Abstract

We tested the hypothesis that *U. paniculata* populations are divided into eastern and western lineages, with the primary geographic break at the southern tip of Florida, as observed in codistributed animal taxa. We asked whether the geographic distribution of chloroplast DNA (cpDNA) variation in *U. paniculata* corresponds to 1) genetic structure in nuclear variation reported in previous studies, and 2) the geographic distribution of morphological adaptive traits reported in previous studies. We sampled 66 populations and performed phylogeographic analyses using sequence variations in maternally inherited cpDNA. We reconstructed the intraspecific phylogenetic network with TCS software and identified phylogeographic breaks in the species using Monmonier's algorithm. Analyses identified 6 cpDNA haplotypes and 2 major lineages: eastern (Atlantic) and western (Gulf), with a phylogeographic break at the southern tip of Florida. The data suggest *U. paniculata* survived the last glacial maximum (LGM) in southern refugia. Following the LGM, differential leading-edge recolonization explains the current distribution of haplotypes into 2 lineages. Populations containing a haplotype from outside its native range are likely due to human-mediated transplantation. The genetic structure of cpDNA variation has weak correlation with nuclear DNA variation, and there is partial concordance between the geographic distribution of cpDNA and morphological variation.

Key words: biogeography, conservation, glaciation, maritime discontinuity, refugium, restoration

Historical climate change has impacted species by causing shifts in their distributions (Hewitt 2000). During the mid-Pliocene (3.3–3 Mya), average global temperatures were 2–3 °C warmer than current conditions and sea level was 25 m higher (Dwyer and Chandler 2009). Global climate oscillations during the Pleistocene (2.6 million to 12 000 ya) led to cycles of glacial advance and retreat in North America. As glaciers advanced, many species shifted their ranges southward; as glaciers retreated, species reradiated northward (Cronin 1988; Delcourt and Delcourt 1993; Morris et al. 2010). Although southeastern North America was unglaciated, climate changes and sea level fluctuations associated with glaciation likely affected the historical distributions of many organisms, including coastal plant species (Bert 1986; Soltis et al. 2006). During periods of glacial advance, more water was confined to glaciers and sea levels fell. During the last glacial maximum (LGM), around 20 000 years BP, lower sea levels caused the coastline in the southeast to resemble the current 200-m depth contour; the coastline was shifted on the Atlantic Coast and throughout the Gulf of Mexico (Jackson et al. 2000).

The distribution of plant species with an exclusively coastal range changed during Pleistocene climate oscillations in different ways than many terrestrial species because of their narrow, linear distribution. Coastal plants are uniquely suited to the ecology of the coastal ecosystem and are found over widespread longitudes and latitudes (Wagner 1964; Barbour and Christensen 1993; Delcourt and Delcourt 1993). Current rapid global climate changes highlight the importance of understanding historical distributions of species and their migration patterns and rates. The ability of species to adjust their ranges or adapt to new climatic conditions affects their survival (Thomas et al. 2004). By studying past distributions and migrations of species, we may infer future geographical ranges of species under predicted models of climate change. It is important to understand processes that determine the geographical distribution of coastal species, as they are more susceptible to sea level fluctuations than inland species.

Several types of data record the historical shift of species' ranges. Fossil pollen and seeds have been used to infer distribution changes of vegetation as the climate changes (Delcourt and Delcourt 1981, 1993; Davis 1983; Jackson 1986; Delcourt and Delcourt 1993). Global climate oscillations lead to cycles of glacial advance and retreat in North America. As glaciers advanced, species shifted their ranges southward and as glaciers retreated, species shifted northward. During periods of glacial advance, more water was confined to glaciers and sea levels fell. During the last glacial maximum (LGM), around 20 000 years ago, lower sea levels caused the coastline in the southeast to resemble the current 200-m depth contour; the coastline was shifted on the Atlantic Coast and throughout the Gulf of Mexico (Jackson et al. 2000).

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Several types of data record the historical shift of species' ranges. Fossil pollen and seeds have been used to infer distribution changes of vegetation as the climate changes (Delcourt and Delcourt 1981, 1993; Davis 1983; Jackson
et al. 2000). However, fossil seed data are scarce and the fossil pollen record can be misinterpreted. Because of the long-distance dispersal ability of pollen, finding fossil pollen does not necessarily confirm a species’ presence (McLachlan and Clark 2004; Davis et al. 2005; Gonzales et al. 2008). Furthermore, the absence of fossil pollen data does not conclusively preclude the historical presence of a species in a region (McLachlan and Clark 2004). Finally, because of high-energy waves and frequent disturbances, coastal ecosystems have a dearth of fossil data (Barbour and Christensen 1993). Molecular markers complement the fossil record to improve understanding of the historical distribution of species (Avise 2000). Chloroplast DNA (cpDNA) is maternally inherited in most angiosperms and only passed on to future generations in seeds (Corriveau and Coleman 1988). Virtually, all Poaceae genera exhibit maternal inheritance; to our knowledge, there have been no documented cases of biparental or paternal inheritance except in interspecific hybridization studies in crop species (Corriveau and Coleman 1988; Pillay and Armstrong 2001). Thus, cpDNA can map seed movement and track thousands of years of maternal lineages because it leaves a genetic footprint detectable by molecular tools (Dorken and Barrett 2004).

The geography of southeastern North America influenced how the ranges of species shifted as climate varied; several patterns have emerged from the evolutionary histories of codistributed taxa (Avise 2000; Soltis et al. 2006). The long, narrow peninsula of Florida divides the ranges of marine and coastal species into 2 units: Atlantic and Gulf of Mexico (Avise 2000). In the mid-Pliocene, warm temperatures caused sea level rises that inundated Florida. During the Pleistocene, the expansion of the Florida peninsula contributed to the isolation of Gulf populations from Atlantic populations (Reeb and Avise 1990; Avise 2000). Additionally, carbonate sediments, ocean currents, and mangrove-dominated ecosystems in southern Florida also prevented migration between Gulf and Atlantic populations of coastal and marine species (Wise et al. 2004). Phylogeographic studies examining the histories of animals based on mitochondrial DNA (mtDNA) variation have documented this separation (Avise et al. 1987; Reeb and Avise 1990; Avise 2000; Soltis et al. 2006). However, there is a dearth of phylogeography studies on many organisms, especially plant species, in coastal southeastern North America (Soltis et al. 2006). To our knowledge, no previously published plant phylogeography studies focus on species with an exclusively coastal distribution in southeastern North America.

The aim of this study is to investigate the phylogeographic history of sea oats, Uniola paniculata L. (Poaceae), to test the hypothesis that there is an east–west disjunction in this species with the southern tip of Florida possibly acting as the primary geographic break, as seen in codistributed animal taxa. Uniola paniculata is a semitropical coastal grass growing in sand dunes from southern Virginia to eastern Mexico, and in Cuba and the Bahamas. It is the dominant plant species on sand dunes and rarely found more than 200 m inland (Wagner 1964; Barbour and Christensen 1993). The species is adapted to a stressful habitat and tolerates high temperatures, unstable substrates, drought conditions, heavy winds, and salt spray (Wagner 1964). Uniola paniculata can reproduce both sexually and clonally; seed heads may be dispersed large distances by ocean currents, wind, and animals. Additionally, the plants can propagate vegetatively through rhizomes (Wagner 1964). Fibrous roots and a rhizomatous growth habit enable the grass to bind sand that builds and sustains coastal sand dunes, preventing erosion (Snyder and Boss 2002). Uniola paniculata provides valuable ecosystem services by building and sustaining coastal dunes that act as a first line of defense to protect land behind the dunes from storm surges (Degner et al. 2007). Uniola paniculata is frequently used in coastal restoration programs following storm damage because it can stabilize dunes and reduce damage arising from erosion and wave action. However, restoration efforts rarely consider the geographic or genetic origin of propagules. Transplantation without regard to origin can lead to the introduction of plants poorly adapted to local conditions, possibly reducing survival. Furthermore, it can harm the genetic integrity of existing populations, leading to problems such as outbreeding depression (Price and Waser 1979; Broadhurst et al. 2008).

To address the main goal of this study, we asked the following questions: 1) What is the geographic/spatial distribution of cpDNA variation, and does it form Gulf/Atlantic lineages as reported in animal studies? 2) Is there concordance between the contemporary geographic distribution of maternal (cpDNA) lineages and genetic structure based on analyses of nuclear genetic markers (Franks et al. 2004; Subudhi et al. 2005)? 3) Is there concordance between the contemporary geographic distribution of maternal (cpDNA) lineages and the geographic distribution of morphological adaptive traits reported in a previous study (Seneca 1972)? 4) What recommendations can be made for restoration practices based on the combined evidence of genetic and morphological investigations that would take into consideration both preserving the evolutionary history of the species and specific adaptive morphological traits?

Methods
Sample Collection
We sampled 66 populations across the range of U. paniculata in the United States and Bahamas (Figure 1; Table 1; Supplementary Table S1 online), spaced 2–50 km apart. At each location, we collected approximately 10-cm² leaf tissue from 2 individuals separated by at least 10 m. Thirteen Florida samples were obtained from tissue cultures grown by Dr M. Kane of the University of Florida. We obtained leaf tissue samples from 2 commercial growers of U. paniculata, Green Seasons Nursery (Parrish, FL) and Oak Island Greenhouse (Oak Island, NC). In Louisiana, where some populations of U. paniculata have been extirpated due to a sand-deficient coastal environment (Hester and Mendelsohn 1987), and in the Bahamas, we used herbarium specimens (1 g of seed head tissue) from the Smithsonian U.S. National Herbarium (Washington, DC) for DNA extraction. All sample
Figure 1. (a) Map of geographical distribution of haplotypes in all populations and the geographic break in the range of *Uniola paniculata* identified by Monmonier’s algorithm using the program BARRIERS 2.2 (Manni et al. 2004). Monochromatic circles represent a population composed solely of individuals of that haplotype. Dichromatic circles represent polymorphic populations, with the haplotypes of the individuals indicated in the circle. Populations with asterisks below them have only one individual – for all other populations n = 2. The dotted line indicates a barrier with 100% bootstrap support when using 100 permutations of a distance matrix that included all the sampled populations. (b) Map of geographical distribution of populations classified as ‘natural’ (n = 2 for all populations). The dotted lines indicate barriers with 100% bootstrap support when using 100 permutations of a distance matrix that included populations classified as ‘natural.’ (c) The cpDNA haplotype network generated with TCS composed of the six *U. paniculata* haplotypes (A–F). The area of each circle is proportional to the haplotype frequency. Each small black circle represents an intermediate, non-sampled or non-existent haplotype. Each line within the *U. paniculata* network represents a mutation (single nucleotide substitution).
Table 1 Geographical regions where U. paniculata populations were sampled, haplotype groups and the percentage of “natural” sampling sites in each region

<table>
<thead>
<tr>
<th>Region</th>
<th>Populations</th>
<th>Individuals</th>
<th>Haplotypes</th>
<th>Percent 'natural'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulf</td>
<td>18</td>
<td>35</td>
<td>A, C, D, E</td>
<td>55.6</td>
</tr>
<tr>
<td>Keys*</td>
<td>8</td>
<td>15</td>
<td>A, C, D, E</td>
<td>50</td>
</tr>
<tr>
<td>Bahamas</td>
<td>40</td>
<td>80</td>
<td>A, B, C, D</td>
<td>37.5</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>130</td>
<td>A, B, C, D</td>
<td>43.9</td>
</tr>
</tbody>
</table>

locations were classified as either “potentially restored” or “natural” based on our knowledge of historical U. paniculata introductions for dune restoration at that location (Figure 1; Table 1; Supplementary Table S1 online). We classified areas with records indicating that no restoration had taken place (e.g., state parks with land management records) as “natural.” All other locations, with records indicating that restoration took place or with no land use records, were defined as “potentially restored.” However, we do not have specific information about all sites. Specimen vouchers are stored in the Appalachian State University Herbarium (Boone, NC; accession numbers 21774-21785).

Laboratory Analyses (DNA Extraction, Amplification, and Sequencing)

Leaf tissue was flash frozen in liquid nitrogen and stored at −80 °C until DNA extraction. Total genomic DNA was extracted from disrupted leaf tissue using the DNeasy Plant Mini (Qiagen, Inc., Valencia, CA) following the protocol of the manufacturer. We amplified noncoding regions of cpDNA using PCR with universal primers (Hamilton 1999; Ebert and Peakall 2009). We carried out PCR in the Biometra T-gradient (Whatman Biometra, Goettingen, Germany) thermoblock. PCR included 12.5 μL goTaq Master Mix (Promega Corporation, Madison, WI), 8.5 μL water, 2 μL DNA, 1 μL forward primer, and 1 μL reverse primer. PCR used was initial denaturation (94 °C for 5 minutes), 40 cycles of denaturation (94 °C for 30 s), annealing (varying annealing temperature for 30 s), and extension (72 °C for 1 min), followed by final extension (72 °C for 7 min). The annealing temperature depended on the primer pair used in the reaction was 1–2 °C below the mean melting temperature of the 2 primers.

Initially, we tested 56 primer pairs on 5 geographically distant samples (Hamilton 1999; Ebert and Peakall 2009) to see if they would amplify cpDNA from U. paniculata. We visualized amplified cpDNA fragments using 1% agarose gel stained with GelRed (Biotium Inc., Hayward, CA). Twenty-five primer pairs yielded a single band and could be successfully sequenced for multiple samples. The fragments amplified by these 25 primer pairs totalled approximately 20 000 bp. Five of the 25 cpDNA fragments showed sequence variation, resulting in 8 variable regions totalling 3870 bp (Supplementary Table S2 online).

We used cpDNA sequence variations to identify distinct haplotypes (Figure 1; Table 1; Supplementary Table S1 online). Amplified fragments were sequenced by Retrogen Inc. (San Diego, CA) and were aligned and compared using Sequencher for Mac version 4.10.1 (Gene Codes Corp., Ann Arbor, MI). DNA sequences were submitted to GenBank (accession numbers JX422730-JX423379). We sequenced 2 individuals from each population except for herbarium specimens (NPBBa and TmILA), where we had 1 individual per population.

Analyses of cpDNA Variation

We reconstructed the evolutionary relationships among cpDNA haplotypes (representing maternal lineages) using statistical parsimony analyses with all nucleotide substitutions weighted equally. We followed the approaches of Clement et al. (2000) and Templeton et al. (1992) using TCS (http://darwin.uvigo.es/software/tcs.html) to create a network of haplotypes with a 95% parsimony criterion. The inferred phylogenetic relationships among cpDNA lineages were combined with their geographical distribution to gain insights into their evolutionary history. The most parsimonious haplotype network combined with the geographic data associated with each haplotype were used to infer phylogeographic relationships. We used BARRIER 2.2 (http://www.mnhn.fr/mnhn/ecoanthropologie/software/barrier.html) to identify geographic barriers using Monmonier’s algorithm, which computes genetic barriers by testing how geographic and genetic distances correspond among populations (Manni et al. 2004; Morris et al. 2010). We performed the BARRIER analysis twice: first using all populations, then using only populations classified as “natural.” For each analysis, we generated 100 bootstrapped genetic distance matrices using cpDNA sequence data and PHYLIP programs DNADIST and SEQBOOT (Felsenstein 1989). When matrices are inputted into BARRIER, they provide bootstrap support for geographic barriers. We tested the identified barriers using AMOVA (analysis of molecular variance) in GenAlEx (http://biology.anu.edu.au/GenAlEx/) and report φPT, a measure of population differentiation for haploid data that calculates the ratio of among-population variance relative to total variance. We conducted AMOVA analyses using all populations and only “natural” populations. For AMOVA analyses, we divided the populations into regions determined by identified barriers. AMOVA calculates the portion of statistically significant genetic variation that exists across the barrier and within each unit on opposing sides of the barrier (Peakall and Smouse 2006).

We calculated total genetic diversity (h1) and genetic diversity within populations (h2) with PERMUT (http://www.pierroton.inra.fr/genetics/labo/Software/Permut/), first using all populations and then using only “natural” populations. For PERMUT analyses, we defined a “population” as a group of sampled populations from a similar
geographic region. We measured haploid diversity (θ) and unbiased haploid diversity (udit) within regions, with all populations and with only “natural” populations, using Allele Frequency by Population in GenAlEx (http://biology.anu.edu.au/GenAlEx/). Additionally, we measured the level of genetic differentiation among “populations” by calculating $G_{ST}$ (Nei 1987) and $N_{ST}$ (Pons and Petit 1995, 1996) in PERMUT, both for all populations and for only “natural” populations. $G_{ST}$ measures genetic differentiation among sample locations using haplotype frequencies. $N_{ST}$ measures genetic differentiation and takes similarities among haplotypes into account, unlike $G_{ST}$ (Petit et al. 2005). PERMUT tests whether $N_{ST}$ is significantly greater than $G_{ST}$ by measuring how many permuted values of $G_{ST}$ are higher than $N_{ST}$. If $N_{ST}$ is significantly greater, we can infer that the distribution of phylogenetically related haplotypes contributes to geographic structure of the species (El Mousadik and Petit 1996).

We conducted a mismatch distribution analysis in ARLEQUIN 3.5 (Excoffier et al. 2005) to estimate the time since population expansion. The mismatch distribution determines whether the observed data are compatible with a unimodal distribution (indicating a rapid, recent population expansion) or a multimodal distribution (indicating demographic equilibrium). Harpending’s raggedness index (r) measures if the distribution of the observed data matches a unimodal distribution (r > 0.05) or a multimodal distribution (r > 0.05). The mismatch distribution estimates the parameter τ, which can be used to estimate the time since population expansion (τ) using the equation $\tau = \frac{\mu}{2} \times \frac{t}{k}$; where μ is the mutation rate and k is number of base pairs. We used τ and the estimated 90% confidence interval (CI) of τ to infer the expansion time in years (τ) and its 90% confidence interval. In our calculations, we used an average value of μ for cpDNA reported in Wolfe et al. (1987).

### Results

**Chloroplast DNA Diversity**

Of the 25 primer pairs that reliably produced a single band in multiple individuals, we found that 20 pairs amplified regions showing no variation among the samples and 5 pairs amplified variable regions (Supplementary Table S2 online). We identified 8 variable characters, all single nucleotide substitutions. Combined, these 8 cpDNA sequence polymorphisms identified 6 distinct haplotypes, labeled A–F (Figure 1). Fifty-four of the 66 populations comprised a single haplotype; 12 populations contained 2 haplotypes (Table 1; Supplementary Table S1 online).

**Geographic Distribution of cpDNA Haplotypes**

Two haplotypes (A and C) appear in more than 20 populations, whereas a third (D) occurs in 10 populations. Haplotype A is dominant on the Atlantic Coast and Bahamas, whereas haplotypes C and D are found predominantly on the Gulf Coast. Haplotype B is rare, occurring in 5 populations in North and South Carolina. Haplotype E was found in 2 sites (the Gulf Coast of Florida and the Florida Keys), whereas haplotype F was unique to 1 population in the Florida Keys. We used cpDNA haplotype data and TCS to generate a phylogenetic haplotype network using the most parsimonious connections (>95%). TCS identified haplotype A as the ancestral root of the network based on haplotype frequency.

BARRIERS identified 1 geographic boundary with bootstrap support of 100% when using all populations in the analysis. This barrier is located at the southern tip of Florida and separates the range of *U. paniculata* into 2 geographic regions (Figure 1a). AMOVA analysis comparing all populations separated into 2 groups (eastern and western) on either side of the barrier at the southern tip of Florida revealed that 54% of the molecular variance existed between the 2 groups, whereas 46% was within groups (P < 0.01; Table 2). When using only

<table>
<thead>
<tr>
<th>Table 2</th>
<th>AMOVA table of molecular variation among and within populations from GenAlEx (Peakall and Smouse 2006)</th>
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</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
<td>df</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>a. All populations</td>
<td>Among populations</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>b. Only “natural” populations, East/West division</td>
<td>Among populations</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>c. Only “natural” populations, East/South/West division</td>
<td>Among populations</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
</tr>
<tr>
<td></td>
<td>Total</td>
</tr>
</tbody>
</table>

Populations were defined as groups occurring on opposite sides of a barrier with 100% bootstrap support in BARRIERS 2.2 (Manni et al. 2004) for analyses in parts a and b. We defined 3 populations for part c: East (Atlantic populations), South (Florida Keys populations), and West (Gulf populations). qPT is reported for each analysis, and an asterisk indicates significance (P < 0.01).
“natural” populations, the BARRIERS analysis found 5 significant barriers with bootstrap support of 100%, all located at the southern tip of Florida (Figure 1b). We performed the AMOVA analysis using the first of the 5 barriers to divide the populations into 2 groups. In this analysis, 76% of the molecular variance existed between the 2 groups, whereas 24% was within groups (P < 0.01; Table 2). We arbitrarily used the first barrier to divide the populations into 2 groups; AMOVA results using the other 4 barriers produced similar results (data not shown). Because 5 barriers were identified in southern Florida, we treated the Florida Keys and the Bahamas as a hotspot of cpDNA biodiversity and treated this hotspot as 1 region. We conducted AMOVA analysis using only “natural” populations by dividing the populations into 3 groups: Atlantic, Gulf, and Florida Keys/Bahamas. When using 3 groups, 88% of the molecular variance existed between the groups, whereas 12% was within groups (P < 0.01; Table 2).

The total genetic diversity within all populations (h_T) was 0.757; average within region genetic diversity (h_S) was 0.591 (Table 3). Using 1000 permutations, N_ST was 0.354, significantly higher than C_ST, which was 0.219 (P < 0.001; Table 3). Similarly, PERMUT analysis using only “natural” populations showed that N_ST (0.704) was significantly greater than C_ST (0.470) (P < 0.05; Table 3). The analysis using only “natural” populations revealed a greater difference between h_T (0.817) and h_S (0.433) than when using all populations (Table 3). Genetic diversity (h) and unbiased genetic diversity (u_h) were highest in region 2, both using all populations and using only “natural” populations (Table 4).

Table 3  Total genetic diversity (h_T), average within region genetic diversity (h_S), N_ST and C_ST computed using all populations and using only “natural” populations

<table>
<thead>
<tr>
<th>Populations included</th>
<th>h_S</th>
<th>h_T</th>
<th>C_ST</th>
<th>N_ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.591</td>
<td>0.757</td>
<td>0.219</td>
<td>0.354*</td>
</tr>
<tr>
<td>Only “natural”</td>
<td>0.433</td>
<td>0.817</td>
<td>0.470</td>
<td>0.704**</td>
</tr>
</tbody>
</table>

N_ST was significantly greater than C_ST in both analyses (*P < 0.05, **P < 0.001).

Table 4  The regions within the range of U. paniculata, their locations, the populations, and individuals contained in these regions, within region genetic diversity (h) and unbiased genetic diversity (u_h) as calculated in GenAlEx (Peakall and Smouse 2006), using (a) all populations and (b) only “natural” populations

<table>
<thead>
<tr>
<th>Region</th>
<th>Location</th>
<th>Populations</th>
<th>Individuals</th>
<th>h (+/− SE)</th>
<th>u_h (+/− SE)</th>
</tr>
</thead>
</table>
| a. All populations
| 1      | Texas, Louisiana, Mississippi, Alabama, Gulf Coast of Florida | 18          | 35          | 0.125 +/- 0.043 | 0.129 +/- 0.044 |
| 2      | Florida Keys, Bahamas             | 8           | 15          | 0.371 +/- 0.057 | 0.398 +/- 0.061 |
| 3      | Atlantic Coast of Florida, Georgia, South Carolina, North Carolina, Virginia | 40          | 80          | 0.248 +/- 0.064 | 0.251 +/- 0.065 |
| b. Only “natural” populations
| 1      | Texas, Louisiana, Mississippi, Alabama, Gulf Coast of Florida | 10          | 20          | 0.053 +/- 0.053 | 0.055 +/- 0.055 |
| 2      | Florida Keys, Bahamas             | 4           | 8           | 0.316 +/- 0.029 | 0.362 +/- 0.033 |
| 3      | Atlantic Coast of Florida, Georgia, South Carolina, North Carolina, Virginia | 15          | 30          | 0.000 +/- 0.000 | 0.000 +/- 0.000 |

The mismatch distribution implemented in ARLEQUIN indicated that the data are compatible with the multimodal distribution expected in a population at demographic equilibrium. The following parameters were estimated under the spatial expansion model: \( \tau = 5.361 \) (95% CI: 2.2706–10.912), Harpending’s raggedness index (\( \delta \)) = 0.1989, with a nonsignificant P value of 0.6173, indicating that the data were not significantly different from the multimodal spatial expansion model assuming demographic equilibrium (Table 5). We calculated the time since population expansion (\( \tau \)) using the estimated \( \tau \) and its 90% confidence interval boundary values, \( \mu = 0.000000002 \), based on the average synonymous nucleotide substitution rate of 0.1–0.3% per million years of cpDNA reported in Wolfe et al. (1987), and \( k = 20 \) 000 bp. These parameters indicate an estimated population expansion time of 67 013 ya, with the 90% CI ranging between 136 400 and 28 383 ya (Table 5).

**Discussion**

**Geographic Distribution of cpDNA Haplotypes**

Our study supported the hypothesis that an east–west phylogeographic break divides the range of U. paniculata into 2 main genealogical lineages (Atlantic and Gulf of Mexico; Figure 1). The east–west disjunction, known as a maritime discontinuity, is congruent with previously reported patterns in codistributed animal species (Avise et al. 1987; Reeb and Avise 1990; Avise 2000; Soltis et al. 2006). Organisms that follow this pattern divide into eastern and western lineages, with the split typically occurring on the southern portion of the Florida peninsula (Avise 2000; Soltis et al. 2006). Many distantly related species, such as the oyster (Crassostrea virginica), dusky seaside sparrow (Ammodramus maritimus), and blacktip shark (Caribarhinus limbatus) show a phylogeographic break at a similar point on the Florida peninsula as U. paniculata (Avise and Nelson 1989; Reeb and Avise 1990; Keeney et al. 2005).

The phylogeographic break was likely caused by historical events, including sea level changes due to glaciation, the associated fluctuations in the size of the Florida peninsula, and...
mangrove-dominated ecosystems in southern Florida (Wise et al. 2004). N_ST was significantly greater than G_ST, indicating the evolutionary history of populations influenced the geographic distribution of cpDNA lineages (El Mousadik and Petit 1996; (Table 3). The patterns of cpDNA variation suggest that U. paniculata survived in southern refugia during the LGM, and subsequent northward migration divided the species into 2 units. One lineage moved northeast to the Atlantic Coast, whereas the other lineage spread northwest into the Gulf of Mexico. The inland habitats of the Florida peninsula, unsuitable for U. paniculata, acted as a barrier to gene flow between Atlantic and Gulf populations. Similarly, the mangrove ecosystems of southern Florida created a barrier between the Atlantic and Gulf populations by preventing sand dunes and beaches from forming in parts of southern Florida.

The majority of populations (54 out of 66) were fixed for a single haplotype. This low level of polymorphism is consistent with other plant phylogeographic studies and the low mutation rate of cpDNA (Wolfe et al. 1987; Avise 2000; Solis et al. 2006; Morris et al. 2010). Surprisingly, of the 66 populations, 17 contained at least 1 haplotype outside of its expected range (i.e., an Atlantic haplotype existing in a population located on the Gulf Coast, or vice versa). However, when “potentially restored” populations were excluded from the analysis, the east–west phylogeographic break became much more evident (Figure 1a). None of the “natural” populations contained any unexpected haplotypes except populations from the Florida Keys, and it may not be appropriate to label these haplotypes “unexpected” since they occur in a southern glacial refugium.

The current distribution of cpDNA haplotypes indicates a hotspot of genetic diversity in the southern portion of the range of U. paniculata in the United States. Five of the 6 haplotypes occur in the Florida Keys, and the highest genetic diversity was found in region 2, which contains the Florida Keys (Table 4). The high diversity suggests there were refugia in the Bahamas and southern Florida, including the Keys, during the LGM. Uniola paniculata may have survived in Cuba and along the Gulf Coast in Florida, Texas, or Mexico. The slow mutation rate of cpDNA and the estimated population expansion time (Table 5) suggest the cpDNA haplotypes predated the LGM (Zurawski et al. 1984; Wolfe et al. 1987; Gaut 1998). As temperatures rose following the LGM, U. paniculata migrated northward, and the unsuitable inland Florida habitats prevented gene flow between the Atlantic and Gulf coasts. Haplotypes A and B would have migrated north along the Atlantic Coast, whereas haplotypes C, D, and E migrated north along the Gulf Coast of Florida, and in the case of haplotype C, eventually west into the Gulf of Mexico.

The only Caribbean island sample is haplotype A, collected east of the southern tip of Florida in the Bahamas, which may have been a refugium for the Atlantic haplotype A. The Bahamas were closer to the Florida peninsula during the LGM than they are today—less than 100 km away (Haq et al. 1987). Uniola paniculata could have migrated northward along the Atlantic Coast from a refugium in the Bahamas due to the Gulf Stream. Our sampling was limited to herbarium specimens in the Bahamas, making it difficult to obtain additional individuals. Populations of Atlantic haplotypes could have survived the LGM in the southern portion of the Florida peninsula. The 7 populations sampled from the Florida Keys contain every haplotype except B, suggesting the Keys may have served as southern refugia and propagule sources for Atlantic haplotype A and Gulf haplotypes C and D. The Florida Keys and Florida peninsula were a continuous landmass during the LGM. As U. paniculata populations from the Keys migrated northward, inland Florida habitats acted as a barrier separating Atlantic populations from Gulf populations, explaining how the Florida Keys could act as a southern refugium for both modern-day Gulf and Atlantic haplotypes. Populations in Cuba, Texas, and Mexico may have persisted along the Gulf Coast during the LGM. However, there were no Cuban or Mexican samples available to investigate this possibility, and we did not find any genetic footprint that would confirm or exclude this scenario.

Anthropogenic introductions may be responsible for 17 populations containing unexpected haplotypes. Interviews with commercial growers of U. paniculata revealed that most greenhouses in Florida grow U. paniculata from seeds collected at Perdido Key State Park on the Gulf Coast of Florida. Genetic analyses indicated plants naturally growing in this location and seedlings commercially grown with seed from Perdido Key were both haplotype C, which had unexpected Atlantic Coast occurrences. Purchased seedlings can be planted hundreds of kilometres away from where seeds were harvested, leading to the introduction of nonnative haplotypes and explaining occurrences of haplotypes C on the Atlantic Coast (personal communication with owner of Green Seasons Nursery). Interestingly, all Atlantic instances of haplotype C occur in populations classified as “potentially restored”; they are in locations with a large amount of coastal real estate development, either because they are resort areas or heavily populated, or both. Humans probably introduced unexpected haplotypes to restore dunes to protect coastal real estate. Atlantic populations comprising the Gulf haplotype D are also in heavily populated locations, where dune restoration projects may have introduced this nonnative

Table 5  The mismatch distribution parameters τ, the 90% confidence interval of τ, t, the 90% confidence interval of t, and Harpending’s raggedness index (r) with corresponding P value, as estimated in ARLEQUIN (Excoffier et al. 2005) using the spatial expansion model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>τ (lower limit of 90% confidence interval)</td>
<td>2.2706</td>
</tr>
<tr>
<td>τ</td>
<td>5.3610</td>
</tr>
<tr>
<td>τ (upper limit of 90% confidence interval)</td>
<td>10.9120</td>
</tr>
<tr>
<td>Expansion time estimate with low value of τ (years)</td>
<td>28 382</td>
</tr>
<tr>
<td>Expansion time estimate with τ (years)</td>
<td>67 012</td>
</tr>
<tr>
<td>Expansion time estimate with high value of τ (years)</td>
<td>136 400</td>
</tr>
<tr>
<td>Harpending’s raggedness index</td>
<td>0.1989</td>
</tr>
<tr>
<td>Harpending’s raggedness index P value</td>
<td>0.6173</td>
</tr>
</tbody>
</table>
haplotype although there is no evidence that haplotype D has been used in restoration like haplotype C. Human transport could also explain the presence of Atlantic haplotypes on the Gulf Coast, where 1 population (Buccaneer State Park, Mississippi) is composed of haplotypes A and C. This sampling location underwent extensive beach restoration after Hurricane Katrina damaged its coastline (personal communication, Mississippi Wildlife, Fisheries and Parks). When we sampled this area, the beach was devoid of any U. paniculata plants more than a meter tall, and the plants were growing in evenly spaced rows. These seedlings were possibly introduced from multiple sources after Hurricane Katrina during large-scale dune restoration.

Synthesis with Nuclear Genetic Diversity Studies

Chloroplast DNA is maternally inherited in grasses and tracks genetic footprints left by seed movement, whereas nuclear DNA markers are biparentally inherited (Corriveau and Coleman 1988; Pillay and Armstrong 2001). We compared our results with 2 studies of U. paniculata that used nuclear markers to characterize genetic diversity across the species’ range. Franks et al. (2004) measured genetic structure and diversity in populations of U. paniculata using allozymes. Their analysis revealed less population genetic structure than cpDNA data, and several geographically disjunct populations were more similar to one another than to neighboring populations. Geographically distant Atlantic Coast populations from Virginia, Georgia, and Florida formed a clade with 2 populations on the western edge of the Gulf Coast in Texas. Another study used amplified fragment length polymorphisms (AFLPs) to assess genetic diversity among populations of U. paniculata (Subudhi et al. 2005). Their data revealed higher population genetic structure among U. paniculata populations than the Franks et al. (2004) allozyme study, which is unsurprising as AFLPs are more sensitive markers than allozymes, and there were larger spatial gaps between sampled populations than in Franks et al. (2004). Like the allozyme study, the AFLP study reported that some geographically distant populations were closely related: Texas, Louisiana, and Virginia populations form a clade with strong bootstrap support. With the exception of this clade, the populations grouped together based on geography. However, Subudhi et al. (2005) did not sample the Florida peninsula or Georgia, making conclusions about congruence among patterns of cpDNA and AFLP markers difficult. Nuclear DNA data do not show a clear east–west phylogeographic break, likely because of anthropogenic introduction of nonnative populations and subsequent long-distance, pollen-mediated gene dispersal among populations.

Synthesis with Morphological Variation Studies

The genetic discontinuity identified by cpDNA variation shows partial congruence with patterns of morphological variation found in a previous U. paniculata study (Seneca 1972). Seneca (1972) observed significant divergence in adaptive traits among plants from different geographic locations: plants differed in germination response following a cold period, and seedlings responded differently to salinity, temperature, and photoperiod treatments. Whereas the cpDNA lineages split into 2 units, Seneca’s (1972) surveyed populations clustered into 3 geographically structured groups based on differences in morphological characters: populations from North Carolina and Virginia grouped together (1), as did populations from the Atlantic Coast of Florida (2), and populations from the Gulf of Mexico formed the final group (3). These results partially correspond to the east–west discontinuity identified by the cpDNA lineages. The western cpDNA lineage correlates with one of the morphological groups (3), indicating historical separation may be accompanied by morphological divergence. Study by Seneca (1972) found that eastern populations (corresponding to Atlantic haplotype A) were further subdivided into a northern and southern groups. The effect of local environmental conditions on the evolution of adaptive traits may explain the subdivision of the eastern lineage; there is only partial congruence between the genetic and morphological structure. However, Seneca’s (1972) sampling was limited, with a large gap between the northern (1) and southern (2) Atlantic groups, and no populations from the Florida Gulf Coast were included in the analysis. Future studies of morphological variation may determine more precisely the relationship between cpDNA lineages and morphological variation.

Evolutionarily Significant Units and Conservation Implications

Uniola paniculata may be especially vulnerable to rapid climate change because the plants can only disperse in 2 directions because of its linear distribution. Uniola paniculata is capable of lateral vegetative spread of up to 2 m per year, whereas its seeds can disperse much greater distances. In the 1900s, the coastline in the western Gulf of Mexico retreated by 1–50 m per year (Hester and Mendelssohn 1987). Unable to vegetatively propagate or disperse quickly enough, U. paniculata populations were extirpated in many areas in Louisiana, where there is presently no suitable sandy beach habitat for the species to colonize. If the rate of sea level change and storm frequency continues to increase, U. paniculata may be extirpated from other locations.

The cpDNA variation detected in this study lays a foundation for defining evolutionarily significant units (ESUs) within U. paniculata by identifying 2 independent evolutionary lineages (Ryder 1986; Moritz 1994; Crandall 2000). One cpDNA unit corresponds geographically to 1 morphology-based cluster, whereas the other cpDNA unit correlates geographically to the other 2 morphology-based clusters. Thus, ecological data (i.e., morphological variation from Seneca, 1972) and genetic data (i.e., cpDNA variations from this study) can be combined to delineate 2 ESUs (sensu Crandall 2000) within the species: an eastern unit and a western unit. More complete nuclear genome data and more information about morphological variations across the entire range of the species are needed to fully grasp how ESUs would be best implemented. At this point, the data suggest the eastern and western maternal lineages may be considered separate ESUs.
However, for restoration purposes, we recommend the eastern lineage be subdivided into 2 management units, a northern and southern unit, based on the geographic distribution of morphological traits (Seneca 1972).

Currently, there are few regulations controlling introductions of \textit{U. paniculata}, seedlings are introduced to nonnative regions (e.g., haplotype C, the Gulf Coast native, being introduced to Atlantic Coast beaches). Thus far, there is no evidence that haplotypes of \textit{U. paniculata} show reduced fitness or survival when planted in nonnative regions, yet the consensus is that using local seeds in restoration better preserves evolutionary potential (Broadhurst et al. 2008). Each morphologically distinct and evolutionarily independent lineage may have unique adaptive variation vital to surviving environmental changes. Based on the distribution of cpDNA lineages, restrictions should be placed on the importation of \textit{U. paniculata} from a different coast (Atlantic or Gulf) for dune restoration to ensure that propagules have an evolutionary history of succeeding in the environment where they are introduced.

\textit{Uniola paniculata} occupies a highly specialized environment, yet environmental conditions throughout its range vary: there is a large difference in temperature between the northern and southern range limits (Seneca 1972; Barbour and Christensen 1993). Controlled reciprocal transplant and common garden experiments are needed to test the success of plants from different lineages in different environmental conditions. Experiments can determine whether the variation within the lineages has adaptive value in the environment (e.g., if lineages native to Texas can survive conditions in North Carolina); then transplantation restrictions for the species can be refined based on experimental results. This study has documented the evolutionary history of different populations of \textit{U. paniculata}; the next step is determining if independent lineages contain necessary adaptive variation and how to best conserve this variation. For now, it is wise to ensure that \textit{U. paniculata} populations conserve an evolutionary history of succeeding in their environment. This approach will ensure that populations of sea oats will maintain sand dunes, providing coastal habitat for themselves and many other species in the future.

**Supplementary Material**


**Funding**

This research was funded by North Carolina Sea Grant (10HRCC-A-2) and Appalachian State University (University Research Council and Office of Student Research).

**Acknowledgments**

We thank Dr Mike Kane, the United States National Herbarium (US), Ciara Lockstadt, and Patrick Sullins for valuable help obtaining samples. We thank Dr Mike Madritch, Dr Howard Neufeld, Dr Doug Soltis, and Dr Pam Soltis for many helpful comments on the article.

**References**


Received September 21, 2012; First decision November 1, 2012; Accepted May 6, 2013

Corresponding Editor: F. Andrew Jones