2012

Conservation And Population Biology: Genetics, Demography And Habitat Requirements Of The Atlantic Coast Beach Mice

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CONSERVATION AND POPULATION BIOLOGY: GENETICS, DEMOGRAPHY AND HABITAT REQUIREMENTS OF THE ATLANTIC COAST BEACH MICE

by

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A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the Department of Biology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer Term
2012

Major Professors: Christopher L. Parkinson and I. Jack Stout

ABSTRACT

The conservation biology field seeks to preserve biodiversity and the processes shaping that variation. Conservation biology is intimately tied to evolutionary research, in order to identify evolutionary distinct lineages that may be in danger of disappearing. Interestingly, patterns and processes of lineage divergence and persistence change with respect to spatial and temporal scale. I seek to evaluate biodiversity, the factors that have shaped this heterogeneity, and how this variability persists. To accomplish this I used a phylogeographic approach as well as niche and population modeling on the *Peromyscus maniculatus* species group found widely distributed in North America. My emphasis was on the southeastern U.S. species *P. polionotus* and its distinct beach forms. At a continental scale, I found that environmental niches are likely involved in generating and/or maintaining genetic lineages within the *P. maniculatus* species group. These findings add to a growing number of studies that have identified lineages occupying different environmental spaces. At a regional scale, I supported the hypothesis that barrier islands on the Atlantic coast of Florida were colonized by an ancestral form of *P. polionotus* by a single colonization, from the central Florida area. Subsequently, at least two distinct lineages diverged (*P. p. phasma* and *P. p. niveiventris*). I also found evidence that suggests that the extinct form of beach mouse (*P. p. decoloratus*) is part of the *P. p. phasma* lineage. At the population level, I evaluated changes in genetic diversity in historical samples compared to those that experienced recent human encroachment on natural habitat I used tissue preserved in natural history collections to compare with live-trapped specimens, and found that *P. p. niveiventris* has maintained historical genetic diversity levels. I suggest that the continuation of historical levels of genetic diversity is due to the presence of a single large area

of continuous habitat in the central portion of the species' current distribution. Finally, I evaluated the importance of scrub and beach habitat to the population dynamics of beach mice. Beach mice have traditionally have been associated with beach dunes rather than with the scrub habitat found more inland on barrier islands. Using almost three years of capture-recapture data from Cape Canaveral Air Force Station (CCAFS), I created a stochastic matrix model to assess the relative contribution of populations from the two different habitats to a variety of demographic measures. Both field data and model results provided evidence that the population dynamics of beach mice may rely much more on scrub habitat than formerly documented. Overall, my research emphasized a hierarchical approach to evaluate biodiversity and the processes shaping differentiation at different spatial and temporal scales. The methods and findings give insight into speciation at different scales, and can be applied to a wide range of taxa for questions related to evolutionary and conservation biology.

To my wife for her support and patience

and to my son for the joy he brings

ACKNOWLEDGMENTS

As I am coming close to the end of one chapter of my life, I realize that many people contributed in diverse ways to bring me to where I am today. In these last few years I have been privileged to work with a wide range of scientists and friends, and all have left their mark on how I view science and nature around us. I want in particular want to thank my committee chairs, Jack Stout and Christopher Parkinson, for providing me with the resources and freedom to work on my own project, and for helping me fine tune my ideas and questions along the way. In particular I want to share my gratitude for Jack's many hours helping me with writing and grammar, and the occasional socio-political discussions. I also want to thank Chris for the opportunities I have had in his lab, such as meeting my wife. I wish to thank my committee members, Eric Hoffman, John Weishample and Terry Doonan, for taking their time to commit to be part of my committee. In particular I want to thank Eric for all his advice on analysis and writing, John for introducing me to population modeling, and Terry for the many samples he has provided for my research.

During my time at the University of Central Florida I have been lucky to be surrounded by peers that have been of great help shaping my research ideas and providing me with invaluable feedback on my work. I want to thank Juan Daza for all his advice and help throughout my doctoral research as a friend and mentor. In addition I want to thank several current and former Parkinson lab members: Todd Castoe, Allyson Fenwick, Josh Reece, Jack Degner, and Greg Territo, for all their help and support in getting me to where I am today. Overall, all members of the pH lab have contributed, with their feedback and suggestions in shaping of my research. I also wish to thank Mary Beth Manjerovic, Lisa McCauley and

Kimberly Medley, for our many discussions on teaching, science, academia and life in general. I want to also express my gratitude to professors David Jenkins and Pedro Quintana-Ascencio for their insights into the quantitative world, and helping me understand appropriate uses of statistics. Additionally, I wish to thank Sara Elliott, Gayle Story and Karen Reinemund for taking care of all the paperwork associated with being a student, especially an international student.

During my time in Orlando I have also gotten the chance to interact with people outside of the University of Central Florida, which I feel should be mentioned. I wish to also share my gratitude to Donna Oddy, Shanon Gann and Alice Bard, for giving me the opportunity to work in the field. Getting the opportunity to learn from their field expertise and chance to test out new field tools and techniques has provided me with new insight and appreciation of field work.

Most of all I want to thank my beautiful wife, Jenna Kalkvik, for all her patience, support, dedication, and most of all love. She has pushed me when I needed pushed, supported me when I needed support, and she has always been there for me. I know that without her I would not be where I am today, and I will be eternally indebted to her for all the time and energy she has put in to ensure I had the chance to move forward. While only recently arrived, I wish also want to thank my incredible son, Elias Kalkvik, because his presence is an inspiration for me, and nothing feels better than to come home to a little boy with a big smile on his face.

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CHAPTER 1. INTRODUCTION

The unifying goal of conservation biology is to preserve biodiversity and the natural processes that shape and maintain that diversity (Moritz 2002, Groom et al. 2005). Therefore, to address questions on how biodiversity is maintained it is necessary to identify patterns of diversity and the processes that have shaped such patterns. Diversity can be organized hierarchically from populations to communities, with each level encompassing a broader spatial scale and posing different challenges and questions. Along this hierarchy we can gain insight into the formation of diversity and persistence at multiple taxonomic, spatial and temporal scales (Noss 1990, 2005). To this end my interests are to understand the evolutionary drivers of biodiversity organization, and how it is maintained at multiple levels. Specifically, I have investigated biodiversity at multiple spatial and taxonomic scales, to better understand how evolutionary process impacts organisms at multiple scales. At a continental spatial scale I investigated the drivers of genetic divergence that occurs in widely distributed taxa, to gain insight into how environmental differences can set the stage for an evolutionary process that shapes their current biodiversity. Evolutionary processes can also influence diversity at a regional scale, e.g., following the isolation of populations exposed to differential selective pressure. I was, in particular, interested in how at a local scale evolutionary processes affect biodiversity subjected to recent colonization. Coastal habitat provides an ideal setting for investigating patterns of speciation at a local scale, as sea level rise has dramatically changed coastal landscapes. At the population level I evaluated how taxa are impacted by historical and current evolutionary processes, and how local diversity may persist in diverse habitats.

Environmental impact on wide spatial scale

The impacts of ecological drivers on evolutionary divergence are not well understood, either as direct selective drivers or as a means of maintaining isolation between geographically divided lineages (Schluter 2001, Wiens and Graham 2005, Schluter 2009). By investigating taxa at a broad spatial scale, ecological drivers should be more evident, as taxa are faced with a wide range of environmental conditions in different habitat settings. The ecological impact on lineage divergence and speciation is closely associated to the niches sister taxa occupy, where niche is defined as the multi-dimensional ecological space that bounds a species' persistence (Hutchinson 1957, MacArthur 1972, Hutchinson 1978). Predictions based on this interpretation of the ecological niche suggest diversity is predominantly explained by two prevailing hypotheses: niche conservatism and niche divergence. Niche conservatism occurs when lineages retain ancestral ecological affiliations and closely related lineages persist in similar ecological niches. This is thought to be caused by stabilizing selection or lack of variation in ancestral traits (Lord et al. 1995, Webb et al. 2002, Wiens and Graham 2005). Several studies have found empirical support for the niche conservatism hypothesis (Peterson et al. 1999, Peterson and Vieglais 2001, Kozak and Wiens 2006). The alternative hypothesis, niche divergence, predicts that sister lineages occupy different niches. Examples are accumulating where phylogenetic relatedness is not related to niche similarity and sister taxa occupy separate niche spaces, suggesting an environmental driver on lineage divergence and the process of speciation (Losos et al. 2003, Graham et al. 2004, Raxworthy et al. 2007, Pyron and Burbrink 2009). Relationships in niche space primarily measures conditions at a fixed point in time; however, an important environmental driver is historical climatic change (Hewitt 2004, Martínez-Meyer et al. 2004, Oberle and Schaal 2011).

Climatic changes and recent habitat: divergence at smaller scales

Biodiversity has been greatly impacted by global events that cause dramatic temporal changes in climatic conditions (Aubry et al. 2009, Wilson and Eigenmann Veraguth 2010). In North America and Europe, glaciation has been shown to impact speciation and population level processes (Hewitt 1996, Lomolino et al. 2005, Aubry et al. 2009, Previšić et al. 2009, Benke et al. 2011). The most recent ice age, which occurred around 20,000 years ago, had a great impact on the distribution and diversity of extant taxa (Hewitt 1996, Hewitt 2000, Hewitt 2004, Lomolino et al. 2005, Morgan et al. 2011). The retreat and displacement of organisms from the glaciated portions of Europe, Asia, and North America are well documented (Hoffman and Blouin 2004, Rowe et al. 2004, Svenning and Skov 2007); whereas, little attention has been given to shifts in the biota of coastal areas as sea levels fluctuated with the end of glaciation (Van Zant and Wooten 2007). In Florida sea levels were 130 meters below current stands at the time of maximum glacial accumulation, with shore lines over 100 km of current shore lines of the Gulf coast (Wanless and Parkinson 1989, Davis 1997, Lambeck and Chappell 2001). Flooding and reshaping of coastal Florida slowed down between 5,000-6,000 years ago as contemporary coast lines stabilized. Barrier islands of the Atlantic and Gulf coasts of Florida were likely in place in the same time period (Wanless and Parkinson 1989, Mayhew and Parkinson 2007, Madsen et al. 2010, Kolditz et al. 2012). Given the complex history of climate change and habitat modification in coastal areas, current biodiversity in these areas must be examined from the viewpoint that both historic and recent evolutionary processes likely explain genetic patterns within taxonomic groups.

Temporal sampling of genetic diversity

In recent time humans have been considered the main culprit in fostering the elevated rate of extinction often associated with habitat loss and genetic deterioration (Schwartz et al. 2007, Wake and Vredenburg 2008, Helm et al. 2009, Barnosky et al. 2011). Humans affect the persistence of populations at a local scale by reducing habitat availability, suitability, and connectivity (Fahrig and Merriam 1994, Fahrig 2003, Dixo et al. 2009). Genetic diversity can be used to index the condition and persistence of threatened and endangered taxa. The reason for this viewpoint is smaller populations often exhibit loss of genetic diversity as a result of genetic drift (Lacy 1987, Frankham 1997), and lowered genetic diversity has been shown to increase the likelihood for a population to go extinct (Saccheri et al. 1998, Reed and Frankham 2003, Lavergne and Molofsky 2007). Loss of genetic diversity is also a sensitive measure of loss of population size (Schwartz et al. 2007).

Several studies have shown that low genetic diversity may be historic and not reflect recent human influences (e.g. Miller and Waits 2003, Taylor et al. 2007, Reding et al. 2010). Thus it is essential to identify what process has shaped current genetic diversity, humans or natural events prior to human influence, before making decisions regarding genetic augmentations or recoveries. To gain insight into the processes that have helped shape genetic diversity we can utilize extensive tissue collections found in museums of natural history (Wandeler et al. 2007, Leonard 2008). Samples collected prior to humans' impacts offer a historic reference condition to compare with the current genetic diversity. In cases where we identify loss of genetic diversity based on natural events, other threats may be more important for the future persistence of a taxa (Miller and Waits 2003). If we can identify human caused loss of

genetic diversity, possible genetic augmentation or other genetic considerations may be necessary to ensure the future of such taxa (Frankham 2010).

Population dynamics in a variable landscape

The landscape a taxon occupies is heterogeneous at many scales (Lord and Norton 1990), and such heterogeneity can possibly influence the persistence of populations. Demographic performance, such as survival and fecundity, can vary based on habitat quality; therefore, a population's risk of experiencing disturbance events also may vary as a function of differences in habitat quality (Sturtevant et al. 1996, Brachet et al. 1999, Heinrichs et al. 2010). Understanding the importance of habitat quality in driving population dynamics, and how these habitats are utilized over time informs our knowledge of management needs (Olson et al. 2004, Heinrichs et al. 2010).

Field data on habitat use provides insight into building more realistic population models that successfully capture and evaluate relative contributions of life stages and habitat features in population persistence (Pascarella and Horvitz 1998, Picó et al. 2002, Olson et al. 2004, Heinrichs et al. 2010). Such quantitative approaches have been recognized as important tools for use in making management choices, and can provide information to justify management of appropriate habitats to ensure persistence of populations (Beissinger and McCullough 2002, Morris and Doak 2002, Olson et al. 2004).

Small rodent as a model system

In my research I use the *P. maniculatus* species group, with special emphasis on the subspecies of *P. polionotus*, as my model system. A number of features of this system make it tractable for testing patterns of diversity and processes shaping diversity at multiple taxonomic

and spatial scales. The *P. maniculatus* species group is distributed throughout North America (Hall 1981, Carleton 1989; Fig. 1.1), with five species recognized within the group: *P. maniculatus*, *P. polionotus*, *P. melanotis*, *P. keeni* and *P. sejugis* (Chirhart et al. 2005). Of these species, *P. maniculatus* is the most widely distributed and occupies a diverse array of habitats (Greenbaum et al. 1978, Carleton 1989). Historically, *P. maniculatus* was used as an example of centrifugal speciation, being a widely distributed central species, with closely related species with more restricted distributions at its periphery (Blair 1950, Brown 1957). This species has also been recognized for its morphological and geographical variation, which is reflected in the recognition of 67 subspecies by Hall (1981). Furthermore, many of these morphological differences have been associated with differences in habitat affinities among the subspecies (Blair 1950, Carleton 1989). Genetic markers have shown further evidence of geographic partitioning of this widely distributed species (Avise et al. 1983, Lansman et al. 1983, Dragoo et al. 2006, Kalkvik et al. 2012). The peripheral species in this group are of more limited distribution. *Peromyscus polionotus* is isolated from *P. maniculatus* and is found in the piedmont and coastal plain of the southeastern U.S.A.; *P. melanotis* is parapatric to *P. maniculatus* in mountain conifer forest areas of Arizona, U.S.A., and central Mexico (Greenbaum et al. 1978). *Peromyscus keeni* is found in northwestern North America, and was recently recognized as a species based on genetic variation (Hogan et al. 1993). *Peromyscus sejugis* is restricted to two islands in Baja California (Fig. 1.1; Alvarez-Castañeda 2001). The diversity within the *P. maniculatus* group makes this an excellent model system for addressing diversification at a broad scale.

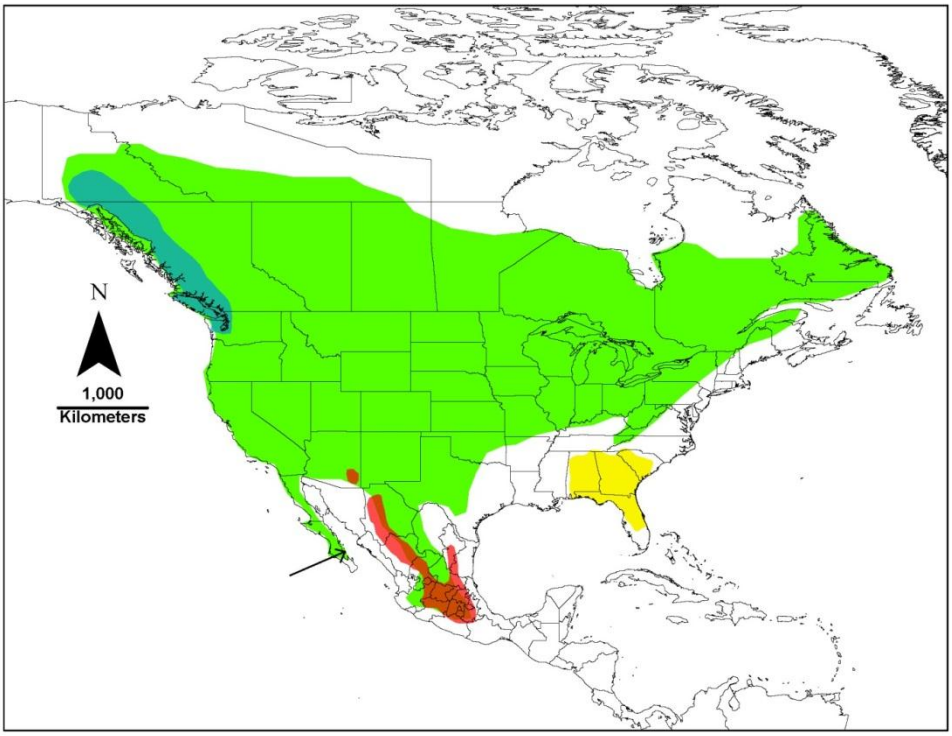

Figure 1.1 Distribution of members of the *Peromyscus maniculatus species* group: *P. maniculatus* (green), *P. keeni* (blue), *P. melanotis* (red), *P. polionotus* (yellow) and *P. sejugis* (black arrow).

Peromyscus polionotus (old field mouse) has been recognized to have 16 subspecies (Hall 1981) and provides a system to address questions on the processes that shape biological diversity at the species and population level. This species is nocturnal, monogamous, and semi-fossorial. The elaborate burrow system limits its distribution to areas with sandy soil in the piedmont and coastal plain, particularly in fallow fields, open scrub, sand hill, and coastal dunes systems (Fig. 1.2; Bowen 1968, Hall 1981, Whitaker and Hamilton 1998). The burrowing behavior has been found to have a strong genetic component in *Peromyscus* species (Weber and Hoekstra 2009). The IUCN red list (accessed July 2012), categorizes subspecies of *P. polionotus* distributed on the mainland of least concern. These subspecies are locally abundant with no recognized threats. In contrast, subspecies of *P. polionotus* distributed along Atlantic and Gulf coastal barrier islands, collectively called beach mice, are under threat primarily from habitat

loss (Ehrhart 1978, Humphrey and Barbour 1981, Humphrey 1992, Stout 1992). Currently seven extant and one recently extinct subspecies are recognized along the barrier islands (Fig. 1.2; Hall 1981). All but one of the extant beach mouse subspecies are listed as threatened or endangered by the U.S. Fish and Wildlife Service. Range reduction and decline in number of populations has been attributed to predation by domestic cats and to loss of coastal habitat as a result of urban development (Ehrhart 1978, Humphrey and Barbour 1981, Humphrey 1992, Stout 1992). In addition, all beach mouse subspecies are vulnerable to severe storms (Holler 1992).

Phenotypically beach mice are distinguished by having lighter pelage color than the mainland subspecies (Sumner 1926, Bowen 1968, Whitaker and Hamilton 1998). The lighter pelage has been attributed to differential selective pressures on the mainland compared to barrier islands, where lighter often white soil is found (Hoekstra et al. 2006, Steiner et al. 2007, Mullen and Hoekstra 2008, Mullen et al. 2009). Some have argued that divergence of these beach mice from an ancestral *P. polionotus* has been driven by this selective pressure (Hoekstra et al. 2006, Steiner et al. 2009). Others have argued that divergence could have been caused by random genetic drift (Van Zant and Wooten 2007). Regardless of the evolutionary mechanism that has led to the formation of these distinct beach subspecies, the processes that shaped the distinct phenotypic variation must have occurred within the last 5000-6000 years, as the coastal habitats they occupy were formed in the same time period (Wanless and Parkinson 1989, Parkinson and White 1994, Mayhew and Parkinson 2007).

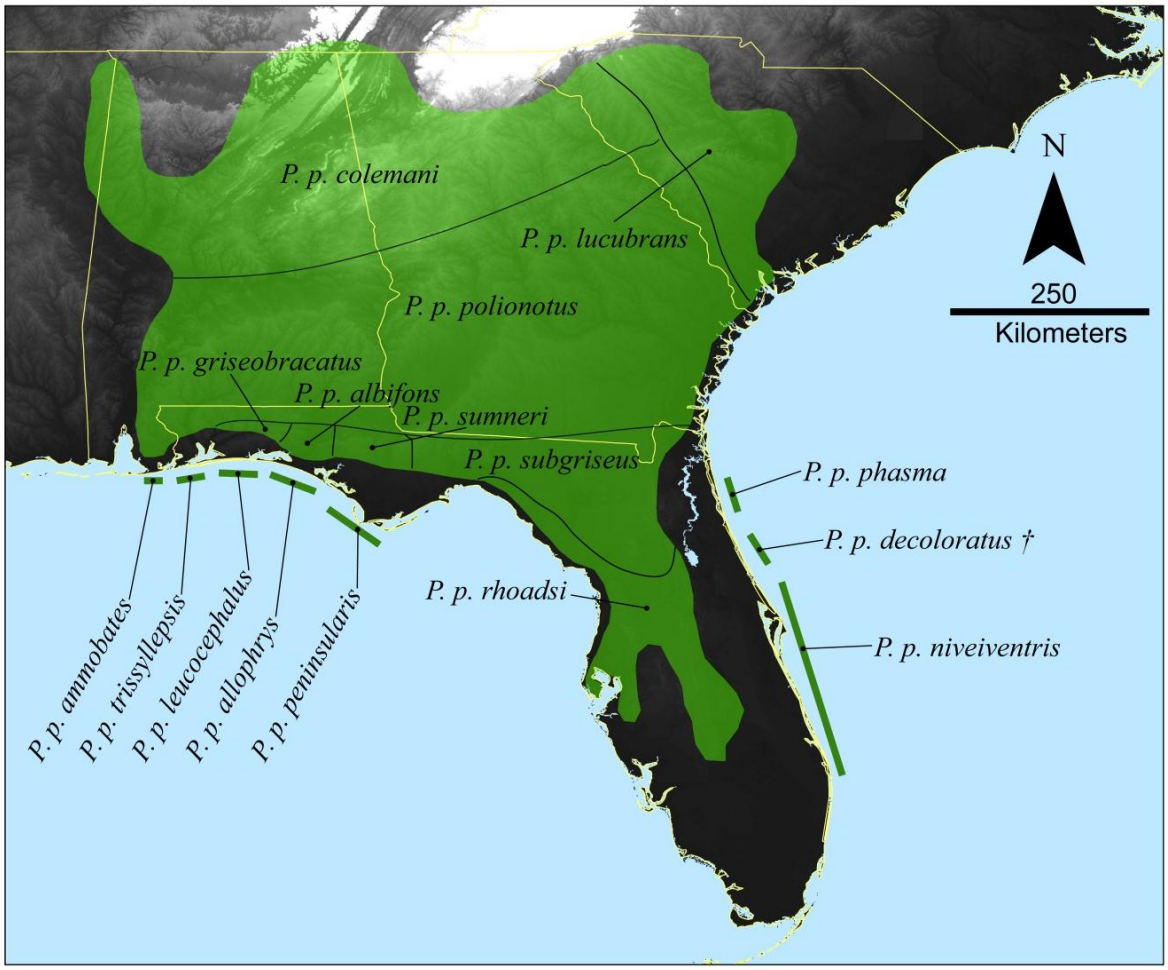

Figure 1.2 Distribution of *Peromyscus polionotus* across south-eastern U. S. Subspecies designations are based on Hall (1981). Beach mice subspecies are distributed along Florida and Alabama barrier islands. † indicates recently extinct taxon.

Goal of study

In my doctoral research I addressed ecological and evolutionary questions related to the origin and persistence of mammal diversity and the effects of niche occupancy and habitat quality at different spatial scales. My research is organized into four chapters, and addresses diversity and persistence at species, subspecies and population levels. I have tested hypotheses related to four goals. First, I began at a larger spatial scale, where I tested the relationships between phylogenetic lineages and climatic environment for the widely distributed *P. maniculatus* species group (Chapter 2). My results showed that diversity within this species

group is greatly associated with differences in the environment they occupy. My second goal was to address the evolutionary history of subspecies found on the Atlantic coast and to determine how they are related to the mainland populations (Chapter 3). This research provided new insight into the formation of the Atlantic coast beach mouse subspecies and the genetic relationship among both extant and extinct subspecies. My third goal was to test the impact of habitat loss and coastal development on the genetic diversity of *P. p. niveiventris* (Chapter 4). This subspecies has experienced an extensive range reduction, and would be a good candidate for showing reduced genetic diversity associated with human influences. I obtained samples of *P. p. niveiventris* collected in the 19th and early 20th centuries from natural history museums to test if genetic diversity prior to major human developmental pressures was greater than we observe today. The results from this study provide greater insight into the historical levels of genetic diversity in this subspecies, as well as providing alternatives for future management of this and potentially other listed taxa. In my fourth goal, I tested the importance of heterogeneous habitat on populations of *P. p. niveiventris* (Chapter 5). Using demographic and model-derived data, my research provides important findings on the likely role of scrub habitat and the population dynamics and persistence of this subspecies. The findings in this chapter will inform the conservation and management of any taxon that occupies juxtaposed habitats with compositional and structural differences.

Overall my research provides insight into the impact of the natural and anthropogenic environment upon which mammal diversity depends. In addition, I provide new information on the evolution and persistence of taxa occupying a dynamic landscape in a changing world.

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CHAPTER 2. INVESTIGATING NICHE AND LINEAGE DIVERSIFCATION IN WIDELY DISTRIBUTED TAXA: PHYLOGEOGRAPHY AND ECOLOGICAL NICHE MODELING OF THE *PEROMYSCUS MANICULATUS* SPECIES GROUP[1]

Introduction

A fundamental question in speciation concerns the influence of the ecological niche on lineage divergence. Most of the current discussion on the relationship between lineage divergence and the ecological niche is dominated by two prevailing hypotheses: niche conservatism and niche divergence. Niche conservatism predicts that closely related taxa retain ancestral ecological affiliations and persist in similar environments. This may be caused by stabilizing selection or lack of variation in ancestral traits (Lord et al. 1995, Webb et al. 2002, Wiens and Graham 2005), but niche conservatism is primarily a pattern of evolution and by itself does not explain causality (Losos 2008). Empirical work has shown that divergence of allopatric sister taxa is often characterized by niche conservatism, whereby geographic isolation influences the pattern of speciation without shifts in niche dimensions (e.g., Peterson et al. 1999, Peterson 2001, Kozak and Wiens 2006). The alternative hypothesis, niche divergence, predicts that sister taxa will occupy different niches. Under this hypothesis, being adapted to different environmental conditions can promote lineage divergence, even in sympatry. Evidence is accumulating that sister taxa often exhibit niche divergence and that niche evolution may be more common than initially assumed (e.g. Losos et al. 2003, Graham et al. 2004, Pyron and Burbrink 2009, Dormann et al. 2010). Differences in niche space can be observed in recently diverged taxa, as researchers have found that niches can shift in as little as 104-105 years (Evans

[1] Kalkvik, H. M. et al. 2012. Investigating niche and lineage diversification in widely distributed taxa: phylogeography and ecological niche modeling of the *Peromyscus maniculatus* species group. — Ecography 35: 54-64.

et al. 2009). In these cases, the environment may function as a barrier to gene flow if two or more allopatric lineages are separated by suboptimal conditions (Rissler and Apodaca 2007). Alternatively, adaptation to different local and regional environmental conditions may prevent lineages from interacting, thus reducing gene flow even when distributed in sympatry or parapatry (Wiens and Graham 2005).

Widely distributed species are ideal for studying the relationship between lineage divergence and niche divergence. Such taxa often show patterns of genetic or phenotypic structure along environmental gradients, as heterogeneous landscapes tend to foster adaptation to distinct niches (Avise et al. 1987). In North America and Europe, many widespread species are divided into distinct lineages that have either allopatric or parapatric distributions (e.g., Taberlet et al. 1998, Hoffman and Blouin 2004, Fontanella et al. 2008). However, little attention has been given to the possible influence that the environment might have on the diversification and maintenance of these lineages.

Phylogeography has been widely used to identify diverging lineages in a spatial context (e.g., Lansman et al. 1983) and is currently the most utilized tool for investigating the connection between micro- and macroevolutionary processes related to speciation (Hickerson et al 2010). This approach identifies evolutionary relationships and the spatial component of the formation and maintenance of biodiversity (Avise et al. 1987, Avise 2000). This spatial component has usually been limited to geographic distances, which unfortunately ignores a great deal of environmental complexity that may impact taxa (Graham et al. 2004). Recent studies have combined phylogeography and ecological niche modeling (ENM) to understand the relationship of distributions and niche spaces with speciation and the maintenance of genetic variation (Graham et al. 2004, Rissler and Apodaca 2007, Jakob et al. 2009, Pyron and Burbrink 2009).

Ecological niche modeling for taxa with wide distributions can help identify contact zones between lineages, quantify their spatial overlap, and explore the possible nature of their isolation (Kozak et al. 2008).

Our application of ENM parallels species distribution models (SDM), and predicts spatial distributions of taxa based on occurrence records and environmental data (Elith and Leathwick 2009). The predicted spatial distribution gives an estimate of the ecological niche: the multi-dimensional environmental space that bounds a species' persistence (Hutchinson 1978). Utilizing the ENM approach facilitates the testing of correlations between lineage diversification and the environmental setting those lineages occupy.

To test for the association between environmental conditions and lineage divergence, we used the widely distributed mammal species *Peromyscus maniculatus* and the more narrowly distributed *P. polionotus* as a model system. These taxa belong to a species group first defined by Osgood (1909) and are distributed throughout North America (Hall 1981, Carleton 1989). Diverse habitat conditions occur over the geographic distribution of *P. maniculatus*, which may have led to isolation of populations. Phylogeographic lineages have previously been identified in *P. maniculatus* (Lansman et al. 1983, Dragoo et al. 2006, Gering et al. 2009), and morphotypes associated with different habitats have also been observed (Blair 1950, Carleton 1989). Morphological variation is further reflected in the recognition of 67 subspecies within *P. maniculatus* (Hall 1981). The closely related species *P. polionotus* is restricted to the southeastern United States, where it is hypothesized to have diverged from a grassland form of *P. maniculatus* (Carleton 1989). Until recently, limited data were available to address the origin of *P. polionotus*. Through the efforts of this and other research (Dragoo et al. 2006, Degner et al. 2007, Van Zant and Wooten 2007, Gering et al. 2009), we compiled a comprehensive dataset for

these two species to evaluate the evolutionary relationships of these taxa and to explore the relationship of environment to lineage divergence between and within these species. Thus we: (1) inferred phylogeographic relationships among individuals of *P. maniculatus* and *P. polionotus*, (2) established whether phylogeographic lineages occupy distinct climatic niches, and (3) predicted distributions of each lineage to determine potential overlap and contact zones.

Materials and Methods

Taxon Sampling

We assembled a cytochrome *b* (cyt *b*) data set using 478 samples that were obtained throughout the known range of *P. maniculatus* and *P. polionotus*. A total of 343 *P. maniculatus* (DQ385628-DQ385827; EF666142-EF666277; EU006766-EU006772), 38 *P. polionotus* (EF216336-EF216347; EU140757-EU140793), 2 *P. keeni* (DQ385716 and EU140797), and 5 *P. melanotis* (DQ385626 and EU574689-EU574701) sequences were obtained from GenBank (Dragoo et al. 2006, Degner et al. 2007, Van Zant and Wooten 2007, Gering et al. 2009). Additionally, 89 tissue samples of *P. polionotus*, representing 9 populations, were collected from peninsular Florida. Published sequences from two additional species were included as outgroups: *P. gossypinus* (DQ385625; Dragoo et al. 2006), and *P. leucopus* (AF131926; Bradley et al. 2000).

DNA Extraction and Sequencing

DNA from collected samples was extracted using a DNeasy tissue kit (Qiagen). We amplified the complete mitochondrial (mtDNA) cyt *b* gene using the primers 14724F and TD-20 (Van Zant and Wooten 2007). PCR conditions followed Herron et al. (2004). Sequencing was conducted by the Nevada Genomics Center (Reno, Nevada, United States) using an ABI 3730

sequencer and chromatograms were edited using Sequencher v.4.7 (Gene Codes Corp.). Alignments of the sequences were made by visual inspection using GeneDoc v.2.6 (Nicholas et al. 1997). GenBank accession numbers for sequences generated by this study are listed in Table 2.1.

Phylogenetic Analysis

We identified haplotypes and reduced redundancy in the data with TCS (Clement et al. 2000), which estimates a haplotype network with maximum parsimony. The output includes a list of individuals with the same haplotypes, and only one representative of each haplotype was included in the final alignment. Phylogenetic analyses were conducted using Bayesian inference (BI; MrBayes v.3.1.2; Huelsenbeck and Ronquist 2001) and maximum likelihood (ML; RAxML v.7.0; Stamatakis et al. 2008). We used Bayes factors to determine the best partitioning strategy in BI (Brandley et al. 2005). Preliminary analyses yielded a two-partition model, with 1^{st} and 2^{nd} codon positions together and the 3^{rd} position separate. Substitution models for each partition in BI and ML were identified by MrModelTest v.2.4 using the Akaike Information Criterion (Nylander 2004). For BI we initiated two independent Markov Chain Monte Carlo (MCMC) runs, each with four chains, and ran them for 10^7 generations, sampling every 1000 generations. Using Tracer v.1.4 (Rambaut and Drummond 2007), we determined stationarity and conservatively discarded 2×10^6 generations as burn-in. We estimated a ML phylogeny using RAxML v.7.0 (Stamatakis et al. 2008), on the Cipres Portal v.2.0 (http://www.phylo.org/portal2). We determined bootstrap values (BS) for ML using 100 pseudo-replicates. We defined lineages based on monophyly and with individuals inhabiting a geographically distinct area (Wiens and Penkrot 2002).

Table 2.1. Cyt *b* haplotypes from the 9 new sampled locations of *P. polionotus* used in phylogeographic analysis. Two haplotypes (EF) were the same as reported by Degner et al. (2007).

Location	Genbank Accession#
Pelican Island National Wildlife Refuge, Indian River County, FL	EF216337
Archbold Biological Station, Highlands County, FL	EF216346, JF322885, JF322886, JF322887
Avon Park Air Force Range, Highlands County, FL	JF322885, JF322887
Suwannee Ridge WEA, Suwannee County, FL	JF322893, JF322894, JF322895, JF322896
Ocala National Forest, Marion County, FL	JF322888, JF322889, JF322890, JF322891, JF322892
Anastasia State Park, St. Johns County, FL	JF322897
Fort Matanzas, St. Johns County, FL	JF322897
Crescent Beach, St. Johns County, FL	JF322897
Frank Butler Park, St. Johns County, FL	JF322897

Taxon and Environmental Sampling for Estimating Climatic Niche

We estimated the niche using two approaches. First we utilized multivariate statistics on climate conditions at lineage occurrence points, which provided information on the climatic niche based on the variables alone. Second we conducted niche modeling, which projects the climatic niche across a geographic region. For both approaches we included the lineages recovered in the phylogenetic analysis of *P. maniculatus* and *P. polionotus* that had more than five location points. We georeferenced our genetic samples based on museum voucher locations, locale descriptions from original papers, and personal communication with the authors of original papers (Dragoo et al. 2006, Degner et al. 2007, Van Zant and Wooten 2007, Gering et al. 2009). Our analysis included only one sample per location.

We utilized climatic variables obtained from the WorldClim database with a resolution of 30 arc-seconds (Hijmans et al. 2005). As temperature and precipitation can impact the metabolic

rate of *Peromyscus* species (MacMillen and Garland Jr 1989), climate could provide insight into the spatial distribution of *Peromyscus* species. Additionally, we incorporated an altitude layer from the Shuttle Radar Topography Mission (SRTM) data set, as *Peromyscus* species exhibit different torpor patterns depending upon the elevation they occupy (Tannenbaum and Pivorun 1984).

Some of the environmental variables are highly correlated and could over-parameterize the models. To reduce over-parameterization we identified and eliminated correlated variables using the methods of Rissler and Apodaca (2007). We extracted climatic and altitude data across North America for 10^5 randomly generated points; for each pair of variables we estimated Pearson correlation coefficients using JMP v.8.0 (SAS Institute Inc.). For those pairs of variables that exceeded our threshold of $r = 0.75$, we included the variable which was most biologically meaningful. We incorporated 12 variables, one quantifying altitude and the others quantifying variation in temperature and precipitation: annual mean temperature, mean diurnal temperature range, isothermality, annual temperature range, mean temperature of wettest quarter, mean temperature of coldest quarter, seasonal precipitation, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter.

Estimation of Climatic Niche by Occurrence Points

We tested for significant differences in the climatic niche based on occurrence points of all lineages using two methods. First, we used parametric multivariate statistics to compare the multi-dimensional climatic values between sample locations. We extracted climatic and altitude values for each georeferenced genetic sample using ArcGIS v.9.2 (ESRI, Redlands, CA, USA). We square-root transformed those variables not found to be normally distributed: altitude, mean

diurnal temperature range, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter. We evaluated homogeneity of variances based on Levene's test conducted in SPSS v.18.0, and all variables met this assumption. Overall significant differences among the climatic spaces that the lineages occupy were determined using a multivariate analysis of variance (MANOVA) in SPSS v.11.0.

As a second approach to compare climatic niches among lineages, we used parametric discriminant analysis (DA). Discriminant analysis maximizes explained variation based on *a priori* defined groups and ordinates the variables at occurrence points. Ordination reduces the number of variables needed to explain the variation among the groups, and removes collinearity among ordination axes for subsequent analysis. For the DA analyses, groups were defined as the lineages inferred by phylogenetic analysis. Canonical scores (CV) for the DA were determined in JMP v.7.0. To determine differences in CV among lineages, we estimated the centroid and 95% confidence interval (CI) for each lineage. Lack of overlap of the 95% CI in at least one of the CV axes suggested deviation in the environmental space we estimated for the lineages.

Testing for Spatial Autocorrelation

Differences in environmental space among phylogenetic lineages can be the result of spatial autocorrelation. We accounted for this potential bias by performing a partial Mantel test to assess the correlation between environmental and genetic differences while controlling for geographic distance. Environmental differences that were positively correlated with genetic divergence, independent of geographic distance, would suggest that genetic divergence is truly associated with changes in the environment. Statistical significance was estimated based on 999 permutations, with $\alpha = 0.05$, using the vegan v.1.13 package in R v.2.6 (Oksanen 2009). Environmental differences were estimated as the Euclidian distance of CVs among sample

locations. We calculated genetic distance with MEGA v.4.0 (Tamura et al. 2007) using a Kimura-2-parameter model and gamma-distributed rate variation. Geographic distance was estimated using the package fields v.4.1 in R (Fields Development Team 2006).

Estimation of Climatic Niche by Niche Modeling

The analyses above are limited to point locations, therefore we used a niche model to project the environmental space utilized by a lineage and to test for environmental and geographic association among and between phylogenetic lineages. We created niche models with Maxent v.3.2, which estimates distributions based upon niche characteristics using a maximum entropy algorithm (Phillips et al. 2006, Phillips and Dudík 2008). Maximum entropy is useful for estimating distributions when absence data are lacking (Phillips et al. 2006) and generally performs better than other distribution modeling approaches (Elith et al. 2006, Wisz et al. 2008). Maxent incorporates a method of regularization for selecting environmental variables when building models. This reduces the need to select environmental variables to avoid over-parameterization, yet some variable selection is recommended to reduce collinearity (Elith et al. 2011). To increase the sample size for creating the model, we included additional locations from natural history museums. Locations were provided by Field Museum of Natural History, Cornell University, Florida Museum of Natural History, Los Angeles County Museum of Natural History, Louisiana State University Museum of Zoology, Museum of Southwestern Biology, Mississippi State University, Museum of Vertebrate Zoology, Paleobiology Database, Santa Barbara Museum of Natural History, University of Alaska Museum of the North, University of Colorado Museum of Natural History, University of Washington, and Yale Peabody Museum (Accessed through GBIF Data Portal, www.gbif.net, 20 April 2009). A minimum convex polygon (MCP) was created using known occurrence points from each phylogenetic lineage, and

museum specimens for locations within each MCP were assigned to the appropriate lineage. A GIS shape file of the locations used in the analysis is available upon request.

The occurrence data were randomly partitioned into training (75%) and test (25%) datasets to evaluate the accuracy of each model. We determined model accuracy by calculating the area under the curve (AUC) of the receiver operating characteristic (ROC) plot (Phillips et al. 2006). In Maxent the AUC is the probability, ranging from 0 to 1, that a random presence location is ranked above a random background site (Phillips et al. 2006). A value of 1 implies a perfect fit, 0.5 no different than random, and < 0.5 suggests the model performs worse than random expectation. Models with AUC values above 0.75 are considered good, and models with AUC > 0.90 are considered excellent (Swets 1988, Elith 2002). We determined the best model for each lineage in Maxent by running iterations until the probability of change was 1.0×10^{-5}, or after a maximum of 500 iterations. To identify contact zones and quantify levels of sympatry or parapatry, we estimated the spatial overlap in predicted distributions between all genetic lineages included in the analysis. We used the minimum training presence, which is the minimum probability of occurrence in the modeled distribution, for each model as the threshold to create maps of suitable climatic niche for each lineage. Overlap between two lineages was calculated as the proportion of cells where conditions were predicted to be suitable for both lineages. All estimates of overlap were conducted using ArcGIS v.9.2.

We utilized the niche equivalency method proposed by Warren et al. (2008) to determine differences in the niche between lineages based on entire distributions. We first measured niche overlap between distributions by calculating two indices: Schoener's *D* (Schoener 1968), and *I* (Warren et al. 2008). Both measures give pair-wise niche overlap with values ranging from 0 (no overlap) to 1 (identical niche models). Then, we used the niche equivalency test in ENMtools

(Warren et al. 2008) to determine niche differences. This is a one-tailed test which determines if niche models are significantly dissimilar from random by comparing the observed *D* and *I* to a null distribution. The null distribution is derived from pseudo-replicate distributions by randomly assigning lineage membership to the occurrence data for two lineages. Subsequently, *D* and *I* are estimated for the resulting models. This is repeated several times to create the null distribution for *D* and *I* (Warren et al. 2008). We compared all pair-wise combinations of lineages to assess differences in both sister and non-sister taxa. We calculated *D* and *I* for each comparison using 100 pseudo-replicates. Because the spatial scale of this study caused computational constraints in estimating the null distributions of *D* and *I*, we reduced the spatial resolution of the environmental layers to 2.5 minutes when inferring the pseudo-replicate niche models as suggested by Pyron and Burbrink (2009). However, changing the scale of the environmental layers has the potential to significantly change the estimated models (Guisan et al. 2007). To ensure that changing the resolution of the environmental layers did not result in overestimating niche similarity of the null distribution, we estimated *D* and *I* between each lineage for niche models created using the lower resolution climatic and altitude data.

Results

Phylogenetic Analysis

The cyt *b* sequence alignment consisted of 1154 base pairs with 274 (23.7%) parsimony informative characters. The sample consisted of 350 haplotypes representing the majority of the geographical distribution of *P. maniculatus* and *P. polionotus*. We identified 13 new cyt *b* haplotypes from the 89 *P. polionotus* individuals sampled for this study (Table 2.1).

GTR+I+G was the optimal nucleotide substitution model for both partitions as determined by MrModelTest. BI and ML methods produced similar topologies, but ML did not resolve deeper divergences in the phylogeny. We identified 6 lineages within *P. maniculatus* (Fig. 2.1) that were distinct monophyletic groups located in geographically distinct areas. Lineage 1 was a well supported clade (posterior probability (PP) = 1.00; bootstrap (BS) = 70) associated with the Pacific coast and Rocky Mountains (lineage 1; Fig. 2.1 and Fig. 2.2), and its sister lineage was restricted to the grasslands of the central United States (lineage 2; Fig. 2.2). Lineage 2 was less well supported (PP = 0.65; BS = 74; Fig. 2.1), but due to their allopatric distributions we consider lineages 1 and 2 as distinct. Two strongly supported sister lineages were identified in northeastern North America (lineage 3; PP = 0.99; BS = 86; lineage 4; PP = 1.00; BS = 77; Fig. 2.1). Clades 3 and 4 overlap in part of their ranges based on sample locations (Fig. 2.2). Lineage 5 was identified in southern New Mexico based on a limited number of sequences (PP = 1.00, BS = 98; Fig. 2.1). An additional clade was inferred by BI that extends from the Baja California peninsula to California and Nevada (lineage 6; PP = 1.00; BS < 50; Fig. 2.1 and Fig. 2.2).

We determined that two of the other members of the species group, *P. polionotus* and *P. keeni*, rendered *P. maniculatus* paraphyletic. *Peromyscus keeni* formed a clade based on BI (PP = 0.97). This lineage seems to be associated with the western lineages (lineage 6), but the relationship is poorly supported (PP = 0.58; BS < 50). *Peromyscus polionotus* forms a strongly supported clade (PP = 1.00; BS = 99) that is nested within *P. maniculatus*. Both BI and ML place *P. melanotis* as the sister species to the remaining members of the *P. maniculatus* species group (PP = 1.00; BS = 100).

Estimated Climatic Niche and Niche Modeling

We modeled *P. polionotus* and the five most widespread lineages of *P. maniculatus* (lineage 1, 2, 3, 4 and 6; Fig. 2.2). Lineage 5 was only identified by 4 sample locations, covering an area of just over 7 km^2. With such limited spatial information we did not include this lineage in the niche modeling because extremely small sample numbers may not produce accurate models (Hernandez et al. 2006, Wisz et al. 2008).

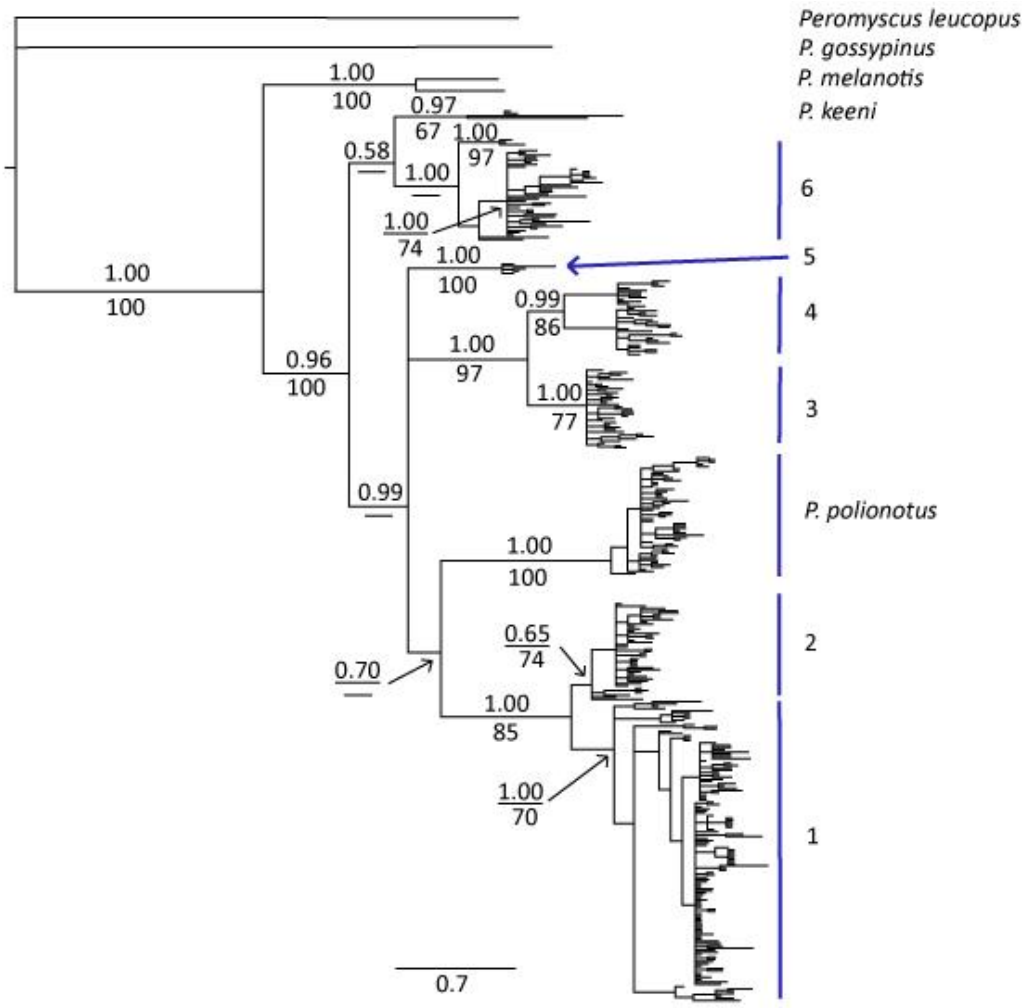

Figure 2.1. Phylogram based on Bayesian inference of cyt *b* sequences of the *P. maniculatus* species group. Nodal support given by Bayesian posterior probability (PP) above the line, and maximum likelihood bootstrap (BS) below line. Values below 0.5 indicated by a dash (–). Numbers indicate lineages of interest within *P. maniculatus*.

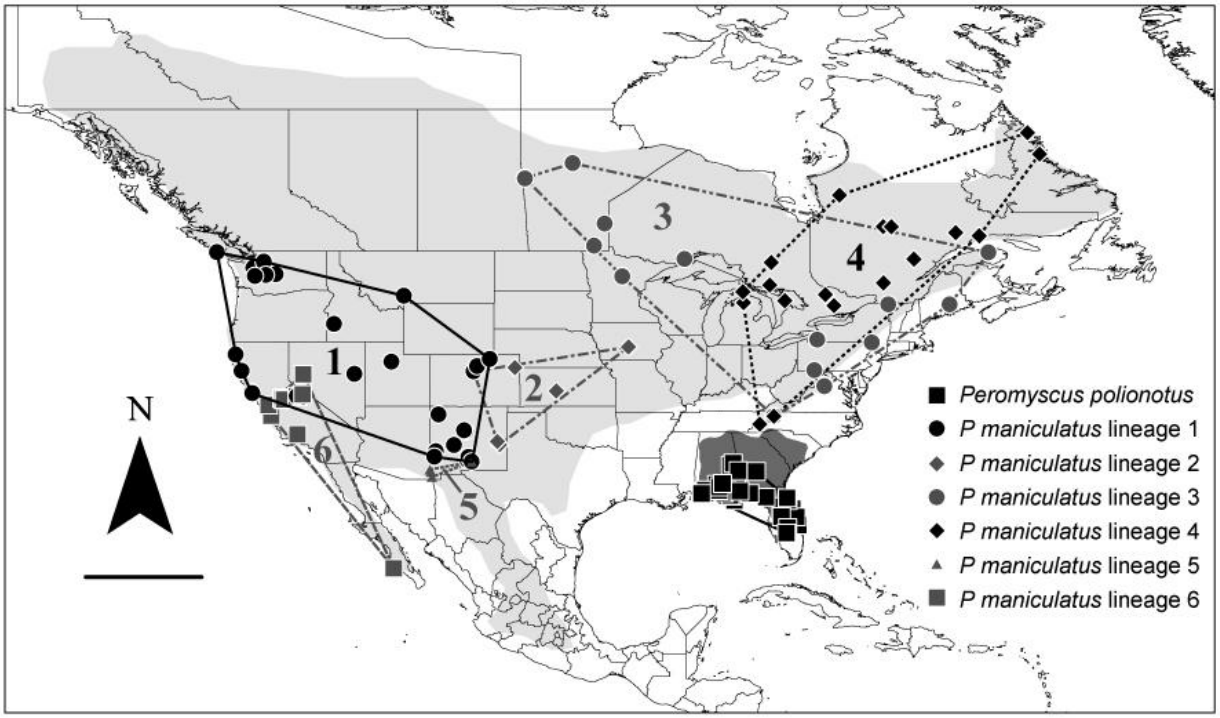

Figure 2.2. Spatial distribution of *P. polionotus* and six monophyletic lineages of *P. maniculatus* based on phylogenetic analysis of cyt *b*. Distribution for the two species shown as shaded area: *P. maniculatus* in light grey and *P. polionotus* in dark grey. Scale bars equal 1000 km.

Among the sample locations of the six lineages included in our analysis, we found a significant overall difference in the climatic and altitude variables at occurrence points (MANOVA, Wilks' lambda = 0.007, $F_{60, 542} = 17.4$, $p < 0.001$). Discriminant analysis indicated 93.4% of environmental variation among the genetic lineages was explained by the first two canonical scores (CV; Fig. 2.3). The first axis explained 74.3% of the variation and was primarily determined by mean diurnal temperature range. The second axis explained 19.1% of the variation and was determined mainly by precipitation of the driest quarter and precipitation of the warmest quarter. On the first axis, 95% confidence intervals overlap for only lineages 3 and 4 (Fig. 2.3). Along the second axis, lineages 1, 2 and 6 formed a group, separate from a second group where lineages 3 and 4 overlap. The *P. polionotus* lineage remained separate on

the second axis (Fig. 2.3). Discriminant analysis supports the MANOVA result of separation in the environmental space among the lineages. Overall the lineages differ in their environmental space, based on the canonical scores. The only lineages without differentiation on either axis were lineages 3 and 4.

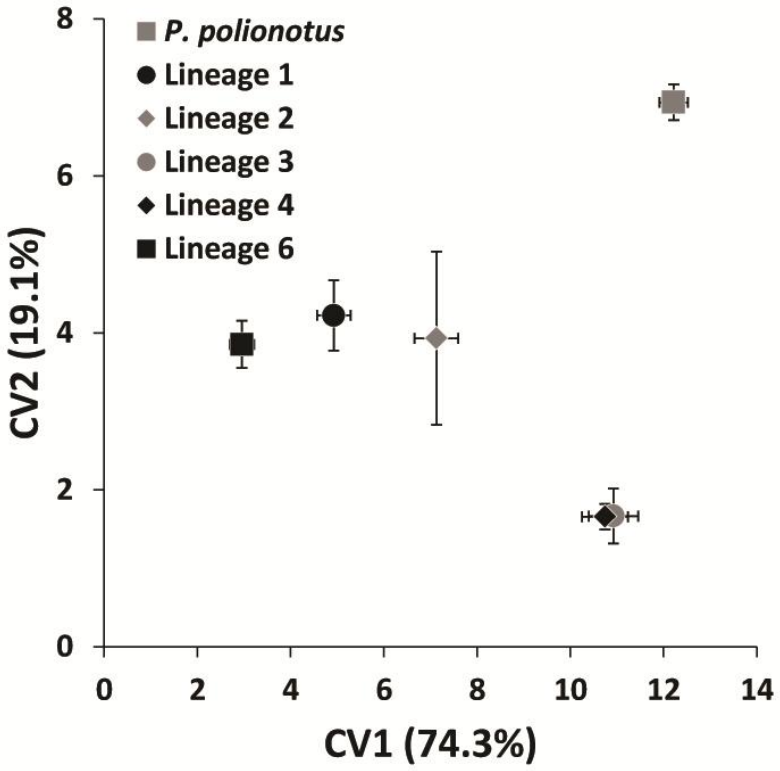

Figure 2.3. Result of discriminant analysis for testing divergence in environmental space for five phylogenetic lineages of *P. maniculatus* and *P. polionotus*, based on 11 climatic layers and altitude. Percent explained variation for each CV is reported for both axes; total variation explained was 93.3%. Each point represents the centroid for each lineage; error bars show the 95 % confidence interval.

Among the lineages we found a positive correlation among the lineages between genetic distance and environmental distance when geographic distance was controlled ($r = 0.562$; $p = 0.001$; Table 2.2). We also observed a significant positive correlation within lineages 3 and 4 (lineage 3, $r = 0.412$, $p = 0.020$; lineage 4, $r = 0.272$, $p = 0.004$; Table 2.2). Within the remaining

lineages (1, 2, 6 and *P. polionotus*) we found no correlation between genetic and environmental distance when we controlled for geographic distance (Table 2.2).

Table 2.2. Estimated correlation coefficients between genetic and environmental distance comparing sample locations of *P. maniculatus* lineages and *P. polionotus*, with geographic distance controlled using a partial Mantel test.

Genetic lineage	r	P-value
All combined	0.562	**0.001***
Lineage 1	0.078	0.258
Lineage 2	0.094	0.296
Lineage 3	0.412	**0.020***
Lineage 4	0.272	**0.004***
Lineage 6	-0.018	0.497
P. polionotus	-0.053	0.628

*significance at $\alpha = 0.05$

We developed distribution models using 189 locations for *P. maniculatus* lineage 1, 45 for lineage 2, 55 for lineage 3, 40 for lineage 4, 107 for lineage 6, and 64 for *P. polionotus*. Each model showed high specificity with AUC values for the test dataset ranging from 0.903 to 0.996. These models exhibited little overprediction outside of the locations included to create the models (Fig. 2.4). The minimum probability of occurrence for training points ranged from 0.066 for lineage 6 to 0.256 for lineage 2. The most important variables for each model were different. Mean temperature for driest quarter was most important for lineages 2 and 3, while precipitation of the warmest quarter was most important for lineage 6 and *P. polionotus*. The remaining models had the following greatest weight of variables: lineage 1 (isothermality), and lineage 4 (precipitation of driest quarter). For each model we determined that four variables were needed to explain 84% or more of the variation, with the remaining variables providing little information to the predicted distributions.

Distributional overlap ranged from 0.0% - 44.4% (Table 2.3). The distribution of *P. polionotus* did not overlap with any other predicted distributions. An overlapping area between

lineages 1 and 2 existed at the eastern edge of the Rocky Mountains that made up 6.7% of the total predicted area (Table 2.2, Fig. 2.5A). Distributions of lineages 1 and 6 overlapped in California (15.5%; Table 2.3; Fig. 2.5B). Overlap between lineages 2 and 4 occurred in the central prairie region of the United States (5.6%; Table 2.3, Fig. 2.5C). Lineages 3 and 4 had the highest overlap (44.4%) of their predicted distributions, encompassing much of the northeastern United States (Table 2.3, Fig. 2.5D).

Based on our estimates of niche overlap, we found *D* to range from 0.001 to 0.609, with lineage 2 and *P. polionotus* showing the largest differences while lineages 3 and 4 are the most similar (Table 2.3). All values are significantly different than predicted by randomly choosing location points for any of the pair-wise comparisons. Values of *I* showed the same pattern observed using *D* and ranged from 0.295 to 0.706 (Table 2.3). Niche overlap according to *I* indicated the lowest overlap between lineages 2 and *P. polionotus* and the highest overlap between lineages 3 and 4. All comparisons remained significant for both *D* and *I* when models were estimated from lower resolution environmental layers. This suggests that changing the resolution did not change our inferences of overlap in niche among lineages. Again, all comparisons show significantly lower values of overlap than expected by random processes (Table 2.3).

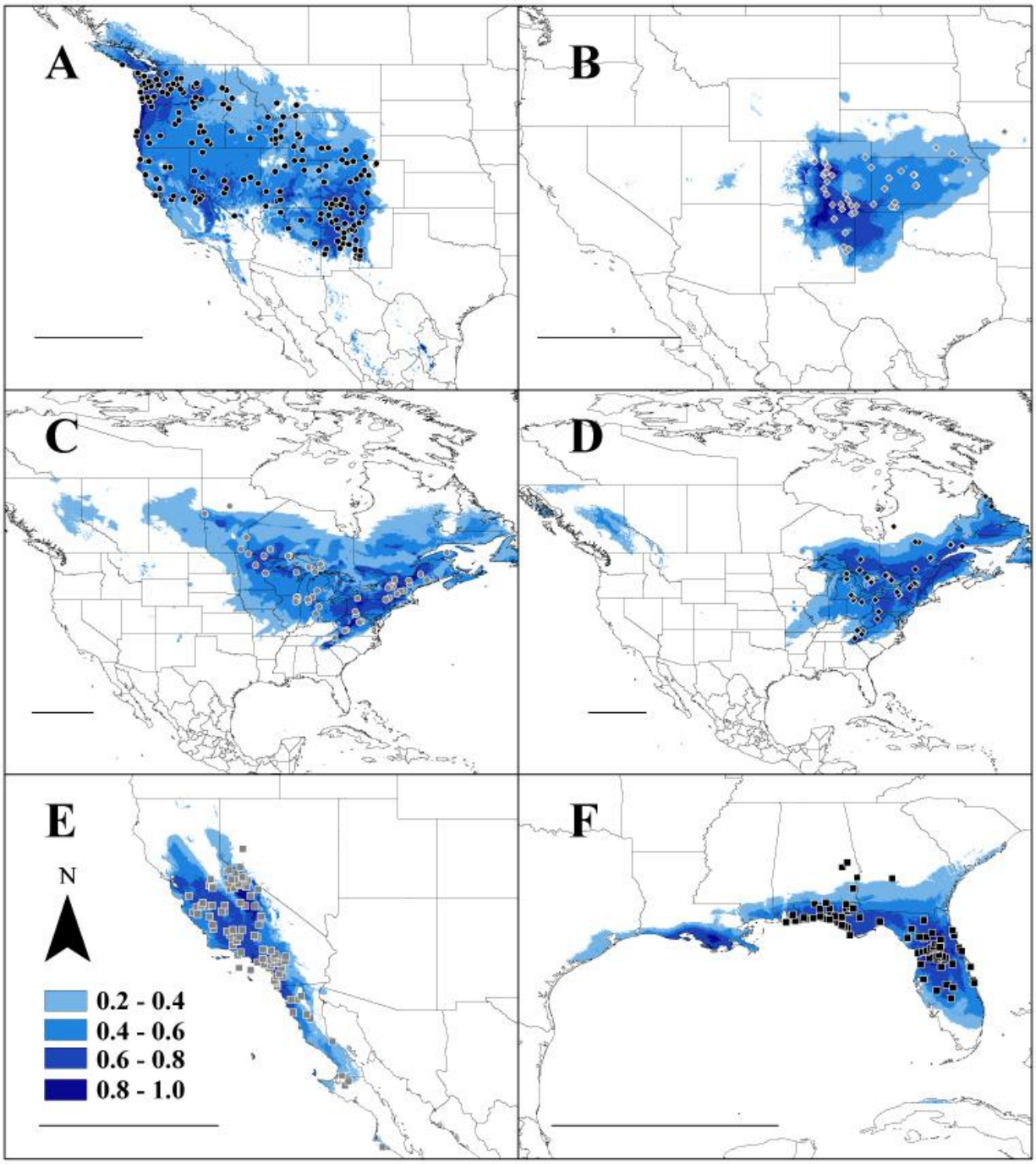

Figure 2.4. Predicted distribution using Maxent for (A) *P. maniculatus* lineage 1, (B) lineage 2, (C) lineage 3, (D) lineage 4, (E) lineage 6, and (F) *P. polionotus*. Lineages were determined based on phylogenetic analysis (see Fig. 2.1). Distribution was determined based on 12 environmental variables. Shades indicate probability of occurrence, with darker shade being higher likelihood. Scale bars show 1000 km on each map.

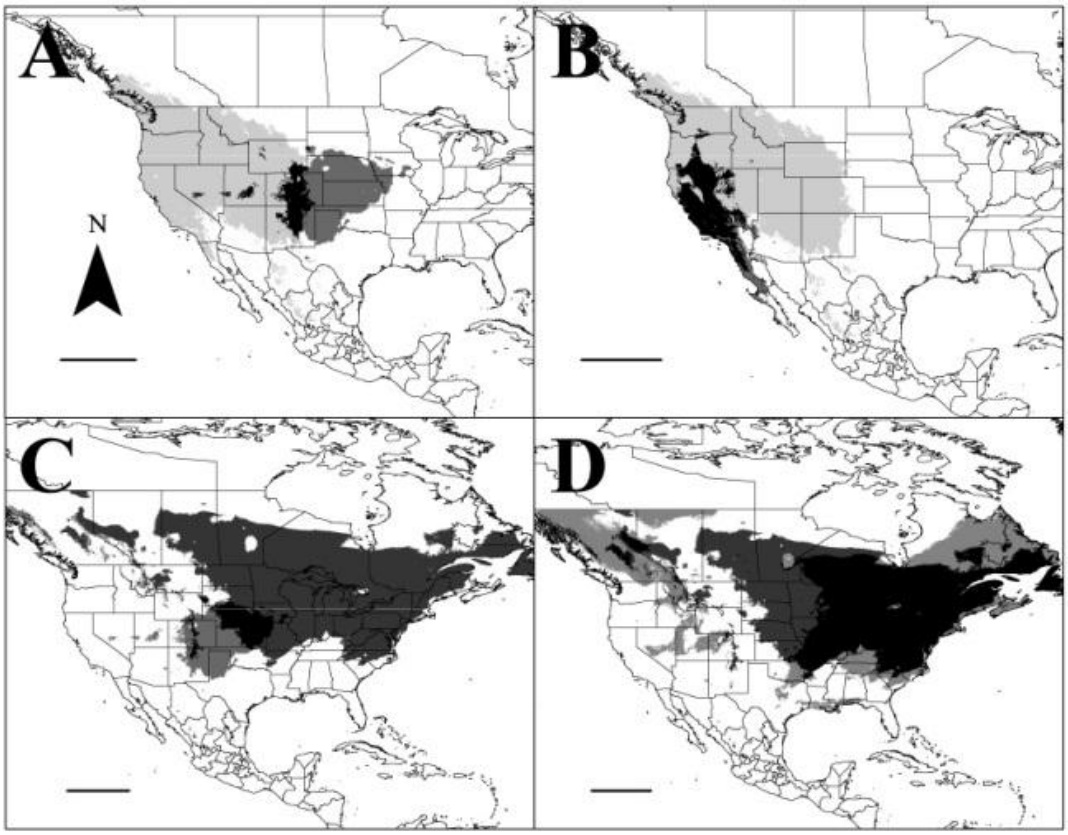

Figure 2.5. Overlap between predicted distributions of lineages using minimum training presence criterion as threshold of presence of suitable niche space. Grey areas are predicted allopatric while black areas are predicted overlap. (A) Predicted distribution of overlap between lineages 1 (light) and 2 (dark), (B) lineages 1 (light) and 6 (dark), (C) lineages 2 (light) and 3 (dark), and (D) lineages 3 (dark) and 4 (light). Scale bars show 1000 km on each map.

Table 2.3. Percent geographic overlap in spatial niche space predicted by Maxent using minimum training presence criterion as thresholds for probability of occurrence of *P. polionotus* and 5 lineages of *P. maniculatus*. In addition, measures of niche overlap between genetic lineages of *P. maniculatus* and *P. polionotus*, using indices *D* and *I* proposed by Warren et al. (2008). Significance for the two indices determined by 100 pseudo-replicates in ENMtools, testing for significant difference in niche space than that expected by chance. Significance determined at $\alpha = 0.05$.

Lineage pair	Percent overlap	*D*	P-value	*I*	P-value
Lineage 1 vs. Lineage 2	6.7%	0.149	< **0.001**	0.419	< **0.001**
Lineage 1 vs. Lineage 3	1.6%	0.049	< **0.001**	0.355	< **0.001**
Lineage 1 vs. Lineage 4	4.7%	0.052	< **0.001**	0.362	< **0.001**
Lineage 1 vs. Lineage 6	15.5%	0.280	< **0.001**	0.533	< **0.001**
Lineage 1 vs. *P. polionotus*	0.0%	0.011	< **0.001**	0.308	< **0.001**
Lineage 2 vs. Lineage 3	5.6%	0.109	< **0.001**	0.403	< **0.001**
Lineage 2 vs. Lineage 4	0.9%	0.042	< **0.001**	0.343	< **0.001**
Lineage 2 vs. Lineage 6	0.0%	0.015	< **0.001**	0.309	< **0.001**
Lineage 2 vs. *P. polionotus*	0.0%	0.001	< **0.001**	0.295	< **0.001**
Lineage 3 vs. Lineage 4	44.4%	0.609	< **0.001**	0.706	< **0.001**
Lineage 3 vs. Lineage 6	0.0%	0.006	< **0.001**	0.303	< **0.001**
Lineage 3 vs. *P. polionotus*	0.0%	0.012	< **0.001**	0.321	< **0.001**
Lineage 4 vs. Lineage 6	0.0%	0.012	< **0.001**	0.307	< **0.001**
Lineage 4 vs. *P. polionotus*	1.1%	0.024	< **0.001**	0.333	< **0.001**
Lineage 6 vs. *P. polionotus*	0.0%	0.007	< **0.001**	0.304	< **0.001**

Discussion

As predicted, our phylogenetic analysis recovered distinct genetic lineages among members of the *Peromyscus maniculatus* species group. We also recovered distinct phylogeographic lineages within *P. maniculatus* similar to those found by other researchers (Lansman et al. 1983, Gering et al. 2009). These data add another example to the library of widely distributed species that show phylogeographic structure across North America (e.g., Hoffman and Blouin 2004, Pyron and Burbrink 2009). It should be noted that our phylogenetic inference relies on a single mitochondrial marker, which has its limitations. Increasingly, the

fields of phylogenetics and phylogeography are utilizing multilocus approaches (Brito and Edwards 2009). Individual loci may not represent the evolutionary history of species as the locus itself is subject to evolutionary forces beyond those affecting the lineage. However, the majority of diversification events within the *P. maniculatus* species group occurred during the Pleistocene (Zheng et al. 2003, Van Zant and Wooten 2007), and adding nuclear gene sequences might not necessarily provide additional information (Martins et al. 2009).

The climatic analysis and ENM results for *P. maniculatus* and *P. polionotus* show that genetic divergence is correlated with niche divergence. Almost all lineages occupy significantly different climatic niches based on multiple lines of evidence. Our two multivariate statistical approaches, MANOVA and DA, support our interpretation that the phylogenetic lineages occupy significantly different climatic envelopes (Fig. 2.3). These two methods both utilize an *a priori* assumption of grouping data (i. e., phylogenetic lineages); however, we came to the same conclusion when using a principal component analysis (results not shown), which have no *a priori* requirement. The MANOVA and DA analyses are limited to known sample locations; however, by incorporating ENM we estimated the climatic and geographic distribution beyond the sample locations, and found lineages to be associated with significantly different climatic niches (Fig. 2.4; Table 2.3). The differences we observed in the climatic niches are not an artifact of geographic distance, as our partial Mantel test showed a positive correlation between genetic and environmental distance when we controlled for geographic distance (Table 2.2).

By evaluating the niche relationship between sister taxa we gained insight into how the environment may influence divergence events. Sister lineages 3 and 4 occupy very similar environmental spaces based on location data, which suggests these lineages have followed the pattern of niche conservatism (Fig. 2.3). If sister taxa are in allopatry, niche conservatism can

reflect a pattern where lineages fail to adapt to the landscape matrix separating populations (Wiens and Graham 2005). Interestingly, the two lineages occupying north-east North America are parapatric based on haplotype distributions (Fig. 2.2), or sympatric based on the level of spatial overlap (Table 2.3; Fig. 2.5D). In either case, due to the extensive spatial overlap found in their estimated distributions, current geographic isolation does not account for the genetic divergence between these two lineages. However, historical spatial isolation could have caused the patterns we observe as the two lineages are currently distributed in an area that was under ice during the last glaciations. Thus, the current pattern could be the result of secondary contact between populations expanding from glacial refugia, a pattern replicated in multiple taxa within this region of North America (Hoffman and Blouin 2004, Rowe et al. 2004). Another line of evidence suggests that niches may not be conserved between these two eastern lineages. Utilizing the methods of Warren et al. (2008), we found that these two lineages exhibit the highest level of niche similarity of any pair-wise comparison of genetic lineages, although they are still less similar to each other than expected by chance (Table 2.3). Lineages 3 and 4 were the only lineages showing a positive correlation between genetic and environmental distance. This suggests that genetic differentiation within these lineages may follow a climatic gradient across their distribution.

Sister lineages 1 and 2 from western North America support the hypothesis of niche divergence. They occupy significantly different climatic niches (Fig. 2.3; Table 2.3), with parapatric distributions (Fig. 2.2 and 2.5A). The overlap forms a potential contact zone between the two sister lineages, and this corresponds to the same area Osgood (1909) proposed to be a zone of integration between two subspecies. These lineages represent two ecomorphs (Blair 1950), with lineage 1 representing a forest type and lineage 2 a grassland type. These ecomorphs

have distinct morphological differences, with the forest type having a long tail, large ears and large feet, while the grassland type is distinct with a short tail, small ears and small feet (Blair 1950). Selective pressure on niche may have influenced this lineage divergence, causing shifts in climatic niche and morphological differences between the two lineages. We cannot refute the possibility that the two lineages are connected by gene flow, which could explain the mixed nodal support for lineage 2 (PP = 0.65; BS = 70). Use of fast evolving nuclear markers such as microsatellites could aid in understanding the current interaction between these two lineages.

We determined that *P. polionotus* has diverged from *P. maniculatus* both genetically and ecologically (Fig. 2.1, 2.3 and 2.4). *Peromyscus polionotus* is hypothesized to have originated from a peripheral population of *P. maniculatus*, in particular from a lineage of short-tailed grassland mice (Blair 1950, Carleton 1989). However, Avise et al. (1983) found that *P. polionotus* clustered with a forest-dwelling form of *P. maniculatus*. Our phylogenetic analysis found low support for a sister relationship between *P. polionotus* and an ancestral *P. maniculatus* lineage (Fig. 2.1); the ancestral lineage diverged into both grassland (lineage 2) and forest-dwelling (lineage 1) forms (Fig. 2.1). While the evolutionary relationships of *P. polionotus* remain unclear, our data suggest that this species occupies a distinct niche compared to its closest relatives (Fig. 2.3). Our distribution models show some overlap among all geographically neighboring lineages for *P. maniculatus*, but overlapping with *P. polionotus* is limited to 1.1% overlap with lineage 4 (Table 2.3). This fits the expected distributions for the two species (Fig. 2.2), but it also suggests that the environmental space between *P. polionotus* and neighboring *P. maniculatus* lineages may have suboptimal conditions and could function as a barrier between the two species.

We also found possible contact zones in our ENM between non-sister lineages of interest. These areas match with proposed contact zones in the western United States between lineages 1 and 6, and in central regions, between lineages 2 and 3 (Fig. 2.5B and 2.5C; Osgood 1909, Hall 1981). Our data show that lineages 1 and 6 occupy significantly different environmental spaces, and our phylogenetic analyses shows they are isolated based on mtDNA. However, it is uncertain if hybridization may occur, or if a factor collinear with our climatic variables separate these two lineages in the contact zone. The contact zone between lineages 2 and 3 could represent another interaction between the long-tailed forest type (lineage 3), and the short-tailed grassland type (lineage 2). Field experiments in parts of *P. maniculatus*' distribution have shown that the two ecomorphs preferentially occupy grassland or forest (Hooper 1942, Wecker 1963), which suggests that these ecomorphs can be isolated by vegetative habitat preference. The divergence in the climatic niche we observe may reflect niche divergence between lineages 2 and 3 because of collinearity with patterns of vegetation separating the different ecomorphs.

Peromyscus polionotus and the lineages within the *P. maniculatus* occupy unique environmental space, which could indicate that natural selection is a factor in lineage divergence, formation, and maintenance of species in a heterogeneous landscape (Rissler and Apodaca 2007). If the landscape had an impact in shaping the current diversity we observe within this group, such divergence would have had to occur recently, as the *P. maniculatus* species group is a recent radiation with divergence occurring in the Pleistocene (Zheng et al. 2003, Van Zant and Wooten 2007). This is possible as studies have found that adaptation to new environmental conditions can occur over short periods of time, with the niche being labile over time (Evans et al. 2009, Dormann et al. 2010). However, to validate niche as a driving factor in lineage divergence, phenotypic traits must be identified related to adaptations for persisting in their niche

and resulting in reproductive isolation between lineages (Graham et al. 2004, Kozak et al. 2008). We do not identify such traits attributed to lineage divergence in our study, but two sister lineages (lineages 1 and 2) and two non-sister lineages (lineages 2 and 3) may represent morphologically different groups: forest and grassland ecomorphs. These morphological differences between lineages 1 and 2 could possibly show differential adaptation to different environments, whereas adaptation to different niche space between lineages 2 and 3 may serve to avoid hybridization between these closely related lineages.

Conclusions

A body of evidence is accumulating that niche divergence is common and can occur over short periods of time (Evans et al. 2009, Dormann et al. 2010). Even within closely related species, evidence for niche conservatism and niche divergence is observed (Evans et al. 2009, McNyset 2009, Pyron and Burbrink 2009). This pattern is evident for *P. maniculatus* and *P. polionotus*, in which we demonstrate niche divergence and niche conservatism in different lineages. Our study suggests a potential for climate, or a collinear variable, to be a relevant component in the diversification of widely distributed taxa. Many taxa inhabit wide distributions and encounter heterogeneous landscapes. This heterogeneity of habitat is often reflected in phylogeographic patterns similar to those we observed (e.g., Hoffman and Blouin 2004, Fontanella et al. 2008). The results of this study suggest that other wide-ranging taxa may also exhibit a mosaic of niche divergence and niche conservatism.

Acknowledgments

Funding for this project was provided by US Fish and Wildlife Service, Florida Fish and Wildlife Conservation Commission, and Patrick Air Force Base, Florida. We want to thank A. Bard, A. DeLong, C. Handrick, H. Hoekstra, M. Keserauskis, D. Oddy, J. Provancha, A. Suazo,

M. Tucker and J. Van Zant, as well as field assistants A. Ashcraft-Cryder, K. Gillespie, M. Green, D. Gunderson, W. Lavigne, S. Letcher, D. Smith and A. Verpoorton, for providing tissue samples for multiple populations utilized for this study. Comments from J. Daza, J. Elith, C. Graham, J. Kalkvik, M. B. Manjerovic, K. Medley, A. Modra, L. Rissler, G. Territo, members of the PH lab, and two anonymous reviewers greatly improved this manuscript. This study was conducted under permit 12-09-4-01 given by the Florida Department of Environmental Protection, Division of Recreation and Parks, WV04065 given by the Florida Fish and Wildlife Conservation Commission, and TE105642-0 issued by USFWS. All animal handling was approved by IACUC protocols # 03-13W and 06-46W from University of Central Florida.

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CHAPTER 3. COLONIZATION AND DIVERGENCE: PHYLOGEOGRAPHY AND POPULATION GENETICS OF THE ATLANTIC COAST BEACH MICE

Introduction

Identifying colonization patterns and the processes driving range changes is fundamental to understanding both the evolutionary history and current patterns of diversity observed in taxa. In newly-formed habitat, colonization is the initial step of speciation (Juan et al. 2000, Grant and Grant 2011). Novel habitats formed by major geological events such as glaciations have been studied to understand the patterns and processes of colonization and speciation in insects (Hochkirch and Görzig 2009, Croucher et al. in press), birds (VanderWerf et al. 2010, Grant and Grant 2011) and marine invertebrates (Tomascik et al. 1996, Bird et al. 2011). However, it is also important to account for how global climatic oscillations have shaped the current distributions and diversity of species. During the last glacial cycle, which reached its maximum around 20,000 years before present (ybp), areas at higher latitudes were directly impacted by a changing landscape, causing species' ranges to shift (Davis and Shaw 2001, Lomolino et al. 2005, Aubry et al. 2009). Evaluating colonization in formerly glaciated areas has provided insight into current patterns of divergence in a wide range of taxa such as mammals (Rowe et al. 2004, Fløjgaard et al. 2009, Grill et al. 2009), amphibians (Hoffman and Blouin 2004, Recuero and García-París 2011), plants (Demesure et al. 1996, Huck et al. 2012), and insects (Mende et al. 2010, Hortal et al. 2011).

Glacial oscillations have also affected taxa beyond the glacial edge (Soltis et al. 2006). Changing sea levels significantly altered coastal areas with an approximately 130 m increase in global sea level since the last glacial maximum (30,000-19,000 ybp; Lambeck and Chappell

2001, Lambeck et al. 2002). Significant research has been done on the impact of sea level fluctuations on coastal marine taxa (Wares and Cunningham 2001, Maggs et al. 2008, Wilson and Eigenmann Veraguth 2010), yet few studies have addressed the impact of sea level oscillation on the biodiversity of terrestrial taxa occupying coastal habitat (but see Van Zant and Wooten 2007).

Islands, especially oceanic islands, have been widely used to evaluate consequences of colonization (MacArthur and Wilson 1967, Cowie and Holland 2006, Losos et al. 2010). While oceanic islands are fully disconnected from the mainland, barrier islands are closely associated with the mainland and are ideal areas to investigate the impacts of colonization on the evolutionary history of recently diverged taxa. These land formations are relatively narrow bands of sand, which are formed parallel to mainland coastline (Johnson and Barbour 1990). Barrier islands in North America (Davis 1997, Bryan et al. 2008) and Europe (Madsen et al. 2010, Kolditz et al. 2012) have their origins during the Holocene. These islands are impacted by high rates of sea level change interupting patterns of sand deposition (Rosati and Stone 2009). With the rapid sea level rise that occurred after the last glacial maximum in North America, conditions were thus poor for barrier islands formation (Davis 1997). As sea level stabilized around 6,000 ybp, near current levels, conditions were again favorable for the formation of barrier islands (MacNeil 1950, Davis 1997). Therefore species inhabiting barrier islands could colonize only very recently.

A species that has been greatly influenced by the recent formation of barrier islands is *Peromyscus polionotus* (old field mouse), which primarily occupies habitat with sandy soil in southeastern U.S.A. (Whitaker and Hamilton 1998). Much attention has been given to subspecies occupying coastal barrier islands of Alabama and Florida, with emphasis on spatial variation of

morphological characters (Sumner 1926, Bowen 1968, Selander et al. 1971, Hoekstra et al. 2006, Van Zant and Wooten 2007, Mullen et al. 2009). These subspecies are collectively called beach mice and each subspecies exhibits lighter pelage color compared to mainland conspecifics (Bowen 1968, Hoekstra et al. 2006). The phenotypic variation in pelage color has been attributed to different selective pressures for crypsis, based on correlations between pelage and soil color found on the different barrier islands (Mullen and Hoekstra 2008). The origins of the beach mice subspecies are hypothesized to be recent events associated with the Holocene formation of the barrier islands (Hoekstra et al. 2006). For the subspecies occupying the Gulf coast barrier islands Bowen (1968) hypothesized, based on pelage color, that the diversity was the result of multiple colonization events from mainland populations after the stabilization of the barrier islands when the Gulf was near current levels. This hypothesis was challenged by molecular data that supported an older establishment of Gulf coast taxa. It was postulated that beach mice tracked the receding shore line and became isolated (Van Zant and Wooten 2007). Others, however, have found evidence of a single colonization of Gulf coast beach mice, but at a much more recent time than previously claimed (Domingues et al. in press). In comparison to the multiple studies conducted and hypotheses generated for Gulf coast beach mice, the evolutionary history and colonization patterns of beach mouse subspecies occupying the Atlantic coast have received less attention in the literature. However, for the Atlantic coast beach mice Bowen (1968) proposed a single colonization event from a mainland source, with subsequent isolation on the barrier islands.

In this study we aimed to determine the evolutionary history of the beach mice occupying the Atlantic coast of Florida, using rapidly evolving genetic markers. First, we sought to evaluate lineage differentiation on the Atlantic coast barrier islands. There are three recognized

subspecies occupying the Atlantic coast (Hall 1981): *P. p. phasma* (Anastasia beach mouse), *P. p. decoloratus* (pallid beach mouse), and *P. p. niveiventris* (southeastern beach mouse). Subspecies are often used as a means of partitioning within species variation, and are frequently based on phenotypic variation (O'Brien and Mayr 1991). However, several studies have shown that phenotypic variation may not represent independent evolutionary trajectories, especially on islands (e.g., Burbrink et al. 2000, Culver et al. 2000, Hull et al. 2008, Tursi et al. in press), and therefore the biological validity of these subspecies should be tested. A study of Gulf Coast beach mice supported the correlation between phenotypic variation as indicated by subspecies designation and genetic differentiation (Mullen et al. 2009). We tested the hypothesis that the three Atlantic coast subspecies each maintain independent evolutionary trajectories, and can be considered as separate taxonomic units. Then, we evaluated the colonization patterns of the Atlantic coast beach mice using sequence and genotype data. We tested Bowen's (1968) single colonization hypothesis, where the Atlantic coast was colonized from a single mainland source, with subsequent processes shaping current diversity. Alternatively, the Atlantic coast beach mice could have colonized the barrier islands from multiple mainland sources, where differences among sources impacted current diversity. Finally, we tested whether the genetic diversity of Atlantic coast beach mice follows the assumptions of island populations, with founder effects and smaller effective population sizes resulting in lower diversity compared to mainland lineages (Frankham 1997). We hypothesized that the Atlantic coast beach mouse subspecies should have lower genetic diversity compared to mainland conspecifics. Of the three Atlantic coast beach mice, one is recently extinct (Humphrey 1992) and the extant subspecies are listed as endangered or threatened (U.S. Fish and Wildlife Service 1989). Therefore, we conclude by discussing implication for conservation efforts related to these taxa and the areas they inhabit.

Methods and Materials

Sampling and DNA extraction

We obtained a total of 492 specimens for this study from 20 locations representing the distribution of *P. polionotus* on peninsular Florida and that of extant Atlantic coast beach mice (Fig. 3.1). Of those, 490 were collected using trapping methods described in Degner et al. (2007). We trapped 69 individuals that we expected to represent two mainland *P. polionotus* subspecies; *P. p. subgriseus* (SRWEA and ONF), and *P. p. rhoadsi* (LARA, LLSP, APAFR, and ABS) (Table 3.1). We collected 77 individuals from four locations representing the range of the Anastasia beach mouse, *P. p. phasma* (Table 3.1). We collected 344 individuals across the current range of the other extant southeastern beach mouse, *P. p. niveiventris* (Table 3.1). We collected 2-4 mm of tail tissue from each individual and stored the samples in 95% ethanol at -20C prior to DNA extraction. We also acquired two museum specimens of the extinct pallid beach mouse, *P. p. decoloratus*, from the Museum of Southwestern Biology (MSB 64761 and MSB 64762). These two specimens were collected on Daytona Beach (DB) in 1946. For each museum specimen, we collected tissue as a 4x4 mm^2 section of skin taken from the venter.

We extracted genomic DNA from all tissue samples using a DNeasy tissue purification kit (Qiagen Inc.), following the manufacturer's protocols. Before extraction, the fresh tissue was lysed for 2-4 hours, until dissolved. The two museum tissues were soaked in 95% ethanol at 4°C for 24 hours to remove any PCR inhibitors (Mullen and Hoekstra 2008), then lysed for 24 hours until dissolved.

Genetic markers

We amplified and sequenced 1100 base pairs (bp) of the mitochondrial cytochrome *b* (*cyt b*) gene for all fresh tissue specimens following the protocol described in Herron et al. (2004).

The DNA from the *P. p. decoloratus* museum specimens was degraded; therefore, we had to amplify seven 200-300 bp amplicons to generate the complete gene sequence. PCR reactions for the museum specimens were done in 25 μL volumes, containing 20-30 ng DNA, 2 mM MgCl, 1X Amplitaq Gold Buffer, 200 μM dNTPs, 0.75 units AmpliTaq Gold (Roche, NJ), and 160 nM primer. Thermocycler conditions were: 94°C for 2 minutes followed by 45 cycles of 94°C for 1 minute, annealing temperature for 1 minute, and 72°C for 2 minutes. Annealing temperatures and primer sequences used for museum specimens can be found in Table 3.2. All sequences were processed on an ABI 3730 DNA analyzer by the Nevada Genomics Center (Reno, NV). Sequences were edited in Sequencher v.4.8 (Gene Codes, Ann Arbor, MI) and aligned by eye in GeneDoc v.2.7 (Nicholas et al. 1997).

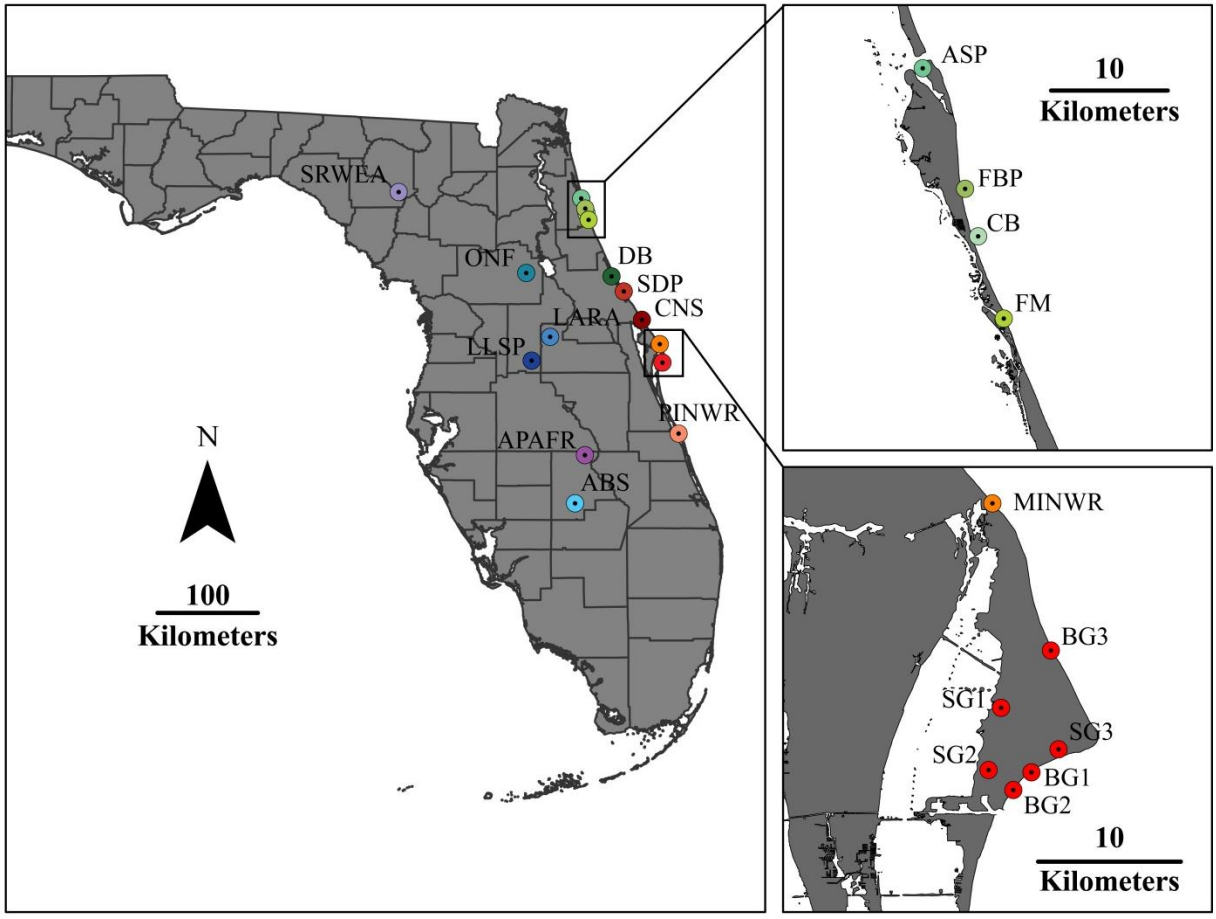

Figure 3.1 Localities of specimens acquired for this study. Circles designate a collection site, and are color coded by taxonomic grouping. Mainland subspecies were represented as blue and purple: *P. p. subgriseus* (SRWEA and ONF), *P. p. rhoadsi* (LARA, LLSP, APAFR, ABS). Atlantic coast beach mice collected were: *P. p. phasma* (ASP, FBP, CB, FM – green), *P. p. decoloratus* (DB – dark green), and *P. p. niveiventris* (SDP, CNS, MINWR, BG1-3, SG1-3, PINWR – red-orange). Abbreviations for sample locations can be found in Table 3.1.

Table 3.1 Samples of *P. polionotus* included in study, with location name, county, sample ID (ID), geographic coordinates (in decimal degrees), sample size, and samples used for cytochrome *b* (*cyt b*) and microsatellite analyses for each sample location.

						Samples used	
Location	County	ID	Latitude	Longitude	n	*Cyt b*	Microsat.
P. p. subgriseus							
Suwannee Ridge Wildlife and Environmental Area	Suwannee Co.	SRWEA	29.9591	-82.9296	10	10	10
Ocala National Forest	Marion Co.	ONF	29.2406	-81.7837	9	7	9
P. p. rhoadsi							
Lake Apopka Restoration Area	Orange Co.	LARA	28.6660	-81.5770	2	2	0
Lake Louisa State Park	Lake Co.	LLSP	28.4517	-81.7388	25	14	25
Avon Park Air Force Range	Highlands Co.	APAFR	27.6104	-81.2591	10	10	10
Archbold Biological Station	Highlands Co.	ABS	27.1833	-81.3493	13	13	13
P. p. phasma							
Anastasia State Park	St. Johns Co.	ASP	29.9018	-81.2910	40	14	40
Frank Butler Park	St. Johns Co.	FBP	29.7723	-81.2483	3	3	0
Crescent Beach	St. Johns Co.	CB	29.8091	-81.2582	1	1	0
Fort Matanzas National Monument	St. Johns Co.	FM	29.7091	-81.2285	33	13	33
P. p. decoloratus							
Daytona Beach	Volusia Co.	DB	29.2106	-81.0231	2	2	0
P. p. niveiventris							
Smyrna Dunes Park	Volusia Co.	SDP	29.0721	-80.9142	19	7	19
Canaveral National Seashore	Volusia Co.	CNS	28.8196	-80.7520	31	8	31
Merritt Island National Wildlife Refuge	Brevard Co.	MINWR	28.6044	-80.5908	32	0	32
Cape Canaveral Air Force Station							
Beach Grid 1	Brevard Co.	BG1	28.4351	-80.5661	35	6	35
Beach Grid 2	Brevard Co.	BG2	28.4239	-80.5776	41	0	41
Beach Grid 3	Brevard Co.	BG3	28.5117	-80.5539	28	0	28
Scrub Grid 1	Brevard Co.	SG1	28.4756	-80.5854	43	0	43
Scrub Grid 2	Brevard Co.	SG2	28.4365	-80.5933	44	0	44
Scrub Grid 3	Brevard Co.	SG3	28.4495	-80.5490	56	0	56
Pelican Island National Wildlife Refuge	Indian River Co.	PINWR	27.7997	-80.4215	15	15	15

Table 3.2 Primer sequences for amplifying *cyt b* in degraded DNA samples of *P. polionotus*.

Sequence	Primer name	Primer sequence	Annealing temperature
Amplicon 1	mt14152F	5' AAC ATC CGA AAA AAA CAC CC 3'	50°C
	mt14341R	5' CTG ATG AGA ATG CTG TAG TTG TG 3'	
Amplicon 2	mt14297F	5' TAG CCA TAC ACT ACA CAT CAG 3'	55°C
	mt14534R	5' CCT ATG AAT GCT GTT GCT ATT AC 3'	
Amplicon 3	mt14471F	5' CTG ATG AGA ATG CTG TAG TTG TG 3'	55°C
	mt14688R	5' AAA TGC GAA GAA TCG TGT TAG G 3'	
Amplicon 4	mt14647F	5' CCT ATG AAT GCT GTT GCT ATT AC 3'	55°C
	mt14894R	5' ATT TTG GTT TTA TTT TTC CCA G 3'	
Amplicon 5	mt14866F	5' ATT TTG GTT TTA TTT TTC CCA G 3'	55°C
	mt15079R	5' GTT TTG AGG TTT GTA GTA GAG G 3'	
Amplicon 6	mt15030F	5' ATT TTG GTT TTA TTT TTC CCA G 3'	55°C
	mt15237R	5' AGA ATA TCT GGG AAA AAT AAA ACC 3'	
Amplicon 7	mt15193F	5' ATT GGA CAA CTA GCC TC 3'	55°C
	mt15377R	5' AGA ATA TCT GGG AAA AAT AAA ACC 3'	

We included rapidly-evolving nuclear markers by genotyping ten microsatellite loci for mainland and Atlantic coast subspecies. We utilized the following ten microsatellite loci: pml-02, pml-06, pml-11 (Chirhart et al. 2000); PO-25, PO-71, PO-105, PO3-68, PO3-85 (Prince et al. 2002); and ppa-01 and ppa-46 (Wooten et al. 1999). We conducted the PCR reactions in 25 μL volumes containing 1-10 ng DNA, 2.5 μL PCR buffer, 0.3 units *Taq* polymerase (Proligo), 0.2 μM of forward and reverse primer, 0.8 mM combined concentration of DNTPs and 1.5-2.5 mM MgCl. We sized the PCR products using a CEQ 8000 Genetic Analysis System (Beckman-Coulter, Fullerton, CA). We scored allele sizes using the CEQ 8.0 software and 400 bp standards (Beckman-Coulter). We tested our microsatellite data for Hardy-Weinberg equilibrium (HWE) and linkage equilibrium in GenePop v.4.0 (François 2008). Significance was estimated using a

Markov chain approach (Dememorization = 10^4, Number of batches = 10^3, Number of iterations per batch = 10^4) for each locus and population.

Data analysis – taxonomic designation

To test our first hypothesis regarding the evolutionary relationships and taxonomic designations of the Atlantic coast beach mouse subspecies we used both phylogenetics and haplotype networks. We estimated the phylogenetic relationship among unique haplotypes using Bayesian Inference (BI; MrBayes v.3.1.2; Huelsenbeck and Ronquist 2001) and maximum likelihood (ML; Garli v.2.0; Zwickl 2006). We rooted our phylogenetic analyses using *cyt b* sequences from *P. melanotis* obtained from Genbank (DQ385626; Dragoo et al. 2006), as this species has been found to be sister to *P. polionotus* and its closest relatives (Kalkvik et al. 2012). In order to evaluate the relationship of *P. polionotus* to its closest relatives we included *cyt b* sequences from each major lineage of *P. maniculatus* (DQ385632, DQ385706, DQ385717, DQ385756, DQ385816, DQ385825; Dragoo et al. 2006) and *P. keeni* (DQ385716; Dragoo et al. 2006) identified by Kalkvik et al. (2012). To provide a complete sampling of *P. polionotus*, we included published *cyt b* sequence data for *P. p. sumneri*, *P. p. albifons*, and *P. p. polionotus*, representing mainland subspecies from the Florida panhandle, Georgia, and Alabama respectively (EU140776,EU140779, EU140770, EU140781, EU140757, EU140767; Van Zant and Wooten 2007). We also included four Gulf coast beach mouse subspecies (*P. p. peninsularis* [EU140791], *P. p. tryssyllepsis* [EU140784], *P. p. leucocephalus* [EU140789], *P. p. allophrys* [EU140778]; Van Zant and Wooten 2007) for complete representation. Following the methods outlined by Brandley et al. (2005) we used Bayes factors to determine the best partitioning strategy for our data set. We determined the best substitution model for the *cyt b* data in

MrModelTest v.2.4 using the Akaike information criterion (Nylander 2004). For the BI we completed two independent Markov chain Monte Carlo (MCMC) runs in MrBayes, with each run having four chains, for 2 x 10^6 generations and sampling every 1,000 generations. We determined stationarity for our runs using Tracer v.1.5 (Rambaut and Drummond 2007) and discarded the first 200,000 generations as burn-in. We used the default parameter settings in Garli to estimate ML topology. Following the recommendations by the program author (Zwickl 2006), we initiated four runs to ensure convergence, where each run was terminated after 20,000 generations with no improvement in the likelihood score of the topology. We assessed the nodal support using bootstrapping, with 1,000 replications. Each replicate was terminated after 10,000 generations with no improvement in likelihood score of the topology. Nodes were considered supported if their posterior probability was above 0.95, as it measures probability of a node representing a true phylogenetic divergence (Huelsenbeck et al. 2001). Supported nodes had bootstrap values above 70% due to conservative estimates of inferring correct clades (Hillis and Bull 1993).We estimated the geographic distribution and frequency of the unique haplotypes for the *cyt b* sequences by constructing a haplotype network following the 95% statistical parsimony method (Templeton et al. 1995). The network was constructed using TCS (Clement et al. 2000).

Data analysis – colonization patterns

We tested Bowen's single colonization hypothesis of the Atlantic coast barrier islands using a haplotype network based on mitochondrial sequence data and measures of genetic structure based on microsatellite data from the Atlantic coast beach mice and mainland subspecies. For analysis of genetic structure using microsatellites we included only sample locations with more than five individuals to provide sufficient population level sampling (Table

3.1). In order to measure genetic structure we estimated genetic differentiation based on microsatellite data among and between mainland and beach mouse subspecies. We determined whether allele size (R_{ST}) or allele state (F_{ST}) best fit our data. In cases where loci are following a stepwise mutation model and have high mutation rates, R_{ST} is expected to be larger than F_{ST} (Hardy et al. 2003). We estimated genetic differentiation using SPAGeDi v.1.3 (Hardy and Vekemans 2002). We tested the null hypothesis of no contribution of allele size on genetic differentiation ($F_{ST} = R_{ST}$) using a permutation test in SPAGeDi, where we created a null distribution of R_{ST} values. We estimated the distribution using 20,000 permutations. We found R_{ST} to be a better predictor across our sample locations as our observed R_{ST} values were significantly larger than the R_{ST} null distribution (one-tailed test) (Hardy et al. 2003; See Resuts). We estimated pair-wise genetic differentiation as pair-wise R_{ST} using SPAGeDi. To test if genetic differentiation is associated with geographic distance (i.e. isolation by distance) we conducted a Mantel test using IBDWS v.3.15 (Jensen et al. 2005), where significance was estimated using 30,000 permutations. We measured geographic distance as Euclidean distance in kilometers between sample locations using the *dist* function in R v.2.12, and genetic differentiation as pair-wise R_{ST} as determined in SPAGeDi described above.

As an additional test of our colonization hypothesis we determined the number of genetically distinct clusters (K) using a Bayesian admixture approach (STRUCTURE v.2.2; Pritchard et al. 2000). STRUCTURE estimates likelihood values [Pr(X|K)] by fitting data to the given K through minimizing HWE and linkage disequilibrium. STRUCTURE also estimates the proportional association for each individual for each inferred K, measured as a membership coefficient. Using the membership coefficient we can identify potential recent migrants or gene

flow. This approach typically identifies the highest order of genetic structure across samples, so we applied a hierarchical approach to test for genetic structure and genetic isolation among the mainland and extant Atlantic beach mouse subspecies. The initial analysis included all individuals, and subsequent clusters were separately analyzed in STRUCTURE to evaluate any lower level genetic structuring.

As we had uneven sampling among our subspecies, which was dominated by *P. p. niveiventris* individuals (n = 344), we tested for the impact of sample bias on our STRUCTURE analysis using ten randomized runs. For each run we included all samples of *P. p. phasma* (n = 73) and all mainland subspecies samples (n = 67). For *P. p. niveiventris* we picked 75 individuals using the random function in Excel 2010 (Microsoft) for each randomized run, ensuring all sample locations were included. To evaluate the sensitivity of STRUCTURE to sample bias we also conducted additional STRUCTURE runs where we included randomly chosen sets of 100, 150 and 200 *P. p. niveiventris* individuals for separate analyses. For all our STRUCTURE analyses we determined the best fit K for our data using the best Pr(X|K), and the procedure of Evanno et al. (2005) based on the second order derivative of Pr(X|K). The second order derivative, called ΔK, shows the rate of change in likelihood between subsequent K. The highest ΔK has been shown to be a good estimate of best K based on given data (Evanno et al. 2005). Most of the parameters were kept at default values as suggested for the STRUCTURE admixture model. Each run had an initial $2x10^4$ generations of burn-in with a subsequent run of $5x10^5$generations used to estimate parameters; we ran 10 independent runs per K. Each analysis was run with K values from one to the number of sample locations included in the analysis. To evaluate geographic distinctiveness, we plotted the membership coefficient values for each

individual for the K determined to provide the best fit to the data. Migrants or recent gene flow was inferred when individuals had membership coefficients more associated to clusters found outside of their sample locations.

Data analysis – island vs. mainland genetic diversity

We tested our last hypothesis of reduced genetic diversity on barrier islands compared to the mainland by determining genetic diversity measures using both *cyt b* and microsatellite data. To compare genetic diversity using our *cyt b* data, we estimated the number of haplotypes, haplotype diversity, average nucleotide differences (k), and nucleotide diversity (π) using DnaSP v.5.10 (Librado and Rozas 2009) for sequences collected from mainland subspecies, and for each Atlantic coast subspecies. To statistically compare the mainland and Atlantic coast subspecies, we generated confidence intervals around our diversity estimates. Computer simulation in DnaSP is based on a coalescent algorithm based on a neutral, infinite-sites model that assumes a large, constant population size (Hudson 1990, Wall 1999). *Peromyscus polionotus* is locally abundant both on the mainland and on the barrier islands, which indicates that our model system does not violate the assumption of large population size (Smith 1968, Extine and Stout 1987, Lynn 2000). We based the simulations on a segregation site in the empirical data set, and we included no recombination in the data because only one mitochondrial locus was considered. Each simulation was based on 10,000 replications, and we generated 95% confidence intervals for estimates of the number of haplotypes, haplotype diversity and π. We interpreted a lack of overlap in confidence intervals as a significant deviation in genetic diversity for *cyt b*.

For the microsatellite data we did pair-wise comparisons of genetic diversity based on samples from mainland and Atlantic coast subspecies. We estimated genetic diversity for each

sampled location as the number of alleles and allelic richness using FSTAT v.2.9.3 (Goudet 2001). Additionally, we estimated observed and expected heterozygosity for each sample location in GenAlEx v.6.4 (Peakall and Smouse 2006). We tested for significant difference in genetic diversity of microsatellites using the group comparison tool in FSTAT, based on allelic richness and observed and expected heterozygosity. Significance was determined based on 15,000 permutations in FSTAT.

Results

Genetic markers

For the mainland subspecies we successfully generated cyt *b* sequence data from 56 individuals representing all mainland sample locations (Table 3.1). For *P. p. phasma* we acquired sequence data for 31 individuals representing the four sample locations, which is a subset of the total number of collected individuals (Table 3.1). We acquired 36 sequences of *P. p. niveiventris* a subset of individuals captured that represented all sample locations (Table 3.1). To ensure that we had sequenced a sufficient number of individuals we conducted an individual-based rarefaction analysis (Gotelli and Colwell 2001). The resulting haplotype accumulation curves suggested we had sample sizes sufficient to identify a majority of the *cyt b* haplotypes.

We generated microsatellite data for all individuals from sample locations where we had collected more than five individuals (Table 3.1). We genotyped a total of 484 individuals that represented five mainland locations (n = 67; Table 3.1), two locations for *P. p. phasma* (n = 73; Table 3.1), and ten locations for *P. p. niveiventris* (n = 344; Table 3.1). We did not include *P. p. decoloratus* in microsatellites analyses, because of low sample size and failure to obtain

microsatellite data as a result of amplification failure DNA. We found ten microsatellite loci to be out of HWE in seven different sample locations after Bonferroni corrections for multiple comparisons. We did not observe a clear pattern of specific loci being consistently out of HWE across sample locations, so we did not expect null alleles to be a major problem for our microsatellite dataset. Of all comparisons between loci within a sample location (765 total comparisons), we determined after Bonferroni correction that only two locus pairs were in linkage in two different sample locations. With so many comparisons we expect some to be significant by chance, so these results suggest our loci are not physically linked. Due to the low number of locus-by-population deviations in HWE and lack of linkage, we did not exclude any populations or loci from our analysis.

Taxonomic designation

The aligned sequence data consisted of 1103 bp with 93 (8.4%) parsimony informative characters. Across our samples we identified a total of 23 unique haplotypes, which correspond to published haplotypes in Degner et al. (2007) and Kalkvik et al. (2012). Preliminary analysis showed that a non-partitioned model was best for estimating phylogenetic relationships from our data set (harmonic mean likelihood [unpartitioned] = -2200.13; harmonic mean likelihood [partitioned] = -2128.39; 2 x ln(Bayes factor) = 0.066). AIC chose GTR+I+Γ (Tavaré 1986) as the best nucleotide substitution model for our data, and it was implemented into both the Bayesian (BI) and maximum likelihood (ML) analyses.

Both BI and ML approaches resulted in similar topologies, with little resolution (Fig. 3.2). The tree estimated from the BI and ML approach resolved a highly supported monophyletic *P. polionotus* lineage (BI = 1.00, ML = 100; Fig. 3.2), but *P. polionotus* haplotypes formed an

extensive polytomy (Fig. 3.2). Among the sampled haplotypes the phylogenies inferred a *P. p. niveiventris* lineage with low support (BI = 0.89, ML = 66; Fig. 3.2). We also identified a single haplotype representing both *P. p. phasma* and *P. p. decoloratus*, and we found no resolution among mainland haplotypes (Fig. 3.2). We identified a strongly supported clade of four haplotypes that represented *P. p. rhoadsi* in part (Ppr7–9; BI = 1.00, ML = 94; Fig. 3.2). These haplotypes were found in south Florida (APAFR and ABS) and in central Florida (LLSP). An additional lineage representing *P. p. rhoadsi* haplotypes from APAFR and LLSP (Ppr4–6) was only supported by BI (BI = 1.00, ML = 67; Fig. 3.2). Finally, we recovered a lineage representing three haplotypes of *P. p. subgriseus* found in ONF (Pps3–5); this was also supported by BI only (BI = 0.97, ML = 67; Fig. 3.2). Overall, there was a lack of resolution using the cyt *b* sequence data to resolve the phylogenetic relationships among *P. p. niveiventris*, *P. p. phasma*, *P. p. decoloratus* and mainland *P. polionotus spp.* with confidence.

We gained additional insight into subspecies relationships through haplotype network analysis of our *cyt b* sequence data (Fig. 3.3). Despite a low number of informative characters leading to low resolution in the BI and ML phylogenies described above, we observed no overlap in haplotypes between mainland subspecies and those found on Atlantic coast barrier islands. We identified a total of 19 unique haplotypes among mainland subspecies. Of these, nine haplotypes represented sample locations within the distribution of *P. p. subgriseus* (Pps1–9; Fig. 3.3), and the remaining ten mainland haplotypes were found in *P. p. rhoadsi* sample locations (Ppr1–10; Fig. 3.3). While the two mainland subspecies do not share haplotypes, we found most *P. p. subgriseus* haplotypes to be more closely associated to the *P. p. rhoadsi* haplotypes Ppr1, than to each other (Fig 3.2). Additionally, there is no overlap of haplotypes between the two sample

locations designated as *P. p. subgriseus* (Fig. 3.3). Of the haplotypes identified among the *P. p. rhoadsi* sample locations, three were shared among some of the sample locations (Ppr1–2, Ppr8; Fig. 3.3). Haplotype Ppr1 is shared between the two sample locations in central Florida (LARA, LLSP), while Ppr2 is found in central Florida (LARA and LLSP) and in south Florida (ABS) (Fig. 3.3). Most of the individuals with haplotype Ppr8 were found in south Florida (APAFR and ABS), but two individuals in central Florida (LLSP) also exhibited this haplotype (Fig. 3.3). All the haplotypes found mainland link up to haplotype Ppr1 with one to seven mutational steps; this haplotype also connects to the haplotypes found in the Atlantic coast beach mice. The beach mice subspecies share a common unsampled or extinct haplotype with the mainland Ppr1 haplotype. For *P. p. niveiventris* we observed three haplotypes with two to three mutational steps from the most similar mainland haplotype. The three haplotypes in *P. p. niveiventris* were unique to the subspecies (Fig. 3.3). The two northern most Atlantic coast beach mouse subspecies, *P. p. phasma* and *P. p. decoloratus*, shared a single haplotype (Fig. 3.3). Our parsimony analysis of the haplotype network indicated that the two extant coastal subspecies are each closely related to but distinct from the mainland subspecies.

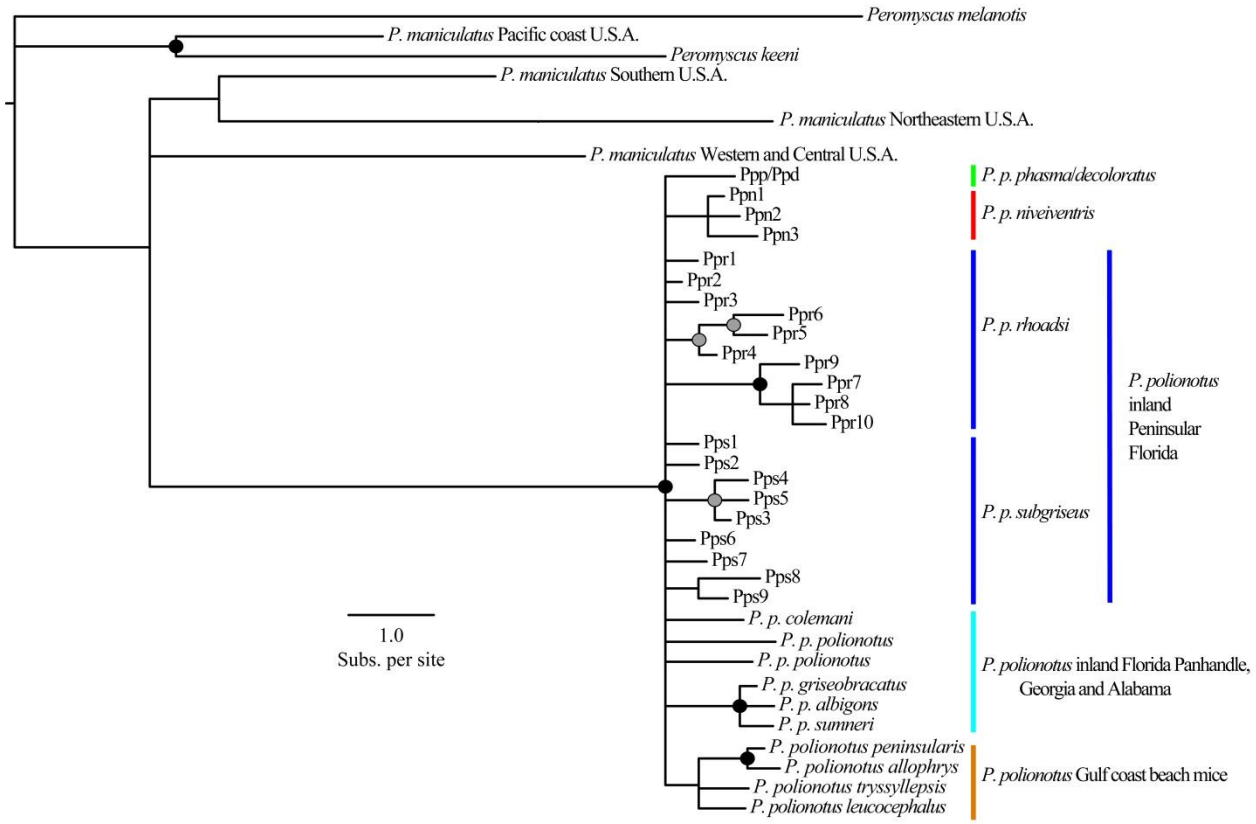

Figure 3.2 Phylogram based on Bayesian inference of *cyt b* haplotypes for Florida peninsula and Atlantic coast beach mice *P. polionotus*. Phylogeny included published haplotypes of Gulf coast beach mice subspecies and mainland *P. polionotus* from Florida panhandle, Alabama and Georgia. Black circles designate strong nodal support based on bootstrap values (> 70), and posterior probability from Bayesian inference (> 0.95). Grey circles mark nodes that are strongly supported by posterior probability (> 0.95) only.

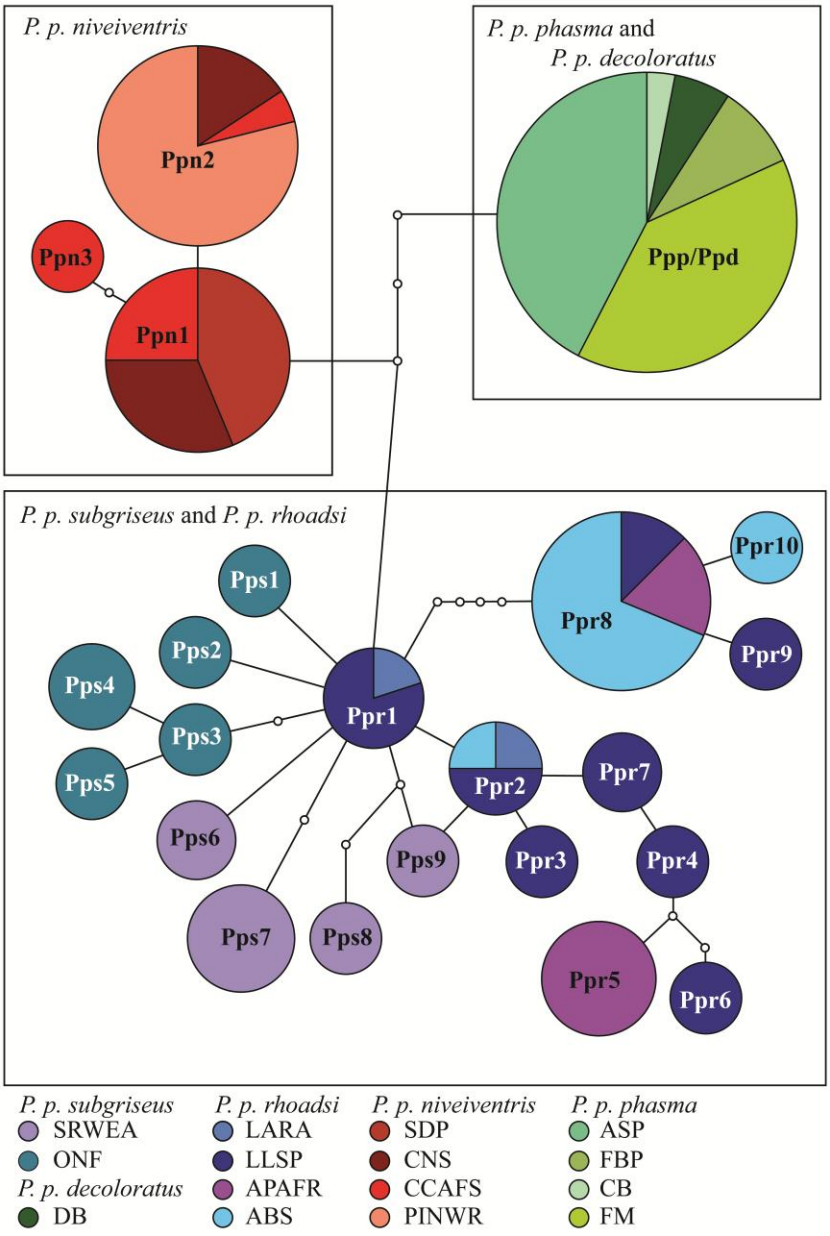

Figure 3.3 Haplotype network based on *cyt b* sequence data from peninsular Florida *P. polionotus spp.* (*P. p. subgriseus* and *P. p. rhoadsi*) and the three Atlantic coast beach mouse subspecies (*P. p. niveiventris*, *P. p. phasma* and *P. p. decoloratus*. Each circle designates a unique haplotype, and size corresponds to frequency of individuals carrying haplotype. Color corresponds to sample locations seen in legend (see Fig. 3.1 for abbreviations). Small white circles designate unsampled or extinct haplotypes.

Colonization patterns

In measuring genetic differentiation we found the observed global R_{ST} value was significantly larger than the permutation distribution (P-value = 0.003). Based on the results of the permutation test, R_{ST} is a better predictor for describing genetic structure across our samples compared to F_{ST}. The global R_{ST} value indicated a high level of genetic structure among mainland *P. polionotus* locations (Global R_{ST} = 0.266 ± 0.050 95% CI). We found the pair-wise R_{ST} values to range from -0.002 (CC IG2 and SDP; CC BG1 and CC BG3) to 0.854 (SDP and FM) (Table 3.3). Pair-wise R_{ST} values among and between Atlantic subspecies and mainland sample locations show that the lowest amount of structure is among *P. p. niveiventris* locations (average R_{ST} = 0.080). Among the mainland locations we observed an average R_{ST} = 0.107, with R_{ST} = 0.133 between the two *P. p. subgriseus* locations and an average of R_{ST} = 0.099 among the *P. p. rhoadsi* locations. We observed the greatest pair-wise structure within *P. p. phasma* (average R_{ST} = 0.319). We found pair-wise R_{ST} values for *P. p. niveiventris* to be lower when compared to mainland locations (average R_{ST} = 0.257) than compared to the *P. p. phasma* locations (average R_{ST} = 0.540). Between the mainland locations and *P. p. phasma* the average pair-wise R_{ST} was 0.447 (Table 3.3). We found a significant positive relationship between genetic differentiation and geographic distance (Mantel test; P = 0.043; Fig. 3.4), but the geographic distance did not explain a majority of the variation in genetic differentiation (R^2 = 0.174; Fig. 3.4).

Table 3.3 Genetic differentiation measured as pair-wise R_{ST} values for sample locations of *P. polionotus* spp., based on ten microsatellite loci (below diagonal). Geographic distance between sample locations are shown as Euclidean distance measured in kilometers (above diagonal).

	Mainland subspecies								Atlantic coast beach mice								
	P. p. subgriseus		*P. p. rhoadsi*			*P. p. phasma*			*P. p. niveiventris*								
	SRWEA	ONF	LLSP	APAFR	ABS	ASP	FM	SDP	CNS	MINWR	CC BG3	CC IG1	CC IG3	CC IG2	CC BG1	CC BG2	PINWR
SRWEA	-	136.7	203.8	308.1	345.5	158.2	166.6	218.8	246.4	272.6	281.5	281.3	285.9	283.3	285.5	285.4	342.9
ONF	0.133	-	87.9	188.6	233.0	87.7	74.9	86.6	110.8	136.1	144.8	144.6	149.2	146.6	148.8	148.7	208.5
LLSP	0.092	0.034	-	104.8	146.3	167.2	148.5	106.1	104.8	113.6	116.1	112.9	116.5	112.1	114.8	113.7	148.3
APAFR	0.189	0.169	0.081	-	48.4	255.1	233.7	166.2	143.5	128.7	121.9	116.9	116.6	112.9	114.3	112.6	85.2
ABS	0.122	0.036	0.076	0.140	-	302.7	281.4	214.6	191.4	174.9	167.3	162.3	161.5	158.1	159.3	157.6	114.5
ASP	0.326	0.169	0.167	0.355	0.176	-	22.3	99.3	131.3	159.6	170.5	172.9	177.0	176.7	177.8	178.5	248.9
FM	0.693	0.719	0.554	0.778	0.535	0.319	-	77.2	109.3	137.7	148.6	150.9	155.0	154.6	155.8	156.5	226.7
SDP	0.302	0.442	0.184	0.249	0.366	0.432	0.854	-	32.2	60.9	71.6	73.8	77.9	77.4	78.6	79.3	149.6
CNS	0.221	0.239	0.197	0.336	0.253	0.322	0.617	0.199	-	28.7	39.4	41.6	45.7	45.4	46.5	47.2	118.1
MINWR	0.283	0.343	0.194	0.274	0.346	0.380	0.732	0.046	0.131	-	10.9	14.4	17.7	18.7	19.0	20.1	91.1
CC BG3	0.241	0.304	0.171	0.210	0.302	0.370	0.719	0.032	0.149	0.022	-	5.1	6.9	9.2	8.6	10.0	80.3
CC SG1	0.180	0.245	0.130	0.174	0.239	0.326	0.641	0.074	0.169	0.078	0.014	-	4.6	4.4	4.9	5.8	76.9
CC SG3	0.223	0.294	0.171	0.251	0.289	0.356	0.633	0.029	0.087	0.018	0.013	0.038	-	4.6	2.3	4.0	73.4
CC SG2	0.213	0.268	0.129	0.186	0.268	0.341	0.647	-0.002	0.128	0.022	0.007	0.027	0.006	-	2.7	2.1	72.9
CC BG1	0.291	0.358	0.206	0.206	0.351	0.424	0.741	0.028	0.201	0.040	-0.002	0.044	0.040	0.023	-	1.7	72.1
CC BG2	0.300	0.360	0.206	0.238	0.364	0.423	0.733	0.004	0.176	0.012	0.007	0.070	0.031	0.013	0.002	-	71.2
PINWR	0.137	0.353	0.194	0.301	0.252	0.375	0.751	0.244	0.134	0.185	0.194	0.161	0.105	0.136	0.230	0.221	-

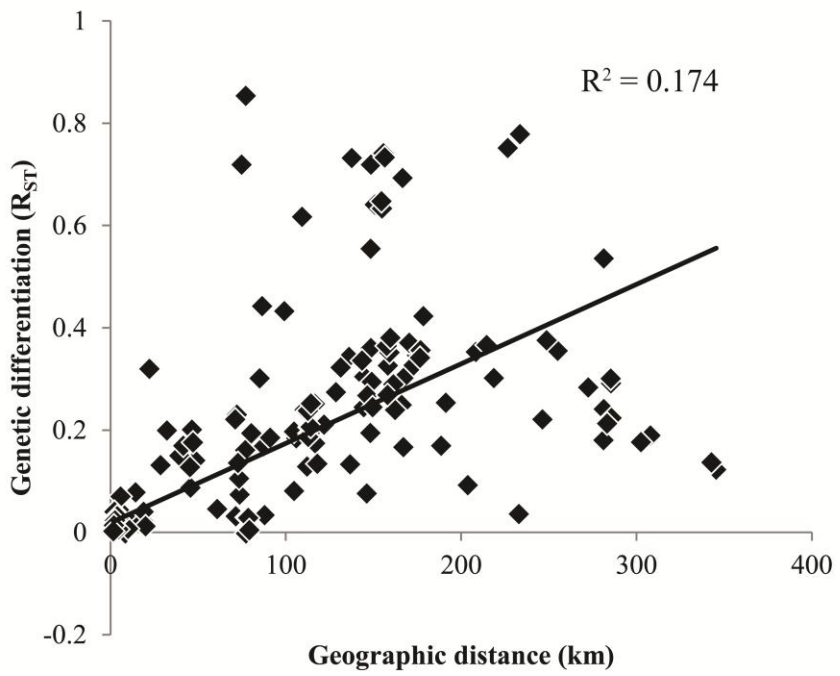

Figure 3.4 Plot of correlation between genetic differentiation and geographic distance. Genetic differentiation was estimated as R_{ST} based on microsatellite data, and geographic distance was Euclidean distance measured in kilometers (km). The relationship between the two axes was significant based on Mantel test (P = 0.043; 30,000 permutations).

When we included all sample locations with microsatellite data in our Bayesian admixture model, the best K describing the genetic structure among all sample locations was two (Fig. 3.5A), based on the methods of Evanno et al. (2005). When all individuals were included *P. p. niveiventris* was assigned to one cluster, and the mainland subspecies and *P. p. phasma* comprised a separate cluster (Fig. 3.6A). We found *P. p. niveiventris* to be strongly associated with a single cluster, with only three individuals having a membership coefficient less than 75% for this cluster. All individuals of *P. p. phasma* were strongly associated (membership coefficient > 97%) with the second cluster (Fig. 3.6A). The individuals captured at mainland locations were primarily associated with the same cluster as *P. p. phasma*, with only 6% of the mainland

individuals more associated with the same cluster as *P. p. niveiventris* (Fig. 3.6A). Such distinct separations between *P. p. niveiventris* and the other two groups in our data set indicate that *P. p. niveiventris* has little to no admixture with *P. p. phasma* or mainland subspecies.

Due to our high sample number of *P. p. niveiventris* relative to the other subspecies we tested if numerical sample bias could impact the STRUCTURE results. Thus we included ten STRUCTURE analyses with randomized sampling of *P. p. niveiventris* samples for more balanced sampling between Atlantic coast and mainland subspecies. We found a consistent pattern suggesting lack of gene flow between the coastal subspecies and mainland, and lack of gene flow between the two extant coastal subspecies, in all analyses. However, numerical sampling bias did influence the hierarchical structure. When including all subspecies with approximately equal sample size we found K = 2 to best fit our data (Fig. 3.7A). With more even sampling one cluster contained all mainland subspecies and *P. p. niveiventris* individuals, and the other cluster contained the *P. p. phasma* individuals (Fig. 3.7B). All individuals were strongly associated with their respective clusters, having membership coefficients over 75% for their respective cluster. Further, over 98% of the individuals had membership coefficients greater than 90% for their respective cluster (Fig. 3.7B). Contrary to our analyses including all individuals, our randomized runs indicated divergence between *P. p. phasma* and the cluster containing the mainland subspecies and *P. p. niveiventris*. With different levels of numerical sample bias we observed consistency in K = 2 best fitting the data (Table 3.4). With approximate even sample size all runs split out *P. p. phasma* as its own cluster, but when we increased our number of *P. p. niveiventris* samples to 100 individuals we found 10% of the runs with *P. p. niveiventris* as its own cluster. By the time we increased *P. p. niveiventris* samples to over twice as many as those

representing mainland subspecies and *P. p. phasma* (n = 200), all runs supported *P. p. niveiventris* forming its own cluster in the STRUCTURE analyses.

When all individuals were included, the mainland individuals primarily associated with the same cluster as *P. p. phasma*. To resolve the relationship between the individuals in the mainland and *P. p. phasma* cluster a separate STRUCTURE analysis with only mainland and *P. p. phasma* individuals. We observed the greatest ΔK at K = 2 for this data subset (Fig. 3.5B). The two clusters separate the mainland individuals from the *P. p. phasma* individuals, with individual membership coefficients >75% for their respective cluster (Fig. 3.6B). With such distinct membership coefficients our data indicates no admixture between mainland subspecies and *P. p. phasma*. For the randomized analyses, we only included the mainland subspecies and the randomly picked *P. p. niveiventris*. For this sample we determined again K = 2 as best fitting the data (Fig. 3.7C), where the two clusters are geographically associated with one cluster containing *P. p. niveiventris* individuals, and the second cluster associated with individuals captured mainland (Fig. 3.7D). Almost all individuals were highly associated with their geographic cluster, with 92% of the individuals having a membership coefficient over 95%. Of all individuals only two individuals had a membership coefficient below 75% (Fig. 3.7D). These two individuals were found mainland, and could reflect limited gene flow. We finally ran STRUCTURE on individuals captured at mainland locations, to evaluate any genetic structure among these populations. When only mainland individuals were included, the greatest ΔK was at K =3 (Fig. 3.5C). Membership was primarily determined by geographic location with one cluster mainly making up the individuals captured in *P. p. subgriseus* sample locations (SWEA and ONF). The remaining clusters consisted of *P. p. rhoadsi* sample locations, with one cluster in

central Florida (LLSP), and a second cluster consisting of primarily individuals from the southern range of *P. p. rhoasi* (APAFR and ABS) (Fig. 3.6C). Close association with specific clusters indicated limited admixture among the sampled mainland locations. However, a few individuals were associated with clusters different from their geographic location, which could indicate current or recent gene flow between populations and between mainland subpsecies (Fig. 3.6C).

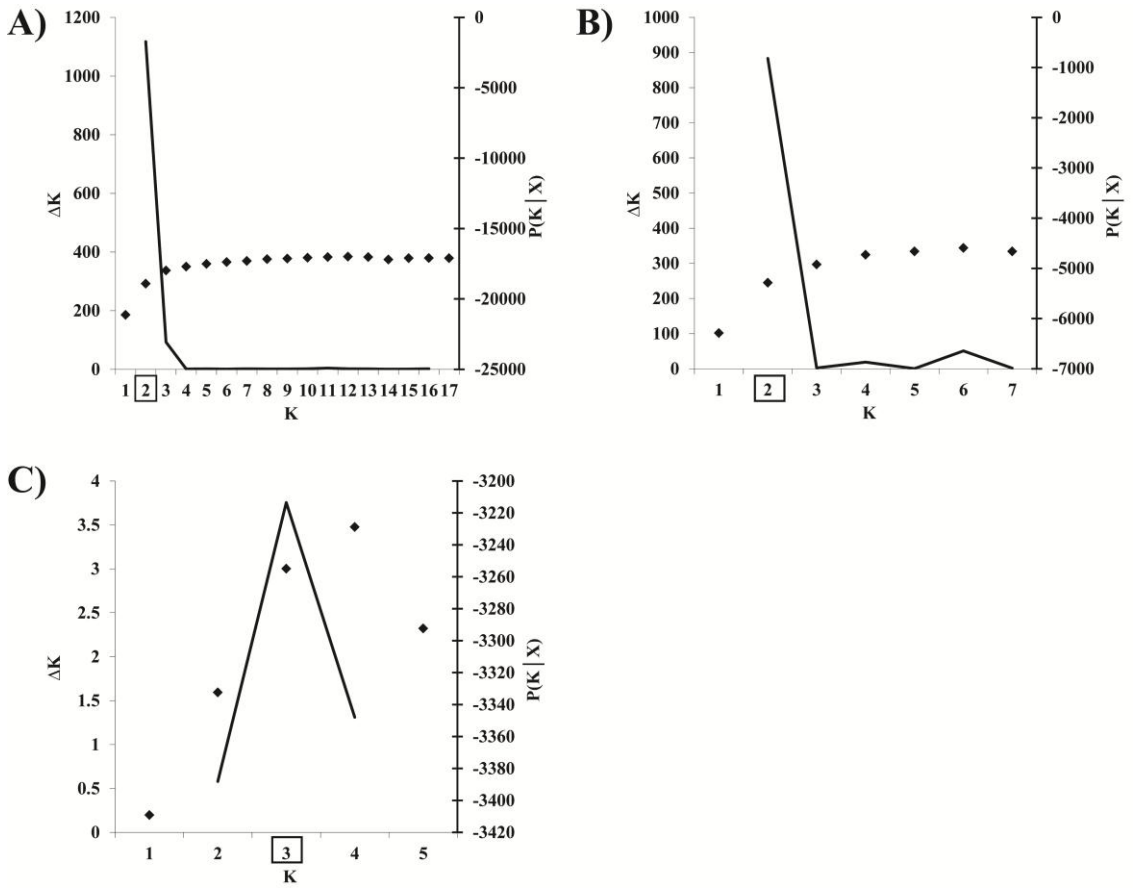

Figure 3.5 Best fit number of clusters (K), shown as black box, for implementing in genetic structure analysis based on likelihood values (diamonds) estimated in STRUCTURE (Pritchard et al. 2000) and ΔK (line) estimated following the methods of Evanno et al. (2005). Three analyses were conducted in STRUCTURE: (A) including all samples of *P. polionotus*, (B) including only individuals captured mainland or *P. p. phasma*, and (C) only *P. polionotus* collected mainland.

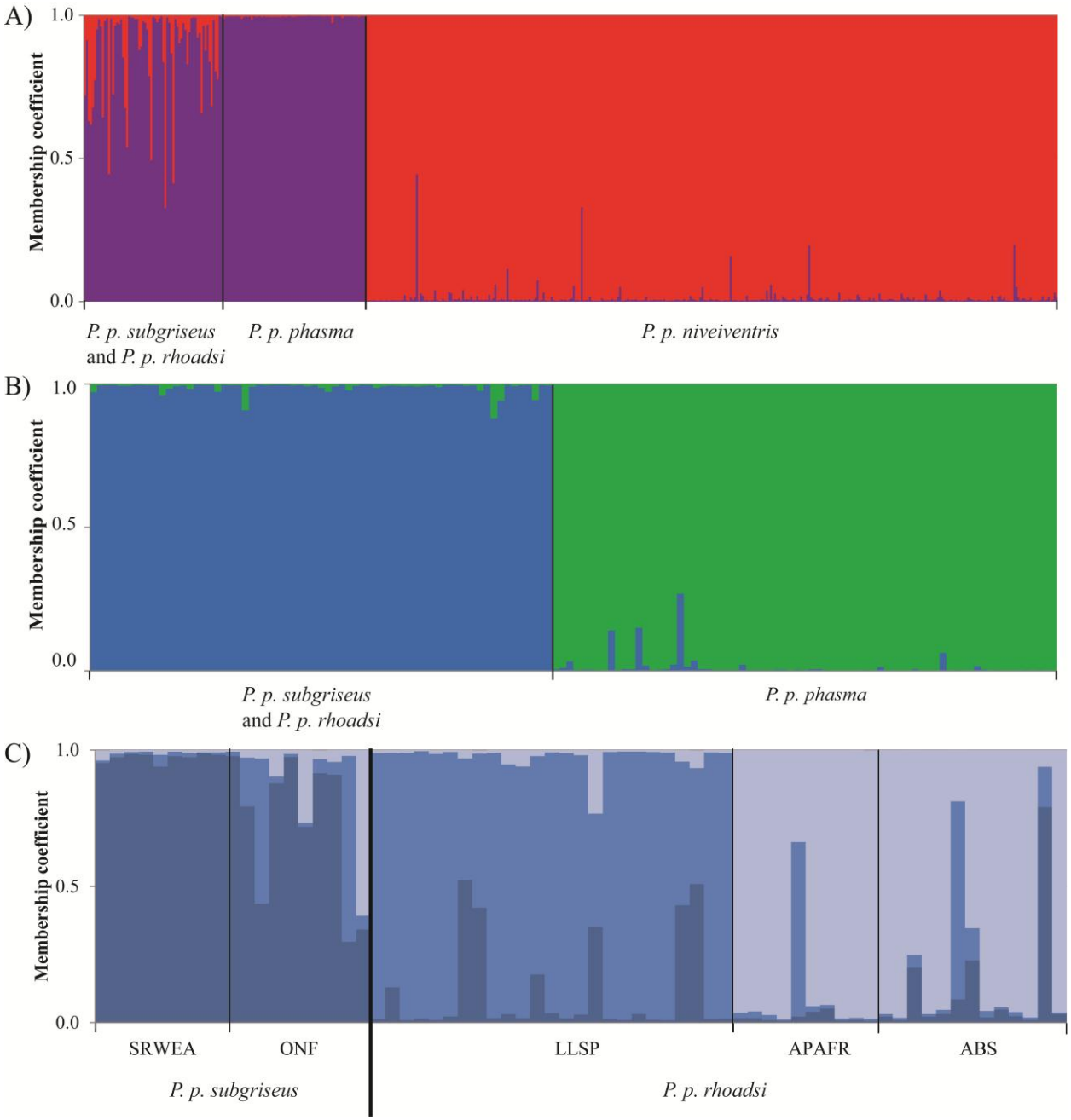

Figure 3.6 Estimated membership coefficients from STRUCTURE analysis based on ten microsatellite loci for *P. polionotus* spp. from peninsular Florida. (A) includes all individuals of mainland spp. (*P. p. subgriseus* and *P. p rhoadsi*) and extant Atlantic coast beach mouse spp. (*P. p. phasma* and *P. p. niveiventris*). (B) includes only individuals defined as *P. p.phasma* or mainland spp. (C) includes only individuals captured mainland, and is divided into sample locations (see Table 3.1 for abbreviations). Colors indicate different clusters: (A) K = 2, (B) K = 2, and (C) K = 3.

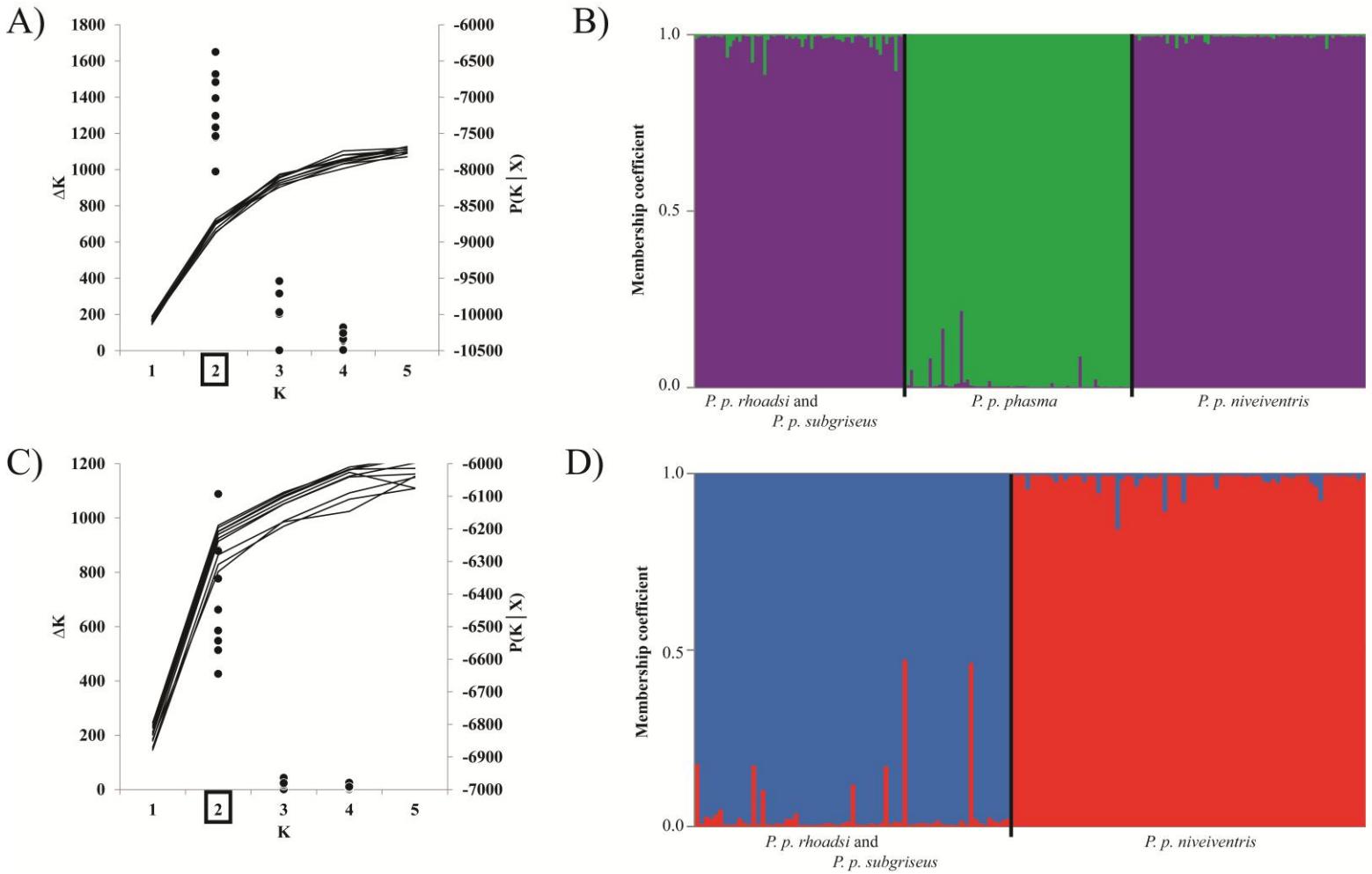

Figure 3.7 Hierarchical STRUCTURE analyses for Peninsular *P. polionotus* spp. using randomized subsampling for even sample size. Best fit number of clusters (black box in A and C) was estimated based on likelihood value (circles) estimated in STRUCTURE (Pritchard et al. 2000) and ΔK (lines) estimated following methods of Evanno et al. (2005) for ten randomized runs for (A) all subspecies, and (C) mainland subspecies and *P. p. niveiventris*. Estimated membership coefficients from STRUCTURE for all subspecies (B) and mainland subspecies and *P. p. niveiventris* (D). Colors indicate different unique clusters.

Table 3.4 Impact of skewed sample size incorporated into STRUCTURE, with different sample sizes for *P. p. niveiventris* (*Ppn*) compared to *P. p. phasma* (*Ppp*) and mainland subspecies, *P. p. subgriseus* and *P. p. rhoadsi*. Best K was determined based on Evanno et al. (2005) criteria. Reported was percent of runs for specific clustering patterns, with "/" indicating same cluster and "+" indicating separate cluster.

Sample size				Percent of runs with specific clustering pattern		
P. p. n.	*P. p. p.*	Mainland	Max ΔK	*Ppp* + *Ppn*/mainland	*Ppn* + *Ppp*/mainland	Mainland + *Ppn*/*Ppp*
75	73	67	2	100%	0%	0%
100	73	67	2	90%	10%	0%
150	73	67	2	30%	70%	0%
200	73	67	2	0%	100%	0%
344	73	67	2	0%	100%	0%

Island vs. mainland genetic diversity

We found the greatest amount of mitochondrial genetic diversity within the mainland locations (Table 3.4). The group with the lowest genetic diversity included *P. p. phasma* and *P. p. decoloratus*, with a single haplotype identified across all samples and locations. Within the *P. p. niveiventris* locations we detected limited genetic diversity, however, none of the measurements of mitochondrial diversity were higher than the overall diversity found in mainland *P. polionotus* (Table 3.4). Each mainland sample location contained more than one haplotype, even with small sample sizes (LARA; N = 2; Table 3.4). We found two locations with more than one haplotype within the *P. p. niveiventris* locations (CNS and CC; Table 3.4), while the remaining locations were fixed for a single haplotype (SDP and PINWR; Table 3.4).

Based on our haplotype and genetic structure data we pooled the mainland samples when comparing genetic diversity between mainland *P. polionotus* and the Atlantic coast subspecies (see Discussion). We found the observed levels of genetic diversity to fall within the 95% confidence interval (CI) of the simulated estimates (Fig. 3.8), suggesting our simulations

provided good estimates of observed diversity. We found the number of haplotypes and π to be significantly higher for the mainland *P. polionotus* subspecies compared to the Atlantic coast subspecies, based on the lack of overlap in the 95% CI (Fig. 3.8). Mainland samples had greater haplotype diversity than *P. p. phasma*, but there was overlap in the 95% CI with *P. p. niveiventris*. This overlap indicates that haplotype diversity is not significantly different between mainland *P. polionotus* and *P. p. niveiventris* (Fig. 3.8). We found *P. p. niveiventris* to have greater genetic diversity than *P. p. phasma*, with no overlap in 95% CI for all measures of diversity (Fig. 3.8).

For our microsatellite data we observed greater genetic diversity within the mainland sample locations compared to the Atlantic coast sample locations (Table 3.5). We observed some of the lowest genetic diversity within the *P. p. phasma* sample locations, however, two of the *P. p. niveiventris* locations (SDP and PINWR; Table 3.5) also exhibited comparable low genetic diversity. The permutation test implemented into FSTAT indicated that the mainland *P. polionotus* had greater genetic diversity than that found in *P. p. phasma* after Bonferroni correction for multiple comparisons (Allelic richness, P-value < 0.001; H_o, P-value < 0.001; H_e, P-value = 0.001; Fig. 3.9). The values of genetic diversity for *P. p. niveiventris* were found to be between the mainland subspecies and *P. p. phasma* for all three measures (Fig. 3.9), and *P. p. niveiventris* was not significantly different from either the mainland subspecies (Allelic richness, P-value = 0.020; H_o, P-value = 0.478; H_e, P-value = 0.050; Fig. 3.9), or *P. p. phasma* (Allelic richness, P-value = 0.038; H_o, P-value = 0.038; H_e, P-value = 0.057; Fig. 3.9), after Bonferroni correction for multiple comparisons.

Table 3.5 Genetic diversity for *cyt b* sequence data across sample locations of *P. polionotus* captured in peninsular Florida. Diversity reported for all mainland subspecies (*P. p. subgriseus* and *P. p. rhoadsi*), all samples of beach mouse subspecies *P. p. phasma* and *P. p. decoloratus*, all beach mouse subspecies *P. p. niveiventris*, and for each sample location for each of these groups (not reported for *P. p. phasma* and *P. p. decoloratus* as they share the same single haplotype). For each location we reported sample size (N), number of haplotypes, number of polymorphic sites, haplotype diversity, nucleotide diversity (π) , and average nucleotide differences (k).

Group	Sample location	N	# haplotypes	# polymorphic sites	haplotype diversity	π	k
Mainland subspecies	All	56	19	26	0.885	0.005	5.098
	SRWEA	10	4	7	0.644	0.002	2.378
	ONF	7	5	6	0.857	0.002	2.381
	LARA	2	2	1	1.000	0.001	1.000
	LLSP	14	9	14	0.934	0.004	4.615
	APAFR	10	2	10	0.467	0.003	4.667
	ABS	13	3	8	0.295	0.002	1.231
P. p. phasma/ *P. p. decoloratus*	All	33	1	0	0.000	0.000	0.000
P. p. niveiventris	All	36	3	3	0.538	0.001	0.624
	SDP	7	1	0	0.000	0.000	0.000
	CNS	8	2	1	0.536	0.004	0.536
	CC	6	3	3	0.600	0.001	1.000
	PINWR	15	1	0	0.000	0.000	0.000

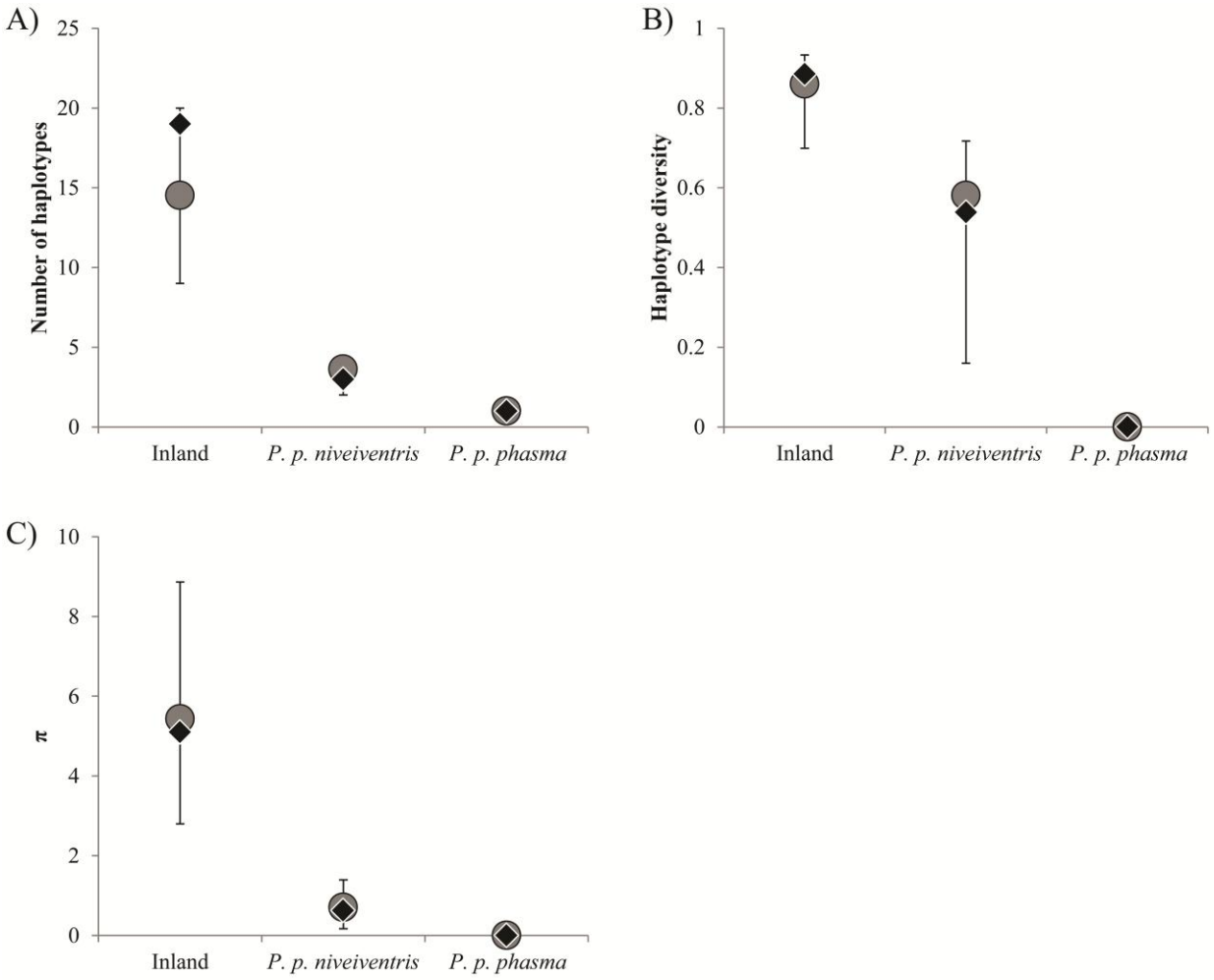

Figure 3.8 Measures of genetic diversity based on *cyt b* sequence data for *P. polionotus* samples: number of haplotypes (A), haplotype diversity (B), and nucleotide diversity (π; C). X-axis shows diversity for mainland subspecies (*P. p. subgriseus* and *P. p. rhoadsi*), *P. p. niveiventris* and *P. p. phasma*. Black diamonds show observed value, and grey circles show estimated values based on coalescence simulation in DnaSP. Error bars show 95% confidence intervals for simulated data.

Table 3.6 Data summary for ten microsatellite loci across the sampled locations of *P. polionotus*; mainland subspecies (*P. p. subgriseus* and *P. p. rhoadsi*), *P. p. phasma*, and *P. p. niveiventris*. For each sample location sample size (n) is reported, in addition to measures of genetic diversity: number of alleles (A), allelic richness (AR), and observed (H_o) and expected (H_e) heterozygosity. For each measure of diversity values are reported as averages and standard deviations.

Group	Sample location	n	A	AR	H_o	H_e
Mainland subspecies	SRWEA	10	8.2 ± 2.6	7.1 ± 2.1	0.764 ± 0.170	0.785 ± 0.166
	ONF	9	7.8 ± 1.9	7.0 ± 1.6	0.760 ± 0.162	0.801 ± 0.067
	LLSP	25	11.7 ± 3.5	7.5 ± 1.2	0.774 ± 0.122	0.855 ± 0.035
	APAFR	10	7.1 ± 2.0	6.3 ± 1.4	0.739 ± 0.218	0.782 ± 0.073
	ABS	13	9.2 ± 2.3	7.1 ± 1.4	0.735 ± 0.203	0.822 ± 0.055
P. p. phasma	ASP	40	5.7 ± 1.8	3.8 ± 1.0	0.478 ± 0.223	0.615 ± 0.144
	FM	33	3.0 ± 1.8	2.2 ± 1.1	0.206 ± 0.233	0.290 ± 0.245
P. p. niveiventris	SDP	19	4.2 ± 1.3	3.5 ± 1.2	0.588 ± 0.237	0.525 ± 0.212
	CNS	31	7.7 ± 3.3	5.1 ± 1.8	0.598 ± 0.232	0.673 ± 0.251
	MINWR	32	7.6 ± 3.4	5.4 ± 1.9	0.671 ± 0.263	0.718 ± 0.201
	BG3	28	8.9 ± 4.1	5.8 ± 2.0	0.709 ± 0.191	0.733 ± 0.187
	SG1	43	8.1 ± 3.0	5.3 ± 1.8	0.707 ± 0.261	0.713 ± 0.235
	SG3	56	9.7 ± 4.3	5.8 ± 1.8	0.711 ± 0.206	0.735 ± 0.204
	SG2	44	9.1 ± 3.7	5.7 ± 2.0	0.691 ± 0.222	0.729 ± 0.217
	BG1	35	9.1 ± 3.0	6.0 ± 1.8	0.706 ± 0.193	0.747 ± 0.191
	BG2	41	9.5 ± 4.0	5.8 ± 2.1	0.716 ± 0.270	0.721 ± 0.252
	PINWR	15	4.2 ± 1.6	3.7 ± 1.3	0.600 ± 0.229	0.583 ± 0.188

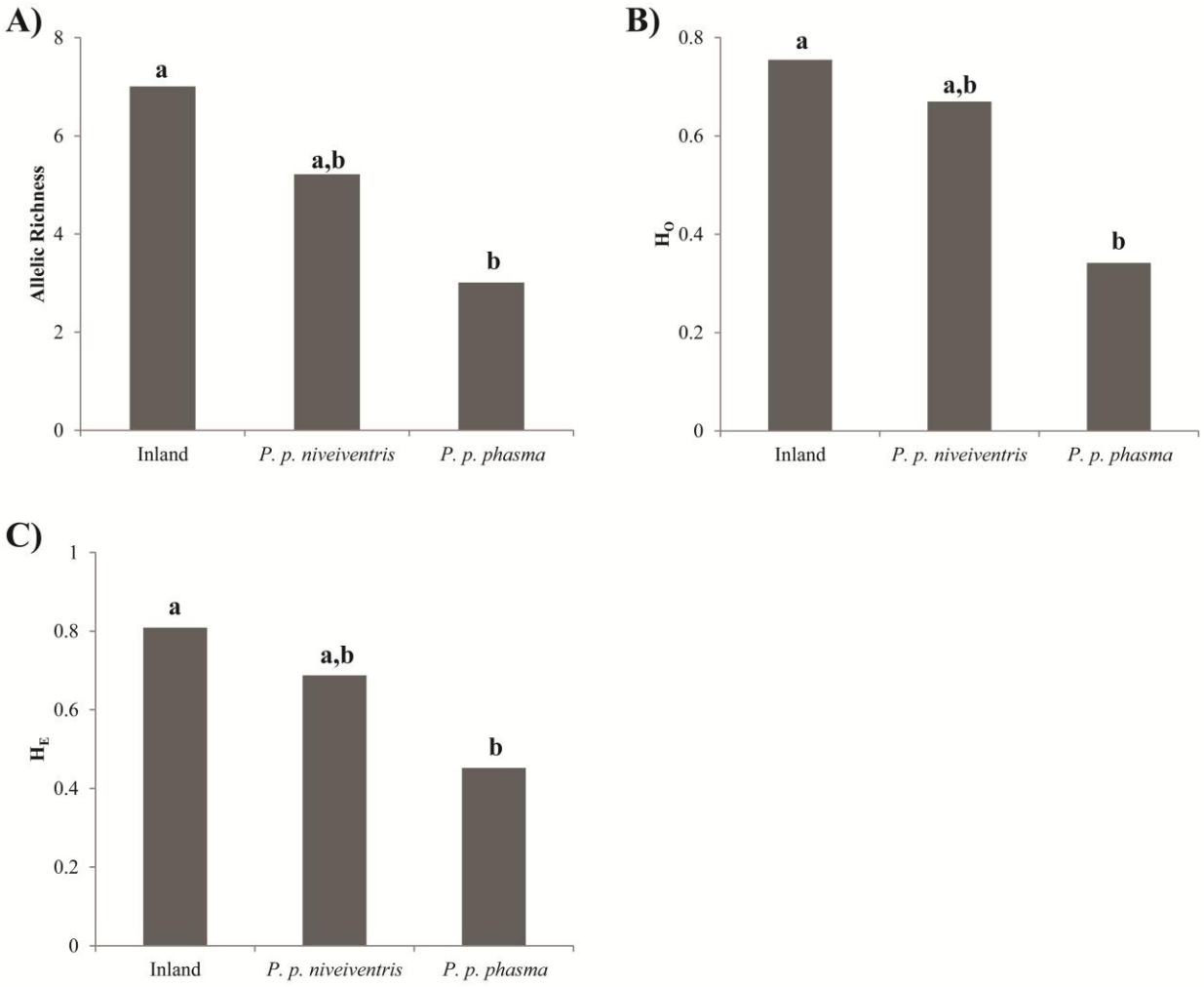

Figure 3.9 Measures of genetic diversity based on ten microsatellite loci for *P. polionotus* across peninsular Florida: allelic richness (A) and observed (B) and expected (C) heterozygosity. Each bar illustrates genetic diversity within mainland subspecies (*P. p. subgriseus* and *P. p. rhoadsi*), *P. p. niveiventris* or *P. p. phasma*. Different letters above bars indicate significant differences between them. After Bonferroni correction $\alpha = 0.017$.

Discussion

We have identified divergence and isolation consistent with rapid evolution in a lineage invading novel coastal habitat. First we found that the two extant Atlantic coast beach mouse subspecies, *P. p. phasma* and *P. p. niveiventris*, represent distinct lineages, supporting the hypothesis that these subspecies constitute unique taxonomic units. The relationship of the extinct subspecies *P. p. decoloratus* to the other subspecies remains unclear, as our limited sample sizes showed it to share a single haplotype with *P. p. phasma*. Second we found that all recognized subspecies appear to have originated from a single mainland source. The haplotypes found on the barrier islands originate from a single haplotype from central Florida. These findings support Bowen's single colonization hypothesis, but we cannot distinguish between a single colonization event with subsequent diversification or multiple colonization events from that shared source. Third we found support for different clustering patterns depending on the level of sample bias (Table 3.4). This can have consequences for future use of STRUCTURE in evaluating genetic structure. Regardless of the route and dynamics of barrier island colonization, the two extant subspecies each have unique phylogenetic trajectories and limited genetic diversity. Finally, as expected for recently established and narrowly-distributed subspecies, we found low genetic diversity in *P. p. phasma* in both mitochondrial and nuclear markers, compared to mainland conspecifics. However, for *P. p. niveiventris* we found similarly low mitochondrial sequence diversity, but nuclear diversity was similar to mainland sample locations (Fig. 5.8 and 5.9), potentially due to large founder populations or multiple founder events.

In evaluating the taxonomic status of the Atlantic coast beach mouse subspecies we found evidence that the two extant subspecies, *P. p. phasma* and *P. p. niveiventris*, show clear genetic differentiation from each other and from the mainland subspecies, with unshared *cyt b* haplotypes and lack of gene flow estimated from microsatellite data. As expected with recently diverged taxa our phylogenetic tree provided little information for discerning evolutionary relationships among *P. polionotus* spp. In recently isolated taxa, divergence can be difficult to detect as a result of incomplete lineage sorting and insufficient time for evidence of phenotypic and genotypic differentiation to manifest in sampled characters (Maddison and Knowles 2006). The remaining analyses suggested that *P. p. phasma* and *P. p. niveiventris* belong to their own distinct taxonomic units. Lack of gene flow has been also documented between the Gulf coast beach mouse subspecies (Mullen et al. 2009, Domingues et al. in press), suggesting that the recognized beach mouse subspecies all represent their own evolutionary trajectories. The only subspecies without support for this evolutionary independence was *P. p. decoloratus*, where both specimens examined had the same haplotype found in *P. p. phasma*. This result suggests these two subspecies may represent the same evolutionary lineage. The different subspecies are identified by their pelage color (Bowen 1968), and the this seems to be a strong predictor for identifying evolutionary lineages for beach mice both on the Atlantic coast, as we have shown here, as well as on the Gulf coast (Mullen et al. 2009, Domingues et al. in press).

Variation within species is widely recognized through the use of subspecies designation. However, many studies have shown discrepancies between evolutionary lineages and taxonomic groupings within species (Burbrink et al. 2000, Zink 2004, Mulcahy 2008, Daza et al. 2009, Newman and Rissler 2011, Tursi et al. in press). Such deviation could reflect influence of clinal

or other environmental variation in morphological characters used to identify (van Valen 1973, Myers et al. 1996, Grieco and Rizk 2010, Svanbäck and Schluter 2012), rather than reflecting evolutionary history. Color patterns have been one trait that has been found to be a poor predictor of intraspecific variation (e.g., Burbrink et al. 2000, Trujano-Alvarez and Álvarez-Castañeda 2007). However, in beach mice pelage-defined color patterns are an ideal character state for determining evolutionary lineages (this study, Mullen et al. 2009, Domingues et al. in press). The possible causal explanations for the correspondence of pelage color to evolutionary lineage in this system maybe the evolutionary processes that impact the beach mice. Extensive research has documented selective differentiation in beach mice, with a selective advantage to matching pelage color to sand substrate (Mullen and Hoekstra 2008, Vignieri et al. 2010). The genes influencing pelage color variation in beach mice differ between Gulf and Atlantic coast subspecies (Hoekstra et al. 2006, Steiner et al. 2007, Steiner et al. 2009), but beach mice differentiation seems to be driven by similar selective pressures on different populations. Comparable results have been reported in *Anolis* species, where dewlap colors denote intraspecific variation (Glor and Laport 2012). In this case dewlap colors may be under selective pressure for species recognition, sexual selection, or both (Losos 1985, Vanhooydonck et al. 2005).

Differential selective pressure for background matching seems to be driving coastal speciation in *P. polionotus*. However, correspondence of taxonomic units to evolutionary lineages is not as clear on the mainland. Mainland *P. polionotus* subspecies are also recognized by phenotypic variation, with eight recognized subspecies (Hall 1981). Our sample locations fell within the distributions of *P. p. subgriseus* and *P. p. rhoadsi*, but our genetic data do not support

the existence of separate evolutionary trajectories for these taxonomic units. The two subspecies do not share haplotypes; however, most of the *P. p. subgriseus* haplotypes directly linked up to a central Florida *P. p. rhoadsi* haplotype (Fig. 3.3). This suggests *P. p. subgriseus* haplotypes are more closely associated with a *P. p. rhoadsi* haplotype than to other *P. p. subgriseus* haplotypes. Additionally, genetic structure does not support clear differentiation between the two subspecies (Fig. 3.6). In order to resolve mainland intraspecific variation further sampling would be needed.

Dispersal to islands has become increasingly recognized as an important process affecting the distribution of biodiversity on islands (de Queiroz 2005, Cowie and Holland 2006), but how islands are initially colonized has received little attention (Cowie and Holland 2006). Several studies have found that diversity of a focal taxon on islands was the result of colonization from a single source that gave rise to adaptive radiations currently seen among island taxa (Grant 1981, Böhle et al. 1996, Burns et al. 2002, Filardi and Moyle 2005). Barrier islands are much more closely associated with continental landmasses than oceanic islands, and could provide a greater opportunity for colonization from multiple sources. Any variation observed among islands could then be a result of variation from different sources. Our study provided an opportunity to test hypotheses of the colonization patterns of *P. polionotus* on to recently formed barrier islands.

One of the most comprehensive studies of beach mouse evolution was based on pelage color by Bowen (1968), and he proposed hypotheses for the establishment and evolution of Gulf and Atlantic coast beach mouse subspecies. On the Gulf coast previous studies have rejected Bowen multiple colonization hypothesis (Van Zant and Wooten 2007, Domingues et al. in

press). On the Atlantic coast, Bowen's hypothesized that the diversity observed is the result of a single colonization event (Bowen 1968). Our haplotype network indicated that the extant subspecies of Atlantic coast beach mice originated from the same source, based on inferred lineages to a haplotype currently found in central Florida (Fig. 3.3), and therefore supports Bowen's single colonization hypothesis. However, our data cannot distinguish between single colonization of the barrier islands or multiple colonization events from the same gene pool.

Our data does suggest a sequence of events regarding the formation of extant Atlantic beach mouse diversity. We found that *P. p. phasma* seems to have been isolated from mainland *P. polionotus* and *P. p. niveiventris* the longest, with the greatest amount of genetic differentiation from other subspecies based on R_{ST} values. When we included even sample sizes we also found support for an initial isolation of *P. p. phasma* from a *P. p. niveiventris*/*P. p. subgriseus*/*P. p. rhoadsi* cluster (Fig. 3.7). These findings suggest that *P. p. phasma* was isolated from other *P. polionotus* populations before *P. p. niveiventris* was isolated from the mainland.

Our numerical sampling bias of *P. p. niveiventris* raises an important question on the sensitivity of STRUCTURE analyses using skewed sample sizes. STRUCTURE is widely used in population genetics studies, illustrated by the over 6,000 times Pritchard et al. (2000) has been cited (Web of Knowledge, accesses 6-July-2012). However, the impact of sample size has not sufficiently addressed in the use of STRUCTURE. With greater trapping intensity of *P. p. niveiventris* we had over three times more samples for this subspecies than for the other subspecies (Table 3.1). With increased sample bias, STRUCTURE tended to attribute all members of the geographical locality with the most samples as being a unique genetic population

(Table 5.4). When we reduced sample bias we increasingly observed a tendency of *P. p. phasma* to form its own cluster, while *P. p. niveiventris* and the mainland subspecies formed a separate cluster. Even with smaller bias (*P. p. niveiventris*; n = 100) we observed runs with contradictory results (90% of runs *P. p. phasma* form its own cluster, 10% of runs *P. p. niveiventris* form its own cluster; Table 3.4). Our findings emphasized the importance of including independent runs for STRUCTURE analysis, but also of considering the possible effects of sampling bias. We found conflicting results due to sampling bias, and our inferences must take such biases into consideration.

Colonizing islands is often associated with the reduction of effective population size through bottlenecks and founder effects. This can lead to loss of genetic diversity through genetic drift and inbreeding (Frankham 1997). Reduced genetic diversity has been reported in many studies of island populations (e.g., Eldridge et al. 1999, Jones et al. 2004, Boessenkool et al. 2007). While barrier islands are often closely associated with the mainland, and therefore may avoid loss of diversity through maintenance of gene flow, we found *P. p. phasma* to conform to the hypothesis of isolation and lower genetic diversity for island populations. Both our *cyt b* and microsatellite data showed significantly lower genetic diversity for *P. p. phasma* compared to mainland genetic diversity (Fig. 3.8 and 3.9). For *P. p. niveiventris* we found genetic diversity to be significantly lower compared to the mainland populations for *cyt b* (Fig. 3.8), but diversity was not significantly different for microsatellite loci (Fig. 3.9). The patterns of genetic diversity suggest that not all island populations are affected equally by initial founder effects and subsequent bottlenecks and isolation. Variation in evolutionary history, ecology and behavior among lineages can cause differences in the impact on genetic diversity (Taylor et al. 2007).

Initial population size can greatly impact genetic diversity for populations (Clegg et al. 2002), so the higher nuclear genetic diversity in *P. p. niveiventris* could be caused by a larger founder population, compared to that of *P. p. phasma*. We did observe a discrepancy between the two genetic markers in *P. p. niveiventris*, which may be due to the nature of the markers. The effective population size for mitochondrial markers is only one quarter of nuclear markers, making genes such as *cyt b* more sensitive to bottlenecks and founder effects (Fay and Wu 1999). If *P. p. niveiventris* was established by a larger founder population, this subspecies could have maintained genetic diversity comparable to what is observed on mainland, but the colonization effects were enough to impact the genetic diversity of the mitochondrial genome.

In summary, we found pelage color to correspond to evolutionary lineages in the Atlantic coast beach mouse subspecies. We also found that the Atlantic coast subspecies originated from the same mainland source. With a large numerical sample bias we were able to show that STRUCTURE analyses can be influenced by numerical sampling bias. And finally, we found *P. p. phasma* to follow the predicted pattern of lower genetic diversity in island population, while *P. p. niveiventris* has maintained nuclear genetic diversity at mainland levels.

Conservation implications

Our understanding of the evolutionary history and population genetics of beach mice can greatly impact conservation efforts. Among the extant beach mouse subspecies, only one is not federally listed as either threatened or endangered. For the extant Atlantic coast beach mice, *P. p. phasma* is listed as endangered and *P. p. niveiventris* is listed as threatened (U.S. Fish and Wildlife Service 1989). In order to protect specific segments of a species the Endangered Species

Act has since 1978 provided protection to populations of terrestrial vertebrates that are considered "distinct population segments" (DPS) (Pennock and Dimmick 1997). Prior research has defined *P. p. niveiventris* as an evolutionarily significant unit (Degner et al. 2007), however, our findings support defining both extant Atlantic coast beach mouse subspecies as two DPS based on the criteria given by the U.S. Fish and Wildlife Service and National Marine Fisheries Services (1996). The criteria for being defined as a DPS is 'discrete', 'significant' and endangered compared to other conspecifics. 'Discrete' refers to being disconnected to conspecifics such as by a lack of gene flow, and 'significant' relates to the use of unique habitat (U.S. Fish and Wildlife Service and National Marine Fisheries Service 1996). The two subspecies are 'discrete' by showing lack of gene flow to mainland conspecifics or between subspecies. The Atlantic coast beach mice are 'significant' as they occupy unique coastal habitat compared to mainland conspecifics. Finally, the Atlantic coast beach mice are federally listed, while the mainland conspecifics are considered of least concern.

Acknowledgments

Funding for this project was provided by US Fish and Wildlife Service, Florida Fish and Wildlife Conservation Commission, and Patrick Air Force Base, Florida. I wish to give my thanks to A. Bard, A. DeLong, T. Doonan., C. Handrick, H. Hoekstra, M. Keserauskis, D. Oddy, J. Provancha, A. Suazo, M. Tucker and J. Van Zant, as well as field assistants A. Ashcraft-Cryder, K. Gillespie, M. Green, D. Gunderson, W. Lavigne, S. Letcher, D. Smith and A. Verpoorton, for providing tissue samples for multiple populations utilized for this study. We greatly appreciate advice and feedback from E. Hoffman and J. Reece on analysis of data used in

this study, as well as interpretation of these results. This study was conducted under permit 12-09-4-01 given by the Florida Department of Environmental Protection, Division of Recreation and Parks, WV04065 given by the Florida Fish and Wildlife Conservation Commission, and TE105642-0 issued by USFWS. All animal handling was approved by IACUC protocols # 03-13W and 06-46W from University of Central Florida.

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CHAPTER 4. UNRAVELING NATURAL VERSUS ANTHROPOGENIC EFFECTS ON GENETIC DIVERSITY WITHIN THE SOUTHEASTERN BEACH MOUSE (*PEROMYSCUS POLIONOTUS NIVEIVENTRIS*)[2]

Introduction

Habitat loss and fragmentation are among the greatest negative impacts humans have on natural populations (Groom et al. 2005, Fischer and Lindenmayer 2007). Reduced habitat and increased fragmentation of populations leads to isolation of populations which are disconnected 'islands' in the landscape. Smaller populations are subject to genetic drift and loss of genetic diversity (Lacy 1987, Frankham 1997, Thalmann et al. 2011, Tracy and Jamieson 2011). Populations that have sustained losses of genetic diversity have an increased probability of extinction (Saccheri et al. 1998, Reed and Frankham 2003, Lavergne and Molofsky 2007). Therefore levels of genetic diversity have been widely used in conservation biology as measures of human impact on taxa of interest and in assessing their future management needs (Miller and Waits 2003, Schwartz et al. 2007, Helm et al. 2009).

Several studies have shown that natural populations can exhibit low genetic diversity independent of human influences (O'Brien 1994, Hedrick 1995). Thus, low levels of contemporary genetic diversity can be explained by historical events acting prior to anthropogenic impacts (Miller and Waits 2003, Taylor et al. 2007, Reding et al. 2010). For example, historical population bottlenecks, gene flow restrictions, and founder effects can account for current levels of genetic structure and diversity (Culver et al. 2000, Paxinos et al.

[2] Kalkvik H.M. et al. (in review) Unraveling natural versus anthropogenic effects on genetic diversity within the southeastern beach mouse (Peromyscus polionotus niveiventris). *Conserv. Genet.*

2002, Miller and Waits 2003, Taylor and Jamieson 2008). Changes in evolutionary pressures may also result in variation in genetic diversity and structure across a species' distribution (Lawton 1993, Eckert et al. 2008) and genome-wide selection sweeps can also cause reduced genetic diversity (Amos and Harwood 1998). These historical impacts on a population can result in adaptation to local environments (Avise et al. 1987, Slatkin 1987, Åbjörnsson et al. 2004).

Continental and insular populations are well known to exhibit differences in genetic diversity (Frankham 1997, Frankham et al. 2002). In a wide range of taxa that occupy landforms such as barrier islands, patterns of reduced genetic diversity relative to congeners on the mainland have been observed (Triggs et al. 1989, Frankham 1997, Bidlack and Cook 2001, MacAvoy et al. 2007). The genetic diversity in these insular populations has been shaped by historical (natural features) forces and more recently subjected to natural and anthropogenic influences. Naturally, populations occupying islands are often founded by few individuals, which has consequences on genetic diversity (Mayr 1942, Tinghitella et al. 2011). Taxa occupying barrier islands most likely dispersed from mainland populations and natural colonization events could account for contemporary genetic diversity. Barrier islands are also unique in that natural disturbance events such as hurricanes and floods may provide additional hardship (selective pressures) for taxa due to reoccurring bottlenecks during such events (Breininger et al. 1999, Oli et al. 2001, Scileppi and Donnelly 2007, Pries et al. 2009, Saha et al. 2011b). Anthropogenic influences manifest as land use conversion in coastal habitats and result in the loss of biotic diversity (Barbier et al. 2008, Mhemmed et al. 2008). One third of earth's human population now occupies coastal areas (Barbier et al. 2008) and this trend seems to be increasing (Small and Nicholls 2003). Thus the genetic diversity of barrier island taxa can be shaped by historical

founder events or bottlenecks acting alone, recent anthropogenic habitat loss, or a combination of historical and contemporary events.

Our purpose here is to examine genetic diversity of *Peromyscus polionotus niveiventris* (southeastern beach mouse) endemic to barrier islands subjected to diverse extrinsic factors, e.g., hurricanes and development pressures. This taxon is one of eight subspecies of *P. polionotus* that occupy barrier islands of the gulf coast of Alabama and Florida and the Atlantic coast of Florida (i.e. beach mice; Hall 1981). Historically ancestral beach mice populations were isolated from mainland conspecifics with the formation of the barrier islands (Hoekstra et al. 2006, Van Zant and Wooten 2007). Within the last few decades *P. p. niveiventris* has experienced a range contraction on the southeast barrier islands of Florida from a historical range of approximately 350 km to an estimated current range of approximately 70 km of continuous habitat as described by Stout (1992). In addition, two disjunct populations remain in a few kilometers of habitat in the northern and southern end of the current distribution (Fig. 4.1). The range contraction of *P. p. niveiventris* can be tracked over the last few decades (Stout 1992), and fits well with a model of decreased habitat associated with increased housing development of the area (Winsberg 1992). Prior work with this subspecies did not determine if genetic diversity was compromised by recent habitat losses (Degner et al. 2007).

To understand the genetic consequences of anthropogenic impacts we must be able to compare pre-development and post-development populations of *P. p. niveiventris*. Recent advances in the use of historical DNA derived from museum collections permit comparisons of historical and contemporary genetic diversity (Wandeler et al. 2007, Leonard 2008). Studies

using this approach have been able to identify taxa where historical processes prior to human influence can explain current low genetic diversity (e.g. Hoffman and Blouin 2004, Chan et al. 2005, Reding et al. 2010), while other studies have documented a reduction in genetic diversity associated with human impacts (e.g. Hauser et al. 2002, Culver et al. 2008, Thalmann et al. 2011).

We predicted a loss of genetic diversity in mitochondrial DNA in the contemporary range of *P. p. niveiventris* compared to the historical samples, based on the extensive loss of habitat for this subspecies. This comparison will identify the degree to which historical diversity has been affected by hypothesized anthropogenic influences over recent decades. Next we examined 10 microsatellite loci to describe genetic diversity and genetic structure within contemporary populations of *P. p. niveiventris*. We expected that extant genetic diversity would be higher in the contiguous tracts of habitat relative to disjunct habitats. The results we report inform future conservation strategies for this taxon based on insights into the historical and contemporary trends in its population genetics.

Methods and Material

Sampling and DNA Extraction

We acquired tissue as 4x4 mm^2 sections of skin taken from the venter of 78 dried museum specimens (Table 4.1). These specimens, categorized as historical, were collected from Volusia to Palm Beach Counties and represent the known historical range of *P. p. niveiventris* (Table 4.2). Our contemporary samples were from Smyrna Dunes Park (SDP), Canaveral National Seashore (CNS), Merritt Island National Wildlife Refuge (MINWR), Cape Canaveral

(CC) and Pelican Island National Wildlife Refuge (PINWR) (Fig. 4.1). Samples from the current distribution were categorized as peripheral (SDP and PINWR), or central (CNS, MINWR and CC), based on location (Table 4.2, Fig. 4.1). In total, 344 contemporary individuals were used in this study. Individuals from contemporary sample locations were live trapped using methods described in Degner et al. (2007) and we collected 2-4 mm of tail tissue and stored the samples in 95% ethanol at -20°C prior to DNA extraction.

Genomic DNA was extracted from all tissue samples using a Qiagen DNeasy tissue purification kit (Qiagen Inc.). Museum tissues were soaked in 95% ethanol at 4°C for 24 hours to remove any salts and PCR inhibitors (Mullen and Hoekstra 2008). These tissues were subsequently lysed for 24 hours until dissolved. Contemporary tissue was lysed for 3-4 hours until completely dissolved.

Table 4.1 Collection data for museum skin specimens of *Peromyscus polionotus niveiventris* used for DNA analysis. Museum samples were acquired from the following museums; American Museum of Natural History (AMNH), Cornell University Museum of Vertebrates (CU), Field Museum of Natural History (FMNH), Florida Museum of Natural History (FLMNH), Harvard Museum of Comparative Zoology (MCZ), University of New Mexico Museum of Southwestern Biology (MSB), University of California Berkley Museum of Vertebrate Zoology (MVZ), and University of Michigan Museum of Zoology (UMMZ).

Museum	Museum ID	Specific Location	County	Year Collected	Haplotype
AMNH	12972	Jupiter Island	Palm Beach Co.	1895	X
AMNH	12973	Jupiter Island	Palm Beach Co.	1895	X
AMNH	12977	Jupiter Island	Palm Beach Co.	1895	X
AMNH	12978	Jupiter Island	Palm Beach Co.	1895	-
AMNH	12981	Jupiter Island	Palm Beach Co.	1895	X
AMNH	12982	Jupiter Island	Palm Beach Co.	1895	X
AMNH	12983	Jupiter Island	Palm Beach Co.	1895	X
AMNH	12984	Jupiter Island	Palm Beach Co.	1895	X
AMNH	166249	Cape Canaveral	Brevard Co.	1923	X
AMNH	166250	Cape Canaveral	Brevard Co.	1923	X
AMNH	166251	Cape Canaveral	Brevard Co.	1923	X
AMNH	166252	Cape Canaveral	Brevard Co.	1923	X
AMNH	166572	Eau Gallie	Brevard Co.	1951	Z
AMNH	166753	Eau Gallie	Brevard Co.	1951	A
CU	3339	Unknown	Brevard Co.	1942	A
CU	3341	Unknown	Brevard Co.	1942	A
CU	3342	Unknown	Brevard Co.	1942	B
CU	3343	Unknown	Brevard Co.	1942	B
CU	3344	Unknown	Brevard Co.	1942	A
CU	3345	Unknown	Brevard Co.	1942	A
CU	3346	Unknown	Brevard Co.	1942	A
CU	3347	Unknown	Brevard Co.	1942	A
CU	3349	Unknown	Brevard Co.	1942	A

Museum	Museum ID	Specific Location	County	Year Collected	Haplotype
CU	3350	Unknown	Brevard Co.	1942	A
CU	3395	Unknown	Brevard Co.	1942	-
CU	3396	Unknown	Brevard Co.	1942	-
CU	3397	Unknown	Brevard Co.	1942	A
CU	8234	New Smyrna	Volusia Co.	1954	A
CU	8235	New Smyrna	Volusia Co.	1954	A
CU	8236	New Smyrna	Volusia Co.	1954	A
CU	8237	New Smyrna	Volusia Co.	1954	A
FMNHC	5321	Jupiter Island	Palm Beach Co.	1895	X
FMNHC	5323	Jupiter Island	Palm Beach Co.	1895	X
FMNHC	5324	Jupiter Island	Palm Beach Co.	1895	X
FLMNH	12534	Merritt Island	Brevard Co.	1942	X
FLMNH	12538	Coronado Beach	Brevard Co.	1946	Y
FLMNH	16389	John's Island Beach	Indian River Co.	1974	A
FLMNH	23730	Canaveral Air Force Base	Brevard Co.	1986	A
FLMNH	23731	Sebastian Inlet	Indian River Co.	1986	B
FLMNH	23732	Sebastian Inlet	Indian River Co.	1986	A
FLMNH	23733	Sebastian Inlet	Indian River Co.	1986	A
FLMNH	23734	Pepper Beach	St. Lucie Co.	1986	B
FLMNH	24370	Fort Pierce	St. Lucie Co.	1988	B
FLMNH	24371	Sebastian Inlet	Indian River Co.	1989	A
FLMNH	2729	Oak Lodge	Brevard Co.	1948	X
FLMNH	2731	Oak Lodge	Brevard Co.	1948	X
FLMNH	2732	Oak Lodge	Brevard Co.	1948	X
FLMNH	2734	Vero Beach	Indian River Co.	1947	Y
FLMNH	5901	Floridana Beach	Brevard Co.	1960	X
FLMNH	63	Sebastian Inlet	Brevard Co.	1948	X
FLMNH	64	Sebastian Inlet	Brevard Co.	1948	X

Museum	Museum ID	Specific Location	County	Year Collected	Haplotype
MCZ	3076	Oak Lodge	Brevard Co.	1893	X
MCZ	3077	Oak Lodge	Brevard Co.	1893	-
MCZ	3078	Oak Lodge	Brevard Co.	1893	-
MCZ	3079	Oak Lodge	Brevard Co.	1893	X
MCZ	3080	Oak Lodge	Brevard Co.	1893	A
MCZ	3081	Oak Lodge	Brevard Co.	1893	Y
MCZ	3082	Oak Lodge	Brevard Co.	1893	-
MCZ	3083	Oak Lodge	Brevard Co.	1893	-
MCZ	3084	Oak Lodge	Brevard Co.	1893	-
MCZ	3085	Oak Lodge	Brevard Co.	1893	-
MCZ	3086	Oak Lodge	Brevard Co.	1893	-
MCZ	3087	Oak Lodge	Brevard Co.	1893	-
MCZ	3088	Oak Lodge	Brevard Co.	1893	-
MCZ	3090	Oak Lodge	Brevard Co.	1893	-
MCZ	3091	Oak Lodge	Brevard Co.	1893	-
MSB	64750	Coronado Beach	Brevard Co.	1946	B
MSB	64755	Coronado Beach	Brevard Co.	1946	A
MSB	64770	Micco	Brevard Co.	1948	A
MVZ	67208	Oak Lodge	Brevard Co.	1895	A
MVZ	67209	Oak Lodge	Brevard Co.	1895	B
UMMZ	104089	Oak Lodge	Brevard Co.	1908	A
UMMZ	104090	Oak Lodge	Brevard Co.	1908	A
UMMZ	104091	Oak Lodge	Brevard Co.	1908	-
UMMZ	104092	Oak Lodge	Brevard Co.	1908	A
UMMZ	104093	Oak Lodge	Brevard Co.	1908	A
UMMZ	104094	Oak Lodge	Brevard Co.	1908	B
UMMZ	104095	Oak Lodge	Brevard Co.	1908	A

Table 4.2. Measures of genetic diversity for *cyt b* sequence data across the contemporary and historical range of *P. p. niveiventris*. For each time scale and each location we report: category (C – C: contemporary – central, C – P: contemporary – peripheral, H: historical), sample size (N), haplotypes, number of polymorphic sites, average nucleotide differences (k), and nucleotide diversity (π).

Time scale	**Location**	**Category**	**N**	**Haplotypes**	**Polymorphic sites**	**k**	**π**
Contemporary	Overall		37	3	3	0.62	0.00056
	Smyrna Dunes Park	C – P	7	1	0	0.00	0.00000
	Canaveral National Seashore	C – C	8	2	1	0.54	0.00050
	Cape Canaveral	C – C	6	3	3	1.00	0.00088
	Sebastian Inlet State Park	C – P	1	1	0	0.00	0.00000
	Pelican Island National Wildlife Refuge	C – P	15	1	0	0.00	0.00000
Historical	Overall		63	5	6	0.91	0.00120
	New Smyrna	H	7	3	2	0.52	0.00071
	Cape Canaveral	H	5	2	1	0.40	0.00113
	Oak Lodge	H	23	4	4	0.84	0.00126
	Jupiter Island	H	10	1	0	0.00	0.00000

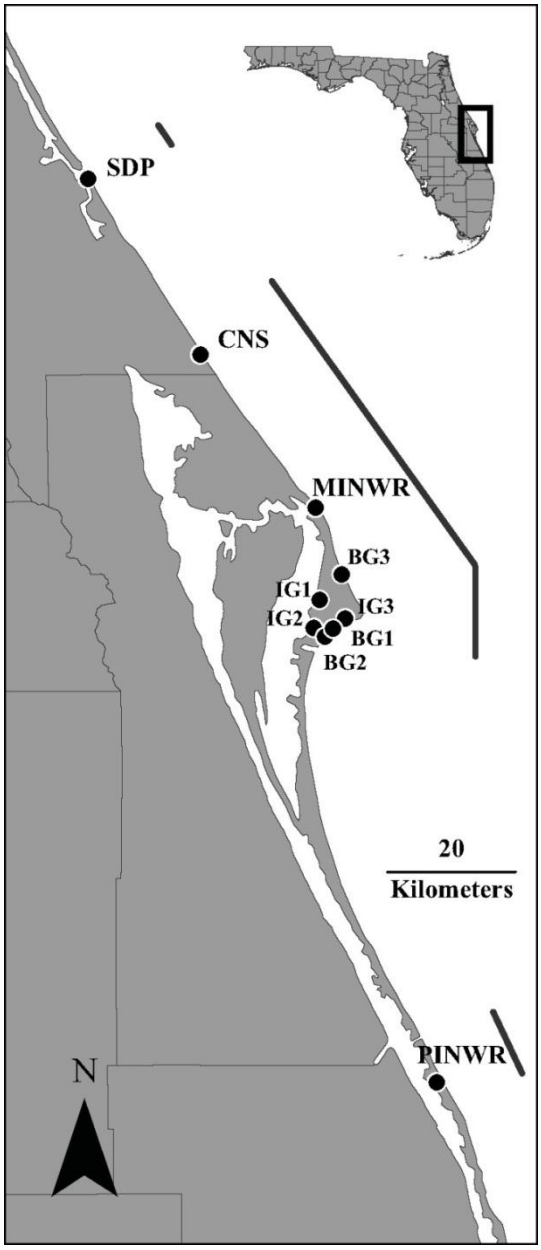

Figure 4.1. Sample locations of *P. p. niveiventris* across their contemporary distribution (black line) along the Atlantic coast of Florida. Tissue samples were collected from Smyrna Dunes Park (SDP), Canaveral National Seashore (CNS), Merritt Island National Wildlife Refuge (MINWR), Cape Canaveral and Pelican Island National Wildlife Refuge (PINWR). Six trapping grids were set up at Cape Canaveral, designated as Beach Grid (BG) 1 to 3 and Inland Grid (IG) 1 to 3.

Evaluating Loss of Genetic Diversity Using mtDNA

We amplified and sequenced 1100 bp of the rapidly evolving mitochondrial gene cytochrome b (*cyt b*), to assess the contemporary and historical genetic diversity of *P. p. niveiventris*. Because historical DNA samples were degraded, we amplified seven 200-300 base pair portions of the gene to recover the entire *cyt b* sequence. PCR reactions were done in 25 μL volumes, containing 20-30 ng DNA, 2 mM MgCl, 1X Amplitaq Gold Buffer, 200 μM dNTPs, 0.75 units AmpliTaq Gold (Roche, NJ), and 160 nM primer. Thermocycler conditions were: 94°C for 2 minutes, 45 cycles of 94°C for 1 minute, annealing temperature for 1 minute, and 72°C for 2 minutes. Annealing temperature and primer sequences used for historical DNA can be found in supplementary materials (Table 4.3). Among the contemporary samples we sequenced between six and 16 individuals for each sample location, a total of 37 individuals, following the same protocol given in Herron et al. (2004). We included MINWR as part of the CC sample for our sequence data. All sequences were processed on an ABI 3730 DNA analyzer by Nevada Genomics Center (Reno, NV). Sequences were edited in Sequencer v.4.8 (Gene Codes, Ann Arbor, MI), and aligned by eye using GeneDoc v.2.6 (Nicholas et al. 1997).

To compare diversity between historical and contemporary samples of *cyt b*, we estimated number of haplotypes, number of polymorphic sites, average nucleotide differences (k), and nucleotide diversity (π) using DnaSP v.4.2 (Rozas et al. 2003). We compared the genetic diversity among historical samples to contemporary samples using a one-tailed Welch's t-test in R v.2.11 *stats* package to test for the loss of genetic diversity. We only included estimated genetic diversity in the t-test for locations with more than five individuals in the sample area.

Table 4.3. Primer sequences for amplifying *cyt b* in degraded DNA samples of *P. polionotus*.

Sequence	Primer name	Primer sequence	Annealing temperature
Amplicon 1	mt14152F	5' AAC ATC CGA AAA AAA CAC CC 3'	50°C
	mt14341R	5' CTG ATG AGA ATG CTG TAG TTG TG 3'	
Amplicon 2	mt14297F	5' TAG CCA TAC ACT ACA CAT CAG 3'	55°C
	mt14534R	5' CCT ATG AAT GCT GTT GCT ATT AC 3'	
Amplicon 3	mt14471F	5' CTG ATG AGA ATG CTG TAG TTG TG 3'	55°C
	mt14688R	5' AAA TGC GAA GAA TCG TGT TAG G 3'	
Amplicon 4	mt14647F	5' CCT ATG AAT GCT GTT GCT ATT AC 3'	55°C
	mt14894R	5' ATT TTG GTT TTA TTT TTC CCA G 3'	
Amplicon 5	mt14866F	5' ATT TTG GTT TTA TTT TTC CCA G 3'	55°C
	mt15079R	5' GTT TTG AGG TTT GTA GTA GAG G 3'	
Amplicon 6	mt15030F	5' ATT TTG GTT TTA TTT TTC CCA G 3'	55°C
	mt15237R	5' AGA ATA TCT GGG AAA AAT AAA ACC 3'	
Amplicon 7	mt15193F	5' ATT GGA CAA CTA GCC TC 3'	55°C
	mt15377R	5' AGA ATA TCT GGG AAA AAT AAA ACC 3'	

To evaluate the evolutionary relationships among haplotypes we constructed haplotype networks for both historical and contemporary *cyt b* sequences. The haplotype networks were generated using Templeton et al. (1992) methodology, following the statistical parsimony approach implemented in TCS v.1.4b1 (Clement et al. 2000). The connection limit for the haplotype network was set to 95%.

Contemporary Genetic Structure and Interconnectivity

We utilized microsatellite loci to assess genetic structure and infer patterns of interconnectivity among sample locations across the current distribution of *P. p. niveiventris*. Microsatellite loci failed to amplify in historical samples, so only contemporary individuals were included. We genotyped all 344 contemporary individuals at ten microsatellite loci: pml-02, pml-06, pml-11 (Chirhart et al. 2000), PO-25, PO-71, PO-105, PO3-68, PO3-85 (Prince et al. 2002), ppa-01, and ppa-46 (Wooten et al. 1999). PCR reactions were conducted in 25 μL volumes containing 1-10 ng DNA, 2.5 μL PCR buffer, 0.3 units *Taq* polymerase (Proligo), 0.2 μM of forward and reverse primer, 0.8 mM combined concentration of DNTPs and 1.5-2.5 mM MgCl. PCR products were sized using CEQ 8000 Genetic Analysis System (Beckman-Coulter, Fullerton, CA). Allele sizes were scored using the CEQ 8.0 software and 400 bp standards (Beckman-Coulter).

We determined if each sample location and locus was in Hardy-Weinberg equilibrium (HWE) and linkage equilibrium using GenePop v.4.0 (François 2008), where significance values were estimated using a Markov chain approach (Dememorization = 10^4, Number of batches = 10^3, Number of iterations per batch = 10^4). We determined the distribution of genetic diversity

across the sample locations in the current range by measuring diversity as the number of alleles and allelic richness using FSTAT v.2.9 (Goudet 2001), in addition to observed and expected heterozygosity, which were estimated using GenAlEx v.6.1 (Peakall and Smouse 2006). Significant differences in genetic diversity between sample locations were determined using the group comparison tool in FSTAT to test for significance at $\alpha = 0.05$ probability level based on 10^4 permutations.

We determined current genetic structure across the range of *P. p. niveiventris* by estimating global F-statistic values following the methods of Weir and Cockerham (1984). To evaluate interconnectivity we estimated differentiation between sample locations as pair-wise F_{ST} (Weir and Cockerham 1984). Both measures of differentiation were estimated in FSTAT. Genetic differentiation is predicted to increase with geographic distance (i.e. Isolation by Distance; Wright 1943), so to test for the relationship between genetic and geographic distances, we ran a Mantel test in IBDWS v.3.15 (Jensen et al. 2005). Significance was estimated using $3x10^4$ randomizations. Pair-wise F_{ST} was used as a measure of genetic distance, and geographic distance was the Euclidiaen distance (km) between sample locations estimated using *dist* functions in R.

Lastly, we estimated the number of genetically distinct clusters (K) as an additional test of genetic structuring across the current range of *P. p. niveiventris* using a Bayesian admixture procedure (STRUCTURE v.2.2; Pritchard et al. 2000). With a Bayesian admixture procedure we can also identify recent gene flow between sample locations. The software STRUCTURE fits the data to a given K minimizing Hardy-Weinberg and linkage disequilibrium, and gives a likelihood

score [Pr(X|K)] for how well the data fit a set K. We applied a hierarchical approach to test the connectivity between the peripheral sample locations and the central part of the current range. First we included all individuals, and then we included only one or the other peripheral sample location (SDP and PINWR, Fig. 4.1) with all central sample locations. Lastly, we included only central sample locations in the STRUCTURE analysis. To determine the best fit K for each analyses, we used the best Pr(X|K) score as well as the method suggested by Evanno et al. (2005), which determines the second order derivative of Pr(X|K). Most parameters were set to the defaults given by STRUCTURE, with an admixture ancestral model. The initial $2x10^4$ MCMC generations were discarded as burn-in, with a subsequent $5x10^5$generations used to estimate parameters. We ran the analyses for the number of clusters ranging from one to ten, with 25 independent runs for each cluster. We plotted the membership coefficient values for each individual included in the analysis to evaluate the genetic structure for the best fit K. Individuals with membership coefficients associated closely to clusters other than those found in its sample location were considered evidence of a migrant or recent gene flow.

Results

Evaluating Loss of Genetic Diversity Using mtDNA

Sixty three of 78 samples provided sufficient (> 75%) *cyt b* sequence data to analyze (Table 4.1). Five haplotypes were identified from the historic samples and two corresponded to published haplotypes of this subspecies (Fig. 4.2A; EF216336 and EF216337; Degner et al. 2007). Of the 63 historical samples, 45 had sufficient spatial information to assign to specific

trapping locations: New Smyrna, Cape Canaveral, Oak Lodge and Jupiter Island (Table 4.2; Fig.4.2A).

A total of 37 *cyt b* sequences were generated from the contemporary sampling of *P. p. niveiventris*. Three haplotypes, corresponding to published haplotypes, were identified in the contemporary distribution (Table 4.2; EF216336-216338; Degner et al. 2007) and two locations had more than one haplotype (Cape Canaveral and Canaveral National Seashore; Fig. 4.2B). All measures of mitochondrial genetic diversity were higher from the historical range than the current range (Table 4.2). However, when the difference in genetic diversity was tested across the sample locations, we found no statistically significant loss of genetic diversity (haplotype: $t = 0.933$, $df = 5.534$, $P = 0.195$; polymorphic sites: $t = 0.677$, $df = 5.798$, $P = 0.262$; k: $t = 0.185$, $df = 5.449$, $P = 0.430$; and π: $t = 1.121$, $df = 5.575$, $P = 0.138$). We did observe a significant loss of nucleotide differences (k: $t = 2.545$, $df = 3$, $P = 0.043$) and nucleotide diversity (π: $t = 2.731$, $df = 3$, $P = 0.036$) when we excluded the central sample locations from the analysis.

The historical haplotype network showed the highest frequency of haplotype A (44%), followed by haplotype X (36%), which was not recovered in the contemporary distribution (Fig. 4.2C). Haplotype B was well represented with eight sequences found across the historical range (Fig. 4.2C), whereas two historical-only haplotypes, Z and Y, were found at lower frequencies. The five haplotypes identified in the historical range were each found in several sample locations, and no area exhibited unique haplotypes (Fig. 4.2A). The resolution of the haplotype network was poor, and does not inform the relationship between the different haplotypes (Fig. 4.2C). All of the haplotypes differed by one to two base pair changes. The contemporary samples

show a majority of individuals were assigned to haplotype B (54%) followed by haplotype A (43%), with only one individual of contemporary-only haplotype C (Fig. 4.2D). Haplotypes A and B differ by one base pair, whereas A and C have two base pair differences separating them (Fig. 4.2D).

Contemporary Genetic Structure and Interconnectivity

Three of the locus-by- sample location comparisons deviated from HWE for the microsatellite data set after the Bonferroni correction for multiple comparisons was applied. The deviation in HWE was found in three separate sample locations. In estimating linkage disequilibrium among all loci within each sample location, two loci combinations significantly deviated from linkage disequilibrium after Bonferroni correction. Based on the limited number of locations with loci out of HWE (N = 3), and only two locus pairs in linkage disequilibrium, we included all sample locations and loci in our analysis.

We found similar genetic diversity among the sample locations from the central part of the current distribution of *P. p. niveiventris* (Fig. 4.1; Table 4.4). The two locations found on the periphery of the range (SDP and PINWR; Fig. 4.1; Table 4.4) had significantly lower allelic richness (P = 0.024) and expected heterozygosity (P = 0.024) than values found for the remaining range of *P. p. niveiventris*.

Global F_{ST} estimated across the current distribution of *P. p. niveiventris* was 0.042 ± 0.004 SE. The pair-wise F_{ST} estimates ranged from 0.004 between the geographically closest locations (BG3 and IG3) to 0.213 between the most distant locations (SDP and PINWR; Table 4.5). We did uncover a significant relationship between geographic distance and genetic distance

throughout the dataset, with increased genetic differentiation following increased geographic distance (Mantel test; $P < 0.0001$, $R^2 = 0.855$; Fig. 4.3).

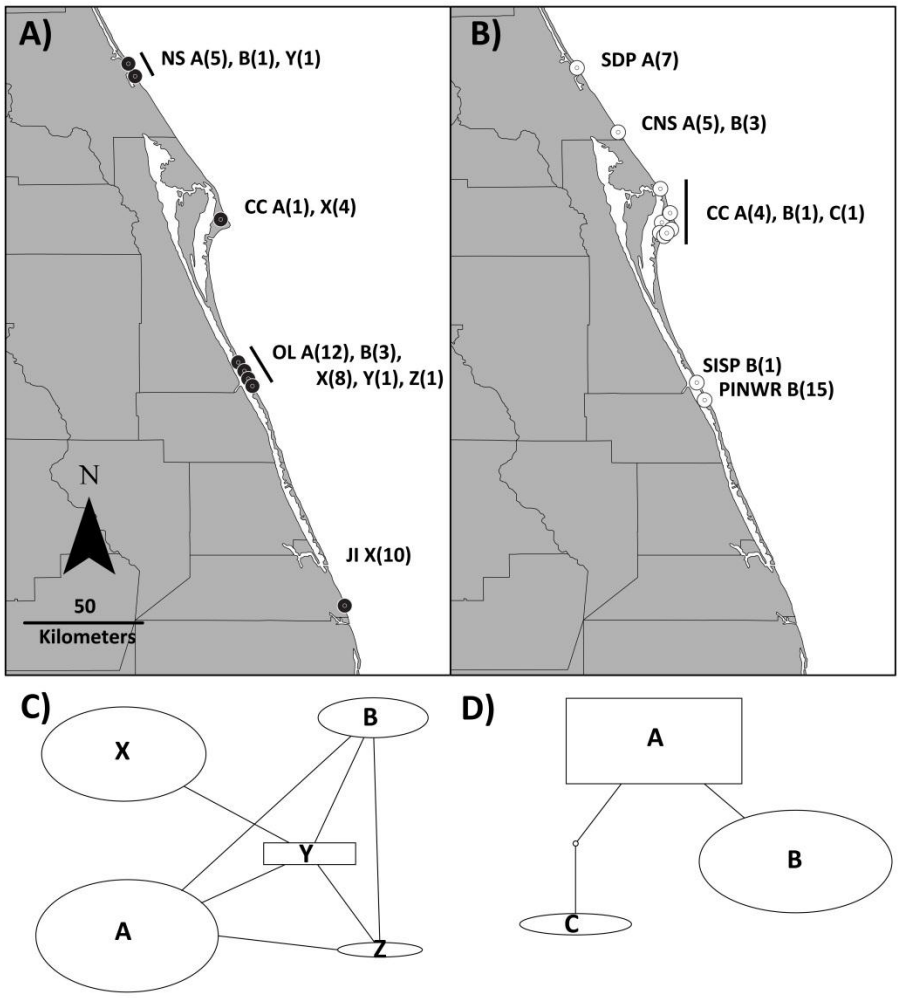

Figure 4.2. Sampling locality of *cyt b* sequence data across the historical (A) and contemporary (B) range of *P. p. niveiventris* with haplotypes labeled by each location (haplotype frequencies are stated in parentheses). Historical samples were collected from four areas; New Smyrna (NS), Cape Canaveral (CC), Oak Lodge (OL), and Jupiter Island (JI). Contemporary samples were collected from Smyrna Dunes Park (SDP), Canaveral National Seashore (CNS), Cape Canaveral (CC), Sebastian Inlet State Park (SISP), and Pelican Island National Wildlife Refuge (PINWR). The relationship among the historical (C) and contemporary (D) haplotypes is shown as haplotype networks, where the relative size of the box or oval illustrates frequency of the haplotype and lines illustrate proposed mutational steps.

Table 4.4. Summary data for 10 microsatellite loci across the contemporary range of *P. p. niveiventris* represented by 344 individuals. Sample location, sample size (N), number of alleles (A), allelic richness (AR), and observed (H_o) and expected (H_e) heterozygosity. Values are reported as averages and standard deviations.

Sample location	N	A	AR	Ho	He
Cape Canaveral					
Beach Grid 1	35.0 ± 0.00	9.4 ± 2.99	8.0 ± 2.54	0.706 ± 0.193	0.759 ± 0.180
Beach Grid 2	40.8 ± 0.42	9.5 ± 3.95	7.7 ± 2.96	0.716 ± 0.270	0.738 ± 0.240
Beach Grid 3	27.6 ± 0.52	8.9 ± 3.98	7.7 ± 3.21	0.709 ± 0.191	0.738 ± 0.185
Inland Grid 1	43.0 ± 0.00	8.6 ± 3.53	7.1 ± 2.90	0.707 ± 0.261	0.731 ± 0.242
Inland Grid 2	44.0 ± 0.00	9.6 ± 4.35	7.8 ± 3.09	0.691 ± 0.222	0.691 ± 0.222
Inland Grid 3	56.0 ± 0.00	9.8 ± 4.26	7.9 ± 2.85	0.711 ± 0.206	0.731 ± 0.242
Canaveral National Seashore	30.5 ± 0.53	7.7 ± 3.30	6.6 ± 2.45	0.598 ± 0.232	0.673 ± 0.251
Merritt Island National Wildlife Refuge	31.7 ± 0.67	7.6 ± 3.41	6.7 ± 2.70	0.671 ± 0.263	0.718 ± 0.201
Smyrna Dunes Park	18.9 ± 0.32	4.2 ± 1.32	4.1 ± 1.34	0.588 ± 0.237	0.525 ± 0.212
Pelican Island National Wildlife Refuge	15.0 ± 0.00	4.2 ± 1.62	4.2 ± 1.62	0.600 ± 0.229	0.583 ± 0.188

Table 4.5. Geographic and genetic distance between sample locations of *P. p. niveiventris*. Below diagonal is pair-wise F_{ST} estimated in FSTAT based on 10 microsatellite loci, while above diagonal is Euclidean distance between sample locations measured in kilometers. Sample location abbreviations are defined in Fig. 4.1.

	BG1	BG2	BG3	CNS	IG1	IG2	IG3	MINWR	SDP	PINWR
BG1	-	4.6	4.6	45.7	4.0	6.9	2.3	17.7	77.1	73.4
BG2	0.012	-	4.4	41.6	5.8	5.1	4.9	14.4	72.9	76.9
BG3	0.005	0.009	-	45.4	2.1	9.2	2.7	18.7	76.6	72.9
CNS	0.032	0.026	0.028	-	47.2	39.4	46.5	28.7	31.4	118.1
IG1	0.021	0.027	0.024	0.046	-	10.0	1.7	20.1	78.5	71.2
IG2	0.012	0.013	0.013	0.033	0.017	-	8.6	10.9	70.8	80.3
IG3	0.009	0.010	0.004	0.023	0.019	0.015	-	19.0	77.8	72.1
MINWR	0.020	0.019	0.013	0.022	0.035	0.019	0.020	-	60.1	91.1
SDP	0.127	0.108	0.109	0.118	0.148	0.116	0.107	0.122	-	148.9
PINWR	0.112	0.125	0.125	0.147	0.149	0.138	0.119	0.130	0.213	-

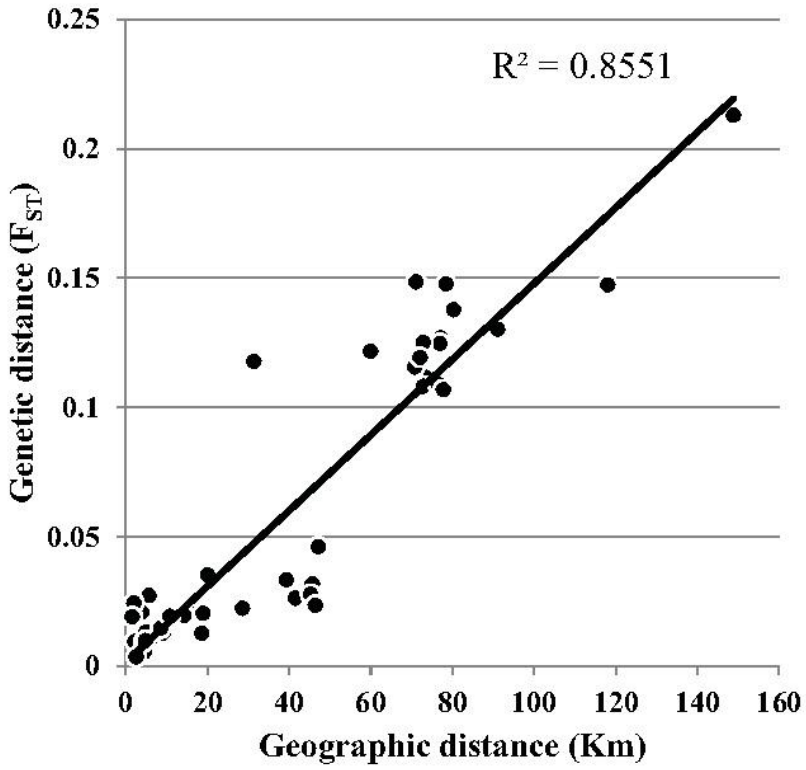

Figure 4.3. Plot of genetic distance (F_{ST} values estimated from microsatellite data) versus Euclidean geographic distance for all contemporary *P. p. niveiventris* sample locations. Mantel test shows a significant relationship between the two variables ($P < 0.0001$, 30000 randomizations).

In the Bayesian admixture model we found a large increase in likelihood scores [P(X|K)] when we included all sample locations with increasing K until reaching a mode of K = 8 (Fig. 4.4A). Based on the procedures by Evanno et al. (2005), the best K value for explaining our microsatellite data was K = 2 (Fig. 4.4A). When only one of the two peripheral sample locations (SDP or PINWR) was included the P(X|K) did not provide a clear mode or optimal K; however, both analyses had K = 3 as best K based on the Evanno et al. (2005) procedures (Fig. 4.4 B and C). We observed a small mode, coinciding with the highest ΔK, at K=7 when we included only the central sample locations (Fig. 4.4D). When all sample locations were included and K = 2,

most locations showed high admixture, with individuals for the sample locations being affiliated with both genetic clusters (Fig. 4.5A). Individuals from the peripheral sample locations favored one cluster, where all individuals were associated to one of the two clusters (Fig. 4.5A). When eight clusters were used, the central sample locations showed admixture, with individuals variably associated with the different clusters (Fig. 4.5B). With K = 8 the two peripheral sample locations had strong membership coefficients for their respective clusters (Fig. 4.5B). The hierarchical analyses provided additional support for these findings, with all individuals in the peripheral sample locations showing high membership coefficients to a single cluster, while the central sample locations showed high levels of admixture (Fig. 4.6A and 4.6B). When only central sample locations were included they all showed admixture (Fig. 4.6C).

Discussion

Evaluating Loss of Genetic Diversity Using mtDNA

Understanding current anthropogenic impact on taxa and ecosystems is central to conservation biology; however, it is vital to understand the historical processes and patterns that have affected these taxa prior to human settlements. Anthropogenic habitat loss has resulted in the recent extirpation or reduction in numbers of *P. p. niveiventris* populations (Stout 1992). Low genetic diversity prior to anthropogenic impacts is also observed. Compared to mainland relatives, where 22 haplotypes have been identified (Degner et al. 2007, Kalkvik et al. 2012), we recovered a total of six haplotypes among both historical and contemporary samples. It is thought that beach mice were isolated by dispersal barriers, i.e., lagoonal systems, when barrier islands became separated from the mainland (Hoekstra et al. 2006, Van Zant and Wooten 2007). It has been suggested that the divergence between beach mice and mainland populations

occurred around 6,000 ybp with the formation of the barriers (MacNeil 1950, Hoekstra et al. 2006), but molecular evidence suggests this divergence occurred around 200,000 ybp (Van Zant and Wooten 2007). An initial founder effect or bottleneck in the early stages of the formation of *P. p. niveiventris* may be the most parsimonious explanation for the historical loss of genetic diversity. Such events can affect taxa over a long period of time with subsequent bottlenecks having little additional impact on the genetic diversity (Taylor and Jamieson 2008). Several studies have found that current, low levels of genetic diversity are explained by historical bottlenecks and founder effects dating to times prior to anthropogenic impacts (Hoffman and Blouin 2004, Chan et al. 2005, Calvignac et al. 2008, Reding et al. 2010).

We were unable to demonstrate a statistically significant loss of genetic diversity in *P.p. niveiventris* over the last century based on *cyt b* sequence data obtained from museum specimens. However, when we removed the contemporary sample locations found in the continuous habitat from the analysis we observed a significant loss of nucleotide differences (k) and nucleotide diversity (π) compared to historical data (Fig. 4.2B). The sample locations within the central range are the only locations that currently exhibit location level genetic variation among *cyt b* sequences (Table 4.2; Fig. 4.2B). These findings indicate that this remaining continuous habitat serves as a refuge of historical genetic diversity. The two areas with the highest historical genetic diversity (New Smyrna and Oak Lodge; Fig. 4.2A), are currently fixed for their haplotypes (described as SDP and PINWR in current distribution; Fig. 4.2B). Thus it seems that these areas have undergone a recent genetic loss most likely due to anthropogenic induced habitat destruction.

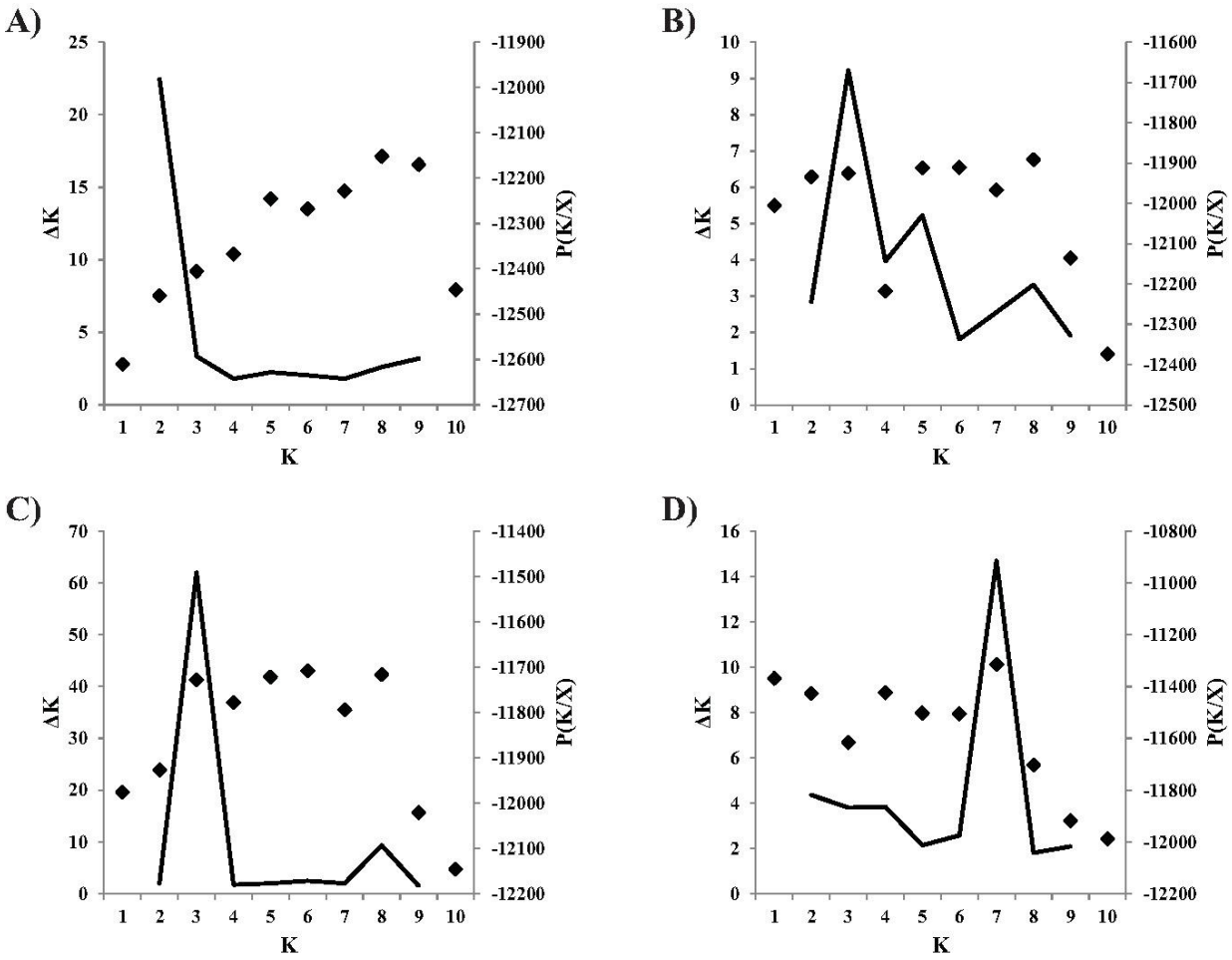

Figure 4.4. Likelihood values (diamonds) estimated in STRUCTURE (Pritchard et al. 2000) and ΔK (line) estimated following the methods of Evanno et al. (2005) for determining best fit numbers of clusters (K) implemented into the STRUCTURE analysis. As described in the methods, structure analysis was conducted hierarchically for contemporary *P. p. niveiventris* sample locations: all sample locations (A), SDP and central sample locations (B), PINWR and central sample locations (C), and only central sample locations (D).

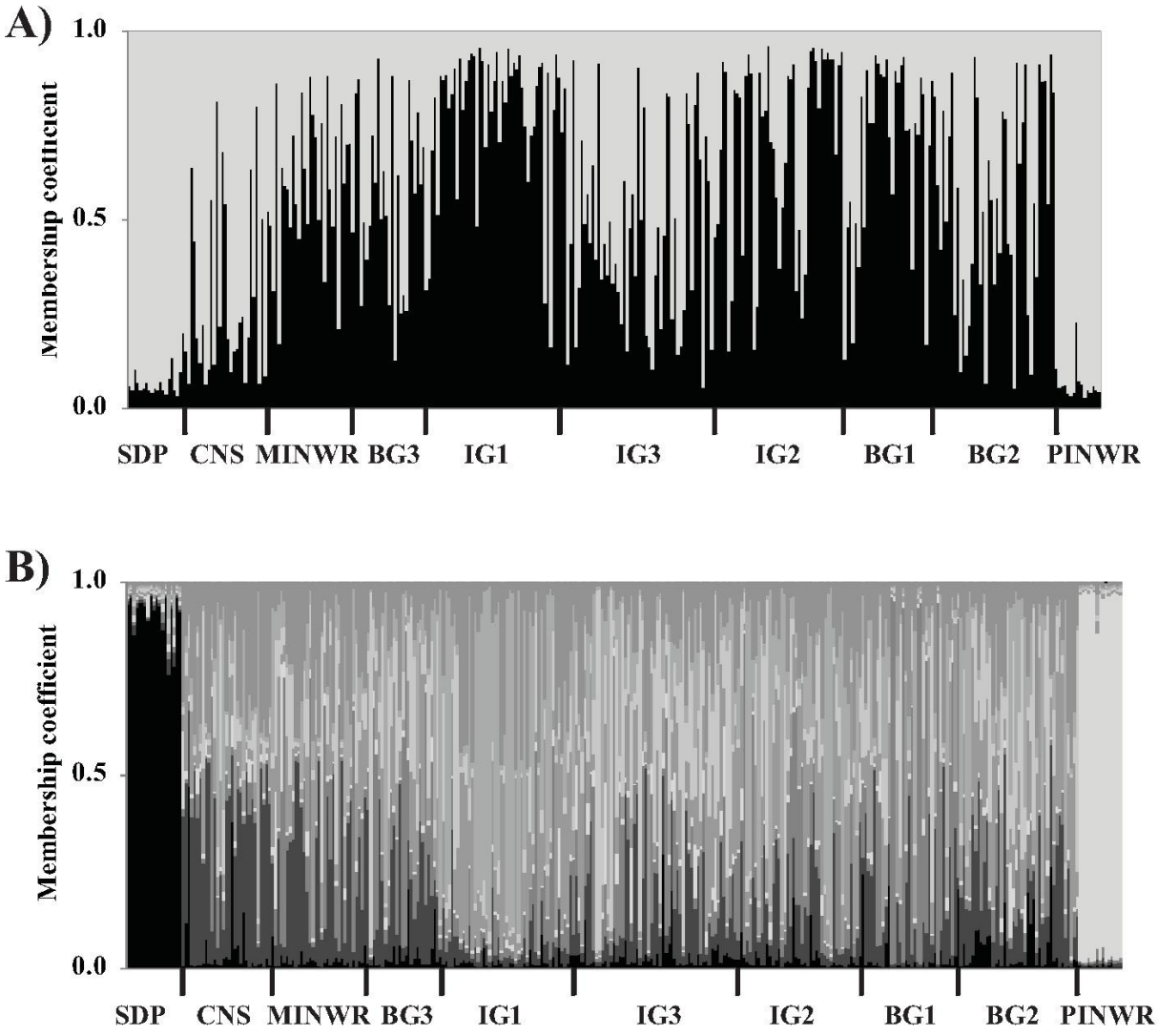

Figure 4.5. Estimated membership coefficients for individuals within the ten sampled locations across the contemporary distribution of *P. p. niveiventris* (based on ten microsatellite loci), for K=2 (A) and K=8 (B) clusters. Sample locations are arranged by decreasing latitude and abbreviations are defined in Fig. 4.1.

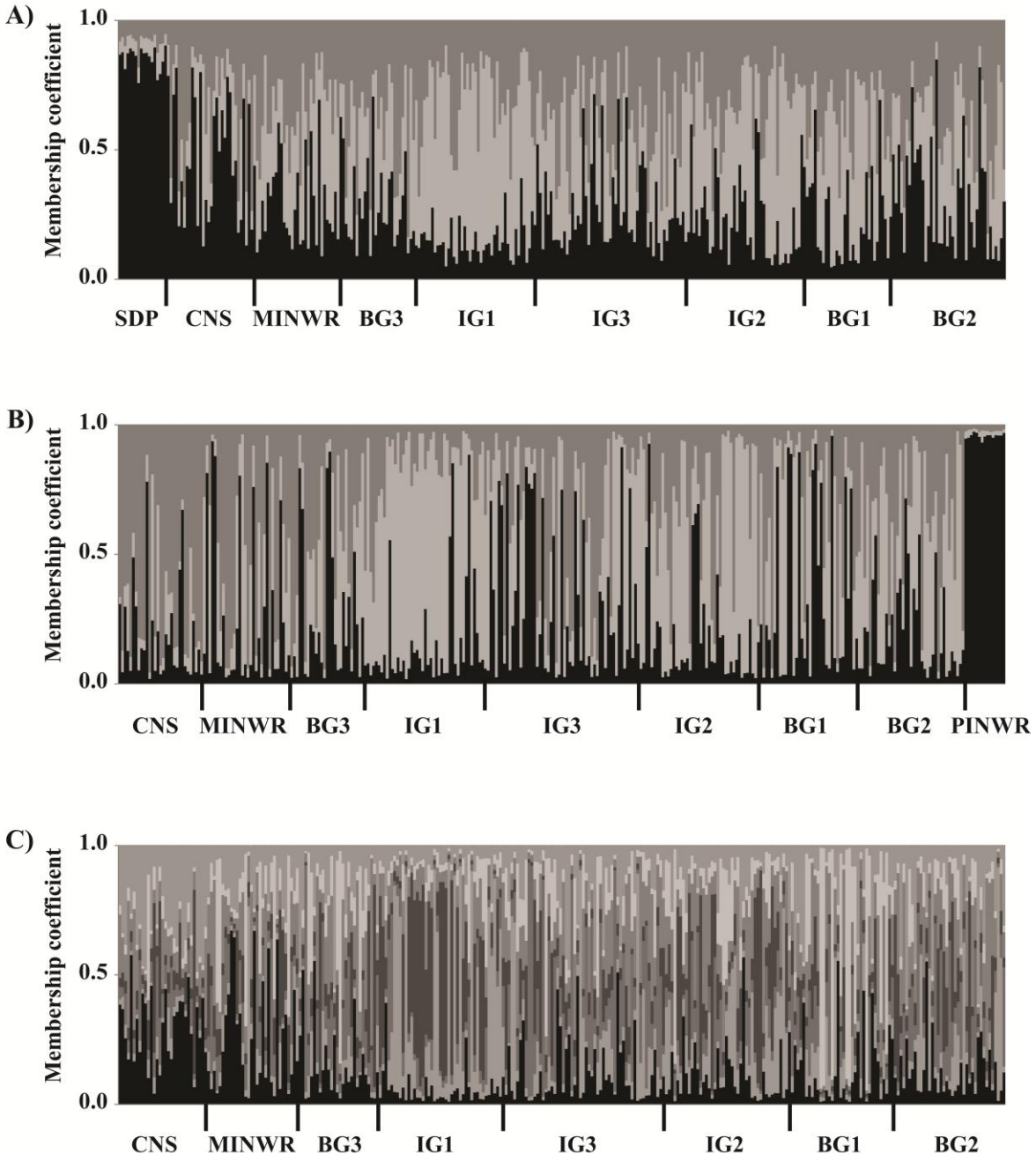

Figure 4.6. Membership coefficients given by STRUCTURE for sampled individuals in sample locations of *P. p. niveiventris* based on ten microsatellite loci. The hierarchical analyses included (A) the peripheral SDP sample location and central sample locations, (B) central sample locations and peripheral PINWR sample location, and (C) only central sample locations. Sample locations are arranged by decreasing latitude and abbreviations are defined in Fig. 4.1.

Contemporary Genetic Structure and Interconnectivity

Studies using temporal sampling have shown that human exploitation and habitat alteration can result in loss of genetic diversity within natural populations of a diverse set of organisms over short periods of time (Pichler and Baker 2000, Hauser et al. 2002, Culver et al. 2008, Thalmann et al. 2011). We predicted a loss of genetic diversity in *P. p. niveiventris* after an approximate 80% reduction in its range over the last few decades. Contrary to our predictions, we observed no statistically significant loss of *cyt b* diversity over the current distribution (Table 4.2), although this locus was not hyper-variable in either sample. We do find a strong geographic pattern of contemporary genetic diversity, which correlates to habitat richness. The central portion of the contemporary range is the most genetically diverse, whereas, the northern and southern disjunct sample locations exhibit statistically lower levels of genetic variation. The importance of continuous habitat for the persistence of biodiversity has been addressed both theoretically (Fahrig 2002) and empirically (Fahrig 2003). It has been shown that continuous habitat is favored for persistence when measured both genetically and demographically in a wide range of taxa, such as reptiles (Johnson et al. 2007, Dixo et al. 2009), birds (Coulon et al. 2010), and mammals (Haag et al. 2010, Holland and Bennett 2010).

Contemporary genetic structure of *P.p. niveiventris* provides insight into the processes that most likely explain the conservation of historical genetic diversity. Central to the outcome of these processes is the continuous, linear configuration of the habitat. We found indirect evidence of dispersal within the 70 km extent of coastal dunes currently occupied by *P. p. niveiventris*. For example, pair-wise F_{ST} values estimated from microsatellite data showed low levels of genetic structuring (Table 4.5), and the STRUCTURE analyses indicated high levels of admixture

among these locations (Fig. 4.5 and Fig. 4.6). These levels of genetic admixture may explain how *P. p. niveiventris* is able to maintain the highest level of genetic diversity observed among beach mice subspecies with an average of 8.0 alleles per loci (Table 4.4), compared to 4.3-6.8 alleles per loci for subspecies on the Gulf coast that typically occupy more fragmented habitat (Mullen et al. 2009).

We identified two peripheral sample locations, SDP and PINWR (Fig. 4.1), as being disjunct from the current central distribution of *P. p. niveiventris*. These sample locations are isolated by urbanized zones that represent barriers to gene flow. The two peripheral sample locations do not share haplotypes, indicating isolation. Further evidence for the lack of connectivity comes from the absence of genetic admixture with the central sample locations (Fig. 4.5 and Fig. 4.6A and 4.6B) and higher levels of genetic differentiation for the disjunct sample locations compared to the central sample locations (Table 4.5). The lack of gene flow may result in further reduction of genetic diversity through genetic drift. We did observe a pattern of isolation by distance (IBD; Fig. 4.2), that is explained by an equilibrium between migration and genetic drift across the range (Wright 1943, Hutchison and Templeton 1999). A lack of gene flow to some of the sample locations would explain a deviation from IBD. However, we may observe IBD across the current range of *P. p. niveiventris* as a result of collinearity between habitat availability and geographic distance, or that the isolated sample locations are peripheral in the current distribution.

Consequences for Management

The relatively undeveloped 70 km stretch of continuous coastal habitat now occupied by *P. p. niveiventris* came under Federal ownership and protection as an indirect result of the cold

war and U.S. space program. Coastal habitats south of Cape Canaveral, with the exception of isolated parcels, have lost beach mice as components of these ecosystems. A combination of land conversion, beach erosion, tropical storms and hurricanes, feral animals, and human activities explain this loss. In the short term, the sequestered Federal lands should continue to support *P. p. niveiventris.* Long term, climate disruption poses an unknown threat given the fact that the critical habitat lies at the interface of land and the Atlantic Ocean (Barbier et al. 2008, Mawdsley et al. 2009).

Overall, our *cyt b* data indicate that genetic diversity has generally been maintained over the past 100 years, even with extensive loss of habitat. However, we postulate that this diversity is only maintained because of the presence of a long section of undeveloped coastal dune habitat, found on protected and managed Federal lands. These findings illustrate the importance of preserving continuous habitat or larger areas for organism to inhabit, to reduce the overall impacts of human interference and allow persistence of the taxon (Breininger et al. 1998, Fischer and Lindenmayer 2007, Medina-Vogel et al. 2008). This area has also been recognized as significant for the conservation of several sea turtle species (Schmid 1995). Going forward, the conservation of the approximately 70 km coast line of intact habitat will be essential to ensure the genetic integrity of *P. p. niveiventris*, and presumably the genetic integrity of other taxa occupying this area.

Given the isolation and associated lower genetic diversity of the two peripheral sample locations (SDP and PINWR), we believe these sample locations (Fig. 4.1) are of immediate conservation concern. Lower genetic diversity can be expected in peripheral sample locations (Lawton 1993, Eckert et al. 2008), but historical data indicate that these two sample locations

had historically higher genetic diversity and the recent range contraction and isolation seem to have resulted in a loss of genetic material from these areas.

Peromyscus polionotus niveiventris is a taxon that most likely will be increasingly impacted by current global warming and sea level rise, as seen and projected in other taxa (Geselbracht et al. 2011, Maschinski et al. 2011, Saha et al. 2011a). Adaptation in response to these changing environmental conditions depends in part on the genetic variation represented in the population at risk (Lavergne and Molofsky 2007). With no evidence of overall loss of mitochondrial genetic diversity over the last few decades, and the highest current nuclear diversity observed among beach mice, *P. p. niveiventris* seems not to have been genetically impacted by human encroachment of coastal habitat elsewhere in its range. However, we show the importance of evaluating changes in the distribution of genetic diversity and isolation of peripheral sample locations from a historical perspective. By including historical and contemporary information we show that the persistence of coastal taxa may depend upon connected habitat with low anthropogenic impacts.

Conclusions

Genetic diversity is associated with persistence of populations (Reed and Frankham 2003, Lavergne and Molofsky 2007), and is therefore an important metric in our conservation of species. Both historical and anthropogenic impacts may be responsible for current levels of genetic diversity and structure. We determined that historical forces are probably responsible for current low levels of genetic diversity rather than recent anthropogenic impacts on habitat of *P. p. niveiventris*. These results are supported by similar studies in different taxonomic groups (e.g. Hoffman and Blouin 2004, Chan et al. 2005, Reding et al. 2010). We determined that *P. p.*

niveiventris has maintained historical levels of genetic diversity in the large federally protected continuous habitat as opposed to the two peripheral sample locations that have reduced diversity. The 70 km long federally protected coastal habitat functions as a refuge for genetic diversity, while lands outside of this area are undergoing anthropogenic change. This study illustrates the importance of evaluating historical genetic diversity in a landscape influenced by both historical events and recent anthropogenic influence. Our results indicate the importance of maintaining continuous habitat for the future persistence of genetic diversity.

Acknowledgments

Our study was funded by the U. S. Fish and Wildlife Service. We thank Alice Bard and Jeffrey Van Zant for aiding in acquiring samples from the current range of *P. polionotus niveiventris*, as well as Ryan Lamers for providing invaluable help in developing primers for the historical samples. We want to acknowledge the museums that provided us with specimens from their collections: American Museum of Natural History, Cornell University Museum of Vertebrates, Field Museum of Natural History, Florida Museum of Natural History, Harvard Museum of Comparative Zoology, University of New Mexico Museum of Southwestern Biology, University of California Berkley Museum of Vertebrate Zoology, and University of Michigan Museum of Zoology. We also thank Jacob Degner, Allyson Fenwick, Eric Hoffman, Jennafer Kalkvik, Mary Beth Manjerovic, Joshua Reece, and the PH lab group for comments that greatly improved this manuscript. Our study was conducted under permits issued by U.S. Fish & Wildlife Service, National Park Service, Florida Fish and Wildlife Conservation Commission, and the Florida Department of Environmental Protection. All live animals were handled according to protocols approved by IACUC# 06-46W from University of Central Florida.

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CHAPTER 5. BEACH MOUSE OR SCRUB MOUSE? CHASING THE TALE OF A MOUSE IN TWO HABITATS

Introduction

Knowledge of population dynamics is essential for informed management to maintain common species or to recover species in decline. Populations vary in space and time both within and between discrete habitats. The importance of spatial habitat heterogeneity on the behavior of individuals in populations has long been recognized in theoretical studies (Holt 1985, Hanski 1998), and an increasing body of literature presents empirical evidence for variation in population dynamics in response to habitat heterogeneity (Kanda et al. 2009, Nystrand et al. 2010, Puzin et al. 2011). In fact, population persistence has been shown to be more dependent on habitat quality (e.g., extent of escape cover or food supply) than other variables, such as small and isolated populations (Thomas et al. 2001). Moreover, some taxa may depend on availability of two or more habitats for long-term persistence (Brambilla et al. 2007). Thus, identifying the relative contributions of different habitats to population dynamics can aid in management of listed species (Sturtevant et al. 1996, Heinrichs et al. 2010) and in the allocation of resources in managing taxa of conservation concern.

Spatial dynamics across landscapes have been observed to vary temporally in red deer (*Cervus elaphus*; Forchhammer et al. 1998), white stork (*Ciconia ciconia*; Sæther et al. 2006), and large mammalian herbivores (Gaillard et al. 2000), providing insight into how populations persist in variable environments (Cáceres 1997). One aspect of temporal variation is seasonality, which has given rise to specific physiological adaptations in vertebrates (Hazlerigg and Loudon 2008). Seasonal variation in population dynamics has been studied in small mammals (Merritt et al. 2001), measles (Ferrari et al. 2008) and green crab (*Carcinus maenas*; Bessa et al. 2010).

Several studies have found correlations between climate and observed seasonal population dynamics of small mammals (O'Connell 1989, Merritt et al. 2001). Seasonal changes can also impact the importance of habitat use for certain taxa (Brambilla and Rubolini 2009). In mammals such as old field mice (*Peromyscus polionotus*), resource availability has also explained seasonal adaptations and differences in vital rates such as survival and reproduction (Smith 1974).

Population modeling has been widely used to evaluate relative contributions of spatial and temporal variation to species' dynamics (e.g., Pascarella and Horvitz 1998, Wemmer et al. 2001, Picó et al. 2002, Olson et al. 2004, Fraterrigo et al. 2009, Heinrichs et al. 2010). Population models have also aided in the development of conservation policies and management of listed taxa (Hedrick et al. 1996, Beissinger and Westphal 1998, Brook et al. 2000). Many of these models incorporate uncertainty and therefore provide accountability for stochastic elements to ensure confidence in the results obtained (Beissinger and McCullough 2002, Morris and Doak 2002, McGowan et al. 2011). Some studies have specifically evaluated the impact of habitat on population dynamics and have employed models to justify particular management choices for protecting appropriate habitat (Olson et al. 2004, Heinrichs et al. 2010). Incorporating habitat differences, temporal variation, and other elements of uncertainly into population models should be expected to greatly improve their precision.

In this study we examined population dynamics of the southeastern beach mouse (*Peromyscus polionotus niveiventris*) with emphasis on spatial and temporal variation in demographic performance measured as abundance, survival and population growth rates. This subspecies is one of eight *P. polionotus* ssp. collectively called beach mice because of the coastal habitats they occupy on the barrier islands of Alabama and Florida (Whitaker and Hamilton

1998). Among these eight subspecies, six are listed as either threatened or endangered, one is not listed, and one is considered extinct (Ehrhart 1978, Humphrey and Barbour 1981, Humphrey 1992a, b, Stout 1992). Habitat loss and fragmentation, driven by land development, are the primary threats to beach mice (Holler 1992a, b, Humphrey 1992a, Stout 1992, Kalkvik et al. in review). Beach mouse habitat was originally considered putatively the narrow zone of sea oats (*Uniola paniculata*) that dominated primary dunes of barrier islands (Ivey 1949, Bowen 1968). Although there are scarce early reports of beach mice occupying denser scrub habitat found further inland on barrier islands (Blair 1951, Pournelle and Barrington 1953, Ivey 1959). However, scrub, secondary dune, and tertiary dune habitats are now recognized as potentially important for beach mice persistence (Extine and Stout 1987, Swilling et al. 1998, Sneckenberger 2001, Pries et al. 2009). Moreover, little is known of the contribution of these habitats to population dynamics of beach mouse subspecies because no studies have addressed temporal variation in occupancy of the different habitat types. Research carried out in a single habitat type has shown mixed results on seasonal variation in *P. polionotus* with some studies showing no seasonal differences in population variables (Davenport 1964, Rave and Holler 1992), while others have reported seasonal and inter-annual variation (Caldwell and Gentry 1965, Gentry 1966).

We evaluated spatial and temporal variation in population dynamics of *P. p. niveiventris* with these objectives: 1) to measure demographic variation of *P. p. niveiventris* across seasons in scrub and beach habitat; 2) to create a stochastic-matrix model reflecting seasonal and habitat variation in population dynamics of *P. p. niveiventris*; and 3) to evaluate the relative impact of habitat, vital rates, and life stages across different seasons using population modeling to

understand the population dynamics of *P. p. niveiventris*. We expected scrub habitat to have a significant influence on the population dynamics of this species in certain coastal settings. Our findings provide insight into how these animals use a spatially heterogeneous landscape across seasons.

Methods and Materials

Study site and data collection

We conducted our study at Cape Canaveral Air Force Station (CCAFS, 28.38°N, 80.42°W), located in Brevard County, Florida, USA (Fig. 5.1). CCAFS covers over 6,000 ha and represents the southern part of a large area of public land comprised of Kennedy Space Center, Merritt Island National Wildlife Refuge, and Canaveral National Seashore. Together these areas make up approximately 70 km of managed barrier island coastal habitat with restricted development. The eastern coast line of CCAFS is predominately relatively low dunes that are dominated by *Uniola paniculata* (sea oats), *Ipomoea pes-caprae* (railroad vine), *Ipomoea imperati* (beach morning-glory), *Panicum amarum* (beach grass) and a variety of herbs and grasses (Kutz 1942). The inland habitats are mainly coastal strand and scrub. Coastal strand includes open sandy areas with patches of scrub dominated by *Serenoa repens* (saw palmetto), *Coccoloba uvifra* (sea grape), *Myrica cerifera* (wax myrtle), *Sideroxylon tenax* (buckthorn), and *Muhlenbergia capillaris* (muhly grass) (Johnson and Barbour 1990, Schmalzer et al. 1999). Coastal scrub is found on inland dunes and is dominated by several oak species (*Quercus geminata*, *Q. chapmanii*, and *Q. myrtifolia*) (Schmalzer et al. 1999), in addition to *S. repens*, *S. tenax*, and *M. capillaris*. Compared to the Gulf coast barrier islands, what is defined as coastal

scrub on the Atlantic coast islands resembles tertiary dunes and interior scrub on the Gulf coast barrier islands (Johnson and Barbour 1990).

We established six study sites at CCAFS to evaluate the population demography and to estimate parameters for our matrix model for *P. p. niveiventris*. Three sites were located in beach habitat, and three in coastal scrub (Fig. 5.1). Habitat structure was shown to differ between beach and scrub grids based on habitat variables such as: bare ground, woody vegetation, non-woody vegetation, height of vegetation, and percent surface course sand (Simmons 2009). Scrub grids were separated from beach grids by 1.7 – 10.0 km (average = 5.1 km), while scrub grids were separated from the beach grids by 2.1 – 9.2 km (average = 4.8 km). We trapped within grids for one night, twice a month from November 2003 through March 2006. Population size estimates typically require trapping between 5 to 7 consecutive nights (Otis et al. 1978); however, beach mice can experience significant weight loss with consecutive trapping (Suazo et al. 2005), so we limited our trapping to one night to avoid detrimental impacts on recaptures. At each site an 8X8 grid was established comprised of 64 trap stations, with rows and columns separated by 15 meters. One Sherman live trap (22.9 x 8.9 x 7.6 cm; H. B. Sherman Traps, Inc., Tallahassee, FL) was set at each station. Traps were baited with sunflower seeds, set in the late afternoon, and checked the next morning. At first capture, each individual was given a numbered ear tag and tail clipped to provide tissue for genetic analysis. At every capture we determined sex and reproductive status (♂: descended or non-descended testes, ♀: pregnant, or lactating). We used pelage color and body mass to determine age class (juvenile, sub-adult or adult) (Layne 1968). All individuals were released at site of capture.

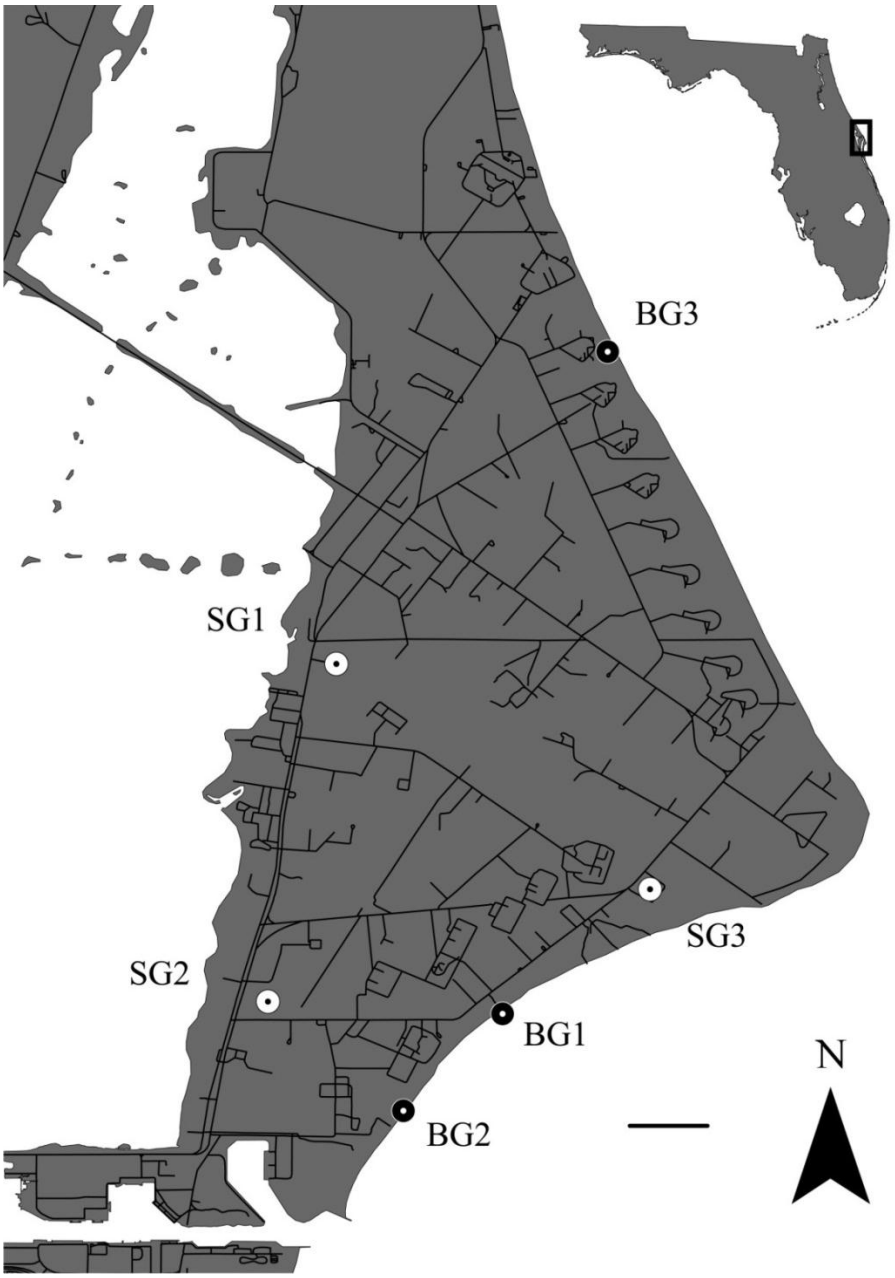

Figure 5.1 Trapping locations at Cape Canaveral Air Force Station, Florida, USA. Three grids were located in scrub habitat (SG;⊙) and three were located in beach habitat (BG;⦿). Scale bar equals 1 km.

Population demography data analysis

We calculated the minimum number alive (MNA – also known as minimum number known alive) for each trap event and grid as an index of abundance. We defined MNA for a given trapping period as the number of individuals captured during a trapping event, in addition to individuals marked previously and not captured during that period but captured during a subsequent event (Krebs 1966, Pocock et al. 2004). Because we trapped on one night per sampling event, we did not have sufficient data for model-based population estimates (Menkens and Anderson 1988), and the grids we trapped cannot be considered closed. While MNA has been shown to underestimate population size, it is proportional to population size and can be used as an index of population size to examine intraspecific patterns of population dynamics (Slade and Blair 2000). We were primarily interested in the relative changes in population size during our trapping period rather than absolute numbers.

For each site and trapping period, we calculated the sex ratio and number of individual for each age classes (juvenile, subadult and adult). *Peromyscus polionotus* is considered to be monogamous (Foltz 1981, Turner et al. 2010), so we expected sex ratios to be 1:1 between adult males and females found in each grid across the sample period. We tested to determine if each grid conformed to the 1:1 ratio based on overall number of males and females using a goodness-of-fit test in R v.2.12.1(R Development Core Team 2010). We tested for temporal variation in sex ratio by pooling data from each trapping event based on habitat (beach or scrub), season and year. We defined seasons as winter (Dec. – Feb.), spring (March – May), summer (June – Aug.), and fall (Sept. – Nov.). To test if we have environmental differences between the seasons, we collected climate data (precipitation and temperature; (Spaceport Weather Data Archive; http://trmm.ksc.nasa.gov/) at CCAFS and Kennedy Space Center collected during the trapping

period 2003-2006. We used the nonparametric Kruskal-Wallis one way analysis of variance to test for significant difference between the seasons.

Habitat differences can impact the abundance and survival of individuals (Manning and Edge 2004, Converse et al. 2006). We estimated adult survivorship and probability of recapture based on the Cormack-Jolly-Seber (CJS) model, which uses mark-recapture data for an open population. CJS estimates survival for a given time interval, but also gives the probability of recapture for an individual in this time interval. This model also allows for considering alternative partitioning of field data (Lebreton et al. 1992). We used program MARK to estimate survival (Φ) and recapture probability (p) using a maximum likelihood approach (White and Burnham 1999, White et al. 2002). We used our mark-recapture data for all trapping grids to estimate survival differences between beach and scrub habitat. We also tested models including temporal variation (e.g. yearly, seasonally and monthly survival). We used the Akaike Information Criterion (AIC) to choose the most informative model as the one with the lowest AIC value and the highest model weight (Burnham and Anderson 2002). We estimated standard deviation and 95% confidence interval for survival values for the most informative model by running a Markov chain Monte Carlo (MCMC) Bayesian parameter estimation procedure in MARK. We used the default settings in MARK with the number of stored samples of 10,000 and a 1,000 burn-in.

We estimated the reproductive rate for the grids as number of reproductive females in the population. Prior studies have shown that *P. polionotus* and close relatives exhibit temporal variation in the number of reproductively active females (Davenport 1964, Smith 1974). To test whether habitat affected reproductive rate we calculated the ratio of reproductive adult females

to the total number of adult individuals captured. We pooled reproductive data within habitat types across season and years because of small sample sizes during monthly trapping periods.

Model structure

To test for effects of habitat on population dynamics of *P. p. niveiventris*, we used a stochastic matrix model with dimensions equal to the product of life stages and habitat types. Such a matrix approach has been called a megamatrix analysis that allows for evaluating the dynamics among patches in a landscape as well as the dynamics of the organisms found within each patch in the landscape (Horvitz and Schemske 1986, Cipollini et al. 1994, Pascarella and Horvitz 1998). In our study, the megamatrix represented population dynamics across a landscape made up of two habitat types: scrub and beach. Part of the matrix represented dynamics within habitat types, while transitions between the two habitat types represented individual movement between the two habitat types.

Our megamatrix model was designed specifically to explore the population dynamics of *P. p. niveiventris* at CCAFS. In the model we included both sexes, with the assumption of a monogamous mating system. We assumed with our model that there was movement between the two habitat types at CCAFS. Mice have been shown to readily move short distances between the habitat types (Extine and Stout 1987), and rare long-distance movement has been documented in this subspecies (4.8 km and 28 km respectively; Bard 1997, Oddy et al. 1999), which suggests that dispersal between and among our sample grids might occur. We assume that in the period of our study, vegetation on the grids remained relatively stable from year-to-year in terms of primary productivity and species composition. Therefore, the habitat is considered static in our

model and transitions in the megamatrix between the two habitats represented movement of animals rather than changes in habitat.

To simplify our analyses we defined two life stages for *P. p. niveiventris* in each habitat: juvenile and adult. Juvenile is defined as sexually immature individuals, whereas individuals capable of reproduction are treated as adults. The juvenile stage is relative short, with females being sexually mature after approximately 30 days (Clark 1938). No clear evidence is available for age at sexual maturity of males in this species, but we assume similar age of sexual maturity in males as in females for our model. Based on molting patterns in *P. polionotus,* subadults are typically >30 days old and would be included in the adult life stage in our matrix model (Golley et al. 1966, Layne 1968). For the life stages we included in the model, juveniles can survive into adulthood, and adults can survive from one season to the next. When an individual reaches adulthood it can contribute to the reproductive output of the population (Fig. 5.2). We assumed that some juveniles migrate between habitat types and are found as adults in the next season in the other habitat (Fig. 5.2). Most reported long-distance migration observed in *P. polionotus* has been by juveniles that were captured as adults in new locations (Smith 1968, Oddy et al. 1999), and other field data show younger mice move longer distances than adults (Swilling and Wooten 2002, Tenaglia et al. 2007).

We used a periodic matrix model to evaluate the impacts of seasonal variation on the population dynamics of *P. p. niveiventris* as other mammals have shown seasonal differences in population dynamics (Caswell and Trevisan 1994, Caswell 2001, Picó et al. 2002). A periodic matrix allows us to consider each season as a separate matrix, where the product of these

matrices is equal to an annual matrix ($\mathbf{A}_{An}$), which projects population dynamics across one year (Caswell and Trevisan 1994, Caswell 2001).

$$(1)\ A_{An} = B_{Sp} \times B_{Su} \times B_{Fa} \times B_{Wi}$$

Based on this approach, we created a megamatrix for each season ($\mathbf{B}_{Sp}$ = spring, $\mathbf{B}_{Su}$ = summer, $\mathbf{B}_{Fa}$ = fall, $\mathbf{B}_{Wi}$ = winter) that represented the population dynamics for each time period.

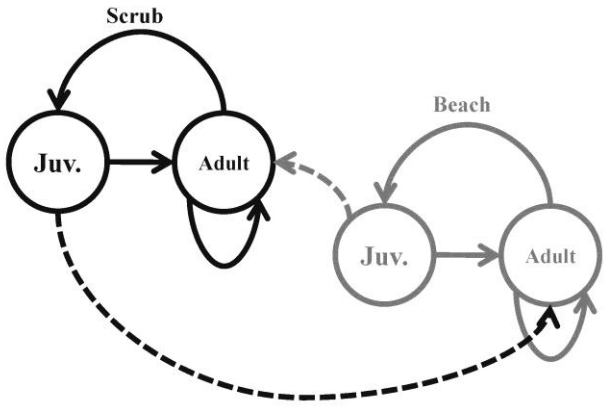

Figure 5.2. Basic life cycle of *P. p. niveiventris* at CCAFS based on known ontogeny and behavior in two different habitats. Basic life stages are non-reproducing juveniles (Juv.) and reproducing adults (Adult). Solid lines show transitions within habitat, and the dashed lines indicate migration between the two habitats

The transition stages in our matrix model were determined by vital rates, which reflected the known ontogeny and behavior of *P. polionotus* (Table 5.1). Most of the life span of *P. polionotus* is spent as an adult in either beach or scrub habitat, which is determined by the adult seasonal survival (S_a). Juveniles surviving to adulthood were determined by juvenile seasonal survival (S_j); however, they become sexually mature after 30 days (Layne 1968), so we included that they have to survive as adults part of the season. A proportion of the juveniles were assumed to migrate from one habitat to the other, so such migration was accounted for in the juvenile and

adult transition (Table 5.1). The fecundity was determined by the survival of adults from one season to the next, and the number of offspring contributed by the adults. Number of offspring was determined as the product of litter size (L), proportion of reproductively active females (Pre) and the survival of the litter to juvenile (S_L):

$$(2)\ \text{Fecundity} = L \times S_L \times \text{Pre}$$

The adult stage is the only life stage contributing juveniles to the next season; however, with rapid sexual maturity and seasonal transition matrices, individuals found as juveniles in one season have time to produce litters that appear as juveniles in the next season (~ 30 days sexual maturity; Layne 1968). We therefore included that juveniles from one season can contribute juveniles to the next season, based on the seasonal survival of juveniles to adulthood (S_j) and then subsequent survival as an adult to the next season (S_a). In order to provide offspring to the next season mice had to survive one third of the season as juvenile, and the remaining two thirds as an adult. Our survival rates reflect seasonal survival, so S_j was multiplied by $\frac{1}{3}$ and S_a was multiplied by $\frac{2}{3}$ (Table 5.1).

A wide range of stochastic elements may impact the outcome of population models (McGowan et al. 2011). We included temporal variation found between seasons; however, when sufficient data were available we incorporated additional temporal variation by including seasonal data from multiple years. We incorporated parametric uncertainty for vital rates for each season by sampling from probability distributions, which characterize uncertainty for specific parameters. When we had sufficient data from multiple years, the model randomly selected one of the years for sampling parameters. The model was then repeated multiple times to capture this

variation (McGowan et al. 2011). For survival (S_a, S_j, and S_L) and migration (m) we used a beta distribution, which is bound by zero and one, and is determined by a defined average and standard deviation (Caswell 2001, Morris and Doak 2002). The distributions for S_a was determined by average and standard deviation (SD) estimates of adult survival from our field data. We acquired estimates for S_j and S_L from other studies. Seasonal S_j has been reported for another beach mouse subspecies (*P. p. ammobates*) as summer survival ($S_j = 0.475 \pm 0.144$ SD), and spring/fall/winter survival ($S_j = 0.638 \pm 0.144$ SD) (Traylor-Holzer et al. 2005). Litter survival was estimated based on field observations of *P. polionotus* whereas of the estimated number of offspring born, only 50% emerged from burrows (Caldwell 1960). Therefore, we set litter survival as an average $S_L = 0.5 \pm 0.1$ SD. We have no estimate of long-distance migration among our sample grids. Genetics studies have shown that there is gene flow across CCAFS (Degner et al. 2007, Kalkvik et al. in review); however, no long-distance migration was observed during trapping events in this study. We included an average seasonal migration rate of $m = 0.1 \pm 0.01$ SD. Preliminary analysis showed average migration rates ranging from m = 0.01 to m = 0.5 did not significantly alter the outcome of the model, so only one migration rate distribution was included in the model. Having limited data on the number of reproductively active females, we used a uniform distribution, which means there is an equal probability to pick any number within a given range. We determined the maximum and minimum proportion of reproductively active females in the population across our sample period for each season. The term for litter size was set as a constant as we did not have data on population level variation in litter size. Based on values from laboratory colonies average litter size was L = 4.117 in *P. polionotus* (Kaufman and Kaufman 1987).

Table 5.1 Population megamatrix model for *P. p. niveiventris*, given by two habitat types (beach and scrub) and two life stages; non-sexually mature juveniles (J) and sexually mature adults (A). Parameters included are: litter size (L), survival of litter (S_L), proportion reproductive females (Pre_{Beach} or Pre_{Scrub}), juvenile survival (S_j), adult survival (S_a) and migration rate (m).

		Beach		Scrub	
		J	A	J	A
Beach	J	$L * S_L * Pre_{Beach} * \left(\frac{2}{3}\right) S_a * \left(\frac{1}{3}\right) S_j$	$L * S_L * Pre_{Beach} * S_a$	0	0
	A	$* \left(\frac{2}{3}\right) S_a * \left(\frac{1}{3}\right) S_j * (1 - m)$	S_a	$* \left(\frac{2}{3}\right) S_a * \left(\frac{1}{3}\right) S_j * m$	0
Scrub	J	0	0	$L * S_L * Pre_{Scrub} * \left(\frac{2}{3}\right) S_a * \left(\frac{1}{3}\right) S_j$	$L * S_L * Pre_{Scrub} * S_a$
	A	$* \left(\frac{2}{3}\right) S_a * \left(\frac{1}{3}\right) S_j * m$	0	$* \left(\frac{2}{3}\right) S_a * \left(\frac{1}{3}\right) S_j * (1 - m)$	S_a

We used MatLab v. 7.4.0 (Mathworks 2007a) for the population model. To provide sufficient sampling of the vital rate distributions, we ran our model with 10,000 replications with independent sampling of the parameter distributions for different seasons for each replicate.

Model Analysis

We calculate the finite rate of increase, lambda (λ), of the annual megamatrix (A_{An}) to evaluate our model output as giving a realistic scenario of the population dynamics of *P. p. niveiventris*. We used sensitivity and elasticity analysis to evaluate relationships between of seasons, habitats, and the population dynamics of *P. p. niveiventris*. Sensitivity analysis of demographic parameters measures the impact that small changes in matrix elements, or vital rates, have on λ (Caswell 2001). Sensitivity can be difficult to interpret, especially where nonlinear relationships between λ and parameters make it more difficult to summarize sensitivity as a single number. Also, sensitivity does not scale the relative impact that changes have on different rates or matrix elements, nor does it exclude matrix elements that are biologically impossible (Morris and Doak 2002). Elasticity ($e_{ij} = s_{ij} \times \frac{a_{ij}}{\lambda}$) rescales sensitivity to make it easier to interpret, and measures proportional changes in λ with small proportional changes in a matrix element or vital rate (de Kroon et al. 1986, de Kroon et al. 2000, Caswell 2001). All elasticity values sum to one in a matrix, and each elasticity value can be interpreted as the relative importance of the vital rate or matrix element on λ (de Kroon et al. 1986, de Kroon et al. 2000).

We determined the impact of individual vital rates by estimating sensitivity and elasticity for each vital rate in each seasonal megamatrix using the *eigenall* function by Morris and Doak

(2002) in MatLab. To evaluate the influence of small changes in the matrix elements, we determined sensitivity and elasticity values for each matrix element for period matrices. Caswell and Trevisan (1994) showed that the sensitivity for a specific periodic matrix [$S_B^{(h)}$] is given by

$$(3)\ S_{B^{(h)}} = \left[B^{(h-1)} \times B^{(h-2)} \cdots B^{(1)} \times B^{(m)} \times B^{(m-1)} \cdots B^{(h+1)}\right]^T \times S_{A^{(h)}}$$

where B_h is a specific periodic matrix (h = 1, 2, 3,…., m), and $S_A^{(h)}$ is the sensitivity matrix for the annual matrix for all periods. The product of the periodic matrices is transposed to the annual sensitivity matrix, denoted by the superscript T. For our study the periodic matrices represent each of our seasonal megamatrix, where the annual matrix is the matrix multiplication of each matrix element for the four seasons. The elasticity [$E_B^{(h)}$] for a the seasonal matrix $B^{(h)}$ was estimated by

$$(4)\ E_{B^{(h)}} = \frac{1}{\lambda} B_h * S_{B^{(h)}}$$

where $E_{B(h)}$ designate the elasticity of each seasonal megamatrix, B_h was the seasonal matrix, and $S_{B(h)}$ was the sensitivity for the respective seasonal megamatrix (Caswell and Trevisan 1994). The asterisk (*) denoted the element-by-element product of the matrices. The sensitivity and elasticity of matrix elements for each seasonal megamatrix revealed the impact of each life-stage transition on population dynamics of *P. p. niveiventris* in the two habitats and four seasons. We evaluated the contribution of the two habitat types and migration between them by summing all elasticity values within each habitat. Sums provide information on the relative importance of events occurring within or between habitats for overall population dynamics (Pascarella and Horvitz 1998).

We estimated sensitivity and elasticity values for each the 10,000 replicates of our model. To compare sensitivity and elasticity we calculated the average and 95% confidence interval across all replicates. We interpreted overlap in confidence intervals to suggest vital rates or matrix elements to be equally influential, while lack of overlap suggested one variable had greater or lesser impact on the population dynamics of *P. p. niveiventris*.

Results

Study site and data collection

We trapped 17,920 trap nights over the period November 2003 – March 2006 across six grids (Table 5.2). Trapping intensity per grid ranged from 2,496 trap nights on one of the beach grids (BG3; Fig. 5.1; Table 5.2) to 3,264 trap nights on one of our scrub grids (SG1; Fig. 5.1; Table 5.2). However, the two habitats were almost equally trapped with a difference of 128 trap nights between scrub and beach (Table 5.2). During the study, 1083 individuals of *P. p. niveiventris* were trapped 3,672 times (0.205 captures/trap night) (Table 5.3). We had greater number of captures in scrub (n = 2683) compared to beach habitat (n = 989). The number of captures per grid was uneven with SG3 having the most with 1,415 captures (0.491 captures/trap night) and the fewest captures in BG3 with 151 captures (0.060 captures/trap night).

We also captured two other small rodents in the trapping grids. Small numbers of cotton mice (*P. gossypinus*) and cotton rats a (*Sigmodon hispidus*) were captured on the grids (Table 5.3). *Peromyscus gossypinus* was captured in all six grids with greater frequency in the beach habitat (115 captures; Table 5.3) than in scrub habitat (43 captures; Table 5.3); however, the number of individuals did not differ substantially between the two habitats (Beach – 54

individuals; Scrub – 41 individuals; Table 5.3). We captured *S. hispidus* in two of three grids in the scrub habitat and at each beach grid, yet neither the number of total captures (Beach – 43 captures; Scrub – 34 captures; Table 5.3), nor the number of individuals captured (Beach – 34 individuals; Scrub – 31 individuals; Table 5.3) differed substantially between the two habitats.

Table 5.2 Trapping efforts defined as trap nights for six grids at CCAFS between November 2003 to March 2006.

Trapping location	Trap nights
All Grids	17920
Scrub Grid	
1	3264
2	2880
3	2880
Total Scrub	9024
Beach Grid	
1	3200
2	3200
3	2496
Total Beach	8896

Table 5.3 Number of *Peromyscus polionotus niveiventris*, *P. gossypinus* and *Sigmodon hispidus* trapped on six grids at Cape Canaveral Air Force Station between November 2003 and March 2006, reported as total captures per species (Captures) and the number of unique individuals per grid (Individuals). Captures per trap night are reported in parentheses.

	P. polionotus niveiventris		*P. gossypinus*		*S. hispidus*	
Grids	Captures	Individuals	Captures	Individuals	Captures	Individuals
All Grids	3672 (0.205)	1083 (0.060)	158 (0.009)	95 (0.005)	77 (0.004)	65 (0.004)
Scrub Grid						
1	746 (0.229)	177 (0.054)	9 (0.003)	7 (0.002)	0 (0.000)	0 (0.000)
2	522 (0.181)	117 (0.041)	5 (0.002)	5 (0.002)	18 (0.006)	15 (0.005)
3	1415 (0.491)	496 (0.172)	29 (0.010)	29 (0.010)	16 (0.006)	16 (0.006)
Total	2683 (0.297)	790 (0.088)	43 (0.005)	41 (0.005)	34 (0.004)	31 (0.003)
Beach Grid						
1	367 (0.115)	90 (0.028)	80 (0.025)	35 (0.011)	5 (0.002)	4 (0.001)
2	471 (0.147)	139 (0.043)	31 (0.010)	15 (0.005)	26 (0.008)	20 (0.006)
3	151 (0.060)	64 (0.026)	4 (0.002)	4 (0.002)	12 (0.005)	10 (0.004)
Total	989 (0.111)	293 (0.033)	115 (0.006)	54 (0.006)	43 (0.005)	34 (0.004)

Population demography data analysis

Temporal variation in MNA of *P. p. niveiventris* showed different trends among grids within habitats and between habitats (Fig. 5.3A and B). Overall, a greater number of individuals was found on the scrub grids than on the beach grids for most of the trapping events (note scale difference between panels; Fig. 5.3A and B). MNA peaked in winter or spring of 2004 on the beach grids and steadily declined until October. It is important to note Hurricane Jeanne reached these grids September 26 and resulted in some flooding and sand deposits up to 30 m inland on the grids. Two of the three grids (BG1 and 2) recovered MNA over fall and winter of 2004-05, whereas BG3 did not recover to it former MNA. The scrub grids peaked in MNA in winter or spring of 2004 and declined through the summer and fall (Fig. 5.3B). Hurricane impacts appeared to be minimal in late summer as MNA on the grids remained relatively stable in contrast with declines in MNA on the beach grids. Nonetheless, the overall trend of MNA on the scrub grids was to gradually decline until November-December 2004 after which SG1 and 3 increased while SG2 remained in decline.

We divided our data into four seasons, and we observed a significant difference in the climatic conditions for the seasons during the period trapped (2003 – 2006) based on data from CCAFS and Kennedy Space Center (Spaceport Weather Data Archive; http://trmm.ksc.nasa.gov/). Specifically, we found precipitation (Kruskal-Wallis; $X^2 = 11.7$, df = 3, P = 0.009) and temperature (Kruskal-Wallis; $X^2 = 29.5$, df = 3, P < 0.001) were significantly different among seasons.

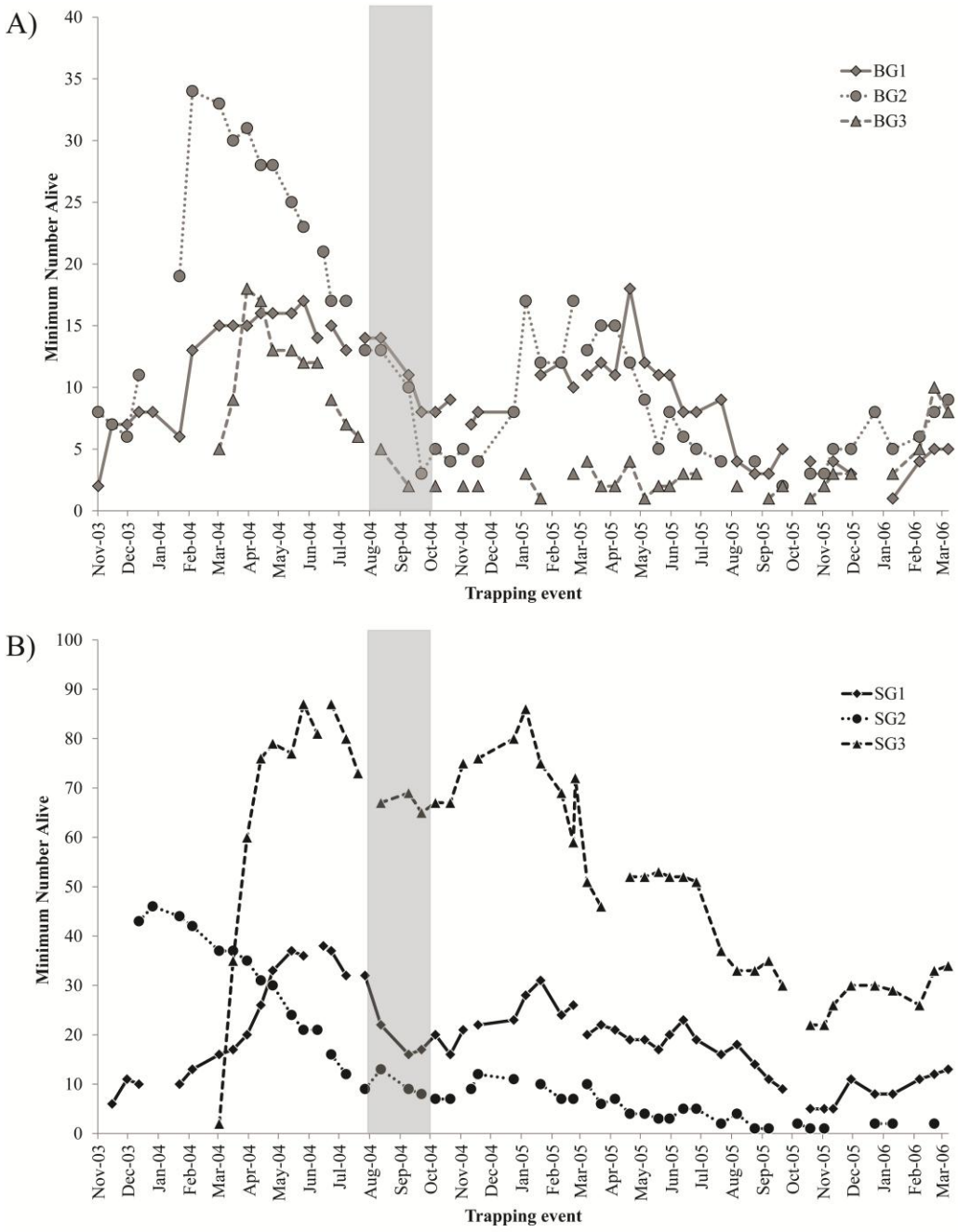

Figure 5.3 Minimum number alive for *P. p. niveiventris* across the trapping period for three grids in (A) beach habitat and three grids in (B) scrub habitat at CCAFS (Fig. 5.1). Gray area indicates a period where three major hurricanes reached Florida; Jeanne impacted the beach grids September 26, 2004.

We captured more individual males than females across the sampling period on four of the six grids, but the differences were not significant ($p > 0.05$) (Table 5.4). When we examined the sex ratio by season, year, and habitat, a significant temporal variation in the 1:1 sex ratio for the beach ($X^2 = 19.0$, df = 8, $p = 0.015$) was indicated (Table 5.5). A reanalysis showed the outcome was explained by one outlier, which when removed reduced the p value to 0.077. We found no significant variation in the 1:1 sex ratio ($X^2 = 7.05$, df = 8, p-value = 0.531) for the scrub grids.

Table 5.4 Individual male and female *P. p. niveiventris* captured in each grid at CCAFS (Fig. 1) during 2003 – 2006, reported as number females, females, ratio between the two sexes, X^2 value for deviation from predicted 1:1 ratio and p-value (P) of goodness-of-fit test.

Grid	Females	Males	Females/Males	X^2	P
BG1	42	48	1.00:1.14	0.40	0.527
BG2	72	67	1.07:1.00	0.18	0.672
BG3	28	36	1.00:1.29	1.00	0.317
SG1	93	84	1.11:1.00	0.46	0.499
SG2	49	68	1.00:1.39	3.09	0.079
SG3	238	258	1.00:1.08	0.81	0.369

Table 5.5 Seasonal variation in number of *P. p. niveiventris* individuals categorized by sex in each habitat at CCAFS based on season and year. Ratio of females and males are reported.

		Sex		
Habitat	Capture period	Female	Male	Female/Male
Beach	Winter 2003	53	34	1.56:1.00
	Spring 2004	141	136	1.04:1.00
	Summer 2004	75	87	1.00:1.16
	Fall 2004	27	36	1.00:1.33
	Winter 2004	30	35	1.00:1.17
	Spring 2005	75	77	1.00:1.03
	Summer 2005	21	35	1.00:1.67
	Fall 2005	8	23	1.00:2.88
	Winter 2005	10	20	1.00:2.00
Scrub	Winter 2003	81	113	1.00:1.40
	Spring 2004	261	249	1.05:1.00
	Summer 2004	243	253	1.00:1.04
	Fall 2004	168	188	1.00:1.12
	Winter 2004	120	139	1.00:1.16
	Spring 2005	151	152	1.00:1.01
	Summer 2005	102	117	1.00:1.15
	Fall 2005	75	69	1.09:1.00
	Winter 2005	53	57	1.00:1.08

Our adult survival estimates were derived from trapping data collected from spring 2004 (March – May 2004) until winter 2005 (December 2005, January – February 2006). The most informative model for survival (Φ) and recapture probability (p) was Φ estimated by year and season, and p by year, season and habitat (Table 5.6). This model (1) suggests there was not a significant difference in survival between the two habitat types. The next best model (2) included differences in survival in the two habitats for estimating Φ (Table 5.6). As an additional test for the hypothesis that Φ differed between habitats we ran a likelihood ratio test which showed no significant contribution of habitat differences in survival rates.

Our data did not support a difference in survival between the two habitats (X^2 = 7.25, p-value = 0.611), therefore we combined the data for further analysis. We observed the highest survival during winter 2004 and 2005 with respective estimated seasonal survival of 0.823 (standard deviation [SD] = 0.027; 95% CI [confidence interval], 0.774 – 0.878) and 0.772 (SD = 0.045; 95% CI, 0.671 – 0.848) (Fig. 5.4). The lowest estimated seasonal survival was observed during fall 2004 (Φ = 0.657; SD = 0.026; 95% CI, 0.603 – 0.707) and 2005 (Φ = 0.667; SD = 0.037; 95% CI, 0.592 – 0.735) (Fig. 5.4). Spring 2004 had higher estimated survival (Φ = 0.751; SD = 0.023; 95% CI, 0.703 – 0.793), than spring 2005 (Φ = 0.661; SD = 0.025; 95% CI, 0.611 – 0.708) (Fig. 5.4). The summer survival estimates were similar, with 0.743 (SD = 0.023; 95% CI, 0.695 – 0.785) for 2004 and 0.755 (SD = 0.044; 95% CI, 0.659 – 0.831) for 2005 (Fig. 5.4).

We observed a great amount of variation in the proportion of reproductive females during our study by season, year, and habitat (Fig. 5.5). The greatest proportions of reproductive females were observed in spring 2004, fall 2005 and winter 2005 (0.28, 0.32, and 0.29, respectively) in scrub habitat. The fewest reproductive females were observed in beach habitat during spring, summer and winter 2004 (0.06, 0.03 and 0.09, respectively). This trend was reversed in 2005 when the proportion of reproductive females on the beach grids was ~20% in each season. We found the proportion of reproductive females in the scrub habitat exhibit more variability among seasons and between years than did females from the beach habitat.

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Table 5.6 Best ten models for estimating survival (Φ) and recapture probability (p) using maximum likelihood in MARK for capture-recapture data for *P. p. niveiventris*. Models were arranged based on Akaike Information Criterion (AICc). Reported is difference in AICc (ΔAICc) for each model compared to model with lowest AICc value (i.e., best model). Additional measures of confidence in model selection are AICc weights and model likelihood. Included is number of parameters required by each model.

Model #	Model	AICc	ΔAICc	AICc Weights	Model Likelihood	Parameters
1	Φ(Year X Season); p(Year X Season X Habitat)	7406.1	0.0	0.995	1.0000	27
2	Φ(Year X Season X Habitat); p(Year X Season X Habitat)	7417.2	11.2	0.004	0.0038	36
3	Φ(Habitat); p(Year X Season X Habitat)	7419.1	13.0	0.002	0.0015	20
4	Φ(Habitat); p(Month X Habitat)	7454.8	48.8	0.000	0.0000	80
5	Φ(.); p(Month X Habitat)	7454.9	48.8	0.000	0.0000	79
6	Φ(Month); p(Season X Habitat)	7458.7	52.7	0.000	0.0000	116
7	Φ(Season); p(Season X Habitat)	7469.5	63.5	0.000	0.0000	12
8	Φ(Year X Season); p(Year X Season)	7470.8	64.7	0.000	0.0000	18
9	Φ(Season X Habitat); p(Season X Habitat)	7470.8	64.7	0.000	0.0000	16
10	Φ(Habitat); p(Season X Habitat)	7474.3	68.2	0.000	0.0000	10

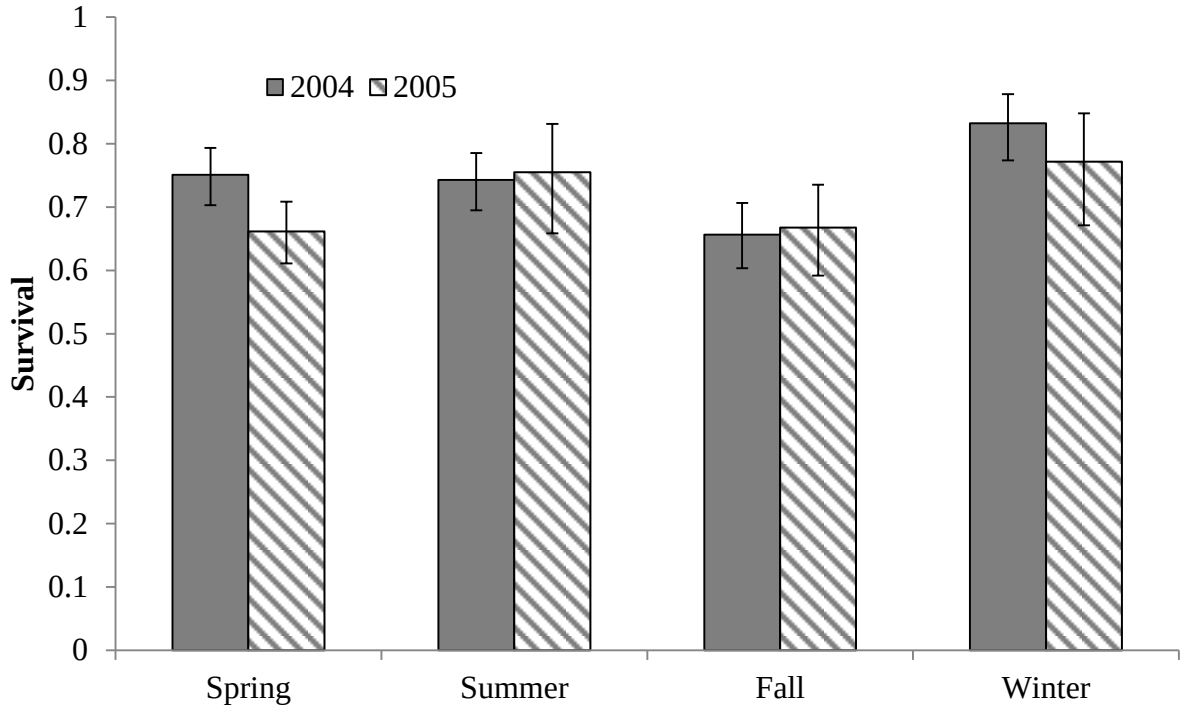

Figure 5.4 Seasonal adult survival estimate for *P. p. niveiventris* based on the best model in MARK, for 2004 and 2005. Bars indicate 95% Confidence interval estimated in MARK.

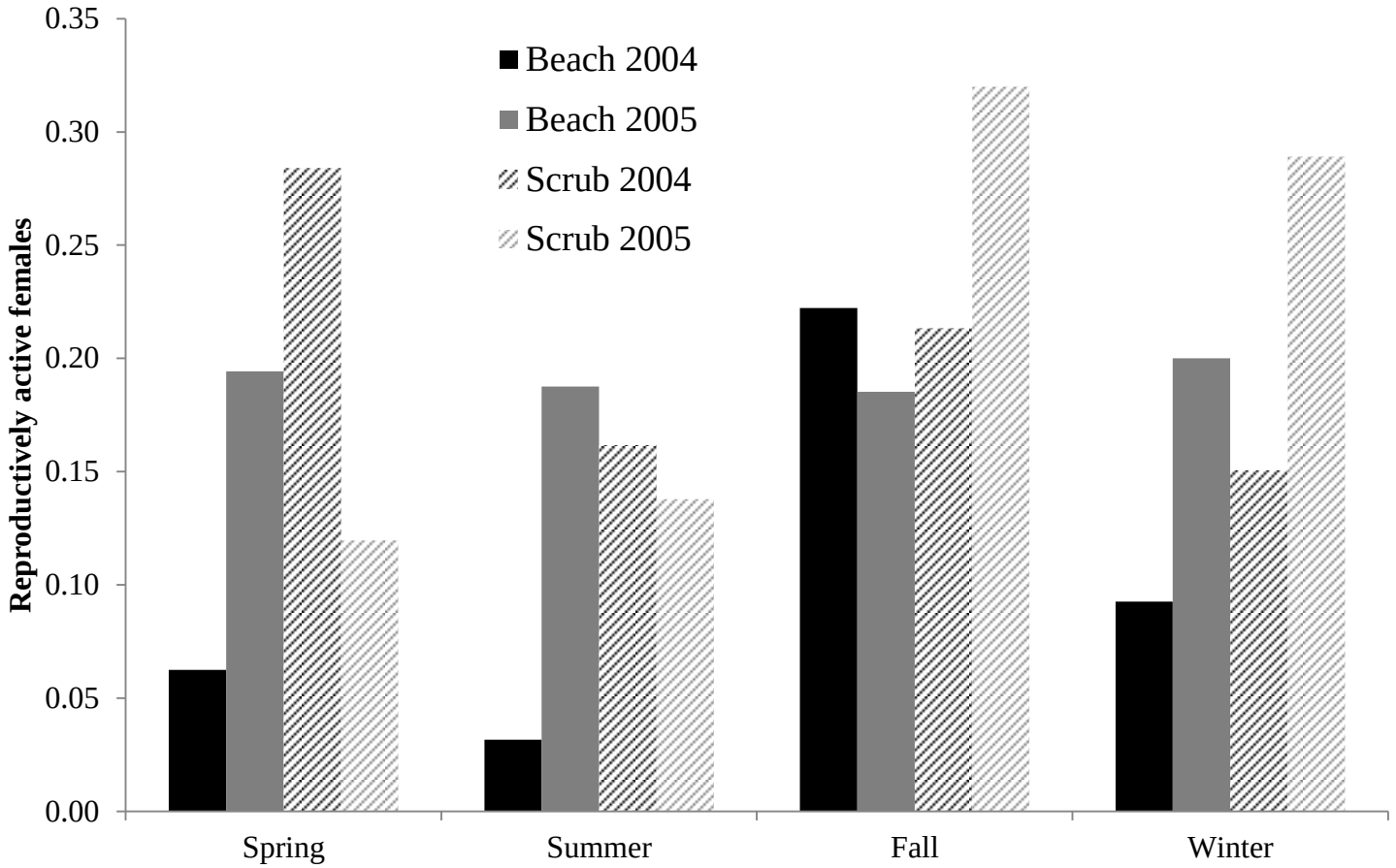

Figure 5.5 Proportion reproductive females in *P. p. niveiventris* population for each season, in beach and scrub habitat for trapping year 2004 and 2005.

Model Analysis

After running our model for 10,000 replications we found the stochastic sampling of parameters gave a finite growth rate, lambda (λ), ranging from population decline (~0.5), to population growth (~2.0). Most replicates were relative close to a stable population (λ =1) with an average λ = 1.041 ± 0.004 95% confidence interval (CI) (Fig. 5.6).

We found that seasonal survival (S_a) of *P. p. niveiventris* had the greatest impact on the population growth in our model for all but one of four seasons, based on sensitivity analysis (Table 5.7) and for all seasons based on elasticity analysis (Table 5.8). For the other vital rates the overall patterns were similar for the sensitivity and elasticity analysis (Table 5.7 and 5.8), and because elasticity gave proportional influence on λ for each vital rate we focus primarily on the elasticity results. Other vital rates indicated as significant in determining λ were proportion of the population that was reproductive females in the scrub (Pre_{Scrub}) and survival of litter (S_L) (Table 5.8). In contrast, reproductive females in the scrub (Pre_{Scrub}) and juvenile survival (S_J) have less impact on the overall population growth rate. Litter size (L) and migration rate (m) have little impact on the growth rate with both values close to zero for all four seasons (Table 5.8). With the exception of the complete overlap of the elasticity of L and $S_{l,}$ no vital rates overlapped in their 95% CI.

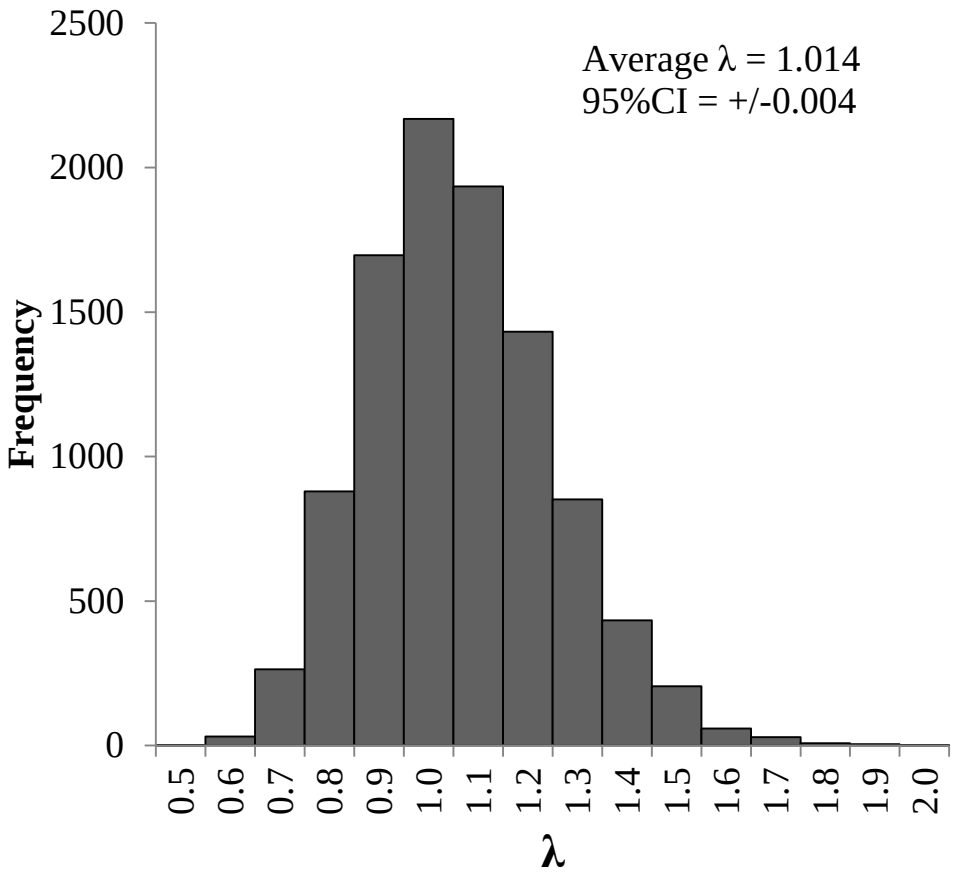

Figure 5.6 Estimate of lambda for stochastic matrix model of *P. p. niveiventris*, based on 10,000 replications. Values reported are average and 95% confidence interval (CI).

Table 5.7 Average sensitivity values and 95% confidence intervals (CI) for each vital rate across the four seasons, based on the model for *P. p. niveiventris*. Bold term shows highest average value for each season. Vital rates are: litter size (L), migration rate (m), proportion reproductive females in beach (Pre_{Beach}) and scrub (Pre_{Scrub}), adult survival (S_a), litter survival (S_L) and juvenile survival (S_j).

Season	L	m	Pre_{Beach}	Pre_{Scrub}	S_a	S_L	S_j
Average							
Spring	0.067	-0.139	0.222	1.146	**1.265**	0.554	0.135
Summer	0.048	-0.088	0.335	0.972	**1.199**	0.398	0.101
Fall	0.084	-0.127	0.150	1.170	**1.346**	0.693	0.177
Winter	0.079	-0.154	0.200	**1.299**	1.285	0.652	0.146
95% CI							
Spring	0.0004	0.0015	0.0071	0.0086	0.0015	0.0025	0.0008
Summer	0.0002	0.0011	0.0081	0.0088	0.0009	0.0009	0.0004
Fall	0.0004	0.0013	0.0028	0.0058	0.0015	0.0019	0.0008
Winter	0.0004	0.0016	0.0061	0.0083	0.0015	0.0024	0.0008

Table 5.8 Average elasticity values for each vital rate across the four seasons, based on the model for *P. p. niveiventris*. Bold term shows highest average value for each season. Also reported is the 95% confidence interval (CI) for each vital rate and season. Vital rates are: litter size (L), migration rate (m), proportion reproductive females in beach (Pre_{Beach}) and scrub (Pre_{Scrub}), adult survival (S_a), litter survival (S_L) and juvenile survival (S_j).

Season	L	m	Pre_{Beach}	Pre_{Scrub}	S_a	S_L	S_J
Average							
Spring	0.278	-0.014	0.037	0.241	**0.913**	0.278	0.087
Summer	0.208	-0.009	0.054	0.154	**0.950**	0.208	0.050
Fall	0.341	-0.012	0.032	0.309	**0.890**	0.341	0.110
Winter	0.286	-0.013	0.031	0.255	**0.919**	0.286	0.081
95% CI							
Spring	0.0012	0.0001	0.0013	0.0019	0.0005	0.0012	0.0005
Summer	0.0008	0.0001	0.0014	0.0013	0.0003	0.0008	0.0003
Fall	0.0011	0.0001	0.0006	0.0014	0.0006	0.0011	0.0006
Winter	0.0011	0.0001	0.0010	0.0017	0.0005	0.0011	0.0005

Our sensitivity analysis showed that the adult stage in the scrub habitat had the greatest impact on the annual λ for modeled populations of *P. p. niveiventris*. Minor changes in adult reproduction (adult to juvenile transition) and adult survival (adult to adult transition) had equal impacts on annual population growth, based on overlap in 95% CI (Table 5.9). This was the case for all seasons, with the highest values in summer (adult-juvenile, 0.654 ± 0.0024 95% CI; adult – adult, 0.744 ± 0.0026 95% CI) and lowest values in winter (adult-juvenile, 0.562 ± 0.0018 95% CI; adult – adult, 0.604 ± 0.0018 95% CI) (Table 5.9). We found adult survival in the scrub habitat to have the greatest proportional impact on the annual λ based on our elasticity analysis. All seasons had the same pattern of greatest elasticity values with adult survival (Table 5.10). In each habitat we found adult survival to have the greatest elasticity values, and these were significantly higher than any other elasticity values within the habitat based on 95% CI for all seasons (Table 5.10). In the scrub and beach habitat, the ability for juveniles to survive to provide offspring for the next season had the lowest elasticity values in each season (Table 5.10). Overall, migration between the two habitats had little impact on the population dynamics of *P. p. niveiventris*, with average elasticity values of 0.004 for the four seasons (Table 5.10).

We found scrub to have an overall higher impact on the population dynamics of *P. p. niveiventris* than the beach, when we summed up the elasticity for each habitat and migration direction. Across all seasons the scrub had the greatest impact on the annual λ (Fig. 5.7). We observed the smallest proportional change of λ with minor change to the migration between the two habitats. We found no seasonal difference between the elasticity of the habitats (Fig. 5.7).

Table 5.9 Average sensitivity values and 95% confidence interval (CI) for each matrix element for the model of *P. p. niveiventris* population dynamics with migration rate between habitats at 1% (m = 0.01). Values reported for each season. Bold terms are the highest values. Values overlapping in the 95% CI were considered not significantly different.

Time t + 1 (by season, habitat and stage)		Average Sensitivity at time t by habitat and stage				95% CI Sensitivity at time t by habitat and stage			
		Beach		Scrub		Beach		Scrub	
SPRING		Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
Beach	Juvenile	0.016	0.061	0.070	0.172	0.0004	0.0010	0.0005	0.0011
	Adult	0.014	0.054	0.058	0.145	0.0004	0.0011	0.0005	0.0013
Scrub	Juvenile	0.049	0.192	0.261	**0.627**	0.0005	0.0012	0.0016	0.0024
	Adult	0.055	0.218	0.297	**0.712**	0.0006	0.0013	0.0018	0.0027
SUMMER									
Beach	Juvenile	0.017	0.061	0.072	0.174	0.0004	0.0010	0.0005	0.0011
	Adult	0.015	0.053	0.059	0.146	0.0004	0.0010	0.0005	0.0013
Scrub	Juvenile	0.051	0.199	0.280	**0.654**	0.0006	0.0011	0.0021	0.0024
	Adult	0.058	0.226	0.318	**0.744**	0.0006	0.0012	0.0024	0.0026
FALL									
Beach	Juvenile	0.016	0.058	0.068	0.165	0.0004	0.0010	0.0005	0.0011
	Adult	0.014	0.051	0.055	0.137	0.0004	0.0011	0.0005	0.0013
Scrub	Juvenile	0.047	0.185	0.259	**0.607**	0.0005	0.0011	0.0019	0.0020
	Adult	0.054	0.210	0.296	**0.691**	0.0006	0.0012	0.0021	0.0022
WINTER									
Beach	Juvenile	0.015	0.055	0.064	0.155	0.0003	0.0009	0.0005	0.0010
	Adult	0.013	0.046	0.050	0.124	0.0003	0.0009	0.0005	0.0011
Scrub	Juvenile	0.044	0.172	0.240	**0.562**	0.0005	0.0010	0.0017	0.0018
	Adult	0.047	0.184	0.258	**0.603**	0.0005	0.0010	0.0019	0.0018

Table 5.10 Average elasticity values and 95% confidence interval (CI) for each matrix element for the model of *P. p. niveiventris* population dynamics with migration rate between habitats at 1% (m = 0.01). Values reported for each season. Bold terms are the highest values. Values overlapping in the 95% CI were considered not significantly different. Matrix elements with '-' indicates biologically impossible transitions based on the assumptions of the model.

Time t + 1 (by season, habitat and stage)		Average Sensitivity at time t by habitat and stage				95% CI Sensitivity at time t by habitat and stage			
		Beach		Scrub		Beach		Scrub	
SPRING		Juvenile	Adult	Juvenile	Adult	Juvenile	Adult	Juvenile	Adult
Beach	Juvenile	0.004	0.012	-	-	$1.0x10^{-4}$	$2.7x10^{-4}$	-	-
	Adult	0.009	0.039	0.004	-	$2.5x10^{-4}$	$8.5x10^{-4}$	$3.5x10^{-5}$	-
Scrub	Juvenile	-	-	0.076	0.176	-	-	$7.1x10^{-4}$	$6.5x10^{-4}$
	Adult	0.004	-	0.177	**0.501**	$4.2x10^{-5}$	-	$6.9x10^{-4}$	$1.7x10^{-3}$
SUMMER									
Beach	Juvenile	0.003	0.012	-	-	$8.5x10^{-5}$	$2.9x10^{-4}$	-	-
	Adult	0.009	0.041	0.004	-	$2.4x10^{-4}$	$8.6x10^{-4}$	$3.4x10^{-5}$	-
Scrub	Juvenile	-	-	0.054	0.148	-	-	$3.4x10^{-4}$	$5.9x10^{-4}$
	Adult	0.004	-	0.182	**0.552**	$4.2x10^{-5}$	-	$9.7x10^{-4}$	$1.3x10^{-3}$
FALL									
Beach	Juvenile	0.004	0.017	-	-	$1.1x10^{-4}$	$3.1x10^{-4}$	-	-
	Adult	0.009	0.035	0.004	-	$2.5x10^{-4}$	$8.0x10^{-4}$	$3.6x10^{-5}$	-
Scrub	Juvenile	-	-	0.089	0.216	-	-	$5.5x10^{-4}$	$8.5x10^{-4}$
	Adult	0.004	-	0.169	**0.455**	$3.9x10^{-5}$	-	$9.1x10^{-4}$	$1.1x10^{-3}$
WINTER									
Beach	Juvenile	0.004	0.014	-	-	$9.6x10^{-5}$	$3.0x10^{-4}$	-	-
	Adult	0.009	0.038	0.004	-	$2.4x10^{-4}$	$8.3x10^{-4}$	$3.4x10^{-5}$	-
Scrub	Juvenile	-	-	0.078	0.200	-	-	$5.2x10^{-4}$	$9.4x10^{-4}$
	Adult	0.004	-	0.169	**0.482**	$4.0x10^{-5}$	-	$9.2x10^{-4}$	$1.2x10^{-3}$

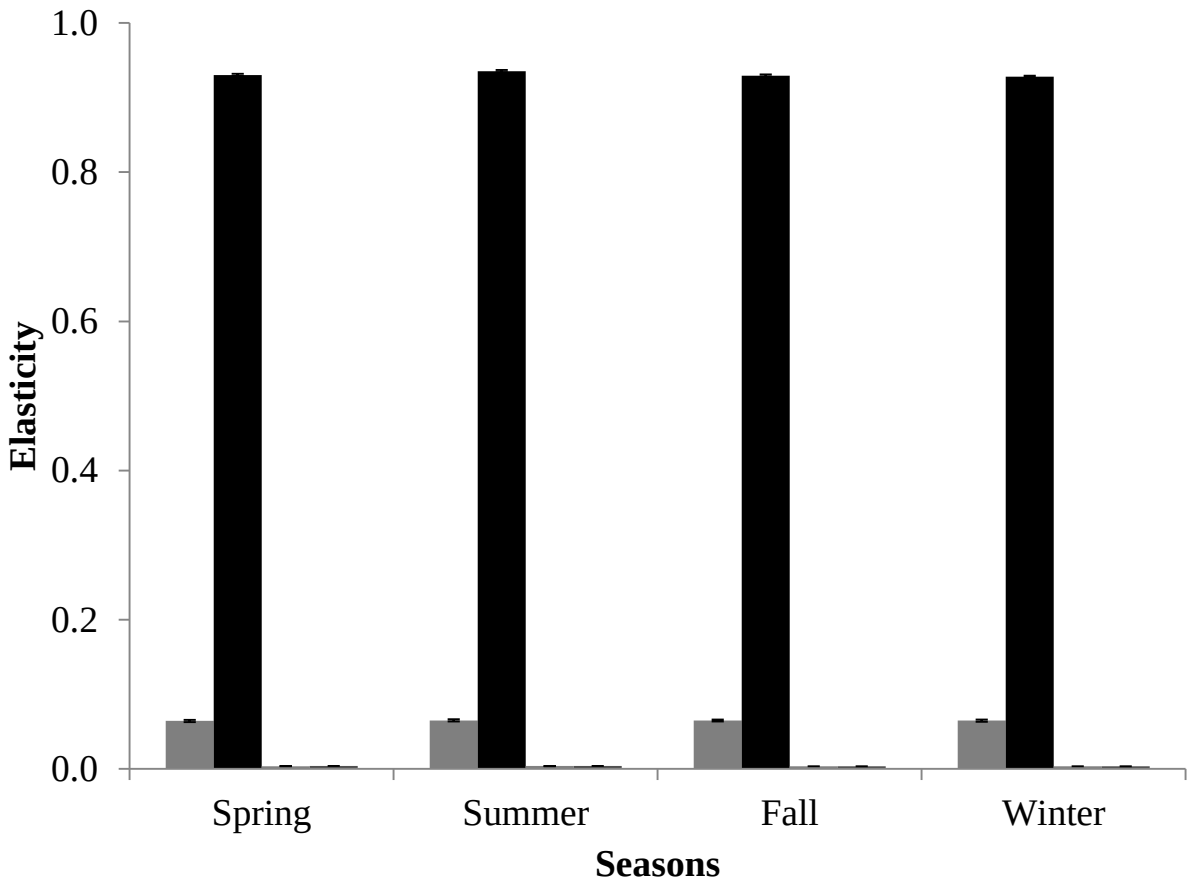

Figure 5.7. Elasticity estimate for each part of the megamatrix, with bars indicating beach (Gray), scrub (Black). Elasticity value is given for the four seasons used in the model. Each bar shows average elasticity based on 10,000 replicates of the model, and the error bar shows the 95% confidence interval. The elasticity values shown in the figure for beach to scrub migration and scrub to beach migration was negligible.

Discussion

Our first objective was to document demographic variation among seasons and between years within two juxtaposed and structurally contrasting habitats occupied by *P. p. niveiventris* on Cape Canaveral, Florida, U.S.A. This area, effectively all of CCAFS, has been identified as critical to the long-term survival of this lineage of *P. polionotus* (Degner et al. 2007, Suazo et al. 2009, Kalkvik et al. in review). Our study strengthened these prior studies by identifying

important differences in the population dynamics of this mouse in adjacent beach and scrub habitat. For example, the joint contributions of these habitats mitigate threats from tropical storms. Therefore population persistence of *P. p. niveiventris* depends locally and perhaps regionally on these habitats remaining intact into the future (Sturtevant et al. 1996, Brambilla et al. 2007, Heinrichs et al. 2010).

Our capture-recapture data indicated important variation in vital rates of *P.p. niveiventris* by grid, habitat, season, and year of study. Minimum number alive on the grids exhibited different trends over the study period. These patterns were driven by capture success in the two habitat types. The number of new individuals and the number of recaptures in the scrub greatly exceeded comparable numbers from the beach habitat. The data did not reveal how the dynamics in MNA was influenced by immigration or emigration. We were able to look at patterns of reproductive efforts and found that most seasons during our trapping period were characterized by a greater proportion of reproductive females in the scrub than were found in the beach habitat (Fig. 5.5). The three highest values of proportion of reproductive females were in spring 2004, fall 2005, and winter 2005 in scrub habitat. These observations suggested the scrub habitat may have contributed more to the population growth of *P.p. niveiventris* than the beach habitat. Nonetheless, reproductive activity of females in the beach habitat for spring and summer of 2005 exceeded that found in the scrub. Dapson (1972) presented indirect evidence that *P.p. niveiventris* at Vero Beach in 1969 ceased reproduction in April-June. Temporal variation in proportion of reproductive females has been observed in close relatives of *P. p. niveiventris* (Davenport 1964, Smith 1974). Seasonal variation in the proportion of reproduction active females clearly occurred on Cape Canaveral in our study. This is contrary to observations of

another beach mouse subspecies, *P. p. ammobates*, where the proportion of reproductively active female mice in scrub habitat apparently tracked similar patterns to those found in females from adjacent beach habitat across time (Swilling et al. 1998).

We expected to find adult survival to show patterns of spatial and temporal variation, but based on our maximum likelihood analysis in MARK, we did not detect habitat differences in survival. Seasonal variation in survival by year averaged > 65% to > 80% in all comparisons with post-hurricane survival the winter of 2004 being significantly improved over the fall ($p < 0.05$) (Fig. 5.4). Seasonal variation in survival has been documented in other beach mice with winter survival as high or higher than other seasons (Swilling et al. 1998). Dapson (1972) estimated the maximum age of *P. p. niveiventris* as 307 days with the implication that some individuals survival up to three seasons. The proximal causes for the temporal variation in survival are not clear, but could be explained by seasonal variation in resources as reported in other small mammal communities (Boutin 1990, Adler 1998).

Hurricanes are a major threat to populations of beach mice on the Gulf and Atlantic coasts (Swilling et al. 1998, Pries et al. 2009). Our grids were subject to indirect effects of two hurricanes (Charley and Frances) and the direct effects of Hurricane Jeanne between August and the end of September 2004 (www.noaa.gov; accessed January 6, 2012). Populations based on MNA were in gradual declines on five of six grids immediately prior to Hurricane Jeanne's landfall September 26 (Fig. 5.3). These trends ceased or began to reverse on the beach and scrub grids in the remaining months of 2004. Some studies have evidence that scrub habitat landward of primary dunes function as a refuge during and after hurricanes (Swilling et al. 1998, Pries et

al. 2009). We expected to see higher survival and numbers in scrub than the beach habitat after Hurricane Jeanne the fall of 2004. MNA on the beach grids were reduced relative to the scrub grids the fall of 2004. The explanation for the lag in population recovery on the beach grids was likely physical damage to the primary dunes and back dune area, e.g., erosion, sand deposition, and flooding. At the population level, we were unable to detect a difference in survival between the two habitats during this period (Table 5.1). Furthermore, recruitment must have failed after the storm damage on the beach grids because the proportion of reproductive females peaked at this time (Fig. 5.5). In contrast to the beach grids, two of our grids in scrub habitat (SG1 and SG3; Fig. 5.3) increased MNA during fall and winter of 2004-2005. We were unable to provide an independent test of the value of the scrub habitat as a refuge and hedge against local extinction as demonstrated by Swilling et al. 1998 and Pries (2009). Our efforts to detect dispersal from beach to scrub or scrub to beach were unsuccessful (unpublished data).

We utilized a population modeling approach to investigate the impact of the different habitats on the overall population dynamics of *P. p. niveiventris* at CCAFS. The use of population modeling has met some criticism in conservation biology, in particular for modeling extinction risk (Beissinger and Westphal 1998, Coulson et al. 2001). Our study does not aim to predict the risk of extinction for the population of *P. p. niveiventris* occupying CCAFS, but rather to utilize modeling approaches to evaluate vital rates of the mouse in the landscape they occupy. Evaluating landscape variation has been successfully used in other taxa without projecting population trends (e.g., Horvitz and Schemske 1986, Cipollini et al. 1994, Pascarella and Horvitz 1998). Our model represents the complex seasonal dynamics of a rapidly developing rodent, where females can theoretically produce offspring of their own in 72 days after their birth

(Layne 1968). In addition, we incorporated stochastic elements by treating vital rates as distributions rather than constants. Temporal variation in vital rates was added when data were available. Incorporating stochasticity in the model provided realism and greater confidence in the results (Beissinger and McCullough 2002, Morris and Doak 2002, McGowan et al. 2011). To evaluate the stability of our model, and to ensure our model was not producing unrealistic scenarios for the population dynamics of *P. p. niveiventris*, we estimated annual λ. With an average λ = 1.014, and a narrow confidence interval (95% CI = 0.004), we believe our model represents a realistic scenario reflecting the population dynamics of *P. p. niveiventris* (Fig. 5.6). Additionally, the confidence interval around our estimated sensitivity or elasticity values accounted for only 5% or less of the estimated average, suggesting even with stochasticity our model produced similar results. Oli et al. (2001) provided estimates of r (intrinsic rate of natural increase), which we transformed to annual increase or λ, for four populations of beach mice (*P. p. ammobates* and *P. p. trissyllepsis*) from the Gulf coast of Alabama and Florida. Their estimates of λ ranged from 2.7511 for Fort Morgan to 1.0704 at Gulf Islands National Seashore. Given the rapid and short-term changes in populations of *P. polionotus* at numerous study sites, major shifts in λ must be the rule (Oli et al. 4 study sites, see Fig. 2; this study, 6 independent grids, see Fig. 5.3).

Our model has two limitations that have not been addressed. First, no explicit effort was made in the model structure to account for catastrophic events. Second, we recognized density-dependence can impact population persistence (Stacey and Taper 1992) through feedbacks on vital rates during various life stages (Morris and Doak 2002). Incorporating density-dependent processes into population models is recommended, but long-term data are necessary for proper

estimation of such processes (Coulson et al. 2001). Oli et al. (2001) were unable to incorporate density dependence in their PVA models and failed to find evidence for density-dependent responses in their field data. Even with the lack of accounting for density-dependence. We believe our model provided useful insights into the population biology of *P. p. niveiventris* in the absence of density-dependent functions.

Our field data suggested temporal and spatial variation of *P. p. niveiventris* at CCAFS, but this does not provide a clear indication of the contribution of the different habitat types on the overall population dynamics. Modeling studies using both theoretical and empirical information have shown that habitats and their configurations can contribute differentially to population dynamics (Fraterrigo et al. 2009, Heinrichs et al. 2010). Using a modeling approach we found scrub habitat to have a greater impact on the overall population dynamics of *P. p. niveiventris* at CCAFS than the beach habitat (Fig. 5.7). With higher numbers of mice found in scrub habitat, as shown in our study (Table 3.3; Fig. 3.3) and in other studies (Extine and Stout 1987), these results seems to fit with observed patterns. We found adult survival in the scrub to have the greatest impact on the population dynamics (Table 5.10), which seems counterintuitive as we found no difference in adult survival between habitats based on our capture-recapture data (Fig. 5.4). Most likely this is a result of greater reproductive effort of females in the scrub habitat relative to females in beach habitat (Table 5.8). While adult survival is the same between scrub and beach habitat, it seems to be the maintenance of the higher reproductive output in the form of juveniles becoming adults , which then survive, that explains the greater influence of adult survival in the scrub habitat.

Our evidence from capture-recapture efforts and modeling strongly indicate the scrub habitat sustained greater numbers of *P. p. niveiventris* at CCAFS than the beach habitat. Only a modest difference in reproductive effort or recruitment by mice in the scrub could account for the apparent success relative to the beach animals. However, this could be a function of the amount of scrub available relative to the beach habitat, that is, an area effect. Other beach mice subspecies has been shown to utilize tertiary dunes and scrub habitat less extensively than documented in this study (Swilling et al. 1998, Sneckenberger 2001, Pries et al. 2009). In other locations within the distribution of beach mice, scrub has also been argued to function as temporary refuge from hurricanes (Pries et al. 2009). We know that within the distribution of *P. p. niveiventris* other populations have persisted for decades with little or no scrub habitat available (Stout 1992). Our study therefore does not suggest that the presence of extensive scrub habitat is essential for the persistence of beach mice; however, when scrub is present it can greatly impact the overall population dynamics of beach mice. If the impact by scrub is purely an area effect, as seen in a wide range of taxa (see review Connor et al. 2000), we would expect population sizes to be correlated with amount of overall habitat (beach and scrub) that is present. To evaluate this prediction, further research is needed on the population dynamics in the known populations of *P. p. niveiventris*, as identified by Kalkvik et al. (in review).

Modeling has provided unique insights into population dynamics of many taxa, which can be directly implemented into management and conservation decisions (Olson et al. 2004, Heinrichs et al. 2010). Conservation of dune systems has received attention in the context of beach mice population management (Jester 1998). Agencies have recognized the need to address the importance of scrub for future beach mice conservation (U.S. Fish and Wildlife Service

1993). Our findings provide further evidence for the need to manage not only beach habitat, but also the adjacent scrub habitat. The data generated from this study suggest higher densities of mice in scrub habitat CCAFS, and our population model provides evidence that mice in scrub habitat can have a significant impact on the overall population dynamics (Fig. 5.7).

In conclusion, our study provides an example of identifying the contribution of different habitat types in the overall population dynamic of an organism. We found that *P. p. niveiventris* at CCAFS occupying scrub habitat have a greater impact on overall population dynamics of the mice than has historically been recognized. Combining both trapping data and population modeling, we gained stronger evidence for the impact that scrub habitat has for this subspecies. While other beach mice populations may not utilize scrub habitat as extensively as the *P. p. niveiventris* population at CCAFS, our study illustrates the need to address conservation of beach mice beyond beaches. Our study does not suggest that beach habitat is incapable of supporting healthy populations; however, we suggest that when extensive scrub habitat is present, it has the potential to influence the overall population dynamics of beach mice. Most likely both habitats would be necessary for long-term persistence of the population as has been seen in other taxa (Brambilla et al. 2007). With our findings of both variation in habitat and seasonal impacts on the population dynamics of *P. p. niveiventris*, we provide further evidence for the need for detailed studies on population dynamics for appropriate management of listed taxa (Gaillard et al. 2000, Heinrichs et al. 2010).

Acknowledgments

Funding for this project was provided by Patrick Air Force Base, Florida. We want to thank A. DeLong, C. Handrick, M. Keserauskis, J. Provancha, A. Suazo, and M. Tucker, as well as field assistants A. Ashcraft-Cryder, K. Gillespie, M. Green, D. Gunderson, W. Lavigne, S. Letcher, D. Smith and A. Verpoorton, for collecting field data. All animal handling was approved by IACUC protocols # 03-13W and 06-46W from University of Central Florida.

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CHAPTER 6. CONCLUDING REMARKS

My aim was to understand diversity and persistence in subspecies of *Peromyscus polionotus* at different spatial and temporal scales. By following this broad based approach I have been able to gain new insight into possible ecological and evolutionary drivers that have shaped the diversity we see today as well as the likely roles played by historic and anthropogenic forces. I provide insight into the association of diversity and the environment at a wide spatial scale (Chapter 2), the formation of the unique coastal diversity we observe in *P. polionotus* (Chapter 3), human impact on genetic diversity (Chapter 4), and how at least one beach mouse population may rely more on scrub habitat than coastal dunes habitat (Chapter 5).

Environmental influence on diversification

Divergence and speciation operate over spatial and temporal gradients in which related taxa respond according to two patterns: niche conservatism and niche divergence. Niche conservatism is associated with sister taxa persisting in similar environmental niches, caused by a restriction via stabilizing selection or by a lack of ancestral variation for natural selection to differentiate (Lord et al. 1995, Webb et al. 2002, Wiens and Graham 2005). An alternative pattern, niche divergence, predicts closely related taxa will occupy different environmental niches (Losos et al. 2003). Contemporary work shows that niches can diverge very rapidly, suggesting that niche divergence can occur in very recently diverged lineages (Evans et al. 2009, Dormann et al. 2010).

My research with the widely distributed *P. maniculatus* species group provides additional support that niche divergence can drive speciation. Using niche based modeling (Phillips et al.

2006, Phillips and Dudík 2008) I observed significant differences in the inferred niche spaces occupied by *P. maniculatus* and *P. polionotus* and found niche distinction between phylogenetically-inferred lineages within *P. maniculatus*. I was even able to determine, using recently developed statistical tools (Warren et al. 2010), that sister taxa with greatly overlapping modeled distributions were occupying environmental spaces more different than would be expected by chance. Most of the lineages identified in my phylogeny for the *P. maniculatus* species group were within *P. maniculatus*. Furthermore, my results support the notion that diversification within widely distributed species, such as *P. maniculatus*, may be dependent upon niche divergence.

My study provided new insight into the identification of contact zones among distinct lineages. I found several potential contact zones between spatially adjacent lineages within *P. maniculatus* using the modeled distributions. These potential contact zones will be important to study to identify possible environmental differences that isolate these lineages at smaller spatial scales. Small scale field studies have already shown habitat preferences between morphologically distinct members of *P. maniculatus* (Hooper 1942, Harris 1954), and my findings provide new areas that warrant research for evaluating local environmental segregation of lineages. My data also suggest that other widely distributed, genetically structured taxa may serve as model organisms for testing the impact the environment has on shaping biodiversity (Hoffman and Blouin 2004a, Fontanella et al. 2008, Vargas-Ramírez et al. 2010, Turmelle et al. 2011).

Climate, sea levels, and young diversity

Historic climatic oscillations and other dramatic environmental fluctuations have impacted modern patterns in biodiversity (Hewitt 1996, Hewitt 2000, Hewitt 2004, Lomolino et al. 2005, Lessa et al. 2010, Hortal et al. 2011). North American biogeographic research has focused on inter- or intraspecific variation observed in taxa that reside at northern latitudes, where glacial sheets covered much of the area during the last ice age (Bermingham et al. 1992, Mila et al. 2000, Shafer et al. 2010). The last ice age left formerly continuous populations to expand from isolated refugia in which distinct lineages had formed; glacial retreat reduced the isolation and these lineages could in time meet forming secondary contact zones. These events during and after the ice age have left distinct genetic patterns in many taxa (Hoffman and Blouin 2004a, Rowe et al. 2004, Rowe et al. 2006, Recuero and García-París 2011). Continental and coastal habitats underwent somewhat similar processes at the end of the Pleistocene as the fauna and flora expanded or retreated with changing conditions.

I chose to study taxa that have a temperate to sub-tropical distribution to investigate the impacts that sea level oscillations (in conjunction with glacial fluctuations) have on coastal diversity. Using two genetic markers, I found the two extant *Peromyscus polionotus* subspecies (*P. p. niveiventris* and *P. p. phasma*) endemic to the Atlantic barrier islands to form two distinct genetic units, with no haplotypes shared between them and distinct differences in microsatellite structure. Both extant subspecies are also genetically distinct from the inland *P. polionotus* populations. These findings support the distinction of the extant Atlantic coast beach mice as their own taxonomic units. Interestingly, the extinct subspecies, *P. p. decoloratus* cannot be

differentiated genetically from the northern subspecies, *P. p. phasma*. Further investigation into the distinctness of *P. p. decoloratus* can help to determine if this subspecies represents lost populations of *P. p. phasma* rather than loss of a whole subspecies. With the exception of *P. p. decoloratus*, my research provides evidence that the extant Atlantic coast beach mouse subspecies are on their own evolutionary trajectory. This is supported by the lack of gene flow and genetic distinctiveness of *P. p. niveiventris* and *P. p. phasma*. The separate evolutionary trajectory is further corroborated by the evidence in literature that the phenotypic differences in pelage coloration are caused by differential selection based on soil coloration (Hoekstra et al. 2006, Mullen and Hoekstra 2008, Vignieri et al. 2010, Domingues et al. In Press)

Bowen (1968) proposed that the Atlantic coast beach mice originated from a single colonization of mice from inland populations that subsequently dispersed along the coastline to diverge into different subspecies. While I cannot reject the possibility of multiple colonization events shaping the current diversity, I found support for the interpretation that all the Atlantic coast beach mice originated from the same source, which currently is located in the interior of central Florida.

Genetic diversity, land management and conservation

Genetic diversity is a widely used tool for assessing the likelihood of population persistence (and therefore conservation concern) of taxa, stemming from the finding that genetically depauperate populations have an increased likelihood of extinction (Frankham 1995, Frankham et al. 2002). Loss of genetic diversity is often associated with anthropogenic influences, but several studies have shown that genetic diversity may be more influenced by

events prior to human alterations of the environment (Hoffman and Blouin 2004b, Taylor and Jamieson 2008, Reding et al. 2010, Tracy and Jamieson 2011). My research demonstrates that *P. p. niveiventris* has maintained the same genetic diversity over the last 100 years in coastal settings where minimal land development pressures have occurred; however, genetic diversity has been reduced in areas with extensive anthropogenic disturbance. My findings suggest that the current genetic diversity observed in *P. polionotus* was shaped by historical events prior to human development of coastal habitat. *P. p. niveiventris* displays a significantly lower signal of genetic diversity when compared with inland populations of *P. polionotus*. It seems likely that founder effects and early bottlenecks resulted in the current genetic diversity we observe in *P. polionotus niveiventris*. Early bottlenecks can greatly reduce genetic diversity, leaving little variation to be acted upon by subsequent bottleneck events (Taylor and Jamieson 2008). Among the coastal populations of *P. polionotus*, *P. p. niveiventris* has maintained higher genetic diversity than any other beach mouse subspecies (see Chapter 3; Mullen et al. 2009).

In land management, the need to protect large spans of continuous habitat has often been associated with charismatic megafauna (Maehr 1990, Quigley and Crawshaw Jr 1992, Noss et al. 1996, Maehr et al. 2002, Leimgruber et al. 2003, Van Aarde et al. 2006). However, smaller organisms can be greatly impacted by the loss of continuous habitat. Currently, *P. p. niveiventris* has approximately 70 km of continuous habitat, making up the central part of their contemporary distribution (Fig. 4.1), which is the longest stretch of continuous habitat found among the beach mice. My research reveals that two disjunct populations of *P. p. niveiventris* show a significant loss of genetic diversity, compared to historical population level genetic diversity. In contrast, the continuous habitat acts as a refuge of historical genetic diversity for *P. p. niveiventris*. With

the genetic diversity found primarily in this large stretch of habitat, my data provide evidence that large areas of continuous habitat may serve to maintain historical genetic diversity. Florida contains large pieces of publically owned land which may contain continuous habitat for a wide range of taxa, including *P. p. niveiventris* (Fig. 6.1). Based on my findings for *P. p. niveiventris*, efforts to maintain these pieces of land may be important for maintaining historical genetic diversity for taxa occupying these areas.

Beach or scrub? That is the question

Habitats can vary in quality, such as presence of cover and food, which can influence population persistence in those habitats (Thomas et al. 2001). However, population persistence can also be influenced by the availability of more than one habitat type (Brambilla et al. 2007). Beach mice have traditionally been associated with primary dunes and sea oat (Uniola paniculata) habitats (Ivey 1949, Bowen 1968), but recent studies have reported several beach mice subspecies occupying scrub habitat, possibly as temporal refugia from natural disturbance (Extine and Stout 1987, Swilling et al. 1998, Pries et al. 2009). I used field data and matrix modeling to show the importance that scrub habitat has on the population dynamics of *P. p. niveiventris*. My field data corroborate the observation by Extine and Stout (1987) that higher numbers of mice can occur in scrub habitat than in juxtaposed beach habitat. I found over a two year period, minimum numbers alive, a proxy of population density, were consistently higher in scrub habitat than in beach habitat.

Population modeling has been shown to be a very useful tool in understanding population dynamics, where we can assess the importance of life stages, habitats, and vital rates on the

overall changes in population numbers (Pascarella and Horvitz 1998, Wemmer et al. 2001, Picó et al. 2002, Olson et al. 2004, Fraterrigo et al. 2009, Heinrichs et al. 2010). Based on matrix modeling, through sensitivity and elasticity analysis, I am able to show that scrub habitat greatly impacts the overall population dynamics of *P. p. niveiventris*. While my research did not seek to project populations into the future, the findings in this study highlight the importance of plant cover other than beach habitat for the persistence of beach mice. To adequately protect the variation in *P. p. niveiventris* and other beach mice, conservation planning needs to take both beach and adjacent scrub habitat into consideration. Scrub habitat may have greater importance for beach mouse population dynamics than solely acting as a refuge from natural disturbances such as hurricanes.

Overall conclusions and future directions

Overall my research has provided new insight into the environmental and climatic influence on speciation and diversification at both a continental and regional scale. In addition, I have provided new research on the impact of human encroachment on natural habitat on populations, and in particular the impact we can have on the genetic diversity in a taxon that has experienced extensive habitat loss. I have also demonstrated the importance of different habitat on the overall population dynamics and possible persistence of a coastal taxon. While my findings can be generally applied to evolutionary biology and ecology, my research has been motivated by conservation. Conservation biology aims not only to identify and preserve biodiversity, but also to identity those processes that shaped biodiversity (Moritz 2002, Groom et al. 2005).

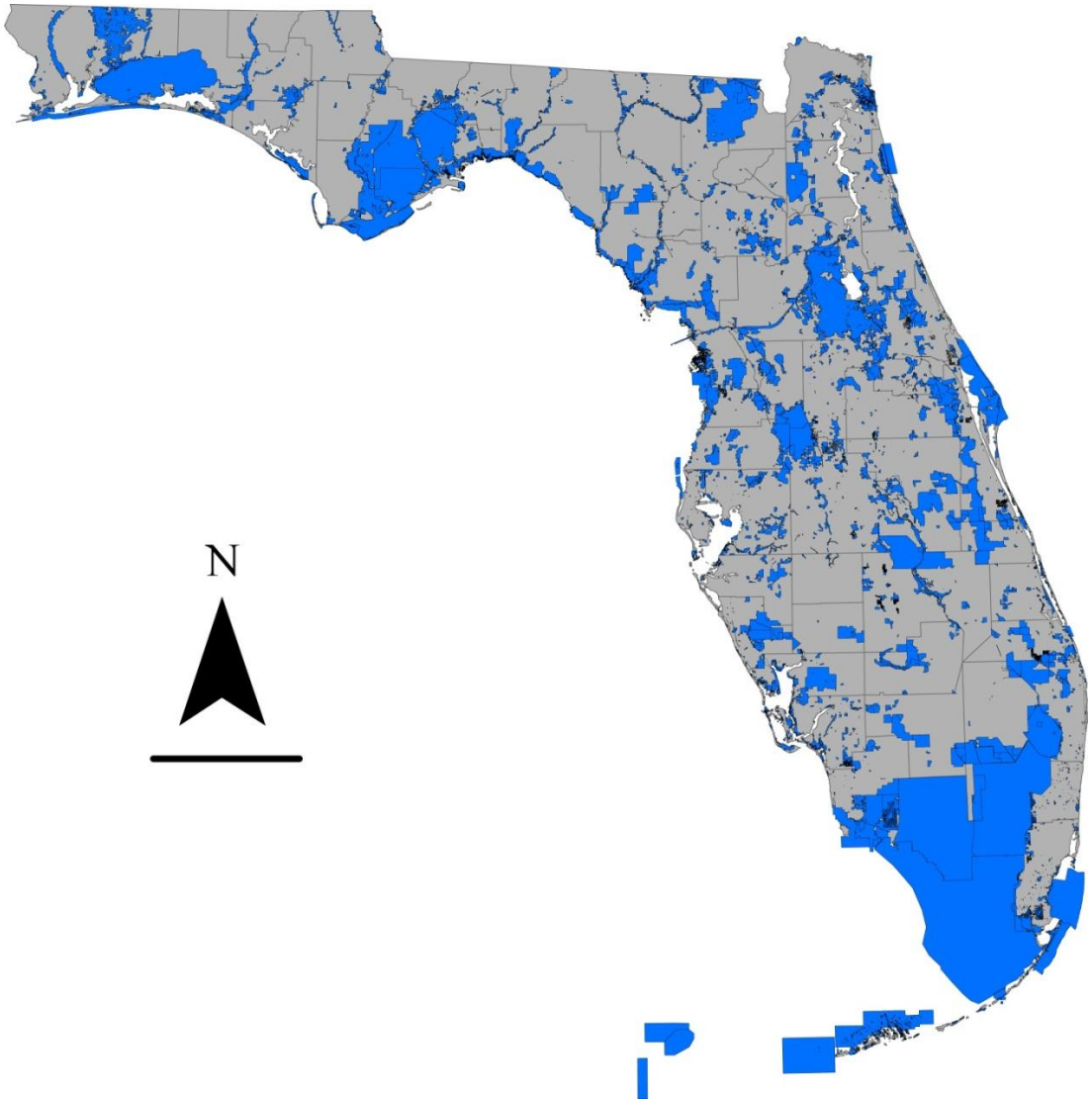

Figure 6.1 Publically owned land across Florida (blue). Scale bar is showing scale to 100 kilometers (Florida Natural Areas Inventory; http://www.fgdl.org).

Future direction in my studies will be to bring my research approach from a single species, or species group, to a multi-taxa sampling strategy. Niche modeling is a correlative analysis and may not provide insight into the mechanistic interaction between the environment and an organism (Kearney 2006). By sampling a wide range of taxa with similar distributions, we may gain evidence of converging environmental features that have a broad impact on biodiversity, rather than species-specific patterns. Other studies have found widely distributed taxa to show evidence of niche divergence (eg. Pyron and Burbrink 2009), so it is clear the *P. maniculatus* species group is not the only North American taxa showing pattern of niche divergence. My predicted distributions from the niche modeling also identify specific areas that sister and non-sister taxa overlap. The areas of overlap I identified could either be hybrid zones between genetic lineages or partitioned by local habitat, so additional field experiments could be executed in these specific areas to identify exact mechanism for interpreting these genetic lineages. I have shown in my research that the coastal *P. polionotus* subspecies may be on their own evolutionary trajectory; furthermore, my data provide support for the possibility of a single colonization event onto the Atlantic coast of Florida, which has given rise to the extant diversity. To provide increased understanding of the dynamics of this young coastal habitat, it would be interesting to investigate the relationship between other taxa found on both mainland and barrier island along the Atlantic coast of Florida. Should we find convergence in the pattern of differentiation in other taxa, it would suggest similar processes shaping the general biodiversity of the barrier islands, whereas a lack of convergence would suggest the beach mice subspecies resulted from a unique pattern of divergence. No other taxa on these islands have been documented to show contrasting phenotype traits as seen in the distinct pelage differences of

beach mice among the barrier islands. Therefore, it would be interesting to further explore why one taxon has undergone such rapid selection, while other have not experienced the same selection regime.

Coastal areas are greatly impacted by human populations (Small and Nicholls 2003), and I have shown that the remaining continuous habitat in the current central portion of the distribution of *P. p. niveiventris* may function as a genetic refuge for the species. This area contains a wide range of listed species (Breininger et al. 1998), and some may be more sensitive to human encroachment than others based on their life-history traits (e.g., generation time, feeding behavior, movement patterns, etc.). By testing temporal changes in genetic diversity and structure of a wide range of taxa in this area we can gain further insight into the impact of such continuous habitat at a community level rather than at a species specific level. Finally, I was able to show the importance of scrub habitat for *P. p. niveiventris* using field data and population modeling. However, many aspects can be further explored for this system, such as how density dependence feedbacks may act on different life stages and their vital rates. Also, with sampling limited to three years, I was not able to capture long term natural fluctuations in the population. Additional questions that would be interesting to resolve might include interspecific competition, resource dependence and the impact of natural (e.g., hurricane) and human caused (e.g., habitat destruction) disasters. By continuing to monitor *P. p. niveiventris* this system can provide additional insight into all of these elements of the population dynamics of a taxon in a dynamics landscape. However, even more important, long term monitoring can also provide insight into the impact of climate changes on a small mammal in a landscape that will be impacted by changes in the air and in the sea.

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