LANDSCAPE AND POPULATION GENETICS OF THE FOUNDATIONAL SALT MARSH PLANT SPECIES BLACK NEEDLERUSH (*JUNCUS ROEMERIANUS*) WITH IMPLICATIONS FOR COASTAL MANAGEMENT AND RESTORATION

by

HAYLEY REID TUMAS

(Under the Direction of Campbell J. Nairn)

ABSTRACT

Salt marshes are ecologically and economically valuable ecosystems in worldwide decline, and are dominated in the southeastern United States by the clonal macrophyte black needlerush (*Juncus roemerianus* Scheele). Clonal and genetic diversity of foundational plant species in monotypic ecosystems, such as the salt marsh, are important for ecosystem processes and resiliency, especially under impending sea level rise and climate change. Gene flow, dependent on population connectivity, maintains genetic diversity in highly fragmented populations and can be affected by anthropogenic land conversion typical of coastal areas. However, no genetic studies have been conducted on *J. roemerianus* despite the species' importance to the salt marsh as a dominant, foundational plant species. We used next-generation sequencing to develop a panel of 19 species-specific microsatellite markers for use in population and landscape genetic studies on *J. roemerianus*. A study on 304 samples from a large, natural population of *J. roemerianus* within the Grand Bay National Estuarine Research Reserve, MS found higher than expected clonal and genetic diversity for a species assumed to have rare sexual reproduction. Patterns of clonal and genetic diversity and population structure were examined in

a population genetic study using 849 samples collected at seventeen sites across a significant portion of the species' range from Mississippi to South Carolina. Clonal and genetic diversity was higher than expected with an average genotypic diversity of 0.67 and average observed heterozygosity of 0.56. Differences in diversity measures across the Gulf and Atlantic coast suggest environmental influences on life history and possible local adaptation. Broad scale structure with samples grouping into three genetic clusters indicated gene flow might be more frequent than suggested by life history literature. A landscape genetic analysis using hierarchical model selection across a least cost transect analysis was performed across 576 samples collected at ten sites in the northeastern Gulf of Mexico to determine the influence of landscape features on population connectivity. Coastal corridors were identified as important for *J. roemerianus* dispersal, and coastal development negatively influenced population connectivity. Results critically contribute to ecological and evolutionary knowledge on *J. roemerianus*, and have important implications for coastal conservation, restoration, and management.

INDEX WORDS: salt marsh, *Juncus roemerianus*, clonal, microsatellite, genetic diversity, genotypic diversity, population structure, landscape genetic, population connectivity

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HAYLEY REID TUMAS

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HAYLEY REID TUMAS

Major Professor:	Campbell J. Nairn
Committee:	Brian M. Shamblin
	Mark S. Woodrey
	Nathan P. Nibbelink
	Richard B. Chandler

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
May 2018

DEDICATION

To the salt marsh, and all the other wild places yet untamed by man.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

I. Salt Marsh

Salt marshes are critically important and highly productive ecosystems that act as the transitional zone between marine and terrestrial environments. The ecosystem provides habitat to a variety of endemic and economically important species including migratory and non-migratory water birds, fishery species, bivalves, and crustaceans (Kennish, 2001). Migratory waterfowl utilize the marsh as feeding grounds, and the young of many fishery species rely on the marsh for nursery habitat. In total, half of all potentially extirpated species of plants and animals in the United States are dependent on wetland ecosystems (Gedan et al., 2009). The salt marsh also supplies a range of ecosystem services vital for human health that have been valued at $10,000 per hectare (Kirwan and Megonigal, 2013). Distributed along coastlines, marshes act as a natural sea barrier that provides storm protection and flood attenuation to coastal communities. Salt marshes help ameliorate the impact of human activities through carbon sequestration, and even act as carbon sinks at higher latitudes (Zedler and Kercher, 2005). Marshes have a marked effect on water quality, improving overall human health by removing sediment and other contaminants from the water. By filtering runoff and retaining nutrients, marshes help to prevent eutrophication, which can cause massive areas of anoxic waters or dead zones that kill fish and other marine species. The water filtration and nutrient retention properties of salt marshes along the Gulf of Mexico are valued at $900-$1,900 per hectare, and help alleviate the impact of nutrient rich inputs from the Mississippi River (Rabotyagov et al., 2014).

Salt marshes form on sheltered shores through sediment accretion and colonization of vascular plants. Pioneer plants aid in continued sedimentation until the surface rises and other plant species take root, a process that can take years to centuries depending on shoreline conditions (Long and Mason, 1983). Contemporary coastal marshes developed when sea levels stabilized approximately 3,000 years ago (Chabreck, 1988). The location of salt marshes causes the ecosystem to possess qualities of both terrestrial and marine environments, placing high physical stress on resident species. Intermittent flooding and high salinity levels have caused species to evolve diverse survival strategies to live in the marsh. The marsh overall is a biogenic community that relies mainly on foundational plant species for formation and continued existence. The variation in flooding and salinity along an elevational gradient has caused zonation of plant species regimes within the marsh, separated into three zones of low marsh, middle marsh, and high marsh. Plant species dominance within each zone is controlled by adaptive superiority and interspecific competition. At lower elevations, plant distributions are limited to species able to withstand high flooding frequency (Pennings and Bertness, 2001). Plant species in the low marsh, such as smooth cordgrass (*Spartina alterniflora*) have evolved various traits such as aerenchyma (air space tissue), and thickened root cell walls to tolerate periods of submergence and re-exposure (Mendelssohn et al., 2006). In most marsh systems, plants are prevented from dispersing into higher elevations by interspecific competition with the dominant plant species in that zone (Pennings and Bertness, 2001).

However, within marshes more temperate marshes, such as those in the southeastern United States, the upper range of a plant species' distribution may also be dictated by soil salinity. Increased temperatures at lower latitudes causes increased evapotranspiration at higher elevations that experience less frequent flooding in the marsh (Pennings and Bertness, 2001). To

survive high salinity conditions, plants have adapted avoidance strategies such as exclusion or excretion of toxic salt ions and tolerance strategies such as osmotic adjustment (Mendelssohn et al., 2006). As a result of the stressors and corresponding adaptations, the low marsh is dominated by plants adapted to submergence such as *S. alterniflora*, the middle marsh is dominated by plants tolerant to higher salinity and irregular submergence such as black needlerush (*Juncus roemerianus*), and the high marsh is dominated by species that can withstand the highest salinities such as American glasswort (*Salicornia virginica*) (Pennings and Bertness, 2001; Stout, 1984). Stress from salinity and flooding is not as severe for animal species that are usually highly mobile, although some endemics have developed traits to cope with marsh conditions (Mendelssohn et al., 2006). As a result, some zonation in animal abundance occurs across the marsh, although the spatial patterns are not as abrupt as those found in plants (Pennings and Bertness, 2001).

For centuries salt marshes were viewed by the public and scientific community alike as barren wasteland, and have only recently been discovered to be important, biologically diverse ecosystems (Gedan et al., 2009). During this time, humans converted salt marshes to "more useful" land types, causing massive destruction and degradation to the ecosystem. As a result, over half the salt marsh habitat has been lost in the United States due solely to human modification, and between 13-30% have been lost worldwide (Kennish, 2001, Valiela et al., 2009). Humans have caused direct declines in marsh distribution through resource exploitation, land conversion, and invasive species introductions. In addition to direct loss of salt marsh, declines are causing fragmentation of a once continuous habitat with unknown impacts on resident species. Indirect impacts such as pollution, eutrophication, and climate change may be less severe, but are also less localized, causing widespread degradation (Gedan et al., 2009). Salt

marshes are designated high risk zones for climate change, the impacts of which are mainly unpredictable, and will vary spatially and temporally (Thorne et al., 2012). Marshes have survived past sea level rise by increasing soil elevation through the sediment accretion and organic biomass accumulation properties of plants (Kirwan and Megonigal, 2013). However, current sea level rise is occurring more rapidly than in the past and may outpace the rate of soil accumulation, drowning marshes. Urban development and steep shorelines will prevent salt marshes from migrating landward to avoid drowning (Gedan et al., 2009). More research is needed to fully understand the impact of sea level rise and climate change on the salt marsh, and the subsequent response and resiliency of marsh communities.

The 1970s brought an end to unregulated marsh destruction both nationally with the 1972 Coastal Zone Management Act and 1977 Clean Water Act, and globally with the 1971 Ramsar Convention. The Ramsar Convention, held in Ramsar, Iran, brought about an international treaty to stimulate wetland conservation efforts on international and national scales. In the United States, the Clean Water Act regulated the destruction of wetlands, and required developers to restore an area of marsh in exchange for converting marsh elsewhere (Zedler and Kercher, 2005). In response to reports released by the United States Fish and Wildlife Service (USFWS) and the National Oceanic and Atmospheric Administration (NOAA) in 1998 and 2004 detailing the loss of coastal wetlands, the United States Environmental Protection Agency (EPA) established the Coastal Wetlands Initiative. The initiative creates a Coastal Wetlands Team formed between EPA and NOAA to review and raise awareness about coastal wetland loss, and forms interagency connections amongst multiple federal agencies to serve in an advisory role (USEPA, 2011). Prior to the Clean Water Act and Coastal Wetland Initiative, the United States Congress passed the Coastal Zone Management Act in 1972 to manage the nation's coastal resources.

Administered by NOAA, the goal of the act is to "preserve, protect, develop and where possible to restore or enhance" the coastal zone through three programs: the National Coastal Zone Management Program, the National Estuarine Research Reserve System, and the Coastal and Estuarine Land Conservation Program. The National Coastal Zone Management Program and Coastal and Estuarine Land Conservation Program form partnerships between the federal and state governments to manage coastal area, while the National Estuarine Research Reserve System creates a network of reserves to serve as field laboratories and improve understanding of estuaries (NOAA, 2016). Restoration efforts within the marsh, including terracing, pollution prevention, and vegetation restoration have had variable success, and no restored marsh has supported more than a single ecosystem service (Zedler and Kercher, 2005).

Salt marshes in the United States are distributed primarily on the east coast and Gulf of Mexico coast, with limited distribution on the west coast of California. The distribution is dependent on the geomorphology of the coasts, with the greatest expanses of marsh forming on areas of flat coastal plains and a gently sloping continental shelf (Chabreck, 1988). The different tidal regimes and climates on the east coast and Gulf coast have caused different marsh communities to form on each coast. On the east coast, salt marshes from northeastern New England to southeastern Florida are dominated by the low marsh species smooth cordgrass (*Spartina alterniflora*). The middle marsh and high marsh plant communities still exist within east coast marshes, however the low marsh occupies the majority of the area. Conversely, the middle marsh is the primary zone within the Gulf of Mexico salt marshes causing the plant species black needlerush (*Juncus roemerianus*) to dominate with only narrow bands of low marsh on the water's edge. The difference in plant communities between the coasts is mainly caused by differences in flooding frequencies due to tidal regimes. *S. alterniflora* is better

adapted to more frequent flooding, prolonged inundation, and anaerobic conditions. *J. roemerianus* has a greater salt tolerance to withstand higher levels of evapotranspiration in areas of less regular flooding (Dardea et al., 1992; Stout, 1984). Tides are semidiurnal on the east coast; with two nearly equal high and low tides occurring daily that are driven by lunar cycles. East coast tides have an overall tidal amplitude of 0.1-2.3 meters so that marshes are frequently flooded (Dardeau et al., 1992). The Gulf of Mexico has less regular tides that are primarily driven by meteorological conditions, specifically seasonally changing winds and storms. Two types of tides occur on the Gulf coast, semidiurnal and diurnal with unequal high and low tides (Stout, 1984). The entire Gulf coast region is considered microtidal with tidal amplitudes ranging from 0.2-0.6 meters, resulting in most marshes being irregularly flooded (Dardea et al., 1992).

The majority of ecological studies have focused on salt marshes along the east coast of the United States. However, the Gulf of Mexico contains approximately 60% of the coastal wetlands in the United States, encompassing a total of 5,480 square miles across five states from eastern Texas to western Florida (Chabreck, 1988). Salt marshes in this region have the largest drainage area in the country with runoff from about two thirds of United States, coming mainly from the Mississippi River (Dardea et al., 1992). In addition to the vital ecosystem services they provide, salt marshes in the Gulf of Mexico have become a part of the culture and a source of recreation in coastal communities. Only 10% of the total area of the Gulf of Mexico states is covered by urban sprawl, compared to 39% on the east coast, but the majority of urban development is along the coast (Valiela et al., 2009). Restoration efforts have been made to alleviate the impact of coastal development and reinforce marshes to withstand sea level rise, specifically by restoring foundational plant communities. The irregularly flooded marshes of the northeastern Gulf of Mexico comprise eight percent of the nation's marsh area, containing 940

square miles. Found along the coasts of Mississippi, Alabama, and northeastern Florida, these ecologically and economically important marshes are dominated by the foundational plant species black needlerush (*Juncus roemerianus)* (Chabreck, 1988; Stout, 1984).

II. Black needlerush (*Juncus roemerianus*)

Black needlerush (*Juncus roemerianus* (Scheele)), commonly referred to as Juncus, is a gynodioecious macrophyte found in the middle marsh zone of marshes from New Jersey to eastern Texas (Eleuterius, 1976). *J. roemerianus* can reproduce either through clonal propagation or sexual reproduction, a characteristic exclusive to *J. roemerianus* amongst other rush species. Genetically identical clonal ramets are produced through rhizomatous growth during vegetative propagation resulting in genets that can cover large expanses of marsh. Resource sharing amongst ramets has been experimentally shown to be important for invading stressful environments such as saltpans and bare areas uninhabited by other plants (Pennings and Callaway, 2000). During sexual reproduction, the plant forms leaves with inflorescences of either exclusively pistallate (female) or perfect (bisexual) flowers, with pistallate plant types relying on perfect flowers for pollination. Dimorphic flowers are unique to *J. roemerianus* within the Juncus genus. Plants only produce a single flower type, and transplanted clonal material from perfect or pistallate flowers produce only the respective flower type for up to five years (Eleuterius, 1974; Stout, 1984). Cold weather initiates flower production, which occurs from late January to early April with peak flowering in March in the northeastern Gulf coast. Plants shed resultant seeds simultaneously in mid-spring and produce a maximum of about sixty seeds per plant (Eleuterius, 1975; Stout, 1984**)**.

Little is known about the true vectors for seed and pollen dispersal, although most wetland plants use wind, water, or animals, usually birds (Cronk and Fennessy, 2001). Seeds are

highly viable for up to one year, and may germinate when submerged or on the surface of water, further indicating possible water dispersal. Furthermore, *J. roemerianus* has small seeds (0.6mm long) (USDA, NRCS, 2017) that would allow for dispersal via wind (Cronk and Fennessy, 2001; Neff and Baldwin, 2005) or on the wings, feet (Cronk and Fennessy, 2001), or in the excrement (Soons et al., 2008) of waterfowl. These seed characteristics, and the fact that the morphologically similar seeds of the related species, common rush (*Juncus effusus* L.) are dispersed using all three vectors (USDA, NRCS, 2017; Neff and Baldwin, 2005; Soons et al., 2008), most likely means *J. roemerianus* uses wind, water, animals, or a combination of the three as seed dispersal vectors. Once established, seedlings require high light and moisture, and are generally only produced on barren, sand substrate. Plants mature at about 12 months and begin flowering at 18-24 months with prolific flower production occurring after 3 years. Shoot longevity varies amongst populations and stands of *J. roemerianus*, and can be up to four years or longer (Eleuterius, 1975; Stout, 1984). Within the middle marsh zone, *J. roemerianus* experiences phenotypic variation based on soil salinity, with taller plants growing in lower salinity soil and shorter plants occurring in high salinities. Transplant studies have revealed possible genetic differences between the phenotypically different plant morphs (Eleuterius, 1989). J. roemerianus tolerates the high salinity soils found at irregularly flooded elevations in low latitude marshes using salt avoidance through decreased stomatal conductance (Touchette et al., 2009).

J. roemerianus is the foundational plant species and an ecosystem engineer in the irregularly flooded marshes along the northeastern coast of the Gulf of Mexico. The species is responsible for creating and maintaining marsh habitat by stabilizing and accreting sediment, and protecting the shoreline from wave stress. Other marsh species utilize *J. roemerianus* as habitat,

and refuge from predators and the physical stress of the environment (Pennings and Bertness, 2001). The resiliency of the marsh to future stressors such as climate change and storm events is dependent on the genetic health of *J. roemerianus*. Research in foundational macrophyte species has found increased clonal diversity was correlated with increased resiliency to environmental stochasticity such as sea level rise through increased shoot density following disturbance (Ehlers et al., 2008; Hughes and Stachowicz, 2004). The habitat uniformity in the salt marsh elevates genetic diversity of the foundation species to a critical role, on par with species diversity in other ecosystems in maintaining ecological health and ecosystem processes (Reusch and Hughes, 2006). Maintaining the genetic diversity of *J. roemerianus* could therefore have far reaching effects for the entire ecosystem. Essentially, marshes would likely not exist without genetically healthy populations of *J. roemerianus*.

Despite the importance of the species in the salt marsh, little research has examined *J. roemerianus* beyond a few early life history studies. These studies assert that the species is mainly clonal with single clones occupying large expanses of salt marsh. *J. roemerianus* is believed to use sexual reproduction primarily to colonize bare substrates, and then chiefly utilizes clonal propagation to maintain the species once established, suggesting the species has low clonal and genetic diversity (Stout, 1984; Eleuterius, 1975). Concepts developed for clonal plant population dynamics and life history supports this conclusion. Clonal plants are classified by rates of seedling recruitment dependent on environmental disturbance and dispersal strategy. Long distance dispersers residing in highly disturbed environments such as the salt marsh are postulated to have a single initial seedling recruitment at the time of colonization (Eriksson, 1989). However, no molecular genetic studies have been conducted on *J. roemerianus* to test this conclusion. The only information on genetic patterns in *J. roemerianus* is from reciprocal

transplant studies that found evidence of genetic differentiation based on soil salinity. Plants from populations in low salinity environments were unable to survive when transplanted to populations growing in higher salinities, while high salinity plants grew well at lower salinities (Eleuterius, 1989). Literature on other clonal plant species indicates that genetic diversity is often higher than expected. A review of clonal plants by Ellstrand and Roose (1987) found most species show intermediate levels of genetic diversity with few to no widespread clones. Similar results have been found for smooth cordgrass (*Spartina alterniflora*), another clonal salt marsh species and the foundational plant species on the east coast. Until recently, populations of *S. alterniflora* were assumed to be dominated by a few large clones. However, genetic analyses revealed the species exhibited higher levels of clonal diversity than expected with only 6% of sampled ramets representing clonal replicates in a study on Sapelo Island, GA (Richards et al., 2004). An examination of the relationship between successional stage and population structure in *S. alterniflora* further disagreed with theory that sexual reproduction is only utilized during colonization. The study compared different aged patches of the clonal species and found younger patches to have the highest rates of selfing. Clonal diversity in patches increased steadily over time until eventually declining (Travis et al., 2004). Conclusions cannot therefore be drawn about the importance of sexual reproduction or patterns of genetic diversity in *J. roemerianus* without adequate molecular genetic studies. The continuous distribution of *J. roemerianus* across large scales lends the species to a landscape scale genetic study.

J. roemerianus is an important species in salt marsh restoration in the Gulf of Mexico, and to a lesser degree the east coast. Restoration has become especially important in coastal areas due to rising sea levels and high human development. Over one third of the world's population reside in coastal areas that make up only 4% of the Earth's total land area (UNEP, 2006). Plant

restoration is the first step in habitat rehabilitation and essential to the overall success of the project. Genetic techniques useful to restoration planning, implementation, and evaluation are becoming less expensive and easier to accomplish. However, ecological restoration practitioners are still undervaluing and overlooking the importance of genetic information (Mijangos et al., 2015). Proper plant restoration techniques are still debated in the field, although the conclusion that decisions should be made on a case-by-case basis is becoming more widely accepted (Kaye, 2001; Zedler and Kercher, 2005). The largest debate over technique is SOMS (single or multiple source), whether transplants or seed sources should be from a single population or multiple populations. The issue surrounding SOMS is that of inbreeding versus outbreeding depression. Selecting source material from a single population mimics a small isolated population, and could result in inbreeding depression through the accumulation of deleterious alleles, increased genetic drift, decreased genetic diversity, and a lowered effective population size. Alternatively, selecting source material from multiple populations could lead to outbreeding depression by losing unique genetic qualities, and therefore local adaptations, in the restored population. Once the technique for selecting source material is determined, practitioners must then also decide source distance, or how close the source population(s) should be to the restored site. Many believe source material should be transplanted from the closest and most ecologically or genetically similar site to prevent outbreeding depression and improve chances of local adaptation (Kaye, 2001). The home-site advantage hypothesis predicts that the success of restored populations will be negatively correlated with genetic or environmental distance from the native population, with higher success rates when populations are more similar. Studies have shown that geographic distance may not be a good predictor for success or degree of genetic distance, and that genetic and environmental similarities need to be considered (Montalvo and

Ellstrand, 2000). Conversely, others argue that distance from the source population should not matter as long as the species is native in order to keep cost of restoration low (Kaye, 2001).

Successful restoration policy considers three basic criteria. First, that sufficient habitat is preserved for the persistence of the species of interest. Second, demographic information is collected to determine the life history stages most important for survival and reproduction. Third, the consideration of genetic variation, especially in population restoration, which plays into the long-term goal of preserved evolutionary potential through maintained genetic variation within and among populations. If genetic diversity, and by extension evolutionary potential, is not adequately conserved the range of the species will be irreversibly reduced (Fenster and Dudash, 1994). Genetically diverse populations have greater adaptive potential resulting in greater resiliency to environmental disturbances such as sea level rise and an increased probability of species persistence (Ehlers et al., 2008; Hughes and Stachowicz, 2004). Restored populations of aquatic macrophytes with higher genetic diversity have been found to be more successful with increased ecosystem services (Reynolds et al., 2012). Despite the experimental and theoretical support for the importance of genetic diversity as a restoration goal, practitioners still argue that genetic diversity is not a critical issue in restored habitats. In general, the field has been reluctant to embrace genetic techniques due to previous research and training focusing on ecosystem functioning (Montalvo et al., 1997). As a result, genetic diversity in restored populations does not always reflect natural levels, especially in clonal species. A study comparing genetic diversity and clonal extent in natural and restored populations of a clonal, coastal species often used in restoration found natural populations were more genetically diverse with fewer extensive clones. Most of the restored populations sampled were entirely monomorphic, consisting of a single clone, and in some cases did not contain local genotypes (Fant et al., 2006). *J.*

roemerianus needs an assessment of natural levels of clonal and genetic diversity, and population structure to adequately plan and implement successful restoration plans.

III. Population Genetics

Population genetics aims to study naturally occurring genetic differences among biological organisms, either at the interspecific or intraspecific level (Hartl, 2000). The purpose of performing a population genetics study can vary widely and includes identifying genetic distinctness between species, understanding population or species history, studying current population demographics, and examining evolutionary mechanisms. The field was initially developed using theoretical models based in mathematical theory, exemplified by the work of Sewell Wright in the 1940s, but has progressed to include laboratory experimental investigation and field studies. The development of new genetic techniques has allowed population genetics to expand and become implemented in a variety of scientific fields. Sir Otto Frankel was the first to recognize the importance of genetic factors and the role of evolution in conservation biology, birthing the field of conservation genetics in the mid 1970s (Frankham, 1995; Frankel, 1974). Conservation genetics has been further developed by Soule and others to solve conservation problems and design management practices for natural populations using genetic theory and techniques (Frankham, 1995). The requirement by the Endangered Species Act to only extend conservation to genetically distinct entities has caused the field to be further utilized by practitioners (Van Dyke, 2008). Rapid changes in environments brought on by urban development and climate change have placed a necessity on incorporating genetic techniques in conservation practice to preserve genetic diversity, and therefore ensure population resiliency. All of this has caused the field of population genetics itself to evolve from theoretical exploration

carried out by statisticians to on the ground analyses utilized by wildlife managers and conservation scientists alike.

As molecular techniques and genetic applications advance, the number and types of conservation problems that can be addressed by genetics grow. Frankham increased the number of major genetic issues in conservation biology from seven in 1995 to eleven in 2002 (Frankham, 1995; Franham et al., 2002), and as next generation sequencing expands and becomes more accessible this number will probably increase again. The eleven major genetic issues in conservation biology as recognized by Frankham et al. (2002) are: (a) deleterious effects of inbreeding depression, (b) loss of genetic diversity and evolutionary potential, (c) population fragmentation and reduced gene flow, (d) genetic drift overcoming natural selection, (e) accumulation and loss of deleterious mutations, (f) genetic adaptation to captivity and resulting reduction in reintroduction success, (g) taxonomic uncertainties and introgression, (h) delineating management units, (i) molecular techniques in forensics, (j) molecular techniques to understand species biology, and (k) outbreeding depression. While all of the listed issues are important in conservation biology, the ongoing and increasing fragmentation of species habitat by human activities and the impending threat of climate change highlight the importance of addressing genetic diversity in conservation practice.

Genetic diversity or genetic variation is the variety of genetic variants, whether alleles or genotypes, present in the unit of study (populations, species, or group of species). This variation in genes is the raw material for adaptive evolution, and necessary for populations and species to evolve in response to environmental change. Fisher's Theorem of Natural Selection states that the rate of evolutionary change is dependent on the amount of available genetic diversity. Furthermore, genetic diversity is highly correlated to population health. Declines in genetic

diversity have been related to reduced reproductive fitness and increased inbreeding depression (Mills, 2007, Frankham et al., 2002). Populations with increased genetic diversity have also been found to be more resilient to environmental disturbances (Ehlers et al., 2008, Hughes and Stachowicz, 2004). Genetic diversity is especially important in conservation efforts because most species of concern are comprised of small, isolated populations vulnerable to inbreeding depression and extinction. Conservation of genetic diversity shifts focus from the short-term management of reproductive fitness to the long-term preservation of evolutionary potential in species, and can be achieved by preserving the evolutionary mechanisms that promote genetic variation.

Genetic diversity within and among populations and species is affected by four main evolutionary processes: selection, gene flow, genetic drift, and mutation (Gillespie, 2004). Selection is differential reproduction or mortality due to differences in phenotype, caused by either environmental processes (natural selection) or human choice (artificial selection) (Frankham et al., 2002). Gene flow is the movement of individuals or genetic material, such as seeds or pollen, between populations that results in reproduction, and therefore an exchange of genes (Gillespie, 2004; Slatkin, 1987). Conversely, genetic drift is a random process, accumulating through unpredictable changes in gene frequency due to finite population size (Gillespie, 2004). Mutation is also sometimes random, and results in a sudden genetic change that causes offspring to differ genetically from parents (Frankham et al., 2002). The contribution of each mechanism to overall genetic diversity within a species or population is dependent on population demographics and theoretical opinion. Large, undisturbed, demographically stable populations tend to follow Fisher's evolutionary theory with mutation and natural selection providing the largest contribution to genetic diversity (Provine, 1985). In these cases, gene flow

and genetic drift acts as a constraining force on local adaptation, having the same average effect on all nuclear genes across the population.

In smaller, fragmented populations that are demographically unstable, gene flow and genetic drift contribute more to genetic patterns. Populations under these conditions more closely follow Wright's shifting balance theory in which gene flow and population fragmentation are fundamental to evolution (Slatkin, 1987). In his theory species exist within an adaptive landscape in which phenotypic traits form the axes and fitness represents the height. Adaptation drives populations to an adaptive peak, at which point genetic drift can fix alleles and shift populations to a higher peak. Gene flow then spreads adaptively superior genes to other populations through dispersal and colonization (Provine, 1985; Slatkin, 1987). The rate at which genetic drift acts upon a population is directly proportional to population size, with smaller populations experiencing drift at a higher rate (Gillespie, 2004). Gene flow will prevent neutral alleles from becoming fixed under genetic drift if an average of one or more individuals are exchanged between populations, independent of population size. In other words, genetic drift will cause significant difference between local populations only if the product of the rate of immigration and population size falls below one (Slatkin, 1987). Most species of conservation concern exist within habitat that has been fragmented by human disturbance, such as the salt marsh, and are most influenced by genetic drift and gene flow. Genetic drift acts to decrease genetic variation in these small, fragmented populations by causing neutral alleles to go to fixation, while gene flow acts as the main source of genetic diversity (Frankham et al., 2002). Highly fragmented populations of species of conservation concern are now being managed as metapopulations, or a population of subpopulations. Population stability and persistence in metapopulations is dependent on constant dispersal and colonization through immigration between subpopulations.

Gene flow contributes more to the maintenance of genetic diversity than mutation or natural selection in metapopulations (Hanski, 1999). For this reason, many conservation efforts seek to preserve gene flow, and subsequently genetic diversity, in fragmented populations through promoting and protecting population connectivity.

In practical applications, genetic diversity and gene flow are measured using a variety of metrics including heterozygosity, allelic richness, and genetic distance that have underlying biological and evolutionary assumptions. The most common assumption in population genetics theory is that populations or loci are in Hardy-Weinberg equilibrium (HWE). The tenets of HWE are that populations be large, undergoing no mutation, selection, or migration, with genotypes that follow Mendelian inheritance and have equal survival and fertility. When populations meet all these assumptions, genotype frequencies follow the following equation:

$$p^2+2pq+q^2=1$$

in which p is the dominant allele frequency and q is the recessive allele frequency. Expected heterozygosity (H_E), a genetic diversity metric, is represented as $2pq$ in the above equation and calculated using given allele frequencies in the population. The observed heterozygosity (H_O) of a population or sample set is the actual proportion of heterozygote individuals or genotypes (Frankham et al., 2002). The two measures can be compared to determine if a population has an excess or lack of heterozygotes compared to that expected of a population in HWE. While most populations violate many of the principles of HWE, the loci used to examine genetic diversity are usually expected to be neutral and at equilibrium. Another common metric to measure genetic diversity is allelic richness or allelic diversity, calculated as the average number of alleles per locus (Frankham et al., 2002). In both heterozygosity and allelic richness, greater values generally indicate higher levels of genetic diversity.

Gene flow between populations or local populations, often referred to as demes, is measured by genetic distance. Genetic distance is a measure of the genetic difference between alleles frequencies across two populations, and is usually reported pairwise in a distance matrix (Frankham et al., 2002). Multiple measures of genetic distance are used in the literature, and each is associated with a different theory and set of assumptions. The most common and traditional measure of genetic distance is Wright's F statistic (F_{ST}). While there are many definitions and statistical interpretations of F_{ST}, the definition that most closely applies to genetic distance is the variation in allele frequencies among populations, standardized by the mean allele frequency at the given locus. Wright's F statistic can also be viewed as measuring population differentiation by measuring the degree of inbreeding resulting from population fragmentation using inbreeding coefficients within and among populations. In this case, F_{ST} is also known as the fixation index, and is the probability that two alleles from a population fragment are identical by descent (IBD). The metric can be calculated using the following equation:

$$F_{ST} = 1 - (H_S/H_T)$$

in which H_S is the expected heterozygosity averaged across all population fragments, and H_T is the expected heterozygosity for the total population. The value of F_{ST} ranges from zero to one, with zero indicating no differences and one indicating total fixation of different alleles in each fragment (Frankham et al., 2002). Wright also linked measures of F_{ST} to the number of migrants a population receives under a model of population structure called the island model. Under this model, the number of migrants (Nm) can be calculated using the following equation:

$$F_{ST} \approx 1/(4Nm+1)$$

However, the island model has a list of assumptions that rarely hold true in natural populations, complicating this simplification. The island model assumes an infinite number of populations

with the same N number of individuals, and the same proportion m of migrants that are given and received. Migrants are also randomized and dispersed without regard to geographic structure so that all populations have an equal probability of giving and receiving migrants (Whitlock and McCauley, 1999). When examining microsatellite data, and to overcome some of these assumptions, practitioners utilize Slatkin's linearized F_{ST} (R_{ST}) (Slatkin, 1995). This measure assumes a much higher mutation rate than previous metrics due to the elevated mutation rate usually associated with microsatellites. Specifically, R_{ST} assumes a generalized stepwise mutation model in which each generation has a probability of mutation (μ), and a mutation increases the allele size by a random variable. The assumption underlying R_{ST} is that the allele size of a new mutant microsatellite is reliant on the size of the original mutated allele (Slatkin 1995). This is because a stepwise mutation model assumes that a mutant allele is either one repeat longer or shorter than the ancestral allele.

As theoretical population genetics progressed from Wright's initial distance metrics, additional measures have been developed with their own sets of underlying biological and evolutionary assumptions. Reynold's distance, or the coansectry distance, assumes that drift is the primary evolutionary force acting on populations and follows an infinite alleles model (Reynolds et al., 1983). In the infinite alleles model an allele can change from any given state to any other given state. Nei's genetic distance (D) calculates the accumulated allele differences per locus (Nei, 1972). The intent of Nei's genetic distance is to measure the number of genetic substitutions that have occurred since the divergence of the two populations, allowing for a biological meaning of the measurement. Due to statistical issues associated with estimation of the exact number of substitution differences, Nei's genetic distance can be estimated as the standard, minimum or maximum number of differences (Nei, 1987). The Cavalli-Sforza chord

distance (D_{ch}) is a geometric distance that measures differences in allele frequencies between populations conceptualized in a dimensional Euclidean space. Chord distance does not have any underlying biological assumptions about population size, but assumes differences in allele frequencies are due purely to genetic drift without any genetic mutations (Cavalli-Sforza and Edwards, 1967). The metric is calculated using the following equation:

$$D_{ch} = (2/\pi r) \sum_j^r \sqrt{2(1 - \sum_i^{m_j} \sqrt{x_{ij} y_{ij}})}$$

in which x_{ij} and y_{ij} are frequencies for the *i*th allele at the *j*th locus in populations x and y (Takezaki and Nei, 1996).

All genetic distance metrics applied to population studies are intended to estimate gene flow between populations by examining the degree of population differentiation and structure. Greater genetic distances imply greater genetic differentiation, and therefore decreased gene flow between populations. In practice, distance metrics have been used to study population connectivity, derive population dynamics, and identify isolated populations. Conservation genetics has utilized genetic diversity measures and genetic distance metrics to identify populations at risk of extirpation. Due to the importance of genetic diversity to fitness and resiliency, populations with lowered levels of heterozygosity or allelic richness, or highly differentiated, isolated populations are generally more likely to become extinct in the future. As a result, practitioners can then choose to implement genetic rescue for these at risk populations, in which individuals from a more genetically diverse population are translocated to the genetically depauperate population (Frankham, 1995).

A central tenet of gene flow theory is that of isolation by distance (IBD) developed by Wright (1943), which follows that adjacent populations should be more genetically similar than geographically distant populations. Under isolation by distance, pairwise genetic distance

between populations increases with pairwise geographic distance between populations. This pattern is a result of geographically-limited dispersal and genetic drift acting more quickly to differentiate allele frequencies than homogenization by gene flow (Aguillon et al., 2017). Isolation by distance is the driving force behind geographically isolated populations experiencing low genetic diversity, due to lower migrants and therefore reduced gene flow. Population genetic investigators are interested in testing for patterns of isolation by distance across sampled populations to understand species evolution and biology. The most common test used is a Mantel test, or partial Mantel test, that correlates two distance matrices, one of genetic distance and one of geographic distance (Diniz-Filho et al., 2013).

Grouping or clustering individuals into populations can provide valuable information on population history, dispersal barriers, and even species biology, and is therefore also a common goal amongst population geneticists and conservation practitioners. Traditionally, biological populations were determined by behavioral, morphological, or geographical similarities. While genetic analyses have allowed population assignment to shift towards more objective methodology, the definition of a population is still highly debated and many times goal or situation dependent. The typical method in population genetics is to probabilistically cluster individuals into populations based on the underlying assumption that allele frequencies in populations will be in Hardy-Weinberg Equilibrium and loci are within linkage equilibrium. One of the more commonly used assignment software packages, STRUCTURE, uses Bayesian clustering to assign individuals to *K* populations based on the individual's genotype while simultaneously estimating population allele frequencies (Pritchard et al., 2000). Other software programs, such as TESS, GENELAND, BAPS, and BAYES, have similar underlying methodology with some key differing assumptions. Spatial genetic structure can also be

examined without underlying biological assumptions using mathematical methodology, most commonly with a principal components analysis (PCA). First implemented by Cavalli-Sforza et al. (1994), a PCA identifies principal directions in which multivariate data varies in multidimensional space. In population genetics, the multivariate data is allele frequencies, with each locus treated as a variable. A PCA is a good way to visualize and interpret large genetic datasets, but have been criticized for being sensitive to misinterpretation due to the fact the analysis is not based on a population genetic model (Novembre and Stephens, 2008).

Clonal species complicate genetic analyses because each genetically unique genet is composed of multiple genetically identical ramets, creating two levels of structure and two metrics of diversity (Eriksson, 1989; Arnaud-Haond, 2007). The metric of diversity unique to clonal species is genotypic diversity, or the number of unique genotypes or clonal variants in a group of samples (Arnaud-Haond, 2007). Genotypic diversity is usually measured by clonal richness and Simpson's diversity (Arnaud-Haond, 2007; Widen et al., 1994). Clonal richness is the proportion of unique genotypes in a sample set, and is calculated using the following equation:

$$R = (G - 1)/(N - 1)$$

where G is the number of unique genotypes, and N is the total number of samples, including all genetically identical ramets (Arnaud-Haond, 2007). Genotypic diversity estimated using Simpson's diversity adjusted for finite sample size is calculated using the following equation:

$$D = 1 - \sum_{i=1}^{k} \frac{ni(ni - 1)}{N(N - 1)}$$

where n_i is the number of individuals in genet i and N is the total number of samples, including genetically identical ramets (Widen et al., 1994; Pielou, 1969). Genetic diversity, the heterozygosity of individuals, is measured using the same metrics as non-clonal species, but

using only a single representative from each genet. All other genetically identical ramets are removed for subsequent genetic diversity analyses. Clonal calculations require that genetically identical individuals be identified to determine numerical size of genets and perform genetic diversity analyses. The ability and accuracy of identifying genetically identical individuals is dependent on the probability of identity of the markers selected for a study, with smaller probabilities of identity indicating greater accuracy and ability. Identification of ramets is somewhat complicated by the possibility of somatic mutations arising during clonal propagation; and the fact that molecular markers only cover a small portion of DNA. This means individuals could be identical at all study markers, but may not be truly genetically identical across the genome. Equations have been devised to help estimate the probability that individuals have been assigned to the correct genet to account for these complications (Widen et al., 1994; Arnaud-Haond, 2007). The dependency of accurate results and interpretations on molecular markers makes marker selection for a study of utmost importance.

Population geneticists use a variety of different genetic makers, but the most common in wildlife and conservation studies are allozymes, mitochondrial, microsatellites (short tandem repeats or STRs), and single nucleotide polymorphisms (SNPs). Allozymes are protein products of different alleles that are measured using gel electrophoresis, and was among the first genetic markers used in wildlife. The disadvantages of allozymes, and the main reason for the development and use of other markers, are the necessity to sometimes kill animal specimens to extract necessary organ tissue coupled with low resolution (Mills, 2007). Mitochondrial markers (mtDNA) examine organellar DNA in the mitochondria of cells, and are well suited for species level analyses. Regions of mitochondrial DNA have variable mutation rates, allowing different regions to be useful for difference applications from intraspecific studies to species

identification. The control region tends to have the highest diversity within species, and is most appropriate for intraspecific studies. The 12s rRNAor protein coding regions are both specific enough to identify specific species. Markers that target mtDNA can be designed to amplify either across multiple species or specifically for a single species, allowing investigators to specify or expand the study as necessary (Wan et al., 2004). Mitochondrial DNA is also inherited uniparentally with maternal inheritance in most plant and animal species, and can therefore be used to study sex biased migration or dispersal. Furthermore, mtDNA can be sampled noninvasively without killing the specimen, making it well suited for studies on endangered or rare species. On the other hand, mtDNA markers are haploid and single locus, which can result in poor resolution within species. Mitochondrial DNA is also does not record contemporary events and traces only maternal or paternal events depending on inheritance (Frankham et al., 2002).

Nuclear genetic markers, such as microsatellites and SNPs, are the most widely used in conservation genetic research. Microsatellites are short tandem repeats of 1-6 nucleotides within the nuclear genome, and are usually associated with high mutation rates. Specifically, mutations generally act on the number of repeats, increasing or decreasing the repeat units so that loci typically vary in length between five to forty repeats, with different numbers of repeats corresponding to different alleles. The resulting high allelic diversity make microsatellite loci well suited for intraspecific studies examining evolutionary processes such as gene flow, kinship, clonal reproduction, and bottlenecks. Microsatellites in noncoding regions are neutral, meaning natural selection does not act upon them, and are assumed to meet Hardy-Weinberg expectations. DNA surrounding microsatellite loci is called the flanking region, and is usually conserved within species and sometimes across different species. Primers, or short stretches of DNA also

called oligonucleotides, can be designed within the flanking region for amplification of microsatellite loci through polymerase chain reaction (PCR) (Selkoe and Toonen, 2006). The benefits of these markers are that microsatellite loci can also be sampled noninvasively, and are fairly inexpensive. Multiple loci can be examined, and loci are co-dominant, allowing for high-resolution studies. On the downside, due to the homozygous nature of small, endangered populations, microsatellites may cause some bias, and microsatellite markers usually need be developed for each species of study, adding to the overall cost and effort (Frankham et al., 2002, Wan et al., 2004).

Compounded with this, microsatellites examine fragment lengths rather than specific sequences, and are therefore subject to a degree of human related genotype scoring error. New techniques that are being developed using next generation sequencing, such as SNPs, may offer more sensitive analyses that are less subjective to operator error. These methods would allow investigators to examine difference in sequences between individuals rather than difference in lengths of sequences (Morin et al., 2004). SNPs are differences between single nucleotides in the nuclear genome, and can exist in neutral and adaptive regions. This allows SNPs to be used to study neutral evolutionary processes such as gene flow as well as adaptive processes such as adaptive variation. However, SNPs have less diversity than microsatellites, meaning studies will require more SNPs to get the same resolution as a set of microsatellite loci (Fernandez et al., 2013). Currently, the cost of such methods makes them unpractical for the majority of studies on non-model organisms, especially species management and conservation research which is limited in funding. Nevertheless, individual assignment techniques will most likely shift to methods that examine large quantities of sequence data rather than length polymorphisms at a limited number of loci.

Case studies

Insular Adriatic Lizards

Soule performed most of the foundational work in conservation genetics, specifically on island populations such as Adriatic lizards. In a 1975 study on three species of lizard (*Lacerta melisellensis, L. sicula*, and *L. oxycephala*), Gorman, Soule, et al. used allozymes to study species differentiation and compare genetic variation across populations. The study utilized 22 allozyme loci on samples across 20 populations to calculate mean heterozygosity by dividing the number of polymorphic loci by the total number of loci, and genetic similarity between populations using Rogers coefficient. The similarity coefficient was used to confirm the morphologically based taxonomy of the three species, although also finding the three species to be highly genetically similar. Measures of heterozygosity were used to compare genetic variation between mainland and island populations, finding the lowest percent polymorphism in mainland populations was still greater than the maximum percent polymorphism of island populations. Genetic variation was also greater on large islands compared to small islands. Multiple regression was also used to attempt to determine the drivers of low levels of heterozygosity on small, distant islands, whether the cause was island size, distance to the mainland, or island geomorphology. The authors found that most of the variance was explained by channel depth, island area, and distance to the mainland (Gorman et al., 1975). The study conclusions demonstrate some of the central tenets of modern population genetics and conservation biology, that of reduced diversity in small, distant populations.

American pika in human-modified landscape

Soule's study of insular Adriatic lizards exemplifies a more basic, traditional population genetics study that could have implications for conservation. However, as conservation genetics

and molecular techniques have progressed, modern conservation genetic studies more resemble that by Waterhouse et al. (2017) on the American pika (*Ochotona princeps*). Pika were sampled at two areas experiencing two different types of human habitat modification, an area adjacent to open-pit copper mine under partial reclamation, and north and south of a bisecting major highway. Eleven polymorphic microsatellite loci were used to genotype samples collected from natural and artificial sample sites around the two study areas. Expected heterozygosity and allelic richness were used to measure genetic diversity and inbreeding coefficients (F_{IS}) were calculated to examine inbreeding. The program STRUCTURE was used to group samples into genetic clusters, and genetic differentiation between sites was calculated to examine population structure. A Mantel test was used to test for isolation by distance. The investigators found no difference in expected heterozygosity or allelic richness between artificial or natural sites, however there was a significant increase in relatedness on artificial sites. Samples clustered into two groups, that corresponded to samples collected north and south of the major highway. Significant genetic differentiation was found between some sites, and a slight pattern of isolation by distance was detected. The results indicate that human modification is significantly impacting distribution of genetic variation, and that the major highway may be driving genetic structure (Waterhouse et al., 2017). The study demonstrates how modern conservation genetic studies use genetic techniques to draw conclusions for conservation practice. In this example, human modified habitat was found to be potentially detrimental to American pika evolutionary potential, even though the species can inhabit those areas.

Conservation genetics has made significant progress during the past fifty years, and upcoming developments in molecular techniques stand to further advance the field. However, the importance of changing landscapes due to human modification and climate change have caused

the needs of conservation genetics to surpass that of existing population genetic theory. Evolutionary mechanisms act across a homogeneous landscape in basic population genetic theory, as shown in Fisher's island model and the foundation of isolation by distance. The natural landscape is heterogeneous, and is becoming more so due to habitat fragmentation. As demonstrated in the American pika study, features on the natural landscape significantly impact genetic patterns. The field of landscape genetics was developed to examine the influence of the heterogeneous landscape on evolutionary mechanisms such as gene flow and selection by integrating population genetics and landscape ecology (Manel et al., 2003).

IV. Landscape Genetics

Landscape ecology was first introduced as a discipline in 1939 by Carl Troll to synthesize the disciplines of regional geography and vegetation science. The main goal of landscape ecology is to understand the relationship between spatial configuration and ecological processes. The ecological processes examined by landscape ecological studies are spatially explicit and occur within a heterogeneous landscape to study how organisms interact with that landscape. Landscape ecology tends to focus on a broader spatial scale than most ecological studies, further differentiating the field from other ecological disciplines (Turner et al., 2001). Scale is a central tenet of landscape ecology, defined by the grain and extent of the object or process of interest (Turner et al., 2001). Studies have both a spatial scale and a temporal scale, or the time period over which the study is conducted.

Natural heterogeneous landscapes represent a middle number system, meaning they have too many components to assign an equation to each (small number system) and too few components to get reliable estimates by averaging (large number system). Hierarchy theory, based on the assertion that interactions in a middle number system can be ordered by interaction

strength and frequency, provides a method to deal with analyses at the landscape scale. The theory orders and partitions a study system into three levels of organization, consisting of a reference or focal level (L), a level below (L-1) and a level above (L+1). The levels are usually nested within one another and interconnected, meaning higher levels constrain lower levels through imposed boundaries and lower levels constrain higher levels through initiated constraints (King, 1997; Tuner et al., 2001). A landscape is defined as being spatially heterogeneous in at least one factor, usually resulting in a mosaic of that factor (Turner et al., 2001). For example, the focal level in most conservation genetic studies is a set of study populations or a single metapopulation. The level below the focal level is the individual population composed of interbreeding individuals, and the genetic diversity across individuals initiates and constrains the variation observed at higher levels. The level above the focal level is the species level, placing a constraining boundary on the amount of genetic diversity in the system by the total amount available within the species.

Unfortunately, there exists no single "correct" scale at which to conduct a study, and the "landscape level" is not a single level but differs across organisms. Instead, the scale at which a study occurs is based on the process of interest and choice of the investigator (Levin, 1992). Scale selection should be a conscious decision made during a study design, but is often overlooked by most investigators or influenced by extrinsic factors such as funding or conservation units. The process and patterns observed rely heavily upon the scale of study, causing many researchers to devote their lives to issues of scale in ecology (Wiens, 1989). Hagget (1963) identified three overarching problems associated with scale that encompass many of the issues experienced by investigators. The first is the scale coverage problem, acknowledging that the world is so vast that there are issues with gathering and understanding

accurate data on the earth's surface. The second is the scale linkage problem, the issues that results when investigators try to link studies conducted at one scale to processes occurring at another scale. This problem is common in ecological studies that extrapolate from small-scale studies to draw conclusions about processes occurring on a broader scale. Landscape ecology studies sometimes practice the reverse, using large scale spatial data to make conclusions about small scale processes. The third is that of scale standardization, the issue that arises from combining different data at different scales such as what occurs when data on different spatial features are collected at different scales (Turner et al., 2001). Despite the technological advances in geographic information systems and other spatial tools that have occurred in the past nearly sixty years since Hagget identified these problems, they are still persistent across ecological fields.

A benefit of examining processes at the landscape scale is the ability to evaluate landscape connectivity, one of the major study areas in landscape ecology. The basis of landscape connectivity is in patch based population models, such as those used in foundational population genetics theory. Assumptions about movement among patches of populations or patches of habitat greatly influence predictions about population dynamics made from these models. However, unlike in population genetics theory, landscape ecology recognizes that movement among patches is a function of the study organism and the intervening heterogeneous landscape. Landscape connectivity is the functional relationship between patches based on spatial configuration of habitat and species movement behavior, and can be broken down into two components. Structural connectivity is the physical relationship between patches in the landscape, and is independent of the movement abilities of a study species. A structural landscape pattern analysis that examines habitat continuity or measures the shortest distance

between two patches can measure structural connectivity. Functional connectivity is the degree to which the landscape facilitates or inhibits the movement of organisms across the landscape, explicitly including the movement behavior of a species. Two patches can have structural connectivity without functional connectivity, depending on the behavior of an organism, meaning no actual movement success occurs. Similarly, different species with the same dispersal habitat may have different functional connectivity causing landscape connectivity to be species specific in most cases. Structural connectivity is relatively easy to measure using landscape ecology technique, while functional connectivity and the realized movement of species through the landscape can be difficult to study (Tischendorf and Fahrig, 2000). Traditionally, the best methods for empirically studying species movement through the landscape were on the ground tracking using radio signals or mark-recapture (Tischendorf and Fahrig, 2000). However, advances in molecular techniques have made genetic data useful for gathering empirical information on dispersal behavior that can be combined with landscape data to understand species' functional connectivity.

The emerging field of landscape genetics, as first described in the flagship paper by Manel et al. (2003), combines the fundamental theories of population genetics and landscape ecology to study the interaction between the landscape and evolutionary mechanisms in species (Manel et al., 2003; Manel and Holderegger, 2013). The field addresses questions that fall into two primary categories; the influence of the heterogeneous landscape on gene flow or, more broadly, dispersal, and the environmental factors driving adaptation. The first question is studied using neutral genetic data (Holderegger et al., 2006), and directly addresses factors influencing functional population connectivity in wildlife. Landscape genetic studies using neutral genetic makers can be used in conservation to guide land management or reserve design, or alternatively

to prevent disease or invasive species spread. Studies on the interaction between the landscape and adaptive variation usually fall under the subfield of landscape genomics, and can be used in conservation to preserve populations or habitats with high adaptive potential. Landscape genomic studies are currently limited, but are becoming more widespread due to the reduced cost and increasing feasibility of genomic data.

The majority of landscape genetic studies have focused on neutral genetic data, examining the influence of landscape factors on gene flow, dispersal, and functional connectivity (Storfer et al., 2010). Results from landscape genetic studies have significant applications for conservation efforts that aim to effectively preserve genetic diversity, and therefore adaptive potential. Landscape genetic analyses could be used to inform land management plans by determining landscape factors facilitating migration or anthropogenic barriers to movement (Keller et al., 2015). A study on a metapopulation of the black-tailed prairie dog (*Cynomys ludovicianus*) found anthropogenic drainage ditches and natural streams acted as dispersal corridors among subpopulations of the species (Antolin et al., 2006). Effectiveness of proposed management plans or the impact of future land development can be evaluated using landscape genetic techniques. Landscape genetic analyses were used to predict the impact of agricultural land development on population structure and genetic diversity on the large marsh grasshopper (*Stethophyma grossum*) in Switzerland (van Strien et al., 2013). Effect of climate change on functional connectivity can also be predicted using landscape genetics, such as the effect of decreased snow pack on functional connectivity in the American Marten (Wasserman et al., 2012). Conversely, landscape genetic analyses can also be used to determine how to sever gene flow for invasive species management or disease containment, such as with infectious cancer in Tasmanian devils (Storfer et al., 2017). Because gene flow is the primary force maintaining

genetic diversity in the small, isolated populations created by habitat fragmentation (Hedrick, 1996; Slatkin, 1987), landscape genetic analyses will become increasingly important for conservation.

Landscape genetic studies using neutral genetic data to examine the influence of landscape and environmental factors on population connectivity follow three main analytical steps: measurement of genetic variation, quantification of spatial heterogeneity, and statistical correlation of genetic and spatial data (Balkenhol et al., 2016; Hall and Beissinger, 2014). Prior to beginning analysis, however, the study objectives must be decided, and a scale and sample design selected to meet those objectives (Hall and Beissinger, 2014). If the study objective focuses on the influence of landscape factors on population connectivity, spatial scale should encompass multiple interacting populations of the study species, and the temporal scale should include or follow the point at which the landscape affects gene flow. Dispersal ability and generation time of the study species affect the scale at which populations interact and the time it takes for genetic data to reflect landscape changes, and can be informative for study scale decisions (Hall and Beissinger, 2014). Spatial distribution and number of samples is a balance between optimizing power and cost of greater numbers of samples (Hall and Beissinger, 2014), and between sampling many individuals in fewer populations versus fewer individuals in many populations. A current debate in landscape genetics is whether to sample populations or individuals. While traditional studies used population-based sampling, simulations and further study has shown individual-based sampling may provide more power and create less bias in genetic differentiation analyses (Balkenhol and Fortin, 2016). In genetic studies, number of loci must also be considered in addition to the number of samples. Spatial distribution should also encompass the environmental or landscape factors of interest, based on a priori hypotheses

surrounding the study objective (Hall and Beissinger, 2014). Spatial sampling design and sample intensity have been found to affect the results of genetic analyses (Balkenhol and Fortin, 2016), and should be well considered prior to beginning a landscape genetics study. Scale selection and sampling design should be conscious decisions made prior to the start of a study based on study objectives and species' life history, but are often overlooked by researchers.

In the first step, genetic variation amongst individual samples or groups of samples is measured using genetic data gathered from genetic markers (Hall and Beissinger, 2014). Microsatellites are the most commonly used markers in landscape genetics (Storfer et al., 2010), and can be used to estimate gene flow either directly or indirectly. Gene flow can be measured indirectly using genetic distance, assuming gene flow is inversely related to genetic distance, or coalescent theory; and directly using assignment tests or parentage analysis (Waits and Storfer, 2016). Genetic distance is commonly used to measure genetic variation, the most common metric used in landscape genetics being Wright's F_{ST} followed by Cavalli-Sforza chord distance (D_{ch}) and Nei's D (Storfer et al., 2010). When using genetic distance as the genetic data in landscape genetic studies, the input usually takes the form of a pairwise genetic distance matrix giving the estimated genetic distance between each pair of samples, sites, or demes.

Spatial input data is usually measured using either transects or resistance surfaces (Hall and Beissinger, 2014). Transects measure landscape heterogeneity by quantifying variables of interest within a buffer around straight lines between pairs of samples or demes. Buffer width around transects should reflect the scale at which the study species encounters the landscape, and some studies use multiple buffer widths to examine the effect on landscape genetic analyses (Hall and Beissinger, 2014). Pairwise measures of the proportion or abundance of landscape variables of interest are used as spatial input for landscape genetic analyses when using transects.

While the buffer width allows transects to consider more than a single migration path among demes, the analysis method does assume the study species uses a recti-linear migration path (van Strien et al., 2012). Such an assumption may be overlooked for species without active dispersal such as wind or water dispersed plants, but is most likely inaccurate for mobile terrestrial animals.

Resistance surfaces are grid representations of the study landscape in which each grid cell is assigned a value symbolic of the predicted permeability of the landscape and environment within the cell (Spear et al., 2010; Zeller at el., 2012). Landscape and environmental variables included in resistance surfaces are based on the study hypotheses and objectives, and resistance surfaces can represent a single or multiple variables (Spear et al., 2016). Spatial data on the landscape and environmental variables is derived from spatial layers such as a digital elevation model (DEM), aerial imagery, or remote sensing, and usually analyzed in a spatial program such as ArcGIS (Zeller et al., 2012). Resistance values are assigned to each variable using expert opinion, empirical data, or model selection. Expert opinion is a common method for parameterizing resistance surfaces, especially for understudied species, and involves an expert on the species estimating resistance values for each landscape variable (Spear et al., 2016; Zeller et al., 2012). However, expert opinion tends to focus more on species' habitat selection rather than movement and has been found to be suboptimal (Zeller et al., 2012). Empirical data in the form of detection data, relocation data, pathway data, or genetic data can also be used to estimate resistance values, and is becoming more widely used due to the lack of bias compared to expert opinion. Model selection of resistance surfaces is based on a two stage process, in which resistance surfaces are first estimated using expert opinion or empirical data, and then the best model is selected based on correlation to genetic data (Spear et al., 2016; Zeller et al., 2012).

Resistance surfaces must be converted to a pairwise measure to compare resistance surfaces to genetic distance data, usually using either least cost paths or circuit theory. Least cost paths are the most popular technique and involve creating a path between pairs of samples or demes that minimizes cumulative movement cost, as measured through resistance values. Length of the least cost path or total resistance cost along the path between pairs is then used as a pairwise measure in landscape genetic analyses. Least cost paths are based on the assumption that the study species has enough knowledge of the landscape that they will follow a single ideal path, and that alternative paths have little effect on genetic structuring. Furthermore, least cost paths can be highly sensitive to resistance surface parameterization and errors in the spatial data layers used for resistance surface creation, which could affect overall study results. Electric circuit theory considers the landscape as nodes connected by resistors, and can also be used to predict dispersal pathways through resistance surfaces. Circuit theory analysis incorporates all possibly pathways between pairs of demes. While circuit theory does not assume a species has total knowledge of a landscape, the analysis still does assume an individual can move throughout the entire landscape. Whether least cost paths or circuit theory will be better correlated to a species' genetic data is highly dependent on the biology of the species and study objectives (Spear et al., 2016). Likewise, whether transect analysis or resistance surfaces are best suited for an analysis is dependent on study species' biology, available data, and study objectives, and how the underlying assumptions and criticisms of each analysis method could affect the results.

The final step in a landscape genetic analysis, statistically comparing genetic and landscape data, is what truly separates the field from population genetics and landscape ecology. Statistical analysis also arguably has the most room for development out of the three steps, as little to no techniques existed prior to landscape genetics. The traditional and most common

method for linking genetic and spatial data is a Mantel or partial Mantel test (Hall and Beissinger, 2014; Storfer et al., 2010), which has been traditionally used in population genetics to test for isolation by distance by correlating genetic distance and Euclidean distance (Diniz-Filho et al., 2013). However, Mantel tests have been criticized for having high type-I error rate (Balkenhol et al., 2009), causing researchers to begin using alternative analyses. Model selection across linear regression models has emerged as an effective method for linking genetic and spatial data without the high rate of type-I error (Wagner and Fortin, 2013). In linear regression models, pairwise genetic distance data acts as the response variable and spatial data measured across transects or resistance surfaces acts as the explanatory variable (Hall and Beissenger, 2014; van Strien et al., 2012). A model selection framework, usually using Akaike's information criterion (AIC), or Bayesian information criterion (BIC) (Burnham and Anderson, 2002), is then used to select the landscape variables that have the greatest influence on gene flow. Signs of slope coefficients associated with each variable is used to infer direction of relationships between landscape variables and gene flow (van Strien et al., 2012). A number of other statistical analysis methods have been used in landscape genetic analyses (Balkenhol et al., 2009), but Mantel tests and linear regression models are currently the most widely used in the field (Storfer et al., 2010).

A new analysis method has been developed to further reduce bias associated with spatial data analysis by combining least cost paths and transects within a model selection framework. Least cost transect analysis (LCTA) entails creating binary resistance surfaces in which each landscape factor of interest is set as migration habitat, regardless of whether the factor is hypothesized as facultative or inhibitive of gene flow, and all other landscape is set as matrix. Regarding every landscape factor as potential migration habitat reduces bias associated with parameterizing resistance surfaces, which can be further reduced by varying the magnitude of

matrix resistance values. From each binary resistance surface, least cost paths are calculated amongst demes and are then buffered as in transect analysis. The buffers increase the likelihood that a species' actual migration habitat is included in the analysis and accounts for potentially important landscape features surrounding the least cost path. Multiple buffer widths can be implemented to encompass a range of scales that may be potentially important to the study species' migration. Length of least cost paths and proportion of each landscape element of interest within the buffer act as explanatory variables in maximum likelihood population effect (MLPE) models (van Strien et al., 2012). MLPE models are linear regression models that, unlike Mantel tests or typical linear regression models, account for the non-independence of pairwise measures in distance matrices (Clarke et al., 2002; van Strien et al., 2012). By using least cost path length and proportion of landscape elements as explanatory variables, both the best migration habitat and the landscape elements most influencing gene flow can be determined through model selection across MLPE models (van Strien et al., 2012). Overall, LCTA reduces bias compared to traditional resistance surface or transect analysis, and may be particularly well suited to understudied species for which there is little to no information on dispersal or species that utilize dispersal strategies other than typical terrestrial dispersal.

For example, LCTA was used to study the effect of agricultural land conversion on functional connectivity in two frugivorous bat species along a biological corridor in Costa Rica (Cleary et al., 2017). The Chestnut short-tailed bat (*Carollia castanea*) and the Jamaican fruit bat (*Artibeus jamaicensis*) are both abundant and widespread bats in tropical forests that are being converted to developed land, pasture, and, more recently, monoculture crops such as pineapple. The study sought to test the effectiveness of a biological corridor using LCTA in a landscape genetic framework, specifically examining the influence of forest, pasture, development, and

pineapple on genetic distance in the two bat species. Through an exemplary method for sample design, 26 sites that were at least 1.5km apart were selected based on forest patch size and percentage of forest, pasture, pineapple, and development in the surrounding landscape. Ten microsatellite loci were used to measure genetic differentiation among sites with G''_{ST}, a pairwise genetic distance metric. Binary resistance surfaces were created with each of the four landscape elements of interest set as migration habitat, and with three varying magnitudes (2^3, 2^6, and 2^9) of resistance for the matrix. Length of least cost paths and proportion of each landscape element across buffers of three different widths (100, 400, and 800m) were used as explanatory variables in MLPE models, and top models were selected using AIC. For both species, lengths of least cost paths from resistance surfaces with forest as the migration habitat were in the top models, meaning forest is the most likely dispersal habitat. Development measured within 100m buffers was the landscape element in top models and had a positive correlation with genetic distance, meaning developed land negatively impacts functional connectivity. Results from the study indicate that linear forest transects in pineapple cropland could positively impact functional connectivity in both species, helping to inform conservation and biological corridor development in Costa Rica (Cleary et al., 2017).

As demonstrated by most of the examples provided, the most popular species of focus in landscape genetic studies are terrestrial vertebrates that are usually endangered or rare (Storfer et al., 2010). Comparatively few studies have focused on freshwater (15%) or saltwater (6%) environments, and plants (14.5%) are still relatively scarce in the landscape genetics literature (Storfer et al., 2010). Furthermore, common, widespread species are understudied despite the fact that common plant species stand to lose as much genetic diversity as rare species under habitat fragmentation (Aguilar et al., 2008; Honnay and Jacquemyn, 2006). In the salt marsh and

other ecosystems, widespread, dominant plant species are important to ecological restoration and maintenance of ecosystem processes (Vellend and Geber 2005; Whitham et al., 2003). Landscape genetic analyses on dominant, widespread plant species in these systems are necessary to adequately inform conservation that aims to preserve functional connectivity and genetic diversity, and ultimately maintain ecosystem processes and species diversity (Ehlers et al., 2008; Hughes and Stachowicz, 2009; Hughes and Stachowicz, 2004; Reusch et al., 2005).

The lack of adequate information on the salt marsh and the resident species has become increasingly apparent under the looming threats of sea level rise and climate change, which will elevate the need by coastal communities for ecosystem benefits associated with a high functioning salt marsh. The objective of this dissertation is to perform population and landscape genetic analyses on the salt marsh foundational species black needlerush (*Juncus roemerianus*) across the southeastern United States to inform coastal conservation, restoration, and management. Analyses and results are presented in the following four empirical chapters. No population genetic analyses have been performed on *J. roemerianus*, so the first chapter details the development of a panel of species-specific microsatellite markers for use in population studies and clonal analyses. Because of the importance of genetic factors to restoration success and ecosystem processes, natural levels of clonal and genetic diversity were determined in Chapter 2 by studying a large, pristine population of *J. roemerianus* preserved in a National Estuarine Research Reserve. Patterns of genetic diversity and structure were examined across populations of *J. roemerianus* in the southeastern United States in Chapter 3 to discover more about the ecology and life history of the species, and inform coastal restoration and conservation. As genetic diversity will be increasingly important for species undergoing environmental change with restricted migration ability, such as *J. roemerianus* and other coastal species, a landscape

genetic analysis was performed across populations in the northeastern Gulf of Mexico in Chapter 4 to determine the influence of landscape factors on population connectivity and inform coastal management that seeks to preserve gene flow. The chapters presented here aim to inform coastal management and restoration to preserve adaptive potential and ecosystem processes, and demonstrate methodology that could be applied to other salt marsh species or other widespread, understudied species that are increasingly needed for ecosystem restoration.

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CHAPTER 2

MICROSATELLITE MARKERS FOR POPULATION STUDIES OF THE SALT MARSH SPECIES *JUNCUS ROEMERIANUS* (JUNCACEAE)

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[1]Microsatellite markers for population studies on the salt marsh species *Juncus roemerianus* (Juncaceae)/ Tumas, H.T., Shamblin, B.M., Woodrey, M.S., Nairn, C.J / *Applications in Plant Sciences* 5 /3.

ABSTRACT

Premise of the study: Juncus roemerianus is a foundational species and ecosystem engineer of salt marshes in the Gulf of Mexico. These ecosystems provide coastal flood attenuation, nurseries for important species, and other ecosystem services, but are experiencing significant decline. Nuclear microsatellite markers were developed for *Juncus roemerianus* to study genetic diversity and population structure for conservation and restoration efforts.

Methods and Results: Illumina NextSeq high-throughput sequencing was utilized to develop a panel of 19 polymorphic microsatellite markers that were tested across individuals from three populations on the Gulf coast. All markers were polymorphic with observed and expected heterozygosities ranging from 0.212 to 0.828 and from 0.362 to 0.873, respectively. Allelic richness ranged from 2 to 13 alleles per locus with an average of 5.737.

Conclusion: The 19 microsatellite markers are useful for population studies throughout the range of *Juncus roemerianus*. Three loci cross-amplified in the related taxa *Juncus effusus.*

INTRODUCTION

Black needlerush (*Juncus roemerianus* Scheele) is a clonal, gynodioecious macrophyte found in salt marshes from the mid-Atlantic in Maryland and Delaware to the western coast of the Gulf of Mexico in Texas (Godfrey and Wooten, 1979). The species has a high salt tolerance and dominates areas of low tidal flux, such as the Gulf coast, forming large monotypic stands through sexual and clonal reproduction (Eleuterius, 1984). *Juncus roemerianus* is an ecosystem engineer and forms the foundation of the salt marsh, creating habitat for other marsh species by accumulating and stabilizing sediment (Pennings and Bertness, 2001). Genetic diversity of foundation species has an elevated importance in maintaining ecosystem health and resiliency in

monotypic ecosystems such as salt marshes (Reusch and Hughes, 2006; Hughes et al., 2008). Restored macrophyte populations with higher genetic diversity are more resilient and have greater overall restoration success (Reynolds et al., 2012). Across the Gulf coast, *J. roemerianus* habitat has been fragmented by human development, and is vulnerable to future losses and degradation from pollution and sea level rise. Information on the genetic diversity and population structure of *J. roemerianus* is essential for salt marsh conservation.

While transplant studies suggest the existence of distinct populations of *J. roemerianus*, no molecular population genetic study has been conducted on the species (Eleuterius, 1989). We address this need by developing and characterizing 19 microsatellite markers for *J. roemerianus* suitable for population studies. Microsatellites are highly variable and useful in characterizing the scale of population structure necessary for successful restoration and management.

METHODS AND RESULTS

Microsatellite markers were developed using an Illumina NextSeq (Illumina, San Diego, California, USA). Genomic DNA was extracted from a leaf sample collected from the same site as the voucher specimen at the Grand Bay National Estuarine Research Reserve (NERR) in Moss Point, MS using a Qiagen DNEasy Plant Maxi kit (QIAGEN, Hilden, Germany) (Table 4). Library preparation was completed using a KAPA LTP Library Preparation Kit (KAPA Biosystems, Wilmington, Massachusetts, USA) for Illumina platforms following manufacturer's protocol. Reads totaling 872,449 sequences were paired by name using Geneious v8.1.2 (Kearse et al., 2012), and archived in GenBank (Bioproject ID: PRJNA356252, Submission ID: SUB2149423). Illumina TruSeq adapters and bases with an error probability limit above 0.04 were trimmed, and de novo assembly was performed on sequences over 150 bases. Unused reads

were extracted to MSATCOMMANDER v1.0.8beta (Faircloth, 2008), and queried for microsatellite loci. MSATCOMMANDER identified 4,237 loci with perfect repeats of 3-6 nucleotides using default minimum lengths and melting temperatures, and combining loci less than 50bp apart. 502 loci had unique sequences surrounding the repeats with sufficient length for primer design. We selected 96 primer pairs with a pair penalty assigned by Primer 3 (Rozen and Skaletsky, 1999) below six that had a diversity of repeat lengths and nucleotide motifs. One primer for each locus was tagged with either a CAG (CAGTCGGGCGTCATCA) or M13 (GGAAACAGCTATGACCAT) sequence addition to the 5' end. Identical nucleotide matches between the 3' end of the tag sequence and the 5' end of the locus specific primer were not duplicated. The corresponding primer for each locus was tagged with a GTTT "pigtail" (Schable et al., 2002).

Amplification through polymerase chain reaction was performed on individual loci in 10 μL reactions containing 0.05 μM CAG (CAGTCGGGCGTCATCA) or M13 (GGAAACAGCTATGACCAT) tagged locus specific primer, 0.5 μM GTTT "pigtailed" locus specific primer (Integrated DNA Technologies, Coralville, Iowa, USA), 0.45 μM fluorescently labeled CAG or M13 tag primer, 0.125 mM dNTPs, 0.1μg/μL Bovine Serum Albumin (New England Biolabs Inc., Ipswich, Massachusetts, USA), 15 mM Tris pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 Units AmpliTaq Gold polymerase (Applied Biosystems, Foster City, California, USA), and 1-10 ng of template DNA. Fluorophores used to label CAG and M13 tag primers included VIC, PET, NED (Applied Biosystems) and FAM (Integrated DNA Technologies) (Table 1). Thermal cycling parameters were: 95℃ for 2 min, 25 cycles of 95℃ for 3 min, 60℃ for 30s, and 72℃ for 20s, then 25 cycles of 95℃ for 3 min, 52℃ for 30s, and 72℃ for 20s with a final extension of 72℃ for 5 min. Two microliters of PCR product was diluted in 50μL USB

nuclease free water (Affymetrix, Santa Clara, California, USA). A 3730*xl* DNA Analyzer (Applied Biosystems) at the Georgia Genomics Facility and GeneScan 500 LIZ size standard (Applied Biosystems) was used to analyze amplicon sizes. GENEMAPPER v4.0 (Applied Biosystems) was used to score allele sizes.

The 96 primer pairs were initially tested for amplification in two individuals from the Grand Bay NERR, and 48 amplified and were polymorphic. The 48 primer pairs were subsequently screened for amplification consistency and polymorphism using 24 individuals that were collected throughout the Grand Bay NERR (Table 2). Nineteen loci consistently amplified, did not significantly deviate from Hardy-Weinberg equilibrium, and had a frequency of null alleles below 0.1 (Table 1). The 19 loci were then tested across two additional populations on the Gulf of Mexico from the Apalachicola NERR and Choctawatchee Bay area to ensure consistent amplification across the range in which the species is dominant (Table 2). No clonal replicates were used to test the microsatellite markers. Three loci consistently cross-amplified in 24 samples of the related taxon, *Juncus effusus* L. collected from Perdido Bay, AL (Table 3).

Allelic data from GENEMAPPER was formatted for analysis using GMCONVERT (Faircloth, 2006). CERVUS v3.0.7 (Kalinowski et al., 2007) was used to calculate allelic richness (k), observed heterozygosity (H_O), expected heterozygosity (H_E), deviations from Hardy –Weinberg equilibrium (HWE), and frequency of null alleles (Table 2). GENEPOP v4.2 (Raymond and Rousset, 1995) was used to calculate linkage disequilibrium (LD). No loci exhibited linkage disequilibrium across or within populations following sequential Bonferroni correction except one pair of loci (Jr03 and Jr29) in the Choctawhatchee Bay population. Allelic richness ranged from 2-13 alleles per locus with an average of 5.737. The panel of 19 microsatellites had a combined non-exclusion probability of identity of 1.009×10^{-15}.

CONCLUSIONS

The 19 polymorphic nuclear microsatellite markers are useful for investigating genetic diversity and population structure in *J. roemerianus* for conservation and restoration efforts. The markers provide sufficient resolution to identify clonal replicates, and to examine the roles of clonal and sexual reproduction in natural populations of *J. roemerianus*.

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Table 2.1: Traits and thermal cycling conditions for 19 microsatellite loci for *Juncus roemerianus*.[a]

Locus	Primer Sequences (5'-3')	Repeat Motif	Allele Size Range (bp)	T_a (°C)	GenBank accession
Jr01[V]	F: [b]GGGTACGTGCGAATTTCCAG	$(AAAG)_9$	226-268	60/52	KX398592
	R: [c]AGCACATTCTTCAGCCCTTG				
Jr02[F]	F: [b]CTCGGTGAAGGCGGTTTC	$(AAAG)_8$	213-237	60/52	KX398593
	R: [d]TTCTTTCAATCCCTGCCCAG				
Jr03[P]	F: [b]ACACCTTACAGACGGGCATC	$(AATT)_8$	112-128	60/52	KX398594
	R: [d]CGACATAGTAAATTGTGCCCAG				
Jr05[P]	F: [d]CCTCTCCATGTTAGCCCTTTC	$(AAAT)_9$	255-271	60/52	KX398595
	R: [b]AGAGTCGATTTGTTTGGCACG				
Jr12[N]	F: [d]CTCTCCTCCGCTTCTGTTCC	$(ACT)_{10}$	200-215	60/52	KX398596
	R: [b]AGGGCTTCACTATCCCACTTC				
Jr13[F]	F: [b]AGCAAAGGTGAAGTCGGAGG	$(AAC)_{10}$	175-193	60/52	KX398597
	R: [d]ATCCGCTCTCACCGTACAC				
Jr16[P]	F: [b]CGGTGCAGGTTTGGATTCAG	$(AAG)_{11}$	192-207	60/52	KX398598
	R: [d]GGATCCTGATTTCAAGCGCC				
Jr19[F]	F: [b]GATCAGGGAGGAGGATTCGG	$(AGG)_{13}$	156-183	60/52	KX398599
	R: [d]CTCCAACTCCTCCGCCAG				
Jr29[N]	F: [b]AACTTGACAAGCGAACAGGC	$(AAT)_{16}$	139-154	60/52	KX398600
	R: [d]TTTGACTAGACAACACCACCC				
Jr33[V]	F: [b]GTTGGGCCTAAACTCTTCCC	$(AAT)_{16}$	179-218	60/52	KX398601
	R: [d]CCTCTGCAACGATCTCAACG				
Jr41[F]	F: [b]AACCCTCCCTTCTCAAACCC	$(AAG)_{23}$	168-204	60/52	KX398602
	R: [d]TTCTTGACCCGGTCCTTCTG				
Jr42[N]	F: [b]GCTCTCTTTACTGCTTGCG	$(ACTGG)_8$	168-208	60/52	KX398603
	R: [d]TGGTAGATAGGCCCGGATTG				
Jr46[V]	F: [c]TCAACATGTCTCCACCCTCC	$(AAAAT)_9$	157-197	60/52	KX398604
	R: [b]CCGACAGTTTACATGTGAAGC				

continued

Jr58[N]	F: [c]TCACTCTGGTCAAGGTTTAGGG	$(AAATC)_6$	149-175	60/52	KX398605
	R: [b]CCGACGACTGCAATCTCAAC				
Jr72[F]	F: [c]AGTGGGCATTATCTTATCACCG	$(AAAT)_8$	333-341	60/52	KX398606
	R: [b]GGCCGTTGTTGGAGTTTG				
Jr73[V]	F: [c]TCTACGTGAGCTACAGTTTCAC	$(AGG)_{11}$	159-180	60/52	KX398607
	R: [b]GTAACTTGGCTGCGGTGC				
Jr80[F]	F: [d]CCAGAAATGAGCACGCTGAAG	$(AAAAG)_7$	133-148	60/52	KX398608
	R: [b]CATGGGCTTGAGAAACCC				
Jr86[P]	F: [d]CCGTGAAGTGTGGCCTTTG	$(AGCAGG)_6$	160-187	60/52	KX398609
	R: [b]ATCCTTGGACGGCTCTGATC				
Jr87[V]	F: [d]ATATATTCGGCCCAGCTCGG	$(ACCTG)_6$	304-314	60/52	KX398610
	R: [b]CCACGTGAAGAGACCGATC				

Note: T_a = annealing temperature

Note: Fluorophore used to label M13 and CAG tag primers: [F]: FAM, [V]: VIC, [N]: NED, [P]: PET

[a] Values are based on 66 samples from the northeastern Gulf of Mexico in North America located in eastern Mississippi and Florida (N=20-24).

[b] GTTT tag addition to 5' terminus.

[c] CAG tag (CAGTCGGGCGTCATCA) addition to 5' terminus.

[d] M13 tag (GGAAACAGCTATGACCAT) addition to 5' terminus.

[*] Thermal cycling conditions were set at 2 annealing temperatures, 60℃ for 25 cycles and 52℃ for 25 cycles.

Table 2.2: Genetic diversity metrics for three populations of *Juncus roemerianus* located in the northern Gulf of Mexico.[a]

	Grand Bay NERR (N=24)			Apalachicola NERR (N=20)			Choctawhatchee Bay (N=22)		
Locus	*A*	H_o	H_e	*A*	H_o	H_e	*A*	H_o	H_e
Jr01	5	0.625*	0.57	7	0.6	0.641	4	0.818	0.735
Jr02	3	0.458	0.393	2	0.1	0.097	3	0.381	0.33
Jr03	3	0.542	0.582	5	0.5	0.518	4	0.636	0.678
Jr05	2	0.458	0.403	3	0.45	0.422	2	0.727	0.507
Jr12	3	0.625	0.664	3	0.15	0.229	3	0.455	0.65
Jr13	5	0.542	0.462	5	0.5	0.687	5	0.773	0.724
Jr16	5	0.583	0.691	4	0.7	0.688	4	0.591	0.576
Jr19	3	0.5	0.526	5	0.7	0.679	5	0.636	0.512
Jr29	5	0.625	0.594	5	0.7	0.672	5	0.636	0.72
Jr33	6	0.833	0.816	7	0.8*	0.808	9	0.85	0.855
Jr41	10	0.958	0.887	6	0.65	0.629	5	0.818	0.779
Jr42	6	0.542	0.566	4	0.55	0.696	4	0.864	0.692
Jr46	3	0.417	0.434	4	0.2	0.191	4	0.727	0.552
Jr58	4	0.458	0.414	4	0.4	0.645	4	0.773	0.591
Jr72	2	0.583	0.507	2	0.1	0.097	2	0.318	0.274
Jr73	5	0.292	0.27	7	0.8	0.767	5	0.636	0.729
Jr80	2	0.333	0.454	1	0	0	3	0.273	0.246
Jr86	4	0.792	0.691	4	0.7	0.721	5	0.818	0.758
Jr87	3	0.375	0.318	3	0.55	0.559	3	0.455	0.54

Note: N= *number of samples, A= number of alleles,* H_o*= observed heterozygosity* H_e*= expected heterozygosity*

[a]: Geographic coordinates for populations are: Grand Bay NERR = N30° 21.865' W88° 26.246' , Apalachicola NERR = N29° 44.177' W84° 53.094', Choctawhatchee Bay = N30° 24.069' W86° 13.834'. Populations were collected from eastern Mississippi and the panhandle of Florida in the United States.

* Significantly deviated from Hardy-Weinberg equilibrium after a sequential Bonferroni correction (P=0.05)

Table 2.3: Characteristics of three primers that cross-amplified in the related species *Juncus effusus*.

Locus	Allele size (bp)	T_a (°C)*
Jr05	263	60/52
Jr46	170-180	60/52
Jr73	129-489	60/52

CHAPTER 3

HOW CLONAL IS CLONAL: CLONAL AND GENETIC DIVERSITY OF THE CLONAL MACROPHYTE BLACK NEEDLERUSH (*JUNCUS ROEMERIANUS* SCHEELE) WITHIN THE GRAND BAY NATIONAL ESTUARINE RESEARCH RESERVE

[1]Tumas H.T., B.M. Shamblin, M.S. Woodrey, C.J. Nairn. Submitted to Estuaries and Coasts.

ABSTRACT

Salt marshes are valuable ecosystems that provide habitat for a range of endemic and economically important species, and supply ecosystem services essential to society. With salt marshes in decline worldwide, Northeastern Gulf of Mexico salt marshes represent a large portion of the remaining salt marsh in the United States and are dominated by the clonal macrophyte black needlerush (*Juncus roemerianus* Scheele). Genetic factors such as clonal and genetic diversity can be critical to restoration success and ecosystem processes, yet no genetic studies have been conducted on large, natural populations of *J. roemerianus* to date. We collected 304 samples from 21 sites within the Grand Bay National Estuarine Research Reserve and used 12 nuclear microsatellite markers to measure levels of clonal diversity in *J. roemerianus*. Clonal diversity was greater than expected based on existing life history literature, which assumes rare sexual reproduction. These findings are inconsistent with assumptions derived from existing literature for the species and can inform the development of science-based conservation and restoration strategies for salt marshes in the Gulf of Mexico.

INTRODUCTION

Salt marshes are valuable ecosystems that provide habitat to ecologically and economically important species (Kennish 2001), and supply ecosystem services to coastal communities valued at $10,000 per hectare (Kirwan and Megonigal 2013). With an over 50% reduction in the United States alone from human modification (Kennish 2001), salt marshes are further threatened by climate change and sea level rise (Thorne et al. 2012). The majority of the remaining salt marsh in the United States, approximately 62% of total marsh area and 22% of global marsh area, exists along the Gulf of Mexico (Greenberg and Maldonado 2006; Greenberg

et al. 2006). These Gulf coast salt marshes provide storm protection and flood attenuation to coastal communities, and have a marked effect on water quality (Zedler and Kercher 2005). Irregularly flooded marshes in the northeastern Gulf of Mexico are dominated by the clonal, gynodioecious, macrophyte black needlerush (*Juncus roemerianus* Scheele), a foundational plant species in salt marshes distributed from eastern Texas to Maryland (Eleuterius 1976A; Godfrey & Wooten 1979; Stout 1984). The species contributes to primary productivity in the marsh (Eleuterius 1976B) and creates habitat for other species by accreting and stabilizing sediment (Pennings and Bertness 2001). Due to the importance of *J. roemerianus* in creating salt marsh habitat and providing the main source of primary productivity, the species is being used in coastal restoration in the Gulf of Mexico and southern Atlantic (Sparks et al. 2013).

However, genetic data for *J. roemerianus* is lacking, which could negatively impact the success of current restoration efforts. Preservation of ecosystem processes and species resilience may be dependent on preserving clonal diversity (number of unique genotypes) and genetic diversity (heterozygosity of individuals) in foundational plant species (Hughes et al. 2008; Reusch and Hughes 2006, Hughes & Stachowicz, 2009; Hughes & Stachowicz, 2004; Reusch et al., 2005; Reynolds et al. 2012). Restored populations with higher levels of genetic and clonal diversity are more resilient to disturbance (Ehlers et al., 2008), support more ecosystem processes (Hughes & Stachowicz, 2009; Hughes & Stachowicz, 2004; Reusch et al., 2005; Reynolds et al. 2012), and have a lower risk of inbreeding depression and other founder effects (Mijnsbrugge et al., 2010; Hufford and Mazer, 2003). Specifically, clonal diversity in foundational plant species positively influences species diversity throughout the ecosystem (Crutsinger et al. 2006; Hughes & Stachowicz 2004). The amount of intraspecific genetic diversity is directly related to adaptive potential and rate of evolutionary change (Mills 2007;

Frankel and Soule 1981; Montalvo et al. 1997), which will be critical for species persistence under natural disturbances such as sea level rise and climate change.

Available literature on *J. roemerianus* perpetuates the idea that the species only uses sexual reproduction in the colonization of new areas, while vegetative propagation is used more often to maintain mature stands (Eleuterius 1975; Eleuterius 1984; Stout 1984). As a result, established populations of *J. roemerianus* are expected to be composed of a few clonal variants or multilocus genotypes (MLGs), and rare sexual reproduction is expected to lead to low levels of genetic diversity. However, the true rate of clonal propagation and amount of standing genetic diversity cannot be determined without a genetic study in a large, natural population of *J. roemerianus*, leaving restoration practitioners to wonder just how clonal is this clonal macrophyte. We begin to address this knowledge gap by conducting an intensive population genetic study on a large *J. roemerianus* dominated salt marsh within a National Estuarine Research Reserve (NERR) to measure clonal and genetic diversity. Results from the study can be used as a benchmark to assess health of disturbed or restored *J. roemerianus* populations and inform coastal restoration.

MATERIALS & METHODS

Study Site and Field Collection

The Grand Bay NERR is part of the National Oceanic and Atmospheric Association (NOAA)'s reserve network established by the Coastal Zone Management Act of 1972 for long-term research, monitoring, education and stewardship. Grand Bay NERR was designated in 1999, and encompasses approximately 7,446 ha of large, pristine estuary located in Moss Point, MS in southeast Jackson County. The reserve supports a variety of plant and animals species

across a range of estuarine and non-estuarine habitats, including beds of submerged aquatic vegetation, salt marsh, freshwater marsh, and maritime forest. Areas of salt marsh are dominated by smooth cordgrass (*Spartina alterniflora* Loisel) in the low marsh, *J. roemerianus* in the mid marsh, and saltmeadow cordgrass (*Spartina patens* Aiton) in the high marsh. Irregularly flooded salt marsh dominated by monotypic stands of *J. roemerianus* covers approximately 2,900 ha, 38% of the reserve (Peterson et al. 2007).

Twenty-five sites at least 1 km apart were randomly selected from within the Grand Bay NERR using the Sampling Tool_10 v2.02012411 created by the NOAA Biogeography Branch in ArcGIS. Twenty-one of the sites were sampled based on accessibility and *J. roemerianus* presence. Fifteen samples were collected at each site in an "X" formation with approximately 9 m between each sample to prevent sampling a single clonal genet (Figure 1). Two sites have less than 15 samples due to human error during collection resulting in a total of 304 samples. A single leaf was collected and deposited in a plastic bag and a GPS waypoint was taken using Garmin GPSMAP 64st for each sample. Leaf tissue was stored at room temperature while in the field, and at -20°C upon return to the lab. Thirty of the samples were used in another study to develop species-specific microsatellite markers for use in population studies on *J. roemerianus* (Tumas et al. 2017). The entire sample set will also be used as part of a larger sample set in landscape genetic and population genetic studies.

Genetic Analysis

Samples were genotyped using 12 microsatellite markers (Jr05, Jr12, Jr13, Jr16, Jr19, Jr33, Jr41, Jr42, Jr46, Jr58, Jr73, Jr80) that had an overall probability of identity of 2×10^{-8} following DNA extraction protocol, PCR reaction procedure, thermo cycling conditions, and genotype scoring as described in Tumas et al. (2017). Missing genotype data results in an

overestimation of genetically distinct clones, and therefore only samples with no missing data were used in clonal analyses. The R-package *poppr* v2.5 (Kamvar et al. 2014; Kamvar et al. 2015; R Core Team 2016) was used to calculate p_{gen} and p_{sex} using the 'pgen' and 'psex' functions, respectively. Number of unique multi-locus genotypes (MLGs), Simpson's index corrected for sample size (λ) (Simpson, 1949), Shannon-Wiener Index of MLG diversity (H), and genotypic evenness (E.5) (Grunwald et al., 2003) were calculated across the reserve and within each sample site using the 'poppr' function in *poppr*. Simpson's index was corrected for sample size by multiplying the statistic calculated in *poppr* by (N/(N-1) where N is the total number of samples. Genotypic diversity (G_D) was calculated using the number of MLGs and the formula (G-1)/(N-1) where G is the number of MLGs and N is the total number of samples (Arnaud-Haond et al. 2007). Average geographic distance between clones within a genet was calculated using the recorded GPS coordinates and the 'gdist' function in the *Imap* package in R (Wallace 2012).The full sample set was used in genetic diversity analyses, and identical genotypes were identified in CERVUS (Kalinowski et al. 2007) using Identity Analysis. A single, randomly selected representative ramet was randomly chosen from each group of identical genotypes. All other genetically identical replicates were excluded from further analyses. Observed heterozygosity (H_O), expected heterozygosity (H_E), allelic diversity (A_D), and the inbreeding coefficient (F_{IS}) were calculated using Arlequin (Excoffier & Lischer 2010).

RESULTS

Of the 304 samples collected, 229 had no missing data for use in clonal analyses and 142 represented unique MLGs, comprising 62% of the analyzed samples (Figure 2). All MLGs had $p_{sex} < 1.48 \times 10^{-5}$ and $p_{gen} < 1.04 \times 10^{-7}$. Total genotypic diversity across samples was 0.62, and

ranged from 0.25 to 1.0 across sample sites. Simpson's index was 0.99 across samples with an average of 0.87 across sites, and Shannon-Wiener Index was 4.76 across samples with an average of 1.72 across sites. Samples were distributed fairly evenly across genets with an average evenness of 0.87 across sites, and no clones were shared between sites (Table 1). Distance between clones within a genet averaged 12.88m across all genets. Across the full set of 304 samples, 158 represented unique genotypes for use in genetic diversity analyses. Observed heterozygosity was 0.55 and expected heterozygosity was 0.56, with an average of 6.75 alleles per locus and a significant ($p=0.03$) inbreeding coefficient of 0.03.

DISCUSSION

The prevailing assumption that mature *J. roemerianus* stands exclude seedlings and rely on vegetative growth (Eleuterius 1975) implies that established populations have low clonal and genetic diversity. However, the results from a genetic analysis on a large, natural population of *J. roemerianus* demonstrate that the species has higher than expected clonal and genetic diversity for a species assumed to have rare sexual reproduction. Over half of the samples represented unique genotypes, a total of 142 MLGs within a single population. Furthermore, no clones were shared across sample points and average distance between clones was under 13m, indicating clonal genets are not geographically widespread. Genetic studies on other clonal plant species have also contradicted life history assumptions, revealing higher than expected clonal diversity and geographically restricted clones across species (Ellstrand and Roose 1987; Widen et al. 1994), including other coastal plant species (Franks et al. 2004; Richards et al. 2004). Greater than expected genetic diversity may indicate this high clonal diversity is due to more frequent sexual reproduction occurring within established populations than previously assumed in life

history literature. A broad scale population genetic study of *J. roemerianus* would be needed to test if patterns of higher than expected clonal and genetic diversity are maintained across populations that vary in size, environment, and geographic location, and if greater rates of sexual reproduction are common across the species' range.

While high overall, clonal diversity varied spatially across sample sites, from sites with proportionally few MLGs to sites composed entirely of unique genotypes. The sample design used could mean differences in clonal diversity are due to random chance, but spatial variation in environmental stressors may also be driving variation in clonal propagation. Even within a single population salt marsh habitat is not uniform, and can experience environmental variations on the scale of one meter (Pennings and Callaway 2000). Although environmental data was not collected at each site, degree of dominance in *J. roemerianus*, elevation, and distance to water varied across sample sites (Figure 3). Spatial variation in stressors such as salinity or wave action could create spatial variation in uninhabited or stressful areas in the marsh. Because *J. roemerianus* can share resources among ramets in a genet, vegetative growth is advantageous for invading stressful habitats or uninhabited areas (Pennings and Callaway, 2000), accounting for variation in clonal propagation across environments. Similarly, variation in clonal diversity across environmentally variable sites was also found in the co-occurring marsh species *S. alterniflora* (Richards et al. 2004), another species that can share resources among ramets (Pennings and Callaway, 2000). The degree to which clonal propagation is phenotypically plastic or genetically linked would determine whether this spatial variation in the trait is due to environmental response or local adaptation as a result of natural selection. Diversifying selection is believed to play an important role in maintaining diversity in clonal plant species (Widen et al. 1994), and may explain why no clones were shared between sites. Furthermore, if local

adaptation is occurring then environmental variation could spatially restrict genotypes within a marsh, allowing for higher rates of sexual reproduction to colonize the multitude of spatially and temporally variable microhabitats. A study on adaptive variation in *J. roemerianus* would be needed to link genotypes to clonal propagation, and determine if clonal propagation is an adaptation to stressful environments.

A lack of seedlings in mature stands of *J. roemerianus* has led to the assumption of rare sexual reproduction in *J. roemerianus* (Eleuterius 1975; Eleuterius 1984), suggesting limited gene flow among populations and low levels of genetic diversity. However, the genetic diversity results contradict this assumption and suggest gene flow, via sexual reproduction, may be occurring at a higher rate. The high disturbance regime in the salt marsh due to daily wave action and intermittent storm events (Pennings and Bertness 2001) may allow for the introduction of new alleles through gene flow creating bare areas of marsh that open up established populations for sexual recruitment. If gene flow is responsible for maintaining the higher than expected levels of genetic diversity, then preserving gene flow among *J. roemerianus* populations should be a priority for coastal managers that seek to ensure species resiliency to climate change and sea level rise. Little is known about dispersal in the species, although seeds in most other wetland plant species, including the morphologically similar seeds of common rush (*Juncus effusus* L.), are dispersed via wind, water, and animals (Cronk and Fennessy 2001; USDA, NRCS, 2017). A landscape genetic study would be useful in determining landscape factors that influence gene flow (Manel et al. 2003), and would inform managers on how to preserve gene flow among *J. roemerianus* populations across the coast.

In addition to adding to current knowledge on *J. roemerianus*, results from this study can be used to inform coastal management and restoration. The GBNERR population represents a

large, healthy population living within a relatively undisturbed salt marsh that may reflect natural levels of diversity. Clonal and genetic diversity results can be used as a benchmark to assess the health of disturbed or other natural *J. roemerianus* populations. Results also indicate that restoration projects should incorporate a greater number of unique genotypes than suggested by current life history literature, and be planned for areas that will allow for gene flow with other populations. Restored populations that reflect levels of genetic and clonal diversity expected from life history literature will lack adequate genetic diversity for adaptation (Frankel and Soule 1981; Montalvo et al. 1997), and will be unable to support the number of ecosystem benefits associated with high clonal diversity (Crutsinger et al. 2006; Hughes & Stachowicz, 2009; Hughes & Stachowicz, 2004; Reusch et al., 2005; Reynolds et al. 2012).

CONCLUSION

Although higher than expected clonal diversity has become a trend across clonal species with the advent of readily available genetic techniques (Ellstrand and Roose 1987), no population genetic study had been conducted on a large, natural population of *J. roemerianus* prior to this study. Higher than expected levels of clonal and genetic diversity indicate sexual reproduction plays a greater role in *J. roemerianus* life history than assumed in current literature. Restoration projects seeking to reestablish ecosystem services and create populations resilient to natural disturbances should incorporate many unique genotypes and source from areas of high genetic diversity. In conclusion, a species that plays such a vital role in an ecologically and economically valuable ecosystem, such as *J. roemerianus*, warrants further investigation.

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Table 3.1: Genotypic diversity of each sample site within GBNERR. For each site, the total number of samples (N) reflects samples with no missing data across a subset of 12 markers. Genotypic diversity was measured by the total number of multilocus genotypes (MLG), genotypic diversity (GD), Shannon-Wiener Index of Diversity (H), Simpson's Index (λ), and evenness (E.5).

Site	N	MLG	GD	H	λ	E.5
GB1	11	8	0.70	1.89	0.89	0.76
GB2	9	3	0.25	0.94	0.64	0.85
GB3	7	7	1.00	1.95	1.00	1.00
GB4	10	5	0.44	1.51	0.84	0.90
GB5	7	6	0.83	1.75	0.95	0.94
GB8	11	5	0.40	1.16	0.62	0.59
GB9	8	5	0.57	1.56	0.89	0.95
GB10	15	8	0.50	1.86	0.87	0.78
GB11	11	9	0.80	2.15	0.96	0.94
GB12	11	5	0.40	1.37	0.76	0.78
GB13	14	9	0.62	2.05	0.91	0.82
GB14	12	4	0.27	1.24	0.74	0.87
GB15	13	7	0.50	1.69	0.83	0.75
GB16	10	10	1.00	2.30	1.00	1.00
GB18	10	10	1.00	2.30	1.00	1.00
GB19	6	4	0.60	1.33	0.87	0.94
GB20	12	4	0.27	1.31	0.77	0.90
GB21	13	6	0.42	1.61	0.83	0.84
GB22	12	9	0.73	2.10	0.94	0.87
GB23	14	9	0.62	2.07	0.92	0.87
GB24	13	9	0.67	2.06	0.92	0.84
Average	10.90	6.76	0.60	1.72	0.87	0.87

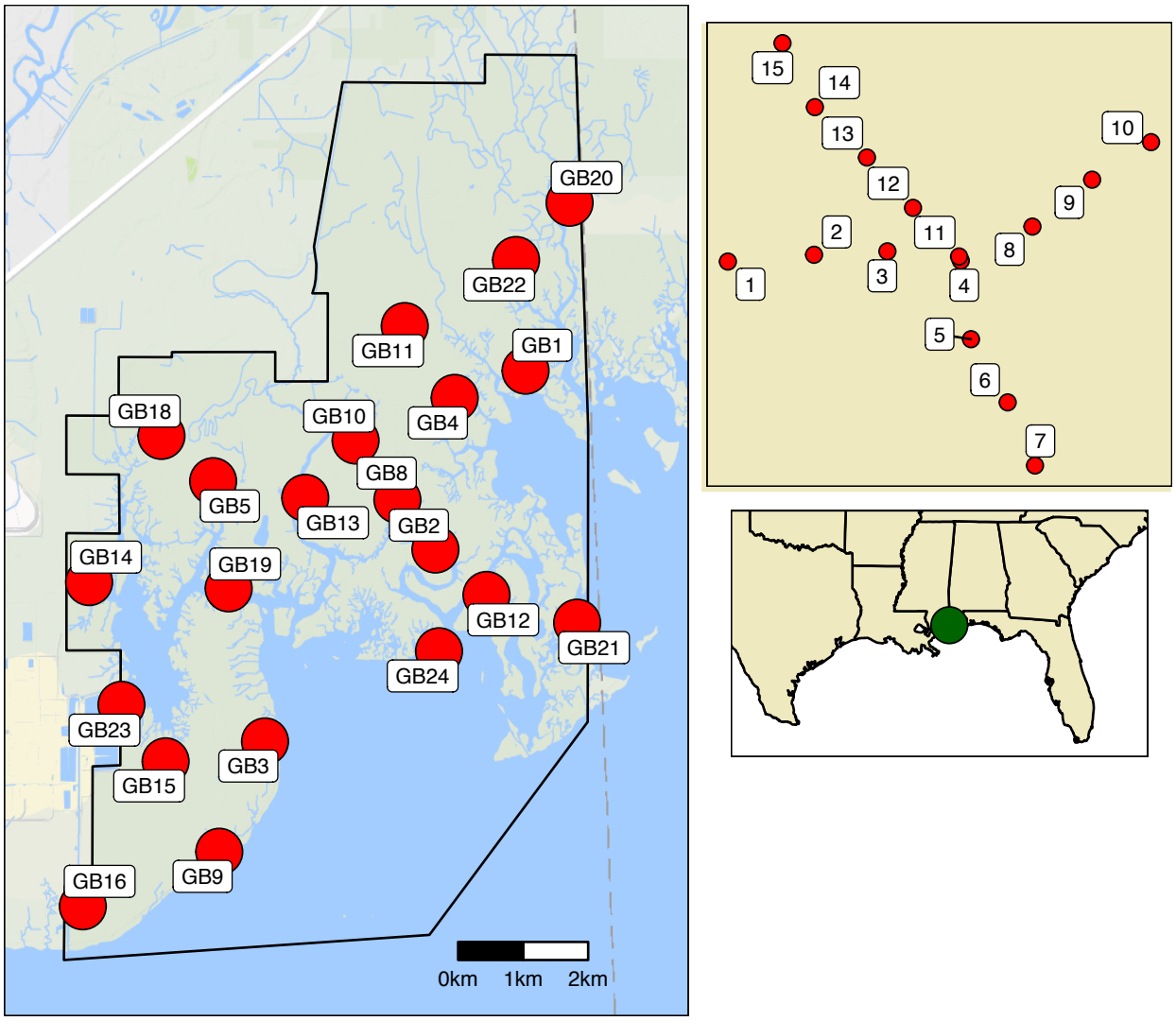

Figure 3.1: Study design of 21 sites (red circles) within the GBNERR (black boundary line) (left), and the sample (red circles) design at each site (top right). GBNERR is located in the northeastern Gulf of Mexico on the border between Mississippi and Alabama (bottom left).

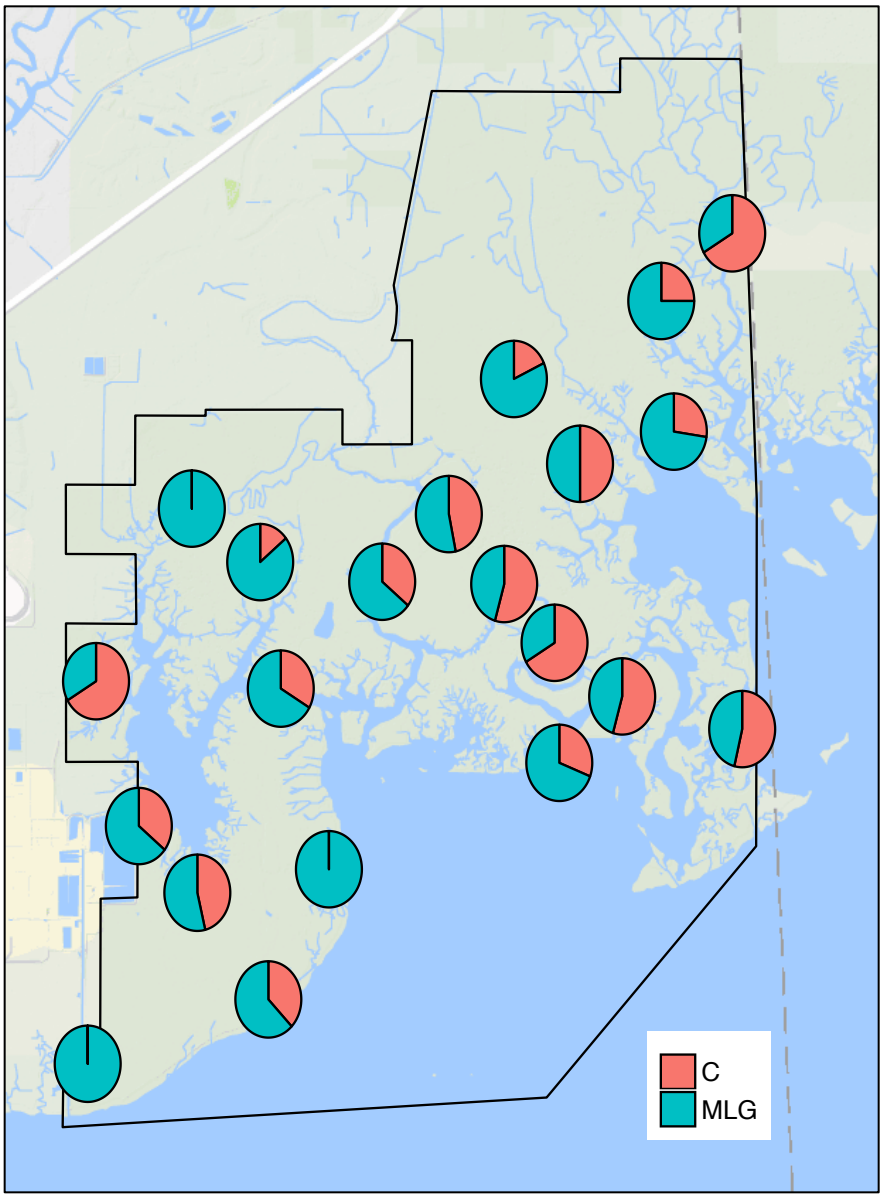

Figure 3.2: Proportion of multilocus genotypes (MLG) shown in blue to clonal replicates (C) shown in red at each sample site within the GBNERR (black boundary line). Note that proportions vary across sites.

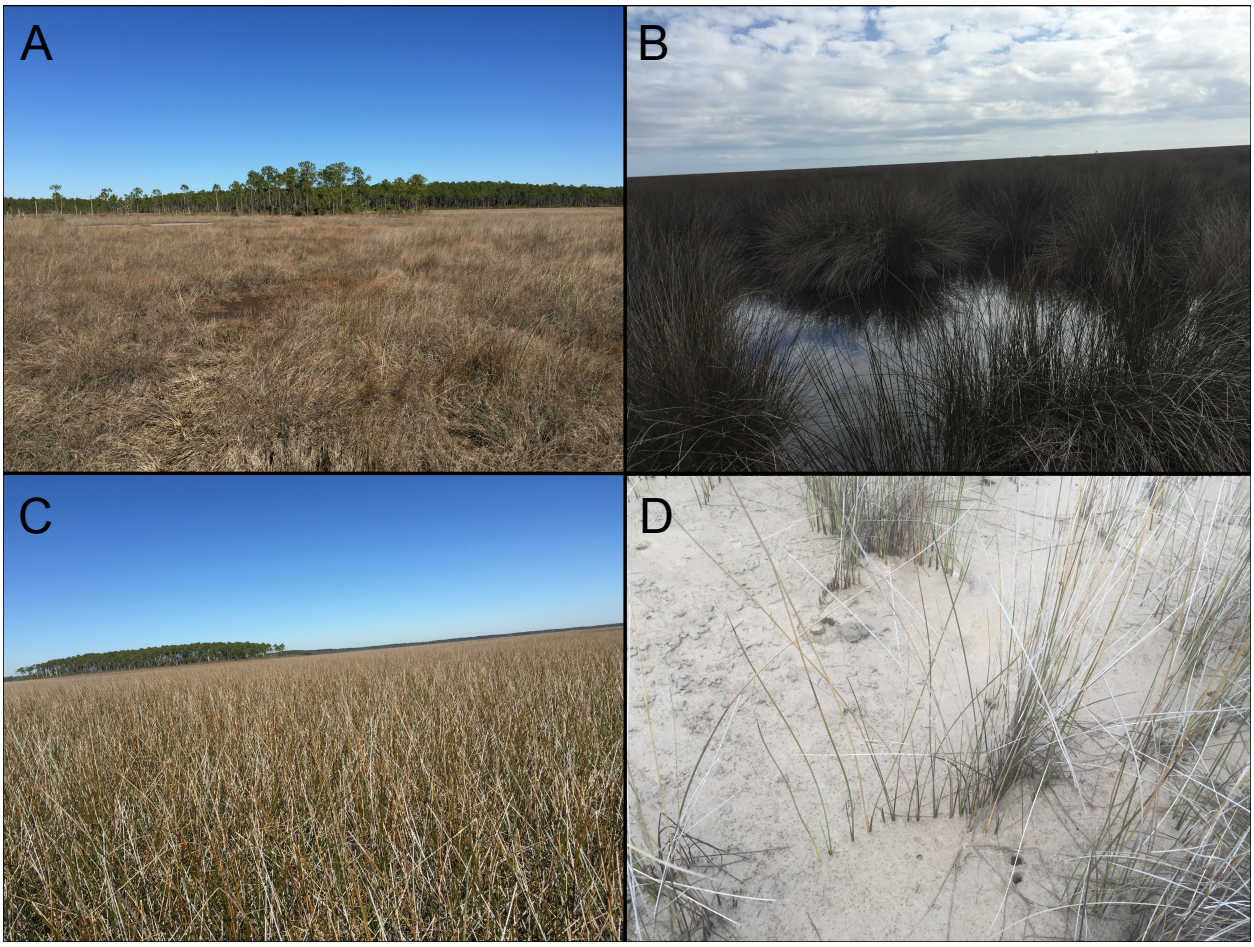

Figure 3.3: Photos that illustrate different environmental conditions across sites from patchily distributed *J. roemerianus* (A) to inundated (B) and irregularly flooded monotypic stands. Photo D illustrates clonal propagation in a salt flat.

CHAPTER 4

PATTERNS OF CLONAL AND GENETIC DIVERSITY IN THE FOUNDATIONAL SALT MARSH PLANT BLACK NEEDLERUSH (*JUNCUS ROEMERIANUS* SCHEELE) ACROSS A MAJORITY OF THE RANGE

[1]Tumas H.T., B.M. Shamblin, M.S. Woodrey, C.J. Nairn. Submitted to Journal of Heredity.

ABSTRACT

Clonal and genetic diversity in foundational plant species are critical for species resiliency and ecosystem processes, both of which contribute to restoration success; however genetic data is often lacking for the common plant species used in many restoration practices. Salt marshes are ecologically and economically valuable ecosystems in decline worldwide that are dominated by a few plant species. Black needlerush (*Juncus roemerianus* Scheele) is an understudied clonal macrophyte and a foundational species in southeastern salt marshes, the largest portion of remaining salt marsh in the United States. We used a panel of 18 microsatellite markers across 849 samples of *J. roemerianus* collected at 17 sites across a majority of the species range from Mississippi to South Carolina to measure clonal and genetic diversity and characterize population structure. Despite expectations of low sexual reproduction from life history literature, we found higher than expected clonal and genetic diversity with an average genotypic diversity of 0.67 and average observed heterozygosity of 0.56. Clonal and genetic diversity differed between the Gulf and Atlantic coast, corresponding to environmental differences in disturbance regimes and plant communities. Genetic structure occurred on a broad scale with samples grouping into three genetic clusters indicating higher than expected rates of gene flow. Although significant pairwise F_{ST} measures and sub-structuring indicate local adaptation may also be an important evolutionary force driving genetic structure. Results from the study add to the knowledge of *J. roemerianus* ecology and have implications for coastal restoration.

INTRODUCTION

Common, foundational plant species are key components of ecological restoration that seeks to reestablish ecosystem processes and benefits (Hughes et al., 2008; Reusch and Hughes,

2006), but have been understudied in conservation genetics (McKay et al., 2005). Native plant restoration success is highly dependent on the genetic composition of source material (Hufford and Mazer, 2003; Mijnsbrugge et al., 2010), specifically the amount of genetic diversity and genetic similarity to the restored area, which can be estimated using neutral genetic markers (McKay et al., 2005). Genetic diversity of dominant plant species in monotypic landscapes is analogous to species diversity in maintaining ecosystem processes (Hughes et al., 2008; Reusch and Hughes, 2006), and contributes to restoration success (Reynolds et al., 2012). In clonal plant species, preserving natural levels of clonal diversity (number of clonal variants) improves plant growth and reproduction (Williams, 2001; Hammerli and Reusch, 2003; Reusch et al., 2005), and increases ecosystem services (Hughes and Stachowicz, 2009; Crustinger at al., 2006). Salt marshes are monotypic landscapes dominated by a few clonal plant species (Stout, 1984) that are undergoing restoration nationally and globally, but have been understudied in the conservation genetic literature.

Decades of destruction and degradation by waste disposal and land conversion (Broome et al., 1988) has caused salt marshes to decline by 13-30% worldwide with an over 50% decline in the United States (Kennish, 2001; Valiela et al., 2009). These habitats are further threatened by climate change and sea level rise (Gedan et al., 2009). More recently, the value of salt marshes to human health and wealth, as well as wildlife, has prompted legislative protection and restoration efforts. Salt marshes supply ecosystem services valued at $10,000 per hectare (Kirwan and Megonigal, 2013; Zedler and Kercher, 2005), and provide critical habitat to a number of endemic, as well as ecologically and economically important species (Kennish, 2001). The southern Atlantic coast and Gulf of Mexico contain a significant portion of the remaining salt marsh habitat in the world, supporting 12,440 km^2 of tidal marsh that makes up 78% of the

United States tidal marsh area and 27% of global marsh area (Greenberg and Maldonado, 2006; Greenberg et al., 2006). In the southeast United States, the two primary target plant species for restoration are smooth cordgrass (*Spartina alterniflora* Loisel) and *J. roemerianus.* Although clonal and population genetic analyses have been conducted on *S. alterniflora* (ex. Novy et al., 2010; Richards et al., 2004), to our knowledge, no such studies have been conducted on *J. roemerianus*.

J. roemerianus is a clonal, gynodioecious macrophyte and a foundation species in salt marshes from eastern Texas to the mid-Atlantic in Maryland (Godfrey and Wooten, 1979; Eleuterius, 1976; Stout, 1984). The species creates marsh area by stabilizing (and thus accreting) sediment, providing habitat for other salt marsh wildlife (Pennings and Bertness, 2001). Life history literature on the species reports *J. roemerianus* as using primarily clonal reproduction with sexual reproduction only occurring during rare recruitment events to colonize new areas (Stout, 1984), implying populations of *J. roemerianus* are composed of only a few clonal variants. Genetic data are needed to inform restoration ecologists about the true rate of clonal reproduction, and how rates vary across environments in natural populations, so that restored ecosystems reflect natural levels of resiliency and diversity.

We performed a broad-scale population genetic analysis on *J. roemerianus* across the majority of the species range in the southeastern United States to inform tidal marsh restoration practices. The objectives of our study were to (i) measure standing levels of clonal and genetic diversity in natural populations, (ii) delineate broad-scale population structure across the majority of the species' range, (iii) characterize fine-scale genetic structure, and (iv) compare genetic patterns between the Atlantic and Gulf coasts. Results from the study will advance the

clonal plant and restoration literature, as well as shed light on an understudied species and ecosystem.

MATERIALS & METHODS

Sample Collection

Seventeen sites were sampled across the northeastern Gulf of Mexico and southeastern Atlantic coast from Moss Point, MS to Awendaw, SC (Figure 1). Ten sites were sampled in the Gulf of Mexico as part of other studies, two of which (GB and AP) are National Estuarine Research Reserves (NERR). A set of 304 samples was collected from Grand Bay NERR (GB) in January and March 2015, and a set of 32 samples was collected from Apalachicola NERR (AP) in May 2015 and March 2016. Thirty samples were collected from eight sites (CS1, CS3, CS5, CS6, CS7, CS8, CS9, CS10) between the two NERRs in March 2016. Forty samples were collected from an additional seven sites in November 2016 for this study to extend the study range in the Gulf coast (EC1 and EC2), and include the Atlantic coast (EC3, EC4, EC6, EC7, EC8) (Table 1). Samples consisted of a single leaf collected and stored in plastic bags, and a GPS waypoint taken using a Garmin GPSMAP 64st.

Microsatellite Amplification

Nineteen species-specific microsatellite markers (Tumas et al., 2017) were used to genotype samples following DNA extraction protocol, PCR reaction conditions, and thermal cycling parameters described in Tumas et al. (2017). Samples were successfully scored across a minimum of 15 loci, or PCR was re-run on the sample. Genotyping error rate was calculated by re-running approximately 17% of samples and dividing the number of mismatched loci identified by Identity Analysis in CERVUS (Kalinowski et al., 2007) by the total number of scored loci.

Clonal Analysis

A subset of twelve markers (Jr03, Jr05, Jr12, Jr13, Jr16, Jr33, Jr41, Jr42, Jr46, Jr58, Jr80, Jr86) with the least missing data were selected and only samples with complete 12 locus genotypes were used in clonal analyses to account for missing data across the full set of microsatellite markers. Unique multilocus genotypes (MLG) were identified, and clonal metrics were calculated using the package *poppr* v2.5 (Kamvar et al., 2014; Kamvar et al., 2015; R Core Team, 2016). The metrics p_{sex} and p_{gen} were calculated across each sample using the 'psex' and 'pgen' functions respectively. Number of MLGs, Simpson's index corrected for sample size (λ), Shannon-Wiener Index of MLG diversity (H), genotypic diversity (G_D), and genotypic evenness (E.5) (Grunwald et al., 2003) were calculated across samples grouped by site and coast of origin (Gulf of Mexico or Atlantic). Genotypic diversity, or the number of unique genotypes, was not reported in *poppr* and was calculated as (G-1)/(N-1) where G is the number of unique MLGs at a site and N is the total number of ramets sampled (Arnaud-Haond et al., 2007). Simpson's index was corrected for sample size by multiplying the reported lambda by (N/(N-1)) where N is the total number of ramets sampled.

Genetic Diversity Analyses

The full panel of markers was used in genetic diversity and structure analyses, following removal of genetically identical clonal replicates identified using Identity Analysis in CERVUS. A single ramet was randomly selected from each group of genetically identical samples, and all other clonal replicates were removed. Hardy-Weinberg Equilibrium was tested at each locus across samples within each site using CERVUS. Linkage disequilibrium was calculated between pairs of loci at each site using Genepop (Raymond and Rousset, 1995).

Expected (H_E) and observed (H_O) heterozygosity, allelic diversity (A_D), and the inbreeding coefficient (F_{IS}) were measured across sites using Arlequin (Excoffier and Lischer, 2010). Allelic richness (A_R) within sites rarefied for sample size was calculated using the *divBasic* function in the 'diveRsity' package in R on the full set of sites (Keenan et al., 2013). The number of private alleles was measured using the *private_alleles* function in *poppr*. Genetic diversity metrics were also calculated across samples grouped by site and coast of origin.

Population Structure Analyses

Genetic clusters were identified using the program STRUCTURE via 20 iterations of 100,000 steps following a burn-in period of 50,000 with admixture, populations with and without prior locations, and correlated allele frequencies for each of K=1-6 (Pritchard et al., 2000). Additional STRUCTURE runs following the same parameters were conducted on samples grouped by genetic cluster to examine hierarchical genetic structure. The optimal number of k clusters was chosen using the Evanno method in STRUCTURE HARVESTER (Earl and vonHoldt, 2012). Principal components analysis (PCA) on the variance-covariance matrix of allele frequencies was conducted using the *dudi.pca* function in the 'ade4' package in R (Dray and Dufour, 2007; Chessel et al., 2004; Dray et al., 2007). PCA examines continuous genetic structure based on genetic similarity unlike STRUCTURE that groups samples into discrete clusters so that frequencies meet Hardy-Weinberg Equilibrium expectations. Pairwise genetic distances between sites were measured as Slatkin's linearized F_{ST} using Arlequin, correcting associated p-values for false discovery rate using the Benjamini and Yekutieli (2001) method (Narum, 2006). A mantel test was run using the *mantel.randtest* function in the R-package 'ade4' to test for significant patterns of isolation by distance.

RESULTS

Clonal Diversity

Across the subset of twelve markers used in clonal analyses, 692 samples had no missing data from which 472 samples were identified as unique MLGs. All MLGs had $p_{sex} < 5.81 \times 10^{-6}$ and $p_{gen} < 1.23 \times 10^{-8}$. Clonal diversity and evenness varied across sites with an average Simpson's Index of 0.92 and average Shannon-Weiner Index of MLG diversity of 2.85. Genotypic evenness ranged from 0.494 at site EC1 to 1.0, with an average genotypic evenness of 0.85. Genotypic diversity ranged from 0.08 at CS8 to 1.0 at two sites (CS10 and EC7) composed of only unique MLGs, and with an average of 0.67 (Table 1). Gulf coast samples had a lower genotypic diversity ($G_D = 0.64$) than the Atlantic coast ($G_D = 0.80$), but an equal Simpson's Index and higher Shannon-Wiener Index ($\lambda = 0.996$, H = 5.56) than the Atlantic coast ($\lambda = 0.996$, H = 4.83). Genotypes were more evenly abundant across Atlantic coast samples (E.5 = 0.84) than across Gulf of Mexico samples (E.5 = 0.67) (Table 1).

Genetic Diversity

A total of 849 samples were typed across a minimum of 15 loci in the full panel of microsatellite markers, and 529 samples were identified as unique genotypes for use in genetic diversity and population structure analyses. Overall genotyping error rate was 2.08%. One marker (Jr73) deviated from Hardy-Weinberg Equilibrium at six sites (EC1, EC2, EC3, EC4, EC7, and EC8), and was subsequently removed from all other analyses. All other loci were retained, and showed no evidence of deviation Hardy-Weinberg Equilibrium except 10 loci at 8 sites (Jr03 and Jr86 in GB, Jr86 in CS5, Jr33 in CS10, Jr19 at EC1, Jr03 at EC2, Jr29 and Jr46 at EC3, Jr80 at EC4, and Jr12 at EC8). Following a sequential Bonferroni correction, all but 14 pairs of loci at 5 sites (Jr13&Jr58, Jr12&Jr72 in GB; Jr29&Jr33, Jr01&Jr80 in CS10; Jr01&Jr02,

Jr01&Jr33, Jr01&jr41, Jr13&Jr86, Jr72&Jr87, Jr19&Jr41 in EC3; Jr33 & Jr42 in EC4; Jr13&Jr19, Jr12&Jr19 in EC6) did not exhibit linkage disequilibrium.

Genetic diversity was moderate across sites, with an average expected heterozygosity of 0.53, an average observed heterozygosity of 0.56, and average allelic richness of 2.33 alleles per locus (Table 2). The Gulf coast had higher values of genetic diversity ($H_E = 0.65$, $H_O = 0.52$, $A_R = 6.96$) than the Atlantic coast ($H_E = 0.53$, $H_O = 0.45$, $A_R = 5.26$). Samples across the Gulf of Mexico also had a greater number of private alleles (32) than samples from the Atlantic coast (4). Levels of inbreeding also varied across sites (average $F_{IS} = -0.02$), but no site had a significant F_{IS} value following a sequential Bonferroni correction. Gulf coast samples had a slightly higher F_{IS} (0.17) than Atlantic coast samples (0.14).

Population Structure

The optimal number of genetic clusters from the STRUCTURE analyses was k = 3 when populations were set as prior locations, grouping sites GB-CS7 into a western Gulf coast (WGC) cluster, sites CS8-EC3 into an eastern Gulf coast (EGC) cluster, and sites EC4-EC8 into a South Atlantic Bight (SAB) cluster (Figure 2). Although, EGC does contain a site located on the Atlantic coast (EC3). When populations were not set as prior locations, the optimal number of genetic clusters was k = 2 with WGC and SAB forming a single cluster. The PCA across all samples reflected the k = 3 result, and demonstrated that SAB is more closely related to WGC than EGC as indicated by the k = 2 result (Figure 3). Additional STRUCTURE runs found the optimal number of clusters was k = 2 for each of the three genetic clusters (Figure 2). All pairwise measures of F_{ST} were significant following correction for false discovery rate, and the pattern of genetic similarity between SAB sites and WGC sites was also reflected in both measures of pairwise genetic distance (Table 3). Values of F_{ST} were smaller between SAB and

WGC than between either of these clusters and EGC sites. The mantel test for isolation by distance was significant (0.55, p=0.001)

DISCUSSION

Despite common species experiencing similar or greater losses in genetic diversity as rare species (Aguilar et al., 2008; Honnay and Jacquemyn, 2006), plant studies have primarily focused on rare species and overlooked widespread common species important for restoration. The present study investigating the foundational and regionally dominant salt marsh species, *J. roemerianus*, contradicts current life history assumptions about the species and structurally conforms to genetic analyses on other species with similar ranges. Across sites, *J. roemerianus* had moderate to high levels of clonal and genetic diversity, although sites across the Gulf of Mexico had higher genetic diversity and lower clonal diversity than sites across the Atlantic coast. Higher than expected levels of genetic diversity combined with significant pairwise F_{ST} measurements suggest repeated, but rare, seedling recruitment in *J. roemerianus*. Samples structured into three genetic clusters and samples from the South Atlantic Bight were more genetically similar to samples from the western Gulf of Mexico portion of the study range. Results have important implications for restoration and management practices of *J. roemerianus*, and highlight the importance of studying common, widespread plant species.

Clonal and Genetic Diversity Across Sites and Coasts

As with many other clonal plant species prior to the availability of molecular markers, *J. roemerianus* life history literature assumed populations of the species were dominated by a few unique MLGs and experienced rare sexual reproduction and seedling recruitment (Eleuterius, 1975). However, this study demonstrates *J. roemerianus* has higher than expected clonal and

genetic diversity. Overall, sites had multiple unique MLGs, with two sites consisting of entirely unique genotypes, and all but a single clone were restricted to a single site. The fewest unique MLGs were found in site CS8, which was likely due a high degree of urban development restricting sampling to a small area (24 m^2). Despite these habitat constraints, CS8 still had three unique MLGs. The only clone shared between sites consisted of samples that were identical across 18 markers, and was shared between sites CS3 and CS5 that are approximately 89 km apart. Heterozygosity was moderate across sites, and many sites had private alleles despite low allelic richness. These genetic patterns are consistent with genetic studies on other clonal plant species, which have also detected higher than expected levels of clonal diversity and few widespread clones (Ellstrand and Roose, 1987; Widen et al., 1994). The co-occurring salt marsh plant species *S. alterniflora*, also expected to be primarily clonal, has similar clonal diversity to *J. roemerianus*, with an average Simpson's diversity of 0.998 across seven sites on Sapelo Island, GA (Richards et al., 2004). However, Richards et al. (2004) followed a traditional gridded format to measure clonal propagation unlike the random sampling scheme used across sites in this study. Such an intensive clonal study on *J. roemerianus* in a large population would provide more information on rates of clonal propagation and size of clones. Genetic studies on clonal plants have contradicted expectations of low genetic diversity (Gabrielsen and Brochmann, 1998; Lloyd et al., 2011; Pluess and Stocklin, 2004), and *J. roemerianus* exhibits higher levels of genetic diversity than would be expected from a species with rare sexual reproduction. Similarly, populations of *S. alterniflora* have also been found to have higher than expected genetic diversity, with average observed heterozygosity ranging from 0.6 to 0.73 across 12 loci in a study on New York populations (Novy et al., 2010).

Higher levels of clonal and genetic diversity, and almost even ratio of unique genotypes to clonal replicates, could suggest a more even balance between clonal propagation and sexual reproduction in *J. roemerianus*. However, the balance shifts across the Atlantic and Gulf coasts. Sites in the Gulf of Mexico have overall greater genetic diversity and lower clonal diversity than sites across the Atlantic coast, which could be driven by differences in plant community and disturbance regime. *J. roemerianus* is dominant in the northeastern Gulf of Mexico (Stout, 1984) where the samples were collected, which means the species covers a greater area and possibly a greater number of microhabitats than on the Atlantic coast. Greater genetic diversity in the Gulf could then be explained by the positive relationship that has been documented between genetic diversity and both population size and area (Frankham, 1996). Availability of microhabitats and area may cause clonal propagation to be more important to *J. roemerianus* growth and survival in the Gulf of Mexico than the Atlantic. *J. roemerianus* has the ability to share resources among ramets, an important trait when invading stressful environments such as salt flats and expanding into uninhabited areas (Pennings and Callaway, 2000). Diversifying selection driven by environmental and ecological factors, which vary on a scale of a meter or less (Pennings and Callaway, 2000) in the salt marsh, would maintain multiple MLGs and create the higher than expected clonal diversity that still exists in the Gulf of Mexico.

Conversely, genetic patterns on the Atlantic coast could be explained by the lack of dominance by the species and a higher disturbance regime. More frequent flooding and competitive exclusion by *S. alterniflora* and other species restricts *J. roemerianus* to the upper marsh on the Atlantic coast (Stout, 1984). The resulting reduced areal coverage and population size of *J. roemerianus* on the Atlantic coast compared to the Gulf of Mexico would correlate to reduced genetic variation (Frankham, 1996). Increased seedling recruitment as a result of

persistent, intermediate disturbance might explain the greater clonal diversity found on the Atlantic coast compared to the Gulf coast (Reusch, 2006). The Atlantic coast experiences greater and more frequent disturbance from flooding caused by higher amplitude diurnal tides compared to the irregularly flooded marshes in the northeastern Gulf of Mexico (Dardeau et al., 1992). The subsequent loss of established genets and recruitment of new genets through sexual reproduction caused by an intermediate disturbance regime could allow for the introduction of novel genotypes and enable the Atlantic coast to maintain higher levels of clonal diversity (Eriksson, 1993; Reusch, 2006).

The two coasts reflect the ends of the clonal plant life history spectrum that transitions from initial seedling recruitment (ISR) with no subsequent seedling recruitment after initial colonization to repeated seedling recruitment (RSR) throughout the lifetime of a population (Eriksson, 1989). Gulf coast populations could use an ISR strategy, causing standing clonal diversity in Gulf populations to depend on a combination of the number of unique MLGs in the founding population and diversifying selection (Eriksson, 1993; Widen et al., 1994). Populations of *S. alterniflora* in the Gulf of Mexico experience declines in clonal diversity with population age following initial establishment, as expected in species that follow an ISR strategy (Travis et al., 2004). Such a pattern may explain the greater number of private alleles found in two of the peninsular Florida sites (EC1 and EC2) that may be younger than other sites in this study due to the emergence of the Florida peninsula in the Miocene Epoch (Avise, 1992). Reduced genotypic evenness across Gulf sites may be an artifact of a few MLGs from the founding population adapting and proliferating in each population and may further indicate an ISR strategy. An intermediate disturbance regime could allow for repeated seedling recruitment in Atlantic populations, more closely reflecting an RSR strategy and causing clonal diversity to be

maintained by sexual reproduction. While determining life history strategy in *J. roemerianus* would require a long-term study, environmental factors driving life history strategy is not unique and other species have also been demonstrated to use different strategies in different habitats (Eriksson, 1993). However, if rate of clonal propagation and sexual reproduction is genetically linked, environmental factors would also be driving adaptation and leading to functional genetic differences between coasts in *J. roemerianus*.

Genetic Structure

Fine-scale genetic structure indicated by significant measures of F_{ST} between all pairs of sties could be a result of restricted gene flow, local adaptation, or genetic drift. While diversity results suggest sexual recruitment in *J. roemerianus* is most likely higher than predicted by current life history literature, clonal propagation may still limit the introduction of novel genotypes into sites by gene flow. The lack of widespread clones may be further indicative of limited gene flow. Local adaptation may also be causing significant genetic differentiation by restricting genotypes to specific environments, and is attributed to maintaining clonal diversity in clonal plants (Widen et al., 1994). Clonal propagation may be driving down the effective population size and increasing the rate of genetic drift (Chung, et al., 2004), which would counteract local adaptation while still causing site differentiation. Most likely a balance is occurring between the three evolutionary forces to create fine scale genetic structure that could be fragile to the effects of ongoing coastal development and sea level rise. Further decline and fragmentation of salt marsh habitat would decrease gene flow while potentially increasing the rate of genetic drift, reducing the introduction of novel genotypes and standing genetic diversity. Marsh conservation and restoration could be necessary for maintaining the balance between the

three evolutionary forces, and therefore preserving genetic variation and local adaptation in the species.

The STRUCTURE analysis revealed that population structure likely occurs on a relatively broad scale in *J. roemerianus*, again indicating some level of gene flow occurs to create large, admixed populations. Many other wind-dispersed plant species also persist in large, effectively panmictic populations even when some population fragments are isolated (Ashley, 2010), such as some salt marsh fragments. Water or bird-mediated dispersal (Huiskes et al., 1995; Soons et al., 2008, respectively) would also allow for longer dispersal distances between fragmented salt marsh populations. Further structure in each genetic cluster indicates genetic structure occurs hierarchically in the species, and sub-clusters may indicate the scale at which genetic neighborhoods are formed. The geographic location of the genetic break between genetic clusters of *J. roemerianus* on the Atlantic coast and Gulf of Mexico, occurring roughly around Cape Canaveral, FL, was similar to that of other estuarine, marine, and freshwater species with geographic distributions that encompass both regions (Avise, 1992). However, unlike other species for which phylogeographic studies on the region have been conducted (Avise, 1992; Blum et al., 2007; Drumm and Kreiser, 2012), the SAB cluster was more closely related to the WGC cluster than the EGC cluster.

Genetic structure in other species that exhibit a genetic break between Gulf of Mexico and Atlantic populations have been attributed to historical factors, specifically the emergence of the Florida peninsula (Avise, 1992). Prior to the emergence of the Florida peninsula in the Miocene epoch (Avise, 1992), populations of *J. roemerianus* in the western Gulf and populations at the upper extent of the southeastern Atlantic coast could have been connected by gene flow into a single admixed population. Low levels of gene flow and clonal propagation could have

allowed the genetic signature from this historical connection to prevail in neutral markers, despite loss of any current gene flow between the SAB and WGC. Genetic distinctiveness of EGC could then be explained by the population being evolutionarily younger than WGC and SAB, potentially also explaining the particular distinctiveness of the peninsular Florida populations (EC1, EC2, EC3) in the PCA. The location of the genetic break between SAB and EGC at Cape Canaveral, FL could be explained by the Gulf Stream, which may facilitate oceanic gene flow in *J. roemerianus* and flows out of the Gulf of Mexico, up the Atlantic coast, leaving land around Cape Canaveral, FL (Avise, 1992). On the other hand, observed genetic structure could be driven by adaptation to climatic factors. WGC and SAB clusters are located in temperate regions while the peninsular Florida cluster is within a tropical region, and the location of the transitional zone between climatic regions, again occurs around Cape Canaveral, FL (Avise, 1992). Use of neutral markers with high mutation rates, however, limits the inferences that can be made about timing of events or adaptation. A study using a more conservative marker, such as cpDNA or mtDNA, could be used to examine the genetic structure and estimate timelines of genetic divergence. However, the observed structure is unlikely random, as geographic locations of genetic breaks reflect historic events and climatic regions, and parallels genetic structure in other species with similar geographic distributions. Overall, genetic structure patterns likely reflect historic gene flow between western Gulf populations and Atlantic populations of *J. roemerianus*, and genetic similarities between the regions have persisted due to more recently limited gene flow among populations and adaptation to similar climates.

Implications for Conservation and Restoration

Despite rarely being accounted for in restoration practices, genetic factors such as genetic diversity, clonal diversity, and population structure have vitally important ramifications for

restoration (Hufford and Mazer, 2003; Montalvo et al., 1997; Kramer and Havens, 2009; Lesica and Allendorf, 1999). Current life history literature underestimates natural levels of clonal and genetic diversity in *J. roemerianus*, and would misinform coastal restoration seeking to preserve resiliency and ecosystem processes. Rather than using one to several clones as suggested by the currently available literature, restoration practitioners should select multiple clonal variants from sites with high genetic diversity for restored populations to reflect natural populations. Regional differences in levels of clonal and genetic diversity could indicate evolutionary responses to environmental factors, stressing the importance of considering the target restoration environment and standing diversity in surrounding populations prior to transplant selection. Failure to include adequate clonal and genetic diversity into restored populations could result in restoration failure in the short term or future die-out due to an inability to adapt to changing environmental and climactic conditions (Kramer and Havens, 2009). Genetic diversity preserves evolutionary potential (Montalvo et al., 1997), and will be increasingly important for plant populations that can only respond to threats, such as climate change and sea level rise, through phenotypic plasticity, migration, or adaptation (Kramer and Havens, 2009). Sea walls and human development will limit inland migration in response to sea level rise for salt marsh species (Kirwan and Megonigal, 2013), meaning many salt marsh plants will have to adapt or go extinct. In restored populations especially, adequate clonal diversity is important for preventing founder effects such as inbreeding depression, low fitness, and low establishment rates (Mijnsbrugge et al., 2010; Hufford and Mazer, 2003), and for preserving ecosystem benefits and processes (Reynolds et al., 2012).

Selecting source populations from within the same genetic cluster as the restored site may better ensure restoration reflects the scale of local adaptation and prevents declines in native and

restored population fitness associated with outbreeding depression, intraspecific hybridization, and genetic swamping (Hufford and Mazer, 2003; Lesica and Allendorf, 1999; Kramer and Havens, 2009). Genetic clusters offer better guidance for source population selection to restoration practitioners than ecoregions or geographic distance between sites, which are often used to guide selection without genetic data. While neutral genetic variation does not necessarily correspond to adaptive variation, structure and differentiation detected by molecular markers could still be indicative of locally adapted populations (Hufford and Mazer, 2003). Hierarchical genetic structure and differences in clonal and genetic diversity suggest local adaptation is occurring at multiple scales across the range of *J. roemerianus*, all of which is encompassed by a single one of Baileys ecoregions. Restoration success tends to be inversely related to the genetic and environmental distance between source and restored populations, which may not always be well correlated to geographic distance (Montalvo and Ellstrand, 2000). Intuitively, restoration practitioners may select a source population from southern Florida for a restoration site in northern Florida, however the results indicate other regions to the north on the Atlantic or even in the western Gulf of Mexico are more genetically similar. Incorporating the genetic data from this study into coastal restoration practices will help to ensure success in the short term and persistence of restored sites into the future.

CONCLUSIONS

J. roemerianus is a foundational, regionally dominant species in a declining ecosystem targeted for restoration efforts, but has been understudied with no broad scale population genetic studies to date. These results reveal that restoration efforts based on current life history literature would create restored populations that are lacking in clonal and genetic diversity compared to natural populations of *J. roemerianus*, and may be maladapted to local environments. Restored

populations with greater genetic diversity and informed source selection will be more successful and have a greater chance of persisting under future conditions (Hufford and Mazer, 2003; Kramer and Havens, 2009; Montalvo et al., 1997; Hammerli and Reusch, 2003; Williams, 2001; Lesica and Allendorf, 1999; Mijnsbrugge et al., 2010). However, such populations cannot be created if genetic studies have not been conducted on the common species frequently used in restoration practices. This study demonstrates the need for a paradigm shift in conservation studies to include widespread, common species alongside the more traditionally studied critically endangered and rare species. Common species such as *J. roemerianus* and other coastal plant species are increasingly important for ecosystem restoration and need to be investigated to ensure such species remain common and that the ecosystems they support persist into the future.

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Table 4.1: Clonal diversity measures for samples with no missing data across a subset of 12 markers. Approximate geographic locations are listed for each sample site. Total number of samples (N), number of multilocus genotypes (MLG), genotypic diversity (G_D), Shannon-Wiener Index of MLG Diversity (H), Simpson's Index corrected for sample size (λ), and evenness (E.5) are reported for samples grouped by site, coast, and cluster.

	N	MLG	G_D	H	$\boldsymbol{\lambda}$	E.5
GB – Moss Point, MS	221	142	0.64	4.788	0.995	0.824
CS1 – Heron Bayou, AL	26	10	0.36	1.955	0.843	0.706
CS3 – Gulf Shores , AL	28	22	0.78	3.016	0.981	0.91
CS5 – Avalon Beach, FL	26	13	0.48	2.451	0.941	0.902
CS6 – Niceville, FL	26	15	0.56	2.611	0.957	0.913
CS7 – Santa Rosa Beach, FL	26	22	0.84	2.992	0.979	0.841
CS8 – West Bay, FL	25	3	0.08	0.708	0.440	0.71
CS9 – Panama City Beach, FL	27	12	0.42	2.198	0.892	0.759
CS10 – Cape San Blas, FL	30	30	1.00	3.401	1.000	1
AP – Apalachicola, FL	27	21	0.77	2.949	0.977	0.882
EC1 – Suwanee, FL	24	13	0.52	2.021	0.797	0.494
EC2 – Crystal River, FL	32	30	0.94	3.379	0.996	0.968
EC3 – Merritt Island NWR, FL	33	17	0.50	2.643	0.945	0.84
EC4 – Fanning Island, FL	35	26	0.74	3.088	0.970	0.777
EC6 – Jekyll Island, GA	36	33	0.91	3.468	0.996	0.961
EC7 – Beaufort, SC	34	34	1.00	3.526	1.000	1
EC8 – Awendaw, SC	36	30	0.83	3.323	0.987	0.894
Average	76.89	52.5	0.67	2.854	0.923	0.846
Gulf Coast	518	332	0.64	5.56	0.9959	0.671
Atlantic Coast	174	140	0.80	4.83	0.9957	0.836
Western Gulf Cluster	352	222	0.63	5.23	0.996	0.807
Eastern Gulf Cluster	199	127	0.64	4.49	0.985	0.551
Southern Atlantic Bight Cluster	141	123	0.87	4.74	0.997	0.875

Table 4.2: Genetic diversity measures for all samples across 18 markers. The total number of samples (N), allelic diversity (A_D), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), number of private alleles (PAS), and inbreeding coefficient (F_{IS}) are reported for samples grouped by site, coast, and cluster. Note that N reflects the total number of samples collected, but only unique genotypes identified across 18 markers were used in analyses.

	N	A_D	A_R	H_O	H_E	PAS	F_{IS}
GB	304	6.22	2.53	0.54	0.56	17	0.02
CS1	30	3.33	2.45	0.6	0.55	0	-0.11
CS3	30	4.22	2.6	0.53	0.58	1	0.08
CS5	30	3.77	2.56	0.6	0.61	1	0.01
CS6	30	3.5	2.26	0.43	0.54	0	0.17
CS7	30	4.11	2.62	0.64	0.6	2	-0.11
CS8	30	2.64	1.75	0.79	0.67	0	-0.24
CS9	30	3.83	2.56	0.59	0.56	1	-0.05
CS10	30	3.88	2.22	0.51	0.5	1	0
AP	32	4.24	2.37	0.9	0.53	1	0.07
EC1	36	4.06	2.23	0.48	0.48	5	-0.05
EC2	40	4.17	2.36	0.48	0.52	3	0.04
EC3	40	3.59	2.12	0.51	0.47	1	-0.14
EC4	40	4.22	2.57	0.54	0.56	1	0.02
EC6	39	3.83	2.36	0.47	0.5	1	0.05
EC7	38	3.18	1.99	0.42	0.42	0	-0.03
EC8	40	3.47	2.01	0.41	0.42	1	0.01
Average/Total	849	3.9	2.33	0.56	0.53	2.12	-0.02
Gulf Coast	652	8.06	6.96	0.52	0.65	32	0.17
Atlantic Coast	197	5.33	5.26	0.45	0.53	4	0.14
Western Gulf Cluster	245	7.11	6.13	0.55	0.61	21	0.12
Eastern Gulf Cluster	147	6.22	5.94	0.48	0.56	12	0.08
Southern Atlantic Bight Cluster	137	5.06	4.96	0.45	0.5	3	0.08

Table 4.3: Pairwise genetic distances among the 17 sites. Chord distances are along the top diagonal, and Slatkin's linearized F_{ST} is along the bottom diagonal. All pairwise F_{ST} measures were significant. Note that distance between western Gulf sites (GB-CS7) and northern Atlantic sites (EC4-EC8) are smaller than between either set of sites and eastern Gulf sites (CS8-EC3).

	GB	CS1	CS3	CS5	CS6	CS7	CS8	CS9	CS10	AP	EC1	EC2	EC3	EC4	EC6	EC7	EC8
GB	*	0.612	0.649	0.657	0.664	0.645	0.740	0.762	0.700	0.718	0.707	0.774	0.744	0.735	0.661	0.677	0.633
CS1	0.075	*	0.336	0.342	0.426	0.417	0.527	0.598	0.499	0.510	0.475	0.629	0.567	0.581	0.404	0.396	0.453
CS3	0.035	0.069	*	0.320	0.443	0.460	0.490	0.592	0.484	0.486	0.447	0.589	0.537	0.581	0.408	0.390	0.453
CS5	0.120	0.131	0.086	*	0.379	0.404	0.478	0.635	0.506	0.506	0.457	0.605	0.551	0.567	0.374	0.376	0.436
CS6	0.166	0.221	0.156	0.112	*	0.383	0.494	0.561	0.497	0.496	0.462	0.529	0.489	0.510	0.405	0.423	0.484
CS7	0.208	0.168	0.150	0.110	0.092	*	0.515	0.560	0.429	0.457	0.442	0.521	0.471	0.476	0.415	0.415	0.483
CS8	0.376	0.366	0.375	0.250	0.275	0.190	*	0.536	0.456	0.500	0.463	0.600	0.570	0.594	0.491	0.497	0.577
CS9	0.262	0.227	0.239	0.190	0.167	0.078	0.152	*	0.464	0.487	0.475	0.551	0.521	0.561	0.542	0.613	0.626
CS10	0.311	0.303	0.322	0.251	0.266	0.173	0.232	0.081	*	0.338	0.328	0.515	0.458	0.515	0.406	0.439	0.488
AP	0.271	0.260	0.264	0.204	0.245	0.158	0.207	0.081	0.046	*	0.270	0.480	0.429	0.471	0.437	0.427	0.498
EC1	0.412	0.442	0.412	0.275	0.310	0.259	0.365	0.290	0.338	0.265	*	0.431	0.385	0.447	0.373	0.391	0.471
EC2	0.331	0.332	0.331	0.220	0.245	0.191	0.227	0.201	0.231	0.166	0.060	*	0.272	0.359	0.493	0.526	0.555
EC3	0.385	0.449	0.419	0.266	0.271	0.249	0.443	0.331	0.333	0.263	0.164	0.083	*	0.313	0.428	0.488	0.520
EC4	0.130	0.155	0.101	0.123	0.162	0.162	0.276	0.152	0.221	0.145	0.303	0.213	0.266	*	0.473	0.520	0.529
EC6	0.161	0.210	0.147	0.161	0.204	0.214	0.464	0.217	0.251	0.191	0.366	0.307	0.347	0.051	*	0.259	0.315
EC7	0.260	0.401	0.271	0.326	0.404	0.405	0.789	0.440	0.496	0.460	0.616	0.506	0.585	0.155	0.112	*	0.292
EC8	0.270	0.392	0.277	0.323	0.401	0.402	0.796	0.446	0.492	0.452	0.633	0.521	0.625	0.150	0.114	0.058	*

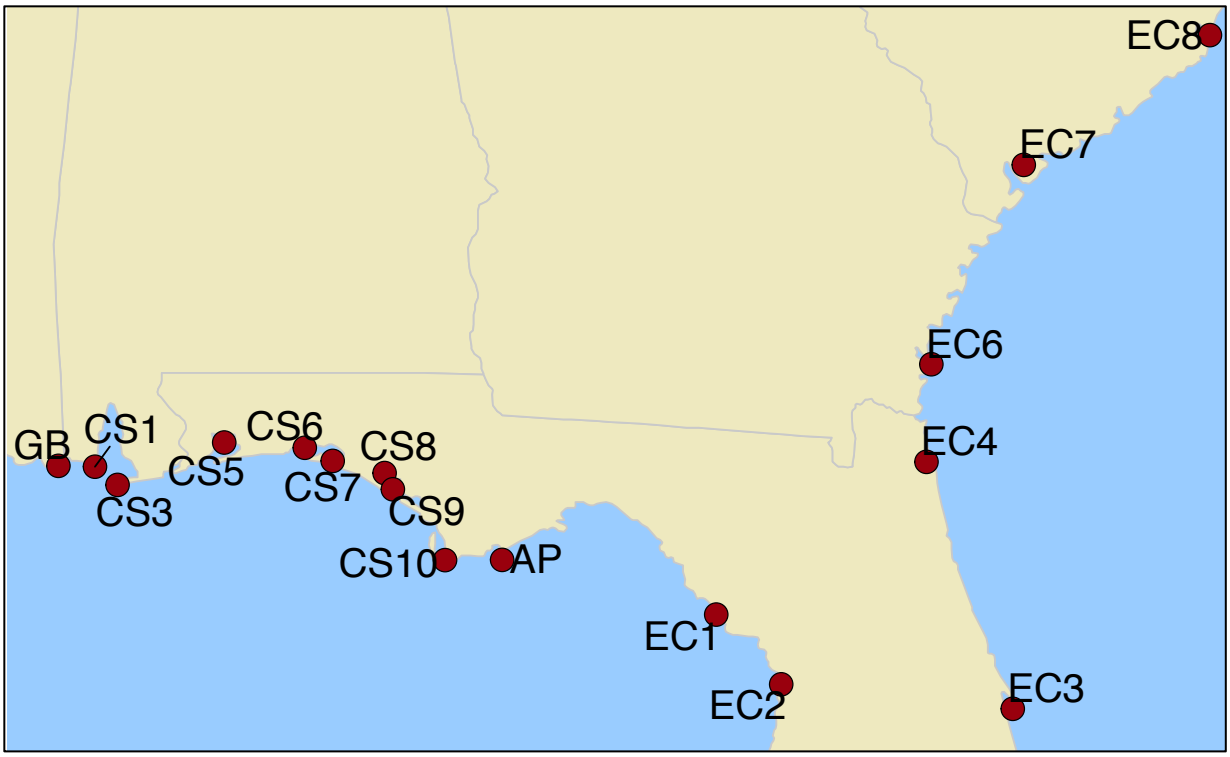

Figure 4.1: Map of the study area showing each of the 17 study sites in red with site labels in black.

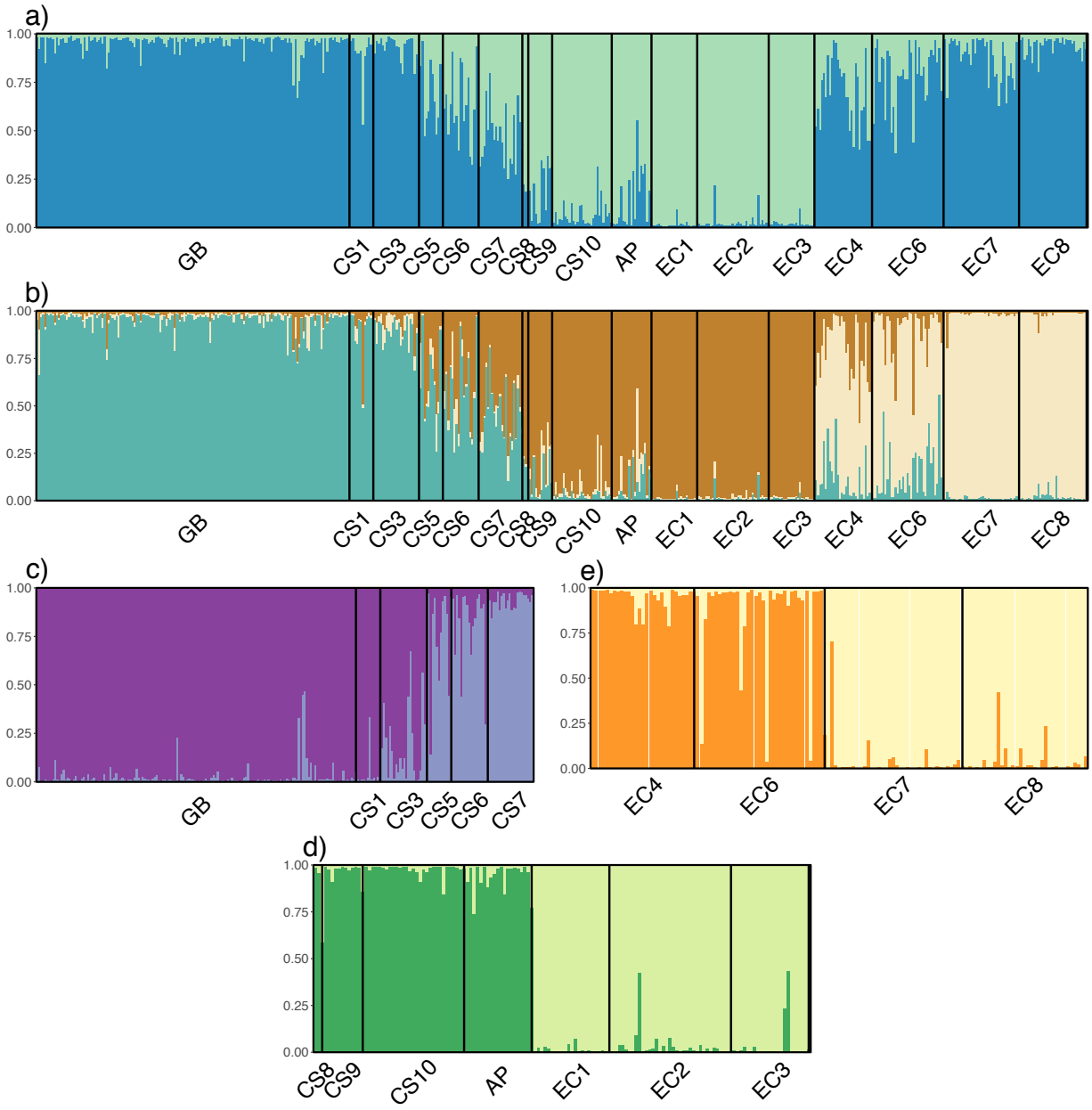

Figure 4.2: Structure plots showing the full set of samples and samples grouped by cluster. The full set is shown without populations set as prior locations when k=2 (a) and with populations set as prior locations when k=3 (b). Samples are grouped by the clusters shown in B and resulted in k=2 with and without populations set as prior locations: Western Gulf (c), Eastern Gulf (d), and Northern Atlantic (e).

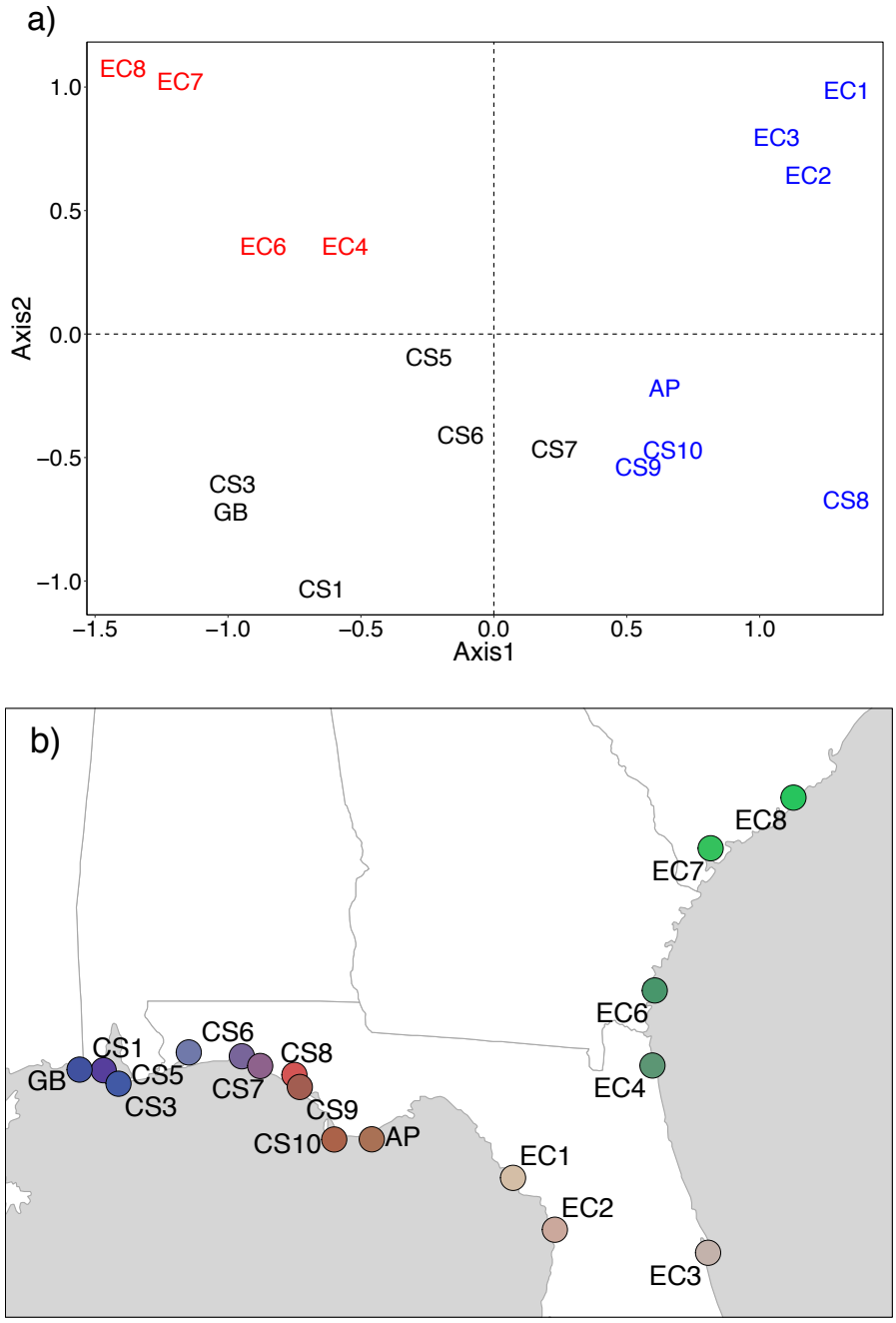

Figure 4.3: Results from a principal component analysis (PCA) on the full set of samples. Sites are plotted based on the first and second axis of the PCA (a). Text color corresponds to the cluster each site was assigned in the structure analysis, with black representing the Western Gulf Coast, blue representing the Eastern Gulf Coast, and red representing the Southern Atlantic Bight. An RGB plot displays results from the first three axes (b). Each site is assigned a color that is a combination of a red color value from the first axis, a green color value from the second axis, and a blue color value from the third axis so that sites more similar in color have more similar allele frequencies.

CHAPTER 5

LANDSCAPE GENETICS OF THE FOUNDATIONAL SALT MARSH PLANT SPECIES BLACK NEEDLERUSH (*JUNCUS ROEMERIANUS* SCHEELE) ACROSS THE NORTHEASTERN GULF OF MEXICO

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[1]Tumas H.T., B.M. Shamblin, M.S. Woodrey, N.P. Nibbelink, R.B. Chandler, C.J. Nairn. Submitted to Landscape Ecology.

ABSTRACT

Common species important for ecosystem restoration stand to lose as much genetic diversity from anthropogenic habitat fragmentation and climate change as rare species, but are rarely studied. Salt marshes, valuable ecosystems in widespread decline due to human development, are dominated by the foundational plant species black needlerush (*Juncus roemerianus* Scheele) in the northeastern Gulf of Mexico. We used nineteen microsatellite markers across 576 samples to measure genetic and genotypic diversity, and characterize population structure of *J. roemerianus* across the study area from Grand Bay National Estuarine Research Reserve (NERR) to Apalachicola NERR. Genetic distances (F_{ST} and D_{ch}) were used in a least cost transect analysis (LCTA) to delineate possible dispersal corridors and identify landscape factors influencing population connectivity. Genetic and genotypic diversity results were higher than expected based on life history literature, and samples structured into two large, admixed genetic clusters across the study area, indicating sexual reproduction may not be as rare as predicted in this clonal macrophyte. Digitized coastal transects buffered by 500m may represent possible dispersal corridors, and developed land may significantly impede gene flow in *J. roemerianus*. Our study expands into an understudied taxon and ecosystem, and uses methodology that could be applied to other common, widespread and understudied species. Results have important implications for coastal restoration and management that seek to preserve adaptive potential by sustaining natural levels of genetic diversity and conserving population connectivity.

INTRODUCTION

The majority of landscape genetic studies to date have focused on rare, threatened, or endangered species that either naturally exist in small, fragmented populations or have done so

for an evolutionarily significant period of time (Storfer et al. 2010). The few genetic studies performed on widespread plant species have found common species experience similar or greater losses in genetic diversity as rare species when populations are dramatically reduced in size and connectivity (Aguilar et al. 2008; Honnay and Jacquemyn 2006). Furthermore, genetic diversity in common, dominant plant species can have important and cascading effects on species diversity and processes throughout the ecosystem (Vellend and Geber 2005; Whitham et al. 2003). Specifically, in monotypic landscapes, genetic diversity of the foundational plant species is analogous to the role of species diversity in maintaining ecological health and ecosystem processes (Hughes et al. 2008; Reusch and Hughes 2006). Such monotypic landscapes are typical in coastal ecosystems that tend to be dominated by single-species macrophyte communities, such as eelgrass (*Zostera marina* L.) in seagrass beds (Reusch and Hughes 2006). Genotypic diversity (number of unique genotypes) in *Z. marina* has been positively correlated to shoot density, which can have a cascading positive effect on faunal abundance and other ecosystem benefits (Ehlers et al. 2008; Hughes and Stachowicz 2004, 2009; Reusch et al. 2005). Similarly, *Z. marina* genetic diversity (heterozygosity of individuals) was positively correlated to shoot density, nutrient retention, faunal abundance, areal productivity (Reynolds et al. 2012), and sexual and vegetative reproduction (Hammerli and Reusch 2003; Williams 2001). Positive effects of genetic diversity are especially important following disturbances, such as transplantation stress during restoration (Reynolds et al. 2012) or a warming event (Ehlers et al. 2008; Reusch et al. 2005).

Foundational plant species in salt marshes are used in ecological restoration efforts to restore coastal ecosystems that are valuable to humans and wildlife, and have been in widespread decline for decades from urban development (Gedan et al. 2009; Kennish 2001). Salt marshes

provide habitat to endemic and economically important species, and supply a range of ecosystem services valued at $10,000 per hectare that include storm protection, flood attenuation, and carbon sequestration (Kennish 2001; Kirwan and Megonigal 2013; Zedler and Kercher 2005). The Gulf of Mexico coastline contains 58% of the remaining salt marsh in the United States, a total of 5,480 square miles of marsh across five states (Chabreck 1988). Gulf coast salt marshes ameliorate eutrophication and have a marked effect on water quality, limiting the hypoxic effects of nutrient rich runoff from the Mississippi River (Zedler and Kercher 2005). The irregularly flooded marshes along the coasts of Mississippi, Alabama, and western Florida are dominated by the mid-marsh species black needlerush (*Juncus roemerianus* Scheele) (Eleuterius 1976; Stout 1984), a target species for restoration in the area (Sparks et al. 2013).

J. roemerianus is a clonal, gynodioecious macrophyte and a foundational species in salt marshes distributed from the western Gulf of Mexico in eastern Texas to the mid-Atlantic in Maryland (Eleuterius 1976; Godfrey and Wooten 1979; Stout 1984). The species plays a crucial role in the salt marsh, accreting and stabilizing sediment to create and maintain marsh habitat for other species (Pennings and Bertness 2001). Although *J. roemerianus* can reproduce both clonally and sexually (Eleuterius 1974, 1984), existing life history literature suggests sexual reproduction is used only in colonization of new areas, and that seedling-mediated gene flow is rare. As a result, established populations of *J. roemerianus* are assumed to be comprised of only a few unique genotypes (Eleuterius 1975; Stout 1984); however to our knowledge no genetic studies have been conducted to confirm these assumptions.

Gene flow among populations of *J. roemerianus* is achieved asexually through division and transport of vegetative ramets during storm events (USDA, NRCS 2017), and sexually through seed and pollen dispersal, of which little is known. Successful gene flow in wetland

plants is dependent on both seed transport, generally via wind, water, or animals, usually birds, and establishment in habitat suitable for germination (Cronk and Fennessy 2001). The small size (0.6mm) (USDA, NRCS 2017) of *J. roemerianus* seeds may allow for wind dispersal, (Cronk and Fennessy 2001; Neff and Baldwin 2005) or dispersal on the bodies, or in the excrement of birds (Cronk and Fennessy 2001; Soons et al. 2008, respectively). *J. roemerianus* seeds can germinate when floating or submerged in water, and are highly viable up to one year (Eleuterius 1975), potentially allowing for long distance oceanic dispersal. While propagule dispersal mechanisms are unknown in *J. roemerianus,* the morphologically similar seeds of the related species, common rush (*Juncus effusus* L.) are dispersed by all three vectors (Neff and Baldwin 2005; Soons et al. 2008; USDA, NRCS 2017). Across the coast, wetland habitat would facilitate seed mediated gene flow by permitting passage of wind and birds and providing suitable habitat for germination of seedlings. Areas of open ocean could facilitate potential long distance dispersal of seeds among salt marshes via tidal currents (Neff and Baldwin 2005). Conversely, developed land and forest cover would limit suitable habitat for germination, and may act as a barrier to wind or bird mediated dispersal (Delaney 2014). Pine plantations along the Gulf coast could mean both developed land and forest cover are anthropogenic barriers to gene flow. In fragmented populations such as *J. roemerianus*, the introduction of new genetic variants by gene flow to maintain genetic and genotypic diversity is dependent on population connectivity (Hedrick 1996; Slatkin 1987).

Landscape genetic techniques can be used to examine the influence of landscape factors on population connectivity by associating measures of gene flow to spatial data with the potential to guide management to maintain or enhance genetic diversity in fragmented populations (Hall and Beissenger 2014; Keller et al. 2015; Manel et al. 2003; Manel and Holderegger 2013; Storfer

et al. 2007). Linear regression models have emerged as an effective way to link genetic and spatial data in landscape genetics (Wagner and Fortin 2013). Gene flow among local populations (demes) or sample sites, measured indirectly using genetic distance, acts as the response variable, and landscape structure, quantified using either landscape resistance surfaces or transects, acts as the explanatory variable (Hall and Beissenger 2014; van Strien et al. 2012). Resistance surfaces are a grid representation of the landscape in which each grid cell is assigned a value symbolic of the predicted permeability of the environment within the cell (Spear et al. 2010; Zeller, McGarigal and Whiteley 2012). One or more least cost paths (LCPs) among demes or sample sites are used to predict organism dispersal through the resistance surface, with either the total cost or length of the LCP used as spatial data in landscape genetic analyses (Hall and Beissenger 2014; Spear et al. 2010; van Strien et al. 2012). Transect analyses quantify landscape structure by measuring landscape composition along a straight line between demes or sites, usually by calculating the abundance of landscape features of interest (Hall and Beissenger, 2014; van Strien et al. 2012). A least-cost transect analysis (LCTA) as described by van Strien et al (2012) combines the two methods to generate LCPs along which landscape composition is quantified, so that the length of the LCP and abundance of one or more landscape features along the LCP are used as explanatory variables in a set of candidate linear models. Model selection is used to determine both potential dispersal corridors and identify landscape features that inhibit or facilitate gene flow (van Strien et al. 2012). The method could be particularly suited for understudied species lacking dispersal information, or species that are not terrestrially dispersed, such as *J. roemerianus*.

We examined patterns of genetic diversity and population connectivity of *J. roemerianus* across irregularly flooded salt marshes in the northeastern Gulf of Mexico using LCTA and

model selection to address knowledge gaps and inform coastal management. We tested the hypotheses that (i) populations of *J. roemerianus* have low genetic diversity and are dominated by few clonal variants, (ii) rare sexual reproduction leads to high population differentiation, and (iii) wetland, open ocean, developed land, and forest cover will influence population connectivity. Contrary to life history literature, we predicted *J. roemerianus* would have greater genotypic and genetic diversity than expected of a predominantly clonal species, and structure into large genetic populations, indicating sexual reproduction plays a greater role in species' life history. Wetland and open ocean were hypothesized to positively influence population connectivity and facilitate gene flow, while developed land and forest cover were predicted to impede gene flow in *J. roemerianus*. Results will provide information on genetic diversity and population connectivity that land managers could use to maintain resilience and evolutionary potential in this important salt marsh plant species.

METHODS

Field Collection

Twelve sites were selected for sample collection across the range in which *Juncus roemerianus* is dominant in the northeastern Gulf of Mexico. A single leaf of *J. roemerianus* was collected and deposited in a plastic bag, and a GPS waypoint was taken using a Garmin GPSMAP 64st at each sample point. Sites at the eastern (Moss Point, MS) and western (Apalachicola, FL) extent of the study area are National Estuarine Research Reserves (NERR). NERRS are established through the Coastal Zone Management Act and maintained by the National Oceanic and Atmospheric Association (NOAA) to study and protect estuarine systems. A total of 304 samples were collected from the Grand Bay NERR, MS in January and March 2015, and 32 samples were collected from the Apalachicola NERR, FL in May 2015 and March

2016 as part of another study. Thirty samples were collected from an additional ten sites between the two NERR sites in March 2016 (Table 1; Fig1). Sites were selected based on the "Tidal Marsh" category of NOAA's Coastal Change Analysis Program land cover atlas, using satellite imagery in Google Earth to verify *J. roemerianus* presence at each point. A uniform distance between sites was not possible due to the irregular distribution of tidal marsh along the coastline, causing distance among sites to vary. Distance among sites ranged from 22.5 – 353 km with an average distance of 158 km.

Microsatellite Amplification

Samples were genotyped using a panel of 19 polymorphic microsatellite markers, previously developed for *J. roemerianus* population studies (Tumas et al. 2017). DNA extractions, PCR reaction conditions, and thermal cycling parameters were as described in Tumas et al (2017). Each sample was genotyped across a minimum of fifteen loci, or were re-genotyped. If more than four loci failed after re-genotyping for a sample, DNA was re-extracted and genotyped again. Eighteen percent of the samples were randomly selected and re-genotyped to test for genotyping error rate, calculated by dividing the number of mismatch genotypes by the total number of genotypes scored.

Genetic Analyses

Samples were assigned to clonal genets by grouping samples with identical genotypes using ID Analysis in CERVUS (Kalinowski et al. 2007). Genotypic diversity (G_D) was calculated for each site as $(G - 1) / (N - 1)$, where G is the number of unique genotypes or clonal genets, and N is the total number of samples (Arnaud-Haond et al. 2007). A single sample was randomly selected from each clonal genet, and all other genetically identical samples were removed for all subsequent genetic analyses. Hardy-Weinberg equilibrium was tested at each

locus for each study site using CERVUS, and GENEPOP (Raymond and Rousset 1995) was used to calculate linkage disequilibrium within sites. Observed heterozygosity (H_O), expected heterozygosity (H_E), and allelic diversity (A_D) were measured for each study site using Arlequin (Excoffier and Lischer 2010). Allelic richness (A_R) standardized to the smallest sample size through rarefaction was calculated using the *divBasic* function in the 'diveRsity' package in R (Keenan et al. 2013; R Core Team 2016). Pairwise genetic distance was measured as Cavalli-Sforza chord distance (D_{ch}) (Cavalli-Sforza and Edwards 1967) using Microsatellite Analyzer (Dieringer and Schlotterer 2002), and as Slatkin's linearized F_{ST} calculated in Arlequin. D_{ch} and F_{ST} were recalculated three times with three different random subsets of 30 individuals from Grand Bay NERR to account for the effect of sample size. Pearson's correlation calculated in R was used to test for correlation between multiple estimates of genetic distance, including D_{ch}, F_{ST}, Reynold's F_{ST}, Nei's genetic distance, and proportion of shared alleles that were calculated using 'hierfstat' in R, Arlequin, and Microsatellite Analyzer (Goudet and Jombart 2015). A Mantel test on D_{ch} and F_{ST} was conducted using the *mantel.randtest* function from the 'ade4' package in R to test for isolation by distance (Chessel et al. 2004; Dray and Dufour 2007; Dray et al. 2007). Population structuring was delineated using the program STRUCTURE, running 20 times for 50,000 steps after a burn-in period of 5,000 steps for each of K=1-6 (Pritchard et al. 2000). Eight sets of STRUCTURE runs were conducted with these parameters to test the effect of using admixture or no admixture models, setting populations with or without prior locations, and correlated or uncorrelated allele frequencies. Hierarchical structure within clusters was identified by performing STRUCTURE analyses on delineated genetic clusters using the above parameters. Population structure results were recalculated with a random subset of 30 individuals from the larger pool of samples collected at the Grand Bay NERR study site. The best number of

genetic clusters (K) was chosen using the Evanno method in STRUCTURE HARVESTER (Earl and vonHoldt 2012). Population differentiation was also measured using a global F_{ST} derived from an AMOVA in Arlequin (Weir and Cockerham 1984).

Spatial Data

A site layer composed of the centroid of all sample points at a collection site was calculated by placing a minimum convex polygon around the GPS waypoint locations of all sampling points within a collection site, and converting polygons to points in ArcGIS (v10.4.1, ESRI). Land cover data was derived from the NOAA's Coastal Change Analysis Program (C-CAP) land cover atlas for Mississippi, Alabama, and Florida (Coastal Change Analysis Program (C-CAP) 2015/2016 Regional Land Cover Data – Contiguous United States, Department of Commerce, NOAA, National Ocean Service, Office for Coastal Management). Spatial layers for the three states were combined and converted to Universal Transverse Mercator (UTM) in datum D_North_American_1983 in ArcGIS. C-CAP land cover categories were grouped to create the four land cover variables (wetland, open ocean, developed land, and forest cover) hypothesized to effect *J. roemerianus* gene flow. Wetland was defined as categories of estuarine or palustrine emergent or scrub/shrub wetland. Open ocean was defined as water and background categories. Forest cover included deciduous, evergreen, and mixed forest; and developed land included developed low (21-49% constructed materials), medium (50-79% constructed materials), and high (80-100% constructed materials) intensity, and developed open space (<20% constructed materials) (Table S1).

Transect Generation

Four sets of transects were created for LCTA, and two additional sets of transects were generated to reflect potential *J. roemerianus* dispersal pathways (Fig2). Basic, straight line

(Euclidean) transects were created in ArcGIS by converting all pairs of points to lines to reflect an isolation by distance pattern with no landscape influence. Digitized coastal transects represent potential routes for wind, waterfowl, or water mediated dispersal, and were created by digitizing the coastline in the C-CAP land cover layers at approximately 60,000m resolution (view scale while digitizing). Transects for LCTA (LCTA: Wetland, LCTA: Ocean, LCTA: Developed, and LCTA: Forest) were created using the Cost Path package in ArcGIS. Cost layers were created for each landscape factor by assigning the category of interest a value of one, and all other categories a value of 100. To minimize computational demand, wetland, developed land, and forest cover layers were clipped to a 60km buffer around the site layer, and open ocean was clipped to a 20km buffer around the site layer. Four binary raster layers were created, one for each land cover category of interest (wetland, open ocean, developed land, and forest cover), by reclassifying the land cover dataset into 1/0 raster datasets where 1 = category of interest and 0 = everything else. The 'raster' and 'rgdal' packages in program R were used to buffer all transects by 500m, 1km, and 2km, and extract the proportion of each land cover type across binary rasters using the mean statistic in the *extract* function (Bivand et al. 2017; Hijmans 2016).

Statistical Analysis

An information theoretic approach to model selection (Burnham and Anderson 2002) was applied to linear mixed effects models within a hierarchical modeling framework to examine the relationship between landscape variables and genetic distance. Due to the lack of independence in pairwise distance matrices, maximum likelihood population effects (MLPE) models were used (Clarke et al. 2002; van Strien et al. 2012). Fixed effects included the landscape variables, transect length, and the Euclidean distances between points. Response variables included pairwise genetic distances, measured as both D_{ch} and F_{ST}. A random effect term was applied to

each population pair to account for the dependency between pairwise distances as a result of two distances with a common node or site (van Strien et al. 2012). MLPE models were run in the R-package 'lme4' (Bates et al. 2015) using code developed by van Strien et al. (2012) and H. Wagner, and adapted by C. Goldberg .

Seven candidate models relating landscape and distance variables to genetic distance were ranked using Akaike's information criterion with second-order bias correction (AIC_c) and the Bayesian information criterion (BIC) (Burnham and Anderson 2002). Response variables and explanatory variables in these models were standardized around their mean to meet normality assumptions. Candidate models consisted of a full model including the four land cover variables and Euclidean distance, five land cover models, one Euclidean distance model, and a null model (Table 3). The five land cover models comprised a model with all four land cover variables, a model of naturally occurring land cover variables (wetland and open ocean), a model of potentially anthropogenic factors (developed land and forest cover), a model of wetland cover, and a model of developed land cover. Euclidean distance was a covariate in all models to account for the effect of isolation by distance on variation in genetic distances. A separate set of four candidate models and a null model were run using the transect length of each transect type as the only explanatory variable (Table 5). The two candidate model sets were run twice using the full sample set, once for each response variable (D_{ch} and F_{ST}). Models sets were rerun using pairwise genetic distances recalculated for three different subsets of 30 samples from the Grand Bay NERR.

Model selection across the seven landscape candidate models was conducted hierarchically across buffer widths and transect types for each land cover variable under the assumption that univariate relationships would not significantly change in a multivariate

framework. First, the best buffer width for each transect type was selected for each land cover variable by running single-variable models across buffer widths within transect types. Then, the best transect type for each variable was selected by running single-variable models across transect types, using the best buffer width from the prior analysis. Selection of best buffer width and transect type was made using BIC and AIC_c. Candidate models were then constructed using variables at the best buffer width and transect type. Signs of slope coefficients and significance in the highest ranked candidate models were used to make inferences about the influence of landscape factors on genetic distance. Variance inflation factors between explanatory variables calculated in the R-package 'usdm' were used to assess multicollinearity, with a maximum factor value of 7 allowed between variables (Naimi 2015).

RESULTS

Genetic Diversity and Structure

A total of 576 samples were genotyped across sites, and 310 samples represented unique genotypes that were used in all subsequent genetic diversity analyses. Samples from two of the twelve study sites (CS2 & CS4) were determined to be the closely related species *Juncus effusus* upon inspection in the lab, and were removed from all analyses. Genotyping error rate was 2.35%. Following a sequential Bonferroni correction, all except four loci (Jr3 & Jr86 in GB, Jr86 in CS5, and Jr33 in CS10) showed no evidence of deviation from Hardy-Weinberg equilibrium within all populations, and all except four pairs of loci (Jr13&Jr58, Jr12&Jr72 in GB; Jr29&Jr33, Jr01&Jr80 in CS10) did not exhibit linkage disequilibrium in any populations, so no loci were excluded from analysis. Genetic diversity indices and genotypic diversity were similar across the ten study sites (Table 1). Genotypic diversity averaged 0.54 across sites, and unique genotypes

comprised approximately half of the samples from each site, except for sites CS3, CS7, CS8, and CS10. Sites CS3, CS7, and CS10 had more unique genotypes than other sites, while CS8 was dominated by only three clonal genets. Genetic diversity was moderate across sites, with an average allelic richness of 2.42 alleles per locus, an average expected heterozygosity of 0.57, and an average observed heterozygosity of 0.58 (Table 1). The optimal number of genetic clusters across STRUCTURE runs was K=2, regardless of admixture, prior population location, or correlated allele frequency settings, and did not change when using a random subset of 30 samples from Grand Bay NERR. Sites clustered into a large western cluster, and a smaller eastern cluster with the division occurring between CS7 and CS8. Additional STRUCTURE analysis on the two clusters revealed a further sub-structuring in the larger western cluster into two genetic clusters, with a division between sites CS5 and CS6, and no further sub-structure in the eastern cluster (Fig3).

The global F_{ST} of 0.165 calculated across the ten sites using an AMOVA was significant ($p < 0.001$). Due to the low number of unique genotypes, CS8 was removed from pairwise genetic distance calculations, and all subsequent analyses on landscape influence. Across the remaining nine sites, all pairwise Slatkin's linearized F_{ST} values were significant (Table 2). Genetic distance metrics calculated across the nine sites, including D_{ch}, F_{ST}, Reynold's F_{ST}, Nei's genetic distance, and proportion of shared alleles, were highly correlated with a minimum Pearson's correlation of 0.82. Mantel tests indicated a significant pattern of isolation by distance for D_{ch} (r=0.79, p=0.003) and F_{ST} (r=0.91, p=0.001).

Landscape Variables Correlated to Genetic Distance

Proportion developed land and Euclidean distance comprised the top model for both response variables (D_{ch} and F_{ST}) (Table 3). However, the highest ranked transect type and buffer

width for developed land (Table 4), and direction of the relationship between developed land and genetic distance differed between response variables (Table 3). When using D_{ch}, proportion developed land was measured across digitized coastal transects buffered by 500m and had a significant positive relationship with D_{ch} in the top model. In the top model for F_{ST}, proportion developed land was measured across LCTA: Forest transects buffered by 2km, and had a significant negative relationship with F_{ST}. Although the direction of the relationship with proportion developed land differed between the response variables in the top model, the relationship was preserved in general based on the transect type used to measure proportion developed land. Proportion developed land had a significant negative relationship with D_{ch} in a model with proportion developed land measured across LCTA: Forest transects buffered by 2km and Euclidean distance as explanatory variables. Similarly, proportion developed land had a significant positive relationship with F_{ST} in a model with proportion developed land measured across digitized coastal transects buffered by 500m and Euclidean distance as explanatory variables. Euclidean distance had a significant positive relationship with both measures of genetic distance across models.

Proportion developed land was also an explanatory variable in the second and third ranked models when using both response variables. However, as in the top model, proportion developed land had a significant positive relationship with D_{ch}, and a significant negative relationship with F_{ST} in all models in which it was an explanatory variable. Proportion forest cover measured across LCTA: Forest transects buffered by 1km was a covariate with proportion developed land and Euclidean distance in the second ranked model, and had a significant positive relationship for both response variables. Proportion wetland measured across LCTA: Forest transects buffered by 500m was a covariate with proportion developed land and Euclidean

distance in the third ranked model for both response variables, and had an insignificant negative relationship with D_{ch} and F_{ST}. Proportion open ocean was in the fourth and sixth models when using D_{ch} and F_{ST}, respectively. When using D_{ch}, proportion open ocean had an insignificant positive relationship in all models in which it was an explanatory variable, while the direction of the relationship changed between models when using F_{ST}. The null model was ranked last for both measures of genetic distance (Table 3).

The top transect length model differed between the genetic distance measures, with digitized coastal transect length ranked highest for D_{ch}, and LCTA: Ocean ranked highest for F_{ST} (Table 5). All transect lengths had a significant positive relationship to genetic distance for both metrics in the set of models examining the top ranked transect length. Top landscape and transect length candidate models, and the relationship with developed land were conserved across three different subsets of thirty samples from Grand Bay NERR (Table S3).

DISCUSSION

Genetic Diversity and Structure

Measures of genetic diversity were moderate across sample sites, and unique genotypes comprised approximately half of the samples at each site on average. The only site with far fewer unique genotypes was CS8, which was likely due to the location and area of the site. Samples from CS8 were collected around a residential dock in a highly developed region in West Bay, FL, and comprised a much smaller area than other study sites. Results aligned with our hypotheses and the greater body of clonal plant literature, but contradicted current *J. roemerianus* life history literature. Despite a priori expectations of investigators, most clonal plant species have been found to have intermediate levels of genotypic diversity, and to rarely

produce geographically widespread clones (Ellstrand and Roose 1987; Silvertown 2008). Genetic analyses on other clonal plants have also contradicted assumptions of low genetic diversity and rare sexual reproduction (Gabrielsen and Brochmann 1998; Lloyd et al. 2011; Pluess and Stocklin 2004) including a study on the co-occurring salt marsh plant smooth cordgrass (*Spartina alterniflora* Loisel) (Richards et al. 2004). Only 6% of *S. alterniflora* samples in a study conducted on Sapelo Island, GA were clonal replicates, when populations of the species were previously assumed to be dominated by a small number of unique genotypes. Similarly, we found a greater number of unique genotypes at each study site than expected from current *J. roemerianus* life history literature, which assumes that the species is predominantly clonal with limited sexual reproduction (Eleuterius 1975). Only one clone, represented by samples identical at 18 markers, was shared between two sites (CS3 and CS5) that are approximately 89 km apart. All other clonal variants in the study were restricted to a single study site, indicating that *J. roemerianus* is like other clonal plant species in that populations are composed of many unique genotypes that are not geographically widespread.

As hypothesized, *J. roemerianus* population structure occurred on a large scale with samples structured into two genetic clusters across the study range. We found no apparent explanation for the division between populations, as there was not an obvious landscape barrier at that location. The genetic division may instead reflect a cline of decreased genetic similarity between samples due to the observed pattern of isolation by distance. The observed genetic structure could be a result of a number of factors, including historical events, biological traits, or environmental or landscape factors not examined within the study. The large scale of genetic structure demonstrates *J. roemerianus* exists within two large, admixed populations, possibly indicating a high degree of intrapopulation dispersal. Long distance dispersal is believed to be

rare in plants, following a leptokurtic curve with seed density declining precipitously with distance (Willson and Traveset 2000). However, genetic studies on wind-dispersed trees found higher pollen dispersal distances than expected based on the leptokurtic curve, suggesting gene flow was high enough to create effectively panmictic populations over large scales in which even isolated fragments may be connected (Ashley 2010). Oceanic (Huiskes et al. 1995) and bird mediated (Soons et al. 2008) dispersal could also allow for more frequent long distance dispersal. If more frequent long distance dispersal is causing observed structure, a higher rate of sexual reproduction would also be necessary for gene flow between habitat fragments. Conversely, the genetic structure could also reflect historic genetic patterns, possibly from a time when *J. roemerianus* habitat was more continuously distributed across the Gulf coast.

More localized hierarchical genetic structure in *J. roemerianus* was demonstrated in the sub-structuring of the large western cluster and the significant pairwise F_{ST} values among study sites. Micro differentiation between local populations is expected in plants species, resulting from local selection pressures and geographically restricted gene flow (Willson and Traveset 2000). Whereas *J. roemerianus* forms genetic populations that meet Hardy-Weinberg Equilibrium assumptions on a large scale, one or more evolutionary mechanisms could be creating fine scale genetic differentiation among sites. Local adaptation has been found to occur on small scales of 500m or less in other plant species (Mjinsbrugge et al. 2010), and could be a force driving genetic differences between sample sites. Diversifying selection is believed to generate genetic variation within clonal plants (Ellstrand and Roose 1987), and could be occurring across sites due to variations in salinity, flooding frequency, and other environmental variables. Local adaptation at the scale of the study site could explain why clonal variants were generally not shared across study sties, a trait common amongst clonal plant species (Ellstrand

and Roose 1987). Adaptation on such a fine scale in *J. roemerianus* would necessitate the conservation of genetic diversity for continued microevolution, and have important implications for transplant selection during restoration to prevent outbreeding depression and intraspecific hybridization. However, our use of neutral markers limits any implications our results have for local adaptation. Alternatively, the reduction in size of many *J. roemerianus* populations due to fragmentation could mean that site differentiation is driven by genetic drift at some or all of the sample sites. The rate of genetic drift is inversely proportional to population size, and can result in neutral alleles becoming randomly fixed in small populations, decreasing genetic diversity (Gillespie 2004). Even low levels of gene flow, estimated at one migrant per generation, can prevent allele fixation from genetic drift (Slatkin,1987; Young et al. 1996), highlighting the importance of population connectivity for maintaining genetic variation. Regardless of the cause of currently observed differentiation, continued fragmentation across coastal ecosystems in the Gulf of Mexico will likely have consequences for the evolutionary mechanisms driving genetic patterns in *J. roemerianus*, especially if population connectivity is not preserved.

Landscape Factors and Population Connectivity

The Mantel test revealed significant isolation by distance in *J. roemerianus*, which is in line with the biology of the species and other plant studies (Fievet et al. 2007; Pollegioni et al. 2014; Trenel et al. 2008). Results from the model selection were somewhat congruent with our hypotheses in that proportion developed land played an important role in population connectivity. However, the relationship between proportion developed land and population connectivity in the top model differed when using D_{ch} and F_{ST} as the response variables due to the transect type used to measure developed land. Differences between the metrics may indicate our models are not robust, and that small changes in model structure results in changes in model

rank and direction of relationships. Although the direction of the relationship between developed land and genetic distance changed in the top model across the two metrics, the direction of the relationship was conserved across transect types and buffer widths for the two metrics. Across both metrics, developed land had a positive relationship with genetic distance when measured across digitized coastal transects buffered by 500m, and a negative relationship when measured across LCTA: Forest transects buffered by 2km. Difference in direction of the relationship across transects could then be reflecting true dispersal biology of the species. In forested transects, developed land may offer open space that increases potential dispersal distance for wind or bird mediated dispersal, thereby causing developed land to have a negative relationship with genetic distance. While in coastal transects developed land limits suitable habitat and potentially inhibits dispersal. Differences in landscape composition along each transect could also be artificially affecting the relationship, as developed land is proportionally greater in digitized coastal transects than LCTA: Forest transects. Either explanation would indicate effect of developed land on gene flow in *J. roemerianus* varies across the landscape, and could mean the direction of the relationship between gene flow and other land cover variables also varies. Other studies have demonstrated landscape genetic results can vary for a single species across different landscapes within the species' range, either due to biological reasons (Trumbo et al. 2013), or degree of variation of land cover variables in the study landscape (Bull et al. 2011). However, greater variation in a land cover variable usually caused models containing the variable to be ranked higher, whereas in our models LCTA: Forest transects with little developed land were the highest ranked model for developed land. Differences in top ranked models between the genetic distance metrics may then be due to the difference in the attributes and assumptions of the genetic distance metrics themselves rather than biological or landscape factors.

While the majority of landscape genetics studies examine only a single genetic distance metric, a study that examined multiple metrics also showed varying results depending on the metric selected (Goldberg and Waits 2010). The differing result is possibly due to the difference in underlying assumptions between the two distance metrics. F statistics are measured using ratios of genetic variance within and among populations, and are based upon the island model, which assumes an infinite number of populations with the same number of individuals that give and receive the same number of migrants without regard to geographic structure (Whitlock and McCauley 1999). Conversely, D_{ch} is a geometric distance that does not have assumptions about population size or geographic configuration, but assumes differences in allele frequencies arise from genetic drift (Cavalli-Sforza and Edwards 1967). The underlying assumptions associated with F_{ST} may make the metric less suited to multiple variable analyses than D_{ch}, and D_{ch} has been shown to be one of the best metrics for denoting relationships among samples (Takezaki and Nei 1996). So while F_{ST} is still the most widely used genetic distance metric in landscape genetics (Storfer et al. 2010) and can be important for comparison purposes across studies, we will make inferences about population connectivity in *J. roemerianus* based on results achieved when using D_{ch} as a response variable.

Based on results from the model selection using D_{ch} as the response variable, the most important factor influencing *J. roemerianus* population connectivity is proportion developed land within a 500m buffer across digitized coastal transects. The negative impact of developed land on *J. roemerianus* population connectivity could be caused by inhibited seed and pollen dispersal, increased fragmentation and interpopulation distance, decreased suitable habitat for germination, or a combination of the three factors. Similar results have been found demonstrating the negative influence of human development on dispersal and species persistence

in other wind-dispersed plant species, especially with increasing fragmentation in urban settings (Soons and Heil 2002; Soons et al. 2004; Williams et al. 2005). Similarly, cities can act as barriers to dispersal for urbanization-sensitive species of bird (Delaney 2014). Increasing fragmentation by human development would also increase distance among *J. roemerianus* populations, causing a decline in gene flow following an isolation by distance pattern. Human development that directly reduces salt marsh area also decreases suitable habitat for germination for *J. roemerianus*, increasing the possibility of seeds establishing in unsuitable areas and decreasing probability of successful gene flow. Plant species have even been found to lose dispersal related traits over time with increasing habitat fragmentation (Riba et al. 2009), due to the adaptive disadvantage of dispersing into unsuitable habitat (Travis et al. 2010). Coastal areas where salt marshes occur have a high degree of human development, with a 50% loss in the last decade due solely to human modification (Kennish 2001) and 40% of the world's human population currently residing on coasts (Gedan et al. 2009). Further habitat fragmentation by human development could have a significant negative impact on *J. roemerianus* population connectivity, and possibly cause the species to lose the selective advantage of long distance dispersal.

The scale at which developed land appears to be most significantly affecting *J. roemerianus* population connectivity is within a 500m buffer zone around digitized coastal transects. Developed land was proportionally high across buffer widths along coastal transects, indicating amount or variation in the land cover variables is likely not driving the high ranking of the 500m buffer. Digitized coastal transects were the highest ranked transect type, so this result could be reflective of true *J. roemerianus* dispersal biology. Coastal winds and tides, and waterfowl dispersal could all cause dispersal to be highly concentrated around the coastline,

limiting dispersal to a small area along the coast and defining a fine scale for managers to target for land management and restoration.

Implications for Restoration and Conservation

Our results indicate that natural populations of *J. roemerianus* have intermediate levels of genotypic and genetic diversity, which has important implications for salt marsh restoration technique and implementation. While there is no universal protocol for *J. roemerianus* restoration (Sparks et al. 2013), any projects that attempt to generate restored populations with natural levels of genetic diversity would be misled by current *J. roemerianus* life history literature. *J. roemerianus* is reported to primarily use clonal propagation to reproduce in established populations (Eleuterius 1975; Stout 1984), implying natural populations are comprised of only a few unique genotypes. Our results suggest that practitioners will need to plant a greater number of unique genotypes than this literature suggests, more on the order of 20 - 30 depending on the area of the restored site, to create restored populations with natural levels of genotypic diversity. Site-specific genetic diversity results can guide selection of naturally sourced transplant stock from areas of high genetic diversity. Restored populations that do not meet this criteria run the risk of founder effects such as inbreeding depression, low fitness, and low establishment rates (Hufford and Mazer 2003; Mijnsbrugge et al. 2010), and lose the positive effects of genetic diversity including resiliency (Ehlers et al. 2008; Hughes and Stachowicz 2004), ecosystem benefits (Reynolds et al. 2012), and evolutionary potential (Frankel and Soule 1981; Mills 2007). Understanding population genetic structure on the different scales explored in our study could aid in selection of genetically similar transplant stock to increase success of *J. roemerianus* restoration and reduce risks to native populations. Local genotypes tend to have a home-site advantage in restored populations, and transplant success has

been found to be inversely related to genetic and environmental distance between the source population and restoration site (Hufford and Mazer 2003; Montalvo and Ellstrand 2000). Planting non-local genotypes in restored populations could have far reaching effects on surrounding native populations through outbreeding depression, and genetic swamping of local genotypes (Hufford and Mazer 2003; Mijnsbrugge et al. 2010). In *J. roemerianus* restoration, practitioners may want to select stock from within the same genetic cluster or sub-cluster to improve restoration success, and prevent outbreeding depression and spread of non-local genotypes in restored areas.

Spatial results from our study can help guide management of coastal areas and *J. roemerianus* restoration to maintain and promote genetic diversity across the landscape. Our models suggest coastal areas within a 500m buffer should be the most targeted by managers to preserve *J. roemerianus* population connectivity by limiting further urban development and implementing marsh restoration efforts. Creating new areas of marsh between existing sites could decrease isolation of extant populations, and further improve population connectivity as indicated by the positive relationship between wetland and gene flow. The significant pattern of isolation by distance we found suggests that decreasing distances between fragments of *J. roemerianus* will improve gene flow and preserve dispersal associated traits (Riba et al. 2009; Travis et al. 2010). Using spatially explicit genetic data to inform *J. roemerianus* restoration and management could help shift the focus of such efforts from triage in the present to conserving persistence and evolutionary potential into the future.

Broader Impacts and Future Directions

The methods developed for this study are applicable to other understudied species important to conservation for understanding population connectivity across the ecosystem and

creating a landscape level management plan for the salt marsh. A number of current landscape genetic studies use resistance surfaces to examine and quantify the effect of landscape factors on movement and population connectivity. While resistance surfaces are useful in the field and provide more spatially explicit information than most other methods, a number of assumptions based on prior knowledge of the species must be made (Zeller et al. 2012), and the method is arguably best suited for mobile land dispersed species. As climate change and anthropogenic habitat alteration affect increasingly more non-model and understudied species, conservation efforts will need methodology that relies on less information than traditional resistance layers. Our use of multiple transect types and buffer widths greatly reduces the number of a priori assumptions needed for resistance surfaces, and requires less knowledge of species life history. Selecting from multiple transect types also allows for greater flexibility across dispersal strategies. LCTA has been successfully applied to tropical bats (Cleary et al. 2017) and common grasshoppers (Keller et al. 2013), which use flight for dispersal. This methodology is more suited to understudied plant and animal species important for conservation and restoration that generally lack necessary dispersal data for resistance surfaces, and use a wide variety of dispersal strategies. The model selection framework implemented in this study provides an improvement over partial mantel tests, which have been criticized for having a high type-1 error rate (Balkenhol et al. 2009). The hierarchical design we used also allows for a simplified and elegant model comparison within and among species. Examining transect types and buffer widths hierarchically reduced the number of candidate models from 177 to eight for our study, which could allow full candidate sets to be compared more easily across species for multispecies studies in the future.

While the field of landscape genetics is growing, plants studies remain relatively rare and few studies have examined salt marsh or estuarine ecosystems (Holderegger et al. 2010; Storfer et al. 2010). A 2010 survey of the landscape genetics literature found 14.5% of studies focused on plants, while only 6% occurred in salt water habitats and estuarine habitats were not listed as a category (Storfer et al. 2010). Many existing plant landscape genetic studies do not actively include landscape elements (Holderegger et al. 2010). The majority of current plant studies use mantel tests or partial mantel tests to correlate genetic measures to geographic distance measures and some measure of ecological distance measure or environmental statistic (Fievet et al. 2007; Hirao and Kudo 2004; Holderegger et al. 2010; Pollegioni et al. 2014; Rico et al. 2014; Trenel et al. 2008). Other studies have used hierarchical genetic structure, assignment tests, or overlays to draw conclusions about the influence of landscape features on gene flow and genetic patterns (Fievet et al. 2007; Kitamoto et al. 2005; Pollegioni et al. 2014). A multiple variable approach, such as that used in this study, has not been widely applied in plant studies, and would allow for greater complexity and fewer a priori assumptions than other approaches. The hierarchical model selection across multiple transect types and landscape variables presented here offers a relatively straightforward and flexible approach that could be applied to plant species with different dispersal strategies, and would allow for a first step beyond tests for isolation by distance in plant landscape genetic studies. This study also expands into an understudied system, both in the field of landscape genetics and conservation genetics. The methods developed here would be suited for continued study in salt marshes and other estuarine landscapes where many of the species lack dispersal data, and use a variety of dispersal strategies including wind, terrestrial, and oceanic. Overall, our study emphasizes the need to apply landscape genetic techniques to

common species that are often understudied, but are increasingly important for conservation and restoration.

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Table 5.1: Approximate geographic location and sample size of the 12 sample sites, and genotypic and genetic diversity results across the ten sample sites with *Juncus roemerianus* samples (Note: Samples from sites CS2 and CS4 were of the related species *Juncus effusus*.)

Site ID	Location	N	N_G	C	G_D	A_D	A_R	H_O	H_E
GB	Moss Point, MS	304	158	146	0.52	6.21	2.49	0.53	0.55
CS1	Heron Bayou, AL	30	12	18	0.38	3.32	2.45	0.6	0.55
CS2	Mobile, AL	30	NA	NA	NA	NA	NA	NA	NA
CS3	Gulf Shores, AL	30	23	7	0.76	4.26	2.62	0.53	0.58
CS4	Lillian, AL	30	NA	NA	NA	NA	NA	NA	NA
CS5	Avalon Beach, FL	30	12	18	0.38	3.78	2.56	0.6	0.61
CS6	Niceville, FL	30	18	12	0.59	3.53	2.29	0.44	0.55
CS7	Santa Rosa Beach, FL	30	22	8	0.72	4.16	2.65	0.64	0.6
CS8	West Bay, FL	30	3	27	0.07	2.67	1.79	0.81	0.68
CS9	Panama City Beach, FL	30	12	18	0.38	3.9	2.61	0.6	0.57
CS10	Cape San Blas, FL	30	30	0	1	3.94	2.27	0.52	0.51
AP	Apalachicola, FL	32	20	12	0.61	4.39	2.42	0.51	0.54
Average	NA	53	31	26.6	0.54	4.02	2.42	0.58	0.57

[a]N = Sample size, N_G = Number of unique genotypes, C = Number of clonal replicates, G_D = Genotypic Diversity, A_D = Allelic Diversity, A_R = Allelic Richness, H_O = Observed Heterozygosity, H_E = Expected Heterozygosity

Table 5.2: Pairwise genetic distance metrics for the nine sites, which excludes site CS08 due to low sample size. Slatkin's linearized F_{ST} is on the top of the diagonal, and Cavalli-Sforza chord distance (D_{ch}) is on the bottom. All pairwise measures of F_{ST} were significant.

	GB	CS1	CS3	CS5	CS6	CS7	CS9	CS10	AP
GB	*	0.0934	0.0385	0.1282	0.1756	0.2124	0.2747	0.3217	0.2996
CS1	0.1145	*	0.0719	0.1221	0.2090	0.1598	0.2301	0.3109	0.2739
CS3	0.0838	0.1331	*	0.0827	0.1486	0.1403	0.2307	0.3090	0.2626
CS5	0.2147	0.2695	0.1558	*	0.1049	0.1038	0.1874	0.2517	0.2136
CS6	0.1952	0.2746	0.1948	0.2122	*	0.0844	0.1579	0.2558	0.2414
CS7	0.2324	0.2799	0.1925	0.1999	0.1379	*	0.0771	0.1702	0.1578
CS9	0.3165	0.3496	0.3130	0.3469	0.2406	0.1992	*	0.0765	0.0789
CS10	0.3538	0.3658	0.3400	0.3701	0.3045	0.2454	0.1526	*	0.0488
AP	0.3074	0.3336	0.2869	0.3322	0.2770	0.2070	0.1430	0.0846	*

Table 5.3: Landscape variables and information theoretic results (AIC_C, BIC, and ΔAIC) for MLPE models using genetic distance metrics (D_{ch} and F_{ST}) as the response variable. Explanatory landscape variables included proportion developed land (dev), proportion forest cover (for), proportion open ocean (opo), and proportion wetland (wet) as measured across buffered transects, and Euclidean distance (euc). Models are ordered based on ranked AIC_C and BIC values from models using D_{ch} as the response variable. Top model values are in bold. Direction of the relationship between each explanatory variable and response variable are in the order listed.

	D_{ch}				F_{ST}			
Explanatory Variables	AIC_C	BIC	ΔAIC_C	Direction	AIC_C	BIC	ΔAIC_C	Direction
dev, euc	47.25	53.74	0	+*,+*	39.96	46.45	0	-*, +*
dev, for, euc	49.51	56.96	2.26	+*,+*,+*	41.85	49.30	1.89	-*, +*, +*
dev, wet, euc	49.57	57.02	2.32	+*,-,+*	42.47	49.92	2.51	-*, -, +*
dev, for, opo, wet, euc	54.99	63.96	7.73	+*,+*,+,-,+*	46.22	55.19	6.26	-*, +, -*, -, +*
euc	58.06	63.47	10.81	+*	42.76	48.16	2.80	+*
wet, euc	60.50	66.99	13.25	+,+*	45.26	51.74	5.30	+, +*
opo, wet, euc	63.11	70.56	15.85	+,+,+*	47.85	55.30	7.89	+, +, +*
Null	107.69	111.90	60.44	NA	107.69	111.90	67.74	NA

* denotes significant relationships

Table 5.4: Highest ranked transect type and buffer width for each landscape variable determined hierarchically that were used to measure each landscape variable in candidate models. Model ranks are based on AIC_C and BIC using D_{ch} and F_{ST} as the response variable.

	D_{ch}		F_{ST}	
Landscape Variable	Transect Type	Buffer	Transect Type	Buffer
dev	Digitized Coastal	500m	LCTA: Forest	2km
for	LCTA: Forest	1km	LCTA: Forest	1km
opo	LCTA: Forest	2km	LCTA: Ocean	2km
wet	LCTA: Forest	500m	LCTA: Forest	500m

Table 5.5: Lengths of transect types and information theoretic results (AIC_C, BIC, and ΔAIC) for MLPE models using genetic distance metrics (D_{ch} and F_{ST}) as response variables. Models using just lengths of transect types as the explanatory variable are ordered based on ranked AIC_C and BIC values from models using D_{ch} as the response variable. Top model values are in bold for each response variable. All lengths had a significant positive relationship with both response variables.

	D_{ch}			F_{ST}		
Transect Type	AIC_C	BIC	ΔAIC	AIC_C	BIC	ΔAIC
Digitized Coastal	49.47	54.88	0	41.63	47.04	4.44
LCTA: Ocean	50.23	55.64	0.76	37.19	42.59	0
LCTA: Wetland	54.25	59.65	4.77	40.56	45.96	3.37
Euclidean	58.06	63.47	8.59	42.76	48.16	5.57
LCTA: Developed	60.74	66.14	11.26	53.23	58.64	16.04
LCTA: Forest	62.26	67.66	12.79	47.94	53.34	10.75

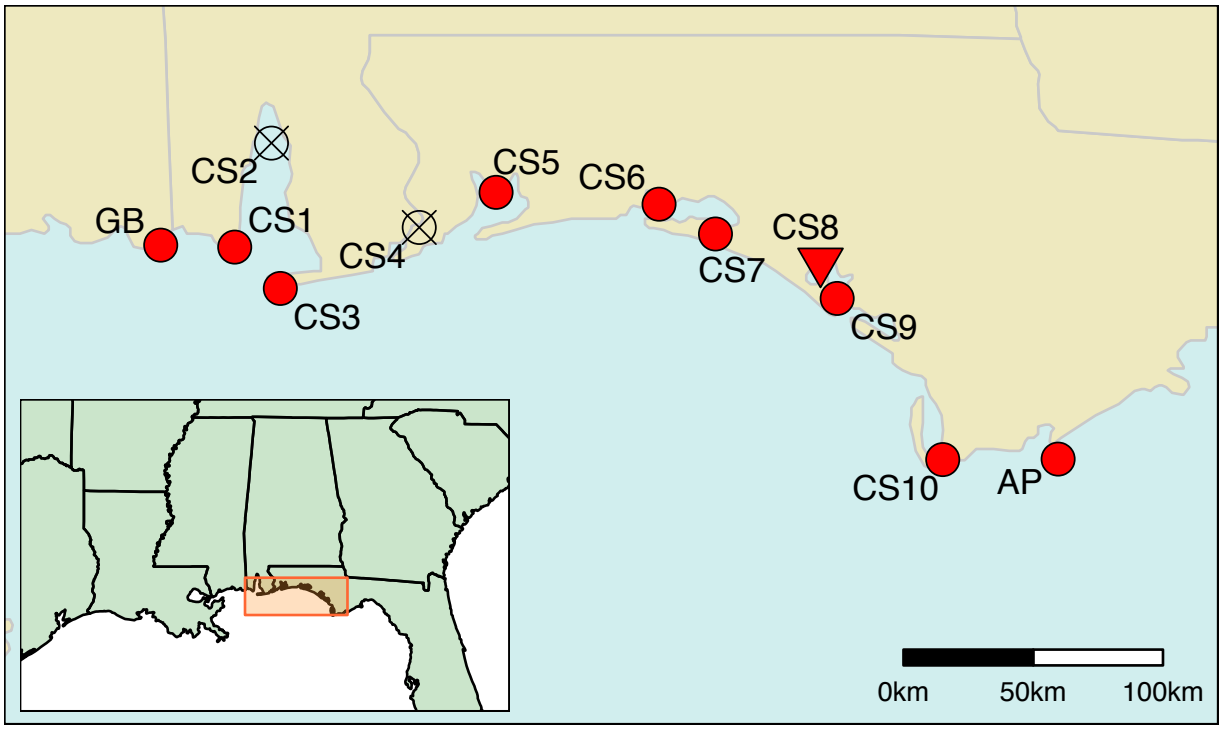

Figure 5.1: Ten *Juncus roemerianus* collection sites across the northeastern Gulf of Mexico from the Grand Bay NERR (GB) in eastern Mississippi to the Apalachicola NERR (AP) in northwest Florida. One site (CS8) was only used in genetic diversity analyses (triangle), nine sites were used in genetic diversity and landscape genetic analyses (circles), and two sites were later determined to be the morphologically similar species *Juncus effusus* (black circle with X).

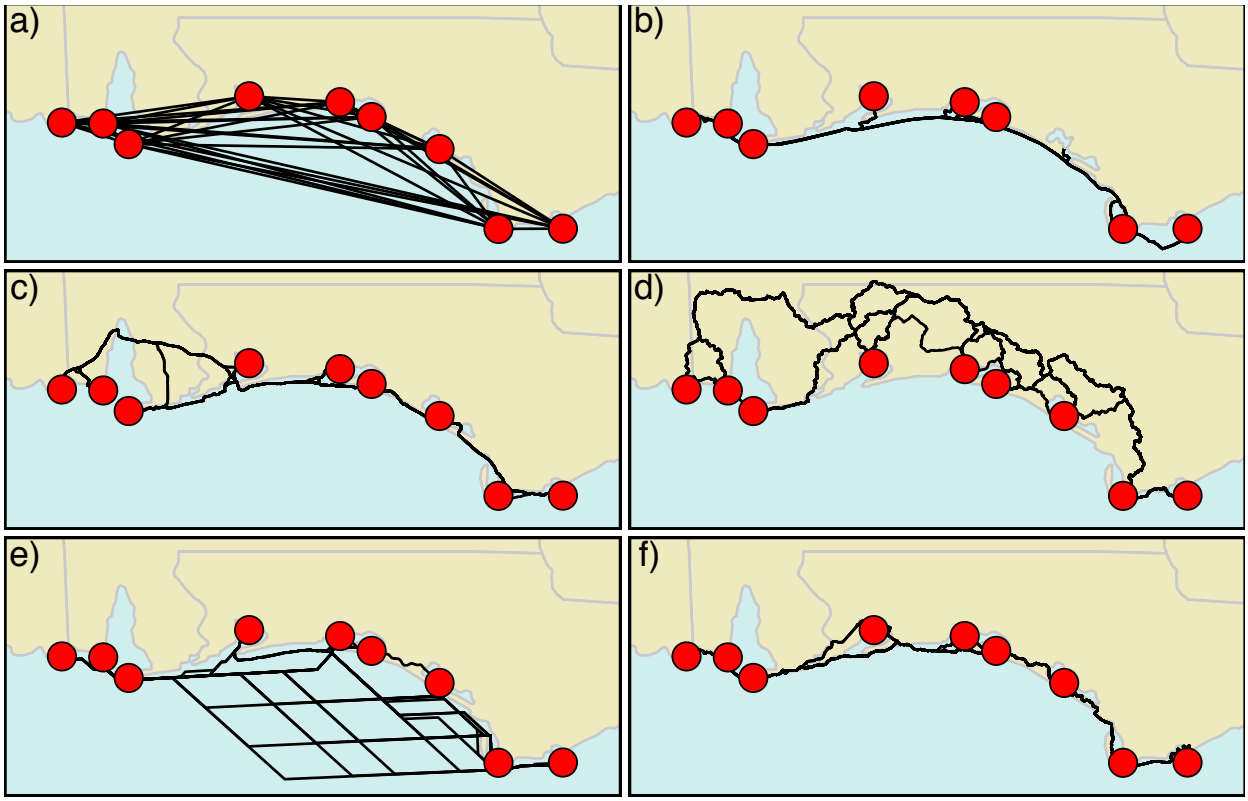

Figure 5.2: Six different transect types (lines) connecting the nine sites (circles) used to measure landscape variables and distances between sites in landscape genetic analyses: (a) Euclidean, (b) digitized coastal, (c) LCTA: Developed, (d) LCTA: Forest, (e) LCTA: Ocean, and (f) LCTA: Wetland connecting pairs of study sites (red circles).

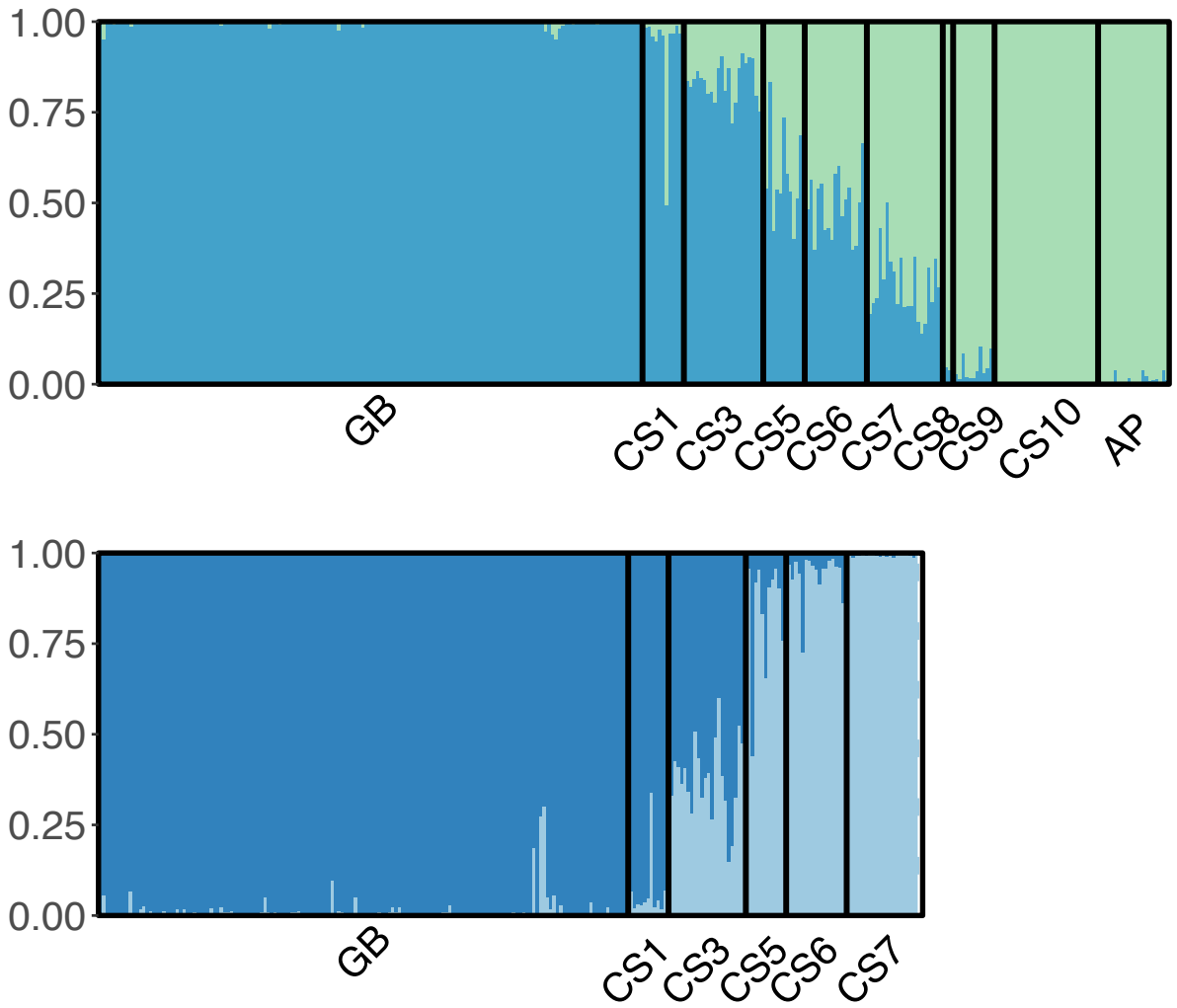

Figure 5.3: Structure plot for k=2 across the ten sites (top) and k =2 for the large western cluster (bottom). Probability of a sample being assigned to each cluster is on the y-axis, and samples grouped by site are on the x-axis. Clusters are delineated by color, and white dashed lines represent site boundaries.

CHAPTER 6

SYNTHESIS AND CONCLUSIONS

The objective of this dissertation was to perform population and landscape genetic analyses on black needlerush (*Juncus roemerianus*) across a majority of the species' range to better understand the ecology and evolution of the species, and to inform conservation, restoration and management that seeks to preserve adaptive potential and ecosystem function. Toward this goal, a panel of nineteen species-specific microsatellite markers were developed that can be used in fine-scale clonal analyses and population studies on *J. roemerianus*. The primers developed here are the only currently available microsatellite markers for the species and have been made publicly available, increasing the likelihood of their use in any subsequent population studies on *J. roemerianus*. This would allow for direct comparison across population studies that will hopefully span regions, environments, and successional stages, further contributing to knowledge on species' ecology and evolution.

The developed markers were used in three population studies that investigated natural levels of clonal and genetic diversity in a large population, examined patterns of diversity and structure across a majority of the species' range, and determined the influence of landscape factors on population connectivity. The prevailing theme across the three studies was higher than expected levels of clonal and genetic diversity for a species assumed to have rare sexual reproduction (Stout 1984). Over half of the samples collected across sites spanning a majority of the species' range represented unique genotypes, and the average observed heterozygosity across all samples was 0.53. However, levels of clonal and genetic diversity differed between the Gulf

coast and Atlantic coast samples, suggesting life history is influenced by environmental conditions. Genetic structure also indicated more frequent sexual reproduction and subsequent gene flow among populations. Samples grouped into three, large genetic clusters from Mississippi to South Carolina, although pairwise measures of F_{ST} were significant among all sample sites. The multiple scales of genetic structure suggest gene flow occurs among sites driving large scale genetic similarity on a regional scale, while local adaptation and drift creates fine-scale genetic differentiation on a site scale. A landscape genetic analysis revealed that *J. roemerianus* population connectivity, and therefore gene flow, occurs at a relatively fine-scale of approximately 500m around coastal corridors and is negatively influenced by coastal development. Results from each chapter advance current knowledge on *J. roemerianus* ecology and evolution, and could be used to inform coastal conservation, restoration, and management.

From the results on clonal and genetic diversity, it can be concluded that *J. roemerianus* has a greater rate of sexual reproduction than reported in current life history literature (Eleuterius 1975; Stout 1984). Current literature implies mature salt marshes are dominated by only a few unique genotypes of *J. roemerianus* with low genetic diversity due to rare sexual recruitment events (Eleuterius 1975). However, the diversity across sample sites measured by proportion of unique genotypes and allelic heterozygosity indicates sexual recruitment is more common and some level of gene flow occurs among populations. Differences in clonal and genetic diversity between regions of different disturbance regimes and dominating plant communities suggest that *J. roemerianus* life history is influenced by environmental factors, and may be affected by climate change and sea level rise. Multiple scale of genetic structure suggests that local adaptation and drift are working within sites to counter balance gene flow among populations. Gene flow in *J. roemerianus* appears to be restricted to coastal corridors, supporting the

hypothesis that seed dispersal is facilitated by oceanic currents, wind, and waterfowl (Cronk & Fennessy, 2001).

Results indicate that conservation efforts for *J. roemerianus* should focus on preserving existing levels of clonal and genetic diversity. Local adaptation, which depends on standing levels of genetic diversity (Frankel and Soule, 1981), most likely occurs in *J. roemerianus* and will be increasingly important for persistence under climate change and sea level rise. Natural levels of genotypic diversity are necessary for ecosystem processes and supporting species diversity in higher trophic levels (Ehlers et al., 2008; Hughes and Stachowicz, 2009; Hughes and Stachowicz, 2004; Reusch et al., 2005; Crustinger at al., 2006). Diversity can be preserved through adequately informed restoration and land management practices. First, restored population of *J. roemerianus* should include many different unique genotypes, more on the order of 10s to 100s depending on the area of the restored site than the few suggested by the literature. Genetic diversity in fragmented populations, such as the salt marsh, is maintained through gene flow (Hedrick, 1996; Slatkin, 1987), which is dependent on population connectivity. Restoration practices can increase gene flow by targeting coastal corridors between existing *J. roemerianus* populations to reduce geographic distance among populations. Managers can preserve existing gene flow by limiting urban development in coastal corridors, especially within 500m of the coast and between salt marsh fragments. Conservation practices that include clonal and genetic diversity shift the objective from triage in the present to long-term persistence through preservation of adaptive potential and ecosystem processes.

This dissertation demonstrates the efficacy of incorporating genetic techniques in designing conservation and restoration plans, and emphasizes the need to include more common, widespread species in current conservation genetic studies. Despite the importance of *J.*

roemerianus to salt marsh survival and function, the species has been understudied in the ecology and genetic literature. One of reasons for the current lack of knowledge on *J. roemerianus* is the tendency to focus on rare, threatened or endangered species that are in immediate danger. However, common plant species stand to lose as much genetic diversity due to habitat fragmentation as rare species (Aguilar et al., 2008; Honnay & Jacquemyn, 2006), and are critically important as foundational species in many ecosystems (Hughes et al., 2008; Reusch & Hughes, 2006). Three studies that used genetic and spatial techniques added a significant amount of knowledge about *J. roemerianus*, and disproved an existing life history assumption that could affect coastal conservation and restoration. The methodology presented in each of the four empirical chapters of this dissertation can be applied to other common and understudied species, potentially revealing results equally as important as those found for *J. roemerianus.* Genetic techniques should be used to study a greater range of species, spanning rare species in immediate danger to common species important to restoration that will still need adaptive potential to be resilient to climate change.

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APPENDIX A

VOUCHER SPECIMEN INFORMATION FOR CHPATER 2

Table A1. Voucher specimen information for the three populations of *Juncus roemerianus* and population of *Juncus effusus*[a].

Species	Collector Number	Location	Population	GPS Coordinates
J. roemerianus	Mark Woodrey 1	Moss Point, MS, USA	Grand Bay NERR	N30°21.761' W88°27.023'
J. roemerianus	Mark Woodrey 2	Moss Point, MS, USA	Grand Bay NERR	N30°22.229' W88°24.429'
J. roemerianus	Mark Woodrey 3	Moss Point, MS, USA	Grand Bay NERR	N30°23.712' W88°23.981'
J. roemerianus	Hayley Tumas 3	East Point, FL, USA	Apalachicola NERR	N29°40.303' W84°51.101'
J. roemerianus	Hayley Tumas 4	East Point, FL, USA	Apalachicola NERR	N29°40.300' W84°51.106'
J. roemerianus	Hayley Tumas 2	Santa Rosa Beach, FL, USA	Choctawhatchee Bay	N30°23.918' W86°13.771'
J. effusus	Hayley Tumas 1	Lillian, AL, USA	Perdido Bay	N30° 25.947' W87°24.843'

[a] Herbarium vouchers are deposited at the University of Georgia Herbarium.

APPENDIX B

HIERARCHICAL MODEL RESULTS FOR CHAPTER 5

Table B1: Reclassification of land cover classes from NOAA's C-CAP land cover atlas into four land cover variables used in the study.

Variable in study	C-CAP Classification
Wetland	Palustrine Scrub/Shrub Wetland Palustrine Emergent Wetland Estuarine Scrub/Shrub Wetland Estuarine Emergent Wetland
Open Ocean	Open Water Background
Forest cover	Deciduous Forest Evergreen Forest Mixed Forest
Developed land	Developed, High Intensity (80-100%) Developed, Medium Intensity (50-79%) Developed, Low Intensity (21-49%) Developed, Open Space (<20%)
Unclassified	Cultivate Crops Pasture/Hay Grassland/Herbaceous Scrub/Shrub Bare Land Palustrine Forested Wetland Estuarine Forested Wetland Unconsolidated Shore Palustrine Aquatic Bed Estuarine Aquatic Bed Unclassified

Table D2: Estimates and standard error for each covariate in all landscape candidate models using D_{ch} and F_{ST} as response variables for the landscape genetic analysis in the study. Models are ordered based on model rank for each response variable.

	Model	Variables	Estimate	Standard Error
D_{ch}	dev, euc	dev	0.3607	0.02059
		euc	0.7768	0.07196
	dev, for, euc	dev	0.3588	0.08871
		for	0.07703	0.01206
		euc	0.7116	0.1955
	dev, wet, euc	dev	0.3666	0.08936
		wet	-0.05618	0.09916
		euc	0.7244	0.1156
	dev, for, opo, wet, euc	dev	0.3619	0.0896
		for	0.07508	0.1656
		opo	0.02889	0.08788
		wet	-0.03487	0.1261
		euc	0.6997	0.1951
	euc	euc	0.9538	0.06839
	wet, euc	wet	0.03792	0.1186
		euc	0.9901	0.125
	opo, wet, euc	opo	0.01091	0.09402
		wet	0.03455	0.1272
		euc	0.9945	0.1264
F_{ST}	dev, euc	dev	-0.1634	0.06612
		euc	0.8869	0.06231
	dev, for, euc	dev	-0.1654	0.06830
		for	0.1067	0.09186
		euc	0.7932	0.09763
	dev, wet, euc	dev	-0.1684	0.06925
		wet	-0.04007	0.09020
		euc	0.8478	0.01032
	euc	euc	0.95	0.06155
	wet, euc	wet	0.001519	0.09273
		euc	0.9586	0.09622
	dev, for, opo, wet, euc	dev	-0.2131	0.07910
		for	0.07531	0.1319
		opo	-0.1058	0.08837
		wet	-0.04798	0.1333
		euc	0.8255	0.1060
	opo, wet, euc	opo	0.01420	0.08414
		wet	0.002916	0.09798
		euc	0.9436	0.0990

Table D3: Landscape model results when using three different subsets of 30 samples from GBNERR to account for sample size. Estimates and standard error for each covariate are reported for when D_{ch} and F_{ST} were used as response variables. Models are ordered based on model rank for each response variable, determined by AIC_C and BIC.

Subset	Metric	Model	AIC_C	Variables	Estimate	Standard Error
1	D_{ch}	dev, euc	42.17	dev	0.3183	0.08326
				euc	0.8260	0.06710
		dev, wet, euc	44.33	dev	0. 3236	0.08281
				wet	-0.06441	0.09205
				euc	0.7668	0.1068
		dev, for, euc	44.51	dev	0.3152	0.08262
				for	0.06475	0.1117
				euc	0.7718	0.1131
		dev, for, opo, wet, euc	48.39	dev	0.3232	0.08205
				for	0.08752	0.1511
				opo	0.1017	0.07946
				wet	-0.07076	0.1142
				euc	0.7520	0.1190
		euc	51.90	euc	0.9802	0.06346
		wet, euc	54.39	wet	-0.008748	0.0.1093
				euc	0.9718	0.1150
		opo, wet, euc	56.15	opo	0.08511	0.08539
				wet	-0.02056	0.1155
				euc	1.022	0.1148
	F_{ST}	dev, euc	44.29	dev	-0.20940	0.07049
				euc	0.83410	0.06651
		dev, for, euc	45.67	dev	-0.2141	0.07235
				for	0.08577	0.09973
				euc	0.75880	0.10540
		dev, wet, euc	46.48	dev	-0.20250	0.07042
				wet	-0.09456	0.08118
				euc	0.76970	0.08480
		euc	49.40	euc	0.91940	0.06756
		dev, for, opo, wet, euc	49.94	dev	-0.2461	0.07740
				for	0.05624	0.1205
				opo	-0.1056	0.08622
				wet	-0.05624	0.09165
				euc	0.7945	0.01103
		wet, euc	50.43	wet	-0.10830	0.08864
				euc	0.84870	0.08788
		opo, wet, euc	52.98	opo	0.02511	0.08687
				wet	-0.10980	0.08840
				euc	0.83260	0.10400
2	D_{ch}	dev, euc	47.58	dev	0.34410	0.08948

				euc	0.79120	0.07239
		dev, for, euc	49.97	dev	0.33990	0.08903
				for	0.06115	0.11990
				euc	0.74110	0.12110
		dev, wet, euc	50.03	dev	0.3468	0.08938
				wet	-0.04185	0.0995
				euc	0.7533	0.115
		dev, for, opo, wet, euc	55.49	dev	0.34190	0.08992
				for	0.06618	0.16680
				opo	0.02833	0.08924
				wet	-0.02353	0.12780
				euc	0.73340	0.12660
		euc	57.46	euc	0.95490	0.06947
		wet, euc	59.95	wet	0.00972	0.11810
				euc	0.96420	0.12420
		opo, wet, euc	62.57	opo	0.00670	0.09632
				wet	0.00812	0.12780
				euc	0.96740	0.12550
	F_{ST}	dev, euc	50.33	dev	-0.19640	0.07921
				euc	0.80780	0.07562
		dev, for, euc	52.19	dev	-0.21110	0.08094
				for	0.09877	0.09956
				euc	0.72590	0.10820
		dev, wet, euc	52.94	dev	-0.19920	0.08264
				wet	-0.01227	0.10210
				euc	0.79620	0.11760
		euc	53.48	euc	-0.19640	0.07921
		wet, euc	55.70	wet	0.06191	0.10640
				euc	0.94530	0.11020
		dev, for, opo, wet, euc	56.47	dev	-0.24540	0.09206
				for	0.12330	0.14550
				opo	-0.11390	0.10200
				wet	0.02361	0.15060
				euc	0.78220	0.11920
		opo, wet, euc	58.29	opo	0.01643	0.09761
				wet	0.06617	0.11090
				euc	0.93850	0.11500
3	D_{ch}	dev, euc	44.43	dev	0.33260	0.08584
				euc	0.80940	0.06925
		dev, for, euc	46.75	dev	0.32930	0.08519
				for	0.06832	0.11500
				euc	0.75250	0.11640
		dev, wet, euc	46.84	dev	0.33650	0.08574
				wet	-0.04549	0.09531
				euc	0.76750	0.11060

		dev, for, opo, wet, euc	51.84	dev for opo wet euc	0.33170 0.09380 0.06121 -0.03027 0.74230	0.08569 0.15850 0.08433 0.12090 0.12190
		euc	54.42	euc	0.97050	0.06577
		wet, euc	56.89	wet euc	0.02709 0.99650	0.11310 0.11900
		opo, wet, euc	59.30	opo wet euc	0.04273 0.01828 1.01800	0.08962 0.12090 0.12000
	F_{ST}	dev, euc	47.30	dev euc	-0.17300 0.86590	0.07397 0.06992
		dev, for, euc	48.67	dev for euc	-0.17640 0.14730 0.73850	0.07659 0.09521 0.10320
		dev, wet, euc	49.58	dev wet euc	-0.18200 -0.07179 0.79580	0.07836 0.09819 0.11280
		wet, euc	49.66	wet euc	-0.02329 0.91250	0.10020 0.10350
		euc	52.05	euc	0.93290	0.06825
		dev, for, opo, wet, euc	52.16	dev for opo wet euc	-0.24810 0.08744 -0.15760 -0.07322 0.79620	0.08627 0.14310 0.09614 0.14540 0.11440
		opo, wet, euc	54.63	opo wet euc	-0.01636 -0.02610 0.92070	0.09301 0.10440 0.10900

APPENDIX C

R CODE FOR MLPE MODELS IN CHAPTER 5

```
#Code for Maximum Likelihood Population Effects Models in Chapter 5
#code adapted from R code provided by Caren Goldberg in R lab for 2016 Landscape Genetic course (week 12 lab)
#code originally provided by Maarten van Strien
#modeling with standardized datasets

#Code shown using Cavalli-Sforza Chord Distance across 9 populations

#In the below code:
#Dch -> chord distance between 9 populations of Juncus roemerianus
#length -> transect length (in this case Euclidean transects between patches)
#wet -> proportion wetland between patches
#dev -> proportion developed between patches
#foe -> proportion forest between patches
#opw -> proportion ocean between patches
#all variables are standardized around the mean

#Using a hierarchical model framework
#find best scale for each variable for each transect type
#then find best transect type for each variable
#then run candidate models using each variable measured across best transect type at best scale

#load package
require(lme4)

#I Best Scale for each transect type for each variable
#Run across each transect type

#Example:  "Basic" Straight Line Euclidean Transects
#load in data
data500 <- read.csv("model_500basic.csv")
data2km <- read.csv("model_2kmbasic.csv")
data1km <- read.csv("model_1kmbasic.csv")

#Testing each variable at each scale
#Developed
#500m
```

```
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data500[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
modd500 <- lFormula(Dch ~ dev + (1|pop1), data = data500, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modd500)
opt <-optimizeLmer(dfun)
mod_d500<-mkMerMod(environment(dfun),opt,modd500$reTrms,fr=modd500$fr)
#In the fitted model replace Zt slot
modd500$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modd500)
opt <-optimizeLmer(dfun)
mod_d500z <- mkMerMod(environment(dfun),opt,modd500$reTrms,fr=modd500$fr)
summary(mod_d500z)
AICdev500 <- AIC(mod_d500z)
BICdev500 <- BIC(mod_d500z)
#1km
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data1km[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 1km
modd1km <- lFormula(Dch ~ dev + (1|pop1), data = data1km, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modd1km)
opt <-optimizeLmer(dfun)
mod_d1km<-mkMerMod(environment(dfun),opt,modd1km$reTrms,fr=modd1km$fr)
#In the fitted model replace Zt slot
modd1km$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modd1km)
opt <-optimizeLmer(dfun)
mod_d1kmz <- mkMerMod(environment(dfun),opt,modd1km$reTrms,fr=modd1km$fr)
summary(mod_d1kmz)
AICdev1km <- AIC(mod_d1kmz)
BICdev1km <- BIC(mod_d1kmz)
#2km
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data2km[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 2km
modd2km <- lFormula(Dch ~ dev + (1|pop1), data = data2km, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modd2km)
opt <-optimizeLmer(dfun)
```

```
mod_d2km<-mkMerMod(environment(dfun),opt,modd2km$reTrms,fr=modd2km$fr)
#In the fitted model replace Zt slot
modd2km$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modd2km)
opt <-optimizeLmer(dfun)
mod_d2kmz <- mkMerMod(environment(dfun),opt,modd2km$reTrms,fr=modd2km$fr)
summary(mod_d2kmz)
AICdev2km <- AIC(mod_d2kmz)
BICdev2km <- BIC(mod_d2kmz)
#making a dataframe of AIC and BIC values
buff.dist <- c("500","1km","2km")
variable <- c("dev","dev","dev")
AIC <- c(AICdev500, AICdev1km, AICdev2km)
BIC <- c(BICdev500, BICdev1km, BICdev2km)
model.selection <- data.frame(buff.dist, variable, AIC, BIC)
#further model diagnosics
CSF.IC <- model.selection
CSF.IC <- cbind(CSF.IC, k = c(attr(logLik(mod_d500z), "df"), attr(logLik(mod_d1kmz), "df"),
attr(logLik(mod_d2kmz), "df")))
AICc <- CSF.IC$AIC + 2*CSF.IC$k*(CSF.IC$k+1)/(48-CSF.IC$k-1)
CSF.IC <- cbind(CSF.IC, AICc = AICc)
#Calculate model weights for AICc
AICcmin <- min(CSF.IC$AICc)
RL <- exp(-0.5*(CSF.IC$AICc - AICcmin))
sumRL <- sum(RL)
AICew <- RL/sumRL
CSF.IC <- cbind(CSF.IC, AICew)
#Calculate model weights for BIC
BICmin <- min(CSF.IC$BIC)
RL.B <- exp(-0.5*(CSF.IC$BIC - BICmin))
sumRL.B <- sum(RL.B)
BICew <- RL.B/sumRL.B
CSF.IC <- cbind(CSF.IC, BICew)
write.csv(CSF.IC, file="~/Documents/Spatial Analyses/Model Transformations/New Chord
Distance/Hierarchical Models/Variable Results/model_basicdev.csv")
#Forest
#500m
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data500[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
modf500 <- lFormula(Dch ~ foe + (1|pop1), data = data500, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modf500)
opt <-optimizeLmer(dfun)
```

```
mod_f500<-mkMerMod(environment(dfun),opt,modf500$reTrms,fr=modf500$fr)
#In the fitted model replace Zt slot
modf500$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modf500)
opt <-optimizeLmer(dfun)
mod_f500z <- mkMerMod(environment(dfun),opt,modf500$reTrms,fr=modf500$fr)
AICfoe500 <- AIC(mod_f500z)
BICfoe500 <- BIC(mod_f500z)
#1km
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data1km[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 1km
modf1km <- lFormula(Dch ~ foe + (1|pop1), data = data1km, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modf1km)
opt <-optimizeLmer(dfun)
mod_f1km<-mkMerMod(environment(dfun),opt,modf1km$reTrms,fr=modf1km$fr)
#In the fitted model replace Zt slot
modf1km$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modf1km)
opt <-optimizeLmer(dfun)
mod_f1kmz <- mkMerMod(environment(dfun),opt,modf1km$reTrms,fr=modf1km$fr)
AICfoe1km <- AIC(mod_f1kmz)
BICfoe1km <- BIC(mod_f1kmz)
#2km
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data2km[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 2km
modf2km <- lFormula(Dch ~ foe + (1|pop1), data = data2km, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modf2km)
opt <-optimizeLmer(dfun)
mod_f2km<-mkMerMod(environment(dfun),opt,modf2km$reTrms,fr=modf2km$fr)
#In the fitted model replace Zt slot
modf2km$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modf2km)
opt <-optimizeLmer(dfun)
mod_f2kmz <- mkMerMod(environment(dfun),opt,modf2km$reTrms,fr=modf2km$fr)
AICfoe2km <- AIC(mod_f2kmz)
BICfoe2km <- BIC(mod_f2kmz)
#making a dataframe of AIC and BIC values
```

```
buff.dist <- c("500","1km","2km")
variable <- c("foe","foe","foe")
AIC <- c(AICfoe500, AICfoe1km, AICfoe2km)
BIC <- c(BICfoe500, BICfoe1km, BICfoe2km)
model.selection <- data.frame(buff.dist, variable, AIC, BIC)
#further model diagnosics
CSF.IC <- model.selection
CSF.IC <- cbind(CSF.IC, k = c(attr(logLik(mod_f500z), "df"), attr(logLik(mod_f1kmz), "df"),
attr(logLik(mod_f2kmz), "df")))
AICc <- CSF.IC$AIC + 2*CSF.IC$k*(CSF.IC$k+1)/(48-CSF.IC$k-1)
CSF.IC <- cbind(CSF.IC, AICc = AICc)
#Calculate model weights for AICc
AICcmin <- min(CSF.IC$AICc)
RL <- exp(-0.5*(CSF.IC$AICc - AICcmin))
sumRL <- sum(RL)
AICew <- RL/sumRL
CSF.IC <- cbind(CSF.IC, AICew)
#Calculate model weights for BIC
BICmin <- min(CSF.IC$BIC)
RL.B <- exp(-0.5*(CSF.IC$BIC - BICmin))
sumRL.B <- sum(RL.B)
BICew <- RL.B/sumRL.B
CSF.IC <- cbind(CSF.IC, BICew)
write.csv(CSF.IC, file="~/Documents/Spatial Analyses/Model Transformations/New Chord
Distance/Hierarchical Models/Variable Results/model_basicfoe.csv")
#Ocean
#500m
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data500[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
modo500 <- lFormula(Dch ~ opw + (1|pop1), data = data500, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modo500)
opt <-optimizeLmer(dfun)
mod_o500<-mkMerMod(environment(dfun),opt,modo500$reTrms,fr=modo500$fr)
#In the fitted model replace Zt slot
modo500$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modo500)
opt <-optimizeLmer(dfun)
mod_o500z <- mkMerMod(environment(dfun),opt,modo500$reTrms,fr=modo500$fr)
AICopw500 <- AIC(mod_o500z)
BICopw500 <- BIC(mod_o500z)
#1km
# Create the Zl and ZZ matrices
```

```
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data1km[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 1km
modo1km <- lFormula(Dch ~ opw + (1|pop1), data = data1km, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modo1km)
opt <-optimizeLmer(dfun)
mod_o1km<-mkMerMod(environment(dfun),opt,modo1km$reTrms,fr=modo1km$fr)
#In the fitted model replace Zt slot
modo1km$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modo1km)
opt <-optimizeLmer(dfun)
mod_o1kmz <- mkMerMod(environment(dfun),opt,modo1km$reTrms,fr=modo1km$fr)
AICopw1km <- AIC(mod_o1kmz)
BICopw1km <- BIC(mod_o1kmz)
#2km
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data2km[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 2km
modo2km <- lFormula(Dch ~ opw + (1|pop1), data = data2km, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modo2km)
opt <-optimizeLmer(dfun)
mod_o2km<-mkMerMod(environment(dfun),opt,modo2km$reTrms,fr=modo2km$fr)
#In the fitted model replace Zt slot
modo2km$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modo2km)
opt <-optimizeLmer(dfun)
mod_o2kmz <- mkMerMod(environment(dfun),opt,modo2km$reTrms,fr=modo2km$fr)
AICopw2km <- AIC(mod_o2kmz)
BICopw2km <- BIC(mod_o2kmz)
#making a dataframe of AIC and BIC values
buff.dist <- c("500","1km","2km")
variable <- c("opw","opw","opw")
AIC <- c(AICopw500, AICopw1km, AICopw2km)
BIC <- c(BICopw500, BICopw1km, BICopw2km)
model.selection <- data.frame(buff.dist, variable, AIC, BIC)
#further model diagnosics
CSF.IC <- model.selection
CSF.IC <- cbind(CSF.IC, k = c(attr(logLik(mod_o500z), "df"), attr(logLik(mod_o1kmz), "df"),
attr(logLik(mod_o2kmz), "df")))
AICc <- CSF.IC$AIC + 2*CSF.IC$k*(CSF.IC$k+1)/(48-CSF.IC$k-1)
CSF.IC <- cbind(CSF.IC, AICc = AICc)
```

```
#Calculate model weights for AICc
AICcmin <- min(CSF.IC$AICc)
RL <- exp(-0.5*(CSF.IC$AICc - AICcmin))
sumRL <- sum(RL)
AICew <- RL/sumRL
CSF.IC <- cbind(CSF.IC, AICew)
#Calculate model weights for BIC
BICmin <- min(CSF.IC$BIC)
RL.B <- exp(-0.5*(CSF.IC$BIC - BICmin))
sumRL.B <- sum(RL.B)
BICew <- RL.B/sumRL.B
CSF.IC <- cbind(CSF.IC, BICew)
write.csv(CSF.IC, file="~/Documents/Spatial Analyses/Model Transformations/New Chord
Distance/Hierarchical Models/Variable Results/model_basicopw.csv")
#Wetland
#500m
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data500[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
modw500 <- lFormula(Dch ~ wet + (1|pop1), data = data500, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modw500)
opt <-optimizeLmer(dfun)
mod_w500<-mkMerMod(environment(dfun),opt,modw500$reTrms,fr=modw500$fr)
#In the fitted model replace Zt slot
modw500$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modw500)
opt <-optimizeLmer(dfun)
mod_w500z <- mkMerMod(environment(dfun),opt,modw500$reTrms,fr=modw500$fr)
AICwet500 <- AIC(mod_w500z)
BICwet500 <- BIC(mod_w500z)
#1km
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data1km[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 1km
modw1km <- lFormula(Dch ~ wet + (1|pop1), data = data1km, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modw1km)
opt <-optimizeLmer(dfun)
mod_w1km<-mkMerMod(environment(dfun),opt,modw1km$reTrms,fr=modw1km$fr)
#In the fitted model replace Zt slot
modw1km$reTrms$Zt<-ZZ
#Refit the model
```

```
dfun <- do.call(mkLmerDevfun,modw1km)
opt <-optimizeLmer(dfun)
mod_w1kmz <- mkMerMod(environment(dfun),opt,modw1km$reTrms,fr=modw1km$fr)
AICwet1km <- AIC(mod_w1kmz)
BICwet1km <- BIC(mod_w1kmz)
#2km
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(data2km[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 2km
modw2km <- lFormula(Dch ~ wet + (1|pop1), data = data2km, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modw2km)
opt <-optimizeLmer(dfun)
mod_w2km<-mkMerMod(environment(dfun),opt,modw2km$reTrms,fr=modw2km$fr)
#In the fitted model replace Zt slot
modw2km$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modw2km)
opt <-optimizeLmer(dfun)
mod_w2kmz <- mkMerMod(environment(dfun),opt,modw2km$reTrms,fr=modw2km$fr)
AICwet2km <- AIC(mod_w2kmz)
BICwet2km <- BIC(mod_w2kmz)
#making a dataframe of AIC and BIC values
buff.dist <- c("500","1km","2km")
variable <- c("wet","wet","wet")
AIC <- c(AICwet500, AICwet1km, AICwet2km)
BIC <- c(BICwet500, BICwet1km, BICwet2km)
model.selection <- data.frame(buff.dist, variable, AIC, BIC)
#further model diagnosics
CSF.IC <- model.selection
CSF.IC <- cbind(CSF.IC, k = c(attr(logLik(mod_w500z), "df"), attr(logLik(mod_w1kmz), "df"),
attr(logLik(mod_w2kmz), "df")))
AICc <- CSF.IC$AIC + 2*CSF.IC$k*(CSF.IC$k+1)/(48-CSF.IC$k-1)
CSF.IC <- cbind(CSF.IC, AICc = AICc)
#Calculate model weights for AICc
AICcmin <- min(CSF.IC$AICc)
RL <- exp(-0.5*(CSF.IC$AICc - AICcmin))
sumRL <- sum(RL)
AICew <- RL/sumRL
CSF.IC <- cbind(CSF.IC, AICew)
#Calculate model weights for BIC
BICmin <- min(CSF.IC$BIC)
RL.B <- exp(-0.5*(CSF.IC$BIC - BICmin))
sumRL.B <- sum(RL.B)
BICew <- RL.B/sumRL.B
```

```
CSF.IC <- cbind(CSF.IC, BICew)
write.csv(CSF.IC, file="~/Documents/Spatial Analyses/Model Transformations/New Chord Distance/Hierarchical Models/Variable Results/model_basicwet.csv")

#substitute basic for digitized coastal and 4 LCTA transects

#Now create new files for each transect type in which each variable is measured across the top scale based on AIC and BIC from the above files
#For each variable, find the best transect using these new data files
#load data
topbasic <- read.csv("model_topbasic.csv", header=T)
topcoastal <- read.csv("model_topcoastal.csv", header=T)
topdev <- read.csv("model_topdev.csv", header=T)
topfoe <- read.csv("model_topfoe.csv", header=T)
topopw <- read.csv("model_topopw.csv", header=T)
topwet <- read.csv("model_topwet.csv", header=T)

#II Best transect across each variable
#Example: Developed Land (dev)
#Across transect types
#basic
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topbasic[[nm]],"d", drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model basic
moddbasic <- lFormula(Dch ~ dev + (1|pop1), data = topbasic, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddbasic)
opt <-optimizeLmer(dfun)
mod_dbasic<-mkMerMod(environment(dfun),opt,moddbasic$reTrms,fr=moddbasic$fr)
#In the fitted model replace Zt slot
moddbasic$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddbasic)
opt <-optimizeLmer(dfun)
mod_dbasicz <- mkMerMod(environment(dfun),opt,moddbasic$reTrms,fr=moddbasic$fr)
AICdevbasic <- AIC(mod_dbasicz)
BICdevbasic <- BIC(mod_dbasicz)
#Coastal
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topcoastal[[nm]],"d", drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model coastal
moddcoastal <- lFormula(Dch ~ dev + (1|pop1), data = topcoastal, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddcoastal)
opt <-optimizeLmer(dfun)
```

```
mod_dcoastal<-mkMerMod(environment(dfun),opt,moddcoastal$reTrms,fr=moddcoastal$fr)
#In the fitted model replace Zt slot
moddcoastal$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddcoastal)
opt <-optimizeLmer(dfun)
mod_dcoastalz <- mkMerMod(environment(dfun),opt,moddcoastal$reTrms,fr=moddcoastal$fr)
AICdevcoastal <- AIC(mod_dcoastalz)
BICdevcoastal <- BIC(mod_dcoastalz)
#LCTA:Developed
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topdev[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model developed
modddev <- lFormula(Dch ~ dev + (1|pop1), data = topdev, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modddev)
opt <-optimizeLmer(dfun)
mod_ddev<-mkMerMod(environment(dfun),opt,modddev$reTrms,fr=modddev$fr)
#In the fitted model replace Zt slot
modddev$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modddev)
opt <-optimizeLmer(dfun)
mod_ddevz <- mkMerMod(environment(dfun),opt,modddev$reTrms,fr=modddev$fr)
AICdevdev <- AIC(mod_ddevz)
BICdevdev <- BIC(mod_ddevz)
#LCTA:Forest
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topfoe[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
moddfoe <- lFormula(Dch ~ dev + (1|pop1), data = topfoe, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddfoe)
opt <-optimizeLmer(dfun)
mod_dfoe<-mkMerMod(environment(dfun),opt,moddfoe$reTrms,fr=moddfoe$fr)
#In the fitted model replace Zt slot
moddfoe$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddfoe)
opt <-optimizeLmer(dfun)
mod_dfoez <- mkMerMod(environment(dfun),opt,moddfoe$reTrms,fr=moddfoe$fr)
AICdevfoe <- AIC(mod_dfoez)
BICdevfoe <- BIC(mod_dfoez)
#LCTA:Ocean
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topopw[[nm]],"d",
drop=FALSE))
```

```
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
moddopw <- lFormula(Dch ~ dev + (1|pop1), data = topopw, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddopw)
opt <-optimizeLmer(dfun)
mod_dopw<-mkMerMod(environment(dfun),opt,moddopw$reTrms,fr=moddopw$fr)
#In the fitted model replace Zt slot
moddopw$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddopw)
opt <-optimizeLmer(dfun)
mod_dopwz <- mkMerMod(environment(dfun),opt,moddopw$reTrms,fr=moddopw$fr)
AICdevopw <- AIC(mod_dopwz)
BICdevopw <- BIC(mod_dopwz)
#LCTA:Wetland
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topwet[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
moddwet <- lFormula(Dch ~ dev + (1|pop1), data = topwet, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddwet)
opt <-optimizeLmer(dfun)
mod_dwet<-mkMerMod(environment(dfun),opt,moddwet$reTrms,fr=moddwet$fr)
#In the fitted model replace Zt slot
moddwet$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddwet)
opt <-optimizeLmer(dfun)
mod_dwetz <- mkMerMod(environment(dfun),opt,moddwet$reTrms,fr=moddwet$fr)
AICdevwet <- AIC(mod_dwetz)
BICdevwet <- BIC(mod_dwetz)

#making a dataframe of AIC and BIC values
transect <- c("Basic", "Coastal", "LCTA:Developed", "LCTA:Forest", "LCTA:Ocean",
"LCTA:Wetland")
AIC <- c(AICdevbasic, AICdevcoastal, AICdevdev, AICdevfoe, AICdevopw, AICdevwet)
BIC <- c(BICdevbasic, BICdevcoastal, BICdevdev, BICdevfoe, BICdevopw, BICdevwet)
model.selection <- data.frame(transect, AIC, BIC)

#further model diagnosics
CSF.IC <- model.selection
CSF.IC <- cbind(CSF.IC, k = c(attr(logLik(mod_dbasicz), "df"), attr(logLik(mod_dcoastalz),
"df"), attr(logLik(mod_ddevz), "df"), attr(logLik(mod_dfoez), "df"), attr(logLik(mod_dopwz),
"df"), attr(logLik(mod_dwetz), "df")))
CSF.IC
AICc <- CSF.IC$AIC + 2*CSF.IC$k*(CSF.IC$k+1)/(48-CSF.IC$k-1)
```

```
CSF.IC <- cbind(CSF.IC, AICc = AICc)
#Calculate model weights for AICc
AICcmin <- min(CSF.IC$AICc)
RL <- exp(-0.5*(CSF.IC$AICc - AICcmin))
sumRL <- sum(RL)
AICew <- RL/sumRL
CSF.IC <- cbind(CSF.IC, AICew)
#Calculate model weights for BIC
BICmin <- min(CSF.IC$BIC)
RL.B <- exp(-0.5*(CSF.IC$BIC - BICmin))
sumRL.B <- sum(RL.B)
BICew <- RL.B/sumRL.B
CSF.IC <- cbind(CSF.IC, BICew)
write.csv(CSF.IC, file="~/Documents/Spatial Analyses/Model Transformations/New Chord
Distance/Hierarchical Models/Variable Results/dev_transectypes.csv")

#substitute “dev” for each landscape variable (forest cover, ocean, wetland)

#Now create a final file that has variables measure across the top transect and scale based on
AIC from the above files
#load this data file and use it to run the candidate models
#load data
topall <- read.csv("model_topall.csv")

Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topall[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])

#Fitting Model 0: Null model
mod0B <- lFormula(Dch ~ 1 +  (1|pop1), data = topall, REML = FALSE)
dfun <- do.call(mkLmerDevfun, mod0B)
opt <-optimizeLmer(dfun)
mod_0B<-mkMerMod(environment(dfun),opt,mod0B$reTrms,fr=mod0B$fr)
#In the fitted model replace Zt slot
mod0B$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,mod0B)
opt <-optimizeLmer(dfun)
mod_0Bz <- mkMerMod(environment(dfun),opt,mod0B$reTrms,fr=mod0B$fr)
summary(mod_0Bz)
AIC0B <- AIC(mod_0Bz)
BIC0B <- BIC(mod_0Bz)

#Model 1: Full Model
mod14 <- lFormula(Dch ~ distance + wet + dev + foe + opw +  (1|pop1), data = topall, REML =
FALSE)
```

```
dfun <- do.call(mkLmerDevfun, mod14)
opt <-optimizeLmer(dfun)
mod_14<-mkMerMod(environment(dfun),opt,mod14$reTrms,fr=mod14$fr)
#In the fitted model replace Zt slot
mod14$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,mod14)
opt <-optimizeLmer(dfun)
mod_14z <- mkMerMod(environment(dfun),opt,mod14$reTrms,fr=mod14$fr)
AIC14 <- AIC(mod_14z)
BIC14 <- BIC(mod_14z)

#Fitting Model 2: Human influence
mod15 <- lFormula(Dch ~ distance + dev + foe +  (1|pop1), data = topall, REML = FALSE)
dfun <- do.call(mkLmerDevfun, mod15)
opt <-optimizeLmer(dfun)
mod_15<-mkMerMod(environment(dfun),opt,mod15$reTrms,fr=mod15$fr)
#In the fitted model replace Zt slot
mod15$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,mod15)
opt <-optimizeLmer(dfun)
mod_15z <- mkMerMod(environment(dfun),opt,mod15$reTrms,fr=mod15$fr)
AIC15 <- AIC(mod_15z)
BIC15 <- BIC(mod_15z)

#Fitting Model 3: Naturally occurring
mod16 <- lFormula(Dch ~ distance + wet + opw +  (1|pop1), data = topall, REML = FALSE)
dfun <- do.call(mkLmerDevfun, mod16)
opt <-optimizeLmer(dfun)
mod_16<-mkMerMod(environment(dfun),opt,mod16$reTrms,fr=mod16$fr)
#In the fitted model replace Zt slot
mod16$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,mod16)
opt <-optimizeLmer(dfun)
mod_16z <- mkMerMod(environment(dfun),opt,mod16$reTrms,fr=mod16$fr)
AIC16 <- AIC(mod_16z)
BIC16 <- BIC(mod_16z)


#Fitting Model 4: Human and nature
mod17 <- lFormula(Dch ~ distance + wet + dev +  (1|pop1), data = topall, REML = FALSE)
dfun <- do.call(mkLmerDevfun, mod17)
opt <-optimizeLmer(dfun)
mod_17<-mkMerMod(environment(dfun),opt,mod17$reTrms,fr=mod17$fr)
```

```
#In the fitted model replace Zt slot
mod17$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,mod17)
opt <-optimizeLmer(dfun)
mod_17z <- mkMerMod(environment(dfun),opt,mod17$reTrms,fr=mod17$fr)
AIC17 <- AIC(mod_17z)
BIC17 <- BIC(mod_17z)

#Fitting Model 5: Wetland
mod18 <- lFormula(Dch ~ wet + distance +  (1|pop1), data = topall, REML = FALSE)
dfun <- do.call(mkLmerDevfun, mod18)
opt <-optimizeLmer(dfun)
mod_18<-mkMerMod(environment(dfun),opt,mod18$reTrms,fr=mod18$fr)
#In the fitted model replace Zt slot
mod18$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,mod18)
opt <-optimizeLmer(dfun)
mod_18z <- mkMerMod(environment(dfun),opt,mod18$reTrms,fr=mod18$fr)
AIC18 <- AIC(mod_18z)
BIC18 <- BIC(mod_18z)

#Fitting Model 6: Developed
mod19 <- lFormula(Dch ~ dev + distance +  (1|pop1), data = topall, REML = FALSE)
dfun <- do.call(mkLmerDevfun, mod19)
opt <-optimizeLmer(dfun)
mod_19<-mkMerMod(environment(dfun),opt,mod19$reTrms,fr=mod19$fr)
#In the fitted model replace Zt slot
mod19$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,mod19)
opt <-optimizeLmer(dfun)
mod_19z <- mkMerMod(environment(dfun),opt,mod19$reTrms,fr=mod19$fr)
AIC19 <- AIC(mod_19z)
BIC19 <- BIC(mod_19z)

#Fitting Model 7: Just distance
mod7 <- lFormula(Dch ~ distance +  (1|pop1), data = topall, REML = FALSE)
dfun <- do.call(mkLmerDevfun, mod7)
opt <-optimizeLmer(dfun)
mod_7<-mkMerMod(environment(dfun),opt,mod7$reTrms,fr=mod7$fr)
#In the fitted model replace Zt slot
mod7$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,mod7)
```

```
opt <-optimizeLmer(dfun)
mod_7z <- mkMerMod(environment(dfun),opt,mod7$reTrms,fr=mod7$fr)
summary(mod_7z)
AIC7 <- AIC(mod_7z)
BIC7 <- BIC(mod_7z)

#making a dataframe of AIC and BIC values
model <- c("Null", "Full","Human Influence (Dev+For)", "Naturally occurring (Wet+Opw)",
"Human and Nature (Wet + Dev)", "Wetland", "Developed", "Distance")
AIC <- c(AIC0B, AIC14, AIC15, AIC16, AIC17, AIC18, AIC19, AIC7)
BIC <- c(BIC0B, BIC14, BIC15, BIC16, BIC17, BIC18, BIC19, BIC7)
model.selection <- data.frame(model, AIC, BIC)

#further model diagnosics
CSF.IC <- model.selection
CSF.IC <- cbind(CSF.IC, k = c(attr(logLik(mod_0Bz), "df"), attr(logLik(mod_14z), "df"),
attr(logLik(mod_15z), "df"), attr(logLik(mod_16z), "df"), attr(logLik(mod_17z), "df"),
attr(logLik(mod_18z), "df"), attr(logLik(mod_19z), "df"), attr(logLik(mod_7z), "df")))
CSF.IC
AICc <- CSF.IC$AIC + 2*CSF.IC$k*(CSF.IC$k+1)/(48-CSF.IC$k-1)
CSF.IC <- cbind(CSF.IC, AICc = AICc)
#Calculate model weights for AICc
AICcmin <- min(CSF.IC$AICc)
RL <- exp(-0.5*(CSF.IC$AICc - AICcmin))
sumRL <- sum(RL)
AICew <- RL/sumRL
CSF.IC <- cbind(CSF.IC, AICew)
#Calculate model weights for BIC
BICmin <- min(CSF.IC$BIC)
RL.B <- exp(-0.5*(CSF.IC$BIC - BICmin))
sumRL.B <- sum(RL.B)
BICew <- RL.B/sumRL.B
CSF.IC <- cbind(CSF.IC, BICew)
write.csv(CSF.IC, file="~/Documents/Spatial Analyses/Model Transformations/New Chord
Distance/Hierarchical Models/across_topall.csv")

#Top Transect Length
#Test using just transect length as explanatory variable
#use transect lengths from each of the top transect files
#transect length does not change based on scale

#load data
topbasic <- read.csv("model_topbasic.csv", header=T)
topcoastal <- read.csv("model_topcoastal.csv", header=T)
topdev <- read.csv("model_topdev.csv", header=T)
topfoe <- read.csv("model_topfoe.csv", header=T)
```

```
topopw <- read.csv("model_topopw.csv", header=T)
topwet <- read.csv("model_topwet.csv", header=T)

#basic
# Create the Zl and ZZ matrices
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topbasic[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model basic
moddbasic <- lFormula(Dch ~ length + (1|pop1), data = topbasic, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddbasic)
opt <-optimizeLmer(dfun)
mod_dbasic<-mkMerMod(environment(dfun),opt,moddbasic$reTrms,fr=moddbasic$fr)
#In the fitted model replace Zt slot
moddbasic$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddbasic)
opt <-optimizeLmer(dfun)
mod_dbasicz <- mkMerMod(environment(dfun),opt,moddbasic$reTrms,fr=moddbasic$fr)
AICdevbasic <- AIC(mod_dbasicz)
BICdevbasic <- BIC(mod_dbasicz)
#Coastal
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topcoastal[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model coastal
moddcoastal <- lFormula(Dch ~ length + (1|pop1), data = topcoastal, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddcoastal)
opt <-optimizeLmer(dfun)
mod_dcoastal<-mkMerMod(environment(dfun),opt,moddcoastal$reTrms,fr=moddcoastal$fr)
#In the fitted model replace Zt slot
moddcoastal$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddcoastal)
opt <-optimizeLmer(dfun)
mod_dcoastalz <- mkMerMod(environment(dfun),opt,moddcoastal$reTrms,fr=moddcoastal$fr)
AICdevcoastal <- AIC(mod_dcoastalz)
BICdevcoastal <- BIC(mod_dcoastalz)
#LCTA:Developed
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topdev[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model developed
modddev <- lFormula(Dch ~ length + (1|pop1), data = topdev, REML = FALSE)
dfun <- do.call(mkLmerDevfun, modddev)
opt <-optimizeLmer(dfun)
```

```
mod_ddev<-mkMerMod(environment(dfun),opt,modddev$reTrms,fr=modddev$fr)
#In the fitted model replace Zt slot
modddev$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,modddev)
opt <-optimizeLmer(dfun)
mod_ddevz <- mkMerMod(environment(dfun),opt,modddev$reTrms,fr=modddev$fr)
AICdevdev <- AIC(mod_ddevz)
BICdevdev <- BIC(mod_ddevz)
#LCTA:Forest
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topfoe[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
moddfoe <- lFormula(Dch ~ length + (1|pop1), data = topfoe, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddfoe)
opt <-optimizeLmer(dfun)
mod_dfoe<-mkMerMod(environment(dfun),opt,moddfoe$reTrms,fr=moddfoe$fr)
#In the fitted model replace Zt slot
moddfoe$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddfoe)
opt <-optimizeLmer(dfun)
mod_dfoez <- mkMerMod(environment(dfun),opt,moddfoe$reTrms,fr=moddfoe$fr)
AICdevfoe <- AIC(mod_dfoez)
BICdevfoe <- BIC(mod_dfoez)
#LCTA:Ocean
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topopw[[nm]],"d",
drop=FALSE))
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
moddopw <- lFormula(Dch ~ length + (1|pop1), data = topopw, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddopw)
opt <-optimizeLmer(dfun)
mod_dopw<-mkMerMod(environment(dfun),opt,moddopw$reTrms,fr=moddopw$fr)
#In the fitted model replace Zt slot
moddopw$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddopw)
opt <-optimizeLmer(dfun)
mod_dopwz <- mkMerMod(environment(dfun),opt,moddopw$reTrms,fr=moddopw$fr)
AICdevopw <- AIC(mod_dopwz)
BICdevopw <- BIC(mod_dopwz)
#LCTA:Wetland
Zl <- lapply(c("pop1","pop2"), function(nm) Matrix:::fac2sparse(topwet[[nm]],"d",
drop=FALSE))
```

```
ZZ <- Reduce("+", Zl[-1], Zl[[1]])
#Model 500m buffer
moddwet <- lFormula(Dch ~ length + (1|pop1), data = topwet, REML = FALSE)
dfun <- do.call(mkLmerDevfun, moddwet)
opt <-optimizeLmer(dfun)
mod_dwet<-mkMerMod(environment(dfun),opt,moddwet$reTrms,fr=moddwet$fr)
#In the fitted model replace Zt slot
moddwet$reTrms$Zt<-ZZ
#Refit the model
dfun <- do.call(mkLmerDevfun,moddwet)
opt <-optimizeLmer(dfun)
mod_dwetz <- mkMerMod(environment(dfun),opt,moddwet$reTrms,fr=moddwet$fr)
AICdevwet <- AIC(mod_dwetz)
BICdevwet <- BIC(mod_dwetz)

#making a dataframe of AIC and BIC values
transect <- c("Basic", "Coastal", "LCTA:Developed", "LCTA:Forest", "LCTA:Ocean",
"LCTA:Wetland")
AIC <- c(AICdevbasic, AICdevcoastal, AICdevdev, AICdevfoe, AICdevopw, AICdevwet)
BIC <- c(BICdevbasic, BICdevcoastal, BICdevdev, BICdevfoe, BICdevopw, BICdevwet)
model.selection <- data.frame(transect, AIC, BIC)

#further model diagnosics
CSF.IC <- model.selection
CSF.IC <- cbind(CSF.IC, k = c(attr(logLik(mod_dbasicz), "df"), attr(logLik(mod_dcoastalz),
"df"), attr(logLik(mod_ddevz), "df"), attr(logLik(mod_dfoez), "df"), attr(logLik(mod_dopwz),
"df"), attr(logLik(mod_dwetz), "df")))
CSF.IC
AICc <- CSF.IC$AIC + 2*CSF.IC$k*(CSF.IC$k+1)/(48-CSF.IC$k-1)
CSF.IC <- cbind(CSF.IC, AICc = AICc)
#Calculate model weights for AICc
AICcmin <- min(CSF.IC$AICc)
RL <- exp(-0.5*(CSF.IC$AICc - AICcmin))
sumRL <- sum(RL)
AICew <- RL/sumRL
CSF.IC <- cbind(CSF.IC, AICew)
#Calculate model weights for BIC
BICmin <- min(CSF.IC$BIC)
RL.B <- exp(-0.5*(CSF.IC$BIC - BICmin))
sumRL.B <- sum(RL.B)
BICew <- RL.B/sumRL.B
CSF.IC <- cbind(CSF.IC, BICew)
write.csv(CSF.IC, file="~/Documents/Spatial Analyses/Model Transformations/New Chord
Distance/Hierarchical Models/Variable Results/length_transectypes.csv")
```