by VORTEX

Parameter	Value	Source
Catastrophes	Number of catastrophes = 2*	*Standard value given by VORTEX
	Frequency = 1†, 50	Modeled range from 1%-50% and showed no change
	Severity – Reproduction = 1*	*(Lopez *et al.* 2003)
	Survival = 1*	*(Lopez *et al.* 2003)
Mate Monopolization	**Males in breeding pool = 25, 100†**	Modeled range from 25%-100% and showed no change
Initial population size	Population size = 1006*	*This study
	Stable Age Distribution with no males surviving after age 3*	*(Klimstra 1992)
Carrying capacity	**250, 607, 1500*†, 2000**	*This value represents a 50% increase over estimated census size
Harvest	None	Phillip Hughes (pers. comm)
Supplementation	None	Phillip Hughes (pers. comm)
Genetics	Additional loci only and 11 neutral loci to be modeled	This study

Table 4. Genetic diversity for five populations of mainland Florida white-tailed deer. Genetic diversity shows number of individuals used (n), number of haplotypes, number of segregating sites, nucleotide diversity (π), haplotype diversity (*h*) and allelic richness. Nucleotide diversity, haplotype diversity, and allelic richness are reported as mean ± standard error.

	Mitochondrial diversity					Microsatellite diversity	
Population	n	No. of haplotypes	No. of segregating sites	Π	*h*	n	Allelic richness
Keys	34	1	0	0	0	185	3.747±0.354
Collier	21	5	44	0.018±0.000	0.714±0.014	30	5.349±0.551
Orange	27	7	47	0.019±0.000	0.769±0.011	30	5.509±0.586
Monroe	4	1	0	0	0	10	5.076±0.435
Palm Beach	7	1	0	0	0	8	5.266±0.452

Table 5. Pairwise F_{ST} values between populations. The Keys have the highest amount of differentiation when compared to other populations. Within the mainland, there is little differentiation between populations. Numbers in bold are significantly greater than zero.

Population	Keys	Collier	Orange	Monroe	Palm Beach
Keys	—	—	—	—	—
Collier	**0.204**	—	—	—	—
Orange	**0.202**	**0.041**	—	—	—
Monroe	**0.207**	0.022	**0.074**	—	—
Palm Beach	**0.155**	**0.052**	**0.040**	**0.057**	—

Table 6. Models tested and AICc scores and weight for census size. The two best models for each method are in bold. See text for contents of these models.

Program	Model	AIC_c	AIC_c weight	Census Estimate
MARK	{N, p(constant)=c(constant)}	-1139.65	0.18	1012.97
MARK	**{N, p(time) = c(time)}**	**-1142.74**	**0.82**	**986.69**
secr	g0	883.98	0.17	1006.93
secr	**g0t**	**880.86**	**0.83**	**1006.93**

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