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The Effects of Climatic and Geographic Events on the Cotton Mouse (Peromyscus gossypinus)

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THE EFFECTS OF CLIMATIC AND GEOGRAPHIC EVENTS ON THE COTTON MOUSE (PEROMYSCUS GOSSYPINUS)

By

Sean M. Beckmann

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THE EFFECTS OF CLIMATIC AND GEOGRAPHIC EVENTS ON THE COTTON MOUSE (PEROMYSCUS GOSSYPINUS)

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Climatic and geographic events such as glaciations, island formation, river formation, and urbanization strongly affect habitat specialist species. By contrast, it is traditionally assumed that these events have little effect on habitat generalists but few studies have tested this assumption. In this study I sought to identify the effects of historic and contemporary biogeographic events on the genetic structure of a habitat generalist, the cotton mouse (*Peromyscus gossypinus*). This species is distributed throughout the southeastern United States, a region that has experienced dramatic geographic changes associated with both historic Pleistocene glacial cycles, as well as contemporary anthropogenic forces. In analyzing the genetic structuring in this species I took a telescopic approach, beginning with the patterns of variation throughout the species range from a phylogenetic standpoint. Using mitochondrial sequence data I identified three reciprocally monophyletic clades of the cotton mouse, a southeastern, northeaster, and western clade. Among these clades I identified eight distinct subspecies, four of which had previously not been identified using morphological characters. Of the four previously identified subspecies, this study resulted in a restructuring of the range of all except the Key Largo cotton mouse, which is restricted to northern Key Largo Florida. Secondly I

employed phylogeographic methods to examine the genetic patterns of the cotton mouse in a geographic context. Phylogeographic breaks in the cotton mouse are largely consistent with those observed in other taxa distributed throughout the southeastern United States. Geographic structuring in this species shows deep patterns associated with glacial maxima and minima of the Pleistocene period. Many of these patterns are maintained by contemporary geographic barriers to gene flow. Thirdly, I identified geographic structuring of genetic variation at a regional and local level. This structuring is partially due to the cotton mouse's limited dispersal ability but is strengthened and reinforced by naturally occurring contemporary barriers to gene flow and contemporary anthropogenic forces which serve to limit dispersal in this species. Finally, I determined that urbanization has a dramatic negative effect on gene flow and genetic variation in this species on a local scale. Over a twenty year period populations in urban areas experienced a marked decrease in genetic variation while populations in non-urban areas experienced an increase in variation. During this time period, gene flow was effectively cut off among populations that had previously been panmictic. This study demonstrates that small mammal habitat generalists can be affected by both historic and contemporary climatic and geographic events at multiple geographic scales. These effects range from large scale geographic structuring throughout the species' range to fine scale structuring associated with contemporary anthropogenic forces.

This dissertation is dedicated to my family:
My parents, who nurtured and supported my love of science from the start;
My wife, who gave me courage to take this path and helped me find myself on the way;
and my children, who gave me the strength to continue when I didn't think I could.
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TABLE OF CONTENTS

Page

LIST OF FIGURES viii

LIST OF TABLES xi

Chapter

1 INTRODUCTION: FACTORS AFFECTING THE PHYLOGEOGRAPHY OF SPECIES 1

2 INTRASPECIFIC SYSTEMATICS OF THE COTTON MOUSE (*PEROMYSCUS GOSSYPINUS*): GENETICALLY DISTINCT EVOLUTIONARY LINEAGES IN A SPECIES WITH HIGH MORPHOLOGICAL PLASTICITY ... 16

3 INFERRING THE EFFECTS OF BIOGEOGRAPHIC EVENTS ON THE GENETIC VARIATION OF THE COTTON MOUSE (*PEROMYSCUS GOSSYPINUS*): A MTDNA APPROACH 68

4 GENETIC VARIATION WITHIN AND AMONG POPULATIONS OF THE COTTON MOUSE (*PEROMYSCUS GOSSYPINUS*): A NUCLEAR APPROACH .. 130

5 THE EFFECTS OF URBANIZATION ON THE GENETIC VARIATION OF THE COTTON MOUSE (*PEROMYSCUS GOSSYPINUS*) 176

6 GENERAL CONCLUSIONS 228

References 234

Appendix I 261

Appendix II 269

LIST OF FIGURES

Page

CHAPTER 2

Figure 2.1. Species' range of the cotton mouse (*Peromyscus gossypinus*) 51

Figure 2.2. Range map of *Peromyscus gossypinus* with ranges of morphologically identified subspecies demarcated with dashed lines 52

Figure 2.3. Range map of *Peromyscus gossypinus* with ranges of hypothesized subspecies, based on the findings of Boone et al. 1999, demarcated by red lines..... 53

Figure 2.4. Field sites for which genetic data was collected for this study 54

Figure 2.5. Phylogenetic tree of (*Peromyscus gossypinus*) reconstructed from control region sequence data using maximum likelihood, Bayesian, and maximum parsimony methods (major clades collapsed)..... 55

Figure 2.6. Phylogenetic tree of (*Peromyscus gossypinus*) reconstructed from control region sequence data using maximum likelihood, Bayesian, and maximum parsimony methods (major clades expanded) 56

Figure 2.7. Maximum likelihood reconstruction of the western clade 57

Figure 2.8. Majority rule consensus tree reconstruction of the western clade 58

Figure 2.9. Maximum likelihood reconstruction of the northeastern clade 59

Figure 2.10. Majority rule consensus tree reconstruction of the northeastern clade .. 61

Figure 2.11. Maximum likelihood reconstruction of the southeastern clade 63

Figure 2.12. Majority rule consensus tree reconstruction of the southeastern clade .. 64

Figure 2.13. Neighbor joining population tree of (*Peromyscus gossypinus*) based on genotype data from seven unlinked polymorphic microsatellite loci 65

Figure 2.14. 50% consensus neighbor joining population tree of (*Peromyscus gossypinus*) based on genotype data from seven unlinked polymorphic microsatellite loci 66

Figure 2.15. Range map of *Peromyscus gossypinus* with ranges of newly identified subspecies demarcated by red lines 67

CHAPTER 3

Figure 3.1. Field sites for which genetic data was collected for this study 116

Figure 3.2. Phylogenetic tree of (*Peromyscus gossypinus*) reconstructed from control region sequence data using maximum likelihood, Bayesian, and maximum parsimony methods (major clades collapsed)..... 117

Figure 3.3. Phylogenetic tree of (*Peromyscus gossypinus*) reconstructed from control region sequence data using maximum likelihood, Bayesian, and maximum parsimony methods (major clades expanded)..... 118

Figure 3.4. Maximum likelihood reconstruction of the western clade 119

Figure 3.5. Majority rule consensus tree reconstruction of the western clade..... 120

Figure 3.6. Maximum likelihood reconstruction of the northeastern clade..... 121

Figure 3.7. Majority rule consensus tree reconstruction of the northeastern clade 123

Figure 3.8. Maximum likelihood reconstruction of the southeastern clade..... 125

Figure 3.9. Majority rule consensus tree reconstruction of the southeastern clade 126

Figure 3.10. Minimum spanning network of control region haplotypes for populations of *Peromyscus gossypinus* in the western clade..... 127

Figure 3.11. Minimum spanning network of control region haplotypes for populations of *Peromyscus gossypinus* in the northeastern clade 128

Figure 3.12. Minimum spanning network of control region haplotypes for populations of *Peromyscus gossypinus* in the southeastern clade 129

CHAPTER 4

Figure 4.1. Range map of *Peromyscus gossypinus* with ranges of subspecies demarcated in red 170

Figure 4.2. Range of the cotton mouse (*Peromyscus gossypinus*) with red circles indicating populations included in this study..... 171

Figure 4.3. STRUCTURE analysis of all 23 populations included in this study..... 172

Figure 4.4. STRUCTURE analysis of the populations in the southeastern region..... 173

Figure 4.5. STRUCTURE analysis of the populations in the western region 174

Figure 4.6. STRUCTURE analysis of the populations in the northeastern region 175

CHAPTER 5

Figure 5.1. Locations where samples of *Peromyscus gossypinus* were collected for inclusion in this study 216

Figure 5.2. Majority rule consensus tree reconstructed of populations included in this study in both 1988 and 2009 217

Figure 5.3. STRUCTURE analysis of the 13 populations sampled in both 1988 and 2009 as well as four populations sampled in only 1988 218

Figure 5.4. Aerial land use maps of the region of Chatham, GA where samples were collected in both 1988 and 2009 219

Figure 5.5. Aerial land use maps of the region of Camden, GA where samples were collected in both 1988 and 2009 220

Figure 5.6. Aerial land use maps of the region of Glynn, GA where samples were collected in both 1988 and 2009 221

Figure 5.7. Aerial land use maps of the region of Cumberland Island, GA where samples were collected in both 1988 and 2009 222

Figure 5.8. Aerial land use maps of the region of Sapelo Island, GA where samples were collected in both 1988 and 2009 223

Figure 5.9. Aerial land use maps of the region of St. Catherines Island, GA where samples were collected in both 1988 and 2009 224

Figure 5.10. Aerial land use maps of the region of Ossabaw Island, GA where samples were collected in both 1988 and 2009 225

Figure 5.11. Aerial land use maps of the region of Jekyll Island, GA where samples were collected in both 1988 and 2009 226

Figure 5.12. Aerial land use maps of the region of St. Simons Island, GA where samples were collected in both 1988 and 2009 227

LIST OF TABLES

Page

CHAPTER 2

Table 2.1. Historic and current island populations of *Peromyscus gossypinus* 44

Table 2.2. Subspecies of *Peromyscus gossypinus* based on current taxonomy 45

Table 2.3. Analysis of molecular variance (AMOVA) for three partitions 46

Table 2.4. Average Tamura Nei genetic distances within all currently recognized subspecies of *P. gossypinus* as well as *P. leucopus* 47

Table 2.5. Average Tamura Nei genetic distances within all proposed taxonomic groups identified by the topologies of this study, as well as *P. leucopus* 48

Table 2.6. Average Tamura Nei genetic distances between all proposed taxonomic groups identified by topologies in this study 49

CHAPTER 3

Table 3.1. Analysis of molecular variance (AMOVA) for each set of population partitions proposed in this study 102

Table 3.2. Average Tamura Nei genetic distances between and within all geographic regions 103

Table 3.3. Tamura Nei corrected population pairwise genetic distances between all population pairs of *Peromyscus gossypinus* in the southeastern clade 104

Table 3.4. Tamura Nei corrected population pairwise genetic distances between all population pairs of *Peromyscus gossypinus* in the northeastern clade 105

Table 3.5. Tamura Nei corrected population pairwise genetic distances between all population pairs of *Peromyscus gossypinus* in the western clade 107

Table 3.6. Average Tamura Nei genetic distances within populations of *Peromyscus gossypinus* in the southeastern clade 108

Table 3.7. Average Tamura Nei genetic distances within populations of *Peromyscus gossypinus* in the northeastern clade 109

Table 3.8. Average Tamura Nei genetic distances within populations of *Peromyscus gossypinus* in the western clade 110

Table 3.9. Population pairwise F_{ST} between all population pairs of *Peromyscus gossypinus* in the southeastern clade..... 111

Table 3.10. Population pairwise F_{ST} between all population pairs of *Peromyscus gossypinus* in the northeastern clade..... 112

Table 3.11. Population pairwise F_{ST} between all population pairs of *Peromyscus gossypinus* in the western clade 114

Table 3.12. Nucleotide diversity and haplotype diversity for all populations of *Peromyscus gossypinus* included in this study with more than one individual and more than one haplotype 115

CHAPTER 4

Table 4.1. Microsatellite loci utilized as part of this study..... 161

Table 4.2. Number of alleles per locus for 23 populations of *Peromyscus gossypinus* included in this study 162

Table 4.3. Unique alleles identified in individual populations or clades..... 163

Table 4.4. Observed and expected heterozygosity of loci that diverged significantly from Hardy-Weinberg Equilibrium 164

Table 4.5. Populations with loci in linkage disequilibrium after applying a Bonferroni correction 165

Table 4.6. Estimates of within population genetic diversity and inbreeding for 23 populations of *Peromyscus gossypinus* included in this study 166

Table 4.7. Population pairwise F_{ST} among 23 populations of *Peromyscus gossypinus* included in this study. Significant F_{ST} vales are indicated in bold 167

Table 4.8. Estimates of number of migrants per generation among 23 populations of *Peromyscus gossypinus* included in this study 168

Table 4.9. Analysis of Molecular Variance (AMOVA) for 23 populations of *Peromyscus gossypinus*, genotyped at seven microsatellite loci..... 169

CHAPTER 5

Table 5.1. List of populations of *Peromyscus gossypinus* sampled for this study..... 208

Table 5.2. Measures of within population genetic variation based on mitochondrial haplotype data for all populations of *Peromyscus gossypinus* included in this study . 209

Table 5.3. Measures of within population genetic variation based on microsatellite genotype data for all populations of *Peromyscus gossypinus* included in this study .. 210

Table 5.4. Tamura Nei corrected population pairwise genetic distances between all population pairs of *Peromyscus gossypinus* sampled 211

Table 5.5. Population pairwise F_{ST} between all population pairs of *Peromyscus gossypinus* sampled..... 212

Table 5.6. Estimates of number of migrants per generation between all population pairs of *Peromyscus gossypinus* included in this study 213

Table 5.7. Measures of effective population size based on microsatellite genotype data for all populations of *Peromyscus gossypinus* included in this study 214

Table 5.8. Analysis of molecular variance (AMOVA) for all population pairs of *Peromyscus gossypinus* sampled in both 1988 and 2009 215

Chapter One

Introduction:
Factors affecting the Phylogeography of species

A thorough knowledge of the evolutionary history of an organism is essential to understand its behavior, morphology, and ecology. Identifying relationships among closely related taxa provides an evolutionary context for observed similarities and differences in behavior, morphology, and ecology. Yet identifying evolutionary relationships does little to explain how those relationships arose. Combining information on evolutionary relationships obtained from genetic data with climatic and geographic data in phylogeographic studies, makes it possible to identify the processes responsible for the geographic distribution of genealogical lineages (Avise 2000).

Phylogeographic studies provide a framework of information about the geographic distribution of genetic variation within and among populations. This makes it possible to determine what historic and contemporary processes have shaped the current distribution of genetic variation within species. Organisms react to geological and climatic events such as glaciation, island formation, mountain formation, and river formation in a variety of ways depending on various life history traits (DiLeo et al. 2010, Griffiths 2010, Sipe and Brown 2004; Soltis et al. 2006, Stewart et al. 2010, Wallis and Trewick 2009). These responses can include migration, adaptation, speciation, and extinction.

Historically, due to advancing ice sheets and colder temperatures, glaciations frequently force populations of the same species to migrate into separate refugia (Avise 2000, Byrne 2008, Sommer and Nadachowski 2006, Stewart et al. 2010). While isolated from each other, these populations may diverge into unique evolutionary lineages due to

different selective pressures in their new environments. After the ice sheets have receded these organisms often come into secondary contact but are no longer able to interbreed, preventing gene flow between populations. As a result, similar patterns of speciation associated with particular glaciations are observed across a wide range of taxa (Gomez and Lunt 2006, Hewitt 2004, Soltis et al. 2006).

The formation of mountains, rivers, and barrier islands also frequently produce barriers to gene flow among populations of the same species. Mountains can affect populations of a species in a variety of ways. These geological features frequently prevent gene flow between populations because few species are adapted to the high altitude climates associated with migrating across mountains. Likewise, organisms that do adapt to mountainous habitats often become too specialized to migrate into lowland areas and become isolated from other lowland taxa (Arbogast et al. 2001, Galbreath et al. 2009). As a result, many taxa exhibit phylogeographic breaks associated with the formation of mountain ranges (Church et al. 2003, Soltis et al. 2006, Zalewski et al. 2009).

Rivers also frequently serve as phylogeographic breaks for terrestrial species (Brant and Orti 2003, Degner et al. 2010, Soltis et al. 2006). While many avian and aquatic species are not limited by these features, the breadth and current of many rivers can inhibit migration for a range of species (Brandley et al. 2010, Cullingham et al. 2008, Vallinoto et al. 2006). Similarly, the formation of barrier islands, associated with a rise in sea level, has resulted in populations of many terrestrial species becoming isolated from their mainland counterparts (Indorf 2010, Van Zant and Wooten 2007). This commonly results in a founder effect (Frankham 1997), which when coupled with the unique

selective pressures on islands can cause the formation of a unique evolutionary lineage. For these reasons, barrier islands contain many endemic species and subspecies of terrestrial organisms (Boone 1995, Chirhart 2001, Degner et al. 2007).

Most frequently it is some combination of these events that result in the contemporary patterns of genetic variation observed among closely related taxa. While populations of organisms frequently diverge in isolation during periods of glacial maxima, rivers and mountains often serve to prevent secondary contact between these organisms during interglacial periods by acting as barriers to gene flow (Jackson and Austin 2010, Soltis et al. 2006). Further, the contemporary course of rivers is often the result of geographic alterations associated with the movements of polar ice sheets (Carter et al. 2006, McKay and Berg 2008), while their flow is the result of the melting of those ice sheets (Alkama et al. 2008). Finally, the rise in sea level which causes the formation of barrier islands, and island endemic species, is the result of melting glacial ice associated with the end of glacial maxima and the start of interglacial periods (Morgan and Emslie 2010).

While historic events have dramatically altered patterns of genetic variation on a geological time scale, contemporary anthropogenic forces can also have similar impacts, and often alter established patterns. Anthropogenic forces such as global climate change, anthropogenic noise, habitat fragmentation, and urbanization have been shown to alter the population dynamics of many species. In particular, habitat destruction associated with urbanization and agricultural use has been shown to negatively impact population size. Likewise, habitat fragmentation has been shown to alter species composition (Bolger et al. 1991, Constantine et al. 2005), habitat usage (Anderson et al. 2003, Mahan

and O'Connell 2005), and patterns of gene flow for many species in both urban and agricultural settings (Bolger et al. 1997, Mabry and Barrett 2002).

The rise in sea level and global temperature associated with climate change also negatively impacts a variety of taxa. Rise in temperature has resulted in alterations in the distribution of marine (Hofstede et al. 2010), terrestrial (Bickford et al. 2010), and avian species (Feehan et al. 2009). This temperature rise is also associated with reduced habitat availability for many montane species (Galbreath et al. 2009) and in some cases extinction (Thomas et al. 2006). Rise in sea level has resulted in reduced habitat availability for both island endemic organisms (Menon et al. 2010) as well as those dependent on marshes and brackish habitats (Williams et al. 1999). A continued rise in sea level is projected to result in the extinction of many of these organisms (Foufopoulos et al. 2011, LaFever et al. 2007).

Conversely, while human activities can create new barriers to gene flow and negatively impact biodiversity, they can also serve to mitigate the effects of many historic barriers to gene flow. Changes in river flow have been shown to alter the patterns of gene flow among populations (Lada et al. 2008). Damming of waterways for resource management alters the breadth, depth, and current of rivers (Corn 2007). By altering these features, terrestrial organisms that could not previously traverse the river, may be capable of migrating across and establishing gene flow with populations on the other side (Cullingham et al. 2008). Building of land bridges between barrier islands and the mainland has also been shown to promote gene flow between populations (Bond and Jones 2008). Additionally, limited data suggest that railway tunnels through mountains may serve as avenues of migration for some species (Goosem et al. 2001). These along

with other anthropogenic effects mentioned above may alter the patterns of genetic variation within a group of organisms, and cloud the effects of historic barriers to gene flow.

Effects of life history traits

While historic and contemporary events have a large impact on the phylogeography of a species, there is still a great deal of variation in the patterns observed among taxa. Some species exhibit a great deal of structuring on a fine scale, while others exhibit structuring associated with large barriers to gene flow, and still others exhibit no structuring throughout their range. How historic and contemporary events affect the patterns of genetic variation in a species is typically determined by two key life history traits: vagility and habitat specificity.

Vagility is an organism's ability to migrate through a given environment and is usually associated with the size of an organism. Large, highly vagile terrestrial species are less likely to be impacted by geographic barriers to gene flow such as rivers. Because of this, larger species commonly exhibit little to no geographic structuring (Hundertmark et al. 2002, Vila et al. 1999). By contrast, small organisms are typically more limited in their dispersal ability. Because of their size, small terrestrial organisms cannot frequently disperse long distances (Gauffre et al. 2008) and are often incapable of traversing geographic barriers to gene flow such as rivers, mountains, and intracoastal waterways (Álvarez-Castaneda 2010, Brant and Orti 2003, Degner et al. 2007). As a result, these organisms are more likely to exhibit geographic structuring of genetic variation (Soltis et al. 2006).

Although vagility is a major factor affecting an organism's phylogeography, there are examples of large organisms exhibiting structuring associated with barriers to gene flow (Ellsworth et al. 1994, Epps et al. 2005), as well as examples of small organisms exhibiting little structuring (Bell et al. 2010, Shipp-Pennock et al. 2005). This is typically a consequence of an organism's habitat specificity. Habitat specialists are typically tied to a particular habitat due to specific dietary needs or reliance on that habitat type for shelter and or reproduction. By contrast, habitat generalists are capable of utilizing a wide range of habitats and surviving on a wide range of dietary sources.

Habitat specialists are more likely to show geographic structuring of genetic variation than habitat generalists. Because of their specific habitat requirements these organisms are frequently incapable of migrating through areas without suitable habitat and commonly exhibit geographic structuring of genetic variation (Arbogast et al. 2001, Galbreath et al. 2009). Depending on their habitat specificity and the availability of that habitat, structuring can often occur on a much finer scale than for other organisms (Álvarez-Castaneda 2010, Hafner and Smith 2010).

The oldfield mouse (*Peromyscus polionotus*) is a habitat specialist that is dependent on sandy soils and is commonly found on the sand dunes of coastal areas throughout the southeastern United States. This species shows strong geographic structuring, with six recognized subspecies found on the dunes of individual islands along the Florida coast (Degner et al. 2007). Another subspecies, *P. p. niveiventris* exhibits such fine scale geographic structuring along the Atlantic coast of Florida that individuals could be assigned to the beach where they were trapped using Bayesian clustering analysis (Degner et al. 2007).

On a more regional scale, comparative studies of co-distributed specialist and generalist species reveal that generalists are unlikely to exhibit structuring (DiLeo et al. 2010, Sipe and Brown 2004). The masked shrew (*Sorex cinereus*) exhibits little to no evidence of geographic structuring in highly fragmented landscapes, while a co-distributed specialist species shows large amounts of structuring (Sipe and Brown 2004). Two generalist rodents, *Oligoryzomys nigripes* and *Euroryzomys russatus*, in Brazil exhibited no geographic structuring of genetic variation. While a 780 meter altitude gradient was present among sites, there was evidence of high levels of gene flow among all populations (Gonçalves et al. 2009).

While generalists traditionally do not exhibit geographic structuring of genetic diversity, there are examples that indicate this is not always the case. The gray wolf (*Canis lupus*) exhibits strong genetic structure among populations in the northern part of its range (Muñoz-Fuentes et al. 2009, 2010). This structuring is highly localized and can occur well within the distance that a single wolf is capable of dispersing. Further, this structuring is likely driven by a combination of ecological and behavioral factors.

Among other generalist species patterns vary depending on the spatial scale. Range-wide patterns of variation in the white-footed mouse (*Peromyscus leucopus*) demonstrate a clear east-west phylogeographic break along the Indiana-Illinois border where the floral composition of forests changes (Rowe et al. 2006). On a regional scale, *P. leucopus* demonstrates little structuring of genetic variation throughout the mid-Atlantic States with the exception of one population associated with an island in North Carolina (Shipp-Pennock et al. 2005). Finally on a local scale, *P. leucopus* exhibits

patterns of fine scale structuring in urban areas where forested areas are limited (Munshi-South and Kharchenko 2010).

The study species

Recent evidence indicates a need to better understand the phylogeographic patterns of habitat generalist species, particularly small mammal habitat generalists, on both a local and range-wide scale. The cotton mouse (*P. gossypinus*) provides an ideal organism to explore these patterns at multiple levels. *Peromyscus gossypinus* is a medium sized rodent that can be found in a variety of habitats including: seasonally flooded bottomland hardwood forests, mesic and hydric hardwood hammocks, and swamps (Wolfe and Linzey 1977). Organisms of the species are most commonly found in medium age successional habitats and prefer edge habitat to interior (Anderson et al. 2003, Keith and Gaines 2002, Sasso and Gaines 2002). The range of *P. gossypinus* encompasses the majority of the southeastern United States and extends north from Key Largo, Florida, to southern Virginia and southern Illinois and westward to eastern Texas. No populations are found in the Appalachian Mountains (Wolfe and Linzey 1977).

Peromyscus gossypinus is capable of diving and swimming short distances, but is not well adapted for swimming across larger bodies of water (Pournelle 1950). The species is well adapted to climbing, tending to nest in trees in areas prone to flooding (Klein and Layne 1978). It has been captured at heights of 4.6 meters and found nesting 6.1 meters above the ground (Wolfe and Linzey 1977). On land, *P. gossypinus* is capable of dispersing large distances and has an exceptional homing ability (Griffo 1961). Individual cotton mice have been shown to successfully return to their home range from distances up to 824 meters, even after being maintained in a laboratory for 12 weeks

(Griffo 1961). They are primarily nocturnal omnivores and have been described as opportunistic feeders (Wolfe and Linzey 1977). Their diet is highly variable, but chiefly composed of animal matter, with item availability ultimately determining their dietary composition (Calhoun 1941). Such high variability in diet allows *P. gossypinus* to persist in a variety of habitats and climatic conditions.

Systematic of Peromyscus gossypinus

The genus *Peromyscus* belongs to the family Muridae, the most speciose family of rodents, and falls into the subfamily Neotominae, which is made up of the North American rats and mice (Bradley et al. 2007). *Peromyscus* is the most populous mammalian genus in the US and occurs exclusively in North America (Hafner et al. 1998). The systematics of the genus have been the subject of much controversy, with numerous groups being elevated to genera (Carelton 1980) or subgenera (Hooper and Musser 1964) while others have been synonymized within *Peromyscus* (Musser and Carleton 1993, 2005). Recent phylogenetic analysis utilizing the gene cytochrome-b has shown that several currently established genera should be synonymized within *Peromyscus* (Bradley et al. 2007).

Within the *Peromyscus* genus, two widespread species groups have been identified, the deer mice (*P. maniculatus* group), composed of eight species and the white-footed mice (*P. leucopus* group) composed of two species, *P. leucopus* and *P. gossypinus*. The range of *P. gossypinus* overlaps extensively with that of *P. leucopus*, and it is often not possible to distinguish between the species morphologically in areas of sympatry (Linzey et al. 1976, McDaniel et al. 1983). Additionally, hybridization between the two species has been observed in a laboratory setting (Dice 1937, 1940) and a few

hybrids have been identified based on morphology in the field (Barko and Feldhamer 2002, Lovecky et al. 1979).

Several subspecies of *P. gossypinus* have been identified on the basis of variation in size and pelage color (Leconte 1853, Osgood 1909). Hall and Kelson (1959) formally recognized seven subspecies of *P. gossypinus*: *P. g. gossypinus* in the eastern portion of the range extending southward into northern Florida; *P. g. megacephalus* throughout the western part of the range; *P. g. palmarius* in mainland peninsular Florida; *P. g. telmaphilus* in Collier County and mainland Monroe County, Florida; *P. g. restrictus* in Chadwick Beach, Florida; *P. g. anastasae* restricted to Anastasia Island, Florida and Cumberland Island, Georgia; and *P. g. allapaticola* currently restricted to northern Key Largo, Florida. Boone et al. (1993) synonymized *P. g. anastasae* with *P. g. gossypinus* after analyzing allozyme data from the population on Cumberland Island and comparing these data to several populations throughout the species' range. Additionally, the last known specimen of *P. g. restrictus* was collected in 1938, and the subspecies is now classified as extinct (Repenning and Humphrey 1986, Hafner et al. 1998).

Several studies have indicated that morphology is highly variable and a poor indicator of underlying genetic variation in *Peromyscus* species (Bradley et al. 2007, Shipp-Pennock et al. 2005). In studies of pelage color in *P. gossypinus*, Batson (1958) identified seasonal variation within populations, and both Howell (1939) and Schwartz (1952) identified ontogenetic changes. Boone (1995) also reported that laboratory populations of *P. gossypinus* produced offspring with a full range of pelage color, regardless of parental coloration. The same study identified that size variation within the species did not correspond to subspecific boundaries. Rather, variation was clinal with

the smallest organisms located in central Florida populations and increasing in size radially in all directions. Additionally, Boone observed that variation of morphological traits within populations frequently exceeded the variation among populations.

In the only genetic study of the entire species, Boone et al. (1999) identified 12 genetically distinct groups based on data from 40 allozyme loci. None of these groups fit the current morphological subspecific classification. Due to the potential drastic change to the current systematics, and because each population in the study harbored unique alleles, Boone (1995) cautioned against revising the current systematics without the support of DNA sequence data. Clearly pelage color and size are highly plastic characters in *P. gossypinus*, making them insufficient to identify distinct evolutionary lineages within this species. In order to achieve a thorough understanding of the genetic variation within *P. gossypinus* and to accurately identify distinct evolutionary lineages, molecular markers from both the mitochondrial and nuclear genomes are needed.

Research objectives

The main objective of my dissertation was to identify the geographic patterns of genetic variation in *P. gossypinus* and determine how those patterns were affected by natural and anthropogenic forces at three levels: 1) the entire species' range, 2) the geographic range of evolutionary units identified with *P. gossypinus*, and 3) the population. I began in Chapter Two by identifying the evolutionary relationships among populations of *P. gossypinus* throughout the species' range. By comparing samples from throughout the entire range, I evaluated the validity of the five extant subspecies of *P. gossypinus* which were identified on the basis of morphological variation. Because morphology does not accurately represent the underlying variation in this species (Boone

et al. 1999), I examined evolutionary relationships by evaluating sequence data from the mitochondrial control region through maximum parsimony, maximum likelihood, and Bayesian methods. Genetic divergence among identified groups was also evaluated by comparing genetic distance estimates obtained using the Tamura-Nei correction (Tamura and Nei 1993).

In Chapter Three I explored the phylogeography of *P. gossypinus* in order to identify how climatic and geographic forces have shaped relationships within and among evolutionary lineages of this species. The southeastern United States has a rich biogeographic history, having been affected by numerous events including glaciations, island formations, mountain formations, and river formations (Briggs et al. 1974). Further, the region is characterized by a variety of climates, ranging from temperate in the north to tropical in southern Florida (Kurten and Anderson 1980). These climatic and geographic features have had a dramatic effect on much of the biota of this region. As a result, organisms across a wide range of taxa exhibit similar phylogeographic patterns throughout the southeastern United States (Avise 2000, Soltis et al. 2006).

In order to identify which climatic and geographic events were responsible for the patterns of variation observed in *P. gossypinus*, I again employed sequence data from the mitochondrial control region. I utilized molecular divergence dating techniques to estimate the age and time of divergence among the evolutionary lineages of *P. gossypinus*. To evaluate the effects of various events on genetic variation I determined estimates of genetic variation within populations and evolutionary lineages. Estimates of gene flow among populations and evolutionary lineages were obtained using both genetic distance data and Wright's F_{ST} (Wright 1965). Finally, I used several methods of

demographic analyses to determine if any populations or lineages had experienced recent population expansions or contractions.

In Chapter Four I evaluated the effects of contemporary barriers to gene flow and urbanization on the patterns of gene flow and genetic variation within and among populations within evolutionary lineages. Populations were evaluated by comparing genetic variation at seven polymorphic microsatellite loci. I identified the degree of gene flow among populations by measuring F_{ST} between all population pairs, including mainland and island pairs to identify contemporary gene flow not observed in the mitochondrial data. I also estimated the number of migrants per generation between nearby populations. Structuring among populations was identified using Bayesian clustering analysis. Microsatellite data were analyzed to estimate the amount of genetic variation within populations as well to identify evidence of inbreeding within populations. Finally, I applied estimates of observed and expected heterozygosity to identify recent population bottlenecks. These data were compared among populations in urbanized and non-urbanized areas to determine if there was any evidence of an effect of urbanization on genetic variation.

In Chapter Five I evaluated the effect of urbanization on both mainland and island populations on a temporal scale. Traditionally, studies that explore the effects of urbanization on a group of organisms typically utilize population demographic data and mark recapture data (Bolger et al. 1997, Nupp and Swihart 1998). Those that do employ molecular markers often evaluate only contemporary populations and then infer the effects of urbanization from observed patterns (Chiappero et al. 2011, Munshi-South and Kharchenko 2010).

I compared populations from urbanized and non-urbanized areas over a twenty year period utilizing mitochondrial control region sequence data, as well as genotype data from seven polymorphic microsatellite markers. I estimated gene flow among population pairs as well as number of migrants per generation between populations both before and after urbanization occurred. Additionally, I estimated genetic variation within populations at both time points and attempted to identify evidence of inbreeding and outbreeding in all populations. Changes in effective population size were determined using information on linkage disequilibrium and observed and expected heterozygosity. I also applied coalescent methods to measure effective population size in all populations across the time period of the study. Finally, I evaluated differences in population structuring by applying Bayesian clustering analyses to samples collected both before and after the development of urbanized areas.

Similar phylogeographic patterns are often observed across a wide range of taxa at the local, regional, and range-wide level (Avise 2000, Soltis et al. 2006). However, patterns can vary dramatically among taxa based on vagility and habitat specificity. Different combinations of these factors can result in organisms with different degrees of susceptibility to climatic and geographic events. As a result, organisms with similar habitat requirements can exhibit dramatically different phylogeographic patterns (Vila et al. 1999, Muñoz-Fuentes et al. 2009). Because of the complex interactions of life history traits, it is essential to not only identify the patterns of genetic variation within and among taxa, but to also evaluate the causes of those patterns at a range-wide, regional, and local level. Insights we gain from such studies can provide valuable information about the interactions of life history traits for a group of taxa, as well as inform management

conservation decisions for a wide range of taxa. In Chapter Six, I summarized the results of each level of this study, and discussed the implications of this research for both *P. gossypinus* and on a broader scale for small mammal habitat generalists as a whole.

Chapter 2:
Intraspecific systematics of the cotton mouse, *Peromyscus gossypinus*: Genetically distinct evolutionary lineages in a species with high morphological plasticity

Linnaean classification effectively identifies species across a wide range of taxa using morphological characters (Waugh 2007). However, this system fails to accurately classify organisms when cryptic variation exists among organisms (Baker and Bradley 2006, Bickford et al. 2006), or when high morphological variation exists within a single taxonomic unit (Park et al. 2004). Rodent taxa are often misclassified due to high plasticity of morphological traits which are dependent on environmental factors (Renaud and Aufrey 2010, McAllan et al. 2008, Patton and Brylski 1987), making morphology a poor indicator of underlying genetic variation for many rodents (Bonvicino and Moreira 2001, Boone 1995).

Among rodents, *Peromyscus* is among the most speciose and taxonomically contentious genera (Bradley et al. 2007). Taxonomic relationships within this genus frequently have been revised on the basis of morphological differences (Osgood 1909, Hooper 1968, Carleton 1980); and several taxa have been either elevated to new species or genera, or synonymized. Two recent studies developed a genetic phylogeny of *Peromyscus* (Bradley et al. 2007, Miller and Engstrom 2008). Both of these studies concluded that morphology was a poor indicator of underlying genetic variation within this genus.

Species of *Peromyscus* often consist of several morphologically identified subspecies. However, genetic studies have shown that many of these subspecies either do not represent unique evolutionary lineages, or include organisms currently assigned to other subspecies (Lucid and Cook 2006, Riddle et al. 2000, Shipp-Pennock et al. 2005).

Two widespread *Peromyscus* species groups are found in North America, the deer mice (*P. maniculatus* group), composed of eight species and the white-footed mice (*P. leucopus* group), composed of two species. Organisms of these species groups often exist in sympatry, where they are difficult to distinguish on the basis of external morphology (Hall 1981, Feldhamer et al. 1998, Sternberg and Feldhamer 1997). Even though these two species groups are morphologically similar they represent unique evolutionary units with >11% mitochondrial sequence between them (Shipp-Pennock et al. 2005). The similarity between two evolutionarily divergent groups highlights the difficulty of species classification in *Peromyscus*. Variation within species often exceeds variation between species (Boone 1995). Within the *P. maniculatus* group several populations have been classified under the wrong species due to cryptic variation. These incorrect classifications were only later identified using genetic markers (Chirhart 2001).

There are two species in the *P. leucopus* species group: *P. leucopus* and *P. gossypinus*. *Peromyscus leucopus* is found throughout the United States with the exception of the Pacific coastal states and Florida (Lackey et al. 1985). Seventeen subspecies of *P. leucopus* have been identified on the basis of morphology and geography; however, genetic studies have indicated several of these subspecies are invalid (Shipp-Pennock et al. 2005).

The range of *P. leucopus* overlaps extensively with that of *P. gossypinus* (Figure 2.1) and it is often not possible to distinguish between the species morphologically (McDaniel et al. 1983, Wolfe and Linzey 1977). Also, hybridization between the two species has been observed in a laboratory setting (Dice 1937, 1940) and morphologically

intermediate individuals have been identified as hybrids in the field (Barko and Feldhamer 2002, Lovecky et al. 1979).

While studies have explored the genetic basis of *P. leucopus* subspecies, the intraspecific systematics of *P. gossypinus* are poorly characterized. *Peromyscus gossypinus* is a habitat generalist found throughout the southeastern United States (Wolfe and Linzey 1977). Its range extends north from Key Largo, Florida (FL) to southern Virginia and southern Illinois and west from the Atlantic Ocean to eastern Texas. Populations have been identified on twelve barrier islands along the Atlantic coast (see Table 2.1) and no populations have been found in the Appalachian Mountains (Figure 2.2). *Peromyscus gossypinus* utilizes a variety of habitats; but prefers mesic and hydric hardwood hammocks (Ivey 1949, Pournelle and Barrington 1953). The species is semi-aquatic and capable of traversing small areas of open water (Pearson 1953).

Since *P. gossypinus* was first described (LeConte 1853) several morphological subspecies have been recognized on the basis of variation in pelage color and size. Studies have shown both morphology (Boone 1995) and pelage color to be poor criteria for identifying subspecies, with the latter varying both seasonally (Batson 1958) and ontogenetically (Howell 1939, Schwartz 1952). In turn, most morphological subspecies of *P. gossypinus* have been synonymized (Boone et al. 1993, Osgood 1909).Currently six recognized morphological subspecies of *P. gossypinus* exist: 1) *P. g. megacephalus*, 2) *P. g. gossypinus*, 3) *P. g. palmarius*, 4) *P. g. restrictus*, 5) *P. g. telmaphilus*, and 6) *P. g. allapaticola* (Wolfe and Linzey 1977) (Figure 2.2). Of these, the first three are widespread and the last three are restricted to small areas or islands of peninsular Florida.

Additionally, *P. g. restrictus* has been classified as extinct since 1986 (Repenning and Humphrey 1986; Hafner et al. 1998).

While morphology has not resolved the current systematics of *P. gossypinus*, genetic studies are more powerful in identifying unique evolutionary lineages. To date, the only study of intraspecific genetic variation in *P. gossypinus* was conducted by Boone et al. (1999). Utilizing variation at 40 allozyme loci, they determined that the genetic variation was inconsistent with the current subspecific classification of the species. The study identified at least three genetic mainland groups of *P. gossypinus* and as many as nine unique island populations (Figure 2.3). Due to the high number of potential new subspecies and because all populations harbored unique alleles, Boone et al. cautioned against revising the species' systematics without a more thorough genetic analysis.

Based on these data and the lack of resolution provided by both allozyme and morphological analyses, the goal of this study was to utilize DNA sequence and microsatellite genotype data to conduct a thorough analysis of the genetic variation of *P. gossypinus*, and determine the validity of the current intraspecific systematics of the species. To accomplish this, I first developed working definitions of a species and subspecies. A species is a group of populations that is reciprocally monophyletic at mtDNA loci. Additionally, the populations must also exhibit significant divergence of allele frequencies at nuclear loci. A subspecies is a population or group of populations that is significantly divergent at mtDNA loci, regardless of the phylogenetic distinctiveness of the alleles. These definitions correspond to those of evolutionary significant units (ESU) and management units (MU) respectively, set forth by Moritz (1994). These definitions were chosen so that valuable genetic variation within a species

would not be overlooked. This allows a monophyletic group of organisms that is within a larger clade to be identified as a subspecies without the requirement of reciprocal monophyly (Avise 2004). As a result, populations that have diverged from conspecific populations, but have not achieved reciprocal monophyly may still achieve taxonomic status.

Other studies in rodents have identified separate species and subspecies based on divergence at the mitochondrial control region (Castro-Campillo et al. 1999, Indorf 2010, Koh et al. 2010, Shipp-Pennock et al. 2005). In these studies, sequence divergence among subspecies ranges from 1.5-5%, while divergence among closely related species' ranges from 3.5-10%. By using these ranges as a general guideline, and combining them with phylogenetic analysis and the geographic and climatic history of the species' range, valid subspecies will be identified.

Utilizing these criteria, I hypothesized that the current subspecific taxonomy of *P. gossypinus* is inaccurate and does not represent the underlying genetic variation of the species. I predicted that DNA sequence and microsatellite genotype data would identify three monophyletic evolutionary lineages: 1) One clade will form west of the Apalachicola-Chattahoochee-Flint River Basin (ACF); 2) another clade will form east of the ACF and south of the Savannah River; and 3) a third clade will form north of the Savannah River. This prediction is based on patterns observed in other species (Soltis et al. 2006) as well as patterns observed in *P. gossypinus* using allozyme variation (Boone et al. 1999). I also predicted that each of these evolutionary lineages would represent unique subspecies. Additionally, I predicted that island populations will form monophyletic groups within the northeastern and southeastern clades, and be identified as

subspecies based on observed patterns in allozymes (Boone et al. 1999) as well as the islands geographic distance from the mainland. Finally, I predicted that the subspecies on Key Largo, FL, *P. g. allapaticola* does represent a unique evolutionary lineage. This is based on the large geographic distance between the mainland and island, making gene flow between the populations unlikely.

Materials and Methods

Sample collection

I collected tissue samples from a total of 177 *P. gossypinus* from island and mainland populations throughout the Atlantic coastal portion of the species' range (see Appendix 1). A total of six mainland and thirteen island sites were trapped (Figure 2.4). Two to twenty individuals were trapped from each field site with three exceptions. No animals were collected from Anastasia Island, FL; Manasota Key, FL; or Skidaway Island, Georgia (GA) and one animal was trapped on Edisto Island, South Carolina (SC). Animals were trapped using a combination of Sherman and Longworth live traps set in grids in hardwood hammocks. Tissue was collected by removing ~1 cm of the tip of the tail from each mouse with scissors. Samples were stored in 1.5 ml tubes containing a 20% DMSO (6 M NaCl) solution. All sampling methods were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Miami and followed methods approved by the American Society of Mammalogists Animal Care and Use Committee (Gannon et al. 2007).

In addition to field samples, 350 museum specimens included in Boone et al. 1999 were obtained (toe bones and liver) to provide thorough sampling of the entire species' range (Figure 2.4; see Appendix 1). These specimens were collected between

1988 and 1990. All liver samples were provided by the Texas Tech University Genetic Resource Collection (TTU). Toe bones were obtained from the mammal collection of the University of Georgia Museum of Natural History (UGMNH). In total, 527 samples from throughout the species' range were analyzed, including samples from all extant taxonomic lineages: *P. g. allapaticola* (n=20); *P. g. gossypinus* (n=400); *P. g. megacephalus* (n=39); *P. g. palmarius* (n=65); *P. g. telmaphilus* (n=3).

Mitochondrial DNA extraction and fragment sequencing

Whole genomic DNA was extracted from tail tips and liver tissue using a standard ethanol precipitation protocol. Qiagen DNeasy® tissue extraction kits (Qiagen Inc., Valenica, California) were used to extract DNA from toe bones following the user-developed protocol *Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit* available on the Qiagen website (http://www.qiagen.com). A 784bp region of the mitochondrial control region was amplified using polymerase chain reaction (PCR) (Saiki et al. 1988). Primers were designed using Primer3 (Rozen and Skaletsky 2000). The forward primer was G-62F 5'-TATCGTACATTAAATTATATTCCCCTA and the reverse primer was G-914R 5'-TATAATATACCACCAGTGTTAAGTG. The total PCR reaction volume was 10 µl, containing 1x buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 1µM of each primer, 0.25 units of Taq polymerase, and 20ng of template DNA. Sequences were amplified using the following parameters: initial denaturation 95^{o}C (15 min), followed by 30 cycles of denaturation 94^{o}C (0.5 min), annealing 48^{o}C (1 min), extension 72^{o}C (1.5 min), followed by final extension 72^{o}C (10 min).

Amplification was verified by running reactions through a 1% agarose gel containing .001% by volume GelRed. All electrophoreses included a size standard to verify correct fragment amplification. Fragments were purified using ExoSAP-IT enzymes (USB corp) at 95^{o}C (15 min) followed by 64^{o}C (15 min). Fragments were then sequenced using ABI Prism Big Dye Terminator v3.1 ready reaction mix (Applied Biosystems) with the same primers from the initial PCR. Cycle sequencing was performed using the following parameters: initial denaturation 95^{o}C (10 min), followed by 40 cycles of denaturation 95^{o}C (1 min), annealing 50^{o}C (.33 min), and extension 60^{o}C (4 min). Reactions were purified using Sephadex columns (Millipore), dried in a vacuum centrifuge for 45 min (60 ^{o}C), and resuspended in 10µl of HiDi Formamide (Applied Biosystems). Sequences were then run on an ABI 3130xl automated capillary sequencer (Applied Biosystems).

Sequence chromatograms were proofread and edited in BioEdit (Hall 1999). Alignments were also performed in BioEdit using ClustalW (Thompson et al. 1994). Haplotype files of aligned sequences were developed in DNAsp v.5 (Librado and Rozas 2009).

Sequence analysis

In order to test the current systematics of the species, phylogenetic trees were reconstructed using the haplotype file. If a single haplotype was found in multiple populations it was retained once for each population. Gaps in the sequence alignment were coded using FastGap (Borchsenius 2007) prior to tree reconstruction. Control region sequences from *P. maniculatus* (GenBank Accession Number: EU170494) and *P. attwateri* (AF081492) were included as outgroups. *Peromyscus leucopus* (AY540410)

was included as an ingroup to account for *P. leucopus* samples that may have been morphologically misidentified as *P. gossypinus*.

Phylogenetic trees were reconstructed using maximum parsimony, maximum likelihood, and Bayesian analyses. Maximum likelihood and Bayesian methods were performed five times to ensure the most likely topology was returned. Maximum parsimony analysis was performed twice due to the time requirements to run this type of analysis. Parsimony analysis was run using PAUP v4.0b10 (Swofford 2002). Nucleotide sites were treated as unordered, equally weighted, discrete characters with four possible character states: A, T, G, or C. Trees were reconstructed using a heuristic search with tree bisection-reconstruction branch swapping. One hundred bootstrap replicates were completed to identify support for the topology (Felsenstein 1985). All reconstructions were limited to a maximum of 10000 trees.

For Bayesian and maximum likelihood analyses, jModeltest was used to identify the model of evolution that best fit the sequence data (Posada 2008). The model chosen using the Akaike Information Criterion with a 95% confidence interval was the General Time Reversible model with rate variation and a proportion of invariable sites (GTR+I+G), with a gamma shape parameter of 0.276 and a proportion of invariable sites of 0.353. RaxML was used to perform maximum likelihood reconstructions due to accuracy and speed (Stamatakis 2006). Support for the identified topology was again determined using 100 bootstrap replicates, using the rapid bootstrap algorithm in RaxML (Stamatakis et al. 2008). A different randomly chosen starting seed was used for each of the five runs to ensure the most likely tree was returned.

Bayesian analysis was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with the aforementioned GTR+I+G model. The program was run with four simultaneous Markov-chains for ten million generations, sampling trees every 500 generations. Log-likelihood scores versus generation were plotted graphically to identify when the run reached stationary. Based on this, the first three million generations (6000 trees) were discarded as burn in. Posterior probabilities calculated in MrBayes were used to determine support for the tree topology.

Analyses of Molecular Variance (AMOVAs) were performed using the program Arlequin v3.5 to identify the proportions of genetic variation attributable to differences within populations, among populations within subspecies, and among subspecies (Excoffier and Lischer 2010). Three separate AMOVAs were performed. The first partitioned populations based on the boundaries of the five extant recognized subspecies; the second partitioned populations based on the hypothesized lineages identified by Boone et al. (1999); the final AMOVA was performed a posteriori by partitioning populations into subspecies based on the tree reconstruction. While this does not test an explicit a priori hypothesis, it is important to identify how variation is structured within the species, and correctly determine operational taxonomic units within the species. If any of these partitioning schemes accurately identify the subspecific classification, most of the variation will be seen among subspecies, rather than among populations within subspecies.

I calculated average genetic distances within currently recognized subspecies and within subspecies indicated by the tree topologies using the Tamura Nei correction (Tamura and Nei 1993) in the software package MEGA v.4 (Tamura et al. 2007). The

Tamura Nei correction was developed for the mitochondrial control region of mammals, making it the ideal for this study. Genetic distances were calculated between all taxonomic lineages identified by the tree topology. Population pairwise genetic distances were calculated within taxonomic groups and between populations of adjacent taxonomic groups to identify differentiation across taxonomic boundaries.

Genotyping of microsatellite loci

In order for population comparisons using microsatellite markers to be statistically meaningful, a minimum of ten samples must be included from each population (Balloux and Lugon-Moulin 2002). A total of 317 samples were included, with between 10 and 28 samples from each of 23 populations. Included populations were: Dade, FL (n=17); Key Largo, FL (n=20); Merritt Island, FL (n=20); Amelia Island, FL (n=10); Alachua, FL (n=10); Nassau. FL (n=15); St. Johns, FL (n=11); Camden, GA (n=27); Jekyll Island, GA (n=23); St. Simons Island, GA (n=12); Glynn, GA (n=10); Sapelo Island, GA (n=11); Skidaway Island, GA (n=10); Chatham, GA (n=11); Cumberland Island, GA (n=28); Clinch, GA (n=11); St. Catherines Island, GA (n=10); Ossabaw Island, GA (n=11); Hunting Island, SC (n=10); New Hanover, North Carolina (NC) (n=10); Santa Rosa, FL (n=10); Perry, MS (n=10); and Tuscaloosa, AL (n=10). Exact sampling locations are listed in Appendix 1.

Genotypes were collected from each sample for seven polymorphic nuclear microsatellites developed in *P. leucopus* (Schmidt 1999) and *P. maniculatus* (Weber et al. 2009). Microsatellite loci were amplified using PCR with 5'-fluorescent dye-labeled forward primers (Applied Biosystems, Foster City, CA). All reactions were carried out in 10 μl volumes containing 1x PCR Buffer, .4mM (GT15, GT66, GATA70) or .15mM

(AC9, AC19, GT21, TA5GT6) dNTPS, 0.5 units Taq DNA polymerase, 0.2μM (GT15, GT66, GATA70) or 0.4μM (AC9, AC19, GT21, TA5GT6) forward and reverse primers, $MgCl_2$, and dH_2O. A total of 40 ng of DNA was included in each reaction and primers AC9, AC19, and TA5GT6 were multiplexed due to similar protocols in an attempt to reduce the total number of reactions.

All thermal profiles used touchdown PCR programs (Don et al. 1991) with an initial denaturation of 95 ^{o}C for 3 min; followed by 5 cycles of 95 ^{o}C for 30 seconds, highest annealing temperature for 30 seconds, and 72 ^{o}C for 30 seconds; 20 cycles of 95 ^{o}C for 30 seconds, highest annealing temperature (decreased 0.25 ^{o}C per cycle) for 30 seconds, 72 ^{o}C for 30 seconds; and 10 cycles of 95 ^{o}C for 30 seconds, lowest annealing temperature for 30 seconds, and 72 ^{o}C for 30 seconds, followed by a final extension step of 72 ^{o}C for 10 minutes. Samples were then genotyped on an ABI3130 xl automated capillary sequencer (Applied Biosystems). Reactions for loci GT15 and GT66 were run in the same capillary, as were reactions for loci GATA70 and GT21. All loci were scored using the software STRand v2.4.21 (Toonen and Hughes 2001).

Microsatellite genotype analysis

In order to identify congruence between the microsatellite and mtDNA sequence data, distance based neighbor joining trees were reconstructed from the microsatellite genotype data. Genetic distances were calculated using the D_A distance (Nei et al. 1983) because this measure has been shown to have the highest probability of returning the correct tree topology (Takezaki and Nei 2008). Trees were then reconstructed from these distances in the program Populations (Langella 1999). One hundred bootstrap replicates were completed in order to identify statistical support for the topology. A 50% major-rule

consensus tree was constructed in the program MEGA 4.0 to visualize which branches had strong statistical support.

Because mtDNA is maternally inherited and male biased dispersal is common among rodent species (Wolff and Sherman 2007), tests for sex-biased dispersal were performed to attempt to explain potential differences between mtDNA and microsatellite tree topologies. The variance of the corrected assignment index (vAIc) and F_{ST} were calculated independently for each sex and compared in the program FSTAT (Goudet 2002). These values were then compared to those obtained from 10,000 randomized datasets created by the program and a p-value was returned (Goudet et al. 2002). A $p<0.05$ indicated that males were significantly more likely to disperse than females, whereas a value above 0.05 indicated that the sexes dispersed equally. F_{ST} and vAIC were chosen over other measures of sex-biased dispersal because they have been shown to be least sensitive to sampling scheme and the degree of sex-biased dispersal (Goudet et al. 2002).

Results

Phylogenetic Analyses

Of the 399 sequences included in this study 226 represented unique haplotypes with a total of 147 parsimoniously informative sites. By coding with FastGap an additional 36 informative sites were identified. The sequences had a transition to transversion ratio of 3.91, and a GC content of 0.341. The overall nucleotide composition was A: 32.05%, T: 33.93%, G: 11.36%, C: 22.66%.

Maximum likelihood, Bayesian, and maximum parsimony analyses reconstructed the same topology (Figure 2.5, 2.6). Twelve samples from South Carolina that were

morphologically identified as *P. g. gossypinus*, grouped with *P. leucopus* as an outgroup to all *P. gossypinus* samples. Three reciprocally monophyletic major clades were resolved in the topology (Figure 2.5, 2.6). One clade contained all samples west of the Ochlockonee River in the Florida panhandle and the lone sample from Edisto Island, SC (clade A), one contained all individuals from northeastern Florida, Georgia, mainland South Carolina, Hunting Island in SC, and North Carolina (clade B), and the final contained all samples from the majority of peninsular Florida (clade C). Clade A was supported with 88% bootstrap support in the likelihood analysis (BSL), 52% bootstrap support in the maximum parsimony analysis (BSP), and a posterior probability (PP) of 1 in the Bayesian analysis; clade B had a BSL of 70%, a BSP of 65%, and PP of 1; clade C had a BSL of 96%, a BSP of 92%, and PP of 1. A PP of 1 is the highest possible value, and a value of 0.95 or higher for a node indicates that node is well supported (Ronquist and Huelsenbeck 2003).

Several monophyletic groups were identified within the three major clades. All samples west of the Mississippi River formed a well supported monophyletic clade within clade A (BSL: 80%, BSP: 80%, PP: 1.00) (Figure 2.7, 2.8). In clade C the population on Key Largo, FL was monophyletic (BSL: 95%, BSP: 92%, PP: 1.00) (Figure 2.11, 2.12). Several island populations within clade B also formed monophyletic groups (Figure 2.9, 2.10). The monophyly of the Jekyll Island, GA and historic St. Simons Island, GA population was supported in all three analyses (BSL: 80%, BSP: 70%, PP: 1.00). Ossabaw Island, GA and St. Catherines Island, GA formed a monophyletic group (BSL: 90%, BSP: 90%, PP: 1.00). Hunting Island also formed a monophyletic

group within clade C (BSL: 70%, BSP: 67%). The PP for Hunting Island was 0.7 which is not considered strong statistical support.

None of the other island populations (i.e. Skidaway, Sapelo, Amelia, Merritt) formed monophyletic groups. Of interest, however, is the population of St. Simons Island, GA. The samples Boone collected from St. Simons Island in 1988 fell well within a monophyletic group of samples from Jekyll Island. The samples I collected from St. Simons Island in 2008 shared a common haplotype and were not most closely related to samples from Jekyll Island, but rather samples from coastal mainland Georgia and northern Florida.

AMOVA

The AMOVA that partitioned populations based on groups identified by Boone et al. (Figure 2.3) showed that 15.3% of the variation was due to differences among hypothesized subspecies and more than 60% of the variation was attributable to differences among populations within subspecies (Table 2.3). Partitioning populations based on the current taxonomy attributed 50.8% of the variation to differences among recognized subspecies and 33% of the variation to differences among populations within subspecies (see Table 2.3). Finally, by partitioning populations based on subspecies identified from the tree topology, 57.2% of the variation was explained by differences among subspecies and the amount of variation among populations within subspecies decreased to 24.4% (see Table 2.3).

Genetic Distances

Genetic distances within the current recognized subspecies, with the exception of *P. g. telmaphilus* which only included one haplotype, ranged from 0.6% in *P. g. allapaticola* to 3.6% in *P. g. palmarius*. The average within group distances for *P. g. gossypinus*, *P. g. megacephalus*, and *P. g. palmarius* were all higher than that for *P. leucopus* which had an average genetic distance of 2.1% (see Table 2.4). Genetic distances within taxonomic lineages identified by the tree topology ranged from 0.4% in the Hunting Island population to 2.7% for the lineage from peninsular Florida (see Table 2.5).

Genetic distances between unique island populations identified in the topology and their most genetically similar mainland lineage were: 1.8% for Hunting Island, 1.9% for Jekyll and St. Simons Island, and 2.4% for Ossabaw and St. Catherines Island. Among mainland lineages, distances ranged from 3% between the lineage west of the Mississippi River and the lineage east of the Mississippi to 5.6% between the lineage including Georgia and the lineage from peninsular Florida (see Table 2.6).

Microsatellite tree reconstructions

The neighbor joining microsatellite tree produced a topology that was markedly different from that of the mtDNA reconstructions (Figure 2.13, 2.14). None of the three major clades identified by mtDNA sequence data appeared in the microsatellite tree. While all included populations from clade A were found in the same group in this analysis, the group also contained the population from Chatham, GA. This group was most closely related to a group containing many of the island population identified as part of clade B in the mtDNA analysis. This group also contained populations from Dade, FL

and Key Largo, FL which appear most closely related to the population from New Hanover, NC, rather than the one from Merritt Island, FL. Finally, the population from Merritt Island, FL formed a separate group with populations from Cumberland Island, GA and St. Johns, FL. However, only two of the nodes in the microsatellite tree had bootstrap values above 50%, and many had values below 25% (Figure 2.14).

Tests of sex biased dispersal

When each population was treated separately the ratio of vAIC for males (8.14) to females (9.13) was 0.89 which was not significant when compared with the values from 10,000 randomized datasets (p=0.255). The difference between the F_{ST} values for males (0.1047) and females (0.1299) was 0.0252 which was also not significant (p=0.170). To determine if sex-biased dispersal was occurring at the regional level I then partitioned organisms into the three clades identified by the mtDNA phylogeny. In this case the vAIC from males (11.67) to females (13.12) was 0.89, a non-signficant value (p=0.701). Likewise, the difference in F_{ST} between males (0.0348) and females (0.0430) was 0.0082 which was also non-significant (p=0.250).

Discussion

The combination of phylogenetic inference, genetic divergence estimates, and biogeographic data support my hypothesis that the current subspecific taxonomy of *P. gossypinus* does not accurately represent the underlying genetic variation of the species. My prediction that sequence data would identify three monophyletic evolutionary lineages was supported. However, the geographic ranges I predicted for these lineages were not supported (Figures 2.3, 2.15). All individuals north and south of the Savannah

River fell into the same monophyletic group. Instead, a monophyletic group formed containing all individuals from central and southern peninsular Florida. Additionally, my prediction that several island populations would form monophyletic groups was supported. One of these monophyletic groups included all samples from the subspecies on Key Largo, Florida, *P. g. allapaticola*, which strongly supports my prediction that this subspecies does represent a unique evolutionary lineage.

Mitochondrial vs. microsatellite analyses

Bootstrap values (BS) and posterior probabilities (PP) indicate strong support for the three reciprocally monophyletic lineages identified by mtDNA analyses (Figures 2.5, 2.6). However, analysis of the microsatellite data identified little structuring of genetic variation in this study (Figures 2.13, 2.14). The observation of different patterns of genetic structuring among genomes is often the result of sex biased dispersal. Sex biased dispersal is common among mammals, with females typically being the more philopatric sex (Wolff and Sherman 2007). However, statistical tests showed no evidence of sex biased dispersal in this study for either sex.

Alternatively, convergent evolution is a logical explanation for the different geographic patterns observed among genomes in this study. Microsatellites evolve by the loss or addition of short sequence repeats during DNA replication. This process typically occurs in a stepwise manner, with the loss or addition of a single repeat per mutation. As a result, microsatellites are highly prone to convergent evolution which can cause unrelated populations to appear similar. Evidence of convergent evolution is prevalent in the analyses included in this study, with several geographically distant populations appearing closely related to each other without the inclusion of geographically

intermediate populations. The populations of New Hanover, NC and Dade, FL form a closely related group not supported in the mitochondrial phylogeny. However, no populations from Georgia or northern Florida fall into this group. It is highly unlikely that these two populations share a more recent common ancestor than either does with populations from Georgia or Florida. Similarly, the population from Chatham, GA is most closely related to populations from Alabama, Mississippi, and western Florida. Again, no populations from northern Florida or Georgia fall in this group suggesting this relationship is also the result of convergent evolution.

In order to account for the high incidence of convergent evolution among microsatellites, studies indicate that at least thirty microsatellite loci are needed to reconstruct an accurate tree topology among populations (Takazaki and Nei 2008). Because this study employed only seven loci, any reconstruction based on microsatellite genotypes was highly susceptible to convergent evolution. However, because sex biased dispersal is not affecting this species, the mtDNA topology should accurately represent the relationships among populations and subspecies of *P. gossypinus*.

Mainland evolutionary lineages

While the three reciprocally monophyletic lineages identified in this study do not correspond to the lineages I predicted (Figures 2.3, 2.15) they do correspond to patterns seen in other organisms. Genetic breaks have been identified in the ACF for a wide range of taxa, with high levels of divergence occurring across small geographic distances (Soltis et al. 2006). Additionally, genetic breaks have also been identified in peninsular Florida for a variety of species (Avise 2000, Hewitt 2001). It has been hypothesized that

this region represents a suture zone where species that diverged during glacial periods come back into contact (Remington 1968).

Although I predicted that the Savannah River would act as a barrier to gene flow for *P. gossypinus*, the phylogenetic trees indicate that this is not the case (Figures 2.5, 2.6, 2.9, 2.10). This prediction was based on limited evidence from allozyme data (Boone et al. 1999) and patterns observed in non-mammalian taxa (Degner et al. 2010). The Savannah River is quite narrow in places, <50 m, and does not have a strong current. Given the semi-aquatic nature of *P. gossypinus,* it is not surprising that this river does not act as a strong barrier to gene flow.

In addition to the three mainland evolutionary lineages, a well differentiated group was identified within the western lineage, and contained all samples west of the Mississippi River. While the existence of this group was not predicted, the Mississippi River has been shown to represent both a contemporary and historic barrier to gene flow in other mammalian species (Burbrink et al. 2008, Brant and Orti 2003, Hoffman and Blouin 2004). The breadth of the river, 1-2 km in Louisiana, and strong current are sufficient to prevent gene flow for even semi-aquatic small mammals such as, *P. gossypinus*. Given that the populations on either side of the river are well differentiated (3%), the Mississippi River has likely served as a barrier to gene flow for a long time.

Mainland subspecies

I originally predicted that each of the mainland evolutionary lineages represent unique subspecies. Based on the criteria previously described, there are four unique mainland subspecies of *P. gossypinus*. The group of populations west of the Mississippi River forms a strongly supported clade within a larger clade containing all western

populations (Figure 2.7, 2.8). Populations west of the Mississippi are well differentiated from the other three mainland groups (3.0%-6.0%) which is consistent with subspecific level differentiation identified in other studies of rodent taxa. Further, because the Mississippi River is a well established barrier to gene flow for many species, the populations west of the Mississippi River represent an as yet unidentified unique subspecies of *P. gossypinus* which should be identified as the Western cotton mouse.

The populations that fall in the range east of the Mississippi River but west of the Appalachian Mountains, including those in the ACF, also form a well supported group in both the maximum likelihood and Bayesian analyses (Figure 2.5, 2.6). The level of divergence between these populations and the other two reciprocally monophyletic lineages (5.5%) is consistent with levels of divergence between closely related *Peromyscus* species identified in other studies (Castro-Campillo et al. 1999). However, the genetic species concept describes a species as a group of genetically compatible interbreeding natural populations that is genetically isolated from other such groups (Baker and Bradley 2006). While the identification of reciprocal monophyly at mtDNA loci is the first step in identifying genetic species, additional evidence of genetic isolation from nuclear loci is necessary to elevate a phylogroup to specific status. Because there is no evidence from the nuclear genome to support the patterns observed in the mitochondrial data, it is not possible to draw any specific level conclusions about any populations in this study.

The ACF is a well documented historic genetic break for a variety of species (Avise 2000, Soltis et al. 2006). It has been proposed that populations of species inhabited refugia on opposite sides of the ACF during glacial maxima resulting in genetic

divergence. Following the glacial maxima this divergence was maintained via a number of mechanisms, resulting in a widespread genetic break in this area. Because *P. gossypinus* also shows a genetic break in this region, populations from this area west to the Mississippi River represent a valid subspecies that should be known as *Peromyscus gossypinus megacephalus*.

The reciprocally monophyletic clade containing all animals from southern peninsular Florida is strongly supported by all three topologies (Figure 2.5, 2.6). This clade is the most highly diverged of the three with average genetic distances of 5.5% from *P. g. megacephalus*, and 5.6% from the northeastern clade. While there are no barriers to gene flow sufficient to explain the divergence between this clade and the northeastern clade, the area where the clades diverge corresponds to Remington's suture zone (Avise 2000, Remington 1968). This suture zone has been hypothesized as an area of secondary contact for more than 50 terrestrial species, resulting from populations being isolated on the Florida peninsula and diverging during glacial maxima and minima (Hewitt 2001, Swenson and Howard 2005, Morgan and Emslie 2010). As a result, the clade containing all populations from southern peninsular Florida represents a unique subspecies known as *Peromyscus gossypinus palmarius*.

The final reciprocally monophyletic clade also exhibits strong statistical support in the three topologies (Figure 2.5, 2.6). This clade contains all remaining populations east of the ACF and the Appalachian Mountains, including populations north and south of the Savannah River. As previously indicated this group is well differentiated from *P. g. megacephalus* (5.5%) and *P. g. palmarius* (5.6%). This level of differentiation is consistent with specific level identification, but because species should not be identified

without support from multiple genomes, this group of populations will be known as the subspecies *Peromyscus gossypinus gossypinus.*

Island subspecies of P. gossypinus

Among island populations of *P. gossypinus*, four groups of islands (Hunting Island, Jekyll Island/St. Simons Island, Ossabaw Island/St. Catherines Island, and Key Largo) formed well supported monophyletic groups (Figures 2.9, 2.10, 2.11, 2.12). Additionally, each of these island groups was sufficiently divergent from its nearest mainland subspecies to warrant subspecific classification. These data support my predictions that several island populations represent unique subspecies and that the population from Key Largo, FL represents a valid subspecies and should retain its taxonomic status.

The populations on St. Catherines Island and Ossabaw Island, GA combine to form a strongly supported monophyletic clade in all topologies (Figure 2.9, 2.10). These populations are well differentiated from the mainland group of *P. g. gossypinus* (2.5%) indicating a lack of current or recent gene flow between the islands and the mainland. The minimum distance between St. Catherines Island and the mainland is 6 km and for Ossabaw Island and the mainland is 6.5 km. Although *P. gossypinus* is semiaquatic, these distances are too large for the species to traverse, especially considering that much of the area is salt water marsh and open salt water. This explains the large differentiation between the islands and the mainland, and supports these populations as an unidentified insular subspecies of *Peromyscus gossypinus* restricted to St. Catherines and Ossabaw islands and should be known as the Ossabaw Island cotton mouse.

Tree topologies strongly support the monophyly of the population from Jekyll Island, GA and the population from St. Simons Island, GA sampled in 1988 (Figure 2.9, 2.10). However, the population of St. Simons Island sampled in 2009 does not fall within this monophyletic group. Based on these data, either the population of St. Simons Island has dramatically diverged in the last 20 years, or *P. gossypinus* has experienced an extirpation on this island followed by recolonization in that same time period.

The Jekyll Island population exhibits subspecific level divergence from the mainland populations of *P. g. gossypinus* (1.9%). This level of differentiation indicates a lack of contemporary gene flow between Jekyll Island and the mainland. While this island is separated from the mainland by 3 km of marsh and open water, there is a narrow land bridge that connects the two. These data suggest that the presence of a land bridge is insufficient to promote gene flow between these populations on its own. In fact, much of this land bridge lacks vegetation, making migrating *P. gossypinus* susceptible to predators. In order for *P. gossypinus* to successfully migrate between island and mainland populations, an appropriate habitat type must be available. Due to this lack of appropriate habitat, the *P. gossypinus* on Jekyll Island have diverged to represent a unique undescribed subspecies of *Peromyscus gossypinus*, the Jekyll Island cotton mouse.

The population of Hunting Island, SC presents a similar situation. Hunting Island is separated from the mainland by 4 km, but this distance is covered by a series of land bridges, marshes, and islands. However, this population forms a monophyletic group that exhibits subspecific level divergence when compared to the mainland populations of *P. g. gossypinus* (1.8%), and the monophyly of this population is supported in both the maximum likelihood and maximum parsimony analyses. Based on these data, the

population of Hunting Island, SC also represents a unique undescribed subspecies of *Peromyscus gossypinus*, the Hunting Island cotton mouse.

The final unique island population is found on Key Largo, FL. This population formed a monophyletic group in all three topologies with the highest statistical support of any island population. Similarly, the population of Key Largo is the most differentiated of all island populations when compared to its nearest mainland subspecies, *P. g. palmarius* (2.8%). This high level of differentiation is consistent with the geography of the area. Key Largo is separated from its nearest mainland population in Dade, FL by several kilometers of open water and marsh. If gene flow were occurring between these populations the expected genetic distance would be much smaller given that both populations are well sampled. Based on these findings the Key Largo population should retain its subspecific status under the name *P. g. allapaticola*, and should retain its listing under the United States Endangered Species Act (Hafner et al. 1998) until a thorough study of the population level processes of this subspecies is completed. Information on all subspecies' ranges and holotypes for undescribed subspecies of *P. gossypinus* can be found in Appendix 2. Formal taxonomic names for undescribed subspecies are awaiting morphological evaluation of museum specimens.

While they are not new subspecies, two other populations show interesting patterns. Samples collected from Amelia Island, FL in 1988 form a monophyletic clade that is genetically distinct from other *P. gossypinus* samples (Figure 2.9, 2.10). However, samples collected from this island in 2009 do not fall into this clade and actually group with mainland samples of *P. g. gossypinus*. This may indicate a similar extirpation and recolonization to that hypothesized for St. Simons Island, GA. Both of these potential

extirpation events can be verified by using microsatellite markers to look at population level processes.

Although the extinct subspecies, *P. g. restrictus*, was not included in this study, the result presented here may provide insight into its taxonomic status. This subspecies was restricted to the southern portion of Manasota Key, FL which is separated from the mainland by 0.1 km of water. This is consistent with the geographic distance between the Amelia Island, Anastasia Island, Merritt Island, and their respective mainland areas. All three of these populations have been shown to not represent distinct subspecies (Boone et al. 1993). This suggests that the semi-aquatic nature of the cotton mouse permits it to traverse short intracoastal waterways. Likewise, *P. g. restrictus* was designated on variation in pelage color which has been shown to be a poor indicator of underlying genetic variation in *P. gossypinus*. Based on this information, it is likely that *P. g. restrictus* never represented a distinct subspecies of *P. gossypinus*. However, this suggestion is simply a hypothesis, and genetic analyses of museum specimens of *P. g. restrictus* are necessary to determine its taxonomic status. Regardless, trapping efforts associated with this study verified that the population of Manasota Key is extinct.

The sample collected from Edisto Island, SC falls within the *P. g. megacephalus* subspecies in all three analyses (Figure 2.7, 2.8). Because this population is represented by a single sample, it is difficult to determine the validity of its placement in the phylogenetic tree. If additional sampling supports this population's placement in the tree, then it represents a unique island subspecies of *P. g. megacephalus*. If this is true, the population is likely a vicariant population of *P. megacephalus* that became isolated on

this island. However, additional sampling of this island and analyses are needed to verify this possibility.

Conclusion

This study demonstrates the utility of genetic markers for resolving evolutionary relationships when high plasticity exists for morphological traits. Of the eight subspecies of *P. gossypinus* identified in this study, only four had previously been identified on the basis of morphological variation. Among those four subspecies, this study resulted in a dramatic restructuring of the geographic range of three of them. The only subspecific range that remained unchanged was that of *P. g. allapaticola*, an island endemic subspecies. Additionally, the population of Edisto Island, SC may still prove to be an unnamed subspecies, which would bring the total to nine subspecies of *P. gossypinus*.

Within this species, several populations and groups of populations appear to be in flux. Nuclear sequence data is necessary to determine whether *P. gossypinus* represents one species with many subspecies, or three unique divergent species. Similarly, it is necessary to determine what forces have led to the rapid differentiation of populations on Amelia Island and St. Simons Island in a twenty year period. We must also determine why some island populations maintain genetic similarity with the mainland in the absence of identifiable paths of gene flow, while islands in closer geographic proximity contain unique subspecies. Given the high level of genetic variation in this species, and the fact that many of these islands are inhabited by humans, *P. gossypinus* may serve as a model for the study of speciation in rodents, as well as the effects of human development on genetic variation.

It is still unclear what historic and contemporary forces led to the formation of multiple mainland subspecies and why some island populations have developed into unique subspecies while others have not. Developing a more thorough understanding of how historic geological and climatic events have shaped the current patterns of variation within this species may provide valuable information about the ecology and life history of this species as well as that of other small mammal habitat generalists. This information may help researchers determine how species will react to future climatic events such as rise in sea level and global warming.

By identifying the intraspecific systematics of *P. gossypinus* this study has opened the door for studies of the effects of both natural and anthropogenic processes on genetic variation in small mammals. By combining the results of this study with others on the population dynamics of *P. gossypinus* it will be possible to gain greater insight into the species' ecology, morphology, and behavior (Avise 2000). As such, the information collected in this study will be valuable to researchers in a variety of fields, and may provide information about how other widespread small mammals evolve in the southeastern United States.

Table 2.1. List of historic and current island populations of *Peromyscus gossypinus*, including island size, distance to the mainland, and the taxonomic and conservation status of the population on the island, as well as any recommended changed made by this study.

Island Name	Size of Island (km^2)	Shortest distance from mainland (km)	Taxonomic or conservation status	Current recommendation
Amelia Island, FL	47.1	1.96	N/A	Island population of *P. g. gossypinus*
Anastasia Island, FL	43	1.05	*P. anastasae* (Bangs 1896), synonymized with *P. g. anastasae* (1909), Synonymized with *P. g. gossypinus* (1993), Extinct (1949)	Extinct population Confirmed 1993/2009
Cumberland Island, GA	147.37	3.3	*P. insulanis* (Bangs 1896), synonymized with *P. g. anastasae* (1909), synonymized with *P. g. gossypinus* (1993).	N/A
Edisto Island, SC	175.1	6.3	N/A	Possible unique subspecies. Additional sampling needed.
Hunting Island, SC	20.23	4.75	N/A	Elevate to subspecies, Hunting Island cotton mouse
Jekyll Island, GA	18	6.63	N/A	Elevate to subspecies, Jekyll Island cotton mouse
Key Largo, FL	77	19.5	*P. g. allapaticola* (Schwartz 1952). Federally endangered (1983). Recommended synonymize with *P. g. palmarius* (1999)	Maintain as subspecies, *P. g. allapaticola*. Retain endangered classification.
Manasota Key, FL	2.8	0.1	*P. g. restrictus* (Howell 1939). Extinct (1992).	Extinct population Confirmed 2009
Merritt Island, FL	45.7	0.06	N/A	N/A
Ossabaw Island, GA	36	5.9	N/A	Elevate to subspecies, Ossabaw Island cotton mouse
St. Catherines Island, GA	59	6.72	N/A	Elevate to subspecies, Ossabaw Island cotton mouse
St. Simons Island, GA	16.6	4.6	N/A	Island population of *P. g. gossypinus*.
Sapelo Island, GA	64.77	7.69	N/A	N/A
Skidaway Island, GA	42.4	0.75	N/A	Possible population reduction or extirpation. No animals trapped in 800 trap nights over two seasons.

Table 2.2. Subspecies of *Peromyscus gossypinus* based on current taxonomy.

Subspecies	Type locality	First described/ Revised	Range	Distinguishing characters	Taxonomic status
P. g. allapaticola	Key Largo, FL	Schwartz 1952	Northern Key Largo, FL	Larger than *P. g. palmarius*, reddish	Recommended synonymized/ Subspecies
P. g. anastasae	Anastasia Island, FL	Bangs 1896/ Osgood 1909/ Boone 1993	Anastasia Island, FL/ Cumberland Island, GA	Smaller than *P. g. gossypinus*, pale coloration	Synonymized/ Extinct
P. g. gossypinus	Riceboro, GA	Leconte/ Osgood 1909	Virginia south to northern Florida, southern Alabama, southern Mississippi, southeastern Louisiana	Darker and less yellow than *P. g. palmarius*	Subspecies
P. g. megacephalus	Woodville, AL	Rhoads 1894/ Osgoos 1909-	Eastern Texas, Oklahoma, Arkansas, Missouri, southern Illinois, Tennessee, northwestern Louisiana, northern Mississippi, northern Alabama, northwestern Georgia	Larger and paler than *P. g. gossypinus*	Subspecies
P. g. palmarius	Oak Lodge, FL	Bangs 1896	Peninsular FL, south out St. Augustine in the East, south of Cedar Key in the West	Paler than *P. g. gossypinus*, smaller hind foot	Subspecies
P. g. restrictus	Manasota Key, FL	Howell 1939	Manasota Key, FL	Smaller than *P. palmarius*, pale coloration	Extinct
P. g. telmaphilus	Royal Palm Hammock, Monroe, FL	Schwartz 1952	Collier and mainland Monroe, FL	Smaller than *P. g. palmarius*, brown	Subspecies

Table 2.3 Analysis of molecular variance (AMOVA) for each set of population partitions.

A. partitioned according to hypothesized subspecies of Boone et al. 1999.
B. partitioned based on current subspecific taxonomy
C. partitioned based on tree topologies

A)

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance
Among subspecies	8	1953.866	2.07084 Va	15.32
Among populations within subspecies	41	2745.639	8.48712 Vb	62.78
Within populations	427	1264.404	2.96113 Vc	21.90
Total	476	5963.910	13.51910	

B)

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance
Among subspecies	4	2093.387	9.29098 Va	50.81
Among populations within subspecies	45	2606.118	6.03375 Vb	33.00
Within populations	427	1264.404	2.96113 Vc	16.19
Total	476	5963.910	18.28587	

C)

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance
Among subspecies	7	3220.095	9.16854 Va	57.16
Among populations within subspecies	42	1479.410	3.91085 Vb	24.38
Within populations	427	1264.404	2.96113 Vc	18.46
Total	478	5963.910	16.04052	

Table 2.4. Average Tamura Nei (Tamura and Nei 1983) genetic distances, reported as percent divergence with standard error (computed by 5000 bootstrap replicates), within all currently recognized subspecies of *Peromyscus gossypinus* as well as *P. leucopus*.

Taxa	**Genetic Distance**
P. g. allapaticola	0.6% ± 0.2%
P. g. gossypinus	2.2% ± 0.3%
P. g. megacephalus	2.5% ± 0.4%
P. g. palmarius	3.6% ± 0.5%
P. g. telmaphilus	0.0% ± 0.0%
P. leucopus	2.1% ± 0.4%

Table 2.5 Average Tamura Nei (Tamura and Nei 1983) genetic distances, reported as percent divergence with standard error (computed by 5000 bootstrap replicates), within all proposed taxonomic groups identified by the topologies of this study, as well as *P. leucopus*.

Taxa	**Genetic Distance**
P. g. allapaticola	0.6% ± 0.2%
Western cotton mouse	2.3% ± 0.5%
P. g. gossypinus	1.8% ± 0.3%
Hunting Island cotton mouse	0.4% ± 0.2%
Jekyll Island cotton mouse	0.5% ± 0.2%
P. g. megacephalus	1.4% ± 0.3%
Ossabaw Island cotton mouse	0.8% ± 0.3%
P. g. palmarius	2.7% ± 0.5%
P. leucopus	2.1% ± 0.4%

Table 2.6. Average Tamura Nei (Tamura and Nei 1983) genetic distances, reported as percent divergence with standard error (computed by 5000 bootstrap replicates), between all proposed taxonomic groups.

Taxa		Genetic Distance
P. g. allapaticola –	Western cotton mouse	6.8% ± 1.2%
	P. g. gossypinus	6.1% ± 1.0%
	Hunting Island cotton mouse	6.3% ± 1.3%
	Jekyll Island cotton	5.5% ± 1.1%
	P. g. megacephalus	6.7% ± 1.3%
	P. g. palmarius	2.8% ± 0.5%
	Ossabaw Island cotton mouse	6.3% ± 1.2%
Western cotton mouse –	*P. g. gossypinus*	5.0% ± 0.9%
	Hunting Island cotton mouse	4.4% ± 0.9%
	Jekyll Island cotton mouse	5.0% ± 1.0%
	P. g. megacephalus	3.0% ± 0.6%
	P. g. palmarius	6.0% ± 0.9%
	Ossabaw Island cotton mouse	5.1% ± 1.0%
P. g. gossypinus –	*Hunting Island cotton mouse*	1.8% ± 0.4%
	Jekyll Island cotton mouse	1.9% ± 0.4%
	P. g. megacephalus	5.5% ± 1.0%
	P. g. palmarius	5.6% ± 0.9%
	Ossabaw Island cotton mouse	2.4% ± 0.5%
Hunting Island cotton mouse –	Jekyll Island cotton mouse	1.5% ± 0.4%
	P. g. megacephalus	4.3% ± 0.9%
	P. g. palmarius	5.3% ± 1.0%
	Ossabaw Island cotton mouse	1.9% ± 0.5%

Jekyll Island cotton mouse-	*P. g. megacephalus*	4.0% ± 0.7%
	P. g. palmarius	4.7% ± 0.9%
	Ossabaw Island cotton mouse	1.7% ± 0.5%
P. g. megacephalus-	*P. g. palmarius*	5.5% ± 1.1%
	Ossabaw Island cotton mouse	3.5% ± 0.7%
P. g. palmarius-	Ossabaw Island cotton mouse	5.2% ± 0.9%
P. leucopus–	*P. g. allapaticola*	11.5% ± 2.0%
	Western cotton mouse	10.8% ± 1.8%
	P. g. gossypinus	11.4% ± 2.0%
	Hunting Island cotton mouse	11.4% ± 2.0%
	Jekyll Island cotton mouse	10.9% ± 2.0%
	P. g. megacephalus	11.8% ± 1.9%
	P. g. palmarius	10.6% ± 1.7%
	Ossabaw Island cotton mouse	10.6% ± 1.9%

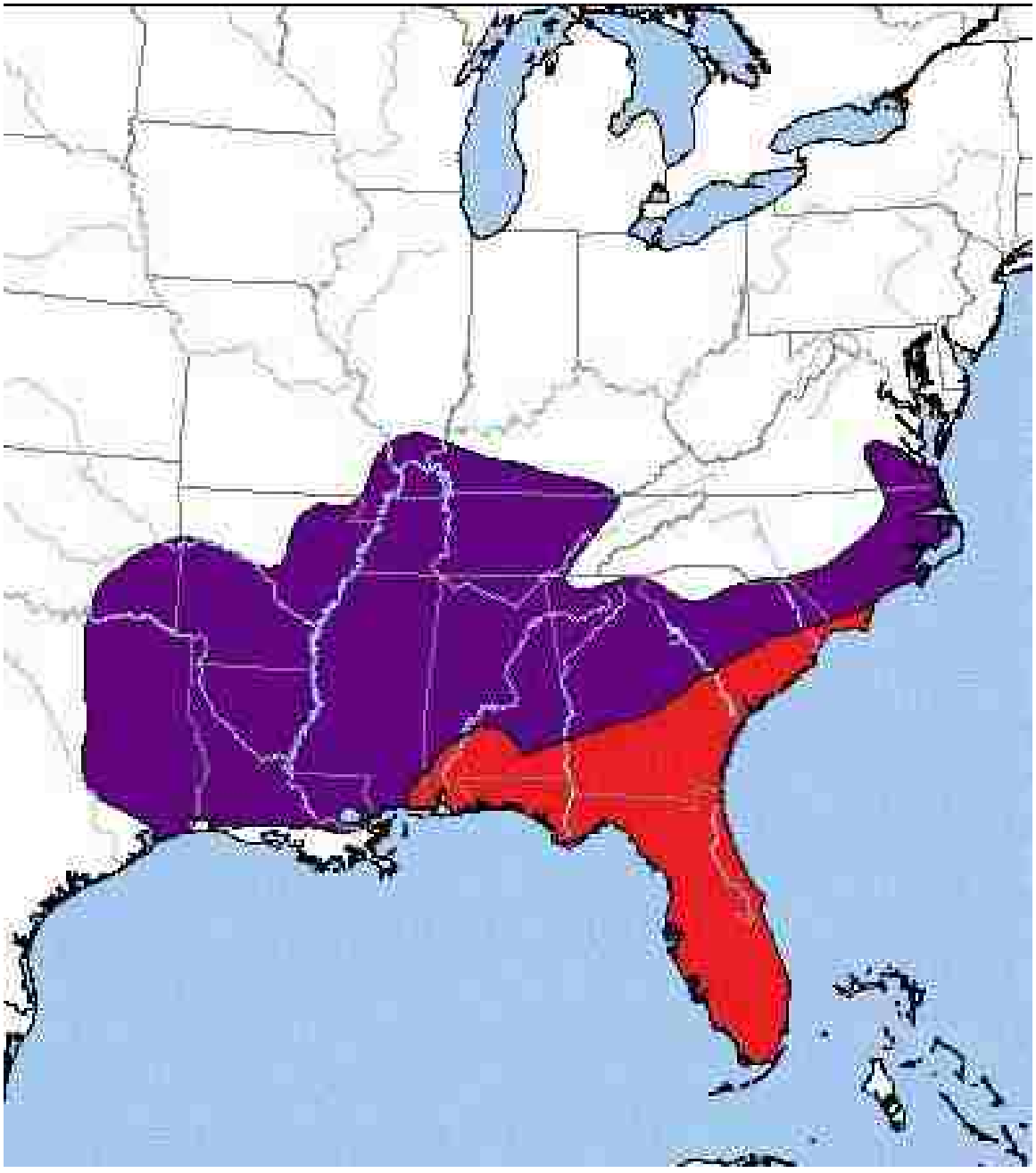


Figure 2.1. Species' range of the cotton mouse, *Peromyscus gossypinus*, with areas of sympatry with *P. leucopus* highlighted in purple, and areas of allopatry in red.

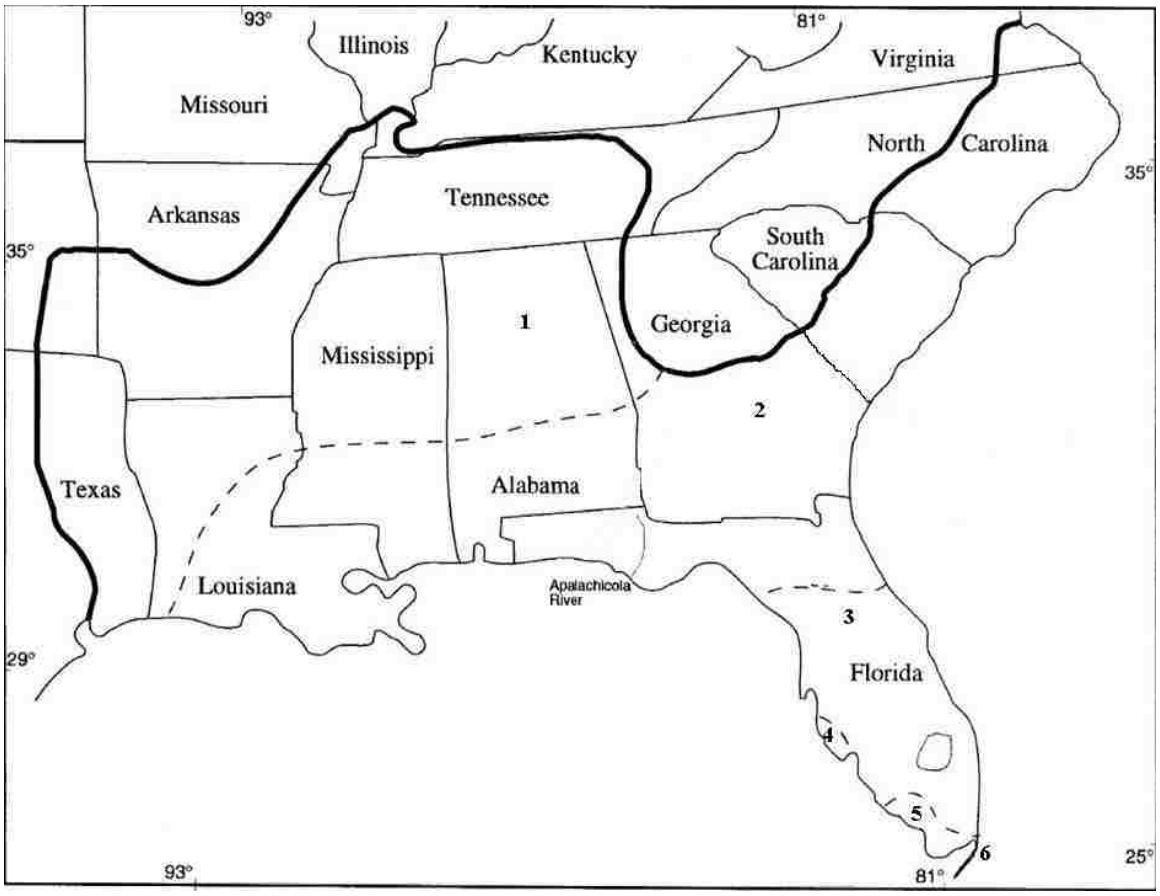


Figure 2.2 Range map of *Peromyscus gossypinus* with ranges of individual subspecies demarcated by dashed lines. 1) *P.g. megacephalus*, 2) *P. g. gossypinus*, 3) *P. g. palmarius*, 4) *P. g. restrictus* (extinct), 5) *P. g. telmaphilus*, 6) *P. g. allapaticola*.

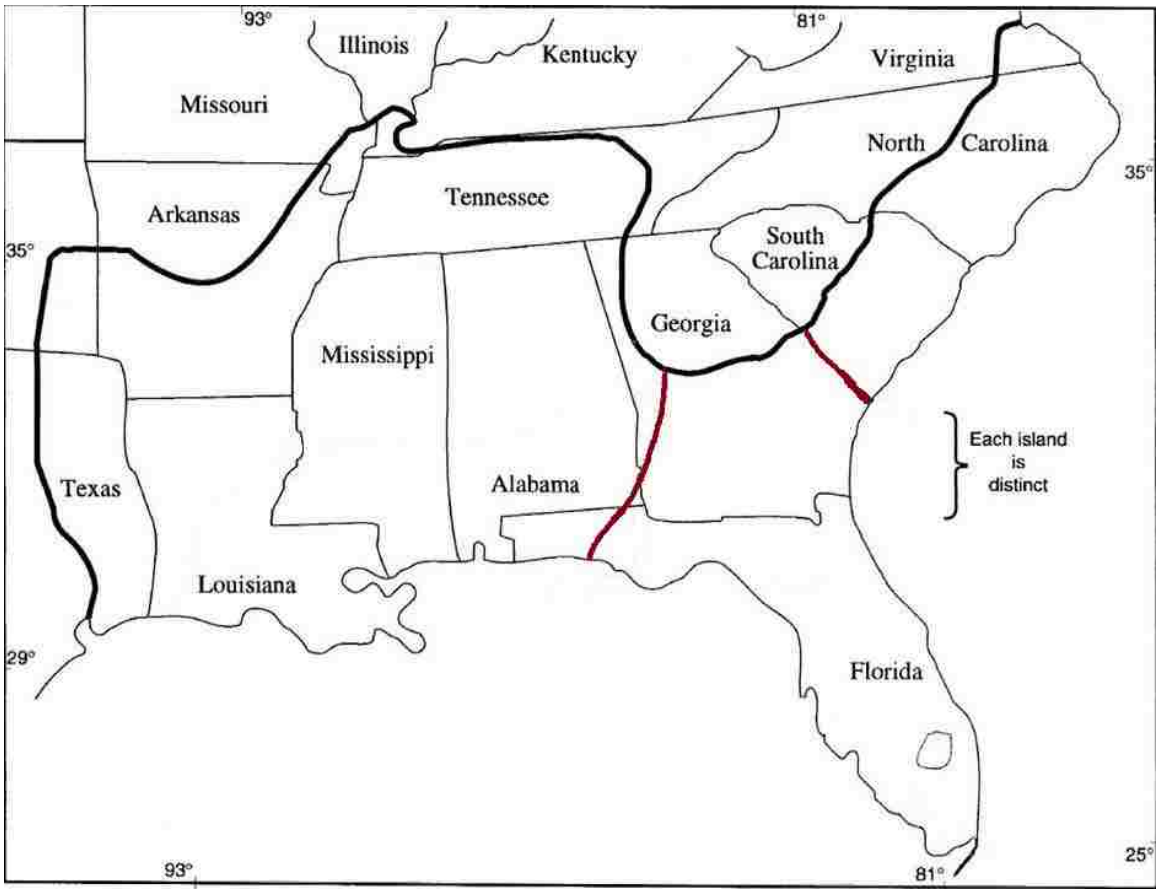


Figure 2.3 Range map of *Peromyscus gossypinus* with ranges of hypothesized subspecies, based on the findings of Boone et al. 1999, demarcated by red lines. Three mainland subspecies were hypothesized. Additionally, nine island populations from coastal South Carolina, Georgia, and Florida were also hypothesized to be unique subspecies (from north to south): Edisto Island, SC; Hunting Island, SC; Ossabaw Island, GA; St. Catherines Island, GA; Sapelo Island, GA; St. Simons Island, GA; Jekyll Island, GA; Cumberland Island, GA; and Amelia Island, FL.

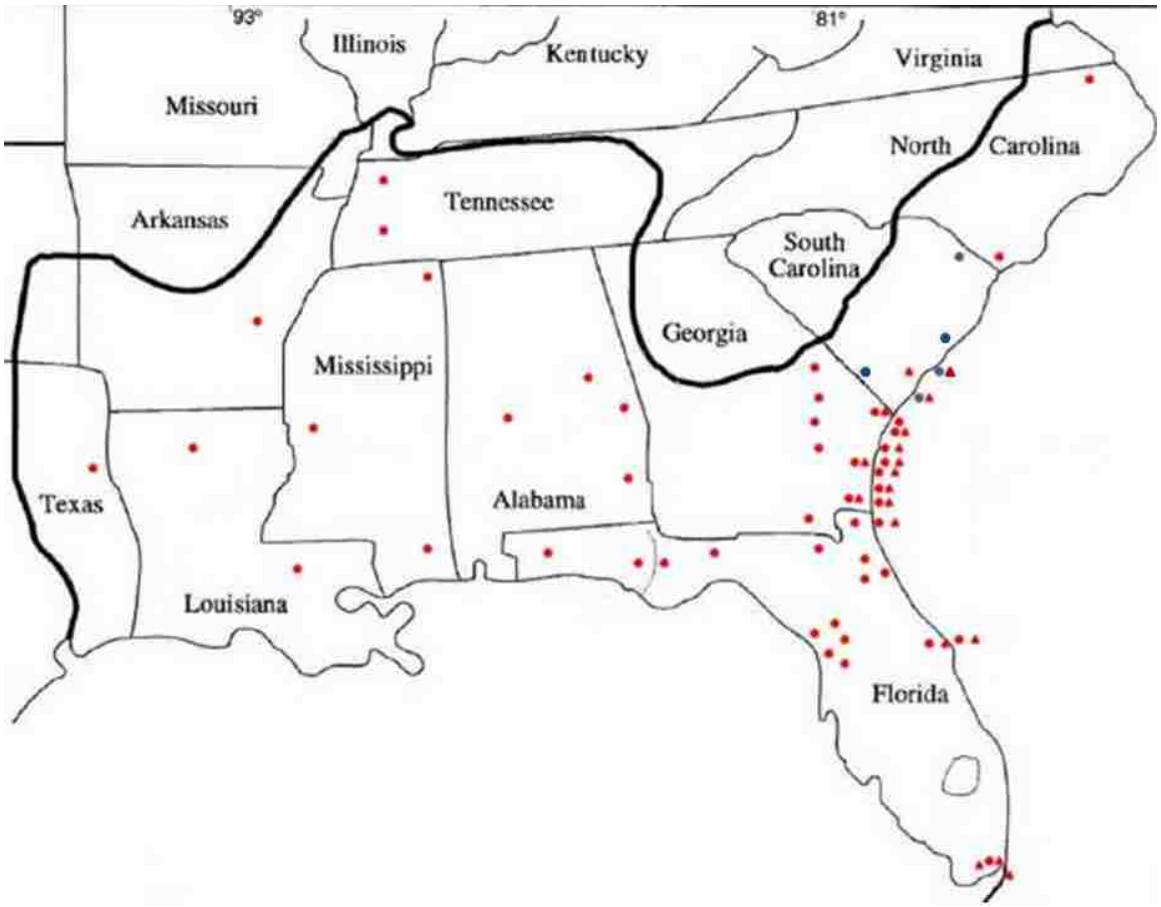


Figure 2.4. Field sites for which genetic data was collected for this study. Circles represent sites of museum samples, triangles represent samples trapped between 2008 and 2010. If both a circle and square are at a site, then both contemporary and museum specimens were collected from that site. Blue indicates samples genetically confirmed as *P. leucopus* and red indicates samples genetically confirmed as *P. gossypinus*.

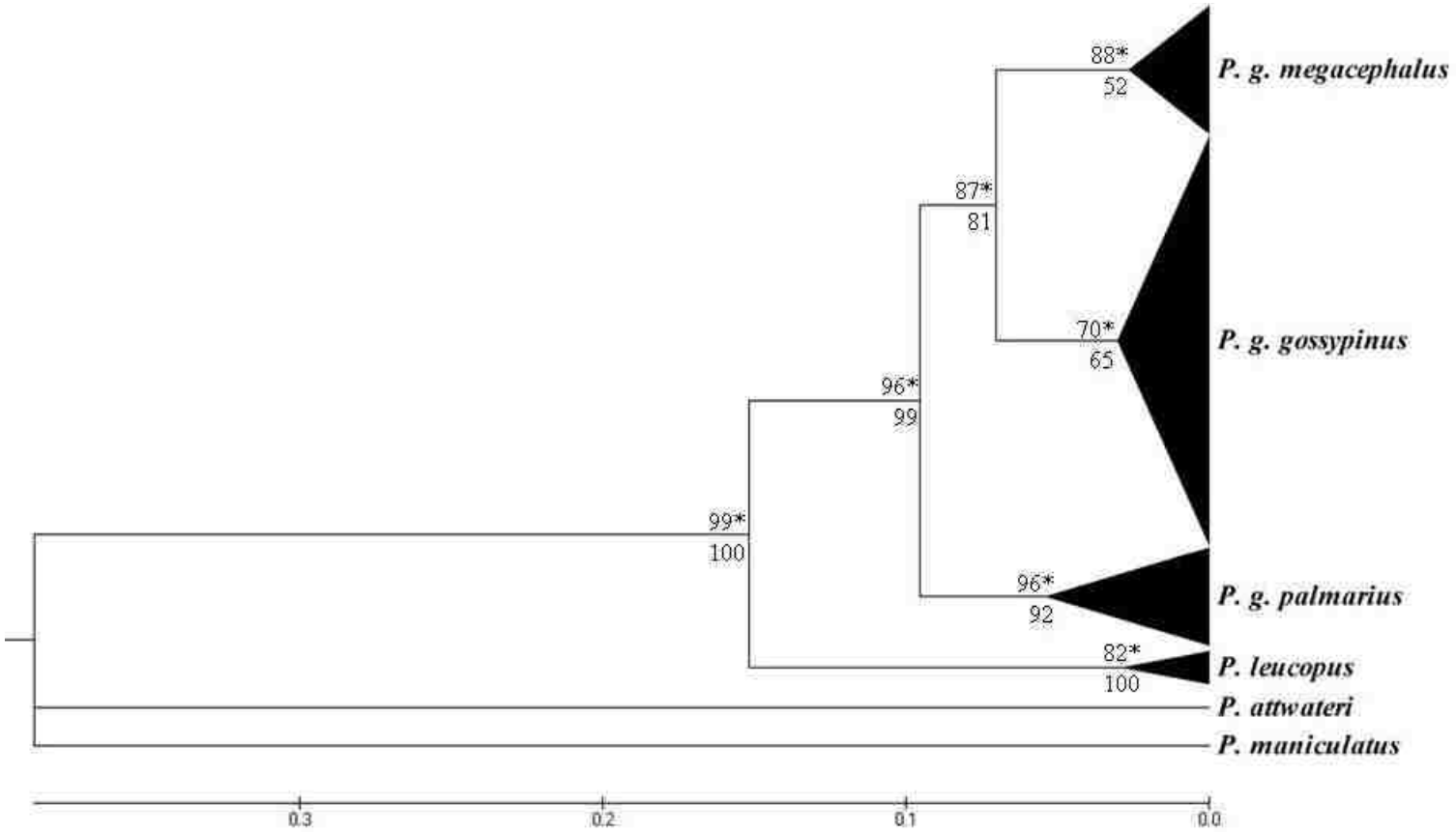


Figure 2.5. Phylogenetic tree of *Peromyscus gossypinus* reconstructed using maximum likelihood, Bayesian, and maximum parsimony methods. *Peromyscus leucopus, P. maniculatus,* and *P. attwateri* were included as outgroups. Maximum likelihood bootstrap values are above the line and maximum parsimony values are below the line if above 50%. A star next to the likelihood bootstrap value indicates a posterior probability of 0.95 or greater in the Bayesian analysis. All three trees returned the same topology and all major clades were collapsed in order to see the overall relationship of the tree.

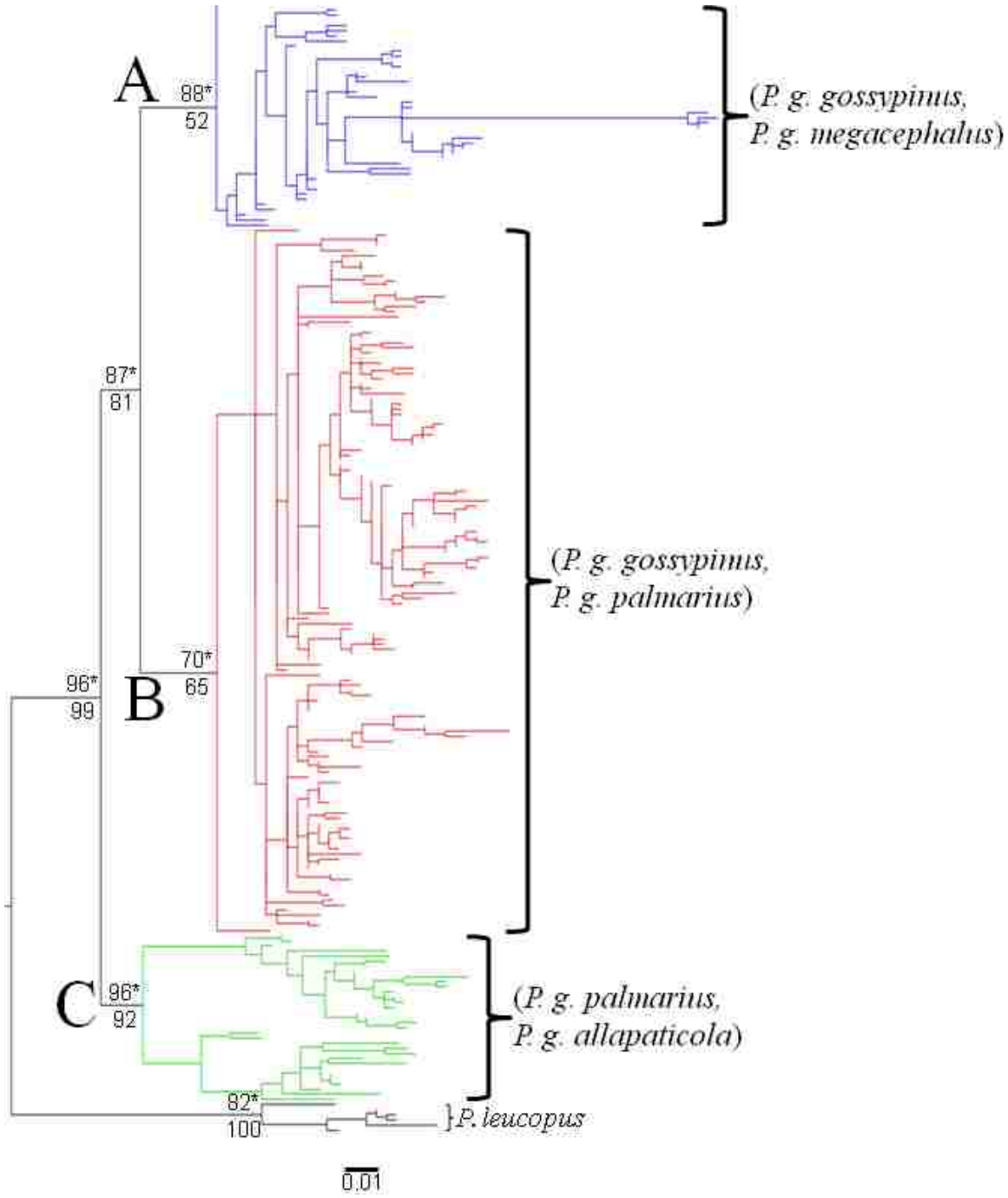


Figure 2.6. Phylogenetic tree reconstructed using maximum likelihood (ML), parsimony (MP), and Bayesian methods with *Peromyscus leucopus* as an outgroup. ML bootstrap values for major clades are above the line and MP values are below the line. Stars indicate a posterior probability of 0.95 or greater in the Bayesian analysis. All three trees returned the same topology. Clade A (blue) contained all samples within the Apalachicola-Chattahoochee-Flint River Basin (ACF) and westward, clade B (red) contained all samples east of the ACF from northern peninsular Florida and northward, clade C (green) contained all samples from southern peninsular Florida. In each clade samples were assignable to multiple recognized subspecies indicated in parentheses.

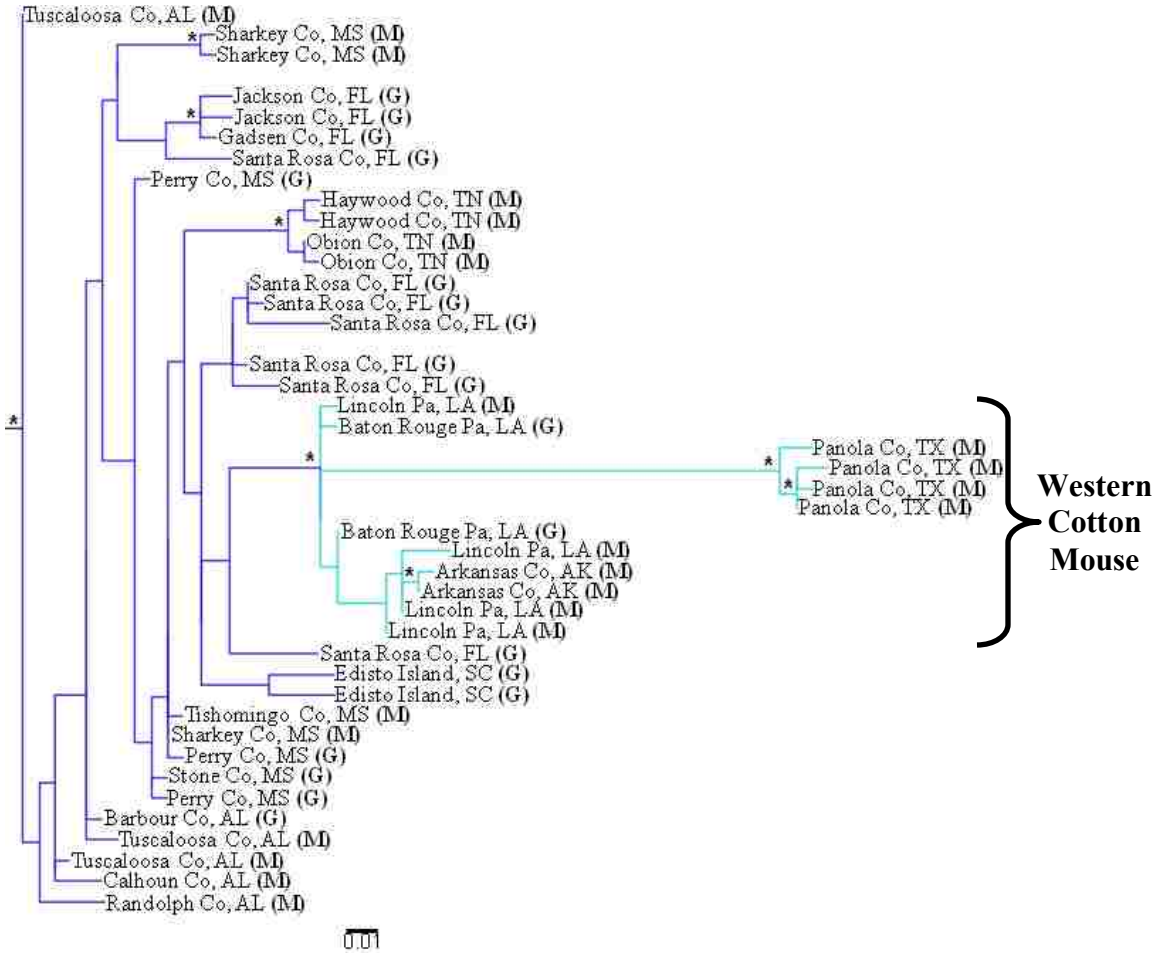


Figure 2.7. Maximum likelihood reconstruction of the western clade (A in Fig. 2.6). Nodes with a * have 0.95 or greater posterior probability based on Bayesian analysis. Letters in parentheses indicate subspecies assignments under current morphological designations: G-*gossypinus*, M-*megacephalus*. Branches in dark blue indicate populations assigned to *P. g. megacephalus* based on sequence data; branches in light blue indicate populations assigned to the Western cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data.

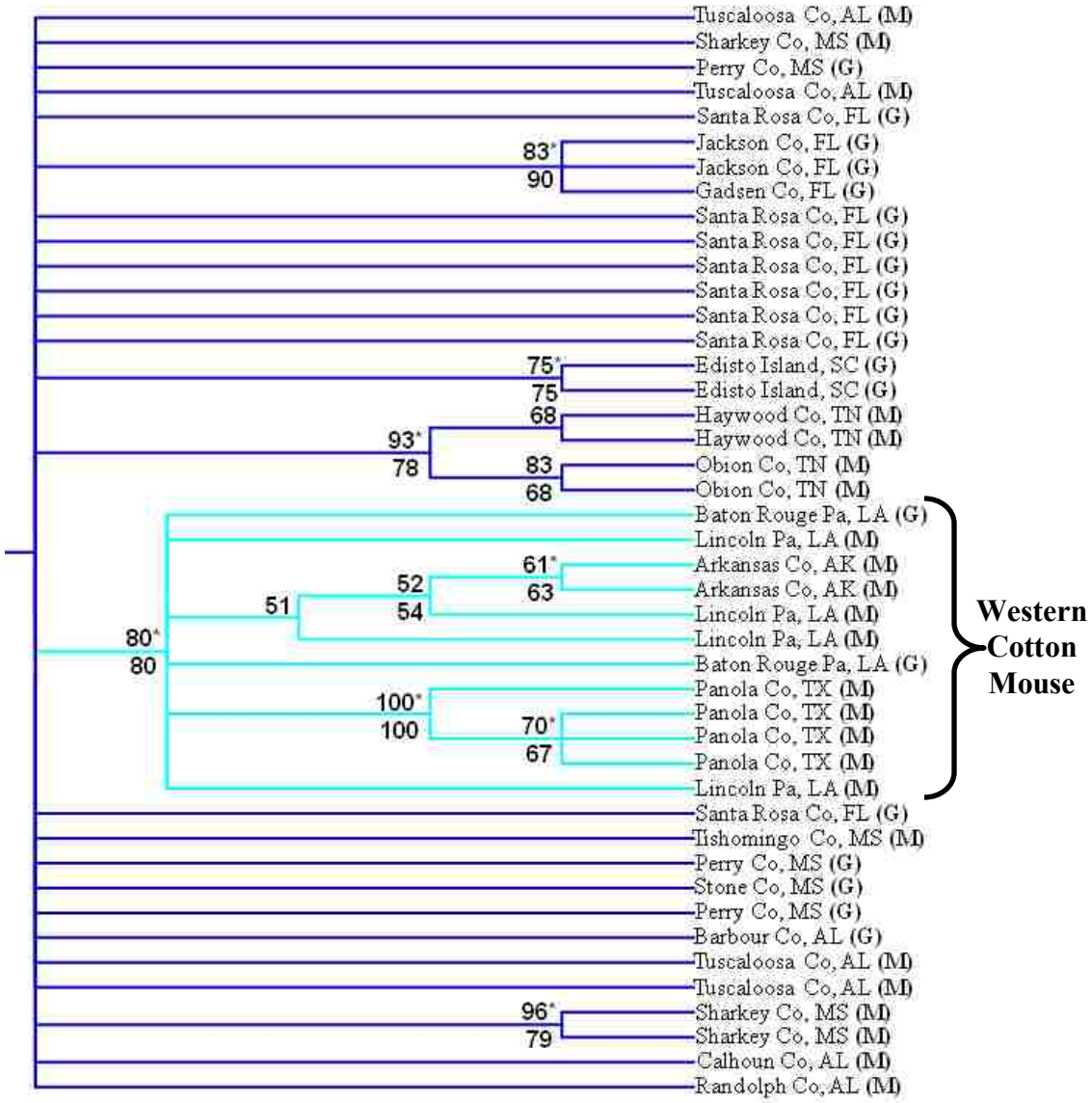
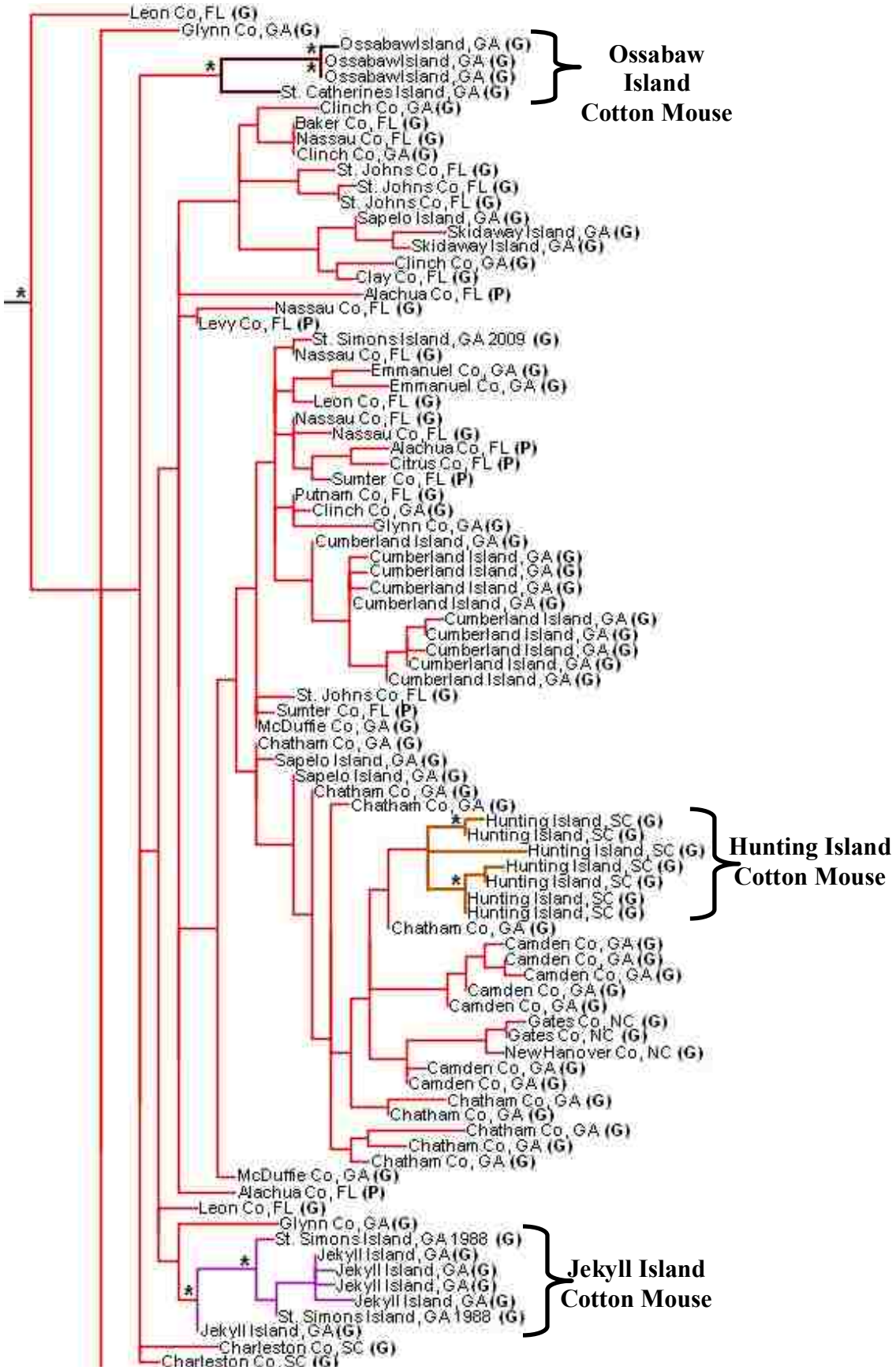


Figure 2.8. 50% majority rule consensus tree of the western clade (A in Fig. 2.6). Bootstrap values above branches are based on maximum likelihood trees. Values below branches are based on maximum parsimony trees. Branches with a * have 0.95 or greater posterior probability based on Bayesian analysis. Letters in parentheses indicate subspecies assignments under current morphological designations: G-*gossypinus*, M-*megacephalus*. Branches in dark blue indicate populations assigned to *P. g. megacephalus* based on sequence data; branches in light blue indicate populations assigned to the Western cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data.


Leon Co, FL (G)
Glynn Co, GA (G)
Ossabaw Island, GA (G)
Ossabaw Island, GA (G)
Ossabaw Island, GA (G)
St. Catherines Island, GA (G)
Ossabaw Island Cotton Mouse
Clinch Co, GA (G)
Baker Co, FL (G)
Nassau Co, FL (G)
Clinch Co, GA (G)
St. Johns Co, FL (G)
St. Johns Co, FL (G)
St. Johns Co, FL (G)
Sapelo Island, GA (G)
Skidaway Island, GA (G)
Skidaway Island, GA (G)
Clinch Co, GA (G)
Clay Co, FL (G)
Alachua Co, FL (P)
Nassau Co, FL (G)
Levy Co, FL (P)
St. Simons Island, GA 2009 (G)
Nassau Co, FL (G)
Emmanuel Co, GA (G)
Emmanuel Co, GA (G)
Leon Co, FL (G)
Nassau Co, FL (G)
Nassau Co, FL (G)
Alachua Co, FL (P)
Citrus Co, FL (P)
Sumter Co, FL (P)
Putnam Co, FL (G)
Clinch Co, GA (G)
Glynn Co, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
St. Johns Co, FL (G)
Sumter Co, FL (P)
McDuffie Co, GA (G)
Chatham Co, GA (G)
Sapelo Island, GA (G)
Sapelo Island, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island Cotton Mouse
Chatham Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Gates Co, NC (G)
Gates Co, NC (G)
New Hanover Co, NC (G)
Camden Co, GA (G)
Camden Co, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
McDuffie Co, GA (G)
Alachua Co, FL (P)
Leon Co, FL (G)
Glynn Co, GA (G)
St. Simons Island, GA 1988 (G)
Jekyll Island, GA (G)
Jekyll Island, GA (G)
Jekyll Island, GA (G)
Jekyll Island, GA (G)
St. Simons Island, GA 1988 (G)
Jekyll Island, GA (G)
Jekyll Island Cotton Mouse
Charleston Co, SC (G)
Charleston Co, SC (G)

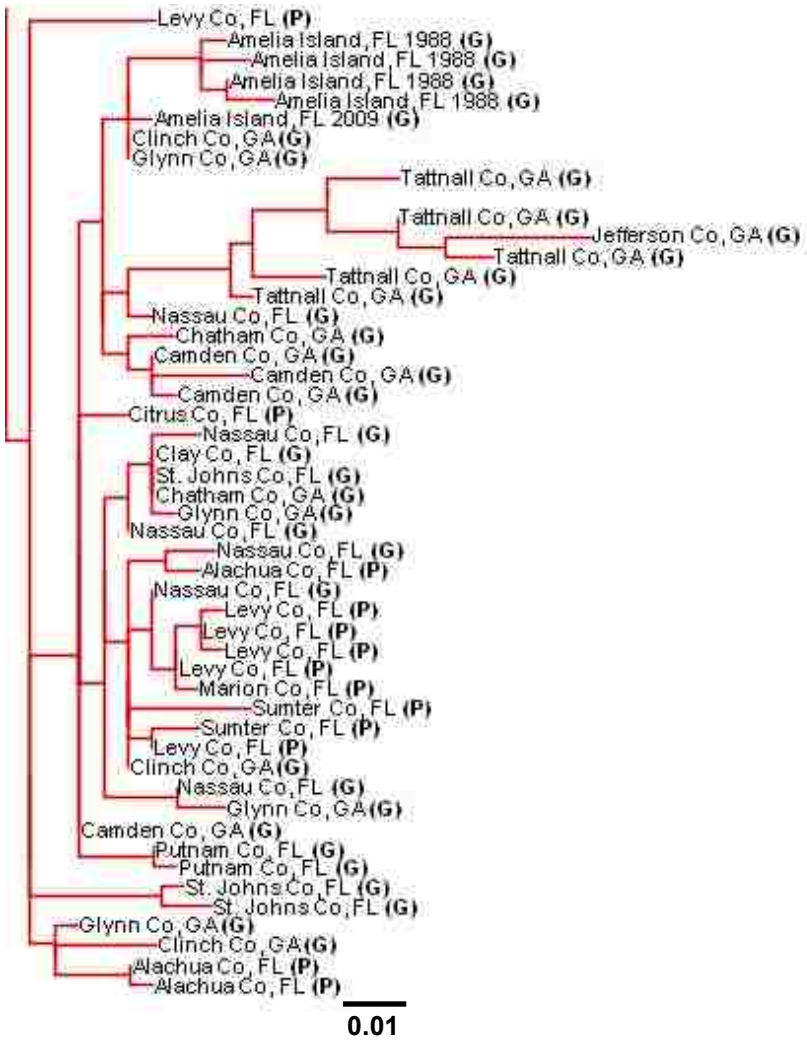
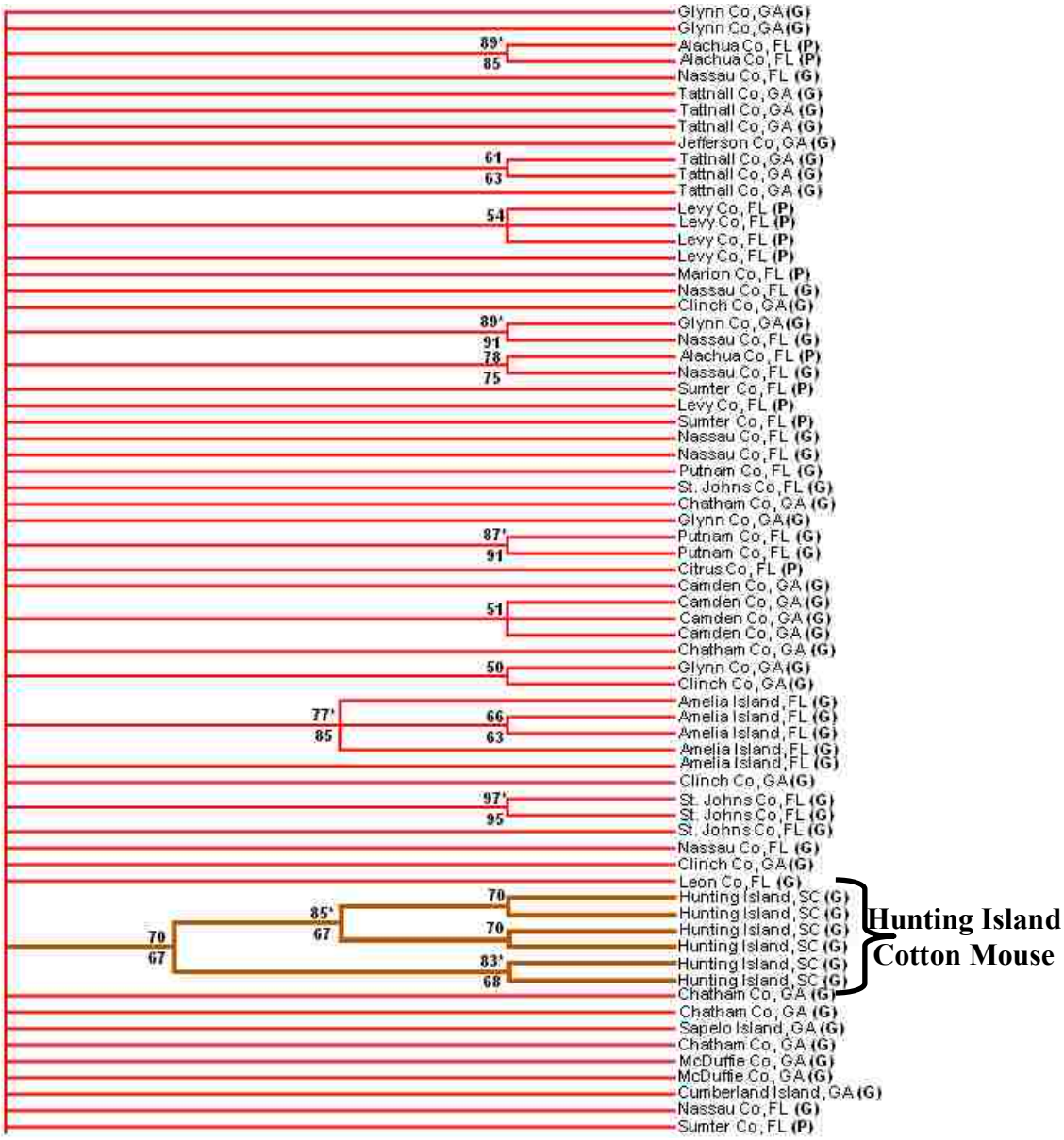


Figure 2.9 (includes previous page). Maximum likelihood reconstruction of the northeastern clade (B in Fig. 2.6). Nodes with a * have 0.95 or greater posterior probability based on Bayesian analysis. Letters in parentheses indicate subspecies assignments under current morphological designations: G-*gossypinus*, P-*palmarius*. Branches in red indicate populations assigned to *P. g. gossypinus* based on sequence data; branches in dark red indicate populations assigned to the Ossabaw Island cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data; branches in brown indicate the population assigned to the Hunting Island cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data; branches in purple indicate populations assigned to the Jekyll Island cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data.


Glynn Co, GA (G)
Glynn Co, GA (G)
89'
Alachua Co, FL (P)
85
Alachua Co, FL (P)
Nassau Co, FL (G)
Tattnall Co, GA (G)
Tattnall Co, GA (G)
Tattnall Co, GA (G)
Jefferson Co, GA (G)
61
Tattnall Co, GA (G)
63
Tattnall Co, GA (G)
Tattnall Co, GA (G)
54
Levy Co, FL (P)
Levy Co, FL (P)
Levy Co, FL (P)
Levy Co, FL (P)
Marion Co, FL (P)
Nassau Co, FL (G)
Clinch Co, GA (G)
89'
Glynn Co, GA (G)
91
Nassau Co, FL (G)
78
Alachua Co, FL (P)
75
Nassau Co, FL (G)
Sumter Co, FL (P)
Levy Co, FL (P)
Sumter Co, FL (P)
Nassau Co, FL (G)
Nassau Co, FL (G)
Putnam Co, FL (G)
St. Johns Co, FL (G)
Chatham Co, GA (G)
Glynn Co, GA (G)
87'
Putnam Co, FL (G)
91
Putnam Co, FL (G)
Citrus Co, FL (P)
Camden Co, GA (G)
51
Camden Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Chatham Co, GA (G)
50
Glynn Co, GA (G)
Clinch Co, GA (G)
77'
Amelia Island, FL (G)
66
Amelia Island, FL (G)
85
63
Amelia Island, FL (G)
Amelia Island, FL (G)
Amelia Island, FL (G)
Clinch Co, GA (G)
97'
St. Johns Co, FL (G)
95
St. Johns Co, FL (G)
St. Johns Co, FL (G)
Nassau Co, FL (G)
Clinch Co, GA (G)
Leon Co, FL (G)
70
Hunting Island, SC (G)
85'
Hunting Island, SC (G)
67
70
Hunting Island, SC (G)
Hunting Island, SC (G)
70
Hunting Island, SC (G)
67
83'
Hunting Island, SC (G)
68
Hunting Island, SC (G)
Chatham Co, GA (G)
Hunting Island
Cotton Mouse
Chatham Co, GA (G)
Sapelo Island, GA (G)
Chatham Co, GA (G)
McDuffie Co, GA (G)
McDuffie Co, GA (G)
Cumberland Island, GA (G)
Nassau Co, FL (G)
Sumter Co, FL (P)

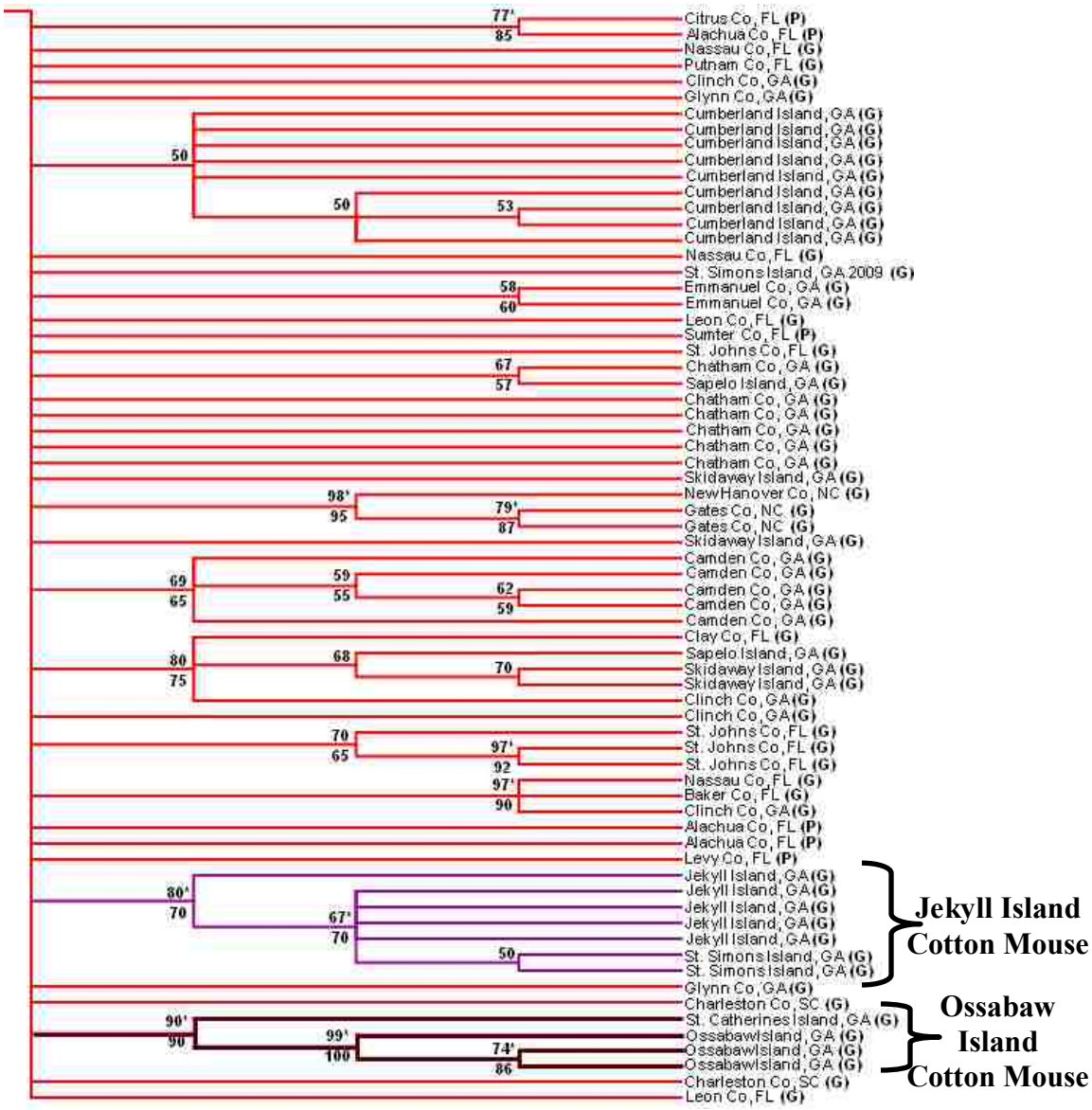


Figure 2.10 (includes previous page). 50% majority rule consensus tree of the northeastern clade (B in Fig. 2.6). Bootstrap values above branches are based on maximum likelihood trees and below branches are based on maximum parsimony trees. Branches with a * have at least a 0.95 posterior probability in Bayesian analyses. Letters in parentheses indicate subspecies assignments under current morphological designations: G-*gossypinus*, P-*palmarius*. Branches in red indicate populations assigned to *P. g. gossypinus* based on sequence data. Branches in other colors indicate populations belonging to previously unidentified subspecies of *P. gossypinus*: Dark Red-Ossabaw Island cotton mouse, Brown- Hunting Island cotton mouse, Purple- Jekyll Island cotton mouse.

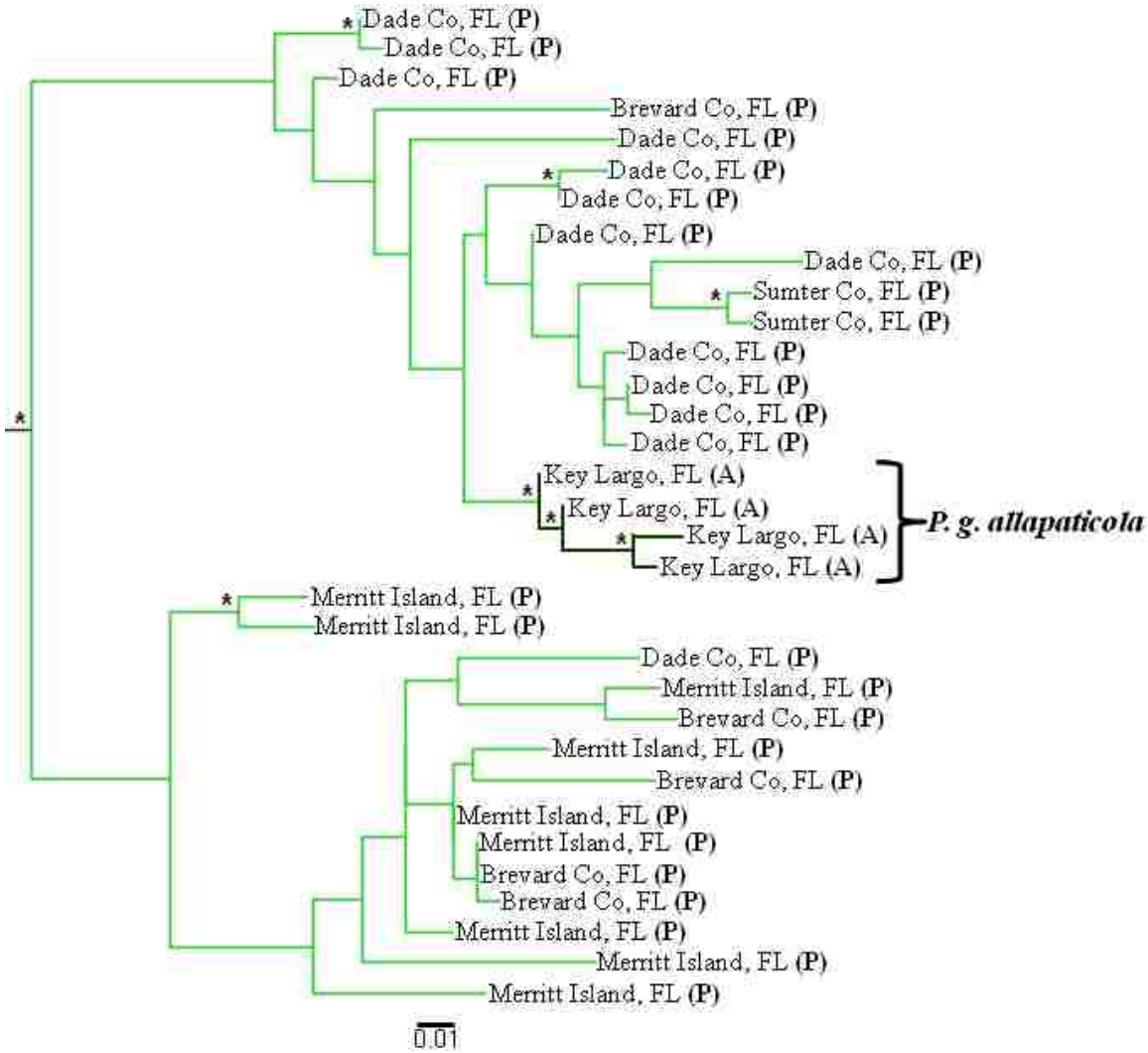


Figure 2.11. Maximum likelihood reconstruction of the southeastern clade (C in Fig. 2.6). Nodes with a * have 0.95 or greater posterior probability based on Bayesian analysis. Letters in parentheses indicate subspecies assignments under current morphological designations: A-*allapaticola*, P-*palmarius*. Branches in light green indicate populations assigned to *P. g. palmarius* based on sequence data; branches in olive green indicate populations assigned to *P. g. allapaticola* based on sequence data.

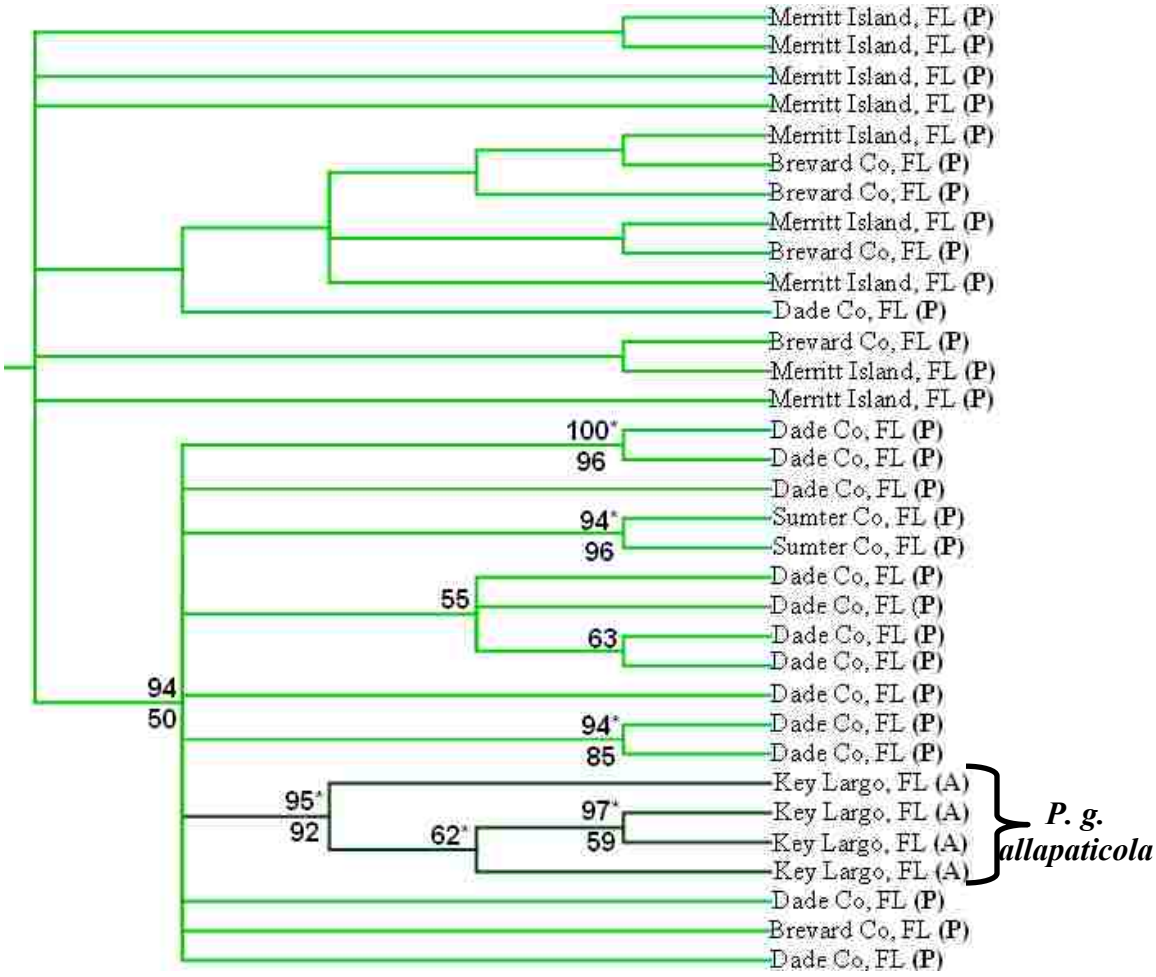


Figure 2.12. 50% majority rule consensus tree of the southeastern clade (C in Fig. 2.6). Bootstrap values above branches are based on maximum likelihood trees and below branches are based on maximum parsimony trees. Branches with a * have at least a 0.95 posterior probability in Bayesian analyses. Letters in parentheses indicate subspecies assignments under current morphological designations: A-*allapaticola*, P-*palmarius*. Branches in light green indicate populations assigned to *P. g. palmarius* based on sequence data; branches in olive green indicate populations assigned to *P. g. allapaticola* based on sequence data.

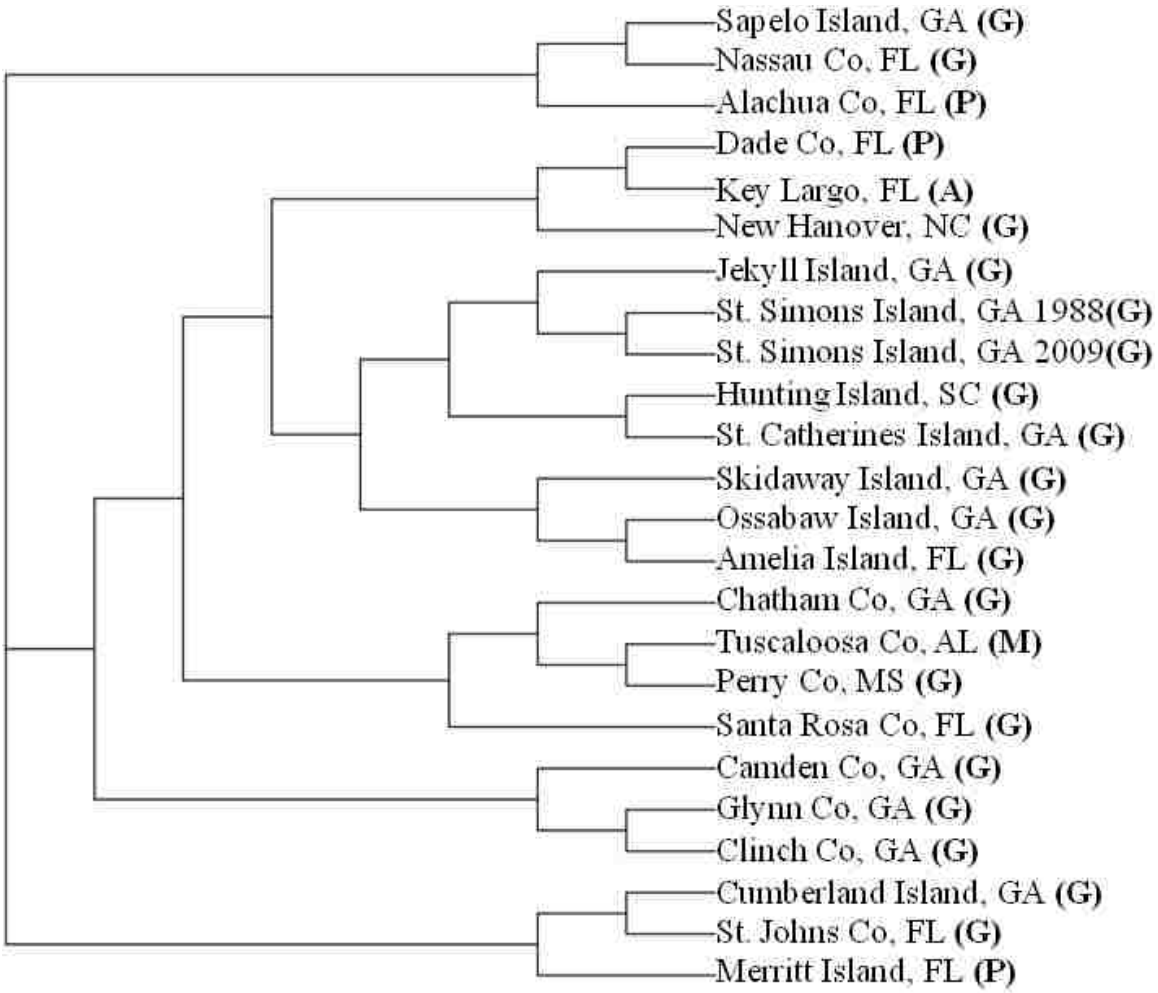


Figure 2.13. Neighbor joining population tree of *Peromyscus gossypinus*. Tree was developed using genotype data from *P. gossypinus* samples at seven unlinked microsatellite loci. The tree is unrooted and little geographic structuring of genetic variation can be identified. Letters in parentheses indicate subspecies assignments under current morphological designations: A-*allapaticola*, G-*gossypinus*, M-*megacephalus*, P-*palmarius*.

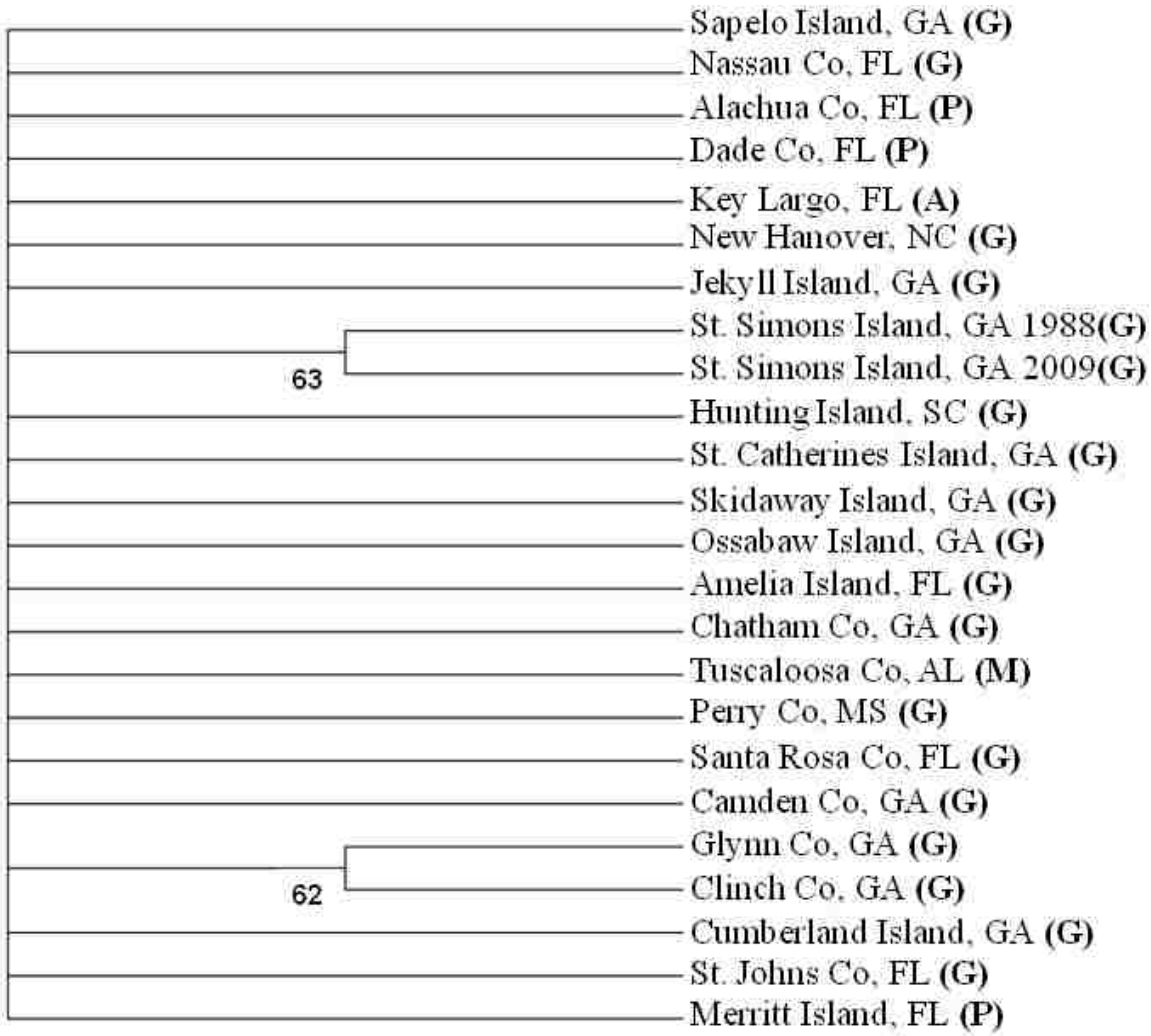


Figure 2.14. 50% consensus neighbor joining population tree of *Peromyscus gossypinus*. Tree was developed using genotype data from *P. gossypinus* samples at seven unlinked microsatellite loci. Bootstrap support of 50% or greater was only identified for two nodes. The tree is unrooted and little geographic structuring of genetic variation can be identified. Letters in parentheses indicate subspecies assignments under current morphological designations: A-*allapaticola*, G-*gossypinus*, M-*megacephalus*, P-*palmarius*.

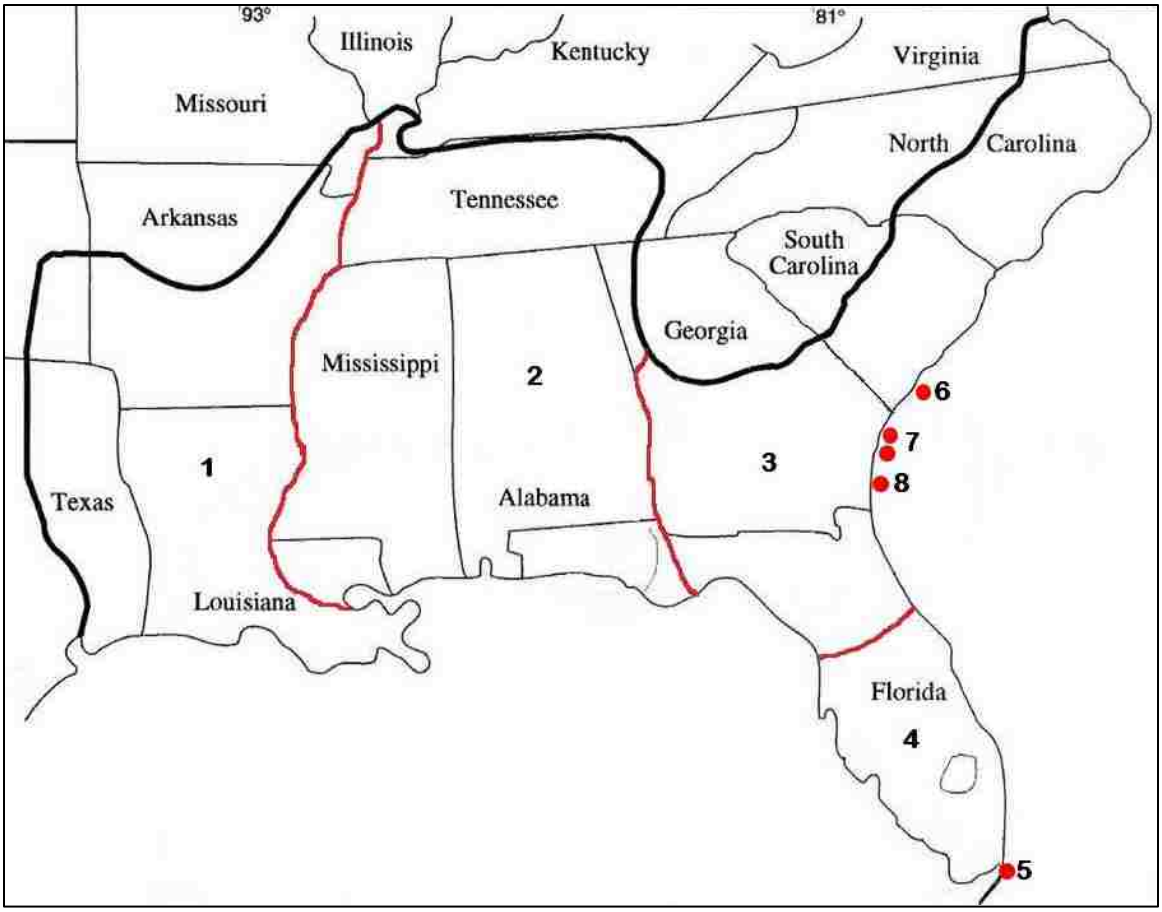


Figure 2.15. Range map of *Peromyscus gossypinus* with ranges of newly identified subspecies demarcated by red lines. 1) Western cotton mouse, 2) *P. g. megacephalus*, 3) *P. g. gossypinus*, 4) *P. g. palmarius*, 5) *P. g. allapaticola*, 6) Hunting Island cotton mouse, 7) Ossabaw Island cotton mouse, 8) Jekyll Island cotton mouse

Chapter 3:
Inferring the effects of biogeographic events on the genetic variation of the cotton mouse, *Peromyscus gossypinus*: a mtDNA approach

Studies of an organism's systematics provide insight into the current structure of genetic variation within or among species (Michener et al. 1970). However, these studies do little to explain how that structure arose because a variety of evolutionary processes can produce similar patterns of genetic variation (Hedrick 2005). Phylogeographic studies combine patterns of genetic variation with information on a species' geographic distribution to infer the processes responsible for these patterns (Avise 2000, Hickerson et al. 2010). These studies identify phylogeographic breaks and seek to explain them in a geographic and climatic context.

Phylogeographic breaks are commonly observed in the southeastern United States (US) (Hewitt 2000, Soltis et. al 2006). The biota of this region have been affected by a variety of unique biogeographic events, including the formation of several potential barriers to gene flow such as mountains, rivers, and islands (Avise 2000, Soltis et al. 2006). A major shift from a temperate to a tropical climate, particularly in peninsular Florida also affects the regional biota (Peel et al. 2007). Also, the geography of the southeastern US has varied dramatically in association with a series of glaciation events and associated interglacial periods (Briggs et al. 1974, Leigh 2008). The combined factors make the biota of the southeastern US a unique study system.

While the advancing ice sheet associated with Pleistocene glaciations, ~2.5 million years ago (mya), did not extend into the southeastern US (Dyke et al. 2002), glacial periods still had a dramatic effect on the region. The formation of glaciers resulted in a dramatic drop in sea level, greatly expanding the coastal region of the southeast,

particularly of the Florida peninsula (Delcourt 1993). During these glacial periods, populations of several temperate species sought refuge on the expanded peninsula (Donovan et al. 2000, Osentoski and Lamb 1995). Many of these populations developed into new species while in reproductive isolation from populations in other refugia (Lewis and Crawford 1995, Zimmerman et al. 1977). During interglacial periods, the sea level rose to as much as 50 m above current levels (Emslie 1998) and Florida became a group of islands isolated from the mainland (Morgan and Emslie 2010). This prevented gene flow between isolated populations and those of the mainland US which may have promoted speciation.

Organisms taking refuge on the peninsula experienced different habitats than those of their mainland counterparts. The tropical climate of Florida associated with interglacial periods gave rise to unique habitats and flora not found in the temperate US (Duever et al. 1994). Peninsular species experienced novel selective pressures and in some cases may have experienced ecological release (Dayan and Simberloff 1998, Lee 1987), resulting in the adaptation of these populations to their new environments. Following the last glacial maximum (LGM: ~20,000 ya), these organisms came into contact with their temperate counterparts in northern peninsular Florida (Avise 2000). This resulted in a suture zone, where closely related temperate and tropical species coexist, and in some cases hybridize (Remington 1968, Hewitt 2000).

During this same time, the rise in sea level resulted in the formation of several coastal barrier islands throughout the southeastern US (Purdue and Reitz 1993). Populations of many terrestrial species became isolated on these islands, and gene flow with mainland populations ceased or was greatly reduced (Ellsworth et al. 1994, Spitzer

and Lazell 1984). As a result, unique species and subspecies of a variety of mainland taxa have been identified on these islands (Brisbin and Lenarz 1984, Hayes and Harrison 1992, Ross and Ruiz 1996, Scott 2004).

Glacial cycles resulted in the formation of a suture zone between the Suwannee River in Florida and the Tombigbee River in Alabama. During periods of glacial maxima, particularly the Wisconsinan, populations of a single species often inhabited refugia on both sides of this suture. After the LGM, populations that came back into contact in this area often had diverged into separate species (Church et al. 2003, Pauly et al. 2007, Walker et al. 2009). Today this 460 km wide range is home to phylogeographic breaks for dozens of plant and animal taxa (Soltis et al. 2006).

Unlike the suture zone in peninsular Florida, most of these phylogeographic breaks are associated with contemporary barriers to gene flow. The Appalachian Mountains have been identified as a barrier to gene flow for several species (Jones et al. 2006, Zamudio and Savage 2003). Likewise, the Suwannee River in Florida (Roman et al. 1999) and the Tombigbee River in Alabama (Gill et al. 1993, Indorf 2010) have been identified as phylogeographic breaks. Finally, organisms across an incredible range of taxa exhibit phylogeographic breaks within the Apalachicola/Chattahoochee/Flint (ACF) River Basin (Avise 2000, Avise et al. 1979, Davis et al. 2002, Liu et al. 2006, Mylecraine et al. 2004, Walker et al. 2009).

Further west, phylogeographic breaks are commonly seen at the Mississippi River (Al-Rabab'ah & Williams 2002, Brant and Ortí, 2003, Howes et al. 2006, Moriarty and Cannatella 2004). Each of these studies has indicated that this break is the result of populations inhabiting separate glacial refugia on opposite sides of the river. The width

and current of the Mississippi River likely is sufficient to maintain the river as a barrier to gene flow for many species if a postglacial dispersal event were to occur.

Clearly, glaciations, climatic events, and historic barriers to gene flow have shaped the genetic diversity of a wide range of species in the southeastern US (Avise 2000). Among them, small mammals consistently demonstrate phylogeographic breaks throughout this region (Brant and Orti 2003, Hayes and Harrison 1992, Indorf 2010). Due to their small size and limited dispersal ability, small mammals often show a high degree of geographic structuring associated with historic climatic and geographic events (Grayson 1987, Riddle et al. 2000). This makes them ideal for phylogeographic studies because it often is possible to infer historic processes that could not be identified in larger, more vagile species that exhibit little geographic structuring (Hundertmark et al. 2003, Lehman and Wayne 1991, Luikart and Allendorf 1996, Vila et al. 1999).

One trait that typically determines how much structuring is expected within a species is habitat usage. Animals that only can utilize specific habitat types (habitat specialists) commonly show a greater degree of structuring than those that utilize more diverse habitats (habitat generalists). Species such as the pocket gopher, *Thomomys* spp. (Belfiore et al. 2008), the pika, *Ochotona princeps* (Galbreath et al. 2010), and the southeastern beach mouse, *Peromyscus polionotus* (Van Zant and Wooten 2007), are habitat specialists that demonstrate fine scale geographic structuring.

In contrast, habitat generalists typically do not show fine scale structuring due to their ability to disperse through a wide range of habitat types (Bradley et al. 2008). An example of this is the white-footed mouse, *Peromyscus leucopus*, a generalist species found throughout much of the US. On a regional scale, low levels of genetic variation

have been identified among mainland populations throughout the mid-Atlantic states (Shipp-Pennock et al. 2005). On a broader scale, Rowe et al. (2006) identified distinct eastern and western clades of *P. leucopus*.

While there is great discord between the patterns shown by habitat generalists and habitat specialists, the majority of studies of small mammals focus on the latter (Galbreath et al. 2009, Indorf 2010, Van Zant and Wooten 2007). In this study I sought to identify the phylogeographic patterns of a habitat generalist species, the cotton mouse, *Peromyscus gossypinus*. This species is found throughout the southeastern US and comprises five extant taxonomic lineages (Wolfe and Linzey 1977) (Figure 3.1). It is distributed throughout peninsular Florida; and is found on both sides of the Suwannee River, the ACF river basin, the Tombigbee River, and the Mississippi River. It also inhabits 13 coastal barrier islands, which became isolated from the mainland following the LGM (Emslie 1998).

Given the abundance of phylogeographic breaks in the southeastern US, I hypothesized that historic climatic and geographic events resulted in geographic structuring of genetic variation in *P. gossypinus*. I predicted that there would be geographic structuring of variation among mainland populations associated with historic barriers to gene flow in other species. Because of the impact of historic events on other co-distributed species, I predicted the populations would form three major clades: 1) a southeastern group in peninsular Florida south of Remington's suture zone, 2) a northeastern group north of Remington's suture zone and east of the ACF, 3) and a western group including the ACF and all populations west. I also predicted that populations west of the Mississippi River and north of the Savannah River would form

minor clades because these rivers have been identified as barriers to gene flow for other terrestrial species (Brant and Ortí 2003, Degner et al. 2010). Given that several island populations of *P. gossypinus* have been identified as unique subspecies (Howell 1939, Osgood 1909, Schwartz 1952), I predicted that several island populations represent Wisconsinan vicariants dating back to the LGM. Finally, based on phylogeographic patterns in other species, as well as the fossil record for *P. gossypinus*, I predicted that the organisms from peninsular Florida are ancestral to all other populations.

Methods

Sample collection and DNA sequencing

I collected tissue samples from a total of 176 *P. gossypinus* from island and mainland populations throughout the Atlantic coastal portion of the species' range (Figure 3.1). Animals were trapped using a combination of Sherman and Longworth live traps set in grids in hardwood hammocks. Tissue was collected by removing ~1 cm of the tip of the tail from each mouse with scissors. Samples were stored in 1.5 ml tubes containing a 20% DMSO (6 M NaCl) solution. All sampling methods were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Miami and followed methods approved by the American Society of Mammalogists Animal Care and Use Committee (Gannon et al. 2007).

In addition to field samples, 338 museum specimens (toe bones and liver) included in Boone et al. 1999 were obtained to provide thorough sampling of the species' entire range (Figure 3.1; see Appendix 1). All liver samples were provided by the Texas Tech University Genetic Resource Collection (TTU). Toe bones were obtained from the mammal collection of the University of Georgia Museum of Natural History (UGMNH).

In total, 514 samples from throughout the species' range were included for sequence analysis. These included samples from all extant taxonomic lineages: *P. g. allapaticola* (n=20); *P. g. gossypinus* (n=387); *P. g. megacephalus* (n=39); *P. g. palmarius* (n=65); *P. g. telmaphilus* (n=3).

Whole genomic DNA was extracted from tail tips and liver tissue using a standard ethanol precipitation protocol. Qiagen DNeasy® tissue extraction kits (Qiagen Inc., Valenica, California) were used to extract DNA from toe bones following the user-developed protocol *Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit* available on the Qiagen website (http://www.qiagen.com). A 784-bp region of the mitochondrial control region was amplified and sequenced using the methods described by Beckmann (in prep).

Sequence chromatograms were proofread and edited in BioEdit (Hall 1999). Alignments were performed using the ClustalW software package included in BioEdit (Thompson et al. 1994). Aligned sequence files were exported to DNAsp v.5 (Librado and Rozas 2009) where a haplotype file was developed.

Tree reconstruction

Phylogenetic trees were reconstructed using maximum parsimony, maximum likelihood, and Bayesian analyses. Control region sequences from *P. maniculatus* (GenBank Accession Number: EU170494) and *P. attwateri* (AF081492) were included as outgroups for all trees. Maximum likelihood and Bayesian methods were performed five times to ensure the most likely topology was returned. Maximum parsimony analysis was performed twice due to the large amount of time required to run this type of analysis. Parsimony analysis was run using PAUP v4.0b10 (Swofford 2002). Nucleotide sites were

treated as unordered, equally weighted, discrete characters with four possible character states: A, T, G, or C. Trees were reconstructed using a heuristic search with tree bisection-reconstruction branch swapping. One hundred bootstrap replicates using the same parameters were completed to identify support for the topology (BSP) (Felsenstein 1985). All reconstructions were limited to a maximum of 10,000 trees.

For Bayesian and maximum likelihood analyses, jModeltest was used to identify the model of evolution that best fit the sequence data (Posada 2008). The model chosen using the Akaike Information Criterion with a 95% confidence interval was the General Time Reversible model with rate variation and a proportion of invariable sites (GTR+I+G), with a gamma shape parameter of 0.276 and a proportion of invariable sites of 0.353. RaxML was used to perform maximum likelihood reconstructions due to accuracy and speed (Stamatakis 2006). Support for the identified topology was again determined via 100 bootstrap replicates (BSL), using the rapid bootstrap algorithm in RaxML (Stamatakis et al. 2008). A different randomly chosen starting seed was used for each of the five runs to ensure the most likely tree was returned.

Bayesian analysis was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with the aforementioned GTR+I+G model. The program was run with four simultaneous Markov-chains for ten million generations, sampling trees every 500 generations. Log-likelihood scores versus generation were plotted graphically to identify when the run reached stationary. Based on this, the first three million generations (6000 trees) were discarded as burn-in. Posterior probabilities (PP) calculated in MrBayes were used to determine support for the tree topology.

Population analyses

Analyses of Molecular Variance (AMOVAs) were performed using the program Arlequin v3.5 to identify the proportions of genetic variation attributable to differences within populations, among populations, within hypothesized lineages, and among hypothesized lineages (Excoffier and Lischer 2010). To better identify geographic structuring two AMOVAs were performed. The first AMOVA partitioned populations based on the three hypothesized major groups and the second AMOVA partitioned populations based on the three hypothesized major groups and two hypothesized minor groups. If the minor groups have recently diverged from their respective major groups I would expect both analyses to show similar levels of variation among regions. However, I would expect a decrease in the proportion of variation among populations within regions between the two AMOVAs.

Average genetic distances within and among the three hypothesized major groups (southeastern, northeastern, and western) were calculated using the Tamura Nei correction (Tamura and Nei 1993) in the software package MEGA v.5 (Tamura et al. 2007). The Tamura Nei correction was developed for the D-loop of the mitochondrial control region of mammals, making it the ideal genetic distance calculation for this study. Genetic distances were recalculated including the hypothesized minor groups west of the Mississippi River and north of the Savannah River. Genetic distances for the northeastern and peninsular Florida groups were calculated twice, once lumping the island populations within the mainland groups, and once without the island populations included.

Population pairwise genetic distances were calculated between all population pairs, allowing for the identification of gene flow among populations within groups and

between hypothesized groups. These measures are particularly important for the island populations, because these measures may identify the source population for these islands, as well as determine if current gene flow is occurring between island and mainland populations. It should be noted that for these calculations, the samples collected on St. Simons Island and Amelia Island in 1989 were treated as separate populations to those collected on these islands in 2009. This was because populations from these two time frames did not form a clade in previous analysis conducted by Beckmann (in prep). Treating populations form these two time periods separately allowed me to determine if extirpations and recolonizations occurred on these islands in the last 20 years.

Wright's F_{ST} (Wright 1965) was calculated between geographic regions and among all population pairs in Arlequin v3.5 (Excoffier and Lischer 2010). F_{ST} is a measure of gene flow between populations (Weir and Cockerham 1984). This statistic measures how genetically differentiated populations are with a value of zero indicating the populations are identical and a value of one indicating they are completely differentiated. A low F_{ST} indicates little divergence among populations, but may also indicate a recent split between two populations (Holsinger and Weir 2009). This could occur if organisms from a mainland population recently colonized an extirpated island and have not diverged from their population of origin.

Nucleotide diversity per site (π) and haplotype diversity were calculated to identify genetic diversity within regions and within populations using Arlequin v3.5 (Excoffier and Lischer 2010). Nucleotide diversity uses the number of pairwise differences between haplotypes in a population and the frequency of those haplotypes in the population to identify the amount of diversity present (Tajima 1983, 1993).

Haplotype diversity looks at the number of haplotypes and their frequencies in a population, but does not take into account the identity of those haplotypes and how different they are from each other (Hedrick 2005). For example, a population with two haplotypes with 45 different base pairs can have the same haplotype diversity as a population with two haplotypes separated by one base pair.

To determine if identified patterns were the result of isolation by distance, a Mantel test was performed in ARLEQUIN v3.5 (Excoffier and Lischer 2010). Mantel tests identify the amount of correlation between population pairwise F_{ST} and geographic distance (Sokal 1979). I measured the distances between populations as straight line distances in Google Earth (Google Inc. 2009). In the event that the straight line distance required movement outside of the species' range, or dispersal across large bodies of water, the shortest over land geographic distance within the species' range was determined using the same software.

A minimum spanning haplotype network (MSN) was constructed to visualize the relationships among haplotypes. The network was constructed using TCS v.1.2.1 (Clement et al. 2000) after importing the sequence alignment. This program identifies the most parsimonious relationship among haplotypes based on the minimum number of mutational steps between them.

To identify if populations have experienced demographic expansions or contractions I created mismatch distributions for all populations, and calculated Fu's F_S in Arlequin v3.5 (Excoffier and Lischer 2010). Mismatch distributions identify demographic changes by calculating the number of pairwise differences between each pair of sequences in a population, and representing this information in a histogram

(Slatkin and Hudson, 1991). If the data produce a unimodal distribution then population expansion is likely. A multimodal distribution indicates the population is stable, and an L-shaped distribution indicates a recent population contraction is likely (Schneider and Excoffier 1999, Harpending 1994). Fu's F_S tests for population expansion and contraction utilizing a coalescent model to test for significant differences. This method is considered more sensitive to population expansions than Tajima's D (Ramos-Onsins and Rozas 2002).

Before testing for population demographic changes it is first necessary to verify that region of DNA is not under selection. Tajima's D traditionally has been used to determine if a region of DNA is evolving neutrally or is under directional or balancing selection (Tajima 1989). This statistic calculates the nucleotide diversity using all pairwise nucleotide differences, $\theta_{\pi,}$ and the nucleotide diversity using only segregating sites, θ_s. The difference between these numbers can identify if a region of DNA is under selection. While the HKA test and McDonald-Kreitman (MK) test are more powerful tests of selection both have requirements beyond the scope of this dataset. The HKA test requires interspecific data from two genomic regions and the MK test requires that either the region of interest be coding, or that the closely linked genomic regions be analyzed (Rozas et al. 1999). Since neither is an option here, it is necessary to rely on Tajima's test.

Dating molecular divergences

In order to determine whether or not Pleistocene glacial events resulted in the patterns of genetic variation seen in *P. gossypinus* today, I estimated the time since molecular divergence among the major groups, among the major groups and minor

groups, and among the island populations relative to the mainland. First, a time estimate based on the fossil record was necessary. A well-established fossil record for *P. gossypinus* does exist in multiple locations. Fossils of *P. gossypinus* that were morphologically indistinguishable from contemporary samples for a number of skeletal traits were identified in Reddick, FL (Gut and Ray 1963). Using a variety of paleontological methods, Pinkham (1971) dated the Reddick, FL site to the early Sangamonian Period, ~120,000 ya (Kurten and Anderson 1980). Additionally, Pinkham noted that due to the high level of similarity between these fossils and contemporary samples, that *P. gossypinus* was already well differentiated from *P. leucopus* by the Sangamonian. This places the divergence between these sister taxa within the glaciation associated with the latter portion of the Illinoian period ~150,000 ya.

In order to test these dates, I first had to determine whether or not a molecular clock could be applied to the data. Because this study is focused on divergence among lineages of *P. gossypinus*, all outgroups used in previous analyses were removed from the dataset with the exception of *P. leucopus*, the sister taxon of *P. gossypinus*. Likelihood scores were calculated in PAUP v4.0b10 (Swofford 2002) under the GTR+I+G model for the dataset, with no molecular clock and with a molecular clock enforced. A likelihood ratio test (LRT) was performed using these likelihood scores. A Chi-square test was used to determine if there was a significant difference between likelihood scores. If the test was not significant, then a strict molecular clock was applied to the data, and rate constancy assumed across the tree. If the statistic was significant, a relaxed molecular clock was considered which allowed rate variation among branches of the tree (Posada 2001).

Initial divergence date estimates were obtained using the program BEAST v1.4.6 (Drummond and Rambaut 2007) using an uncorrelated relaxed molecular clock model. The relaxed molecular clock program assumes no a priori correlation between a lineages evolutionary rate and that of its ancestors (Drummond et al. 2006). BEAST identifies the divergence date of each node separately and independently of its ancestral nodes (Drummond et al. 2006, 2007). A substitution rate for the sequence was estimated by comparing sequences from *P. gossypinus* and *P. leucopus* to identify average number of substitutions between the two. This rate was then applied to the data. The program BEAUTi v.1.4.8 (Drummond and Rambaut 2007) was used to create input files for BEAST utilizing the GTR+I+G evolutionary model. BEAST estimates divergence times using a Bayesian implemented Markov Chain Monte Carlo (MCMC) method and allows for tuning of the MCMC parameters. This option was set to auto-optimize and all chains were started from a random tree. Each chain ran for 10 million generations and was sampled every 500 generations. Four runs were performed and results were analyzed using TRACER v1.5. Runs were combined using LOGCOMBINER v1.4.3 (Rambaut and Drummond 2007) and trees were summarized using the TREEANOTATOR package included in BEAST with a 2 million generation burnin. Trees with dated nodes were then exported to FigTree v1.0 for visualization (Rambaut 2006).

While BEAST is commonly used for molecular divergence dating using mitochondrial loci, any method based on a single locus is prone to overestimate divergence dates due to ancestral lineage sorting. Ancestral lineage sorting results from alleles at a polymorphic locus sorting into different lineages when they diverge from their shared most recent common ancestor (Hedrick 2005). Because this polymorphism

predates the divergence of the lineages, molecular divergence dating estimates identify that the lineages diverged earlier than they actually did. As a result, the gene tree does not demonstrate the same divergence pattern as the species tree. By incorporating sequences from multiple loci, the discrepancies between the gene tree and species can be marginalized. However, programs that utilize coalescent based methods to obtain divergence estimates can at least, partially account for ancestral lineage sorting at a single locus.

To obtain a more accurate estimate of divergence dates using a coalescent based approach, the data were analyzed using the program BPP v2.0 (Rannala and Yang, 2003; Yang and Rannala, 2010). This method accommodates the species phylogeny as well as lineage sorting due to ancestral polymorphism. A gamma prior $G(33, 1437)$ was applied to the population size parameter (θ). The age of the root in the species tree ($\tau 0$) was assigned the gamma prior $G(25, 333)$, while the other divergence time parameters are assigned the Dirichlet prior by the program (Yang and Rannala, 2010: equation 2). Each analysis was run four times to confirm consistency among runs.

Results

Phylogenetic analyses

The sequence dataset had a nucleotide composition of A: 32.2%, T: 31.2%, G: 12.2%, and C: 24.4%, with a transition to transversion ratio of 3.51. A total of 183 variable sites were identified among the sequences, with 146 of those being parsimoniously informative. Coding the alignment in FastGap added an additional 18 informative sites. A total of 226 ingroup haplotypes and two outgroup haplotypes were identified, with only four haplotypes found in multiple populations. Haplotype 38 was

found in both mainland Brevard, FL and nearby Merritt Island, FL; haplotype 90 was found in Nassau, FL, Alachua, FL, and Clinch, GA; haplotype 116 was found in Clay, FL and St. John's, FL on opposite sides of the St. John's River; and haplotype 166 was found in populations from Jackson, FL on both sides of the Apalachicola River.

All three tree reconstruction methods produced the same topology, although there was weaker support for nodes in the parsimony analysis (Figure 3.2, 3.3). Three reciprocally monophyletic clades were identified: 1) Clade A (BSL: 88 PP: 1.00) contained all samples from the ACF and west, clade B (BSL: 70 PP: 0.97) contained samples east of the ACF and north of peninsular Florida, and clade C (BSL: 96 PP: 1.00) contained samples from most of peninsular Florida (Figure 3.2, 3.3). In clade A all samples west of the Mississippi River formed a monophyletic group (BSL: 80 PP: 1.00) (Figure 3.4, 3.5). Three island groups formed well-supported monophyletic groups in clade B: Hunting Island (BSL: 70 PP: 0.96), Jekyll Island (BSL: 75 PP: 1.00), and Ossabaw/St. Catherines Island (BSL: 90 PP: 1.00) (Figure 3.6, 3.7). In clade C, the population on Key Largo, FL (BSL: 95 PP: 1.00) formed a monophyletic group (Figure 3.8, 3.9).

The minimum spanning networks (MSN) also identified the same patterns as the phylogenetic analyses (Figures 3.10, 3.11, 3.12). All samples were combined into a single MSN, as well as clade-specific MSNs (Figures 3.10, 3.11, 3.12). A minimum of 14 mutational steps separated populations from clade A and clade B, while a minimum of 29 mutational steps separated populations from clade B and clade C (Figures. 3.10, 3.11, 3.12). Within clade A there were between one and 27 minimum steps between haplotypes (Figure 3.10). However, among groups east of the Mississippi there were between one

and seven steps. Ten steps separated groups on opposite sides of the Mississippi River. Additionally, there were a minimum of 26 steps between the population from Panola, TX and any other population. There were one to seven minimum steps between haplotypes in clade B with one exception (Figure 3.11). The population from Ossabaw Island, GA had a 27-bp insertion not found in any other population, resulting in a large number of steps between haplotypes of this population and nearby St. Catherines Island. Within clade A there were one to 16 minimum steps between haplotypes (Figure 3.12).

A high degree of variability was found within and among clades, with clade B exhibiting much less variability than either clade A or C. There were twice as many (14) steps separating clade A from clade B as there are steps separating populations within clade A east of the Mississippi River. Likewise, there were nearly twice as many (29) steps separating clade B and C as there were steps separating populations within clade C (16).

Genetic diversity among clades

The AMOVA that partitioned populations into the three major groups indicated that 58.24% of the variation was due to differences among clades, while 26.85% was due to variation among populations within groups. The AMOVA that included the minor groups indicated that the amount of variation among groups was 55.66%. (Table 3.1).

Based on these results a third AMOVA was run that partitioned the western group at the Mississippi River, but did not partition the northeastern group at the Savannah River. In this four-group AMOVA, 58.98% of the variation was among groups, while 26.12% was due to differences among populations within groups.

Genetic distance data also support the formation of three distinct clades. The average genetic distance between the northeastern and southeastern clades was 0.056 or 5.6% sequence divergence (Table 3.2). This was much higher than the average distance among populations within either clade. Within the northeastern clade the average distance was 0.018 (1.8%) and within the southeastern clade the average distance was 0.027 (2.7%) (Table 3.2). Similarly, the average distance between the northeastern and western clade is 0.042 (4.2%) and between the southeastern and western clade is 0.055 (5.5%). Within the western clade, the average genetic distance is 0.021 (2.1%) and the populations east and west of the Mississippi River are separated by an average genetic distance of 0.030 (3.0%) (Table 3.2).

F_{ST} values also indicate there is little gene flow among the three clades. The lowest F_{ST} between northeastern and southeastern populations was 0.65 ($p<0.001$) (Table 3.9). Similarly, the smallest distance between the northeastern and western populations was 0.6 ($p=0.002$) and between the southeastern and western populations was 0.65 ($p<0.001$). A similar pattern was seen when populations along the geographic borders of each clade were compared. The F_{ST} between the Sumter North population (northeastern) and Sumter South population (southeastern) was 0.84 ($p=0.017$) over a geographic distance of 20 km. When the Leon population (northeastern) and the Jackson East population (western) were compared, populations separated by a distance of 20 km, the F_{ST} was 0.78 ($p=0.026$). Finally, when populations on opposite sides of the Mississippi River were compared, the smallest F_{ST} was 0.49 ($p=0.023$).

These patterns of divergence among clades are further promoted by an isolation by distance (IBD) effect. The Mantel tests identified a significant positive correlation

between the pairwise F_{ST} and the geographic distances between all population pairs ($r=0.3914$, $p<0.001$). This indicates that as geographic distance increases between populations, so does genetic distance.

Molecular divergence dating

The application of a strict molecular clock was not supported by the likelihood ratio test ($\chi^2=359.33$, df=214, $p<0.001$). Rowe et al. (2006) identified a mutation rate for the control region of $1x10^{-7}$ mutations/site/year based on the fossil record and molecular divergence between *P. leucopus* and *P. maniculatus* (Rowe et al. 2006). Because the control region is highly variable across a range of taxa (Li et al. 1990, Sbisà et al. 1997), I attempted to verify this rate by calculating a mutation rate for *P. gossypinus* using the sequence data from this study and the fossil record. The average percent sequence divergence between *P. gossypinus* and *P. leucopus* over the 784bp of the control region sequenced in this study was 10.9%. Combining this with an estimated fossil divergence for *P. gossypinus* of 150,000 years ago (Pinkham 1968), produced a mutation rate of $3.5x 10^{-7}$ mutations/site/year. To accommodate the discrepancy in rate among closely related taxa, as well as the possibility of divergence among species prior to that indicated by the fossil record, a mean evolutionary rate prior was applied to the data in BEAST which included both estimates of mutation rate in the 95% confidence interval.

BEAST

Using a relaxed molecular clock BEAST indicated that *P. leucopus* and *P. gossypinus* diverged approximately 0.190 mya (CI: 0.154-0.237). The molecular clock places this divergence in the Illinoian and is consistent with the fossil record (McKay and

Berg 2008). The southeastern clade of *P. gossypinus* diverged from all other *P. gossypinus* ~0.128 mya (CI:0.094-0.150). Among remaining populations, the western clade and northeastern clade diverged ~0.091 mya (0.072-0.107). Within the western clade the populations west of the Mississippi diverged ~0.041 mya (0.022-0.063).

The time to the most recent common ancestor (tmrca) for the southeastern group was 0.098 mya (CI: 0.063-0.147); for the northeastern group was 0.066 mya (CI: 0.044-0.107); and for the western group was 0.055 mya (CI: 0.030-0.072). This indicates that the southeastern clade is older than the northeastern clade, which is older than the western clade.

In the southeastern clade, the Key Largo population diverged at a minimum ~0.016 mya (CI: 0.010-0.019) based on the tmrca of all individuals sampled on Key Largo and ~0.021 mya (CI: 0.016-0.023) at a maximum, based on the age of most closely related bifurcating node. In the northeastern clade, the divergence time from the mainland for the Hunting Island population is 0.014 mya (CI: 0.008-0.019); for Ossabaw and St. Catherines Islands is 0.020 mya (CI: 0.010-0.027); and for Jekyll Island is 0.019 mya (CI: 0.010-0.024). These times occur after the LGM ~20,000 years ago, and are associated with a period when marine incursions from melting glacial ice resulted in the isolation of many barrier islands off the southeastern coast (McKay and Berg 2008).

BPP 2.0

Patterns observed in BEAST were generally supported by those obtained from coalescent based methods in BPP with regard to geological time period of divergences. However, dates obtained from BPP were typically more recent than those in BEAST, particularly at deeper nodes. This suggests that estimates in BEAST were affected by

ancestral lineage sorting. Coalescent methods indicate that *P. gossypinus* diverged from *P. leucopus* ~0.152 mya (CI: 0.120-0.187), during the Illinoian Period (McKay and Berg 2008). Within *P. gossypinus*, the southeastern clade diverged from the remainder of the species ~0.102 mya (CI: 0.080-0.121). The populations of the northeastern and western clades diverged from each other ~0.072 mya (0.052-0.090).

Within the western clade, the populations west of the Mississippi River diverged from those populations east of the river ~0.040 mya (0.020-0.060). In the southeastern clade, the population of Key Largo, FL diverged from the mainland portion of the clade ~0.017 (CI: 0.014-0.025). Several island populations in the northeastern clade also diverged from the mainland during this same time period. The Hunting Island, SC population diverged ~0.013 mya (0.009-0.020); the populations of Ossabaw Island and St. Catherines Island diverged ~0.014 mya (0.009-0.017); and the Jekyll Island population diverged ~0.015 mya (0.011-.020). As with BEAST these divergence times occurred during the period following the LGM, during the time when these islands became isolated from the mainland.

Genetic diversity within clades

The southeastern clade showed the highest average genetic distance among populations within a clade at 0.027 (2.7%). Within the northeastern clade the average distance was 0.018 (1.8%) and within the western clade the average distance was 0.021 (2.1%) (Table 3.2). Pairwise genetic distances within the southeastern clade ranged from 0.014 (Collier, FL to Sumter South, FL) to 0.039 (Key Largo, FL to Merritt Island, FL) (Table 3.3). The smallest genetic distance within the northeastern clade was 0.003 (Gates, NC to New Hanover, NC) and the largest was 0.029 (Ossabaw Island, GA to Leon, FL)

(Table 3.4). Genetic distances in the western clade ranged from 0.003 (Jackson West, FL to Jackson East, FL) to 0.054 (Randolph, AL to Panola, TX) (Table 3.5). However, if the Texas population is removed due to its high level of divergence, the largest distance was 0.026 between Randolph, AL and Arkansas, AK. These data demonstrate that the southeastern clade harbors a higher degree of genetic diversity than any other clade, within a much smaller geographic range.

The southeastern clade also contained the populations with the highest within-population genetic distances. Within this clade, three of the five populations with more than one sample had genetic distances of 0.015 or higher: Merritt Island, FL (0.015), Dade, FL (0.019), and Brevard, FL (0.021) (Table 3.6). By comparison, the northeastern clade only had two populations with within-population genetic distances of 0.015 or higher: Clinch, GA (0.016) and Alachua, FL (0.018). Additionally, five populations had genetic distances of zero: Amelia Island, FL, St. Catherines Island, GA, New Hanover, NC, Baker, FL, and Leon, FL. Of these, only the Baker, FL population was represented by less than five individuals (Table 3.7).

Overall, the lowest within-population variation was seen within the western clade, where no population had a genetic distance over 0.01 (Santa Rosa, FL) and all but two populations had genetic distances of 0.005 or lower. Additionally, four populations had genetic distances of zero: Calhoun, AL; Barbour, AL; Jackson East, FL; and Tishomingo, MS. However, less than five individuals were sampled from two of these populations: Calhoun (2) and Jackson (3) (Table 3.8).

Variation within clades was further analyzed by calculating pairwise F_{ST} values between all population pairs within clades. Within the southeastern clade F_{ST} values

ranged from 0 to 0.87. A value of 0 between Collier and Dade indicates that they are one panmictic population. Likewise, a value of 0.08 between Merritt Island and Brevard, FL indicates a high degree of contemporary gene flow between these populations. Significant F_{ST} values within this clade ranged from 0.24 between South Sumter, FL and Dade, FL (p=0.024) to 0.68 between South Sumter and Merritt Island (p=0.001). The population from Key Largo, FL was significantly different from all other populations sampled, including the closest geographic population (Dade, FL) (Table 3.9).

Within the northeastern clade, F_{ST} ranged from 0 to 1, with significant values ranging from 0.10 (Clinch, GA to Glynn, GA, p=0.049) to 1 (Gates, NC to Baker, FL, p=0.018). All F_{ST} values between Gates, NC and other populations were significantly high which may be due to isolation by distance given that Gates is the most northern population and it is distant from all other sampled populations (Table 3.10). Among island populations, the lowest F_{ST} between either St. Catherines or Ossabaw Island and the mainland was 0.58 (Alachua, FL); the lowest F_{ST} between Hunting Island, SC and the mainland was 0.44 (Alachua, FL); and the lowest F_{ST} between Jekyll Island and the mainlandwas 0.65 (Alachua, FL).

When comparing island populations on a temporal scale, the current population on St. Simons Island, GA differed significantly from the population of that island in 1989 (F_{ST}=0.97). The St. Simons Island population in 2009 was least differentiated from Nassau, FL (F_{ST}=0.52). The current population on Amelia Island also is significantly different from the population on that island in 1989 (F_{ST}=0.84). At either time point, the Amelia Island population is least differentiated from Nassau, FL (F_{ST}=0.34).

Finally, F_{ST} values in the western clade ranged from 0.28 (Jackson West, FL to Jackson East, FL) to 1 (several population pairs) (Table 3.11). The large number of significantly high F_{ST} values in this clade indicate a high degree of genetic structuring with low gene flow throughout the region. For the Panola, TX population, the lowest F_{ST} was 0.84 (Santa Rosa, FL) supporting that this group is distinct from all others. The smallest F_{ST} across the Mississippi River was 0.49 between West Baton Rouge LA and Santa Rosa, FL.

Nucleotide and haplotype diversity were calculated as a measure of genetic variation within clades and populations when more than one individual was sampled. The overall nucleotide diversity (π) for all samples was 0.028, and the overall haplotype diversity (h) was 0.991. Within clades, the southeastern group had the highest π (0.031918), followed by the northeastern clade (0.018357), and finally the western clade (0.015760). In contrast, the largest h was in the northeastern clade (0.9845), followed by the western clade (0.9819), and finally the southeastern clade (0.09451) (Table 3.12). The population with the highest π was Dade, FL (0.018552). All samples from this county were collected within the eastern portion of Everglades National Park, suggesting that this population has been well-established and stable for a long time. A π=0 was found in ten populations: the current population of St. Simons Island, GA; the current population of Amelia Island, FL; Baker, FL; St. Catherines Island, GA; New Hanover, NC; Calhoun, AL; Jackson East, FL; Barbour, AL; Tishomingo, MS; and Jefferson, GA. This lack of diversity may have been an artifact of sample size for four of these populations, Baker, Calhoun, Jackson, and Jefferson which were represented by less than five samples (Table 3.12). Consequently, these populations also exhibited no haplotype diversity.

Several populations had an h=1 (St. Johns, FL; Tattnall, GA; Baton Rouge, LA; Lincoln, LA; Panola, TX), but only Panola, TX and Tattnall, GA had n>3. Six individuals were sampled from the Tattnall population, and five were sampled from Panola, all of which represented unique haplotypes. The second highest haplotype diversity was within the Clinch, GA population (0.9732), followed by Chatham, GA (0.9670), and Dade, FL (0.9591). All of these populations were characterized by at least nine samples (Table 3.12).

Population size changes

Tajima's D (-1.594) was not significant, supporting that the control region is evolving neutrally. Because the data did not violate the assumption of neutral evolution, Fu's F_S (F) and mismatch distributions (MM) were calculated to identify recent changes in the demography of clades and populations. MM did not identify signatures of population expansion or contraction in any of the clades, but the northeastern clade (F=-23.69026, p=0.004) and the western clade (F=-10.03604, p=-0.008) had significant F values indicating signatures of population expansion.

One population (Ossabaw Island, GA) showed a significant F value (F=-1.61464, p=0.017). Mismatch distributions identified several populations that significantly differed from expected values based on raggedness indices (r). Three of these populations had multimodal distributions indicating stable populations: Glynn, GA (r=0.186, p=0.02), Camden, GA (r=0.213, p<0.001), and Perry, MS (r=0.363, p=0.03). Another population (Skidaway Island, GA) had a bimodal distribution (r=0.274, p=0.05), suggesting a recent contraction, followed by gene flow from an outside source. Finally, two populations had L-shaped distributions indicating population contractions: Jekyll Island, GA (r=0.189,

p=0.05) and the population from St. Simons Island from 1989 (r=0.787, p=0.03). Several populations could not be tested because MM cannot be performed on populations with only one haplotype.

Discussion

Major mainland clades correspond to established phylogeographic breaks

Several lines of evidence collected in this study support my prediction that there would be geographic structuring of variation among mainland populations of *P. gossypinus* associated with historic barriers to gene flow in other species. Phylogenetic analyses (Figure 3.2, 3.3) identified three major clades (southeastern, northeastern, and western) with boundaries that occur in well established suture zones for other species, as I predicted.

The northern boundary of the southeastern clade occurs in an area of northern peninsular Florida that has been established as a hybrid zone and phylogeographic break for a variety of taxa with differing life history traits (Burbrink et al. 2000, Douglas et al. 2009, Hull et al. 2008, Speller et al. 2010, Walker and Avise 1998). The western boundary of the northeastern clade occurs within the ACF. This region spanning from the Suwannee River in Florida to the Tombigbee River in Alabama has been identified as a suture zone where phylogeographic breaks and secondary contact among divergent sister taxa are abundant (Soltis et al. 2006).

While the ACF has been indicated as a phylogeographic break for a variety of species, most breaks occur across the Apalachicola River (Burbrink et al 2000, Ellsworth et al. 1994, Mylecraine et al. 2004, Swift et al. 1985). In the case of *P. gossypinus*, the break actually occurs east of the river. This appears to be the first instance of a

phylogeographic break in the ACF that does not occur across one of the three rivers of this system. One explanation for this observation is that reduced water flow over the last hundred years may have mitigated the effectiveness of the Apalachicola as a barrier to gene flow. In that time period several dams were built throughout the ACF for water management purposes which have reduced water flow down the Apalachicola River. As recently as 2007, drought conditions resulted in water flow in the river reaching record lows (Corn 2007). It is reasonable to assume that a reduction in water level and flow associated with dams and drought conditions at various times during the 20th and 21st centuries have permitted gene flow across the river. The low genetic distance (0.003) between the populations on opposite sides of the river, and the moderate F_{ST} (0.23) indicate that recent but irregular gene flow has occurred across the river. A pattern of limited dispersal during periods of reduced water flow likely is responsible for the maintaining these patterns.

Additional evidence from minimum spanning networks (Figures 3.10, 3.11, 3.12), genetic distance data, and F_{ST} suggest that these three clades are well differentiated. The average genetic distances between the three major clades are consistent with the amount of variation between closely related species of other *Peromyscus* groups (Castro-Campillo et al. 1999). Genetic distance data also indicate that there is more variation between neighboring clades than within any individual clade. Likewise, populations from different clades that lie in close geographic proximity to each other exhibit higher pairwise genetic distances than between any pair of mainland populations within a clade (Table 3.4). Further, the high F_{ST} values between the northeastern and southeastern

clades, and between the northeastern and western clades indicate low levels of gene flow among the three clades supporting these groups as well defined evolutionary lineages.

A minor clade exists west of Mississippi River but not north of the Savannah River

As predicted, a minor clade formed within the western clade containing all samples west of the Mississippi River (Figure 3.4, 3.5). The average genetic distance between populations east and west of the river (0.030) is more than double the genetic distance between any populations east of the river, providing further support for this grouping. Similarly, gene flow estimates obtained from F_{ST} values between populations on opposite sides of the river also are consistently higher than among populations on either side. These data demonstrate that the Mississippi River is a barrier to gene flow for *P. gossypinus*. These patterns are consistent with those observed in other small terrestrial fauna (Brant and Ortí 2003, Moriarty and Cannatella 2004) and can likely be attributed to the wide breadth of the Mississippi River, which is the widest river in the US.

The data are inconsistent with my prediction that the Savannah River acts as a barrier to gene flow. None of the phylogenetic analyses supported a monophyletic group north of the river (Figure 3.6, 3.7). Further, F_{ST} values and genetic distances between populations on opposite sides of the river were consistent with those among mainland Georgia populations (Tables 3.4 and 3.10). AMOVA data also demonstrate that while the Mississippi River acts as a barrier to gene flow, the Savannah River does not (Table 3.1). This is likely the result of the Savannah River's small breadth and *P. gossypinus*' semi-aquatic nature. The Mississippi and Apalachicola Rivers are several times wider than the Savannah River. In some spots the Savannah River is less than 50 m wide. Because *P. gossypinus* is capable of swimming small to moderate distances, it is likely able to

traverse this distance. As a result, while the Savannah River prevents gene flow for some species (Degner et al. 2010), it does not for *P. gossypinus.*

Florida populations are ancestral

Among mainland clades identified in this study, divergence dating, estimates of genetic diversity and evidence from the fossil record support my prediction that the southeastern clade is ancestral to all others. The oldest known *P. gossypinus* fossils (Ray 1958, Pinkham 1971) were found in Florida and date to ~120,000 ya during the Sangamonian period (Kurten and Anderson 1980). Fossil evidence indicates that by this point *P. gossypinus* were well differentiated from *P. leucopus* and demonstrated morphological characters consistent with contemporary populations. This suggests that *P. gossypinus* and *P. leucopus* likely diverged earlier than this period, and is consistent with molecular divergence estimates obtained using both BEAST and BPP. These estimates indicate that the divergence occurred well within the Illinoian period. During this time polar ice had advanced into the continental United States, likely resulting in populations of the *P. leucopus* species group occupying separate refugia and subsequently diverging into distinct species (McKay and Berg 2008).

Divergence estimates indicate that the southeastern clade diverged ~102,000 ya. This date corresponds with the Sangamonian interglacial period. During this time period, populations of cotton mice in peninsular Florida likely became isolated from those on the mainland, as rising sea levels resulted in flooding of portions of peninsular Florida. This resulted in the separation of populations in southern Florida from those in the mainland United States. While inhabiting this refuge, the peninsular Florida populations diverged from mainland populations to form the oldest of the three clades (98,000 years old).

Molecular divergence estimates indicate that the northeastern group is the second oldest clade (~65000 ya). This timing also is supported by both the nucleotide and haplotype diversity data which indicate that the northeastern clade is more diverse than the western clade. Coalescent based estimates place the divergence between the northeastern and western groups in the first half of the Wisconsinan, during the Tahoe glaciation (Schaffer 1997). While the divergence between these groups occurred ~72,000 ya, the tmrca for populations in the western clade was ~55,000 ya. This large difference suggests that the oldest lineages of *P. gossypinus* in the western clade went extinct at some point; a suggestion that is further supported by the results of Fu's F_S which indicate that the western clade went through a recent population expansion.

Island populations are Wisconsinan vicariants

Within the southeastern and northeastern clades, several island populations exhibit patterns associated with late Pleistocene glacial events. These data support my prediction that many of these populations represent vicariants from the LGM. Within the southeastern clade, phylogenetic analyses and minimum spanning networks indicate that the population on Key Largo, FL forms a genetically distinct group relative to all other populations. The smallest pairwise genetic distance for this population is 2.2% with nearby Dade, FL indicating that the island population has been isolated from the mainland for a long time. Similarly, significantly high F_{ST} values with all other populations throughout the southeastern clade indicate a lack of contemporary gene flow and long term isolation.

Molecular divergence dating supports that the Key Largo population diverged from the mainland ~17,000 ya using coalescent based methods and between 16,000 and

21,000 ya using BEAST. This time period marks the end of the Pleistocene and the beginning of the Holocene. At this time, the ice sheet associated with Pleistocene glaciations receded and the resulting rise in sea level caused numerous marine incursions in the southeastern US (McKay and Berg 2008). These incursions resulted in the isolation of organisms on barrier islands throughout the region. Key Largo is one such barrier islands; as such this population is well supported as a Wisconsinan vicariant.

Within the northeastern clade four islands show a similar pattern to that of Key Largo. Phylogenetic analyses indicate that these islands form three distinct monophyletic groups: Hunting Island, Ossabaw/St. Catherines Island, and Jekyll Island. Estimates of genetic distance for each of these island populations indicate that they are highly diverged from the mainland (Hunting Island: 1.8%; Ossabaw/St. Catherines Island: 2.5%; Jekyll Island: 1.9%). These distances are consistent with the amount of genetic distance between divergent subspecies identified in several other taxa (Castro-Campillo et al. 1999, Koh et al. 2000, Shipp-Pennock et al. 2005, Thomas et al. 1990, Indorf 2010). This suggests that these island populations have been isolated from the mainland for an extended period of time.

Estimates of contemporary gene flow based on F_{ST} values also indicate that the island populations are not experiencing gene flow with any mainland populations. The lowest F_{ST} for Hunting Island was 0.45 with Chatham, GA, which is consistent with the F_{ST} between Key Largo and Dade, FL. Likewise the smallest FST for either St. Catherines Island or Ossabaw Island was 0.58 between St. Catherines Island and Alachua, FL. The smallest F_{ST} for Jekyll Island was 0.65, also with Alachua, FL. Interestingly, while they formed a distinct group in the phylogenetic analysis there is no

contemporary gene flow between St. Catherines Island and Ossabaw Island as indicated by a markedly high F_{ST} (0.99) and the presence of a 27-bp insertion in the Ossabaw Island population that is not found anywhere else.

Coalescent based molecular divergence dating for each of the island groups supports their long term isolation from the mainland and indicates that the populations on these islands represent Wisconsinan vicariants which became isolated on the islands following the LGM. The divergence time between the Hunting Island population and the mainland is ~13,000 ya. The populations from St. Catherines Island and Ossabaw Island diverged from the mainland ~14,000 years ago. Finally, the population from Jekyll Island also became isolated from the mainland for ~15,000 years ago. The divergence times for each of these islands are consistent with the timing of the marine incursions that occurred at the end of the Pleistocene as a result of receding polar ice.

The results of this study indicate strong geographic structuring of genetic diversity throughout the species' range. On the mainland several lines of evidence indicate this structuring is well defined by a series of suture zones associated with Pleistocene geological events. Further, the differentiation of each major clade is associated with a Pleistocene glaciation or interglacial event. Likewise, the origin of several island populations of *P. gossypinus* can be traced to marine incursions that occurred at the end of the Pleistocene and the start of the Holocene. Together, these results support my hypothesis that historic climatic and geographic events resulted in geographic structuring of genetic variation in *P. gossypinus*.

Conclusions

This is the first study to explore the phylogeography of *P. gossypinus* using DNA sequence data. Previous work on this species has relied exclusively on morphological data with one exception (Boone et al. 1999). Utilizing allozyme markers, Boone et al. identified several unique alleles within populations and limited large scale differentiation. Allozymes represent a small part of the underlying genetic variation within a species (Graur and Li, 2000), and their diversity is rarely sufficient to identify intraspecific level variation (Avise 2000, Boone 1995).

This study demonstrated that the genetic diversity of *P. gossypinus* has been shaped by a variety of events throughout the Pleistocene period, including the advance and retreat of glacial ice, rise in sea level, and formation of rivers. As a result, three major clades are present within this species, each associated with different glacial refugia. On a finer level, large scale genetic differentiation was identified across small geographic ranges of less than 20 km.

The results of this study also provided support for the possibility that phylogeographic breaks may occur along suture zones in the absence of physical barriers to gene flow. Specifically, *P. gossypinus* provides evidence of the suture zone in peninsular Florida first identified by Remington (1968). Additionally, a previously unknown phylogeographic break was identified east of the Apalachicola River in the Florida panhandle. Future studies in this area should seek to identify if the reduction of water flow in the Apalachicola has resulted in the eastward migration of *P. gossypinus* from the western clade. If not, the Apalachicola likely never served as a barrier to gene

flow in this species, and research is needed to identify what forces are maintaining this phylogeographic break.

A thorough understanding of a species' phylogeography is valuable from both an evolutionary and conservation point of view. Phylogeographic studies provide information about how historic climatic and geographic events have affected the genetic diversity of groups of organisms. Understanding the patterns of genetic diversity within a species can inform decisions on how best to conserve that genetic diversity. This is of particular consequence given the limited funding available for conservation, and the widespread observation of anthropogenic effects on a variety of taxa. Moreover, this study serves to highlight an important fact; that a species' phylogeography does not exist in a static state. Rather, it is constantly in flux responding to changes in climate and geography, which may be the result of either natural or anthropogenic events.

Table 3.1 Analysis of molecular variance (AMOVA) for each set of population partitions.

A. partitioned into Clades southeastern, northeastern, and western clades.
B. additional partition of western clade at the Mississippi River
C. additional partition of the northeastern clade at the Savannah River

A)

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance
Among groups	2	2509.824	10.54044 Va	58.24
Among populations within groups	53	2367.222	4.85978 Vb	26.85
Within populations	447	1205.400	2.69664 Vc	14.90
Total	502	6082.445	18.09686	

B)

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance
Among groups	3	2593.183	10.67404 Va	58.98
Among populations within groups	52	2283.862	4.72796 Vb	26.12
Within populations	447	1205.400	2.69664 Vc	14.90
Total	502	6082.445	18.28587	

C)

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance
Among groups	4	2686.786	9.19778 Va	55.66
Among populations within groups	51	2190.260	4.63107 Vb	28.02
Within populations	447	1264.404	2.69664 Vc	16.32
Total	502	6082.445	16.52550	

Table 3.2. Average Tamura Nei (Tamura and Nei 1983) genetic distances, reported as percent divergence with standard error (computed by 5000 bootstrap replicates), between and within all geographic regions

Population(s)	**Genetic Distance**
Northeast to Southeast	5.6% ± 0.9%
Northeast to West	4.2% ± 0.7%
Southeast to West	5.5% ± 1.1%
E of Mississippi River to West of Mississippi River	3.0% ± 0.6%
Within Populations	
Northeast	1.8% ± 0.3%
Southeast	2.7% ± 0.5%
West	2.1% ± 0.28%

Table 3.3. Tamura Nei corrected population pairwise genetic distances between all population pairs of *P. gossypinus* in the southeastern clade. Highest and lowest values are in bold. Population acronyms are: Dade, FL (DAD); Collier, FL (COL); Key Largo, FL (KLF); Brevard, FL (BRE); Merritt Island, FL (MER); South Sumter, FL (SSF).

	DAD	COL	KLF	BRE	MER	SSC
DAD						
COL	1.6					
KLF	2.2	1.6				
BRE	3.2	3.4	3.4			
MER	3.6	3.9	3.9	1.9		
SSF	1.8	**1.4**	3.3	3.5	**4.0**	

Table 3.4. Tamura Nei corrected population pairwise genetic distances between all population pairs of *P. gossypinus* in the northeastern clade. Population acronyms are: Glynn, GA (GLY); Chatham, GA (CHA); Camden, GA (CAM); Jekyll Island, GA (JEK); St. Simons Island, GA 2009 (SS9); Charleston, SC (CSC); Hunting Island, SC (HUN); Cumberland Island, GA (CUM); Amelia Island, FL 2009 (AM9); Ossabaw Island, GA (OSS); St. Catherines Island, GA (SCI); Sapelo Island, GA (SAP); St. Simons Island, GA 1989 (SS8); Skidaway Island, GA (SKI); New Hanover, NC (NHN); Nassau, FL (NAS); Alachua, FL (ALA); Clinch, GA (CLI); Baker, FL (BAK); Levy, FL (LEV); North Sumter, FL (NSF); Marion, FL (MAR); Citrus, FL (CIT); St. Johns, FL (SJF); Amelia Island, FL 1989 (AM8); Leon, FL (LEO); McDuffie, GA (MCD); Gates, NC (GAT); Tattnall, GA (TAT); Emanuel, GA (EMA); Jefferson, GA (JEF). Highest and lowest values are in bold.

	GLY	CHA	CAM	JEK	SS9	CSC	HUN	CUM	AM9	OSS	SCI	SAP	SS8	SKI	NHN	NAS
GLY																
CHA	1.4															
CAM	1.2	1.4														
JEK	1.9	1.8	1.8													
SS9	1.5	1.3	1.7	2												
CSC	1.4	1.3	1.5	1.2	1.2											
HUN	1.9	1.4	1.8	1.5	1.5	1.5										
CUM	1.7	1.2	1.7	1.9	0.8	1.4	1.7									
AM9	0.9	1.5	1.1	2.2	1.9	1.5	2.1	2.1								
OSS	2.4	2.4	2.5	1.7	2.4	1.7	2	2.6	2.3							
SCI	1.8	1.9	1.9	1.7	1.8	1.4	1.7	2	1.6	1.1						
SAP	1.7	1.1	1.5	1.7	1.1	1.3	1.5	0.9	1.9	2.4	1.9					
SS8	2	1.6	1.8	0.5	2.1	1.3	1.4	1.9	2.3	1.8	1.8	1.7				
SKI	2.2	1.7	1.9	2	2.1	1.8	1.7	1.9	2.5	2.3	2.1	1.5	1.8			
NHN	2.1	1.7	1.9	1.5	2.4	1.5	1.6	2.2	2.3	2.1	1.6	1.8	1.3	1.5		
NAS	1.3	1.6	1.5	2	1.4	1.4	1.9	1.6	1.2	2.3	1.8	1.7	2.1	2.3	2.3	
ALA	1.7	1.8	1.8	1.8	1.7	1.5	1.9	1.8	1.7	2.3	1.9	1.9	1.9	2.3	2.3	1.7
CLI	1.5	1.5	1.5	2	1.5	1.4	1.8	1.6	1.4	2.3	1.7	1.6	2	1.9	2.1	1.5
BAK	2.1	1.7	1.9	1.8	1.3	1.5	1.9	1.3	2.1	1.9	1.4	1.4	1.9	1.5	1.9	1.8
LEV	1.2	1.5	1.3	1.9	1.8	1.4	1.9	1.7	0.9	2.4	1.8	1.7	2	2.2	2.1	1.2
NSF	1.4	1.6	1.6	1.9	1.3	1.3	1.7	1.5	1.2	2.2	1.6	1.6	2	2.4	2.3	1.3
MAR	1.2	1.6	1.4	2.1	1.9	1.5	2	1.9	0.6	2.2	1.6	1.9	2.2	2.5	2.3	1.2
CIT	1.5	1.7	1.8	2.1	1.3	1.4	2	1.4	1.5	2.4	2	1.8	2.2	2.4	2.4	1.4
SJF	1.4	1.5	1.5	1.9	1.3	1.3	1.9	1.4	1.5	2.4	1.8	1.5	2	2.2	2.2	1.5
AM8	1.3	1.8	1.5	2.4	2.1	1.7	2.3	2.1	0.8	2.8	2.1	2.1	2.5	2.8	2.6	1.5
LEO	1.8	2	1.9	2	2	1.7	2.1	2	1.7	2.9	2.2	2.1	2.1	2.6	2.5	1.9
MCD	1.3	0.9	1.3	1.4	0.6	0.9	1	0.7	1.5	2	1.4	0.7	1.4	1.6	1.7	1.3
GAT	2.2	1.7	2	1.4	2.4	1.4	1.6	2.2	2.3	2.1	1.6	1.9	1.2	1.5	**0.3**	2.3
TAT	1.7	1.8	1.7	2.3	2.1	1.8	2.1	2.1	1.4	2.7	2.4	1.9	2.4	2.5	2.5	1.8
EMA	1.3	1.3	1.5	2.2	1.1	1.5	1.8	1.3	1.2	2.7	2	1.6	2.2	2.3	2.5	1.4
JEF	2.1	1.9	2.2	2.4	1.5	1.8	2.1	1.6	2.4	**2.9**	2.2	1.7	2.6	2.7	**2.9**	2.1

	ALA	CLI	BAK	LEV	NSF	MAR	CIT	SJF	AM8	LEO	MCD	GAT	TAT	EMA	JEF
ALA															
CLI	1.8														
BAK	1.9	1.4													
LEV	1.7	1.5	2.2												
NSF	1.7	1.5	1.8	1											
MAR	1.8	1.5	2.1	0.5	0.9										
CIT	1.9	1.7	1.8	1.3	1.3	1.4									
SJF	1.7	1.5	1.6	1.5	1.5	1.6	1.6								
AM8	2.1	1.8	2.6	1.3	1.6	1.3	1.6	1.8							
LEO	2.1	1.9	2.2	1.6	1.8	1.7	2	1.8	2.3						
MCD	1.4	1.2	1.1	1.4	1.1	1.5	1.3	1.1	1.7	1.6					
GAT	2.3	2.1	1.9	2.1	2.3	2.3	2.3	2.3	2.6	2.3	1.7				
TAT	2	1.8	2.3	1.8	1.9	1.8	2.1	1.9	1.7	2	1.6	2.4			
EMA	1.8	1.5	1.5	1.6	1.4	1.7	1.3	1.4	1.5	2	0.9	2.5	2		
JEF	2.3	2.1	2.1	2.2	1.9	2.4	2.1	1.9	2	2.7	1.1	**2.9**	1.7	1.8	

Table 3.5. Tamura Nei corrected population pairwise genetic distances between all population pairs of *P. gossypinus* in the western clade. Population acronyms are: Tuscaloosa AL (TUS); Sharkey, MS (SHA); Santa Rosa, FL (SRF); Perry, MS (PER); Calhoun, AL (CAL); West Jackson, FL (WJF); East Jackson, FL (EJF); Barbour, AL (BAR); Tishomingo, MS (TIS); Randolph, AL (RAN); Baton Rouge, LA (BRL); Panola, TX (PAN); Lincoln, LA (LIN); Arkansas, AK (ARK); Obion, TN (OBI); Haywood, TN (HAY). Highest and lowest values are in bold.

	TUS	SHA	SRF	PER	CAL	WJF	EJF	BAR	TIS	RAN	BRL	PAN	LIN	ARK	OBI	HAY
TUS																
SHA	1.1															
SRF	1.4	1.3														
PER	0.9	0.7	1.2													
CAL	0.8	1.4	1.7	1.1												
WJF	1.3	1.3	1.3	1.2	1.4											
EJF	1.3	1.1	1	1	1.4	**0.3**										
BAR	0.5	0.9	1.2	0.6	0.8	1.1	1									
TIS	0.9	0.7	1.1	0.4	1	1.2	0.9	0.7								
RAN	1	1.8	2	1.5	1.1	1.9	1.9	0.8	1.3							
BRL	2	2	1.7	1.6	1.9	2.2	1.9	1.9	1.3	2.5						
PAN	4.8	5.1	4.9	4.9	5	4.9	4.8	4.6	4.6	**5.4**	3.8					
LIN	1.9	2.1	1.9	1.8	1.6	2.3	2.1	2	1.5	2.4	0.7	4				
ARK	2.1	2.2	2.2	1.9	1.7	2.5	2.3	2.3	1.7	2.6	0.9	4.4	0.5			
OBI	1.7	1.7	1.7	1.5	1.5	1.8	1.5	1.5	1	2.1	1.6	4.7	1.6	1.9		
HAY	1.6	1.7	1.7	1.5	1.1	1.8	1.6	1.4	1.1	2	1.7	4.6	1.7	2	0.5	

Table 3.6. Average Tamura Nei (Tamura and Nei 1983) genetic distances, reported as percent divergence with standard error (computed by 5000 bootstrap replicates), within populations of *P. gossypinus* in the southeastern clade. Distances were only calculated within populations with more than one individual. Number of individuals are indicated in parentheses.

Population	Genetic Distance
Dade, FL (23)	1.9% ± 0.3%
Key Largo, FL (20)	0.3% ± 0.1%
Brevard, FL (5)	2.1% ± 0.4%
Merritt Island, FL (23)	1.5% ± 0.3%
South Sumter, FL (3)	0.2% ± 0.1%

Table 3.7. Average Tamura Nei (Tamura and Nei 1983) genetic distances, reported as percent divergence with standard error (computed by 5000 bootstrap replicates), within populations of *P. gossypinus* in the northeastern clade. Distances were only calculated within populations with more than one individual. Number of individuals are indicated in parentheses.

Population	Genetic Distance
Glynn, GA (20)	1.1% ± 0.3%
Chatham, GA (16)	1.1% ± 0.3%
Camden, GA (37)	1.0% ± 0.3%
Jekyll Island, GA (39)	0.1% ± 0.1%
St. Simons Island, GA 2009 (12)	0.0% ± 0.0%
St. Simons Island, GA 1989 (12)	0.1% ± 0.1%
Charleston, SC (6)	0.4% ± 0.2%
Hunting Island, SC (10)	0.4% ± 0.2%
Cumberland Island, GA (44)	0.4% ± 0.1%
Amelia Island, FL 2009 (10)	0.0% ± 0.0%
Amelia Island, FL 1989 (5)	0.4% ± 0.2%
Ossabaw Island, GA (21)	0.0% ± 0.0%
St. Catherines Island, GA (15)	0.0% ± 0.0%
Sapelo Island, GA (17)	0.6% ± 0.2%
Skidaway Island, GA (10)	0.9% ± 0.3%
New Hanover, NC (10)	0.0% ± 0.0%
Nassau, FL (15)	1.4% ± 0.3%
Alachua, FL (10)	1.8% ± 0.3%
Clinch, GA (11)	1.6% ± 0.3%
Baker, FL (2)	0.0% ± 0.0%
Levy, FL (7)	0.5% ± 0.2%
North Sumter, FL (5)	1.2% ± 0.3%
Citrus, FL (3)	1.2% ± 0.3%
St. Johns, FL (11)	1.4% ± 0.3%
Leon, FL (4)	0.0% ± 0.0%
McDuffie, GA (4)	0.3% ± 0.2%
Gates, NC (3)	0.1% ± 0.1%
Tattnall, GA (6)	0.9% ± 0.2%
Emanuel, GA (4)	0.5% ± 0.2%
Jefferson, GA (2)	0.0% ± 0.0%

Table 3.8. Average Tamura Nei (Tamura and Nei 1983) genetic distances, reported as percent divergence with standard error (computed by 5000 bootstrap replicates), within populations of *P. gossypinus* in the western clade. Distances were only calculated within populations with more than one individual. Number of individuals are indicated in parentheses.

Population	Genetic Distance
Tuscaloosa, AL (10)	0.5% ± 0.2%
Sharkey, MS (4)	0.8% ± 0.3%
Santa Rosa, FL (10)	1.0% ± 0.2%
Perry, MS (10)	0.3% ± 0.1%
Calhoun, AL (2)	0.0% ± 0.0%
West Jackson, FL (4)	0.4% ± 0.2%
East Jackson, FL (3)	0.0% ± 0.0%
Barbour, AL (5)	0.0% ± 0.0%
Tishomingo, MS (5)	0.0% ± 0.0%
Baton Rouge, LA (2)	0.3% ± 0.2%
Panola, TX (4)	0.4% ± 0.2%
Lincoln, LA (4)	0.5% ± 0.2%
Arkansas, AK (2)	0.1% ± 0.1%
Obion, TN (3)	0.1% ± 0.1%
Haywood, TN (2)	0.3% ± 0.2%

Table 3.9. Population pairwise F_{ST} between all population pairs of *P. gossypinus* in the southeastern clade. Significant values ($p<0.5$) are indicated by bold numbers. Negative values have been changed to zero. Population acronyms are: Dade, FL (DAD); Collier, FL (COL); Key Largo, FL (KLF); Brevard, FL (BRE); Merritt Island, FL (MER); South Sumter, FL (SSF).

	DAD	BRE	MER	COL	SSF	KLF
DAD						
BRE	**0.40**					
MER	**0.54**	0.08				
COL	0.00	0.39	0.62			
SSF	**0.24**	**0.60**	**0.68**	0.87		
KLF	**0.49**	**0.81**	**0.76**	**0.85**	**0.84**	

Table 3.10. Population pairwise F_{ST} between all population pairs of *P. gossypinus* in the northeastern clade. Significant values ($p<0.5$) are indicated by bold numbers. Negative values have been changed to zero. Population acronyms are: Glynn, GA (GLY); Chatham, GA (CHA); Camden, GA (CAM); Jekyll Island, GA (JEK); St. Simons Island, GA 2009 (SS9); Charleston, SC (CSC); Hunting Island, SC (HUN); Cumberland Island, GA (CUM); Amelia Island, FL 2009 (AM9); Ossabaw Island, GA (OSS); St. Catherines Island, GA (SCI); Sapelo Island, GA (SAP); St. Simons Island, GA 1989 (SS8); Skidaway Island, GA (SKI); New Hanover, NC (NHN); Nassau, FL (NAS); Alachua, FL (ALA); Clinch, GA (CLI); Baker, FL (BAK); Levy, FL (LEV); North Sumter, FL (NSF); Marion, FL (MAR); Citrus, FL (CIT); St. Johns, FL (SJF); Amelia Island, FL 1989 (AM8); Leon, FL (LEO); McDuffie, GA (MCD); Gates, NC (GAT); Tattnall, GA (TAT); Emanuel, GA (EMA); Jefferson, GA (JEF).

	GLY	SKI	CHA	CAM	SS9	NAS	BAK	ALA	LEV	SJF	CLI	MCD	TAT	EMA	JEF
GLY															
SKI	**0.55**														
CHA	**0.23**	**0.40**													
CAM	**0.16**	**0.50**	**0.24**												
SS9	**0.61**	**0.80**	**0.55**	**0.61**											
NAS	0.07	**0.51**	**0.24**	**0.25**	**0.52**										
BAK	**0.57**	**0.51**	**0.47**	**0.56**	**1.00**	**0.44**									
ALA	**0.17**	**0.42**	**0.19**	**0.28**	**0.51**	0.07	**0.26**								
LEV	**0.28**	**0.67**	**0.43**	**0.36**	**0.89**	**0.17**	**0.80**	**0.28**							
SJF	**0.21**	**0.44**	**0.22**	**0.27**	**0.57**	**0.15**	0.23	0.04	**0.38**						
CLI	**0.10**	**0.37**	**0.11**	**0.14**	**0.54**	0.03	0.23	0.02	**0.23**	0.06					
MCD	**0.37**	**0.57**	0.11	**0.38**	**0.90**	**0.25**	0.81	0.13	**0.69**	0.16	0.13				
TAT	**0.40**	**0.65**	**0.45**	**0.44**	**0.86**	**0.36**	**0.71**	**0.30**	**0.62**	**0.37**	**0.28**	**0.61**			
EMA	**0.34**	**0.66**	**0.34**	**0.44**	**0.90**	**0.27**	0.78	**0.28**	**0.68**	**0.31**	**0.21**	**0.60**	**0.65**		
JEF	**0.57**	**0.72**	**0.52**	**0.61**	**1.00**	**0.49**	1.00	**0.37**	**0.81**	**0.42**	**0.40**	0.82	**0.60**	0.81	
SAP	**0.50**	**0.51**	**0.23**	**0.44**	**0.69**	**0.46**	**0.62**	**0.40**	**0.67**	**0.40**	**0.35**	**0.32**	**0.65**	**0.64**	**0.69**
CUM	**0.64**	**0.73**	**0.49**	**0.61**	**0.66**	**0.58**	**0.74**	**0.58**	**0.77**	**0.58**	**0.57**	**0.52**	**0.79**	**0.70**	**0.79**
LEO	**0.23**	**0.49**	**0.17**	**0.30**	**0.68**	0.16	0.40	0.08	**0.48**	0.07	0.03	0.16	**0.45**	**0.31**	0.50
JEK	**0.76**	**0.84**	**0.75**	**0.69**	**0.95**	**0.74**	**0.93**	**0.65**	**0.90**	**0.73**	**0.74**	**0.90**	**0.89**	**0.93**	**0.95**
SS8	**0.65**	**0.72**	**0.58**	**0.60**	**0.97**	**0.62**	**0.93**	**0.50**	**0.85**	**0.57**	**0.59**	**0.87**	**0.83**	**0.90**	**0.95**
CLA	0.03	**0.55**	**0.30**	0.18	**0.84**	0.01	0.56	0.07	**0.36**	0.10	0.00	**0.46**	**0.40**	**0.37**	0.63
PUT	**0.33**	**0.64**	**0.28**	**0.43**	**0.67**	**0.25**	0.66	**0.23**	**0.61**	**0.26**	**0.19**	**0.32**	**0.60**	**0.50**	**0.68**
CIT	**0.28**	**0.59**	**0.34**	**0.43**	**0.84**	0.06	0.59	0.15	**0.44**	0.22	0.12	**0.48**	**0.53**	**0.39**	0.64
AM8	**0.38**	**0.74**	**0.52**	**0.45**	**0.95**	**0.34**	0.89	**0.40**	**0.66**	**0.47**	**0.36**	**0.81**	**0.63**	**0.72**	**0.86**
AM9	**0.30**	**0.80**	**0.57**	**0.39**	**1.00**	**0.34**	**1.00**	**0.43**	0.75	**0.53**	**0.35**	**0.94**	**0.74**	**0.88**	**1.00**
MAR	0.12	0.63	0.33	0.29	1.00	0.00	1.00	0.00	0.00	0.14	0.00	0.82	0.52	0.72	1.00
NSF	**0.16**	**0.57**	**0.27**	**0.32**	**0.71**	0.00	**0.49**	0.07	**0.17**	**0.16**	0.04	0.28	**0.45**	**0.38**	0.52
SCI	**0.68**	**0.81**	**0.70**	**0.65**	**1.00**	**0.63**	**1.00**	**0.58**	**0.90**	**0.65**	**0.60**	**0.96**	**0.89**	**0.95**	**1.00**
OSS	**0.77**	**0.84**	**0.79**	**0.74**	**1.00**	**0.73**	**0.99**	**0.68**	**0.93**	**0.76**	**0.73**	**0.97**	**0.91**	**0.96**	**0.99**
HUN	**0.60**	**0.64**	**0.45**	**0.56**	**0.87**	**0.53**	**0.82**	**0.44**	**0.77**	**0.53**	**0.46**	**0.65**	**0.73**	**0.77**	**0.84**
CSC	**0.39**	**0.61**	**0.35**	**0.45**	**0.89**	**0.32**	**0.78**	**0.20**	**0.66**	**0.25**	**0.25**	**0.57**	**0.65**	**0.71**	**0.81**
NHN	**0.70**	**0.69**	**0.63**	**0.64**	**1.00**	**0.68**	**1.00**	**0.61**	**0.90**	**0.65**	**0.63**	**0.96**	**0.87**	**0.95**	**1.00**
GAT	**0.61**	**0.55**	**0.50**	**0.58**	**0.99**	**0.56**	0.97	**0.43**	**0.82**	**0.46**	**0.46**	**0.88**	**0.74**	**0.88**	0.98

	SAP	CUM	LEO	JEK	SS8	CLA	PUT	CIT	AM8	AM9	MAR	NSF	SCI	OSS	HUN	CSC	EDI	GAT
SAP																		
CUM	**0.52**																	
LEO	**0.39**	**0.54**																
JEK	**0.83**	**0.86**	**0.83**															
SS8	**0.76**	**0.83**	**0.72**	**0.74**														
CLA	**0.59**	**0.75**	0.19	**0.87**	**0.79**													
PUT	**0.47**	**0.52**	0.20	**0.89**	**0.83**	**0.44**												
CIT	**0.59**	**0.66**	0.21	**0.90**	**0.83**	0.24	**0.34**											
AM8	**0.75**	**0.83**	**0.57**	**0.94**	**0.91**	**0.45**	**0.72**	**0.60**										
AM9	**0.80**	**0.86**	**0.71**	**0.95**	**0.96**	**0.51**	**0.81**	**0.80**	**0.84**									
MAR	0.69	0.81	0.23	0.94	0.93	0.11	0.60	0.16	0.71	1.00								
NSF	**0.51**	**0.64**	0.17	**0.85**	**0.74**	0.12	0.28	0.06	**0.48**	**0.59**	0.00							
SCI	**0.82**	**0.86**	**0.82**	**0.95**	**0.96**	**0.84**	**0.87**	**0.90**	**0.96**	**1.00**	1.00	**0.78**						
OSS	**0.87**	**0.90**	**0.88**	**0.95**	**0.96**	**0.90**	**0.92**	**0.93**	**0.97**	**1.00**	0.99	**0.86**	**0.99**					
HUN	**0.67**	**0.78**	**0.61**	**0.87**	**0.80**	**0.69**	**0.72**	**0.73**	**0.84**	**0.89**	0.81	**0.62**	**0.89**	**0.91**				
CSC	**0.58**	**0.74**	**0.34**	**0.86**	**0.81**	**0.45**	**0.49**	**0.51**	**0.77**	**0.88**	0.72	**0.39**	**0.91**	**0.93**	**0.73**			
NHN	**0.80**	**0.87**	**0.82**	**0.94**	**0.94**	**0.86**	**0.89**	**0.90**	**0.96**	**1.00**	1.00	**0.82**	**1.00**	**0.99**	**0.87**	**0.90**		
GAT	**0.73**	**0.85**	0.60	**0.91**	**0.88**	0.69	**0.81**	0.73	**0.90**	**0.99**	0.96	**0.65**	**0.99**	**0.99**	**0.80**	**0.78**	**0.94**	

Table 3.11. Population pairwise F_{ST} between all population pairs of *P. gossypinus* in the western clade. Significant values ($p<0.5$) are indicated by bold numbers. Negative values have been changed to zero. Population acronyms are: Tuscaloosa AL (TUS); Sharkey, MS (SHA); Santa Rosa, FL (SRF); Perry, MS (PER); Calhoun, AL (CAL); West Jackson, FL (WJF); East Jackson, FL (EJF); Barbour, AL (BAR); Tishomingo, MS (TIS); Randolph, AL (RAN); Baton Rouge, LA (BRL); Panola, TX (PAN); Lincoln, LA (LIN); Arkansas, AK (ARK); Obion, TN (OBI); Haywood, TN (HAY). Highest and lowest values are in bold.

	SRF	PER	JAW	JAE	TIS	TUS	HAY	OBI	SHA	CAL	RAN	BAR	ARK	LIN	BAT	PAN
SRF																
PER	**0.41**															
WJF	**0.37**	**0.71**														
EJF	**0.31**	**0.76**	0.28													
TIS	**0.39**	**0.50**	**0.83**	**1.00**												
TUS	**0.46**	**0.55**	**0.64**	**0.70**	**0.61**											
HAY	**0.49**	**0.79**	0.80	0.94	0.94	**0.70**										
OBI	**0.54**	**0.82**	**0.85**	0.97	**0.96**	**0.75**	0.67									
SHA	**0.29**	**0.30**	**0.54**	**0.56**	**0.43**	**0.45**	0.62	**0.70**								
CAL	**0.51**	**0.77**	0.79	1.00	1.00	**0.47**	0.88	0.96	0.58							
RAN	0.48	0.79	0.79	1.00	1.00	0.51	0.87	0.96	0.56	1.00						
BAR	**0.45**	**0.68**	**0.84**	**1.00**	**1.00**	**0.33**	**0.96**	**0.98**	0.61	**1.00**	1.00					
ARK	**0.66**	**0.87**	**0.90**	**0.98**	**0.97**	**0.81**	0.93	**0.95**	**0.80**	0.96	0.97	**0.98**				
LIN	**0.54**	**0.79**	**0.80**	**0.86**	**0.83**	**0.74**	0.73	**0.79**	**0.68**	0.76	0.78	**0.89**	**0.38**			
BAT	**0.49**	**0.81**	0.84	0.95	0.94	**0.77**	0.84	0.91	0.67	0.93	0.89	**0.97**	0.85	0.34		
PAN	**0.83**	**0.93**	**0.92**	**0.95**	**0.96**	**0.90**	0.92	0.94	**0.88**	0.94	0.92	**0.96**	**0.94**	**0.89**	0.90	

Table 3.12. Nucleotide diversity and haplotype diversity for all populations of *P. gossypinus* with more than one individual and more than one haplotype. Number of haplotypes in the population are indicated in parentheses.

Population	Nucleotide Diversity	Haplotype Diversity
Glynn, GA (7)	0.011	0.825
Chatham, GA (11)	0.011	0.967
Camden, GA (9)	0.009	0.779
Jekyll Island, GA (5)	0.001	0.595
St. Simons Island, GA 1989 (2)	0.002	0.5333
Charleston, SC (2)	0.004	0.600
Hunting Island, SC (6)	0.004	0.894
Cumberland Island, GA (10)	0.004	0.872
Amelia Island, FL 1989 (4)	0.004	0.900
Ossabaw Island, GA (3)	0.001	0.242
Sapelo Island, GA (3)	0.006	0.544
Skidaway Island, GA (4)	0.008	0.733
Nassau, FL (11)	0.013	0.967
Alachua, FL (7)	0.017	0.910
Clinch, GA (8)	0.015	0.973
Putnam, FL (3)	0.006	0.524
Clay, FL (2)	0.010	0.500
North Sumter, FL (4)	0.012	0.900
Citrus, FL (2)	0.011	0.556
St. Johns, FL (8)	0.015	1.00
Leon, FL (3)	0.012	0.833
McDuffie, GA (2)	0.003	0.667
Gates, NC (2)	0.001	0.667
Tattnall, GA (6)	0.008	1.00
Emanuel, GA (2)	0.667	0.005
Perry, MS (4)	0.003	0.806
Dade, FL (13)	0.019	0.9591
Key Largo, FL (4)	0.003	0.439
Brevard, FL (5)	0.019	1.00
Merritt Island, FL (9)	0.014	0.844
Levy, FL (6)	0.005	0.952
South Sumter, FL (2)	0.002	0.667
Tuscaloosa, AL (4)	0.005	0.822
Sharkey, MS (3)	0.008	0.833
Santa Rosa, FL (8)	0.010	0.956
West Jackson, FL (3)	0.004	0.833
Lincoln, LA (4)	0.081	1.00
Baton Rouge, LA (2)	0.002	1.00
Panola, TX (4)	0.004	1.00
Haywood, TN (2)	0.003	1.00
Obion, TN (2)	0.001	0.667
Arkansas, AK (2)	0.001	0.667

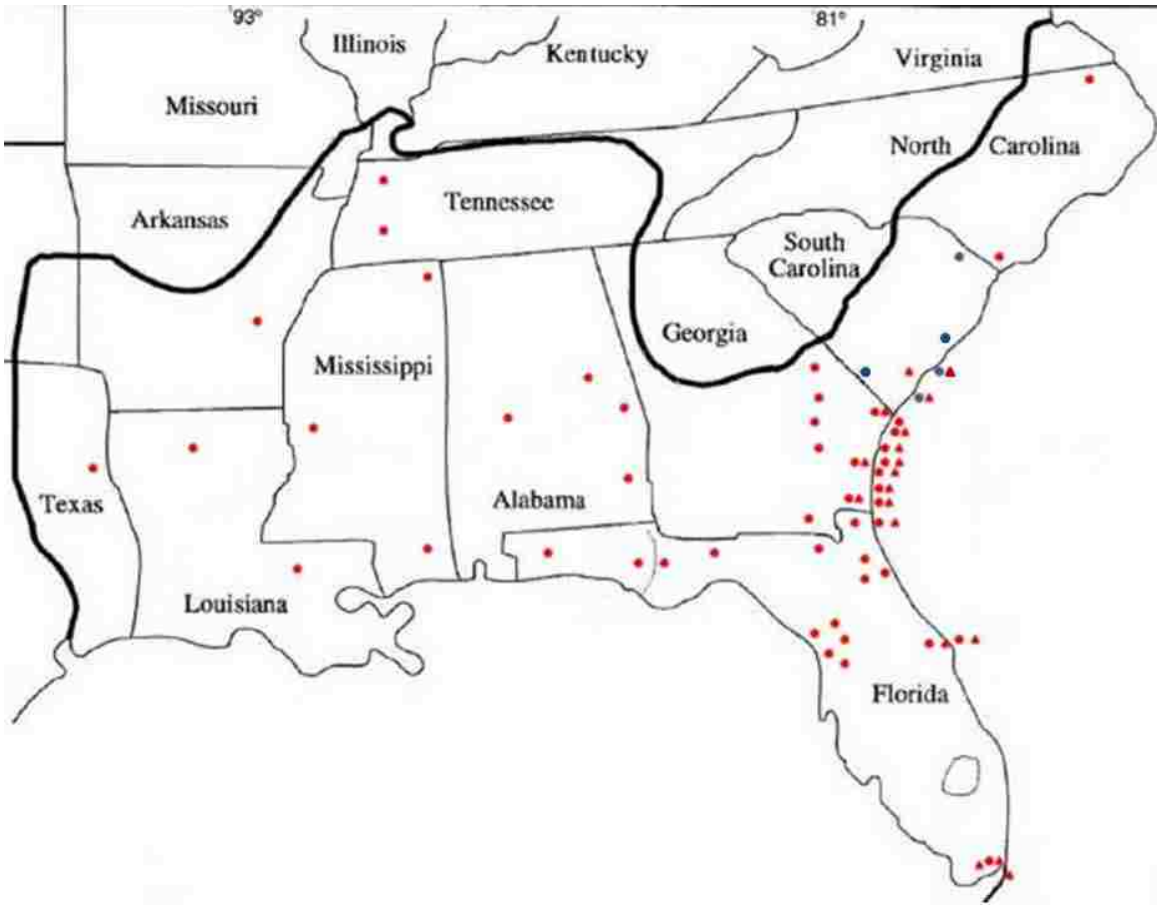


Figure 3.1. Field sites for which genetic data was collected for this study. Circles represent sites of museum samples, triangles represent samples trapped between 2008 and 2010. If both a circle and square are at a site, then both contemporary and museum specimens were collected from that site. Blue indicates samples genetically confirmed as *P. leucopus* and red indicates samples genetically confirmed as *P. gossypinus*.

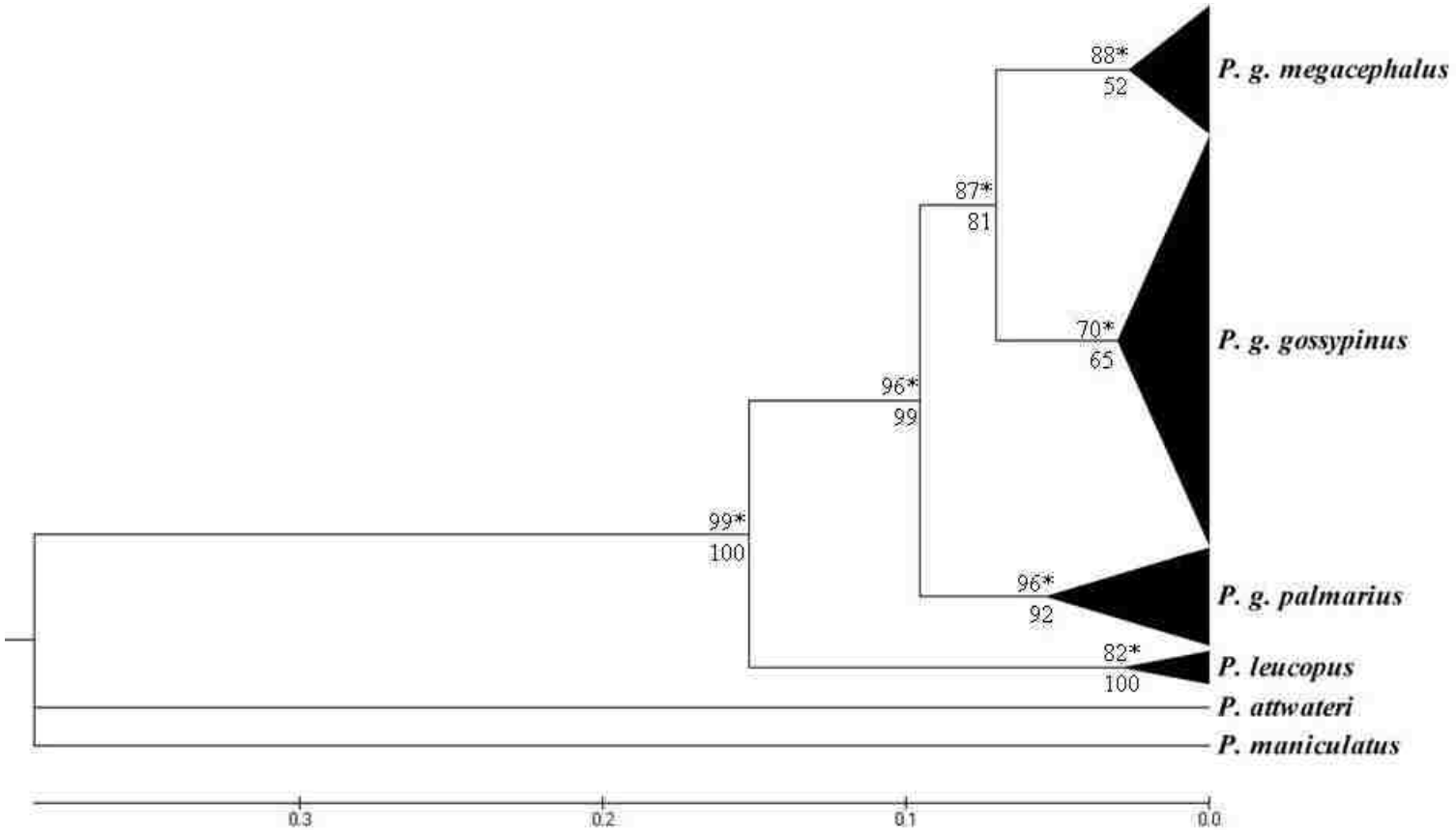


Figure 3.2. Phylogenetic tree of *Peromyscus gossypinus* reconstructed using maximum likelihood, Bayesian, and maximum parsimony methods. *Peromyscus leucopus, P. maniculatus,* and *P. attwateri* were included as outgroups. Maximum likelihood bootstrap values are above the line and maximum parsimony values are below the line if above 50%. A star next to the likelihood bootstrap value indicates a posterior probability of 0.95 or greater in the Bayesian analysis. All three trees returned the same topology and all major clades were collapsed in order to see the overall relationship of the tree.

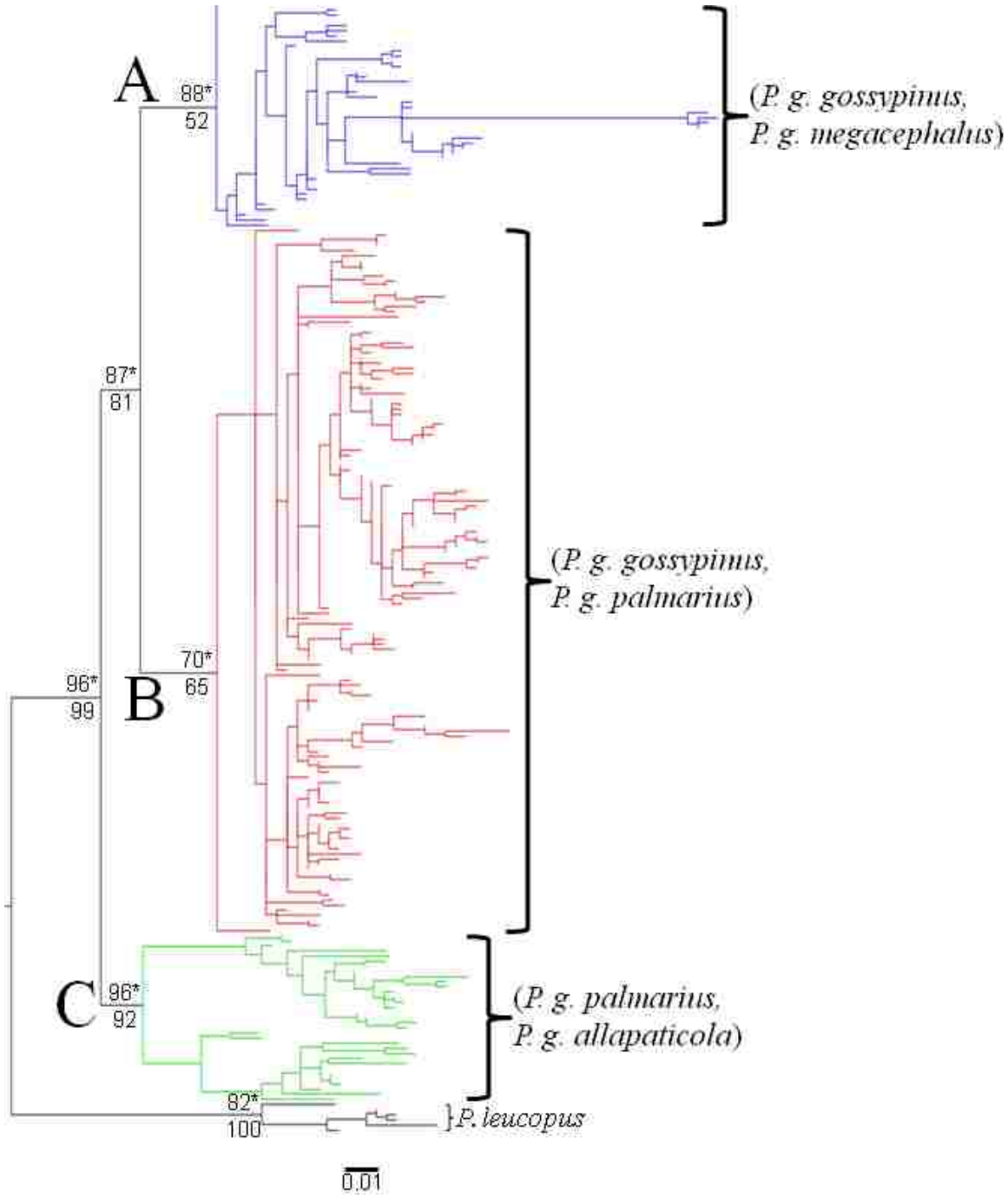


Figure 3.3. Phylogenetic tree reconstructed using maximum likelihood (ML), parsimony (MP), and Bayesian methods with *Peromyscus leucopus* as an outgroup. ML bootstrap values for major clades are above the line and MP values are below the line. Stars indicate a posterior probability of 0.95 or greater in the Bayesian analysis. All three trees returned the same topology. Clade A (blue) contained all samples within the Apalachicola-Chattahoochee-Flint River Basin (ACF) and westward, clade B (red) contained all samples east of the ACF from northern peninsular Florida and northward, clade C (green) contained all samples from southern peninsular Florida. In each clade samples were assignable to multiple recognized subspecies indicated in parentheses.

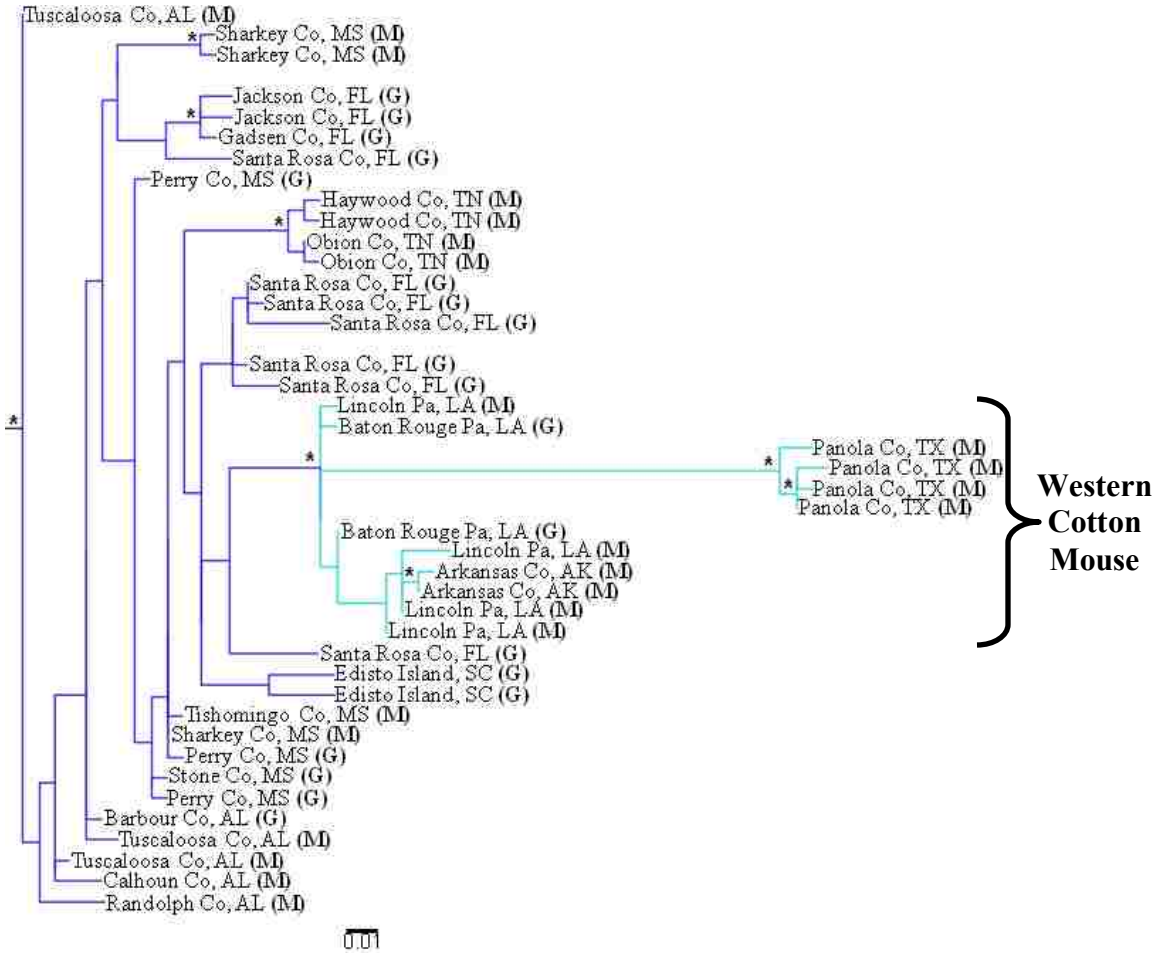


Figure 3.4. Maximum likelihood reconstruction of the western clade (A in Fig. 2.6). Nodes with a * have 0.95 or greater posterior probability based on Bayesian analysis. Letters in parentheses indicate subspecies assignments under current morphological designations: G-*gossypinus*, M-*megacephalus*. Branches in dark blue indicate populations assigned to *P. g. megacephalus* based on sequence data; branches in light blue indicate populations assigned to the Western cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data.

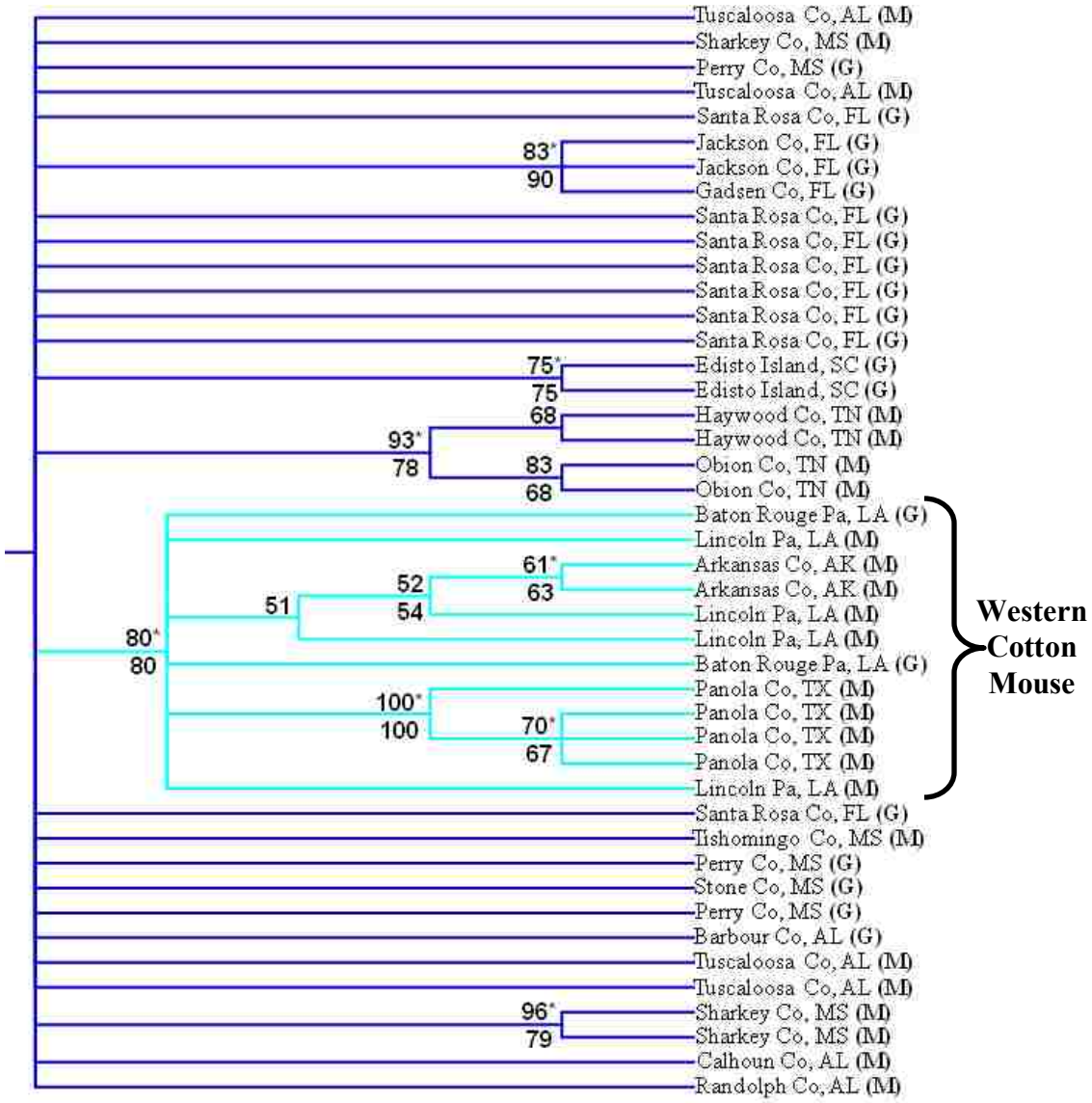
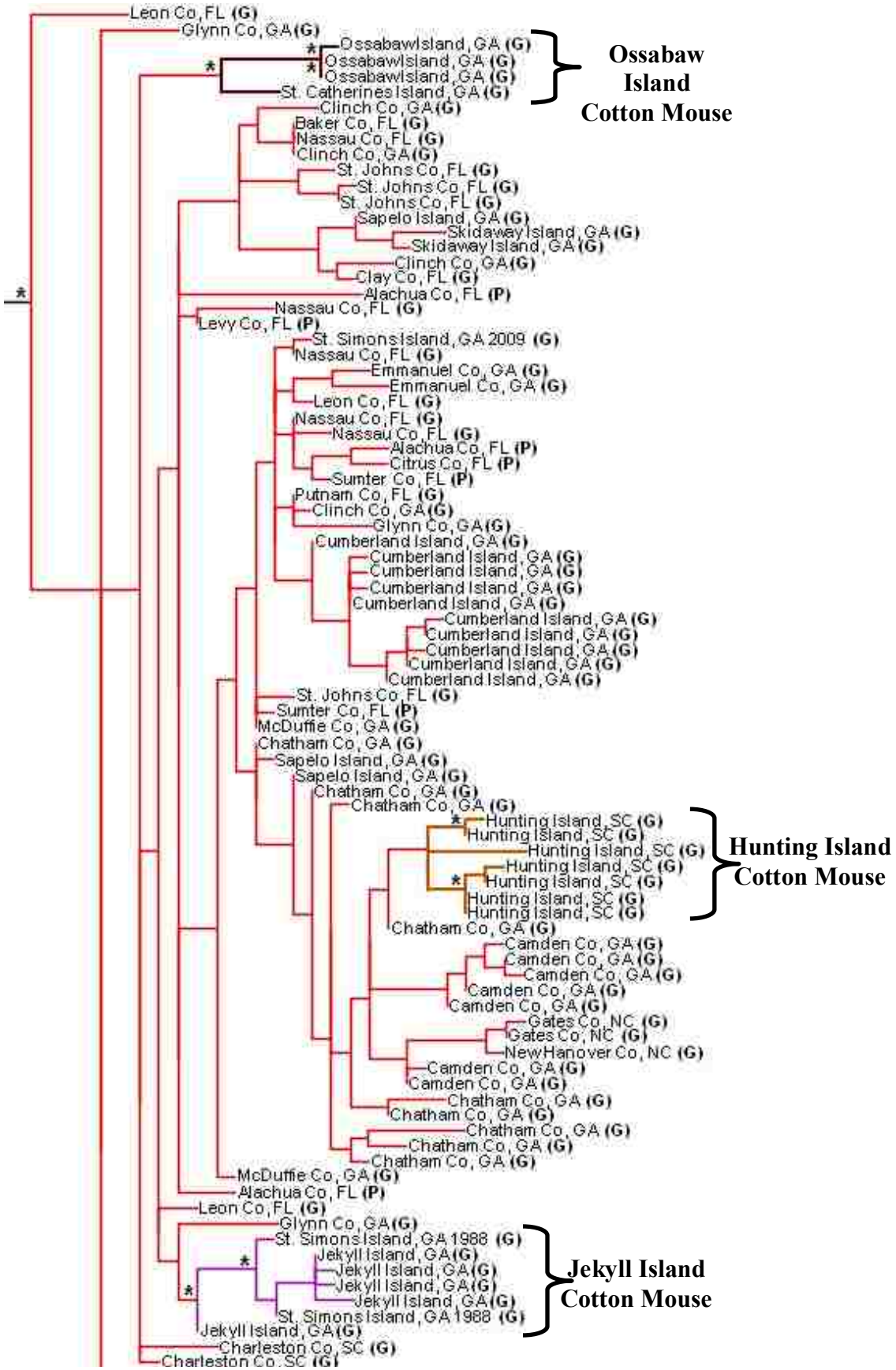


Figure 3.5. 50% majority rule consensus tree of the western clade (A in Fig. 2.6). Bootstrap values above branches are based on maximum likelihood trees. Values below branches are based on maximum parsimony trees. Branches with a * have 0.95 or greater posterior probability based on Bayesian analysis. Letters in parentheses indicate subspecies assignments under current morphological designations: G-*gossypinus*, M-*megacephalus*. Branches in dark blue indicate populations assigned to *P. g. megacephalus* based on sequence data; branches in light blue indicate populations assigned to the Western cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data.


Leon Co, FL (G)
Glynn Co, GA (G)
Ossabaw Island, GA (G)
Ossabaw Island, GA (G)
Ossabaw Island, GA (G)
St. Catherines Island, GA (G)
Ossabaw Island Cotton Mouse
Clinch Co, GA (G)
Baker Co, FL (G)
Nassau Co, FL (G)
Clinch Co, GA (G)
St. Johns Co, FL (G)
St. Johns Co, FL (G)
St. Johns Co, FL (G)
Sapelo Island, GA (G)
Skidaway Island, GA (G)
Skidaway Island, GA (G)
Clinch Co, GA (G)
Clay Co, FL (G)
Alachua Co, FL (P)
Nassau Co, FL (G)
Levy Co, FL (P)
St. Simons Island, GA 2009 (G)
Nassau Co, FL (G)
Emmanuel Co, GA (G)
Emmanuel Co, GA (G)
Leon Co, FL (G)
Nassau Co, FL (G)
Nassau Co, FL (G)
Alachua Co, FL (P)
Citrus Co, FL (P)
Sumter Co, FL (P)
Putnam Co, FL (G)
Clinch Co, GA (G)
Glynn Co, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
Cumberland Island, GA (G)
St. Johns Co, FL (G)
Sumter Co, FL (P)
McDuffie Co, GA (G)
Chatham Co, GA (G)
Sapelo Island, GA (G)
Sapelo Island, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island, SC (G)
Hunting Island Cotton Mouse
Chatham Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Gates Co, NC (G)
Gates Co, NC (G)
New Hanover Co, NC (G)
Camden Co, GA (G)
Camden Co, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
Chatham Co, GA (G)
McDuffie Co, GA (G)
Alachua Co, FL (P)
Leon Co, FL (G)
Glynn Co, GA (G)
St. Simons Island, GA 1988 (G)
Jekyll Island, GA (G)
Jekyll Island, GA (G)
Jekyll Island, GA (G)
Jekyll Island, GA (G)
St. Simons Island, GA 1988 (G)
Jekyll Island, GA (G)
Jekyll Island Cotton Mouse
Charleston Co, SC (G)
Charleston Co, SC (G)

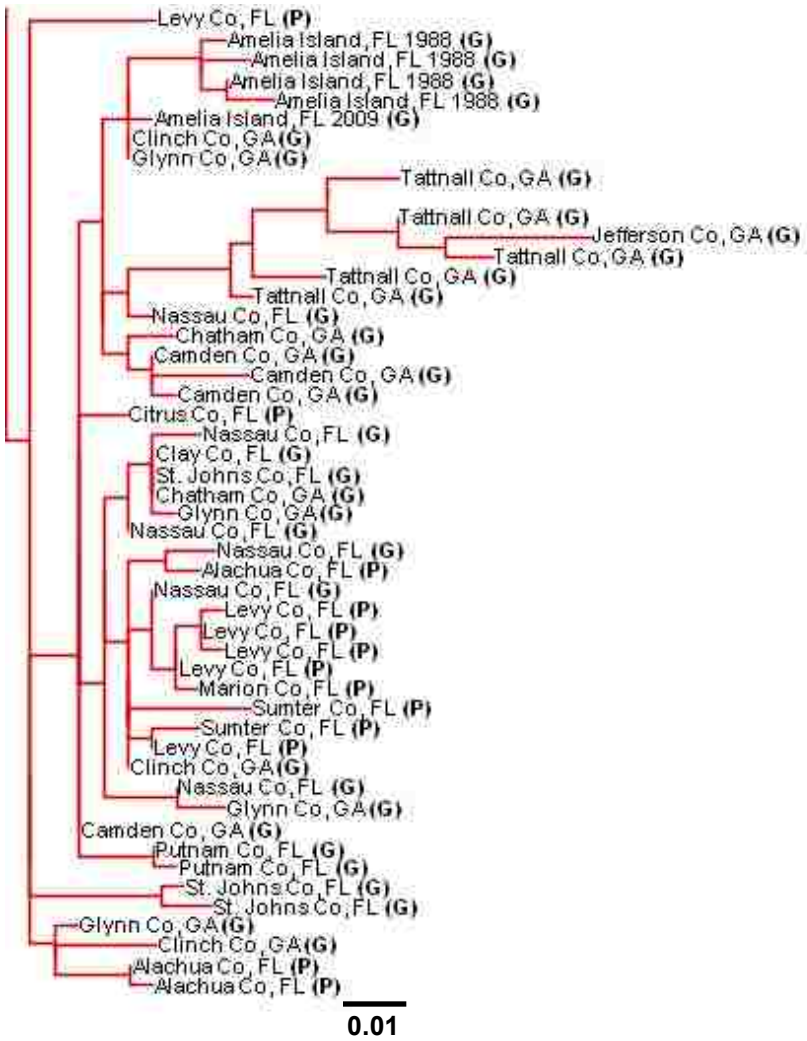
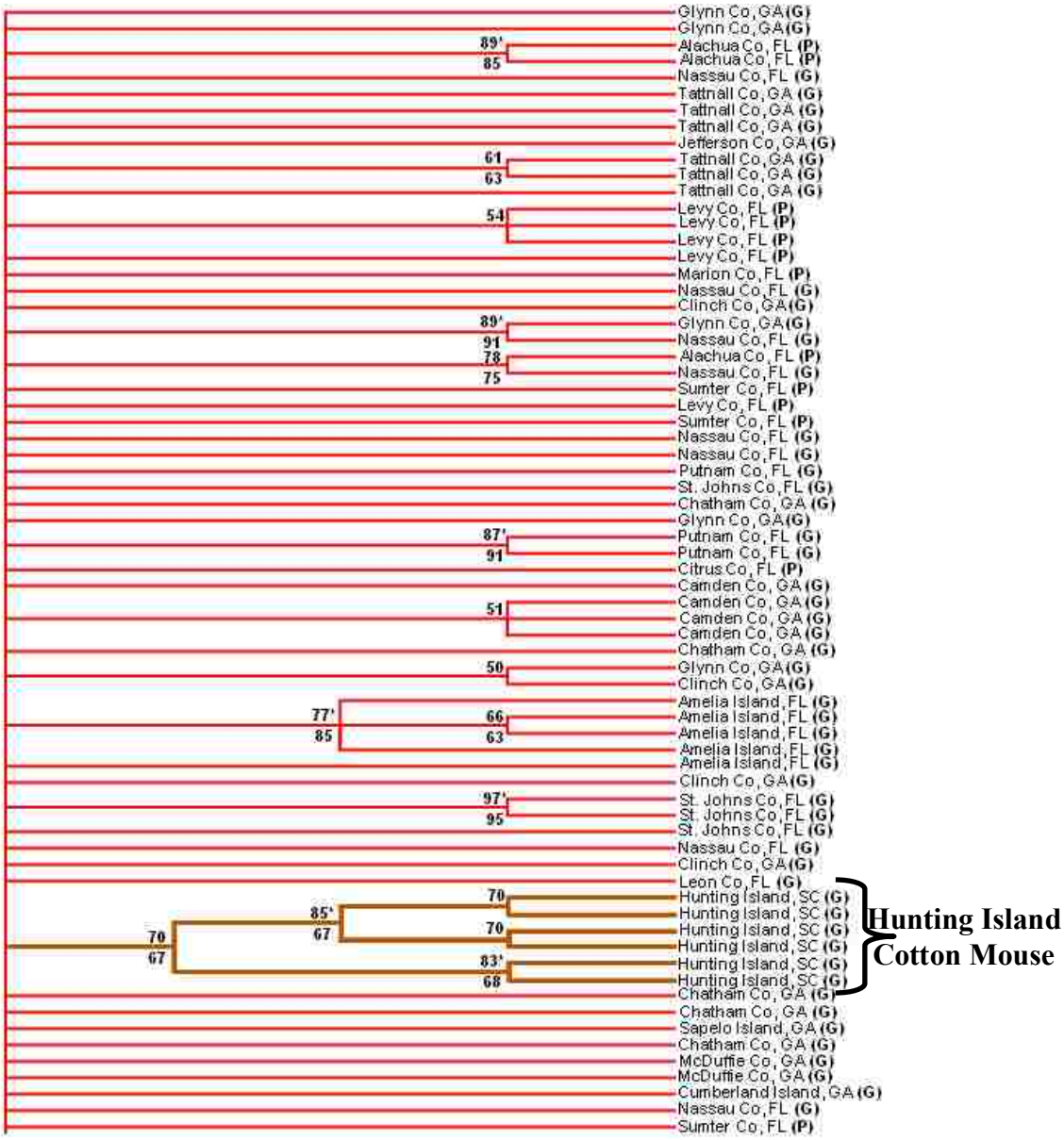


Figure 3.6 (includes previous page). Maximum likelihood reconstruction of the northeastern clade (B in Fig. 2.6). Nodes with a * have 0.95 or greater posterior probability based on Bayesian analysis. Letters in parentheses indicate subspecies assignments under current morphological designations: G-*gossypinus*, P-*palmarius*. Branches in red indicate populations assigned to *P. g. gossypinus* based on sequence data; branches in dark red indicate populations assigned to the Ossabaw Island cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data; branches in brown indicate the population assigned to the Hunting Island cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data; branches in purple indicate populations assigned to the Jekyll Island cotton mouse, a previously unidentified subspecies of *P. gossypinus*, based on sequence data.


Glynn Co, GA (G)
Glynn Co, GA (G)
89'
Alachua Co, FL (P)
85
Alachua Co, FL (P)
Nassau Co, FL (G)
Tattnall Co, GA (G)
Tattnall Co, GA (G)
Tattnall Co, GA (G)
Jefferson Co, GA (G)
61
Tattnall Co, GA (G)
63
Tattnall Co, GA (G)
Tattnall Co, GA (G)
54
Levy Co, FL (P)
Levy Co, FL (P)
Levy Co, FL (P)
Levy Co, FL (P)
Marion Co, FL (P)
Nassau Co, FL (G)
Clinch Co, GA (G)
89'
Glynn Co, GA (G)
91
Nassau Co, FL (G)
78
Alachua Co, FL (P)
75
Nassau Co, FL (G)
Sumter Co, FL (P)
Levy Co, FL (P)
Sumter Co, FL (P)
Nassau Co, FL (G)
Nassau Co, FL (G)
Putnam Co, FL (G)
St. Johns Co, FL (G)
Chatham Co, GA (G)
Glynn Co, GA (G)
87'
Putnam Co, FL (G)
91
Putnam Co, FL (G)
Citrus Co, FL (P)
Camden Co, GA (G)
51
Camden Co, GA (G)
Camden Co, GA (G)
Camden Co, GA (G)
Chatham Co, GA (G)
50
Glynn Co, GA (G)
Clinch Co, GA (G)
77'
Amelia Island, FL (G)
66
Amelia Island, FL (G)
85
63
Amelia Island, FL (G)
Amelia Island, FL (G)
Amelia Island, FL (G)
Clinch Co, GA (G)
97'
St. Johns Co, FL (G)
95
St. Johns Co, FL (G)
St. Johns Co, FL (G)
Nassau Co, FL (G)
Clinch Co, GA (G)
Leon Co, FL (G)
70
Hunting Island, SC (G)
85'
Hunting Island, SC (G)
67
70
Hunting Island, SC (G)
Hunting Island, SC (G)
70
Hunting Island, SC (G)
67
83'
Hunting Island, SC (G)
68
Chatham Co, GA (G)
Chatham Co, GA (G)
Sapelo Island, GA (G)
Chatham Co, GA (G)
McDuffie Co, GA (G)
McDuffie Co, GA (G)
Cumberland Island, GA (G)
Nassau Co, FL (G)
Sumter Co, FL (P)
Hunting Island
Cotton Mouse

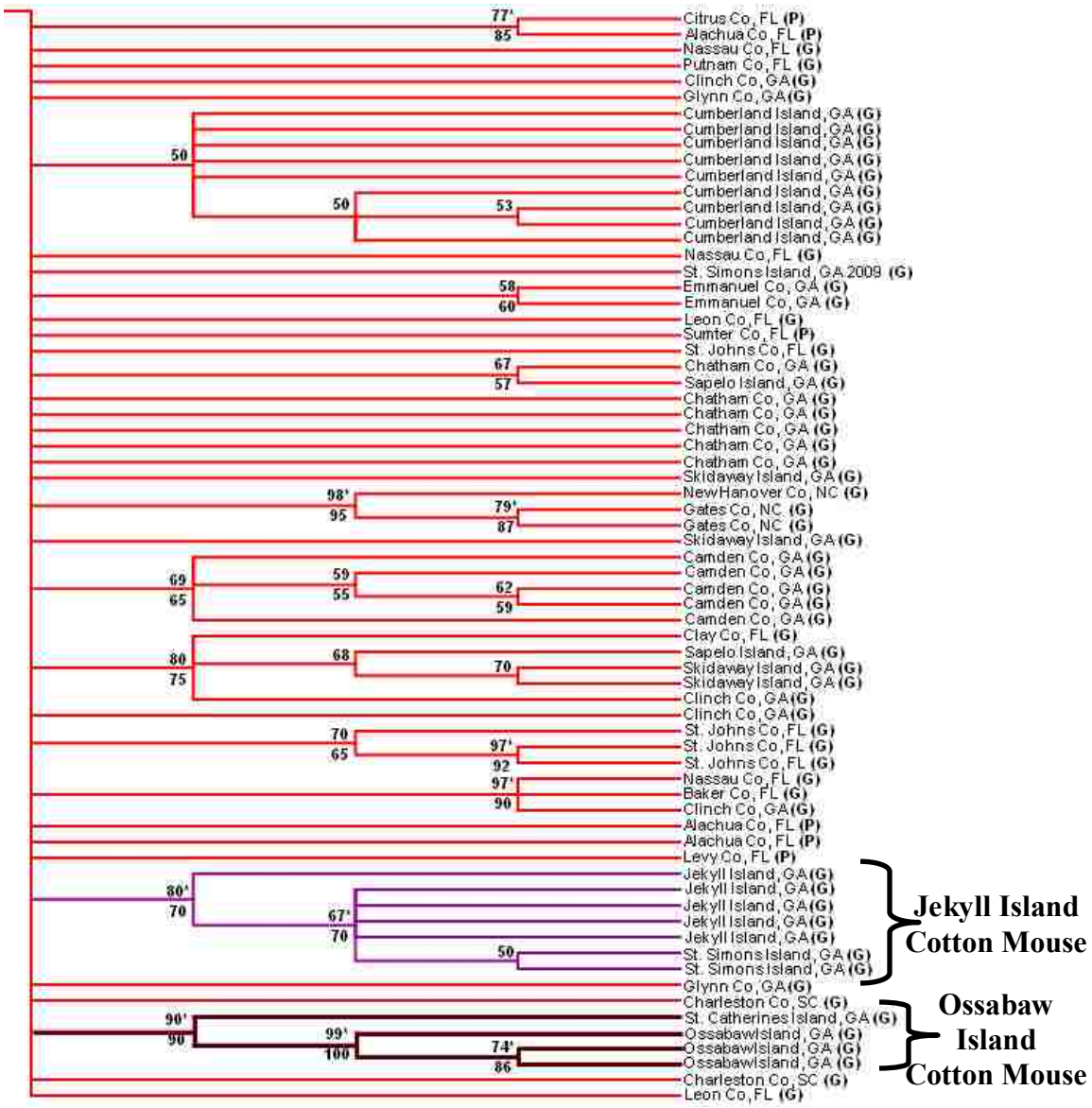


Figure 3.7 (includes previous page). 50% majority rule consensus tree of the northeastern clade (B in Fig. 2.6). Bootstrap values above branches are based on maximum likelihood trees and below branches are based on maximum parsimony trees. Branches with a * have at least a 0.95 posterior probability in Bayesian analyses. Letters in parentheses indicate subspecies assignments under current morphological designations: G-*gossypinus*, P-*palmarius*. Branches in red indicate populations assigned to *P. g. gossypinus* based on sequence data. Branches in other colors indicate populations belonging to previously unidentified subspecies of *P. gossypinus*: Dark Red-Ossabaw Island cotton mouse, Brown- Hunting Island cotton mouse, Purple- Jekyll Island cotton mouse.

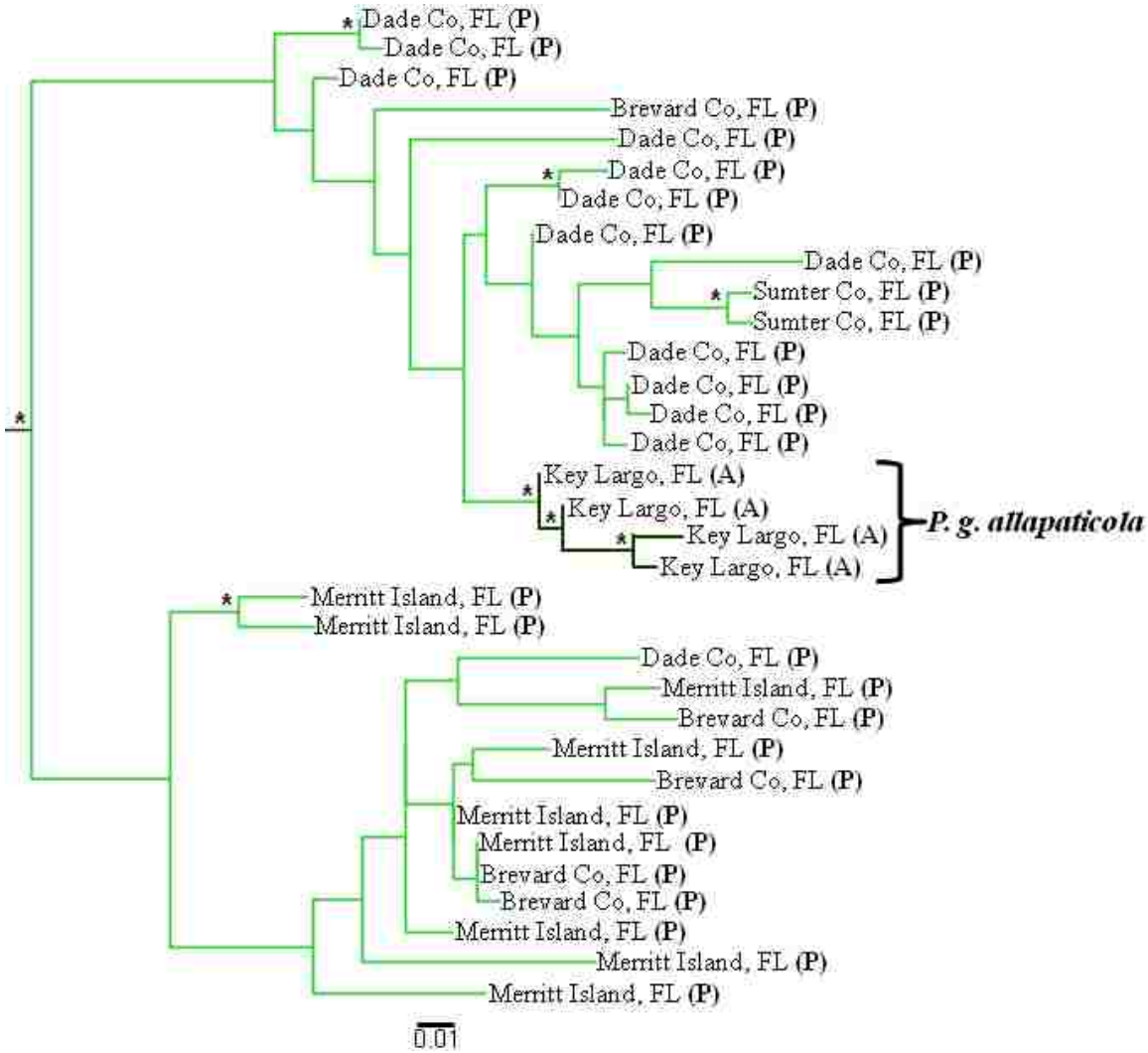


Figure 3.8. Maximum likelihood reconstruction of the southeastern clade (C in Fig. 2.6). Nodes with a * have 0.95 or greater posterior probability based on Bayesian analysis. Letters in parentheses indicate subspecies assignments under current morphological designations: A-*allapaticola*, P-*palmarius*. Branches in light green indicate populations assigned to *P. g. palmarius* based on sequence data; branches in olive green indicate populations assigned to *P. g. allapaticola* based on sequence data.

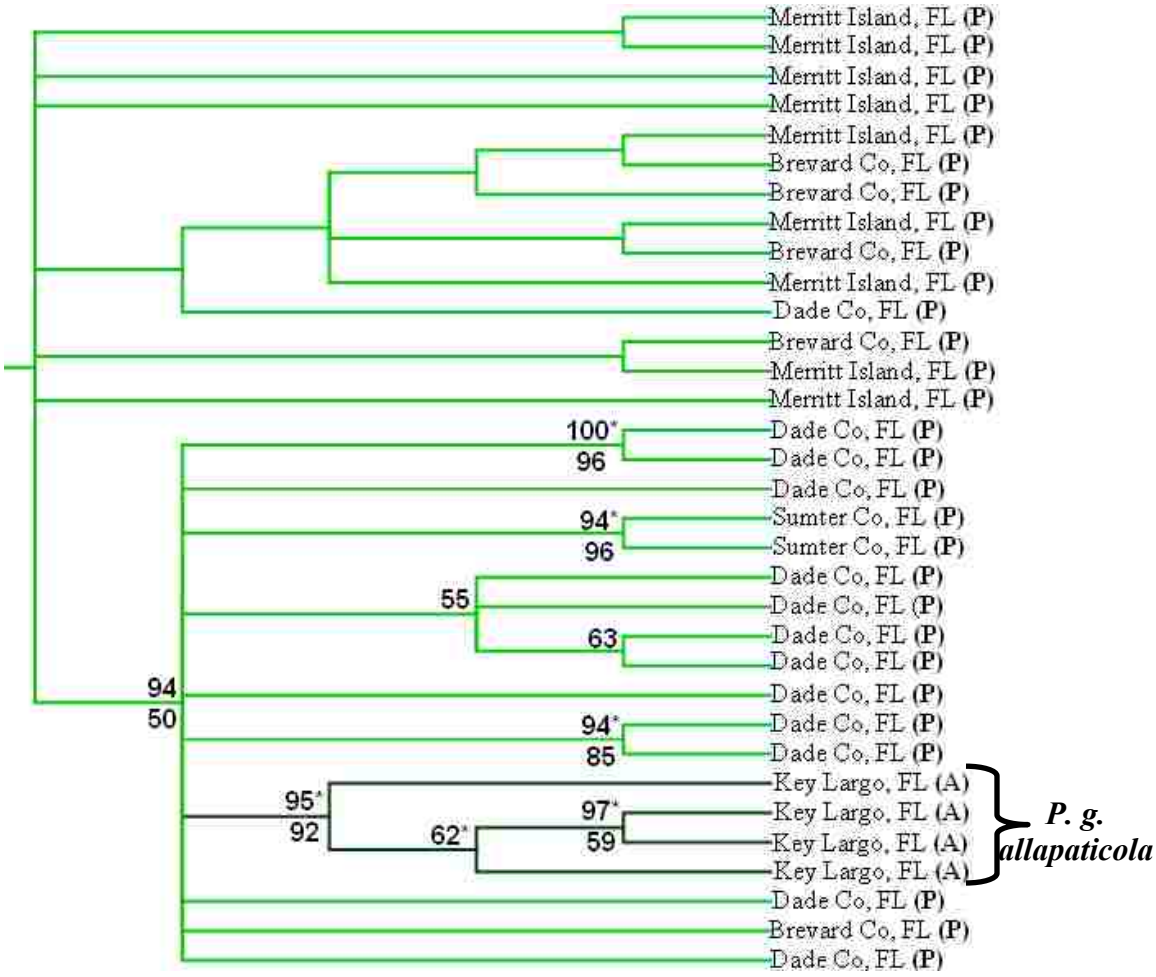


Figure 3.9. 50% majority rule consensus tree of the southeastern clade (C in Fig. 2.6). Bootstrap values above branches are based on maximum likelihood trees and below branches are based on maximum parsimony trees. Branches with a * have at least a 0.95 posterior probability in Bayesian analyses. Letters in parentheses indicate subspecies assignments under current morphological designations: A-*allapaticola*, P-*palmarius*. Branches in light green indicate populations assigned to *P. g. palmarius* based on sequence data; branches in olive green indicate populations assigned to *P. g. allapaticola* based on sequence data.

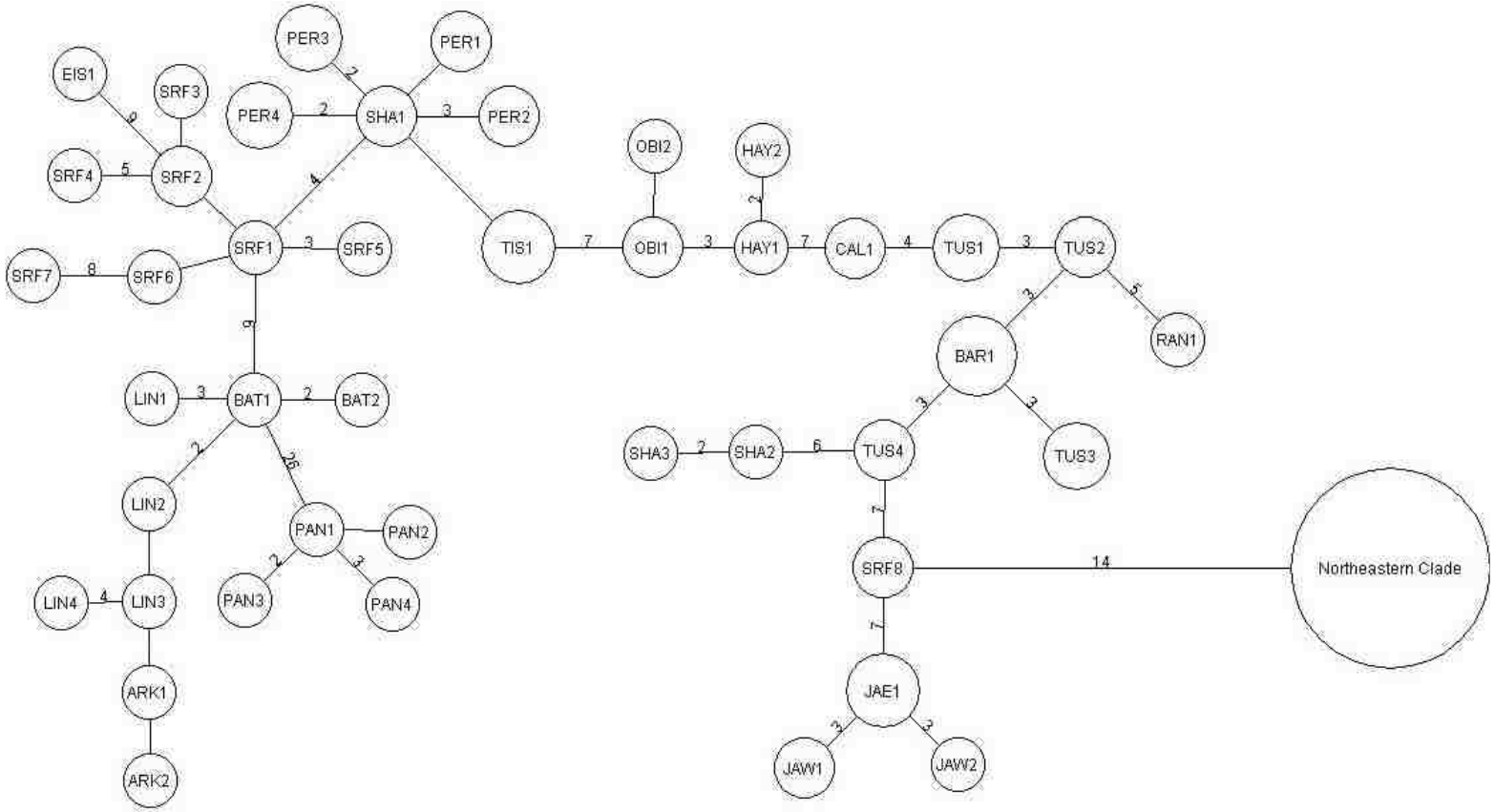


Figure 3.10. Minimum Spanning Network of control region haplotypes for populations of *Peromyscus gossypinus* in the western clade. Circles indicate haplotypes, with larger circles containing more individuals. Labels identify populations and haplotype number within the population. Numbers along branches indicate the minimum number of mutation steps between haplotypes. Population abbreviations are: ARK (Arkansas Co., AK), BAR (Barbour Co., AL), BAT (west Baton Rouge Parish, LA), CAL (Calhoun Co., AL), EIS (Edisto Isl., SC), HAY (Haywood Co., TN), JAE (eastern Jackson Co., FL), JAW (western Jackson Co., FL), LIN (Lincoln Parish, LA), OBI (Obion Co., TN), PAN (Panola Co., TX), PER (Perry Co, MS), SHA (Sharkey Co., MS), SRF(Santa Rosa Co., FL), TUS (Tuscaloosa Co., AL)

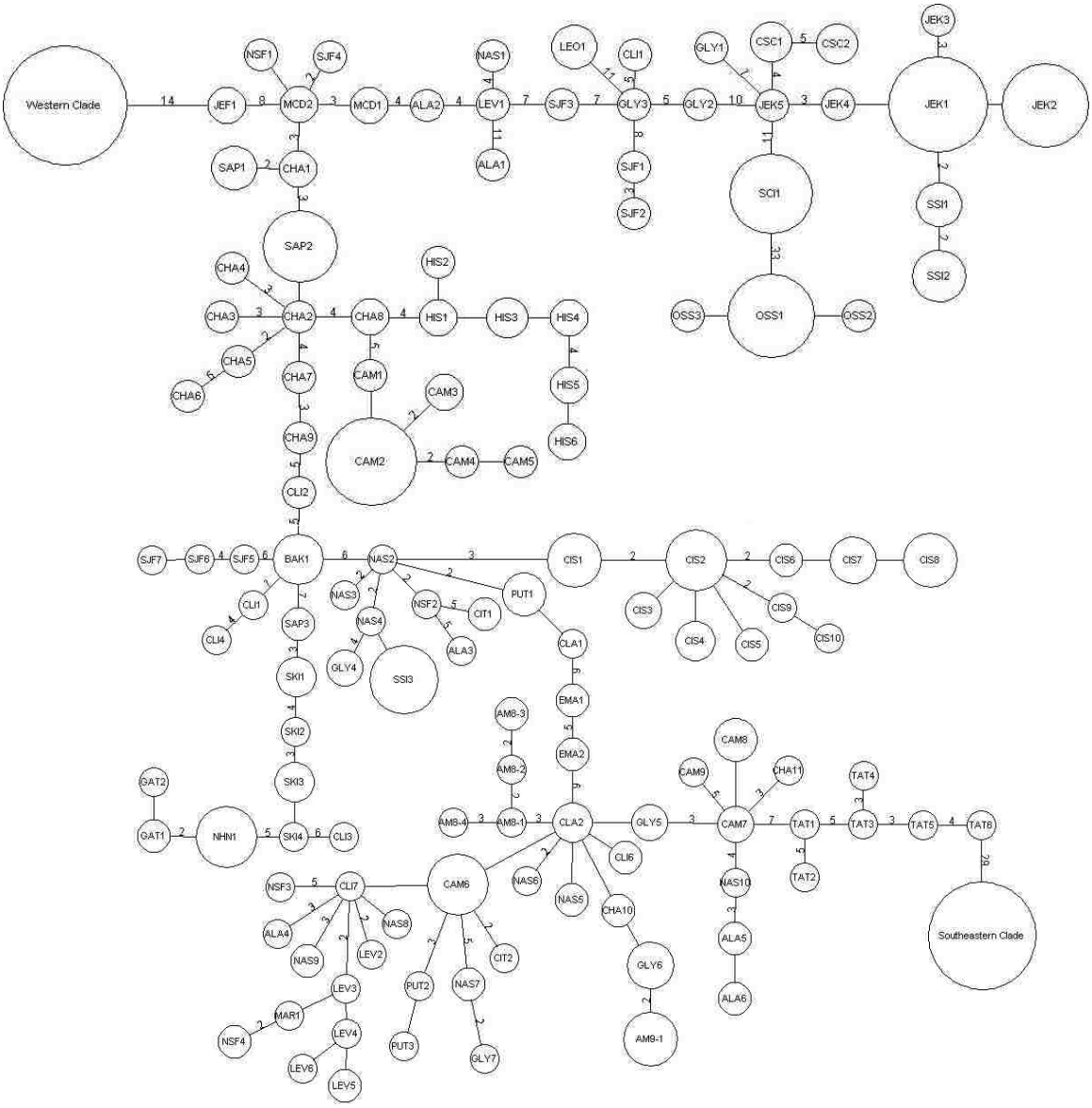


Figure 3.11. Minimum Spanning Network of control region haplotypes for populations of *Peromyscus gossypinus* in the northeastern clade. Circles indicate haplotypes, with larger circles containing more individuals. Labels identify populations and haplotype number within the population. Numbers along branches indicate the minimum number of mutation steps between haplotypes. Population abbreviations are: ALA (Alachua Co., FL), AMI (Amelia Isl., FL), CAM (Camden Co., GA), CHA (Chatham Co., GA), CIS (Cumberland Isl., GA), CLA (Clay Co., FL), CLI (Clinch Co., GA), CSC (Charleston Co., SC), EMA (Emanuel Co., GA), GAT (Gates Co., NC), GLY (Glynn Co., GA), HIS (Hunting Isl., SC), JEF (Jefferson Co., GA), JEK (Jekyll Isl., GA), LEO (Leon Co., FL), LEV (Levy Co., FL), MCD (McDuffie Co., GA), NAS (Nassau Co., FL), NHN (New Hanover Co., NC), NSF (northern Sumter Co., FL), OSS (Ossabaw Isl., GA), SAP (Sapelo Isl., GA), SJF (St. Johns Co., FL), SKI (Skidaway Isl., GA), TAT (Tattnall Co., GA).

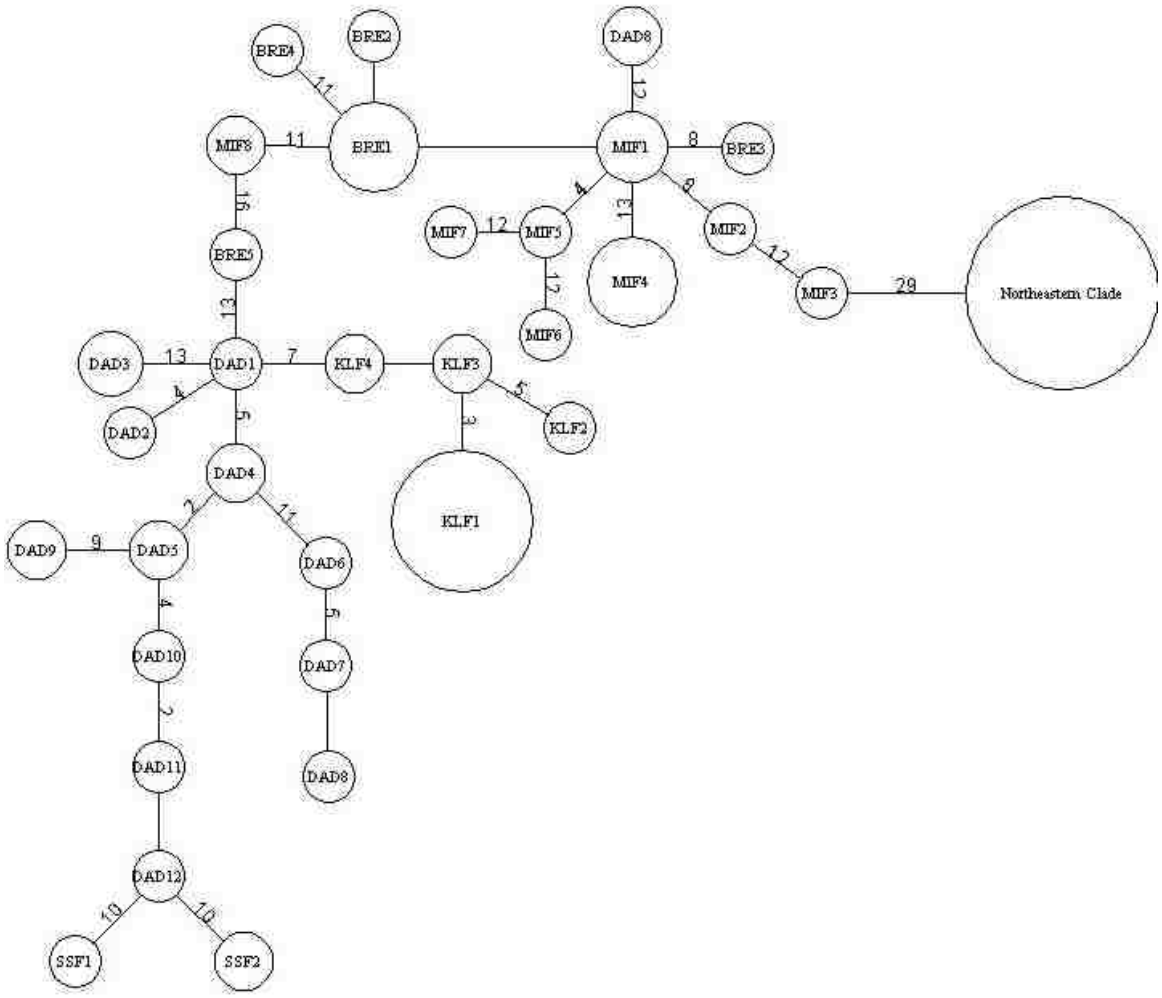


Figure 3.12. Minimum Spanning Network of control region haplotypes for populations of *Peromyscus gossypinus* in the southeastern clade. Circles indicate haplotypes, with larger circles containing more individuals. Labels identify populations and haplotype number within the population. Numbers along branches indicate the minimum number of mutation steps between haplotypes. Population abbreviations are: BRE (Brevard Co., FL), COL (Collier Co., FL), DAD (Dade Co., FL), KLF (Key Largo, FL), MER (Merritt Isl., FL), SSF (southern Sumter Co., FL).

Chapter 4:
Genetic variation within and among populations of the cotton mouse, *Peromyscus gossypinus*: a nuclear approach

Genetic processes at the population level are responsible for the geographic distribution of genetic diversity throughout a species' range (Bowler and Benton 2005, Clobert et al. 2001). Gene flow and genetic drift play critical roles in shaping the patterns of diversity within and among populations. The effects of these processes on a population depend largely on an organism's dispersal ability; however, available habitat, geography, and climatic events, can also inhibit or promote gene flow and genetic drift. In order to understand the geographic patterns of genetic variation within a species, it is first necessary to identify how environmental factors affect dispersal.

An organism's dispersal ability is typically tied to the size of the organism. Large, highly vagile mammals can disperse long distances, and typically exhibit a lack of patterning of genetic diversity (Fernando et al. 2000, Hofreiter et al. 2004, Lehman and Wayne 1991). In contrast, small mammals are less vagile and disperse shorter distances (Indorf 2010), often resulting in a pattern of isolation by distance (Wright 1943) where genetic similarity between populations decreases with geographic distance (Mora et al. 2010).

While dispersal ability can dictate range wide patterns of diversity, other life history traits affect patterns at a local level. A species' habitat requirements determine where populations can occur and often what habitats a species can migrate through (Castleberry et al. 2002). Small mammals often require specific habitats and can only disperse between areas of suitable habitat. If the distance between habitats exceeds an

animal's dispersal ability then there will be structuring among geographically close populations (Degner et al. 2007, Van Zant and Wooten 2007).

Geographic barriers to gene flow also inhibit an organism's ability to migrate between populations. Depending on an organism's dispersal ability, mountains, rivers, and intracoastal waterways may all act as barriers to gene flow. While mountains and rivers may not affect dispersal for a bird (Vallianatos et al. 2001) or a large mammal (Lehman and Wayne 1991), they may represent a significant barrier to dispersal for small mammals (Brant and Ortí 2003, Chambers and Garant 2010, Root et al. 2003). Intracoastal waterways also commonly prevent gene flow between island and mainland populations of small mammals (Calhoun and Greenbaum 1991, Indorf 2010). New alleles that arise in a population are unlikely to be passed across these barriers due to reduced gene flow, promoting structuring of genetic diversity on a local scale.

In addition to the effects of environmental factors, anthropogenic forces also shape patterns of genetic diversity within a species. The building of dams that slow the flow of rivers and the construction of land bridges to connect islands to the mainland can mitigate the effects of barriers to gene flow for a species (Bond and Jones 2008, Corn 2007, Hinten et al. 2003), and the destruction of habitat for urbanization and agriculture can reduce gene flow among previously connected populations. Studies indicate that organisms across a variety of taxa are incapable of migrating through urbanized areas (Bolger et al. 1997, Davidson et al. 2009) and that urbanization greatly reduces gene flow in small mammals. Other research suggests that the presence of roads greatly reduces dispersal ability for several taxa (Fahrig and Rytwinski 2009, McGregor et al. 2008, Papouchis et al. 2001, Rytwinski and Fahrig 2007). This reduction in gene flow often

results in the isolation of several previously connected populations (Chiappero et al. 2011). Additionally, urbanization often reduces the amount of available habitat in an area which may result in a reduction in population size.

In isolated populations, a reduction in population size makes organisms more susceptible to genetic drift, which results in the random loss of alleles that cannot be replaced due to a lack of gene flow with neighboring populations (Frankham 1997, Wang et al. 2009). As new alleles arise in isolated populations, their transmission to other nearby populations is prevented, which strengthens patterns of genetic diversity on a local scale. These patterns are often strongest in highly isolated populations with small amounts of available habitat such as in barrier islands (Garner et al. 2005). Barrier islands are commonly separated from the mainland by large areas of brackish and salt water, which prevents many small mammals from dispersing between islands and the mainland (Duesser et al. 1979, Hice and Schmidly 2002). Due to this isolation, and the small amount of available habitat in islands, urbanization has a greater impact on genetic diversity in island areas.

The southeastern United States (US) has been affected by all of these factors, and patterns of genetic diversity are documented for a variety of species throughout this region (Pauly et al. 2007, Soltis et al. 2006). Intracoastal waterways (Degner et al. 2007), mountains (Austin et al. 2004, Zamudio and Savage 2003), and rivers (Brant and Ortí 2003, Ellsworth et al. 1994, Hayes and Harrison 1992) have been identified as barriers to gene flow in the southeastern US. Among mammals, most studies of genetic diversity have focused on habitat specialists (Brant and Ortí 2003, Degner et al. 2007, Hayes and Harrison 1992, Indorf 2010), which are expected to show strong geographic structuring

due to their specific habitat requirements. Habitat generalists are expected to disperse more easily through unfavorable habitat and show less structuring than habitat specialists (Pandit et al. 2009). However, a few studies have identified structuring in both small (Edwards et al. 2011) and large mammals (Ellsworth et al. 1994) that are habitat generalists. Research also indicates that small mammal habitat generalists are often incapable of migrating through urban areas (Bolger et al. 1997).

The cotton mouse (*Peromyscus gossypinus*) is a habitat generalist rodent found throughout the southeastern US. It inhabits both sides of several barriers to gene flow for other species: the Mississippi River (Bryant 2007, Cullingham et al. 2008), the Tombigbee River (Indorf 2010), the Apalachicola River (Avise et al. 1979, 1983), the Savannah River (Degner et al. 2010), and the Appalachian Mountains (Walker et al. 2009). The species is also found on several barrier islands in South Carolina, Georgia, and Florida (Figure 4.1). *Peromyscus gossypinus* is semiaquatic (Pournelle 1950) but is unlikely to cross large rivers and intracoastal waterways due to their size, current, and salinity. *Peromyscus gossypinus* has a maximum observed dispersal distance of under one kilometer (Pournelle 1950, Griffo 1961), making local structuring likely. Also, other generalist *Peromyscus* species have been shown to be incapable of migrating through urban areas (Bolger et al. 1997) making local structuring in *P. gossypinus* more likely. Based on these factors, *P. gossypinus* serves as a unique system to study the dispersal ability of a habitat generalist species with respect to a variety of barriers to gene flow.

Studies of *P. gossypinus* have identified range wide geographic structuring and isolation by distance in the species (Beckmann in prep., Boone et al. 1999). The Beckmann study also identified four genetically distinct lineages associated with

common phylogeographic breaks for a variety of species (Figure 4.1). However, no study has explored local geographic structuring in this species. Additionally, Beckmann's study utilized mitochondrial DNA which is maternally inherited and only identifies structuring associated with the dispersal of females (Avise 2000). The goal of this study was to identify if local geographic structuring of genetic diversity is present in *P. gossypinus.* I sought to identify if intracoastal waterways and rivers represented barriers to gene flow for this species and if urbanization limited *P. gossypinus*' dispersal ability. This would provide information as to how small mammal habitat generalists may respond to local climatic and geographic events, as well as anthropogenic forces. By utilizing microsatellite loci I was also able to determine if there is current gene flow not seen in mitochondrial analyses.

I hypothesized that microsatellite loci would identify geographic structuring of genetic diversity throughout the species range. I predicted that due to *P. gossypinus'* limited dispersal ability, there would be local structuring of genetic diversity within three of the mainland lineages of *P. gossypinus* identified by Beckmann (2010) (Figure 4.1). I also predicted that urbanization in mainland areas would strengthen local patterns of genetic diversity. Finally, I predicted that several island populations are genetically isolated from their nearest mainland populations.

Methods

Sample collection and DNA extraction

Tissue samples or toe bone samples were collected from 23 populations throughout the range of *P. gossypinus* (Figure 4.2). Eighteen populations included samples collected between 1988 and 1990. An additional five populations (Amelia

Island, FL; Key Largo, FL; Merritt Island, FL; Dade, FL; Hunting Island, SC) were included which were sampled in 2009. The samples from 2009 were included in order to give an accurate representation of genetic diversity throughout the species' range including all island populations. There were not enough samples available from any of these five populations collected between 1988 and 1990. For population comparisons using microsatellite markers to be statistically meaningful, a minimum of ten samples must be included from each population (Balloux and Lugon-Moulin 2002).

A total of 317 samples were included in this study, with between 10 and 28 samples from each of the 23 populations. Included populations were: Dade, FL (n=17); Key Largo, FL (n=20); Merritt Island, FL (n=20); Amelia Island, FL (n=10); Alachua, FL (n=10); Nassau. FL (n=15); St. Johns, FL (n=11); Camden, GA (n=27); Jekyll Island, GA (n=23); St. Simons Island, GA (n=12); Glynn, GA (n=10); Sapelo Island, GA (n=11); Skidaway Island, GA (n=10); Chatham, GA (n=11); Cumberland Island, GA (n=28); Clinch, GA (n=11); St. Catherines Island, GA (n=10); Ossabaw Island, GA (n=11); Hunting Island, SC (n=10); New Hanover, NC (n=10); Santa Rosa, FL (n=10); Perry, MS (n=10); and Tuscaloosa, AL (n=10). Exact sampling locations are listed in Appendix 1.

DNA extraction and genotyping of microsatellite loci

Whole genomic DNA was extracted from tissue using a standard ethanol precipitation protocol. Qiagen DNeasy® tissue extraction kits (Qiagen Inc., Valencia, California) were used to extract DNA from toe bones following the user-developed protocol *Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit* available on the Qiagen website (http://www.qiagen.com).

Genotypes were collected for seven polymorphic nuclear microsatellites developed in *P. leucopus* (Schmidt 1999) and *P. maniculatus* (Weber et al. 2009). Microsatellite loci were amplified using PCR with 5'-fluorescent dye-labeled forward primers (Applied Biosystems, Foster City, CA). Dye labels for each forward primer are listed in Table 4.1. All reactions were carried out in 10 μl volumes containing 1x PCR Buffer, .4mM (GT15, GT66, GATA70) or .15mM (AC9, AC19, GT21, TA5GT6) dNTPS, 0.5 units Taq DNA polymerase, 0.2μM (GT15, GT66, GATA70) or 0.4μM (AC9, AC19, GT21, TA5GT6) forward and reverse primers, $MgCl_2$ (see Table 4.1 for concentrations), and dH_2O. A total of 40 ng of DNA was included in each reaction and primers AC9, AC19, and TA5GT6 were multiplexed due to similar protocols in an attempt to reduce the total number of reactions.

All thermal profiles used touchdown PCR programs (Don et al. 1991) with an initial denaturation of 95 ^{o}C for 3 min; followed by 5 cycles of 95 ^{o}C for 30 seconds, highest annealing temperature for 30 seconds, and 72 ^{o}C for 30 seconds; 20 cycles of 95 ^{o}C for 30 seconds, highest annealing temperature (decreased 0.25 ^{o}C per cycle) for 30 seconds, 72 ^{o}C for 30 seconds; and 10 cycles of 95 ^{o}C for 30 seconds, lowest annealing temperature for 30 seconds, and 72 ^{o}C for 30 seconds, followed by a final extension step of 72 ^{o}C for 10 minutes. Reactions were conducted over a 5 ^{o}C range of annealing temperatures and ranges for individual reactions are listed in Table 4.1. Samples were then genotyped on an ABI3130 xl automated capillary sequencer (Applied Biosystems). Reactions for loci GT15 and GT66 were run simultaneously in the same capillary, as were reactions for loci GATA70 and GT21. All loci were then scored using the software STRand v2.4.21 (Toonen and Hughes 2001).

Genotype verification

To determine if all samples correctly amplified, the data were checked with the software Micro-Checker v.2.2.3 (Van Oosterhout et al. 2004). Micro-Checker identifies deviations from Hardy-Weinberg equilibrium (HWE) at individual loci and determines if it is the result of a biological process or technical error. It identifies the presence of null alleles, large allele dropout, and scoring errors at individual loci which can lead to an overestimate of homozygosity. Null alleles are alleles which fail to amplify during PCR amplification, resulting in the false identification of an individual as a homozygote (Van Oosterhout et al. 2004). Overestimates of homozygosity can lead to the false identification of biological processes such as inbreeding in a population (Dewoody et al. 2006). Through a series of algorithms, Micro-Checker is able to identify technical errors that only affect a subset of loci, whereas real processes such as inbreeding should affect all loci equally.

In addition to identifying the presence of null alleles, Micro-Checker also accounts for them as well. Using the methods of Brookfield (1996), Micro-Checker estimates the true allele frequencies of the population at the locus containing null alleles. These corrected allele frequencies can then be used to identify if a population is in HWE. However, since Micro-Checker estimates true allele frequencies for the population, it does not assign alleles to specific individuals. As a result, the corrected allele frequencies cannot be used in multi-locus analyses, where the genotype of each individual must be known.

Effects of selection on microsatellite loci

For microsatellite loci to accurately identify geographic structuring of genetic diversity, they must meet the assumption that they are not under the influence of natural selection. If any of the loci are under selection, they may falsely identify heterozygote deficiency or excess in populations, which may be indicative of population processes that are not really occurring. Selection may also alter allele frequencies in such a way that the populations appear more similar or different with regard to other populations than they would if neutral markers were employed. In order to account for this, a test of selection was performed on each individual locus in Arlequin v.3.5 (Excoffier and Lischer 2010) via coalescent simulation using a finite island model, with 50,000 simulations of 100 demes (Excoffier et al. 2009).

Genetic variation within populations

I assessed genetic variation within populations by calculating a series of statistics in the software package Arlequin v.3.5 (Excoffier and Lischer 2010). Deviations from HWE were calculated for all populations using both the uncorrected allele frequencies and the frequencies identified by Micro-Checker. Tests of linkage disequilibrium were also performed for all pairs of loci in all populations using the uncorrected allele frequencies. Levels of inbreeding within each population were identified by calculating the inbreeding coefficient (F_{IS}), which is a measure of the amount of homozygosity within a population (Wright 1951). A high degree of inbreeding in a population may be indicative of a small isolated population or a population that is undergoing genetic drift (Hedrick 2005). I also calculated Nei's mean gene diversity (H_E) and mean allelic

richness (R) for each population using FSTAT v.2.9.3.2 (Goudet 2001). When calculating R, FSTAT corrects for the sample size in each population (El Mousadik and Petit 1996).

Genetic variation among populations

By quantifying the amount of genetic variation among populations it may be possible to identify whether or not gene flow is occurring among populations. However, if gene flow is indicated between populations, it does not imply that individuals are dispersing directly between populations. Rather, gene flow across large geographic distances commonly occurs through a stepping stone model (Kimura and Weiss 1964). In order to assess gene flow among populations, I first calculated the amount of genetic variation for all populations by identifying the mean number of alleles, observed heterozygosity, and expected heterozygosity for each microsatellite locus in Arlequin v3.5. I then calculated F_{ST} values among all population pairs using the adjusted allele frequencies identified by Micro-Checker. Van Oosterhout et al. (2006) found that adjusted allele frequencies, accounting for null alleles, can be used to estimate $F_{ST;}$ and Chapuis and Estoup (2007) identified that this correction does not significantly affect the results. R_{ST} is more commonly used as a measure of genetic differentiation than F_{ST} in studies utilizing microsatellite loci (Balloux and Lugon-Moulin 2002). However, when sample sizes and number of loci are low, as in this study, F_{ST} is a more accurate measure of genetic distance (Gaggiotti et al. 1999).

Number of migrants per generation was identified using the program Migrate-n v.3.2.6 between all pairs of populations (Beerli 2010). Migrate-n implements maximum likelihood and Bayesian methods to estimate Θ (the mutation scaled population size for a population) and M (the mutation scaled immigration rate) from microsatellite genotypes.

The product of Θ (4Neμ) and M (m/μ) divided by four is an estimate of the number of migrants per generation from population A to population B. The program was using the recommended initial settings until each run achieved convergence and Θ and M were then recorded for all populations.

Population size reductions

Recent population size reductions were identified by using the program BOTTLENECK v.1.2.02 (Piry et al. 1999). In a stable population, the expected heterozygosity (H_E) should be equal to that under mutation-drift equilibrium (H_{eq}). When there is a reduction in population size, there is an associated reduction in both the number of alleles in a population and the expected heterozygosity (H_E). However, the reduction in allele number occurs more rapidly than the reduction in H_E, leaving an H_E that is significantly larger than H_{eq} (Cornuet and Luikart 1996).

BOTTLENECK utilizes a two-phase mutation model that simultaneously incorporates the infinite allele model (IAM) and the stepwise mutation model (SMM). The IAM assumes that any number of changes in number of repeats between alleles is possible in a single mutational step (Nei et al. 1976). As a result, two alleles that are separated by twenty repeats are as likely to occur as two alleles that are separated by two repeats. The SMM accounts for the size in basepairs of each allele and assumes mutations only occur by the addition or subtraction of a single repeat (Kimura and Ohta 1978); so that alleles separated by twenty repeats require twenty mutational steps, and alleles separated by two repeats only require two mutational steps. Under the two phase mutation model in BOTTLENECK, the majority of mutations may occur in a stepwise manner while a small proportion of mutations may occur under the IAM. This is a more

realistic model of microsatellite evolution than either the SSM or IAM alone (Piry et al. 1999). I implemented the TPM on the data allowing 5% of mutations to occur under the IAM and ran the data for 2000 replicates.

Population structuring

In order to identify the amount of genetic diversity at various hierarchical levels I performed an analysis of molecular variance (AMOVA) on the data in Arlequin v3.5 (Excoffier and Lischer 2010). Populations were partitioned based on the three major clades identified by Beckmann (2010): southeastern (Dade, FL; Key Largo, FL; Merritt Island, FL), northeastern (Amelia Island, FL; Alachua, FL; Nassau, FL; St. Johns, FL; Clinch, GA; Camden, GA; Chatham, GA; Glynn, GA; Cumberland Island, GA; Jekyll Island, GA; St. Simons Island, GA; Sapelo Island, GA; St. Catherines Island, GA; Ossabaw Island, GA; Skidaway Island, GA; Hunting Island, SC; New Hanover, NC), and western (Santa Rosa, FL; Perry, MS; Tuscaloosa, AL). Genetic variation was estimated within individuals, among individuals within populations, among populations, and among the three geographic regions.

Local structuring among populations was identified using STRUCTURE v.2.3.3 (Falush et al. 2003, Hubisz et al. 2009, Pritchard et al. 2000). By using a Bayesian clustering method this software organizes samples into the most likely number of clusters (K). Populations were assigned to clusters without prior information about geographic location and admixture was allowed among populations. Individual Markov Chain Monte Carlo simulations were run with a 50,000 iteration burnin followed by 450,000 additional iterations. Five independent simulations were run for each possible value of K from 1 to

26. This is three more clusters than the number of populations in the study, allowing for potential population substructuring (Evanno et al. 2005).

Previous research indicates all three clades have been isolated for more than 50,000 years (Beckmann 2010). In that time, homoplasy is likely to arise among populations from different clades, which may incorrectly identify structuring among populations from different clades. To account for the high levels of homoplasy associated with microsatellite loci, each of the three clades was also independently analyzed in STRUCTURE using the same parameters described above with the exception of K: southeastern (K=1-6), northeastern (K=1-20), western (K=1-6).

Two conflicting approaches are suggested for identifying the most likely value of K. Pritchard et al. (2000) indicates that the log probability output by STRUCTURE (ln P(D)) accurately represents the most likely estimate of K. However, Evanno et al. (2005) found that this was not the best method when K was greater than 2. That study suggested that the change in the likelihood relative to K (ΔK) better determined the most accurate value for K. As such, both methods were used to identify the optimal value of K for all four STRUCTURE analyses.

Results

Of the seven loci included in this study, none exhibited signs of selection. A total of 104 alleles were identified among all populations, with an average of 14.86 alleles per locus (Table 4.2). The fewest alleles were identified at locus TA5GT6 (5) and the most were identified at GT15 (20). All loci were polymorphic in all populations except for the Cumberland Island population where allele 227 was fixed for locus TA5GT6. Eight populations harbored unique alleles, ranging from one (Camden, GA; Ossabaw Island,

GA; Santa Rosa, FL; Merritt Island, FL) to six (Key Largo, FL). A total of 15 unique alleles were identified across six loci and no unique alleles were identified at locus TA5GT6 (Table 4.3). A unique allele was shared among all populations in the southeastern clade (153 at locus GT66); no unique alleles were shared in the western clade, and eight unique alleles were shared among populations in the northeastern clade (Table 4.3). One allele (178 at locus AC19) was found in both the western and southeastern clades, but not the northeastern clade.

Micro-Checker identified null alleles in four populations: Camden, GA (AC19, 0.0965); Cumberland Island, GA (GT66, 0.0977) (AC19, 0.0974); Alachua, FL (GT21, 0.1501); and St. Johns, FL (GT66, 0.1538). (Numbers in parentheses represent the locus and the frequency of null alleles at that locus). Few populations contained null alleles, indicating that the amplification procedures used were effective across all loci. Micro-Checker also identified no evidence of large allele dropout or scoring errors.

Genetic variation within populations

Deviations from HWE were identified in 19 of the 23 populations analyzed. Of those 19 populations, eleven showed deviations at one locus, five had deviations at two loci, two had deviations at three loci (Hunting Island, SC and St. Johns, FL), and one had deviations at six loci (Cumberland Island, GA) (Table 4.4). After correcting for null alleles one locus became consistent with HWE (St. Johns, FL, GT66). I assumed all other deviations from HWE were due to biological processes and not the result of technical error.

All but two populations (Dade, FL and Clinch, GA) showed evidence of linkage disequilibrium (LD) among loci at a significance level of $p< 0.05$. Linkage disequilibrium

between loci can occur due to gene flow, genetic drift, or physical linkage between loci. In the case of physical linkage, recombination does not occur between loci and they are inherited as a single unit. If this is the case, then genotypes at these loci are not independent, and one locus must be removed from the dataset in order to accurately analyze variation within and among populations. However, testing multiple hypotheses using the same dataset greatly increases the likelihood of falsely identifying a significant difference among groups (Type I error) (Abdi 2007). To account for this, I applied a Bonferroni correction to the data which accounts for an increased likelihood of Type I error. Based on 21 comparisons within each population, the corrected p value was $p<0.00244$. This correction, although contentious, is widely used when calculating LD (Nakagawa 2004). After applying the correction, only five populations contained loci with significant tests of LD: Camden, GA; St. Simons Island, GA; Ossabaw Island, GA; Cumberland Island, GA; and Key Largo, FL (Table 4.5). Because no pair of loci consistently demonstrated LD with or without a Bonferroni correction all loci were used in the remaining analyses (Selkoe and Toonen 2006).

Mean gene diversity and allelic richness varied considerably among populations (Table 4.6). The lowest He (0.536) and R (3.143) were found in the Skidaway Island, GA population and the largest He (0.832) and R (7.714) were found in Glynn, GA. Among mainland populations, the lowest He (0.745) and R (6) were found in the Tuscaloosa, AL population (Table 4.6). Five populations also had significantly high values of F_{IS} indicative of inbreeding (Table 4.6): Merritt Island, FL (F_{IS}: 0.075), Saint Johns, FL (F_{IS}: 0.236), Santa Rosa, FL (F_{IS}: 0.124), Perry, MS (F_{IS}: 0.114), and Tuscaloosa, AL (F_{IS}: 0.075).

Genetic variation among populations

Among all population pairwise comparisons, only 13 F_{ST} values were not significant (Table 4.7). Of these, 12 were among populations from mainland Georgia and Florida. The remaining non-significant value was between Santa Rosa, FL and Perry, MS (0.012). The F_{ST} values between Clinch, GA and either Glynn, GA or Nassau, FL were zero. The highest F_{ST} value was between Hunting Island, SC and Skidaway Island, GA (0.355). The largest F_{ST} not between two island populations was between Skidaway Island, GA and Tuscaloosa, AL (0.227).

All island populations were significantly divergent from all mainland populations based on F_{ST}. Six of the 11 island populations also had F_{ST} values greater than 0.10 when compared with any mainland population. Of those with values less than 0.10, the Jekyll Island, GA population was most similar to the Santa Rosa, FL (0.05) and Perry, MS (0.071) populations. Given the large distances between these populations, and that similar values are not seen between Jekyll Island and mainland Georgia populations, this is likely due to homoplasy.

The Merritt Island, FL population exhibited F_{ST} values that were similar to those of Dade, FL. Cumberland Island, GA had values below 0.10 when compared to St. Johns, FL (0.053) and Nassau, FL (0.059). The Sapelo Island, GA population was also more similar to Nassau, FL than any other mainland population (0.065). Finally, the St. Simons Island, GA population had several values below 0.10 including: Glynn, GA (0.052), Clinch, GA (0.065), and Camden, GA (0.085).

Migrate-n identified five mainland populations exhibiting low levels of immigration and emigration (Tuscaloosa, AL; Perry, MS; Santa Rosa, FL; St. Johns, FL;

and New Hanover, NC). The lack of migration involving the New Hanover population is likely the result of isolation by distance. Of the six populations, three of them comprise all of the populations sampled from the western region. Within the western region, the Tuscaloosa, AL population exhibited the fewest number of migrants per generation (ΘM) in or out of the population, and few migrants were observed among any of the three populations (Table 4.8)

In the northeastern region, the population from St. Johns, FL had the lowest ΘM. Large ΘM were observed among many of the mainland populations in this region with the exception of the populations from St. Johns and New Hanover. Of the nine island population in this region, five had extremely low ΘM indicative of isolated populations (Hunting Island, SC; Ossabaw Island, GA; St. Catherines Island, GA; Jekyll Island, GA; and St. Simons Island, GA). Among the remaining four islands, Skidaway Island, GA had the largest ΘM.

Within the southeast region, the Key Largo population had very low estimates of ΘM indicative of long standing isolation. Both Dade and Merritt Island showed similar patterns of ΘM. Estimates also indicate that migration between Dade and Merritt Island is occurring at a rate of approximately one migrant every other generation. Estimates also indicate there may be some gene flow between Dade, FL and the population from Alachua, FL in the northeastern region. Other than the potential migration between Dade and Alachua, estimates of ΘM among populations from different geographic regions are low.

Reductions in population size

Using one-tailed Wilcoxon sign-rank tests, BOTTLENECK identified significant excess heterozygosity in seven populations: St. Simons Island, GA (p=0.004); Sapelo Island, GA (p=0.004); Cumberland Island, GA (p=0.04); Chatham, GA (p=0.008); Clinch, GA (p=0.04); Alachua, FL (p=0.008); and Nassau, FL (p=0.008). These values indicate that these populations likely experienced a recent population bottleneck. While the Wilcoxon sign-rank test provides a quantitative measure of excess heterozygosity, BOTTLENECK also performs a qualitative test to graphically identify mode shifts associated with population bottlenecks. While six additional populations showed mode shifts consistent with a population bottleneck (Hunting Island, SC; New Hanover, NC; St. Catherines Island, GA; Amelia Island, FL; Tuscaloosa, AL; Perry, MS), the authors of the program caution that mode shift distortions are not sufficient to identify a real population bottleneck unless a minimum of 30 individuals and 10 microsatellite loci are sampled (Piry et al. 1999). Due to low sample sizes, I must assume that only bottlenecks identified by Wilcoxon sign-rank tests represent real biological events.

Population structuring

The AMOVA analysis identified that most of the genetic variation in this study was due to variance within individuals (83.94%) (Table 4.9). A significant proportion of the variation (11.27%) was also attributable to variance among populations within the three previously identified clades. However, little variation was due to variance within populations (3.87%) or among clades (0.92%). This suggests that there is not much genetic variation within populations or among clades.

In the STRUCTURE analysis of all 23 populations the ln P(D) method of Pritchard et al. (2000) indicated K=14, while the ΔK method of Evanno et al. (2005) indicated K=3. Because there is a running debate over which method to utilize (Pritchard and Wen 2003, Evanno et al. 2005, Hubisz et al. 2009), I compared both results to other available data as suggested by Pritchard et al. (2007). When K=3, the clusters did not correspond to the three clades previously identified (Beckmann 2010) and did not identify any unique island populations which is inconsistent with the F_{ST} values in this study (Table 4.7). Also, the ln P(D) method indicates that K=3 is extremely unlikely, so K=14 was accepted as the correct number of clusters (Figure 4.3).

Assuming K=14, several island populations formed unique clusters (Skidaway Island, GA; St. Simons Island, GA; Jekyll Island, GA; Hunting Island, SC; Ossabaw Island, GA: St. Catherines Island, GA; Cumberland Island, GA; Key Largo, FL; Amelia Island, FL). The populations of Merritt Island, FL and Glynn, GA fell into the same cluster, which is likely due to homoplasy, given that no populations located between them fell into this cluster. The population of Chatham, GA clustered with the populations from the western clade, which is also likely due to homoplasy, as no populations from southern Georgia or northern Florida fell into this cluster. In the state of Florida, the populations of Dade, FL and Alachua, FL formed a cluster. Because these populations are from separate clades, this is either the result of homoplasy or recent gene flow between these populations. However, I can make no definite conclusion because no populations located geographically between Alachua and Dade were included in this analysis.

When the southeastern clade was analyzed separately, K=3 was supported by both the ln P(D) method and the ΔK method (Figure 4.4). The clusters indicated admixture

between Dade, FL and Merritt Island, FL indicating gene flow may be occurring throughout peninsular Florida. However, little to no admixture existed between either of these populations and the Key Largo, FL population, indicating it has been isolated from mainland Florida for a long period of time.

The analysis of the western clade, provided conflicting results between the ln P(D) method (K=1) and the ΔK method (K=2). However, the ΔK method is incapable of correctly identifying cases where all populations form a single cluster, and Evanno et al. (2005) state that the ln P(D) method is the better method when K<3. As a result, K=1 was accepted for the western clade. Each population was then forced into a unique cluster to identify degree of admixture among clusters (K=3), and complete admixture was identified among the three populations, suggesting high levels of gene flow throughout the western clade (Figure 4.5).

When the northeastern clade was analyzed separately both methods indicated K=12. All nine island populations in this region formed unique clusters (Figure 4.6). Among those, the populations of Jekyll Island, GA; Sapelo Island, GA; Skidaway Island, GA; St. Catherines Island, GA; Ossabaw Island, GA; Amelia Island, FL; and Hunting Island, SC had low levels of admixture with other populations. The population of St. Simons Island, GA exhibited admixture from both the Jekyll Island population and from the mainland clusters. Admixture with the mainland was also identified in the Cumberland Island, GA population.

The mainland populations fell into three clusters, with Chatham and Glynn, GA forming one cluster, Camden, GA forming its own cluster, and a third cluster forming with the remaining populations (New Hanover, NC; Clinch, GA; Alachua, FL; Nassau,

FL; St. Johns, FL). This last cluster was also identified in the combined analysis of all 23 populations; excluding the Alachua, FL population. Given the short geographic distance and low F_{ST} values between Alachua and these other populations, this clustering is more logical than a cluster including only Alachua and Dade (Table 4.7). All mainland clusters showed evidence of admixture with each other however the cluster containing Chatham and Glynn, GA and the cluster containing only Camden, GA both harbored unique alleles not present in other clusters. The Chatham/Glynn cluster contained allele 244 at the GT15 locus, while the Camden cluster contained allele 157 at the GT66 locus and allele 229 at the GT21 locus.

Discussion

Structuring among geographic regions

The results of this study provide support for my hypothesis that there is geographic structuring of genetic variation throughout the species' range. Unique alleles, specific to particular regions suggest that there is structuring of genetic variation among the three regions identified by Beckmann (2010). All populations in the southeastern region harbor an allele that was not found in either the western or northeastern regions (Table 4.3). Also, both the southeastern and western regions contain an allele that was not found in any population in the northeastern region. Given that the northeastern region was better sampled than either of the other two regions, the presence of these alleles suggests reduced gene flow among the three regions. While the northeastern region harbors eight unique alleles, it is not possible to determine whether this is due to geographic isolation from the other two regions, or the result of poor sampling in both the southeast and west. However, estimates of ΘM indicate there is little to no gene flow

between populations from the western region and either the northeastern or southeastern regions (Table 4.8).

STRUCTURE analyses also provide evidence for differentiation of the western region from the other two regions. In the analysis that included all populations, the samples from the western region fell into the same cluster along with the samples from Chatham, GA (Figure 4.3). However, none of the populations geographically located between Chatham, GA and the western region fell into this cluster. This suggests that similarities among these populations are likely the result of size homoplasy among microsatellite loci. Additionally, when the samples from the northeastern clade were analyzed separately, the Chatham, GA population grouped into a cluster with Glynn, GA (Figure 4.6). If the relationship between the western clade and Chatham were due to common ancestry, I would expect the Chatham population to form a unique cluster when the western samples were removed. Instead, the population groups with Glynn, GA, a cluster that is consistent with expectations based on the population pairwise F_{ST} values.

Similarly, when comparing the northeastern and southeastern regions, the populations from Merritt Island, FL and Glynn, GA form a cluster in the combined analysis (Figure 4.6). All populations geographically located between them fall into a cluster with other populations from the northeastern region. As was the case with Chatham, GA and the western region, this suggests homoplasy between Glynn and Merritt Island. The populations from Alachua, FL and Dade, FL also form a cluster in the combined analysis. Given that no geographically intermediate populations were sampled between Alachua and Dade, it is not possible to determine if this is due to homoplasy or recent gene flow between the populations. Further, recent gene flow is a strong

possibility, because these two mainland populations are not separated by any known geographic barriers to gene flow. However, estimates of ΘM indicate less than one migrant per generation between these two populations, suggesting that the level of gene flow is not sufficient to maintain similarities between the populations (Table 4.8). Additional sampling is needed throughout the southeastern region to determine whether there is actually contemporary gene flow between these populations, or if homoplasy is responsible for this pattern.

Structuring within geographic regions

Multiple lines of evidence support my prediction that there would be local structuring of genetic diversity within the three mainland lineages of *P. gossypinus* identified by Beckmann (2010) (Figure 4.1). STRUCTURE analyses and pairwise F_{ST} values identified structuring of populations within all three geographic regions. In the southeastern region, the Dade, FL and Merritt Island, FL populations fell into different clusters in the STRUCTURE analysis (Figure 4.4). While this is initially expected because Merritt Island is technically an island, it is separated from the mainland by less than 60m and previous studies indicate populations on the island are panmictic with nearby mainland populations (Beckmann 2010, Boone et al. 1999). Because of this, the two populations falling into separate clusters demonstrates structuring within the southeastern region.

A high F_{ST} value between Dade and Merritt Island also indicates reduced gene flow between these populations, as does a low estimate of ΘM. Given *P. gossypinus*' limited dispersal ability, this may be due to isolation by distance. Alternatively, evidence of inbreeding in the Merritt Island population suggests that organisms from Merritt Island

and nearby Brevard, FL may be isolated from other populations (Table 4.6). This is also supported by the presence of a unique allele in this population (Table 4.3). More thorough sampling of populations from central Florida is necessary to determine which mechanism is responsible for this divergence.

Geographic structuring is also present in the northeastern region. While STRUCTURE analyses, F_{ST} values and ΘM indicate a high degree of gene flow among mainland Georgia and northern Florida populations, several populations provide evidence of geographic structuring. The New Hanover, NC population exhibits significantly high F_{ST} values consistent with isolation by distance as well as low estimates of ΘM. This is expected given that this population is over 1000 km from the nearest sampled population in Georgia. Although it is in close geographic proximity to other populations in coastal Georgia and northern Florida, the St. Johns, FL population also shows evidence of isolation and differentiation.

The St. Johns, FL population also exhibits extremely high F_{ST} values relative to other mainland populations from this region. The St. Johns population also harbored unique alleles suggesting it has limited gene flow with other nearby populations. Additionally, this population had the lowest estimates of ΘM among mainland populations from the northeastern region (Table 4.8). Dispersal of organisms involving this population is likely limited by rivers which may act as geographic barriers to gene flow with other nearby populations. Rivers have been identified as barriers to gene flow in several other small mammal species (Degner et al. 2010; Indorf 2010, Soltis et al. 2006). This population provides evidence that limited dispersal ability and geographic

barriers to gene flow can combine to strengthen structuring of genetic variation within a region.

Although all populations in the western region fall into a single cluster in the STRUCTURE analysis, other lines of evidence indicate geographic structuring in this region (Figure 4.5). The Santa Rosa, FL population contains a unique allele not found in either of the other sampled western populations. All three western populations are characterized by a significant inbreeding coefficient indicating reduced gene flow in this region and providing evidence of local structuring (Table 4.6). Additionally, the F_{ST} values between the Tuscaloosa, AL population and both the Santa Rosa, FL (0.083) and Perry, MS (0.062) are five to seven times greater than the F_{ST} values between Santa Rosa and Perry (0.012). While these populations are farther from Tuscaloosa (270 km) than they are from each other (200 km), an isolation by distance model is not sufficient to explain such a large increase F_{ST} over this distance. Rather, a better explanation is that migration out of this population is limited by urbanization.

Urbanization strengthens patterns of diversity

Evidence from several populations, including the one from Tuscaloosa, support my prediction that urbanization strengthens local geographic structuring of genetic variation in mainland populations. In the case of Tuscaloosa, the population was sampled on the north side of downtown Tuscaloosa in an urbanized area. High F_{ST} values between the Tuscaloosa population and other western populations as well as low estimated ΘM between Tuscaloosa and either of the other western populations suggest a negative effect of urbanization on gene flow (Table 4.8). A likely explanation for the low levels of gene flow between this population and the other two from this region is that the urban matrix

of the downtown area is limiting the dispersal ability of *P. gossypinus*. Other small mammals, including *Peromyscus* species have been shown to exhibit reduced dispersal ability through urban matrices (Bolger et al. 1997, Davidson et al. 2009).

An effect of urbanization on genetic variation is further indicated in the case of the Tuscaloosa population because it harbors the lowest level of genetic diversity among all mainland populations in this study, and has a heterozygote deficiency at six of the seven loci sampled. Heterozygote deficits can be attributed to selection, genetic drift, inbreeding, or a Wahlund effect (Hedrick 2005). A Wahlund effect occurs when multiple non-interbreeding populations are sampled and analyzed as if they were one population. In the case of Tuscaloosa a Wahlund effect is unlikely. All individuals from this area were genetically and morphologically identified as members of the same species and were sampled from the same trapping grid in a single forested fragment. Also, previous mitochondrial analysis indicates less genetic variation is present in this population than would be expected if two non-interbreeding populations were sampled as one. As a result, the reduced heterozygosity is likely the result of genetic drift and/or inbreeding associated with urbanization.

In the northeastern region, the St. Johns, FL population exhibited heterozygote deficits at six of the seven loci. This was the only mainland populations outside of the western region to exhibit a significantly high inbreeding coefficient, with a coefficient that was 70% higher than that of the Tuscaloosa population and more than double that of either of the other western populations (Table 4.6). Inbreeding coefficients this high indicate that this population may be isolated from other nearby mainland populations. This is further supported by extremely low ΘM into this population (Table 4.8). Further,

this population was sampled from an area that has undergone recent large scale urbanization (Mulkey 2007).

In addition to Tuscaloosa and St. Johns, several other mainland populations show evidence of recent population bottlenecks which are often characteristic of the effects of urbanization (Noel et al. 2007). Recent bottlenecks in Alachua, FL ($p=0.008$); Nassau, FL ($p=0.008$); Clinch, GA ($p=0.04$); and Chatham, GA ($p=0.008$) indicate that some recent event caused a marked decrease in population size for each of these populations. The most likely explanations are the effects of climatic events or habitat destruction associated with urbanization. If climatic events are responsible, then all populations in an area should be affected. Because the bottlenecks are scattered across a large geographic area and all populations in the southeastern US experience the same climate, climatic events are unlikely to explain the pattern in these populations. On the other hand, two of the populations Nassau, FL and Chatham, GA are in close proximity to areas where recent widespread development has occurred (Jacksonville, FL and Savannah, GA). As such, these bottlenecks may be the result of urbanization. However, it is not possible to distinguish between these two possibilities without studies of variation genetic variation at the population level over time.

Structuring among island populations

Several measures of genetic diversity and population structuring support my prediction that some island populations are genetically distinct from the mainland. Of the eleven island populations included in this study, only Merritt Island and Sapelo Island did not form unique clusters in the STRUCTURE analysis (Figure 4.3). Further, every island except Merritt Island and Jekyll Island showed low levels of allele richness which is

consistent with an isolated island population (Table 4.6). The populations of Ossabaw Island, Jekyll Island, and Key Largo all contained unique alleles which also suggests a lack of gene flow with nearby mainland populations. Finally, seven of the island populations showed little evidence of admixture with the mainland in the STRUCTURE analyses (Figure 4.3). Lack of admixture indicates that these islands are genetically distinct and isolated from the mainland, which is consistent with previous findings using both allozyme and mitochondrial markers (Boone 1995, Beckmann 2010).

Four island populations did exhibit evidence of recent gene flow from the mainland. Cumberland Island, Sapelo Island, St. Simons Island, and Merritt Island all showed some degree of admixture with mainland populations in the STRUCTURE analysis (Figure 4.6). F_{ST} values and estimates of ΘM indicate higher levels of gene flow between the mainland and these island populations than for any other islands. While St. Simons Island and Merritt Island are connected to the mainland by a land bridge, both Sapelo and Cumberland Islands are separated from the mainland by several kilometers of open water making evidence of gene flow surprising. However, both islands are serviced several times a day by ferries which transport people and goods between the islands. Given *P. gossypinus*' inability to disperse across large bodies of water (Pearson 1950) these ferries are the most likely avenue of migration between the island and mainland.

Conclusion

Based on the results of this study, gene flow and genetic drift have shaped the distribution of genetic variation throughout the species' range of *P. gossypinus*. Given the high degree of similarity among population separated by large distances, gene flow is not

limited by the species' low vagility, but rather by a variety of physical barriers to gene flow, as well as climatic and anthropogenic events.

Evidence indicates there is a division between the northeastern and western clades that is consistent with patterns identified using allozyme and mitochondrial data (Boone 1995, Beckmann 2010). While this division likely occurs in the Apalachicola/Chattahoochee/Flint River Basin (ACF), this cannot be clearly identified in this study. Additional sampling from geographic locations between Santa Rosa, FL and Alachua, FL is necessary to verify if structuring in this region is consistent with the results of previous studies. Likewise, increased sampling is needed throughout peninsular Florida to effectively conclude if gene flow is occurring between populations in northern Florida and populations in peninsular Florida. While F_{ST} values and range wide STRUCTURE analyses support the presence of gene flow between southeastern and northeastern clades, region specific analyses and the presence of unique alleles within regions indicate these similarities may be the result of homoplasy. Without further sampling of populations throughout the peninsula this question remains unanswered.

Within regions, intracoastal waterways and rivers act to reduce or eliminate gene flow between populations. As a result, there is local structuring of genetic diversity, which can be seen by the separation of several island populations into unique clusters (Figures 4.3-4.6). In addition, rivers appear to act as effective barriers to gene flow in this species, as evidenced by the low levels of gene flow with the St. Johns, FL population, even over short distances.

Natural barriers to gene flow are not the only barriers responsible for current structuring of variation. Reduced gene flow and recent population bottlenecks involving

populations in recently urbanized areas can likely be attributed to anthropogenic effects. However, to conclude that these patterns are due to anthropogenic effects, an analysis of genetic variation within and among populations before and after urbanization occurred is necessary. This analysis will also determine if high levels of inbreeding in some populations is the result of urbanization.

The results of this study indicate that barriers to gene flow and anthropogenic effects combine to shape the geographic structure of genetic diversity on a regional scale. Further, this study identified several island and mainland populations that are currently experiencing genetic drift and inbreeding. Conservation biologists can utilize these data to prevent local extinctions, maintain gene flow among populations, and preserve the overall genetic diversity of the species.

In particular the conservation of island population experiencing these processes should be a priority. Island populations are more likely to go extinct as a result of genetic drift, due to a lack of gene flow from the mainland. Extinction of small mammal species in island populations without recolonization can greatly disrupt an island's ecosystem, as these species commonly serve as a food source for a large number of carnivorous species.

The effects of natural and anthropogenic barriers to gene flow on this species provide a more thorough understanding of how small mammal habitat generalists respond to these barriers. These findings may drive research into how other small mammal species are affected by both aquatic and terrestrial barriers to gene flow. Additionally, this study also provides support for previous research, which suggests that small mammals are negatively impacted by urbanization, regardless of habitat specificity (Bolger et al. 1997). While this research improves knowledge of how species respond to

urbanization, further studies are needed to explore the degree to which urbanization impacts genetic diversity, and how best to mitigate its effects on small mammals.

Table 4.1. Microsatellite loci utilized as part of this study. Locus name and repeat type are reported, as well as the paper where they were first published. Weber indicates the locus was taken from Weber et al. 2009, Schmidt indicates the locus was taken from Schmidt 1999. Also reported are the dye label, $MgCl_2$ concentration, and annealing temperature range used in this study. Numbers in parentheses are the original loci names from the source publication.

Locus	Repeat type	Source publication	Dye-Label	$MgCl_2$ Concentration	Annealing Temperature
GT15	Dinucleotide	Schmidt	NED yellow	1.5mM	53-58^o
GT66	Dinucleotide	Schmidt	VIC green	1.5mM	50-55^o
GATA70	Tetranucleotide	Schmidt	6-FAM blue	2.5mM	53-58^o
TA5GT6 (14)	Dinucleotide	Weber	VIC green	2mM	55-60^o
AC9 (35)	Dinucleotide	Weber	NED yellow	2mM	55-60^o
AC19 (49)	Dinucleotide	Weber	6-FAM blue	2mM	55-60^o
GT21 (80)	Dinucleotide	Weber	PET red	2mM	55-60^o

Table 4.2. Number of alleles per locus for 23 populations of *Peromyscus gossypinus* included in this study. At total of 104 alleles were identified among all populations. Population abbreviations are: DAD (Dade, FL), KLF (Key Largo, FL), MER (Merritt Island, FL), CAM (Camden, GA), JIG (Jekyll Island, GA), SSI (St. Simons Island, GA), GLY (Glynn, GA), HIS (Hunting Island, SC), SKI (Skidaway Island, GA), SAP (Sapelo Island, GA), CHA (Chatham, GA), NHN (New Hanover, NC), CIS (Cumberland Island, GA), NAS (Nassau, FL), ALA (Alachua, FL), SJF (St. Johns, FL), CLI (Clinch, GA), SCI (St. Catherines Island, GA), OSS (Ossabaw Island, GA), AMI (Amelia Island, FL), TUS (Tuscaloosa, AL), SRF (Santa Rosa, FL), PER (Perry, MS). Numbers of individuals sampled per population are in parentheses.

	GT15	GT66	GATA70	TA5GT6	AC9	AC19	GT21	Mean per pop
DAD (18)	10	7	5	2	10	12	6	7.429
KLF (20)	7	8	5	3	6	8	5	6.000
MER (20)	10	10	4	4	10	12	10	8.571
CAM (27)	9	9	6	3	4	10	11	7.429
JIG (23)	9	7	5	4	13	10	7	7.857
SSI (10)	6	6	4	4	6	5	5	5.143
GLY (10)	13	9	5	4	6	10	7	7.714
HIS (10)	4	4	3	3	3	2	2	3.143
SKI (10)	2	3	4	2	3	4	4	3.143
SAP (10)	7	5	5	3	5	5	4	4.857
CHA (11)	9	8	5	4	7	9	6	6.857
NHN (10)	7	7	5	3	5	7	5	5.571
CIS (28)	8	7	6	1	4	8	8	6
NAS (15)	12	8	5	4	8	8	7	7.429
ALA (10)	6	6	5	3	8	8	5	5.857
SJF (11)	11	7	6	3	6	9	7	7.000
CLI (11)	10	8	5	4	6	9	6	6.857
SCI (10)	6	4	4	2	3	5	5	4.143
OSS (11)	4	5	6	3	3	5	5	4.429
AMI (10)	2	4	2	3	4	4	4	3.286
TUS (10)	7	7	4	4	6	8	6	6.000
SRF (10)	12	8	4	4	6	9	6	7.000
PER (10)	9	6	4	4	5	9	5	6.000
Mean per locus	**15.667**	**13.333**	**7.667**	**4.333**	**12.667**	**15.333**	**10.667**	**14.857**
Total per locus	**20**	**17**	**13**	**5**	**17**	**18**	**14**	**104**

Table 4.3. Unique alleles identified in individual populations or clades. The locus where the alleles were identified is listed in column 2 and the size of the allele in bp is listed in column 3. Northeaster, Southeastern, and Western represent the three clades identified in Chapters 2 and 3.

Population	Locus	Allele size in bp
Camden, GA	GT66	157
Glynn, GA	GT15	226 234
Jekyll Island, GA	AC9	186 188 192
Ossabaw Island, FL	GT21	231
St. Johns, FL	AC19	164
Santa Rosa, FL	GT15	230
Merritt Island, FL	TA5GT6	219
Key Largo, FL	GT66	125
	GATA70	265 269 273 277 281
Southeastern Clade	GT66	153
Northeastern Clade	GT66	155
	GATA70	259 283 287
	AC19	176 206
	GT21	207 209
Southeastern and Western Clades only	AC19	178

Table 4.4. Observed and expected heterozygosity of loci that diverged significantly from Hardy-Weinberg Equilibrium. Numbers in bold indicate loci with null alleles. An asterisk indicates a locus that no longer diverged from Hardy-Weinberg Equilibrium after accounting for null alleles. Population abbreviations are: DAD (Dade, FL), KLF (Key Largo, FL), MER (Merritt Island, FL), CAM (Camden, GA), JIG (Jekyll Island, GA), SSI (St. Simons Island, GA), HIS (Hunting Island, SC), SKI (Skidaway Island, GA), SAP (Sapelo Island, GA), CIS (Cumberland Island, GA), NAS (Nassau, FL), ALA (Alachua, FL), SJF (St. Johns, FL), CLI (Clinch, GA), SCI (St. Catherines Island, GA), OSS (Ossabaw Island, GA), AMI (Amelia Island, FL), TUS (Tuscaloosa, AL), PER (Perry, MS).

	GT15	GT66	GATA70	TA5GT6	AC9	AC19	GT21
DAD	-	-	-	-	0.100/0.895	-	-
KLF	-	-	0.950/0.787	-	-	-	-
MER	-	-	-	-	0.750/0.827	-	0.700/0.871
CAM	-	-	-	-	-	**0.593/0.783**	-
JIG	-	-	0.826/0.723	-	0.913/0.906	-	-
SSI	0.700/0.789	-	-	-	-	-	1.000/0.784
HIS	-	0.500/0.647	1.000/0.611	-	0.300/0.584	-	-
SKI	-	-	0.700/0.574	-	-	-	-
SAP	-	-	0.900/0.779	-	-	-	-
CIS	-	**0.643/0.790**	0.893/0.748	No het	0.786/0.681	**0.607/0.787**	0.786/0.779
NAS	-	-	-	-	0.800/0.851	-	-
ALA	-	-	-	-	**0.600/0.847**	-	-
SJF	-	**0.545/0.857***	-	0.182/0.437	0.545/0.805	-	-
CLI	-	-	-	-	0.636/0.818	-	-
SCI	-	-	-	-	0.100/0.426	-	-
OSS	-	-	0.727/0.799	-	-	-	-
AMI	-	-	-	-	0.400/0.758	-	0.800/0.758
TUS	0.500/0.800	-	-	-	-	-	0.700/0.832
PER	0.700/0.905	-	-	-	-	-	-

Table 4.5. Populations with loci in linkage disequilibrium after applying a Bonferroni correction. The Bonferroni correction lowered the p-value for significance to $p<0.0024$.

Population	Loci in disequilibrium
Camden, GA	GT15 and GT66 GATA70 and AC19 AC9 and GT21
Cumberland Island, GA	GT15 and GT66
St. Simons Island, FL	GT15 and GT66 GT15 and AC19
Ossabaw Island, GA	GT66 and GT21
Key Largo, FL	GT15 and GT66

Table 4.6. Estimates of within population genetic diversity and inbreeding for 23 populations of *Peromyscus gossypinus* included in this study. Measures of genetic diversity are mean gene diversity (H_E) and mean allelic richness (R). Population specific inbreeding coefficients (F_{IS}) are also included. Significant inbreeding coefficients are indicated in bold ($P < 0.05$). H_E is also included for each of the three previously identified clades at the bottom of the table. Population abbreviations are: DAD (Dade, FL), KLF (Key Largo, FL), MER (Merritt Island, FL), CAM (Camden, GA), JIG (Jekyll Island, GA), SSI (St. Simons Island, GA), GLY (Glynn, GA), HIS (Hunting Island, SC), SKI (Skidaway Island, GA), SAP (Sapelo Island, GA), CHA (Chatham, GA), NHN (New Hanover, NC), CIS (Cumberland Island, GA), NAS (Nassau, FL), ALA (Alachua, FL), SJF (St. Johns, FL), CLI (Clinch, GA), SCI (St. Catherines Island, GA), OSS (Ossabaw Island, GA), AMI (Amelia Island, FL), TUS (Tuscaloosa, AL), SRF (Santa Rosa, FL), PER (Perry, MS).

Population	Mean Gene Diversity (H_E)	Mean Allelic Richness (R)	Inbreeding Coefficient (F_{IS})
DAD	0.785	6.396	-0.079
KLF	0.737	5.267	0.021
MER	0.782	7.685	**0.075**
CAM	0.774	6.042	0.071
JIG	0.770	6.314	0.032
SSI	0.772	5.143	0.001
GLY	0.832	7.714	0.021
HIS	0.556	3.286	-0.012
SKI	0.536	3.143	0.015
SAP	0.734	4.857	0.027
CHA	0.816	6.634	-0.034
NHN	0.759	5.571	-0.017
CIS	0.662	4.903	0.013
NAS	0.815	6.722	-0.005
ALA	0.803	5.857	0.096
SJF	0.797	6.773	**0.236**
CLI	0.825	6.682	0.008
SCI	0.632	4.143	**0.231**
OSS	0.619	4.347	0.078
AMI	0.594	3.286	-0.156
TUS	0.745	6.000	**0.136**
SRF	0.816	7.000	**0.124**
PER	0.791	6.000	**0.114**

H_E for the northeastern clade: 0.821
H_E for the southeastern clade: 0.828
H_E for the western clade: 0.808

Table 4.7. Population pairwise F_{ST} among 23 populations of *Peromyscus gossypinus* included in this study. Significant F_{ST} vales are indicated in bold. Population abbreviations are: DAD (Dade, FL), KLF (Key Largo, FL), MER (Merritt Island, FL), CAM (Camden, GA), JIG (Jekyll Island, GA), SSI (St. Simons Island, GA), GLY (Glynn, GA), HIS (Hunting Island, SC), SKI (Skidaway Island, GA), SAP (Sapelo Island, GA), CHA (Chatham, GA), NHN (New Hanover, NC), CIS (Cumberland Island, GA), NAS (Nassau, FL), ALA (Alachua, FL), SJF (St. Johns, FL), CLI (Clinch, GA), SCI (St. Catherines Island, GA), OSS (Ossabaw Island, GA), AMI (Amelia Island, FL), TUS (Tuscaloosa, AL), SRF (Santa Rosa, FL), PER (Perry, MS).

	DAD	KLF	MER	SSI	GLY	HUN	SKI	SAP	CHA	NHN	CIS	JEK	ALA	SJF	CLI	SCI	OSS	NAS	CAM	SRF	AMI	PER
DAD																						
KLF	**0.115**																					
MER	**0.061**	**0.137**																				
SSI	**0.075**	**0.174**	**0.095**																			
GLY	**0.050**	**0.160**	**0.056**	**0.052**																		
HUN	**0.210**	**0.272**	**0.202**	**0.205**	**0.159**																	
SKI	**0.202**	**0.290**	**0.210**	**0.224**	**0.189**	**0.355**																
SAP	**0.089**	**0.175**	**0.087**	**0.155**	**0.124**	**0.272**	**0.253**															
CHA	**0.058**	**0.127**	**0.049**	**0.076**	0.016	**0.107**	**0.179**	**0.104**														
NHN	**0.074**	**0.115**	**0.064**	**0.141**	**0.068**	**0.208**	**0.235**	**0.118**	**0.044**													
CIS	**0.115**	**0.160**	**0.066**	**0.184**	**0.147**	**0.240**	**0.273**	**0.145**	**0.100**	**0.102**												
JEK	**0.104**	**0.144**	**0.083**	**0.079**	**0.101**	**0.156**	**0.212**	**0.129**	**0.102**	**0.140**	**0.139**											
ALA	**0.026**	**0.136**	**0.036**	**0.094**	0.021	**0.182**	**0.170**	**0.084**	**0.031**	**0.042**	**0.101**	**0.112**										
SJF	**0.034**	**0.112**	**0.031**	**0.088**	**0.040**	**0.159**	**0.235**	**0.104**	0.014	**0.043**	**0.053**	**0.108**	0.024									
CLI	**0.044**	**0.140**	**0.035**	**0.065**	0.000	**0.151**	**0.156**	**0.087**	0.014	**0.056**	**0.122**	**0.153**	0.014	**0.038**								
SCI	**0.168**	**0.233**	**0.186**	**0.183**	**0.128**	**0.205**	**0.290**	**0.200**	**0.139**	**0.181**	**0.260**	**0.171**	**0.117**	**0.164**	**0.108**							
OSS	**0.151**	**0.230**	**0.123**	**0.174**	**0.159**	**0.285**	**0.255**	**0.215**	**0.109**	**0.167**	**0.124**	**0.150**	**0.148**	**0.146**	**0.148**	**0.290**						
NAS	**0.047**	**0.116**	**0.025**	**0.092**	**0.025**	**0.139**	**0.156**	**0.065**	0.002	**0.025**	**0.059**	**0.137**	0.010	0.018	0.000	**0.140**	**0.119**					
CAM	**0.100**	**0.189**	**0.079**	**0.085**	**0.042**	**0.145**	**0.130**	**0.110**	0.017	**0.111**	**0.120**	**0.107**	**0.063**	**0.069**	**0.037**	**0.161**	**0.129**	**0.042**				
SRF	**0.083**	**0.148**	**0.113**	**0.087**	**0.077**	**0.184**	**0.148**	**0.097**	**0.064**	**0.073**	**0.113**	**0.050**	**0.067**	**0.074**	**0.067**	**0.145**	**0.137**	**0.079**	**0.066**			
AMI	**0.142**	**0.273**	**0.178**	**0.146**	**0.153**	**0.234**	**0.289**	**0.214**	**0.158**	**0.220**	**0.263**	**0.179**	**0.170**	**0.188**	**0.132**	**0.236**	**0.237**	**0.177**	**0.158**	**0.183**		
PER	**0.076**	**0.156**	**0.107**	**0.088**	**0.084**	**0.177**	**0.139**	**0.095**	**0.071**	**0.075**	**0.115**	**0.071**	**0.084**	**0.094**	**0.075**	**0.106**	**0.133**	**0.082**	**0.066**	0.012	**0.155**	
TUS	**0.095**	**0.168**	**0.134**	**0.136**	**0.093**	**0.241**	**0.227**	**0.124**	**0.085**	**0.162**	**0.170**	**0.159**	**0.093**	**0.109**	**0.088**	**0.182**	**0.218**	**0.093**	**0.090**	**0.083**	**0.220**	**0.062**

Table 4.8. Estimates of number of migrants per generation among 23 populations of *Peromyscus gossypinus* included in this study. Populations in columns are populations of immigration and populations in rows are populations of emigration. Cells with a dash indicate no migration. Population abbreviations are: DAD (Dade, FL), KLF (Key Largo, FL), MER (Merritt Island, FL), CAM (Camden, GA), JIG (Jekyll Island, GA), SSI (St. Simons Island, GA), GLY (Glynn, GA), HIS (Hunting Island, SC), SKI (Skidaway Island, GA), SAP (Sapelo Island, GA), CHA (Chatham, GA), NHN (New Hanover, NC), CIS (Cumberland Island, GA), NAS (Nassau, FL), ALA (Alachua, FL), SJF (St. Johns, FL), CLI (Clinch, GA), SCI (St. Catherines Island, GA), OSS (Ossabaw Island, GA), AMI (Amelia Island, FL), TUS (Tuscaloosa, AL), SRF (Santa Rosa, FL), PER (Perry, MS).

	SRF	ALA	SJF	CHA	CLI	CAM	NAS	GLY	NHN	TUS	PER	HIS	SKI	SCI	OSS	SAP	JIG	SSI	AMI	DAD	KLF	MER	CIS
SRF	-	-	-	**0.34**	-	-	-	-	-	**0.1**	**1**	-	-	-	-	-	-	-	-	**0.05**	-	-	-
ALA	**0.2**	-	**0.3**	**1.6**	**2**	-	**3**	-	**0.3**	-	-	-	**0.4**	**0.1**	-	**0.6**	**0.2**	-	**0.4**	**0.56**	-	**0.5**	**0.5**
SJF	-	**0.07**	-	**0.2**	-	-	**1**	-	-	-	-	-	-	-	-	-	-	-	-	**0.05**	-	**0.1**	**0.1**
CHA	-	**0.8**	-	-	**1**	**1.5**	-	**1**	**0.2**	**0.5**	-	-	-	-	-	**0.2**	**0.2**	0.1	-	-	-	-	**0.2**
CLI	-	**2.6**	**0.3**	**1.7**	-	-	**3**	**1.8**	-	-	-	-	**2.2**	-	-	-	**0.07**	**0.1**	**0.4**	-	-	-	**0.1**
CAM	**0.3**	**0.25**	-	**1**	**1**	-	**0.5**	**1.5**	-	-	**0.2**	-	**0.3**	-	-	**0.1**	**0.3**	**0.4**	-	-	-	-	**1**
NAS	-	**1.2**	-	**1.2**	**4**	**0.3**	-	-	**0.2**	-	-	**0.08**	-	**0.1**	-	**0.6**	**0.2**	**0.4**	**1**	**0.3**	-	**0.5**	**0.5**
GLY	**0.2**	-	-	**1**	-	**2**	-	-	-	-	-	-	**0.2**	-	-	**0.1**	**0.2**	-	-	-	-	-	**1**
NHN	-	**0.6**	-	-	-	-	**1**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TUS	-	**0.1**	-	-	-	**0.1**	-	-	-	-	**0.5**	-	-	-	-	-	-	-	-	-	-	-	-
PER	**1**	-	-	**1.6**	-	-	-	-	-	**0.5**	-	-	-	-	-	-	-	-	-	-	-	-	-
HIS	-	-	-	-	**0.4**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SKI	-	-	-	**1**	**2**	-	**3**	**0.3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SCI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OSS	-	-	-	-	-	**0.1**	-	**0.1**	-	-	-	-	-	**0.2**	-	-	-	-	-	-	-	-	-
SAP	-	**0.1**	-	**2**	**0.4**	**0.8**	**1**	**0.3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.1**
JIG	-	**0.1**	-	**0.3**	-	**0.1**	-	**0.1**	-	-	-	-	-	-	-	-	-	**1.3**	-	-	-	-	-
SSI	-	**0.4**	-	**2**	**0.5**	-	**1**	**0.2**	-	-	-	-	-	-	-	-	**0.23**	-	-	-	-	-	**0.1**
AMI	-	-	-	**2**	-	**2.7**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.5**
DAD	**0.1**	**0.7**	-	-	-	-	**0.3**	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.05**	**0.4**	-
KLF	-	**0.05**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**0.05**	-	-	-
MER	**0.1**	**0.7**	-	-	-	-	**0.3**	-	-	-	-	-	-	-	-	-	-	-	-	**0.5**		-	-
CIS	-	**0.3**	**0.3**	-	**0.4**	**0.5**	**1**	**0.1**	-	-	-	-	-	-	-	**0.2**	-	-	0.2	-	-	**0.1**	-

Table 4.9. Analysis of Molecular Variance (AMOVA) for 23 populations of *P. gossypinus*, genotyped at seven microsatellite loci. Populations represent all three major clades identified in mitochondrial analysis in Chapters 2 and 3. Populations were partitioned into Northeastern (Camden, GA; Jekyll Island, GA; St. Simons Island, GA; Glynn, GA; Hunting Island, SC; Skidaway Island, GA; Sapelo Island, GA; New Hanover, NC; Chatham, GA; Cumberland Island, GA; Nassau, FL; Alachua, FL; St. Johns, FL; Clinch, GA; St. Catherines Island, GA; Ossabaw Island, GA; Amelia Island, FL), Southeastern (Merritt Island, FL; Dade, FL; Key Largo, FL); and Western (Tuscaloosa, AL; Perry, MS; Santa Rosa, FL) regions for analysis.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance
Among groups	2	32.365	0.02702 Va	0.92
Among populations within groups	20	233.199	0.33230 Vb	11.27
Among individuals within populations	292	789.330	0.11429 Vc	3.88
Within individuals	315	779.500	2.47460 Vd	83.94
Total	629	1834.394	2.94822	

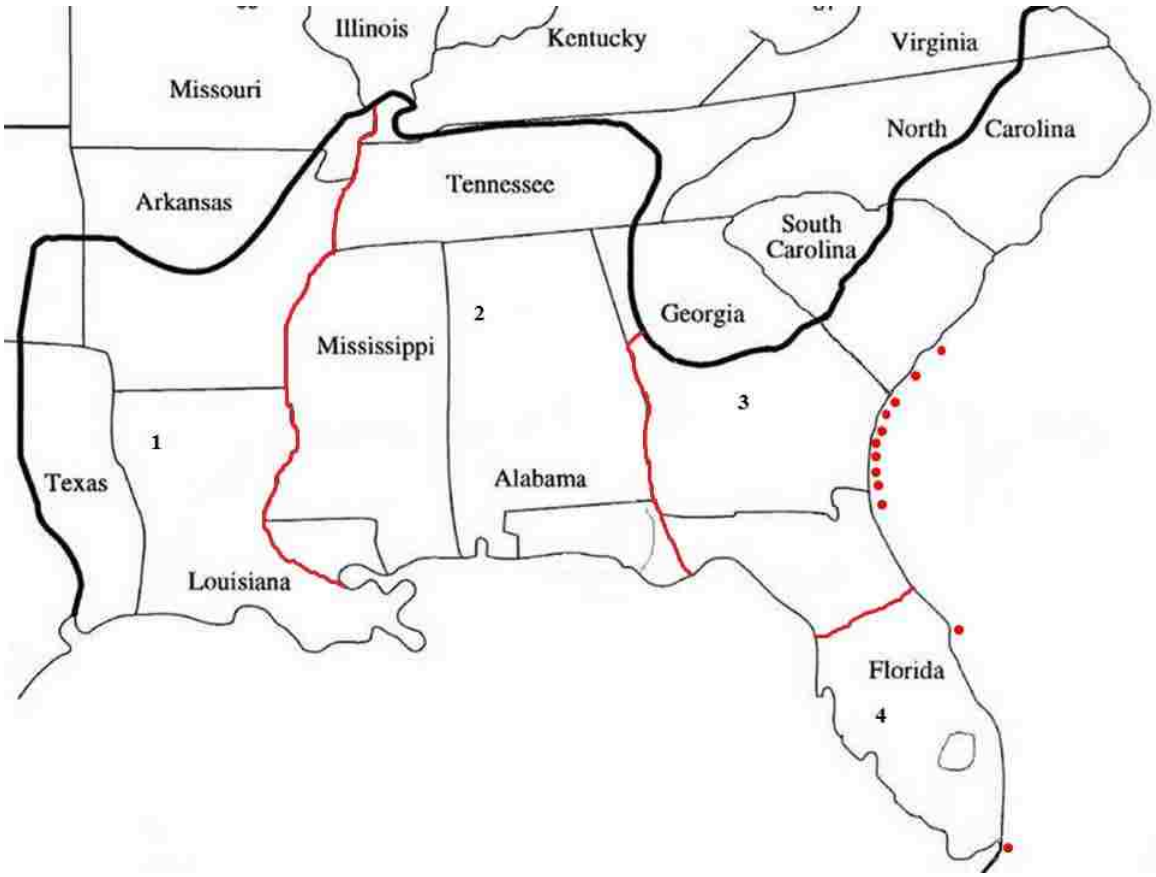


Figure 4.1. Range of the cotton mouse, *Peromyscus gossypinus* (area south and east of black line). Locations of island where *P. gossypinus* is found are highlighted with red circles. From north to south the islands are: Edisto Island, SC; Hunting Island, SC; Skidaway Island, GA; Ossabaw Island, GA; St. Catherines Island, GA; Sapelo Island, GA; St. Simons Island, GA; Jekyll Island, GA; Cumberland Island, GA; Amelia Island, FL; Merritt Island, FL; and Key Largo, FL. Red lines indicate borders between mainland subspecies. Mainland subspecies are indicated by numbers: 1) *P. g. booni*, 2) *P. g. megacephalus*, 3) *P. g. gossypinus*, 4) *P. g. palmarius.*

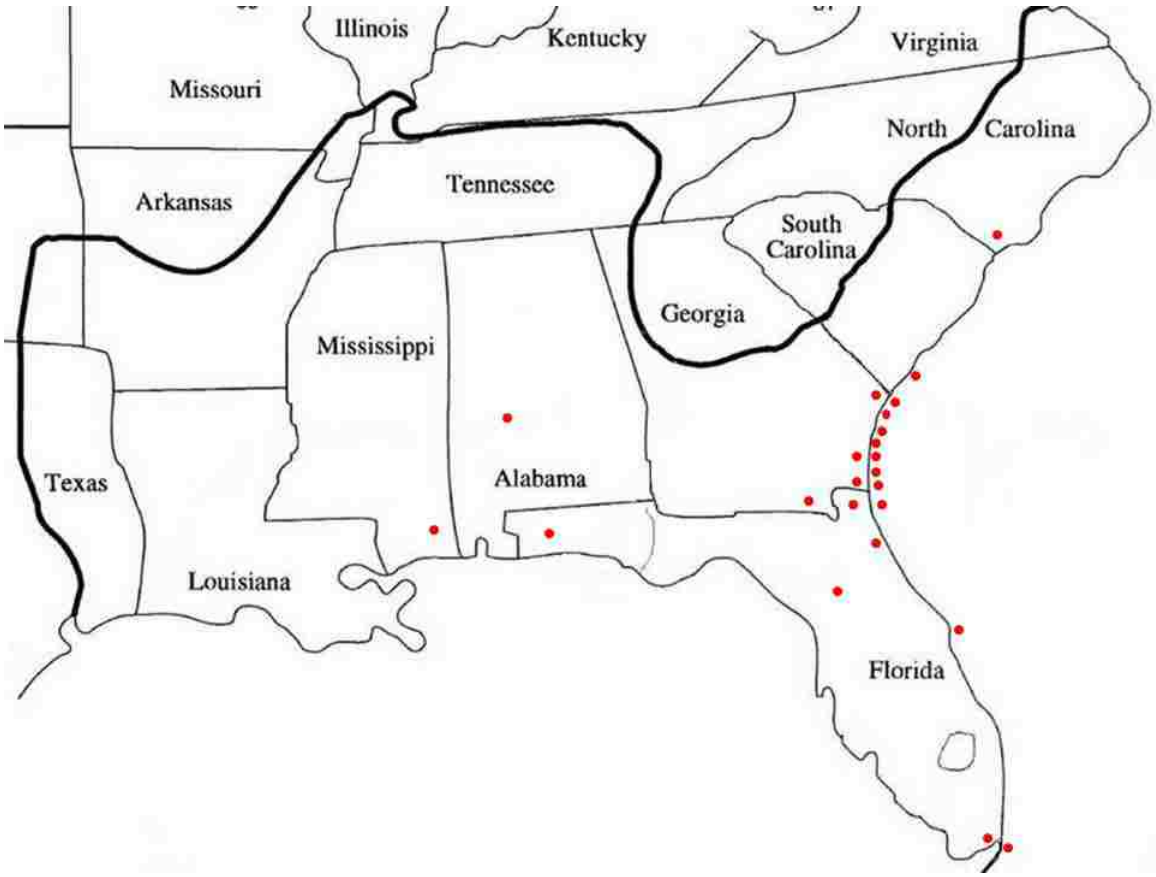


Figure 4.2. Range of the cotton mouse, *P. gossypinus*, (area south and east of black line) with red circles indicating populations included in this study.

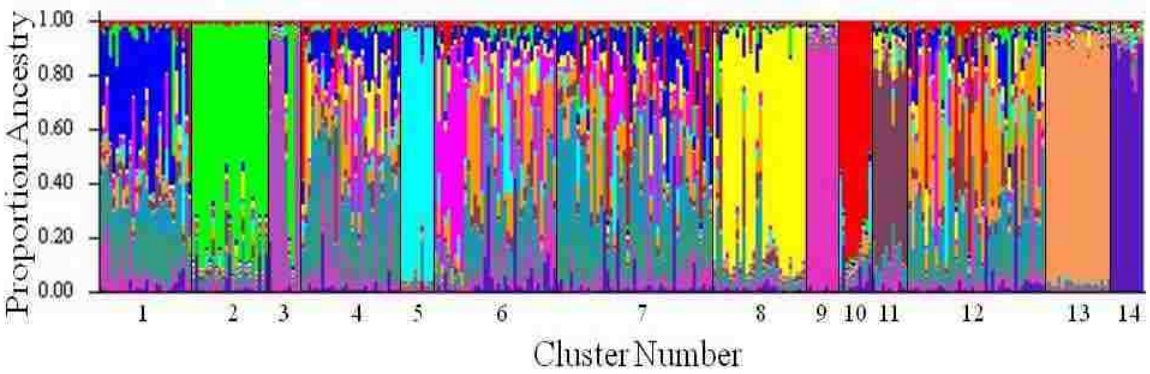


Figure 4.3. STRUCTURE analysis of all 23 populations included in this study. Populations formed 14 distinct clusters using the ln P(D) method of Pritchard et al. 2000. Cluster 1 contains all individuals from Dade, FL and Alachua, FL; cluster 2 contained all samples from Jekyll Island, GA; cluster 3 contained all samples from St. Simons Island, GA; cluster 4 contained all samples from Glynn, GA and Merritt Island, FL; cluster 5 contains all samples from Skidaway Island, GA; cluster 6 contained all samples from Sapelo Island, GA and Camden, GA; cluster 7 contained all samples from New Hanover, NC, St. Johns, FL, Nassau, FL, and Clinch, GA; Cluster 8 contained the samples from Cumberland Island, GA; cluster 9 contained the samples from Amelia Island, FL; cluster 10 contained all samples from St. Catherines Island, GA; cluster 11 contained all samples from Ossabaw Island, GA, cluster 12 contained the samples from Chatham, GA, Santa Rosa, FL, Perry, MS, and Tuscaloosa, AL; cluster 13 contained all samples from Key Largo, FL; and cluster 14 contained all samples from Hunting Island, SC. All cluster containing mainland populations showed a high degree of admixture. All island populations showed little to no admixture with the exceptions of Cumberland Island, Sapelo Island, and St. Simons Island. St. Simons Island showed admixture with both the mainland and Jekyll Island.

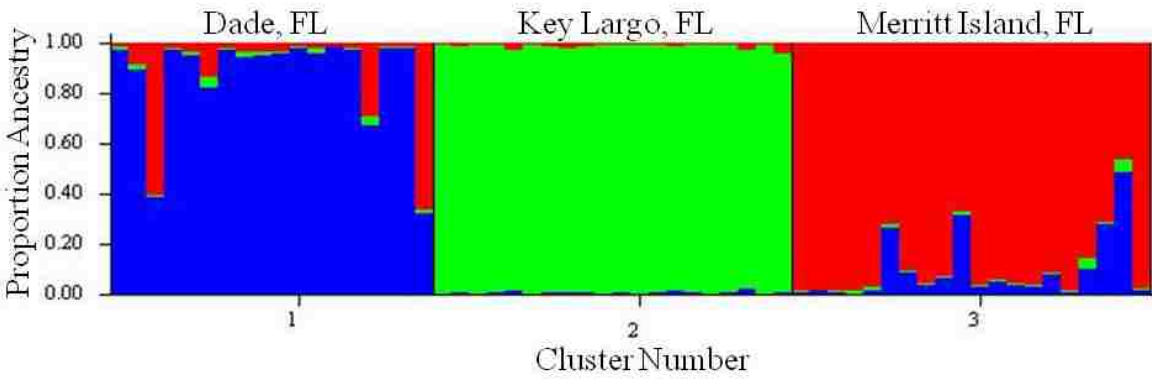


Figure 4.4. STRUCTURE analysis of the three populations in the southeastern regions. Populations formed three distinct clusters using the ln P(D) method of Pritchard et al. 2000 and the ΔK method of Evanno et al. (2005). A small amount of admixture was indicated between the Dade, FL and Merritt Island, FL population. No admixture was indicated between the Key Largo, FL population and any other population. This supports this populations status as a unique subspecies.

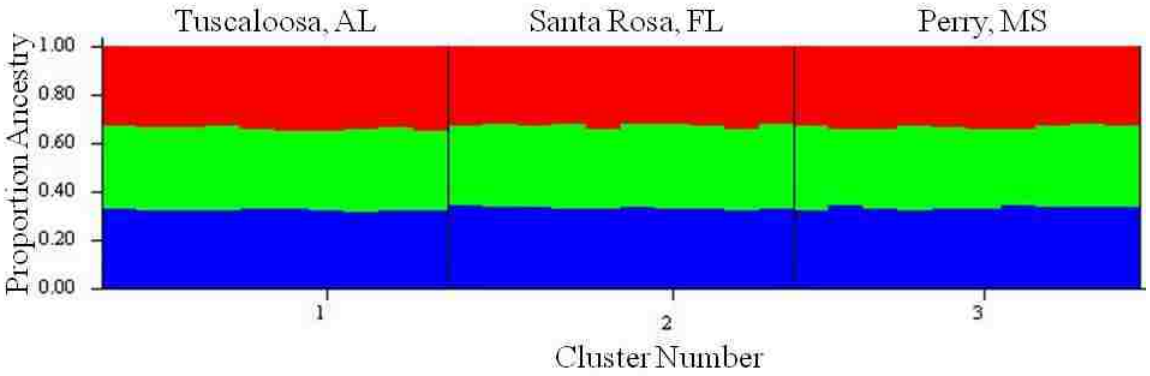


Figure 4.5. STRUCTURE anlaysis of the three populations in the western region. All populations fell into one cluster using the ln P(D) method of Pritchard et al. 2000. The ΔK method of Evanno et al. (2005) is not appropriate for identifying the number of clusters when K<3. Although all three populations fell into a single cluster, the populations were forced into three clusters to identify the degree of admixture. Based on this all three populations are equally admixed indicating a high degree of gene flow among populations.

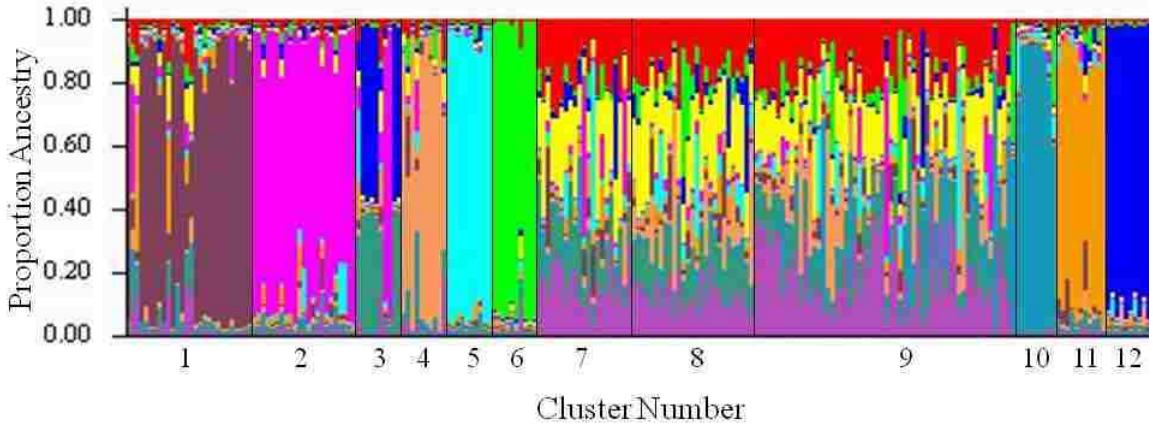


Figure 4.6. STRUCTURE analysis of the 17 populations in the northeastern region. Populations formed 12 distinct clusters using the ln P(D) method of Pritchard et al. 2000 and the ΔK method of Evanno et al (2005). Cluster 1 contains all individuals from Cumberland Island, GA; cluster 2 contained all samples from Jekyll Island, GA; cluster 3 contained all samples from St. Simons Island, GA; cluster 4 contained all samples from Sapelo Island, GA; cluster 5 contained all samples from Hunting Island, SC; cluster 6 contains all samples from Skidaway Island, GA; cluster 7 contained the samples from Chatham, GA and Glynn, GA; cluster 8 contained all samples from Camden, GA; cluster 9 contained all samples from New Hanover, NC, St. Johns, FL, Nassau, FL, Alachua, FL, and Clinch, GA; Cluster 10 contained the samples from St. Catherines Island, GA; cluster 11 contained the samples from Ossabaw Island, FL; and cluster 12 contained all samples from Amelia Island, FL. All cluster containing mainland populations showed a high degree of admixture. All island populations showed little to no admixture with the exceptions of Cumberland Island and St. Simons Island. St. Simons Island showed admixture with both the mainland and Jekyll Island.

Chapter 5:
The effects of urbanization on the genetic variation of the cotton mouse, *Peromyscus gossypinus*

Anthropogenic forces negatively impact taxa with a variety of life history traits (Davidson et al. 2009, Keyghobadi 2007, Trombulak and Frissell 2000). Studies of aquatic and avian fauna have identified demographic and behavioral changes, as well as changes in habitat usage associated with anthropogenic change (Francis et al. 2009, Waples et al. 2009). Among terrestrial species, research has identified a negative impact of habitat fragmentation on migration (Diffendorfer et al. 1995, Dixon et al. 2006, Schwab and Zandbergen 2010) and habitat usage (Crooks 2002). In recent years there has been an increase in the use of molecular markers to infer effects of anthropogenic forces on genetic variation and connectivity (Keyghobadi 2007). Little research has focused on the effects of anthropogenic forces on small mammal habitat generalists which are more likely to be negatively impacted than larger species due to their reduced vagility.

Among commonly studied anthropogenic forces—global climate change (Carey and Alexander 2003), anthropogenic noise (Francis et al. 2009), habitat fragmentation (Coulon et al. 2004), and urbanization (Bolger et al. 1991, 1997, 2008)—have been shown to affect organisms in a variety of habitats. While terrestrial species are impacted by all of these forces, the most pertinent to population level processes are urbanization and habitat fragmentation associated with the development of roads and agricultural lands. Habitat fragmentation results when areas of contiguous habitat, such as large forests are modified, resulting in a series of smaller, disconnected habitat fragments. Fragmentation is commonly associated with the development of land in suburban and rural communities.

Modifications associated with fragmentation commonly result in reduced gene flow among fragments (Diffendorfer et al. 1995), and alterations to habitat usage within fragments. Species often prefer either edge or interior habitats in forests, which are characterized by different vegetation types and susceptibility to outside environmental forces (Anderson et al. 2003). Fragmentation increases the amount of edge habitat, which can alter the distribution and abundance of organisms within fragments. An associated reduction in the size of forest fragments often reduces their carrying capacity for terrestrial fauna (Vieira et al. 2009).

As with fragmentation, habitat destruction associated with urbanization can also impact migration and population demographics. While many organisms are adapted to urban areas (Rose et al. 2006, Traweger et al. 2006), the majority of species that have been studied are negatively impacted by urbanization (Bolger et al. 2008, Davidson et al. 2009). Among small mammals, several studies have identified a negative impact of urbanization on habitat specialists (Bock et al. 2002, Degner et al. 2007, Price et al. 1994). It is generally assumed that habitat generalist species are more capable of utilizing habitat types associated with urban landscapes and migrating through urban areas to reach other areas of suitable habitat. The few studies of the effects of urbanization on native habitat generalists have found widely varying results.

Bolger et al. (1997) identified that many native rodent species, including habitat generalists, are incapable of migrating through urban areas, and that the majority of habitat fragments in urbanized areas are incapable of supporting native rodent populations. Soulé et al. (1992) also found that most native rodents are highly susceptible to extinction in urbanized areas. Genetic analyses of the habitat generalist *Peromyscus*

leucopus have found local isolation of populations in urban greenspaces (Munshi-South and Kharchenko 2010), suggesting that species that are capable of penetrating and colonizing an urban matrix may experience reduced gene flow once they do. In other studies, mark-recapture methods have shown that *P. leucopus* reaches higher densities in habitat fragments in urbanized areas (Mahan and O'Connell 2005, Nupp and Swihart 1998), and is capable of moving through modified habitats and colonizing small patches (Rizkalla et al. 2009, Swihart et al. 2006). While studies have provided markedly different results, data suggest that *P. leucopus* is the exception and not the rule (Swihart et al. 2006), indicating small mammal habitat generalists are likely susceptible to urbanization.

In addition to urbanization, the presence of roads also fragments habitats. While roads are associated with urban areas, they can extend far from urban centers often passing through large areas of otherwise pristine habitat. Research across a wide range of taxa indicates that the presence of roads negatively impacts gene flow among habitat fragments (Dixon et al. 2007, Epps et al. 2005, Simmons et al. 2010), and may have a strong negative influence on the abundance of already threatened species (Coffin 2007, Fahrig and Rytwinski 2009). Within *P. leucopus*, each successive road between habitats has been shown to reduce migration by ~50% regardless of the amount of traffic on the road (McGregor et al. 2008, Rytwinski and Fahrig 2007), suggesting roads have a strong negative effect on gene flow in small mammals.

While the effects of anthropogenic forces are widespread and dramatic in mainland populations, they are even more dramatic in island populations. In mainland areas, when populations go extinct due to fragmentation resulting from urbanization, they

are often replenished via source-sink dynamics, and little overall genetic variation is lost (Fahrig and Merriam 1994). Island populations often lack gene flow with nearby mainland populations and source-sink dynamics are insufficient to replenish these populations. As a result, island populations are more susceptible to extinction associated with anthropogenic forces (Amori et al. 2008, Knowlton et al. 2007).

Studies (Alvarez et al. 1996, Amori et al. 2008, Knowlton et al. 2007, Smith et al. 1993 Whitten et al. 1987) have identified several instances of island extirpations or population declines associated with the effects of anthropogenic activities, many of which have affected habitat generalists. Additional research by Smith et al. (1993) indicated that anthropogenic forces are likely to cause rodent and non-rodent populations to become endangered or extinct on several islands in the Gulf of California.

Based on these findings, it is critical to identify the effects of anthropogenic forces on genetic variation in small mammal habitat generalists in both island and mainland populations. Few studies have utilized molecular markers to identify these effects (Dixon et al. 2007, Simmons et al. 2010), and those that have, looked at genetic variation at a single point in time. While this allows for the inference of anthropogenic effects, it does not directly test if the patterns seen are the result of anthropogenic forces. In order to determine if anthropogenic forces are responsible for declines in genetic variation, and eventual population extinctions, it is necessary to study genetic variation in populations both before and after urbanization occurs.

The cotton mouse (*P. gossypinus*) is an ideal system to study the effects of urbanization on a temporal scale. It is a small mammal habitat generalist found throughout the southeastern United States and can be found in a variety of habitats

(Wolfe and Linzey 1977). The species is semi-aquatic (Pournelle 1950) and can migrate over 0.5 km between mainland habitats (Griffo 1961), and is capable of migrating short distance over water as well (Beckmann in prep.).

Peromyscus gossypinus is found on several barrier islands, two of which are in close proximity to the mainland (Figure 5.1). Others are separated from the mainland by several kilometers of open water and marsh (Boone et al. 1999). Some of these islands are under state or federal protection and have experienced little urbanization, while others have experienced recent widespread urbanization (NARSAL 2010). Many of the mainland areas where *P. gossypinus* is found have also undergone recent urbanization. Because there is a good collection of museum specimens that predates this recent urbanization, *P. gossypinus* is an excellent species to explore the effects of urbanization on genetic variation on a temporal scale. By comparing genetic variation across time points in urbanized and non-urbanized areas it is possible to identify the effects of anthropogenic forces on genetic variation and gene flow in this species.

Based on this information, I hypothesized that anthropogenic forces have affected genetic variation within populations and gene flow among populations of *P. gossypinus*. Based on patterns seen in other species, I predicted that mainland populations in urbanized areas would experience reduced genetic variation within populations. I also predicted that island populations that have been urbanized would exhibit reduced genetic variation when compared to populations on non-urbanized islands. Similarly, I predicted that populations in urbanized areas would exhibit reduced gene flow with nearby populations. Finally, because anthropogenic habitat destruction can negatively impact

small mammal abundance, I predicted that several populations would show signs of reduced population size following urbanization.

Methods

Sample collection and DNA extraction

A total of 298 tissue and toe bone samples were collected from 13 populations from the eastern portion of the range of *P. gossypinus* (Figure 5.1). Each population was sampled at two time points, once between 1988 and 1990 and a second time in 2009 (Table 5.1). In addition, 45 samples were included from mainland populations only sampled in 1988 (Figure 5.1) to identify the level of gene flow among populations at that time.

Because microsatellite analyses require many samples to provide strong statistical support (Balloux and Lugon-Moulin 2002), populations with less than five samples for a single time frame were excluded from the microsatellite analyses. This eliminated populations from Brevard, FL and Merritt Island, FL. While a minimum of 10 samples is common for microsatellite analyses, populations with five to nine samples for a single time frame were included to identify trends in the data. The exact locations where organisms were sampled are listed in Appendix 1.

Whole genomic DNA was extracted from tissue using an ethanol precipitation protocol. Qiagen DNeasy® tissue extraction kits (Qiagen Inc., Valencia, California) were used to extract DNA from toe bones following the user-developed protocol *Purification of total DNA from compact animal bone using the DNeasy® Blood & Tissue Kit* available on the Qiagen website (http://www.qiagen.com). A 784-bp region of the mitochondrial control region was amplified and sequenced using methods described in Beckmann 2010.

Additionally, genotypes were collected for seven polymorphic nuclear microsatellites using methods described by Beckmann (in prep).

Tree reconstruction

Phylogenetic trees were reconstructed using maximum parsimony, maximum likelihood, and Bayesian analyses. Control region sequences from *Peromyscus maniculatus* (GenBank Accession Number: EU170494) and *P. attwateri* (AF081492) were included as outgroups for all trees. Maximum likelihood and Bayesian methods were performed five times to ensure the most likely topology was returned. Maximum parsimony analysis was performed twice due to the large amount of time required to run this type of analysis. Parsimony analysis was run using PAUP v4.0b10 (Swofford 2002). Nucleotide sites were treated as unordered, equally weighted, discrete characters with four possible character states: A, T, G, or C. Trees were reconstructed using a heuristic search with tree bisection-reconstruction branch swapping. One hundred bootstrap replicates using the same parameters were completed to identify support for the topology (BSP) (Felsenstein 1985). All reconstructions were limited to a maximum of 10,000 trees.

For Bayesian and maximum likelihood analyses, jModeltest was used to identify the model of evolution that best fit the sequence data (Posada 2008). The model chosen using the Akaike Information Criterion with a 95% confidence interval was the General Time Reversible model with rate variation and a proportion of invariable sites (GTR+I+G), with a gamma shape parameter of 0.228 and a proportion of invariable sites of 0.356. RaxML was used to perform maximum likelihood reconstructions due to accuracy and speed (Stamatakis 2006). Support for the identified topology was again

determined via 100 bootstrap replicates (BSL), using the rapid bootstrap algorithm in RaxML (Stamatakis et al. 2008). A different randomly chosen starting seed was used for each of the five runs to ensure the most likely tree was returned.

Bayesian analysis was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with the aforementioned GTR+I+G model. The program was run with four simultaneous Markov-chains for ten million generations, sampling trees every 500 generations. Log-likelihood scores versus generation were plotted graphically to identify when the run reached stationary. Based on this, the first three million generations (6000 trees) were discarded as burn-in. Posterior probabilities (PP) calculated in MrBayes were used to determine support for the tree topology.

Mitochondrial population analyses

Average genetic distances within and among all populations were calculated using the Tamura Nei correction (Tamura and Nei 1983) in the software package MEGA v.5 (Tamura et al. 2007). This permits the identification of which populations are most similar within and among times frames. Nucleotide diversity per site (π) and haplotype diversity were calculated at both time points for each population using Arlequin v3.5 (Excoffier and Lischer 2010) to identify genetic diversity within populations before and after urbanization. Nucleotide diversity identifies the level of diversity based on the number of pairwise differences between haplotypes in a population and the frequency of those haplotypes in the population (Tajima 1983, 1993). Haplotype diversity on the other hand, looks at the number of haplotypes and their frequencies in a population, but does not take into account the number of differences between haplotypes (Hedrick 2005), as a result this measure is much more susceptible to sample size. If recent urbanization has

affected a population, a reduction in nucleotide and haplotype diversity would be expected from 1988 to 2009.

Microsatellite population analyses

An Analysis of Molecular Variance (AMOVA) was performed on the microsatellite data in Arlequin v3.5 to identify if anthropogenic forces had affected populations over the last twenty years (Excoffier and Lischer 2010). The AMOVA partitioned populations into eight groups with each group containing samples from a single population for both time periods. If anthropogenic forces have affected some of the populations, a larger percentage of variation should be due to differences among time points within populations than between populations.

Levels of inbreeding and outbreeding were identified for all populations at all timepoints by calculating inbreeding coefficients (F_{IS}) (Wright 1951). A significantly positive value of F_{IS} is indicative of inbreeding in a population, while a significantly negative value of F_{IS} indicates outbreeding. Outbreeding can result from the introduction of new alleles into a population with low levels of genetic variation. If inbreeding was identified in a population in 2009 but not in 1988, this would suggest a reduction in abundance of *P. gossypinus* associated with urbanization.

Nei's mean gene diversity (H_E) and mean allelic richness (R) were calculated using microsatellite genotypes in the program FSTAT v.2.9.3.2 (Goudet 2001). When calculating R, FSTAT corrects for the sample size in each population (El Mousadik and Petit 1996). These statistics are indicative of the amount of genetic variation within a population and are akin to nucleotide diversity and haplotype diversity in the mitochondrial analyses. I also determined the mean number of alleles using Arlequin v3.5

to further compare populations across time points. These values were only useful if populations had at least ten samples for both time points. If a population from 2009 had less than 10 samples but showed at least the same number of alleles as a population from 1988, urbanization was unlikely to have affected this population.

To identify levels of gene flow among populations I used Arlequin v3.5 to calculate F_{ST} (Wright 1951) between all population pairs at both time points using corrected allele frequencies identified by Micro-Checker. While R_{ST} is more commonly used as a measure of population differentiation for microsatellites (Balloux and Lugon-Moulin 2002), when sample sizes and number of loci are low, F_{ST} has been shown to be a more accurate measure (Gaggiotti et al. 1999).

The program Migrate-n v.3.2.6 (Beerli 2010) was used to estimate number of migrants per generation between all population pairs at both time points. This program uses coalescent methods to estimate Θ (the mutation scaled population size for each population) and M (the mutation scaled immigration rate for a population) from microsatellite genotypes. By multiplying M by Θ and dividing by four it was possible to estimate the number of migrants per generation between population pairs. Migrate-n was also used to determine the effective population size (N_e) for each population at both time points. By comparing estimates of Θ for each population at both time points it was possible to identify changes in N_e over the last twenty years.

Changes in population size were also identified using the program LDNe (Waples and Do 2008). This program utilizes information on linkage disequilibrium, and a bias-correction method described by Waples (2006), to identify N_e for a population using samples from a single point in time. Further, this program runs the data several times

excluding alleles with frequencies below 0.02, 0.05, and 0.1 in successive runs. This allows for the identification of bias associated with the inclusion of these alleles. By implementing this program on samples from each population at each of the two time points it is possible to identify reductions in effective population size which may be associated with anthropogenic forces.

To identify significant reductions in N_e I analyzed both the contemporary and historic data from each population with the program BOTTLENECK v.1.2.02 (Piry et al. 1999). BOTTLENECK incorporates a two-phase mutation model (TPM) allowing the majority of mutations to occur under a stepwise mutation model (SSM), while a small proportion of mutations occur under the infinite allele model (IAM). This is likely a more realistic model of microsatellite evolution than either the SSM or IAM alone (Piry et al. 1999). The TPM was implemented on the data allowing 5% of mutations to occur under the IAM and the data was run for 2000 replicates. Given the short time frame between sampling points, if a genetic bottleneck was identified in a population in both the historic and contemporary samples it is possible the same event was identified twice. If a bottleneck was identified only in the 2009 samples then it was likely associated with anthropogenic forces.

To identify structuring among populations among time periods, I analyzed the data using STRUCTURE v.2.3.3 (Falush et al. 2003, Hubisz et al. 2009, Pritchard et al. 2000). By using a Bayesian clustering method this software organizes samples into the most likely number of clusters based on allele frequencies within populations. The program was run assuming correlated allele frequencies and allowing admixture among populations. Markov Chain Monte Carlo simulations were run for 400,000 iterations with

a 40,000 iteration burnin. Five simulations were run for each possible value of K from 1 to 27. The ideal value of K was determined by utilizing both the ln P(D) method of Pritchard et al. (2000) and the ΔK method of Evanno et al. (2005). Additionally, if a population was assigned to different clusters at each time point, the amount of admixture between the two clusters was identified.

Results

Phylogenetic reconstructions

All three reconstruction methods returned the same topology with varying degrees of support (Figure 5.2). Several populations formed monophyletic groups across both time periods. All samples collected from Dade, FL in both 1988 and 2009 formed a monophyletic group with high bootstrap support in both the parsimony (BSP:100) and maximum likelihood analyses (BSL:90). This group was also well supported in the Bayesian analysis (PP: 0.97). The samples from Merritt Island, FL and Brevard, FL also formed a monophyletic group (BSP:73, BSL:81). Samples from these populations were well mixed within this group, indicating gene flow between the island and mainland.

In Georgia, three island populations formed monophyletic groups across both time periods. The populations of Ossabaw Island (BSP:100, BSL:100, PP:1.00) and St. Catherines Island (BSP:100, BSL:98, PP:1.00) both formed strongly supported groups. Similarly, all samples from Jekyll Island formed a monophyletic group with the samples collected on St. Simons Island in 1988 (BSP:100, BSL:95, PP:1.00). Yet, the samples collected on St. Simons Island in 2009 formed a group with populations from southern mainland Georgia (BSP:63, BSL:90, PP:0.95). This group fell within a larger group containing all the samples collected from Cumberland Island across both time periods

(BSP:88, BSL:90, PP:0.95). Within this lineage, all haplotypes from Cumberland Island formed a monophyletic group with the exception of one haplotype from 1988 that was more closely related to mainland samples.

The other two island populations in this study also failed to form monophyletic groups across time periods. The population sampled on Sapelo Island in 1988 was most closely related to samples collected from Chatham, GA in both 1988 and 2009 (BSP:70, BSL:66), while the samples collected on Sapelo Island in 2009 grouped together with a more basal group of samples from Chatham, GA collected in 1988 (BSP:63, BSL:85). In the case of Amelia Island, FL, samples collected in 1988 formed a monophyletic group that was most closely related to samples from Tattnall, GA (BSP:67, BSL:70). Samples collected from Amelia Island in 2009 did not fall into this group. Rather, they formed a monophyletic group with contemporary samples from Glynn, GA (BSP:70, BSL:90, PP:0.95).

Of the three mainland Georgia populations that were sampled at both time points (Camden, Chatham, Glynn) none formed monophyletic groups in 1988, suggesting gene flow was prevalent among mainland populations at the time. The contemporary Chatham population also failed to form a monophyletic group, but the populations of Glynn and Camden did form monophyletic groups. The contemporary samples from Glynn formed a group with contemporary samples from Amelia Island, FL and Chatham, GA (BSP:70, BSL:90, PP:0.95), and the contemporary population from Camden, GA formed a monophyletic group most closely related to samples from Glynn, GA and Nassau, FL collected in 1988 (BSP:63, BSL:87, PP:0.97).

Variation within populations

Several populations in this study showed little change in nucleotide diversity (π) or average genetic distance (Di) from 1988 to 2009 (Chatham, GA; Brevard, FL; Merritt Island, FL; Cumberland Island, St. Catherines Island, and Ossabaw Island, GA) (Table 5.2). While Brevard, St. Catherines Island and Ossabaw Island also showed no change in haplotype diversity (H), the populations from Merritt Island, FL (1.0 to 0.773), Chatham, GA (0.978 to 0.667) and Cumberland Island, GA (0.796 to .633) showed a decrease in H during this study.

Among the remaining populations, all but two showed a decrease in genetic variation from 1988 to 2009. The mainland populations from Camden, GA and Glynn, GA lost all genetic variation from 1988 to 2009 as did the populations from Jekyll Island, GA, St. Simons Island, GA, and Amelia Island, FL.

Of the 13 populations sampled at both timepoints, two showed an increase in genetic variation over the past 20 years. The Dade, FL population exhibited an increase in π (0.007 to 0.022) and Di (0.007 to 0.018), but demonstrated little change in H (1.0 to 0.94). Similarly, the population from Sapelo Island, GA exhibited an increase in π (0 to 0.009), Di (0.0 to 0.01), and H (0 to 0.533). This increase occurred in spite of a smaller samples size in 2009 relative to 1988.

Mean gene diversity (He) and mean allelic richness (R) for microsatellite loci showed similar patterns to the mitochondrial data (Table 5.3). Several populations exhibited little change in genetic variation from 1988 to 2009 (Cumberland Island, Sapelo Island, Ossabaw Island, and Camden, GA). Four other populations exhibited a decrease in genetic variation: Amelia Island, FL (R:3.52 to 3.01, He:0.646 to 0.594), St.

Simons Island, GA (R:4.62 to 3.17, He:0.783 to 0.610), Glynn, GA (R:5.49 to 2.90, He:0.832 to 0.660), and Jekyll Island, GA (R:5.23 to 4.67). Unlike the other populations, the Jekyll Island population showed little change in He. The three remaining populations exhibited an increase in the amount of genetic variation from 1988 to 2009: St. Catherines Island, GA (R:2.52 to 3.50, He:0.514 to 0.636), Chatham, GA (R:5.01 to 5.43, He:0.819 to 0.860) and Dade, FL (R:3.50 to 5.20, He:0.675 to 0.768).

Among the populations sampled in this study, several had signatures consistent with either inbreeding or outbreeding (Table 5.3). Outbreeding is indicated by a significantly negative inbreeding coefficient (F_{IS}), while inbreeding is indicates by a significantly positive F_{IS}. The populations of Cumberland Island, GA (p=0.008) and Jekyll Island, GA (p=0.006) had significant inbreeding coefficients (F_{IS}) in the contemporary population, but not the historic population. The St. Simons Island, GA population had a significant F_{IS} (p=0.009) in 1988, but showed no evidence of inbreeding in 2009. The Camden, GA populations also had a significant F_{IS} in 1988 (p=0.007), but in 2009, the population showed evidence of outbreeding (F_{IS}:-0.151). Outbreeding is also suggested by the presence of eight new alleles in 2009 that were not previously seen in this population. The contemporary population from Amelia Island, FL also exhibited evidence of outbreeding (F_{IS}:-0.155).

Variation among populations

Genetic distance data indicates that several populations sampled in 1988 are genetically most similar to populations sampled from the same location in 2009 (Dade, FL; Brevard, FL; Merritt Island, FL; Cumberland Island, GA; Jekyll Island, GA; Sapelo Island, GA; St. Catherines Island, GA; Ossabaw Island, GA) (Table 5.4). Neither of the

remaining island populations showed high levels of similarity across time points. While the St. Simons Island, GA population sampled in 1988 was most similar to the Jekyll Island, GA population at that time point (0.6%), the contemporary population from St. Simons Island was most similar to the contemporary population from Chatham, GA (1.2%). Also, in 2009 the St. Simons Island population was highly diverged from the Jekyll Island population (2.1%) and the historic St. Simons Island population (2.1%). In 1988, the Amelia Island, FL population was most similar to the population from Nassau, FL (1.5%). In 2009, the Amelia Island population was most nearly identical to the 2009 Glynn, GA population (0.1%). By comparison, the 1988 population from Amelia Island was 18 times more different from the Glynn population at that time point (1.8%).

Patterns also varied among the three mainland GA populations sampled at both timepoints. In 1988 the Camden population was most similar to the populations of Glynn, GA (1.0%) and Chatham, GA (0.9%). Likewise, the 1988 Glynn population was most similar to the Chatham, GA population (0.07%) and the Camden, GA population (1.0%), The 1988 Chatham population was most similar to Sapelo Island, GA (0.07%) and Glynn, GA (0.07%). By 2009, the Camden population (0.4%) and Glynn population (0.1%) were most similar to the Amelia Island, FL population, while the Chatham population was still most similar to the Sapelo Island population (1%). During that same time the three mainland populations also diverged from each other.

F_{ST} values from the microsatellite data demonstrate many of the same patterns seen in the mitochondrial analyses (Table 5.5). The lowest F_{ST} for many populations was with that same population at a different time point: Dade, FL (0.069), Amelia Island (0.121), Jekyll Island (0.052), Cumberland Island (0.018), Ossabaw Island (0.101),

Sapelo Island (0.116) and St. Catherines Island (0.109) (Table 5.3). In 1988, the Sapelo Island population was also closely related to the Chatham population (0.117) but by 2009 had diverged (0.219). As with the mitochondrial data, the 1988 sample from St. Simons Island was most similar to the 1988 sample from Jekyll Island (0.063). In 2009 those two populations were three times more different (0.225), and the contemporary population from St. Simons Island was most similar to the historic St. Simons Island population (0.130).

Among the three mainland GA populations that were sampled at both timepoints, the 1988 populations of both Camden, GA (0.024) and Glynn, GA (0.009) were most similar to the 1988 population from Chatham, GA, as was the contemporary Chatham population (0.076). The contemporary Camden population was most similar to the population from Nassau, FL (0.043) and the contemporary Glynn population was most similar to the population sampled from Glynn in 1988 (0.117).

The number of migrants per generation (ΘM) estimated by Migrate-n indicated a decrease in the amount of gene flow among populations from 1988 to 2009 (Table 5.6). The populations of Glynn, GA, Camden, GA, and Chatham, GA all experienced at least a ten-fold decrease in ΘM among those three populations. Similarly, ΘM between island and mainland populations also decreased markedly. While in 1988 the Chatham population received approximately two migrants per generation from Sapelo Island, GA, this number was reduced to 0.2. Additionally, in 1988 Sapelo Island received one migrant every five generations from Chatham, but this gene flow had been effectively cut off by 2009.

Changes in population size

Utilizing one-tailed Wilcoxon signed rank tests in the program BOTTLENECK, population bottlenecks were clearly identified in four populations. Three of the populations were sampled in 2009: Camden, GA (p=0.027), Glynn, GA (p=0.004), and St. Simons Island, GA (p=0.039). The fourth bottleneck was identified in the 1988 sample from Sapelo Island (p=0.004). It was not possible to test for bottlenecks in the 2009 population from St. Catherines Island, GA, Chatham, GA, Sapleo Island, GA or the 1988 population from Amelia Island, FL.

Using information on linkage disequilibrium and a bias correction, LDNe identified estimates of the effective population size (N_e) for all populations at both time periods (Table 5.7). Because of the large confidence intervals associated with many of the values due to low sample size in some populations, it was not possible to make direct inferences about N_e, but it was possible to make comparisons within populations on a temporal scale. Among mainland populations, the contemporary N_e of Camden, GA was approximately fifteen times smaller than it was in 1988. Similarly, the Glynn, GA population was also markedly smaller than it was 20 years ago. On the other hand, the Dade, FL population exhibited an increase in N_e during the course of this study.

Within the Jekyll Island, GA population the contemporary N_e (13.2) was less than half of what it was in 1988 (29.1). Similarly, the N_e of St. Simons Island (0.8) is approximately 50 times smaller than it was in 1988 (38.4). The population of Amelia Island also had a nearly ten-fold decrease in N_e (11.1 to 1.2). Two populations also showed an increase in N_e during the course of this study. The population of Cumberland

Island, GA in 1988 had an estimated N_e of 56.8, but in 2009 the estimate was 72.7. The population on St. Catherines Island also experienced an increase in N_e from 4.6 to 7.2.

MIGRATE-n indicated many of the same patterns identified by both LDNe and BOTTLENECK (Table 5.7). The mainland population from Camden, GA experienced a four-fold decrease in N_e during this study, while the Glynn, GA population experienced a seven-fold decrease. As in the other analyses, the Dade, FL population also experienced an increase in N_e over the last twenty years, albeit much smaller than indicated by LDNe (Table 5.7).

The size of the contemporary Jekyll Island population was approximately half of what it was in 1988 and the contemporary St. Simons Island population was one quarter of what it was in 1988. On the other hand, the populations of Cumberland Island, GA (0.99 to 1.23) and St. Catherines Island, GA (0.46 to 0.72) experienced increases in N_e over that same time period. MIGRATE-n also indicated an increase in the population size for both Sapelo Island (0.88 to 1.67) and Ossabaw Island (0.88 to 1.59).

Population structuring

The results of the AMOVA indicate that most of the genetic variation occurs within individuals (79.85%) (Table 5.8). Of the remaining variation, more is due to differences among time periods within populations (8.38%) than is due to differences among populations (7.40%), indicating that changes within populations over time have impacted the amount of genetic variation in the populations studied.

The STRUCTURE analysis indicated that populations fell into a total of fourteen clusters using both the ln P(D) method of Pritchard et al. (2000) and the ΔK method of Evanno et al. (2005) (Figure 5.3). Among these clusters, seven populations fell into the

same cluster at both time points (Amelia Island, FL; Cumberland Island, GA; Sapelo Island, GA; Ossabaw Island, GA; Jekyll Island, GA; St. Catherines Island, GA; and Dade, FL). Of the remaining populations, the 1988 populations from Glynn, GA, Camden, GA, and Chatham, GA fell into a cluster with the samples collected from several other mainland populations in 1988. The 2009 populations from Glynn, Camden, and Chatham each formed unique clusters. This indicates that the 2009 populations from each of these locations are distinct from each other. Additionally, the 1988 and 2009 populations from St. Simons Island, GA also each formed unique clusters, suggesting they are not closely related.

Since STRUCTURE allows for admixture among populations, it is possible to determine the proportion of each population assigned to a given cluster. By comparing these values for a population it may be possible to identify what if any evolutionary processes have affected each population. The percentage of individuals from Ossabaw Island assigned to cluster 14 increased from 49.2% in 1988 to 82.1% in 2009. Similarly, the proportion of individuals from Amelia Island assigned to cluster nine also increased dramatically between 1988 and 2009 (51.4% to 90.9%). Less dramatically, the populations of Jekyll Island (34.5 to 45.6) and St. Catherines Island (83.9 to 93.2) also showed an increase in the percentage of individuals assigned to their primary cluster over time.

The Cumberland Island, Sapelo Island, and Dade, FL populations appear to be stable based on the STRUCTURE analysis. On Cumberland Island there was a slight decrease in the proportion of individuals assigned to cluster five (72.5% to 67.3%), while there was a slight increase in the proportion of individuals from Sapelo Island assigned to

cluster 11 (80.2% to 88.9%). The Dade, FL population also showed a slight decrease in the proportion of individuals assigned to cluster six from 1988 to 2009 (55.0% to 46.5%). In 1988, the St. Simons Island populations was spread out among many clusters with the largest percentage of individuals (24.0%) assigned to cluster three. By 2009, 78.2% of the individuals sampled on St. Simons Island were assigned to cluster two and only 7.5% of individuals were assigned to cluster three.

On the mainland, the Glynn, GA population from 1988 had 26.6% of individuals assigned to cluster one and only 5.0% assigned to cluster seven. In 2009, 93.7% of individuals were assigned to cluster seven and only 0.5% were assigned to cluster one. In the Camden, GA population 25.9% of individuals were assigned to cluster one in 1988 and 5.8% were assigned to cluster eight, but in 2009 38.3% of individuals were assigned to cluster eight and only 8.0% were assigned to cluster one. The population of Chatham, GA presents a similar situation. Of the samples collected there in 1988, 25.7% were assigned to cluster one while only 6.5% were assigned to cluster 12. Among the contemporary samples from Chatham, 82.2% were assigned to cluster 12 and only 2.8% were assigned to cluster one. It should be noted that the differences in the Chatham population may be inflated given that only five individuals were sampled in 2009. While all three of these populations were assigned to cluster one in 1988, none of them had more than 1% of their contemporary populations assigned to a cluster containing the contemporary population of one of the other two populations.

Discussion

Variation in mainland populations

Data from both the mitochondrial and nuclear genome support my prediction that populations in urbanized mainland areas exhibit reduced genetic variation. Estimates of genetic variation in populations from both urbanized and non-urbanized areas indicate a reduction of variation in populations from urbanized areas that is not seen in non-urbanized areas. Of the five mainland areas sampled in this study, three are from areas that did not experience urbanization in the last twenty years.

The Dade, FL population was sampled within Everglades National Park (ENP) and the Brevard, FL population was sampled within Merritt Island National Wildlife Refuge (MINWR), indicating that neither population has been affected by urbanization. Similarly, the Chatham population was also sampled from a region that has experienced little to no urbanization over the last twenty years (Figure 5.4). Estimates of average genetic distance within populations (Di) and nucleotide diversity (π) (Table 5.2) in these populations show that levels of variation have either been maintained or increased during the course of this study. Additionally, while Dade, FL was the only non-urbanized mainland population with sufficient samples to measure diversity at microsatellite loci, it showed an increase in both mean gene diversity (He) and allelic richness (R) over the last twenty years.

The two remaining mainland populations in this study (Camden, GA and Glynn, GA) were both sampled from areas that have experienced widespread urbanization over the last twenty years (Figures 5.5, 5.6). While in 1988 both population exhibited some degree of nucleotide diversity and high levels of haplotype diversity (H), in 2009 each

population was characterized by a single mitochondrial haplotype. Measures of variation in microsatellite loci also indicate a marked decrease in variation in the Glynn population, with a ~21% decrease in He and a 50% decrease in R. By comparison, the Camden population only demonstrated an ~5% decrease in both He and R. This is partially due to a recent introduction of new alleles as indicated by strong evidence of outbreeding in this population (Table 5.3). In reality, 20 alleles that were identified in this population in 1988 were not present in 2009, indicating this population experienced a marked loss of variation which it may now be recovering from.

Comparing genetic variation among urbanized and non-urbanized populations further demonstrates the impact of urbanization on *P. gossypinus*. Among non-urbanized populations in 2009, π varied from 0.010 to 0.022, whereas in urbanized populations π was 0. These combined data demonstrate a decrease in genetic variation that is associated with recent urbanization in the Glynn and Chatham areas. Other studies demonstrating low levels of variation in urbanized populations have attributed this to isolation or reduced population size resulting from habitat destruction (Munshi-South and Kharchenko 2010, Trizio et al. 2005, Wandeler et al. 2003). In both Camden and Glynn, large scale urbanization has disrupted and decreased the size of suitable habitats for *P. gossypinus* (NARSAL 2010). This appears to have resulted in a dramatic loss of variation for these populations, relative to those in non-urbanized areas.

Variation in island populations

Several urbanized island populations also exhibit a reduction in genetic variation. These data support my prediction that populations on urbanized island would exhibit reduced variation compared to those on non-urbanized islands. Of the eight island

populations included in this study, five are from protected areas that did not experience urbanization in the last 20 years (Merritt Island, FL; Cumberland Island, GA; Sapelo Island, GA; St. Catherines Island, GA; and Ossabaw Island, GA) (Figures 5.7-5.10). In the case of Merritt Island, the southern portion of the island is extensively urbanized but the larger northern portion and the nearby mainland fall within MINWR which has not experienced urbanization over the last 20 years. All animals trapped on Merritt Island were trapped well within MINWR.

Among these non-urbanized populations, estimates of Di and π indicate that all but one of the populations showed either a stable or increased amount of genetic variation during this study (Table 5.2). The one non-urbanized island that experienced a loss of variation was Ossabaw Island, which had a decrease in Di from 0.0001 to 0 as well as a decrease in π from 0.0001 to 0. This decrease is the result of two haplotypes that were each present in one individual in 1988 which were not found in 2009. Given the low π in this population in 1988 it is likely that either these haplotypes were not sampled in 2009 by random chance due to their low frequency in the population, or that that they were lost via genetic drift.

Measures of genetic variation at microsatellite loci (He and R) demonstrate a slight increase in genetic variation in the Ossabaw Island population (Table 5.3), indicating that this population has not recently experienced genetic drift and suggesting that the lack of variation in mitochondrial data is the result of random sampling and not drift. Among the remaining non-urbanized populations, Sapelo Island and Cumberland Island experienced no change in either He or R, and St. Catherines Island demonstrated an increase in both He and R during this study. These patterns agree with those identified

in non-urbanized mainland populations and demonstrate an overall maintenance of variation in non-urbanized populations.

The remaining three island populations (Amelia Island, FL; Jekyll Island, GA; and St. Simons Island, GA) experienced increased urbanization during the last twenty years (pers. comm. Frangiamore and Simmons) (Figures 5.11, 5.12). Over that same time period all three populations exhibited a complete loss of genetic variation as measured by π and Di. Similarly, all three populations demonstrated a decrease in variation at microsatellite loci as well. Most notably, the St. Simons Island population exhibited a loss of 23 alleles across seven microsatellite loci demonstrating a marked loss of variation associated with urbanization. Less extreme was the loss of eleven alleles in the Amelia Island population. Although, given that only five samples were available from this island in 1988, it is logical that a greater loss of variation would have been observed over the last twenty years if sampling was better in 1988. Evidence of contemporary outbreeding in this population also suggests that the loss of variation is more severe than the numbers indicate.

While the Jekyll Island population demonstrated the smallest loss of variation over the twenty year period of this study, it also experienced the smallest degree of urbanization during that same time period. Portions of both the northern and southern thirds of the island are under the protection of the Jekyll Island Authority (JIA) and have experienced little urbanization during this study (Spears, in prep.). These areas are dotted by areas of dense urbanization, and are separated by a large area of widespread commercial and residential urbanization (NARSAL 2010). As a result, a large portion of

the diversity at highly variable microsatellite loci has been preserved while diversity has been lost in the less variable mitochondrial control region.

Given that a significant F_{IS} value demonstrates the Jekyll Island population is currently experiencing inbreeding (Table 5.3), and the marked loss of variation in the mitochondrial control region, it is clear that the urbanization on this island has negatively impacted this population. Likewise, large scale urbanization on both St. Simons Island (Figure 5.12) and Amelia Island (pers. comm. Simmons) has dramatically decreased the amount of habitat available to *P. gossypinus* in a twenty year period. While outbreeding may be maintaining the population on Amelia Island in the short term, it is clear that the population of St. Simons Island is in danger of potential extinction if urbanization continues on this island at its current rate, unless gene flow can be established with a nearby mainland population.

Gene flow among nearby populations

Phylogenetic and population structure analyses, estimates of genetic variation and number of migrants per generation indicate a marked reduction in gene flow among populations during the course of this study. This supports my prediction that urbanization has a negative impact on gene flow among nearby populations. Results of all three phylogenetic analyses show that while gene flow was widespread among mainland Georgia populations in 1988, this is no longer the case (Figure 5.1). Over the last twenty years the populations of Glynn, Camden, and Chatham have differentiated both from each other and themselves, indicating a lack of gene flow maintaining similarities among these populations. This conclusion is also supported by population structure analysis.

STRUCTURE grouped all mainland Georgia populations into a single cluster in 1988 indicating high levels of gene flow among these populations (Figure 5.3). By 2009 the populations of Camden, Glynn, and Chatham each formed a unique cluster, with low levels of admixture among them indicating these populations are no longer regularly exchanging migrants. During this time, coastal Georgia has experienced widespread urbanization (Figures 5.4, 5.5) resulting in decreased dispersal ability for *P. gossypinus*. This is consistent with patterns of dispersal observed in other small mammal species in urban areas (Bolger et al. 1997, Munshi-South and Kharchenko 2010, Simmons et al. 2010).

Decreased dispersal among populations is further indicated by an increase in the genetic distance and F_{ST} values among these three populations over the last twenty years (Tables 5.4, 5.5). Over the course of this study genetic distances among these three populations have approximately doubled, while F_{ST} values have increased dramatically. The contemporary values between Camden and Glynn or Chatham are four and eight times larger than they were in 1988 respectively. Similarly, the contemporary value between Glynn and Chatham is 20 times larger than it was in 1988. While the markedly high numbers for Chatham may be partially due to the low contemporary sample size there, it still demonstrates a marked decrease in gene flow among these populations.

A lack of contemporary gene flow is also indicated by estimates of number of migrants per generation between populations (Table 5.6). These data demonstrate that while at least one migrant per generation was exchanged between mainland populations in 1988, there is virtually no gene flow among the contemporary populations of Camden, Glynn, and Chatham. Likewise, while the populations of Sapelo Island, Cumberland

Island, and St. Simons Island occasionally exchanged migrants with these three mainland populations in 1988, by 2009 urbanization had resulted in those avenues of gene flow being completely cut off. On the same note, large scale urbanization on St. Simons Island and Amelia Island also eliminated gene flow between those populations and their neighboring islands during the same time period.

These combined data demonstrate a marked decrease in gene flow among both mainland and island populations as a result of urbanization. In many cases this decrease in gene flow has resulted in the differentiation of populations that were formerly closely related. This differentiation has occurred in both mainland populations (Camden, Chatham, Glynn) and island populations (Jekyll and St. Simons Island) and is consistent with the results of other research using mitochondrial markers (Beckmann 2010). On islands this reduction in gene flow resulted in the increased isolation of populations, and in at least one case (Cumberland Island) is associated with a marked increase in the amount of inbreeding in the population (Table 5.3). Such isolation of populations on the mainland, and more so on islands is typically associated with an increased susceptibility to genetic drift and population size reductions (Garner et al. 2005, Wang et al. 2009).

Reduced effective population size

Various estimates of effective populations size (N_e) provide strong evidence to support my prediction that urbanized populations have experienced a reduction in population size. Using coalescent methods each of the non-urbanized populations analyzed demonstrated an increase in N_e over the last 20 years (Table 5.7). Also, estimations based on linkage disequilibrium identified an increase in N_e for Dade, FL, Cumberland Island, and St. Catherines Island, but identified no change in the populations

of Sapelo Island and Ossabaw Island (Table 5.7). By comparison, both mainland populations (Camden and Glynn) and two of the island populations (St. Simons and Jekyll) that have experienced urbanization in the last 20 years exhibited marked decreases in N_e. The population from Amelia Island also exhibited a marked decrease using estimates based on linkage disequilibrium, but only showed a very small decrease when coalescent methods were applied.

The three populations that showed the largest decrease in N_e (Camden, Glynn, and St. Simons Island) also demonstrated evidence of population bottlenecks since 1988. Further, the contemporary populations from each of these locations fell into unique clusters which were well differentiated from the clusters containing the historic populations from these locations (Figure 5.3). Given that urbanized populations were the only ones to demonstrate a decrease in N_e, this data provides strong evidence of an association between increased urbanization and a decrease in population size. Further, levels of admixture among these clusters demonstrate that population bottlenecks associated with urbanization can dramatically alter the structure of genetic variation within a population.

The results of this study indicate a strong negative impact of urbanization on populations of *P. gossypinus.* Several island and mainland populations show a marked reduction in genetic variation among mitochondrial haplotypes over the last 20 years which is associated with urbanization. Further, many of these urbanized populations also show a decrease in genetic variation at microsatellite loci. There is also a temporal reduction in N_e associated with an increase in urbanization in both island and mainland populations. By comparison, none of these patterns are seen in populations that did not

experience urbanization during this study. Together, these results support my hypothesis that anthropogenic forces affect the amount of genetic variation within and the amount of gene flow populations of *P. gossypinus*.

Conclusion

To my knowledge this is the first study to identify the effects of urbanization on genetic variation by comparing naturally occurring populations in urbanized and non-urbanized areas on a temporal scale. Because all populations sampled in this study are in close geographic proximity it is reasonable to assume that all mainland and all island populations have experienced the same non-anthropogenic forces during the course of this study. Previous studies of the effects of urbanization on rodent species have drawn conclusions on the effects of urbanization by looking at either genetic variation (Munshi-South and Kharchenko 2010) or migration and distribution (Bolger et al. 1997) in populations after urbanization has already occurred. This study is unique in that it compares genetic variation and effective population size within populations and migration among populations both before and after urbanization has occurred. The inclusion of populations that have not experienced urbanization provides a control group for comparison of changes in genetic variation and population size over the same time period.

My results demonstrate that populations in urbanized areas have experienced a marked reduction in genetic variation over a time period when populations in protected areas have experienced either a lack of change or an increase in variation. This reduction in variation has been shown to affect both island and mainland populations. As a result,

there has been a reduction in, and in many cases a complete loss of, gene flow among mainland populations in urbanized areas.

Several island and mainland population pairs that occasionally exchanged migrants are now isolated from one another. Likewise, as urbanization has increased, island populations that formerly exchanged migrants no longer do, increasing this isolation. In combination with a decrease in N_e, also associated with urbanization, several of these islands have experienced marked changes in their genetic structure over the last 20 years, and in at least one case have become highly inbred. This marked decrease in N_e has been observed in both island and mainland populations, with mainland populations being more affected than islands.

These data add to an increasing literature base which indicates that small mammal habitat generalists are affected by urbanization in many of the same ways as habitat specialists (Bolger et al. 1997, McGregor et al. 2008, Munshi-South and Kharchenko 2010). It is necessary to understand how several species with a variety of life history traits are affected by habitat destruction and urbanization. Understanding these effects can provide valuable information about how to prioritize which habitat types to protect in order to conserve the greatest number of species. Additionally, by understanding how individual species respond at the population level it will be possible to develop plans to preserve genetic variation and promote gene flow. Given the continually increasing prevalence of urban areas, it is paramount that we identify ways to maintain variation and gene flow so as to attempt to mitigate the effects of urbanization on organisms across a wide range of taxa. By incorporating this information into governmental land use

guidelines, it may be possible to promote the development of urban areas that also promote the sustainability of biodiversity.

Table 5.1. List of populations of *P. gossypinus* sampled for this study, including the number of samples collected in 1988 and 2009. Populations without sample data for 2009 were only sampled in 1988 and included to provide data on gene flow among mainland populations.

Population	Number of samples (1988)	Number of sample (2009)
Dade County, FL	5	17
Brevard County, FL	2	3
Merritt Island, FL	3	20
Amelia Island, FL	5	10
Camden County, GA	27	10
Glynn County, GA	10	10
Chatham County, GA	11	5
Cumberland Island, GA	28	16
Jekyll Island, GA	23	16
St. Simons Island, GA	12	12
Sapelo Island, GA	11	6
St. Catherines Island, GA	10	5
Ossabaw Island, GA	11	10
Alachua County, FL	10	-
South Sumter County, FL	3	-
Nassau County, FL	15	-
Clinch County, GA	11	-
Tattnall County, GA	6	-

Table 5.2. Measures of within population genetic variation (nucleotide diversity, haplotype diversity, average Tamura-Nei genetic distance) based on mitochondrial haplotype data for all populations of *Peromyscus gossypinus* included in this study which were sampled in both 1988 and 2009.

Population	Nucleotide diversity 1988	Nucleotide diversity 2009	Haplotype diversity 1988	Haplotype diversity 2009	Genetic distance 1988	Genetic distance 2009
Dade, FL	0.007	0.022	1.000	0.942	0.007±0.003	0.018±0.004
Brevard, FL	0.032	0.030	1.000	1.000	0.017±0.008	0.016±0.004
Merritt Island, FL	0.019	0.020	1.000	0.773	0.020±0.004	0.019±0.003
Amelia Island, FL	0.004	0.0	0.900	0.0	0.004±0.002	0.0
Camden, GA	0.008	0.0	0.722	0.0	0.008±0.003	0.0
Glynn, GA	0.013	0.0	0.867	0.0	0.013±0.003	0.0
Chatham, GA	0.009	0.010	0.978	0.667	0.009±0.002	0.010±0.004
Cumberland Island, GA	0.002	0.002	0.796	0.633	0.002±0.001	0.001±0.001
Jekyll Island, GA	0.001	0.0	0.320	0.0	0.001±0.000	0.0
St. Simons Island, GA	0.002	0.0	0.533	0.0	0.001±0.001	0.0
Sapelo Island, GA	0.0	0.009	0.0	0.533	0.0	0.01±0.003
St. Catherines Island, GA	0.0	0.0	0.0	0.0	0.0	0.0
Ossabaw Island, GA	0.0002	0.0	0.346	0.0	0.0001±0.0	0.0

Table 5.3. Measures of within population genetic variation (mean gene diversity, mean allelic richness, inbreeding coefficient) based on microsatellite genotype data for all populations of *Peromyscus gossypinus* included in this study with at least five samples which were sampled in both 1988 and 2009.

Population	Mean gene diversity 1988	Mean gene diversity 2009	Mean allelic richness 1988	Mean allelic richness 2009	Inbreeding coefficient 1988	Inbreeding coefficient 2009
Dade, FL	0.675	0.768	3.50	5.20	0.097	0.092
Amelia Island, FL	0.646	0.594	3.52	3.01	0.277	-0.156
Camden, GA	0.774	0.733	4.73	4.56	0.097	-0.151
Glynn, GA	0.832	0.660	5.49	2.90	0.021	0.091
Chatham, GA	0.819	0.860	5.01	5.43	0.87	0.80
Cumberland Island, GA	0.662	0.693	3.99	4.05	0.030	0.136
Jekyll Island, GA	0.783	0.770	5.23	4.67	0.032	0.168
St. Simons Island, GA	0.783	0.610	4.62	3.17	0.154	0.005
Sapelo Island, GA	0.549	0.537	3.97	4.03	0.027	-0.096
St. Catherines Island, GA	0.514	0.636	2.52	3.50	0.030	-0.019
Ossabaw Island, GA	0.619	0.630	3.51	3.58	0.078	0.035

Table 5.4. Tamura Nei corrected population pairwise genetic distances between all population pairs of *Peromyscus gossypinus* sampled in A)1988 and B) 2009. Numbers in bold along diagonal indicate the genetic distance between populations from the same location across time points.

A)

	Glynn	Chatham	Camden	Dade	Jekyll Island	St. Simons Island	Cumberland Island	Amelia Island	Ossabaw Island	St. Catherines Island	Sapelo Island	Brevard	Merritt Island
Glynn	**0.012**												
Chatham	0.007	**0.014**											
Camden	0.010	0.009	**0.014**										
Dade	0.053	0.056	0.056	**0.022**									
Jekyll Island	0.013	0.015	0.018	0.046	**0.002**								
St._Simons Island	0.019	0.016	0.019	0.046	0.005	**0.021**							
Cumberland Island	0.015	0.012	0.016	0.056	0.018	0.019	**0.005**						
Amelia Island	0.018	0.020	0.017	0.055	0.025	0.026	0.022	**0.009**					
Ossabaw Island	0.024	0.026	0.028	0.050	0.018	0.018	0.028	0.029	**0.00**				
St._Catherines Island	0.019	0.020	0.021	0.050	0.017	0.018	0.021	0.022	0.012	**0.00**			
Sapelo Island	0.015	0.007	0.015	0.052	0.016	0.016	0.010	0.020	0.026	0.019	**0.01**		
Brevard	0.055	0.056	0.056	0.029	0.045	0.048	0.061	0.056	0.046	0.048	0.056	**0.022**	
Merritt Island	0.052	0.051	0.052	0.033	0.039	0.040	0.057	0.054	0.041	0.045	0.051	0.026	**0.021**

B)

	Glynn	Chatham	Camden	Dade	Jekyll Island	St. Simons Island	Cumberland Island	Amelia Island	Ossabaw Island	St. Catherines Island	Sapelo Island	Brevard	Merritt Island
Glynn	**0.012**												
Chatham	0.014	**0.014**											
Camden	0.016	0.016	**0.014**										
Dade	0.056	0.056	0.052	**0.022**									
Jekyll Island	0.020	0.017	0.017	0.048	**0.002**								
St._Simons Island	0.018	0.012	0.015	0.063	0.021	**0.021**							
Cumberland Island	0.019	0.012	0.016	0.062	0.019	0.020	**0.005**						
Amelia Island	0.001	0.010	0.004	0.059	0.022	0.02	0.02	**0.009**					
Ossabaw Island	0.025	0.023	0.022	0.056	0.016	0.025	0.025	0.023	**0.00**				
St. Catherines Island	0.018	0.016	0.015	0.055	0.016	0.018	0.019	0.016	0.012	**0.00**			
Sapelo Island	0.021	0.014	0.018	0.062	0.018	0.013	0.012	0.023	0.023	0.019	**0.01**		
Brevard	0.055	0.058	0.055	0.035	0.048	0.065	0.065	0.057	0.051	0.054	0.063	**0.022**	
Merritt Island	0.044	0.05	0.044	0.038	0.041	0.057	0.057	0.046	0.042	0.045	0.057	0.016	**0.021**

Table 5.5. Population pairwise F_{ST} between all population pairs of *Peromyscus gossypinus* sampled in A) 1988 and B) 2009. Numbers in bold along diagonal indicate the F_{ST} value between populations from the same location across time points.

A)

	Glynn	Chatham	Camden	Jekyll Island	St. Simons Island	Cumberland Island	Amelia Island	Ossabaw Island	St. Catherines Island	Sapelo Island
Glynn	**0.117**									
Chatham	0.009	**0.073**								
Camden	0.042	0.024	**0.111**							
Jekyll Island	0.081	0.095	0.085	**0.052**						
St._Simons Island	0.082	0.096	0.084	0.063	**0.130**					
Cumberland Island	0.149	0.108	0.125	0.138	0.163	**0.018**				
Amelia Island	0.132	0.169	0.162	0.179	0.146	0.302	**0.121**			
Ossabaw Island	0.159	0.121	0.128	0.150	0.156	0.124	0.232	**0.101**		
St._Catherines Island	0.128	0.140	0.161	0.171	0.178	0.258	0.255	0.289	**0.109**	
Sapelo Island	0.124	0.116	0.121	0.129	0.146	0.146	0.232	0.215	0.200	**0.116**

B)

	Glynn	Chatham	Camden	Jekyll Island	St. Simons Island	Cumberland Island	Amelia Island	Ossabaw Island	St. Catherines Island	Sapelo Island
Glynn	**0.117**									
Chatham	0.228	**0.073**								
Camden	0.160	0.161	**0.111**							
Jekyll Island	0.168	0.147	0.104	**0.052**						
St._Simons Island	0.284	0.246	0.240	0.225	**0.130**					
Cumberland Island	0.225	0.190	0.087	0.129	0.259	**0.018**				
Amelia Island	0.301	0.254	0.223	0.137	0.274	0.249	**0.121**			
Ossabaw Island	0.229	0.212	0.183	0.158	0.252	0.222	0.271	**0.101**		
St._Catherines Island	0.290	0.215	0.270	0.232	0.342	0.291	0.357	0.270	**0.109**	
Sapelo Island	0.297	0.213	0.223	0.205	0.291	0.263	0.285	0.339	0.429	**0.116**

Table 5.6. Estimates of number of migrants per generation between all population pairs of *Peromyscus gossypinus* included in this study with at least five samples which were sampled in both 1988 and 2009. Estimates are based on microsatellite genotypes at seven unlinked loci and were determined using coalescent methods in the program MIGRATE-n. Source populations are indicated in columns and receiving populations are in rows. Island to island estimates were only calculated between neighboring islands

	Glynn	Chatham	Camden	Jekyll Island	St. Simons Island	Cumberland Island	Amelia Island	Ossabaw Island	St. Catherines Island	Sapelo Island
Glynn 1988	-	1	1.5	0.1	0.2	0.07	0	0.1	0	0.25
Glynn 2009	-	0	0.01	0	0	0	0.2	0	0	0
Chatham 1988	1	-	1	0.38	2	0	0	0	0	2
Chatham 2009	0	-	0	0	0.02	0	0	0	0	0.2
Camden 1988	2	1.5	-	0.1	0	0.51	2.7	0.08	0	0.8
Camden 2009	0.2	0	-	0	0.02	0	0	0	0	0
Jekyll Island 1988	0.23	0.2	0.28	-	0.23	0	-	-	-	-
Jekyll Island 2009	0	0	0.04	-	0	0	-	-	-	-
St. Simons Island 1988	0	0.1	0.4	1.3	-	-	-	-	-	-
St. Simons Island 2009	0	0	0.05	0	-	-	-	-	-	-
Cumberland Island 1988	0	0.17	1	-		-	0.5	-	-	-
Cumberland Island 2009	0	0	0	-		-	0.04	-	-	-
Amelia Island 1988	0	0	0	-	-	0.2	-	-	-	-
Amelia Island 2009	0.2	0	0.1	-	-	0.05	-	-	-	-
Ossabaw Island 1988	0	0	0	-	-	-	-	-	0	-
Ossabaw Island 2009	0	0	0	-	-	-	-	-	0	-
St. Catherines Island 1988	0	0	0	-	-	-	-	0	-	0
St. Catherines Island 2009	0	0	0	-	-	-	-	0	-	0
Sapelo Island 1988	0.1	0.2	0.08	-	0	-	-	-	0	-
Sapelo Island 2009	0.05	0	0	-	0	-	-	-	0	-

Table 5.7. Measures of effective population size based on microsatellite genotype data for all populations of *Peromyscus gossypinus* included in this study with at least five samples which were sampled in both 1988 and 2009. Estimates were obtained from the programs LDNe and MIGRATE-n. Measurement are not raw numbers and can only be compared within populations.

Population	LDNe (1988)	LDNe (2009)	MIGRATE (1988)	MIGRATE (2009)
Dade, FL	62.4	98.7	1.08	1.49
Camden, GA	31.6	2.2	1.66	0.45
Glynn, GA	91.0	13.6	8.00	1.27
Chatham, GA	80.7	95.3	1.00	1.12
Amelia Island, FL	11.1	1.2	1.07	0.98
Cumberland Island, GA	56.8	72.7	0.99	1.13
Jekyll Island, GA	29.0	13.2	0.56	0.27
St. Simons Island, GA	38.4	0.8	0.81	0.24
Sapelo Island, GA	42.2	74.6	0.88	1.67
St. Catherines Island, GA	4.6	7.2	0.46	0.72
Ossabaw Island, GA	8.6	17.3	0.88	1.59

Table 5.8 Analysis of molecular variance (AMOVA) for all population pairs of *Peromyscus gossypinus* sampled in both 1988 and 2009. Populations are partitioned into 13 groups with each group containing the samples from a given location at both time points.

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variance
Among groups	7	144.699	0.21711 Va	7.40
Among populations within groups	8	68.859	0.24589 Vb	8.38
Among individuals within populations	196	509.161	0.12789 Vc	4.36
Within individuals	212	496.500	2.34198 Vd	79.85
Total	423	1219.219	2.93287	

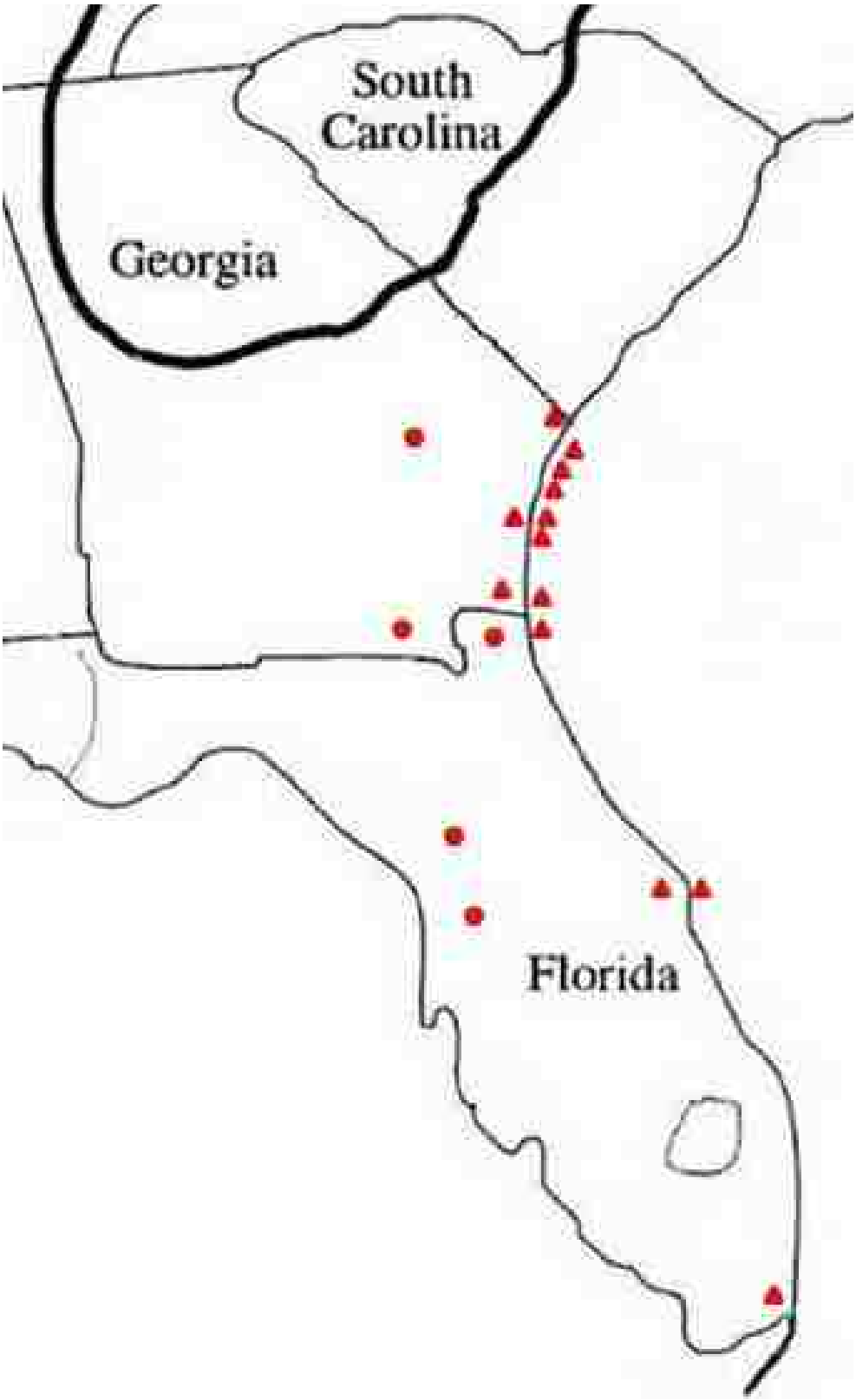


Figure 5.1. Locations where samples of *Peromyscus gossypinus* were collected for inclusion in this study. Locations where samples were collected in both 1988 and 2009 are indicated by a triangle, locations only trapped in 1988 are indicated by a circle.

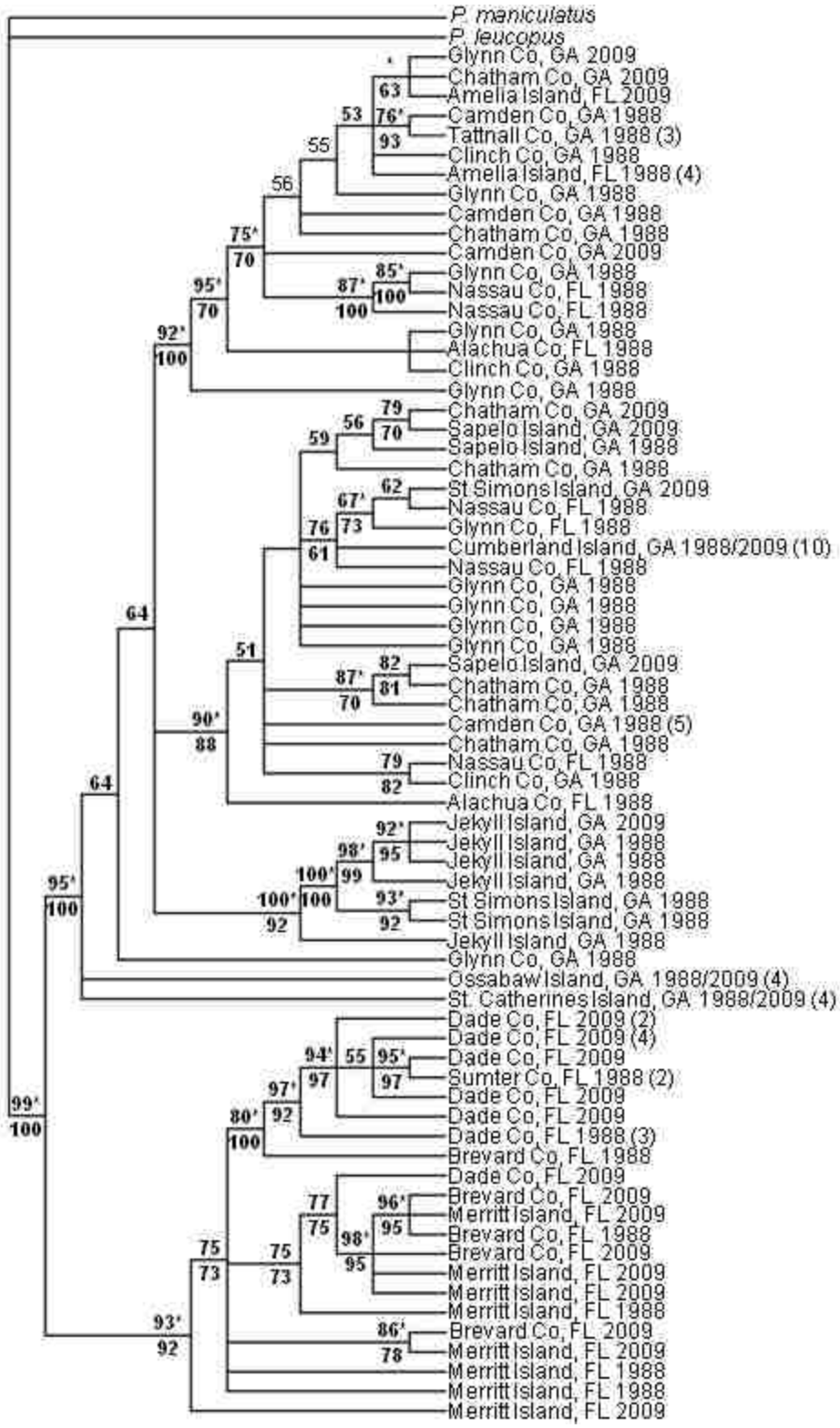


Figure 5.2. Phylogenetic tree reconstructed from populations included in this study. Maximum likelihood bootstrap values are above the line and maximum parsimony values are below the line if above 50%. A * next to a node indicates a posterior probability of 0.95 or greater in the Bayesian analysis. Tips with parentheses indicate monophyletic groups that were collapsed to see the overall relationship of the tree. Numbers in parentheses indicate number of haplotypes.

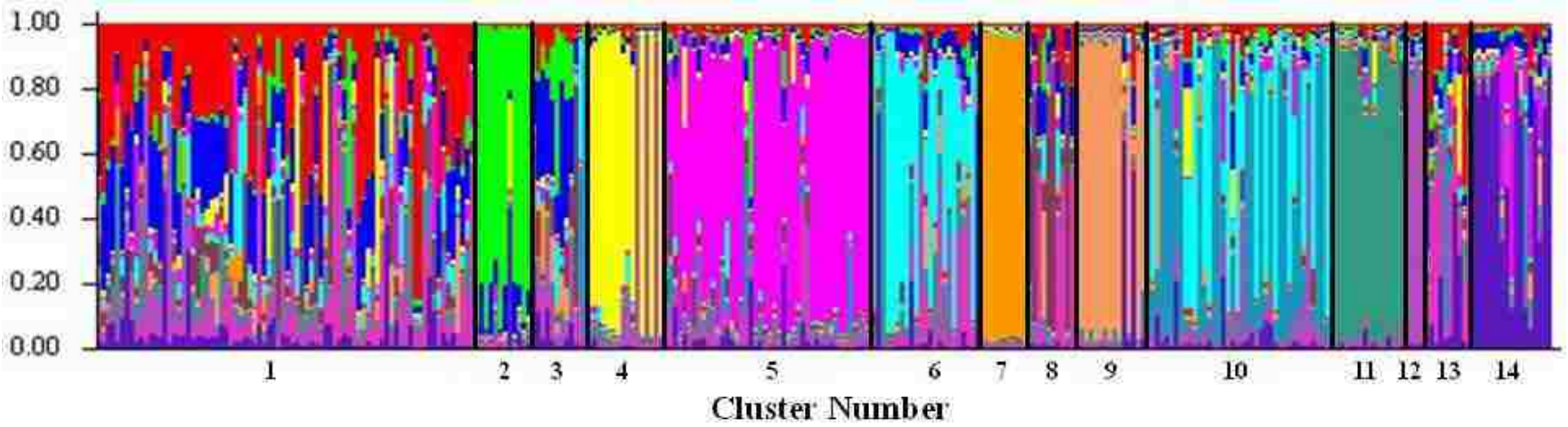
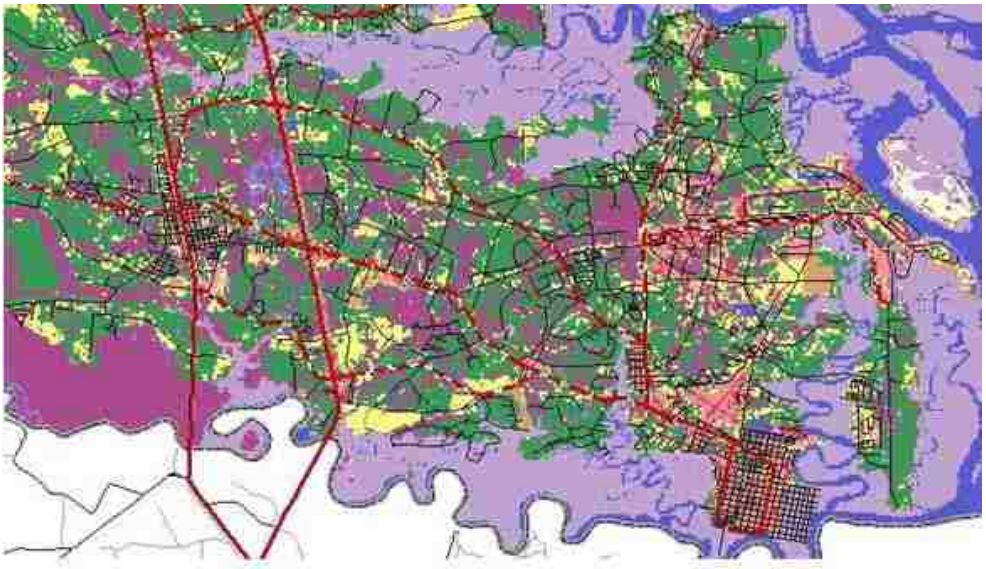


Figure 5.3. STRUCTURE analysis of the 13 populations sampled in both 1988 and 2009 as well as four populations sampled in only 1988. Populations formed 14 distinct clusters using the ln P(D) method of Pritchard et al. 2000 and the ΔK method of Evanno et al (2005). Cluster 1 contains the 1988 populations of Nassau, FL, Clinch, GA, Tattnall, GA, Camden, GA, Glynn, GA, and Chatham, GA; cluster 2 contained the 2009 population of St. Simons Island; cluster 3 contained the 1988 population from St. Simons Island, GA; cluster 4 contained the 1988 and 2009 populations from St. Catherines Island, GA; cluster 5 contained the 1988 and 2009 populations from Cumberland Island, GA; cluster 6 contained the 1988 and 2009 populations from Dade, FL; cluster 7 contained the 2009 population from Glynn, GA; cluster 8 contained the 2009 population from Camden, GA; cluster 9 contained the 1988 and 2009 populations from Amelia Island, FL; cluster 10 contained the 1988 and 2009 populations from Jekyll Island, GA; cluster 11 contained the 1988 and 2009 populations from Sapelo Island, FL; cluster 12 contained the 2009 population from Chatham, GA; cluster 13 contained the 1988 population from Alachua, FL; and cluster 14 contained the 1988 and 2009 populations from Ossabaw Island, GA.

Figure 5.4. Aerial land use maps of the region of Chatham, GA where samples were collected in both 1988 and 2009. The photo on the left is from 1985 and the photo on the right is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.



Camden, GA 1985

Camden, GA 2009

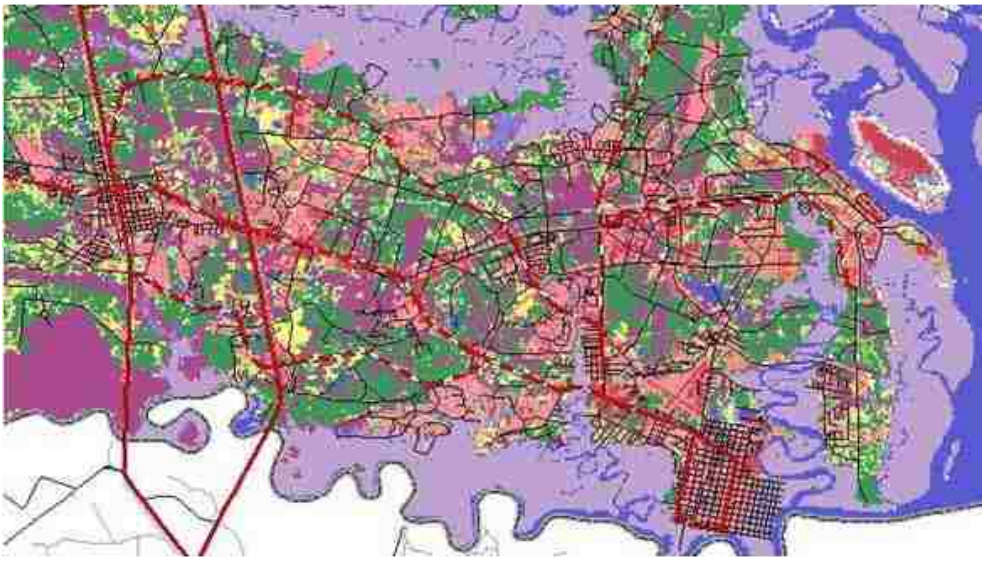
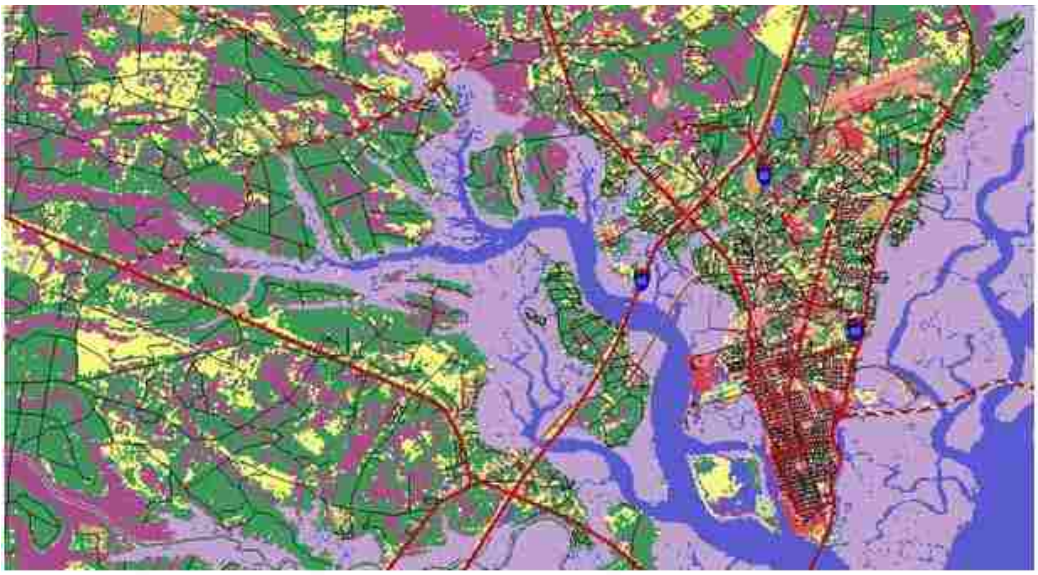


Figure 5.5. Aerial land use maps of the region of Camden, GA where samples were collected in both 1988 and 2009. The photo on the top is from 1985 and the photo on the bottom is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.



Glynn, GA 1985

Glynn, GA 2009

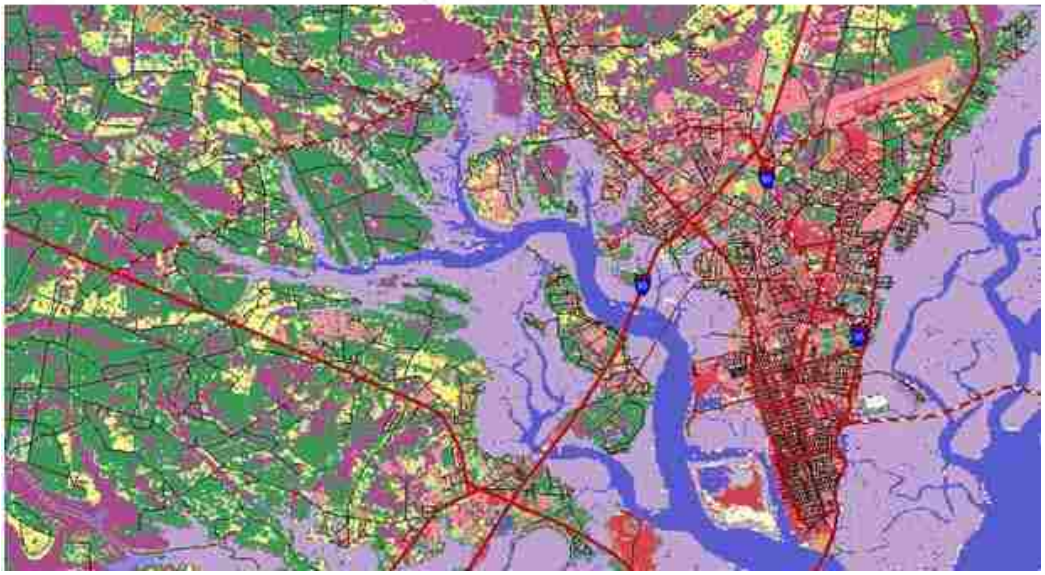


Figure 5.6. Aerial land use maps of the region of Glynn, GA where samples were collected in both 1988 and 2009. The photo on the top is from 1985 and the photo on the bottom is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.

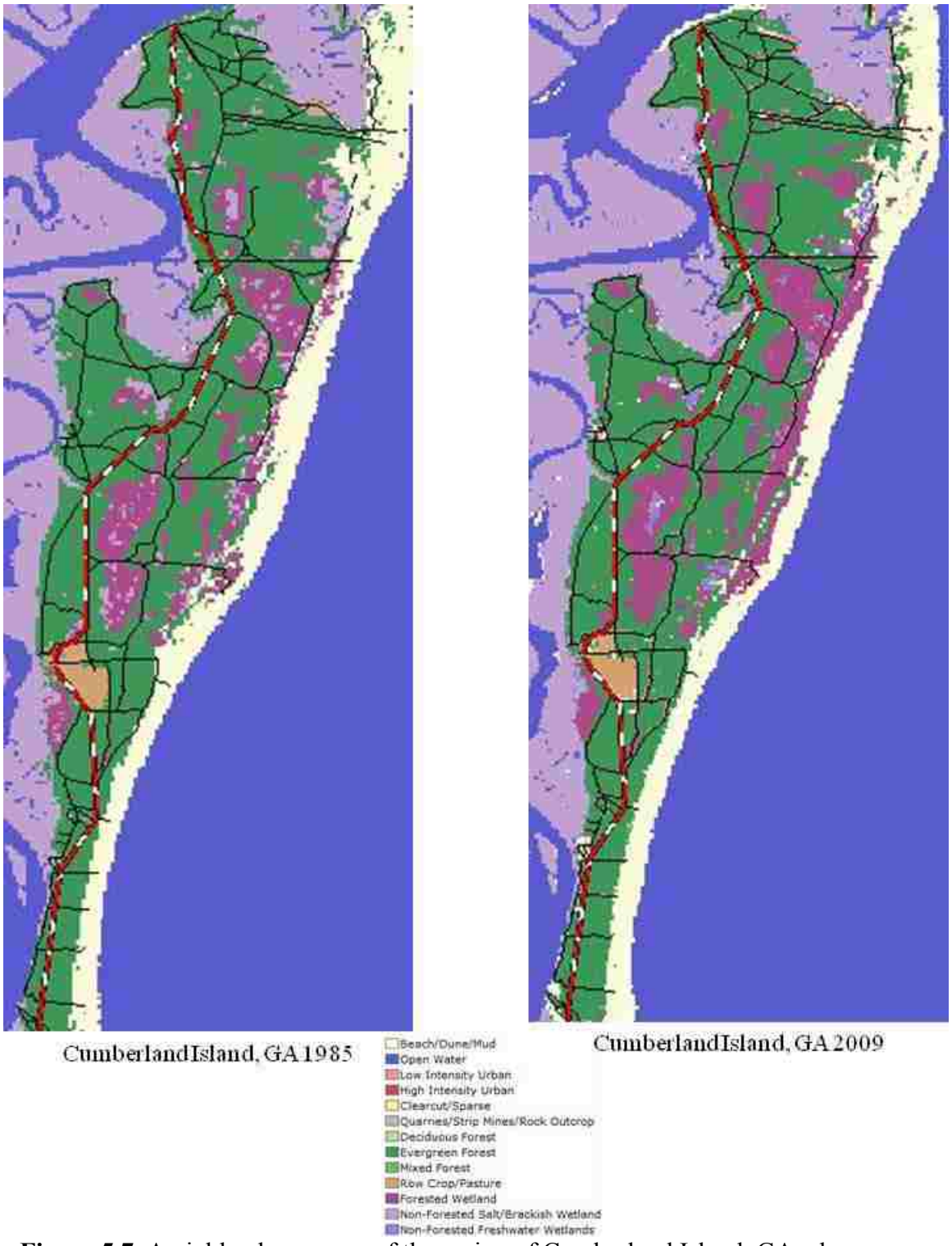


Figure 5.7. Aerial land use maps of the region of Cumberland Island, GA where samples were collected in both 1988 and 2009. The photo on the left is from 1985 and the photo on the right is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.

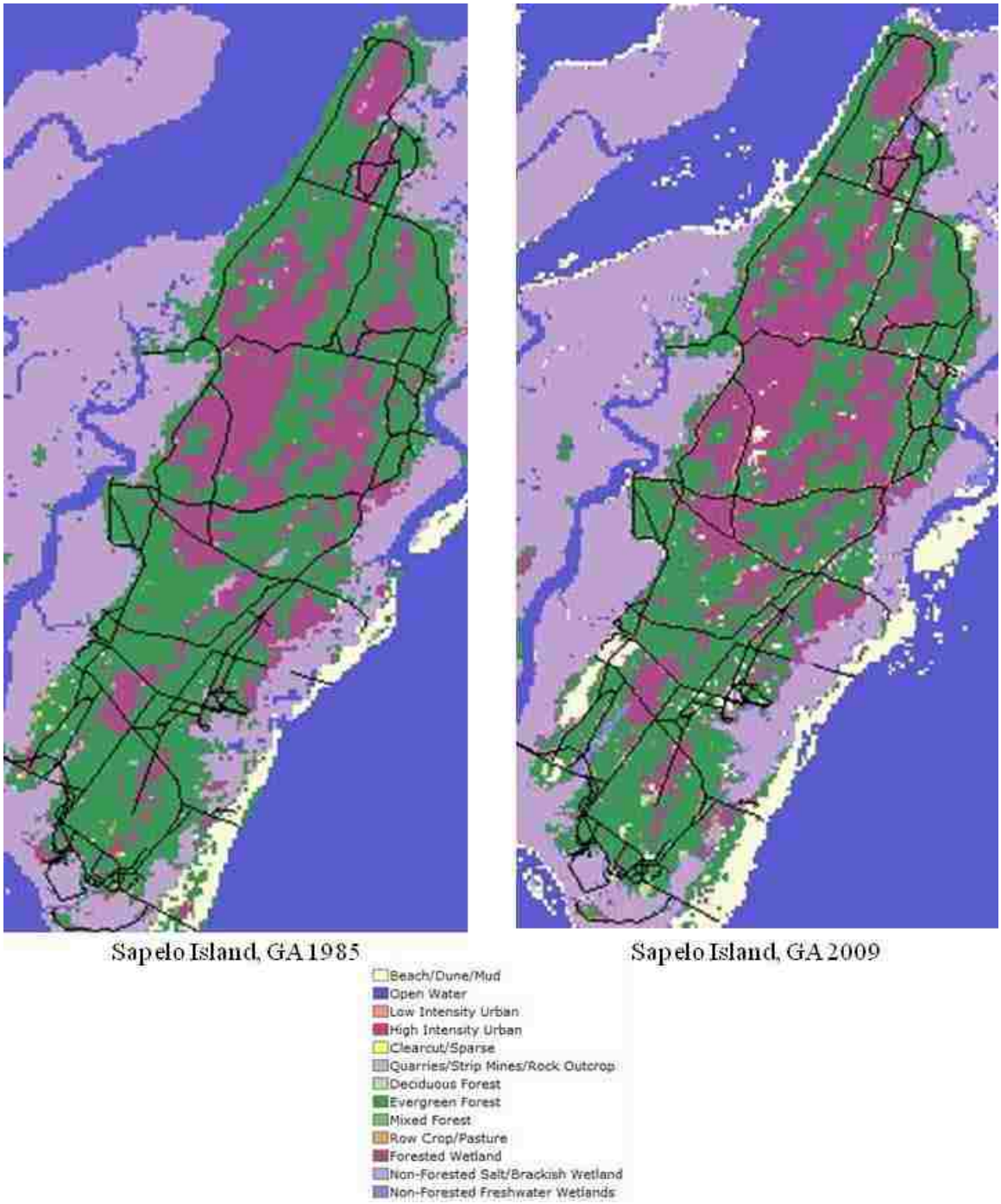


Figure 5.8. Aerial land use maps of the region of Sapelo Island, GA where samples were collected in both 1988 and 2009. The photo on the left is from 1985 and the photo on the right is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.

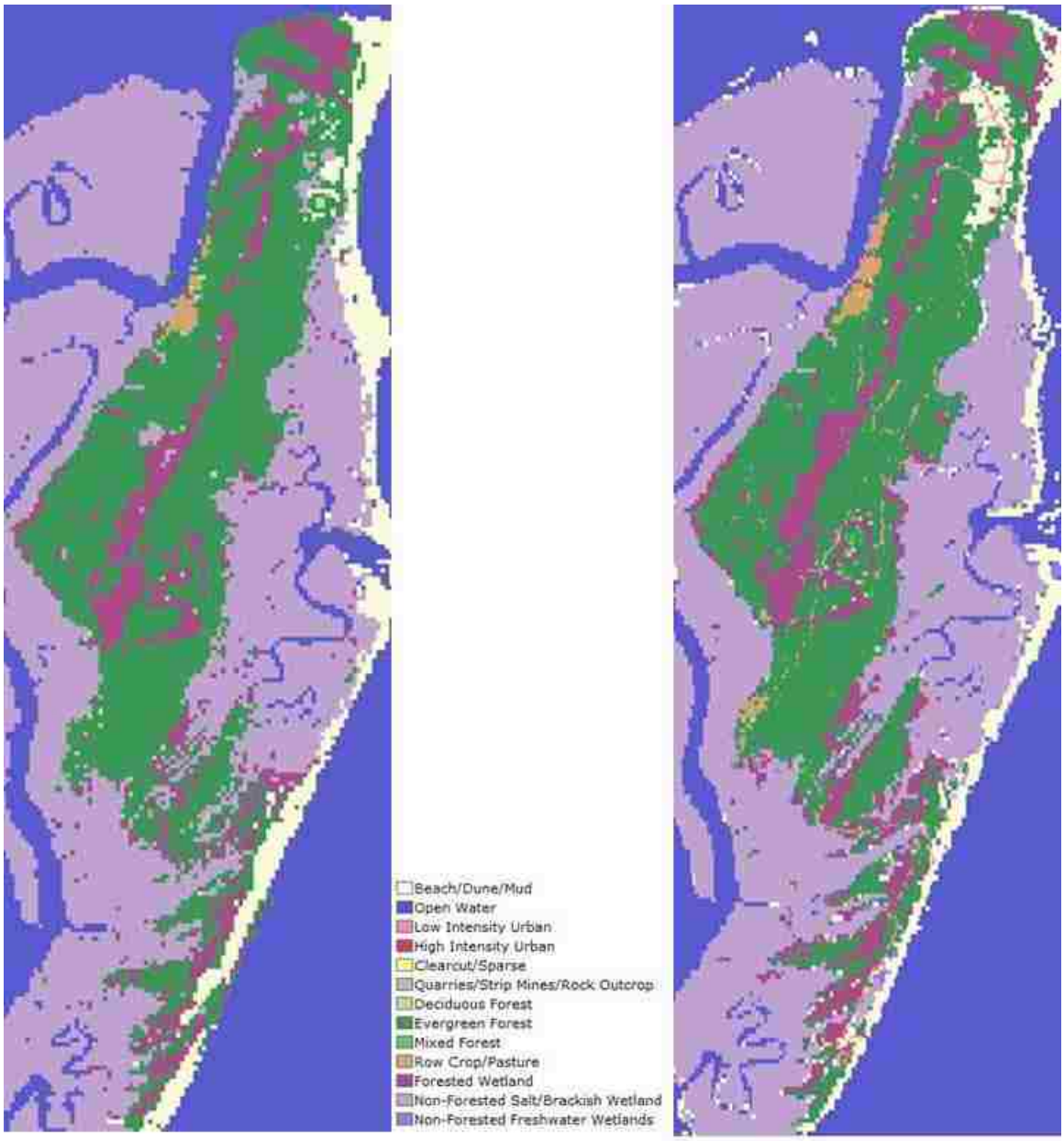


Figure 5.9. Aerial land use maps of the region of St. Catherines Island, GA where samples were collected in both 1988 and 2009. The photo on the left is from 1985 and the photo on the right is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.

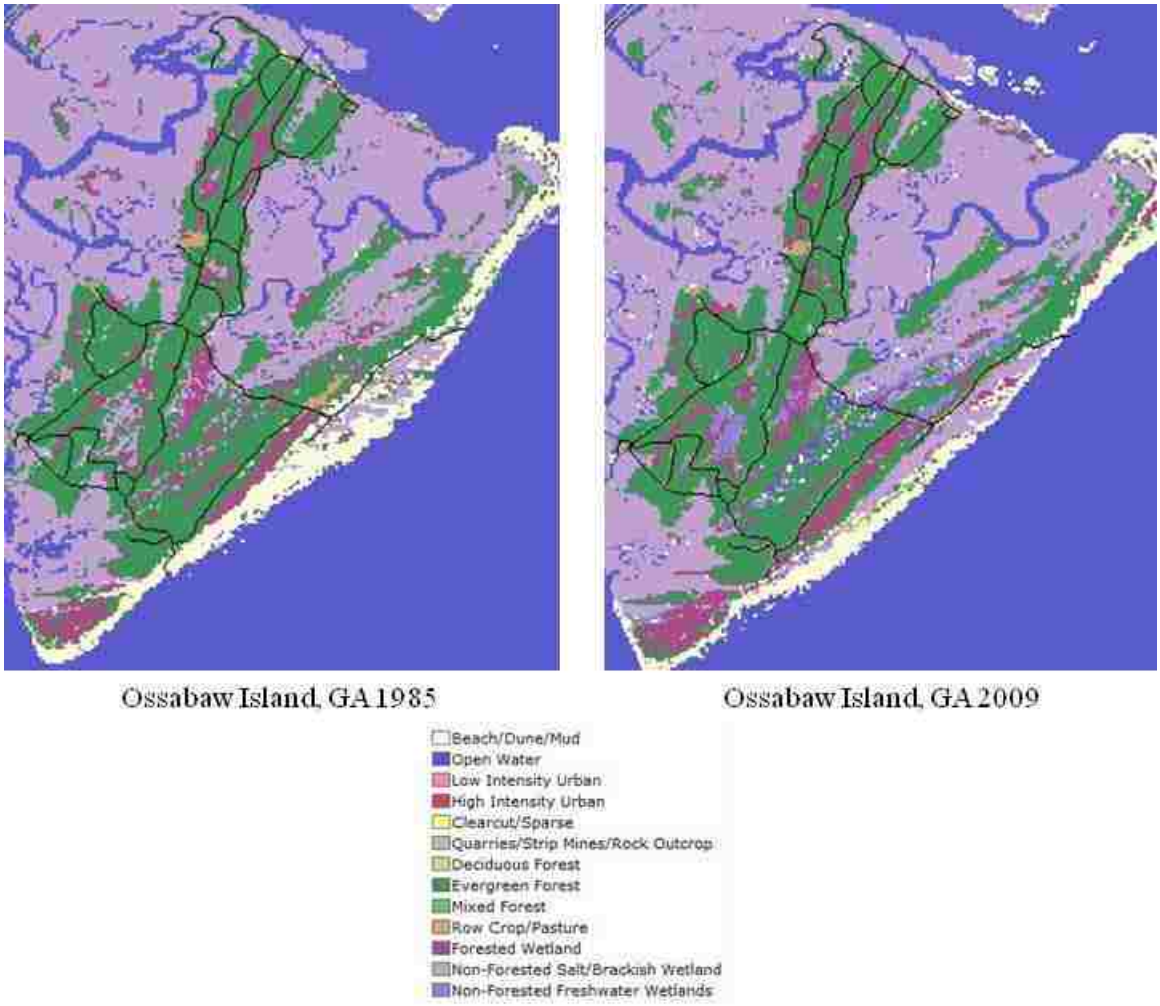


Figure 5.10. Aerial land use maps of the region of Ossabaw Island, GA where samples were collected in both 1988 and 2009. The photo on the left is from 1985 and the photo on the right is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.

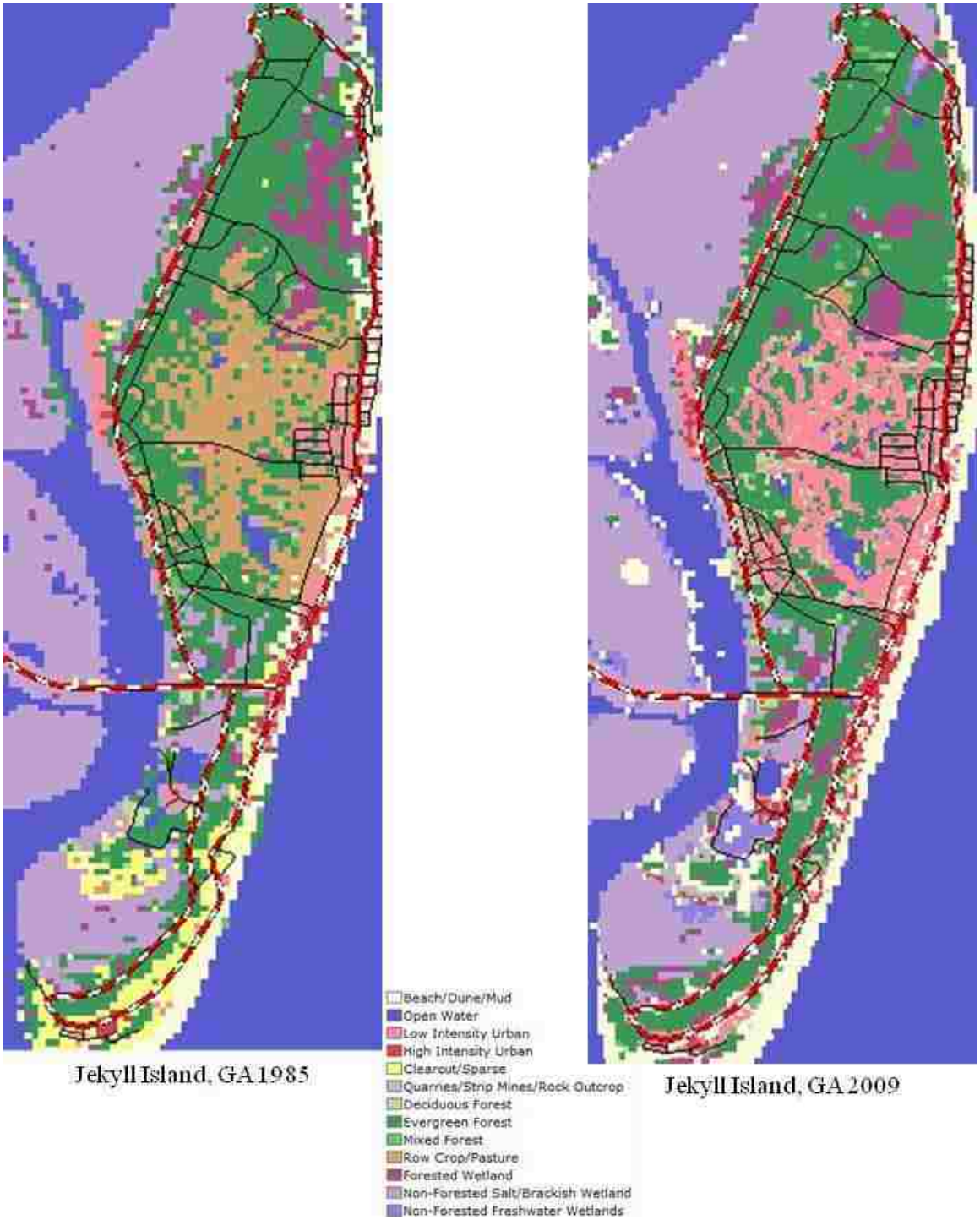


Figure 5.11. Aerial land use maps of the region of Jekyll Island, GA where samples were collected in both 1988 and 2009. The photo on the left is from 1985 and the photo on the right is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.

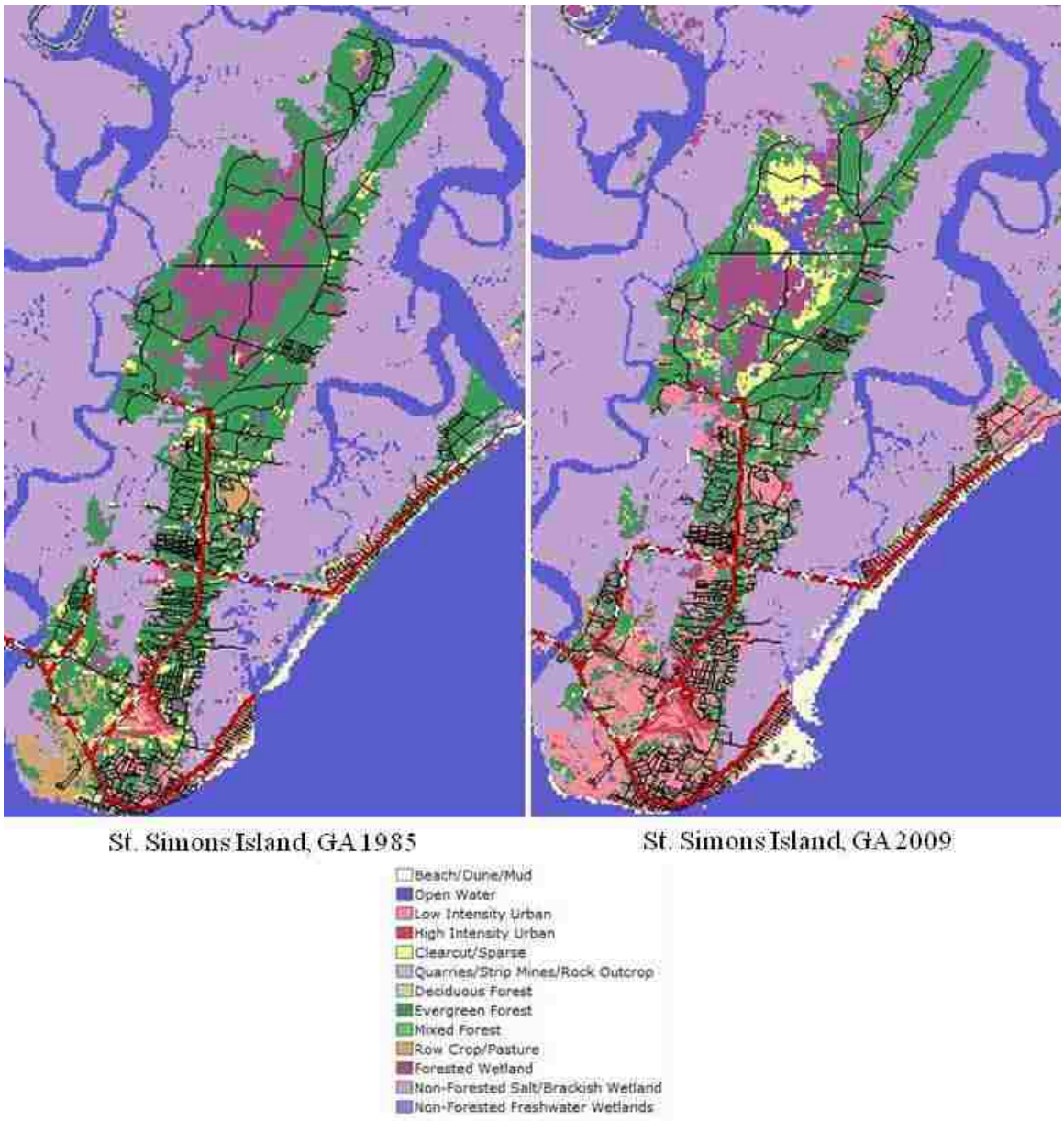


Figure 5.12. Aerial land use maps of the region of St. Simons Island, GA where samples were collected in both 1988 and 2009. The photo on the left is from 1985 and the photo on the right is from 2009. Areas of low intensity urbanization are indicated in pink and areas of high intensity urbanization are in red. Photos courtesy of the Natural Resources Spatial Analysis Lab (NARSAL) at the University of Georgia.

Chapter Six

General conclusions

The geographic patterns of genetic variation within and among closely related species are the consequence of complex interactions among life history traits as well as historic and contemporary geographic and climatic events. Traditionally, data suggest that habitat generalists (Bradley et al. 2008, Hundertmark et al. 2003) are less susceptible to the effects of climatic and geographic events such as glaciations, island formation, river formation, and mountain formation. As a result they demonstrate less geographic patterning than habitat specialists (Galbreath et al. 2009, Van Zant and Wooten 2007). However, these data are based primarily on large vagile species which are intuitively less susceptible to these effects than organisms with limited dispersal. The primary goals of this research were to: 1) identify the patterns of genetic variation in a habitat generalist with limited dispersal, the cotton mouse, *Peromyscus gossypinus*' and 2) determine the climatic and geographic events that were responsible for those patterns. Each chapter focused on variation at a different organizational level, from species to population, utilizing molecular markers to identify and explain the patterns of genetic variation in *P. gossypinus*.

In chapter two, I explored the large-scale patterns of genetic variation throughout the entire species' range in order to identify unique evolutionary lineages within *P. gossypinus*. Using mitochondrial control region sequence data I found three reciprocally monophyletic lineages, as well as five additional distinct lineages within the reciprocally monophyletic groups. I also proposed recognizing each lineage as a separate subspecies. While the genetic divergence between the three reciprocally monophyletic groups is

consistent with specific level variation in other *Peromyscus* species (Castro-Campillo et al. 1999), it is necessary to verify the mitochondrial patterns with nuclear sequence data before elevating these lineages to new species.

The first subspecies, *P. g. palmarius* is found in peninsular Florida and has a range consistent with unique subspecies of several other taxa (Douglas et al. 2009, Hull et al. 2008, Speller et al. 2010, Walker and Avise 1998). A second subspecies, *P. g. gossypinus* inhabits the remainder of the mainland Atlantic region extending westward to the Appalachian Mountains and the Apalachicola-Chattahoochee-Flint River Basin (ACF) which has been identified as a break between species and subspecies across a range of life history traits (Soltis et al. 2006). Two additional subspecies occur west of the ACF. *Peromyscus gossypinus megacephalus* is found between the ACF and the Mississippi River which has been shown to be a barrier to gene flow for both mammalian (Brant and Ortí 2003) and non-mammalian taxa (Howes et al. 2006), and the Western cotton mouse is found west of the Mississippi River. In addition to the four mainland subspecies, four island subspecies were also identified: the Hunting Island cotton mouse *(*Hunting Island, SC), the Ossabaw Island cotton mouse (Ossabaw and St. Catherines Island, GA), the Jekyll Island cotton mouse (Jekyll Island, GA)*,* and *P. g. allapaticola* (Key Largo, FL).

In chapter three, I utilized mitochondrial sequence data to examine the phylogeographic patterns of *P. gossypinus*. These data allowed me to identify the rate of gene flow among populations and subspecies of *P. gossypinus* as well as the time of divergence of each of the unique evolutionary lineages. The results indicated that the genetic variation in *P. gossypinus* were shaped by a series of glaciation events and

associated interglacial periods, resulting in a group of well differentiated subspecies, as identified in chapter two, with little contemporary gene flow between groups.

The major glaciations of the Illinoian and Wisconsinan periods resulted in the formation of several unique evolutionary lineages in *P. gossypinus*. The oldest lineage, *P. g. palmarius* diverged during the Sangamonian interglacial period, while *P. g. gossypinus* and *P. g. megacephalus* diverged during the Tahoe glaciation of the Wisconsinan. Finally, the populations west of the Mississippi diverged during the Tenaya glaciation. During glacial maxima, advancing ice sheets and cooling temperatures commonly resulted in populations of the same species utilizing different geographic refugia (Avise 2009, Donovan et al. 2000). Throughout these periods of isolation, populations diverged, eventually developing into new subspecies or species. Following the glaciation, these populations either came into secondary contact in suture zones (Remington 1968, Hewitt 2000) or remained isolated from one another by rivers and other geographic barriers to gene flow (Soltis et al. 2006).

The island subspecies of *P. gossypinus* formed as an indirect result of the Tioga glaciation of the Wisconsinan. Each of the four island subspecies diverged from the mainland approximately 10,000-20,000 years ago during a time period when melting glacial ice resulted in the formation of several barrier islands off the coast of the southeastern United States (Morgan and Emslie 2010). Subspecies endemic to barrier islands have frequently been identified in other terrestrial organisms throughout the southeastern United States (Degner et al. 2007, Hayes and Harrison 1992, Indorf 2010). Populations on these islands commonly became isolated from mainland populations due

to an inability to disperse across large areas of salt water. Over time they diverged due to a lack of gene flow and have come to represent unique evolutionary lineages.

In chapter four I analyzed variation at microsatellite loci to explore connectivity among populations within evolutionary lineages. This information allowed me to assess whether geographic features such as rivers, and anthropogenic features, such as urbanization acted to reduce gene flow among populations of *P. gossypinus* on a regional level. The results indicated that patterns of gene flow within regions were the result of the species' vagility in combination with naturally occurring barriers to gene flow and anthropogenic forces. Populations in close geographic proximity exhibited high levels of gene flow, in some cases showing no differentiation at all. However, populations separated by large distances exhibited less similarity consistent with an isolation by distance model already identified in *P. gossypinus* (Boone et al. 1999).

In chapter five I took advantage of a naturally occurring experiment to test the effects of urbanization on genetic variation at a finer scale than the regional level in chapter four by assessing variation within populations and gene flow among populations. Utilizing both mitochondrial and microsatellite data, I was able to compare populations in urbanized and non-urbanized areas both before and after urbanization occurred. These data demonstrated that urbanization resulted in a reduction in gene flow among population as well as reduction in genetic variation within populations. Among mainland populations, those in urbanized areas experienced a marked decrease if not a total loss of genetic variation during the last twenty years, while populations in non-urbanized areas experienced no loss or an overall gain of variation. A similar pattern was also apparent among island population.

As was the case for the regional level analysis there was a marked reduction in gene flow also associated with an increase in urbanization. This reduction in gene flow, along with reductions in population size, frequently resulted in urbanized populations diverging from populations they were once closely related to. Reduced gene flow and divergence among populations in urban areas has been observed in a variety of taxa (Delaney et al. 2010, Vandergast et al 2007). These patterns have been attributed to an inability to migrate through urban matrices as well as habitat alteration, fragmentation, and loss associated with urbanization (Anderson et al. 2003, Bolger et al. 1991, 1997, 2008, Munshi-South and Kharchenko 2010, Wandeler et al. 2003).

The combined results of this research indicate that the geographic patterns of genetic variation in *P. gossypinus* have been dramatically shaped by both historic and contemporary climatic and geographic events. This indicates that dispersal limited habitat generalists can be as susceptible to natural and anthropogenic events as habitat specialists. At the local level, these results provide valuable information about the effects of habitat loss, alteration, and fragmentation for native habitat generalist species.

Understanding the patterns of genetic variation in *P. gossypinus* at both the local and range-wide level may provide valuable insight into the patterns of genetic variation in other habitat generalist species. As a result, information on patterns of variation in multiple species makes it possible to identify geographic areas of high conservation priority. These areas must be established in order to maintain genetic variation and the evolutionary processes responsible for this variation in *P. gossypinus* as well as other species. Due to limitations placed on habitat preservation, taking evolutionary processes

into account when preserving habitats is more likely to ensure the long term persistence of genetic variation within a species (Mace and Purvis 2008).

Placing the patterns identified in this study into a historic context may also provide insight into how this and other habitat generalist species may respond to future geographic events and impending global climate change. In particular, island populations and populations in urbanized areas are most susceptible to these events due to reduced habitat availability and a reduction or loss of gene flow with neighboring populations. These areas must be given high priority for conservation in order to preserve the genetic diversity of this and other species. While the southeastern United States is home to an abundant biodiversity, unless increased priority is given to maintaining the genetic variation of both habitat generalist and habitat specialist species at both a local and range-wide level, much of this diversity may soon be lost.

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Appendix I:

Geographic Locations and Accession Number of Samples

Specimens examined- All samples utilized as part of this are listed below by species, subspecies, and geographic location. Study specific identification numbers and institutional identification numbers for each sample are given in parentheses (Study ID, Institutional ID). Museum and collection abbreviations are: Georgia Museum of Natural History Mammal Collection (GMNH), Texas Tech University Genetic Resource Center (TK), Sean Beckmann-University of Miami (BAMI, BARS, BBCS, BBRE, BCAM, BCHA, BCIS, BDAD, BEIS, BGLY, BHIS, BJIG, BKLF, BMIF, BOSS, BSAP, BSCI, BSSI), James L. Boone-University of Georgia (JLB), D. D. Platt-University of Georgia (DDP).

Peromyscus leucopus-South Carolina; Beaufort Co., Beaufort, SR S 7-20 0.8mi N of Hwy170 (BBCS1; BBCS2), Hunting Island (JLB704, GMNH7363; JLB706, GMNH7359; JLB709, GMNH7358; JLB711, GMNH7349; BHIS2; BHIS5; BHIS6; BHIS7; BHIS9; BHIS11); Charleston Co., Adams Run, Moffett Rd. (BARS2; BARS3), Edisto Island (JLB702, GMNH7285); Dillon Co., Little Pee Dee River at Hwy 41 (JLB967, GMNH7499; JLB968, GMNH7500; JLB970, GMNH7501); Marion Co., Highway 41 at Latta Rd. (JLB952, GMNH7463; JLB958, GMNH7446; JLB961, GMNH7445; JLB963, GMNH7448)

Peromyscus gossypinus allapaticola-Florida; Monroe Co., Key Largo (BKLF1; BKLF2; BKLF3; BKLF4; BKLF5; BKLF6; BKLF7; BKLF8; BKLF9; BKLF10;

BKLF11; BKLF12; BKLF13; BKLF14; BKLF15; BKLF16; BKLF17; BKLF18; BKLF19; BKLF20)

Peromyscus gossypinus gossypinus-Florida; Alachua Co., Gainesville (JLB308, TK157308; JLB309, TK157309; JLB310, TK157310; JLB311, TK157311; JLB312, TK157312; JLB313, TK157313; JLB314, TK157314; JLB315, TK157315), Windsor City (JLB316, TK157316; JLB317, TK157317); Baker Co., 0.25 mi W of Montiac GA, Moccasin Creek (JLB306, TK157306; JLB307, TK157307); Citrus Co., Dunnellon (JLB369, TK157369; JLB370, TK157370; JLB371, TK157371); Clay Co., Tacoi Rd. 6mi. S of CR-226 (JLB379, TK157379; JLB380, TK157380; JLB381, TK157381; JLB383, TK157383); Leon Co., Ochlockonee River at Hwy 12 (JLB688, GMNH6982; JLB691, GMNH6983), 2 mi W of Tall Timbers (JLB696, GMNH6984; JLB697, GMNH6985); Levy Co., Gulf Hammock, Goethe State Park (JLB338, TK157338; JLB339, TK157339; JLB340, TK157340; JLB341, TK157341; JLB342, TK157342; JLB343, TK157343; JLB344, TK157344); Marion Co., Hwy 484 ¼ mi W of Hwy 475 (JLB367, TK157367); Nassau Co., Amelia Island (JLB645, GMNH6934; JLB646, GMNH6935; JLB647, GMNH6936; JLB648, GMNH6937; JLB649, GMNH6938), Amelia Island, Amelia Island State Park (BAMI1, BAMI2, BAMI3, BAMI4, BAMI5, BAMI6, BAMI7, BAMI8, BAMI9, BAMI10), Hilliard, CR-121, North of Hwy 108 (JLB414, TK157414; JLB415, TK157415; JLB416, TK157416; JLB417, TK157417; JLB418, TK157418; JLB419, TK157419; JLB420, TK157420; JLB421, TK157421; JLB422, TK157422; JLB423, TK157423; JLB424, TK157424), Kent, FL-2 at bridge over St. Marys River (JLB302, TK157302; JLB303, TK157303; JLB304, TK157304; JLB305, TK157305); Putnam Co., CR 209 6mi E of US17 (JLB372, TK157372; JLB373,

TK157373; JLB374, TK157374; JLB375, TK157375; JLB376, TK157376; JLB377, TK157377; JLB378, TK157378); St. Johns Co., Hwy 13 at Six Mile Creek (JLB384, TK157384; JLB385, TK157385; JLB386, TK157386; JLB387, TK157387; JLB388, TK157388), Hwy 208 5mi E of Hwy 13 (JLB398, TK157389; JLB390, TK157390; JLB391, TK157391; JLB392, TK157392; JLB393, TK157393; JLB402, TK157402); Sumter Co. North, Rt. 44 1mi. E of Withlacoochee River (JLB362, TK157362; JLB363, TK157363; JLB364, TK157364; JLB365, TK157365; JLB366, TK157366); Georgia; Camden Co., entrance to Crooked River State Park (JLB1, TK157001; JLB2, TK157002; JLB3, TK157003; JLB4, TK157004; JLB5, TK157005; JLB6, TK157006; JLB8, TK157008; JLB9, TK157009; JLB11, TK157011; JLB12, TK157012; JLB13, TK157013; JLB14, TK157014; JLB15, TK157015; JLB16, TK157016; JLB17, TK157017; JLB18, TK157018; JLB19, TK157019; JLB20, TK157020; JLB21, TK157022; JLB23, TK157023; JLB24, TK157024; JLB25, TK157025; JLB26, TK157026; JLB27, TK157027; JLB28, TK157028; JLB31, TK157031; BCAM1, BCAM2, BCAM3, BCAM4, BCAM5, BCAM6, BCAM7, BCAM8, BCAM9, BCAM10), Cumberland Island (JLB247, TK157247; JLB248, TK157248; JLB249, TK157249; JLB250, TK157250; JLB251, TK157251; JLB252, TK157252; JLB253, TK157253; JLB254, TK157254; JLB255, TK157255; JLB256, TK157256; JLB257, TK157257; JLB258, TK157258; JLB259, TK157259; JLB260, TK157260; JLB261, TK157261; JLB262, TK157262; JLB263, TK157263; JLB264, TK157264; JLB265, TK157265; JLB266, TK157266; JLB267, TK157267; JLB268, TK157268; JLB270, TK157270; JLB271, TK157271; JLB272, TK157272; JLB273, TK157273; JLB274, TK157274; JLB275, TK157275; BCIS1; BCIS2; BCIS3; BCIS4; BCIS5; BCIS6; BCIS7;

BCIS8; BCIS9; BCIS10; BCIS11; BCIS12; BCIS13; BCIS14; BCIS15; BCIS16); Chatham Co., Hwy 204 4-6mi W of I-95 (JLB186, TK157186; JLB187, TK157187; JLB188, TK157188; JLB189, TK157189; JLB190, TK157190; JLB191, TK157191; JLB192, TK157192; JLB193, TK157193; JLB194, TK157194; JLB195, TK157195; JLB196, TK157196; BCHA1; BCHA2; BCHA3; BCHA4; BCHA5), Skidaway Island (JLB138, TK157138; JLB139, TK157139; JLB140, TK157140; JLB141, TK157141; JLB142, TK157142; JLB143, TK157143; JLB144, TK157144; JLB145, TK157145; JLB146, TK157146; JLB147, TK157147); Clinch Co., Okeefenokee Swamp (JLB432, TK157432; JLB433, TK157433; JLB434, TK157434; JLB435, TK157435; JLB436, TK157436; JLB437, TK157437; JLB438, TK157438; JLB439, TK157439; JLB440, TK157440; JLB441, TK157441; JLB442, TK157442); Emanuel Co., Blundale, Hwy 1 9mi S of Wadley (DDP120, GMNH7982; DDP121. GMNH7983); Glynn Co., Everett, Altamaha Campground (JLB109, TK157109; JLB110, TK157110; JLB111, TK157111; JLB112, TK157112; JLB113, TK157113; JLB114, TK157114; JLB115, TK157115; JLB116, TK157116; JLB117, TK157117; JLB118, TK157118), Brunswick, US17 1mi. S of Hwy 520 (BGLY1; BGLY2; BGLY3; BGLY4; BGLY5; BGLY6; BGLY7; BGLY8; BGLY9; BGLY10); Jefferson Co., Old Town Plantation (DDP118, GMNH8099; DDP119, GMNH8100); St. Simons Island (BSSI1; BSSI2; BSSI3; BSSI4; BSSI5; BSSI6; BSSI7; BSSI8; BSSI9; BSSI10; BSSI11; BSSI12); McDuffie Co., Thompson, I-20 0.8-2.5mi W of Hwy 150 (JLB810, GMNH7294; JLB817, GMNH7398; JLB825, GMNH7395; JLB827, GMNH7396); McIntosh Co., Sapelo Island (JLB158, TK157158; JLB159, TK157159; JLB160, TK157160; JLB161, TK157161; JLB162, TK157162; JLB163, TK157163; JLB164, TK157164; JLB165, TK157165; JLB166, TK157166;

JLB167, TK157167; JLB169, TK157169; BSAP1; BSAP2; BSAP3; BSAP4; BSAP5; BSAP6); Tattnall Co., Brazellis Creek 1mi N of Reidsville (JLB1397, GMNH7901; JLB1402, GMNH7902), Ohoopee River at Rt 292 (JLB1403, GMNH7893; JLB1404, GMNH7892; JLB1407, GMNH7895; JLB1409, GMNH7891); Upson Co., Adams Rd. .4mi N Delray Rd. (JLB828, GMNH7451); North Carolina; Gates Co., Dismal Swamp (JLB842, GMNH7485), Gatesville, Chowan Swamp (JLB1228, GMNH7508; JLB1230, GMNH7509; JLB1234, GMNH7507); New Hanover Co., Wilmington, Hwy 421 1/2mi N of Cape Fear River (JLB212; TK157212, JLB213, TK157213; JLB214, TK157214; JLB215, TK157215; JLB216, TK157216; JLB217, TK157217; JLB218, TK157218; JLB219, TK157219; JLB220, TK157220; JLB221, TK157221); South Carolina; Aiken Co., Savannah River Plant (JLB1602, GMNH8050; JLB1605, GMNH8049); Berkeley Co., Hwy 41 SW side of Santee River (JLB1567, GMNH8204; JLB1570, GMNH8203; JLB1572, GMNH8205); Charleston Co., Adams Run, Moffett Rd. (BARS1; BARS4; BARS5; BARS6), Hwy 45 at Berkeley Co. Line (JLB1583, GMNH8082; JLB1586, GMNH8081)

Peromyscus gossypinus megacephalus-Alabama; Barbour Co., Blue Springs, Rt. 53 1mi. N of Hwy 10 (JLB1051, GMNH7567; JLB1052, GMNH7568; JLB1054, GMNH7569; JLB1055, GMNH7569; JLB1057, GMNH7570); Calhoun Co., Choccollocco Game Management Area (JLB615, GMNH6997; JLB618, GMNH7042); Randolph Co., Tallapoosa River at Hwy 49 (JLB1274, GMNH7513); Tuscaloosa Co., Moody Swamp (JLB502, TK157502; JLB503, TK157503; JLB504, TK157504; JLB505, TK157505; JLB506, TK157506; JLB507, TK157507), Hwy 82 at Sipsey River (JLB508, TK157508; JLB509, TK157509; JLB511, TK157511; JLB512, TK157512); Florida;

Gadsen Co., Chattahoochee, East Bank of the Apalachicola River (JLB677, GMNH6943; JLB680, GMNH6946; JLB681, GMNH6947); Jackson Co., West Bank of the Apalachicola River (JLB663, GMNH7070; JLB664, GMNH7071; JLB668, GMNH7069; JLB669, GMNH7072); Santa Rosa Co., Black Water State Forest (JLB556, TK157556; JLB557, TK157557; JLB558, TK157558; JLB559, TK157559; JLB560, TK157560; JLB561, TK157561; JLB562, TK157562; JLB563, TK157563; JLB564, TK157564; JLB565, TK157565); Mississippi; Perry Co., DeSoto National Forest (JLB583, TK157583; JLB584, TK157584; JLB585, TK157585; JLB586, TK157586; JLB587, TK157587; JLB588, TK157588; JLB589, TK157589; JLB590, TK157590; JLB591, TK157591; JLB592, TK157592); Prentiss Co., Hwy 4 4.7mi W of Tombigbee River (JLB1195, GMNH8020; JLB1200, GMNH8022; JLB1201, GMNH8015; JLB1202, GMNH8017; JLB1203, GMNH8080); Sharkey Co., Delta National Forest (JLB525, TK157525; JLB527, TK157527; JLB538, TK157538L JLB539, TK157539); Tennessee; Haywood Co., Hwy 54 at Forked Deer River (JLB1932, GMNH8489; JLB1933, GMNH8490); Obion Co., Reelfoot Lake (JLB1961, GMNH8346; JLB1962, GMNH8348; JLB1970, GMNH8347)

Peromyscus gossypinus palmarius-Florida; Brevard Co., Merritt Island National Wildlife Refuge 4-8mi. N of Haulover Canal (JLB717, GMNH7080; JLB718, GMNH7081; BBRE1; BBRE2; BBRE3), Merritt Island, 1-4mi S of Haulover Canal (JLB719, GMNH7083; JLB724, GMNH7087; JLB726, GMNH7085; JLB728, GMNH7086; BMIF1; BMIF2; BMIF3; BMIF4; BMIF5; BMIF6; BMIF7; BMIF8; BMIF9; BMIF10; BMIF11; BMIF12; BMIF13; BMIF14; BMIF15; BMIF16; BMIF17; BMIF18; BMIF19; BMIF20); Collier Co., Collier-Seminole State Park (JLB1725,

GMNH8263; JLB1726, GMNH8267); Miami-Dade Co., Everglades National Park (JLB1776, GMNH8437; JLB1778, GMNH8438; JLB1779, GMNH8439; JLB1780, GMNH8440; JLB1781, GMNH8441; BENP1; BENP2; BENP3; BENP5; BENP6; BENP7; BENP8; BENP9; BENP10; BENP11; BENP12; BENP13; BENP14; BENP15; BENP16; BENP17); Monroe Co., Everglades National Park (BENP4; BENP18); Sumter Co., Rt. 44 at the Withlacoochee River (JLB360, TK157360; JLB361, TK157361; JLB1366, GMNH12973)

Hunting Island cotton mouse-South Carolina; Beaufort Co., Hunting Island (BHIS1; BHIS3; BHIS4; BHIS8; BHIS10; BHIS12; BHIS13; BHIS14; BHIS15; BHIS16)

Jekyll Island cotton mouse-Georgia; Glynn Co., Jekyll Island (JLB33, TK157033; JLB53, TK157053; JLB55, TK157055; JLB56, TK157056; JLB57, TK157057; JLB60, TK157060; JLB61; TK157061; JLB62, TK157062; JLB63, TK157063; JLB64, TK157064; JLB65, TK157065; JLB66, YK157066; JLB67, YK157067; JLB68, TK157068; JLB69, TK157069; JLB70, TK157070; JLB71, TK157071; JLB72, TK157072; JLB73, TK157073; JLB74, TK157074; JLB75, TK157075; JLB76, TK157076; BJIG1; BJIG2; BJIG3; BJIG4; BJIG5; BJIG6; BJIG7; BJIG8; BJIG9; BJIG10; BJIG11; BJIG12; BJIG13; BJIG14; BJIG15; BJIG16; BJIG17), St. Simons Island (JLB78, TK157078; JLB79, TK157079; JLB81, TK157081; JLB82, TK157082; JLB83, TK157083; JLB84, TK157084; JLB85, TK157085; JLB86, TK157086; JLB87, TK157087; JLB88, TK157088; JLB89, TK157089)

Ossabaw Island cotton mouse-Georgia; Chatham Co., Ossabaw Island (JLB476, TK157476; JLB477, TK157477; JLB478, TK157478; JLB479, TK157479; JLB480, TK157480; JLB481, TK157481; JLB482, TK157482; JLB483, TK157483; JLB484, TK157484; JLB485, TK157485; JLB486, TK157486; BOSS1; BOSS2; BOSS3; BOSS4; BOSS5; BOSS6; BOSS7; BOSS8; BOSS9; BOSS10), Liberty Co., St. Catherines Island (JLB448, TK157448; JLB449, TK157449; JLB450, TK157450; JLB451, TK157451; JLB452, TK157452; JLB453, TK157453; JLB454, TK157454; JLB455, TK157455; JLB456, TK157456; JLB457, TK157457; BSCI1; BSCI2; BSCI3; BSCI4; BSCI5)

Western cotton mouse-Arkansas; Arkansas Co., Bayou Meto WMA, Long Bell Access (JLB1893, GMNH8486; JLB1894, GMNH8485); Louisiana; Lincoln Pa., Hwy.151 at Bayou D;Arbonne (JLB1552, GMNH8003; JLB1554, GLMN8006; JLB1555, GMNH8004; JLB1559, GMNH8005); West Baton Rouge Pa., Brusly, South River Rd. (JLB1437, GMNH8474; JLB1439, GMNH8475); Texas; Panola Co., Hwy 2517, East side of Sabine River (JLB1499, GMNH8109; JLB1500, GMNH8110; JLB1501, GMNH8111; JLB1503, GMNH8113)

Appendix II:
Geographic Ranges of Subspecies of *Peromyscus gossypinus*

P. g. gossypinus

Currently accepted range

Based on current taxonomy, the range of *P. g. gossypinus* includes the majority of the range of *P. gossypinus*. This includes all populations found in Virginia, North Carolina, and South Carolina, as well as all populations in Georgia found south and east of the Chattahoochee River. The range of this subspecies also encompasses populations in southern Alabama and Mississippi, as well as the southeastern 2/3 of Louisiana. In Florida, the range extends southward to approximately Gulf Hammock, FL near Cedar Key in the west, and just south of St. Augustine, FL in the east (Bangs 1896).

Newly suggested range

The results of this study indicate that this lineage is limited to the eastern portion of the current range of *P. g. gossypinus*. This includes all populations found in Virginia, North Carolina, and South Carolina, as well as all populations in Georgia found south and east of the Chattahoochee River. In contrast to the current taxonomy, all populations west of the Georgia-Alabama border are not included in the range of *P. g.* gossypinus. Likewise, in the state of Florida, the western border of *P. g. gossypinus* is roughly equivalent to the Ochlockonee River with all individuals sampled west of the Ochlockonee belonging to another subspecies. The southern border of *P. g. gossypinus* occurs in Sumter County, FL and is approximately associated with the Withlacoochee River. This includes all populations sampled in Citrus and Marion counties and represents a southward expansion of the range of this subspecies. Because the current

type locality for this lineage is Riceboro, GA, which falls within the geographic limits of the new subspecies' range, the name *P. g. gossypinus* will be retained.

P. g. palmarius

Current accepted range

Based on current taxonomy, the range of *P. g. palmarius* includes the majority of peninsular Florida. This subspecies extends southward from approximately Gulf Hammock, FL near Cedar Key in the west and St. Augustine, FL in the east (Bangs 1896). The southern range of this subspecies includes Miami-Dade County in eastern Florida but does not include Collier or Monroe counties. The populations from Manasota Key, Englewood, FL is recognized as a separate subspecies, *P. g. restrictus*, which is currently recognized as extinct. The mainland populations from Collier and Monroe counties are also currently recognized as a separate subspecies *P. g. telmaphilus*. Finally, the population of north Key Largo, FL is also recognized as a separate subspecies, *P. g. allapaticola*.

Newly suggested range

The results of this study indicate that the northern edge of this subspecies' range actually occurs further south than the current taxonomy suggest. The northern edge occurs at approximately the Withlacoochee River in Sumter County, with all individuals found south of this river belonging to *P. g. palmarius*. The range of this subspecies extends southward to include all of mainland peninsular Florida not inhabited by *P. g. gossypinus*. Extensive trapping did not result in a single sample being collected from Manasota Key, indicating that this population is extinct, and preventing the assessment of the subspecies found there. This study found no evidence supporting the recognition of *P.*

g. telmaphilus as a valid subspecies. As a result, *P. g. telmaphilus* should be synonymized with *P. g. palmarius*, indicating the range of *P. g. palmarius* includes both mainland Collier and Monroe counties. However, samples collected from Key Largo, Fl were genetically distinct from mainland samples, indicating this population represents a separate subspecies. The mainland Florida group includes the type localities for both *P. g. palmarius* and *P. g. telmaphilus* (Bangs 1896, Schwartz 1952). However, since the naming of *P. g. palmarius* is the older of the two, the subspecies shall retain the name *P. g. palmarius*.

P. g. allapaticola

All samples collected from Key Largo, Florida form a monophyletic group within the larger clade containing *P. g. palmarius*. Phylogenetic inference and genetic distance data support that this is a well differentiated evolutionary lineage that has been isolated from *P. g. palmarius* for approximately 10,000 years. As a result, this study supports the assertion that this population is a separate subspecies. The populations from this area are already recognized under the current taxonomy as *P. g. allapaticola*.

P. g. megacephalus

Current accepted range

Based on current taxonomy, the range of *P. g. megacephalus* includes all populations from Illinois, Missouri, Tennessee, Arkansas, and Texas. Additionally, populations in Louisiana, Mississippi, Alabama, and Georgia which are not included in the range of *P. g. gossypinus* also are currently recognized as *P. g. megacephalus*.

Newly suggested range

This study found little evidence to support the current subspecies range of *P. g. megacephalus*. Based on these findings, the eastern boundary of the range of *P. g. megacephalus* is the Ochlockonee River in Florida and Chattahoochee River in Georgia. Data indicate the western boundary of the range occurs along the Mississippi River. This range encompasses western Florida and extreme northwestern Georgia, as well as all populations from Tennessee, Alabama, Mississippi, Illinois, and the populations of Louisiana north and east of the Mississippi River. This represents a southward range expansion for this subspecies, but also an eastward compression of the range. The area inhabited by this lineage includes portions of the range of *P. g. gossypinus* and *P. g. megacephalus*. However, the range includes the type locality of *P. g. megacephalus*. As such, it will retain this subspecific name.

Undescribed western subspecies

This study identified a new mainland subspecies of *P. gossypinus* that includes all populations west of the Mississippi River. The samples from these populations formed a monophyletic group within a larger clade containing all samples from *P. g. megacephalus.* However, genetic distance data and phylogenetic inference support the conclusion that these populations represent a distinct subspecies. The range of this subspecies includes nearly all of the populations sampled in Louisiana, as well as the populations of Missouri, Arkansas, and Texas. Since the type localities of both *P. g. gossypinus* and *P. g. megacephalus* lie east of the Mississippi River, the lineage west of the Mississippi represents an as yet unnamed subspecies, which I propose designating the Western cotton mouse.

Undescribed subspecies from Jekyll Island, GA

Based on the results of this study the population on Jekyll Island, GA represents a distinct subspecies of *P. gossypinus*. It should be noted that while this subspecies was found on both Jekyll and St. Simons Island in Georgia as recently as 1988, this is no longer the case. Samples collected in 2008 from St. Simons Island are not monophyletic with the samples from Jekyll Island. Rather, they are most closely related to a population from mainland Clinch, GA. Genetic distance data support that the population of Jekyll Island is distinct enough from the populations of *P. g. gossypinus* to warrant subspecific designation and this is further supported by phylogenetic inference. Additionally, estimates of genetic divergence indicate this island has been isolated from the mainland for several thousand years. Given that this island falls within the range of *P. g. gossypinus* a new designation is needed for this subspecies, which can be named the Jekyll Island cotton mouse.

Undescribed subspecies from Ossabaw Island, GA

Based on the results of this study, I also recommend elevating the populations from St. Catherines Island and Ossabaw Island, GA to subspecific status. Estimates of genetic divergence indicate these populations have been separated from mainland populations of *P. g. gossypinus* over 10,000 years, during which time they have diverged significantly. Additionally, phylogenetic inference and genetic distance data also support the elevation of this group to subspecific status. I propose identifying this undescribed subspecies as the Ossabaw Island cotton mouse.

Undescribed subspecies from Hunting Island, SC

Data collected in this study also support the identification of the population of Hunting Island, SC as a distinct subspecies. Divergence dating indicates this population has been isolated from mainland South Carolina populations of *P. g. gossypinus* for several thousand years. Genetic distance data and phylogenetic inference also support the elevation of this population to subspecific status. Based on these data, I recommend classifying this population as a distinct subspecies known as the Hunting Island cotton mouse.

Morphological comparisons of populations of all newly recognized subspecies are necessary in order to determine if any of them are characterized by unique morphological characters. However, given the high morphological plasticity within *P. gossypinus* morphological identification of these subspecies is unlikely.