# FIFTY YEARS OF ANTHROPOGENIC PRESSURE: TEMPORAL GENETIC VARIATION OF THE ENDEMIC FLORIDA MOUSE (*Podomys floridanus)*

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2010

To God, who gave me a wonderful family.

ACKNOWLEDGMENTS

I am especially thankful to my advisor David Reed for his continued guidance, support and encouragement over the course of this project. I would also like to thank the members of my committee Lyn Branch and James Austin for their comments.

I would like to thank my entire family for their support: my husband for being part of this adventure and his suggestions and support, my parents, my brother and sister for their support throughout the years and my sons for giving me inspiration.

Without the continued support from several organizations and people, this project would not be possible. Funding and logistic support was provided by University of Florida-School of Natural Resources and Environment and Florida Museum of Natural History. I thank the San Felasco Hammock Preserve for allowing me to do fieldwork in the Preserve and for providing scientific information. The following people must be thanked for their help with information, laboratory support and field work : Candace McCaffery, Larry Harris, Hopi Hoeskstra, Jesse Weber, Julie Allen, Jorge Pino, Angel Soto-Centeno, Lisa Barrow, Chelsey Spirson, Bret Pasch, Sergio Gonzalez, Daniel Pearson and Luis Ramos.

I would also like to extend my gratitude to my friends: Candace McCaffery, Caroll Mercado, Marie Claude Arteaga, Heidy Resnikowski, Jorge Pino, Pilar Fuente Alba, Pablo Pinedo, Luis Ramos, Laura Magana, Antonio Rocabado and Mario Villegas for the encouragement given.

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May 2010

Chair: David Reed
Major: Interdisciplinary Ecology

Habitat fragmentation is one of the most important threats to global biodiversity. Although, some of Florida's ecosystems are naturally fragmented, human development has greatly increased this fragmentation and reduced available habitat for many species. Habitat fragmentation may impede gene flow between adjacent populations and may have significant consequences for population genetic structure.

The Florida Mouse (*Podomys floridanus*) is the only mammal genus endemic to the Florida peninsula. It is considered a *vulnerable species* by the IUCN, based on the extensive loss of habitat in the last fifty years. Its narrow habitat specificity makes it especially vulnerable to habitat loss. Florida mouse habitat is in high demand for human development because of its high, dry, and well-drained soils, suitable for building homes and for agricultural uses.

The objective of this study is to determine whether reduction in populations identified by different researchers over the last fifty years as a consequence of habitat loss can be identified through change in genetic variability. Using molecular markers, I compared genetic variation in two geographic areas, Highlands County (Archbold Biology Station) from 1957, 1983 and 2006, and Alachua County (San Felasco

Hammock Preserve) from 1958 and 2009. Fourteen microsatellite loci were genotyped from museum skins and recently caught specimens from The Florida Museum of Natural History. These microsatellites are presented in this study as a tool for genetic population analysis and sixty-two additional microsatellites are described for future use.

In Highlands County, population diversity declined from 1957 to 1983. Reduction in genetic diversity (Heterozygosity) suggested that this population underwent a bottleneck. In 2006, dramatic increases in heterozygosity suggest the possibility of a restoration of genetic diversity through gene flow.

In San Felasco Preserve, I documented a reduction in the effective number of alleles and a reduction in effective population size over the last 50 years. San Felasco is identified as an area with limited opportunity for recolonization; therefore a decrease in population size appears to be driving a reduction in genetic variability through genetic drift.

Comparing current populations of Florida mice from Highlands County (2009) and Alachua County (2006), I found that the Alachua population has fewer alleles, lower effective population size and lower heterozygosity than the Highlands population. This difference might be explained by the observation that San Felasco has limited opportunity for recolonization from neighboring populations. Further, differences in habitat in Highlands and Alachua counties may account for some of the differences in genetic variability at the two sites.

# CHAPTER 1
# INTRODUCTION

The Florida Mouse (*Podomys floridanus*) is the only mammal genus endemic to the Florida peninsula (Layne 1992). It is considered a *vulnerable species* by the IUCN, *threatened* by the Florida Committee on Rare and Endangered Plants and Animals (Kirkland 1998) and is considered a *Species of Special Concern* by the Florida Game and Fresh Water Fish Commission (Wood 1996, Kirkland 1998). Its narrow habitat specificity makes it especially vulnerable to habitat loss (Layne 1992). Florida mouse habitat is in high demand for human development because of its high, dry, and well-drained soils, suitable for building homes and for agricultural uses (Layne 1992).

*P. floridanus* was originally described in the genus *Hesperomys* (Chapman 1889). Later it was moved to the genus *Peromyscus* as *Peromyscus floridanus* (Bangs 1898) and was placed in the subgenus *Podomys* (Osgood 1909). Carleton (1980) elevated *Podomys* to the generic rank after a major revision of the genus *Peromyscus*. *P. floridanus* is very similar in external morphology to *Peromyscus* but they are most closely related to the genera *Habromys* and *Neotomodon* (Layne 1992). A recent study of *Peromyscus* phylogenetics based on mitochondrial cytochrome-*b* sequence data differs significantly from the most current taxonomic arrangement, placing *Podomys, Habromys, Megadontomys, Neotomodon, Osgoodomys* within *Peromyscus* (Bradley et al. 2007). Of the other rodents within the geographic range of *Podomys*, it is most likely to be confused with *Peromyscus gossypinus* (the cotton mouse). They are very similar in appearance but the Florida mouse can be distinguished by the presence of five well–developed plantar tubercles on the soles of the hind feet in contrast to six in the cotton mouse. Typical of all *Peromyscus* relatives, *P. floridanus* has a fragile tail sheath, but

larger eyes and ears than species of *Peromyscus* in Florida (Layne 1992) (Figure 2-1) Moreover *P. floridanus* has low levels of genetic variability compared with other species of *Peromyscus* (Smith 1973).

The habitat for *P. floridanus* is primarily scrub and sandhill associations, restricted to fire-maintained, xeric and upland vegetation. Scrub may be the original and primary habitat of the Florida mouse, while sandhill vegetation is a secondary habitat that may not have been generally occupied until historic times when the original habitat was degraded (Layne 1990, Layne 1992). These two vegetation types are closely linked ecologically and historically, however they are different in species composition and physiognomy (Myers 1990): Sandhill, is dominated by open stands of longleaf pine (*Pinus palustris*) or south Florida slash pine (*Pinus elliottii*), deciduous oaks, turkey oak (*Quercus laevis*), and ground cover composed primarily of wire grass (*Aristida stricta*). On the other hand, Sand pine scrub, is dominated by a closed overstory of sand pine (*Pinus clausa*), rosemary (*Ceratiola ericoides*) with an understory of evergreen scrub oaks and a little or no herbaceous ground cover. Scrub habitat occurs on sand ridges or old dunes with deep, fine sand and consists of a closed to open canopy of sand pines (*P. clausa*) (Enge 1999).

Many former sandhill and scrub sites have been converted to pine plantations, citrus groves and urban areas (Layne 1992, Myers 1990, Kirkland 1998). Human activities have reduced high pine areas by more than 90%, as well as the number and size of scrub patches (Myers 1990). As a result, Florida's scrub is one of the most endangered ecosystems in the southeastern United States. Thirteen species of scrub plants and five vertebrates are listed as endangered or threatened under the U.S.

Endangered Species Act (Clark et al. 1999). In addition, much of the sand pine scrub association along the Atlantic coast has been destroyed with resultant loss of *Podomys* populations (Layne 1992).

From the early 1940s to 1980s, 64% of the xeric upland habitat suitable for *Podomys* in Highlands County was destroyed, and an additional 10% was disturbed (Peroni 1983, Layne 1992). In the late 1990s, Lake Wales Ridge, the oldest of Florida's ridges, had been reduced to a mere 15% of its original upland habitat (Menges 1999). In addition, suppression of fire, and the resultant habitat conversion, has reduced *P. floridanus* populations in many of the remaining sandhill and scrub habitats (Layne 1992). A long–term monitoring study on Florida mouse populations on Smith Lake sandhill in Putman County (Newman 1997) reported a population decline beginning in the 1980s. Similarly, trapping success of Florida mice showed the magnitude of the reduction. Eisenberg and Franz had trapping success of 43% in 1983, whereas Jones had trapping success of 12% from 1984-1988 and Newman had 1% success in 1996. All three studies use the same trapping technique in the same area of Smith Lake sandhills. Additionally, Smith (1973) reported low population densities in 26 localities in Florida. However, it should be noted that there are few studies of this type, which can assess long-term trends in population density or relative abundance of Florida mice. The commonality or rarity of *P. floridanus* in any given part of its range is largely unknown.

Other scrub species, such as the Gopher Tortoise (*Gopherus polyphemus*), have been affected by extensive land modification and have seen population reductions and extirpations. Gopher Tortoise populations have been drastically reduced in the last

decade, with losses of 33% in some Florida counties (Auffenberg et al. 1981). In the same way, populations of Florida Scrub Jays (*Aphelocoma coerulescens*) have declined statewide by 50% over the last 100 years (Fitzpatrick et al. 1991). Scrub-jay population loss along the Lake Wales Ridge is estimated at 80% or more since pre-European settlement (Fitzpatrick et al. 1991).

Some of Florida's ecosystems, such as scrub, are naturally fragmented and many species have fragmented distributions within scrub habitat (Menges 1999); however human development has further increased this fragmentation and reduced the habitat for many species. Fragmentation and loss of habitat is a major problem facing biodiversity worldwide (Noss et al. 2006). Fragmentation can lead to isolation of populations, reduction in population size, and reduced gene flow, all of which can decrease genetic diversity (Frankham 1996). Loss of genetic diversity can decrease the ability of an organism to cope with novel environmental challenges (Scribner et al. 2006). Small and isolated populations tend to lose genetic variation over time through genetic drift. Inbreeding can result in lower survival and reproduction (Frankham 1995; Reed and Frankham 2003) as well as increasing risk of extinction (Amos and Balmford 2001; Lowe et al. 2004, Saccheri et al. 1998; Westemeier et al. 1998).

Based on the reported population declines of *P. floridanus*, the objective of this study is to determine whether there has been a significant change in genetic diversity in populations of *P. floridanus* over the last 50 years and whether significant reductions in population size can be detected with molecular data. This thesis is divided in four chapters; the first chapter is the introduction, Chapter 2 is a description of 14 microsatellites markers for use in studies of the Florida mouse (*Podomys floridanus*).

Chapter 3 is a comparison of genetic variability over a fifty-year time span in two Florida counties, and finally Chapter 4 is the conclusion of this study.

# CHAPTER 2
# FOURTEEN MICROSATELLITE LOCI FOR GENETIC POPULATION STUDIES OF THE ENDEMIC FLORIDA MOUSE (*Podomys floridanus*)

## 2.1 Introduction

Genetic variation is the raw material of evolution. Species that maintain a large degree of genetic diversity within populations have a greater chance to persist through times of adverse conditions. In recent years many molecular markers have been developed to measure genetic variation within and among populations. There are two categories of markers: Co-dominant markers that allow us to identify all the alleles that are present at a particular locus; and dominant markers that reveal only a single dominant allele (Freeland 2005). Microsatellite loci (Co-dominant markers) were selected in this study, on the basis of their effectiveness in population genetic studies. Microsatellites are short tandem repeats of nucleotides located throughout nuclear and chloroplast genomes and have been found in the mitochondrial genomes of some species (Freeland 2005).

Microsatellites are one of the most popular molecular markers used in population genetics. They have high levels of polymorphism that result from a high mutation rate of around $10^{-3}$ or $10^{-4}$ events per locus per replication in mice (Dallas 1992). This high polymorphism makes them very desirable for inferring relatively recent population genetic events (Freeland 2005, Allendorf and Luikart 2008). Moreover, homologous microsatellites can often be easily assayed across a wide number of closely related species (Weber et al. 2009, Prince et al. 2002). Homologous sequences in different

species often show a degree of similarity to one another because they are descended from the same ancestral gene. Therefore, looking for molecular markers for one species by examining the genome of a closely related species is rapidly increasing the efficiency of finding new markers for population-level studies. The genus *Podomys* is nested within the genus *Peromyscus* based on molecular data (Bradley et al. 2007). Therefore, I evaluated 526 primer pairs that amplify microsatellite DNA loci for *Peromyscus maniculatus bairdi* (Weber et al. 2009). From this set I selected primers that successfully amplified microsatellites in *P. floridanus*. A panel of fourteen microsatellites described in this chapter as well as a longer list of 62 additional primer pairs, can be very helpful for future population genetic studies of *P. floridanus*.

## 2.2 Methods

### 2.2.1 Primer Selection

Primers used for DNA amplification were originally developed for *Peromyscus maniculatus bairdii* by Weber and colleagues from Harvard University (Hoeskstra laboratory). They described 526 primer pairs (Weber et al. 2009) that amplify microsatellite DNA loci for *P. maniculatus bairdii*. From this set of 526, 480 primers were tested for three individual Florida mice (from DNA extracts of fresh tissue at approximately 40-50 ng/uL).

In collaboration with Hoekstra Laboratory, PCR was performed in a reaction volumes of 15uL: 1uL template, 1.5uL of 10x buffer, 0.3uL of 10mM total dNTPs, 0.6uL forward primer (1um), 0.6uL reverse primer (10um), labeled CAG Primer (5'CAGTCGGGCGTCATCA 3'), 0.3uL of $MgCl_2$, 0.15uL taq and 10.01uL of water. The amplification had an initial denaturation step at 94˚C for 2 min, followed by 20 cycles of 20s at 94˚C, 20s at 60˚C (decreasing 5 ˚C ) and 30s at 72˚C. Then followed by 15

cycles of 20s at 94˚C; 20s at 50˚C, 30s at 72˚C and a final extension step of 72˚C for 5 minutes. Amplifications were visualized with agarose gel electrophoresis. In total, 76 microsatellite loci successfully amplified for *Podomys floridanus*.

After several tests of these 76 microsatellites in the Reed Laboratory (University of Florida), I selected fourteen microsatellites to analyze temporal and geographic genetic variation in populations of *P. floridanus* (Chapter 3).

### 2.2.2 DNA Amplification (PCR) of 14 Microsatellite Loci

PCR amplification was performed in a final volume of 25uL containing 12.5uL Microsatellite master mix (Qiagen), 9uL of water, 0.5uL of forward primers (1uM) and 0.5uL reverse primers (10uM), 0.5uL of dye 6-FAM (10uM) and 2uL of DNA template (40-50ng/uL). Forward primers in each pair were modified on the 5' end to include a sequence tag M-13 (5'-CACGACGTTGTAAAACGAC-3') allowing the use of a third oligonucleotide in the PCR that is fluorescently labeled for detection.

The amplification was carried out under the following condition: initial denaturalization step at 95˚C for 5 min, followed by 20 cycles of 30s at 95˚C, 1min 30s at 60˚C (decreasing 0.5˚C each time) and 30s at 72˚C. Then followed by, 15 cycles of 30s at 95˚C; 1min 30s at 48˚C, 30s at 72˚C and a final extension step of 72˚C for 30 min.

## 2.3. Results

In Table 2-1 are fourteen primer pairs for microsatellites useful for population studies of *P. floridanus*. The size of microsatellites ranged from 120-396 base pairs. Microsatellite repeats were di and tetra-nucleotide.

These microsatellites were used to study the population genetics of Florida mice in Highlands County at 3 periods of time and in Alachua County at two periods of time

(Chapter 3). All microsatellites were polymorphic, however two loci (pmbw392, pmbw400) were monomorphic for two populations. The overall number of alleles (na) ranged from 4 to 27. The mean expected heterozygosity is 0.79 and the mean observed heterozygosity is 0.66 (Table 2-2).

Sixty-two additional primer pairs that amplified in Florida mice are available in this chapter (Table 2-3). They were not used for population analysis, but they are a potential resource for future studies.

## 2.4. Discussion

The use of primers developed for *Peromyscus maniculatus bardii* to amplify microsatellites in *Podomys floridanus* was sufficient to acquire enough variable markers for a population genetic study of *P. floridanus*. The ability of these markers to achieve amplification beyond *Peromyscus* to *Podomys* extends the utility of the work by Weber et al. (2009), who also identified amplification in *Peromyscus polionotus subgriseus*.

Primer pairs developed in one species can often be used in closely related species because priming sites are generally chosen in highly conserved regions of sequence data (Allendorf et al. 2008). Many studies have reported cross-species amplification of microsatellites. For example, of 18 microsatellite loci developed for *Panthera tigris sumatrae*, 16 of these markers produced a single band in all three tiger and ten non-tiger felid species (Williamson et al. 2002). Along the same lines, cross-species amplification of 6 microsatellite loci in big brown bats (*Eptesicus fuscus*), was successfully performed in species of the family Vespetilionidae and Antrozoidae (Vonhof et al. 2002).

The primer pairs presented in this study offer the first list of microsatellite markers that can be used for population genetics or genetic mapping studies in *P. floridanus*. Many population analyses require large numbers of loci to be statistically sound, such as measuring the effects of bottleneck events, which require at least 10-20 polymorphic microsatellites (Lowe et al. 2006). There are other genetic markers available for Florida mice, such as mitochondrial markers (cytochrome *b*) and proteins at the National Center for Biotechnology Information (Genbank; http://www.ncbi.nlm.nih.gov). However microsatellites are very suitable for inferring relatively recent population events. They can easily discriminate genetically between individuals and populations (Freeland 2005).

Table 2-1. Fourteen microsatellite primer pairs for Florida mouse population studies.

| Locus Pmbw | Forward Primers | Reverse Primers | Size Range |
|---|---|---|---|
| 52 | ACTGTGCAATCAGCCTAC | ATGTTCCCCTTCTACCTC | 272-304 |
| 206 | CTTGTTGTGACTAGTGGAGG | TCTTGATACGAAGGCAGC | 220-250 |
| 217 | ACACCATAAGATGGGCAGAC | AGCTGAATTGGTCCAGTGAC | 274-314 |
| 219 | TGTCAAGGGTCCTCTATCTG | TAACCCAAGCATTCTCACTG | 278-336 |
| 220 | TAATCCACTCACCTCATCTG | TTAAGTTGAAGACCAACCTG | 254-296 |
| 252 | AGCTTCCCCCATTATTTG | ACAACAGCCAGGAAATGC | 184-296 |
| 264 | TGGTTACAATCTCCCCTTTC | TCCTGCTTTGCCTTTATCTC | 196-396 |
| 274 | GCTCAGTAAAAGAGCCTTGC | CCAGCCAAACCTAGTCAGTG | 218-264 |
| 294 | AAATTCAGGCCAAGTGTG | AACAGGAAAGCAGCAATG | 122-170 |
| 392 | TCTCTGGTCCAAACCTTTC | TCTACTGTCACCTTGCTGTG | 120-130 |
| 400 | AATCTGGGTTTACAAAGGATAC | ATGGCAGACATTACAAGAGC | 146-154 |
| 403 | AGACCCACCTACCCTTGC | TACCAATAGTTCCCTAAACAC | 156-238 |
| 421 | TGTTTGCTTCAAGCTCGC | GCCCTCTTACCTTACACCAC | 166-284 |
| 428 | TGAAGTGAGTAAAGGAAGCAG | GTCCTCCAAGATTGAATGC | 155-193 |

Table 2-2. Observed and expected heterozygosity and number of alleles (Na) for 14 microsatellite loci surveyed in 93 Florida mice.

| Locus | He observed | He expected | Na |
|---|---|---|---|
| Pmbw 52 | 0.75 | 0.87 | 14 |
| Pmbw 206 | 0.76 | 0.85 | 13 |
| Pmbw 217 | 0.80 | 0.92 | 18 |
| Pmbw 219 | 0.85 | 0.93 | 24 |
| Pmbw 220 | 0.75 | 0.90 | 19 |
| Pmbw 252 | 0.72 | 0.90 | 18 |
| Pmbw 264 | 0.75 | 0.90 | 18 |
| Pmbw 274 | 0.75 | 0.89 | 16 |
| Pmbw 294 | 0.29 | 0.65 | 9 |
| Pmbw 392 | 0.16 | 0.18 | 4 |
| Pmbw 400 | 0.27 | 0.49 | 4 |
| Pmbw 403 | 0.88 | 0.88 | 14 |
| Pmbw 421 | 0.76 | 0.93 | 27 |
| Pmbw 428 | 0.68 | 0.82 | 16 |
| Mean | 0.66 | 0.79 | 15 |

Table 2-3. Sixty-two primer pairs that amplify microsatellites in Florida mice (Size range for *Peromyscus*)

| Locus Pmbw | Forward Primers | Reverse Primers | Size Range |
|---|---|---|---|
| 12 | TTCTGTGGCCTCTGTGAACG | ACATTGGCTGTAAGTCTGGG | 302-328 |
| 59 | TCAGTGAGTAAAGGTGTTC | AGTACATTGGATAGCATAC | 308 |
| 89 | ACTGATGCAAGCATAACC | ACTTTCTGTGCCTTTGAG | 235 |
| 91 | CAAGTTTGGTGGGAAGGC | TTGAGATGCAAATGGGTG | 242 |
| 95 | AAATTGGACTGTGGGTAG | ATAGCCAGTGCATTCTTC | 273-277 |
| 197 | CACCCGATTGGTCTAATAAC | TTCTAGCTCCAGGCATCTTC | 186-279 |
| 210 | TTCAGGCATGTCACAGATTC | AGCATTCGGTAGCATAATTG | 240-262 |
| 227 | CCCAAGGCAAAGCGTATC | ATCCCAGCAATGGAAACC | 273-390 |
| 247 | TAAAAGAGGCAAACCAAATG | CTCCCAAAGGTGGCAGTG | 305-314 |
| 251 | AGCATTGATATGAGGGTTTG | ATCTAGAACTGCTAAGGATTCC | 271-279 |
| 260 | GCAAGGGGAGAAGGCTAG | GAAACCTTCCCATACCAGAG | 279 |
| 263 | CCTGGCTTCTCAGAGTGGTG | TGCTGTTTAGAGGCATTTGG | 305 |
| 272 | AGACATAGAGTTTACCACAATC | GTGTAGCTGAAAGTGGCAC | 182-190 |
| 300 | TTCCTTTTGTTCTCCCTG | GATCTGTTAGTTTCTGACCTAC | 247-307 |
| 302 | GAACAACAAGGGACAAGC | ATGCCATTTATTCATACCC | 265-274 |
| 304 | TGATCCCAACAGCTTACAC | GAAGCCTAAGTCCATTCTTC | 270-278 |
| 346 | CCCTGAGGAAGACAATTTC | GCCTAACAGGAGCTGAAC | 145-179 |
| 348 | TTAAAGCCAGTTACTCATCC | CCAAAGTTCCAGGACATATC | 156 |
| 349 | TGAAAAGGCAGACTGGAAG | AACTCTGAGAAGCTGTGAGG | 273-283 |
| 356 | CTCTGCTCTTCTTTGCCTAC | CCTGTTCTGATGGGTTGG | 299 |
| 363 | GAACAATACCAAAGACTATCC | TCAGTGTAACTCTACAGCATC | 291 |
| 364 | AGGTCGTGGCTGCTTATG | TGTGCACAGAATGACTTTATC | 153 |
| 393 | TGCCTGTGACATCACCATC | GCTGGAGGGGATGTATGAG | 294-306 |
| 399 | CACTGGGTGAACTACAACCTC | ACCTCAGGGTTCCTACCTG | 230-264 |
| 402 | GAAAACCAGTTCTCCCATTC | TTATCTGAGCAGTTAGGCAAG | 254 |
| 406 | CCTAACATCAACTGAAGTCTCC | GCCAAAGTTTAGTGTTTATCTC | 221-237 |
| 416 | TCCACAAAGTTCCCTCTG | ACCTACATGGTAACTGTCTTAC | 109-118 |
| 429 | AGTTTGAGGCCAACTTAGG | TCAATTGATTTGTCCAAAAG | 302-328 |
| 430 | TGGACAGCCAGTTGATACAC | AAGTGGACAGGGAAAAGC | 175-186 |
| 431 | ACTGTTAGCAGCACGAGC | AGAGATGCCATCTGTCTGTC | 281-285 |
| 433 | CACTCCAGAAACAGAGGTAGG | AAGCTTTGAACTCCTTATCAG | 147-155 |
| 436 | CAGAGGCAGGTGGGTCTC | TCTCGCTCAGTTTCATTCC | 220-250 |
| 448 | TTTCTTTCACTTTCTTTGCC | CATGATGTCAACCTCTGGTG | 234 |
| 449 | TCAGTGGCCTTTCTGTATC | TTCCAGCTTCTAGTGTCTTC | 251 |
| 455 | GTTCTTTAACTCAGTCCCAAG | CTTTACCCACCCACCTTC | 190-228 |
| 457 | ATGGCTGGCTTAGACTGG | CTCTTTGAATTTATGGCCTC | 259 |
| 467 | CTCCCTGTCTGGGGTGAG | GTTCAGTTCCCTGCTTGC | ~ 100 |
| 469 | TTTCAACAACTTCCAGGTC | AGGCAGGTTTCATCATAAC | 229 |
| 478 | ATTAGAGGCCCAACAATG | CTCCAGGTCAGTTAGACAGC | 205 |
| 479 | TGGGACTGTTTGGGTAGC | CAAAATTACTGTGGTTCATCC | 135-141 |
| 489 | TTCAAAATCACCCTGATACTC | ACAGAAAGACAGAAAGGACAG | 264-276 |
| 491 | ACACCTGGTTCCCTCCTC | AACTGCTAAGTCAACAGAAAAG | 242-251 |
| 492 | ACTTCCTAGTAAGCCCTATTTC | CTGCTCCCAAATTATCTGTC | 141-145 |
| 500 | GTAGCAGTAAACATTCCAGATC | GGTATACAGCCTAGGTGGAG | 272 |

Table 2-3. Continued

| Locus Pmbw | Forward Primers | Reverse Primers | Size Range |
|---|---|---|---|
| 506 | CTCATTCCTCAGCCATTTAC | AGCCATGATATTTCTGTTCC | 254-275 |
| 508 | AGGGACACTGAGATGGCTAC | GGAAATCTACTGATGGGCAC | 223-233 |
| 518 | AAAGGATGACAGGTTGGAAC | AATACCCAGGGAAGAAGAAC | 187 |
| 523 | CAGACACTGCCAAGGAGG | GCTAGAGCGGGTGGTTAG | 260 |
| 526 | TCACATTGATCTTGACAACTG | CAAGCACTTTATCAAATAAGC | 249-326 |
| 527 | ATGGGTAAGAGGGAAAGG | AGAAATTCTGATCCGAAATAG | 274-282 |
| 533 | TGAATCAGAGGGCAGAGC | TGGAGAAGTGGCAAAGAAG | 190-253 |
| 534 | GCTTATTCCTCGGCCTCTAG | GGTGGCTCCTCATGCTTC | 118-127 |
| 574 | TTCTTGCAGAGGTAATGAAGTC | TCTCTTGAGGGTATGGTTATTC | 242 |
| 581 | ATACCACAGGTGAGGCTCTTC | TTCTGACTCTTTCTTCCCAAC | 298 |
| 586 | CCTTAGTTTGTGATGATGG | GCTGAGACAGGAGGATTGC | 212-252 |
| 599 | AGATTGTGAGCAACTGTATGG | ACTGCCCTCTCAAGACAAAAG | 239-251 |
| 604 | ACTCAAACCCAGGTCATCG | CTGCCCTCTCAAGACAAAAG | 212-219 |
| 635 | GGGGAGAATAGATTTGGTTAG | CTGTGCGTCTGCCTGTC | 312-327 |
| 639 | GACAACAGACACCCCTAATC | GTTCCCAGCACCCAAATAG | 254-307 |
| 662 | AGCCACCTCAAGAGTCATC | CTTGGGACTTGCCATACAG | 297 |
| 665 | GGGCACACAAGCACTATTTC | CCAGGCTGACCTCAAACTC | 195 |
| 667 | GCTGCCTGCTGAGAATC | GAAGCCTGGTCTCAAGTAAC | 229-269 |

Figure 2-1. Photo of *Podomys floridanus* trapped in San Felasco Hammock Preserve

# CHAPER 3
# FIFTY YEARS OF ANTHROPOGENIC PRESSURE: TEMPORAL GENETIC VARIATION OF THE ENDEMIC FLORIDA MOUSE (*Podomys floridanus)*

## 3.1 Introduction

The Florida mouse (Fig. 2-1), the only endemic mammal of Florida, has undergone population declines lately. In the last 50 years researchers reported a high reduction in habitat suited for this species and as consequence a decrease in population levels (Newman 1997, Layne 1992, Peroni 1983). In an effort to identify the degree to which this reduction has caused a loss in genetic diversity, I examined allele variation in museum and recently caught specimens from the Florida Museum of Natural History (FLMNH, Gainesville, Florida).

Specimens stored in museum collections represent the genetic diversity of past populations often predating anthropogenic changes. Thus, museum specimens present the remarkable opportunity to step back in time and assess pre-disturbance levels of genetic diversity in populations that may be presently depleted or extirpated. Ancient DNA techniques allow the measurement of temporal changes in allele frequencies and gene coalescence that at present can only be estimated from spatially distributed living populations (Roy et al. 1994).

The FLMNH has a large collection of *P. floridanus* specimens. There are more than 1900 specimens collected by different researchers since 1925 in twenty Florida Counties: Alachua, Brevard, Citrus, Clay, Gilchrist, Hernando, Highland, Taylor, Indian River, Lake, Levy, Marion, Nassau, Orange, Palm Beach, Pinellas, Putnam, St. Jones, St. Lucie, and Sumter (Figure 3-1). This collection represents an important genetic resource that will permit reconstruction of past population structure, the estimation of past bottlenecks and other population genetic estimates.

Genetic diversity is one of the three forms of biodiversity recognized by the IUCN as deserving conservation, along with species and ecosystem diversity (McNeely et al. 1990). The amount of genetic variation within a population gives insight into the demographic structure and evolutionary history of a population. For instance, lack of genetic variation may indicate that the population has gone through a recent, dramatic reduction in size (Allendorf and Luikart 2008).

Understanding how much genetic variation has been lost over time in populations of Florida mice can play an important role in their conservation and management. Furthermore, as its habitat has become highly developed, the data generated in this study will be useful for inferring possible loss of genetic variation in other areas with similar biologic and anthropogenic characteristics.

## 3.2. Methods

### 3.2.1 Sample Selection

The FLMNH has *P. floridanus* specimens from twenty counties in Florida. Highlands and Alachua counties were selected for this study because they contained many geographically close samples that ranged in collection dates from the 1950s to the present. Samples from other counties contained too few specimens from any single time period to be of use.

A total of 93 specimens of Florida mice were studied from populations in Archbold Biological Station (ABS) in Highlands County from 1957, 1983, 2006, and two populations from 1958 and 2009 from San Felasco Hammock Preserve (SFH) in Alachua County. All Samples from Archbold Biological Station were taken from the FLMNH collections, twenty specimens were taken from 1957, twenty from 1983 and nineteen specimens from 2006 (collected by different researchers).

For San Felasco, twenty specimens from 1958 were taken from FLMNH, and fourteen individuals were trapped and released alive in 2009. I trapped within the trapping grid used by James Layne: Stations 21 and 31 (Figure 3.2) during his extensive studies of *Podomys* (Layne's field notes are held at the FLMNH). I trapped in collaboration with people from FLMNH for 8 nights between April 17$^{th}$ and May 7$^{th}$. Four hundred traps were set every night and a total of 14 Florida mice were captured. The distal portion of their tail (sans vertebrae) was removed from each individual. Their tails were marked with permanent ink, treated with an antibiotic to stave off infection, then the mice were released within an hour of capture. No individual mouse was sampled more than once (recapture was plainly evident by their tail tips being missing and ink on their tail). Lastly tails clips from fresh specimens were stored at -20C until DNA extraction.

Liver tissue was taken from specimens collected in Highlands 2006 (10 mg) during the preparation of voucher specimens at the FLMNH. Tissue samples from historical museum specimens were taken from skin (10 mg), which was cut from the suture line on the ventral side of the prepared specimen and stored at -20C.

### 3.2.2 DNA Extraction

The total DNA extraction was carried out using the Qiagen Kit for animal tissue following the manufacture's instructions. In the case of museum skin samples we used the same protocol but tissues were first soaked in 250µl 1X PBS overnight in the refrigerator. DNA extraction was suspended in a final volume of 200uL of elution buffer (for fresh tissue) and 60uL (for museum samples) and stored at -20˚C.

To reduce the chance of contamination, DNA extractions from historical skins were carried out in the ancient DNA laboratory at the FLMNH. All materials (tips, tubes, etc.) were UV irradiated prior to labwork.

### 3.2.3 DNA Amplification (PCR)

Primers used for DNA amplification were originally developed for *Peromyscus maniculatus bairdii* by Weber and colleagues from Harvard University (see Chapter 2 of this thesis). For the present study I used 14 microsatellite loci: pmbw52, pmbw206, pmbw217, pmbw219, pmbw220, pmbw252, pmbw264, pmbw274, pmbw294, pmbw392, pmbw400, pmbw403, pmbw421, pmbw428 (Table 2.1).

PCR amplification was performed in a final volume of 25uL containing 12.5uL Microsatellite master mix (Qiagen), 9uL of water, 0.5uL of forward primers (1uM) and 0.5uL reverse primers (10uM), 0.5uL of dye and 2uL of DNA template (40-50 ng/mL). Forward primers in each pair were modified on the 5' to include a sequence tag M-13 (5'-CACGACGTTGTAAAACGAC-3') allowing the use of a third oligo in the PCR that is fluorescently labeled for detection.

The amplification was carried out under the following conditions: initial denaturation step at 95˚C for 5 min, followed by 20 cycles of 30s at 95˚C, 1 min 30s at 60˚C (decreasing 0.5˚C each time) and 30s at 72˚C. Then followed by 15 cycles of 30s at 95˚C, 1 min 30s at 48˚C, 30s at 72˚C and a final extension step of 72˚C for 30 min.

All PCR products were visualized on a 2% agarose gel for verification of amplification. In order to monitor contamination, negative controls were run for PCR and run on electrophoresis gels along with samples. Finally, 6uL of PCR product was sent for genotyping (ICBR Genetic Laboratory- University of Florida).

### 3.2.4 Data Treatment and Statistics

This study considered a total of five samples (three samples from Highlands County and two samples from Alachua County, collected at different periods of time). To assign individuals to populations, I used the Bayesian clustering software STRUCTURE v. 2.2 (Pritchard et al. 2007). This software assigns individuals to 1 of *k* populations, allowing for admixture, without a priori knowledge of the source population. The value "*k*" is defined by the user and I evaluated values of *k* from 1 to 7. I allowed a burn-in of 10,000 replicates and sampled from the subsequent 2 million generations.

Microsatellite alleles sometimes do not amplify during PCR (null alleles) and heterozygotes can be genotyped erroneously as homozygotes. To determine presence of null alleles, GENEPOP v.4 software was used. In addition, linkage disequilibrium was tested in order to identify non-random association of alleles among loci using GENEPOP v.4.

Loci were analyzed using Genemarker software (Softgenetics). This program calculates *allele (microsatellite)* lengths. Fourteen microsatellite loci were successfully amplified for four of the five populations; only 7 loci could be genotyped for the 1958 population from San Felasco. Six genetic diversity parameters were estimated using the program PopGene (Yeh et al. 1999): allele frequencies, percent polymorphic loci (P), number of alleles per locus (Na), effective number of alleles per locus (ne), observed heterozygosity (Ho) and expected Heterozygosity (He, computed using Levene1949). Genetic distance among populations was also analyzed with PopGene. Significant differences among populations for He, Na and ne were tested with a paired t-test. Tests for deviations from Hardy–Weinberg equilibrium (HWE) were performed across all loci using GENEPOP 4.0 (Rousset, 1997), applying the exact test with default

settings of the Markov chain (Dememorization: 10000, Batches: 20, Iterations per batch: 5000). To estimate population differentiation from allele frequency (Fst) and allele size (Rst), GENEPOP 4.0 software was used. GENEPOP was also used to estimate pairwise differentiation and degree of inbreeding (Fis) (Weir and Cockerham 1984).

Effective population size (Ne) was calculated with NeEstimator version 1.3 (Peel et al. 2004) using linkage disequilibrium method (Hill 1981), and LDNE version 1.31 (Waples et al. 2008).

## 3.3 Results

For the fourteen polymorphic loci surveyed, 214 alleles were observed among 93 individuals. The total number of alleles per locus ranged from 4 to 27 (see Table 2-2). There was significant departure from Hardy Weinberg Equilibrium (HWE) detected in the five populations analyzed (see below). Locus pmbw294 showed evidence of null alleles in 4 of the 5 populations studied, and significant linkage disequilibrium was detected between locus pmbw428 and 4 other loci (pmbw206, pmbw264, pmbw294, pmbw400). Thus, loci pmbw294 and pmbw428 were removed from further analysis.

### 3.3.1 Hardy-Weinberg Equilibrium

#### 3.3.1.1 Archbold Biological Station's population, Highlands County

The three populations from Highlands (1957, 1983 and 2006) deviated from Hardy-Weinberg Equilibrium at $\alpha = 0.05$ (Tables 3-1 through 3-3). However for each population we can see that just a few loci show significant departure from HWE, suggesting that these loci may be responsible for the previous significant result when comparing all loci. In the population from 1957 three of 12 loci were out of HWE, the removal of one locus (pmbw392), produced HWE estimates that were not significant (P=0.17). In 1983, I found similar results, two of 12 loci are highly significant however removal of these loci

(pmbw217, pmbw252) produced a non-significant test of HWE (P=0.08). In the same way, three loci from the 2006 population showed significant departure from HWE but removing these loci ( pmbw206, pmbw400, pmbw392) led to a non-significant test of HWE (P=0.19).

For the 1957 population, three loci (pmbw252, pmbw392, and pmbw421) showed significant heterozygote deficiency (Fis) and all loci were polymorphic (Table 3-1). For 1983, four loci (pmbw52, pmbw217, pmbw264, pmbw421) had significant heterozygote deficiency and two loci were monomorphic (pmbw392, pmbw400) (Table 3-2). In 2006, one locus (pmbw220) had a significant deficit of heterozygosity and all loci are polymorphic (Table 3-3).

#### 3.3.1.2 San Felasco Hammock Preserve's populations, Alachua County

The population from 1958 was found to be out of HWE, however a single locus was driving this departure from HWE. After removing this locus (pmbw252), the population was within HWE expectations (P=0.06). Two loci were found to be monomorphic and Fis showed significant deficit of He for two loci (Table 3-4).

Similar to the 1958 population, the current population (2009) had one locus (pmbw421) that was out of HWE. The total population was also out of HWE but in this case removing locus pmbw421 resulted in significant difference from HWE. There was not a significant deficit of He in 2009 and all loci were polymorphic (Table 3-5).

### 3.3.2 Genetic Diversity

The overall Fst for the five populations studied (Highlands and Alachua) was Fst=0.13. Fst values ranged from 0.05-0.25, which indicated moderate genetic differentiation (Freeland 2005). The percent polymorphism per population ranged from 71.4% to 100%. The mean value of observed heterozygosity was Ho = 0.68 and the

expected was He=0.80. All loci were polymorphic except for locus pmbw392 and locus pmbw400 from population ABS-Highlands 1983 and SFH-Alachua 1958, respectively.

#### 3.3.2.1 Genetic diversity in Archbold Biological Station, Highlands County

The total number of alleles in Archbold Station was 166. 120 alleles were identified in the Archbold population of 1957, 103 alleles in 1983 and 119 alleles in 2006. The maximum number of alleles per locus was 24 (pmbw421) and the minimum was 3 (pmbw392). All 12 loci were polymorphic for the 3 populations except two loci (pmbw392 and pmbw400) that were monomorphic in the 1983 population.

In Table 3-6 we see that from 1957 to 1983 there was a significant decline in heterozygosity (P=0.026) and a significant increase from 1983 and 2006 (P=0.049). Interestingly, there was no significant difference in heterozygosity between 1957 and 2006. Although number of alleles and effective number of alleles show the same pattern of reduction from 1957 to 1983 and increase in 2006, there was no significant difference among the values from the three time periods. In addition, private alleles and percent polymorphism exhibit the same pattern.

Levels of genetic diversity averaged across loci in Highlands County indicated moderate genetic differentiation, Fst = 0.07 (Rst=0.005). However, pairwise Fst values indicated little genetic differentiation between 1957 and 1983 (Fst=0.03; Rst=-0.01), moderate genetic differentiation between 1957 and 2006 (Fst=0.08; Rst= 0.02); and moderate differentiation between 1983 and 2006, (Fst= 0.08; Rst=0.002)

#### 3.3.2.2 Genetic diversity in San Felasco Hammock Preserve, Alachua County

From the analysis of five microsatellite loci, 30 alleles were identified in the 1958 population and 21 alleles in 2009. The maximum number of alleles per locus was 13 (pmbw421) and the minimum was 2 (pmbw392, pmbw400). Two loci were

monomorphic (pmbw392 and pmbw400) in the 1958 population and the remaining loci were polymorphic in both populations. Genetic differentiation between past and present populations was pronounced; Fst = 0.36, Rst=0.22 (>0.25, Freeland 2005).

Table 3-7 shows a significant decrease in the effective number of alleles (P= 0.023) from 1958 to 2009. There was an increase in private alleles and polymorphic loci, however the there was neither a significant difference in Heterozygosity (p= 0.13) nor number of alleles (p=0.054).

#### 3.3.2.3 Genetic diversity comparison between Highlands and Alachua populations

In 1957 and 1958 the populations from Highlands and Alachua counties do not showed significant differences in Heterozygosity (P=0.20) or total number of alleles (P=0.078), but they did differ significantly in the number of effective alleles (P=0.01). In contrast, the current population from Highlands had significantly greater levels of heterozygosity (P=0.022), numbers of alleles (P < 0.001) and effective number of alleles (P < 0.001) compared to the current population from Alachua county (Tables 3-8 and 3-9).

### 3.3.3 Genetic Distance

Nei's genetic distance values showed greater distance between populations from 1957 and 2006 than from 1957 and 1983 in Highlands County (Table 3-10). Pairwise Fst indicated little differentiation between1957 and 1983 and moderate differentiation between1957 and 2006 (Table 3-11). The greatest genetic distance observed was between the 1958 and 2009 populations in Alachua County (Table 3-10). This comparison also had the largest Fst estimate.

### 3.3.4 Estimates of Effective Population Size (Ne)

In Highlands County, there was inconsistency in estimates of effective population size over the three times periods and between the two methods used for estimation. Negative values estimate by LDNE for 1983 and 2006 are interpreted as infinitely large effective populations. In contrast, NeEstimator showed a reduction in effective population size from 1957 to 1983 and failed to estimate Ne in the population from 2006 (Table 3-12). The estimates for Alachua County, however, were more consistent between analytical methods. Both software programs estimated a reduction in population size from 1958 and 2009 (Table 3-12).

### 3.3.5 Allele Frequency

In Figure 3-3 we can see the allele frequency distribution for Archbold Biological Station (Highlands). Three periods of time (1957, 1983, and 2006) were compared by locus. Over time the allele frequencies changed in all loci. In total, 26 alleles since 1957 were lacking in 2006 and 19 new alleles were present in 2006.

The 1958 population at San Felasco Preserve also contained alleles lacking in the 2009 population (e.g., in locus pmbw252 six alleles are lost over time). In total for 5 loci, 18 alleles were absent in 2009 population and the current population contained 8 new alleles. Allele frequencies changed for all loci when comparing 1958 to 2009 (Figure 3-4). The current populations from Alachua and Highlands counties had different allele frequencies. Highlands had more alleles than Alachua (Figure 3-5).

### 3.3.6 Population Structure

Structure analysis evaluated values of *k* (number of populations) from 1 to 7 with the highest likelihood associated with *k*= 5 populations (Ln P(D) = -3853.3; Table 3-13 . The populations correspond with the 5 populations we defined *a priori*. Structure

attempts to assign individuals to populations without knowledge of where (or in this case, when) they were collected. The yellow, blue, and green bars represent individuals from the Archbold Biological Station in Highlands County, whereas the purple and red bars represent San Felasco Preserve in Alachua County (Figure 3-6). The output from Structure shows clear distinction between Highlands County and Alachua County with few individuals showing any affinity for the opposite locality. However, there was more continuity across time within a given locality. For example, several of the individuals from the 1957 Highlands population (yellow) had a high probability of being assigned to the 1983 Highlands population (blue). If these were spatially segregated populations, we would say that those individuals showing blue affinities in the 1957 population might be recent migrants. However, because we are talking about temporally sampled populations, we might conclude that a number of haplotypes from the 1983 population were retained through time from the earlier 1957 population. Visually, there seems to be more similarity between 1957 and 1983 than any other two comparisons.

## 3.4. Discussion

### 3.4.1 Historical and Contemporary Genetic Diversity

#### 3.4.1.1 Archbold Biological Station, Highlands County

From 1957 to 1983 the population declined in genetic diversity (heterozygosity, number of polymorphic alleles and private alleles). Genetic diversity may have been reduced by events such as habitat fragmentation where previously widespread populations are divided and effectively reduced in size (Lowe 2006). These results are coincident with the time (1980s) when researchers reported a reduction in populations of Florida mice (Peroni 1983, Layne 1992). In Highlands County, Layne (1992) reported that 64% of suitable habitat was destroyed for *Podomys* from 1940-1981. Similar to the

observations in Highlands County, Newman (1997) identifies a decline in 1980s in Smith Lake sandhill in Putnam County.

Estimates of effective population size (from LDNE and NeEstimator) were inconsistent for Highlands County. Although when a population undergoes a bottleneck its allelic diversity usually decreases and this can lead to reduced expectations of heterozygosity under HWE (Lowe 2006), which is what we see from 1957 to 1983. Given the overall loss of genetic diversity from 1957 to 1983, it is likely that the population experienced a bottleneck and that our analytical methods (LDNE and NeEstimator) were incapable of estimating Ne with enough accuracy to show this. It is possible that the poor estimates of Ne are the result of low sample sizes and could be improved greatly in future studies.

Samples taken from 1957 and 1983 are geographically closer (0.2 miles), than either is to the collecting site from 2006 (7 and 8 miles away, respectively). It is critical when studying population genetic variation through time that each temporal sample is taken from the same geographic locality to avoid sampling from distinctly different populations, and thus inferring genetic change over time erroneously. Populations from Highlands County are very close geographically with no obvious barriers between sites. Additionally, the average home range of Florida mice is 4,042 $m^2$ for males and 2,601 $m^2$ for females, and female home ranges do not overlap, except occasionally with juveniles (Jones 1995). Therefore, I am confident that differences between temporally sampled populations in Highlands County represent temporal changes in allele richness and frequency rather than spatial ones. Nevertheless, the genetic distance between populations from 1957 and 2006 is large (0.49) with moderate differentiation (Fst = 0.08)

between them. These differences could be explained either by spatial variation across known populations over a 7-8 mile distance or by temporal variation of a 50-year time span.

After the decline in genetic diversity from 1957 to 1983 in Highlands County, we see that the 2006 population has a greater heterozygosity, new private alleles and polymorphic alleles. It is possible that gene flow with neighboring populations restored previous levels of genetic variation. In addition, I identified several new alleles present in the current (2006) population that were not found in previous populations. Whilst a population may rapidly recover in size after a bottleneck, the levels of genetic variation do not recover until restored by mutation or gene flow (Lowe 2006).

Fluctuation in heterozygosity from 1957 to 2006 can be also related to changes in habitat quality through time, in response to the natural dynamic of fire. The rate and magnitude of post-fire recovery of reproduction by plants producing seeds or fruits used by animal species has important implications at the community level (Abrahamson and Layne 2002). For example, acorns appear to be a major food source for Florida mouse, when available, and generally higher acorn production correlates with greater abundance of *Podomys* (Layne 1990). Therefore, it is possible that we are seeing population cycling at one time scale (changes in *Podomys* abundance driven by food availability or the affects of fire on habitat) and population decline over a much longer time scale (due to habitat degradation). Teasing these two apart would require far greater sampling than was attempted in this study.

#### 3.4.1.2 San Felasco Hammock Preserve, Alachua County

The pronounced levels of genetic differentiation (Fst = 0.36) as well as the large genetic distance (1.15) between present and historic populations are perhaps surprising

given that I was able to collect in precisely the same area as the 1957 population (less than 0.2 miles). The large genetic differentiation over time, the small effective population size (Ne) and significant reduction in effective number of alleles suggests that the original 1957 population may have undergone a bottleneck in some point in the past (similar to populations from Highlands County in 1983). After a bottleneck, populations from 1957 may have had limited opportunity for recolonization (Layne 1991) with less opportunity for an influx of genes from neighboring populations, to restore genetic diversity. This could lead to a population in 2009 with lower genetic variability. The high levels of genetic differentiation and distance between these populations can be the result of genetic drift, the effect of which is more profound in small populations (Freeland 2005).

James Layne began working in San Felasco Hammock Preserve in 1957. He reported a reduction in Florida mouse populations beginning in 1980 at these sites. Specifically, for Station 6 (sta. 6 is close to my study area) he mentioned that “it appears that the Florida mouse population in this isolated sandhill site has gone extinct” (Layne 1991). Thus, it is likely to expect local extinctions or reductions occurred in other stations in San Felasco.

The two primary effects of genetic drift are changes in allele frequency and loss of genetic variation. The smaller the population’s size, the greater the changes in allele frequency, because the new population will carry only a portion of the genetic diversity that was present in the source population (Lowe 2006, Freeland 2005, Allendorf and Luikart 2008). Reduced levels of genetic diversity may render the population unable to adapt to a changing environment.

### 3.4.2. Contemporary Population's Diversity

Results suggest that the current populations from San Felasco Hammock Preserve (Alachua County) and Archbold Biological Station (Highlands County) have undergone a reduction in population size (bottleneck) in the past. However populations sampled from Alachua County contain fewer alleles (a=70) and less heterozygosity (Ho=0.56) than those of Highlands County (a=119; Ho=0.81). Isolation and limited opportunity for recolonization in San Felasco (station 6) reported by Layne (1991) likely contributed to the low diversity found in Alachua County. Isolated populations will have greater effects from genetic drift and lower genetic variation compared to populations that have greater gene flow with neighboring populations (Freeland 2005).

Patches of habitat suitable for Florida mice are often naturally fragmented. As a consequence of patchiness, habitat quality varies spatially, and many species are distributed as metapopulations linked by occasionally dispersal (Groom 2006). If dispersal between patches becomes impossible for *Podomys* due to distance or lack of corridors, then the populations may decline with less chance of source populations providing new migrants.

Habitat differences in the two counties may also explain differences in genetic variability. Florida mice in Alachua County are found in sandhill habitats whereas the mice from Highlands are found in scrub habitat. Mice in sandhills are usually found in lower in abundance compared to populations in scrub. Layne (1990) identified higher population densities in scrub and smaller home range size. Scrub habitat may have a greater carrying capacity, perhaps because oaks are more abundant. Moreover, Layne attributed the decline of Florida mice in San Felasco to the chronically low carrying capacity of sandhill habitat (Layne 1991).

For the conservation of species inhabiting fragmented habitats, securing dispersal corridors between local habitat patches appears to be a challenge. Connection between areas through biological corridors seems to be important for both study areas here, but especially important for San Felasco Hammock Preserve, where genetic diversity is low and local extinctions and limited opportunity for recolonization were identified previously Layne (1991). Perhaps one area to take into consideration is the west side of the 75 highway recommended by Layne (1991), who indicated that this area could serve as a source of new animals to replenish declines he observed at San Felasco Hammock Preserve.

The Florida mouse is narrowly restricted to fire-maintained habitat. Populations of *Podomys* decline as the habitat becomes dense and shady (Layne 1992). Thus, it is also recommended to continue with periodic burns; burned areas were found with an increase of open areas and more seedling stems (Jones 1990, Newman 1997). Jones (1990) referred to *Podomys* as a good colonist on high sandhills, (e.g., the Ordway property in Putnam County) consequently; an increase of habitat quality and connectivity may facilitate recolonization of *Podomys* populations in the Preserve.

It is suggested for future studies to increase sample sizes and the number of microsatellite loci examined. For this study, I examined 14-20 specimens per population, which was sufficient for the primary questions addressed in this thesis. However, it is likely that better parameters estimates could have been achieved by increasing the number of individuals per population and the number of loci examined (especially for the population from Alachua county in 1958) to better locate rare alleles. Figure 3-7 shows the number of alleles observed by locus in relation to sample size

used in this study (20 individuals, Highlands 1957). For the six loci shown, 100% of the alleles were identified in a sample of 12-18 individuals. However, in one locus (pmbw52) a new allele was found in the 20$^{th}$ individual examined suggesting that more rare alleles could be found in the population with greater sampling effort. I suggested for future research an increase in both sample size and the number of loci examined especially for tests of bottlenecks.

# CHAPTER 4
# CONCLUSION

This study provides evidence that in Highlands County, populations of Florida mice likely declined from the 1950s to the 1980s as evidenced by a large decline in genetic diversity during that time period. However, estimates of genetic diversity returned to 1950s levels by 2006, likely through the process of gene flow with neighboring populations. Thus, there is no significant difference in genetic diversity when comparing the current populations of Highlands County to those of the 1950s. In Alachua County, I found greater genetic differentiation and genetic distance over the same 50 year time span. Clear evidence of a population decline appears to be driving genetic differentiation in Alachua County through genetic drift, likely because of reduced gene flow with neighboring populations, in contrast to the pattern in Highlands County.

When we compare the current populations of Alachua County and Highlands County to one another, we see a significant difference in genetic diversity. The population from Alachua County has fewer alleles, lower heterozygosity and lower effective population size than the Highlands population. This difference can be explained by the fact that San Felasco is identified as an area with limited opportunity for recolonization (Layne 1991) and perhaps by the fact that these populations have

different habitats. Florida mouse density is reportedly lower in sandhills like San Felasco, and population isolation has been reported there previously.

Seventy six primer pairs for *P. floridanus* DNA microsatellites described in this study will constitute a great tool for future population genetics and genetic mapping studies for *P. floridanus*. Fourteen loci were used to conduct population genetic studies.

More studies are needed on the population genetics of *P. floridanus*, in order to know historic and current population structure better. Understanding population genetic change over time can guide management and conservation decisions for this species. To accomplish this I suggest to consider the importance of scientific collection that museums provide, which allows reconstruction of past events though the use of historical specimens.

Table 3-1. Hardy Weinberg Equilibrium test for population ABS-1957. P-value (Fisher's method), standard error and inbreeding coefficient (Fis)

| Locus | P-value | S.E. | Fis W&C | Fis P-value |
|---|---|---|---|---|
| Pmbw 52 | 0.1828 | 0.0215 | -0.0441 | 0.19 |
| Pmbw 206 | 0.8245 | 0.0105 | -0.0772 | 0.88 |
| Pmbw 217 | 0.0445 | 0.0104 | 0.0366 | 0.08 |
| Pmbw 219 | 0.1703 | 0.0330 | 0.0216 | 0.08 |
| Pmbw 220 | 0.4085 | 0.0296 | -0.0688 | 0.80 |
| Pmbw 252 | 0.2106 | 0.0266 | 0.1679 | 0.01 |
| Pmbw 264 | 0.3366 | 0.0308 | -0.0588 | 0.43 |
| Pmbw 274 | 0.2093 | 0.0254 | -0.0570 | 0.72 |
| Pmbw 392 | 0.0255 | 0.0007 | 1.0000 | 0.02 |
| Pmbw 400 | 1.0000 | 0.0000 | -0.1515 | 1.00 |
| Pmbw 403 | 0.9436 | 0.0087 | -0.0688 | 0.84 |
| Pmbw 421 | 0.0454 | 0.0167 | 0.1437 | 0.02 |
| Probability | 0.0399 | | | |

Table 3-2. Hardy Weinberg Equilibrium test for population ABS-1983. P-value (Fisher's method), standard error and inbreeding coefficient (Fis)

| Loci | P-value | S.E. | Fis W&C | Fis P-value |
|---|---|---|---|---|
| Pmbw 52 | 0.0962 | 0.0063 | 0.2199 | 0.004 |
| Pmbw 206 | 0.3238 | 0.0276 | 0.0943 | 0.31 |
| Pmbw 217 | 0.0078 | 0.0037 | 0.1483 | 0.01 |
| Pmbw 219 | 0.4552 | 0.0324 | 0.1000 | 0.27 |
| Pmbw 220 | 0.1940 | 0.0213 | 0.1565 | 0.09 |
| Pmbw 252 | 0.0057 | 0.0024 | 0.0603 | 0.18 |
| Pmbw 264 | 0.4076 | 0.0250 | 0.1510 | 0.03 |
| Pmbw 274 | 0.2191 | 0.0315 | 0.0471 | 0.36 |
| Pmbw 392 | monomorphic | | | - |
| Pmbw 400 | monomorphic | | | - |
| Pmbw 403 | 0.9433 | 0.0035 | -0.2245 | 1.00 |
| Pmbw 421 | 0.0890 | 0.0169 | 0.2177 | 0.002 |
| Probability | 0.0031 | | | |

Table 3-3. Hardy Weinberg Equilibrium test for population ABS-2006. P-value (Fisher's method), standard error and inbreeding coefficient (Fis)

| Loci | P-value | S.E. | Fis W&C | Fis P-value |
|---|---|---|---|---|
| Pmbw 52 | 0.4098 | 0.0200 | 0.0847 | 0.39 |
| Pmbw 206 | 0.0096 | 0.0050 | 0.2707 | 0.05 |
| Pmbw 217 | 0.3484 | 0.0336 | 0.0270 | 0.39 |
| Pmbw 219 | 0.4048 | 0.0433 | 0.0286 | 0.64 |
| Pmbw 220 | 0.1313 | 0.0201 | 0.1751 | 0.01 |
| Pmbw 252 | 0.1296 | 0.0133 | 0.1330 | 0.10 |
| Pmbw 264 | 0.8347 | 0.0113 | 0.0319 | 0.32 |
| Pmbw 274 | 0.8476 | 0.0202 | 0.0033 | 0.47 |
| Pmbw 392 | 0.0383 | 0.0013 | -0.5652 | 1.00 |
| Pmbw 400 | 0.0004 | 0.0003 | -0.5510 | 1.00 |
| Pmbw 403 | 0.4365 | 0.0323 | -0.0426 | 0.65 |
| Pmbw 421 | 0.2518 | 0.0387 | -0.0189 | 0.47 |
| Probability | 0.0013 | | | |

Table 3-4. Hardy Weinberg Equilibrium test for population of SFH-1958. P-value (Fisher's method), standard error and inbreeding coefficient (Fis)

| Loci | P-value | S.E. | Fis W&C | Fis P-value |
|---|---|---|---|---|
| Pmbw 220 | 0.0682 | 0.0092 | 0.1795 | 0.02 |
| Pmbw 252 | 0.0000 | 0.0000 | 0.5412 | 0.00 |
| Pmbw 392 | Monomorphic | | | - |
| Pmbw 400 | monomorphic | | | - |
| Pmbw 421 | 0.2136 | 0.0204 | 0.1258 | 0.05 |
| Probability | Highly significant | | | |

Table 3-5. Hardy Weinberg Equilibrium test for population of SFH-2009. P-value (Fisher's method), standard error and inbreeding coefficient (Fis)

| Loci | P-value | S.E. | Fis W&C | Fis P-value |
|---|---|---|---|---|
| Pmbw 220 | 0.0758 | 0.0088 | 0.0209 | 0.60 |
| Pmbw 252 | 0.0731 | 0.0038 | -0.0954 | 0.45 |
| Pmbw 392 | 0.1133 | 0.0018 | 0.6486 | 0.11 |
| Pmbw 400 | --- | | | - |
| Pmbw 421 | 0.0056 | 0.0010 | 0.2753 | 0.12 |
| Prob. Fisher's m. | 0.0015 | | | |

Table 3-6. Genetic diversity per population (1957, 1983 and 2006) in Highlands County

| Parameters | ABS 1957 | ABS 1983 | ABS 2006 |
|---|---|---|---|
| Number of different alleles (na) | 10 | 8.5 | 9.9 |
| Number of effective Alleles (ne) | 6.2 | 5.9 | 6.3 |
| No. Private Alleles | 1.5 | 0.47 | 1.0 |
| % Polymorphic loci | 100% | 71,4% | 100% |
| Heterozygosity observed (Ho) | 0.75 | 0.65 | 0.81 |
| Unbiased expected Heteroz.(He) | 0.75 | 0.73 | 0.81 |

Table 3-7. Genetic diversity per population (1958 and 2009) in Alachua County

| Parameters | SFH1958 (5 loci) | SFH 2009 (5 loci) |
|---|---|---|
| Number of different alleles (na) | 6 | 4.2 |
| Number of effective Alleles( ne) | 4.5 | 2.6 |
| No. Private Alleles | 1.2 | 0.4 |
| % Polymorphic loci | 71,4% | 100% |
| Heterozygosity observed (Ho) | 0.38 | 0.44 |
| Unbiased expected Heteroz.(He) | 0.52 | 0.49 |

Table 3-8. Genetic diversity comparison between historical populations (1957-1958) and current populations (2006-2009) in Highlands and Alachua counties.

| Parameters | ABS1957 (5 loci) | SFH1958 (5 loci) | ABS2006 (12 loci) | SFH2009 (12 loci) |
|---|---|---|---|---|
| Number of different alleles (na) | 8.6 | 6 | 9.9 | 5.8 |
| Number of effective Alleles( ne) | 5.3 | 4.5 | 6.3 | 2.9 |
| No. Private Alleles | 1.4 | 0.5 | 1.0 | 1.2 |
| % of Polymorphic loci | 100% | 71,4% | 100% | 100% |
| Heterozygosity observed (Ho) | 0.55 | 0.38 | 0.81 | 0.56 |
| Unbiased expected Heteroz.(He) | 0.60 | 0.52 | 0.81 | 0.59 |

Table 3-9. T-test: paired two samples for means in Heterozygosity (He), number of alleles (na) and number of effective alleles (ne) by locus.

| Populations | p-value (He) | p-value (na) | p-value (ne) |
|---|---|---|---|
| ABS1957-ABS1983 (12 loci) | 0.026 | 0.11 | 0.25 |
| ABS1983-ABS2006 (12 loci) | 0.049 | 0.12 | 0.21 |
| ABS1957-ABS 2006 (12 loci) | 0.22 | 0.43 | 0.34 |
| SFH1958-SFH2009 (5 loci) | 0.13 | 0.054 | 0.023 |
| ABS1957-SFH1958 (5 loci) | 0.20 | 0.078 | 0.01 |
| ABS2006-SFH2009 (12 loci) | 0.022 | 0.00083 | 0.0007 |

Table 3-10. Nei's unbiased measured of genetic distance using 12 loci and 5 loci

| Comparison | Genetic distance (12 loci) | Genetic distance (5 loci) |
|---|---|---|
| ABS 1957-1983 | 0.17 | 0.05 |
| ABS 1983-2006 | 0.36 | o.41 |
| ABS 1957-2006 | 0.44 | 0.39 |
| SFH 1958-2009 | 1.13 | 0.80 |
| SFH 1957-ABS 1958 | 0.30 | 0.10 |
| SFH 2009-ABS 2006 | 0.68 | 0.35 |

Table 3-11. Genetic differentiation, pairswise Fst comparison using 12 loci and 5 loci

| Comparison | Pairwise Fst (12 loci) | Pairwise Fst (5 loci) |
|---|---|---|
| ABS 1957-1983 | 0.03 | 0.03 |
| ABS 1983-2006 | 0.08 | 0.13 |
| ABS 1957-2006 | 0.08 | 0.16 |
| SFH 1958-2009 | 0.36 | 0.36 |
| SFH 1957-ABS 1958 | 0.05 | 0.05 |
| SFH 2009-ABS 2006 | 0.17 | 0.15 |

Table 3-12. Effective population size from Highlands and Alachua counties using Linkage disequilibrium method by two different programs (LDNe and NeEstimator)

| Population | Ne Estimator Ne | Lower 95%CI | Upper 95%CI | LDNe Ne | Lower 95%CI | Upper 95%CI |
|---|---|---|---|---|---|---|
| ABS 1957 | 32.4 | 26.6 | 41.0 | 32.2 | 21 | 57 |
| ABS 1983 | 11.9 | 10.1 | 4.3 | -9.7 | -11.2 | Large |
| ABS 2006 | NaN | NaN | NaN | -300.9 | 133.2 | Large |
| SFH 1958 | 22.4 | 9.2 | Large | -56.4 | 8 | Large |
| SFH 2009 | 4.2 | 3.8 | 4.8 | 2.8 | 1.9 | 3.3 |

NaN =not available (NeEstimator). Lowest allele frequency used was 0.05 (LDNE)

Table 3-13. Structure simulation summary

| K | Ln P(D) |
|---|---|
| 1 | -4604.5 |
| 2 | -4225.0 |
| 3 | -4146.5 |
| 4 | -3963.5 |
| 5 | -3853.3 |
| 6 | -3866.3 |
| 7 | -3975.3 |

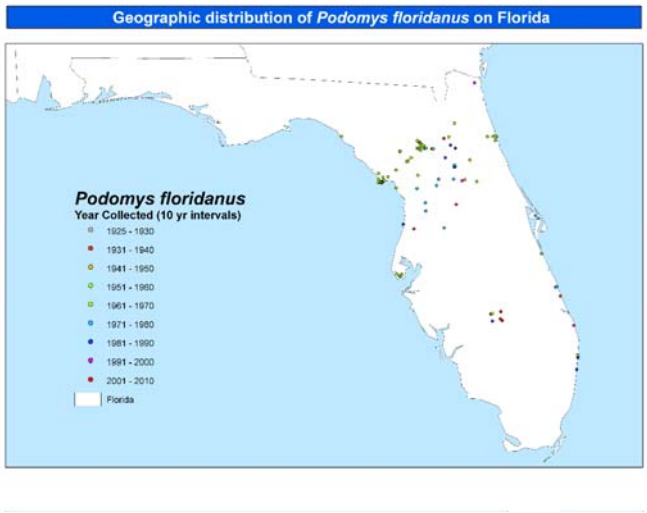


Figure 3-1. Distribution map of *P. floridanus* specimens collected in Florida at FLMNH

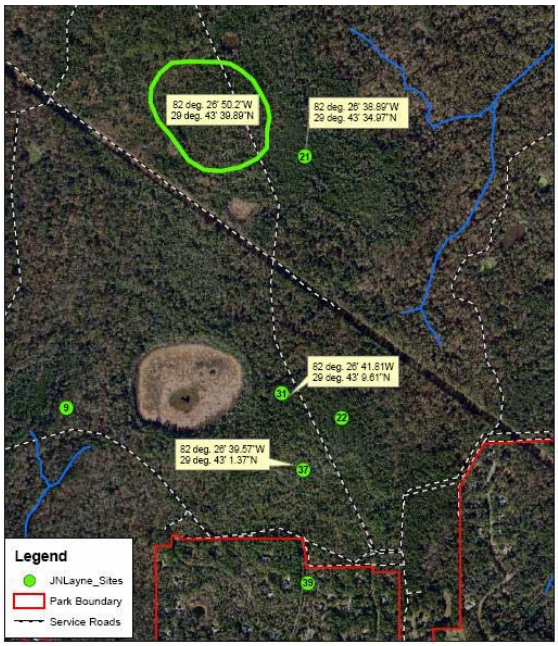


Figure 3-2. Area of Study in San Felasco Hammock Preserve

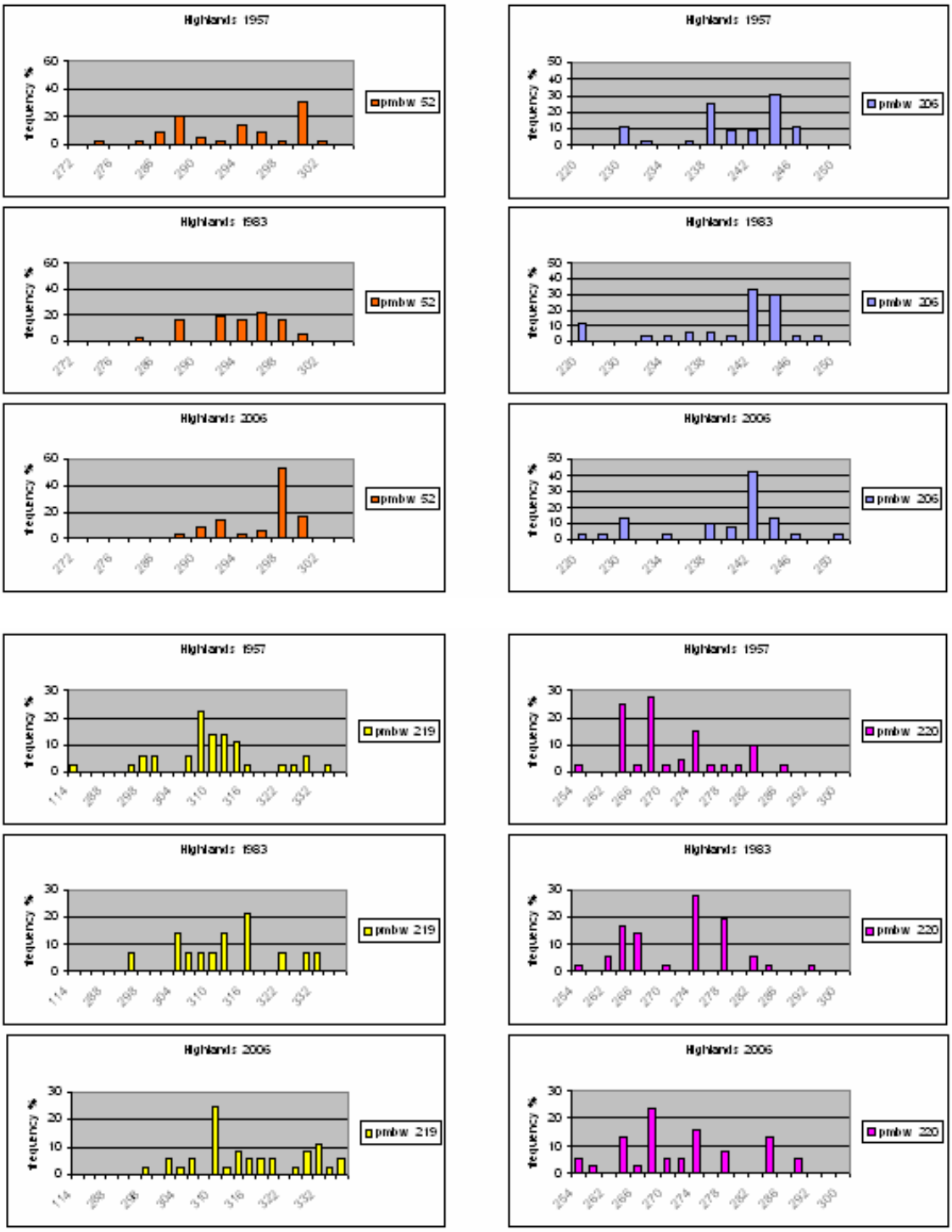


Figure 3-3. Allele frequencies for 12 loci from Highlands County from 1957, 1983 and 2006.

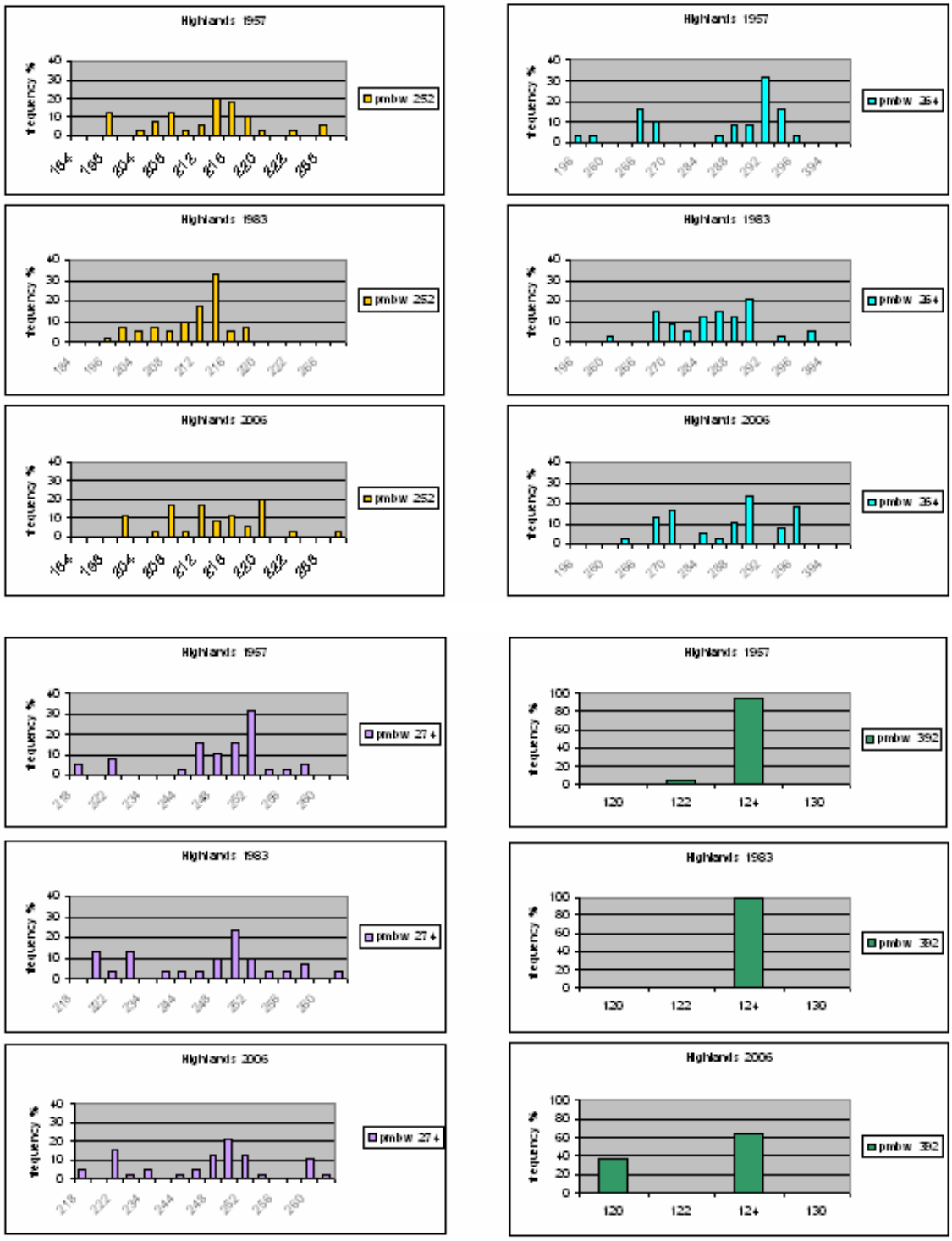


Figure 3-3. Continued

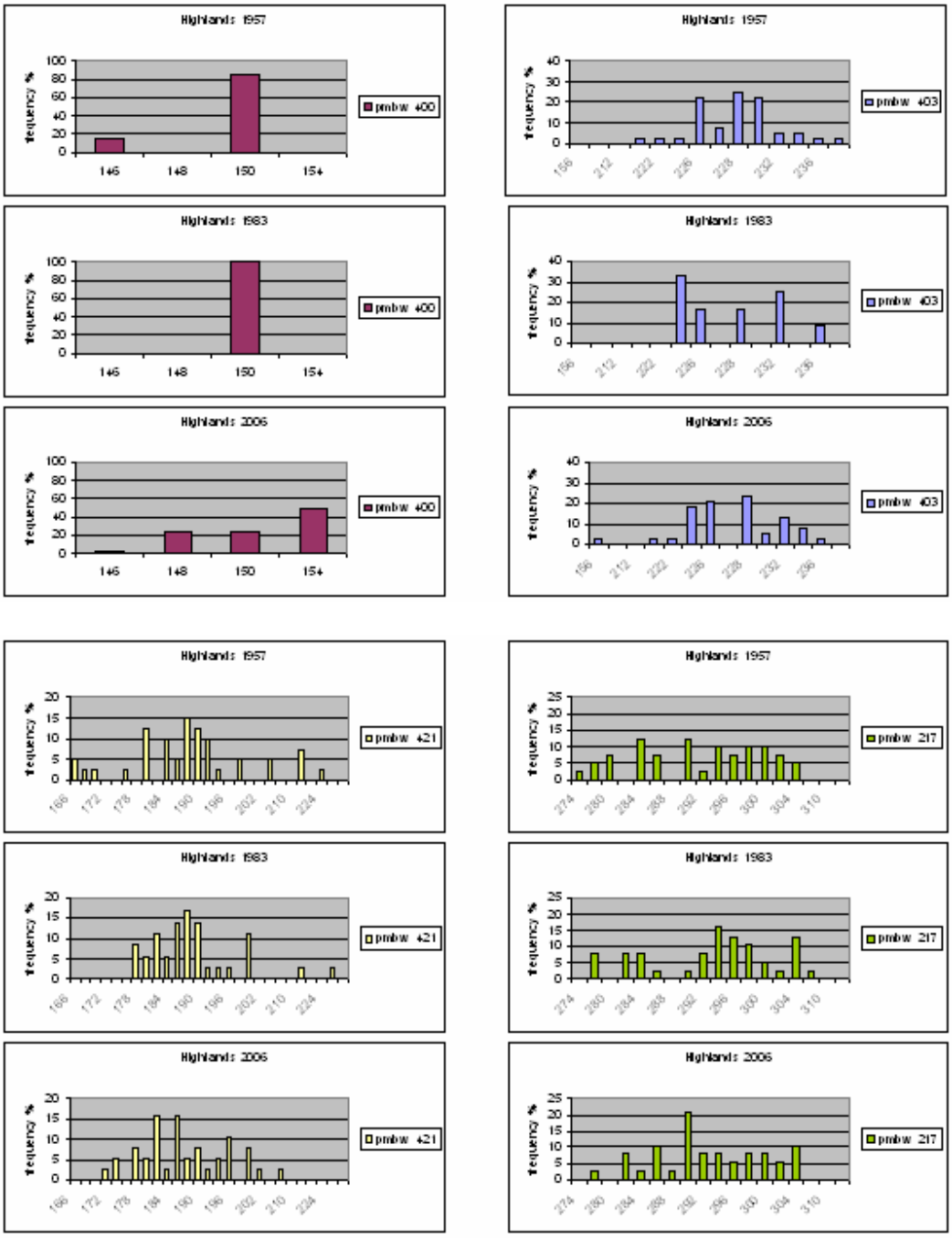


Figure 3-3. Continued

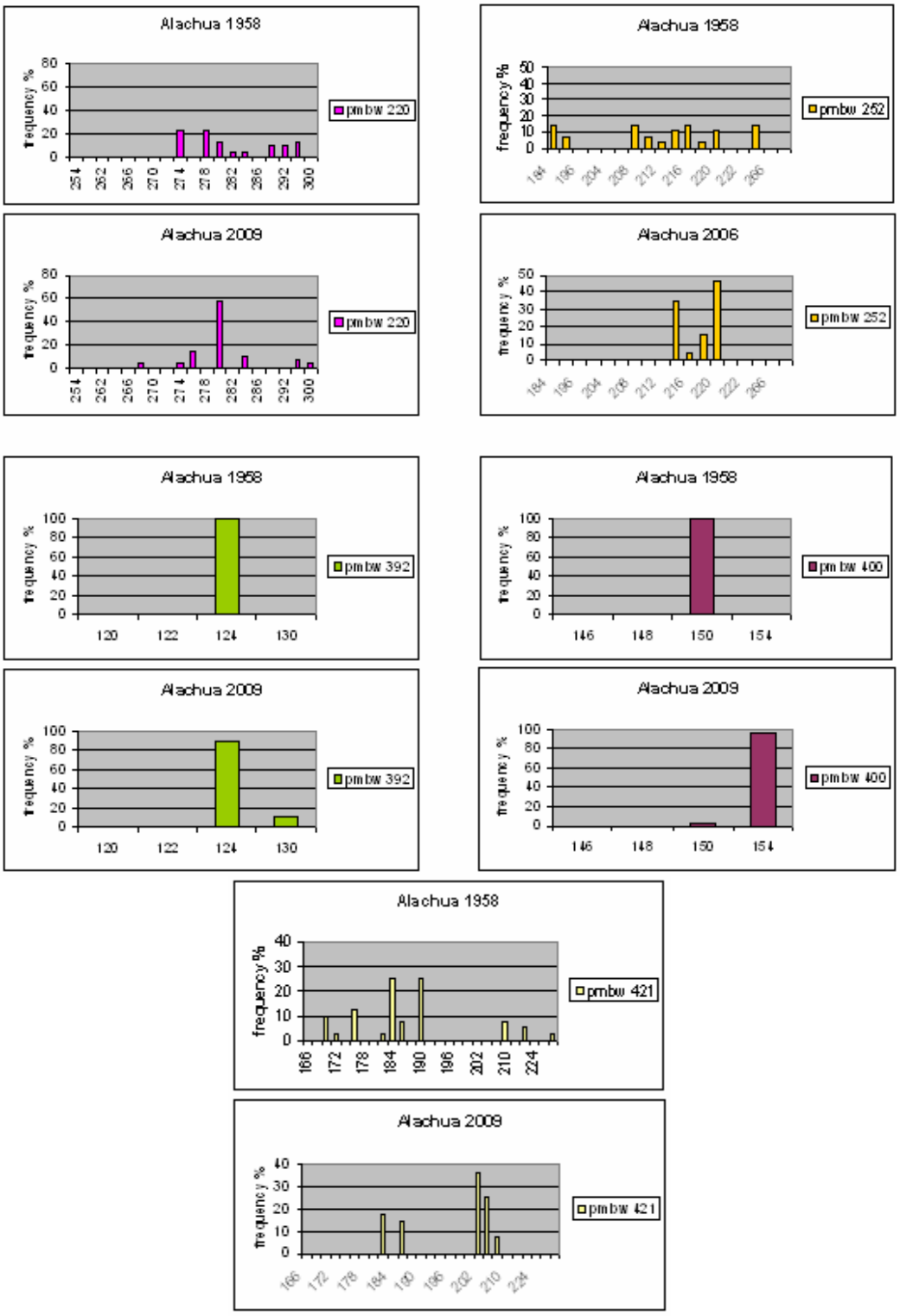


Figure 3-4. Allele frequencies for 5 loci from Alachua County from 1958 and 2006.

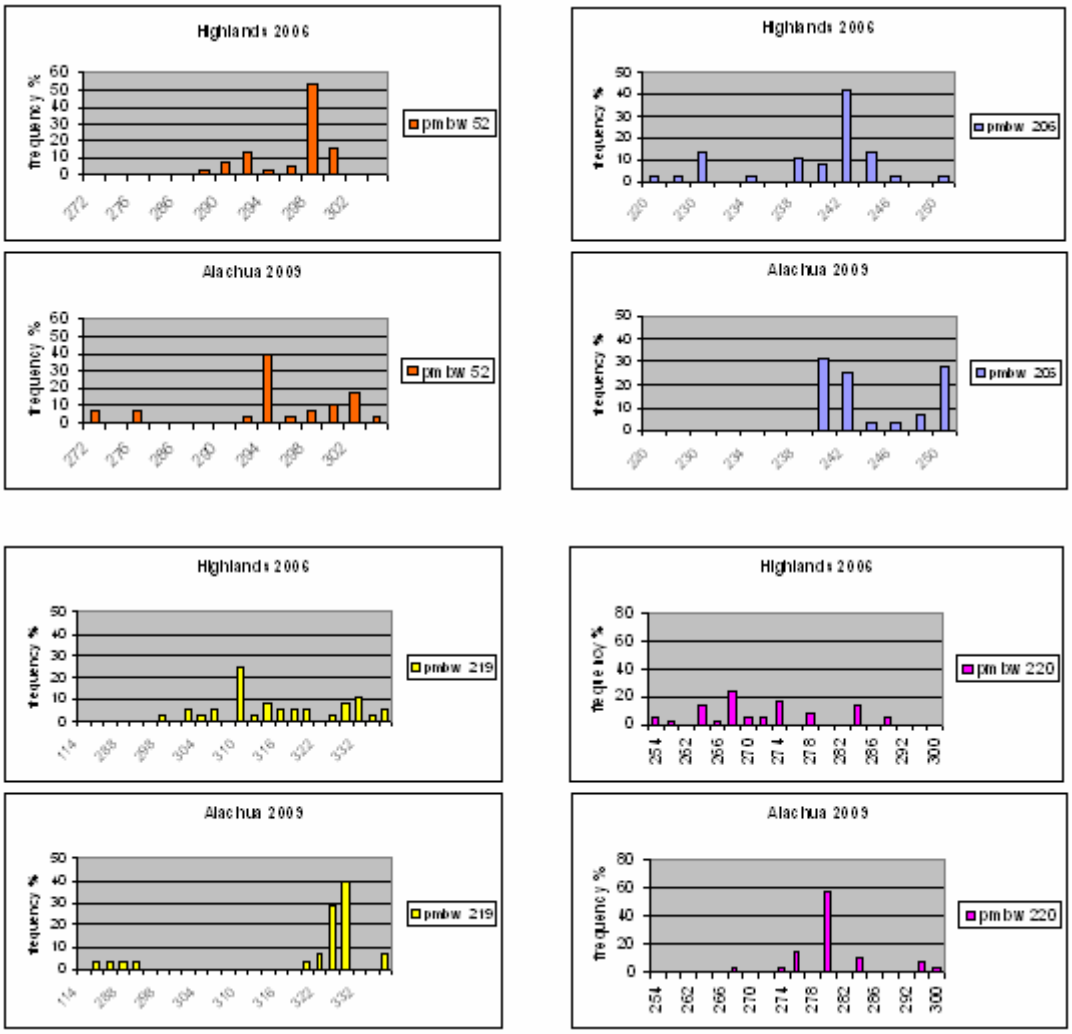


Figure 3-5. Comparison of allele frequencies for 12 loci between current populations from Highlands and Alachua counties.

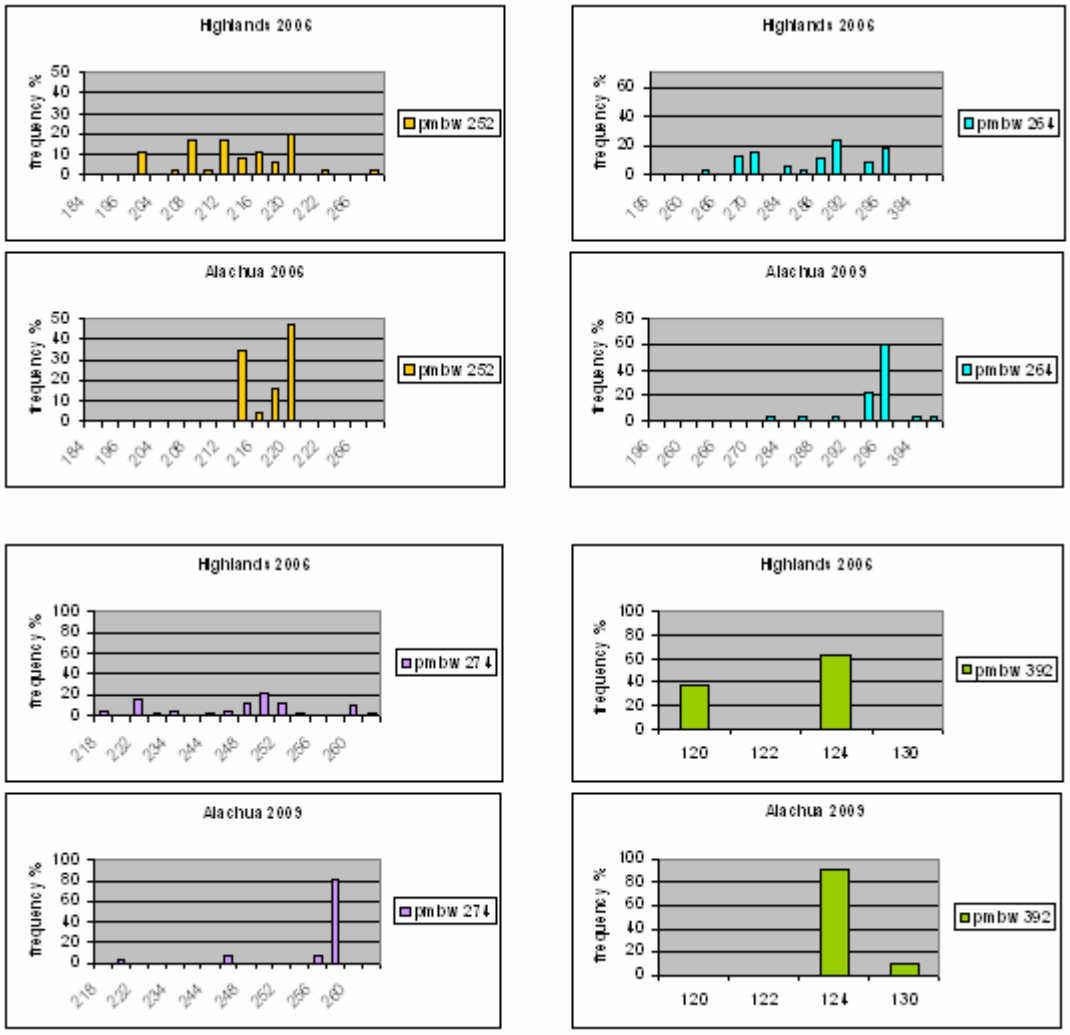


Figure 3-5 continued

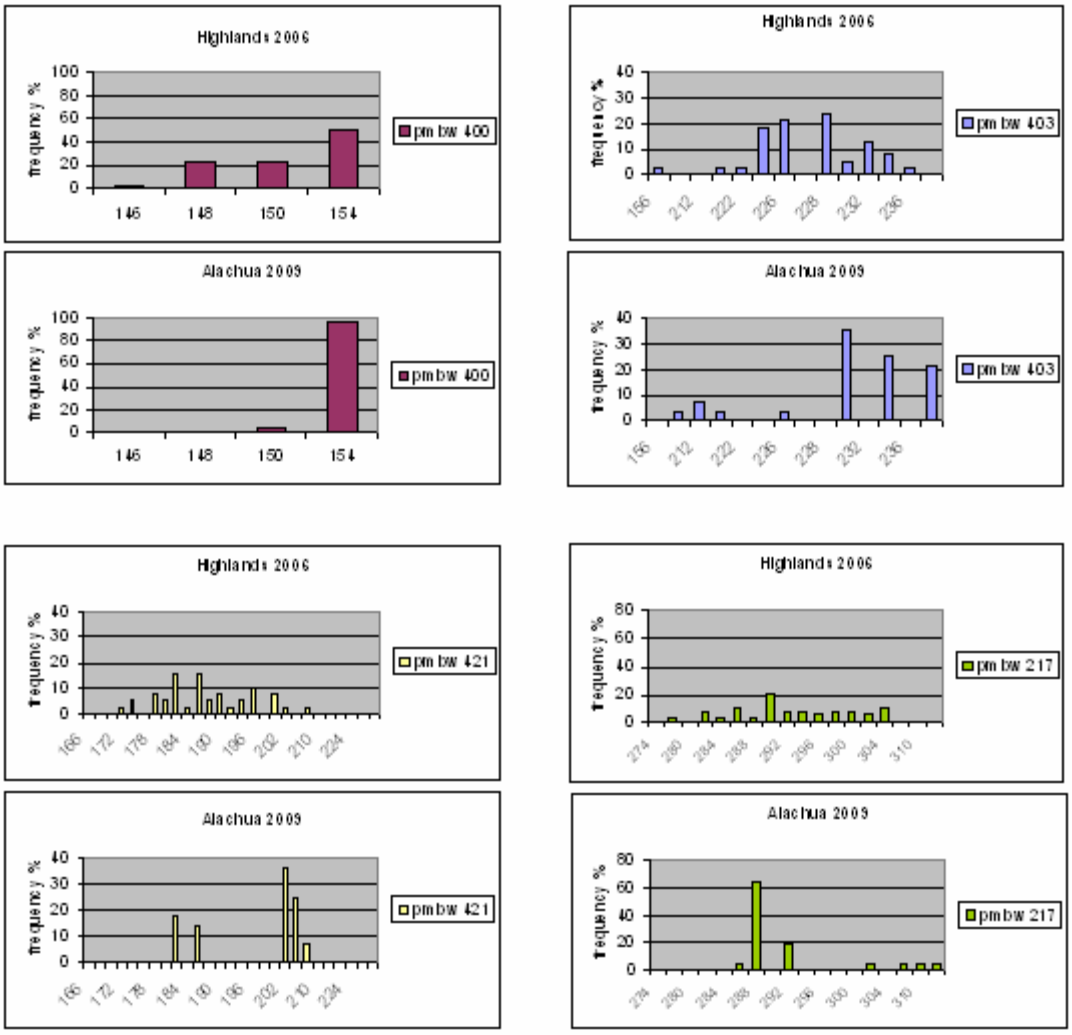


Figure 3-5. Continued

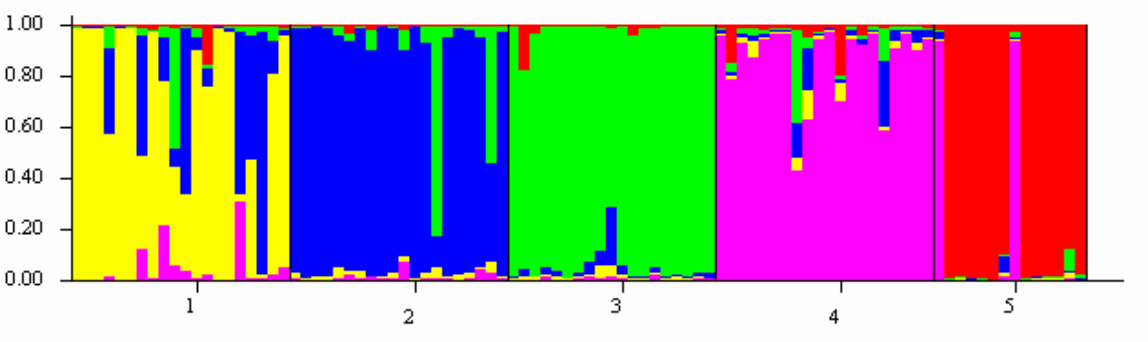


Figure 3-6. Plot of population assignments and coancestry coefficients (*k*=5) generated by STRUCTURE for *P. floridanus*. The five populations are ABS 1957 (yellow), ABS 1983 (blue), ABS 2006 (green), SFH 1958 (purple), and SFH 2009 (red).

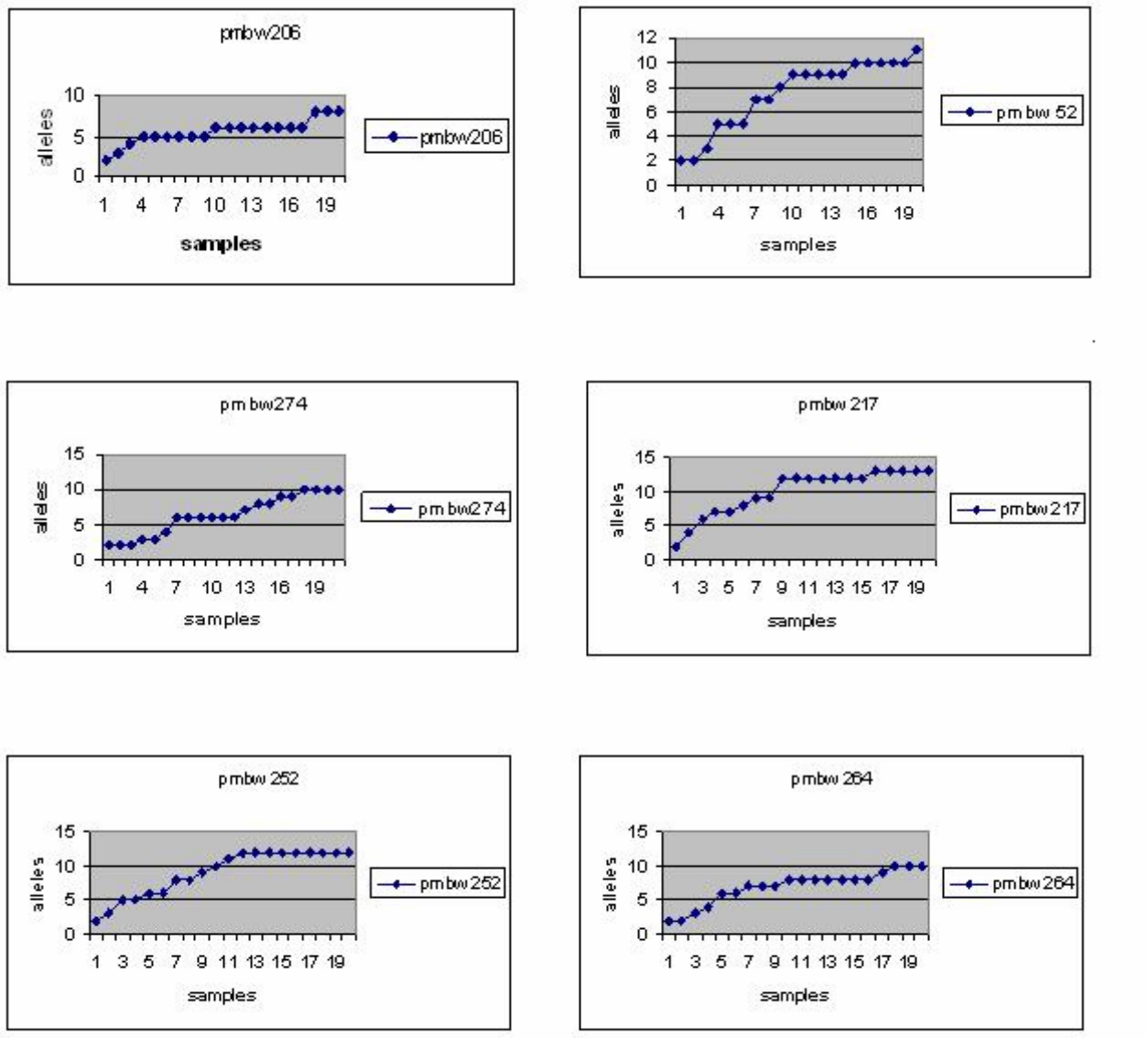


Figure 3-7. Rarefaction curves showing the number of alleles found in one population as a function of increasing sample size.

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# BIOGRAPHICAL SKETCH

Catalina Rivadeneira Canedo was born in Mexico on February of 1975, but moved to La Paz, Bolivia at the age of three; she spent most of her youth there. In January 1993, she began college at Universidad Mayor de San Andres in La Paz-Bolivia, where she received her degree in biology (Licenciada en Biología). She then moved to Santa Cruz-Bolivia and worked for a Bolivian NGO in biodiversity conservation (Fundación Amigos de la Naturaleza) for three years. She began graduate school in January of 2008 at The University of Florida, School of Natural Resource and Environment, and received her Master of Science degree in Interdisciplinary Ecology in May of 2010.