2013

Bachman's Sparrow (Peucaea aestivalis) population structure across the southeastern USA

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BACHMAN'S SPARROW (*PEUCAEA AESTIVALIS*) POPULATION STRUCTURE ACROSS THE SOUTHEASTERN USA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Renewable Natural Resources

by
Blain A. Cerame
B.S., Southeastern Louisiana University, 2005
B.S., Louisiana State University, 2008
December 2013

ACKNOWLEDGEMENTS

I would like to express my profound gratitude to my advisor, Dr. Sabrina S. Taylor, who has provided unwavering support during the course of my graduate career. She has provided training, funding, and most importantly guidance, for which I am sincerely grateful. She has always made herself available to me, even when short on time. Most of all, I am especially grateful for her encouragement and genuine concern for my progress and well-being over the past three years. With such excellent support, she has helped me grow as a scientist. Sabrina's passion to further her own knowledge and research has inspired me to continue learning beyond my time as a graduate student, which is the greatest gift I take from my experience as her student. I would also like to thank the rest of my committee, Robb Brumfield and Phil Stouffer, who have been generous with their time and knowledge. Their efforts in contributing ideas and recommendations have helped me write the best thesis possible.

I want to acknowledge Louisiana State University (LSU) and the LSU AgCenter. Funding for this project was provided by the Louisiana Board of Regents through a Research Competitiveness Subprogram Grant. I would also like to thank the Louisiana Department of Wildlife and Fisheries, US Forest Service, US Army, Money Hill Real Estate Group, The Nature Conservancy and The Girl Scouts of the USA for allowing us to collect samples on their lands. My field season would not have been as successful without the help of my field technicians, Catherine Normand, Kiah Williams, and Chris Mariani. I am very grateful for their hard work and long hours of dedication to my project.

A large portion of my samples were provided by James "Jim" Cox from Tall Timbers Land Conservancy and Research Station located in northern Florida and James Tucker who sampled at Avon Park Air Force Range in southern Florida. I'm very grateful for their valuable advice on capturing and sampling this amazing, yet often overlooked species, but mostly for their inspiration and dedication to working with such an amazing bird. Without their contribution, my project would not have been as successful or informative.

I would like to extend my sincerest thanks to my lab mates, all of whom have given me friendship and much priceless academic support through their own graduate experiences: Kristin Brzezki, Jean Elbers, Andrew Rodriguez and Robert Ford. It has been wonderful to get to know them. I am especially appreciative for the guidance from Dr. Stefan Woltmann, our Post-Doc. He always had time and answers to my seemingly endless list of questions. I owe him a huge debt for helping me accomplish as much as I have.

No amount of words can express how thankful I am to my family and my closest friends. These people have been instrumental in my life, and whose love and support I greatly value. Foremost of all are my mother and father, Barbara N. McLeod and Joseph P. Cerame Jr., for whom I am overwhelmingly grateful for standing by me through anything I have encountered. Their constant words of encouragement have instilled in me the drive to better myself through any means necessary, despite the difficulty, and have prepared me for whatever may lie ahead. I want to thank my brothers Preston Cerame and Anthony Cerame, my sister Blair C. Coxe and their families for giving me places to lean for support, even though most times they were unaware of my need and my nephews and niece for being able to brighten my days with unconditional love. I am also especially grateful to my closest and dearest friends: Breanna Sullivan, Liana Coleman, Bonnie Britt and Hannah Everritt. They forgave the distance created in our friendship by the pressures of school. These women provided a sounding board for any frustrations when I desperately needed a sympathetic ear and were always up for fun times and ample amounts of laughter. To all my friends I have the deepest gratitude. Finally, I am thankful to Jeremy Miller. His kindness, understanding, and love helped me to grow as a person, and in my graduate career. I cannot express enough how he touched my life.

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ABSTRACT

Understanding gene flow and population structure in wildlife populations helps managers to protect distinct genetic lineages and genetic variation in small, isolated populations at high risk of extinction. I assessed genetic diversity in Bachman's Sparrows (*Peucaea aestivalis*) to evaluate the role of natural barriers in shaping evolutionarily significant units as well as the effect of anthropogenically-caused habitat loss and fragmentation on population differentiation and diversity. Genetic diversity was assessed across the geographic range of Bachman's Sparrow by genotyping 226 individuals at 18 microsatellite loci and sequencing 48 individuals at nuclear and mitochondrial DNA genes. Multiple analyses consistently demonstrated high levels of gene flow, which appear to have maintained high levels of genetic variation and panmixia in populations throughout the species' range. Based on these genetic data, separate management units/subspecies designations or artificial gene flow among populations in habitat fragments do not seem necessary. High vagility in Bachman's Sparrow may be an adaptation to colonize ephemeral, fire-mediated longleaf pine habitat, but in recent times, it also appears to have reduced inbreeding and loss of genetic diversity in habitat fragments.

1. INTRODUCTION

Population structure in wildlife populations may be caused by natural processes or by anthropogenically-caused habitat loss and fragmentation. Population differentiation caused by natural processes may produce distinct evolutionarily lineages that may deserve protection to ensure evolutionary potential and maintenance of biodiversity. In contrast, population differentiation caused by habitat loss and fragmentation may cause loss of genetic variation and inbreeding in habitat fragments, an outcome that may require management actions such as translocations to ensure evolutionary potential and reduce extinction risk. Below, I describe the evolutionary forces that affect population structure, discuss natural and anthropogenic habitat fragmentation generally, and finally, discuss genetic variation and habitat fragmentation in Bachman's Sparrow, the subject of this study.

1.1 Evolutionary Forces that Effect Genetic Diversity

Genetic variation is the raw material for evolutionary change allowing species and/or populations to evolve in response to environmental changes ranging from new or changed diseases, pests, parasites, competitors or predators, climate change, habitat loss or pollution (Frankham 1996). To successfully protect genetic variation, an understanding of the factors that affect genetic variation in natural populations is necessary. Genetic variation is driven by four evolutionary forces: mutation, natural selection, genetic drift, and migration. Mutation is the ultimate source of genetic diversity, but it occurs at such a low rate that it typically takes thousands to millions of generations to produce variation (Frankham et al. 2004), and so, it is important for generating new variation and long-term population differentiation, but is not normally considered in studies examining the effect of recent environmental changes on population differentiation.

Natural selection can change the genetic composition of populations by either eroding variation via the fixation of alleles through directional or stabilizing selection or promoting its retention as a result of balancing selection (Frankham 1996). Selection works by acting on existing genetic variation to perpetuate phenotypes and the underlying genotypes that confer a fitness advantage for individuals (Frankham et al. 2004). For

example, Fjeldså (1983) found that Silvery Grebe (*Podiceps occipitalis*) populations inhabiting Lake Junin in the Andes evolved shorter beaks in comparison to other Silvery Grebe populations in response to food resource competition with the larger, flightless Junin Grebes (*Podiceps taczanowskii*) utilizing the same habitat. Natural selection primarily acts on phenotypes that increase the survival and reproduction of individuals within populations, but because most phenotypes are determined by underlying genotypes, natural selection can change the frequency of alleles in a population. In some situations, natural selection acts directly on specific genes in order to maintain high levels of polymorphism in the population because it confers an increased resistance to parasites and disease. For instance, Westerdahl et al. (2004) looked at whether selection or random demographic change was the cause of allele frequency fluctuations in the major histocompatibility complex (MHC), a gene which plays an important role in an individual's immune response, in nine cohorts of great reed warblers (*Acrocephalus arundinaceus*) in Sweden. The study found that the fluctuations in MHC allele frequencies between cohorts was not a result gene flow or any other demographic event, but rather an effect of balancing selection favoring individuals with polymorphic MHC genes that could better cope with pressures from parasites and pathogens found in the environment from year to year (Westerdahl et al. 2004). Studies such as these show that natural selection plays a large role in the amount of variation within populations and over time can produce differentiation among populations.

Genetic drift can affect population genetic variation and differentiation by causing the loss of alleles through random sampling during transmission from one generation to the next. Under genetic drift, allele frequencies increase or decrease from generation to generation, and with enough time, alleles become fixed or lost, leading to reduced heterozygosity and creating significant genetic differences among populations. The effects of drift are more apparent in small populations because there is a finite number of alleles that can be passed to the next generation, making genetic drift more important than selection, and allowing deleterious mutations to accumulate and become fixed by chance (Keller and Waller 2002). Stochastic events can create special cases of genetic drift when populations experience severe bottlenecks and founder effects that reduce the

size of populations to a very small number, abruptly changing allelic frequencies and ultimately leading to loss of genetic variation and possible population differentiation. Importantly, reduced genetic diversity appears to be associated with elevated extinction rates. For instance, a study by Newman and Pilson (1997) found that decreased genetic diversity in small populations of the annual evening primrose (*Clarkia pulchella*) resulted in an increased probability of population extinction above the extinction rates attributed to random demographic changes alone. Negative effects of increased genetic drift in small populations of this plant species resulted in significantly lower mean fitness levels leading to a lower probability of population survival and the random loss of different alleles through the process of drift created significant between-population genetic differentiation (Newman and Pilson 1997). Similar effects of genetic drift were also seen in Glanville fritillary butterfly (*Melitaea cinxia*) populations when Saccheri et al. (1998) was able to directly correlate population size, increased genetic drift and reduced genetic variation with elevated extinction rates. Multiple populations of the Glanville fritillary butterfly had a high genetic load perpetuated by drift within and gene flow among local populations that carried numerous deleterious alleles, making selection relatively inefficient in eliminating the harmful alleles and ultimately effecting the overall survival of each individual population (Saccheri et al. 1998).

Finally, migration or gene flow strongly affects genetic variation and differentiation among populations. Individual populations have varying degrees of contact with each other, from frequent genetic interchange to complete isolation. High gene flow maintains high genetic diversity within individual populations (Moritz 1994) and helps to prevent rare alleles, which may be advantageous, from disappearing in the larger population. However, high gene flow may also prevent adaptation to differing environmental conditions among populations, which may reduce fitness. In addition, a small amount of gene flow can be instrumental in "rescuing" small populations that have been extirpated or are at risk of extinction by providing immigrants harboring new alleles that boost population numbers and fitness. Genetic rescue has been documented in fish, reptile, mammal and bird species (Evans and Sheldon 2008) where natural populations that have experienced local extinction events or high levels of inbreeding are rescued by immigrants from neighboring habitats,

thereby increasing fitness or reestablishing the population (Allendorf et al. 2013). However, recolonization can only occur when there is the possibility of migration among populations, and for many species listed as rare, threatened or endangered, habitat loss and fragmentation have reduced or prevented gene flow (e.g. *Ovis canadensis nelson*, Epps et al. 2005; *Puma concolor*, Ernest et al. 2003; *Perognathus longimembris pacificus*, Swei et al. 2003).

1.2 Natural Habitat Fragmentation

Fragmented habitats created by natural barriers (e.g. rivers, oceans, deserts and mountain ranges) have major effects on population differentiation (Hanski & Gaggiotti 2004) and species-level diversity among various taxonomic groups including birds, amphibians, reptiles, fish and plants (Brunsfeld et al. 2001, Soltis et al. 2006, Jackson and Austin 2010, McKay 2009). Rivers in particular may create impenetrable barriers to gene flow that ultimately result in discontinuity in genetic variation between populations located on either side of the river, especially in areas far from the headwaters where the barrier is typically much larger (Haffer 1997). In the southeastern U.S., rivers like the Mississippi, Apalachicola, and Tombigbee produce significant changes in the topography, hydrology, and habitat types in the areas surrounding the river, creating significant genetic and biological differences between populations of the same species, which potentially warrant separate management or conservation priorities (Crandall et al. 2000, Fraser and Bernatchez 2001, Allendorf et al. 2013).

Any population that shows distinct genetic and phenotypic variation may be considered a separate evolutionary significant unit (ESU) and managed as such (Crandall et al. 2000). The concept of an ESU was proposed as a unit of conservation for populations that harbor unique characteristics, which should be protected. The development of ESUs arose with the aim of avoiding many of the conservation issues associated with using Biological Species Concept definitions that in many cases could be vague or difficult to apply to a wide variety of taxa. ESU designations have become an important determinant in whether distinct population segments should receive protection under the Endangered Species Act (ESA). Some researchers have suggested that evolutionary significant unit designations should be based on genetic markers that show differentiation between

populations no matter the effects the markers analyzed ultimately have on an individual's ability to adapt to the surrounding environment (Moritz 1994; Avise 2000; Zink 2004). In contrast, another theory suggests that ESUs should be identified by differences in traits that are ecologically important and represent a population's adaptability to stochastic changes in the environment despite the degree, if any, of genetic differentiation (Crandall et al. 2000; Fraser & Bernatchez 2001). Despite the specific definitions, separate units can be difficult to determine when differences among populations are cryptic or inconsistent, and any change in ESU designations could cause a species or subspecies to become ineligible or lose existing protection under the ESA. For example, populations of Carolina Chickadee (*Poecile carolinensis*) on either side of the Tombigbee River in Alabama have significant genetic differences, but are morphologically indistinguishable from one another (Gill et al. 1993, 1999). Similarly, Yellow-throated Warbler (*Setophaga dominica*) populations on opposite sides of the Tombigbee are morphologically distinct with differing habitat preferences and winter migration routes, but are genetically similar (McKay 2009). These morphological differences, along with observed differences in life history traits, have resulted in three subspecies designations. However, the lack of genetic differences has called into question the need for three subspecies classifications (McKay 2009). Understanding the genetic structure of populations is important in identifying genuine evolutionarily significant units of conservation in ecosystems with large, natural land features because morphological or behavioral features may not provide enough evidence to indicate genetically unique populations.

The Mississippi River and surrounding bottomland hardwood forests in Louisiana provide a good example of how a major geological barrier can produce disjunct habitat. The Mississippi River and its adjacent bayous and swamps, including the vast Atchafalaya swamp, act to bisect longleaf pine (*Pinus palustris*) savanna habitat (Figure 1.1), along with longleaf pine associated taxa (Sorrie and Weakley 2006). For example, population subdivision in the North American racer (*Coluber constrictor*; Burbrink et al. 2007), the North American rat snake (*Elaphe obsolete*; Burbrink et al. 2000) and the cornsnake (*Elaphe guttata*; Burbrink 2002) is associated with the Mississippi River. Similarly, the Mississippi River prevents seed dispersal between

populations of pitcher plant colonies, (*Sarracenia* spp.), found in longleaf pine savannahs on opposite sides of the river, resulting in genetic subdivision of the species (Koopman and Carstens 2010).

1.3 Anthropogenic Habitat Fragmentation

Anthropogenic habitat fragmentation, degradation and loss have been identified as major threats to global biodiversity (IUCN 2011), and a large body of research has sought to better understand and mitigate their effects on biodiversity in various ecosystems. The overarching conclusion of these studies is that habitat loss has large negative effects on biodiversity while fragmentation has both negative and positive effects on ecosystems and species found in habitat remnants (Fahrig 2003). Negative effects are attributed to significant ecosystem alterations that usually produce poorer-quality habitat with fewer resources and increased edge effects (Harrison and Bruna 1999). This occurs because large, continuous tracts of habitat are progressively fragmented to a point where the remaining patch can no longer support a number of diverse species, multiple populations of a single species or even the territory of a single individual because basic biological requirements previously provided within the ecosystem are no longer available. As adverse landscape alteration occurs, the probability of persistence for many species declines from either lack of essential resources to survive in the fragment or reduced population numbers due to increased mortality (Fahrig 2002). Overall, habitat loss and fragmentation are found to significantly alter species richness, abundance and distribution of species (Shmida and Wilson 1985, Flather and Bevers 2002), population social structure (Ims and Andreassen 1999, Cale 2003) and trophic webs (Komonen et al. 2000), and adversely affect important life history traits such as lowered foraging success (Mahan and Yahner 1999), modified dispersal rates (Brown and Kodric-Brown 1977), decreased reproductive success (Kurki et al. 2000), slowed population growth (Bascompte et al. 2002) and increased mortality rates (Jules 1998).

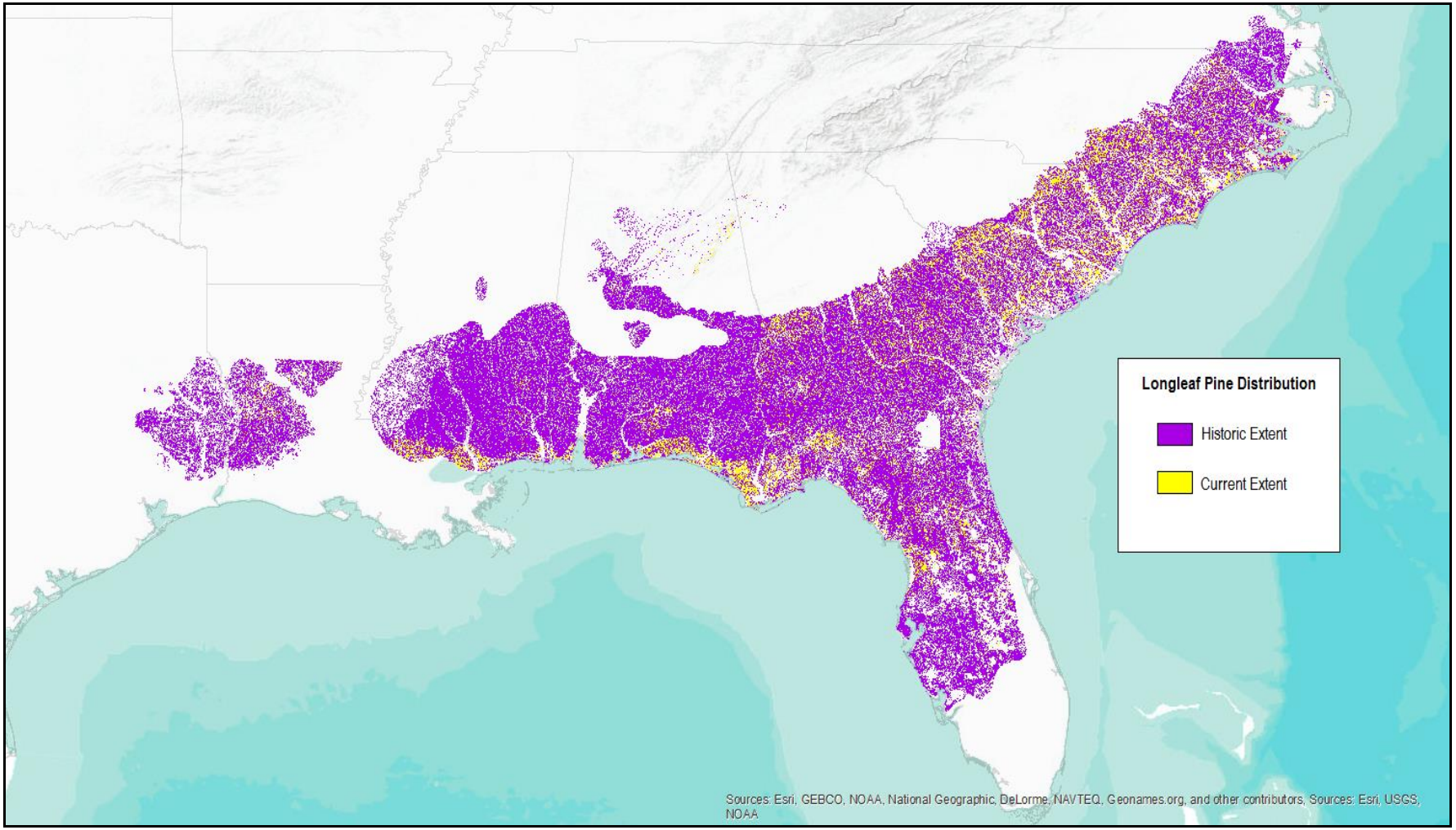

Figure 1.1 Map of historic and current longleaf pine (*Pinus palustris*) habitat in the southeastern United States created using data provided by NatureServe© and LandScope America©.

In addition to altering biological and ecological processes, fragmentation can also reduce population connectivity by disrupting gene flow among populations (Petren et al. 2005). Without connectivity, the likelihood and rate of extinctions rise while subsequent recolonizations are reduced. Many species that were historically distributed continuously across broad geographic areas have become restricted to increasingly smaller and more isolated patches (IUCN 2011) by loss and fragmentation, creating habitat ‘‘islands’’ that reduce the size of remaining populations and prevent genetic contact with conspecifics from adjacent areas (Templeton et al. 2001). As population size decreases, genetic drift also increases, causing allele fixation and loss, which ultimately reduces genetic variation, an outcome that could have adverse consequences for fitness, and subsequently for population demography, in small isolated populations (Lande 1988).

Multiple studies suggest that populations occupying large contiguous habitat fragments will be characterized by high gene flow and high genetic variation while small, isolated fragments typically show low levels of genetic variation, and increased levels of inbreeding and population differentiation (Willi et al. 2006, Allendorf et al. 2013). For example, Johannson et al. (2007) found that agriculturally-induced habitat fragmentation within the range of the European common frog (*Rana temporaria*) increased genetic drift and consequently, the frequency of recessive deleterious mutations to such an extent that genotypic variability and phenotypic traits related to fitness were significantly reduced in comparison to larvae from populations from continuous landscapes; specifically larval size and survival rates were reduced. Both larval size and survival in amphibians are positively correlated with fitness, so the negative effects of drift in the European common frog habitat suggests that the larvae from the fragmented areas will have lower fitness than larvae from continuous landscape (Johannson et al. 2007). Similar reductions in genetic diversity and fitness have been documented in other species including snakes (*Vipera berus*; Madsen et al. 1996), insects (*Polyommatus coridon*; Vandewoestijne et al. 2008) and plants (*Swainsona recta*; Buza et al. 2000).

In birds, early studies suggested that high mobility and seasonal migratory behavior resulted in high gene flow and large effective population sizes (Barrowclough 1983). Hence birds should show an overall lower

degree of population differentiation than other less mobile vertebrates (Winkler et al. 2000). In actuality, avian species demonstrate considerable variation in their responses to habitat connectivity (McCulloch 2012). Studies have found genetic structure reflecting restricted gene flow in both non-migratory and migratory species (Arguedas and Parker 2000), illustrating the potential for restricted movement, due to fragmentation, even in animals that are thought to have high dispersal capability.

1.4 Inbreeding

Habitat fragmentation can also result in inbreeding because populations become small, and so many individuals may mate with related individuals. Inbreeding may lead to inbreeding depression, or reduced fitness, including decreased reproductive success and survivorship (Benedick et al. 2007, Frankham 2002), which can further reduce population sizes. Inbreeding depression is especially important in populations whose sizes have been severely reduced by fragmentation. For example, Karlsson and Van Dyck (2005) investigated the effects of fragmentation on the reproduction of a woodland butterfly species, *Pararge aegeria,* whose habitat was fragmented by agriculture. They compared populations in contiguous and fragmented areas and found that females from the small, isolated habitats had reduced fecundity, egg number, weight and size compared to females in the contiguous forests. Similarly, Saccheri et al. (1998) directly correlated fragment size, inbreeding, and reduced genetic diversity with elevated extinction rates in Glanville fritillary butterfly (*Melitaea cinxia*) metapopulations. Inbreeding has also been linked to low sperm quality in large carnivores and ungulates (O'Brien et al. 1983, Roldan et al. 1998), higher proportions of unhatched eggs in avian species (Kempenaers et al. 1996) and high offspring mortality (Keller et al. 2002), all of which decrease population numbers.

1.5 Bachman's Sparrow and Habitat Fragmentation

One avian species potentially affected by both natural and anthropogenic fragmentation is the Bachman's Sparrow (*Peucaea aestivalis*) (Dunning 2006, Sibley 2000). There are three recognized subspecies

(Figure 1.2) of Bachman's Sparrow listed by the American Ornithologists' Union (AOU): *P. a. illinoensis* occupies the northern and westernmost areas of Bachman's Sparrow range including Texas, Louisiana, Indiana, Illinois and Missouri; *P. a. aestivalis* occupies Longleaf Pine east into Florida, Georgia and South Carolina; and, *P. a. bachmani* occupies habitat in North Carolina and Virginia (AOU 1957, Dunning 2006; Figure 1.2).

These designations conflict with morphological observations published by Sibley (2000) who has identified distinct morphological differences between individuals on either side of the Mississippi river. Despite these groupings by Sibley (2000) and the AOU no genetic data on population structure exist for Bachman's Sparrows, but these data would help to resolve the identity of genuine subspecies potentially created by geological barriers found throughout this species geographic range. Basing subspecies and management priorities on morphological traits alone could result in the loss of a genetically unique population (Agapow et al. 2004). For example, population genetic structure has been used to determine subspecies classifications for threatened and endangered species like the Northern Sportive Lemur (*Lepilemur septentrionalis*; Ravaoarimanana et al. 2004) and Spotted Owl (*Strix occidentalis*; Barrowclough *et al.* 1999). Both of these species' subspecies were originally based on morphological characteristics only, until modern genetic analyses were used to identify genetically distinct populations that required separate management.

In addition to large natural barriers, habitat loss and fragmentation through human-induced changes in longleaf pine habitat may have reduced gene flow among Bachman's Sparrow populations inhabiting remnant longleaf pine tracts. The Bachman's Sparrow is a species endemic to the southeastern United States and is closely associated with mature, pine woods savannas typically dominated by a longleaf pine overstory and an understory consisting of a diverse assemblage of warm season grasses (Dunning 2006, Gilliam and Platt 2006). In the early 1900s Bachman's Sparrow range expanded dramatically northward as far as Illinois and Pennsylvania due to increases in suitable habitat created by abandoned farms, fields and clearcuts that produced early successional habitat that mimicked the grass-dominated ground cover found in longleaf ecosystem (Dunning 2006).

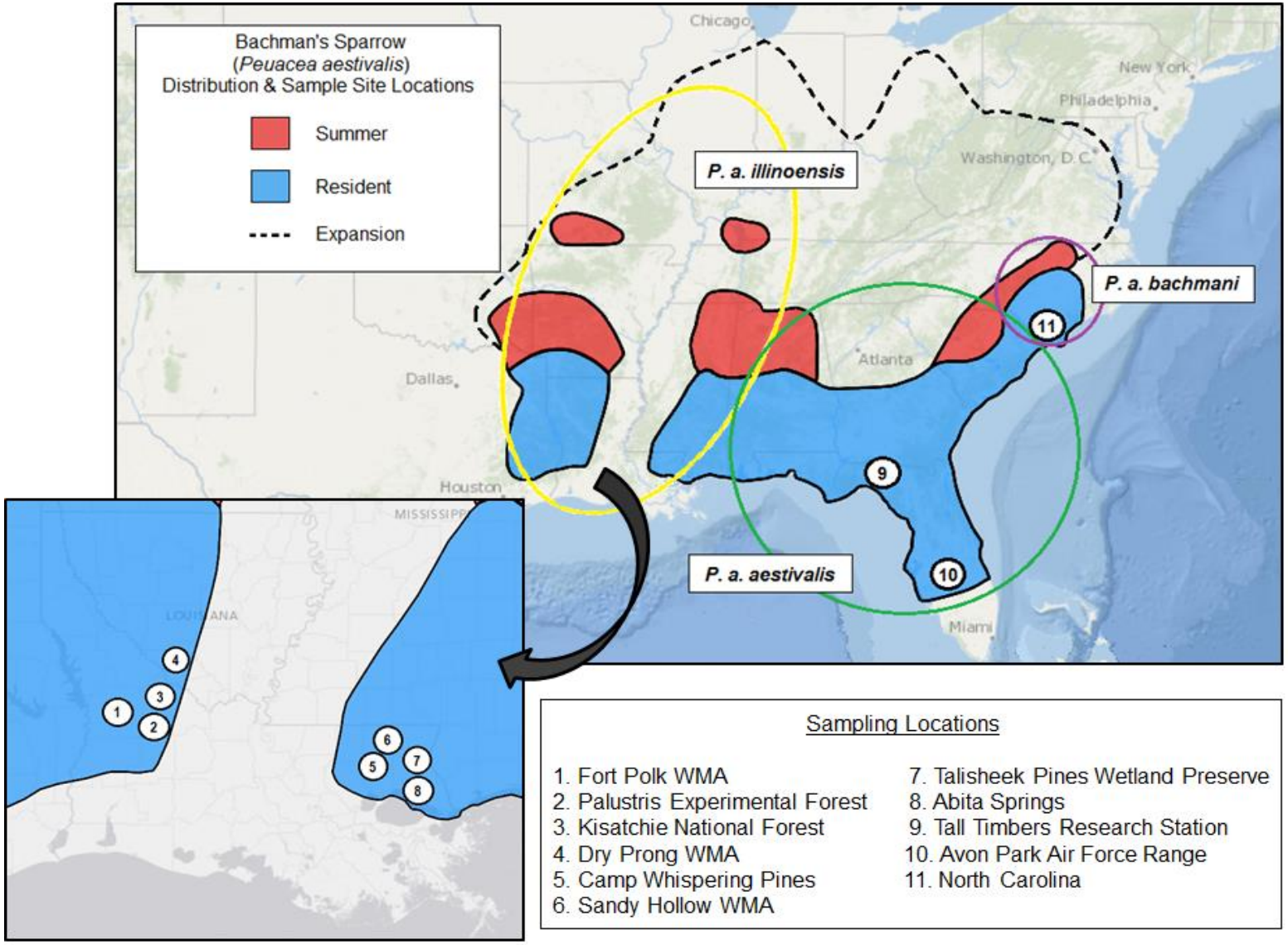

Figure 1.2 Bachman's Sparrow (*Peucaea aestivalis*) North American distribution including historic range expansion, subspecies, and sampling locations created using the AOU 1957 Check-list of North American Birds and Dunning (2006) Bachman's Sparrow (*Aimophila aestivalis*) in The birds of North America.

However, since the 1930s, populations have begun contracting back toward the core historical range in the southeast as secondary succession has created plant communities that cannot support Bachman's Sparrows. Importantly, population declines of Bachman's Sparrows within the center of the breeding range have been attributed to loss of habitat and habitat degradation (Tucker et al. 2004). Fire suppression, timber harvesting, and fragmentation of open longleaf pine savannahs have resulted in over 95% loss of the total area of this once extensive ecosystem (Tucker et al. 2004). As the amount of remnant longleaf pine habitat declines, it is not surprising that Bachman's Sparrow populations and distribution have also declined (Dunning 2006). The overall, range-wide decline in population sizes has caused many organizations to add Bachman's Sparrows to lists for rare, threatened and endangered species in the United States. The species has been designated a Near Threatened species by the International Union for Conservation of Nature (BirdLife International 2012), Vulnerable S3 species in Louisiana (Louisiana Department of Wildlife and Fisheries) and a Species of Special Management Concern by the U.S. Forest Service (USDA). Conner et al. (2005) has listed Bachman's Sparrow among the species of highest management concern within the southeastern United States.

Other longleaf pine associated species have experienced population subdivision and decline associated with natural and anthropogenic habitat fragmentation and, in some cases, it has resulted in unique conservation units established for population management. Gopher tortoise (*Gopherus polyphemus*), Red-cockaded Woodpecker (*Picoides borealis*), Louisiana pine snake (*Pituophis ruthveni*), and the eastern indigo snake (*Drymarchon couperi*) are other longleaf pine specialists that have all experienced similar population declines and subdivision as Bachman's Sparrows through the loss of longleaf pine ecosystems throughout the southeastern United States. For all three species, a primary technique for management is translocation of individuals between populations with the goal of bolstering population numbers and increasing genetic diversity (Stangel et al. 1998, Kwiatkowski et al. 2010, Clostio et al. 2010), so correct identification of genetically distinct populations is key to successful population management. For the gopher tortoise, conservation units in the western portion of the species' range are currently defined using natural river barriers that bisect suitable

habitat creating four distinct units that are all federally listed by the United States Fish and Wildlife Service (USFWS) (U.S. Fish and Wildlife Service, 2009); eastern region populations are not currently listed. These designations are based on the assumption that rivers have created significant barriers to gene flow, and thus genetically differentiated populations, by preventing movement between populations on either side of the river (Clostio et al. 2012). However, a recent genetic analysis of gopher tortoise populations by Clostio et al. (2012) found no genetic evidence to support the four conservation units currently held for populations in the western region, suggesting the smaller riverine system were not restricting gene flow (Clostio et al. 2012).

Similarly, Red-cockaded woodpecker (RCW) populations have also experienced division and decline due to longleaf fragmentation and loss. The initial recovery plan for RCWs called for the establishment of 16 populations, of >250 breeding pairs, located throughout the historic range and to accomplish this managers relied heavily on translocation of individuals. Stangel et al. (1998) conducted a genetic analysis of multiple RCW populations to determine genetic variability and population structure across the southeastern United States to identify distinct populations. Despite fragmentation of populations and reduced sizes, overall heterozygosity was high in the populations studied (Stangel et al. 1998). Their results indicated somewhat reduced genetic diversity in smaller populations, and genetic differentiation as a function of geographic distance (Stangel et al. 1998).

Understanding genetic variation of Bachman's Sparrow populations is important in helping managers identify different genetic lineages as well as maintain genetic variation and reduce inbreeding depression in remnant populations, actions that should help to ensure that populations of high genetic value are conserved. The objectives of this study are to: 1) examine Bachman Sparrow population differentiation across its range to help evaluate whether current subspecies designations are valid, and; 2) evaluate gene flow among habitat fragments and genetic diversity within habitat fragments to identify areas of restricted gene flow and populations with inbreeding and low levels of genetic diversity. The results of this study will help to identify potential ESUs and populations with high, low or unique genetic variation.

2. MATERIALS AND METHODS

2.1 Study Sites

Sampling sites were identified by locating mature longleaf pine stands featuring the open canopy and dense herbaceous understory preferred by Bachman's Sparrows (Plentovich et al. 1998, Tucker et al. 2004) as well as using sightings recorded by biologists and the general public through eBird, a real-time online checklist created by a partnership between the Cornell Lab of Ornithology and the National Audubon Society. I sampled four study sites on the west side and three study sites on the east side of the Mississippi River in Louisiana (Figure 1.2). Western Louisiana has larger, contiguous longleaf pine tracts while eastern Louisiana has smaller and more fragmented patches of longleaf pine. Collaborators from Louisiana State University Museum of Natural Science and two locations, Tall Timbers Land Conservancy and Research Station and Avon Park Air Force Range in Florida generously provided additional samples from Louisiana, Florida and North Carolina (Figure 1.2, Table 2.1).

2.2 Field Protocols

Individuals were captured using song playback and 6 m, 36 mm mist nets (Cox and Jones 2004). All populations were sampled from February through June in 2011 (n = 26) and 2012 (n = 88). Each bird was banded with a Size 1 USFWS aluminum numbered band and a unique color combination of 2.3mm Darvic or Acetal leg bands to ensure individual bird identification and to prevent sampling individuals more than once. Breeding characteristics such as the presence/absence of a cloacal protuberance or brood patch were used to determine sex in the field because male and female Bachman's Sparrows cannot be distinguished through plumage coloration. Blood samples (<100 µl) were collected using venipuncture of the brachial vein and stored in 1.0 mL of Queen's lysis buffer (Seutin et al. 1991) at 10 ºC until they could be processed. A handheld Garmin GPSmap 60CSx GPS unit was used to mark capture locations.

Table 2.1 Study site, geographic location, ownership and managing entity, and provenance with sample size for 226 Bachman's Sparrow (*Peucaea aestivalis*) samples used in the study.

Study Site	Location	Ownership & Managing Entity	Provenance and Sample Size (*n*)
Fort Polk WMA[1]	Vernon Parish, LA Calcasieu Ranger District, KNF[2]	U.S Army; U.S. Forest Service; LDWF[3]	Field[4] = 25
Dry Prong	Grant Parish, LA Catahoula Ranger District, KNF	U.S. Forest Service	Field = 20 LSUMZ[5] = 5
Kisatchie National Forest	Rapides Parish, LA Kisatchie Ranger District, KNF	U.S. Forest Service	Field =14 LSUMZ = 1
Palustris Experimental Forest	Rapides Parish, LA Kisatchie Ranger District, KNF	U.S. Forest Service	Field = 10 LSUMZ = 3
Sandy Hollow WMA	Tangipahoa Parish, LA	Tangipahoa Parish School Board; LDWF	Field = 23 LSUMZ = 6
Lee Memorial Forest	Washington Parish, LA	Louisiana State University Agricultural Center	Field = 2
Camp Whispering Pines	Tangipahoa Parish, LA	Girl Scouts of the USA	Field = 14
Talisheek Pine Wetlands Preserve	St. Tammany Parish, LA	Money Hill Real Estate Group; TNC[6]	Field = 5
Abita Springs	St. Tammany Parish, LA		LSUMZ = 15
Florida	Madison County, FL		LSUMZ = 1
North Carolina	Brunswick and Columbus County, NC		LSUMZ = 3
Tall Timbers Land Conservancy and Research Station	Madison County, FL	Tall Timbers Land Conservancy	TTRS[7] = 32
Avon Park Air Force Range	Polk and Highlands County, FL	U.S. Air Force	AVON[8] = 47

1. Wildlife Management Area
2. Kisatchie National Forest
3. Louisiana Department of Wildlife and Fisheries
4. 2011-2012 Louisiana Field Seasons
5. Louisiana State University Museum of Natural Science
6. The Nature Conservancy
7. Tall Timbers Conservancy and Research Station
8. Avon Park Air Force Range

2.3 Molecular Methods

DNA was extracted from blood samples from a total of 226 individuals, using DNeasy Blood and Tissue kits (Qiagen, Valencia, CA) following the manufacturer's instructions and amplified using polymerase chain reaction (PCR) with an Eppendorf Mastercycler pro S thermal cycler. A total of 23 nuclear microsatellite loci developed in other avian species were tested in Bachman's Sparrows (Appendix). PCR reactions consisted of 1.0 µl DNA, 1X buffer, 2.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.10 µM for each forward and reverse primers, 0.50 µl of 100% dimethyl sulfoxide (DMSO), 1M betaine, 0.03 nmol M13 fluorescent tag, and 2.0 units *Taq* DNA polymerase (New England BioLabs, Ipswich, MA) and water to a final volume of 10µl. PCR amplification conditions were as follows: 95 ºC for 30 seconds followed by 34 cycles of 95 ºC for 1 minute, 48-60 ºC (see Appendix for annealing temperatures) for 1 minute, 72 ºC for 1 minute and a final extension step of 72 ºC for 4 minutes. Forward or reverse primers were labeled at the 5' end with M13 tags (LI-Cor Biosciences) to allow the DNA amplicons to be detected by infrared laser fluorescence. For each amplified sample, 0.8µl of product was resolved by electrophoresis on a 25-cm, 7% polyacrylamide gel and genotyped on a LI-Cor 4200 Gene ReadIR DNA Analyzer (LI-Cor Biosciences) with 50-350 bp IRDye 700 and 800 frequency size standards (LI-Cor Biosciences). In conjunction with the size standards, Bachman's Sparrow samples representing all allele sizes for each locus were added to gels as additional size markers to ensure accurate genotyping. Allele sizes were estimated using Saga v3.2 (LI-Cor Biosciences) and verified by eye.

Sequence data was obtained for one mitochondrial locus, the nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2) (Johnson and Sorenson 1998, DaCosta et al. 2009), and one nuclear locus, the transforming growth factor β-2 (TGFβ2) intron 5 (Primmer et al. 2002). Both genes were sequenced for 15 individuals at each of the following locations: Tall Timbers Research Station (Northern FL), Avon Park Air Force Base (Southern FL), and eastern and western populations in Louisiana. Three individuals from Columbus County (NC) were also sequenced at these genes. PCR reactions consisted of 1µl DNA, 1X buffer, 1.50 mM $MgCl_2$, 8.0 mM of dNTPs, 1.25 µM of each forward and reverse primers, 2.0 units *Taq* DNA polymerase (New

England BioLabs, Ipswich, MA), and water for a final volume of 25 µl. PCR amplification conditions were as follows: 95 ºC for 30 seconds followed by 34 cycles of 94 ºC for 30 seconds, 50 ºC (ND2)/ 60 ºC (TGFβ2) for 30 seconds, 72 ºC for 1 minute, and a final extension step of 72 ºC for 7 minutes. PCR products were sent to Beckman Coulter Laboratories (Danvers, MA) for Sanger single-pass sequencing. Forward and reverse strands were aligned for each sample and corrected using SEQUENCHER 5.0 (Gene Codes Corp.) All sequence data will be deposited in GenBank.

Individual birds lacking distinct cloacal protuberances or brood patches were sexed through amplification of the chromo-helicase DNA-binding genes using P2 and P8 primers (Griffiths et al. 1998). PCR reactions were performed using 13.0 µl reactions that included 9.30µl DNA, 1X buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.30 µM of each forward and reverse primers, and 2.0 units *Taq* DNA polymerase (New England BioLabs, Ipswich, MA). PCR amplification conditions were as follows: 94 ºC for 1 minute followed by 40 cycles of 94 ºC for 30 seconds, 48 ºC for 30 seconds, 72 ºC for 30 seconds and a final extension step of 72 ºC for 5 minutes. PCR products were separated by electrophoresis for 45–60 minutes at 116-120 volts in a 2% agarose gel.

2.4 Data Analysis

2.4.1 Population Molecular Variation

Microsatellite data were checked for genotyping errors such as stutter bands, large allele dropout and null alleles using MICROCHECKER V 2.2.3 (Van Oosterhout et al. 2004). Significant deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium were assessed using GENEPOP V 4.1.4 (Raymond and Rousset 1995, Rousset 2008). Due to low sample size, samples from Lee Memorial Forest (n = 2) and Madison County, Florida (n = 1) were combined with the nearest sampling locations: Talisheek Pine Wetlands Preserve and Tall Timbers Land Conservancy and Research Station, respectively. Exact P-values for HWE were computed using the complete enumeration method for loci with fewer than four alleles (Louis and Dempster 1987) and the Monte Carlo Markov chain (MCMC) method (dememorization 10000; batches 1000; iterations

per batch 10000) for loci with more than four alleles (Guo and Thompson 1992). Global deviation from HWE for populations was calculated using the same parameters listed previously. Significance values were adjusted using a Bonferroni sequential correction for multiple comparisons (Rice 1989) to maintain an experiment-wise error rate of $\alpha = 0.05$.

Population genetic variation was measured as observed average heterozygosity (H_O), expected average heterozygosity (H_E), the average number of alleles per locus (A) and allelic richness (AR), which controls for variation in sample size using rarefaction, with GENETIX V 4.03 (Belkhir et al. 1996-2004) and FSTAT V 2.9.3 (Goudet 1995). Initial allelic richness calculations included all populations; however low sample sizes in North Carolina and Talisheek Pine Wetlands Preserve substantially reduced AR across populations, so these two populations were dropped and allelic richness was calculated again for the remaining populations. GENEPOP was used to calculate F_{IS}, the inbreeding coefficient (Weir and Cockerham 1984).

For nuclear and mitochondrial DNA sequence data, samples were grouped into five regional populations, western Louisiana, eastern Louisiana, northern Florida, southern Florida and North Carolina, to compare genetic differences among Bachman's Sparrows subspecies. A 1038 base pair sequence for the ND2 gene from 47 sampled individuals and a 570 base pair sequence from TGFβ gene for 43 individuals were examined. Slightly different sets of idividuals were sequenced at each gene because some individuals did not amplify well at TGFβ. Nucleotide diversities (π), number of haplotypes and haplotype diversities (Nei 1987) were calculated for each population using DNASP V 5.10.1 (Librado and Rozas 2009). Estimates of sequence divergence between populations was also calculated using DNASP, which included the number of net nucleotide substitutions per site between populations (D_a) and the average number of nucleotide substitutions per site between populations (D_{xy}).

2.4.2 Population Genetic Structure

Genetic differentiation among sampling sites using calculated global F_{ST} (θ), as well as pairwise F_{ST} (Weir and Cockerham 1984) and R_{ST} (ρ) (Michalakis and Excoffier 1996) values from microsatellite data were calculated using GENEPOP. Both R_{ST} and F_{ST} were used because each can be applied at different evolutionary time scales. F_{ST} values are based on an infinite alleles model that states that there are an infinite number of states that an allele can mutate to in a single mutation event, hence each mutation is assumed to be unique, making this value more appropriate for studying recent patterns of genetic differentiation. A stepwise mutation model is applied to calculate R_{ST} values under the assumption that there is only one step per mutation, with equal probability of increasing or decreasing the number of repeats of a microsatellite marker by one. This makes R_{ST} more suited for determining population differentiation at microsatellite markers over longer evolutionary time scales (Balloux and Lugon-Moulin, 2002).

Patterns of population structure were analyzed for all microsatellite data using multiple methods: (1) the Bayesian clustering approach of STRUCTURE V 2.3.2 (Pritchard et al. 2000); (2) a spatial analysis of molecular variance using the program GENELAND V 4.0 (Guillot et al. 2005); and (3) a multivariate analysis using a factorial correspondence analysis (FCA) in the program GENETIX V 4.05. Using multiple analytical methods is recommended because it can lead to less biased assessments of population structure (Francois and Durand 2010). Also, multivariate analyses such as FCA are useful for comparison to Bayesian clustering approaches like STRUCTURE and GENELAND because of their ability to identify genetic structure in very large datasets, with negligible computational time, and without the required underlying assumptions of Bayesian models, such as setting an *a priori* maximum number of populations, and assuming that all possible populations have been sampled and are represented in the dataset (Patterson et al. 2006, Jombart et al. 2010).

The Bayesian assignment approach developed by Pritchard et al. (2000) in the program STRUCTURE V 2.3.2, assesses whether the sampled genotypes are substructured into multiple ($K > 1$) clusters or constitute a single population ($K = 1$). In this program, individuals are iteratively clustered based on a user defined number

of populations where log-likelihood ratios from Monte Carlo Markov Chain (MCMC) sampling provide the basis for deciding which number of clusters best fits the data. Analysis to determine population genetic structure was implemented with and without using the LocPrior clustering algorithm that incorporates user-defined sampling location information into determining the appropriate number of population clusters (Pritchard et al. 2000, Hubisz et al. 2009). The LocPrior model accounts for sampling locations and assumes that the probability that an individual is assigned to a cluster varies among locations. This method is appropriate for detecting weak genetic structure and is desirable in that it does not find structure where it does not exist (Hubisz et al. 2009). Five runs for each *K* between 1 and 11 were conducted with each run consisting of a burn-in period of 50,000 followed by 50,000 iterations. The admixture model, which calculates admixture proportions assuming that all individuals originated from the admixture of *K* parental populations (Pritchard et al. 2000) was also used and assumed allele frequencies were correlated (Falush et al. 2003). Using the output from STRUCTURE, the best estimate of the number of clusters *K* was determined using log-likelihood ratios from STRUCTURE and the method of Evanno et al. (2005; STRUCTURE HARVESTER), which identifies the most likely *K* as that which corresponds to the maximum change in the log probability of the data for successive values of *K*. The resulting most likely *K* indicated during initial runs was rerun in STRUCTURE for an additional 25 runs and averaged results were calculated across runs to obtain an average value of *r*, the parameter that estimates the informativeness of the sampling location data in the LocPrior model. Values of r close to or less than 1 indicate that the inclusion of sampling locations is informative, whereas values of $r >> 1$ imply that location data is uninformative when inferring ancestry (Hubisz et al. 2009)

Genetic structure as calculated by GENELAND was implemented using the package "Geneland" in R V 3.0. This program is a spatially explicit model to detect population subdivision and barriers to gene flow, which incorporates geographic data into the analysis of genetic structuring at a stage that defines and incorporates geographic boundaries among populations (Latch et al. 2008). GENELAND then uses the spatial coordinates, coupled with genetic marker data, to optimize the delineation of subpopulations under the assumption that the

more geographically isolated populations are, the more genetically differentiated they will be (Dore et al. 2009). This is in contrast with the STRUCTURE approach, where all clustering solutions are equally probable. In GENELAND, the spatial-D model was used to infer the number of subpopulations, *K*. Initial runs allowed *K* to vary under the following conditions; 10,000 stored iterations of the Markov chain, maximum rate of Poisson process set at the default value of 100, minimum population number set to 1 and maximum to 11, and the number of thinnings set to 10. Because individual GPS coordinates were available for each sample at the location of capture, the uncertainty of coordinates value was set to zero. A Correlated Allele Frequency model, a true Spatial model and a false Null Allele model were used in the analysis. Five independent runs of the above parameters were run for each potential *K*.

A 2D factorial correspondence analysis (FCA) was the final test, run in GENETIX, to determine population structure among sampling locations. The 2D FCA shows the relationship between each individual genotype in a two dimensional plot using a multivariate technique summarizing large datasets into informative multidimensional subsets representing the trends of the original multivariate data set (e.g., multiple loci and multiple samples).

Mantel tests were used to identify the presence of isolation by distance (IBD) across the study area. IBD is the theory that genetic distances between populations increase as geographic distances increase. Frantz et al. (2009) found that isolation by distance can confound the results from various Bayesian clustering programs such as STRUCTURE and GENELAND which may overestimate the degree and number of distinct populations by detecting artificial population clusters when there is an isolation-by-distance cline among the sampled populations. IBD was tested with a Mantel test (Mantel 1967) in program IBDWS v 3.23 for correlation between pairwise genetic (Nei 1972) and pairwise geographical distances. A reduced major axis regression (RMA) with 10,000 randomizations calculated the slope/correlation between genetic variation and geographic distances (Jensen et al, 2005). Unlike ordinary least-squares regression methods, RMA is less sensitive to error because it optimizes the "best-fit" line by reducing error for both variables simultaneously in the regression, a

more powerful statistical way to test for IBD (Hellberg, 1994; Jensen et al., 2005). Geographic distances between all sample locations were calculated as the average longitude and latitude coordinates associated with samples from each region.

Genetic structure was examined with nuclear and mitochondrial DNA sequence data by calculating an estimate of global F_{ST} using an analysis of molecular variance (AMOVA) implemented in the program ARLEQUIN V 3.11 (Excoffier et al. 2005) with statistical significance tested using 10,000 randomizations of the data. Pairwise F_{ST} estimates were also calculated using 10,000 randomizations using ARLEQUIN. The significance level was set at $p \leq 0.05$ for all tests.

To investigate phylogeographic structuring, relationships among nuclear and mitochondrial DNA haplotypes were constructed using the method of statistical parsimony (Templeton 1998, 2004) using TCS v 1.13 (Clement et al. 2000). Networks were used because they can give a better representation of the phylogenetic relationship among haplotypes in cases in which sequences are very similar and the strength of the historical inferences increase as genetic variation decreases (Dor et al. 2012). The program considers that a single polymorphic site in a sample, with a single variant allele, was derived and occurred as a result of a single mutation. The probability of parsimony (Templeton et al. 1992) is calculated for DNA pairwise differences until the probability exceeds, by default, 0.95. The number of mutational differences associated with the probability just before this 95% cutoff is then the maximum number of mutational connections between pairs of sequences justified by the "parsimony" criterion. MEGA5 (Tamura et al. 2011) was also used to construct Neighbor Joining trees using mitochondrial and nuclear DNA sequences in order to visualize the evolutionary relatedness between sampled populations. An unrooted neighbor joining tree was constructed after running 2000 replications of the bootstrap method to test for phylogeny. The Maximum Composite Likelihood substitution model including transitions and transversions with the substitution rate set at the default of uniform rates. Because the mitochondrial and nuclear sequences did not have any missing nucleotide bases, the gaps/missing data option was set for complete deletion and all three codon positions were used to build the tree.

After the tree was constructed, nodes with less than 50% support were condensed due to the uncertainty of the branching order.

2.4.3 Population Bottlenecks and Connectivity

Several methods were used to elucidate the effects of historic population declines and the degree of present day connectivity. Program BOTTLENECK v 1.2.02 (Piry et al. 1999) was used to evaluate evidence for recent population bottlenecks within several dozen generations (Cornuet and Luikart 1996) for each sampled population. During founder events, rare alleles are lost from the population more quickly than heterozygosity and, thus populations that have recently experienced a bottleneck will tend to show an apparent heterozygosity excess (Nei et al. 1975). Two estimates of expected heterozygosity are compared, one based on allele frequencies (H_e) assuming HWE and another based on the number of alleles and sample size (H_{eq}) assuming mutation-drift equilibrium. At equilibrium both estimates should be similar, but if a population has experienced a bottleneck, H_{eq} will decrease faster than H_e. The reverse could suggest population expansion. Estimates of heterozygosity were calculated using the two-phase model (TMP), which has been suggested as a better model for microsatellites than the other models possible in BOTTLENECK (Cornuet and Luikart 1996). TMP requires two parameters to be set: (1) the percentage of mutations that follow a strict stepwise mutational process and (2) the variance in size of multistep mutations. The variance parameter was set at the default setting of 30 and the stepwise mutation rate was set to 70% with the analysis set to run 10,000 iterations. A 70% stepwise mutation rate was used because recent research focused on mutational dynamics of avian microsatellites suggest ~60% to 80% of mutations involve a single-step change (Miller et al. 2012). The Wilcoxon signed-ranks procedure was used to test whether observed heterozygosity exceeded that expected at mutation-drift equilibrium, as it is robust to the effects of both small sample size (<30) and a small number of loci (<20) (Piry et al. 1999). The Wilcoxon test provides relatively high power and it can be used with as few as four polymorphic loci and any number of individuals; 15-40 individuals and 10-15 polymorphic loci is recommended to achieve high power (Luikart et al. 1997).

To examine whether sampled populations might contain individuals that were first generation (F_0) immigrants that originated from other geographically distinct populations, we used the Bayesian assignment procedure of Rannala and Mountain (1997), as implemented in GENECLASS 2.0 (Piry et al. 2004). The 'detect migrants' function was selected in GENECLASS as it is explicitly designed to identify F_0 (Piry et al. 2004) using the Paetkau et al.'s (2004) method to compute probabilities from 10,000 simulated genotypes, creating a test distribution of simulated individuals by drawing haplotypes, rather than alleles, from the observed data and thus preserving the partial linkage disequilibrium present in genotypes that have immigrant ancestry (Paetkau et al. 2004). The L_h/L_{max} likelihood test statistic, which is the ratio of the likelihood computed from the population where the individual was sampled (L_{home}) over the highest likelihood value among all population samples including the population where the individual was sampled (L_{max}), was used to identify migrants. An alpha level of 0.01 was selected to determine critical values and reduce the chance of Type I errors (Rannala and Mountain 1997).

3. RESULTS AND DISCUSSION

3.1 Population Molecular Variation

A total of 226 Bachman's Sparrows from 11 different sampling sites were genotyped at 19 microsatellite loci (Appendix). MICROCHECKER V. 2.2.3 analysis suggested the presence of null alleles for one locus, Zole F11. This locus also showed consistent deviations from HWE across all populations, and so it was dropped from the analyses. After Bonferroni correction, significant global deviations from HWE ($P < 0.05$) were found for three loci, Am 08, Am 18 and Am 20, however the deviations were not consistent across populations, so these loci were kept for subsequent analysis. Linkage disequilibrium analysis indicated evidence for linkage between Aca 01 and Aca 17 and Asμ09 and Zole E11. However, these associations were not present in all populations, suggesting these pairs of loci are not linked. Individual loci were polymorphic with 2-60 alleles per locus. Average allelic richness (*AR*) was 8.6 (Table 3.1). Expected heterozygosity was similar among populations, and in all but North Carolina, the average observed heterozygosity (H_O) was slightly lower than average expected heterozygosity (H_E) (Table 3.1). The inbreeding coefficient F_{IS} ranged from -0.0130 to 0.0678 and was positive in all but the North Carolina population (Table 3.1).

DNA sequence analysis at TGFβ showed a total of 27 haplotypes across all study populations. Eleven of these haplotypes were private and found within one population and no other. At ND2 there was a total of 19 haplotypes, 15 which were private and found within a single population. Overall sequence diversity within populations was low with nucleotide diversity (π) ranging from 0.0044-0.0076 for TGFβ and 0.0015-0.0026 for ND2 sequences (Table 3.2). Sequence divergence between populations was also low for both genes (Table 3.3). Despite low nucleotide diversity, both nuclear markers showed multiple haplotypes within individual populations and high haplotype diversity that ranged from 0.692-1.000 for ND2 and 0.925-1.00 for TGFβ (Table 3.2).

Table 3.1 Molecular variation of 226 *Peucaea aestivalis* individuals sampled from 11 study sites across the Southeastern United States including sample size (*n*), observed (H_O*; mean ± std. error)* and unbiased expected (H_E*; mean ± std. error*) heterozygosity, average number of alleles/locus (*A*), allelic richness (*AR*), and inbreeding coefficient (F_{IS}).

Population	***n***	H_O	H_E	***A***	***AR* (populations with n < 10)**	***AR* (populations with n > 10)**	F_{IS}
Abita Springs	15	0.7320 (± 0.2703)	0.7664 (± 0.2435)	9.167	3.006	8.246	0.0447
Avon Park Air Force Range	47	0.7524 (± 0.2292)	0.7801 (± 0.2342)	14.556	3.047	8.540	0.0355
Dry Prong	25	0.7358 (± 0.2537)	0.7716 (± 0.2549)	11.722	3.042	8.543	0.0470
Fort Polk	25	0.7307 (± 0.2838)	0.7740 (± 0.2481)	11.556	3.043	8.426	0.0572
Kisatchie National Forest	15	0.7199 (± 0.2347)	0.7563 (± 0.2518)	8.722	2.971	7.818	0.0489
North Carolina	3	0.7222 (± 0.3284)	0.7148 (± 0.3015)	3.667	2.822	-	-0.0130
Palustris Experimental Forest	13	0.7279 (± 0.2744)	0.7789 (± 0.2356)	8.778	3.046	8.236	0.0678
Sandy Hollow	29	0.7148 (± 0.2762)	0.7522 (± 0.2706)	10.778	2.980	8.066	0.0494
Tall Timbers Research Station	33	0.7314 (± 0.2484)	0.7732 (± 0.2502)	12.333	3.039	8.343	0.0529
Talisheek Pine Wetland Preserve	7	0.7460 (± 0.2477)	0.7807 (± 0.2361)	6.444	3.044	-	0.0489
Camp Whispering Pines	14	0.7145 (± 0.2600)	0.7425 (± 0.2211)	7.889	2.885	7.234	0.0394
Mean		**0.7298**	**0.7628**	**9.601**	**2.993**	**8.161**	**0.0435**

Table 3.2 Genetic diversity measures at ND2 and TGFβ for five regional *Peucaea aestivalis* populations including sample size (*n*), nucleotide diversity (π), number of haplotypes, and haplotype diversity with standard deviation.

	ND2				TGFβ-5			
Population Grouping	***n***	**π**	**# of Haplotypes**	**Haplotype Diversity**	***n***	**π**	**# of Haplotypes**	**Haplotype Diversity**
Western Louisiana	7	0.0022	5	0.857 (±0.137)	7	0.0050	10	0.925 (±0.047)
Eastern Louisiana	8	0.0021	5	0.857 (±0.108)	6	0.0044	9	0.939 (±0.058)
Northern Florida	14	0.0015	7	0.692 (±0.137)	14	0.0067	17	0.960 (±0.019)
Southern Florida	15	0.0017	8	0.867 (±0.067)	13	0.0055	16	0.945 (±0.027)
North Carolina	3	0.0026	3	1.000 (±0.272)	2	0.0076	4	1.000 (±0.177)

Western Louisiana population grouping: Fort Polk WMA, Dry Prong, Kisatchie National Forest and Palustris Experimental Forest sampling locations. Eastern Louisiana population grouping: Camp Whispering Pines, Sandy Hollow WMA, Abita Springs, Talisheek Pines Wetlands Preserve and Lee Memorial Forest. Northern Florida population: Tall Timbers Research Station. Southern Florida population: Avon Park Air Force Park.

Table 3.3 Estimates of mitochondrial (ND2) and nuclear (TGFβ) DNA sequence divergence between five regional *Peucaea aestivalis* populations. Number of net nucleotide substitutions per site between populations (D_a) located above the diagonal. Average number of nucleotide substitutions per site between populations (D_{xy}) located below the diagonal.

	ND2					TGFβ-5				
	Western Louisiana	**Eastern Louisiana**	**Northern Florida**	**Southern Florida**	**North Carolina**	**Western Louisiana**	**Eastern Louisiana**	**Northern Florida**	**Southern Florida**	**North Carolina**
Western Louisiana		0.00003	0.00072	-0.00002	-0.00037		-0.00017	0.00002	-0.00009	-0.00049
Eastern Louisiana	0.00217		0.00005	0.00003	-0.00015	0.00452		0.00012	0.00002	-0.00034
Northern Florida	0.00196	0.00061		0.00002	-0.00011	0.00590	0.00566		0.00002	-0.00057
Southern Florida	0.00192	0.00053	0.00159		0.00084	0.00517	0.00495	0.00613		-0.00057
North Carolina	0.00202	0.00217	0.00190	0.00075		0.00584	0.00566	0.00661	0.00600	

Western Louisiana population grouping: Fort Polk WMA, Dry Prong, Kisatchie National Forest and Palustris Experimental Forest sampling locations. Eastern Louisiana population grouping: Camp Whispering Pines, Sandy Hollow WMA, Abita Springs, Talisheek Pines Wetlands Preserve and Lee Memorial Forest. Northern Florida population: Tall Timbers Research Station. Southern Florida population: Avon Park Air Force Park.

3.2 Population Genetic Structure

Global F_{ST}, the measure of population subdivision across all populations, using microsatellite data was 0.012 (± 0.002), indicating slight genetic structure. Small but significant differences in pairwise genetic differentiation (F_{ST}) were detected for approximately half of the sampled populations, with values ranging from 0.0001 to 0.0574 (Table 3.4). R_{ST} ranged from -0.0003 to 0.1893 (Table 3.4). Pairwise F_{ST} and R_{ST} indicated that gene flow was highest between Fort Polk WMA and both Kisatchie National Forest and Palustris Experimental Forest, whereas North Carolina and Camp Whispering Pines had the lowest amount of gene flow (Table 3.4). Camp Whispering Pines was divergent from most populations with the highest significant pairwise F_{ST} and R_{ST} estimates for 10 and 8 population pairs, respectively (Table 3.4).

The AMOVA global test of differentation among samples was nonsignificant and suggested no population structure when examining both nuclear (P = 0.9261 ± 0.0206) or mitochondrial sequences (P = 0.2498 ± 0.0964). Nearly all the genetic diversity in sequence data was attributed to within-population variation: 95.07% from nuclear (ND2) haplotypes and 103.41% from mitochondrial (TGFβ-5) haplotypes (Table 3.5). Results of an AMOVA analysis greater than 100% can occur when there is no genetic structure because the true value of the estimated parameter is zero (Schneider et al. 2000).

STRUCTURE assigned the highest likelihood to a model with K = 1 populations when geographic location data was incorporated in the analysis (Ln *P(D)* for K = 1: -17429.3; Table 3.6), suggesting a single population. However, when information on sampling location was provided using the LocPrior model, as suggested for data sets with relatively weak structure (Hubisz et al. 2009), the model with the highest Ln *P(D)* and ΔK was obtained for K = 2 (Ln *P(D)* for K = 2 : -17338.7; Table 3.6). One cluster identified in STRUCTURE consisted of two of the four eastern Louisiana populations, and the Florida and North Carolina populations (Figure 3.1). The second cluster consisted of the remaining two eastern Louisiana populations (Figure 3.1). All remaining populations appeared to be a mixture of the two clusters (Figure 3.1).

Table 3.4 Pairwise estimates of F_{ST} (below diagonal) and R_{ST} (above diagonal) for eleven study sites, arranged from western to eastern, using 226 *Peucaea aestivalis* samples. Significant *P*-values ($p < 0.05$) indicated in bold.

	DP	**FP**	**KNF**	**PEF**	**WP**	**SH**	**TNC**	**AS**	**TTRS**	**AP**	**NC**
DP		-0.0058	-0.0003	-0.0106	0.0915	0.0301	0.0082	-0.0164	0.0042	-0.0039	-0.0410
FP	0.0007		0.0058	0.0060	**0.0968**	**0.0311**	0.0171	-0.0052	**0.0105**	-0.0005	-0.0498
KNF	0.0063	0.0001		0.0047	0.0390	0.0126	0.0505	-0.0116	-0.0042	0.0108	-0.0165
PEF	0.0026	0.0001	0.0029		**0.1348**	0.0318	0.0603	-0.0130	0.0076	-0.0010	-0.0232
WP	**0.0342**	**0.0255**	**0.0255**	**0.0231**		**0.0614**	**0.1893**	**0.0920**	**0.0892**	**0.1185**	**0.1584**
SH	**0.0098**	**0.0062**	**0.0137**	0.0081	**0.0332**		**0.1264**	-0.0000	**0.0314**	**0.0483**	-0.0032
TNC	0.0108	0.0063	0.0101	0.0035	**0.0422**	**0.0160**		0.0401	**0.0604**	0.0212	0.0077
AS	0.0038	0.0002	0.0091	0.0027	**0.0391**	**0.0130**	0.0108		-0.0029	-0.0036	-0.0432
TTRS	**0.0095**	0.0021	**0.0110**	0.0051	**0.0364**	**0.0138**	0.0092	0.0069		0.0050	-0.0291
AP	**0.0113**	0.0032	**0.0139**	**0.0067**	**0.0347**	**0.0188**	**0.0188**	**0.0132**	0.0018		-0.0505
NC	0.0153	0.0115	0.0306	0.0162	**0.0574**	0.0208	0.0183	0.0209	0.0110	0.0047	

Populations abbreviated as follows: Abita Springs (AS), Avon Park (AP), Dry Prong (DP), Fort Polk WMA (FP), Kisatchie National Forest (KNF), North Carolina (NC), Palustris Experimental Forest (PEF), Sandy Hollow WMA (SH), Tall Timbers Research Station (TTRS), Talisheek Pine Wetlands Preserve (TNC), Camp Whispering Pines (WP).

Table 3.5 AMOVA results using nuclear (TGFβ) and mitochondrial (ND2) sequences from five regional *Peucaea aestivalis* populations.

Source of Variation	d.f.		Sum of Squares		Variance Components		Percentage of Variation	
	TGFβ	ND2	TGFβ	ND2	TGFβ	ND2	TGFβ	ND2
Among Groups	4	4	5.518	1.543	0.0870	-0.0552	5.40	-13.45
Among Populations Within Groups	6	7	7.125	3.571	-0.1419	0.07532	-8.82	18.47
Within Populations	75	35	124.833	13.567	1.6644	0.38762	103.41	95.07
Total	85	46	137.477	18.681	1.6095	0.40772		

Table 3.6 Average log likelihood probability of *Peucaea aestivalis* microsatellite data between successive *K* values for groups ranging from 1 to 11 using the LocPrior algorithum in program STRUCTURE.

# *K*	Reps	Mean LnP(*K*)	Standard Deviation LnP(*K*)	Ln'(*K*)	|Ln''(*K*)|	Δ*K*
1	5	-17430.5	2.7601	--	--	--
2	5	-17338.7	9.7006	91.82	113.46	11.69616
3	5	-17360.4	62.8522	-21.64	42.36	0.673962
4	5	-17339.6	66.6611	20.72	50.66	0.759963
5	5	-17369.6	68.5804	-29.94	255.72	3.728764
6	5	-17655.2	235.3642	-285.66	473	2.009651
7	5	-17467.9	144.8908	187.34	482.2	3.328023
8	5	-17762.8	229.3732	-294.86	420.26	1.83221
9	5	-17637.4	144.0174	125.4	312.6	2.170571
10	5	-17824.6	351.7327	-187.2	250.24	0.711449
11	5	-18262	430.5205	-437.44	NA	NA

STRUCTURE HARVESTER results suggested $K = 2$ populations, however, these results are based on the greatest change in the average likelihood score (ΔK) between suggested K values (Figure 3.2), and therefore, $K = 1$ cannot be calculated (Table 3.6). However, mean Ln $P(D)$ values for $K = 1$ and $K = 2$ populations were very similar when using sampling location information (Table 3.6), suggesting little improvement when $K = 2$. Additionally, the inference of two population clusters in STRUCTURE and STRUCTURE HARVESTER could be

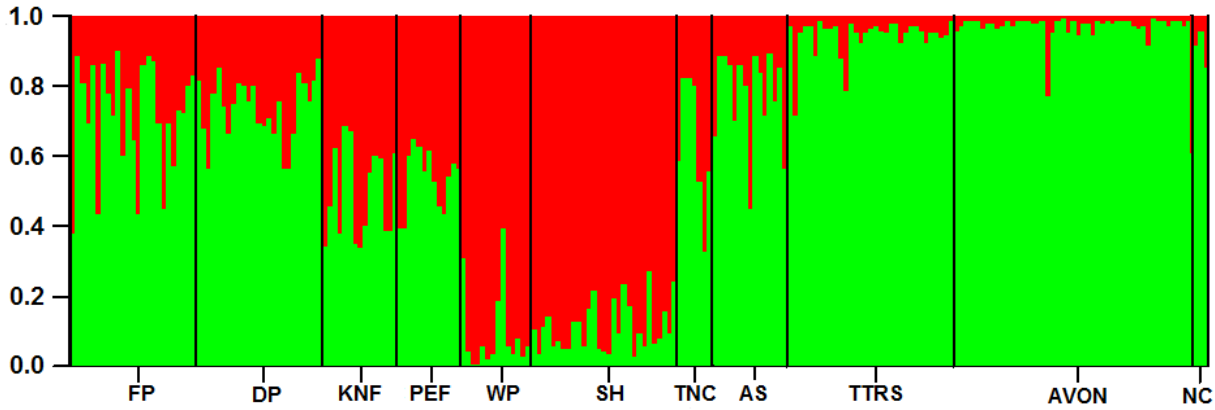

Figure 3.1 STRUCTURE boxplot for K_{max} = 11 based on 18 microsatellite loci for 226 *Peucaea aestivalis* individuals sampled during the study. Population abbreviations are as follows: Abita Springs (AS), Avon Park (AP), Dry Prong (DP), Fort Polk WMA (FP), Kisatchie National Forest (KNF), North Carolina (NC), Palustris Experimental Forest (PEF), Sandy Hollow WMA (SH), Tall Timbers Research Station (TTRS), Talisheek Pine Wetlands Preserve (TNC), Camp Whispering Pines (WP).

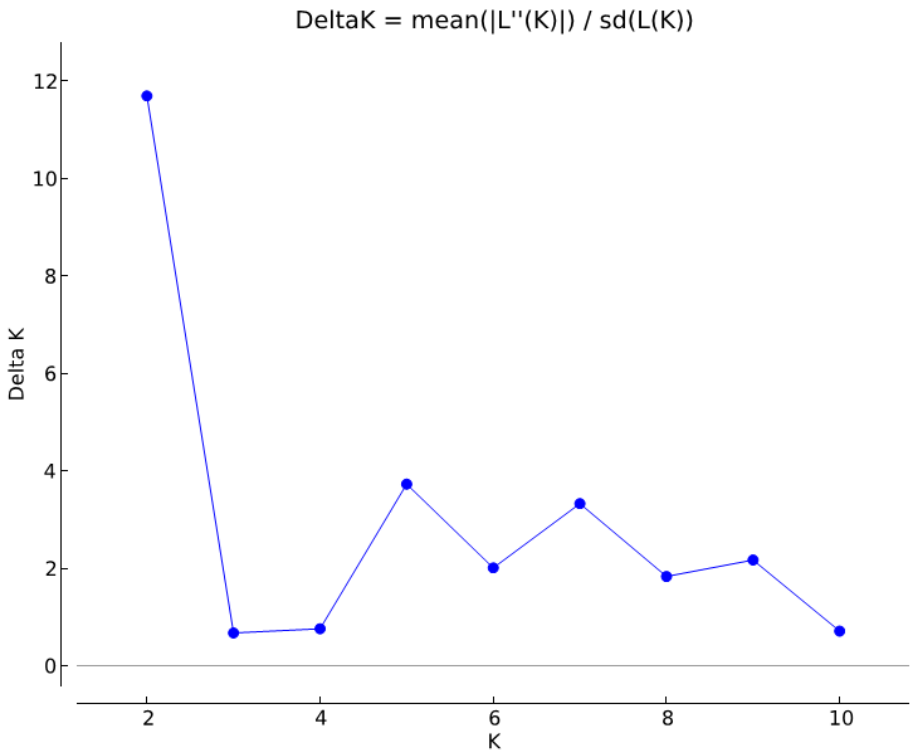

Figure 3.2 STRUCTURE HARVESTER calculated rate of change in the log likelihood probability (Δ*K*) of *Peucaea aestivalis* microsatellite data between successive *K* populations ranging from 2 to 11.

explained by the reduced ability of these two methods to detect the correct number of population clusters when F_{ST} values are low (Latch *et al.* 2008, Kalinowski 2011). The average value of the *r* parameter for 25 runs of *K* = 2 was 0.73, indicating that location data coupled with genotype data may be more informative in inferring ancestry than genetic information alone.

Similarly, GENELAND results suggested a single population (Figure 3.3a) with no barriers to gene flow as given by the map of posterior probability (Figure 3.3b). Black dots on the map are geo-referenced individual genotypes while color corresponds to population membership as well as the estimated number of populations; a single color suggests a single population (Figure 3.3b). The factorial correspondence analysis 2D plot calculated using program GENETIX also suggested little structure: axis one and two, which represent the degree of separation among individual Bachman's Sparrows, explained only 2.80% of the variation and individuals were tightly grouped with no discernible separation among the geographic areas (Figure 3.4). Finally, isolation by distance (IBD) analysis of Bachman's Sparrow populations showed no significant relationship between geographic distance and genetic distance ($r^2 = 5.752e^{-03}$, Figure 3.5), with the y-intercept not differing from zero (y-intercept = -0.04114 ± 0.00777). A Mantel test found no relationship between geographic distance and genetic distance matrices (r = 0.0758, $p = 0.3140$).

Parsimony haplotype networks created using program TCS suggested some structure among Bachman's Sparrow populations (Figures 3.6, 3.7). ND2 sequences contained 19 haplotypes, with 15 (83%) of the haplotypes unique to particular regional populations. The most common haplotype was shared by 42.5% of the 47 sampled individuals. The highest frequency of a single, unique haplotype was in Southern Florida, and was present in three (6.4%) of the 47 individuals. Similar structure was found with nuclear, TGFβ-5, sequence data. There were 27 haplotypes, with 11 (40.7%) of the haplotypes unique to particular regional populations and the most common haplotype shared by 48.1% of the 43 sampled individuals. Overall there was no clear geographical pattern in the distribution of haplotypes. The statistical parsimony tree for both ND2 and TGFβ-5 were both star-like (Figures 3.6, 3.7), suggesting a possible range expansion from a single refugium. Neighbor

joining trees built in MEGA5 using ND2 sequence data produced a cladogram that divided sampled individuals into two separate clades (Figure 3.8), however these clades included individuals from all sampled sites and had no clear geographic pattern. The neighbor joining tree built with TGFβ-5 sequence data produced a cladogram with a single polytomy (Figure 3.9), with all sequenced individuals creating a single clade. Polytomies can suggest multiple, simultaneous speciation events, but in this case the resulting cladogram is likely suggesting an absence of data to resolve the tree any further due to the low number of polymorphic sites within the sequences. Both neighbor joining trees suggest no genetic differentiation among the sampled populations.

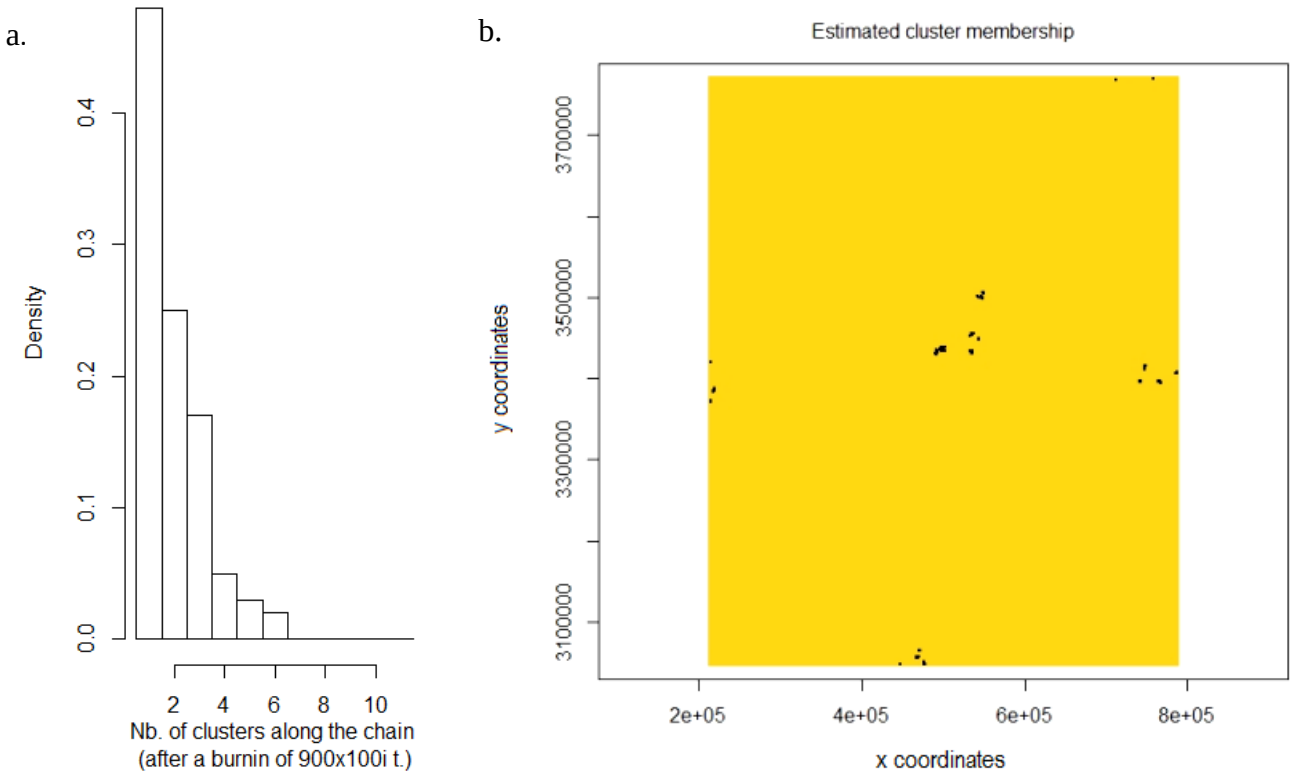

Figure 3.3 GENELAND analysis (a) Most likely number of *Peucaea aestivalis* populations. (b) Map of population subdivision data based on mode of posterior probabilities calculated using microsatellite and geographic population data.

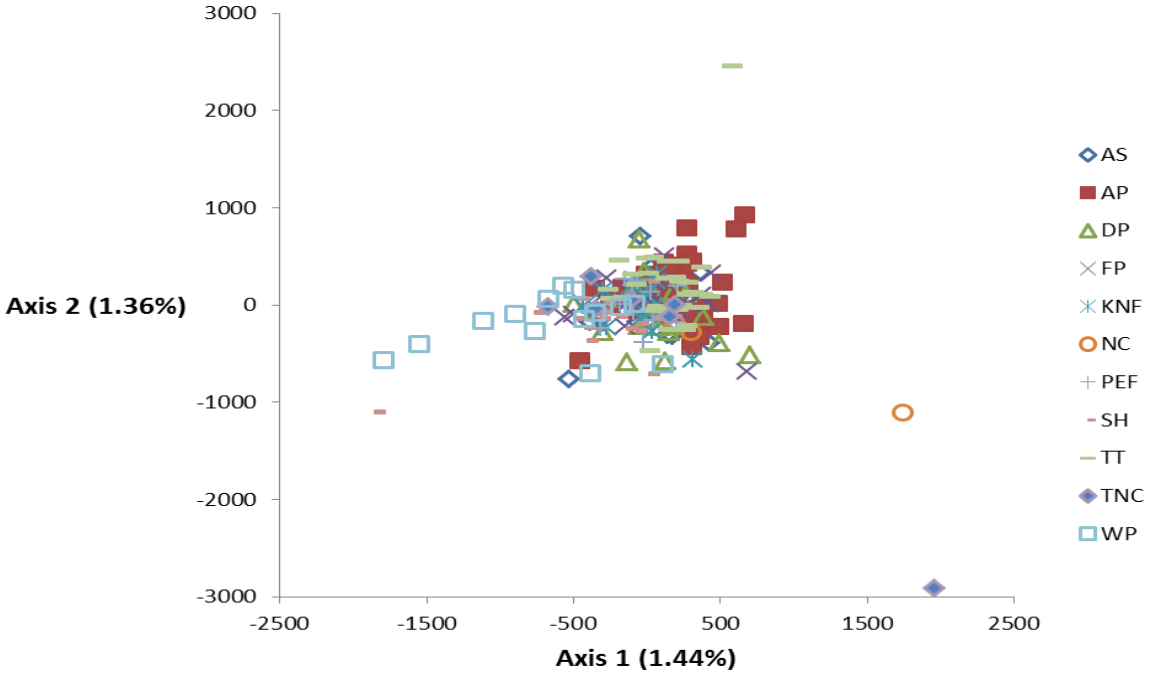

Figure 3.4 Factorial correspondence analysis (FCA) of 226 *Peucaea aestivalis* individuals sampled from eleven study sites.

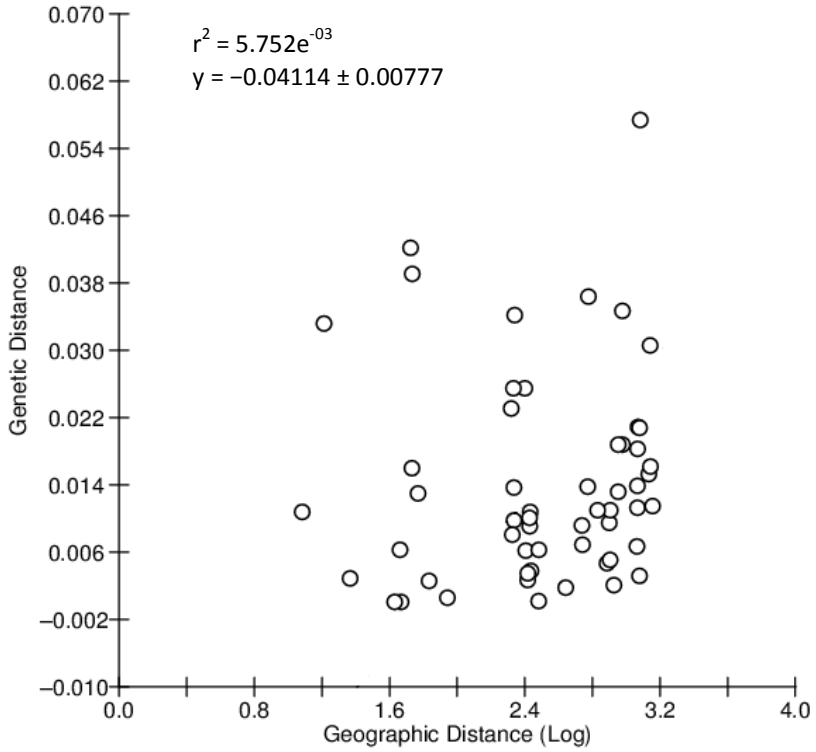

Figure 3.5 Isolation by distance relationship between pairwise genetic vs. pairwise geographical distances in 226 *Peuacea aestivalis* samples using a reduced major axis regression calculated from a Mantel test for matrix correlations.

3.3 Population Bottlenecks and Connectivity

Analyses in BOTTLENECK showed significant heterozygosity excess in four populations: Fort Polk (p = 0.037), North Carolina (p = 0.025), Talisheek Pine Wetlands Preserve (p = 0.049), and Camp Whispering Pines (p = 0.030), under the two-phase mutation model, indicating evidence of recent bottlenecks in those, although low sample size for Talisheek Pine Wetlands Preserve and North Carolina could create a significant P value indicating a false positive for a bottleneck. Pope et al. (2000) also found that false bottleneck signals could be observed in populations experiencing high rates of migration, which may be relevant to Bachman's Sparrow. GENECLASS was able to detect 15 first generation (F_0) migrants that were assigned to areas other than their sampling location (Table 3.7).

Table 3.7 Results of migrant detection analysis by GENECLASS showing individuals with significant assignment probabilities ($P < 0.01$) suggesting population origins other than the study site in which they were sampled.

Sample	Geographic origin	GENECLASS locality of highest probability assignment	GENECLASS highest assignment probability
LSUMZ 2470	Abita Springs	Kisatchie National Forest	0.0026
11009	Avon Park	Fort Polk	0.0039
11011	Avon Park	Tall Timbers	0.0041
58407	Fort Polk	Kisatchie National Forest	0.0096
58481	Fort Polk	Abita Springs	0.0069
58497	Dry Prong	Sandy Hollow	0.0098
58428	Kisatchie National Forest	Avon Park	0.0012
58429	Kisatchie National Forest	Palustris Experimental Forest	0.0022
58468	Sandy Hollow	Fort Polk	0.0094
07738	Tall Timbers	Sandy Hollow	0.0039
07813	Tall Timbers	Avon Park	0.0046
47760	Tall Timbers	Abita Springs	0.0061
58450	Talisheek Pine Wetlands	Palustris Experimental Forest	0.0077
58447	Camp Whispering Pines	Kisatchie National Forest	0.0034
58448	Camp Whispering Pines	Fort Polk	0.0019

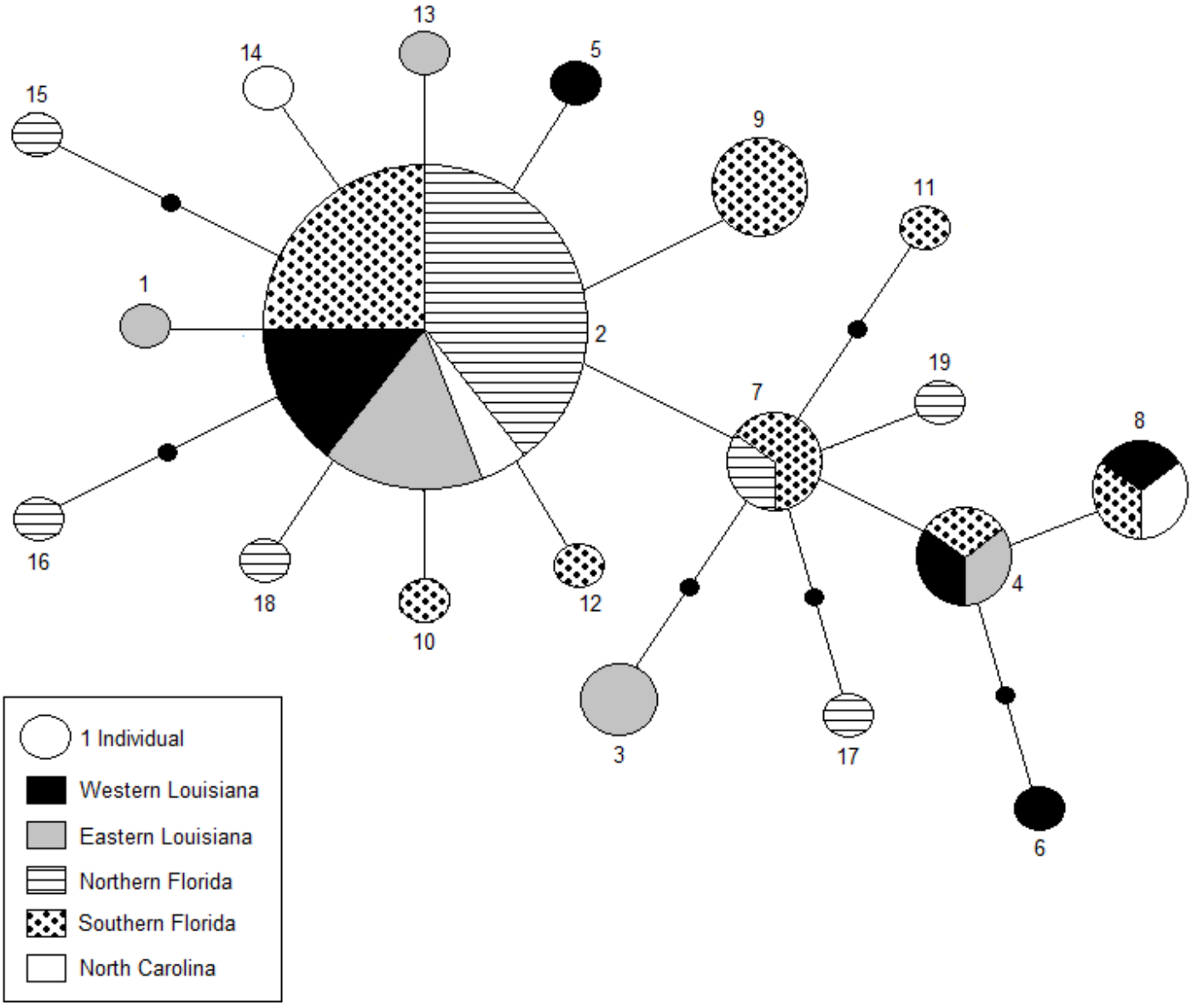

Figure 3.6 Unrooted parsimony haplotype network for mitochondrial sequence data (ND2) as computed using TCS V 1.21 for five regional populations of *Peucaea aestivalis*. Areas of circles are proportional to the number of individuals with that haplotype and haplotype number is listed next to circles. A haplotype found in only one individual is given as a size reference in the legend. Small black circles indicate a missing haplotype (one that either was not recovered during sampling or is extinct).

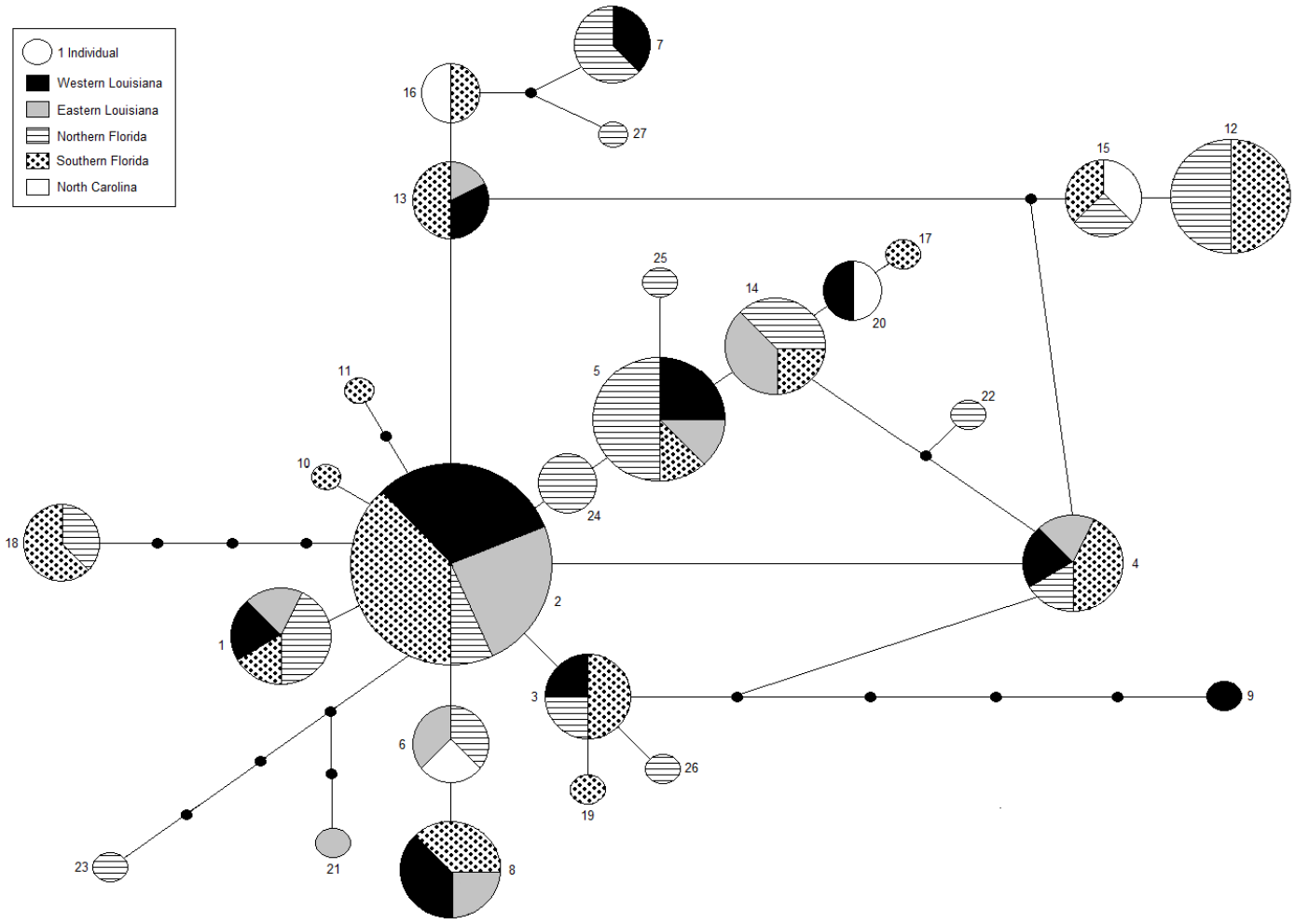

Figure 3.7 Unrooted parsimony haplotype network for nuclear sequence data (TGFβ) as computed using TCS V 1.21 for five regional populations of *Peucaea aestivalis*. Areas of circles are proportional to the number of individuals with that haplotype and haplotype number is listed next to circles. A haplotype found in only one individual is given as a size reference in the legend. Small black circles indicate a missing haplotype (one that either was not recovered during sampling or is extinct).

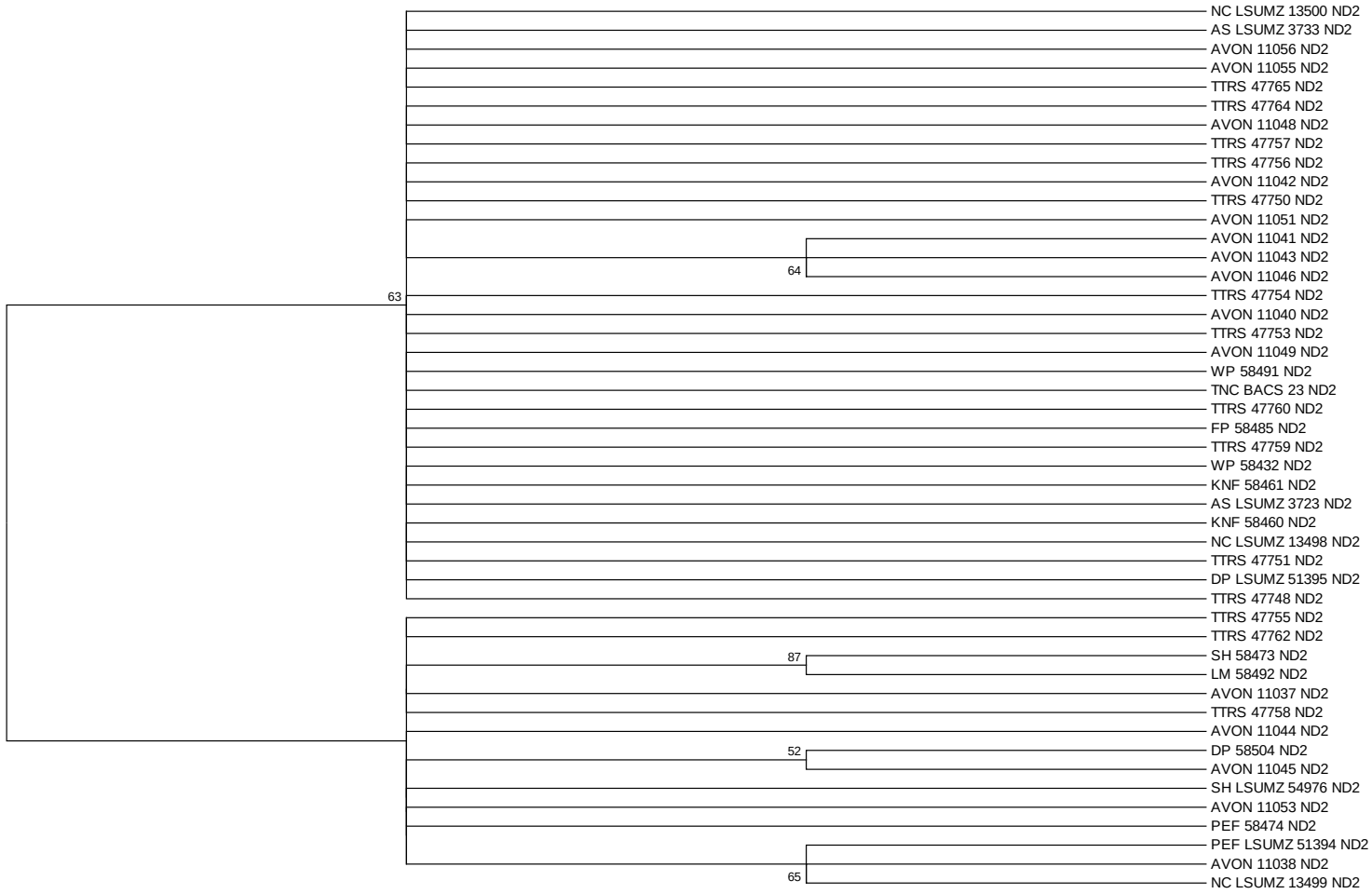

Figure 3.8 Evolutionary relationships of five regional *Peucaea aestivalis* populations using a mitochondrial gene, ND2, inferred using the Neighbor-Joining method in MEGA v 5.2. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) is shown next to the branches. Population abbreviations are as follows: Abita Springs (AS), Avon Park (AP), Dry Prong (DP), Fort Polk WMA (FP), Kisatchie National Forest (KNF), North Carolina (NC), Palustris Experimental Forest (PEF), Sandy Hollow WMA (SH), Tall Timbers Research Station (TTRS), Talisheek Pine Wetlands Preserve (TNC), Camp Whispering Pines (WP).

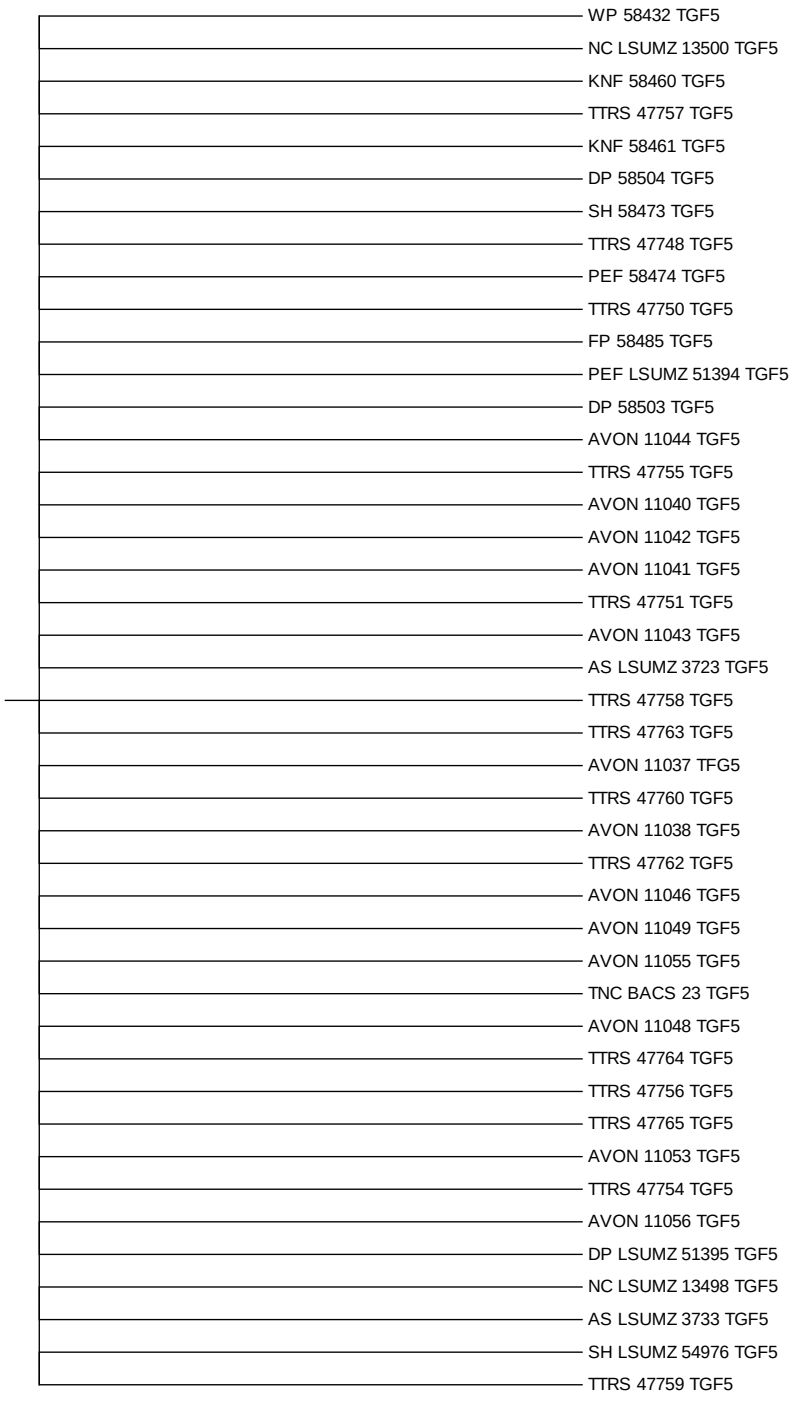

Figure 3.9 Evolutionary relationships of five regional *Peucaea aestivalis* populations using a nuclear gene, TGFβ-5, inferred using the Neighbor-Joining method in MEGA v 5.2. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) is shown next to the branches. Population abbreviations are as follows: Abita Springs (AS), Avon Park (AP), Dry Prong (DP), Fort Polk WMA (FP), Kisatchie National Forest (KNF), North Carolina (NC), Palustris Experimental Forest (PEF), Sandy Hollow WMA (SH), Tall Timbers Research Station (TTRS), Talisheek Pine Wetlands Preserve (TNC), Camp Whispering Pines (WP).

4. CONCLUSIONS AND RECOMMENDATIONS

Genetic studies can provide important information about the connectivity of populations and can have management implications when they uncover significant genetic differentiation or provide no evidence of distinct population units. Genetic studies are especially informative in identifying distinct populations or populations of high genetic value that may be used as a source to bolster populations at risk from inbreeding and low genetic diversity, which can occur after population declines. In this study, Bachman's Sparrow populations were examined for range-wide genetic structure and diversity using nuclear and mitochondrial DNA sequences and microsatellite data. Most analyses indicate a single, panmictic population of Bachman's Sparrows in the southeastern US. That so little spatial genetic structure was detected across such a large area is contrary to expectations given the different subspecific descriptions, the patchy distribution of longleaf pine savannahs in which Bachman's Sparrows are primarily found, and the widely presumed low dispersal rates of non-migratory populations (Dunning 2006).

4.1 Population Variation, Structure, and Viability

Bachman's Sparrow populations show high genetic diversity, little to no inbreeding, and weak genetic population structure; results that indicate considerable gene flow among populations. Our results are consistent with many migratory (Frankham et al. 2002) passerines such as Emberizidae and Neotropical songbird species, which have high levels of gene flow even among distantly located populations (Lee *et al.* 2001). For example, genetic differentiation is both small and non-significant among fragmented populations of Brewer's Sparrow (*Spizella breweri breweri*; Croteau *et al.* 2007), Reed Buntings (*Emberiza schoeniculus*; Mayer *et al.* 2009), Song Sparrows (*Melospiza melodia*; Wilson *et al.* 2011) and Cerulean Warbler (*Setophaga cerulean*; Veit *et al.* 2005), species that are all known to have either north-south or east-west patterns of seasonal migration. Non-migratory species are generally expected to show spatial genetic structure over large spatial scales because they may have low gene flow, which causes genetic differentiation, and in some instances where dispersal distances are small, the landscape matric between habitat patches could be perceived by many species as so inhospitable

that dispersal is severely limited or completely lost, creating significant genetic structure (de Ita *et al.* 2012). On the other hand, if long-distance dispersal occurs regularly, no genetic structure, isolation-by-distance (IBD) or spatial changes in genetic diversity are expected across the species' range (Bialozyt *et al*. 2006). A prime example of genetic effects created by limited dispersal can be found in the House Wren, which consists of migratory northern populations (*Troglodytes aedon*) and sedentary southern populations (*Troglodytes aedon musculus*) located across the species' North American range. House Wren populations show differing levels of genetic structure based on their migratory nature. Arguedas and Parker (2000) found high genetic diversity and less population substructure in northern House Wren populations that have seasonal north-south migration compared to southern sedentary populations. In this species, dispersal through seasonal migration is enough to reduce genetic differentiation.

In Bachman's Sparrow, the Fort Polk and North Carolina populations were the most geographically distant populations (~1,500 km), located at the western and eastern extremes of the range, but pairwise F_{ST} values for these two populations were low and non-significant, though non-significance can likely be attributed to low sample size for the North Carolina population. Significant, but low pairwise F_{ST} values were calculated between Fort Polk and Avon Park Air Force Range, which are geographically separated by similar distances (~1,200 km) to North Carolina. Low differentiation and no evidence of isolation-by-distance suggest significant connectivity between populations located at the extremes of Bachman's Sparrow range, a much higher level of dispersal than expected given that southern populations are considered sedentary throughout the year while populations found in the northern most regions of the range are known to migrate to southern latitudes during the winter months (Dunning 2006). Interestingly, the most differentiated populations, Sandy Hollow WMA and Camp Whispering Pines, were located closer to the center of the species' range. This is not entirely surprising since these sites are both in the isolated and highly fragmented longleaf pine habitat found in southeastern Louisiana. In this area of the state, the majority of longleaf communities have been lost or degraded by increased ecosystem alterations through human-use changes or fire suppression significantly

reducing or completely eliminating contiguous forests. This fragmentation creates smaller, isolated populations due to the inhospitable habitat matrix created between neighboring populations that could reduce gene flow, ultimately increasing the chance of genetic variation among neighboring populations. Low pairwise F_{ST} values between populations east and west of the Mississippi River in Louisiana also suggest, on a local level, the absence of genetic structure and any unsuitable habitat created by both the Mississippi River and longleaf pine fragmentation has not deterred individuals from dispersing across the state. Over the entire geographic range, lack of isolation-by-distance and evidence of individuals originating from populations other than the one in which they were sampled, is consistent with long distance dispersal that has prevented any structure from arising.

Although differentiation among most sampling locations was significant, F_{ST} values were generally low and within the range of drift connectivity ($F_{ST} < 0.1$). Populations that have similar allelic frequencies indicate substantial genetic connectivity in the order of greater than 10 migrants per generation (Lowe and Allendorf 2010). However, low population differentiation does not necessarily imply contemporary genetic connectivity in all situations. Low genetic differentiation between populations lacking connectivity could be attributed to populations having either a large effective population size created by recent expansion from a single, refugial population or recent isolation in which factors like genetic drift, selection, and mutation have not had sufficient time, even in small populations, to produce significant genetic differences (Brown et al. 2013). Rapid population expansion is suggested in the star-like DNA haplotype networks but does not appear to be consistent with what is known about population range expansion and contraction.

Little research has been done to elucidate the dispersal habitats of Bachman's Sparrows, but what has been observed suggests that this species has the ability to move large distances and adapt to a constantly changing landscape. In the southern states, Bachman's Sparrow is considered to be a year-round resident during the winter while northern populations are more migratory in the winter months moving large distances south from North Carolina, Kentucky, and Arkansas down along the Atlantic coast to southern Florida and then

westward into the Gulf States (Mitchell 1998). Bachman's Sparrows have also shown high vagility during the past century when their range size increased dramatically during an expansion north into Pennsylvania and Illinois when large areas of abandoned farms and degraded pastures left fallow created suitable habitat that mimicked the savannah-like understory of longleaf pine stands (Watts *et al.* 1998). In fact, this species is so well adapted to landscapes resembling longleaf pine habitat that Bachman's Sparrows have also been observed using human-created clearcuts and utility right-of-ways (Dunning 2006) suggesting that this species is adaptable and much more mobile than perhaps researchers originally acknowledged. Individuals have also been observed establishing new territories or reestablishing and defending previously held territories immediately following fire. Such individuals remain on these territories through the remainder of the breeding season, despite the impoverished habitat quality temporarily created by the fire (Shriver and Vickery 2001; Tucker et al. 2006; Cox and Jones 2007; Brown 2013; Jones et al. 2013; personal field observation). Currently recognized habitat characteristics that promote Bachman's Sparrow use frequently include the presence of dense grass and forb ground cover and low-statured hardwoods (Tucker et al. 1998) created by frequent (≥3 years) burning that provide concealment cover from predators and increased food supplies (Plentovich et al. 1998). Nesting characteristics have been based primarily on habitat traits where territorial, singing, males were observed rather than female habitat preference because of the difficulty in finding the well concealed nests (Jones et al. 2013). Despite the majority of Bachman's Sparrow literature suggesting these habitat attributes are required for Bachman's Sparrow habitation, more recent studies have found other characteristics may be more important in territory establishment than ground cover. A study by Brooks and Stouffer (2010) suggested that males selected territory sites based in part on available singing perches, while Jones et al. (2013) found the amount of bare ground, created in part by prescribed fires, was an important structural component for nest location. Given that Bachman's Sparrow habitat is ephemeral and only suitable within three years of a burn (Watts *et al.* 1998), it is perhaps not surprising that this species is adapted to colonize suitable habitat quickly, even over long distances.

Overall, the low genetic population structure found in this study may have occurred because Bachman's Sparrow are adapted to the spatial and temporal habitat fragmentation patterns produced by natural fire, a disturbance that produces high quality longleaf pine savannahs (Jones et al. 2013). Dispersal modeling in several studies has suggested that higher dispersal capability should be maintained in species inhabiting landscapes that have frequent temporal and spatial changes while species found in less disturbed and more contiguous habitat would have less pronounced dispersal (McPeek and Holt, 1992; Paradis, 1998). Longleaf pine communities historically burned frequently and had one of the lowest fire return intervals of ecosystems globally, ranging from 1 to 10 years, with an average of every three years, maintaining both high species richness and the open-canopy structure (Mitchell et al. 2006) preferred by Bachman's Sparrows (Watts *et al.* 1998). However, fires are suppressed on most remnant stands of longleaf pine and now produce insufficient fire return intervals. In some areas, prescribed burns have replaced the naturally occurring fires that were once the main drivers in maintaining this ecosystem. Along with prescribed fire, another common longleaf pine management strategy is even-aged stand management where uniform or irregular shelterwood cuts are performed to closely mimic stand replacing hurricane-type disturbances that historically occurred in longleaf pine stands across the southeast (Brockway et al. 1997). Both management techniques lead to a patch-work type management regime, with managers burning or cutting various sized sections of contiguous longleaf pine stands while leaving the adjacent areas of mature trees and grasses as seed banks for future regeneration. For example, on some federally managed lands such as the Apalachicola National Forest, management requires burning over 40,000 ha each year and is only accomplished using prescribed fires that encompass very large tracts of longleaf pine, on average, 550 ha or more (Jones 2008), creating large gaps between unburned fragments that can support an individual's territory. Without regular disturbance regimes, habitat patches suitable for this species become patchily distributed with large areas of habitat between potential territory sites that could affect rates, distance and the resulting gene flow between populations.

Bachman's Sparrows require frequent fire and are typically absent in pine forests where fire has been excluded for >3 years (Engstrom et al. 1984, Jones et al. 2013). Numerous studies suggest that their survival rates may be influenced by the timing and frequency of prescribed burns (Tucker 1998; Seaman and Krementz 2000; Stober and Krementz 2006). Typical longleaf pine habitat management focuses on growing season burns applied from April to August because this decreases hardwood regeneration and improves grass and forb cover (Seaman and Krementz 2000), that in turn has been attributed to increased density of Bachman's Sparrows occupying the area the following year. Using prescribed burns during this part of the year also mimics the natural fire season of longleaf pine brought about by lightning-set fires that peak during April through July (Brockway et al. 2005). One drawback to this particular management strategy is that growing season burns coincide with the Bachman's Sparrow breeding season which spans late February to July (Dunning 2006), but little research has been done to determine how individuals may move in response to fire regimes, especially after breeding territories and nests have been established. Dispersal after fire may also be affected by the distance to the nearest suitable habitat. If established territories and/or nests are destroyed by fire, males could disperse to the nearest suitable habitat for breeding, which may be miles away, artificially creating gene flow to areas that might not otherwise receive migrants. In a study to understand Bachman's Sparrow movement in relation to prescribed fire, Seaman and Krementz (2000) reported that nearly all radio-tagged sparrows dispersed from sites where growing-season burns were applied and did not return. During a similar study, Brown (2013) found that daily post-burn movements were significantly larger than pre-burn movements (255.9 m versus 485.3 m). Average displacement distances also increased (733.4 m) and 64% of individuals left the study site, though researchers partially attributed increased movement distance to a severe drought occurring on the study area. The use of prescribed fire may be causing individuals to disperse farther to establish replacement breeding territories or support offspring by utilizing resources in areas further away from the nest. As a shifting patch mosaic of unsuitable and suitable habitat is created through prescribed fire and typical natural community succession, these events could facilitate individual movement and thus gene flow across

larger areas. This increased gene flow may prevent population differentiation by homogenizing genetic structure. Similar instances of apparent genetic connectivity, low population differentiation and weak genetic structure in fire-prone habitats by *in-situ* repopulation have been recorded in other avian species adapted to living in frequently fragmented landscapes of burned and unburned areas, such as the Mallee Emu-wren (*Stipiturus mallee*) and the Eastern Bristlebird (*Dasyornis bachypterus*) of Australia and the Blue Chaffinch (*Fingilla teydea polatzeki*) of Spain (Brown et al. 2013).

4.2 Implications for Conservation and Management

The apparent high genetic connectivity of Bachman's Sparrow populations is a positive outcome for the conservation of this species. The capacity for high dispersal by Bachman's Sparrows recorded over the past century, coupled with these genetic results suggest panmixia and provide evidence that neither natural nor anthropogenic fragmentation has caused population differentiation. As a result, these findings challenge existing ideas about dispersal rate and distance in Bachman's Sparrow. The lack of differentiation across the species' geographic range means that for management purposes this species could probably be treated as a single evolutionary significant unit (ESU). However, an examination of morphological differences may be necessary to confirm this conclusion: the three AOU subspecies designations of Bachman's Sparrow are based on plumage differences and geographic location. Sibley (2000) also describes morphological differences with "eastern" populations as having strong black streaks along the back and an overall dark gray coloration and "western" populations as having a bright rufous and gray pattern, however the exact geographic range for eastern and western populations are not elaborated. Moreover, translocations to provide gene flow among populations and counteract the negative effects of genetic drift and inbreeding depression do not appear to be necessary as all populations had high levels of diversity and low levels of inbreeding. Although our results imply that habitat fragmentation and loss had little effect on the erosion of genetic diversity of Bachman's Sparrow populations, it is still important to consider habitat in the management of this species. Bachman's

Sparrows may be adapted to ephemeral habitat through high vagility, but they nevertheless require sufficient suitable habitat to persist over the long term.

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APPENDIX: MICROSTATELLITE CHARACTERISTICS

Characteristics of 23 microsatellite loci used for genotyping 226 *Peucaea aestivalis* individuals. Information is given on the base repeat motif and forward and reverse primer sequence, annealing temperature in Celsius degrees (T_A) for optimized amplification, size range (bp) of alleles, and number of alleles (N_A).

Locus	Repeat Motif	Primer sequence	T_A	Size range	N_A	GenBank accession no.	Reference
*Mme 12	$(CCCACA)_{13}$	F: AGGGACTGTCACTGTGGGACTGAAG R: TGGCTTTATGGAACAAGGCATC	48	199-203 bp	2	AF127385	Jeffery *et al.* 2001
*FhU2	$(CT)_{12}$	F: GTGTTCTTAAAACATGCCTGGAGG R: GCACAGGTAAATATTTGCTGGGCC	48	144-184 bp	18	X84361	Primmer *et al.* 1996
*Asμ09	$(CA)_{25}$	F: CTTTGATTACAGAAATATGTCTTCT R: GAAAGAGGCATGCTCGTAT	48	137-161 bp	11	AY172992	Bulgin *et al.* 2003
*SOSP 01	$(GGAT)_{17}GCAT$-$(GGAT)_2$	F: GCCAACACCCTCAACAAGAT R: ACCAACTGATGCACCTTCTG	48	219-259 bp	11	GU301255	Sardell *et al.* 2010
*SOSP 02	(CTGT)6(GT)3	F: AAACTCGCGTCTTTGCTAGG R: CAGGTGTCCTGCAGATGTTG	48	179-219 bp	18	GU301256	Sardell et al. 2010
*SOSP 04	$(TGTC)_6$	F: GGTTGATGGGGATGTTTCTG R: CTTCTTGAGCTTGGGGTCAC	48	186-232 bp	22	GU301258	Sardell *et al.* 2010
*SOSP 14	$(CTAT)_{16}$	F: GGGCTTTCTGGCAAAGATATG R: AAAAAGGGGCTTAGGTCCAG	48	187-287 bp	33	GU301268	Sardell *et al.* 2010

*Loci used in final analyses

Locus	Repeat Motif	Primer sequence	T_A	Size range	N_A	GenBank accession no.	Reference
*Aca 01	$(TCTA)_{14}(TCA)_2$-TCTATCA$(TCTA)_{13}$	F: AGCCCACTAATGGGTTTTCC R: TGAGTGTTCAAAGTTGCCAGA	58	164-224 bp	24	EF447093	Hill *et al.* 2008
*Aca 05	$(TGTC)_2(TATG)_7$-$(TATC)_{14}$	F: CCTGCTAGGCTGCATCTTCT R: GAGTGTCATCACATTTGTACTTTGG	58	204-306 bp	40	EF447095	Hill *et al.* 2008
*Aca 17	$(TCTA)_{13}(TC)_9$	F: GGAGCATGTGACAATGGAGT R: TCTGTGCTGTTCCAAGCAGA	58	251-339 bp	23	EF447100	Hill *et al.* 2008
*Am 02	$(CTCA)_{13}$	F: CTGCAAAATGTTCAGGCC R: GTTTACTGGAACCTTGCATGCAAC	58	246-262 bp	5	JQ845066	Lehmicke *et al.* 2012
*Am 08	$(AGGT)_{13}$	F: GTTTGGGACATGAAAAGCTGGCAG R: GGTCATCGGTGGGTTG	58	212-354 bp	60	JQ845069	Lehmicke *et al.* 2012
*Am 12	$(AGAT)_{15}$	F: GTTTCCCCACCCATTTTCACCATC R: GAACTTCCAAACACAAAGGC	58	239-411 bp	34	JQ845070	Lehmicke *et al.* 2012
*Am 14	$(ATAG)_{10}$	F: GACCTGCAAGAGAGGTGTC R: GTTTAGTTGAGTTGTTTGATCCAGGC	58	141-145 bp	2	JQ845071	Lehmicke *et al.* 2012
*Am 18	$(ATAG)_{15}$	F: GTTTCACCAGGAAACCCTTGCAAC R: GTCTCTGCCTGCATCTTCAG	58	146-260 bp	18	JQ845073	Lehmicke *et al.* 2012
*Am 20	$(ATAG)_{12}$...-$(AGAC)_5$	F: GTTTGGCTTTTCAAGGGTCTGTCC R: AACCCCAACCTGTCCCATG	58	156-296 bp	35	JQ845074	Lehmicke *et al.* 2012

*Loci used in final analyses

Locus	Repeat Motif	Primer sequence	T_A	Size range	N_A	GenBank accession no.	Reference
*Zole C11	$(ATCT)_{14}$	F: TCCATGCTTCTGAACTGCC R: ACACCTGCTTTTCCTGACTG	58	149-203 bp	16	EU410392	Poesel *et al.* 2009
*Zole E11	$(ATCT)_{13}$	F: AGAATGCTCTGGAACCGGC R: AGGACCTGTGTGCCAATTAAG	58	175-219 bp	18	EU410395	Poesel *et al.* 2009
*Zole F11	$(ATCC)_{10}$	F: AACCAAGCCACCACAATGC R: GACAGGCACTAGGATGGGAG	58	232-336 bp	25	EU410397	Poesel *et al.* 2009

*Loci used in final analyses

VITA

Blain Cerame, a native of Baton Rouge, Louisiana, received her first bachelor's degree at Southeastern Louisiana University in Organismal Biology in 2005. She returned to Louisiana State University to complete a second bachelor's degree in 2008 in Wildlife Ecology. Thereafter, she worked as a wildlife technician for the Louisiana Department of Wildlife and Fisheries Natural Heritage Program. As her interest in wildlife conservation grew, she made the decision to enter graduate school in the School of Renewable Natural Resources at Louisiana State University. She will receive her master's degree in December 2013 and upon graduation, plans to begin a career in the natural resources field applying the knowledge gained during her time as a graduate student.