ECOLOGICAL AND EVOLUTIONARY RESPONSES OF PLANT POPULATIONS TO THE COASTAL DUNE ENVIRONMENT

by

CARA LYNN GORMALLY

(Under the Direction of Lisa A. Donovan)

ABSTRACT

Phenotypic trait variation is ubiquitous among plant populations. Multiple evolutionary and ecological processes may produce trait variation, including divergent selection due to heterogeneous environmental conditions driving local adaptation, phenotypic plasticity, historical genetic structure, genetic drift, and barriers to gene flow. Consequently, disentangling the principal cause driving trait variation is challenging, since multiple processes may result in similar outcomes. Understanding the underlying source of phenotypic trait variation is particularly critical for informing restoration and conservation decisions. *Uniola paniculata* is a perennial grass that occurs on southeastern U.S. coastal dunes and is federally protected due to its role in stabilizing dune habitats. Across a single dune system, *U. paniculata's* range spans a localized environmental gradient from the dynamic dunes closest to the shoreline to the more stabilized dunes farther inland. We characterize the shoreline-to-landward environmental gradient and determine that variation in morphological and physiological traits in *U. paniculata* mirrors variation in the underlying abiotic conditions in a mensurative field study. We find no evidence that populations are locally adapted to microhabitats along the environmental gradient using reciprocal transplants of individuals from each habitat. We employ a comparative approach

using a greenhouse common garden to examine variation in quantitative traits and genetic analyses (allozymes) to examine neutral genetic variation. This study includes populations from four Georgia barrier islands. We find evidence of divergent selection on both aboveground and total biomass which appears to be driven primarily by inter-island differences rather than intra-island differences from habitats along the shoreline-to-landward environmental gradient. Lastly, we explore the genetic structure of a widely dispersed beach annual, *Cakile edentula*, which is comprised of three subspecies associated with particular geographic distributions. This study has three key findings to contribute to our understanding of *C. edentula*: genetic diversity (H_{ep}) is fairly low; taxonomic subspecies designations are supported by UPGMA-dendrogram clusters and AMOVA analysis indicating that variation among regions explains 42% of total variation; and pairwise F_{ST} estimates suggest that there are significant rates of migration between populations.

INDEX WORDS: environmental gradients, natural selection, phenotypic plasticity, population genetics, coastal dunes, *Uniola paniculata, Cakile edentula*.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Coastal dunes are well-established systems for ecological research due to the dynamic nature of the habitat and the successional patterns of vegetation zonation (Cowles 1899, Clements 1904, Oosting & Billings 1942). Coastal sand dune habitats are spatially and temporally heterogeneous. This is in part due to frequent disturbances that can result in the removal of low-lying foredunes, partial destruction of larger dunes, the erosion of dune profiles and even complete habitat destruction (e.g., submerging of an entire barrier island) (Claudino-Sales et al. 2007). In addition to physical disturbance, environmental conditions may differ dramatically across relatively small distances across these narrow, fragmented ecosystems, driving changes in plant community composition and species' distributions in as little as 0.5 m (van der Valk 1974, Barbour *et al.* 1985, Doing 1985, Moreno-Casasola & Espejel 1986, Moreno-Casasola 1988, Maun & Perumal 1999, Martinez *et al.* 2001). The plant community response to these environmental gradients is often cited as the textbook example for primary succession and vegetation zonation (Cowles 1899, Clements 1904, Barbour *et al.* 1987, Barbour *et al.* 1999, Smith & Smith 2001).

The relative importance of abiotic factors implicated in changes in species' distributions and vegetation zonation continues to be debated. These factors include salt spray (Oosting & Billings 1942, Boyce 1954, Barbour 1978, Wilson & Sykes 1999); soil nutrients and chemistry (Wagner 1964, Lane *et al.* 2008); sand burial and movement (Maun & LaPierre 1984, Moreno-Casasola 1986, Seliskar 1994, Maun & Perumal 1999, Martinez *et al.* 2001, Owen *et al.* 2004);

soil moisture (Griffiths 2006); wind (Lubke 1983, Lortie & Cushman 2007), and combinations of the factors (Lee & Ignaciuk 1985, Olff *et al.* 1993, Dilustro & Day 1997, Houle 1997, Griffiths 2006). However, most studies examine one or a limited number of abiotic factors, rather than a suite of environmental traits, which would allow for a more complete characterization of the coastal dune environment.

On many coastal dunes, the primary dune communities are nearly monospecific, with only one grass species building these dunes. Some dominant species occur across the entire primary dune complex, from the dynamic embryonic dunes closest to the shoreline to the more stabilized dune. Within this primary dune complex, environmental conditions may vary greatly. While the role of environmental gradients in structuring zonation is well-documented, we know relatively little about the intraspecific ecological and evolutionary responses of plant populations to the environmental heterogeneity of coastal dune systems (Knight & Miller 2004).

Natural selection can drive local adaptation when plant species encounter heterogeneous environmental conditions (Turesson 1922, Clausen *et al.* 1940, Hereford 2009), whether at the landscape level across the span of their ranges, or at finer, microhabitat scales (Antonovics & Bradshaw 1970). Local adaptation is the association of certain genotypes with particular habitats, with the locally adapted genotypes outperforming "foreign" genotypes (Linhart & Grant 1996, Kawecki & Ebert 2004). Most studies of local adaptation target plant species growing in environments characterized by abiotic spatial heterogeneity due to conditions such as toxic soils, fertilizers, herbicides, elevation, light conditions, temperature, and soil water availability, rather than environments that are frequently disturbed (Linhart & Grant 1996, Leimu & Fischer 2008) (but see (Galloway & Fenster 2000), a study of an annual legume grown in disturbed sites). However, much less is known about whether long-lived plant species located in frequently

disturbed environments tend to be locally adapted or respond plastically to temporal heterogeneity (Gregor 1930, Miller & Fowler 1994, Leimu & Fischer 2008).

Tests of local adaptation in coastal dunes are affected not only by the dynamic coastal dune environment, which is frequently altered by sand erosion and accretion, but also by the growth habit of dune plants, most of which are long-lived and clonal (Gray 1985, Cheplick & White 2002, Knight & Miller 2004). Plant cover is relatively sparse on the coastal dunes, in particular on the dynamic dunes closest to the shoreline. Sand burial limits species' distributions as well as abundance, with perennial plants tolerating the most burial (van der Valk 1974, Maun & Perumal 1999, Owen *et al.* 2004). Perennial clonal dune grasses dominate areas typified by burial and erosion (Garcia-Mora et al. 1999). These plants have traits which may facilitate both tolerance to burial (van der Valk 1974, Maun & Perumal 1999, Owen *et al.* 2004); and survival (Maun 1998), such as the ability to grow upward rapidly, produce extensive rhizomes, and propagate vegetatively (Johnson & Barbour 1990).

We chose to use *Uniola paniculata* to investigate population-level evolutionary responses, since its natural history, as a rhizomatous perennial grass capable of both sexual and clonal reproduction, is representative of many dune plant species (Wagner 1964). The distribution of *U. paniculata* ranges along the southeastern Atlantic coast from Virginia to the Bahamas and the Gulf coast to Veracruz, Mexico (Wagner 1964). At the geographic scale, there are no strong regional patterns of genetic structure among populations of *U. paniculata* (Franks *et al.* 2004) (but see (Subudhi *et al.* 2005)), but fine-scale clonal structure and diversity has been shown to vary widely even in relatively small areas (Franks *et al.* 2004). *Uniola paniculata*'s habitat spans a localized dune gradient from the dynamic embryonic foredunes to the more stabilized dunes farther inland, and it is the dominant plant species on the primary dunes.

Frequently used in dune restoration projects, *U. paniculata* is federally protected because it stabilizes dune habitats.

Colonizing species are ecologically important as they represent the first stage of succession (Clements 1904, Feagin *et al.* 2005). Recent work by Feagin *et al* (2005) indicates that when establishment of colonizing dune plants is inhibited by habitat loss and sea level rise, the successional community fails to develop. Annuals represent a significant component of the vegetation community on coastal strandlines (Lee & Ignaciuk 1985). Populations in these dynamic habitats are ephemeral. The unique aspects of the strandline habitat, combined with its narrow, fragmented, and linear distribution, may have interesting consequences for the population genetic structuring of strandline species.

The genus *Cakile* is distributed worldwide, primarily on coastal strandlines (Rodman 1974). *Cakile edentula* (Brassicaceae), commonly known as sea rocket, is an annual beach colonizer which grows on strandlines throughout North America (Rodman 1974, Duncan & Duncan 1987). The species is subdivided into three subspecies whose distributions correlate with major biogeographic boundaries: *ssp. edentula var. lacustris*, found on the Great Lakes; *ssp. edentula*, found on the Atlantic coast from North Carolina northward; and *ssp. harperi*, found on the Atlantic coast from North Carolina southward. Populations of *ssp. edentula* and *ssp. harperi* are sympatric on the North Carolina coast (Rodman 1974). *Cakile edentula ssp. edentula* was also introduced to the Pacific coast, reportedly documented in the late 1800s (Rodman 1974). However, it is unclear how genetic variation among regions and populations is related to these subspecies designations.

Genetic structuring in *C. edentula* may also be influenced by its unique dispersal biology. Within a single flower, *C. edentula* produces heteromorphic fruit segments, proximal and distal, each of which typically contains one seed (Rodman 1974). All fruits have a thick, buoyant pericarp, which floats, allowing for long-distance dispersal by wind and water. Distal fruit segments are easily detached from the maternal plant and usually disperse independently whereas seeds from proximal fruit segments have limited dispersal capacities since proximal fruits often remain attached to the maternal plant (Zhang & Maun 1992). In spatially and temporally heterogeneous environments such as the coastal dunes, plant survival may be facilitated by strategies such as seed dimorphism, as this phenomenon can allow multiple opportunities for seed dispersal and germination (Venable & Levin 1985, Telenius & Torstensson 1989) as well as for establishment and survival (Philipupillai & Ungar 1984). Differential dispersal of these heteromorphic seeds may have important ecological and evolutionary implications for populations of *C. edentula*.

The genetic structuring of this species may reflect the influences of its fragmented habitat as well as its unique dispersal syndrome. Since *Cakile* is widely distributed and the species is classified into three subspecies that are associated with distinct geographic junctures, we investigated the geographic distribution of genetic variation to determine whether genetic structure aligns with the taxonomic subspecies delineations.

We ask the following series of interrelated questions in order to understand the ecological and evolutionary responses of *U. paniculata* to the coastal environment:

1. What is the relationship between environmental variation and intraspecific trait variation?
2. Are populations locally adapted to microhabitats on the coastal dunes?

3. What are the relative roles of natural selection and neutral genetic processes in shaping populations of *U. paniculata*?

An additional question was developed to explore the population genetics of a widely distributed annual colonizer, *C. edentula*, found on coastal strandlines We asked specifically:

4. Is the structuring of genetic diversity in *C. edentula* related to the geographic distribution of the subspecies?

To address these questions, we combine techniques from ecology, ecophysiology, and population genetics to explore the interrelated pieces of the larger puzzle of whether environmental gradients on the coastal dunes drive population differentiation. Addressing several interrelated questions—characterizing the relationship between intraspecific trait variation and environmental variation, and determining the role of natural selection and other evolutionary processes in shaping natural populations—offers clues to answer the larger question of this dissertation—to understand the evolutionary processes and outcomes of coastal dune plant populations. Finally, we use population genetic techniques to investigate the underlying genetic structure of the biogeographic patterns of a widespread coastal annual colonizer.

While coastal dunes are frequently disturbed habitats, dunes now face anthropogenic development which encroaches on fragile dune habitats, and environmental change, such as increased storm surge from severe weather and sea level rise ((IPCC) 2001, Feagin *et al.* 2005). At this point, there are no general guidelines for restoration about how localized the origin of seed or plant material should be relative to the restored habitat. Coastal dune plants have a long, narrow, fragmented distribution. How and whether this unusual geographic distribution, combined with microenvironmental factors, affects the underlying genetic structure of

populations is largely unknown since little genetic work has been conducted on coastal dune species. Understanding patterns of environmental variation and population differentiation in response to environmental variation is crucial for successful restoration and conservation efforts.

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CHAPTER 2

RESPONSES OF *UNIOLA PANICULATA* L. (POACEAE), AN ESSENTIAL DUNE-BUILDING GRASS, TO COMPLEX CHANGING ENVIRONMENTAL GRADIENTS ON THE COASTAL DUNES[1]

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Abstract

Coastal dunes are well known for plant species zonation but less is known about species-specific responses to underlying environmental gradients. We investigated variation in morphological traits and tissue nutrient concentration in *Uniola paniculata*, along a shoreline-to-landward gradient (transects spanning from the dunes directly behind the high tide mark to 40-100 m inland) in the southeast U.S. Several environmental factors decreased with distance from the shoreline (soil B, K, Mg, Na; salinity, pH, and sand accretion), and differences were most pronounced between the 10 m closest to the shoreline and the remainder of the transect. In the 10 m closest to the shoreline, 94% more sand accumulated, which was 31% more saline. Additionally, plants here were taller, contained higher aboveground tissue N and K, and a higher percentage tended to flower. This contrasts with patterns found in salt marshes and saline desert dunes, where plant size is often negatively correlated with salinity. During the two years following the planned study, storms washed out ≤ 25 m of the transects. Re-sampling of the remaining sites demonstrated that even after erosion of the dune profile, a higher percentage of the plants in the 10 m closest to the shoreline tended to flower, relative to populations located further from the shore. Our findings suggested that the environment and plant response in the shoreward 10 m can re-establish relatively quickly.

Introduction

Plant populations often encounter heterogeneous environmental conditions across their ranges and at finer microhabitat scales. On the coastal sand dunes, environmental conditions may differ dramatically across relatively small distances, driving changes in plant community composition and species' distributions in as little as 0.5m (Doing 1985, Moreno-Casasola & Espejel 1986, Moreno-Casasola 1988, Martinez *et al.* 2001). The plant community response to

these environmental gradients is often cited as the textbook example for primary succession and vegetation zonation (Cowles 1899, Clements 1904, Barbour *et al.* 1987, Barbour *et al.* 1999, Smith & Smith 2001). The relative importance of the many abiotic factors implicated in changes in species' distributions and vegetation zonation continues to be debated. These abiotic factors include salt spray (Oosting & Billings 1942, Boyce 1954, Barbour 1978, Wilson & Sykes 1999), soil nutrients and chemistry (Willis 1963, Wagner 1964, Lane *et al.* 2008), sand burial and movement (Maun & LaPierre 1984, Moreno-Casasola 1986, Seliskar 1994, Maun & Perumal 1999, Martinez *et al.* 2001, Owen *et al.* 2004), soil moisture (Griffiths 2006), wind (Lubke 1983, Lortie & Cushman 2007), and combinations of the factors (Lee & Ignaciuk 1985, Olff *et al.* 1993, Dilustro & Day 1997, Houle 1997, Griffiths 2006). However, most studies examine one or a limited number of abiotic factors, rather than a suite of environmental traits, which would allow for a more complete characterization of the coastal dune environment.

In contrast to the well-documented role of environmental gradients in structuring species zonation, intraspecific responses to environmental gradients across the entire dune complex have received much less attention (Knight & Miller 2004). Although plant communities closest to the shoreline are often nearly monospecific with only one grass species (Doing 1985), there are dominant species that occur across the entire primary dune complex, from the dynamic dunes closest to the shoreline to more stabilized dunes further inland. Several manipulative studies have investigated intraspecific plant responses to sand burial and salt spray, which are arguably the most important environmental gradients on the coastal dunes (Cowles 1899, Oosting & Billings 1942, Maun & Perumal 1999, Wilson & Sykes 1999) although each may elicit different responses (Cheplick & Demetri 1999). Dune species tend to respond to sand burial by increasing in size through shifts in resource allocation (e.g. shifting resources from roots to aboveground

biomass) (Zhang & Maun 1992, Martinez & Moreno-Casasola 1996, Brown 1997, Perumal & Maun 2006), possibly requiring multiple years (Seliskar 1994). Following salt spray treatments, however, dune species generally show decreased growth (Sykes & Wilson 1988, Griffiths 2006). Sand burial and salt spray are also intertwined, since salt spray deposited on vegetation can be washed into the soil and then be affected by sand movement. Thus, both soil salinity and sand burial need to be assessed in relation to intraspecific variation in plant traits.

Nutrient and soil moisture availability are also expected to affect coastal dune plant traits. Dune soils are expected to be nutrient-limited due to low organic content (Barbour *et al.* 1985), however results from experimental additions of nitrogen (N) and phosphorus (P) on the coastal dunes are mixed as to whether they indicate plant growth limitation by these macronutrients (Willis 1963, Wagner 1964, Pemadasa & Lovell 1974, Olff *et al.* 1993, Houle 1997) . Soil moisture availability and salt spray are also expected to interact with nutrient use in coastal dune plants (Boyce 1954, Houle 1997). Plant growth on the stabilized dunes may be limited by potassium (K) since it quickly leaches out of sandy soils (Willis 1963, van der Valk 1974b). We chose to focus on soil moisture availability, soil N, P, K, and other nutrients (B, Ca, Mg, Na) that are ionic components of seawater and sea spray.

We investigated the relationship between environmental factors and morphological and physiological traits in *Uniola paniculata* L. (Poaceae). It is a perennial grass that reproduces both clonally and sexually and is the dominant plant species on the primary dunes. The distribution of *U. paniculata* ranges from the southeastern Atlantic coast from Virginia to the Bahamas and the Gulf coast to Veracruz, Mexico (Wagner 1964). It spans a localized dune gradient from the dynamic dunes closest to the shoreline to the more stabilized dunes farther inland. *Uniola paniculata* is a federally protected plant that stabilizes dune habitats and is frequently used in

dune restoration projects. A previous allozyme study demonstrated that there is no strong regional pattern of genetic structure among populations of *U. paniculata*, but fine-scale clonal structure and diversity vary widely (Franks *et al.* 2004).

The primary objectives of this study were: (1) to characterize the abiotic gradients across the primary dunes and (2) to determine whether this environmental variation is associated with intraspecific variation in morphology, physiology, demography and phenology in *U. paniculata* on Sapelo Island, Georgia. Based on the extensive coastal dune ecology literature, we predicted that abiotic conditions would change linearly, rather than in patchy distributions, with the shoreline end of the gradient experiencing more severe conditions, in particular, more saline soil, greater sand accretion, and greater soil nutrient availability. However, we had no *a priori* expectations as to whether the gradient would be continuous or exhibit clear break points. Additionally, we predicted that plant trait variation in *U. paniculata* would mirror the underlying environmental variation.

Multiple storms altered the dune profile during the two years following our initial study. The dunes closest to the shoreline, initially 2-3 m meters tall, experienced approximately 1-2 m of vertical erosion, leaving bare beach and washing away several of our sites closest to the shoreline (sites located ≤25 m from the shoreline) (Gormally, pers. observ.). Consequently, the dune profile shifted, and sites that had been previously more protected further inland (>10 m from the shoreline) were exposed to the high tide mark (Gormally, pers. observ.). On the Atlantic coast, the dynamic dunes closest to the shoreline frequently experience this kind of erosion from storms. We documented the plant response to a shift in the dune profile to determine if newly exposed dune sites took on the characteristics of the sites that were washed away.

Materials and Methods

All fieldwork was conducted on the coastal sand dunes at the National Estuarine Research Reserve on Sapelo Island, Georgia. The Sapelo Island beach dunes are protected through the National Estuarine Research Reserve System (NERRS), as well as through the Long Term Ecological Research Network (LTER). Thus, these dunes are ideal settings to document natural coastal dune ecology, as they have not been impacted by anthropogenic development or foot traffic. Plant community composition on the Sapelo Island dunes is typical for southeastern U.S. coastal dunes. The relatively sparse plant cover, which ranged from 14% on the dynamic dunes closest to the shoreline to 49% on the stabilized dunes further inland, is mostly comprised of clonal perennials, including *U. paniculata*, *Panicum amarum*, *Distichlis spicata*, and *Hydrocotyle bonariensis* (unpublished data). Precipitation during the April-October 2006 growing season was 77.6 cm, ~ 68% of annual precipitation, typical of long term averages for this site (2000-2006, UGA Marine Institute Climate Monitoring Station, Georgia Coastal Ecosystems LTER Network).

In May 2006, we established six transects perpendicular to the shoreline that began at the leading edge of vegetation and spanned the range of *U. paniculata* from the dynamic dunes closest to the shoreline to the stabilized dunes farther inland. Transects were located at least 300 m apart and ranged from 40-100 m in length, according to the presence of *U. paniculata*, which varied with island geomorphology (Stallins & Parker 2003). Each transect was sampled at 20 equidistant points, resulting in distances between sampling points ranging from 2 m on the shortest transect (40 m), to 5 m on the longest transect (100 m). Sites from each transect were then categorized into distance classes away from the shoreline (0-10 m, 11-20 m, 21-30m, 31-40 m, 41-50 m, 51-60 m, 61-70 m, 71-80 m, 81-90 m, 91-100 m) for analyses.

In June 2006, soil samples were collected for the determination of soil moisture, nutrients, pH, and salinity. Soils were collected at 25 cm depth intervals from 0-100 cm. Samples were weighed and oven dried at 60°C until constant weight to determine gravimetric soil moisture. Samples collected at 25-50 cm depth were analyzed for soil nutrients and pH since the majority of roots were found at this depth, although plants may additionally access the soil profile above and below this depth. Dried soils were sieved to remove organic matter and ball-milled prior to analysis for total nitrogen (N) (NA1500 C/H/N Analyzer, Carlo Erba Strumentazione, Milan) and for the following soil nutrients using ICP analysis: boron (B), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), and sodium (Na) (Thermo Jarrell-Ash Enviro I ICAP Spectrometer). Relative soil salinity of 25-50 cm depth samples was assessed following the LTER protocol (Robertson *et al.* 1999), by measuring the electrical conductivity (EC) of a rehydrated soil solution.

In June 2006, we surveyed dune elevation, to the nearest cm, using a theodolite (Topcon GTS-212), at each site along each transect. The change in sand accretion from June 2006 to June 2007 was measured to the nearest cm at each site on every transect using marked PVC pipes (Franks 2003). Unfortunately, we were unable to collect sand accretion data from the remaining transects as markings on PVC proved to be impermanent.

In June 2006, we assessed plant morphological variation using three randomly chosen plants, each (semi-permanently tagged), at each sampling site along each transect. Each plant was assessed for plant height measured from the base of the plant, stem diameter measured at the base of the plant, number of leaves, internode length (distance between the two uppermost nodes), and number of nodes. The aboveground biomass of one of the randomly chosen plants per sampling site was harvested, dried at 60°C until constant weight and ground for analysis of

the same nutrients as detailed above for soil samples. We also counted both the number of plants and number and percent that were flowering in a $3m^2$ area at each sampling site.

After our initial characterization of the dune environment and plant trait measurements in June 2006, multiple storm over-wash events (Fall 2006- Fall 2008) resulted in the erosion of the most shoreward ≤ 25m of dunes along some transects. Thus, the mean tideline was ≤ 25m further inland than it was prior to over-wash. This meant that some sites that were previously sheltered farther inland on stabilized dunes (≥11- 20 m from the high tide mark) now occupied the 10 m of land closest to the shoreline, since the sites located directly behind the high tide mark (0-10 m from the high tide mark) were washed away (Gormally, pers. observ.). In November 2008, we investigated whether this dune profile shift resulted in a change in the underlying abiotic factors. We used our earlier findings (described below) to inform our resampling of established sites: the new 10 m closest to the shoreline (0-10 m) and the sites directly inland (11-20 m) along each transect. We measured soil salinity and sand accretion, and counted the number of plants present and the number and percent that were flowering in a $3m^2$ area at each site.

We used analyses of variance (ANOVAs) to determine how environmental factors and *U. paniculata* morphological and physiological traits varied across the shoreline-to-landward gradient. The main effect was the distance class from the shoreline (0-10 m, 11-20 m, 21-30m, 31-40 m, 41-50 m, 51-60 m, 61-70 m, 71-80 m, 81-90 m, 91-100 m). Transects were treated as blocks. When there was a significant effect for distance class, Fisher's protected least significantly different (LSD) test was used to examine pairwise differences. Dependent variables were log transformed to meet assumptions of normality when necessary.

We also used a principal components analysis (PCA) followed by a protected Multivariate Analysis of Variance (MANOVA) of the three principal components generated by

the PCA to explore the underlying pattern of environmental factors shaping the dune gradient (Proc Factor and Proc GLM, SAS 9.1). The first three principal components explained 56% of the variance. Results from the PCA and MANOVA were similar to the results from the individual ANOVAs, and so are not included here.

For the fall 2008 sampling following storm over-wash, we used ANOVAs to compare two distance classes (0-10 m closest to the shoreline versus the inland 11-20 m) for sand accretion and soil salinity. We also used ANOVAs to compare the same two distance classes for number of individual plants, and number of flowering plants, for both the initial June 2006 sampling and the Fall 2008 sampling. We included a habitat *x* transect interaction term in order to detect whether effects were more pronounced along certain transects.

Results

Most of the environmental factors differed by distance class along the shoreline to landward transect: soil B, Ca, K, Mg, Na, N, soil moisture at 75-100 cm, soil pH, soil salinity, and sand accretion (Table 2.1). Sites closer to the shoreline generally had higher soil nutrient concentrations (except Ca and N), soil pH, soil salinity and sand accretion (Figs. 2.1, 2.2). The differences were most pronounced between the first 10 m closest to the high tide mark and the sites further inland for soil B, K, salinity and sand accretion (Figure 2.1, 2.2). Soils at sites in the 10 m closest to the high tide mark were 31% more saline than the average for the remaining sites. Sand accumulation at sites in the 10 m closest to the shoreline was 23 cm/year (June 2006-June 2007), which was ~94% more than the average for the remaining sites. Soil P and soil moisture at 0-25, 25-50 and 50-75 cm depth did not differ by distance class (data not presented).

Two plant aboveground nutrient concentrations (N, K) differed by distance class on the dunes (Figure 2.2). Tissue N concentrations differed by a maximum of 30%, with higher

concentrations in plants located in the 10 m closest to the shoreline ($F_{(9,77)}$=2.46, p=0.016). Tissue K concentrations differed as much as 92%, with higher concentrations in plants located in the 10 m closest to the shoreline ($F_{(9,76)}$=5.24, p<0.0001). There was no effect of distance class on the plant tissue of concentrations of B, Ca, Mg, Na, and P (data not presented).

Morphological traits in *U. paniculata* generally differed in the first 10 m closest to the shoreline compared to individuals located along the remainder of the transect (Figure 2.3). Shoot height was the most dramatically different ($F_{(9, 444)}$=4.63, p<0.0001) with plants located in the first 10 m closest to the shoreline nearly 30 cm (14-39%) taller than plants located at sites on the stabilized dunes. Stem diameter was larger in the same plants ($F_{(9, 443)}$=3.38, p=0.0005), as were the number of nodes ($F_{(9, 443)}$=2.04, p=0.034) and number of leaves ($F_{(9, 444)}$=2.11, p=0.027). However, number of leaves did not vary in a consistent pattern between dune locations. Internode length was the only morphological trait measured which did not vary between dune locations ($F_{(5, 106)}$=0.85, p=0.517). Although traits were larger in size, aboveground biomass did not differ by dune location, though biomass tended to be greater at sites closest to the shoreline ($F_{(9, 77)}$=0.95, p=0.484).

In November 2008, following the dune profile shift, the soil was more saline (20% more) in the 10 m closest to the shoreline than at 11-20 m (Table 2.2). There also was more sand accretion in the 10 m closest to the shoreline than at 11-20 m (22 cm/yr versus 6 cm/yr, respectively). We were unable to quantify disturbance due to erosional (horizontal) processes, beyond observation that between 5-20 meters of our transects eroded (horizontal erosion) in the two years following our June 2006 measurements (Gormally, pers. obs.).

There were no differences between the number of individuals located in the 10 m closest to the shoreline versus 11-20 m from the shoreline in either Summer 2006 prior to the storm

over-wash, or Fall 2008 after the dune profile shift (Table 2.3). However, at both sampling times, twice as many plants flowered in the 10 m closest to the shoreline than in the 11-20 m farther inland (2006: 10 versus 5 plants—21% versus 13%, respectively, $F_{(1, 13)}$=3.42, p>0.0873, N=24; 2008 10 versus 4 plants—11% versus 7%, respectively, $F_{(1, 13)}$=5.17, p>0.0406, N=24).

Discussion

The habitat comprising the 10 m of dunes located closest to the shoreline was environmentally distinct from the remainder of the gradient across the primary coastal dunes. Soil nutrients (B, K, Mg, Na), soil pH, soil salinity, and sand accretion generally decreased with increasing distance from the shoreline, with the most pronounced differences occurred between 10 m closest to the shoreline and the remainder of the transects. Both sand accretion and salt spray likely contributed to the soil salinity gradient (Oosting & Billings 1942, Boyce 1954, Barbour *et al.* 1985, Wilson & Sykes 1999) and thus contributed to a greater abundance of nutrients such as B and Mg, as well as a higher pH (Boyce 1954). Nutrient availability also may be greater on the shoreline due to onshore wrack deposition (Orr et al. 2005). Although there were no differences in soil N and total P across the transects, the differences in pH may have affected plant nutrient availability by altering the form of inorganic ions (e.g. nitrate versus ammonium, monovalent versus divalent or trivalent othophosphate ions) (Lambers *et al.* 1998, Larcher 2003). Soil pH may also affect a plant's ability to obtain nutrients from mycorrhizal associations (Habte & Soedarjo 1995). Although Na is not considered an essential element for all plants, it may be needed for *U. paniculata* as a C_4 grass (Marschner 2002). These gradients in sand accretion and soil salinity and chemistry are consistent with those found in other coastal dune studies (Oosting & Billings 1942, Wagner 1964, Barbour *et al.* 1985, Doing 1985).

Uniola paniculata plants located in the 10 m closest to the shoreline had higher aboveground tissue concentrations of N and K, but not P. This is consistent with previous work on the Sapelo Island dunes which found higher concentrations of leaf N in both *U. paniculata* and another grass (*Panicum amarum*) located near the shoreline as compared to 25 m inland (Dubois 1977). Depending on its salt tolerance strategy (i.e., exclusion or inclusion of Na^+ and Cl^-), *U. paniculata* may have an increased mineral nutrient demand for potassium and calcium (Marschner 2002). The increased sand deposition in the 10 m closest to the shoreline may have elicited an additional physical response from plants. Since sand burial effectively reduces the total amount of photosynthetic area and aboveground biomass (Brown 1997, Perumal & Maun 2006), some plants initiate compensatory growth after burial (Martinez & Moreno-Casasola 1996), which requires nutrients to produce photosynthates.

We found that intraspecific trait variation in *U. paniculata* tended to mirror environmental variation. Plants in the first 10 m of the gradient were generally taller, wider in stem diameter and had more nodes per culm than plants along the remainder of the gradient. Although these larger plants in the first 10 m also tended to have greater aboveground biomass, the biomass differences were not significant, possibly due to the smaller sample size. While the number of individuals did not differ between shoreline (0-10 m) and inland dune (11-20 m) locations, there tended to be nearly twice as many *U. paniculata* reproductive culms with flowering inflorescences in the 10 m closest to the shoreline, which is a reasonable indication of vigor. This increased shoreward vigor is consistent with a study of *U. paniculata* on the dynamic dunes in Bogue Bank, North Carolina (Wagner 1964) which found a significant increase in plant vigor (number of spikelets per panicle, flowering plant height, culm diameter) in individuals located closest to the shoreline. Although other studies have found that perennials may require

multiple seasons to respond to sand burial (Seliskar 1994, Dech & Maun 2005), it is possible that responses may be detected sooner.

Our results indicate that plants located in the 10 m closest to the shoreline where soil salinity is highest are larger (in trait size) and more vigorous. This contrasts with patterns in more saline habitats such as salt marshes and saline desert dunes where plant size is often negatively correlated with soil salinity (Lubke 1983, Haines & Dunn 1985, Donovan *et al.* 1997, Griffiths 2006). The pattern in coastal dunes may differ from these other habitats because coastal dune soil salinity rarely approaches toxic levels (Barbour *et al.* 1976), and other factors such as responses to sand burial may be more important in controlling species-specific responses (Houle 2002). Further, there may be trade-offs in plant responses to abiotic factors, as found for the perennial dune grass *Leymus mollis*: near-shore ramets had a higher relative growth rate allowing plants to withstand sand deposition, but at the expense of lowered salt tolerance (Houle 2002). Additionally, *Uniola paniculata* and other clonal perennials that dominate dunes have traits that facilitate tolerance to burial, such as the ability to grow upward rapidly, produce extensive rhizomes, and propagate vegetatively (Johnson & Barbour 1990). Thus, relatively low soil salinities (compared to other habitats) and tolerance of burial may partially account for *U. paniculata* success close to the shoreline where soil salinity is highest.

Increased sand deposition near the shoreline may also contribute to the increased vigor of *U. paniculata* by decreasing interspecific competition. Plant cover is relatively sparse on the coastal dunes, particularly the sites closest to the shoreline where *U. paniculata* dominates the plant cover. If sand burial is limiting the abundance of other species in this area, then decreased competition for water and nutrients may be an indirect benefit for *U. paniculata* (van der Valk 1974a).

Over the course of the two years following our characterization of the dune environment and plant traits, multiple storm over-wash events changed the dune profile along some transects. The underlying abiotic factors characterizing this area quickly changed, with the amount of sand accretion and soil salinity matching the environmental characterization of the former sites located in the10 m closest to the shoreline. Soil was more saline in the newly exposed sites located in the 10 m closest to the shoreline. While the density of *U. paniculata* plants did not differ between shoreline (0-10 m) and inland dune (11-20 m) locations, neither prior to nor following the dune profile shift, twice as many individuals flowered in the 10 m closest to the shoreline both prior to and following the dune profile shift. This suggests that *U. paniculata* is capable of responding to the changed habitat in as few as two growing seasons. Though the 10 m of the dunes closest to the shoreline sustained frequent environmental change due to periods of sand deposition and erosion, *U. paniculata* was most successful in this microhabitat, growing larger and tending to flower at twice the rate of individuals located farther inland.

Additional manipulative experiments are needed to determine whether these environmental differences drive trait variation in *U. paniculata*, as this experiment was designed only as a mensurative field study. Reciprocal transplants and common garden experiments could be used to investigate whether long-lived perennials such as *U. paniculata* respond to microhabitat differences through the process of local adaptation versus phenotypic plasticity (Kawecki & Ebert 2004, Richards *et al.* 2010). The responses we documented following the storm over-wash events may have been driven by selective conditions affecting recruitment and establishment of particular genotypes. It is also possible that there is variation in ramets due to age, or that genotypes themselves are phenotyically plastic. Since *U. paniculata* is a long-lived perennial, multiple years may be required to disentangle these hypotheses. In addition, the

majority of coastal dune plant species and community studies, including our own, are limited by the exclusive focus on aboveground biomass. Although measuring belowground biomass and traits would be challenging for extensively clonal plants, these are important measurements related to plant-environment interactions that should be considered in future studies. Our findings that substantial intraspecific trait variation occurs in *U. paniculata* at the oceanic edge of its range, where environmental factors also differ, is an important first step in understanding species-level responses to the coastal dune environment. This baseline ecological knowledge of the underlying environmental variation which influences natural populations' distributions and responses is an integral component for developing successful conservation strategies (Pywell *et al.* 2003, Wikelski & Cooke 2006).

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Table 2.1. ANOVA characterization of environmental factors (Summer 2006 study) which differed by distance class (10 m intervals) along the shoreline-to-landward costal dune gradient. Dependent variables which were transformed are indicated by crosses (†). Significant *p* values are in bold. Distance class means and means comparisons can be found in Figures 2.1 and 2.2. Soil P and soil moisture at 0-25, 25-50, and 50-75 cm depths did not differ by distance class.

Dependent variable	**Effects**	***F* (df)**	***p***
Soil B†	Distance class	5.26 (9, 105)	**<0.0001**
Soil Ca	Distance class	2.12 (9, 105)	**0.0339**
Soil K†	Distance class	10.6 (9, 105)	**<0.0001**
Soil Mg†	Distance class	7.9 (9, 105)	**<0.0001**
Soil Na†	Distance class	2.75 (9, 105)	**0.0064**
Soil N	Distance class	2.88 (9, 105)	**0.0044**
Soil moisture 75-100 cm	Distance class	2.19 (9, 103)	**0.0285**
Sand accretion	Distance class	4.36 (5, 33)	**0.0037**
Soil pH	Distance class	9.73 (9, 105)	**<0.0001**
Soil salinity†	Distance class	2.75 (9, 105)	**0.0064**

Table 2.2. ANOVA characterization of environmental factors following dune profile shift (Fall 2008 sampling). The distance class effect compares the sites in the 0-10 m closest to the shoreline to the sites 11-20 m farther inland. Dependent variables which were transformed are indicated by crosses (†). Significant *p* values are in bold.

Dependent variable	**Effects**	***F* (df)**	***p***
Sand accretion†	Distance class	13.69 (1, 26)	**0.001**
	Transect	3.43 (5, 26)	**0.0164**
	Distance class x transect	4.72 (5, 26)	**0.0033**
Soil salinity†	Distance class	6.16 (1, 29)	**0.0191**
	Transect	1.16 (5, 29)	0.3502
	Distance class x transect	1.40 (5, 29)	0.2519

Table 2.3. ANOVA characterization of number of *U. paniculata* individuals and number of reproductive (flowering) individuals (Summer 2006 and Fall 2008 studies). The distance class effect compares the sites in the 0-10 m closest to the shoreline to the sites 11-20 m farther inland. Dependent variables which were log transformed are indicated by crosses (†). Significant *p* values are in bold.

Dependent variable	**Effects**	***F* (df)**	***p***
Summer 2006			
Number of individuals†	Distance class	1.23 (1, 24)	0.2779
	Transect	0.76 (5, 24)	0.5895
	Habitat x transect	2.38 (5, 24)	0.0686
Number flowering†	Distance class	3.42 (1, 13)	0.0873
	Transect	1.11 (5, 13)	0.4012
	Habitat x transect	0.82 (4, 13)	0.5361
Fall 2008			
Number of individuals†	Habitat	0.54 (1, 20)	0.4690
	Transect	6.63 (5, 20)	**0.0009**
	Habitat x transect	0.34 (5, 20)	0.8811
Number of flowering†	Habitat	5.17 (1, 13)	**0.0406**
	Transect	2.54 (5, 13)	0.0817
	Habitat x transect	0.86 (4, 13)	0.5131

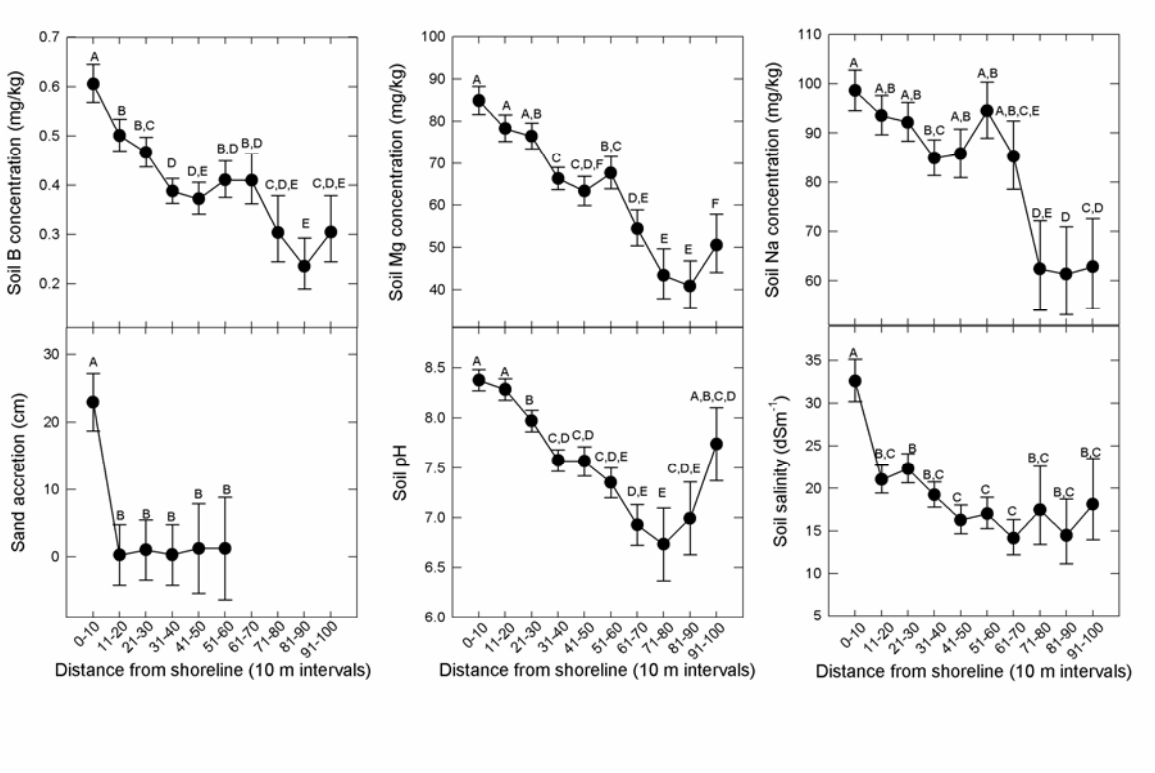

Figure 2.1. Environmental variables which varied significantly with distance class from shoreline (Summer 2006 study). LS means ± 1 standard error are presented. Sand accretion was only measured to 60 m along the transects. Different letters indicate significantly different means. Environmental variables which had no clear pattern related to distance from shoreline (Ca, soil moisture 75-100 cm) and which did not vary significantly with distance (P, soil moisture 0-25, 25-50, 50-75 cm) are not shown.

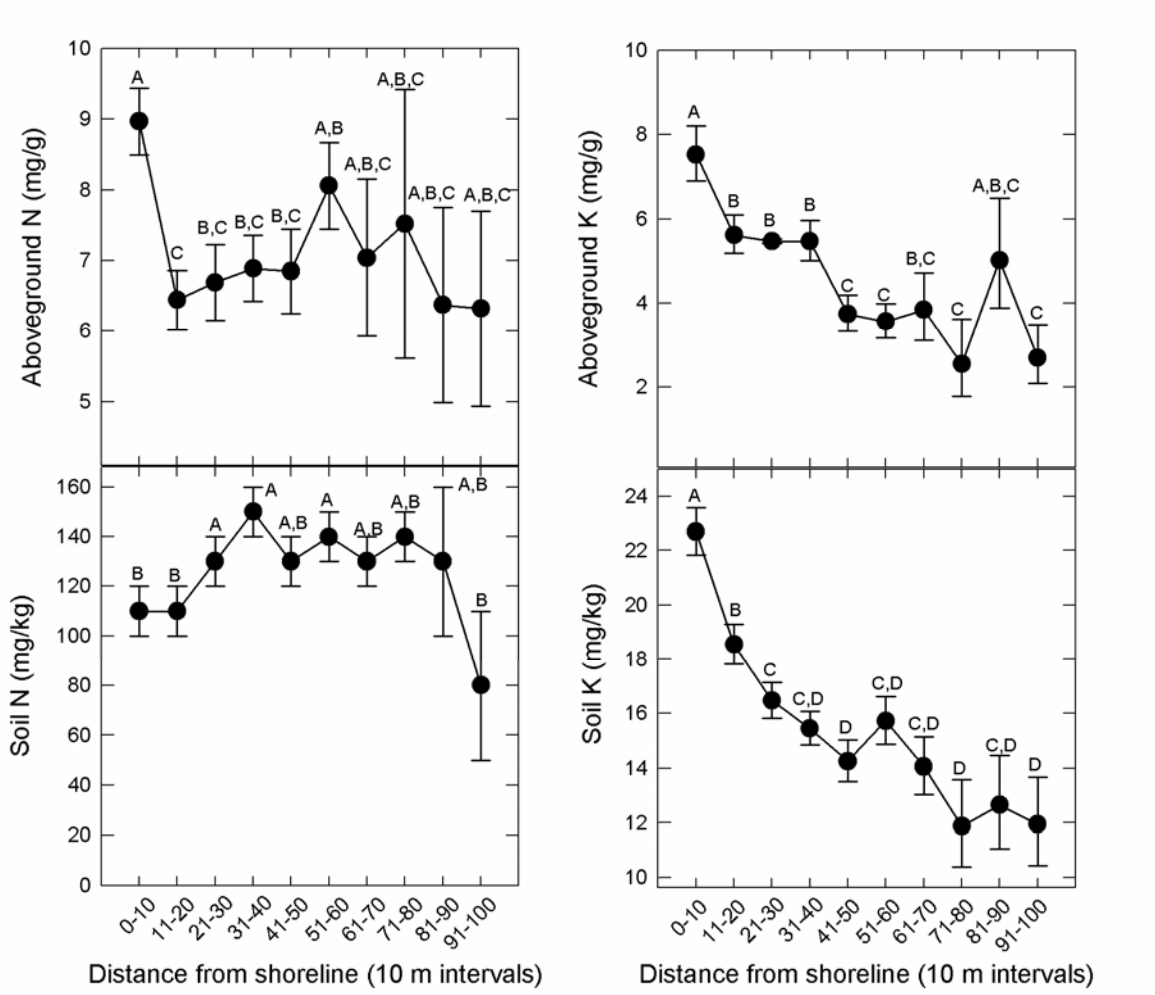

Figure 2.2. *Uniola paniculata* tissue nutrient concentrations which varied significantly with distance from shoreline, shown with corresponding soil nutrient concentrations (Summer 2006 study). LS means ± 1 standard error are presented. Different letters indicate significantly different means.

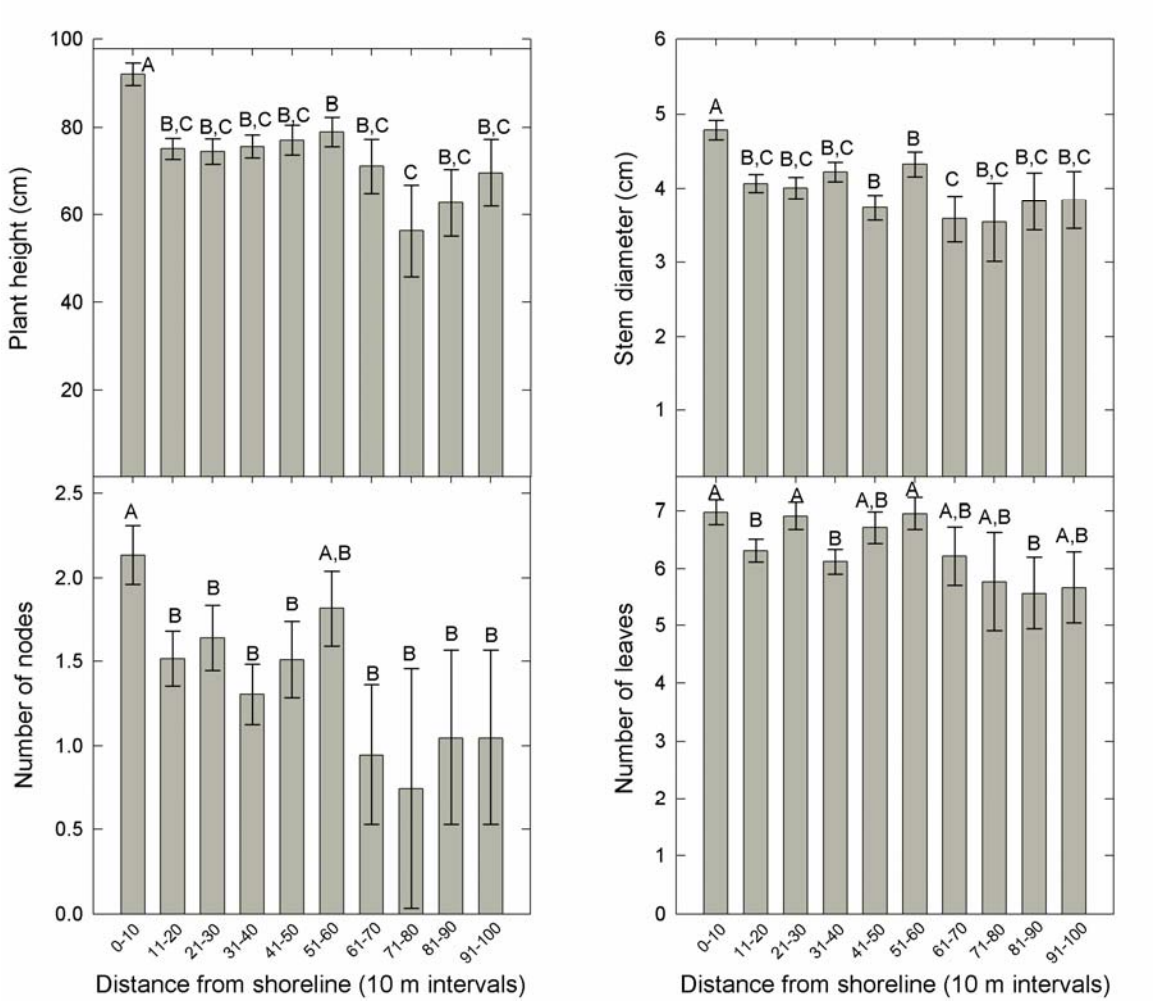

Figure 2.3. *Uniola paniculata* morphological traits which varied significantly with distance from shoreline (Summer 2006 study). LS means ± 1 standard error presented. Different letters indicate significantly different means.

CHAPTER 3

NO EVIDENCE OF LOCAL ADAPTATION IN *UNIOLA PANICULATA* L. (POACEAE), A COASTAL DUNE GRASS[1]

[1] Gormally, C.L. and L.A. Donovan. Submitted to *Southeastern Naturalist*, 1/8/2010.

Abstract

Studies of local adaptation generally investigate plants growing in relatively stable habitats. We asked whether populations of the long-lived clonal grass, *Uniola paniculata*, are locally adapted to microhabitats in the southeastern U.S. coastal dunes, a habitat characterized by dynamic environmental gradients spanning relatively small distances. Although vegetative zonation is well characterized across these gradients, little is known about intraspecific evolutionary responses of species spanning the gradients. Plants from the foredune and backdune areas of the gradient (<10m and 40-60 m from the shoreline, respectively) were reciprocally transplanted into experimental plots in both habitats. Although foredune plots were washed away by storms before harvest, early morphological growth measures indicated no local adaptation for foredune plants. At harvest, we found no evidence of local adaptation for backdune plants in the backdune plots, with no advantage in survival, growth or total biomass. In frequently disturbed environments such as the coastal dunes, plants may be more likely to respond with phenotypic plasticity than through local adaptation.

Introduction

Natural selection can drive local adaptation when plant species encounter heterogeneous environmental conditions (Clausen et al. 1940, Hereford 2009, Turesson 1922,), both at the landscape level across the span of their ranges, as well as at finer, microhabitat scales (Antonovics and Bradshaw 1970). Local adaptation is the association of certain genotypes with particular habitats, with the locally adapted genotypes outperforming "foreign" genotypes (Kawecki and Ebert 2004, Linhart and Grant 1996). Most field-based reciprocal transplant experiments of local adaptation target plant species growing in environments characterized by abiotic spatial heterogeneity due to conditions such as toxic soils, fertilizers, herbicides,

elevation, light conditions, temperature, and soil water availability, rather than environments that are frequently disturbed (Leimu and Fischer 2008, Linhart and Grant 1996) (but see Galloway and Fenster 2000, a study of an annual legume grown in disturbed sites). However, much less is known about whether long-lived plant species located in frequently disturbed environments tend to be locally adapted or respond plastically to temporal heterogeneity (Gregor 1930, Leimu and Fischer 2008, Miller and Fowler 1994).

Coastal sand dunes are spatially and temporally heterogeneous. This is in part due to frequent disturbances that can result in the removal of low-lying foredunes, partial destruction of larger dunes, the erosion of dune profiles and even complete habitat destruction (e.g. submerging of an entire barrier island) (Claudino-Sales et al. 2007). In addition to physical disturbance, environmental conditions—including salt spray, sand accretion, and soil nutrients— may differ dramatically across distances as small as 0.5 m, driving changes in plant community composition and species' distributions (Barbour et al. 1999, Boyce 1957, Cowles 1899, Doing 1985, Maun and Perumal 1999, Oosting and Billings 1942, Smith and Smith 2001, Wagner 1964,). Plants in these habitats demonstrate intraspecific variation in morphological, physiological, and phenological traits (Gormally and Donovan, in review, Jerling 1985, Wagner 1964). Among the grasses studied, as the distance from the shoreline increases, plant size generally decreases, and proportions of flowering, seeding, and establishment decline (Gormally and Donovan, in review, Jerling 1985, Wagner 1964). While extensive ecological research has been conducted in order to understand the role of abiotic conditions in species zonation and primary succession on coastal dunes (Barbour et al. 1999, Boyce 1957, Cowles 1899, van der Valk 1974, Wilson and Sykes 1999), we know relatively little about the evolutionary responses of plant populations to the environmental heterogeneity of coastal dune systems.

Tests of local adaptation in coastal dunes are affected not only by the dynamic coastal dune environment, which is frequently altered by sand erosion and accretion, but also by the growth habit of dune plants, most of which are long-lived and clonal (Cheplick and White 2002, Gray 1985, Knight and Miller 2004). Vegetative reproduction may reinforce local adaptation, through the level of clonal diversity as well as through clonal growth patterns, e.g. the establishment of independent daughter ramets in the same habitat as the maternal plant. Alternatively, guerrilla clonal growth patterns—spreading rather than clumping placement of ramets— may prevent the association of particular genotypes with certain habitats, decreasing the potential for population differentiation and local adaptation.

Phenotypic plasticity is another response to heterogeneous environmental conditions (Schlichting and Pigliucci 1998, Via and Lande 1985), which may evolve when selective pressures fluctuate frequently (Schlichting and Pigliucci 1998). The ability to respond plastically is a means to deal with environmental variability, as adjusting its phenotype in response to particular environmental cues may allow an individual to maximize its fitness potential (Schlichting and Pigliucci 1998). Disturbances to the coastal dune habitat are frequent relative to its plant inhabitants' long life spans. Erosion can result in the removal of foredune habitat, exposing plant populations previously situated on the backdunes to the unprotected foredune conditions. Alternately, sand accretion can result in the building of new dunes along the shoreline, so that established plant populations experience changed conditions when new dunes act as buffers from salt spray and sand movement. If the time scale of disturbance is shorter than the lifespan of an individual genet, one individual may produce multiple phenotypes—through clonal reproduction of daughter ramets—in response to fluctuating environmental conditions (van Kleunen and Fischer 2001). Thus, processes such as phenotypic plasticity may facilitate an

individual's success in environments which experience frequent disturbances or are spatially heterogeneous.

We chose to use *Uniola paniculata* L. (Poaceae) (Sea Oats) to investigate population-level evolutionary responses, since its natural history, as a rhizomatous perennial grass which reproduces both clonally and sexually, is representative of many dune plant species (Wagner 1964). *Uniola paniculata* is a federally protected plant due to its role in stabilizing dune habitats, and is frequently used in dune restoration projects. *Uniola paniculata* is the dominant plant species on the primary dunes where it occurs, from Virginia southward along the Atlantic coast to the Bahamas and along the Gulf coast to Veracruz, Mexico (Wagner 1964). On the dunes, *U. paniculata*'s habitat spans a localized dune gradient from the dynamic foredunes (embryonic dunes) to the older, stabilized backdunes situated farther inland.

We investigated whether foredune and backdune populations of *U. paniculata* were locally adapted to microhabitats across its shoreline-to-landward range on the coastal sand dunes. In a previous mensurative field study, we documented intraspecific trait variation in populations of *Uniola paniculata* located in the dynamic foredunes and on the stabilized backdunes that mirrored the underlying habitat variation (Gormally & Donovan, in review). However, that study could not identify the underlying process driving this variation. A previous allozyme study demonstrated that there is no strong regional pattern of genetic structure among populations of *U. paniculata*, but fine-scale clonal structure and diversity vary widely, perhaps resulting from localized microhabitat differences (Franks et al. 2004). In this manipulative study, we asked whether populations of *U. paniculata* were locally adapted to foredune and backdune microhabitats as evidenced by ramets transplanted from their source habitats outperforming ramets from the other habitat.

Materials and Methods

All fieldwork was conducted on the coastal sand dunes at the National Estuarine Research Reserve System (NERRS) on Sapelo Island, located on the Atlantic coast of Georgia. Sapelo Island beach dunes are protected through the NERRS, as well as through the Georgia Coastal Ecosystems Long Term Ecological Research Network (GCE-LTER). Thus, these dunes are ideal settings to document the evolutionary responses of natural coastal dune plant populations, as the dune habitats are not currently impacted by anthropogenic development or foot traffic, although cattle were regularly allowed to graze on the dunes until 1970.

Vegetation on the Sapelo Island dunes is typical of southeastern Atlantic coastal dunes, consisting primarily of clonal perennial grass and vine species. *Uniola paniculata* reproduces both vegetatively and sexually, although vegetative reproduction may contribute more to fitness than seed production, as a high percentage of ovules are aborted (Hester and Mendelssohn 1987). On Sapelo, *U. paniculata*'s growing season begins in late March, when plants develop new leaves to replace the previous year's leaves, and ends in October and plants are quiescent during the winter (Wagner 1964). On average, annual precipitation is 115 cm, with about 80% occurring during the growing season (2000-2005, Georgia Coastal Ecosystems Long-Term Ecological Research network data). From July 2006 until November 2008, conditions in coastal Georgia ranged from a moderate to severe drought (National Oceanic Atmospheric Administration 2009).

We conducted two reciprocal transplant experiments, designated as Summer 2006 and Fall 2007 (referring to the date plants were transplanted into experimental plots). The foredune habitat was defined as the first 10 meters closest to the shoreline and the backdune habitat was defined as the farthest inland edge of the species' range, based on previous characterization of the environment at this study site (Gormally & Donovan, in review). The foredune habitat is

characterized by greater levels of soil boron, potassium, magnesium, manganese, as well as higher soil salinity, pH, and sand accretion, than the backdune habitat, which experiences less sand movement in terms of accretion and erosion and is consequently a more stable habitat (Gormally & Donovan, in review). For both the Summer 2006 and Fall 2007 experiments, we set up three, 3 m^2 reciprocal transplant plots in each habitat (foredune and backdune), with the plots located at least 300 m apart within each of the habitats (corresponding to 3 of the transects in Gormally & Donovan, in review). Plot locations in the backdune habitat varied from 40 to 60 m inland from the foredune, according to the presence of the species, which varied with island geomorphology (Stallins and Parker 2003). Existing vegetation was cleared from each plot at least four months prior to transplanting. Plots were weeded to remove other vegetative growth throughout the course of both experiments.

The *U. paniculata* ramets were collected from the foredune and backdune habitats and are referred to as the foredune (FD) and backdune populations (BD), respectively. To ensure that ramets represented distinct genets, they were always dug up from locations separated by at least 3 m (Franks et al. 2004). When collected, the ramet consisted of a tiller—an aboveground stem formed from an axillary bud on a maternal ramet—and a variable amount of roots. For the Summer 2006 experiment, we collected 412 total ramets in June 2006 (N=207 FD ramets and N=205 BD ramets). The ramets were initially planted in 25.4 cm^3 pots containing sand, and then maintained in a open-air shaded greenhouse on Sapelo to facilitate root growth and minimize environmental or maternal effects (Roach and Wulff 1987). In August 2006, the surviving ramets were transplanted into experimental plots, 30 ramets per plot (N=89 FD ramets and N=91 BD ramets), for a total of N=180 ramets in the Summer 2006 experiment. Ramets were watered for two weeks following transplant to promote establishment. We measured the basal stem diameter

and height of the Summer 2006 ramets at transplant (August 2006) to account for initial size, and then at six months (February 2007) to assess growth. The Summer 2006 foredune plots were completely washed away during storms in April 2007. Backdune plots remained and surviving ramets were measured for height and stem diameter at 1 year (August 2007) and harvested at 2 years (September 2008). At harvest, ramets were assessed for stem diameter, height, number of tillers, and total dry biomass (dried at 60°C).

The Fall 2007 experiment was set up after the Summer 2006 foredune plots were washed away. For this experiment, we adjusted the experimental design to include more ramets and collected two ramets per genet in order to place the same genotype into plots in each habitat (360 ramet pairs for a total of N=720 individual ramets). The ramets were initially planted in 25.4 cm^3 pots of sand and maintained in the University of Georgia Plant Biology greenhouses in Athens, Georgia, for approximately 4.5 months to facilitate root growth and minimize environmental or maternal effects (Roach and Wulff 1987). In November 2007, the 326 surviving ramets (161 FD and 165 BD ramets) were transplanted into experimental plots, and were watered for two weeks to promote establishment. We measured the wet biomass of the Fall 2007 ramets at transplant to account for initial size. The Fall 2007 foredune plots washed away during storms in December 2007, so no growth measurements were possible for these plots. The Fall 2007 backdune plots remained and surviving ramets were harvested after 1 year (September 2008). At harvest, ramets were assessed for stem diameter, height, number of tillers, and dry biomass (dried at 60° C).

For the Summer 2006 experiment, we compared survival and growth of FD and BD ramets in foredune and backdune plots at 6 months, and in backdune plots at 1 year and at harvest. For both the Summer 2006 and Fall 2007 experiments, only backdune plot data were available for comparison of survival and growth of FD and BD ramets at 1 year and at harvest. Survival was

analyzed with a chi square (χ^2) test. Growth (stem diameter, height, and biomass) of surviving ramets was analyzed with analysis of covariance (ANCOVAs), with source population as the main predicting variable, and using stem diameter or height at transplant as covariates for the Summer 2006 experiment and wet biomass at transplant as the covariate for the Fall 2007 experiment. To determine whether the amount of vegetative reproduction, as indicated by number of tillers, differed between populations, we used analysis of variance (ANOVAs).

Results

At six months after transplantation into reciprocal transplant plots, there was no evidence that either FD or BD ramets were locally adapted to their respective home habitats, using non-destructive growth measurements of height and stem diameter (Tables 3.1 and 3.2). Although there was a significant population effect, FD plant stem diameter increased more than that of BD ramets during this interval—the reverse of the pattern that would support local adaptation in the backdune habitat. Nor was there evidence of differential survival in either habitat (Foredune χ^2=0.37, df=3, N=89; Backdune χ^2=0, df=3, N=90).

After a year of growth in the backdune plots, there were no differences between FD and BD ramets for survival (χ^2=0.79, df=3, N=90), height or stem diameter (Table 3.2). At harvest, only 15 ramets were alive (7 FD and 8 BD ramets), and there was no differential survival of FD and BD ramets (χ^2=0.00891, df=3, N=90). There were also no differences in size of surviving FD and BD ramets at harvest: stem diameter, height, total dry biomass, and vegetative reproduction (estimated as number of tillers produced).

Unfortunately, history repeated itself in December 2007 and nor'easters—storms known for coastal erosion and flooding which occur in winter and early spring, with hurricane-force winds originating from the northeast—completely destroyed the second set of the recently transplanted

foredune plots, leaving only the backdune plots intact. At harvest, only 13 ramets (7 FD and 6 BD ramets) remained alive in the backdune plots, with no differential survival by population (χ^2=0.05829, df=3, N=156). At harvest, there were no differences between populations in stem diameter or height, total dry biomass or number of tillers produced (Table 3.3).

Discussion

Our study contributes to the small but growing body of evidence that clonal plants are more likely to respond through the strategy of phenotypic plasticity than local adaptation in frequently disturbed habitats. Using measurements of growth in the field and, most significantly, survival in the field during an extreme drought, we found no evidence that populations of *U. paniculata* were locally adapted to specific microhabitats across a shoreline-to-landward environmental gradient. This finding helps to explain the results from a previous mensurative field study, that FD ramets were morphologically and physiologically different (larger in height, stem diameter, number of nodes, with higher aboveground tissue concentrations of N and K) and more likely to flower than ramets located farther inland (Gormally & Donovan, in press). The phenotypic variation detected in the previous study mirrored the underlying environmental variation, with increased soil salinity, sand accretion, soil pH, and concentrations of soil nutrients (B, K, Mg, and Na) in the 10 m interval closest to the shoreline. To demonstrate that populations were locally adapted, transplant populations would have to outperform populations from other sites in the reciprocal transplant plots located in their source habitat (Kawecki and Ebert 2004, Linhart and Grant 1996). However, there was no evidence from our reciprocal transplant experiments to support the hypothesis that the trait variation previously documented in naturally occurring populations was due to local adaptation or genetic differentiation of any kind.

Early growth measurements from both the foredune and backdune plots provided no evidence of population differentiation. At harvest, there was no differential survival by populations in the backdune plots. Though the plots in the backdune habitat were unaffected by the nor'easters, mortality was substantial, with <10% of ramets from both the Summer 2006 and Fall 2007 experiments surviving to the time of harvest. Growth and survival following transplantation were likely negatively impacted by the moderate to extreme drought conditions which lasted from May 2006 to August 2008. Nearly 30% of the ramets in the Summer 2006 experiment died during the six month period of May-December 2007, characterized as a severe to extreme drought, according to the NOAA Palmer Drought Index (National Oceanic Atmospheric Administration 2009).

Results from the few studies that not only document variation in natural populations but also explicitly test for local adaptation of coastal perennial grasses are consistent with our findings. The two other studies reported variation in phenotypic responses to abiotic conditions but, consistent with our findings, populations of *Triplasis purpurea*, located 15 m and 80 m from the shoreline (Cheplick and White 2002), and foredune and mature dune populations of *Ammophila arenaria* (Gray 1985), were not locally adapted. A study of a coastal perennial vine, *Hydrocotyle bonariensis*, did find evidence of local adaptation, but these populations were adapted to microhabitats located at high and low dune heights (Knight and Miller 2004), different from the microhabitats tested in our study. Additionally, local adaptation of *H. bonariensis* appeared to be associated with interactions of the surrounding vegetation at each microhabitat (Knight and Miller 2004).

Most dune plant species are clonal perennials, propagating both by sexual and vegetative reproduction. Though clonal reproduction does not include recombination, clonal plants are not

less genetically diverse (Hamrick and Godt 1989). One expectation is that clonality would increase the likelihood of local adaptation through the reduction of gene flow and increased placement of a genet's ramets into preferentially locally adaptive sites. However, clonality may reduce local adaptation if genets are adapted to past environmental conditions, so that populations might be genetically differentiated, but not adapted to their current habitat (Callaghan et al. 1996). Local adaptation may also be constrained when strong gene flow prevents differentiation, when selection is constrained by low amounts of genetic variation, or when natural selection fluctuates due to strong spatial variability. Given the changeable nature of the coastal sand dune environment, one genet might experience multiple environments over the course of its life. Under fluctuating environmental conditions, plasticity may be more likely to have evolved than local adaptation (Mitchell-Olds 1992, Richards et al. 2010). In a greenhouse study of the clonal salt marsh perennial, *Borrichia frutescens*, Richards et al. (2010) determined that trait variation along a salinity gradient was due to phenotypic plasticity (2010). It seems likely that the trait variation we previously documented (Gormally & Donovan, in review) was a result of phenotypic plasticity rather than local adaptation (Schlichting and Pigliucci 1998, Via and Lande 1985).

Our study highlights the challenges implicit in addressing the question of how long-lived plants respond to selection in a variable environment that may change through the course of an individual's lifetime. Since *U. paniculata* is a long-lived perennial grass, identifying the environments to which these populations may be adapted is difficult. On the coastal dunes, selective pressures may fluctuate frequently, particularly due to periods of sand accretion and erosion, sometimes resulting in the removal of foredunes, exposing populations of plants previously situated on the backdunes to increased disturbance from sand accretion and erosion.

Further, as a clonal plant capable of sexual and vegetative reproduction, *U. paniculata* may respond to its environment at both the level of the genet—through the processes of local adaptation or phenotypic plasticity—and the ramet—through physiological integration, developmental plasticity, and selective placement of daughter ramets (van Kleunen and Fischer 2001). We used ramets rather than seedlings due to concerns about seedling survival following transplantation, but it is possible that seedlings might respond differently than ramets to transplantation and might differ in average lifespan. We lack specific knowledge about the average lifespan of an individual ramet, as well as average lifetime of a genet.

Our finding of no evidence of local adaptation in *Uniola paniculata* may have positive implications for conservation and restoration efforts. Despite dramatic environmental differences across the coastal dunes, we found no evidence that populations are locally adapted across dune microhabitats. This means that plant material for this species can be sourced without concern for the visible intraspecific variation that exists in these microhabitats, since genotypes from each microhabitat should be equally likely to flourish in any habitat. However, studies of populations across the species' entire range are needed in order to understand larger geographic patterns. Current conservation and restoration considerations underscore the necessity for acquiring a better understanding of the evolutionary responses of natural plant populations on the coastal dunes, despite the challenges implicit in addressing these questions.

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Table 3.1. *Uniola paniculata* growth for Summer 2006 experiment foredune plots measured at 6 months after transplant. LSmeans ± 1 SE are presented. (***=$p \leq 0.0001$)

	Foredune Plot		ANCOVA Effects	
	BD ramets	FD ramets	*Covariate (initial size)*	*Population*
Height (cm)	14.173 ± 0.952	13.422 ± 0.967	$\boldsymbol{F_{(1,62)}=45.44^{***}}$	$F_{(1,62)}=0.29$
Stem diameter (cm)	2.450 ± 0.165	2.898 ± 0.187	$F_{(1,68)}=2.05$	$F_{(1,68)}=3.17$

Table 3.2. *Uniola paniculata* growth (measured in cm) for Summer 2006 experiment backdune plots measured at 6 months, 1 year, and harvest (2 years). LSmeans ± 1 SE are presented. (*=$p<0.05$, **=$p<0.01$, ***=$p\leq0.0001$)

	Backdune Plot		ANCOVA Effects	
Measurements at 6 mon.	BD ramets	FD ramets	*Covariate (initial size)*	*Population*
Height	11.821 ± 1.086	13.058 ± 1.073	$\mathbf{F_{(1,82)}=39.26***}$	$F_{(1,82)}=0.61$
Stem diameter	2.327 ± 0.187	3.051 ± 0.182	$F_{(1,81)}=0.69$	$\mathbf{F_{(1,81)}=7.34**}$
Measurements at 1 year				
Height	53.872 ± 2.691	54.228 ± 2.812	$\mathbf{F_{(1,72)}=9.08*}$	$F_{(1,72)}=0.01$
Stem diameter	3.808 ± 0.264	3.774 ± 0.272	$F_{(1,67)}=0.03$	$F_{(1,67)}=0.01$
Measurements at harvest				
Biomass	9.899 ± 1.752	10.431 ± 1.874	$F_{(1,12)}=1.01$	$F_{(1,12)}=0.04$
Height	50.333 ± 3.817	57.705 ± 4.083	$F_{(1,12)}=1.79$	$F_{(1,12)}=1.72$
Stem diameter	3.37 ± 0.323	3.838 ± 0.426	$F_{(1,10)}=0.02$	$F_{(1,10)}=0.65$
Tillers	2.375 ± 0.676	2.429 ± 0.723	*n/a*	$F_{(1,13)}=0$

Table 3.3. *Uniola paniculata* measurements at harvest (1 year) for the Fall 2007 experiment. LSmeans ± 1 SE are presented. (*=$p<0.05$)

	Backdune Plot		ANCOVA Effects	
	BD ramets	FD ramets	*Covariate (initial size)*	*Population*
Biomass (g)	4.756 ± 0.80	3.22 ± 0.738	$\mathbf{F_{(1,10)} = 7.66^{*}}$	$F_{(1,10)}=1.91$
Height (cm)	33.259 ± 7.743	32.564 ± 7.144	$F_{(1,10)}=1.33$	$F_{(1,10)}=0$
Stem diameter (cm)	2.686 ± 0.395	2.259 ± 0.365	$F_{(1,10)}=3.22$	$F_{(1,10)}=0.60$
Tillers (#)	1.333 ± 0.182	1.143 ± 0.169	$F_{(1,10)}=0.05$	$F_{(1,10)}=0.41$

CHAPTER 4

COMPARISON OF QUANTITATIVE TRAIT VARIATION AND ALLOZYME DIVERSITY WITHIN AND AMONG POPULATIONS OF SEA OATS, *UNIOLA PANICULATA* L. (POACEAE), ACROSS AN ENVIRONMENTAL GRADIENT[1]

[1] Gormally, C.L., J.L. Hamrick, and L.A. Donovan. *To be submitted to* Conservation Genetics.

Abstract

Understanding the underlying cause of phenotypic trait variation among populations is important for informing conservation decisions. This knowledge can be used to determine whether locality matters when sourcing populations for transplantation in habitats undergoing restoration. *Uniola paniculata* is a federally protected coastal dune grass native to the southeastern Atlantic and the Gulf coasts which is often used to stabilize restored dune habitats. This study uses neutral genetic markers (allozymes) and greenhouse common garden approaches to determine the relative contributions of neutral evolutionary processes and natural selection to phenotypic variation among natural populations of *U. paniculata*. Seeds were collected from foredune and backdune populations spanning shoreline-to-landward environmental gradients on each of four Georgia barrier islands. Based on previous work, we expected to find evidence of divergent selection among populations located on the shoreline-to-landward environmental gradient. Differences among islands, rather than intra-island habitat differences, have resulted from divergent selection on aboveground and total biomass. Previously documented intra-island trait variation is likely due to phenotypic plasticity. Our findings have implications for conservation and restoration efforts involving *U. paniculata*, as there is evidence for divergent natural selection even among populations located on closely neighboring islands.

Introduction

Determining the underlying mechanism driving trait variation among populations is particularly critical for informing restoration and conservation decisions, e.g., choosing plant material for transplanting (Lynch 1996). The source of plant material to be transplanted may have a profound effect on the success of transplant establishment, due to a population's evolutionary history and factors such as genotype-environment matching, i.e., local adaptation

(Rice et al. 1997, McKay et al. 2005). However, multiple evolutionary and ecological processes may produce trait variation, including divergent selection due to heterogeneous environmental conditions driving local adaptation (Turesson 1922, Clausen et al. 1948, Hereford 2009), phenotypic plasticity (Via & Lande 1985, Schlichting & Pigliucci 1998), historical genetic structure (Batista *et al.* 2004), genetic drift, and barriers to gene flow (Briggs & Walters 2001). Consequently, identifying the principal cause driving trait variation is challenging, since multiple processes may result in similar outcomes.

An effective way to unravel these multiple hypotheses is to compare patterns of quantitative trait variation with neutral genetic variation. Greenhouse common garden experiments are used to assess the genetic basis of variation in quantitative traits, under environmental conditions that minimize environmentally induced trait variation, and genetic markers are used to measure neutral genetic variation (Merila & Crnokrak 2001). Wright's F_{ST} can be used to examine the distribution of neutral genetic variation within and among populations (Wright 1965). There is a comparable measure for quantitative traits, Q_{ST}, which can be directly compared to F_{ST} (Spitze 1993). F_{ST} is based on genetic markers that are subject to drift, mutation, and gene flow, but not selection, while Q_{ST} is estimated from quantitative traits that are potentially subject to all evolutionary pressures, including selection (Workman and Niswander 1970; McKay and Latta 2002). There are three possible outcomes resulting from the comparison of the two analyses (Merila & Crnokrak 2001):

1. If Q_{ST} is not significantly different from F_{ST}, the degree of trait differentiation is what is expected by genetic drift and gene flow.
2. If Q_{ST} is significantly greater than F_{ST}, selection on the trait is divergent across the sampled range of populations.

3. If Q_{ST} is significantly less than F_{ST}, selection is uniform across the sampled range of populations.

Coastal dunes are spatially and temporally heterogeneous habitats. Along a localized environmental gradient spanning the dynamic embryonic foredunes to the more stabilized dunes situated further inland, environmental conditions can differ across distances as small as 0.5 m, driving changes in plant community composition and species' distributions (Cowles 1899, Oosting & Billings 1942, Boyce 1954, Wagner 1964, Doing 1985, Barbour *et al.* 1999, Maun & Perumal 1999, Smith & Smith 2001). Dune habitats are often detrimentally impacted by human activities, including dune destruction due to construction (Davis Jr & Fitzgerald 2004), changes in on- and off-shore sand movement due to hard protection structures (e.g. jetties, seawalls, groins) (Davis Jr & Fitzgerald 2004), changes in plant species distribution resulting from human trampling (Acosta et al. 2000) and changes to dune form and plant community as a result of planting exotics, ostensibly to stabilize dunes (Wiedemann & Pickart 1996). Understanding the responses of natural populations in undisturbed habitats to microgeographic variation across this shoreline-to-landward environmental gradient, as well as population genetic structure, can provide input for restoration and conservation decisions (Lynch 1996). Considering that coastal dune habitats are dynamic and frequently disturbed, understanding the underlying genetic responses to heterogeneous environmental factors may inform management strategies for the long-term persistence of these populations (Franks 2009).

We estimated neutral genetic variation using allozymes and examined trait variation in *Uniola paniculata*, a dominant perennial coastal dune grass on primary dunes. *Uniola paniculata* is protected by law because it stabilizes dune habitats, and is frequently used in dune restoration projects. We chose to use *U. paniculata* to investigate population-level evolutionary responses,

because its natural history as a rhizomatous perennial grass capable of both sexual and clonal reproduction is representative of many dune plant species (Duncan & Duncan 1987). Its habitat on the dunes spans a localized dune gradient from the foredunes to dunes situated farther inland. The distribution of *U. paniculata* ranges along the southeastern Atlantic coast from Virginia to the Bahamas and the Gulf coast to Veracruz, Mexico (Wagner 1964). At the geographic scale, there are only weak regional patterns of genetic structure among populations of *U. paniculata* (Franks *et al.* 2004) (but see (Subudhi et al. 2005)).

Since coastal dunes are spatially and temporally heterogeneous habitats, we designed this study expecting that divergent selection pressures along the environmental gradient were responsible for documented patterns of phenotypic variation among populations that mirrored the underlying habitat variation along the dune gradient (Gormally and Donovan, *in press*). In a reciprocal transplant experiment, we found no evidence of local adaptation; however, the test for local adaptation to each microhabitat was compromised as foredune plots were washed away prior to harvest (Gormally and Donovan, *in review).* Other research findings suggested that localized microhabitat differences might drive trait variation among *U. paniculata* populations, as fine-scale clonal structure and diversity have been shown to vary widely even in relatively small areas (Franks *et al.* 2004, Bush & Stelato 2007). Here, comparative studies of quantitative genetic trait variation and neutral allozyme variation, considered in light of these previous results, are used to determine whether phenotypic trait variation among populations of *U. paniculata* is due to phenotypic plasticity, divergent selection, or genetic drift.

Materials and Methods

In November 2006, we collected panicles from four barrier islands on the Georgia coast: Nannygoat Beach on Sapelo, Cabretta, Jekyll, and Sea Island (Table 4.1). Cabretta and Sea

Island were formed 4,000-5,000 years ago during the Holocene with the rise of sea level following the last glacial maximum. Sapelo Island was formed during the Pleistocene, 35,000-40,000 years ago, before the last glacial maximum. Nannygoat Beach is technically a separate island that was formed during the Holocene. Parts of Jekyll Island were formed during both the Pleistocene and Holocene.

On each island, panicles were collected from two populations, dynamic foredunes and stabilized backdunes. In each population, panicles were collected from 20 individuals located at least 3 m apart to increase the likelihood that seeds from unique genetic individuals were sampled (Franks et al. 2004).

In January 2008, 1624 seeds were removed from panicles, scarified by nicking the seed coat with a razor blade, and germinated in Petri dishes in a growth chamber (95°F for 7 hours/ 65°F for 17 hours). Since some panicles contained few or no seeds, several populations represented in the study contain fewer families and individuals within families. Seedlings were transplanted into 2.5 x 10 inches (40 $inch^3$ capacity) Deepots (Steuwe and Sons) and grown under ambient conditions in the University of Georgia Plant Biology greenhouses.

We measured 10 quantitative traits. These traits, describing plant morphology and phenology, were measured either during plant growth (time to germination; seedling height, measured in March 2008; adult stem diameter, measured in May 2008) or at harvest in November 2008 (average root diameter of the three largest roots; number of ramets, rhizomes, and roots; and above-, below-, and total biomass, dried to constant weight). Since *U. paniculata* is a grass, we were limited in our choice of quantitative traits to measure. We chose to measure traits that might be ecologically important to the species, and which might affect plant success depending on variation in localized abiotic conditions, e.g., sand burial. As *U. paniculata*

reproduces both sexually and clonally, we measured number of ramets, since reproductive strategy might differ by habitat. We measured biomass partitioning (above- and below- ground biomass; number of ramets and rhizomes) since dune species tend to respond to sand burial by increasing size through shifts in resource allocation (e.g., shifting resources from roots to aboveground biomass) (Zhang & Maun 1992, Martinez & Moreno-Casasola 1996, Brown 1997, Perumal & Maun 2006).

A total of 117 seedlings were crushed for protein extraction using liquid nitrogen, sea sand, and Camelia crushing buffer (Wendel & Parks 1982). These 117 seedlings included 53 germinated from panicles collected from backdune populations and 64 from foredune populations from Jekyll, Sapelo, Cabretta, and Sea Island, Georgia, following the collection protocol described above. Extracted proteins were run on 9.5% starch gels and fourteen enzymes were examined: asparate amino transferase (*Aat*), diaphorase (*Dia*), glutamate dehydrogenase (*Gdh*), malic enzyme (*Me*), and triose-phosphate isomerase (*Tpi*) on buffer system 8-; menazion reductase (*Mnr*), phosphoglucoisomerase *(Pgi)*, and phosphoglucomutase (*Pgm*) on buffer system 6; 6-phosphogluconate dehydrogenase (*Pgd*), F-1,6-diphosphate (*F1,6*), and malate dehydrogenase (*Mdh*) on buffer system 4; and adenylate kinase (*Ak*), isocitrate dehydrogenase (*Idh*), and shikimate dehydrogenase (*Skdh*) on buffer system 11 (Soltis *et al.* (1983) for all buffers and stains except: *Aat*, *Ak*, *Dia*, and *Mnr* (Cheliak & Pitel 1984)). Following the established protocols cited above, gels were stained with enzyme-specific stains and scored for allozyme banding patterns, with interpretation guided by Weeden and Wendel (1989) and Kephart (1990).

For each quantitative trait, variance components were used to estimate Q_{ST} following Spitze (1993). For each quantitative trait, variance was partitioned into population, family nested

within population, and error components (PROC MIXED, SAS 9, Cary, NC). The population variance component was used as the estimate of among population variance (σ^2_b) and four times the family within-population variance component was used as the estimate of within-population variance (σ^2_w) to account for the half-sibling (i.e., open pollinated) design (Lynch & Walsh 1998). For each Q_{ST} value, standard error was estimated as the standard deviation of Q_{ST} estimates calculated from 1,000 iterations bootstrapped over all samples. The model structure described above was used to test three comparisons: (1) comparison of individual populations, accounting for both island origin and habitat type (n=8 populations); (2) comparison of foredune and backdune habitats, with populations pooled across islands (n=2 populations); and (3) comparison of islands, with populations pooled across habitats within each island (n=4 populations). We present the results of the latter two comparisons to explicate our primary findings from the comparison of individual populations, with the caveat that the latter two models may have resulted in artificially inflated or deflated estimates, depending upon the manner in which populations were pooled.

For each quantitative trait, narrow-sense heritabilities (h_n^2) were estimated for each population using family and residual variance components from a separate random model (PROC MIXED, SAS 9, Cary, NC). To account for the half-sibling design, h_n^2 was calculated as four times the within family variance component divided by the sum of the family and residual variance components (Falconer & Mackay 1996).

F_{ST} was calculated following Wright (1965) with the program GDA (Weir 1996, Lewis & Zaykin 2001), and bootstrapped confidence intervals were estimated over 1,000 iterations. Values of F_{ST} were calculated to test three comparisons: (1) comparison of individual populations, accounting for both island origin and habitat type (n=8 populations); (2) comparison

of foredune and backdune habitats, with populations pooled across islands (n=2 populations); and (3) comparison of islands, with populations pooled across habitats within each island (n=4 populations). The following genetic diversity parameters were estimated for each locus and for the three comparisons of populations described above, as well as pooled across all populations, using the program GDA (Lewis & Zaykin 2001): the percentage of polymorphic loci (*P*), the mean number of alleles per locus (*A*), the mean number of alleles per polymorphic locus (*Ap*), and observed (H_o) and expected heterozygosity (H_e). Subscript *p* and *s* are used to designate population-level and pooled effects, respectively.

Results

Here, we report results from the primary analysis of interest: comparison of individual populations, accounting for both island origin and habitat type (n=8 populations). Estimates of Q_{ST} varied from 0.005 for time to germination to 0.116 for aboveground biomass at harvest, with a mean Q_{ST} of 0.055 (Figure 4.1a, Table 4.2). Populations were significantly differentiated for 5 of the 10 quantitative traits measured (number of roots, mean root diameter, above- and below-ground biomass, total biomass), with bootstrapped confidence intervals revealing Q_{ST} values significantly different from zero. There was some variation among traits for heritability, ranging from 0.114 for number of ramets, to 0.393 for time to germination (Table 4.2). Estimates of heritability for all traits were less than 1, which is considered to be the upper limit for "permissible" heritability estimates, except for number of ramets in the Sapelo backdune population, which was excluded from calculations. By utilizing results from Franks *et al.* (2004) to inform our sampling strategy for seed collection, we had hoped to avoid collecting seeds ramets belonging to the same clone. However, if clonality occurred on a larger scale, inadvertent

sampling within genets may have occurred. The estimate of heritability for number of ramets in this population fell within permissible bounds when estimated using a full-sibling design.

Sixteen of the 23 allozyme loci analyzed were polymorphic in multiple populations (Table 4.3). The most variable loci were *Pgm-1* and *Tpi-3*, with 4 and 3 alleles each, respectively. For the 16 polymorphic loci, a total of 35 alleles were found among the individuals sampled. Seven loci (*Aat, Ak-1, F16, Mdh-1, Pgi-2, Pgi-3, and Skdh*) were monomorphic in all populations. Across pooled populations, there was an average of 2.23 alleles per polymorphic locus (AP_s) and genetic diversity (H_{es}) was 0.158. Within populations (habitat and island comparison), there was an average of 2.06 alleles per polymorphic locus (AP_p) and mean genetic diversity was 0.147 (Table 4.3).

F_{ST} was estimated to be 0.032. The Q_{ST} values for all traits, except two, fell within the confidence intervals of the estimated value of F_{ST} (Figure 4.1). The Q_{ST} values for aboveground and total biomass fell above the confidence interval of the F_{ST} estimate, suggesting that divergent selection is acting among populations on aboveground biomass, which likely drives differences in total biomass. Results from the comparisons of islands and habitats indicate that selection among populations likely differs due to differences among islands, rather than due to variation in abiotic conditions along the localized environmental gradient (Figure 4.1).

Discussion

Most comparative studies of neutral marker variation and quantitative genetic variation report estimated values of Q_{ST} which often far exceed F_{ST} (Merila & Crnokrak 2001, Leinonen *et al.* 2008). It is expected that quantitative traits show higher levels of differentiation among populations than neutral markers due to divergent selection, resulting in different optimal phenotypes for populations in different habitats. We found evidence of divergent selection

among populations for 2 of the 10 measured quantitative traits, aboveground biomass and total biomass. Divergent selection on aboveground biomass likely contributed to selection on total biomass. Estimated Q_{ST} values for other traits were not significantly different from F_{ST}, indicating that for those traits, the structuring of quantitative trait variation and neutral genetic variation among populations are similar. Based on the wide overlap between confidence intervals, there is no evidence to suggest that divergent selection plays a stronger role in shaping differences among populations than genetic drift for traits other than aboveground biomass and total biomass.

Often, it is expected that populations are genetically differentiated and that local adaptation is ubiquitous—an assumption that is made because this is often the case for populations occurring across environmental gradients (McKay et al. 2005). Based on our own (Gormally and Donovan, *in press,* Gormally and Donovan, *in review*) and others' work on *U. paniculata* (Franks *et al.* 2004, Bush & Stelato 2007), we had expected to find that previously observed phenotypic variation resulted from divergent selection resulting from differences between habitats across the shoreline-to-landward environmental gradient. However, results from further analyses comparing populations pooled by island and by habitat type indicate that divergent selection is likely driven by inter-island differences rather than intra-island differences (Figure 4.1). While we sampled populations from islands formed during the Holocene with similar geomorphologies, there may be considerable inter-island variation in dune topography. These differences may result from island orientation relative to prevailing winds, affecting overwash patterns and sand accretion and erosion, as well as local beach and dune sediment budgets (Stallins & Parker 2003). Consequently, divergent selection among populations appears

to result from inter-island differences rather than differences among habitats located along an intra-island shoreline-to-landward environmental gradient.

Differences between habitats along the environmental gradient may only minimally contribute to divergent selection among populations. Consequently, at the intra-island scale, the previously observed phenotypic variation among natural populations located across the shoreline-to-landward environmental gradient on the Sapelo Island dunes likely results from phenotypic plasticity (Gormally and Donovan, *in press*). This is an important reminder that phenotypic trait variation does not always result from population differentiation. Environmental conditions across a single dune system fluctuate frequently due to cyclic patterns of sand accretion and erosion. In dynamic and frequently disturbed habitats such as the coastal dunes, plant species often respond to heterogeneous environmental factors through phenotypic plasticity (Schlichting & Pigliucci 1998). An individual genotype of *U. paniculata* may experience the entire spectrum of conditions along the localized environmental gradient as dunes build and erode over the course of its lifetime, since it is a relatively long-lived clonal grass. The ability to respond plastically to this locally heterogeneous environment may be a favorable strategy contributing to the long-term persistence of these populations. Individuals may successfully respond to changed habitat conditions, such as following major changes to dune topography as a result of overwash and erosion.

Sufficient genetic variation is required for populations to evolve in response to environmental change (Jump *et al.* 2008, Kramer & Havens 2009). Possessing sufficient genetic variation is particularly important for species such as *U. paniculata*. *Uniola paniculata's* habitat is naturally fragmented and may be impacted by further fragmentation and reduced population size, due both to natural and man-made disturbances. Our measures of heritability indicate that

these populations should have the genetic capacity to respond to changes in environmental conditions.

The possession of sufficient genetic variation is considered to be a measure of the adaptive potential of a species or population (Frankham et al. 2002). It should be noted that our estimates of heritability may include non-additive genetic components such as environmental maternal effects, since plants used in this study were grown from field-collected seeds. Since paternity of our field-collected seeds is unknown, it is possible that some families contain both half and full siblings, resulting in both heritability and Q_{ST} values that do not reflect expected genetic relatedness. Additionally, though 95% confidence intervals are more conservative tests of significance than difference testing at α=0.05, there are methodological difficulties with the construction of confidence intervals on variance component ratios for Q_{ST} which are still under development and debate (Bonnin *et al.* 1996, O'Hara & Merila 2005, Whitlock 2008).

Recently questions have been raised about the $Q_{ST} - F_{ST}$ comparison (Miller *et al.* 2008, Pujol *et al.* 2008, Santure & Wang 2009). Of particular importance is that the research design utilizes a common garden approach so that the comparison does not confound additive genetic divergence among populations with environmental effects (Pujol et al. 2008). Assumptions of F_{ST} and Q_{ST} under neutrality have been called into question (Miller et al. 2008), and inbreeding and dominance have been shown, in theory, to affect the comparison (Santure & Wang 2009). However, avoiding bias (e.g., in selection of traits measured) and accounting for sampling error remain the most critical concerns. In particular, questions have been raised concerning our ability to not simply compare mean estimates of each parameter, but to compare the parameters' distributions themselves. To account for sampling error, bootstrapping approaches are generally

recommended (Whitlock 2008). Though there are valid concerns about $Q_{ST} - F_{ST}$ comparison, it remains a valuable technique for making evolutionary inferences.

Our findings of similar values of F_{ST} and Q_{ST} for many traits, as well as low amounts of among-population genetic structure, are also consistent with results from studies of other plant species with distributions that are either geographically or ecologically narrow (Waldmann & Andersson 1998, Widen *et al.* 2002, Jorgensen *et al.* 2006, Badri *et al.* 2008, Helsen *et al.* 2009). Species with life history traits similar to *U. paniculata* have G_{ST} values similar to our F_{ST} estimate, e.g., long-lived outcrossing perennials (0.094) and long-lived perennials with wind-dispersed seeds (0.086) (Hamrick & Godt 1996). Nei's G_{ST} (Nei 1973) and Wright's F_{ST} (Wright 1965) are comparable genetic diversity statistics for partitioning neutral genetic variation, which rely on different underlying assumptions, but result in similar values, given large sample sizes and several populations. Our F_{ST} estimate is lower than allozyme-based estimates of G_{ST} for Poaceae (0.284) (Hamrick & Godt 1996) and for other outcrossing grasses (0.112) (Godt & Hamrick 1998), as well as estimates for *U. paniculata* reported by Franks *et al.* (2004). However, Franks *et al.* (2004) sampled populations across the species' geographic range, as did an AFLP-based survey which found higher levels of genetic diversity (Subudhi et al. 2005). Our genetic diversity parameter measurements, including P_p, and H_{op}, are similar to those reported by Franks *et al.* (2004).

Understanding the spatial scale at which populations are genetically differentiated is critical for informing restoration decisions, i.e., determining the scale at which locality is important when sourcing populations for transplantation (Frankham et al. 2002, McKay et al. 2005). Genotype-environment matching, resulting from local adaptation or historical genetic structure, can profoundly influence the success of restoration efforts (Rice et al. 1997). The

design of our study, combined with results from prior studies, allows us to disentangle the underlying sources of phenotypic variation, and interpret evidence of genetic differentiation among populations to infer the degree to which locality is critical to *U. paniculata* populations. This finding may have implications for conservation and restoration efforts involving *U. paniculata*. Given our results, it is likely that *U. paniculata* individuals will be successful across the range of conditions in a single dune system. This is promising, since coastal dunes undergoing restoration efforts have often experienced permanent and substantial habitat loss of the shoreline-to-landward environmental gradient due to beach erosion or subsidence (Feagin et al. 2005). However, conservation efforts should use care in seed or ramet sourcing for populations located on different dune systems, since natural selection appears to be divergent even among closely neighboring islands. Differences in quantitative traits, particularly such as the ones documented here—aboveground biomass and total biomass—may impact the successful translocation of individuals from one environment to another (Frankham et al. 2002). An important next step would be to conduct inter-island reciprocal transplants of populations to understand whether divergent selection among populations drives local adaptation, as well as to determine the environmental conditions associated with selection favoring certain phenotypes. Together, comparative studies of neutral genetic variation and quantitative trait variation reveal the complex interactions occurring within and among populations and provide the kind of genetic information that can be used to guide plant restoration efforts.

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Table 4.1. Populations sampled are presented with their GPS coordinates and number of individuals sampled, including the number of half-sibling families sampled in parentheses.

Island	*Population abbreviation*	*Latitude*	*Longitude*	*N (families)*	
				Backdune	*Foredune*
Cabretta Island	Cab B/Cab F	31°25'57.85"	81°14'19.89"	189 (17)	194 (20)
Nannygoat Beach, Sapelo Island	Sap B/Sap F	31°23'25.67"	81°15'50.51"	200 (18)	201 (16)
Sea Island	Sea B/Sea F	31°10'43.93"	81°20'48.66"	209 (16)	203 (17)
Jekyll Island	Jek B/Jek F	31°04'23.77"	81°24'09.26"	197 (18)	231 (18)

Table 4.2. Traits measured. Sample size (N), mean narrow-sense heritabilities (h_n^2), among- (σ_b^2) and within- (σ_b^2) population variances, and Q_{ST} values are presented.

Trait	**N**	h_n^2	σ_b^2	σ_w^2	Q_{ST}
Time to germination	761	0.393	0.03752	1.036	0.004507
Seedling height	668	0.214	0.18	0.2276	0.089964
Adult stem diameter	643	0.150	0.000054	0.0011	0.006039
Number of ramets	644	0.114	0.1164	0.2398	0.057205
Number of rhizomes	644	0.249	0.000822	0.008142	0.012462
Number of roots	644	0.359	4.4136	6.3811	0.079578
Root diameter	642	0.153	0.000435	0.001465	0.035788
Aboveground biomass	643	0.200	1.1652	1.1121	0.115802
Belowground biomass	643	0.166	0.04568	0.1076	0.050393
Total biomass	643	0.200	1.4893	1.6458	0.101619

Table 4.3. Genetic diversity parameters derived from 23 allozyme loci in *U. paniculata* presented for each model comparison as described in the methods (a) island and habitat comparison, (b) habitat comparison, (c) island comparison. Sample size (N), percentage of polymorphic loci (P_p), number of alleles per locus (A_p), mean number of alleles per polymorphic locus (AP_p), expected heterozygosity (H_{ep}) and observed heterozygosity (H_{op}) are presented.

(a)

Population	**N**	$\boldsymbol{P_p}$ **(%)**	$\boldsymbol{A_p}$	$\boldsymbol{AP_p}$	$\boldsymbol{H_{ep}}$	$\boldsymbol{H_{op}}$
CabB	2	30.4	1.30	2.00	0.188	0.261
CabF	8	47.8	1.48	2.00	0.143	0.174
JekB	13	56.5	1.61	2.08	0.179	0.197
JekF	22	56.5	1.61	2.08	0.169	0.184
SapB	20	47.8	1.52	2.09	0.152	0.196
SapF	10	39.1	1.44	2.11	0.132	0.204
SeaB	18	43.5	1.44	2.00	0.163	0.225
SeaF	24	39.1	1.44	2.11	0.137	0.199
Mean	14.63	45.1	1.48	2.06	0.147	0.205
Pooled	117	56.5	1.83	2.23	0.158	0.199

(b)

Population	**N**	P_p **(%)**	A_p	AP_p	H_{ep}	H_{op}
Backdune	53	56.5	1.74	2.154	0.163	0.208
Foredune	64	56.5	1.74	2.231	0.153	0.192
Mean	117	56.5	1.74	2.192	0.158	0.200

(c)

Population	**N**	P_p **(%)**	A_p	AP_p	H_{ep}	H_{op}
Cab	10	47.8	1.52	2.091	0.147	0.191
Jek	35	65.2	1.74	2.133	0.175	0.189
Sap	29	47.8	1.52	2.091	0.144	0.198
Sea	43	47.8	1.57	2.182	0.149	0.210
Mean	117	52.2	1.59	2.124	0.154	0.197

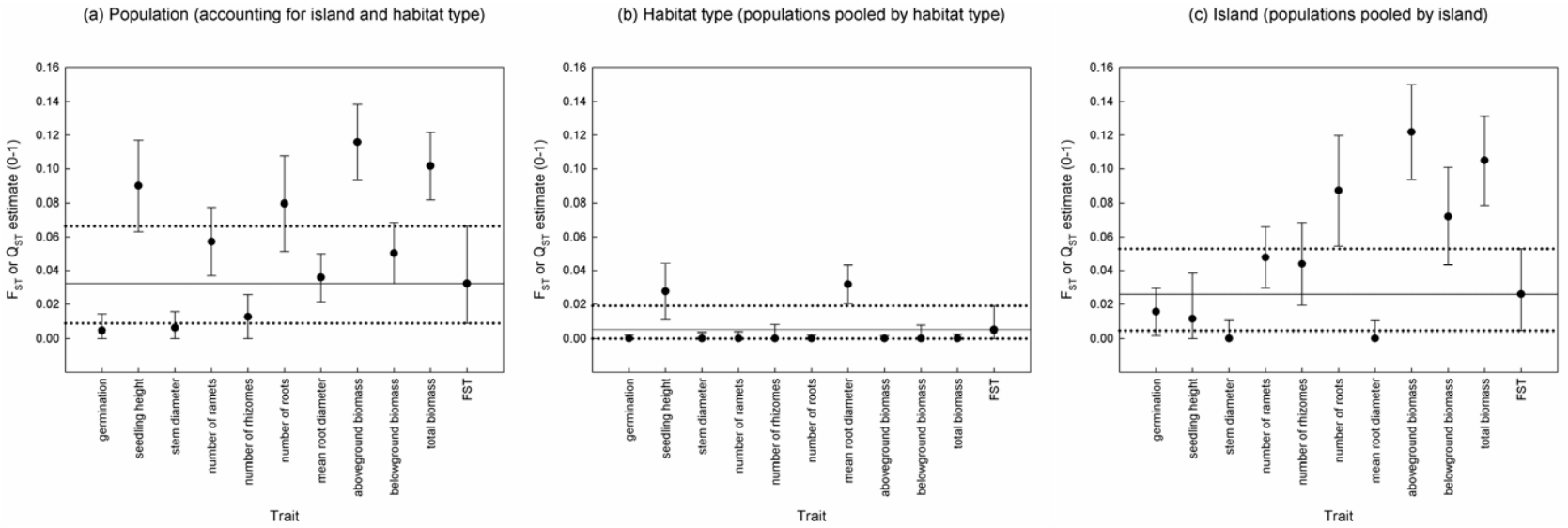

Figure 4.1. Point estimates and bootstrapped 95% confidence intervals for quantitative traits measured and estimated F_{ST}. The values represent the proportion of total heritable variance that is partitioned among populations. The solid line indicates the estimated value of F_{ST}. The dashed lines indicate the upper and lower bounds of the confidence interval around the estimated value of F_{ST}. (a, b, c) Values estimated for each model as described in the methods.

CHAPTER 5

GENETIC STRUCTURE OF A WIDELY DISPERSED BEACH ANNUAL, *CAKILE EDENTULA* (BRASSICACEAE)[1]

[1] Gormally, C.L., L.A. Donovan, and J.L. Hamrick. *To be submitted to* American Journal of Botany.

Abstract

We investigated the geographic distribution of genetic variation in three subspecies of *Cakile edentula*, an annual colonizer of coastal strandlines throughout North America, as well as inland beaches of the Great Lakes. The subspecies, *ssp. edentula var. edentula*, *ssp. harperi*, and *ssp. edentula var. lacustris*, are associated with particular regional distributions: the northern and southern Atlantic coast, and the Great Lakes, respectively. Of 24 allozyme loci surveyed, 29.2% were polymorphic (P_s) in the 608 individuals sampled. We found fairly low genetic diversity (H_e) at the species, region, and population level in *C. edentula.* Taxonomic subspecies designations are supported by UPGMA-dendrogram clusters and an AMOVA analysis indicates that variation among regions explains 42% of the total variation. Pairwise F_{ST} estimates suggest that there are moderate rates of migration between populations, but there was no evidence of significant isolation by distance. The genetic structure of this coastal species is likely influenced by its narrow, fragmented geographic distribution, as well as its mating system and unique dimorphic dispersal ecology, with buoyant propagules capable of long-distance dispersal by sea to colonize new habitats.

Introduction

The genetic structure of coastal species is of particular interest to evolutionary biologists because the distribution of genetic variation within and among populations may be influenced by several factors. These factors include the geographic distribution of the coastal habitat, and the species' mating system and its dispersal ecology. Most population genetic studies of coastal plant species target island endemics, often with a strong emphasis on the conservation genetics of rare species (Maki et al. 2003, Batista et al. 2004, Franks 2009). However, little is known about the distribution of genetic variation in widely dispersed coastal annuals, which play a significant role

in establishing healthy dune communities (Feagin et al. 2005). This categorization includes the early successional species colonizing the strandline, the dynamic habitat located immediately behind the high tideline, which is characterized by flotsam and tidal wrack.

The genetic structure of coastal plant species may be affected by the narrow, linear and fragmented geographic distribution of the coastal habitat. Species occurring across discontinuous ranges tend to have greater genetic structure among populations than congeners with more continuous ranges (Hamrick & Godt 1996). This has been shown for coastal plant species, which generally have high genetic heterogeneity among populations, likely the result of low or restricted gene flow between naturally fragmented coastal habitats (Maki et al. 2003, Batista et al. 2004, Castillo-Cardenas et al. 2005, Franks 2009).

In addition to habitat discontiguity, the genetic structure of a coastal species may be affected by other factors including its mating system and dispersal capabilities (Hamrick & Godt 1996). Many coastal dune annuals self-fertilize (Maun 2009), which impacts genetic structure (Loveless & Hamrick 1984). Self-fertilization typically results in less gene flow and lower levels of genetic diversity, especially at the within population level (Loveless & Hamrick 1984). Additionally, new populations of selfing species may derive from a single founder, resulting in extremely low levels of genetic variation within populations. Alternatively, if there are multiple founding individuals, inbreeding can result in intrapopulation genetic subdivision (Loveless & Hamrick 1984).

Many coastal plant species produce seeds with morphological features that confer buoyancy; some seeds can survive immersion in sea water for more than 100 days (Maun 2009). This capacity for long distance dispersal can maintain gene flow between discontiguous populations and facilitate the colonization of newly available, far-flung habitats. Relatively high

rates of migration and successful establishment between populations can result in extremely low levels of genetic variation among populations (Slatkin & Maruyama 1975). Consequently, if a species has the potential for genetic mobility across long distances, there may be relatively little differentiation among non-contiguous populations.

Cakile edentula (Brassicaceae), commonly known as sea rocket, is an annual beach colonizer that grows on strandlines throughout North America (Duncan & Duncan 1987). Populations of *Cakile* are ephemeral, in keeping with the dynamic nature of the strand habitat. The species is subdivided into three subspecies whose distributions correlate with major biogeographic regions: *ssp. edentula var. lacustris*, found on the Great Lakes; *ssp. edentula var. edentula*, found on the Atlantic coast from North Carolina northward; and *ssp. harperi*, found on the Atlantic coast from North Carolina southward. Populations of *ssp. edentula var. edentula* and *ssp. harperi* are sympatric on the North Carolina coast (Rodman 1974). *Cakile edentula ssp. edentula var. edentula* was also introduced from the Atlantic coast to the Pacific coast of California, reportedly first documented around 1888 (Rodman 1974).

Climatic conditions resulting from the Laurentide ice sheet during the last glacial period limited the northern range of *C. edentula's* distribution to south of Long Island until ~20,000 years ago (Rodman 1974). Following the end of the last glacial maximum, there was a northward range expansion of the species, as well as to the inland dunes on the Great Lakes. Range expansion to the Great Lakes is estimated to have occurred ~10,000 years ago, when Lake Ontario was connected to the Gulf of Lawrence by the Champlain Sea. The Great Lakes populations likely derive from populations of *ssp. edentula var. edentula* (Rodman 1974). Multiple subsequent introductions to the Great Lakes have likely occurred, as accounts of propagule dispersal by ship ballast have been documented (Rodman 1974). Both *ssp. edentula*

var. edentula and *ssp. edentula var. lacustris* have been recorded on freshwater inland dunes. Of the two varieties, *C. edentula ssp. edentula var. edentula* is rarer, and probably was introduced during more recent historical times, transported accidentally by ship ballast (Rodman 1974). It is unclear how underlying genetic variation within and among populations is related to these subspecies designations. Here, we investigate the geographic distribution of genetic variation in a widely dispersed beach annual and determine whether genetic structure matches taxonomic subspecies delineations.

Materials and Methods

Cakile edentula is primarily self-fertilizing (Donohue 1998). Within a single flower, *C. edentula* produces heteromorphic fruit segments, proximal and distal, each of which typically contains one seed (Rodman 1974). Distal and proximal propagules each have a distinctive dispersal ecology: distal propagules are well adapted to long-distance dispersal, easily detaching from the maternal plant and usually dispersing independently, while proximal propagules have limited dispersal capacities, typically remaining attached to the maternal plant (Maun et al. 1990, Zhang 1994). Propagules have a thick, buoyant pericarp which facilitates dispersal by water. *Cakile edentula* has a transient seed bank (Planisek & Pippen 1984, Looney & Gibson 1995).

Three subspecies of *C. edentula* are distinguished by fruit morphological characters, leaf characters, as well as qualitative and quantitative patterns of glucosinolate composition (Rodman 1974). Phenology also varies between subspecies: Great Lakes and northern Atlantic populations are summer annuals, while southern Atlantic populations are winter annuals (Gormally, pers. observ. and seed collection volunteers, pers. comm.). There are finer clinal differences in phenology within regions (Rodman 1974).

Fruits were collected from 22 populations on coastal sand dunes on the Atlantic and Pacific coasts, as well as from inland sand dunes on the Great Lakes with extensive help from volunteers (Table 5.1, Figure 5.1, Acknowledgements). These sites spanned the majority of the range of *C. edentula* in North America, excluding the California coast. Fruits were collected from at least twenty maternal plants in most populations.

The pericarp was removed and each seed was scarified by nicking the seed coat using a razor blade before being placed into Petri dishes to germinate. Seeds coats were removed by hand after one day to promote germination. Seedlings from unique maternal plants were crushed for protein extraction using liquid nitrogen, sea sand, and the crushing buffer of Wendel and Parks (1982) and the extract was absorbed into 4 x 6 mm filter paper wicks. Extracted proteins were run on 9.5% starch gels and 12 enzymes were examined: aconitate hydratase (*Aco*), isocitrate dehydrogenase (*Idh*), F-1,6-diphosphate (*F1,6*), malate dehydrogenase (*Mdh*), phosphoglucoisomerase (*Pgi*), and UDPglucose pyrophosphorylase (*Ugpp*) on buffer system 4; fluorescent esterase (*Fe*), leucine amino peptidase (*Lap*), and triose-phosphate isomerase (*Tpi*) on buffer system 6; and asparate amino transferase (*Aat*), diaphorase (*Dia*), and menazion reductase (*Mnr*), on buffer system 7 (Soltis *et al.* (1983) for all buffers and stains except: *Aat*, *Dia*, and *Mnr* (Cheliak & Pitel 1984)). Following the established protocols cited above, gels were stained with enzyme-specific stains and scored for allozyme banding patterns, with interpretation guided by Wendel and Weeden (1989) and Kephart (1990). Loci and alleles were numbered consecutively beginning with the most anodal form.

The following genetic diversity indices were calculated for each locus, and at the species, region, and population levels using the program GDA (Lewis & Zaykin 2001): the percentage of polymorphic loci (*P*); the mean number of alleles per locus (*A*); the mean number of alleles per

polymorphic locus (*AP*); observed (H_o) and expected heterozygosity (H_e); the number of private alleles; and the inbreeding coefficient (F_{is}), which was calculated only at the population level. Subscript *s* is used to designate species-level parameters, *r* is used to designate regional-level values, and *p* is used to designate population-level values.

Population genetic structure was evaluated using an analysis of molecular variance (AMOVA) in the program GenAlEx6 (Peakall & Smouse 2006). Total genetic diversity was partitioned into three levels: among regions, among populations within regions, and among individuals within populations. Regions were defined as: Northern Atlantic (ME, NH, MA, RI, DE); Southern Atlantic (NC1-4, SC1-4, GA); Pacific (OR); and Great Lakes (WI, IL, IN, MI, OH1-2, ON). Genetic distances between all possible pairwise combinations between populations, as well as between populations within regions, were calculated following Nei (1978). To examine patterns of genetic association among populations, a dendrogram was constructed using the matrix of genetic distances using the UPGMA cluster method in GDA (Lewis & Zaykin 2001) and TreeView (Page 1996). After examining the dendrogram, a second AMOVA was performed. Total genetic diversity was partitioned into three levels: among regions, among populations within regions, and among individuals within populations. Regions and populations within regions were defined using dendrogram results: North1, North2, and South (Figure 5.1). Values of F_{ST} were calculated between each pair of populations within regions and a Mantel test was performed as a means of testing for significant isolation by distance (IBD), using GenAlEx6 (Peakall & Smouse 2006).

Results

Thirteen of the 24 allozyme loci analyzed were polymorphic in at least one population (Table 5.2). The other 11 loci were monomorphic in all sampled populations. For the 24 loci, a

total of 40 alleles were found among the individuals sampled. The most variable loci were *Fe-1* and *Lap*, with 3 and 4 alleles each, respectively. Both *Tpi-1*and *Fe-1* were only present in a single individual in the OR population. *Aco-2* was nearly completely monomorphic, with only two heterozygous individuals in ME; likewise, *Dia-3* was nearly completely monomorphic, with a single polymorphic individual in NC4. There were six private alleles, one each found in NC4 (*Dia-3*) and ME (*Aco-2*), and the remaining four found in OR (2 alleles at locus *Fe-1* and 2 alleles at locus *Tpi-1*). At the species level, the mean percentage of polymorphic loci (P_s) was 29.2%, with an average of 2.29 alleles per polymorphic locus (AP_p), and genetic diversity (He_s) was estimated to be 0.118 (Table 5.2). Among regions, the mean percentage of polymorphic loci (P_r) was 37.5%, with an average of 2.24 alleles per polymorphic loci (AP_r), and genetic diversity (H_{er}) was estimated to be 0.059. Among populations, the mean percentage of polymorphic loci (P_p) was 18.9%, with an average of 2.19 alleles per polymorphic loci (AP_p), and genetic diversity (He_p) was estimated to be 0.041. Genetic diversity was highest in NC4 and lowest in DE. Nearly every population had a deficiency of heterozygosity, with values of F_{is} ranging from -0.043 to 0.94, with a mean inbreeding coefficient (F_{is}) of 0.522.

To explore population genetic structure in *C. edentula*, we used an AMOVA, which revealed significant genetic variation among regions (Northern Atlantic, Southern Atlantic, Great Lakes, and Pacific) (42.2%, $p<0.01$), as well as among populations within regions (28.1%, $p<0.01$) (Table 5.3). The dendrogram constructed from the pairwise genetic distances between populations (Appendix B, Table B.2), determined the presence of three primary groups of populations (Figure 5.2). The first group consists of the two populations of *ssp. edentula var. lacustris* from Lake Erie (OH1, OH2) the population from Lake Ontario (ON), and two northern Atlantic populations (ME and NH) of *ssp. edentula var. edentula*. The second group consists of

populations from Lake Michigan (IN, MI, WI), northern Atlantic populations (DE, MA, and RI), and the Pacific coast population (OR). The third group is comprised of two subgroups that are comprised entirely of southern populations (NC1-4, SC1-4, GA). Populations from South Carolina and Georgia are classified as *ssp. harperi*, but in North Carolina, *ssp. harperi* and *ssp. edentula var. edentula* are considered to be sympatric. Further examination of allelic frequencies supports the constructed UPGMA-dendrogram, as allelic frequencies for *Aat-2, Aco-3,* and *Lap* differentiate the two clusters of northern and Great Lakes populations from the cluster of southern populations. There are differences in allelic frequencies between the two sub-clusters of southern populations (NC1, NC2, SC3, GA; and NC3, NC4, SC1, SC2, SC4). Loci *Aco-4* and *Fe-3* are responsible for differentiation of the two clusters containing the northern Atlantic and the Great Lakes populations. There was no evidence of significant IBD among populations within regions (North1, $p>0.071$; North2, $p>0.109$; South, $p>0.390$) (Appendix B, Tables B.3-B.6). The post-hoc AMOVA structured following the dendrogram revealed significant genetic variation among regions as defined by the dendrogram (53.9%, $p<0.01$), as well as among populations within regions (18.2%, $p<0.01$) (Table 5.3).

Discussion

In this study, we surveyed variation in 24 allozyme loci in 22 populations of *Cakile edentula* spanning the entire species' range, with the exception of the Pacific coast of California. Populations were included from each of the three subspecies associated with particular geographic distributions. Our study has three key findings: genetic diversity (H_e) is fairly low at the population, regional, and species levels; taxonomic subspecies designations are supported by UPGMA-dendrogram clusters as well as by AMOVA analysis indicating that there is greater

variation among regions than among or within populations within regions; and the AMOVA results indicate that there is also moderate gene flow among populations within regions.

Genetic diversity (H_{es}) is fairly low in *C. edentula* when compared to estimates for selfing species with similar life history traits (Hamrick & Godt 1996). This is not surprising, as *C. edentula* is restricted to a linear, narrow, and fragmented range given its habitat preferences of coastal and inland dune strandlines. Furthermore, populations of *C. edentula* may result from only a few immigrating propagules, contributing to lower levels of genetic diversity at the population level and greater among population genetic heterogeneity. Since populations of *C. edentula* are often ephemeral and small, random genetic drift, which affects smaller populations more severely, may contribute to the low level of genetic diversity.

Our AMOVA results indicate that there is significant genetic structuring among regions, and among populations within regions. There is greater genetic structuring (total F_{ST}=0.703) in *C. edentula* than other self-fertilizing annuals (G_{ST}=0.553), while genetic diversity in *C. edentula* (0.118) is similar to that reported by Hamrick and Godt (1996) (H_{es}=0.131). Genetic structuring is also greater in *C. edentula* as compared to reports by Mhemmed *et al.* (2008) for a closely related species, *Cakile maritima*, which had pairwise estimates of F_{ST} ranging from 0.05 to 0.285, and a value of F_{ST} of 0.465 for the species. It is important to note that while both *Cakile* species are ephemeral annuals which colonize the strandline, *C. edentula* is primarily self-fertilizing whereas *C. maritima* has a mixed mating system and is typically self-incompatible, pollinated by wind and insects (Thrall et al. 2000). Differences in mating systems may contribute to differences in genetic structure between the two *Cakile* species, since plant species with selfing breeding systems typically have greater genetic structure, in particular, widespread selfing colonizers such as *C. edentula* (Hamrick & Godt 1996).

Our finding of greater variation among regions than among populations within regions or within populations is likely related to the distribution of *Cakile edentula* subspecies. The three taxonomically distinct subspecies are associated with particular geographic regions (Rodman 1974). Results from the UPGMA cluster analysis indicate that there are three major clusters of populations. Geographically proximate populations on the southern Atlantic coast clustered together. The one population representing the Pacific coast clusters with populations from the northern Atlantic coast and the Great Lakes. This suggests that the OR population was founded by individuals that either originated or descended from populations on the northern Atlantic coast. Unsurprisingly, the northern Atlantic and Great Lakes populations are not discretely clustered. Populations from the two regions cluster together, likely as a result of frequent translocation of propagules by ship ballast, perhaps reflecting multiple and continuous founding events.

Migration events between populations of *C. edentula* on a regional scale are probably the result of seed dispersal rather than pollen-mediated, since C. edentula is a predominantly selfing species and any outcrossing events are unlikely to occur across the long distances between fragmented habitats (Tero et al. 2003). A good correlation between genetic distance and geographic distance in populations of *C. maritima* indicates that the dispersal capacities of *Cakile* are considerable (Clausing et al. 2000). The migration rate estimated for *C. maritima*, Nm=1.65, is comparable to our pairwise estimates of F_{ST} between geographically close populations (Clausing et al. 2000). Research on *C. maritima* found that seeds can survive up to four months immersed and as long as one year floating in sea water (Gandour et al. 2008). Consequently, since seed survival is possible (Maun 2009), propagules may be dispersed by ocean currents, and carried even longer distances accidentally by ship ballast. In some cases,

long distance migration is probably unidirectional, with immigrating individuals but few or no emigrants, as private alleles were identified in several populations of *C. edentula* (ME, NC4, OR). Unidirectional migration patterns may be the consequence of sea current patterns, which can exert a directional influence on gene flow, thus affecting genetic structure (Mhemmed *et al.* 2008, Westberg & Kadereit 2009). Migration patterns may also be related to frequently used shipping routes. While there is some genetic structuring among populations of *C. edentula*, the species' distinctive dispersal ecology, combined with human-mediated movement of propagules, likely contributes to moderate amounts of gene flow between many pairs of populations.

Cakile edentula is common and widely distributed species across the North American strandline habitat, which is narrow and fragmented. While most coastal plant species show evidence of restricted gene flow among populations due to their narrow, linear distributions, there is a moderate rate of migration between populations of *C. edentula*, particularly among populations within regions. While individual populations of *C. edentula* are ephemeral due to the dynamic strandline habitat, the species' genetic structure is impacted by its distinctive dimorphic dispersal ecology, which facilitates long distance migration to colonize new habitats and at the same time promotes the continuation of extant populations.

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Table 5.1. Geographic locations and regional distributions of the 22 populations of *C. edentula* collected for this study.

Site	***Code***	***Latitude***	***Longitude***
Wells National Estuarine Research Reserve (NERR), Maine (A)	ME	43°19’51”	70°32’36”
Wallis Sands State Beach, New Hampshire (A)	NH	43°01’36”	70°43’44”
Waquoit Bay NERR, Massachusetts (A)	MA	41°32’56”	70°31’49”
Narragansett Bay NERR, Rhode Island (A)	RI	41°37' 34"	71°19' 37"
Bethany Beach, Delaware (A)	DE	38°32’14”	75°03’15”
Currituck Bay NERR, North Carolina (A)	NC1	36°22’31”	75°49’50”
Masonboro, North Carolina (A)	NC2	34°10’53”	77°48’45”
Zeke’s Island, North Carolina (A)	NC3	33°56’17”	77°55’59”
Bird Island, North Carolina (A)	NC4	33°51’40”	78°31’16”
Baruch NERR, South Carolina (A)	SC1	33°22’57”	79°08’49”
Sullivan’s Island, South Carolina (A)	SC2	32°45’32”	79°49’52”
Folly Island, South Carolina (A)	SC3	32°39’35”	79°55’41”
Jones Island, South Carolina (A)	SC4	32°03’34”	80°56’14”
Nannygoat Beach, Sapelo Island NERR, Georgia (A)	GA	31°23’25”	81°15’50”
South Slough NERR, Point Adams, Charleston, Oregon (P)	OR	43°20'54"	124°19'16"
Whitefish Dunes State Park, Wisconsin (Lake Michigan)	WI	44°55’08”	87°12’01”
South Boulevard Beach, Evanston, Illinois (Lake Michigan)	IL	42°01’36"	87°40’00”
Indiana Dunes National Lakeshore, Indiana (Lake Michigan)	IN	41°39’52"	87°03’39”
Orchard Beach State Park, Manistee, Michigan (Lake Michigan)	MI	44°16’50”	86°19’04”
Old Woman Creek NERR, Ohio (Lake Erie)	OH1	41°23’03”	82°30’52”
Conneaut, Ohio (Lake Erie)	OH2	41°57’51”	80°33’52”
Hamilton Harbour, Ontario, Canada (Lake Ontario)	ON	43°17’47”	79°47’35”

Table 5.2. Genetic diversity parameters derived from 24 allozyme loci in 22 populations of *C. edentula*, and calculated at regional and species levels. Sample size (N), percentage of polymorphic loci (P_p), number of alleles per locus (A), mean number of alleles per polymorphic locus (AP), expected heterozygosity (H_e) and observed heterozygosity (H_o), and inbreeding coefficients (F_{is}) are presented.

(a)

Population	**N**	***P* (%)**	***A***	***AP***	H_e	H_o	F_{is}
ON	9	33.3	1.50	2.50	0.051	0.014	0.742
NH	31	16.7	1.17	2.00	0.044	0.003	0.94
ME	33	16.7	1.21	2.25	0.030	0.014	0.537
OH2	37	16.7	1.17	2.00	0.012	0.003	0.718
OH1	16	8.3	1.08	2.00	0.020	0.021	-0.043
MI	23	20.8	1.21	2.00	0.036	0.013	0.650
IN	22	20.8	1.25	2.20	0.057	0.017	0.703
RI	30	16.7	1.17	2.00	0.025	0.024	0.039
IL	29	16.7	1.21	2.25	0.052	0.003	0.423
WI	31	16.7	1.25	2.50	0.038	0.026	0.337
DE	18	8.3	1.08	2.00	0.009	0.005	0.493
OR	30	33.3	1.50	2.50	0.063	0.040	0.361
MA	32	20.8	1.25	2.20	0.025	0.024	0.282
SC2	30	25.0	1.29	2.17	0.063	0.033	0.475
NC4	30	29.2	1.33	2.14	0.068	0.032	0.531
NC3	30	25.0	1.25	2.00	0.050	0.019	0.618
SC1	29	8.3	1.17	3.00	0.019	0.013	0.316
SC4	30	16.7	1.21	2.25	0.027	0.011	0.594
GA	28	33.3	1.38	2.13	0.051	0.018	0.652
SC3	29	12.5	1.17	2.33	0.046	0.020	0.566
NC1	31	20.8	1.25	2.20	0.046	0.015	0.679
NC2	30	20.8	1.21	2.00	0.037	0.017	0.553
$Mean_p$	27.64	18.9	1.22	2.19	0.041	0.019	0.522

(b)

Region	**N**	***P* (%)**	***A***	***AP***	H_e	H_o
North1	126	29.2	1.33	2.20	0.036	0.009
North2	215	41.7	1.58	2.29	0.067	0.026
South	267	41.7	1.50	2.22	0.075	0.020
Mean	202.67	37.5	1.47	2.24	0.059	0.018
Species	608	29.2	1.71	2.29	0.118	0.020

Table 5.3. Analysis of molecular variance (AMOVA) for 608 individuals of *C. edentula* collected from 22 populations across North America. (a) Populations were grouped into four regions: Northern Atlantic (including populations from Delaware northward); Southern Atlantic (including populations from North Carolina southward); Great Lakes; Pacific. Analysis consistency was determined by 1000 permutations of the distance matrix. (b) Post-hoc AMOVA with populations grouped into three regions as designated by UPGMA dendrogram results: North1, North2, South (Figure 5.2).

(a)

Source of variation	***df***	***Sum of squares***	***Variance component***	***Total (%)***
Among regions	3	656.296	0.700	42.15
Among populations within regions	18	467.642	0.467	28.11
Within populations	1194	589.790	0.494	29.74
Total	1215	1713.728	1.661	100

(b)

Source of variation	***df***	***Sum of squares***	***Variance component***	***Total (%)***
Among regions	2	779.802	0.954	53.92
Among populations within regions	19	344.136	0.321	18.17
Within populations	1194	589.790	0.494	27.92
Total	1215	1713.728	1.769	100

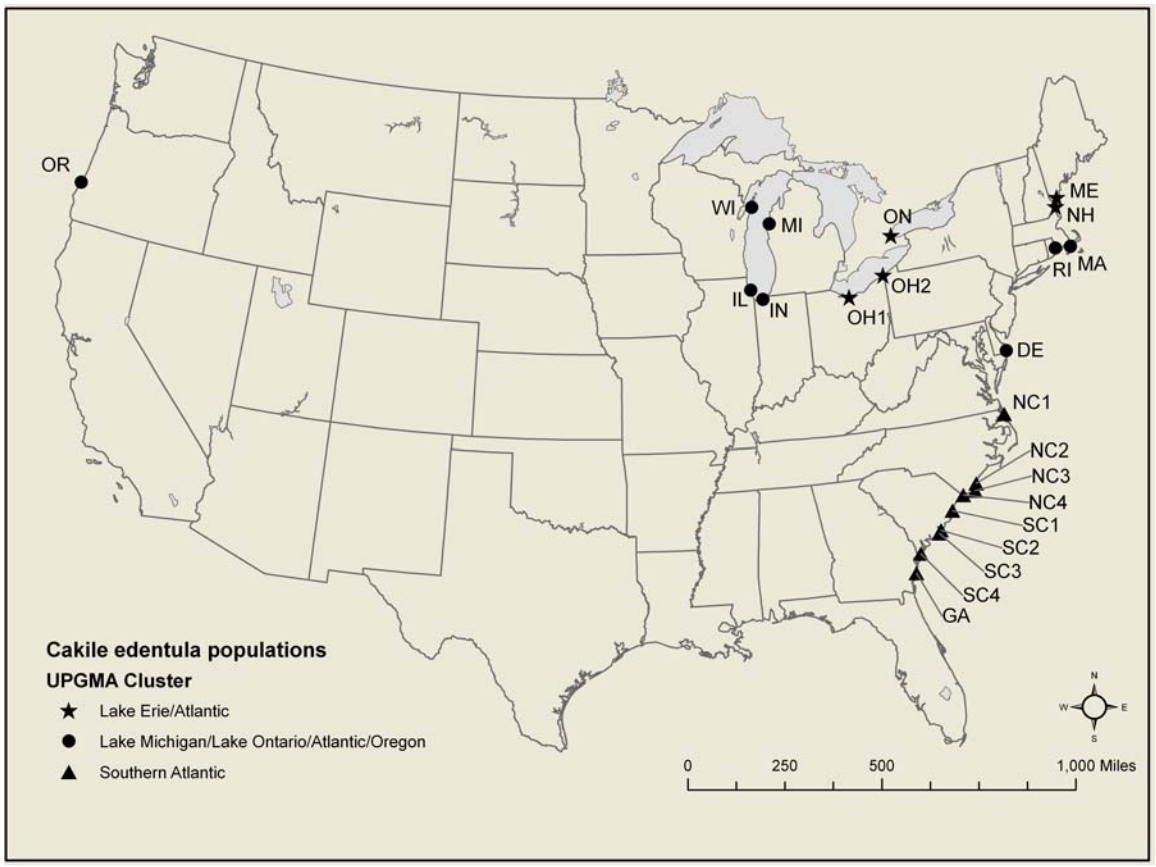

Figure 5.1. Distribution of 22 *C. edentula* populations sampled across North America. The population codes are shown in Table 5.1. Marker shape indicates UPGMA cluster, shown in Figure 5.2.

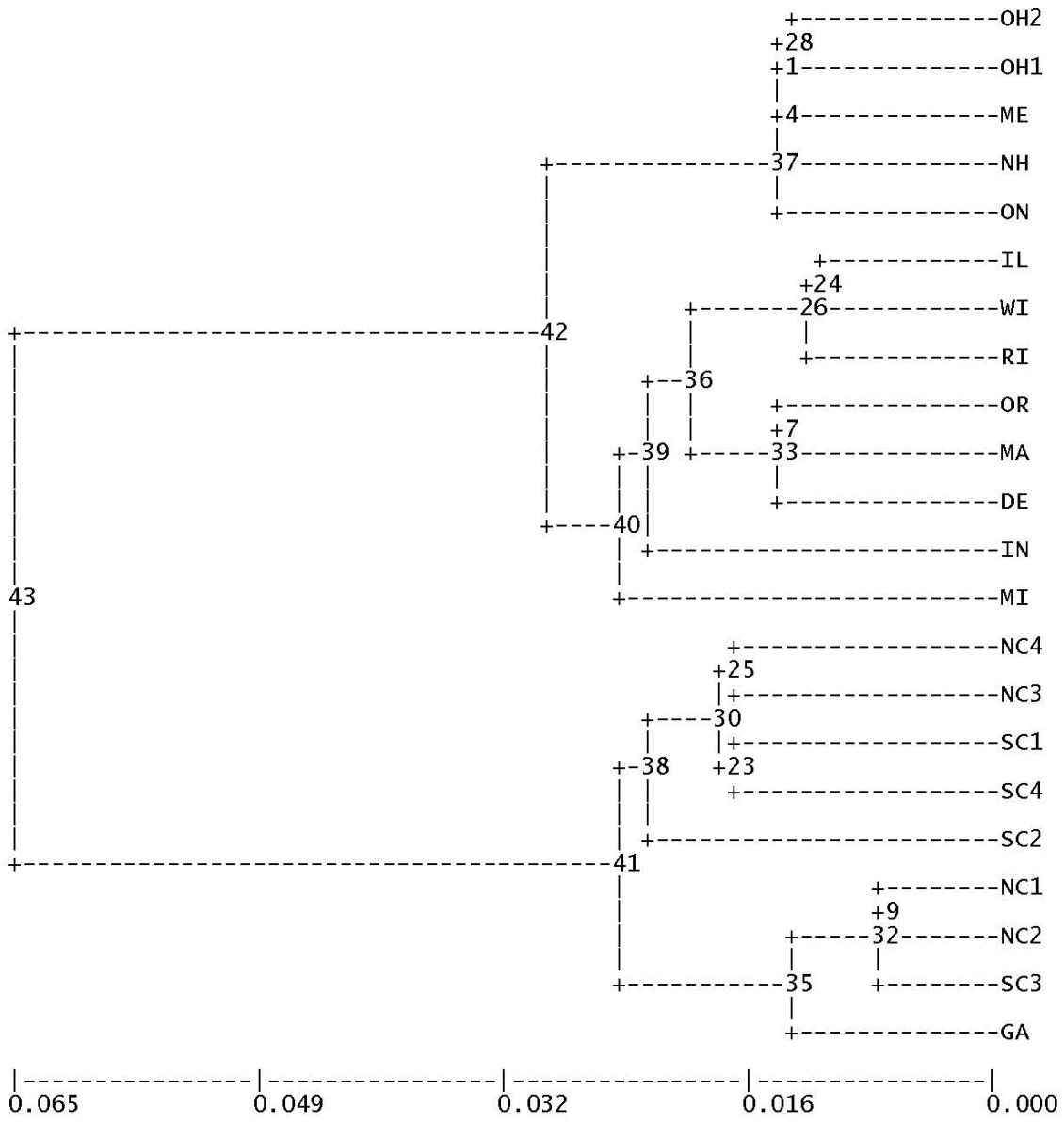

Figure 5.2. Dendrogram of *C. edentula* populations constructed with UPGMA cluster method. Population codes are shown in Table 5.1.

CHAPTER 6

CONCLUSIONS

In the first chapter, we posed a series of questions that were addressed by the studies described in this dissertation. In this chapter, we reconsider those questions and summarize our findings.

What is the relationship between environmental variation and intraspecific trait variation in *Uniola paniculata*?

We characterized the shoreline-to-landward abiotic gradients across the primary dunes and investigated patterns of trait variation in *Uniola paniculata*. Several environmental factors decreased with distance from the shoreline (soil B, K, Mg, Na; salinity, pH, and sand accretion), and differences were most pronounced between the 10 m closest to the shoreline and the remainder of the transect. In the 10 m closest to the shoreline, 94% more sand accumulated, which was 31% more saline. Likewise, we found that intraspecific trait variation in *U. paniculata* tended to mirror environmental variation. In the 10 m closest to the shoreline, plants were taller, contained higher aboveground tissue N and K, and a higher percentage tended to flower. During the two years following the planned study, storms washed out ≤ 25 m of the transects. Re-sampling of the remaining sites demonstrated that even after erosion of the dune profile, a higher percentage of the plants in the 10 m closest to the shoreline plants tended to flower, relative to populations located further from the shore. Our findings suggested that the environment and plant response in the shoreward 10 m can re-establish relatively quickly. Our findings that substantial intraspecific trait variation occurs in *U. paniculata* at the margin of its range, where environmental factors also differ, is an important first step in understanding

species-level responses to the coastal dune environment. The role of environmental gradients in structuring coastal dune vegetation zonation is well-documented (Cowles 1899, Clements 1904, Barbour *et al.* 1999) and our study adds to the few studies that have documented intraspecific responses across the entire primary dunes (Knight & Miller 2004).

Are populations of *Uniola paniculata* locally adapted to microhabitats on the coastal dunes?

Plants from the foredune and backdune habitats were reciprocally transplanted into experimental plots in both habitats. Using measurements of growth in the field and, most significantly, survival in the field during an extreme drought, we found no evidence that populations of *U. paniculata* were locally adapted to specific microhabitats. Although foredune plots were washed away by storms before harvest, early morphological growth measures indicated no local adaptation for foredune plants. At harvest, we found no evidence of local adaptation for backdune plants in the backdune plots, with no advantage in survival, growth or total biomass. Given the changeable nature of the coastal sand dune environment, one genet might experience multiple environments over the course of its life, so plasticity may be more likely to evolve than local adaptation (Mitchell-Olds 1992).

What are the relative roles of natural selection and neutral genetic processes in shaping populations of *U. paniculata*?

To disentangle the relative roles of natural selection and neutral evolutionary processes contributing to phenotypic variation among populations of *U. paniculata*, we compared quantitative trait and neutral marker differentiation (Merila & Crnokrak 2001). We estimated neutral genetic variation using allozymes and examined trait variation in a greenhouse common

garden experiment. Seeds were sourced from both foredune and backdune populations on four barrier islands located on the Georgia coast. Based on previous work, we expected to find evidence of divergent selection among populations located on the shoreline-to-landward environmental gradient. We found evidence of divergent selection on aboveground and total biomass. Unexpectedly, divergent selection appears to be driven by inter-island differences, rather than intra-island habitat differences. Consequently, previously documented intra-island trait variation is likely due to phenotypic plasticity.

Is the structuring of genetic diversity in *C. edentula* related to the geographic distribution of the subspecies?

We investigated the geographic distribution of genetic variation in 22 populations of *C. endentula*, a widely dispersed beach annual and determined whether genetic structure matches taxonomic subspecies delineations. Of the 24 allozyme loci surveyed, 54% were polymorphic in the 608 individuals sampled. Our study has three key findings: genetic diversity (H_{ep}) is fairly low in *C. edentula* populations; there is greater variation among regions than among or within populations within regions; and there are moderate rates of migration between populations but no evidence of significant isolation by distance. Though individual populations of *C. edentula* are ephemeral as a result of the dynamic nature of the strandline habitat, the species has a distinctive dimorphic dispersal ecology with propagules that are capable of surviving dispersal by sea, which facilitates long-distance migration across open water to colonize new habitats.

Future Directions

Results from our studies with *U. paniculata* may have implications for its management, as well as for restoration efforts. Given our results, it is likely that *U. paniculata* individuals will

be successful across the range of conditions in a single dune system. This is promising, since coastal dunes undergoing restoration efforts have often experienced permanent and substantial habitat loss of the shoreline-to-landward environmental gradient due to beach erosion or subsidence (Feagin *et al.* 2005). However, conservation efforts should use care in seed or ramet sourcing for populations located on different dune systems, since natural selection is divergent even among closely neighboring islands. Differences in quantitative traits, particularly such as ones documented here—aboveground biomass and total biomass—may impact the successful translocation of individuals from one environment to another (Frankham et al. 2002). An important next step would be to conduct inter-island reciprocal transplants of populations in order to understand whether divergent selection among populations drives local adaptation, as well as to begin to determine the environmental conditions associated with selection favoring certain phenotypes. Together, field-based studies and comparative studies of neutral genetic variation and quantitative trait variation can reveal the complex interactions occurring within and among populations and provide the kind of genetic information that can be used to guide plant restoration efforts.

More questions are raised from the findings concerning the genetic structuring of *C. edentula*. While we sampled populations encompassing much of its range, the inclusion of multiple populations from the Pacific Coast, including additional populations from Oregon, as well as populations from the California coast, might provide more information about long-distance founding events. Since individual populations of C. *edentula* are ephemeral, tracking genetic structuring of populations over time would offer clues about the population dynamics and establishment. A field-based study to determine whether there is a persistent seed bank for this species would provide additional insights about how ephemeral populations really are.

Paternity analyses could be used to determine whether populations are derived from one or multiple founders, as well as to examine whether there are particular migration patterns reoccurring through time. This may also elucidate our understanding of the genetic structure of metapopulations in dynamic habitats such as the coastal strandline.

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APPENDIX A

ADDITIONAL DATA SUPPORTING CHAPTER 4

Table A.1. Allozyme marker loci, sample size (N), number of alleles (*A*), expected heterozygosity (H_e) and observed heterozygosity (H_o).

Locus	***N***	***A***	H_e	H_o
Aat	117	1	0	0
Ak-1	117	1	0	0
Ak-2	117	2	0.120502	0.059829
Dia	117	2	0.494956	0.401709
F16	117	1	0	0
Idh	117	2	0.501559	0.914530
Gdh	117	2	0.342357	0.350427
Mdh-1	117	1	0	0
Mdh-2	117	2	0.033748	0.034188
Me	117	2	0.017021	0.017094
Mnr	117	2	0.311984	0.230769
Pgi-1	117	2	0.042001	0.042735
Pgi-2	117	1	0	0
Pgi-3	117	1	0	0
Pgi-4	117	2	0.501999	0.931624
Pgm-1	117	4	0.226221	0.247863
Pgm-2	117	2	0.017021	0.017094
Skdh	117	1	0	0
Tpi-1	117	2	0.477349	0.777778
Tpi-2	117	2	0.135322	0.145299
Tpi-3	117	3	0.339239	0.358974
Pdg-1	117	1	0.058288	0.042735
Pdg-2	117	2	0.008547	0.008547

APPENDIX B

ADDITIONAL DATA SUPPORTING CHAPTER 5

Table B.1. Characteristics of polymorphic allozyme loci in sampled populations, presented with sample size (N), number of alleles (*A*) and expected (H_T) heterozygosities.

Enzyme	**N**	***A***	$\boldsymbol{H_T}$
Aco-1	608	2	0.435
Aco-2	608	2	0.003
Aco-3	608	2	0.499
Aco-4	608	2	0.499
Aat-2	608	2	0.474
Dia-3	608	2	0.002
Fe-1	608	3	0.003
Fe-3	608	2	0.434
Lap	608	4	0.351
Mnr	608	2	0.094
Tpi-1	608	3	0.003
Ugpp-1	608	2	0.013
Ugpp-2	608	2	0.037
Mean	608	2.31	0.217

Table B.2. Matrix of Nei's (1978) unbiased genetic distance index between pairs of populations of *C. edentula*.

	ME	NH	MA	RI	DE	NC1	NC2	NC3	NC4	SC1	SC2	SC3	SC4	GA	OR	WI	IL	IN	MI	OH1	OH2	ON
ME	0																					
NH	0.0092	0																				
MA	0.0279	0.0344	0																			
RI	0.0549	0.0789	0.0247	0																		
DE	0.048	0.051	0.0078	0.0381	0																	
NC1	0.0644	0.059	0.0916	0.1417	0.1084	0																
NC2	0.0913	0.0822	0.1133	0.1626	0.1265	0.0033	0															
NC3	0.113	0.1015	0.1594	0.1492	0.1911	0.0627	0.0583	0														
NC4	0.1141	0.0915	0.1517	0.1549	0.1818	0.0546	0.0512	0.0008	0													
SC1	0.1538	0.1356	0.1972	0.1817	0.233	0.0827	0.072	0.0036	0.0069	0												
SC2	0.1193	0.0784	0.1444	0.2054	0.1638	0.036	0.0315	0.0385	0.0247	0.0483	0											
SC3	0.0961	0.0883	0.1215	0.1702	0.1469	0.0082	0.0053	0.0547	0.0473	0.0624	0.031	0										
SC4	0.1475	0.1276	0.1897	0.1794	0.2242	0.0768	0.0669	0.0027	0.0048	0.0624	0.0417	0.0577	0									
GA	0.0838	0.0925	0.1215	0.1266	0.1496	0.0204	0.0198	0.0303	0.0327	0.0384	0.0569	0.0161	0.037	0								
OR	0.0402	0.0396	0.0396	0.0414	0.0062	0.0935	0.112	0.1737	0.2024	0.2114	0.1616	0.1216	0.204	0.1355	0							
WI	0.0327	0.078	0.0197	0.0011	0.0307	0.1394	0.1591	0.1545	0.1578	0.1856	0.1997	0.1647	0.1826	0.1286	0.0327	0						
IL	0.0429	0.0629	0.0148	0.002	0.0274	0.1257	0.1474	0.1455	0.1472	0.1782	0.1845	0.1525	0.1745	0.1192	0.0275	0.0004	0					
IN	0.0717	0.0669	0.049	0.0298	0.0691	0.157	0.1727	0.1016	0.1022	0.1221	0.1461	0.1694	0.1193	0.1374	0.0613	0.0294	0.0287	0				
MI	0.0810	0.0935	0.0496	0.0355	0.0546	0.0863	0.1004	0.1071	0.1064	0.1375	0.1401	0.1112	0.1338	0.081	0.0628	0.0349	0.0352	0.0287	0			
OH1	0.008	0.0083	0.0273	0.0826	0.0464	0.063	0.0906	0.148	0.1358	0.1863	0.1105	0.0927	0.177	0.1057	0.0311	0.0788	0.0788	0.0946	0.1037	0		
OH2	0.0036	0.00065	0.0288	0.0745	0.043	0.0591	0.0857	0.1359	0.1271	0.1761	0.1117	0.0952	0.1677	0.097	0.0355	0.0738	0.0585	0.0922	0.0928	0.003	0	
ON	0.0228	0.0164	0.057	0.0645	0.0799	0.0972	0.1194	0.0736	0.0714	0.1011	0.0981	0.1224	0.0966	0.0965	0.0708	0.0678	0.0548	0.035	0.0962	0.0415	0.0338	0

Table B.3. Matrix of pairwise F_{ST} values estimated between *C. edentula* populations.

	ME	NH	MA	RI	DE	NC1	NC2	NC3	NC4	SC1	SC2	SC3	SC4	GA	OR	WI	IL	IN	MI	OH1	OH2	ON
ME	-																					
NH	0.069	-																				
MA	0.110	0.144	-																			
RI	0.231	0.326	0.130	-																		
DE	0.206	0.231	0.080	0.252	-																	
NC1	0.267	0.289	0.273	0.453	0.368	-																
NC2	0.319	0.322	0.327	0.512	0.422	0.039	-															
NC3	0.387	0.391	0.437	0.472	0.596	0.242	0.209	-														
NC4	0.292	0.273	0.335	0.386	0.447	0.168	0.170	0.013	-													
SC1	0.496	0.513	0.531	0.564	0.712	0.335	0.292	0.049	0.059	-												
SC2	0.320	0.251	0.366	0.493	0.415	0.126	0.123	0.124	0.085	0.186	-											
SC3	0.377	0.400	0.344	0.533	0.485	0.073	0.047	0.231	0.177	0.373	0.141	-										
SC4	0.473	0.484	0.510	0.555	0.687	0.309	0.271	0.034	0.04	0.013	0.160	0.278	-									
GA	0.219	0.265	0.303	0.323	0.377	0.073	0.067	0.097	0.094	0.132	0.178	0.061	0.119	-								
OR	0.128	0.127	0.028	0.134	0.047	0.241	0.281	0.412	0.317	0.496	0.278	0.307	0.475	0.274	-							
WI	0.230	0.278	0.101	0.026	0.199	0.446	0.505	0.477	0.379	0.575	0.474	0.517	0.557	0.320	0.110	-						
IL	0.186	0.272	0.084	0.039	0.197	0.412	0.472	0.455	0.356	0.557	0.444	0.484	0.537	0.300	0.093	0.021	-					
IN	0.256	0.269	0.196	0.153	0.353	0.493	0.527	0.334	0.269	0.403	0.365	0.518	0.391	0.340	0.182	0.141	0.155	-				
MI	0.269	0.366	0.205	0.184	0.270	0.292	0.341	0.351	0.277	0.454	0.363	0.373	0.435	0.212	0.178	0.151	0.181	0.280	-			
OH1	0.074	0.086	0.156	0.474	0.299	0.262	0.315	0.548	0.385	0.670	0.367	0.370	0.638	0.309	0.108	0.365	0.376	0.436	0.482	-		
OH2	0.031	0.061	0.135	0.354	0.209	0.245	0.298	0.511	0.367	0.646	0.337	0.379	0.618	0.285	0.113	0.295	0.291	0.367	0.423	0.049	-	
ON	0.122	0.115	0.253	0.345	0.484	0.397	0.404	0.302	0.220	0.421	0.305	0.471	0.395	0.282	0.224	0.282	0.278	0.206	0.382	0.304	0.193	-

Table B.4. Matrix of pairwise F_{ST} values estimated between *C. edentula* populations within North 1 region.

	ON	**NH**	**ME**	**OH2**	**OH1**
ON	0				
NH	0.115	0			
ME	0.122	0.069	0		
OH2	0.193	0.061	0.031	0	
OH1	0.304	0.086	0.074	0.049	0

Table B.5. Matrix of pairwise F_{ST} values estimated between *C. edentula* populations within North 2 region.

	MI	**IN**	**RI**	**IL**	**WI**	**DE**	**OR**	**MA**
MI	0							
IN	0.280	0						
RI	0.184	0.153	0					
IL	0.181	0.155	0.039	0				
WI	0.151	0.141	0.026	0.021	0			
DE	0.270	0.353	0.252	0.197	0.199	0		
OR	0.178	0.182	0.134	0.093	0.110	0.047	0	
MA	0.205	0.196	0.130	0.084	0.101	0.080	0.028	0

Table B.6. Matrix of pairwise F_{ST} values estimated between *C. edentula* populations within South region.

	SC2	**NC4**	**NC3**	**SC1**	**SC4**	**GA**	**SC3**	**NC1**	**NC2**
SC2	0								
NC4	0.085	0							
NC3	0.124	0.013	0						
SC1	0.186	0.059	0.049	0					
SC4	0.160	0.040	0.034	0.013	0				
GA	0.178	0.094	0.097	0.132	0.119	0			
SC3	0.141	0.177	0.231	0.373	0.278	0.061	0		
NC1	0.126	0.168	0.242	0.335	0.309	0.073	0.073	0	
NC2	0.123	0.170	0.209	0.292	0.271	0.067	0.047	0.039	0