WETLANDS, Vol. 19, No. 2, June 1999, pp. 352–358
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GENETIC DIVERSITY WITHIN TALL FORM *SPARTINA ALTERNIFLORA* LOISEL. ALONG THE ATLANTIC AND GULF COASTS OF THE UNITED STATES

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Abstract: *Spartina alterniflora* is the dominant flowering plant of regularly flooded salt marshes along the Atlantic and Gulf coasts of the United States. Studies have suggested that there may be a genetic basis for the morphological, physiological, and phenological differences observed in plants sampled over a broad geographic range. Randomly amplified polymorphic DNA (RAPD) analysis was employed to assess the genetic variability in tall form *S. alterniflora* from the Atlantic and Gulf coasts. Twenty-nine RAPD primers produced 300 scoreable electrophoretic bands, of which 225 were polymorphic (75%). An UPGMA (unweighted pair-group method using an arithmetic average) phenogram based on Jaccard's genetic distances showed three clusters of plants: New England/New Jersey, North Carolina/South Atlantic, and Gulf coast. Analysis of molecular variance (AMOVA) was used to estimate how genetic variability is partitioned among regions, areas, and individuals. The resulting variance components were highly significant at all hierarchical levels for the sampling regime employed. The correlation between genetic and estimated coastal geographic distance was positive and highly significant based on Mantel's non-parametric test. Although direct gene flow among plants from geographically separate areas is not probable due to differences in flowering phenology, a total barrier to the exchange of genetic information is not likely. Present data and results of previous studies suggest a genetic continuum for this species rather than discrete, isolated populations.

Key Words: AMOVA, Genetic diversity, PCR, Population structure, RAPD, *Spartina alterniflora*

INTRODUCTION

The important salt marsh plant *Spartina alterniflora* Loisel. shows a range of morphological and physiological variation over both geographic and within-marsh scales. A large number of studies have investigated within-marsh growth forms of *S. alterniflora* and the ecophene versus ecotype question (see Gallagher et al. 1988). Evidence from a 9-year common garden study indicates that these growth forms have a genetic basis, either in the basic coding information or regulatory mechanisms that become permanently set at an early developmental stage (Gallagher et al. 1988).

In addition to morphological differences between growth forms, differences have been observed between different geographic populations of *S. alterniflora* (Seneca 1974, Somers and Grant 1981). In a two-year common garden study, morphological and physiological differences between separate geographic populations of *S. alterniflora* prompted Seneca (1974) to conclude that four broadly distributed populations exist along the Atlantic and Gulf coasts of the United States. These populations include individuals from (1) New England (Massachusetts, Rhode Island, and Connecticut), (2) the Mid-Atlantic (New York, Virginia, and North Carolina), (3) the South Atlantic (Georgia and Florida), and (4) the Gulf coast (Mississippi and Texas). Somers and Grant (1981) reported substantial differences in the timing of flowering for plants originating from seeds collected from Maine to Virginia that were grown in common gardens. They concluded that the differences in flowering phenology were due to inherent genetic variation, which evolved in populations experiencing different environmental conditions.

It has been suggested that additional studies be conducted with specimens collected from a variety of marshes along the Atlantic coast so that conclusions on the genetic variability between populations can be made (Anderson and Treshow 1980). A positive correlation between nuclear genome size and latitude was found in a subsequent study (Freshwater 1988), suggesting that a genetic continuum exists within the species rather than genetic discontinuity associated with distinct geographic populations.

Polymerase chain reaction (PCR) based molecular markers are valuable tools in the characterization of genetic diversity within and between populations. Randomly amplified polymorphic DNA (RAPD) analysis

Figure 1. Map of Atlantic and Gulf coasts of the United States showing collection locations for 25 tall form *Spartina alterniflora* plants used in this study.

employs short random sequence primers (generally 10 base pairs) to amplify regions of genomic DNA using PCR (Welsh and McClelland 1990). Mutations at and between priming sites may cause variation in the number and size of amplified regions, which can be detected by separation on agarose gels.

Previously, RAPDs have been used by Stiller and Denton (1995) to investigate the genetic relatedness of *S. alterniflora* tussocks in Willapa Bay, Washington, USA. Their findings indicate that the Willapa population was established from a single genet. The purpose of this study was to determine the extent of genetic diversity within tall form *Spartina alterniflora* along the Atlantic and Gulf coasts of the United States and whether a genetic basis exists to support the four geographic groupings of *S. alterniflora* as identified by Seneca (1974).

MATERIALS AND METHODS

Five individual plants from each of five geographic areas along the Atlantic and Gulf coasts of the United States were used for this study (Figure 1). The geographic areas sampled included (1) New England (Maine, New Hampshire, and Massachusetts), (2) New Jersey, (3) North Carolina, (4) South Atlantic (southern South Carolina, Georgia, and northeast Florida), and (5) Gulf coast (Florida, Mississippi, and Louisiana). These areas, circumscribed here and in Seneca (1974) by political boundaries, represent different general coastal systems defined by geology, tides, and wave energy. Sampled culms were collected geographically distant from one another to exclude clones. Plant material was collected during the summers of 1995 and 1996. All *S. alterniflora* samples collected were taken from tall form plants along the marsh edge. The youngest 4–5 leaves were cut from each plant, placed with a damp paper towel into a resealable plastic bag, and kept on ice or refrigerated until the DNA was extracted. Following DNA extractions, samples were pressed and dried as voucher specimens.

DNA extractions were made using a modification of the methods of Doyle and Doyle (1987) and Freshwater and Rueness (1994). DNA concentrations were determined using a DNA fluorometer (Hoeffer Scientific Instruments, San Francisco, CA, USA).

The protocol used for the amplification of RAPD products was optimized during preliminary studies (unpublished data). The 10-mer oligonucleotide primers (Operon Technologies Inc., Alameda, CA, USA)

Table 1. Jaccard's genetic distance (below diagonal) and estimated geographic coastal distance (Km; above diagonal) for 25 tall form *Spartina alterniflora* plants sampled from 5 geographic areas.

Collection	1	2	3	4	5	6	7	8	9
1. Freeport, ME	—	23	125	198	260	670	713	731	763
2. Portland, ME	0.198	—	102	175	237	647	690	708	740
3. Portsmouth, NH	0.276	0.264	—	73	135	545	588	606	638
4. Gloucester, MA	0.236	0.257	0.252	—	62	472	515	533	565
5. Cohassett, MA	0.248	0.284	0.298	0.290	—	410	453	471	503
6. Sandy Hook, NJ	0.350	0.353	0.397	0.330	0.357	—	43	61	93
7. Pt. Pleasant, NJ	0.318	0.291	0.336	0.327	0.324	0.288	—	18	50
8. Seaside Heights, NJ	0.327	0.359	0.431	0.393	0.377	0.328	0.309	—	32
9. Long Beach, NJ	0.400	0.402	0.496	0.421	0.433	0.385	0.357	0.322	—
10. Great Egg Harbor, NJ	0.395	0.388	0.487	0.436	0.421	0.425	0.412	0.348	0.420
11. Morehead City, NC	0.559	0.524	0.569	0.532	0.521	0.459	0.462	0.441	0.455
12. Snead's Ferry, NC	0.588	0.557	0.591	0.555	0.544	0.472	0.512	0.528	0.504
13. Wrightsville Beach, NC	0.523	0.500	0.532	0.508	0.472	0.398	0.463	0.468	0.457
14. Federal Basin, NC	0.586	0.574	0.574	0.583	0.628	0.512	0.529	0.483	0.520
15. Oak Island, NC	0.516	0.540	0.562	0.537	0.584	0.500	0.504	0.496	0.496
16. Isle of Palms, SC	0.524	0.500	0.521	0.496	0.496	0.472	0.475	0.492	0.504
17. Beaufort, SC	0.606	0.605	0.629	0.581	0.638	0.567	0.574	0.553	0.586
18. Tybee Island, GA	0.496	0.522	0.543	0.519	0.519	0.450	0.545	0.457	0.504
19. Jekyll Island, GA	0.566	0.520	0.565	0.585	0.597	0.527	0.520	0.524	0.500
20. St. Augustine, FL	0.548	0.515	0.546	0.556	0.556	0.466	0.515	0.496	0.496
21. St. Marks Reserve, FL	0.597	0.565	0.607	0.594	0.606	0.540	0.534	0.569	0.568
22. Hollywood, FL	0.650	0.639	0.652	0.676	0.678	0.596	0.623	0.624	0.660
23. Waveland, MS	0.685	0.645	0.716	0.710	0.667	0.621	0.609	0.630	0.648
24. Bay St. Louis, MS	0.691	0.671	0.712	0.689	0.692	0.639	0.648	0.658	0.682
25. Grand Isle, LA	0.674	0.672	0.697	0.710	0.704	0.637	0.687	0.676	0.702

used in this study were selected based on the clarity and repeatability of amplified products. PCR reactions were set up in 20 μL volumes containing 2 units AmpliTaq DNA polymerase Stoffel Fragment and 10× buffer as per manufacturer specifications (Perkin Elmer, Norwalk, CT), 3.2 mM $MgCl_2$, 0.16 mM each dNTP, 12 ng primer, and 24 ng of template DNA. To check banding pattern reproducibility, each 20 μL reaction was either set up in duplicate or divided in half before thermocycling to provide two 10 μL replicate reactions. All reactions were run in a PTC-100™ thermocycler (MJ Research, Inc., Watertown, MA, USA) programmed for 2 min at 95°C followed by 40 cycles of 15 sec at 95°C; 30 sec at 40°C; 60 sec at 72°C. A negative control reaction, run without DNA template, was included with the reactions for each primer. After size separation on agarose gels, RAPD profiles were photographed and scored from negatives projected with a photographic enlarger.

One hundred twenty primers were screened for their ability to provide clear, reproducible, banding patterns. The 28 primers selected for use in this study (OPB-3,4,8,20; OPG-1,7,8,10; OPJ-6,7,9–18,20; OPP-14; OPX-4,6,7,9,15,18,20) produced a total of 300 scoreable bands, of which 225 (75%) were polymorphic. The gels were scored conservatively, whereby only the brightest visible bands were recorded, to reduce the amount of 'noise' that can result from scoring faintly visible bands. Amplified bands were treated as individual loci and scored as either present or absent. Data matrices were constructed using MacClade (Maddison and Maddison 1992). Distance matrices based on these data were generated using both the Jaccard (Jaccard 1908) and the Euclidean (Excoffier et al. 1992) distance coefficients as implemented in the GENESTAT-3 program (Kapraun 1997).

An UPGMA (unweighted pair-group method using an arithmetic average) phenogram was constructed using PHYLIP version 3.5c (Felsenstein 1993) and TREEVIEW (Page 1996). To estimate how phenotypic variability is partitioned among regions, areas, and individuals, the genetic distance data were analyzed by analysis of molecular variance (AMOVA) using WINAMOVA version 1.55 (provided by L. Excoffier, University of Geneva). A Mantel's test (Mantel 1967) was run using the program distributed with GENEPOP version 1.2 (Raymond and Rousset 1995) to determine whether genetic distance and estimated coastal geographic distance between plants were correlated. Distances calculated using both Jaccard's and Euclidean methods were similar for these data, and no differences were found in the significance of the variance com-

Table 1. Extended.

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
821	1411	1476	1534	1566	1576	1808	1907	1967	2098	2223	3577	3819	4165	4158	4459
798	1388	1453	1511	1543	1553	1785	1884	1944	2075	2200	3554	3796	4142	4135	4436
696	1286	1351	1409	1441	1451	1683	1782	1842	1973	2098	3452	3694	4040	4033	4334
623	1213	1278	1336	1368	1378	1610	1709	1769	1900	2025	3379	3621	3967	3960	4261
561	1151	1216	1274	1306	1316	1548	1647	1707	1838	1963	3317	3559	3905	3898	4199
151	741	806	864	896	906	1138	1237	1297	1428	1553	2907	3149	3495	3488	3789
108	698	763	821	853	863	1095	1194	1254	1385	1510	2854	3106	3452	3445	3746
90	680	745	803	835	845	1077	1176	1236	1367	1492	2846	3088	3434	3427	3728
58	648	713	771	803	813	1045	1144	1204	1335	1460	2814	3056	3402	3395	3696
—	590	655	713	745	755	987	1086	1146	1277	1402	2756	2998	3344	3337	3638
0.496	—	65	123	155	165	397	496	556	687	812	2166	2408	2754	2747	3048
0.520	0.384	—	58	90	100	332	431	491	622	747	2101	2343	2689	2682	2983
0.472	0.415	0.417	—	32	42	274	373	433	564	689	2043	2285	2631	2624	2925
0.561	0.400	0.470	0.484	—	10	242	341	401	532	657	2011	2253	2599	2592	2893
0.549	0.496	0.483	0.496	0.432	—	232	331	391	522	647	2001	2243	2589	2582	2883
0.483	0.425	0.426	0.345	0.496	0.442	—	99	159	290	415	2184	2426	2772	2765	3066
0.582	0.555	0.542	0.492	0.534	0.482	0.422	—	60	191	316	1670	1912	2258	2251	2552
0.484	0.455	0.456	0.433	0.472	0.446	0.362	0.454	—	131	256	1610	1852	2198	2191	2492
0.528	0.487	0.462	0.463	0.466	0.425	0.435	0.487	0.426	—	125	1479	1721	2067	2060	2361
0.500	0.472	0.435	0.425	0.451	0.476	0.447	0.508	0.389	0.350	—	1354	1596	1942	1935	2236
0.585	0.455	0.527	0.526	0.554	0.531	0.492	0.627	0.515	0.523	0.507	—	242	588	581	882
0.650	0.576	0.645	0.603	0.612	0.602	0.638	0.700	0.621	0.603	0.564	0.519	—	346	339	640
0.657	0.614	0.662	0.629	0.669	0.669	0.644	0.727	0.664	0.630	0.600	0.545	0.364	—	7	294
0.664	0.603	0.678	0.637	0.657	0.685	0.623	0.740	0.671	0.667	0.638	0.536	0.333	0.259	—	301
0.674	0.641	0.689	0.664	0.677	0.656	0.662	0.737	0.662	0.656	0.625	0.580	0.357	0.402	0.294	—

ponents determined using AMOVA for either distance method. Only results based on Jaccard's distances are presented here.

RESULTS

The genetic distance between all individuals (Table 1) ranged from a low of 0.198 (Freeport, ME and Portland, ME) to a high of 0.740 (Beaufort, SC and Bay St. Louis, MS). The smallest range of variation occurred within the New England area (0.198–0.298; Δ0.100), whereas the greatest range of variation occurred within the Gulf area (0.294–0.580; Δ0.286). In a pairwise comparison of the five Gulf coast samples, the St. Marks Reserve individual was the most unrelated to any of the other Gulf coast samples. Excluding the St. Mark's Reserve plant from the pairwise analysis, the range of variation within the Gulf coast samples is reduced to 0.294–0.402; Δ0.108, which is similar to the variation observed within the four Atlantic coast areas.

Jaccard's distances were used to construct an UPGMA phenogram (Figure 2) in which the 25 samples segregate into three clusters: New England/New Jersey, North Carolina/ South Atlantic, and the Gulf coast. Segregating samples into two broad geographic regions, Atlantic and Gulf coasts, an AMOVA was conducted to determine the significance of the variance among these regions, among areas within regions, and within areas (Table 2). The variance components at all levels were highly significant ($p<0.001$; Table 2). To determine whether a correlation exists between genetic distance and estimated coastal geographic distance (Table 1), a Mantel's non-parametric test was conducted. The ranked regression was positive and highly significant.

DISCUSSION

Three of the geographic areas sampled in this study (New England, South Atlantic, and Gulf coast) closely approximate three of Seneca's (1974) broad geographic populations. The northern (New Jersey) and southern (North Carolina) portions of the large mid-Atlantic region were sampled extensively. By including samples from near the limits of this region, any divergence between Seneca's (1974) proposed Atlantic coast populations should be resolved.

Most RAPD studies have shown a significant level of polymorphism at the individual level (e.g., Huff et al. 1993, Stiller and Denton 1995). AMOVAs produced variance components that are significant at all

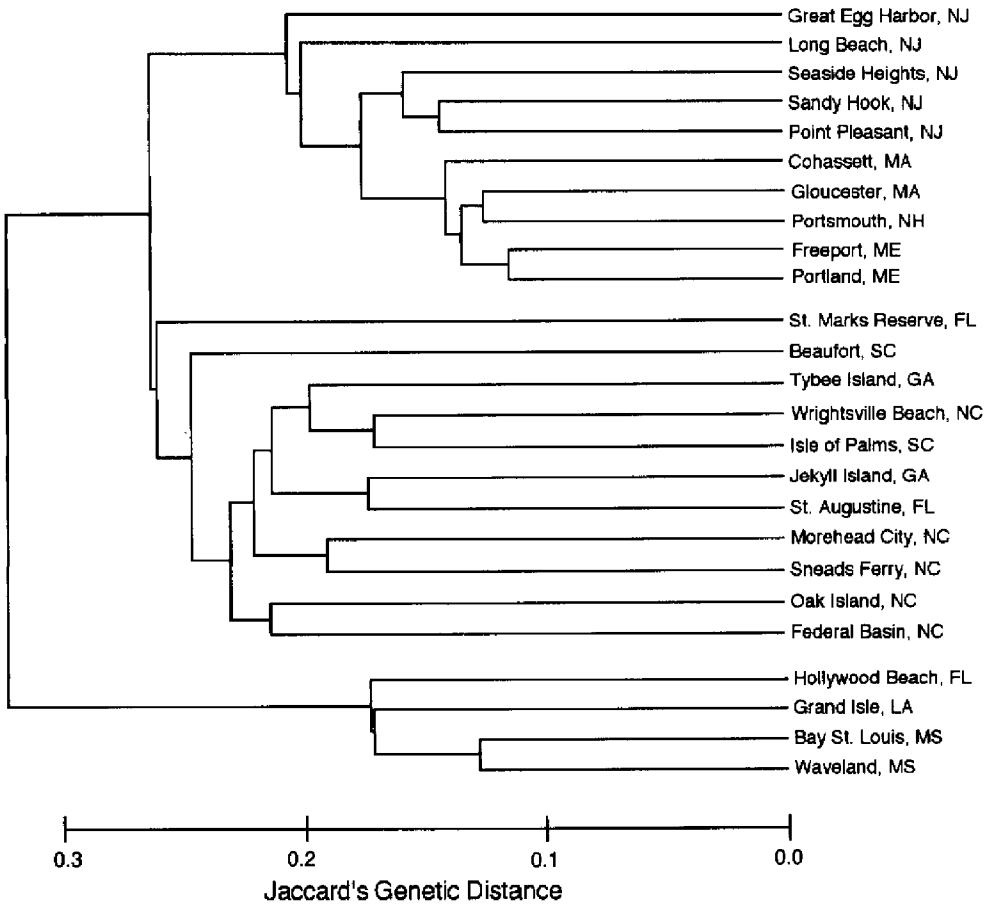

Figure 2. UPGMA (unweighted pair-group method using an arithmetic average) cluster analysis of Jaccard's genetic distance for 25 tall form plants of *Spartina alterniflora* sampled from 5 geographic areas: New England, New Jersey, North Carolina, South Atlantic, and the Gulf coast.

hierarchical levels in this study. Van Oppen et al. (1996) reported that variation between individuals can become as large as variation between populations at arbitrarily small scales. The high levels of variation within individuals found here may be a result of open pollination in *S. alterniflora* (Somers and Grant 1981). Although the majority of the variance occurred at the individual level within areas, a statistically significant amount of variance is attributed to differences among areas (NE, NJ, NC, SE) and among regions (Atlantic, Gulf) (Table 2). The variances attributed to differences among regions and differences among areas within regions can be combined to account for 39% of the total variation.

Table 2. Analysis of molecular variance (ANOVA) for 25 tall form *Spartina alterniflora* plants from 5 geographic areas calculated using Jaccard's genetic distance. Statistics include sums of squared deviations (SSD), mean squared deviations (MSD), variance components estimates, the percentages of total variance (% total) contributed by each component, and the probability of obtaining a more extreme variance component estimate by chance alone compared to a null distribution generated from 1000 random permutations of the data matrix.

Source of Variation	df	SSD	MSD	Variance Component	% Total	P-Value
Among 2 regions: Atlantic and Gulf	1	0.987	0.987	0.063	20.3	<0.001
Among areas within regions	3	1.441	0.480	0.058	18.5	<0.001
Within areas	20	3.829	0.191	0.191	61.3	<0.001
Among 3 regions: NE/NJ, NC/SA, and Gulf	2	1.812	0.906	0.075	25.8	<0.001
Among areas within regions	2	0.616	0.308	0.023	8.05	0.010
Within areas	20	3.829	0.191	0.191	66.13	<0.001

The phenogram produced for the 25 tall form plants sampled from 5 geographic areas (Figure 2) shows three geographically separate clusters: New England/ New Jersey, North Carolina/South Atlantic, and the Gulf coast. These groups comprise samples associated by geographic distance, with the exception of the St. Marks Reserve, FL sample, which was connected weakly to the North Carolina/ South Atlantic group. This individual may represent an unusual genotype not typical of the area population, or it could represent the past presence of a contiguous population connecting the Atlantic and Gulf regions during pre-Eocene periods of high eustatic sea level (Webb 1990). Additional sampling is necessary to address this question.

The two clusters of Atlantic coast plants in Figure 2 are likely an artifact of the sampling regime. Although areas such as New Jersey and North Carolina were heavily sampled, a large area between New Jersey and North Carolina was not included in this study. This sampling regime was intentional in attempting to identify where genetic change is occurring along the Atlantic coast in relation to Seneca's (1974) four broad geographic populations. If population subdivision between the three geographic areas along the Atlantic coast does exist, samples from New Jersey and North Carolina should cluster together rather than with geographically closer samples from outside of the mid-Atlantic region. The AMOVA based on two regions identifies a significant division between plants of the Atlantic and Gulf coast regions ($P<0.001$; Table 2), which was also observed by Seneca (1974). A significant division among areas along the Atlantic coast is also identified; however, this analysis does not indicate where the genetic divergence is occurring. For this reason, a second AMOVA was conducted based on the three clusters defined in the phenogram (Figure 2). Grouping the samples in this way increased the variance among regions to 25.82% ($P<0.001$) and reduced the variance among areas within regions to 8.05% ($P=0.01$). By grouping the samples into three regions, over one quarter of the total genetic variation can be attributed to occurring among these three regions, while the differences among areas within regions becomes less significant. These results indicate that variation among areas along the Atlantic coast is not the result of there being three distinct geographic populations but, instead, suggest that variation along the Atlantic coast might best be described as a genetic continuum.

The genetic continuum observed in tall form *S. alterniflora* along the Atlantic coast is likely the result of reduced gene flow due to distance and subtle selection pressure provided by environmental stresses along the latitudinal gradient. Various measures of genetic variation have shown that the extent of differentiation between populations usually increases with geographic distance (Slatkin 1993). The positive correlation between genetic and geographic distances found using the Mantel's non-parametric test supports this idea. Environmental based selection pressures have been found to strongly influence genetic structure in *S. patens* (Aiton) Muhl. (Silander 1979, 1984, 1985). Environmental changes based on latitudinal differences may be working similarly in *S. alterniflora.*

Although environmental selection pressures, distance, and physiological differences between geographically separate populations of *S. alterniflora* may hinder or restrict gene flow, a total barrier to this process is not likely to exist. The overlapping flowering phenologies between adjacent populations (Somers and Grant 1981) and the ability for long distance seed dispersal (Vivian-Smith and Stiles 1994) can effectively achieve gene flow between geographically separate populations over time.

ACKNOWLEDGMENTS

The authors thank Drs. D.F. Kapraun, D.E. Padgett, T.H. Shafer, and J.B. Geller for critical comments, and the late K.T. Bird for his support of this study. This study was funded in part by North Carolina Sea Grant (project no. R/MER-28), The Center for Marine Science Research, and the Department of Biological Sciences, University of North Carolina at Wilmington. This is contribution number 217 from the Center for Marine Science Research.

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Manuscript received 15 January 1998; revisions received 27 July 1998 and 5 November 1998; accepted 16 February 1999.