Genetic Structure of Native and Restored Populations of American Beachgrass (*Ammophila breviligulata* Fern.) along the New Jersey Coast

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**ABSTRACT**

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*Ammophila breviligulata* Fern. (American beachgrass) is planted extensively along the Atlantic coast of North America and in the Great Lakes region to stabilize damaged and constructed coastal dunes. Most *A. breviligulata* restorations are planted with a single cultivar for rapid dune stabilization. Restoration practice, however, is increasingly focused on maintaining native genetic diversity and restoring ecological services and function. We used intersimple sequence repeat (ISSR) markers to characterize the genetic structure of four native and four restored *A. breviligulata* populations along the coast of New Jersey on the northeastern Atlantic coast of the United States. Native populations had high levels of genotypic diversity for a clonal species, whereas restored populations on constructed dunes had low diversity or were monotypic. Commercial varieties used in dune restoration were not found in native populations. Native foredune populations were composed of many small- to medium-sized clones, while a rear-dune population was dominated by a single large clone. Genetic differentiation was low among native foredune populations. These results, discussed in the context of other clonal and coastal dune species, suggest that sexual recruitment plays an important role in determining the genetic structure of *A. breviligulata* populations, that gene flow has occurred among populations along the New Jersey coast, and that native New Jersey populations could provide genotypically diverse plant material for local restoration efforts.

**ADDITIONAL INDEX WORDS:** Clonal plants, ISSR, genotypic diversity, molecular population genetics, coastal dune restoration.

**INTRODUCTION**

Coastal dunes protect coastal infrastructure from storm damage, provide natural beauty for recreational users and residents, and provide critical habitat for threatened and endangered plant and animal species (U.S. Fish and Wildlife Service, 2006). Because of their importance, damaged dunes are repaired and replaced at substantial expense to government and private agencies. Along much of the eastern United States and in the Great Lakes region, American beachgrass, *Ammophila breviligulata* Fern., is planted on constructed dunes for sand stabilization and to initiate community succession (Miller and Peterson, 2006; Skaradek, Miller, and Hocker, 2003), paralleling the use of *Ammophila arenaria* in Europe (Rodriguez-Echeverria, Freitas, and van der Putten, 2008).

*Ammophila breviligulata* is also naturally occurring along the northern Atlantic coast of North America and the Great Lakes (Maun and Baye, 1989; Miller and Peterson, 2006). *A. breviligulata* acts as a pioneer species that colonizes bare beach and facilitates dune development and ecosystem succession toward maritime forest (Maun, 2009). Colonization of dunes occurs through water dispersal of rhizome fragments, seed, and asexual reproduction along rhizome nodes (Maun, 1984, 1985, 2009). The aboveground biomass of *A. breviligulata* captures and accumulates windblown sand, promoting dune formation, while underground biomass prevents dune erosion. As dunes accrete, additional plant species colonize, and *A. breviligulata* prevalence gradually declines due to a mix of factors, including reduced sand accretion, root pathogen load, and shifts in mycorrhizal interactions (Maun, 2009).

Restoration plantings of *A. breviligulata* are typically conducted using single cultivar plantings of asexually propagated nursery stock, often of nonlocal provenance. In New Jersey, located on the northeastern Atlantic coast of the United States, the “Cape” variety (originally collected from Cape Cod, Massachusetts) is most commonly planted, following its selection for aboveground vigor, large leaf size, and ease of propagation in the 1970s by the U.S. Department of Agriculture (USDA), Natural Resources Conservation Service, Cape May Plant Materials Center (Gaffney, 1977). However, success with “Cape” plantings has been mixed; for example, a beach renourishment project in the early 1990s in Avalon, New Jersey, saw a return of maritime forest (*Nordstrom et al.,* 2002) to 100% plant mortality after 2 years at Sandy Hook National...
Recreation Area (NRA), New Jersey (Miller and Skaradé, undated). Lack of sand accretion is often cited as the reason for restoration failure, but a combination of biotic and abiotic factors (Maun, 2009) and the use of genetically inappropriate cultivars in these plantings (Falk et al., 2006; Montalvo et al., 1997; Rice and Emery, 2003) might also play a role.

While dune restorations in New Jersey have emphasized rapid stabilization of constructed dunes, restoration practice is increasingly focused on restoring ecological services and function (Palmer, 2009; Richie and Krauss, 2012; Suding, 2011; Wortley, Hero, and Howes, 2013; Zedler, Doherty, and Miller, 2012). Utilizing and conserving native genetic diversity within restoration populations are central components of this shift. Intrapopulation genetic diversity, particularly in foundation or dominant species, enhances population performance, community-level diversity, and the development of ecosystem services (Hughes et al., 2008; Montalvo et al., 1997; Rice and Emery, 2003). Genetically diverse populations have outperformed monotypic and low-diversity populations on measures such as aboveground biomass (Cook-Patton et al., 2011; Crawford and Rodgers, 2012; Crutsinger et al., 2006; Kotowska, Cahill, and Keddie, 2010; Wang et al., 2012), survival time (Kotowska, Cahill, and Keddie, 2010; Reynolds, McGlathery, and Waycott, 2012), rate of multiplication and spread (Reynolds, McGlathery, and Waycott, 2012; Wang et al., 2012; Williams, 2001), and patch size and competitive ability (Wang et al., 2012). On a community level, intrapopulation genetic diversity in dominant or foundation plant species enhanced plant diversity (Fridley and Grime, 2010; Fridley, Grime, and Bilton, 2007) and invertebrate density and diversity (Cook-Patton et al., 2011; Hughes et al., 2008; Kotowska, Cahill, and Keddie, 2010; Moreira and Mooney, 2013; Reynolds, McGlathery, and Waycott, 2012), increased resistance to stress and disturbance (Hughes and Stachowicz 2004, 2009, 2011), accelerated ecosystem recovery after climate shifts (Reusch et al., 2005), and enhanced ecosystem services such as nutrient cycling, decomposition, and nutrient retention (Hughes et al., 2008; Reynolds, McGlathery, and Waycott, 2012). In addition, coastal sand dunes are spatially heterogeneous environments (Maun, 2009), prone to disturbance (Ehrenfeld, 1990), and sensitive to climate change (National Research Council, 2010; van der Meulen and Salman, 1996). Intrapopulation genetic diversity might increase the ability of plant populations to adapt and persist under these conditions (Gibson et al., 2012; Montalvo et al., 1997; Rice and Emery, 2003).

Another important consideration in ecological restoration is the use of locally sourced restoration propagules to maintain locally adapted genotypes and avoid introducing maladapted or overly competitive nonlocal genotypes (Bischoff, Steinger, and Müller-Scharer, 2010; McKay et al., 2005; Montalvo et al., 1997). In restorations of Ammophila sp., displacement of native genotypes through intraspecific competition is a serious concern. Michigan A. breviligulata plants used to restore Minnesota dunes outperformed Minnesota plants in field and common-garden experiments on a number of growth and sexual reproductive measures, including aboveground growth rate and size, fertile culm size, and flowering frequency (Hols trom, Et terson, and Schimpf, 2010). This is particularly important for A. breviligulata populations in Minnesota and Illinois, as well as populations of Ammophila chaplainensis Seymour in New York and Vermont, where these species are listed as threatened and endangered, respectively—a status justified in part by displacement of local genotypes by nonlocal strains introduced through restoration efforts (Minnesota Department of Natural Resources, undated; New York Natural Heritage Program, 2013). On a community level, studies with the European congener A. arenaria found that invertebrate diversity decreased as the distance from the plants’ location of origin increased, suggesting that nonlocal sourcing of propagules can reduce community-level diversity (Vandeggehuchte et al., 2012).

Restoring genetic diversity requires prior characterization of genetic structure within and among local native populations (Fant et al., 2008; Franks et al., 2004; Novy et al., 2010; Stingenmore and Krauss, 2013; Utomo et al., 2009). In the Great Lakes region, Fant et al. (2008) used intersimple sequence repeat (ISSR) markers to detect substantial genotypic diversity in native A. breviligulata populations in Minnesota and Illinois, in contrast to restorations planted with commercial nursery stocks, which remained monotypic. Crawford and Rodgers (2012, supplement), also using ISSR markers, noted that while most Michigan A. breviligulata populations were genetically distinct from one another, genetic similarity was greater within populations than between them.

Restorations mimicking local diversity have the potential to be more adaptive, resilient, and sustainable, and to support more complex and stable ecosystem functions and services. When available and practical, locally sourced propagules should be used to create restorations that mimic the genetic structure of local native populations. Before the present study, genetic diversity within A. breviligulata populations based on high-resolution molecular markers has only been published for the Great Lakes region (Fant et al., 2008). Here, we report on the genetic structure of native and restored A. breviligulata populations from northern, central, and southern coastal locations in New Jersey, using ISSR markers.

**METHODS**

Ramets from four native and four restored A. breviligulata populations were sampled along the New Jersey coast. Leaf tissue from ramets was genotyped using ISSR markers, and data were analyzed for genotypic and genetic diversity, clonal structure, and population genetic differentiation. Detailed procedures for each step are as follows.

**Sample Collection and Handling**

Leaf samples of A. breviligulata were collected between June 2010 and September 2012 from four native and four restored populations in New Jersey, U.S.A. (Figure 1). Native populations were located at (1) the north end of North Beach, Sandy Hook Unit, Gateway NRA (SHBN-N; 40°28′37.1604″ N, 74°0′34.2246″ W and 40°28′36.7314″ N, 74°0′30.7074″ W), (2) Southern Natural Area, Island Beach State Park (IBSP-N; 39°47′15.885″ N, 74°5′39.0192″ W), (3) Little Beach Island, Forsythe National Wildlife Refuge (NWR) (FORS-N; 39°29′36.7548″ N, 74°19′8.5404″ W), and (4) Two Mile Beach Unit, Cape May NWR (CMRF-N; 38°56′56.6118″ N, 74°51′24.231″ W). Native populations were located on fore-
dunes, except IBSP-N, which was located on a rear dune ~50 m inland from the foredune in that system. Restored populations were located at (5) the south end of North Beach, Sandy Hook Unit, Gateway NRA (SHNB-R; 40°28′2.1138″ N, 73°59′48.57″ W; planted 1991/1992), (6) Spermaceti Cove Visitor’s Center, Sandy Hook Unit, Gateway NRA (SHSC-R; 40°25′35.6622″ N, 73°59′0.3408″ W and 40°25′36.6996″ N, 73°58′59.9046″ W; planted prior to 2000), (7) Brigantine Township (BRIG-R; 39°24′31.5282″ N, 74°21′38.2314″ W; planted in mid-1990s), and (8) Cape May Point State Park (CMSP-R; 38°55′52.068″ N, 74°57′41.5836″ W; planted in early 2000s). Approximate planting dates were provided by site personnel (see Acknowledgments). Restored populations were planted on constructed dunes that were part of beach nourishment projects, except BRIG-R, where the “Cape” variety was planted into an existing natural dune following storm erosion (Ed Stinson, Township Engineer, Brigantine, NJ, personal communication).

Leaf samples consisted of one healthy mature leaf collected from 150 ramets in each native population, and from 50 ramets in each restored population. Where possible, three parallel 45 m transects spaced 6 m apart were sampled in restored populations. However, some transect lengths and arrangements varied to accommodate population shape and/or piping plover exclosures. All transects were straight lines with fine-scale sampling quadrants at 5 m intervals. At each sampling quadrant, five samples were collected 1 m apart in a centered cross formation (Figures 2 and 3). Leaves were cut at the blade base, folded into labeled 50 mL plastic screw-cap tubes, and immediately placed on ice. Samples remained on ice no more than 24 hours prior to storage at ~8°C. Leaf samples of commercial varieties “Cape,” “Hatteras,” and “Bogue” were collected from greenhouse-grown plants originally provided as culms by Chris Miller, Manager and Plant Materials Specialist, USDA Cape May Plant Materials Center, Cape May, New Jersey.

Deoxyribonucleic Acid (DNA) Extraction
Genomic DNA was extracted from approximately 2 cm² of leaf tissue homogenized by hand with a plastic pestle in 0.3 mL Plant DNAzol (Life Technologies, Grand Island, New York, U.S.A.) in a 1.5 mL microcentrifuge tube, according to...
manufacturer instructions. DNA was quantified using a BioSpec-nano spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

Polymerase Chain Reaction (PCR) and Data Collection

The three primers that generated up to 10 polymorphic loci in Great Lakes populations (Fant et al., 2008) gave only six polymorphic loci in a 20 sample test population from SHNB-N. Because five of the six polymorphic loci were from one primer (UBC810), we screened 100 primers from the University of British Columbia's microsatellite-anchored Primer Set #9 (University of British Columbia, discontinued) and selected six primers giving a total of 19 polymorphic loci and 32 nonpolymorphic loci with consistent, easily scored bands. ISSR primers used in this study were UBC810 (GA<sub>9</sub>T), UBC842 (GA<sub>9</sub>YG), UBC844 (CT<sub>6</sub>RC), UBC845 (CT<sub>9</sub>RG), UBC846 (CA<sub>9</sub>RT), and UBC846 (CA<sub>9</sub>RG); R is a purine (A or G), and Y is a pyrimidine (C or T).

Individual PCR reactions consisted of 12.5 μL of 2X Promega PCR Master Mix (Promega Corporation, Madison, Wisconsin, U.S.A.), 1.2 μL of 20 μM primer, 20 ng DNA, and sterile distilled water (SDW) to 25 μL final volume. Pre-mixes containing Master Mix, primer, and SDW were aliquoted to reaction tubes prior to adding DNA. Reactions were initiated for 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 44°C, and 2 min at 72°C, and ended with a 10 min extension at 72°C. Each set of PCR reactions included an internal “Cape” control, and all PCR reactions were run in duplicate with duplicate reactions set up and run independently.

PCR products were visualized alongside a 50 bp DNA ladder (New England Biolabs, Inc., Ipswich, Massachusetts, U.S.A.) on 1.5% agarose gels in 1XTris-Borate-EDTA (TBE) containing 0.3 μg/mL ethidium bromide. Images were captured using a Bio-Rad ChemiDoc XRS+ with Image Lab 4.0.1 software (Bio-Rad, Hercules, California, U.S.A.). Polymorphic loci were scored manually as band presence (1) or absence (0), and binary data sets including both polymorphic and monomorphic loci were created. Each unique multilocus banding profile was considered a distinct multilocus genotype.

Data Analysis and Clone Maps

The number of distinct multilocus genotypes (G) was obtained using the Multilocus Matches function in GenAIEx 6.5 (Peakall and Smouse, 2012). Genotype richness was calculated as (G – 1)/(N – 1), the probability of sampling a different genet with each newly sampled ramet, where G is the number of multilocus genotypes distinguished and N is the number of ramets sampled. (G – 1)/(N – 1) will be zero for a monotypic population, and it will equal 1.0 when every sampled ramet has a distinct genotype (Arnaud-Haond et al., 2005). We also calculated Simpson’s diversity index corrected for finite sample size, or genotypic (clonal) diversity, D = 1 – Σ[ni(ni – 1)]/[N(N – 1)], where ni is the number of distinct multilocus genotypes, and N is the total number of individuals in a population (Pielou, 1969). D is useful for comparison across clonal plant studies, since it is only weakly affected by marker resolution and the number of loci sampled (Honnay and Jacquemyn, 2008). When D = 0, one dominant clone is present, while a value of 1.0 suggests every sample has a distinct genotype (Meloti et al., 2013). GenAIEx Multilocus Matches data were also used to create clone maps, which were further used to determine the number of unique genotypes per square meter (G/m²), an index of fine-scale clonal structure, within each population. G/m² values were limited to sample quadrants where all five samples were successfully genotyped. Percent polymorphic loci (P) was also determined.

RESULTS

Here, we present genotypic and genetic diversity measures for four native and four restored A. breviligulata populations located in New Jersey along the Atlantic coast of the United States based on 19 polymorphic ISSR loci. We also present clonal structure within populations and characterize the distribution of genetic variation within and among native populations.

Genotypic Diversity in Native and Restored Populations

The three native foredune populations CMRF-N, FORS-N, and SHNB-N contained from 56 to 91 distinct genotypes per population (G) and showed high levels of genotype richness [(G – 1)/(N – 1) = 0.42–0.61] and genotypic (clonal) diversity (D = 0.97–0.98) (Table 1). The fourth native population, IBSP-N, was located on a rear dune where beachgrass was no longer dominant. IBSP-N contained 21 distinct genotypes and had substantially lower genotypic diversity than foredune populations [(G – 1)/(N – 1) = 0.13; D = 0.48]. None of the four native populations contained the “Cape” variety, which is commonly used in New Jersey restorations, nor did they contain “Hatteras” or “Bogue.” Hatteras and Bogue are commonly planted in North Carolina, and both have been planted in New Jersey.
The four restored populations were planted with "Cape" variety on constructed dunes as part of beach nourishment projects (CMSP-R, SHSC-R, and SHNB-R) and contained zero or very low genotype richness \([(G - 1)/(N - 1) = 0.0–0.04]\) and zero to moderate genotypic diversity \((D = 0.0–0.31)\) (Table 1). In CMSP-R (planted early 2000s) and SHSC-R (planted prior to 2000), only the "Cape" variety was found. In SHNB-R (planted 1991/1992), 82% of the samples were "Cape" genotypes. The fourth restored population, BRIG-R, was located on a natural dune supplemented with "Cape" following storm damage in the mid-1990s. Thirty-two percent of BRIG-R samples were "Cape," and 18% were composed of two non-"Cape" genotypes. The fourth restored population, BRIG-R, was located on a natural dune supplemented with "Cape" following storm damage in the mid-1990s. Thirty-two percent of BRIG-R samples were "Cape," and 18% were composed of two non-"Cape" genotypes. BRIG-R had genotypic diversity measures similar to those seen in native populations \((D = 0.16; D = 0.80)\).

The number of distinct genotypes per square meter \((G/m^2)\) is a measure of fine-scale clonal structure within a population (Fant et al., 2008). With the exception of BRIG-R, \(G/m^2\) values were higher in native than restored populations (Table 1). In native foredune populations CMRF-N, FORS-N, and SHNB-N, \(G/m^2\) values ranged from 2.8 to 3.5 \(G/m^2\), whereas the rear-dune population IBSP-N had a lower value of 1.9 \(G/m^2\). Restored populations that remained "Cape" monocultures had 1.0 \(G/m^2\), but SHNB-N, which was also planted with "Cape" on a constructed dune, had 1.4 \(G/m^2\). The "Cape"-supplemented restoration population BRIG-R had 2.6 \(G/m^2\).

Finally, the number and percentage of polymorphic loci \((P)\), a measure of genetic diversity, were higher in native than restored populations (Table 1). In total, 51 loci, including 19 polymorphic loci, were scored in this study. Native populations contained from 12 to 18 polymorphic loci per population, with percent polymorphic loci \((P)\) ranging from 24% to 35%. Restored populations contained from 0 to 5 polymorphic loci per population, with \(P\) ranging from 0% to 10%.

### Clonal Structure in Native and Restored Populations

In native foredune populations SHNB-N, FORS-N, and CMRF-N, clones were distributed fairly evenly throughout the population (Figures 2A, C, and D). In restored populations where non-"Cape" genotypes were observed, these genotypes were also well distributed within the population (Figure 3). The native rear-dune population IBSP-N, however, contained a single dominant clone (Figure 2B) that represented 72% of the ramets sampled in that population. Aside from the dominant IBSP-N genotype and "Cape," clones from all populations ranged in size from 1 to 13 ramets, averaging 2.1 ramets per genet \((R/G)\) within a population. Excluding "Cape" and genotypes that occurred only once in a population, the average clone spread was 8.1 m, with the longest clone spreading 53 m between two end-to-end transects in SHNB-N (shown as two unconnected \(\triangle\)-labeled genets in Figure 2A due to spatial constraints). On average, 63% of genotypes in native populations were sampled only once within a population.

Eighteen of 214 (8%) distinct genotypes were found in more than one of the native populations. Sixteen of these were found in two native populations, and two were found in all four of the native populations. Of the 16 genotypes found in two populations, one was shared between CMRF-N and the rear-dune population IBSP-N, four were shared between CMRF-N and FORS-N, five were shared between CMRF-N and SHNB-N, and six were shared between FORS-N and SHNB-N. Among restored populations, one non-"Cape" genotype was found in two restored populations: BRIG-R and SHNB-R. No genotypes were found in common between native and restored populations.

### Genetic Structure within and among Populations

A UPGMA dendrogram based on Nei's (1978) unbiased pairwise population genetic distances discerned two distinct groupings among the eight populations sampled (Figure 4). One group included all four native populations, and the second group included all four restored populations. Among native populations, the largest difference was between the rear-dune

![Figure 4. UPGMA dendrogram for eight New Jersey A. breviligulata populations based on Nei's (1978) unbiased genetic distances from 19 polymorphic ISSR markers.](image)
population IBSP-N, which was dominated by a single clone, and the three foredune populations, which were composed of many smaller genets and had substantially higher genotypic diversity.

To further assess the genetic structure of native beachgrass populations, Phi-PT was calculated to estimate the degree of genetic variation within and among native populations. Phi-PT for all four native populations was 0.19 (p = 0.001 based on 999 permutations). This suggests that a modest and statistically significant amount of interpopulation genetic differentiation has occurred, with 19% of variation observed among populations, and 81% of the variation observed within populations. However, when IBSP-N was removed from the analysis, Phi-PT dropped to 0.09 among the three native foredune populations (p = 0.001 based on 999 permutations). This suggests that much of the interpopulation differentiation is due to the rear- dune population IBSP-N, and that little differentiation has occurred among foredune populations that span the full length of the New Jersey coast (Figure 1). This trend is confirmed by pairwise Phi-PT values, where Phi-PTs involving IBSP-N were 2.3- to 5.9-fold higher than those between foredune populations (Table 2).

**DISCUSSION**

Native New Jersey *A. breviligulata* populations had high average genotype richness ([\(G - 1\)/[\(N - 1\)] = 0.41] and genotypic diversity (\(D = 0.86\)). Populations of many clonal plant species contain levels of genotypic diversity comparable to purely or predominantly sexual species (Ellstrand and Roose, 1987; Honnay and Jacquemyn, 2008; Widen, Cronberg, and Widen, 1994), including many coastal dune species. For example, a Welsh population of *A. arenaria*, the European congener of *A. breviligulata*, was shown using Amplified Fragment Length Polymorphism (AFLPs) to have a G/N value of 0.48 (Hol et al., 2008), and the dominant coastal dune grass of the southeastern U.S. and Gulf Coast states, *Uniola paniculata* (sea oats), averaged G/N = 0.131 and D = 0.60 across two sites—one in North Carolina and a second on the Gulf Coast of Florida (Franks et al., 2004), both based on isozyme analysis. A second study of *U. paniculata*, using Random Amplified Polymorphic DNAs (RAPDs), estimated a high ([\(G - 1\)/[\(N - 1\)] 1 value of 0.70 and \(D = 0.95\) for a well-established population, but substantially lower values of ([\(G - 1\)/[\(N - 1\)] = 0.11 and \(D = 0.23\) for a newly established population, both on Waites Island, South Carolina (Bush and Stelato, 2007). While Subudhi et al. (2005) suggested that the number of isozyme loci analyzed by Franks et al. (2004) might have underestimated diversity in *U. paniculata* relative to DNA-based molecular markers, Sánchez-Vilas, Philipp, and Retuerto (2010) observed little difference between estimates of ([\(G - 1\)/[\(N - 1\)] (0.22 and 0.36) and \(D = 0.65\) and 0.68) for Honkenya peplusoides, another clonal, coastal dune species, based on four polymorphic isozyme loci and 51 polymorphic AFLP loci, respectively. Additional clonal, coastal dune species assessed for genotypic diversity include *Glomera hederacea* (G/N = 0.45, D = 0.98) (Widen, Cronberg, and Widen, 1994), *Carex kobomugi* (mean G/N = 0.20) (Ohsako, 2010), *Calystegia soldanella* (mean G/N = 0.57, D = 0.88), and *Ipomoea stolonifera* (mean G/N = 0.20, D = 0.76) (Kim and Chung, 1995). While it is clear from previous studies that clonal plant populations contain substantial genotypic diversity (Ellstrand and Roose, 1987; Honnay and Jacquemyn, 2008; Widen, Cronberg, and Widen, 1994), it is also evident that genotypic diversity is not adversely affected by restriction of a plant species to the relatively narrow, linear, and often fragmented coastal dune habitat.

Interestingly, average diversity measures in native New Jersey *A. breviligulata* populations ([\(G - 1\)/[\(N - 1\)] = 0.41; \(D = 0.86\)] were higher than those observed in native Great Lakes populations (average ([\(G - 1\)/[\(N - 1\)] = 0.17, \(D = 0.62\)) (Fant et al., 2008). This discrepancy may occur in part because Great Lakes populations are at *A. breviligulata*s range margin, where greater isolation and smaller effective population sizes are expected to reduce within-population genetic diversity (Eckert, Samis, and Lougheed, 2008). Sixty-four percent of studies reviewed by Eckert, Samis, and Lougheed (2008), covering 67 plant species, reported lower within-population genetic diversity at range margins. In addition, Great Lakes populations may have experienced founder effects during postglaciation recolonization of the Great Lakes region from Atlantic coast refugia following retreat of the Laurentide ice sheet (Walker, 1998). The difference in diversity between native New Jersey and Great Lakes populations might, however, also reflect a difference in resolving power of the marker systems used. The most diverse Great Lakes population was assessed with 10 polymorphic loci and had high genotypic diversity measures ([\(G - 1\)/[\(N - 1\)] = 0.57 and \(D = 0.96\)) (Fant et al., 2008). However, most native Great Lakes populations were assessed with six or fewer polymorphic loci, compared to 12–18 polymorphic loci in native New Jersey populations. Differences in resolving power might also explain the high proportion of distinct genotypes found in more than one Great Lakes population (45% in native Illinois populations and 42% in native Minnesota populations), compared to 8% of genotypes among native New Jersey populations. In addition, Fant et al. (2008) noted that many of their native populations contained a known commercial genotype and suggested that undocumented restoration efforts may have occurred at those sites. Those populations might be roughly equivalent to our population BRIG-R, where “Cape” was planted into a storm- damaged natural dune, and moderate diversity was observed ([\(G - 1\)/[\(N - 1\)] = 0.16, \(D = 0.80\)]. Such “supplemental” restorations highlight the threat of cryptic invasion and displacement of native genotypes by commercial cultivars (Holstrom, Eterson, and Schimpf, 2010). While *A. brevili- guleta* restorations in New Jersey have typically relied on “Cape” plantings, the genotypic diversity seen in native New Jersey populations suggests that native populations could serve as propagule sources for local restoration efforts. For example, in the Gateway NRA, Sandy Hook Unit, native

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<th>Population</th>
<th>CMRF-N</th>
<th>FORS-N</th>
<th>SHNB-N</th>
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Table 2. Pairwise population Phi-PT values for native New Jersey *A. breviligulata* populations.
genotypes were collected from the SHNB-N population and used to establish a native nursery stock for local restoration efforts. Unfortunately, this nursery stock was lost during tropical storm Sandy in October 2012 (G. Frame, personal communication).

While seedling recruitment is thought to occur at a very low rate in A. breviligulata (Lichter, 2000; Maun, 1985), low levels of sexual reproduction can maintain fairly high levels of genotypic diversity in clonal plant populations (Watkinson and Powell, 1998). High genotypic diversity and a predominance of small genets observed in native New Jersey populations of A. breviligulata, and similar patterns in the Great Lakes region (Funt et al., 2008), suggest that seedling recruitment is an important determinant of genetic structure in A. breviligulata populations (Eriksson, 1989). Successful sexual recruitment in A. breviligulata may be episodic, occurring only under rare favorable conditions (Eriksson and Fröborg, 1996), such as confinement to favorable microhabitats with increased soil moisture (Jonsson and Prentice, 2000). Episodic seedling recruitment and limited field observations (Maun, 1985) may have underestimated the importance of sexual recruitment in some clonal plants (Eriksson, 1989). Episodic seedling recruitment has been put forward to explain within-population genotypic diversity in other clonal species with little observed seedling recruitment, such as U. paniculata (Franks et al., 2004), Carex arenaria (Jonsson and Prentice, 2000), Saxifraga cernua (Gabrielsen and Brochmann, 1998), and Vaccinium stamineum (Kreher, Foré, and Collins, 2000; Yakimowski and Eckert, 2008). However, somatic mutation can also contribute to genetic diversity in clonal plant populations (Chapman, Parh, and Oraguzie, 2000; Honnay et al., 2006; Klekowski, 1997; Kreher, Foré, and Collins, 2000; Rottenberg and Parker, 2004; Sánchez-Vilas, Philipp, and Retuerto, 2010; Tuskan et al., 1996). For example, in the oldest (~20 year old) “Cape”-restored population, SHNB-R, the two non-“Cape” genotypes varied from “Cape” at a single locus, suggesting that these genotypes may have arisen from somatic mutation during vegetative reproduction (Chapman, Parh, and Oraguzie, 2000; Kreher, Foré, and Collins, 2000). However, distinguishing somatic variation from sexually derived allelic variation is difficult (de Witte and Stöcklin, 2010) and could not be distinguished in our analysis, so crosses between local native genotypes and planted “Cape” cultivar cannot be ruled out. While somatic mutation may contribute to genotypic variation in A. breviligulata populations, the high levels of genotypic diversity and the preponderance of small genets within native populations are most readily explained by episodic sexual reproduction (Chen et al., 2006; Eriksson, 1989; Kreher, Foré, and Collins, 2000; Sánchez-Vilas, Philipp, and Retuerto, 2009; Widen, Cronberg, and Widen, 1994).

Current understanding is that A. breviligulata colonizes bare beach in part through rhizome fragments washed up in the surf (Maun, 1984), and small numbers of genotypes were shared among native populations in our study. Furthermore, the low level of genetic differentiation among native foredune populations suggests that gene flow has occurred along the length of the New Jersey coast. Long-distance dispersal of a small number of rhizome fragments among populations and the contribution of those genets to sexual reproduction within the population could be sufficient to prevent genetic differentiation (Slatkin, 1985). For example, rhizome dispersal, a strategy potentially used by many coastal plant species (Maun, 2009), is thought to contribute to low genetic differentiation among European populations of Carex arenaria (Jonsson and Prentice, 2000). More commonly, however, ocean transport of buoyant fruits and seeds has been suggested to explain gene flow and low levels of genetic differentiation among populations of coastal dune species, including U. paniculata (Franks et al., 2004), Calystegia soldanella (Arefeh and Kadereit, 2006; Kim and Chung, 1998), Carex arenaria (Jonsson and Prentice, 2000), and Crambe maritime (Bond, Daniels, and Bioret, 2005). A. breviligulata caryopses are buoyant (Maun, 1985, 2009), suggesting their dispersal by water could play a role in long-distance gene flow, but additional genetic and seed survival studies are required to directly assess this possibility.

Little is known about the way in which genetic diversity influences performance of A. breviligulata populations, or community development and ecosystem function. Emery and Rudgers (2010) compared A. breviligulata populations restored with a single genotype ~25 years ago to natural reference populations and found no differences between the associated communities in plant species diversity, invertebrate diversity, or mycorrhizal diversity. This is in contrast to studies where intrapopulation genetic diversity in the dominant plant species enhanced plant diversity (Fridley and Grime, 2010) and invertebrate density and diversity (Cook-Patton et al., 2011; Hughes et al., 2008; Kotowska, Cahill, and Keddie, 2010; Moreira and Mooney, 2013; Reynolds, McGlathery, and Waycott, 2012). Performing such studies under a variety of stress conditions common to dune ecosystems might demonstrate direct benefits of genetic diversity in A. breviligulata, as seen in eelgrass (Zostera marina), where clonal diversity correlated to shoot density, but only under winter stress conditions (Hughes and Stachowicz, 2009). However, the benefits of genetic diversity may not always be direct. Crawford and Rudgers (2012) showed that genetic diversity in A. breviligulata on Michigan dunes had no direct effect on community species diversity or biomass production, but genetic diversity did promote a positive relationship between those two variables, highlighting the fact that genetic diversity can benefit a species in complex and indirect ways.

CONCLUSIONS

Native New Jersey populations of American beachgrass (A. breviligulata) exhibit substantial within-population genotypic diversity. On average, genotypic diversity in native New Jersey populations exceeds the diversity in restored New Jersey populations and native and restored Great Lakes populations. Patterns of diversity within native New Jersey populations, as in the Great Lakes region, suggest that sexual and vegetative reproduction both play important roles. Patterns of diversity among native New Jersey populations suggest that gene flow has occurred along the length of the New Jersey coast. These results suggest that native New Jersey populations are a potentially valuable source of genetically diverse propagules for future dune restoration efforts. Common-garden experiments to assess variation in plant performance, and further
characterization of genetic diversity along the Atlantic coast are recommended.

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LITERATURE CITED


