

RESEARCH ARTICLE

Dune Restoration Introduces Genetically Distinct American Beachgrass, *Ammophila breviligulata*, into a Threatened Local Population

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Abstract

There is mounting concern over the source and genetic history of plant propagules used for habitat restoration because introduced genotypes may destabilize remnant local populations through competition and introgression. We examined whether introduced dune grass, *Ammophila breviligulata*, from Michigan is genetically distinct from a threatened local Minnesota population by comparing local and nonlocal genotypes in well-established stands in the field and in experimental common gardens. Both observational and experimental studies suggest that Michigan plants differed genetically and had an advantage over local plants in terms of vegetative spread and sexual reproduction. Well-established restoration populations composed of Michigan plants produced fertile culms that were 1.8 times taller than Minnesota plants and more than twice as fecund. Introgression is unlikely, however, because Minnesota genotypes flowered on average 6.7 weeks earlier

than Michigan plants and only 20% of Minnesota pollen remained viable after 4 weeks. In the common gardens, Michigan plants from two sources were larger in size, grew faster, and flowered more frequently than Minnesota plants. Plant surveys across the study area suggest that nonlocal genotypes have spread beyond documented restoration areas and into local stands, particularly in foredunes. Even if gene flow between local and nonlocal plants is limited due to differences in flowering phenology, Michigan genotypes may out-compete plants in the threatened Minnesota population through greater vegetative and sexual reproduction. The fitness consequences of this change in the genetic composition of the local population have yet to be determined.

Key words: *Ammophila breviligulata*, common garden, genetic divergence.

Introduction

Restoration ecologists are becoming increasingly concerned about the source and genetic characteristics of seeds or clones used to supplement and replace degraded local populations (Hufford & Mazer 2003). Nonlocal sources of plant material are often relied upon although these propagules may differ from local genotypes in ways that may ultimately undermine the success of the restoration project. In the short-term, nonlocal plants may fail to become established if they are maladapted to their new environment (Montalvo et al. 1997). Alternatively, nonlocal plants may persist in their new environment with positive or negative impacts depending on the factors that underlie their success.

Restoration propagules that were planted and became established during a period of favorable climatic conditions may suffer high mortality or reproductive failure during extreme weather events in subsequent years that are tolerated by the local population. Nonlocal plants may also escape damage by local herbivores and pests because their flowering and fruiting phenologies do not correspond to those of other members of the local community. However, phenological disjunction between community members can also have negative fitness consequences particularly on codependent relationships, such as pollination. If nonlocal propagules have a fitness advantage, either through vegetative spread or sexual reproduction, they may obtain a numerical advantage over the local population especially if restoration plantings were extensive relative to the size of remnant local populations (Rhymer & Simberloff 1996).

Genetic attributes of restoration propagules may confer fitness advantages over local populations. Restoration populations may be composed of particularly robust genotypes selected from the original natural populations (e.g., “plus-trees” in forestry). These plants may have experienced further inadvertent or intentional artificial selection in propagation gardens (Montalvo et al. 1997). However, this genetic material

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may be inherently depauperate in genetic diversity if the original collections from native populations were limited (Williams 2001). Furthermore, propagation techniques, such as collecting plant material from small areas or from a limited number of selected clones may further reduce the genetic diversity of restoration material (Williams 2001). Random genetic processes may be especially strong under these circumstances where the effective population size is small and the plants are isolated from gene flow.

Nonlocal plants may negatively impact remnant local populations if they persist and interbreed with local plants causing outbreeding depression in the hybrid progeny (Lynch 1991; Waser & Price 1994; Etterson et al. 2007). Outbreeding depression can occur if local and nonlocal plants differ for traits that are associated with local adaptation, such as morphology or phenology, and hybrids express intermediate phenotypes that are less fit (Templeton 1986). Alternatively, outbreeding depression may occur due to a break-up of positive cytonuclear or epistatic genetic interactions when hybridized ("intrinsic" outbreeding depression; e.g., Whitlock et al. 1995). Theoretical and empirical studies support the hypothesis that gene flow between distinct genetic entities may degrade local populations and threaten persistence (Lynch 1991; Rhymer & Simberloff 1996; Quilichini et al. 2001). The threat these factors pose is generally not known because baseline genetic studies are rarely conducted and long-term effects of restorations are rarely monitored.

Gene flow can also have positive effects. If the local population is small and genetically isolated or was founded by a limited number of individuals, it may have reduced diversity due to genetic drift and inbreeding (Barrett & Kohn 1991; Ellstrand & Elam 1993). Inbred populations exhibit reduced fitness because of greater homozygosity leading to the expression of deleterious recessive alleles. Under these circumstances, gene flow may increase population fitness by masking deleterious alleles and/or restoring heterozygote advantage and favorable allele combinations (Lynch 1991). Heterosis has been observed for intraspecific F_1 hybrids in a number of studies (Sheridan & Karowe 2000; Luijten et al. 2002).

This study focuses on a widespread grass species, *Ammophila breviligulata* (American beachgrass), which is planted for dune stabilization and restoration. Various strains and local populations have been propagated and sold commercially for restoration (Maun & Baye 1989). Because of these practices, plants used for restoration are likely to differ genetically from local populations both in terms of the composition of genotypes represented and of the overall levels of genetic diversity. Concern has been raised by state agencies over the potential impact of introducing imported genetic stock into local populations, especially when those populations are threatened or endangered.

In the study area at Park Point in Duluth, Minnesota, landowners and local organizations have planted *A. breviligulata* from Michigan for three decades to restore dune vegetation and reduce erosion. This is the only Minnesota site that supports a native population of *A. breviligulata* (Lakela 1965;

Ownbey & Morley 1991) and it is also the most western location of the species natural range (Maun & Baye 1989). Because of its limited distribution, geographical location, and important ecological role, the Park Point population of *A. breviligulata* is on the state's threatened species list.

The goal of this work was to determine whether imported genotypes of *A. breviligulata* are genetically distinct and have a fitness advantage compared with plants from the threatened Minnesota population by: (1) observing morphological and phenological differences between plants in indigenous stands and plants in well-established restorations composed of propagules imported from Michigan, (2) experimentally testing whether differences between local and restoration plants are genetically based by rearing propagules in a common garden, and (3) using distinguishing morphological traits to determine whether nonlocal genotypes have spread into areas where no previous restorations are documented. These results are discussed in relation to companion studies that compare differences in molecular variation between these source populations (Fant et al. 2008) and the impact of interpopulation hybridization (Anderson 2006).

Methods

Study Species

Ammophila breviligulata Fern. (American beachgrass) is a long-lived, clonal, perennial, grass species that occurs in coastal dunes along the Atlantic coast from the Belle Isle Straits to North Carolina and inland along the Great Lakes (Maun & Baye 1989). Vegetative stock of this species is often used for restoration because it rapidly colonizes bare areas and aides in dune stabilization. Restorations using both native lineages and selected cultivars of *A. breviligulata* have been attempted throughout much of the species range. Scattered populations of unknown origin extend as far south as Florida and range expansion has occurred to the west along the Pacific (Maun & Baye 1989; Seabloom & Wiedemann 1994; Page 2001).

Ammophila breviligulata is a tetraploid species with no known ploidy variation (Church 1929) that produces both asexual and sexual progeny that may contribute to the establishment of new populations. Once established, individuals expand by plagiotropic rhizomes, often ranging from one to several meters in all directions from parental genets (Maun 1984; Maun & Baye 1989). Rhizomes are commonly fragmented and dispersed by wind and wave action. The seeds float and can also disperse long distances. However, establishment by seed may be more limited because of seedling sensitivity to drying, excessive sand burial, and erosion by wind and water (Laing 1958; Maun 1984; Maun & Lapierre 1986). *Ammophila breviligulata* is wind-pollinated, outcrossing, and largely self-incompatible (Krajnyk & Maun 1982; Anderson 2006). Sexual reproduction is positively correlated with sand deposition and rooting depth (Eldred & Maun 1982).

This study was conducted at the periphery of the natural distribution of *A. breviligulata* in North America at the western

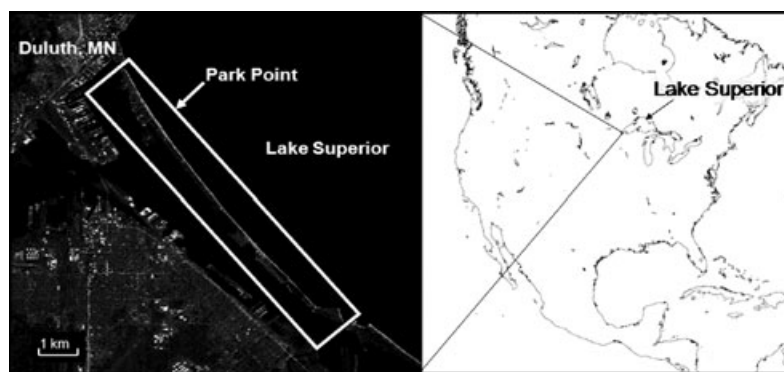


Figure 1. Location of the study area at Park Point in Duluth, Minnesota, on the western tip of Lake Superior.

tip of Lake Superior (Fig. 1). This species occurs at a single location in Minnesota on Park Point (Duluth, MN) where it dominates the low dunes with a near continuous distribution that is interrupted by development and erosion at the northern end and by a natural pine woodland at the southern end. Restorations are relatively well-documented in the southern portion and have relied heavily upon material from a commercial supplier in MI (Mason-Lake Conservation District, MLDC, Mason County, Anderson 2006). These propagules are presumed to be native to MI because MLCD claims to harvest and propagate genotypes from local populations. Park Point is approximately 3°N and 6°W from the MI site and is cooler (−2.8 and −6.1°C in July and January, respectively), drier (18 cm less annual precipitation), and has a shorter growing season (110 vs. 145 days, Albert 1995).

Observation of Field Populations

Differences in phenology and reproduction between well-established restored (MI) and indigenous stands (MN) of *A. breviligulata* at Park Point were examined by tagging 30 MI and 30 MN plants and monitoring them for 2 months. Restoration areas were at least 5 years old and were documented to have been barren expanses of dune revegetated exclusively with propagules purchased from MLCD. Local MN populations are within a preserved area on the outermost 3 km of Park Point where no restorations are known to have occurred.

Plants with emerging fertile culms were selected at least 3 m apart but otherwise at randomized distances along two transects through areas of dense flowering (total distance MI 0.6 km, MN 1.6 km). At anthesis, the number of open florets and their location on the panicle were recorded daily (07:00–10:00). Anthers on open florets were clipped to avoid recounting on subsequent days. Height (sand surface to tip) and diameter of fertile culm, panicle length (lowermost panicle branch to tip), the number of ergot sclerotia (*Claviceps purpurea*), and percentage of the panicle length damaged by insects were recorded 45 days after the peak of flowering.

A distinct set of 20 unmanipulated and naturally pollinated panicles (8 MI and 11 MN) no closer than 3 m apart were haphazardly collected from the same areas to assess seed set. Seed was manually extracted and classified as “viable” if there

were no visible deformities and seed length was greater than or equal to 2 mm (Krajnyk & Maun 1982).

To determine whether the populations differed in pollen production and viability, three anthers were collected as they emerged from a single floret (70 MI and 50 MN plants) and were stained in 50 µL of lactophenol aniline blue solution (Kearns & Inouye 1993). Fifteen microliters of solution was pipetted into a hemocytometer and viable and nonviable pollen grains were counted on five quadrats per sample. To determine whether the pollen from the earlier-flowering MN plants remained viable until the MI plants bloomed, 10 samples were collected from each of 10 MN plants and were stored at ambient temperature for 0, 1, 3, 5, 7, 9, 21, and 31 days after which 50 µL of the staining solution was added and pollen viability assessed.

The distribution of local and nonlocal genotypes at Park Point was delineated by walking transects through the length of the Point and classifying stands as “pure MN” or “mixed MN and MI.” These classifications were based on phenological and morphological differences that distinguished the populations in the observational and common garden studies and included height, panicle length, and flowering phenology.

Two Common Gardens

Common garden 1 was established in spring and included plants from MN and MI-C. Five hundred culms were collected from MN by sampling 2–3 culms per plant along transects 10 m apart with a minimum distance of 5 m between individuals. MN plants were harvested on the same day that 500 culms of MI-C were shipped from the MLCD nursery and both were planted in a sparsely vegetated area of foredune in a randomized block design with five blocks. Experimental units consisted of three culms randomly selected from a single source population (not necessarily a single genotype). To the extent possible, roots were trimmed to standardize below-ground biomass. Experimental units were weighed and planted 20 cm deep in an area devoid of vegetation (45 cm radius). A total of 287 experimental units (approximately 140 from each of MN and MI-C) were planted in 5 days and watered as necessary for 17 days. One block was later eliminated due to encroachment of existing vegetation.

In the fall, a second common garden was established adjacent to the previous one that included plant material from MN, MI-C, and a native MI source. Native MI plants (MI-L) were harvested from the nearest local stand at Ludington State Park in Ludington, MI (approximately 10 km from the MI-C commercial supplier). Plants were sampled from native MN and MI-L populations as described above. A total of 354 experimental units were planted into a randomized block design with five blocks (approximately 117 from each of MN, MI-C and MI-L) and watered once at the time of planting.

Traits were measured at the beginning and end of the following growing season in both gardens including: survivorship, number of culms per experimental unit, height of tallest culm; and on the tallest culm: number of green blades, length and width of the bottom blade, and culm diameter. Growth rate was calculated on plants that survived as the difference between late-season and early-season height during a single growing season. Measures of reproductive success included presence of fertile culms, week of first flowering, number of flowering culms, fertile culm height, panicle length, and fertile culm diameter. At the end of the growing season, a random subset of experimental units was destructively harvested for biomass measurements (garden 1: above- and belowground biomass, $N_{MN} = 35$, $N_{MI-C} = 36$; garden 2: aboveground biomass $N_{MN} = 26$, $N_{MI-C} = 25$, $N_{MI-L} = 25$). Aboveground material, including tillers emerging from horizontal rhizomes, was clipped at the soil surface, rinsed with water, and bagged. Belowground parts were completely excavated by hand and the number of tillers, and the number and length of 1° and $2/3^\circ$ rhizomes was recorded. Biomass was dried and weighed.

Data Analysis

Observational data were analyzed by analysis of variance (ANOVA) (SAS Institute 1999) with the origin of the stand considered a fixed effect. Traits were transformed as necessary to meet standard assumptions of ANOVA (shown on tables).

Presence or absence of ergot sclerotia was analyzed by nominal logistic regression and Wald's chi-square values are reported.

Data from gardens 1 and 2 were analyzed separately by analysis of covariance (ANCOVA) with population as a fixed effect, block and interactions with block as random effects, and initial fresh weight as a covariate. Data were transformed as necessary except for the number of 1° and $2/3^\circ$ rhizomes for which no suitable transformations could be found. Survivorship and flowering were analyzed using nominal logistic regression with population and block as main effects and initial fresh weight as a covariate; Wald chi-square values are reported. Linear contrasts were done between all comparisons of the three populations assessed in the ANCOVA. Contrast results were adjusted using sequential Bonferroni tests for k comparisons by the Dunn-Sidak method at a specified experiment-wise error rate of α (Sokal & Rohlf 1995).

Results

Observations of Field Populations

Well-established beachgrass stands restored using MI genotypes differed strikingly in phenology, reproduction, and susceptibility to local pests compared with native MN stands. Overall, MI genotypes were larger and more fecund producing fertile culms that were 1.4 times greater in diameter at the base and were 1.8 times taller with proportionally larger panicles (Table 1). During the peak of flowering, MI plants had more florets at anthesis than MN (2,098 vs. 1,442). MI florets on average produced 1.5 more pollen grains than MN florets and their pollen had a greater percent viability (Table 1). Peak flowering for MN was 6.7 weeks earlier than MI ($F_{1,57} = 963.66$, $p < 0.0001$; Fig. 2) and was of shorter duration (20 vs. 36 days). The viability of MN pollen decayed rapidly after anthesis with only 20% remaining viable after 4 weeks ($\beta = -1.14$, $F_{1,99} = 29.53$, $p < 0.0001$; Fig. 3). Dissection of a smaller number of naturally pollinated panicles showed that MI plants produced 2.7 times more florets per

Table 1. Differences in reproduction and insect damage between local MN populations and well-established restored populations composed of MI plants of *Ammophila breviligulata* measured in the field.

	Least Squares Mean (SE)		Test Statistics	
	MN	MI	df	F/X^2
Fertile culm diameter at base ^a (mm)	1.57 (0.05)	2.15 (0.05)	1, 54	65.28***
Fertile culm height (cm)	65.85 (1.86)	117.59 (1.89)	1, 57	382.14***
Panicle length ^a (cm)	19.73 (0.60)	35.63 (0.61)	1, 57	386.96***
Number of florets per panicle ^a	195.17 (20.32)	523.00 (24.89)	1, 18	109.42***
Pollen production per floret	8,251.27 (620.82)	12,698.29 (550.30)	1, 123	28.73***
% viable pollen	67.16 (1.93)	75.32 (1.70)	1, 122	10.09**
% seed set per panicle	23.73 (6.60)	59.28 (8.08)	1, 18	11.60**
% viable seed per panicle	18.33 (5.53)	53.08 (6.77)	1, 18	15.84**
% of panicle damaged by insects	38.82 (0.04)	1.50 (0.04)	1, 54	38.27***
Presence/absence of ergot	7.14	25.00	1	2.95 ⁺

Least squares means (not transformed, standard errors in parentheses) and percentages for phenotypic traits shown with ANOVA/Nominal logistic regression test statistics.

Transformations for analyses: ^a natural log.

⁺ $p < 0.09$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$.

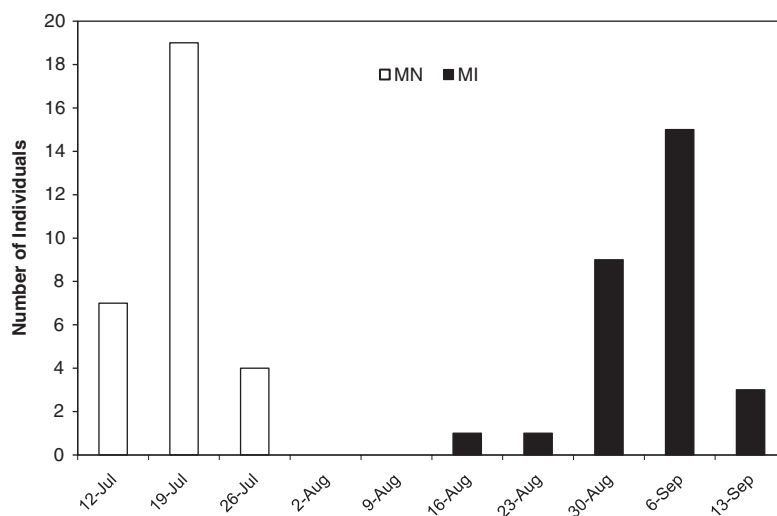


Figure 2. Week of first flower of plants in a local MN population and introduced MI plants of *Ammophila breviligulata* in Minnesota.

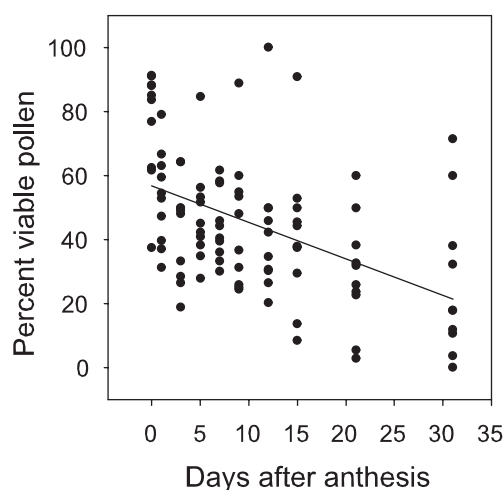


Figure 3. Change in the percentage of viable pollen from the day of anthesis until 31 days post-anthesis in a natural population of *Ammophila breviligulata* in Minnesota.

panicle; a significantly greater percentage of these florets ultimately produced viable seed (Table 1).

MI plants suffered less insect damage than local MN plants both in terms of the number of damaged panicles (29 vs. 89%) and the extent of damage to any given panicle (Table 1). Insect damage was due primarily to Thripidae that fed on floral parts and ultimately led to flower abortion. Ergot fungus was more widespread among MI panicles although the difference between populations was only marginally significant ($p = 0.09$) and the infestation of MI plants largely restricted to one part of the study area.

Proximity of Local and Nonlocal Stands

Nonlocal beachgrass plants, putatively from MI, are interspersed with local MN beachgrass stands throughout the study

area, even where no restorations have occurred in the past. At the northern end of Park Point, where human activity is most intense, nonlocal stands dominate the foredunes, whereas local stands occupy the reardune (Fig. 4A). Nonlocal stands also dominate the mid-section of the point until the end of the residential area (Fig. 4B). At the interface with the city park, there is an abrupt transition to local grass in both foredunes and reardunes (Fig. 4C). However, even in these foredune areas isolated individuals occur that have phenotypes and phenologies that match MI grass. Native grass dominates the southern end of the point where only one intentional introduction of MI grass is documented (Fig. 4D). Nevertheless, isolated nonlocal individuals are present which may indicate recent colonization events from more northern areas.

Common Gardens

After 2 years of growth in common garden 1, restoration propagules from MI-C survived at greater rates and had greater vegetative and sexual reproduction than propagules sampled from the local MN population. Although all plants survived at high rates in the common garden (89.6%), MI-C experienced significantly less mortality than MN (Table 2; Fig. 5A). At the end of the second growing season, MI-C were larger than the MN for 11 of 14 measurements of plant size after accounting for differences in the initial fresh weight which were also significant (Table 2). MI-C produced 2.1 times as many culms that were wider at the base and 1.4 times taller than MN. Although there was no difference in the number of leaf blades produced, the leaves of MI-C plants were on average longer and wider than MN. During the second year, MI-C grew 2.5 faster than MN and ultimately produced 3.2 times more aboveground biomass (Fig. 5B). None of the MN plants produced fertile culms in contrast to 11.4% flowering among MI-C plants (Fig. 5C).

Belowground, MI-C and MN did not differ for the number of tillers or primary rhizomes, although MN produced 1.7

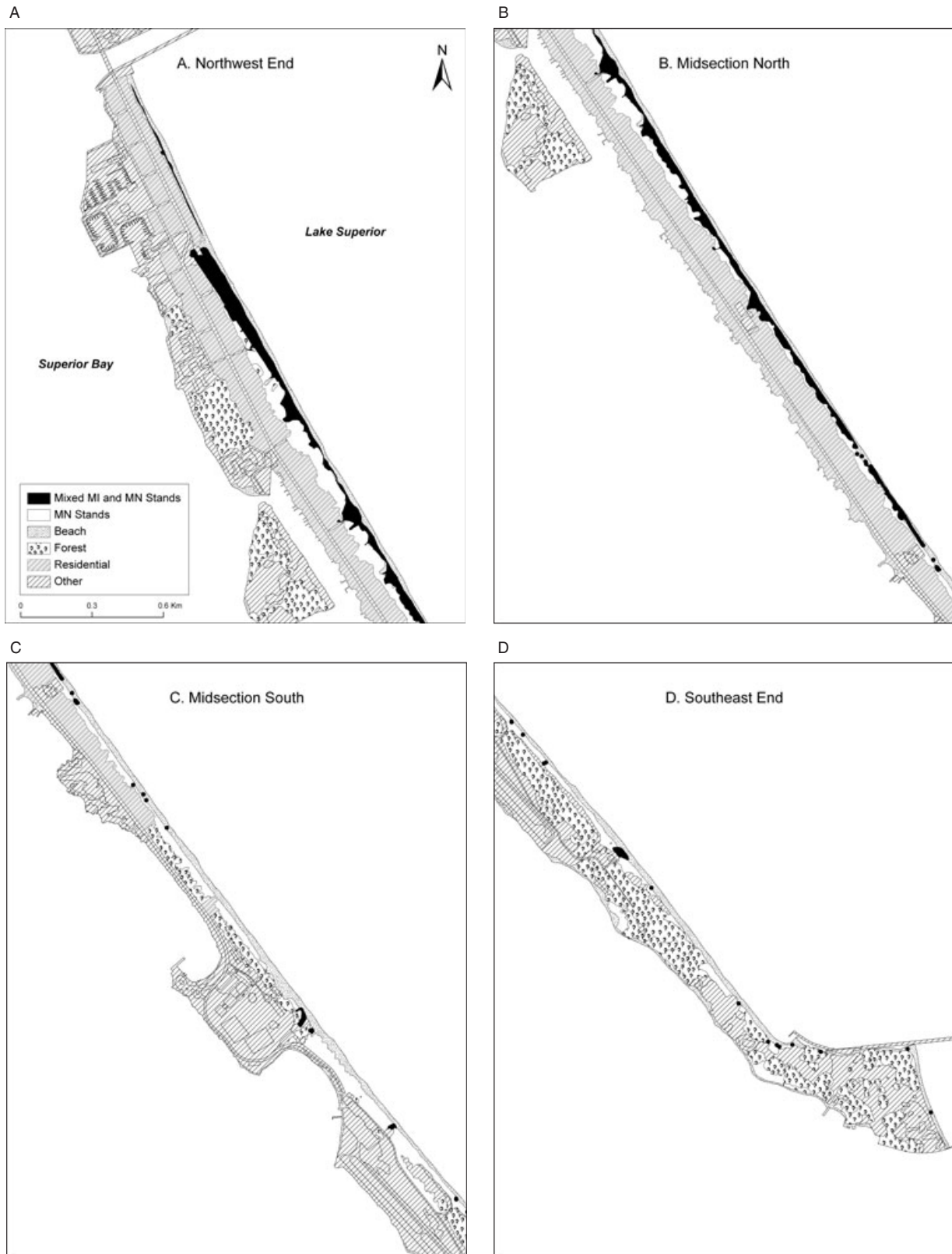


Figure 4. Distribution of *Ammophila breviligulata* stands composed exclusively of local plants or composed of a mix of local and nonlocal plants based on morphological and phenological differences at the (A) Northwest end, (B) midsection North, (C) midsection South, and (D) Southeast end of Park Point in Duluth, MN (supporting landuse data; NRRI Technical Report 1999).

Table 2. Differences among local MN genotypes and nonlocal genotypes from MI of *Ammophila breviligulata* grown in two common gardens.

	Least Square Means (SE)						ANCOVA/Logistic Regression					
				Population			Block		Population × block		Initial weight	
	MV	MI-C	MI-L	df	F / X ²	df	F / X ²	df	F	df	F / X ²	
Garden 1												
Initial fresh weight (g)	9.04 (0.23)	10.25 (0.24)		1, 259	13.03***							
Mortality (%)	18.52	1.59		1	10.93**	3	2.53	—	—	1	3.03 ⁺	
Culm number ^a	3.28 (0.60)	6.81 (0.59)		1, 4	55.70**	3, 3	12.23*	3, 225	0.93	1, 225	10.31**	
Culm diameter (mm)	3.91 (0.10)	4.30 (0.10)		1, 4	7.85*	3, 3	9.51*	3, 223	0.82	1, 223	4.93*	
Height (cm)	46.64 (2.21)	66.09 (2.20)		1, 3	38.53**	3, 3	4.44	3, 224	5.06**	1, 224	9.80**	
Leaf number	4.79 (0.11)	4.89 (0.10)		1, 5	0.48	3, 3	24.85*	3, 223	0.66	1, 223	1.25	
Blade length (cm)	35.52 (0.87)	40.36 (0.86)		1, 4	15.34*	3, 3	1.74	3, 223	1.11	1, 223	1.49	
Blade width (mm)	6.07 (0.11)	6.62 (0.11)		1, 5	12.97*	3, 3	20.66**	3, 223	0.61	1, 223	8.57**	
Growth rate (cm per growing season)	10.18 (2.36)	25.79 (2.34)		1, 3	21.86*	3, 3	2.81	3, 224	9.53***	1, 224	0.08	
AG biomass ^a (g)	4.70 (2.63)	15.14 (2.60)		1, 3	88.60**	2, 3	19.19*	2, 64	0.43	1, 64	0.32	
Tiller number	0.57 (0.15)	0.92 (0.15)		1, 4	2.71	2, 3	3.53	2, 64	0.29	1, 64	0.16	
1° rhizome number	1.66 (0.45)	2.48 (0.45)		1, 2	1.57	2, 2	1.86	2, 64	2.21	1, 64	0.01	
2°/3° rhizome number ^b	0.49 (0.09)	0.29 (0.09)		1, 5	10.83*	2, 3	7.13 ⁺	2, 64	0.22	1, 64	0.22	
1° rhizome length ^c (cm)	8.76 (0.87)	13.46 (0.97)		1, 3	11.93*	2, 2	0.00	2, 48	0.79	1, 48	0.39	
BG biomass ^a (g)	1.80 (0.09)	2.87 (0.09)		1, 3	69.42**	2, 2	7.17	2, 64	0.66	1, 64	0.43	

Table 2. Continued.

	Least Square Means (SE)			ANCOVA/Logistic Regression								
	MN	MI-C	MI-L	Population		Block		Population × block		Initial weight		
				df	F / X ²	df	F / X ²	df	F	df	F / X ²	
Garden 2												
Initial fresh weight (g)	14.36 _b (0.48)	14.44 _b (0.48)	22.18 _a (0.47)	2, 350	89.05***							
Mortality (%)	5.93	0.86	23.53	2	15.62**	4	10.77*			1	2.36	
Culm number ^a	3.05 _{ab} (0.16)	3.44 _a (0.17)	2.73 _{bc} (0.22)	2, 15	5.26*	4, 8	2.31	8, 301	1.07	1, 301	16.91***	
Culm diameter (mm)	3.84 (0.13)	3.90 (0.14)	4.06(0.18)	2, 11	0.44	4, 8	0.86	8, 299	2.16*	1, 299	3.12 [†]	
Height (cm)	48.90 _b (1.08)	61.52 _a (1.16)	60.50 _a (1.49)	2, 14	38.61***	4, 8	5.50*	8, 301	1.20	1, 301	4.32*	
Leaf number	4.67 (0.14)	4.46 (0.15)	4.88(0.20)	2, 12	1.35	4, 8	6.00*	8, 301	1.65	1, 301	0.10	
Blade length (cm)	33.88 _c (0.74)	44.89 _a (0.79)	39.40 _b (1.02)	2, 19	53.84***	4, 9	1.82	8, 300	0.70	1, 300	1.16	
Blade width (mm)	6.21 (0.13)	6.02 (0.14)	6.07(0.18)	2, 14	0.53	4, 8	1.24	8, 299	1.20	1, 299	12.76*	
Growth rate (cm per growing season)	17.14 _c (0.83)	25.23 _b (0.87)	29.05 _a (1.14)	2, 23	42.31***	4, 9	12.03**	8, 296	0.52	1, 296	0.70	
AG biomass (g)	3.59 _b (0.69)	6.73 _a (0.67)	5.39 _{ab} (0.76)	2, 2	5.80	1, 2	0.17	2, 69	1.87	1, 69	5.19*	

Garden 1 included: (1) local MN genotypes (MN) and (2) restoration propagules purchased from a commercial source in MI (MI-C). Garden 2 included: (1) MN, (2) MI-C, and (3) local MI genotypes sampled from a native population close to the MI-C commercial source near Ludington, MI (MI-L). Least squares means (not transformed, standard errors in parentheses) and percentages shown with ANCOVA/Nominal logistic regression test statistics. Shared letters indicate contrasts that were not statistically significant after sequential Bonferroni adjustments.

+ $p < 0.08$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$. Transformations for analyses: ^anatural log, ^bnatural log($x + 0.1$), ^csquare root.

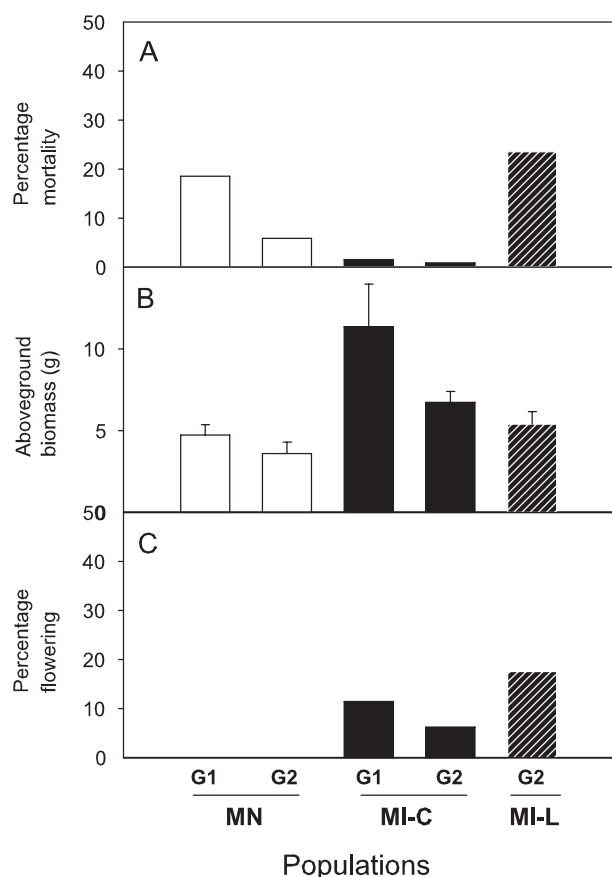


Figure 5. Comparison among *Ammophila breviligulata* plants sampled from a local Minnesota population (MN), a commercial supplier in Michigan (MI-C), and a native population in Ludington, Michigan (MI-L) when grown in two common gardens for (A) percentage mortality, (B) aboveground biomass, and (C) percentage flowering. Garden 1 (G1) was planted in the spring and measured at the end of the second growing season. Garden 2 (G2) was planted in the fall and measured at the end of a single growing season. Percentages and least squares means (not transformed, with standard errors) are shown.

times more secondary and tertiary rhizomes. Despite the greater branchiness of MN rhizomes, MI-C plants produced 1.6 times more belowground biomass, presumably due to the greater length of their primary rhizomes (Table 2). Overall, the total length of horizontal rhizome spread of MI-C was 43.1% greater than MN (MI – C = 208.5 cm, MN = 89.9 cm).

After 1 year of growth in the second common garden, MI (MI-C and MI-L) individuals were larger than local MN plants and exhibited greater sexual reproduction. Survival rates were high (89.8%) with very low mortality for MI-C and MN but significantly higher mortality for the MI-L (Table 2; Fig. 5B). Survival was not influenced by initial fresh weight, which differed significantly among the populations (Table 2). MI-C plants produced 1.3 times more culms than MI-L but not significantly more than MN (Table 2). In contrast to the first garden, culm diameter did not differ among the populations. Plants from both MI source populations were significantly taller than MN plants (approximately 1.25 times taller). The

populations did not differ for leaf production or leaf blade width, but MI-C produced longer leaf blades, followed by MI-L and MN. Plants sampled from the native MI-L population exhibited the fastest growth rates followed by MI-C and MN. At the end of the growing season, MI-C had accumulated significantly more aboveground biomass than MN and MI-L was intermediate. MI-L plants had the highest percentage of flowering individuals followed by MI-C. As in garden 1, no MN plants flowered (Fig. 5C).

Discussion

For three decades, propagules of the beachgrass, *Ammophila breviligulata*, have been imported to Park Point (Duluth, MN) from a commercial source in Michigan for restoration within a local population that is on the state threatened species list. The two primary goals of this work were to determine whether these nonlocal plants are genetically distinct from the local population and to assess the potential impact that these importations may have on the threatened local population.

This study overwhelmingly demonstrated that local MN plants have distinct phenotypes and phenologies compared with imported MI plants. Observations of well-established populations at Park Point showed that plants in the restored populations were larger, flowered later, and were more fecund than plants in the local population both for offspring numbers and viability. When exposed to the same environmental conditions in the common gardens, MI and MN plants differed for 76% of the traits measured, providing evidence for the genetic basis of the ecotypic variation. These differences parallel those observed in the established populations with MI plants being larger and allocating more resources for sexual and asexual reproduction. It is possible that some of the vigor of MI plants resulted from positive environmental effects that carried over from MI propagation gardens, such as regular watering and fertilization (e.g., Hume & Cavers 1981). However, we attempted to minimize any such effects in this study by standardizing root biomass before planting and accounting for initial differences in ramet fresh weight in the statistical analyses. Furthermore, any advantage from environmental carry-over from MI propagation gardens would not be expected to persist after several years of establishment at Park Point.

The genetic distinctiveness between the source populations was further supported by a companion study that compared molecular diversity using ISSR markers (Fant et al. 2008). That study showed that the MN population harbored substantial ISSR diversity, exceeding the levels reported for other clonal species (Widen et al. 1994; Brzosko et al. 2002). In contrast, MI propagules obtained from the commercial supplier and harvested from populations in MN that were restored with MI beachgrass 5–14 years ago were completely lacking ISSR diversity. In fact, all of these MI plants had the same multilocus genotype and this single genotype did not occur in any local MN population. This extreme lack of molecular diversity could indicate that few genotypes were used to

found commercial populations or that restoration orders are filled by sampling from a limited number of clones. Alternatively, commercial plants in MI may be under “unconscious selection” (Montalvo et al. 1997) or intentional artificial selection in the propagation gardens that favors only the most robust and vigorous clones. Although this practice may erode genetic variation in the original base population, it is advantageous from the perspective of the commercial supplier if it results in lineages that spread rapidly and are highly effective at dune stabilization. However, other studies have shown that restored populations harbor less genetic variation than natural populations (Williams & Davis 1996; Helenurm & Parsons 1997; Williams & Orth 1998) and that this can negatively impact population growth (Williams 2001).

What impact will the importation of genetically distinct MI plants have on the threatened local MN population? Native populations may be affected by nearby restoration populations by two primary mechanisms. First, gene flow from restoration propagules may “swamp out” the local gene pool through introgression. Our study found that gene flow between these distinct ecotypes will not readily occur because MN plants flowered on average 6 weeks earlier, and their pollen did not remain viable until the MI plants were in flower. However, it should be noted that this study included only 30 MN and MI plants and a more extensive survey of flowering phenology yielded a set of particularly late-flowering MN plants and early-flowering MI plants that were successfully cross-pollinated and produced viable seed (Anderson 2006). Although the potential for gene flow is limited, it is nevertheless possible. Theoretical studies suggest that even low levels of gene flow can homogenize populations (Slatkin 1985).

Competition is the second mechanism by which nonlocal plants may impact the threatened native MN population of beachgrass. If plants used for restoration have a competitive advantage over plants in the local population, they may ultimately become numerically dominant. Surveys of the field populations reported in this study indicate that MI plants are already in the process of spreading at Park Point. Plants possessing traits that are characteristic of the MI ecotype occur in places where no previous restorations have been documented. MI plants may out-compete the local plants because of their higher survival rates, more rapid growth including vegetative spread via rhizomes, and larger overall size. MI plants also exceeded the MN plants in terms of sexual reproduction by producing larger panicles with greater percentages of viable seed. Finally, the later flowering phenology of MI plants may also confer an advantage at the MN site if it allows these plants to escape insect damage that was extensive among native plants. Ultimately, this may mean that plants in the threatened MN population will be displaced by a genetically depauperate but very robust ecotype from MI.

On the basis of the data obtained in this study, one might conclude that the plants imported from MI are better adapted to the Park Point environment than the indigenous plants. However, it is not certain that the traits expressed by the MN population are maladaptive over the longer-term, especially in harsh northern climates. Natural selection in

cold northern climates with short growing seasons may favor traits that would not typically indicate high fitness, such as small plant size, infrequent flowering, and slow growth rates. This apparent lack of “vigor” may actually reflect natural selection constrained by underlying genetic tradeoffs between different life history attributes. For example, selection may strongly favor early flowering, which necessitates smaller plant size and lower seed production (Bennington & McGraw 1995). Selection may also favor cold tolerance, which has been hypothesized to be negatively genetically correlated with growth rate and, consequently, competitive ability (Rehfeldt et al. 1999). The advantages of smaller size and earlier flowering may be more evident during a more typical colder and shorter growing season.

The MI plants may have experienced greater fitness in the years in which this study was conducted because of unusually mild weather. The growing seasons during the study were warm by historical standards, especially September 2004 (Minnesota State Climatology 2004), when MI seeds were maturing. Michigan plants may not experience the same degree of reproductive success in more average climate conditions in northern Minnesota where the first frost typically occurs earlier. Nevertheless, the warming trends predicted with climate change may enhance the advantage to the MI plants given the expectation that the growing season in northern Minnesota will be extended for 3–6 weeks by the end of the 21 century (UCSUSA 2003).

Barring any intervention, this study suggests that a single vigorous genotype of MI beachgrass may dominate Park Point in the future. This is significant because populations that harbor little genetic diversity have a limited potential to adapt to ongoing environmental change. However, it is not certain that a lack of molecular diversity at neutral markers corresponds to lack of genetic diversity in phenotypic traits that would be important for adaptation to a changing environment. Previous studies have reported both correspondence and contradiction between measures of molecular and quantitative trait variation (Knapp & Rice 1998; Pfrender et al. 2000). Future studies will need to be conducted to determine whether the imported MI propagules are also lacking in genetic diversity in quantitative traits that are the targets of natural selection.

Implications for Practice

- Beachgrass planting stock introduced from a nonlocal source may be competitively superior to a remnant local population, even if from a seemingly different environment.
- A higher level of molecular genetic diversity may not be a reliable predictor of better phenotypic performance in the field.
- Differences in flowering time between local and introduced beachgrass may minimize interbreeding where the two are in spatial proximity.
- The extra effort needed to propagate local beachgrass for use in restoration plantings can be more readily justified

when restoration efforts are likely to be repeated at the same location over time because of ongoing recreational trampling, or when preservation of local genotypes, co-dependent relationships, or biodiversity is a restoration priority.

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