GENETIC ANALYSIS OF THE ENDANGERED SILVER RICE RAT (*Oryzomys palustris natator*) AND LOWER KEYS MARSH RABBIT (*Sylvilagus palustris hefneri*)

A Thesis

by

AMANDA LOUISE CROUSE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2005

Major Subject: Zoology

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Approved by:

Chair of Committee,	Rodney L. Honeycutt
Committee Members,	Roel R. Lopez
	John W. Bickham
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ABSTRACT

Genetic Analysis of the Endangered Silver Rice Rat (*Oryzomys palustris natator*) and Lower Keys Marsh Rabbit (*Sylvilagus palustris hefneri*). (December 2005)

Amanda Louise Crouse, B.S., Kansas State University

Chair of Advisory Committee: Dr. Rodney L. Honeycutt

Genetic analyses of two endangered species of mammals in the Lower Keys of Florida (Lower Keys marsh rabbit, LKMR, *Sylvilagus palustris hefneri*; silver rice rat, SRR, *Oryzomys palustris natator*) were performed to evaluate the genetic structure of their populations. Mitochondrial sequence data (control region; 763 base pairs (bp), LKMR; 788 bp, SRR) were used to explore patterns of genetic variation within and among island populations in both species. Analysis of the SRR also included 8 polymorphic nuclear microsatellite loci (9 to 16 alleles). Phylogenetic analyses of mitochondrial sequence data for both species revealed two main lineages corresponding to eastern and western localities, with high levels of genetic structuring (LKMR F_{ST} = 0.982, SRR Φ_{ST} = 0.916). The two species differed in the level of sequence divergence between eastern and western populations (LKMR, 19 bp; SRR 4 bp). In addition to an overall similar pattern of genetic subdivision, populations of both species possessed low levels of mtDNA variation (haplotypic diversity in the LKMR = 66.1%, SRR = 58.6%). Microsatellite analyses of the SRR revealed subdivision between eastern and western regions. Although less pronounced than the structure observed in mtDNA, the overall

pattern was still apparent. Additional examination of divergence between mainland and Lower Keys rice rats revealed a genetic division that indicated a lack of recent gene exchange between the regions (i.e. no shared haplotypes, the presence of private alleles, and distinctive separation in numerous analyses). Although this degree of division does not warrant species designation, the levels and patterns of divergence, both morphological and genetic, do suggest genetic isolation of mainland and island forms. This fact, along with restricted gene flow between the Lower Keys and the Everglades, suggests that the SRR is on an evolutionary trajectory separate from its mainland counterparts and validates its identification as a separate subspecies, *Oryzomys palustris natator*. Finally, the genetic division between eastern and western populations of the SRR and LKMR suggests that populations of both species in these two regions of the Lower Keys should be treated as separate management units, especially when considering the enhancement of populations via translocations.

ACKNOWLEDGMENTS

There are a number of people that helped make the completion of this thesis possible. First and foremost, Bob McCleery, who has provided excellent advice and encouragement on all levels from writing, editing, and map making, to survival in the Keys. His ability to provide perspective when I had lost most of mine made the completion of this project possible.

Special thanks to my committee, especially Rodney Honeycutt, who gave me the opportunity to work this project and provided advice and encouragement along the way. Thanks also to committee member Roel Lopez who gave this geneticist a chance to get off the lab bench and work in the field and for his extensive knowledge of Florida Keys. I also want to thank Nova Silvy for taking the time to make valuable editing comments and for sitting in as a substitute committee member for my defense, and John Bickham for helpful comments.

Craig Faulhaber and Neil Perry kindly provided hair samples from Lower Keys marsh rabbits and Neil Perry also provided silver rice rat tissue samples. In addition, Craig and Neil generously shared their knowledge on the biology of both species. Other samples were provided by the Texas Cooperative Wildlife Collection.

A big thanks to everyone that helped me with lab work: Mindy Walker who taught me everything I know in the lab and patiently answered my constant barrage of questions, Glenn Proudfoot for training on the ABI 377, Jan Janecka for instruction on the use of museum samples, Colleen Ingram for running the mtDNA samples on the ABI 3100, Jason Summers for instruction on software programs, and John Patton for

development of several control region primers and his helpful advice. I also want to thank Sandra Pina for her help during the final stages of lab work. The advice of Marianne Litvaitis was also pivotal in the sequencing of the rabbit DNA, her generous knowledge of the *Sylvilagus* genome provide the information to get the project up and running.

NOMENCLATURE

AMOVA	Analysis of Molecular Variance
BA	Bayesian-based Analysis
FA	Frequency-based Analysis
ha	Hectares
HWE	Hardy-Weinberg Equilibrium
IAM	Infinite Alleles Model
LKMR	Lower Keys Marsh Rabbit
ML	Maximum Likelihood
MP	Maximum Parsimony
mtDNA	Mitochondrial DNA
NJ	Neighbor Joining
SMM	Step-wise Mutation Model
SRR	Silver Rice Rat
TBE	Tris, Borate, EDTA buffer
USFWS	U.S. Fish and Wildlife Service

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CHAPTER I

INTRODUCTION

Island populations provide an intuitive model for the study of complex genetic relationships that occur in continental populations (Mayr 1963; Hinten et al. 2003). The island model is characterized by smaller population sizes, reduction in the exchange of individuals between subpopulations, and an often datable geologic history. These features can create an environment that is conducive to revealing, through genetic analysis, the sequential relationships that connect intraspecific island populations both spatially and temporally (Malone et al. 2003). As a consequence, one can evaluate the influence of dispersal, local extinction, colonization, and fragmentation of habitats on the genetic structure within populations and the genetic connectedness between populations. Understanding such processes and population interactions is fundamental to the field of conservation biology, as knowledge of such factors allows for initiatives that can offset the loss of genetic variation in natural populations occupying islands. Additionally, the study of genetic variation in an island system is applicable to mainland populations, which, through loss and fragmentation of available habitat, are being converted into habitat islands (Hinten et al. 2003).

The same characteristics that make islands good models for studying population interactions (i.e., small population numbers, low migration rates, and narrow geographic range) also place these populations at greater risk of extinction than their mainland

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This thesis follows the style of *Conservation Genetics*.

counterparts (Vitousek 1988; Case et al. 1992; Smith et al. 1993; Frankham 1997, 1998). In addition, island populations, especially endemic forms, often lack the capability to adapt genetically to environmental changes (Frankham 1997). This can result from a variety of genetic factors including: inbreeding depression, loss of genetic variation, accumulation of mildly deleterious mutations, and adaptation to island environments (i.e., flightlessness, recession of predator avoidance traits, and diseases; Frankham 1996). Populations can become susceptible to these genetic limitations once their numbers have been reduced by human perturbations such as habitat loss, introduced species, overexploitation, and pollution. Indeed, over the past 50,000 years, human activities such as these have been the major cause of species extinctions on islands (Olson 1989).

The Florida Keys archipelago provides an opportunity to study a recently developed island system and its endemic inhabitants. These unique islands extend in a narrow arc (6.4 km average width) for 240 km to the south and west of mainland Florida. Florida Bay, 60 km of relatively shallow (average depth approximately 1.5 m) open water, separates the islands from the mainland. On an evolutionary timescale, the islands have only recently been isolated from mainland Florida. During the last glacial maximum, which reached its peak approximately 18,000 years before present (YBP; Florida Geological Survey 1994), large amounts of water were trapped in glacial form causing a dramatic reduction in ocean levels. As a consequence, Florida Bay existed as a vast plain and the Keys were geographically contiguous with the mainland. Although there is some controversy surrounding the exact events of the last 10,000 years, the rise

in sea level that followed the glacial melt eventually caused flooding of the South Florida shelf, creating Florida Bay. Radiocarbon dating of sediments from Florida Bay confirms that deposition began 4,000 years ago (Hoffmeister 1974), providing strong evidence for the formation of the islands at this time. Thus, any inhabitants of the islands have been isolated since this time, continuing on a separate evolutionary trajectory from their mainland counterparts.

The Keys are divided into three sections based on geological composition; the Upper Keys and Middle Keys (177 km in length) that possess fauna similar to mainland Florida and the Lower Keys (64 km in length) that are effectively isolated from the Upper Keys by the 11 km Moser channel. This isolation has led to the development of a distinct endemic mammal community in the Lower Keys, which includes the Key deer (*Odocoileus virginianus clavium*), the Lower Keys marsh rabbit (*Sylvilagus palustris hefneri*; Lazell 1984), and the silver rice rat (*Oryzomys argentatus = Oryzomys palustris natator*; Spitzer and Lazell 1978).

As with other island fauna and flora, endemic species and populations are threatened by human population growth. For instance, since the opening of U.S. Highway 1 in 1938, which connected mainland Florida and the Keys, human populations in the Keys have shown a steady increase. In the last century alone, the number of people living on the islands has risen from 6,000 to 80,000 (Monroe County Growth Management Division 1992), and this number does not include the over three million tourists that visit the region each year (Leeworthy and Wiley 1996). Subsequent commercial and residential developments have resulted in both the loss of habitat and its

subdivision by road systems, leading to an increase in the isolation and fragmentation of the Lower Keys fauna (Forys et al. 1996). Although further habitat destruction has been limited by recent conservation legislation, indirect anthropogenic effects remain a threat to the survival of endemic species (Forys et al. 1996; U.S. Fish and Wildlife Service (USFWS) 1999). These include roadway mortalities (USFWS 1999; Harveson et al. 2004), increased predation by feral cats (*Felis domesticus*; Forys and Humphrey 1999), competition from black rats (*Rattus rattus*; Goodyear 1992; Forys et al. 1996), and increased mortality of neonates as a result of imported fire ants (*Solenopsis invicta*; Forys et al. 2002), a recent colonizer of the Florida Keys.

As a result of concern over the loss of native endemic land mammals in the Lower Keys, the USFWS listed three species as endangered, including the Key deer, the Lower Keys marsh rabbit, and the silver rice rat. Genetic analysis of the Key deer populations have revealed considerably lower levels of mitochondrial haplotype diversity and heterozygosity at nuclear microsatellite loci than levels of diversity seen in mainland populations (Ellsworth et al. 1994a; Banks 2001). Additionally, these genetic data reveal a unique phylogenetic position of Key deer relative to mainland populations, thus adding support to their distinctiveness as ascertained from morphological studies (Dickson 1955; Hardin et al. 1984; Maffei et al 1988). Within the Keys examined, however, genetic subdivision among deer populations is not apparent (Banks 2001). Unlike the Key deer, little genetic information is available for the other two endemic mammals, yet such information is essential for determining the taxonomic uniqueness of these island forms as well as the genetic structure of remaining populations in and

between islands. In particular, important dispersal corridors, impediments to gene flow (natural and anthropogenic), and cases of extreme inbreeding can be identified. If deemed necessary, this information can then be used to guide translocations of animals between isolated sub-populations to promote gene flow and improve the genetic health of isolated populations (Frankham 1998; Hendrick and Kalinowski 2000).

Genetic analysis can further benefit conservation efforts by clarifying uncertain taxonomic status. The significance of this issue should not be minimized, for it shapes the perception of an organism's biotic complexity, regardless of the information used to establish its taxonomic status (Avise 1989; Mace 2004). In a conservation context, this means that management efforts are established on what is perceived, based on taxonomy, as the best way to preserve biological diversity. This is of even greater importance when the taxonomic status of an endangered species is in question, as is the case with the silver rice rat (Mace 2004). Resources for species preservation are limited and need to be allocated based on the most complete scientific information available.

Due to the naturally patchy quality of both Lower Keys marsh rabbit and silver rice rat habitat, their population structure is governed by the amount migration that connects local breeding populations, which are requisite for a metapopulation. The amount of gene flow connecting subpopulations will depend on each species' ability to disperse between local populations. As insular populations, dispersal ability may be especially vital for the continued existence of both species (Lomolino 1986; Krohne 1997; Hendrick and Kalinowski 2000). Additionally, gene flow can result when the extinction and recolonization of subpopulations are frequent (Slatkin 1985). The

homogenizing effect of a high level of gene flow will result in little genetic variation across the entire population.

Genetic analyses, particularly those that have employed mitochondrial DNA (mtDNA) sequence and nuclear microsatellite data, have been increasingly used in the management of endangered species (Avise 1994). For example, genetic data obtained from the analysis of these molecular markers have been used to estimate inbreeding (Wielebnowski 1996; Ellegren 1999; Kalinowski et al. 1999), gene flow between populations (Avise 1994; Seppä and Laurila 1999), and the genetic distinctiveness of taxonomic units (Laerm et al. 1982; Avise and Nelson 1989).

Both mtDNA sequence and microsatellite data will be employed within the study of the silver rice rat and an analysis of mtDNA will be employed to study the Lower Keys marsh rabbit. The data from these analyses will be used to characterize the metapopulation dynamics of both the silver rice rat and the Lower Keys marsh rabbit. Specifically, describe dispersal patterns between sub-populations, identify barriers to dispersal, and analyze levels of genetic variation within each population. In addition, the genetic distance between the Lower Keys population of rice rats and its mainland counterpart will be quantified. This information, combined with existing morphological data, will clarify the taxonomic status of the silver rice rat.

The following chapters will be devoted to the specific status of the silver rice rat (Chapter II) and the Lower Keys marsh rabbit (Chapter III) followed by a conclusion of the information gathered from the genetic analyses of these two populations (Chapter IV).

CHAPTER II

GENETIC ANALYSIS OF THE SILVER RICE RAT (*Oryzomys palustris natator*)

INTRODUCTION

The islands of the Florida Keys extend in a narrow arc (6.4 km average width) for 240 km to the south and west of mainland Florida, and are divided into three sections based on geological composition (Figure 2.1). The Upper and Middle Keys (177 km in length) possess fauna similar to mainland Florida, whereas the Lower Keys (64 km in length) are effectively isolated from the Upper and Middle Keys by the 11 km wide Moser channel and are separated from mainland Florida by 60 km of shallow, open water of Florida Bay. This isolation has led to the development of a distinct endemic terrestrial mammal community in the Lower Keys, which includes the Key deer (*Odocoileus virginianus clavium*), the Lower Keys marsh rabbit (*Sylvilagus palustris hefneri*; Lazell 1984), and the silver rice rat (*Oryzomys argentatus* = *Oryzomys palustris natator*; Spitzer and Lazell 1978). Due to increasing human populations and subsequent commercial and residential developments, habitats supporting these endemic species have declined in area, thus creating concern for continued conservation of the Lower Key's fauna. Factors including loss of habitat, habitat fragmentation by road systems and multiple indirect anthropogenic effects (Goodyear 1992; Forys et al. 1996; Forys and Humphrey 1999; USFWS 1999; Forys et al. 2002; Harveson et al. 2004) have all contributed to the demise of these three endemic mammal species.

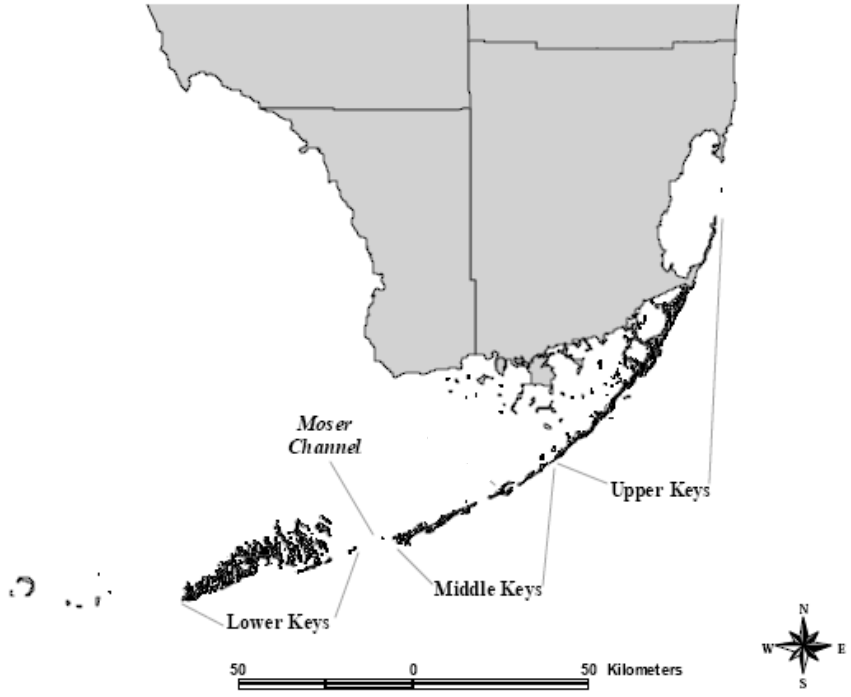

Figure 2.1. The Florida Keys: (1) Upper Keys, (2) Middle Keys, and (3) Lower Keys. The 11 km Moser Channel isolates the Lower Keys producing the geographic isolation necessary for the divergence of the Lower Keys fauna to their current forms. Map adapted from Faulhaber 2003.

The silver rice rat (*Oryzomys palustris natator*) is a small, nocturnal rodent restricted in distribution to 14 islands in the Lower Florida Keys (Vessey et al. 1976; Goodyear 1984, 1987; Wolfe 1986, 1987; Goodyear 1992; Forys et al. 1996; Mitchell 1996). Rice rats are dependent on both saline and freshwater wetland habitats, which appear to be lacking from the Upper and Middle Keys (Goodyear 1987). Currently, the marsh habitat preferred by rice rats exists as a system of habitat patches distributed throughout the Lower Keys. At least one cause for this patchiness is increased dredge and fill operations targeting mangrove, freshwater, and salt marsh areas where rice rats

reside. Presumably as a result of habitat loss, surveys conducted between 1970 and 1990 have revealed an overall decline in densities of rice rats in the Lower Keys (Vessey et al. 1976; Goodyear 1987, 1993; Wolfe 1986, 1987). As a consequence of habitat fragmentation and decline in numbers, the U.S. Fish and Wildlife Service (USFWS, 1991) listed the silver rice rat as an endangered population of the subspecies *Oryzomys palustris natator*.

Forys (1994) proposed that large, contiguous areas of mangrove and salt marsh habitat should be maintained to sustain viable populations of silver rice rats. This argument appears to be based on the assumption that metapopulation dynamics of species rely on movement of individuals between either neighboring patches on an island or between adjacent islands. If barriers to dispersal exist as a result of either recent environmental perturbations or historical effects, then such barriers contribute directly to the metapopulation as a whole (Johst et al. 2002). Based on several ecological studies, silver rice rats occupy larger ranges (2.0 to 8.5 ha for females and 3.4 to 11.0 ha for males, Mitchell 1996) in comparison to mainland populations (average 0.33 ha for females and 0.25 ha for males, Birkenholz 1963). Therefore, rice rats in the Keys have rather high dispersal capability among habitat patches on islands. In addition, members of the genus *Oryzomys* are noted for both their swimming capability (Esher et al. 1978) and ability to colonize islands (Heller 1904).

Given the current data on range size and dispersal abilities over land and water, one might predict little genetic differentiation among populations within the Keys as well as high levels of similarity between mainland populations and those restricted today

to the Lower Keys. Unfortunately, little is known about the genetic structure and patterns of gene flow within and between populations of rice rats in the Florida Keys, yet there are at least two ways that genetic information can contribute to on-going conservation efforts directed towards small mammals in the Keys. First, genetic data can provide an independent test of taxonomic decisions based on morphological comparisons. Spitzer and Lazell (1978) initially described the silver rice rat as a new species, *Oryzomys argentatus*, characterized by physical differences in the skull, hind feet, and pelage coloration relative to rice rats from mainland Florida. Since the inception of this taxonomic decision, the species-level status of the silver rice rat has been debated (Humphrey and Barbour 1979; Barbour and Humphrey 1982; Goodyear and Lazell 1986; Humphrey and Setzer 1989; Goodyear 1991; Humphrey 1992), with the primary argument related to the uniqueness of populations in the Keys in comparison to mainland populations in Florida. To date, the only comparative genetic study on the silver rice rat is that of Gaines et al. (1997), who sequenced a 291 base-pair (bp) fragment of the mitochondrial control region for small numbers of rice rats obtained from one island in the Lower Keys and the mainland Everglades. Based on these data, they concluded that island and mainland rice rat populations did not possess species level differences relative to mitochondrial DNA (mtDNA) variation. Nevertheless, their study was limited in terms of geographic sampling and the molecular markers used.

Second, combined studies, employing both nuclear and mitochondrial genetic markers, provide more accurate assessments of gene flow and genetic substructuring within and between populations, especially when the goal is to implement conservation

efforts designed to enhance the viability of declining populations (O'Brien 1994**)**. For populations that have experienced more recent changes as a result of barriers to gene flow, genetic data obtained from faster evolving elements of mitochondrial (i.e., the control region) and nuclear (i.e., DNA microsatellites) genomes have proven useful in examining the effects of both historical and more recent events (Hinten et al. 2003; Zenger et al. 2003, 2005; Randi et al. 2004).

Specifically, this study employed analyses of nuclear and mitochondrial molecular markers to estimate levels of genetic variation within and between populations of the silver rice rat, evaluate population structure and gene flow throughout the Lower Keys, and analyze phylogenetic relationships among rice rat populations of the Keys as well as between the Keys and mainland Florida. These genetic data, in combination with on-going ecological studies can contribute to management practices by enhancing our understanding of the structure of rice rat populations in the Lower Keys, while providing evidence related to the current taxonomic status of populations in the Keys.

METHODS

Sample Collection and DNA Extraction

Individuals were collected with Sherman live traps (Sherman Traps Incorporated, Tallahassee, Florida, USA) baited with peanut butter. Trapping was performed under Animal Use Protocol #2003-271, as approved by the Texas A&M University Animal Care and Use Committee. Specimens were trapped on 11 islands and at 18 trap sites

(see page 21) Water Key (*n* = 2), Saddlebunch Keys (3 trap sites, *n* = 28), Big Pine Key (*n* = 1), Big Torch Key (3 trap sites, *n* = 11), Middle Torch Key (2 trap sites, *n* = 2), Lower Sugarloaf Key (*n* = 1), Upper Sugarloaf Key (2 trap sites, *n* = 3), Cudjoe Key (2 trap sites, *n* = 3), Howe Key (*n* = 5), Ramrod Key (*n* = 1), and Summerland Key (*n* = 3), for a total of 70 Lower Keys samples. Tail clips from rice rats were collected and preserved in 0.5 mL of Longmire's solution (Longmire et al. 1988) at room temperature until extraction. DNA was extracted from tissue samples using the Qiagen DNeasy™ Tissue Kit (Qiagen Incorporated, Valencia, California, USA) following the supplier's standard protocol for DNA extraction from tail clips.

To quantify the level of genetic variation existing between populations in the Keys and those occurring on the mainland, isolated DNA samples also were obtained from 10 *Oryzomys palustris* specimens collected in the Everglades. In addition, rice rat sequences from central Texas (n = 6, *Oryzomys palustris palustris*, Humphrey and Setzer 1989) were included in the phylogenetic analysis for outgroup purposes. These samples were extracted from liver tissue following the same protocol as described for tail clips.

Mitochondrial DNA Amplification and Screening

For 85 individuals (Everglades *n* = 9, Lower Keys *n* = 70, Texas *n* = 6), 788 bp of the 5' end of the mtDNA control region were amplified by the polymerase chain reaction (PCR, Saiki et al. 1988). Amplification of this region incorporated the following PCR primers, 1115R (5'-ATGACCCTGAAGAARGAACCAG-3', Bickham et al. 1995) and ORY 283 (5'-CCCAACTCCTATACTGAATTTTCG-3', developed for

this project). PCR reactions (MyCycler™ Thermal Cycler, Bio-Rad Laboratories, Inc., Hercules, California, USA) were conducted in 25 µL reaction volumes with the following components and concentrations: 1µL DNA, 12.85 µL ddH_2O, 2.5 µL 10X PCR buffer (Takara Shuzo, Shiga, Japan), 1.25 µL 10X BSA, 2.25 µL of 10mM dNTPs (Takara Shuzo, Shiga, Japan), 2.5 µL each of forward and reverse primers (2pmol/µL), and 0.75 U *Taq* polymerase (Takara Shuzo, Shiga, Japan). The amplification protocol consisted of an initial denaturation at 94 °C for 4 min, followed by 35 cycles of 45 sec each at 94°C, 46°C, and 72°C, and a final extension cycle of 10 min at 72°C. All products were visualized on a 1% agarose and TBE gel matrix. Amplified products were purified using a combination of Exonuclease I and Shrimp Alkaline Phosphatase (ExoSAP, USB, Cleveland, Ohio, USA).

Sequencing reactions were performed using Big Dye termination chemistry (Applied Biosystems Inc. (ABI), Foster City, California, USA) in a GeneAmp® PCR System 2700 Thermal Cycler (ABI). Sequences were obtained on an ABI 3100 automated sequencer following protocols of the manufacturer (Perkin-Elmer Biosystems, Wellesley, Massachusetts, USA). Fragments were sequenced in the forward and reverse directions; sequence alignments and the formation of contigs were accomplished with the Sequencher 4.2 software program (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Microsatellite Amplification and Screening

A total of 79 individuals (Everglades $n = 10$, Lower Keys $n = 69$) was genotyped for a panel of eight polymorphic microsatellite markers. This panel was developed *de*

novo for use in the silver rice rat by Wang et al. (2000). For each primer pair, a fluorescent dye was incorporated onto the 5' end of the forward primer, and PCR conditions were optimized prior to genotyping (Table 2.1). Amplification reactions (MyCycler™ Thermal Cycler, Bio-Rad Laboratories, Inc., Hercules, California, USA) were conducted in 10 µL reaction volumes with the following components and concentrations: 1µL DNA, 6.62 µL ddH_2O, 1.60 µL 10X PCR buffer (Takara Shuzo, Shiga, Japan), 0.20 µL of 10mM dNTPs (Takara Shuzo, Shiga, Japan), 0.24 µL each of forward and reverse primers (10µM), and 0.50 U *Taq* polymerase (Takara Shuzo, Shiga, Japan). PCR amplifications included an initial denaturation cycle of 1 min at 94°C, followed by 30 cycles of 15 sec at 94°C, 30 sec at 50-55°C (following annealing temperatures of Wang et al. 2000, Table 2.1), and an extension for 1 min at 72°C, followed by a final extension for 1 min at 72°C. Samples were genotyped on an Applied Biosystems Inc. 377 automated sequencer (ABI, Foster City, California, USA). Genotypes were determined with Genotyper, version 2.5 software programs (ABI).

Table 2.1. Annealing temperatures and fluorescent dye labels for the 8 microsatellite loci used in this study.

Locus	Dye Label	Annealing Temperature	Locus	Dye Label	Annealing Temperature
AAT03	HEX-green	55°C	AAT26	6FAM-blue	50°C
AAT10	6FAM-blue	53°C	AAT28	6FAM-blue	55°C
AAT16	HEX-green	50°C	AAT40	NED-yellow	55°C
AAT21	HEX-green	55°C	AAT60	6FAM-blue	53°C

Diversity Indices

Standard measures of genetic variability for microsatellite loci, specifically, heterozygosity (observed, H_O and Hardy-Weinberg expected, H_E), allelic diversity (*A*), and locus size range were estimated in GENEAlEx 5.0 (Peakall and Smouse 2001). Exact tests for Hardy-Weinberg equilibrium (HWE) were calculated across all microsatellite loci using the Markov chain method (Guo and Thompson 1992) with 1,000 iterations in GENEPOP version 3.4 (Raymond and Rousset 1995). In addition, GENEPOP 3.4 was employed to test the assumption of linkage disequilibrium by Fisher's exact test and the Markov chain method, as well as genic and genotypic differentiation (Goudet et al. 1996) among populations. When performing multiple simultaneous comparisons, the significance level was adjusted by means of the sequential Bonferroni procedure (Rice 1989) with $\alpha = 0.05$.

Estimates of mtDNA haplotypic diversity (*h*), nucleotide diversity (π), number of segregating sites (*S*), average number of nucleotide substitutions per site (Dxy), and net number of nucleotide substitutions per site between populations (D_a) were calculated from Nei (1987) with the software package DnaSP version 4.0.6 (Rozas et al. 2003). This program also was used to test the assumption of neutrality of mutations by Tajima's D (Tajima 1989).

Population Structure

Given the potential dispersal capabilities of the silver rice rat and the short distances between several of the sampling localities, it was apparent the 18 trap sites were not representative of 18 distinct populations. In order to obtain a more realistic

estimate of how the populations were structured, multilocus genotype data were used to infer the number of populations via a model-based Bayesian clustering algorithm, determined from posterior probabilities (STRUCTURE 2.0, Pritchard et al. 2000). The Markov chain simulation was set at 10^5 iterations, following a burn-in period of 10^4 simulations. Possible population numbers (k) were tested at values from 1 through 10. The test was then repeated with the Everglades samples removed to detect the presence of potential structure within the Lower Keys region (assessed with k set at values from 1 through 9). Presence of additional structure within groups defined in the Lower Keys was evaluated in the same manner.

To obtain a more detailed assessment of relationships between sampling locales, the program MICROSAT (Minch 1995) was used to calculate *F*-statistics (Wright 1951, 1965), R_{ST}-statistics (Slatkin 1995), and proportion of shared alleles (PSA, Bowcock et al. 1994). Distance estimates based on proportion of shared alleles and the Neighbor-joining (NJ) method (Saitou and Nei 1987) were used to construct phylograms in PAUP* 4.0b2 (Swofford 2002). This information was analyzed in conjunction with the STRUCTURE (Version 2.0, Pritchard et al. 2000) analysis to specifically characterize patterns of genetic subdivision among populations in the Lower Keys.

Population-level relationships between trap localities for silver rice rats also were analyzed by using among population distances and the NJ procedure. *F*-statistics (Wright 1951, 1965) and *R*st-statistics (Slatkin 1995) were both used to calculate distances between localities and unrooted NJ trees were produced from pairwise comparisons of these values (calculations performed with the MICROSAT software

program, Minch 1995). Assignment tests, both frequency-based (Paetkau et al. 1995, 2004) and Bayesian-based (Rannala and Mountain 1997), were carried out in GeneClass2 (Piry et al. 2004) under Monte-Carlo simulation with 10,000 permutations. Proposed populations for assignment were based on the three Lower Keys populations ascertained in both the STRUCTURE (Version 2.0, Pritchard et al. 2000) analysis and the phylogenetic analyses based on proportion of shared alleles. Individuals were assigned to specified populations based on the log likelihood of their genotype originating from that pool of genotypes. High levels of misassignment are indicative of extensive gene flow between populations.

Patterns of mtDNA geographical structuring were analyzed using a nested hierarchical analysis of genetic differentiation via an analysis of molecular variance (AMOVA; Excoffier et al. 1992) performed in the ARLEQUIN, version 2.0 (Schneider et al. 2000) software package. The AMOVA was calculated with Φ-analogues of Wright's (1951, 1965) *F*-statistics under three models: (1) the geographic separation observed in the analysis of microsatellite data (a northern, eastern, and western population), (2) a variation of this pattern, with only two populations (eastern and western), and (3) the Lower Keys population split into three regions based on geographic location (i.e., east, central, and west). Significance was tested by means of a non-parametric permutation of haplotypes among populations among groups (10,100 permutations).

Gene Flow

Migration estimates were calculated from both microsatellite and mitochondrial sequence data. Estimates of female migration (N_fm) were obtained from pairwise Φ_{ST} (Excoffier et al. 1992) estimates calculated from mtDNA data and the relationship: $N_fm = [(1/\Phi_{ST})-1]/2$ (Slatkin 1993, Baker et al. 1994) in the program ARLEQUIN, version 2.0 (Schneider et al. 2000). Additionally, estimates were calculated from Nei's (1973, 1982) coefficient of gene differentiation, γ_{ST}, with the DnaSP version 4.0.6 software package (Rozas et al. 2003).

Two calculations also were used for estimates of net migration rates (N_em) from microsatellite data. First, pairwise θ_{ST} values were estimated and used to calculate N_em from the relationship: $N_em = [(1/\theta_{ST})-1]/4$ (Wright 1951) in the software program ARLEQUIN, version 2.0 (Schneider et al. 2000). Values of N_em were also calculated based on the mean frequencies of private alleles with corrections for sample size (Barton and Slatkin 1986) in GENEPOP version 3.4 (Raymond and Rousset 1995). All migration estimates were made among the Lower Keys subpopulations and between the Everglades and the Lower Keys.

Phylogenetic Analysis

Evolutionary relationships among mitochondrial DNA haplotypes of the Keys and the Everglades were analyzed by both maximum parsimony (MP) and maximum likelihood (ML) analyses. A heuristic search option (random addition sequence, 10 replications, TBR branch swapping, PAUP* 4.0b2, Swofford 2002) with gaps identified as a fifth state was employed for both searches. Bootstrapping was performed to

provide support for branching topology (1,000 replications; Felsenstein 1985). Prior to the ML analysis, ModelTest version 3.06 (Posada and Crandall 1998) was used to obtain the appropriate parameters for the search. The analysis employed *Oryzomys palustris palustris* sequences from Texas for outgroup purposes. This same procedure was used in a ML analysis that included all samples from mainland Florida.

RESULTS

Patterns of Microsatellite Genetic Variation

A panel of eight microsatellite loci was used to obtain genotypes for 79 individuals from the Keys ($n = 69$) and Everglades ($n = 10$) populations. Bonferroni corrections for multiple comparisons (Rice 1989) were applied to tests for HWE, linkage disequilibrium, genic, and genotypic differentiation. In addition, Lower Keys populations were divided into subpopulations to prevent artificial significance of results due to the Wahlund effect. Two loci in the eastern Lower Keys population (AAT10 and AAT21) and one locus (AAT16) in the Everglades were not in equilibrium, but after Bonferroni correction, no significant deviation from equilibrium was observed (Appendix 2). Analysis of linkage disequilibrium over all loci, revealed evidence of linkage equilibrium in one pair (AAT03 and AAT28, Appendix 3), this remained highly significant ($p < 0.0001$) when tested within each population and after Bonferroni correction. Pairwise analyses of genotypic and genic differentiation among the three Lower Keys populations were all highly significant ($\chi^2 = \infty$, df = 16, $p < 0.001$, Appendix 4) when tested across all loci combined. When analyzed separately, five of

the eight loci (AAT03, 16, 21, 26, and 28, Appendix 4) demonstrated lack of genotypic and genic differentiation between the northern and eastern populations. All tests of differentiation between the Everglades and the Keys subpopulations were highly significant ($p \leq 0.001$).

All loci were polymorphic, with an average of 9.88 (± 0.35) alleles per locus over all samples from both the Keys and the mainland. Overall observed heterozygosity (H_O) in the Keys was 0.677 ± 0.16 compared to 0.850 ± 0.14 for the Everglades (Table 2.2). The number of private alleles per locus observed in the Everglades population averaged 2.75 ± 0.49 alleles/locus (range of 1 to 5 alleles, $n = 10$), while the entire Keys population averaged 2.13 ± 0.52 (range of 0 to 4, $n = 69$) private alleles per locus. When divided into the four populations identified in analyses of population structure, (northern, eastern, and western Lower Keys populations and the Everglades), allelic diversity (A) averaged 4.9-7.8 alleles per locus and observed heterozygosity ranged from 0.606 to 0.850 (Table 2.2). Within the Keys, the northern population had the highest observed heterozygosity ($H_O = 0.708$, Table 2.2) and the greatest average number of private alleles per locus (0.63 ± 0.26).

The model-based clustering method in STRUCTURE (Pritchard et al. 2000) denoted four distinct populations, with three occurring in the Lower Keys and one in the Everglades (P = 0.9996). Removal of the Everglades resulted in a probability of 1.0 for the three major subpopulations in the Keys, which occur in northern, eastern, and western regions (Figure 2.2). A separate analysis of each of these groups by this method did not reveal further structuring. A phylogenetic approach, employing neighbor-joining

and estimates of the proportion of shared alleles across all individuals, revealed distinct groupings of individuals from the eastern (Big Pine, Ramrod, Middle Torch, Summerland, and Cudjoe keys) and western (Lower Sugarloaf and all Saddlebunch keys) clades, with a large amount of mixing with individuals from the northern portion of islands between the eastern and western regions (Howe, Water, Big Torch, and Upper Sugarloaf keys; Figures 2.2 and 2.3).

Table 2.2. Microsatellite diversity indices for rice rats from each region (Lower Keys and Everglades) and the three subpopulations of the Lower Keys (northern, western, and eastern).

Population	n	H_O (± sd)	H_E (± sd)	No. Alleles	Alleles/ Locus	Private alleles per locus	Fixation Index
Lower Keys	69	0.677 (±0.16)	0.720 (±0.03)	57	7.13	2.13 (±0.52)	0.057
Northern Keys	20	0.708 (±0.17)	0.703 (±0.11)	46	5.75	0.63 (±0.26)	0.003
Eastern Keys	20	0.606 (±0.17)	0.623 (±0.11)	39	4.88	0.00	0.021
Western Keys	29	0.716 (±0.13)	0.639 (±0.12)	44	5.50	0.13 (±0.13)	-0.127
Everglades	10	0.850 (±0.13)	0.834 (±0.02)	62	7.75	2.75 (±0.49)	-0.021

Assignment tests ($\alpha = 0.05$, Appendix 5) of Rannala and Mountain (1997, Bayesian analysis (BA), 10,000 permutations) and Paetkau et al. (1995, 2004, frequency analysis (FA), 10,000 permutations) also showed differentiation among these three regions, with 79.7% of all rice rats correctly assigned in both tests. As can be seen in Figure 2.3, most inconsistencies involved individuals from the northern region, with the majority (92% with BA and 85% with FA) of misassignments occurring between the eastern and

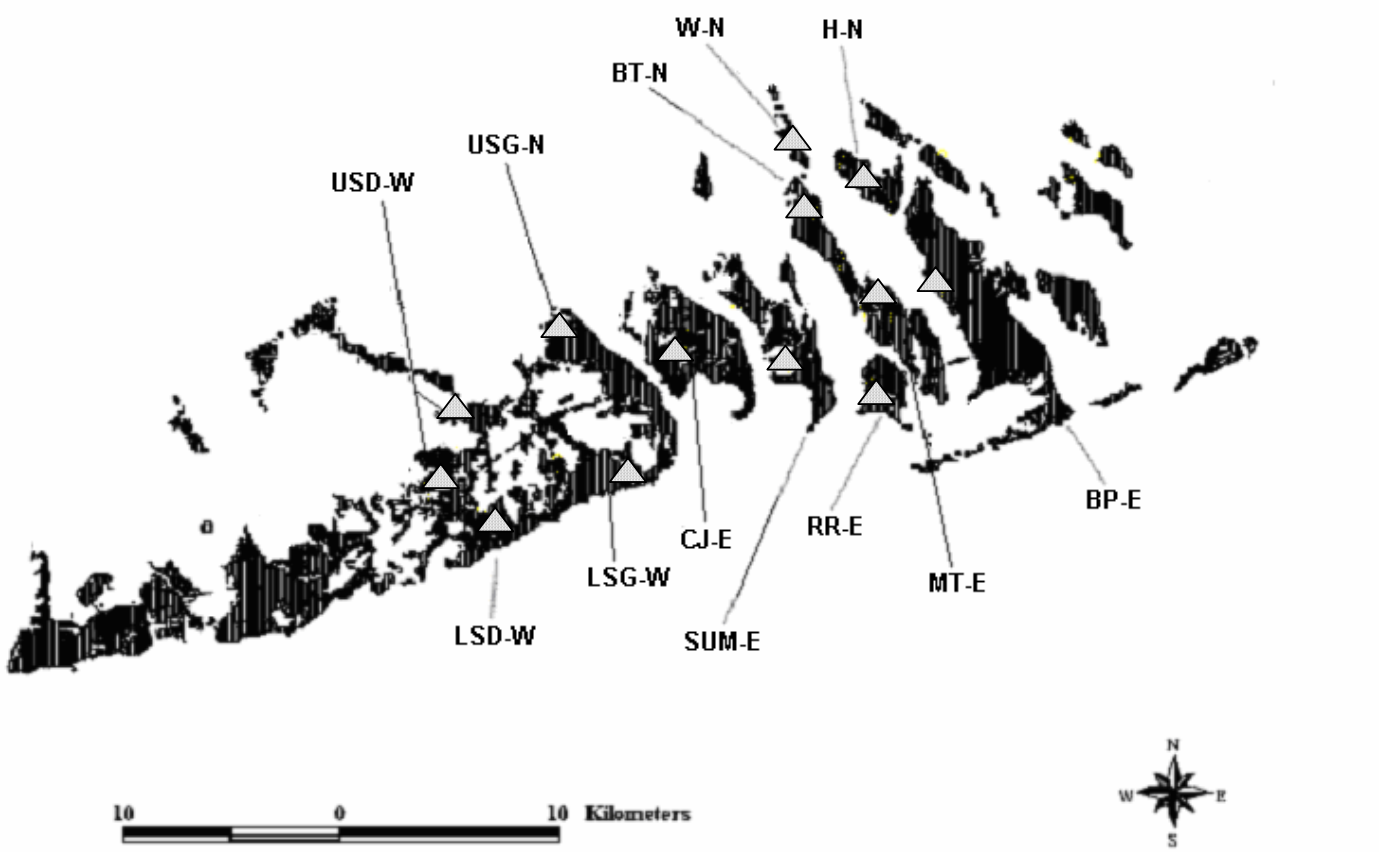

Figure 2.2. Map of Lower Keys depicting the three subpopulations defined by microsatellite analysis: (N) northern (Water-W, Howe-H, Big Torch-BT, and Upper Sugarloaf-USG keys), (E) eastern (Big Pine-BP, Ramrod-RR, Middle Torch-MT, Summerland-SUM, and Cudjoe-CJ keys), and (W) western (Lower Sugarloaf-LSG and Lower-LSD and Upper-USD Saddlebunch keys).

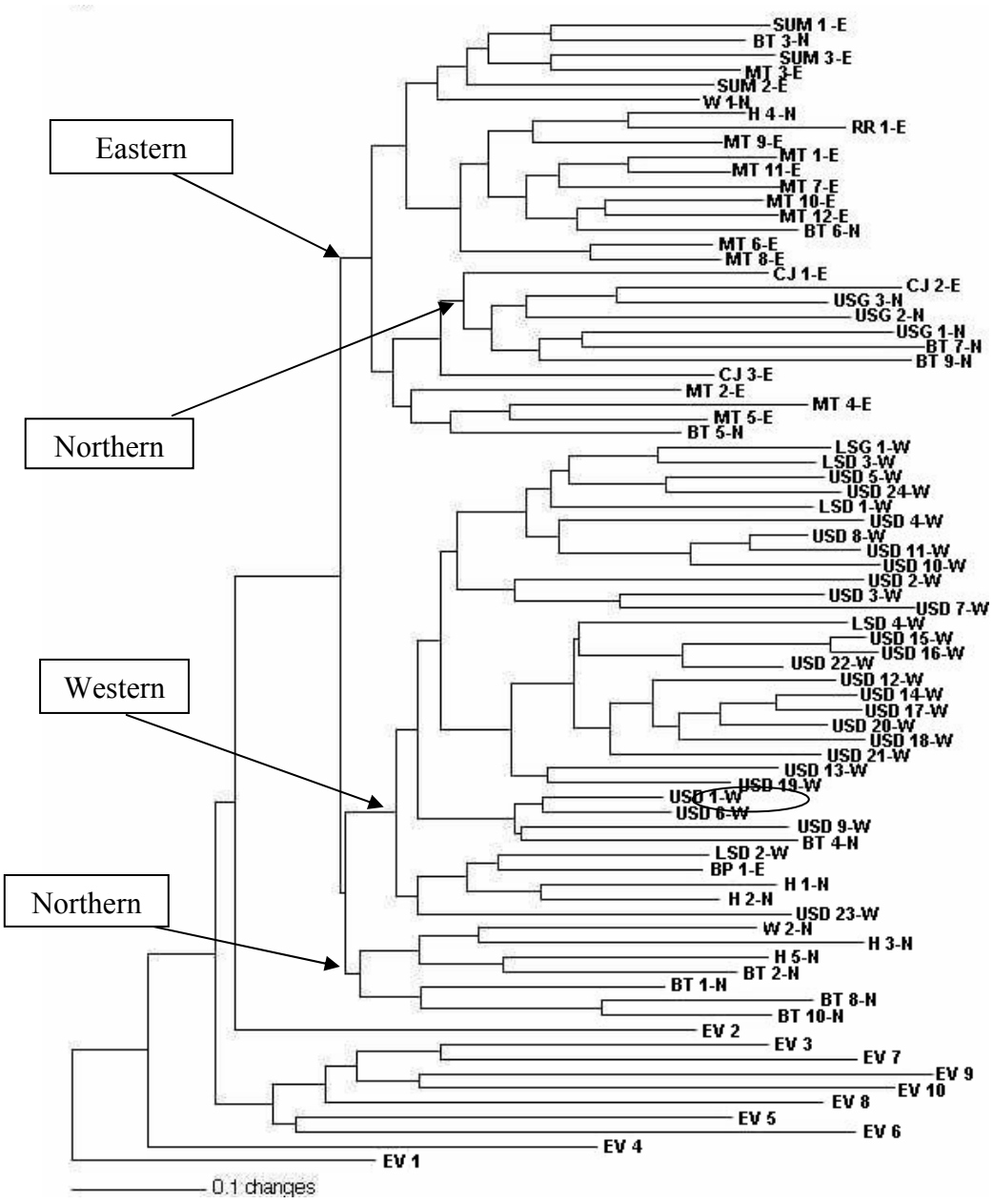

Figure 2.3. Neighbor-joining tree based on pairwise comparisons of proportion of shared alleles between individual silver rice rats from the Lower Keys, with Everglades samples set as the outgroup. Trapping localities are denoted by letter prefix (SUM = Summerland, BT = Big Torch, MT = Middle Torch, W = Water, H = Howe, RR = Ramrod, CJ = Cudjoe, USG = Upper Sugarloaf, LSG = Lower Sugarloaf, USD = Upper Saddlebunch, LSD = Lower Saddlebunch, BP = Big Pine, EV = Everglades), numbers represent individuals. Subpopulations are designated by the letters following the dash (W = western, E = eastern, N = northern). One individual (BP 1-E, circled) is misassigned between the western and eastern clades, multiple northern individuals are found in both clades.

northern regions. When the eastern and northern regions were combined, the level of correct assignment increased (93.7% BA and 92.4% FA). No individuals from the Keys were mistakenly assigned to the Everglades' population or vice versa, indicating distinct genotypes in these regions. Neighbor-joining trees derived from pairwise comparisons of the two distance algorithms, F_{ST} and R_{ST}, reconstructed similar relationships between sampling localities in the Lower Keys (Figure 2.4). Both defined a separation from east to west. A more distinct separation between the four westernmost trapping localities and the remaining localities was formed from the F_{ST} comparisons. The NJ tree of the R_{ST} values included Upper and Lower Sugarloaf Keys with the other western localities, while the F_{ST} comparison did not.

Patterns of Mitochondrial DNA Variation

Nucleotide sequences of the mitochondrial control region were obtained for 79 individuals from the Florida Keys (n = 70) and the Everglades (n = 9). Eleven unique haplotypes were observed, with four restricted to the Florida Keys and seven occurring in the Everglades. Variation among these haplotypes involved 36 segregating sites, with an average nucleotide diversity of 1.337 ± 0.125%, corrected to 1.350% with the Jukes and Cantor (Table 2.3). The four haplotypes restricted to the Florida Keys differed at six segregating sites. Haplotypic diversity (h±sd) within the Florida Keys was 58.6% (± 2.9%) and overall nucleotide diversity was 0.481% ± 0.128%, corrected to 0.483% with Jukes and Cantor (Table 2.3). Of the four haplotypes restricted to the Keys, two had frequencies of 47% and 44%, respectively. These two haplotypes were partitioned into two distinct geographic areas. One occurred predominately in the west (94%) and the

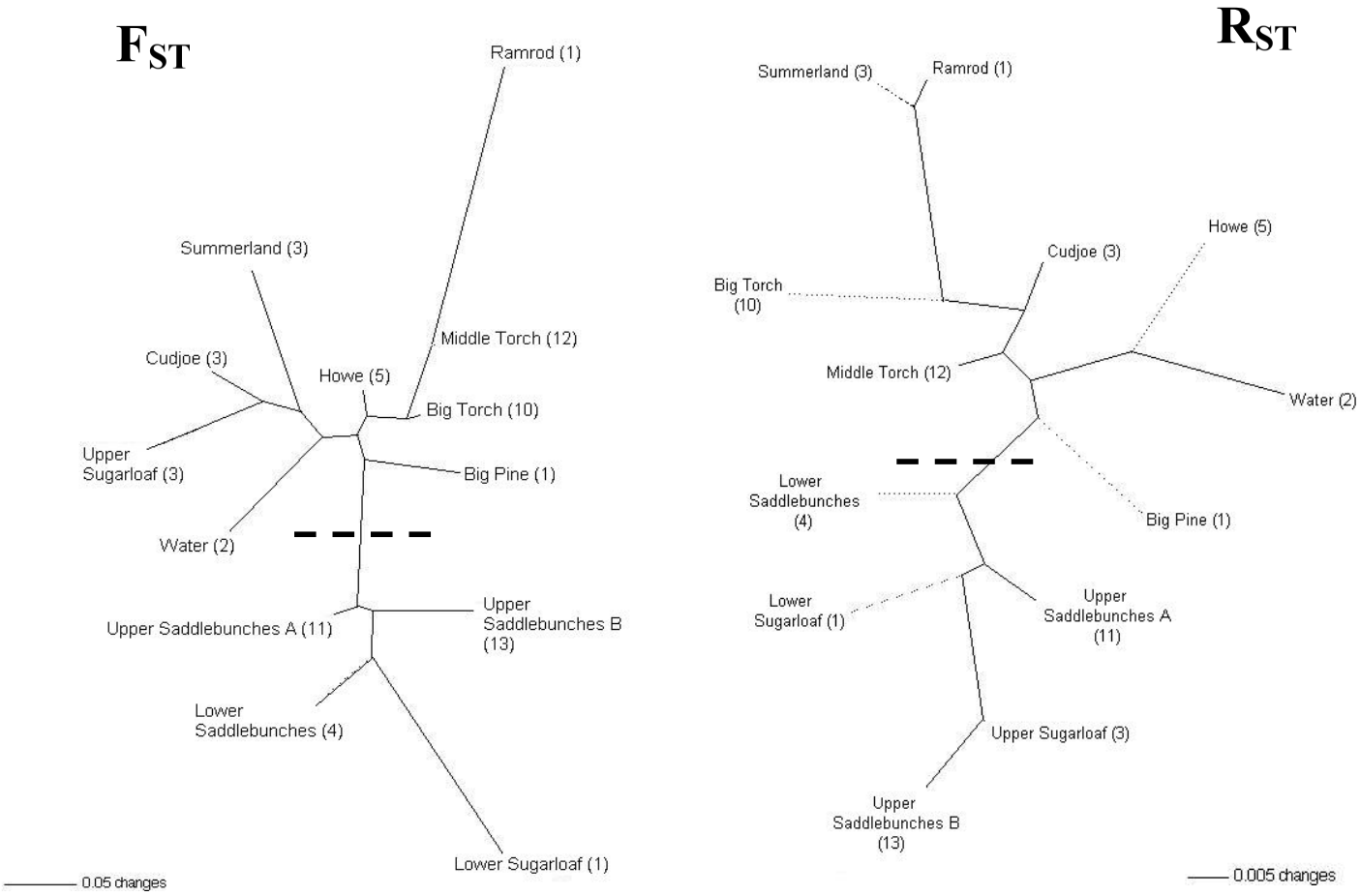

Figure 2.4. Unrooted neighbor-joining phylograms showing the genotypic relationships among different sampling localities in the Lower Keys. Trees are reconstructed from pairwise comparisons of two distance algorithms, F_{ST} (left) and R_{ST} (right). Sample sizes are in parentheses; eastern and western islands are separated by dashed lines.

Table 2.3 . Standard diversity indices from mitochondrial DNA analyses of the silver rice rat for each region (Lower Keys and Everglades) and for the three subpopulations of the Lower Keys defined by the microsatelite analysis (northern, western, and eastern).

Population	**n**	**No. haplotypes**	**% h (±*sd*)**	**% πJC**
Lower Keys	70	4	58.6 (±2.9)	0.483
Northern Keys	21	3	26.7 (±12.0)	0.438
Eastern Keys	20	3	41.6 (±11.6)	0.431
Western Keys	29	1	0	0
Everglades	9	7	94.4 (±7.0)	1.388
All Data	79	11	67.5 (±3.4)	1.350

other was found exclusively in eastern localities (Figure 2.5). The two low frequency haplotypes were found in the central range of the Lower Keys.

In contrast to the small number of haplotypes observed in the Keys, the 7 unique haplotypes found on the mainland (varying at 28 segregating sites) were observed for the 9 individuals examined. Nucleotide diversity averaged 1.374 ± 0.184% (π_{JC} = 1.388%) and haplotypic diversity (94.4 ± 7.0 %) was considerably higher than observed in the Lower Keys (Table 2.3). No haplotypes were shared between the Lower Keys and Everglades, and haplotypes found in the island and mainland populations differed by an average of 11.357 nucleotide differences. Net nucleotide divergence ($d_A \pm sd$) between the Keys and the Everglades haplotypes was 0.00566 ± 0.00384, and the average number of nucleotide substitutions per site between populations ($D_{xy} \pm sd$) was 0.0150 ± 0.00373 (both calculated with Jukes-Cantor correction). Tajima's test for neutrality of the data was negative (D = -0.66751) but not significant ($p > 0.10$).

Both maximum likelihood and maximum parsimony were used to examine relationships among mtDNA haplotypes observed in the Florida Keys and mainland

Everglades populations, and identical results were obtained (Figure 2.6). There was strong support for a monophyletic group containing haplotypes from the Keys, with an eastern and western subdivision as demonstrated by information on haplotype frequencies. The most divergent haplotype was from the mainland site in the Everglades.

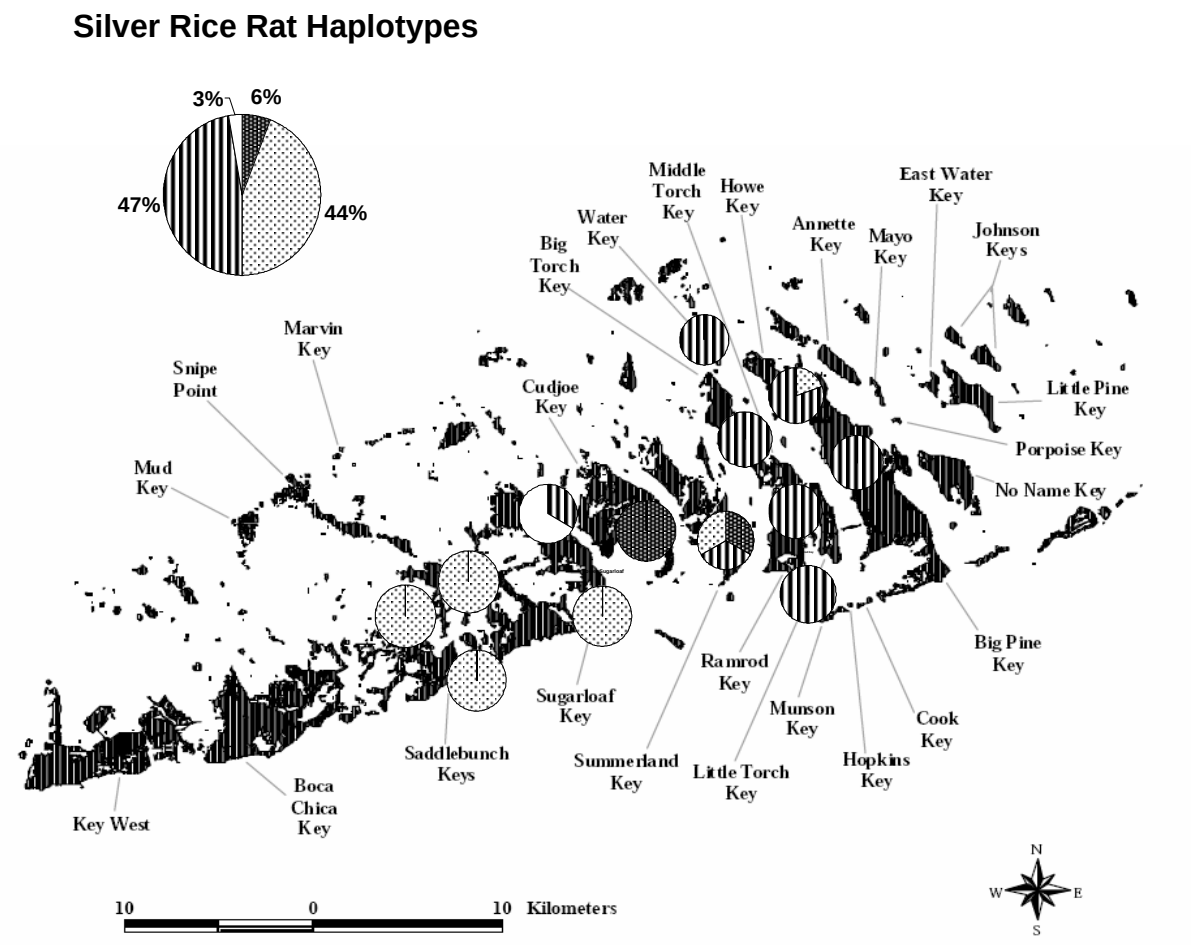

Figure 2.5. Pie charts of silver rice rat mtDNA haplotypes by sampling locality, superimposed on geographic layout of the Lower Keys.

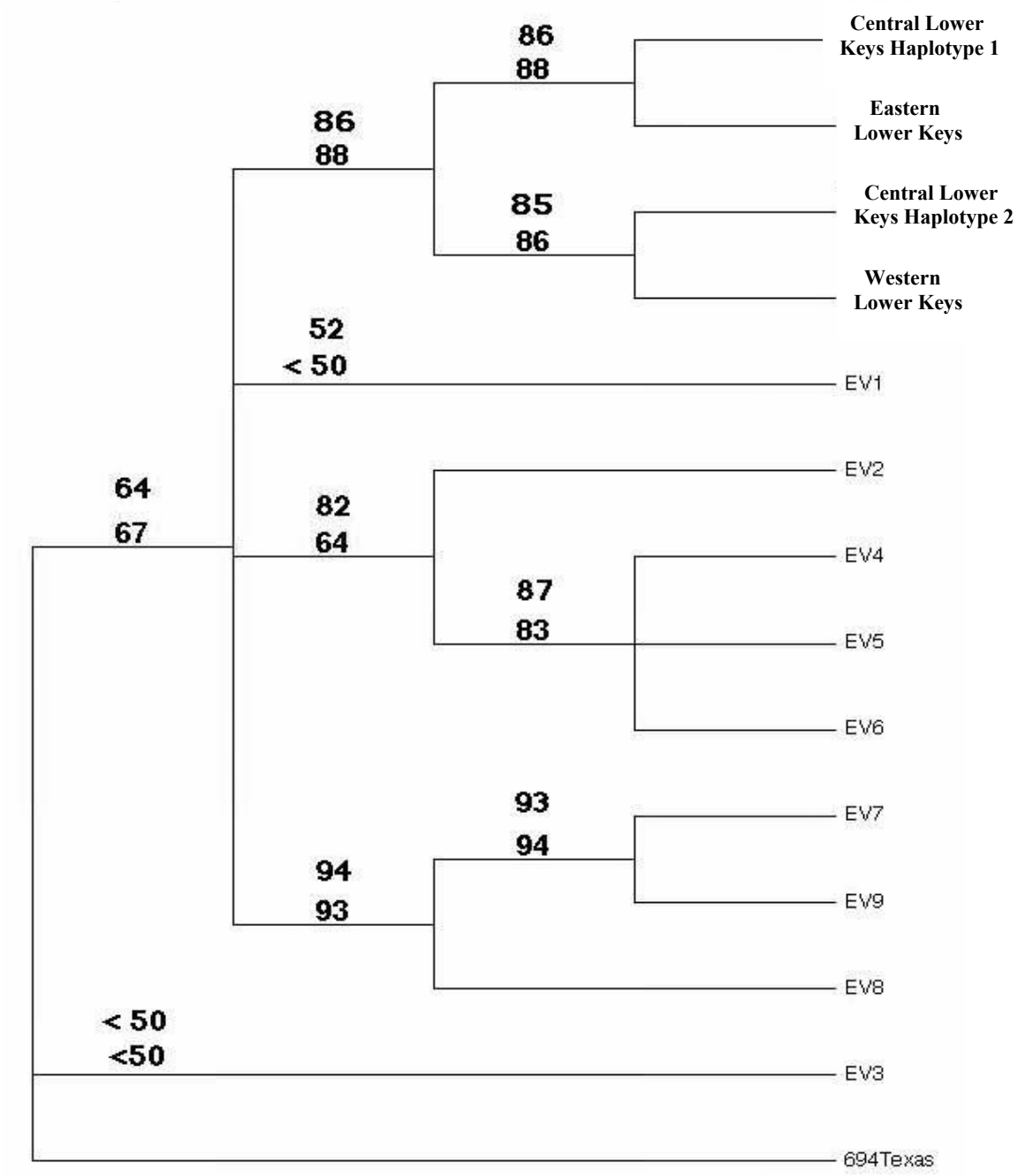

Figure 2.6. Phylogenetic analysis of Everglades and Keys mtDNA haplotypes, upper and lower numbers represent bootstrap values (1000 replications) for ML and MP analyses, respectively. Maximum likelihood analysis was performed using the General Time Reversible model with a gamma distribution for variable sites ($\alpha = 0.1388$), as suggested by ModelTest. Two most parsimonious trees with a tree length of 102 were found with maximum parsimony, with a consistency index (CI), excluding uninformative characters of 0.7778, and a retention index (RI) of 0.8298. This tree represents a strict consensus of the two most parsimonious trees. A haplotype from Texas was used as an outgroup.

Based on the structure revealed by the microsatellite data, populations of rice rats were subdivided into three groups (eastern, northern, and western, Figure 2.2), and an AMOVA (Excoffier et al. 1992) was used to test for significant differences in haplotypic variation. Results of this test revealed 60.49% of total molecular variation ($\Phi_{CT} = 0.605$; $p < 0.05$) partitioned among groups, 31.10% ($\Phi_{SC} = 0.787$; $p < 0.001$) among populations within these groups, and 8.4% ($\Phi_{ST} = 0.916$; $p < 0.001$) within the populations. These values increased with the comparison of a combined eastern and northern group with the western group (70% of variation partitioned among groups; $\Phi_{CT} = 0.70$; $p < 0.01$).

Patterns of Gene Flow

Within the Lower Keys, both estimates for the total number of migrants (N_em) obtained from microsatellite data and the levels of female migration (N_fm) calculated from mtDNA demonstrated low levels of gene flow between the western region and the rest of the Keys (Table 2.4). Mitochondrial gene flow between the Lower Keys and the Everglades was estimated to be 0.3 and 0.65 females per generation (N_fm from Φ_{ST} and γ_{ST}, respectively). When based on frequency of private alleles, adjusted for sample size (Barton and Slatkin 1986), net migration rate (N_em) between the Keys and the mainland was estimated to be 0.438 individuals per generation.

Table 2.4. Migration estimates between Lower Keys subpopulations based on microsatellite data (θ and private alleles methods) and mtDNA sequence data (Φ and γ_{ST})

Lower Keys Subpopulation		
Eastern	**Northern**	**Eastern**
N_em (from θ)	5.768	-
N_em (from private alleles)	2.276	-
N_fm (from Φ)	4.250	-
N_fm (from γ_{ST})	5.460	-
Western		
N_em (from θ)	1.448	1.360
N_em (from private alleles)	0.924	0.878
N_fm (from Φ)	0.033	0.172
N_fm (from γ_{ST})	0.060	0.340

DISCUSSION

Genetic Diversity

In comparison to mainland populations in the Everglades, populations of silver rice rats in the Lower Keys show considerably lower levels of genetic variation. For instance, average observed heterozygosity for the eight microsatellite loci is 0.677 for the Lower Keys populations versus 0.85 for the Everglades (Table 2.2). Similarly, populations in the Lower Keys have even lower levels of haplotypic diversity (58.6%) in comparison to the 94.4% observed for Everglades (Table 2.3).

The reduced levels of genetic variation observed in the Keys relative to populations on the Florida mainland are consistent with other studies involving mammalian species distributed in both the Keys and mainland Florida. Ellsworth et al.

(1994a, b) examined both nuclear (allozyme) and mtDNA (RFLPs) variation in the Key deer and other populations of white-tailed deer distributed throughout the southeastern U.S. Heterozygosity estimated from allozymes averaged 6.4% for populations in the Keys and 7 to 10% in mainland populations, and mtDNA haplotype diversity for the Keys and south Florida was 0% and 70.6%, respectively. A more recent study by Banks (2001) examined variation at seven microsatellite loci and also found lower levels in populations from the Keys relative to those in the Everglades. For instance, Keys populations averaged 2.14 alleles per locus with no unique alleles, whereas mainland populations averaged 7.13 alleles per locus. In addition, average heterozygosity for the Keys (0.242) was considerably less than seen for the Everglades (0.495).

Several factors probably account for patterns of variation seen in these island endemics relative to mainland forms. First, both the silver rice rat and the Key deer represent island populations that were probably established as a result of founder events. Like other island forms, these two species reveal reduced levels of variation as a result of founder effects and presumably drift in small initial populations (Frankham 1997, Garner et al. 2004). Second, mtDNA haplotype and nuclear DNA phylogenies for mainland populations of both the silver rice rat and the Key deer show an association between populations in southern Florida and those in the Keys, suggesting founding of these populations from mainland sources in south Florida. Finally, the lack of shared mtDNA haplotypes and differences in allele frequencies between these mainland and island populations suggest isolation and restricted gene flow over enough generations for variation in these two groups to coalesce. The higher degree of difference between

mainland and island forms for mtDNA markers is consistent with expectations for uniparentally inherited markers (Avise 1994).

Population Structure and Dispersal

One surprising result from this study is the observed level of genetic subdivision among particular islands in the Lower Keys. As indicated earlier, rice rats are generally effective at colonizing islands via over water dispersal, yet both microsatellite and mtDNA markers show distinct genetic differences between eastern and western populations. Of the four mtDNA haplotypes observed, eastern and western populations were almost fixed for alternate haplotypes, and even the central population had haplotypes not found in the islands to the east and west (Figure 2.5). The degree to which these three regions differ can be seen by estimates of differentiation based on Φ_{ST}, which averaged 0.916 ($p < 0.0001$). Likewise, microsatellite DNA analyses based on STRUCTURE revealed three genetically distinct populations in the Lower Keys that are very similar to patterns reflected by mtDNA. Although the average F_{ST} of 0.174 ($p < 0.0001$) is lower than that observed for mtDNA, it still supports considerable population subdivision, especially between eastern and western populations. This is confirmed by both assignment tests and phylogenetic comparisons of individuals (Appendix 6 and Figure 2.3).

Relationships between localities were measured by both F_{ST} and R_{ST}, which vary in the assumptions of microsatellite evolution upon which they are based. Weir and Cockerham's (1984) θ is an analogue to Wright's (1951, 1965) F_{ST} and is calculated under the infinite alleles model (IAM, Kimura and Crow 1964), which assumes all

alleles are equally likely and equally distant from one another. Under IAM, distances between two alleles are treated dichotomously as either different (1) or the same (0), regardless of the fact that similar alleles have been shown to arise independently (Levinson and Gutman 1987, Weber and Wong 1993). Hence, IAM may not the most appropriate model for microsatellite evolution (Kirchman et al. 2000, Maroja et al. 2003). Alternatively, Slatkin's (1995) R_{ST}, an F_{ST} analogue based on the stepwise mutation model (SMM, Kimura and Ohta 1978), assumes that mutations arise from slippage during replication, resulting in the gain or loss of a single repeat. Thus, unlike IAM where alleles are similar or not, each mutation under the SMM creates an allele that retains a history of previous alleles. One principle of the SMM is that only a single repeat is added or removed with any given mutation. Nevertheless, this assumption is violated when alleles mutate by multiple repeats, as has been shown to occur, albeit rarely (Primmer et al. 1996). Additionally, Gaggiotti et al. (1999) demonstrated that F_{ST} is more effective than R_{ST} in indicating gene flow when working with moderate sample sizes ($n \leq 50$) and a low number of alleles. Both parameters apply to this study, implying that the F_{ST} comparison may be a more reliable estimate of locality relationships. Given that microsatellites do not demonstrate strict adherence to either set of assumptions, relationships between localities were analyzed by both calculations for comparative purposes (Figure 2.4). The separation of eastern and western localities is evident in trees constructed from both measurements.

How does one explain the apparent genetic subdivision seen among populations of rice rats in the Lower Keys? One historical explanation may be that currents and

depth and width of channels present barriers to dispersal, thus limiting the degree to which individuals can be exchanged between the eastern and western populations. Although the silver rice rat is known to be an excellent swimmer, strong currents and/or wider crossings may impede dispersal. This may explain why populations in the central portion of the Lower Keys share more microsatellite alleles (as revealed by assignment tests) in common with those located to the east. In this particular area intertidal flats, representing areas of shallow water barely covered at low tide, occur more often along the northern side of the islands, thus providing an avenue of exchange. There is some evidence that these intertidal areas were more recently submerged by rising oceans, thus increasing the probability of exchange between central and eastern populations (Lidz and Shinn 1991).

More recent events, associated with the loss of habitats as a result of human encroachment, also may have created barriers to dispersal, thus interrupting the overall metapopulation structure of silver rice rats. As wetlands continue to be removed, suitable habitat for rice rats is becoming patchily distributed, thus potentially reducing the overall size of populations. For instance, presumably as a result of habitat loss, surveys conducted between 1970 and 1990 have revealed an overall decline in densities of rice rats in the Lower Keys (Vessey et al. 1976; Goodyear 1987, 1993; Wolfe 1986, 1987), and this, in combination with fragmentation, might create overall smaller effective population sizes and more rapid rates of change in the frequency of mtDNA haplotypes and microsatellite alleles.

Regardless whether one can explain current levels of genetic subdivision on the basis of either historical or more recent events, the overall pattern of higher levels of differentiation for mtDNA versus those seen for microsatellite loci are consistent with expectations associated uniparentally versus biparentally inherited genetic markers (Hartl and Clark 1989). Furthermore, overall differences among the four rice rat haplotypes (π_{JC} = 0.483%, average of 3.67 nucleotide differences between haplotypes) found in the Keys suggest a more recent separation of populations. This level of differentiation is at least consistent with data suggesting that fragmentation of islands in the Lower Keys resulted from channel formation occurring in the last 2,000 to 3,000 years (Lidz and Shinn 1991).

Phylogeographic Patterns and Taxonomic Implications

Although once geographically contiguous with the mainland, the Florida Keys have been isolated for at least 4,000 years (Hoffmeister 1974), and several distinct lineages of mammals (e.g., silver rice rat, Key deer, Lower Keys marsh rabbit, and Key Largo woodrat) have been assigned taxonomic distinction relative to lineages on the mainland. The three species in the Keys, for which there are genetic data, are similar in showing genetic distinction relative to mainland forms. This similarity in terms of phylogeographic pattern is probably the result of similar vicariant events that created barriers to dispersal between the Keys and south Florida (Avise 2000). For instance, mtDNA haplotypes of *Neotoma floridana smalli* from Key Largo (Upper Keys, Figure 2.1) form a monophyletic group relative to haplotypes from mainland Florida and differ by 0.9% (Hayes and Harrison 1992). Key deer show a unique haplotype that differs by

0.14% from those found in south Florida, and based on phylograms derived from microsatellites both mtDNA, allozymes, and microsatellites, the Key deer lineage is distinct from populations in mainland Florida (Ellsworth et al. 1994 a,b; Banks 2003). Unlike the preliminary study by Gaines et al. (1997), silver rice rat populations in the Keys also are genetically and phylogenetically divergent from those seen in south Florida. MtDNA divergence between the Keys and mainland Florida averaged 1.5% (D_{XY}), a value closer to that seen for the woodrat, and both mtDNA and microsatellite markers revealed restricted gene flow between populations in the Keys and mainland Florida. In addition, Keys rice rats form a monophyletic group relative to their mainland neighbors (Figure 2.6).

In many cases subspecies designations derived from morphology do not always coincide with patterns observed with genetic markers (Honeycutt 2000). In the case of the silver rice rat, Spitzer and Lazell (1978) used evidence of morphological differences to advocate taxonomic distinction for the Keys populations. Although others (Humphrey and Setzer 1989) have questioned this morphological and taxonomic distinction, analyses of both mitochondrial and nuclear DNA clearly indicate that populations of the silver rice rat located in the Lower Keys are genetically distinct from mainland populations, and are therefore on a separate evolutionary trajectory.

Implications to Conservation Efforts

Increased human population growth and on-going development in the Keys is altering native habitat and threatening many endemic species of vertebrates. Although the silver rice rat is currently not under active management, it is considered an

endangered mammal in the Keys. Our data support the uniqueness of these populations in the Keys, and the congruent patterns observed for both mtDNA and microsatellites suggest that the silver rice rat is a distinct 'evolutionary significant unit' (as per Moritz 1994). Although some have challenged the concept of an evolutionary significant unit (Bowen 1998; Crandall et al. 2000; Mace 2004), all the genetic data reveal genetic separation between the Keys and mainland. Furthermore, subdivision within the Lower Keys, as revealed by separation between groups of populations on different islands, indicates that management decisions designed to restore wetland habitats and viable populations of rice rats should at least treat eastern and western populations in the Lower Keys as separate management units. These genetic subdivisions should be considered in any management scheme that involves translocations and/or restocking from other source populations.

CHAPTER III

MITOCHONDRIAL DNA ANALYSIS OF THE LOWER KEYS MARSH RABBIT *(Sylvilagus palustris hefneri)*

INTRODUCTION

The Lower Keys marsh rabbit (*Sylvilagus palustris hefneri*) is listed as a federally endangered subspecies by the U.S. Fish and Wildlife Service (USFWS, 1990). The smallest of the three marsh rabbit subspecies, this subspecies is distinguished from marsh rabbits occupying the mainland (*S. p. palustris*) and Upper Keys (*S. p. paludicola*) by distinct cranial characteristics, including a shorter molariform tooth row, a broader skull with a higher, more convex frontonasal profile, and a longer dentary symphysis (Lazell 1984). Although marsh rabbits are found throughout the southeastern U.S., the range of the Lower Keys marsh rabbit is limited to particular islands in the Lower Keys. Like the Key deer and the silver rice rat, marsh rabbits were probably isolated from the mainland as ocean levels rose and separated the Keys from the mainland approximately 10,000 years ago (Lazell 1984). A restricted range, coupled with loss and fragmentation of habitat by commercial and residential development in the Keys, has led to the decline of marsh rabbits in the Lower Keys. A population viability analysis (PVA) carried out by Forys (1995) projected that under current conditions the subspecies could become extinct in as little as 50 years.

This critically endangered rabbit exhibits a strong preference for coastal marsh habitats that exist as areas of transition between mangrove swamps and upland areas throughout the Lower Keys (Faulhaber 2003). These patches include zones of lower

elevation, most of which have been filled and developed in the recent past. Although remaining marsh is now protected due to its wetland status, adjacent uplands remain a target for human development, and consequently, are currently under threat by developers (Williams 1991). The grassy marshes of the transition area currently exist as a mosaic of suitable habitat, both native and disturbed, with sub-populations of the marsh rabbit restricted to small, disjunct patches (Forys and Humphrey 1999). Few areas of contiguous habitat greater than 5 hectares (ha) remain, and a majority of patches are surrounded by urban development, making dispersal between populations unlikely (Forys et al. 1996). Although local extinctions of sub-populations are a natural occurrence in a metapopulation, if patches become too isolated, extinctions cannot be balanced by recolonizations, and the entire population becomes less sustainable (Hanski and Simberloff 1997). This level of patch isolation is the most critical threat that faces the continued existence of the Lower Keys marsh rabbit. The serious nature of this problem is realized by the fact that such isolation may already be occurring in an area known as the Gap Islands Complex (Neil Perry, unpublished data). This group of islands, located in the center of the marsh rabbit's range (extending from Middle Torch Key to Cudjoe Key; Figure 3.1), contains patches of suitable marsh rabbit habitat, yet there are no recorded rabbit sightings on the Gap Islands. Marsh rabbits are found on islands throughout the Lower Keys from Big Pine Key (and surrounding islands) to Boca Chica Key, but the most recent study by Faulhaber (2003) found no evidence of their presence in the Gap Islands. The only verification of their past existence in this area is in the form of anecdotal evidence from historic interviews with local inhabitants

(Lazell 1989). It is important to note that geographic variation in pelage color has been observed between marsh rabbits captured on Big Pine Key and those captured in the area west of the Gap Island Complex (Lazell 1989). This may be an indication of a more historical separation between these two areas.

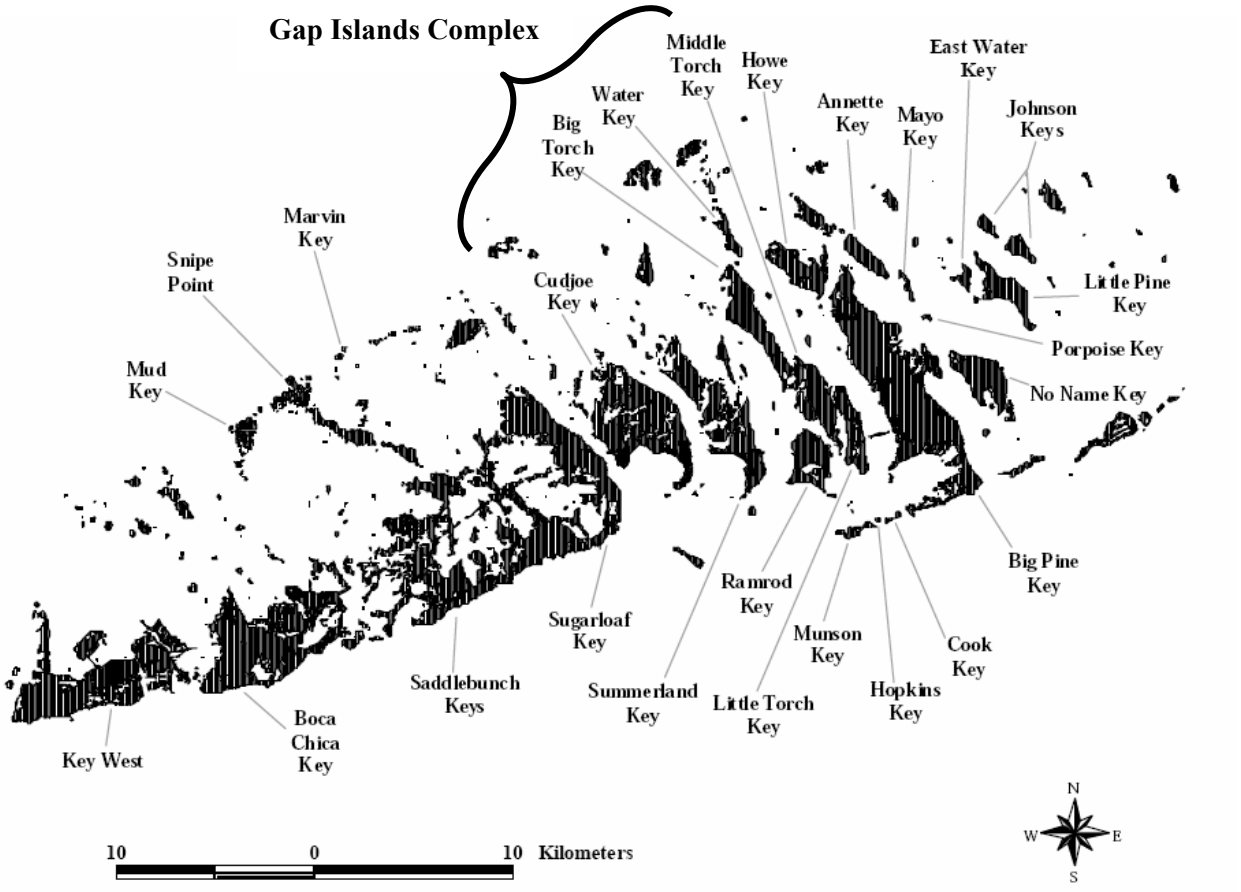

Figure 3.1. Map of the current range of the Lower Keys marsh rabbit, from Big Pine Key and its surrounding islands to Boca Chica Key. The Gap Islands Complex is an area uninhabited by marsh rabbits, although it contains patches of suitable LKMR habitat (adapted from Faulhaber 2003).

In addition to habitat quality, social behavior plays a critical role in shaping the genetic structure of a population (Sugg et al. 1996; Ross 2001). A radio-telemetry study of the Lower Keys marsh rabbit by Forys and Humphrey (1996) revealed several behaviors that presumably could influence patterns of genetic variation in the Lower Keys marsh rabbit: (1) Although capable of moving, adult rabbits remain in one patch for a lifetime. (2) Rabbits born in a patch remain there until sexual maturity, upon which time many then undertake a relatively long one-way movement (≤ 2 km). (3) Males move a greater distance from their natal patch than females, which tend to remain in their natal patch throughout their adult life. This study also found the average home range size of an adult marsh rabbit to be 3.96 ha (average core area 1.21 ha).

The ability of an animal to disperse between patches of habitat also plays a significant role in the determination of population structure (Johst et al. 2002). In the island habitat of the Lower Keys, water serves as the decisive barrier between most populations. Marsh rabbits are known to be relatively good swimmers (Tomkins 1935; Ivey 1959; Padgett 1989), but swimming frequency between islands is unknown. Other studies (Blair 1936) have noted that marsh rabbits will enter water only under extreme conditions; hence, it is possible that even small channels may impede dispersal. The Lower Keys marsh rabbit has been recorded swimming across ditches, canals, and even larger bodies of water (> 3 m), although swimming was a rare occurrence in relation to the presence of water in the environment (Humphrey and Forys 1996).

Although information on the ecology and behavior of marsh rabbits is available, no studies of genetic variation in populations located in the Lower Keys have been

conducted. As with other studies of threatened and endangered populations (Avise 1989; Kalinowski et al. 1999; Malone et al. 2003), a genetic survey of the Lower Keys marsh rabbit can provide meaningful data on the structure of local populations and the presence or absences of barriers to gene flow between existing populations. Such information is fundamental to management efforts of this endangered subspecies, and it can be used to identify source populations, for potential translocation of individuals where marsh rabbits have become rare. Such efforts are currently underway. Therefore, this study will provide preliminary information on the genetic structure of marsh rabbits in the Lower Keys through an analysis of variation at the mitochondrial control region. Given the ecological and behavioral evidence for female philopatry, one might expect more evidence for substructure with this maternally inherited marker (Avise 1994).

METHODS

Data Collection

Hair samples were collected from individual rabbits as part of a population survey (Faulhaber, 2003) conducted from November 2001 through July 2004 at the following localities: Boca Chica (n = 19), Sugarloaf (n = 13), Saddlebunch (n = 16), Big Pine (n = 9), and Geiger (n = 2) keys (Figure 3.1). In addition, one mainland sample from Lover's Key, located on the southwestern coast of Florida, was obtained and used as an outgroup. Rabbits were trapped and handled in accordance with Animal Use Protocol #2001-109, as approved by the Texas A&M University Institutional Animal Care and Use Committee. The trapping protocol consisted of unbaited Tomahawk Traps

(Tomahawk Live Traps, Tomahawk, Wisconsin, USA) lined with grasses and placed in vegetation tunnels through which rabbits travel. Traps were then covered with vegetation to simulate a natural passageway. In addition, drift fences made of chicken wire were used in an attempt to drive rabbits into the traps. Prior to analysis all hair samples were stored in either 15 mL of lysis buffer (Longmire's solution, Longmire et al. 1988) or plastic bags.

A Gentra Puregene™ DNA isolation kit (Gentra Systems, Minneapolis, Minnesota, USA) was used to extract DNA from individual hair samples. A total of 763 base pairs (bp) of the mitochondrial cyt *b* gene and the control region was amplified by the polymerase chain reaction (PCR, Saiki et al. 1988). The analysis was limited to the first half of the mtDNA control region due to extensive length variation, resulting from tandem repeats, located in the second half of the control region (Biju-Duval et al. 1991). The PCR primer set used in the initial amplification was L15774 (5'-TGAATTGGAGGACAACCAGT-3') and H16498 (5'-CCTGAAGTAGGAACCAGATG-3') (Shields and Kocher 1991). An additional internal primer set, L15934 (5'-CCCTGGTCTTGTAAGCCAGAAATGG-3') and H16431 (5'-GGGCGGGTTGCTGGTTTCACG-3') (Litvaitis et al. 1997), was used in sequencing. Amplifications were conducted in 50 μL reactions with the following components and concentrations: 2 μL DNA, 25.7 μL ddH_2O, 5 μL 10X PCR buffer (Takara Shuzo, Shiga, Japan), 2.5 μL 10X BSA, 4.5 μL of 10mM dNTPs (Takara Shuzo, Shiga, Japan), 5 μL each of forward and reverse primers (2pmol/μL), and 1.5 U *Taq* polymerase (Takara Shuzo, Shiga, Japan). Amplifications were carried out

(MyCycler™ Thermal Cycler, Bio-Rad Laboratories, Inc., Hercules, California, USA) in three stages that included an initial denaturation cycle at 95 °C for 4 min, followed by 35 cycles of 30 sec at 95°C, 1 min at 40°C, and 2 min at 72°C, and a final extension cycle of 10 min at 72°C. PCR products were visualized on a 1% agarose and TBE gel matrix. Amplified products were purified using a combination of Exonuclease I and Shrimp Alkaline Phosphatase (ExoSAP, USB, Cleveland, Ohio, USA).

Each sequencing reaction was performed using Big Dye termination chemistry (Applied Biosystems Inc. (ABI), Foster City, California, USA) in a GeneAmp® PCR System 2700 Thermal Cycler (ABI). Sequences were obtained on an ABI 3100 automated sequencer following protocols of the manufacturer (Perkin-Elmer Biosystems, Wellesley, Massachusetts, USA). Fragments were sequenced in the forward and reverse directions; sequence alignments and the formation of contigs were accomplished with the Sequencher 4.2 software program (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Statistical Analysis

Traditional population statistics (Nei 1987), including nucleotide diversity (π), haplotypic diversity (h), number of segregating sites (S), average number of nucleotide substitutions per site between populations (D_{xy}), and the average number of nucleotide differences between populations were calculated with the DnaSP version 4.0.6 (Rozas et al. 2003) software package. Estimates of γST (Nei 1982) and F_{ST} (Hudson et al. 1992) were also calculated in this program and used to estimate gene flow (N_fm).

Additionally, DnaSP was used to test the assumption of neutrality of mutations by Tajima's D (Tajima 1989).

The significance of haplotypic variation between populations was calculated using analysis of molecular variance (AMOVA; Excoffier et al. 1992). AMOVA was calculated with Φ-analogues of Wright's (1965) F-statistics in the ARLEQUIN version 2.0 software package (Schneider et al. 2000). This program estimates the proportion of genetic variation present at different hierarchical levels based on haplotype distribution and pairwise distance. The analysis was calculated with the Lower Keys population divided into two regions based on geographic location (i.e., east and west of the Gap Islands Complex). Significance was tested by means of a non-parametric permutation of haplotypes among both populations and groups (10,100 permutations).

Relationships between haplotypes were resolved by both maximum likelihood (ML) and maximum parsimony (MP) methods for phylogenetic reconstruction (PAUP*4.0b10, Swofford 2002). Both analyses used the single mainland sample as an outgroup and employed a heuristic search option with gaps identified as a fifth state (random addition sequence, 100 replications, TBR branch swapping). Bootstrap values were obtained to provide statistical support for branching topology (1,000 replications; Felsenstein 1985). Prior to the ML analysis, ModelTest version 3.06 (Posada and Crandall 1998) was used to obtain the appropriate parameters for the search.

RESULTS

MtDNA sequences derived from the 59 Lower Keys marsh rabbits revealed five unique haplotypes differing at 23 segregating sites. These five haplotypes represented two distinct clades (Western Clade and Eastern Clade, Figure 3.2) separated by 19 nucleotide substitutions. Haplotypic diversity (h±sd) was low (66.1 ± 3.4%) and haplotypes were distributed unevenly, with a majority of the samples represented by three types (42%, 39%, and 14%, Figure 3.3). Tajima's test for neutrality of the data, was positive (D = 1.722), but not significant ($p > 0.10$). The among group variation (AMOVA) between the two regions tested was 97.53% (among populations within groups 0.20% and within populations 2.28%), indicating a substantial separation between the two regions. Genetic variation was significantly partitioned among the two groups, with $F_{ST} = 0.98198$ (Hudson et al. 1992) and $\gamma_{ST} = 0.91811$ (Nei 1982; both calculated in DnaSP, Rozas et al. 2003).

Nucleotide diversity ($\pi \pm$ sd) over all Lower Keys samples was 1.782 ± 0.481% (with the Jukes and Cantor correction (π_{JC}) 1.816%). The eastern and western haplotypes differed by an average of 0.0288 nucleotide substitutions per site (D_{xy}, with Jukes and Cantor correction, 0.0294) and an average 22 nucleotide differences per sequence. Gene flow estimates were very low under both measures (γ_{ST}, $N_f m = 0.04$, Nei 1982; F_{ST}, $N_f m = 0.01$, Hudson et al. 1992).

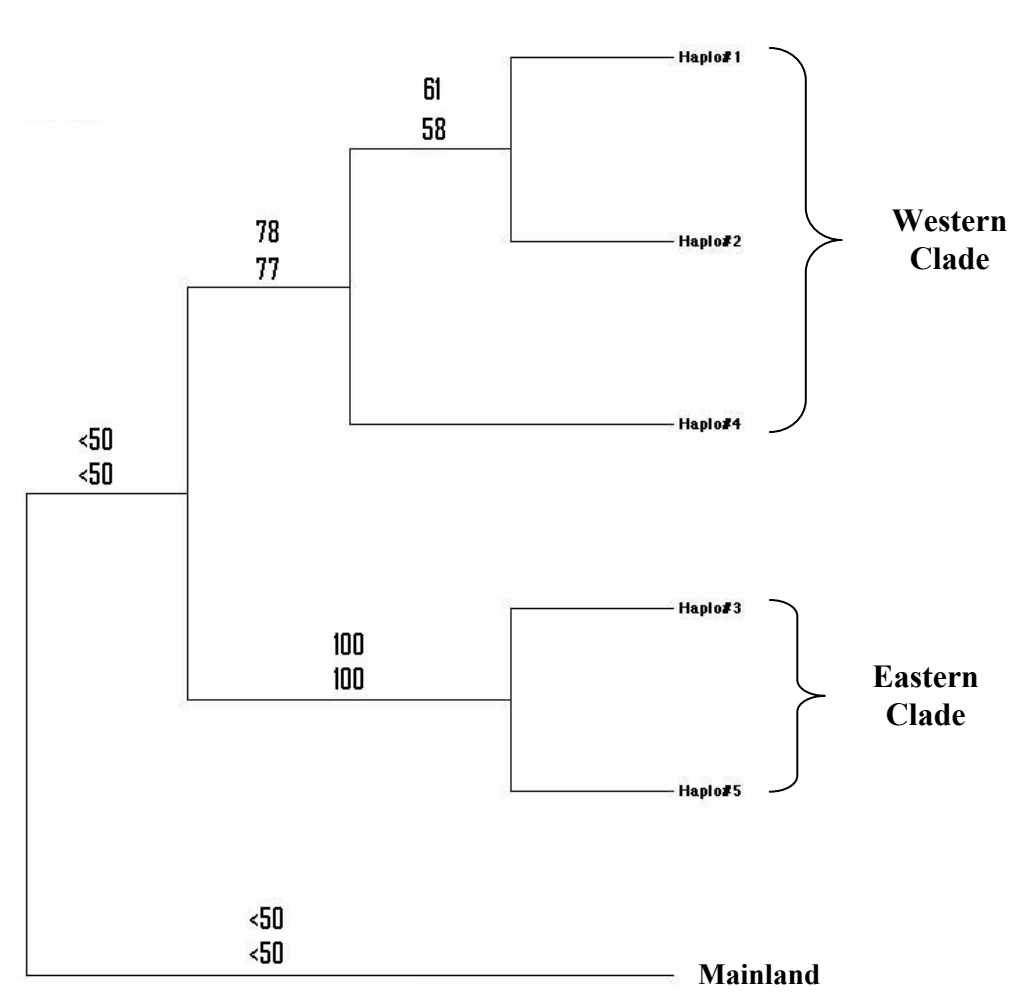

Figure 3.2. Phylogenetic tree constructed by maximum parsimony (MP) and maximum likelihood (ML) analysis of Lower Keys marsh rabbit mitochondrial sequence data. The Western clade refers to all trap sites west of the Gap Islands Complex; the Eastern clade is composed of all samples from Big Pine Key. Bold numbers represent bootstrap values, MP values below and ML values above.

To account for unequal base frequencies and allow for transition and transversion rate bias, the model HKY85 (Hasegawa et al. 1985) was used in the ML analysis. Nucleotide frequencies over all data were adenine (0.3028), guanine (0.1311), thymine

(0.2725), and cytosine (0.2936). Both the MP and the ML analyses produced identical trees (Figure 3.2; bootstrap values ≥ 50 shown, ML values above, MP values below). For the tree constructed by the MP analysis, the consistency index (CI), excluding uninformative characters, was 0.9565, the retention index (RI) was 0.9600, and the rescaled consistency index (RC) was 0.9200.

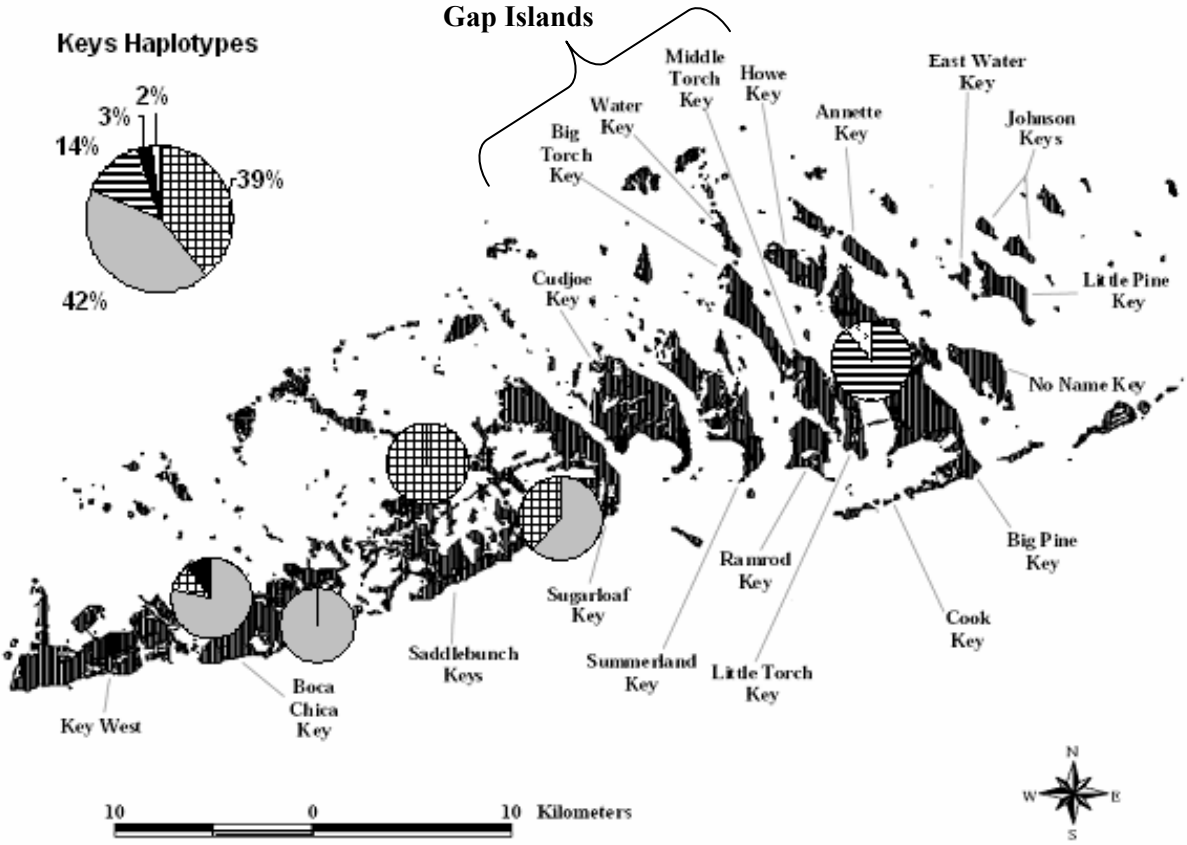

Figure 3.3. Pie charts of Lower Keys marsh rabbit mtDNA haplotypes by sampling locality, superimposed on geographic layout of the Lower Keys.

DISCUSSION

Phylogenetic analyses identified two main haplogroups occurring within the Lower Keys. These two groups are clearly separated geographically, with an Eastern Clade (trap sites east of the Gap Island *n* = 9) and a Western Clade (all trap sites west of the Gap Island complex *n* = 50, Figure 3.3). Patterns of strong genetic differentiation (F_{ST} = 0.98198, γ_{ST} = 0.91811) indicate little gene flow between eastern and western localities, which are separated by a region unoccupied by marsh rabbits, the Gap Islands Complex. Previous studies on the presence of marsh rabbit habitat throughout the Lower Keys have identified suitable sites within the Gap Island Complex but only in small disjunct patches (Faulhaber 2003). The lack of gene flow observed between the eastern and western regions (N_fm from F_{ST} = 0.01, N_fm from γ_{ST} = 0.04) is likely due to the fact that individuals attempting to traverse the Gap Islands Complex experience high mortality rates.

Moritz (1994) advocated using distinct separation of mtDNA (reciprocal monophyly) to identify historically isolated populations that potentially have distinct evolutionary potential. Populations that do not show clear separation, yet are functionally independent due to limited gene flow, may be designated as "management units," a still relevant designator for conservation efforts. The distinct genetic separation observed in the Lower Keys marsh rabbit, in addition to the geographic variation noted by Lazell (1989) provides strong evidence for the recognition of these two regions as separate management entities. Although the corroboration appears to verify this view, the small sample size from the eastern region makes it difficult to discern if the presence

of a single haplogroup over the entire area east of the Gap Islands Complex is merely an artifact of small sample size. Further sampling of Big Pine Key and outer islands would provide a more complete picture of geographic separation.

The Lower Keys marsh rabbit is currently under active management that includes translocations to suitable habitat patches throughout the Lower Keys. Translocations have already occurred on Little Pine, Big Torch (Faulhaber 2003), and Water keys involving rabbits from source populations throughout both the eastern and western regions of the Lower Keys (Faulhaber 2003). Although further study is warranted, implications of defining management units will significantly affect these efforts, as combining individuals from eastern and western islands would no longer be recommended.

CHAPTER IV

CONCLUSION

Genetic analyses of two endangered species of mammals in the Lower Keys (Lower Keys marsh rabbit, *Sylvilagus palustris hefneri*; silver rice rat, *Oryzomys argentatus* = *Oryzomys palustris natator*) were performed to address unknown features of their population structure. Striking similarities between the mitochondrial DNA (mtDNA) analyses of the Lower Keys marsh rabbit and the silver rice rat were revealed, most notably, the genetic subdivision between eastern and western populations. Over their approximately 48 km range within the Lower Keys, gene flow has been restricted to the extent that some haplotypes appear fixed within populations. This separation may have been established by the earlier formation of several channels that divide the Lower Keys. As ocean levels rose after the last ice age, the Keys were slowly transformed from a single landmass into the collection of islands that exist today. These historical barriers to dispersal would have helped shape the genetically structured population observed today. Future studies on dispersal in the silver rice rat include a landscape analysis which will examine factors such as channel depth and currents, human development, and ocean levels at low tide for possible effects on dispersal of the rice rat.

The genetic separation between mtDNA haplotypes of rabbit populations (19 bp) was more extreme than the division between the rat populations (4 bp). This disparity is likely due to a large area of unoccupied habitat (Gap Islands, Neil Perry unpublished data) present in the center of the Lower Keys marsh rabbit range that separates the distinctive haplogroups and impedes dispersal between these two regions. Presumably,

this phylogeographic pattern is the consequence of historical events that created effective barriers to dispersal of marsh rabbits. Conversely, the silver rice rat population is continuously present throughout the Lower Keys. In addition to an overall similar pattern of genetic subdivision, populations of both species possess low levels of mtDNA variation (haplotypic diversity in the Lower Keys marsh rabbit = 66.1%, silver rice rat = 58.6%), a common occurrence among island populations.

Both studies within the Lower Keys would benefit from additional sampling. For the Lower Keys marsh rabbit, further sampling from the eastern region of the Lower Keys would provide a more complete picture of geographical separation. Although morphological evidence corroborates the marsh rabbit division (Lazell 1989), small sample size from this area makes it difficult to determine if a single haplogroup predominates over the entire area east of the Gap Islands Complex, or if this is merely an artifact of sample size. Similarly, more samples from the center islands of the silver rice rat range would more accurately verify the frequency of the two unique haplotypes observed in this region.

The addition of microsatellite analyses of the silver rice rat allowed a more in depth look at the structuring of this population. Although subdivision between eastern and western regions is more pronounced in terms of mtDNA, this overall pattern is still apparent. This is not unexpected, because the mode of inheritance of mtDNA is such that we would expect more geographic subdivision. Over the relatively brief 4,000 years that the Lower Keys have been isolated from the mainland, mtDNA haplotypes may have had sufficient time to coalesce among these isolated populations. Conversely,

nuclear microsatellites may have retained enough residual variation to reveal alleles shared between geographic regions that existed prior to rising ocean levels and the subsequent creation of obstacles to dispersal. Alternatively, the difference in variation between nuclear and mtDNA may be the result of sex biased dispersal. Species that demonstrate pronounced female philopatry and male dispersal should show more genetic subdivision when maternally inherited markers are used. Therefore, the lack of structure observed in analyses of nuclear DNA may be a result of male dispersal. However, ecological studies have not recorded any evidence of sex biased dispersal in the silver rice rat. In addition to a difference in the level of population structure observed, the microsatellite analyses suggested that rice rats were using the shallower waters on the north side of the islands as a dispersal corridor.

The examination of divergence between mainland and Lower Keys rice rats revealed a genetic division that indicated a lack of recent gene exchange between the regions (i.e., no shared haplotypes, the presence of private alleles, and distinctive separation in numerous analyses). Although this degree of division does not warrant species designation, the level and pattern of divergence, both morphological and genetic, does suggest genetic isolation of mainland and island forms. This fact, along with clearly restricted gene flow between the Lower Keys and the Everglades, clearly positions the silver rice rat on an evolutionary trajectory separate from its mainland counterparts and validates identification as the subspecies, *Oryzomys palustris natator*.

REFERENCES

Avise JC (1989) A role for molecular genetics in the recognition and conservation of endangered species. *Trends Ecol. Evol*., **4**, 279-281.

Avise JC (1994) *Molecular Markers, Natural History and Evolution.* Chapman & Hall, New York.

Avise JC (2000) *Phylogeography: The History and Formation of Species.* Harvard University Press, Cambridge, Massachusetts.

Avise JC, Nelson WS (1989) Molecular genetic relationships of the extinct dusky seaside sparrow. *Science*, **243**, 646-648.

Banks KR (2001) *Conservation Genetics of the Endangered Florida Key Deer*. M.S. Thesis, Texas A&M University, College Station.

Baker CS, Slade RW, Bannister JL, Abernethy RB, Weinrich MT, Lien J, Urban J, Corkeron P, Calmabokidis J, Vasquez O, Palumbi SR (1994) Hierarchical structure of mitochondrial DNA gene flow among humpback whales (*Megaptera novaeangliae*) worldwide. *Mol. Ecol*., **3**, 313-327.

Barton NH, Slatkin M (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, **56**, 409-415.

Barbour DB, Humphrey SR (1982) Status of the silver rice rat (*Oryzomys argentatus*). *Fla. Sci.*, **45**,106-112.

Bickham JW, Wood CC, Patton JC (1995) Biogeographic implications of cytochrome *b* sequences and allozymes in sockeye (*Oncorhynchus nerka*). *J. Hered*., **86**, 140-141.

Biju-Duval C, Ennafaa H, Dennebouy N, Monnerot M, Mignotte F, Soriguer RC, El Gaaied A, El Hili A, Monnolou JC (1991) Mitochondrial DNA evolution in lagomorphs: Origin of systematic heteroplasmy and organization of diversity on European rabbits. *J. Mol. Evol*., **33**, 92-102.

Birkenholz DE (1963) Movement and displacement in the rice rat. *Qt. J. Fla. Acad. Sci*., **26**, 269-274.

Blair FW (1936) The Florida marsh rabbit. *J. Mammal*., **17**, 197-207.

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368**, 455-457.

Bowen BW (1998) What is wrong with ESUs? The gap between evolutionary theory and conservation principles. *J. Shellfish Res.*, **17**, 1355-1358.

Case TJ, Bolger DT, Richman AD (1992) Reptilian extinctions: The last ten thousand years. In: *Conservation Biology: The Theory and Practice of Nature Conservation, Preservation and Management* (eds. Fielder PL, Jain SK), pp. 90-125. Chapman and Hall, New York.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.*, **15**, 290-295.

Dickson JD (1955) An ecological study of the Key deer. Technical Bulletin 3. Florida Game and Fresh Water Fish Committee, Tallahassee, Florida.

Ellegren H (1999) Inbreeding and relatedness in Scandinavian grey wolves (*Canis lupus*). *Hereditas*, **130**, 239-244.

Ellsworth DL, Honeycutt RL, Silvy NJ, Bickham JW, Klimstra WD (1994a) Historical biogeography and contemporary patterns of mitochondrial DNA variation in white-tailed deer from the southeastern United States. *Evolution*, **48**, 122-136.

Ellsworth DL, Honeycutt RL, Silvy NJ, Smith MH, Bickham JW, Klimstra WD (1994b) White-tailed deer restoration to the southeastern United States: Evaluating genetic variation. *J. Wildl. Manag.*, **58**, 686-697.

Esher RJ, Wolfe JL, Layne JN (1978) Swimming behavior of rice rats (*Oryzomys palustris*) and cotton rats (*Sigmodon hispidus*). *J. Mammal.*, **59**, 551-558.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA hapoltypes: Application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479-491.

Faulhaber CA (2003) *Updated Distribution and Reintroduction of the Lower Keys Marsh Rabbit*. M.S. Thesis, Texas A&M University, College Station.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* **39**, 783-791.

Florida Geological Survey (1994) *Special Publication No. 35: Florida's Geological History and Geological Resources* (ed. Lane E) Tallahassee, Florida.

Forys EA (1994) Gene flow among island populations of rats. *Va. J. Sci*., **45**, 3-11.

Forys EA (1995) *Metapopulations of Marsh Rabbits: A Population Viability Analysis of the Lower Keys Marsh Rabbit (Sylvilagus palustris hefneri).* Ph.D. Dissertation, University of Florida, Gainsville, Florida.

Forys EA, Allen CR, Wojcik DP (2002) Influence of the proximity and amount of human development and roads on the occurrence of the red imported fire ant in the lower Florida Keys. *Biol. Conserv*., **108**, 27-33.

Forys EA, Frank PA, Kautz RS (1996) Recovery actions for the Lower Keys marsh rabbit, silver rice rat, and Stock Island tree snail. Final Report to Florida Game and Freshwater Fish Commission, Tallahassee, Florida, Cooperative Agreement No. 1448-0004-94-9164.

Forys EA, Humphrey SR (1996) Home range and movements of the Lower Keys marsh rabbit in a highly fragmented habitat. *J. Mammal*., **77**, 1042-1048.

Forys EA, Humphrey SR (1999) Use of population viability analysis to evaluate management options for the endangered Lower Keys marsh rabbit. *J. Wildl. Manag*., **63**, 251-260.

Frankham R (1996) Relationships of genetic variation to population size in wildlife. *Conserv. Biol*., **10**, 1500-1509.

Frankham R (1997) Do island populations have less genetic variation than mainland populations? *Heredity*, **78**, 311-327.

Frankham R (1998) Inbreeding and extinction: Island populations. *Conserv. Biol*., **12**, 665-675.

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating levels of gene flow using microsatellite data. *Mol. Ecol*., **8**, 1513-1520.

Gaines MS, Diffendorfer JE, Tamarin RH, Whittam TS (1997) The effects of habitat fragmentation on the genetic structure of small mammal populations. *J. Hered*., **88**, 294-304.

Garner TWJ, Pearman PB, Angelone S (2004) Genetic diversity across a vertebrate species' range: A test of the central-peripheral hypothesis. *Mol. Ecol*., **13**, 1047-1053.

Goodyear NC (1984) Final report on the distribution, habitat, and status of the silver rice rat *Oryzomys argentatus*. Unpublished report prepared for the U.S. Fish and Wildlife Service under contract No. 14-16-0604-83-57. Jacksonville, Florida.

Goodyear NC (1987) Distribution and habitat of the silver rice rat, *Oryzomys argentatus*. *J. Mammal*., **68**, 692-695.

Goodyear NC (1991) Taxonomic status of the silver rice rat, *Oryzomys argentatus*. *J. Mammal*., **72**, 723-730.

Goodyear NC (1992) Spatial overlap and dietary selection of native rice rats and exotic black rats. *J. Mammal*., **73**, 186-200.

Goodyear NC, Lazell JD (1986) Relationships of the silver rice rat *Oryzomys argentatus* (Rodentia: Muridae). *Postilla*, **198**, 1-7.

Goudet J, Raymond M, De Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933-1940.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, **48**, 1933-1940.

Hanski IA, Simberloff D (1997) The metapopulation approach, its history, conceptual domain, and application to conservation. In: *Metapopulation Biology: Ecology, Genetics, and Evolution.* (eds. Hanski IA, Gilpin ME), pp 5-26. Academic Press, San Diego, California.

Hardin JW, Klimstra WD, Silvy NJ (1984) Florida Keys. In: *White-tailed Deer: Ecology and Management* (ed. Halls LK), pp 381-390. Stackpole Books, Harrisburg, Pennsylvania.

Hartl DL, Clark AG (1997) *Principles of Population Genetics*. 3rd ed. Sinauer Associates, Sunderland, Massachusetts.

Harveson PM, Lopez RR, Silvy NJ, Frank PA (2004) Source-sink dynamics of Florida Key deer on Big Pine Key, Florida. *J. Wildl. Manag*., **68**, 909-915

Hasegawa M, Kishino H, Yano TA (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol*., **22**, 160-174.

Hayes JP, Harrison RG (1992) Variation in mitochondrial DNA and the biogeographic history of woodrats (Neotoma) of the eastern United States. *Syst. Biol*., **41**, 331-344.

Heller E (1904) Mammals of the Galapagos Archipelago, exclusive of the cetacea. San Francisco: *Calif. Acad. Sci*., **3**, 233-251.

Hendrick PW, Kalinowski S (2000) Inbreeding depression in conservation biology. *Ann. Rev. Ecol. Syst*., **31**, 139-162.

Hinten G, Harriss F, Rossetto M, Braverstock PR (2003) Genetic variation and island biogeography: Microsatellite and mitochondrial DNA variation in island populations of the Australian bush rat, *Rattus fuscipes greyii*. *Conserv. Genet*., **4**, 759-778.

Hoffmeister JE (1974) *Land from the Sea: The Geologic Story of South Florida*. University of Miami Press, Coral Gables, Florida.

Honeycutt RL (2000) Genetic applications for large mammals. In: *Ecology and Management of Large Mammals in North America* (eds. Demarais S, Krausman PR), pp. 233–252. Prentice-Hall, Upper Saddle River, New Jersey, USA.

Hudson RR, Slatkin M, Maddison WP (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics*, **132**, 583-589.

Humphrey SR (1992) Rare: Lower Keys population of rice rat, *Oryzomys palustris natator* (in part). In: *Rare and Endangered Biota of Florida, Volume I. Mammals* (ed. Humphrey SR), pp. 300-309. University Press of Florida, Gainesville, Florida.

Humphrey SR, Barbour DB (1979) Status and habitat of eight kinds of endangered and threatened rodents in Florida. Special Scientific Report No. 2. Office of Ecological Services, Florida. Florida State Museum, Gainesville, Florida.

Humphrey SR, Setzer HW (1989) Geographic variation and taxonomic revision of rice rats (*Oryzomys palustris* and *O. argentatus*) of the United States. *J. Mammal*., **70**, 557-570.

Ivey RD (1959) The mammals of Palm Valley, Florida. *J. Mammal*., **65**, 424-432.

Johst K, Brandl R, Eber S (2002) Metapopulation persistence in dynamic landscapes: The role of dispersal distance. *Oikos*, **98**, 263-270.

Kalinowski ST, Hedrick PW, Miller PS (1999) No inbreeding depression observed in Mexican and red wolf captive breeding programs. *Conserv. Biol*., **13**, 1371-1377.

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. *Genetics*, **49**, 725-738.

Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in a finite population. *Proc. Nat. Acad. Sci. USA*, **75**, 2868-2872.

Kirchman JJ, Whittingham LA, Sheldon FH (2000) Relationships among cave swallow populations (*Petrochelidon fulva*) determined by comparisons of microsatellite and cytochrome b data. *Mol. Phylogenet. Evol*., **14**, 107-121.

Krohne DT (1997) Dynamics of metapopulations of small mammals. *J. Mammal*., **78**, 1014-1026.

Laerm J, Avise JC, Patton JC, Lansman RA (1982) Genetic determination of the status of an endangered species of pocket gopher in Georgia. *J. Wildl. Manag.*, **46**, 513-518.

Lazell JD (1984) A new marsh rabbit (*Sylvilagus palustris*) from Florida's Lower Keys. *J. Mammal*., **65**, 26-33.

Lazell JD (1989) *Wildlife of the Florida Keys: A Natural History*. Island Press, Washington D.C.

Leeworthy VR, Wiley PC (1996) Visitor profiles: Florida Keys, Key West. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service, Office of Ocean Resources Conservation and Assessment, Strategic Environmental Assessments Division, Silver Spring, Maryland.

Levinson G, Gutman GA (1987) High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. *Nucleic Acids Res.*, **15**, 5323-5338.

Lidz BH, Shinn EA (1991) Paleoshorelines, reefs, and a rising sea: South Florida, USA. *J. Coastal Res.*, **7**, 203-229.

Litvaitis MK, Litvaitis JA, Woo-Jai L, Kocher TD (1997) Variation in the mitochondrial DNA of *Sylvilagus* complex occupying the northeastern United States. *Can. J. Zoo*., **75**, 595-605.

Lomolino MV (1986) Mammalian community structure on islands: The importance of immigration, extinction and interactive effects. *Biol. J. Linn. Soc*., **28**, 1-21.

Longmire JL, Lewis AK, Brown NC, Buckingham JM, Clark LM, Jones MD, Meincke LJ, Meyne J, Ratliff RL, Ray FA, Wagner RP, Moyzis RK (1988) Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics*, **2**, 14-24.

Mace GM (2004) The role of taxonomy in species conservation. *Phil. Trans. R. Soc. Lond. B*, **359**, 711-719.

Maffei MD, Klimstra WD, Wilmers TJ (1988) Cranial and mandibular characteristics of Key deer (*Odocoileus virginianus clavium*). *J. Mammal*., **69**, 403-407.

Malone CL, Knapp CR, Taylor JF, Davis SK (2003) Genetic consequences of Pleistocene fragmentation: Isolation, drift, and loss of diversity in rock iguanas (*Cyclura*). *Conserv. Genet*., **4**, 1-15.

Maroja LS, Almeida FC, Cerqueira R, Seuanez HN, Moreira MAM (2003) Nectomys squamipes microsatellites and homologous loci in sigmodontine rodents. *J. Hered*., **94**, 171-174.

Mayr E (1963) *Animal Species and Evolution*. Belknap, Cambridge, Massachusetts.

Minch E, Ruiz-Linares A, Goldstein D, Feldman M, Cavalli-Sforza LL (1996) MICROSAT (version 1.5b): A computer program for Calculating Various Statistics on Microsatellite Allele Data (program available from: http://hpgl.stanford.edu/projects/microsat/).

Mitchell NC (1996) Silver rice rat status. Draft final report to Florida Game and Fresh Water Fish Commission, Tallahassee, Florida.

Monroe County Growth Management Division (1992) Monroe County year 2010 comprehensive plan. Monroe County Planning Department, Key West, Florida.

Moritz C (1994) Defining "evolutionary significant units" for conservation. *Trends Ecol. Evol*., **9**, 373-377.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA*, **70**, 3321-3323.

Nei M (1982) Evolution of human races at the gene level. In: *Human Genetics, Part A: The Unfolding Genome* (eds. Bonne-Tamir B, Cohen T, Goodman RM), pp. 167-181. Alan R. Liss, New York.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

O'Brien SJ (1994) Genetic and phylogenetic analyses of endangered species. *Annu. Rev. Genet*., **28**, 467-489.

Olson SL (1989) Extinction on islands: Man as a catastrophe. In: *Conservation for the Twenty First Century* (eds. Western D and Pearl MC), pp. 50-53. Oxford University Press, New York.

Padgett TM (1989) A range extension of the marsh rabbit, *Sylvilagus palustris*, from southeastern Virginia. *Va. J. Sci*., **40**, 177.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol*., **4**, 347-354.

Paetkau D, Slade R, Burdens M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: A simulation-based exploration of accuracy and power. *Mol. Ecol*., **13**, 55-65.

Peakall R, Smouse PE (2001) GeneAlEx V5: Genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GeneClass2: A software for genetic assignment and first-generation migrant detection. *J. Hered.*, **95**, 536-539.

Posada D, Crandall KA (1998) ModelTest: Testing the model of DNA substitution. *Bioinformatics*, **14**, 817-818.

Primmer CR, Ellegren H, Saino N, Møller AP (1996) Directional evolution in germline microsatellite mutations. *Nat. Genet.*, **13**, 391-393.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using mulitlocus genotype data. *Genetics*, **155**, 945-959.

Randi E, Alves PC, Carranza J, Milošević-Zlatanović S, Sfougaris A, Mucci N (2004) Phylogeography of roe deer (*Capreolus capreolus*) populations: The effects of historical genetic subdivisions and recent nonequilibrium dynamics. *Mol. Ecol*., **13**, 3071-3083.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proc. Natl. Acad. Sci. USA*, **84**, 9197-9221.

Raymond M, Rousset F (1995) GENEPOP (version 3.4): Population genetics software for exact tests and ecumenicism. *J. Hered*., **86**, 248-249.

Rice WA (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.

Ross KG (2001) Molecular ecology of social behavior: Analysis of breeding systems and genetic structure. *Mol. Ecol*., **10**, 265-284.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analysis by the coalescent and other methods. *Bioinformatics*, **19**, 2496-2497.

Saiki KD, Gelfand H, Stoffel S, Scharf S, Higucho R, Horn G, Mullis K, Erlich H (1988) Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. *Science*, **239**, 487-491.

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406-425.

Schneider S, Roessli D, Excoffier L (2000) Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Seppä P, Laurila A (1999) Genetic structure of island populations of the anurans *Rana temporaria* and *Bufo bufo*. *Heredity*, **82**, 309-317.

Shields GF, Kocher TD (1991) Phylogenetic relationships of North American ursids based on analysis of mitochondrial DNA. *Evolution*, **45**, 218-221.

Slatkin M (1985) Gene flow in natural populations. *Ann. Rev. Ecol. Syst*., **16**, 393-430.

Slatkin M (1993) Isolation by distance in equilibrium and nonequilibrium populations. *Evolution*, **47**, 264-279.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457-462.

Smith FDM, May RM, Fellow R, Johnson TH, Walter KR (1993) How much do we know about the current extinction rate? *Trends Ecol. Evol.*, **8**, 375-378.

Spitzer NC, Lazell JD (1978) A new rice rat (Genus: Oryzomys) from Florida's Lower Keys. *J. Mammal*., **59**, 787-792.

Sugg DW, Chesser RK, Dobson FS, Hoogland JL (1996) Population genetics meets behavioral ecology. *Trends Ecol. Evol.*, **11**, 338-342.

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony and other methods, Version 4.0b2. Sinauer Associates, Sunderland, Massachusetts.

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585-595.

Tomkins IR (1935) The marsh rabbit: An incomplete life history. *J. Mammal*., **16**, 201-205.

US Fish and Wildlife Service (1991) Final Rule: Endangered status for the Lower Keys population of the rice rat (silver rice rat). *Federal Register*, **56** (8), 19809-19814.

US Fish and Wildlife Service (1999) South Florida multi-species recovery plan. US Fish and Wildlife Service, Atlanta, Georgia.

Vessey SH, Meikle DB, Spaulding SR (1976) Biological survey of Raccoon Key Florida: A preliminary report to the Charles River Breeding Labs, Wilmington, Massachusetts.

Vitousek PM (1988) Diversity and biological invasions of oceanic islands. In: *Biodiversity* (eds Wilson EO, Peters FM), pp. 181-189. National Academy Press, Washington, D.C.

Wang Y, Hughes CR, Gines-Candelaria EA, Gaines MS (2000) Polymorphic microsatellite loci of *Oryzomys palustris*, the marsh rice rat, in South Florida detected by silver staining. *Mol. Ecol*., **9**, 1931-1932.

Weber JL, Wong C (1993) Mutation of human short tandem repeats. *Human Mol. Genet.*, **2**, 1123-1128.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358-1370.

Wielebnowski N (1996) Reassessing the relationship between juvenile mortality and genetic monomorphism in captive cheetahs. *Zoo Biol*., **15**, 353-369.

Williams A (1991) Transitional wetlands. In: *The Monroe County Environmental Story* (ed. Gato J), pp. 32-33. The Monroe County Environmental Education Task Force, Big Pine Key, Florida.

Wolfe JL (1986) Survey for silver rice rats (*Oryzomys argentatus*) on Racoon Key, Monroe County, Florida, 12-15 December. Unpublished report to Charles River Laboratory, Wilmington, Massachusetts.

Wolfe JL (1987) Survey for silver rice rat on US Naval property in the Lower Florida Keys, Monroe County, Florida. Unpublished report to Southern Division, Naval Facilities Engineering Command, Charleston, South Carolina.

Wright S (1951) The genetical structure of populations. *Ann. Eugenics*, **15**, 323-353.

Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution*, **19**, 395-420.

Zenger KR, Eldridge MDB, Cooper DW (2003) Intraspecific variation, sex-biased dispersal and phylogeography of the eastern grey kangaroo (*Macropus giganteus*). *Heredity*, **91**, 153-162.

Zenger KR, Eldridge MDB, Johnston PG (2005) Phylogenetics, population structure and genetic diversity of the endangered southern brown bandicoot (*Isoodon obesulus*) in south-eastern Australia. *Conserv. Genet*., **6**, 193-204.

APPENDIX 1

GENOTYPES

	AAT_03		AAT_10		AAT_16		AAT_21		AAT_26		AAT_28		AAT_40		AAT_60	
REF#	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
US1	120	135	138	141	106	106	174	180	109	112	109	124	138	156	160	163
US101	114	120	141	144	106	115	174	177	109	112	103	109	150	156	160	160
US102	120	135	141	144	94	106	183	186	109	109	109	124	138	150	160	160
SU103	114	120	141	144	94	94	168	168	109	121	103	109	138	138	157	157
SU104	117	120	141	144	94	115	168	180	109	109	106	109	138	138	145	157
CJA105	120	120	141	144	94	106	168	183	112	118	109	109	132	138	157	160
SU106	114	120	141	144	106	109	180	183	109	109	103	109	132	138	157	157
CJB108	120	126	144	144	94	106	183	183	109	109	109	115	132	141	160	160
LSU414	117	126	141	141	106	106	177	183	112	112	106	115	138	138	154	157
BP416	117	132	144	144	94	106	168	183	109	112	106	121	138	150	160	166
WATER425	114	120	144	144	94	94	177	183	109	109	106	109	138	150	157	157
ANTB431	126	132	144	147	94	106	168	183	112	118	115	121	144	144	157	163
WATER443	117	129	135	138	94	94	183	183	109	118	106	118	141	150	145	157
SAC447	114	117	141	141	106	106	168	183	112	112	103	106	132	147	157	160
SAC448	117	132	138	144	94	106	168	183	112	112	106	121	141	150	154	157
SAC452	117	126	141	141	106	106	168	177	109	112	106	115	141	147	154	157
SAC454	132	132	138	144	94	106	174	183	112	112	118	121	132	144	157	160
HOWE464	117	132	135	138	106	106	168	177	109	112	106	121	129	150	157	166
HOWE466	117	132	141	147	94	94	168	177	109	112	106	121	150	156	157	166
HOWE475	129	132	135	138	94	94	177	180	109	109	118	121	129	150	160	163
HOWE476	114	117	144	144	94	106	177	177	109	118	103	106	138	150	160	160
HOWE484	108	114	138	141	94	94	177	177	109	118	100	103	129	150	157	163
BTD532	116	116	141	144	94	94	168	168	118	118	109	112	141	150	145	163

	AAT_03		AAT_10		AAT_16		AAT_21		AAT_26		AAT_28		AAT_40		AAT_60	
REF#	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
BTC692	119	134	141	141	94	94	168	183	109	118	109	124	129	129	163	163
CJA2107	114	120	138	141	94	106	183	183	109	112	106	109	138	141	154	157
MTB2110	132	132	144	144	94	106	177	177	109	118	118	121	138	138	157	160
MTB2111	114	120	138	141	94	94	168	177	112	118	106	109	144	150	157	157
MTB2112	114	120	141	141	94	106	177	177	109	109	103	109	138	138	157	157
MTB2186	117	135	138	141	94	106	177	177	112	112	106	124	144	144	157	160
MTB2187	116	119	144	144	94	106	177	177	112	112	106	109	141	150	157	160
MTB2188	117	117	144	144	94	94	177	180	109	118	106	109	138	138	157	160
MTB2192	132	132	141	144	94	94	168	177	109	118	121	121	138	138	157	157
MTB2193	117	120	144	144	94	94	177	180	118	118	106	109	138	141	157	160
MTB2194	117	132	144	144	94	106	177	177	109	118	106	121	132	138	157	157
RR2196	111	117	144	144	94	106	177	177	118	118	103	106	132	141	160	160
MTA2197	120	132	144	144	94	106	177	177	118	118	109	121	138	141	157	160
MTB2198	114	132	144	144	94	106	168	177	109	118	103	121	138	138	157	160
MTB2199	117	132	144	144	94	94	177	177	118	118	106	121	138	138	157	160
ANTA10404	114	117	141	144	94	106	174	183	109	112	103	106	144	150	157	163
ANTA10405	114	129	141	144	106	106	177	180	112	124	103	118	147	150	157	163
ANTA10407	113	131	141	141	94	106	174	183	109	124	103	121	144	147	157	157
ANTA10408	116	116	141	147	106	106	168	174	112	112	106	106	150	153	157	166
ANTA10409	116	116	141	141	94	106	177	183	124	124	106	106	144	147	154	157
ANTA10410	114	117	141	141	94	106	177	183	109	112	103	106	144	150	154	157
ANTA10412	114	135	141	144	106	115	168	183	115	124	103	124	144	147	157	157
ANTA10414	117	132	141	147	106	106	177	183	109	112	106	121	147	147	157	157
ANTA10415	117	129	141	141	94	106	174	180	109	112	106	118	144	150	154	163
ANTA10416	116	131	147	147	106	106	174	183	112	112	106	121	144	147	157	157
ANTA10417	117	132	141	147	106	106	174	174	109	112	106	121	147	147	157	157

	AAT_03		AAT_10		AAT_16		AAT_21		AAT_26		AAT_28		AAT_40		AAT_60	
REF#	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
ANTB10418	117	126	141	144	94	106	180	183	112	112	106	115	144	156	154	157
ANTB10419	126	132	141	147	94	106	168	180	112	112	115	121	144	144	157	157
ANTB10420	132	132	141	147	94	106	180	180	112	112	121	121	144	147	157	160
ANTB10424	132	132	141	147	94	106	180	180	112	112	121	121	144	147	157	157
ANTB10425	126	132	144	147	94	106	180	183	112	112	115	121	144	144	157	157
ANTB10427	126	132	141	144	106	106	180	183	112	112	115	121	144	147	157	163
ANTB10429	117	132	141	144	94	106	168	180	112	118	106	121	144	156	157	163
ANTB10431	126	132	141	147	94	106	168	180	112	112	115	121	144	147	157	160
ANTB10432	120	132	144	147	94	106	180	183	112	112	109	121	144	147	157	157
ANTB10433	117	132	138	141	94	106	180	180	112	112	106	121	144	147	157	160
ANTB10434	117	132	138	141	94	115	174	183	109	118	106	121	147	147	154	157
ANTB10435	116	116	141	141	106	106	168	183	112	124	106	106	141	144	154	157
BTB10436	116	116	141	141	94	94	168	183	109	118	106	106	138	150	157	160
BTA10439	113	116	144	144	94	94	168	177	109	118	103	106	129	150	163	163
BTB10441	114	120	138	141	94	94	168	180	109	118	109	109	138	138	157	160
BTA10446	113	116	141	141	94	118	-	-	109	118	103	106	144	159	160	163
BTB10447	116	119	138	144	94	106	168	177	109	112	106	109	150	150	145	157
BT10454	114	132	141	144	94	94	177	177	118	118	103	121	138	141	145	157
BTB10457	120	135	123	138	94	115	177	177	109	118	109	124	138	141	160	160
BTD10460	117	117	123	141	94	115	-	-	118	118	109	112	141	150	157	160
TX31907	129	129	138	141	109	115	165	171	123	132	112	118	129	141	139	157
TX31908	129	132	144	150	100	115	165	168	132	132	118	121	129	141	148	160
TX31909	126	132	141	144	100	115	171	177	120	126	115	121	135	147	154	166
EV1	120	123	141	147	109	118	165	180	97	121	109	112	144	150	154	157

	AAT_03		AAT_10		AAT_16		AAT_21		AAT_26		AAT_28		AAT_40		AAT_60	
REF#	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
EV2	126	129	132	144	106	109	165	174	103	118	115	118	141	144	151	160
EV3	120	129	121	147	94	94	165	174	97	130	109	118	141	150	139	148
EV4	129	135	147	150	103	103	177	183	118	121	118	124	138	144	157	166
EV5	123	138	132	135	103	112	168	177	121	121	112	127	138	141	145	145
EV6	123	126	147	150	97	109	174	177	97	127	112	115	135	144	151	160
EV7	129	129	132	132	106	106	159	180	103	124	118	121	138	141	151	154
EV8	111	126	135	150	100	100	180	183	97	97	100	115	153	153	148	166
EV9	111	129	132	135	100	115	165	174	127	133	100	118	147	150	151	154
EV10	126	126	144	147	94	106	168	171	124	124	115	118	138	150	148	157

APPENDIX 2

HARDY-WEINBERG EQUILIBRIUM TESTS

Hardy-Weinburg Equilibrium

	Locus							
Population	AAT_03	AAT_10	AAT_16	AAT_21	AAT_26	AAT_28	AAT_40	AAT_60
Lower Keys-Eastern	0.273 (0.014)	0.031 (0.002)	0.099 (0.006)	0.024 (0.002)	0.310 (0.008)	0.526 (0.016)	0.085 (0.007)	0.864 (0.007)
Lower Keys-Western	0.562 (0.016)	0.783 (0.005)	0.055 (0.003)	0.255 (0.007)	0.160 (0.011)	0.699 (0.014)	0.282 (0.020)	0.848 (0.007)
Lower Keys-Northern	0.081 (0.009)	0.173 (0.010)	0.290 (0.009)	0.535 (0.012)	0.148 (0.004)	0.308 (0.016)	0.320 (0.013)	0.163 (0.007)
Everglades	0.968 (0.004)	0.611 (0.014)	0.055 (0.008)	0.279 (0.016)	0.379 (0.020)	0.993 (0.002)	0.874 (0.008)	0.225 (0.014)

Heterozygote Deficit

	Locus							
Population	AAT_03	AAT_10	AAT_16	AAT_21	AAT_26	AAT_28	AAT_40	AAT_60
Lower Keys-Eastern	0.500 (0.019)	0.382 (0.006)	0.988 (0.002)	0.056 (0.003)	0.042 (0.003)	0.875 (0.011)	0.127 (0.007)	0.702 (0.020)
Lower Keys-Western	0.858 (0.012)	0.825 (0.006)	0.994 (0.001)	0.792 (0.007)	0.233 (0.011)	0.904 (0.010)	0.051 (0.008)	1.000 (0.001)
Lower Keys-Northern	0.829 (0.011)	0.365 (0.013)	0.146 (0.006)	0.259 (0.011)	0.771 (0.004)	0.844 (0.013)	0.603 (0.016)	0.187 (0.007)
Everglades	0.463 (0.017)	0.788 (0.013)	0.010 (0.004)	1.000 (0.000)	0.074 (0.009)	1.000 (0.000)	0.360 (0.015)	0.286 (0.017)

Heterozygote Excess

	Locus							
Population	AAT_03	AAT_10	AAT_16	AAT_21	AAT_26	AAT_28	AAT_40	AAT_60
Lower Keys-Eastern	0.516 (0.020)	0.631 (0.006)	0.045 (0.005)	0.947 (0.003)	0.975 (0.002)	0.133 (0.012)	0.878 (0.006)	0.464 (0.018)
Lower Keys-Western	0.238 (0.018)	0.203 (0.006)	0.016 (0.001)	0.214 (0.007)	0.787 (0.010)	0.125 (0.011)	0.936 (0.008)	0.029 (0.005)
Lower Keys-Northern	0.211 (0.011)	0.623 (0.013)	0.901 (0.006)	0.759 (0.013)	0.240 (0.005)	0.231 (0.016)	0.440 (0.017)	0.819 (0.008)
Everglades	0.714 (0.015)	0.485 (0.016)	1.000 (0.000)	0.343 (0.018)	0.935 (0.009)	0.202 (0.015)	0.853 (0.011)	0.758 (0.016)

APPENDIX 3

TESTS FOR LINKAGE DISEQUILIBRIUM

Locus Pair		Chi-squared	df	p-value
AAT_03	AAT_10	4.191	6	0.651
AAT_03	AAT_16	1.128	6	0.980
AAT_10	AAT_16	5.067	6	0.535
AAT_03	AAT_21	2.061	6	0.914
AAT_10	AAT_21	5.832	8	0.666
AAT_16	AAT_21	12.431	6	0.053
AAT_03	AAT_26	6.928	6	0.328
AAT_10	AAT_26	6.669	6	0.353
AAT_16	AAT_26	9.478	6	0.148
AAT_21	AAT_26	13.392	6	0.037
AAT_03	AAT_28	∞	6	≤ 0.001
AAT_10	AAT_28	5.985	8	0.649
AAT_16	AAT_28	0.754	6	0.993
AAT_21	AAT_28	2.737	8	0.950
AAT_26	AAT_28	7.511	6	0.276
AAT_03	AAT_40	8.421	6	0.209
AAT_10	AAT_40	5.099	8	0.747
AAT_16	AAT_40	8.679	6	0.192
AAT_21	AAT_40	3.462	8	0.902
AAT_26	AAT_40	12.399	6	0.054
AAT_28	AAT_40	12.001	8	0.151
AAT_03	AAT_60	11.126	6	0.085
AAT_10	AAT_60	13.570	8	0.094
AAT_16	AAT_60	2.945	6	0.816
AAT_21	AAT_60	8.309	8	0.404
AAT_26	AAT_60	6.689	6	0.351
AAT_28	AAT_60	7.232	8	0.512
AAT_40	AAT_60	8.560	8	0.381

APPENDIX 4

TEST OF GENIC AND GENOTYPIC DIFFERENTIATION

BY LOCUS:

Locus	Populations		Genic Differentiation Probability	Genotypic Differentiation P-value
AAT_03	Keys East	Keys West	0.000 (0.000)	0.000 (0.000)
AAT_03	Keys East	Keys North	**0.227 (0.008)**	**0.188 (0.006)**
AAT_03	Keys East	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_03	Keys West	Keys North	0.000 (0.000)	0.000 (0.000)
AAT_03	Keys West	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_03	Keys North	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_10	Keys East	Keys West	0.000 (0.000)	0.000 (0.000)
AAT_10	Keys East	Keys North	0.002 (0.001)	0.006 (0.001)
AAT_10	Keys East	Everglades	0.000 (0.000)	0.001 (0.000)
AAT_10	Keys West	Keys North	0.001 (0.000)	0.000 (0.000)
AAT_10	Keys West	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_10	Keys North	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_16	Keys East	Keys West	0.001 (0.000)	0.000 (0.000)
AAT_16	Keys East	Keys North	**0.322 (0.008)**	**0.289 (0.006)**
AAT_16	Keys East	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_16	Keys West	Keys North	0.000 (0.000)	0.000 (0.000)
AAT_16	Keys West	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_16	Keys North	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_21	Keys East	Keys West	0.000 (0.000)	0.000 (0.000)
AAT_21	Keys East	Keys North	**0.637 (0.006)**	**0.601 (0.006)**
AAT_21	Keys East	Everglades	0.000 (0.000)	0.002 (0.000)
AAT_21	Keys West	Keys North	0.001 (0.000)	0.000 (0.000)
AAT_21	Keys West	Everglades	0.003 (0.001)	0.002 (0.001)
AAT_21	Keys North	Everglades	0.006 (0.001)	0.011 (0.001)
AAT_26	Keys East	Keys West	0.000 (0.000)	0.000 (0.000)
AAT_26	Keys East	Keys North	**0.577 (0.006)**	**0.609 (0.004)**
AAT_26	Keys East	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_26	Keys West	Keys North	0.000 (0.000)	0.000 (0.000)
AAT_26	Keys West	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_26	Keys North	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_28	Keys East	Keys West	0.000 (0.000)	0.000 (0.000)
AAT_28	Keys East	Keys North	**0.527 (0.009)**	**0.414 (0.008)**
AAT_28	Keys East	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_28	Keys West	Keys North	0.000 (0.000)	0.000 (0.000)
AAT_28	Keys West	Everglades	0.000 (0.000)	0.000 (0.000)

Locus	Populations		Genic Differentiation Probability	Genotypic Differentiation P-value
AAT_28	Keys North	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_40	Keys East	Keys West	0.000 (0.000)	0.000 (0.000)
AAT_40	Keys East	Keys North	0.000 (0.000)	0.000 (0.000)
AAT_40	Keys East	Everglades	0.003 (0.001)	0.000 (0.000)
AAT_40	Keys West	Keys North	0.000 (0.000)	0.006 (0.001)
AAT_40	Keys West	Everglades	0.001 (0.000)	0.004 (0.001)
AAT_40	Keys North	Everglades	0.010 (0.002)	0.007 (0.001)
AAT_60	Keys East	Keys West	0.000 (0.000)	0.000 (0.000)
AAT_60	Keys East	Keys North	0.001 (0.000)	0.003 (0.001)
AAT_60	Keys East	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_60	Keys West	Keys North	0.000 (0.000)	0.000 (0.000)
AAT_60	Keys West	Everglades	0.000 (0.000)	0.000 (0.000)
AAT_60	Keys North	Everglades	0.000 (0.000)	0.000 (0.000)

OVER ALL LOCI:

Genic Differentiation

Population Pair		Chi-squared	df	p-value
Keys East	Keys West	8	16	= 0.001
Keys East	Keys North	8	16	= 0.001
Keys East	Everglades	8	16	= 0.001
Keys West	Keys North	8	16	= 0.001
Keys West	Everglades	8	16	= 0.001
Keys North	Everglades	8	16	= 0.001

Genotypic Differentiation

Population Pair		Chi-squared	df	p-value
Keys East	Keys West	8	16	= 0.001
Keys East	Keys North	8	16	= 0.001
Keys East	Everglades	8	16	= 0.001
Keys West	Keys North	8	16	= 0.001
Keys West	Everglades	8	16	= 0.001
Keys North	Everglades	8	16	= 0.001

APPENDIX 5

ASSIGNMENT TESTS

TEST 1:
Silver Rice Rat Data: Assignement to Three Keys Subpopulations and Everglades
Criterion: Rannala & Mountain (1997) Bayesian Analysis
Simulation algorithm: Paetkau et al. (2004)
Number of simulated individuals: 10000

Quality Index: 67.87%
Correctly Assigned: 79.7% (63 individuals)

Locality of Individual	Eastern Subpopulation	Western Subpopulation	Northern Subpopulation	Everglades
East	0.1647	0.0013	0.3639	0.0569
East	0.0440	0.0018	0.7053	0.0319
East	0.0827	0.0038	0.0487	0.0176
East	0.5574	0.0062	0.3285	0.1096
East	0.0135	0.0041	0.0102	0.1130
East	0.0623	0.0443	0.2735	0.0112
East	0.1289	0.1280	0.5939	0.0075
East	0.1110	0.0011	0.0922	0.0226
East	0.3701	0.0218	0.5080	0.1261
East	0.2019	0.0533	0.7764	0.0279
East	0.5787	0.0108	0.8532	0.0394
East	0.0015	0.1385	0.2555	0.0126
East	0.4781	0.0428	0.7725	0.0601
East	0.8767	0.0158	0.9295	0.0490
East	0.6833	0.0835	0.6108	0.0626
East	0.8089	0.0051	0.8794	0.1598
East	0.8861	0.0920	0.3516	0.0079
East	0.8912	0.0049	0.7196	0.2840
East	0.8213	0.0326	0.7651	0.0260
East	0.9302	0.0199	0.8162	0.0486
West	0.0600	0.0782	0.0047	0.0632
West	0.0322	0.4079	0.0270	0.0005
West	0.1286	0.3916	0.1494	0.0073
West	0.0086	0.3858	0.0014	0.0296

Locality of Individual	Eastern Subpopulation	Western Subpopulation	Northern Subpopulation	Everglades
West	0.0203	0.1642	0.0135	0.0185
West	0.0145	0.4467	0.5543	0.0020
West	0.0000	0.0648	0.0119	0.0520
West	0.0019	0.5328	0.0109	0.0179
West	0.0002	0.0332	0.0264	0.0276
West	0.0019	0.3992	0.0018	0.0628
West	0.1381	0.4865	0.2946	0.0033
West	0.0000	0.0001	0.0007	0.0279
West	0.0088	0.8965	0.0296	0.0045
West	0.0001	0.1485	0.0516	0.0255
West	0.0003	0.9481	0.0026	0.0220
West	0.0002	0.7827	0.0079	0.0055
West	0.0026	0.3765	0.0029	0.1169
West	0.0057	0.5752	0.0015	0.0313
West	0.0054	0.8801	0.0011	0.1642
West	0.0032	0.8066	0.0055	0.0531
West	0.0029	0.9376	0.0037	0.0529
West	0.0077	0.8319	0.0006	0.1895
West	0.0012	0.8620	0.0003	0.0477
West	0.0225	0.4066	0.3434	0.0028
West	0.0018	0.7803	0.0003	0.1337
West	0.0154	0.0902	0.0159	0.1059
West	0.0252	0.8184	0.0410	0.0039
West	0.0012	0.1260	0.0134	0.0030
West	0.0113	0.5647	0.0119	0.0317
North	0.0043	0.0105	0.1676	0.0015
North	0.0118	0.1851	0.1415	0.0028
North	0.0000	0.0002	0.1704	0.0123
North	0.6777	0.0158	0.9237	0.0103
North	0.0003	0.0001	0.1409	0.0005
North	0.8026	0.0155	0.8907	0.0499
North	0.0019	0.0008	0.1870	0.0581
North	0.4359	0.1405	0.9566	0.0224
North	0.0102	0.0048	0.8614	0.0003
North	0.5261	0.0017	0.7604	0.0546
North	0.0015	0.0038	0.0086	0.0000
North	0.2316	0.0108	0.8283	0.0135

Locality of Individual	Eastern Subpopulation	Western Subpopulation	Northern Subpopulation	Everglades
North	0.4278	0.0047	0.5093	0.0504
North	0.0192	0.0000	0.3123	0.0418
North	0.0053	0.0002	0.5028	0.0809
North	0.0003	0.0001	0.4352	0.0075
North	0.0080	0.0002	0.2382	0.0783
North	0.0003	0.0026	0.0987	0.0064
North	0.0078	0.0054	0.2838	0.0063
North	0.0483	0.0002	0.2949	0.0670
Everglades	0.0000	0.0000	0.0000	0.1925
Everglades	0.0000	0.0009	0.0000	0.3218
Everglades	0.0000	0.0000	0.0000	0.7202
Everglades	0.0000	0.0000	0.0000	0.0899
Everglades	0.0000	0.0000	0.0000	0.1255
Everglades	0.0000	0.0000	0.0000	0.0150
Everglades	0.0000	0.0000	0.0000	0.2079
Everglades	0.0000	0.0000	0.0000	0.2126
Everglades	0.0000	0.0000	0.0000	0.1050
Everglades	0.0000	0.0000	0.0000	0.0904

TEST 2:
Silver Rice Rat Data: Assignement to Three Keys Subpopulations and Everglades
Criterion: Paetkau et al. (1995)
Simulation Algorithm: Paetkau et al. (2004) Frequency Based
Number of Simulated Individuals: 10000

Quality Index: 68.95%
Correctly Assigned: 81.0% (64 individuals)

Locality of Individual	Eastern Subpopulation	Western Subpopulation	Northern Subpopulation	Everglades
East	0.2181	0.0005	0.4021	0.0583
East	0.0882	0.0007	0.6406	0.0256
East	0.0993	0.0018	0.0667	0.0187
East	0.5569	0.0007	0.3696	0.1076
East	0.0202	0.0007	0.0190	0.0643
East	0.0706	0.0268	0.2855	0.0234
East	0.1602	0.1008	0.5210	0.0196
East	0.1459	0.0004	0.1454	0.0256
East	0.5091	0.0068	0.3823	0.0812
East	0.1716	0.0329	0.7247	0.0419
East	0.5518	0.0024	0.7972	0.0218
East	0.0013	0.1052	0.1162	0.0124
East	0.4634	0.0232	0.7006	0.0448
East	0.8684	0.0048	0.8998	0.0329
East	0.6507	0.0490	0.4812	0.0283
East	0.8102	0.0009	0.8481	0.1480
East	0.8991	0.0649	0.3969	0.0168
East	0.8962	0.0007	0.6506	0.2312
East	0.8266	0.0137	0.7065	0.0401
East	0.9279	0.0059	0.7527	0.0438
West	0.0313	0.0960	0.0071	0.0426
West	0.0337	0.3865	0.0313	0.0017
West	0.0880	0.3881	0.1377	0.0125
West	0.0073	0.3752	0.0055	0.0426
West	0.0175	0.1404	0.0064	0.0125
West	0.0257	0.4438	0.4812	0.0081
West	0.0001	0.0404	0.0127	0.0861
West	0.0041	0.5375	0.0117	0.0232

Locality of Individual	Eastern Subpopulation	Western Subpopulation	Northern Subpopulation	Everglades
West	0.0003	0.1010	0.0130	0.0082
West	0.0008	0.3646	0.0020	0.0205
West	0.1007	0.4953	0.3167	0.0112
West	0.0001	0.0005	0.0013	0.0345
West	0.0102	0.8981	0.0115	0.0090
West	0.0001	0.1226	0.0327	0.0466
West	0.0002	0.9392	0.0006	0.0205
West	0.0001	0.7838	0.0016	0.0098
West	0.0024	0.3515	0.0020	0.1626
West	0.0047	0.5783	0.0024	0.0368
West	0.0029	0.8716	0.0005	0.1177
West	0.0037	0.7837	0.0013	0.0171
West	0.0034	0.9332	0.0009	0.0154
West	0.0051	0.8346	0.0003	0.1410
West	0.0008	0.8654	0.0006	0.0384
West	0.0421	0.4052	0.2512	0.0097
West	0.0011	0.7864	0.0006	0.1058
West	0.0288	0.2917	0.0078	0.0774
West	0.0245	0.8214	0.0224	0.0058
West	0.0004	0.0945	0.0091	0.0070
West	0.0099	0.5538	0.0103	0.0128
North	0.0067	0.0205	0.0844	0.0060
North	0.0213	0.1632	0.1211	0.0095
North	0.0001	0.0002	0.0831	0.0160
North	0.6784	0.0052	0.8836	0.0200
North	0.0009	0.0001	0.2314	0.0040
North	0.8043	0.0048	0.8310	0.0329
North	0.0013	0.0007	0.0861	0.0861
North	0.4017	0.1156	0.9253	0.0085
North	0.0116	0.0029	0.8085	0.0016
North	0.5135	0.0001	0.6806	0.0583
North	0.0053	0.0072	0.0433	0.0007
North	0.1823	0.0079	0.7873	0.0295
North	0.4032	0.0022	0.4078	0.0546
North	0.0167	0.0000	0.2017	0.0426
North	0.0074	0.0001	0.3761	0.0426
North	0.0001	0.0000	0.3078	0.0033

Locality of Individual	Eastern Subpopulation	Western Subpopulation	Northern Subpopulation	Everglades
North	0.0107	0.0003	0.1963	0.0434
North	0.0003	0.0005	0.0400	0.0155
North	0.0136	0.0009	0.1788	0.0155
North	0.0446	0.0000	0.2905	0.0413
Everglades	0.0000	0.0000	0.0000	0.2022
Everglades	0.0000	0.0009	0.0000	0.2504
Everglades	0.0000	0.0000	0.0000	0.7096
Everglades	0.0000	0.0000	0.0000	0.0935
Everglades	0.0000	0.0000	0.0000	0.1156
Everglades	0.0000	0.0000	0.0000	0.0097
Everglades	0.0000	0.0000	0.0000	0.2350
Everglades	0.0000	0.0000	0.0000	0.2057
Everglades	0.0000	0.0000	0.0000	0.0432
Everglades	0.0000	0.0000	0.0000	0.1162

TEST 3:
Assignment of Individuals
Title: Silver Rice Rat Data: Assignment to Two Keys Subpopulations and Everglades
Criterion: Ranala & Mountain (1997)
Simulation Algorithm: Paetkau et al. (2004)
Number of Simulated Individuals: 10000

Quality Index: 81.79%
Correctly Assigned: 93.70% (74 individuals)

Locality of Individual	North & East Combined	Western Subpopulation	Everglades
East	0.3187	0.0015	0.0544
East	0.5620	0.0021	0.0278
East	0.1351	0.0034	0.0176
East	0.6769	0.0043	0.1103
East	0.0200	0.0034	0.1140
East	0.1895	0.0441	0.0109
East	0.5567	0.1258	0.0073
East	0.1963	0.0013	0.0203
East	0.6734	0.0178	0.1276
East	0.6325	0.0525	0.0240
East	0.8411	0.0075	0.0349
East	0.1283	0.1365	0.0122
East	0.7780	0.0428	0.0570
East	0.9697	0.0115	0.0466
East	0.7670	0.0829	0.0604
East	0.9219	0.0041	0.1603
East	0.8566	0.0936	0.0074
East	0.9026	0.0039	0.2811
East	0.8930	0.0318	0.0224
East	0.9417	0.0157	0.0463
East	0.1486	0.0069	0.0010
East	0.1060	0.1894	0.0024
East	0.0580	0.0006	0.0119
East	0.9313	0.0114	0.0102
East	0.0340	0.0001	0.0007
East	0.9611	0.0104	0.0473
East	0.0761	0.0008	0.0551

Locality of Individual	North & East Combined	Western Subpopulation	Everglades
East	0.8440	0.1396	0.0200
East	0.5333	0.0038	0.0004
East	0.7879	0.0019	0.0523
East	0.0094	0.0040	0.0004
East	0.6784	0.0074	0.0132
East	0.6419	0.0038	0.0482
East	0.1911	0.0000	0.0361
East	0.2382	0.0001	0.0810
East	0.1191	0.0001	0.0072
East	0.1156	0.0004	0.0771
East	0.0529	0.0024	0.0060
East	0.1733	0.0041	0.0054
East	0.1936	0.0001	0.0657
West	0.0647	0.0782	0.0616
West	0.0713	0.4033	0.0006
West	0.2805	0.3943	0.0071
West	0.0118	0.3842	0.0252
West	0.0841	0.1640	0.0178
West	0.3901	0.4503	0.0013
West	0.0031	0.0628	0.0498
West	0.0071	0.5373	0.0176
West	0.0080	0.0312	0.0236
West	0.0010	0.4067	0.0606
West	0.3615	0.4897	0.0026
West	0.0000	0.0001	0.0240
West	0.0250	0.8949	0.0038
West	0.0325	0.1503	0.0221
West	0.0017	0.9492	0.0199
West	0.0027	0.7868	0.0047
West	0.0227	0.3775	0.1171
West	0.0227	0.5818	0.0276
West	0.0111	0.8801	0.1649
West	0.0076	0.8004	0.0511
West	0.0063	0.9387	0.0507
West	0.0116	0.8325	0.1918
West	0.0036	0.8618	0.0462
West	0.3527	0.4147	0.0024
West	0.0027	0.7756	0.1347

Locality of Individual	North & East Combined	Western Subpopulation	Everglades
West	0.0339	0.0911	0.1064
West	0.0531	0.8152	0.0029
West	0.0084	0.1235	0.0025
West	0.0216	0.5682	0.0276
Everglades	0.0000	0.0000	0.1833
Everglades	0.0000	0.0011	0.3076
Everglades	0.0000	0.0000	0.7102
Everglades	0.0000	0.0000	0.0888
Everglades	0.0000	0.0000	0.1273
Everglades	0.0000	0.0000	0.0144
Everglades	0.0000	0.0000	0.2050
Everglades	0.0000	0.0000	0.2217
Everglades	0.0000	0.0000	0.0982
Everglades	0.0000	0.0000	0.0861

TEST 4:
Assignment of Individuals
Title: Silver Rice Rat Data: Assignment to Two Keys Subpopulations and Everglades
Criterion: Paetkau et al. (1995)
Simulation Algorithm: Paetkau et al. (2004) Frequency Based
Number of Simulated Individuals: 10000

Quality Index: 82.67%
Correctly Assigned: 92.4% (73 individuals)

Locality of Individual	North & East Combined	Western Subpopulation	Everglades
East	0.4571	0.0006	0.0582
East	0.5131	0.0008	0.0276
East	0.1724	0.0022	0.0187
East	0.6512	0.0008	0.1070
East	0.0451	0.0008	0.0645
East	0.2435	0.0267	0.0250
East	0.5136	0.1014	0.0200
East	0.2665	0.0004	0.0276
East	0.6226	0.0073	0.0782
East	0.5966	0.0333	0.0417
East	0.8231	0.0024	0.0229
East	0.0552	0.1060	0.0125
East	0.7611	0.0236	0.0448
East	0.9640	0.0049	0.0350
East	0.7223	0.0484	0.0308
East	0.9215	0.0014	0.1425
East	0.8407	0.0637	0.0170
East	0.8906	0.0011	0.2281
East	0.8940	0.0148	0.0406
East	0.9315	0.0058	0.0443
East	0.0812	0.0209	0.0067
East	0.1120	0.1633	0.0096
East	0.0169	0.0003	0.0164
East	0.9221	0.0055	0.0206
East	0.0790	0.0002	0.0043
East	0.9577	0.0052	0.0350
East	0.0291	0.0010	0.0816

Locality of Individual	North & East Combined	Western Subpopulation	Everglades
East	0.8248	0.1150	0.0090
East	0.4728	0.0029	0.0015
East	0.7674	0.0002	0.0582
East	0.0391	0.0065	0.0012
East	0.6683	0.0081	0.0319
East	0.6023	0.0023	0.0533
East	0.1104	0.0000	0.0427
East	0.1525	0.0002	0.0427
East	0.0536	0.0000	0.0030
East	0.0782	0.0003	0.0412
East	0.0154	0.0005	0.0153
East	0.0982	0.0016	0.0153
East	0.2401	0.0002	0.0417
West	0.0198	0.0979	0.0427
West	0.0768	0.3853	0.0016
West	0.2003	0.3822	0.0126
West	0.0062	0.3696	0.0427
West	0.0342	0.1394	0.0126
West	0.3210	0.4445	0.0090
West	0.0057	0.0460	0.0816
West	0.0134	0.5424	0.0245
West	0.0036	0.1011	0.0090
West	0.0016	0.3619	0.0210
West	0.2846	0.4859	0.0114
West	0.0008	0.0010	0.0361
West	0.0173	0.9021	0.0093
West	0.0078	0.1247	0.0475
West	0.0004	0.9440	0.0209
West	0.0007	0.7703	0.0101
West	0.0037	0.3460	0.1575
West	0.0040	0.5765	0.0379
West	0.0012	0.8814	0.1159
West	0.0034	0.7905	0.0172
West	0.0025	0.9318	0.0152
West	0.0012	0.8351	0.1367
West	0.0012	0.8593	0.0387
West	0.2817	0.4030	0.0100
West	0.0009	0.7821	0.1051

Locality of Individual	North & East Combined	Western Subpopulation	Everglades
West	0.0291	0.2874	0.0748
West	0.0509	0.8147	0.0065
West	0.0036	0.1000	0.0078
West	0.0150	0.5533	0.0130
Everglades	0.0000	0.0000	0.2020
Everglades	0.0000	0.0014	0.2539
Everglades	0.0000	0.0000	0.6897
Everglades	0.0000	0.0000	0.0931
Everglades	0.0000	0.0000	0.1198
Everglades	0.0000	0.0000	0.0104
Everglades	0.0000	0.0000	0.2385
Everglades	0.0000	0.0000	0.2039
Everglades	0.0000	0.0000	0.0417
Everglades	0.0000	0.0000	0.1103

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