Ecology Letters, (2004) **7**: 1155–1162 doi: 10.1111/j.1461-0248.2004.00674.x

REPORT

Unexpectedly high clonal diversity of two salt marsh perennials across a severe environmental gradient

Christina L. Richards,[1]* J. L. Hamrick,[2] Lisa A. Donovan[1] and Rodney Mauricio[3]

[1]Department of Plant Biology, 2502 Miller Plant Sciences Building, University of Georgia, Athens, GA 30602, USA

[2]Departments of Plant Biology and Genetics, 2502 Miller Plant Sciences Building, University of Georgia, Athens, GA 30602, USA

[3]Department of Genetics, Davison Life Sciences Complex, University of Georgia, Athens, GA 30602-7223, USA

**Correspondence: E-mail: richards@life.bio.sunysb.edu*

Abstract

In salt marsh habitats, noted for their extreme environments, a widely held assumption is that a few large clones dominate plant populations. Using a large number of polymorphic genetic markers, we were able to test this assumption for two salt marsh plants known to span extreme salinity gradients. For both species, clonal diversity was surprisingly high across populations: Simpson's diversity indices were 0.96 and 0.99. Although clonal diversity was high, there was no pattern of association between specific clones or alleles with salt microhabitat. Our findings suggest that sexual reproduction and recruitment from seeds may have been generally underappreciated as an important ecological force in the salt marsh. Furthermore, clonal diversity has implications for conservation and restoration of these critical coastal habitats, particularly with regard to buffering environmental change or disease. Recent studies also suggest that high levels of intraspecific diversity can affect a variety of community and ecosystem processes.

Keywords

Allozymes, *Borrichia frutescens*, clonal structure, fine-scale differentiation, salt marsh plants, Sapelo Island, sea ox-eye daisy, smooth cord grass, *Spartina alterniflora*.

Ecology Letters (2004) 7: 1155–1162

INTRODUCTION

Clonal plants predominate in many challenging environments (Turkington & Harper 1979; Silander 1985b). Salt marshes are extremely stressful habitats where clonal plant species span major environmental gradients. Salt marsh ecologists have long assumed that salt marsh plant populations are dominated by a few large clones which allows them to tolerate these environmental gradients (Hartman 1988; Adam 1990). This assumption is supported by the many reports that salt marsh perennials have limited seedling establishment because of intense flower and seed predation (Bertness *et al.* 1987; Bertness & Shumway 1992), little or no seed bank (Hartman 1988), inhibited germination (Shumway & Bertness 1992) and high seedling mortality (Hopkins & Parker 1984; Ungar 1987). Besides low levels of recruitment, studies have shown that ramets survive high salt bare patches or salt pans by rhizome connections to nearby vegetation in less stressful habitat (Shumway 1995; Pennings & Callaway 2000). The pervasiveness of extensive clonal spread in these communities is further evidenced by isolated clones of *Spartina* species invading bare mud flats (Callaway & Josselyn 1992; Castellanos *et al.* 1994, 1998; Daehler & Strong 1994; Sanchez *et al.* 2001). Nevertheless, the assumption that populations are dominated by a few large clones has not been tested in natural standing populations of salt marsh plants.

If clonal reproduction is extensive in salt marsh plants, a corollary prediction is that there should be no association between clonal genotypes or alleles at specific loci and microhabitat: individual clones should be able to span environmental gradients and share resources across rhizomes (Steufer *et al.* 1996; Jonsdottir & Watson 1997; Pennings & Callaway 2000). This ability to overcome environmental heterogeneity obviates the need to evolve locally adapted or specialized genotypes and, therefore, lends insight into the ongoing debate about the genetic basis of different height forms of *S. alterniflora* at the ends of environmental gradients (Shea *et al.* 1975; Valiela *et al.* 1978; Gallagher *et al.* 1988).

Population genetic techniques allow us to test these important hypotheses about salt marsh plants by identifying different genotypes and mapping their spatial distribution. Not only do patterns of molecular markers reveal the diversity of clones in a population, but also they allow us to observe the interactions between genotype and environment at a fine geographic scale. In essence, this approach allows

us to address the question of whether environmental variation is reflected in genotypic diversity.

In this study, we used a large number of polymorphic allozyme loci to identify clonal structure with a high degree of certainty in two dominant salt marsh perennials in a south-eastern US salt marsh: *Borrichia frutescens* (L) DC. (Asteraceae: sea oxeye daisy) and *Spartina alterniflora* Loisel. (Poaceae: smooth cord grass). Both of these species live across an especially broad range of environmental conditions. Clonal spread by rhizomes is thought to be extensive with purported low rates of seedling establishment in the field for both species (Antlfinger 1981; Hartman 1988; Adam 1990; Stiling 1994). Although extensive clonality is thought to be an important strategy allowing salt marsh plants to deal with the steep gradients in the salt marsh (Hartman 1988; Shumway 1995; Pennings & Callaway 2000), little is known about the clonal structure and genetic make-up of these species (but see Silander 1984, 1985a). While these species have some characteristic differences, we expected that clonal reproduction would lead to similar patterns of clonal structure and microhabitat association. For both species, we tested the expectation that populations are dominated by a few large clones. We also tested the consequential prediction that large clones span the environmental gradient and that there is no association between clone genotype and microhabitat type.

METHODS

Study site and study species

The study was conducted at six sites on Sapelo Island, GA, USA (31° 28′ N, 81° 14′ W). Vegetation patterns in Sapelo Island marshes are typical of south-eastern marshes in the United States (Pomeroy & Wiegert 1981). Lower elevations of the marsh are subject to daily tidal submergence and are dominated by *S. alterniflora*. Higher elevations of the marsh are flooded irregularly and are often characterized by highly saline salt pans and associated salt-tolerant species such as *B. frutescens* (Antlfinger 1981).

We focused our study on two dominant salt marsh species with clonal reproduction: *S. alterniflora* and *B. frutescens*. These species represent a contrast in growth form, habitat preference, and physiology. *Spartina alterniflora* is widely recognized as an important salt marsh perennial both in its native east coast habitat and as a cosmopolitan invasive (Callaway & Josselyn 1992). It grows in thick, monospecific stands in the low marsh. *Borrichia frutescens* is also a dominant, perennial shrub that grows in the upper, more saline, parts of the salt marsh but has a narrower distribution geographically along the eastern US coast (Virginia–Florida). The two species represent a significant contrast in basic physiology: *S. alterniflora* is a C4 monocot while *B. frutescens* is a C3 dicot.

Borrichia frutescens and *S. alterniflora* occur across a broad range of environments, with soil salinities ranging from 20 to > 100 ppt (Richards *et al.* 2004). Both species also exhibit extreme phenotypic variation, with *B. frutescens* heights ranging from > 100 to < 10 cm and *S. alterniflora* heights ranging from > 200 to < 20 cm (Richards *et al.* 2004). Edaphic factors alone significantly predict 20% and over 50% of the variation in height in natural populations of *B. frutescens* and *S. alterniflora*, respectively (Richards *et al.* 2004).

Sampling design

We designed a sampling scheme to examine clonal structure at several spatial scales for *B. frutescens* and *S. alterniflora* across microhabitats at five marsh sites separated by approximately 3–5 km on Sapelo Island. Because salinity is significantly correlated with height for both species, we used height as a proxy for salinity microhabitat (Richards *et al.* 2004). At each site, we collected live leaf tissue from 96 ramets.

Although clone size in natural standing populations of *B. frutescens* has not been examined in detail, clonal spread by rhizomes is thought to be extensive and isolated clones of *B. frutescens* colonizing 'spoil' islands have been reported to be > 3 m in diameter (Stiling 1994). We used two sampling schemes to represent three microhabitats (high, intermediate and low salt) designated at each site. At Marsh Landing, Light House and Hunt Camp sites, we sampled at every 1 m mark on grids spanning a mound dominated by *B. frutescens*. Sampled ramets were assigned more or less equally to each of three microhabitats: (1) high salt, which were the shortest plants found adjacent to salt pans, (2) intermediate salt, which were intermediate height plants found between the salt pan and the middle of the stand and (3) low salt, which were the tallest plants found in the middle of the stand. At the Cabretta and Shell Hammock sites, the populations covered significantly more area. We, therefore, collected leaf tissue from 32 ramets on separate grids within each microhabitat. The details of plant height and salinity range for each of these three microhabitats varied from site to site and, therefore, the assignments of plants to microhabitats were relative at each site.

The *S. alterniflora* populations cover a much more extensive area than those of *B. frutescens*. Clonal spread by rhizomes is also thought to be extensive in native populations of *S. alterniflora* (Hartman 1988) and isolated clones of invading *Spartina* species have been reported to be > 3 m in diameter (Callaway & Josselyn 1992; Castellanos *et al.* 1994, 1998; Daehler & Strong 1994). We had the same sampling scheme at each site to represent three microhabitats (high, intermediate, and low salt). We collected plant tissue from 32 ramets each from (1) high salt, which were the shortest plants found adjacent to salt pans, (2) intermediate salt,

which were intermediate height plants found between the salt pan and the creek bank, and (3) low salt, which were the tallest plants found immediately adjacent to the creek bank. Microhabitats were separated by 20–50 m depending on the site. Within each microhabitat, we sampled plants with four sets of paired transects. The four transect pairs were separated by 10 m and ran perpendicular to the shoreline. The two transects within a pair were separated by 1 m and plants were sampled every 4 m.

Electrophoresis

Leaf tissue samples were kept on ice and crushed within 24 h for protein extraction using sea sand and the buffer of Alvarez-Buylla & Garay (1994) for *B. frutescens* and the buffer of Wendel & Parks (1982) for *S. alterniflora*. Extracted material was absorbed onto Whatman 3 mm filter paper wicks and then stored at −80 °C until needed for electrophoresis. Extracted proteins were run on 9.5% starch gels. We used standard recipes for enzyme buffers and stains (Soltis *et al.* 1983; Cheliak & Pitel 1984). Seventeen allozyme loci were resolved for *B. frutescens*: ALD, IDH, 6-PGD and PGI (2) on buffer system 4; AAT and MNR on buffer system 7; FE (4), GDH and ME on buffer system 8-; F1,6 on buffer system 11 and CE, DIA, and PGM on buffer system 6. Twenty-seven loci were resolved for *S. alterniflora*: MDH (3), 6-PGD and UGPP (4) on buffer system 4; AAT (3) on buffer system 7; ACO, AK, F1,6 (2), IDH, MPI and SKDH on buffer system 11 and DIA(2), FE, LAP, PER, PGI, PGM and TPI (2) on buffer system 6. Twenty-two of the 27 *Spartina* loci were scored as regular diploid loci. Two loci (IDH1 and PGI) displayed tetraploid inheritance patterns and were scored as four alleles per ramet and analysed as two loci each. Three (TPI2, PGM and PER) loci displayed complex polyploid banding patterns and were scored as phenotypes. These three loci were excluded from the analysis of general population genetics statistics, but they were used to identify multilocus genotypes and clonal structure.

Analyses

To visualize clonal structure and diversity, we mapped multilocus genotypes (genets) for each ramet within each patch. We assessed clonal diversity first as the number of ramets per number of genets sampled. Our second measure of diversity is Simpson's index: $D = 1 - \Sigma\ [n_i(n_i - 1))/(N(N - 1))]$, where n_i is the number of ramets of the ith genet and N is the total number of ramets (Pielou 1969). We calculated the probability of exclusion to determine the likelihood that two ramets did not share a multilocus genotype by chance alone using the Probability of Identity for each locus $= \Sigma\ p_i^4 + \Sigma\ (2p_ip_j)^2$ where p_i and p_j are the frequencies of the ith and jth alleles (Paetkau & Strobeck 1994). The overall probability of identity for a multilocus genotype is the product of all the separate probabilities across loci. The probability of exclusion for a multilocus genotype is then 1−overall probability of identity.

Population genetic statistics were calculated using the program LYNSPROG developed by M. D. Loveless (College of Wooster, Wooster, OH, USA) and A. Schnabel (Indiana University, South Bend, IN, USA). At the species and population levels, we estimated percent polymorphic loci (*PL*) and expected heterozygosity ($H_e = 1 - \Sigma p_i^2$, where p_i is the frequency of allele i), which estimates genetic diversity. Standard errors for within population parameters were obtained by averaging across all populations.

We explored associations between clone genotype and microhabitat in two ways. First, we looked for the presence of multiple ramets of a clone within a single microhabitat both within and among sites for both species. If ramets from the same genet were present across different microhabitats, we would take that as evidence against an association of clone with microhabitat. Second, we looked for specific associations of allele frequencies with microhabitat at each polymorphic locus for both species. We ran a separate one-way analysis of variance (ANOVA) for each allele at polymorphic loci with microhabitat as the main effect (PROC GLM in the SAS statistical package, version 8.02 for Windows; SAS Institute, Cary, NC, USA). Allele frequencies were arcsine square root transformed to meet statistical assumptions. A significant effect of microhabitat would suggest that allele frequencies at a given locus were significantly associated with microhabitat across sites.

RESULTS

Clonal diversity

Clonal diversity in both species was high. Our ten polymorphic loci for *B. frutescens* and 17 polymorphic loci for *S. alterniflora* resulted in a high probability of exclusion, which allowed us to distinguish unique multilocus genotypes with a high degree of certainty (Table 1). For *B. frutescens*, 74 unique multilocus genotypes out of 480 ramets were identified across the five sites. Of these, 34% (25 genotypes) were represented by only one ramet in one site and 20% (15 genotypes) were represented by multiple ramets within only one site. Clonal structure and diversity varied among the five sites (Table 1a). The average number of ramets/genet was 4.1 and average clonal diversity (measured as Simpson's diversity index) was 0.92 (Table 1a). The number of unique genotypes per site ranged from 16 at Shell Hammock to 31 at Marsh Landing. The majority of the 31 genotypes at Marsh Landing were represented by one to six ramets (Fig. 1). One obvious exception was one

Table 1 Statistics of clonal structure and diversity of (a) *Borrichia frutescens* sites and (b) *Spartina alterniflora* sites

Site	Ramets/Genet (SE)	Clonal diversity	Probability of exclusion
(a) *B. frutescens*			
Cabretta	3.00 (0.49)	0.953	0.95
Hunt Camp	4.57 (1.07)	0.885	0.97
Lighthouse	3.69 (0.94)	0.896	0.93
Marsh Landing	3.10 (0.62)	0.980	0.87
Shell Hammock	6.00 (1.70)	0.872	0.90
Overall	5.9 (1.03)	0.96	
Average	4.1 (0.21)	0.92	
(b) *S. alterniflora*			
Cabretta	1.04 (0.02)	0.999	> 0.999
Hunt Camp	1.05 (0.03)	0.999	> 0.999
Island Apex	1.12 (0.05)	0.996	> 0.999
Lighthouse	1.14 (0.04)	0.997	> 0.999
Marsh Landing	1.03 (0.02)	0.999	> 0.999
Overall	1.08 (0.02)	0.999	
Average	1.08 (0.02)	0.998	

Each site is represented by 96 ramets collected across three microhabitats. Average number of ramets per unique multilocus genet and standard error between genets are presented. Clonal diversity = Simpson's diversity index. Probability of exclusion = 1−product of Probability of Identity (Paetkau & Strobeck 1994). See Methods for details.

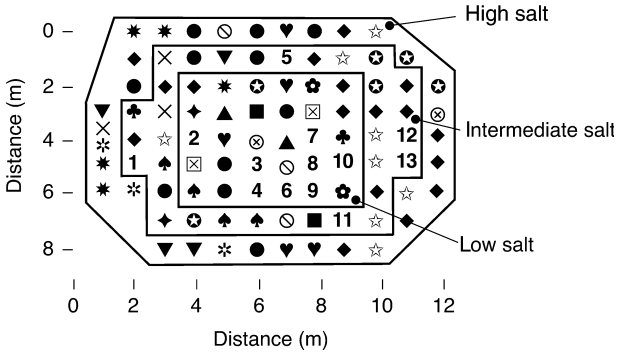

Figure 1 Map of the clonal structure of a population of *Borrichia frutescens* spanning a mound at the Marsh Landing site. The edges of the mound are adjacent to salt pan with high salinity. Salinity decreases as one moves to the top of the mound in the center. Salt microhabitat was assigned based on the height of the plant growing in that position. Each symbol represents one individual ramet. Different symbols represent unique multilocus genotypes for allele combinations across 10 polymorphic loci. Genets with only one ramet are represented by Arabic numerals, 1–13.

genotype, which represented 18% (17 of 96 ramets) of the population (diamonds in Fig. 1). Similarly, one genotype in the Shell Hammock site represented 38% (27 of 96) of the ramets sampled.

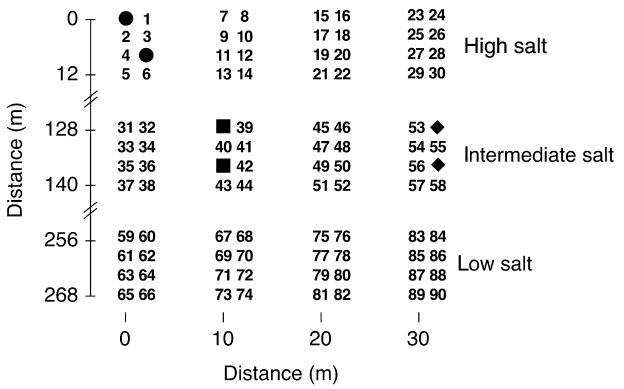

Figure 2 Map of the clonal structure of *Spartina alterniflora* at the Marsh Landing site. Salinity decreases as one moves towards the ocean (highest in the salt pans). Salt microhabitat was assigned based on the height of the plant growing in that position. Each symbol represents one individual ramet. Different symbols represent unique multilocus genotypes for allele combinations across 17 polymorphic loci. Genets with only one ramet are represented by Arabic numerals, 1–90.

The *S. alterniflora* sites displayed an even higher level of clonal diversity (Table 1b and Fig. 2). Out of 480 ramets sampled, only 6% (30 genets) were represented more than one time for a total of 447 unique multilocus genotypes. All of the 30 genets with multiple ramets were found in only one site and 90% of these (27 of the 30) were represented by only two ramets. The average number of ramets/genet and average clonal diversity were therefore very close to 1 (Table 1b). The number of unique genotypes per site ranged from 88% (84 out of 96 ramets sampled) at Lighthouse to 97% (93 out of 96 ramets sampled) at Marsh Landing.

Genetic diversity as measured by expected heterozygosity (H_e) was 0.089 for *B. frutescens* and 0.205 for *S. alterniflora* (Table 2), indicated that both species were about average compared to species with similar life history characteristics (Hamrick & Godt 1996, Franks *et al.* 2004). *Borrichia frutescens* had typical levels of polymorphism (58.8% polymorphic loci), while *S. alterniflora* was well above average with 81% polymorphic loci (Table 2). Within sites pooled across microhabitats, observed and expected heterozygosities were not significantly different (G-test, $P > 0.05$), indicating allele frequencies were in Hardy–Weinberg equilibrium for both species.

Distribution of clones

To investigate the relationship between microhabitat and clonal structure, we examined the distribution of multiple ramets for a given clone across the three microhabitat zones corresponding to low, intermediate and high salinity levels for both species. For the majority of replicated *B. frutescens*

Table 2 Within population estimates of genetic diversity and structure across microhabitats within five sites for (a) *Borrichia frutescens* and (b) *Spartina alterniflora*

Site	*PL* (%)	H_e
(a) *B. frutescens*		
Cabretta	53.9	0.126
Hunt Camp	41.2	0.058
Lighthouse	50.0	0.084
Marsh Landing	53.9	0.117
Shell Hammock	35.3	0.066
Mean	46.8	0.090
SD	5.7	0.018
Pooled values	58.8	0.089
(b) *S. alterniflora*		
Cabretta	65.4	0.220
Hunt Camp	65.4	0.209
Island Apex	61.5	0.189
Lighthouse	61.5	0.197
Marsh Landing	61.5	0.192
Mean	63.1	0.201
SD	4.2	0.019
Pooled values	80.8	0.205

PL (%), percent polymorphic loci, H_e, mean Hardy–Weinberg expected heterozygosity. The species level expected heterozygosity is that given as the pooled values.

clones, multiple ramets were found in more than one microhabitat (Table 3). Clones that were associated with only one microhabitat represented 3–19% of the ramets at a given site or 8.5% of total ramets sampled across sites. In the *S. alterniflora* sites, all replicated clones were associated with only one microhabitat, however most replicated clones were represented by only two ramets. These replicated ramets represented only 6–24% of the total ramets sampled at a given site or 13% over all 480 ramets sampled (Table 3b).

Table 3 Total number of genets with more than one ramet at each site, number of genets with ramets found in only one microhabitat within that site and percentage of all ramets sampled that are replicated in only one microhabitat for (a) *Borrichia frutescens* and (b) *Spartina alterniflora*

Site	Genets with multiple ramets	Genets with ramets found in one microhabitat	% of total ramets
(a) *B. frutescens*			
Cabretta	17	6	18.8
Hunt Camp	12	2	4.2
Lighthouse	14	4	9.4
Marsh Landing	18	3	7.3
Shell Hammock	11	1	3.1
(b) *S. alterniflora*			
Cabretta	5	5	10.4
Hunt Camp	4	3	7.3
Lighthouse	7	7	17.7
Marsh Landing	11	11	24.0
Shell Hammock	3	3	6.3

We also examined the distribution of alleles at polymorphic loci across the five sites for each species. One-way ANOVA's revealed that microhabitat did not explain the distribution of allele frequencies at any of the polymorphic loci for either *B. frutescens* or *S. alterniflora* ($P > 0.05$, for example Fig. 3).

DISCUSSION

Salt marsh ecologists have long speculated that extensive clonal reproduction is essential for survival across the severe environmental gradients of the marsh (Shumway 1995; Pennings & Callaway 2000) and therefore most dominant marsh plants consist solely of a few large clones (Hartman

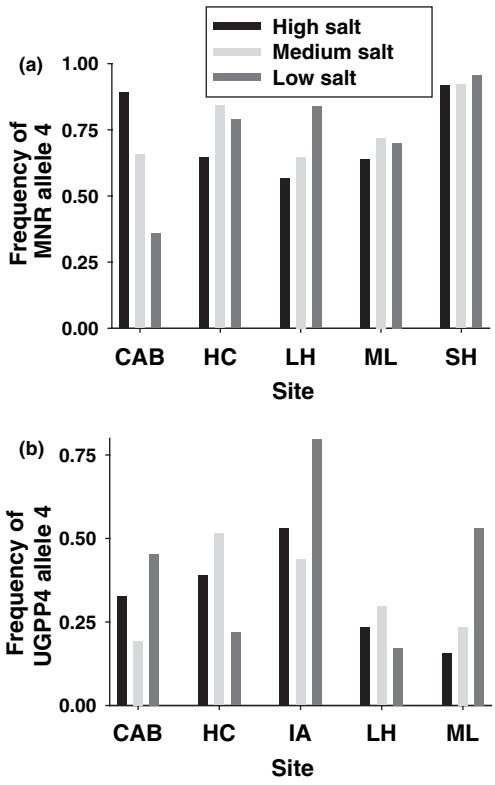

Figure 3 Allele frequencies at the (a) MNR locus for each of the five populations by high, medium and low salt microhabitat type of *Borrichia frutescens* and (b) UGPP4 locus for each of the five populations by high, medium and low salt microhabitat type of *Spartina alterniflora*. Site abbreviations: CAB, Cabretta; HC, Hunt Camp; LC, Light House; ML, Marsh Landing; SH, Shell Hammock; IA, Island Apex. See Methods for sampling details.

1988; Adam 1990). In general however, we found that clonal diversity across the salt marsh was very high for both species. Our clonal maps demonstrate that there are a small number of large clones in *B. frutescens* populations (see the two large genets represented by filled circles and filled diamonds in Fig. 1). Nevertheless, the pattern across all sites suggests that there were many multilocus genotypes indicating high clonal diversity. We have very little evidence for the existence of extensive clone size in the *S. alterniflora* sites given that most genets were represented only once. Of the genets with multiple ramets, all but three genets were represented by just two ramets that were typically only 1 or 2 m apart. Only one exceptionally large *S. alterniflora* genet was detected. This genet was represented by five ramets that extended over 16 m at Island Apex in the low salt microhabitat. In general, overall clonal diversity was much higher than expected for both species. *Borrichia frutescens* ramets spaced more than 2 m apart and *S. alterniflora* ramets spaced 1 m apart were unlikely to belong to the same clone, indicating that sexual reproduction may be important for these species.

The high clonal diversity was surprising given that clonal spread is known to be extensive in these species (Callaway & Josselyn 1992; Daehler & Strong 1994; Stiling 1994) and salt marsh perennials generally have limited seedling establishment (Hopkins & Parker 1984; Bertness *et al.* 1987; Ungar 1987; Hartman 1988; Bertness & Shumway 1992; Shumway & Bertness 1992). However, at least one other study found high clonal diversity in another salt marsh grass *Distichlis spicata*, but reported half the level of diversity that we measured in *Spartina* (Eppley *et al.* 1998). In addition, there are reports that seedling establishment can be the predominant form of recruitment into bare substrates for *S. alterniflora* invasions in the western US (Daehler 1998; Davis *et al.* 2004) and recolonization along the Gulf Coast US (Proffitt *et al.* 2003; McKee *et al.* 2004). Occasional disturbance events like that of the recent large scale die-back in the Gulf Coast and south-eastern US marshes may result in localized flushes of seedling establishment which could explain these patterns of high clonal diversity (Proffitt *et al.* 2003, Franks *et al.* 2004). Clearly the importance of sexual reproduction and seedling establishment has been underestimated in these dominant salt marsh plant species.

The high levels of genetic diversity suggest that these plants do not deal with the extreme salt marsh environment solely by growing as large clones that span the environmental gradient. We, therefore, considered alternative strategies these plants might employ to deal with the environmental gradients of the marsh. Studies of clonal species have found that different genets vary in their ability to discriminate between patches and proliferate in favourable habitat (Silander 1979; Salzman 1985; Bazzaz 1991). This type of habitat foraging would lead to an association of clone genotype with microhabitat. However, we did not find evidence for this type of microhabitat association. In fact, our clonal maps suggest that a small number of *B. frutescens* clones are very large, but tend to traverse the entire gradient through high, intermediate and low salt microhabitats (see genets indicated by filled diamonds and filled circles in Fig. 1), suggesting that physiological integration instead of preferential foraging may be a factor for the success of these few genotypes.

Another strategy for coping with environmental variation is the maintenance of specialized genotypes that are adapted to different microhabitats. There has been considerable interest in whether natural selection in these relatively harsh habitats has resulted in local adaptation, but the question has never been conclusively resolved (Shea *et al.* 1975; Valiela *et al.* 1978; Silander 1979, 1985a; Antlfinger 1981; Gallagher *et al.* 1988). Studies have demonstrated that natural selection in different microhabitats can result in associations of genotypes or alleles with habitat type (Hamrick & Allard 1972; Silander 1984; Salzman 1985; Heywood 1991; Schmidt & Rand 1999). This does not appear to be the case for these two salt marsh species: we found no significant associations between specific marker loci and the salt environment. Although we did not find these patterns at specific allozyme loci, selection may be acting on ecologically important traits that are not simply represented by or linked to any one locus. Reciprocal transplants of field collected *B. frutescens* reveal that there is genetic variation for many traits related to salt tolerance and that some of these traits demonstrate patterns consistent with local adaptation (Richards 2004). In addition, selection may be acting on a much finer scale or on a much more dynamic scale than can be addressed with our sampling scheme.

In sum, we found that clonal diversity was high in both *B. frutescens* and *S. alterniflora*. Given this high clonal diversity, we additionally asked whether the clonal structure in these species was consistent with local adaptation that might result from either clonal foraging or natural selection. However, we found no association of genotypes or alleles with microhabitat, which would provide evidence for one or both of these processes. We conclude that while integration may be important for some clones on a scale of < 1 m (Shumway 1995; Pennings & Callaway 2000), on a larger scale clone size is limited. In addition, we have no evidence that *B. frutescens* and *S. alterniflora* accommodate the broad range and variation of salt marsh environments by microhabitat association of genotypes in response to selection. Variation in size among clones suggests that genets may have different strategies for dealing with environmental gradients of the marsh (Silander 1979; Brewer & Bertness 1996). Although our study does not address these different strategies, our data reveal that sexual reproduction and recruitment from seeds

are likely important in maintaining diversity even in these stressful habitats.

The unexpectedly high levels of clonal diversity have far reaching implications for conservation and restoration of these important coastal habitats. Maintaining high levels of diversity in natural populations and restoring high levels of diversity in disturbed marshes will be important for the ability of these populations to respond to environmental change or disease. Given that these habitats are quite dynamic, this high level of clonal diversity may be an important component for the long-term persistence of these populations. These previously unknown levels of diversity also have consequences for manipulative studies in salt marsh ecology because most of these studies do not consider the importance of genetically based variation in responses. Recent interest in this type of approach has revealed that heritable genetic variation within species does indeed have community and ecosystem consequences (Whitham *et al.* 2003; Schweitzer *et al.* 2004). Understanding the genetic make-up of natural populations is therefore an important link to understanding complex interactions and processes within species as well as at the community level.

ACKNOWLEDGEMENTS

The authors thank Regina Baucom, Amy Bouck, Janine Cousins, Jenny Cruse-Saunders, Cecile Deen, Grant Farmer, Steven Franks, Rebecca Pappert, Chelly Richards, Jeffrey Ross-Ibarra and Dorset Trapnell for assistance in completing this project. Regina Baucom, Amy Bouck, Susan Dudley, Maheteme Gebremedhin, Vanessa Koelling, Steven Pennings, David Rosenthal and Jeffrey Ross-Ibarra provided helpful comments on earlier versions of the manuscript. We thank the Georgia Coastal Ecosystems LTER (OCE 99-82133), SINERR (NOAA-GRF program) and the University of Georgia Plant Biology Department's Palfrey Fund for financial support. This is contribution number 937 for the University of Georgia Marine Institute Collected Reprints.

REFERENCES

Adam, P. (1990). *Saltmarsh Ecology*. Cambridge University Press, Cambridge, UK.

Alvarez-Buylla, E.R. & Garay, A.A. (1994). Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer tree species. *Evolution*, 48, 437–453.

Antlfinger, A.E. (1981). The genetic basis of microdifferentiation in natural and experimental populations of *Borrichia frutescens* in relation to salinity. *Evolution*, 35, 1056–1068.

Bazzaz, F.A. (1991). Habitat selection in plants. *Am. Nat.*, 137, S116–S130.

Bertness, M.D. & Shumway, S.W. (1992). Consumer driven pollen limitation of seed production in marsh grasses. *Am. J. Bot.*, 79, 288–293.

Bertness, M.D., Wise, C. & Ellison, A.M. (1987). Consumer pressure and seed set in a salt marsh perennial plant community. *Oecologia*, 71, 190–200.

Brewer, J.S. & Bertness, M.D. (1996). Disturbance and intraspecific variation in the clonal morphology of salt marsh perennials. *Oikos*, 77, 107–116.

Callaway, J.C. & Josselyn, M.N. (1992). The introduction and spread of smooth cordgrass (*Spartina alterniflora*) in south San Francisco Bay. *Estuaries*, 15, 218–226.

Castellanos, E.M., Figueroa, M.E. & Davy, A.J. (1994). Nucleation and facilitation in saltmarsh succession: interactions between *Spartina maritima* and *Arthrocnemum perenne*. *J. Ecol.*, 82, 239–248.

Castellanos, E.M., Heredia, C., Figueroa, M.E. & Davy, A.J. (1998). Tiller dynamics of *Spartina maritima* in a successional and non-successional Mediterranean salt marsh. *Plant Ecol.*, 137, 213–225.

Cheliak, W.M. & Pitel, J.A. (1984). *Techniques for starch gel electrophoresis of enzymes from forest tree species*. Petawawa National Forestry Institute, Information Report P1-X-42. Chalk River, Ontario: Canadian Forestry Service, Agriculture.

Daehler, C.C. (1998). Variation in self-fertility and the reproductive advantage of self-fertility for an invading plant (*Spartina alterniflora*). *Evol. Ecol.*, 12, 553–568.

Daehler, C.C. & Strong, D.R. (1994). Variable reproductive output among clones of *Spartina alterniflora* (Poaceae) invading San Francisco Bay, California: the influence of herbivory, pollination, and establishment site. *Am. J. Bot.*, 81, 307–313.

Davis, H.G., Taylor, C.M., Civille, J.C. & Strong, D.R. (2004). An Allee effect at the front of a plant invasion: *Spartina* in a Pacific estuary. *J. of Ecol.*, 92, 321–327.

Eppley, S.M., Stanton, M.L. & Grosberg, R.K. (1998). Intrapopulation sex ratio variation in the salt grass *Distichlis spicata*. *Am. Nat.*, 152, 659–670.

Franks, S.J., Richards, C.L., Gonzales, E., Cousins, J.E. & Hamrick, J.L. (2004). Multi-scale genetic analysis of *Uniola paniculata* (Poaceae): a coastal species with a linear, fragmented distribution. *Am. J. Bot.*, 91, 1345–1351.

Gallagher, J.L., Somers, G.F., Grant, D.M. & Seliskar, D.M. (1988). Persistent differences in two forms of *Spartina alterniflora*: a common garden experiment. *Ecology*, 69, 1005–1008.

Hamrick, J.L. & Allard, R.W. (1972). Microgeographical variation in allozyme frequencies in *Avena barbata*. *Proc. Natl. Acad. Sci. USA*, 69, 2100–2104.

Hamrick, J.L., & Godt, M.J.W. (1996). Effects of life history traits on genetic diversity in plant species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 351, 1291–1298.

Hartman, J.M. (1988). Recolonization of small disturbance patches in a New England salt marsh. *Am. J. Bot.*, 75, 1625–1631.

Heywood, J.S. (1991). Spatial analysis of genetic variation in plant populations. *Annu. Rev. Ecol. Syst.*, 22, 335–355.

Hopkins, D.R. & Parker, V.T. (1984). A study of the seed bank of a salt marsh in northern San Francisco Bay. *Am. J. Bot.*, 71, 348–355.

Jonsdottir, I.A. and Watson, M.A. (1997). Extensive physiological integration: an adaptive trait in resource poor environments? In: *The Ecology and Evolution of Clonal Plants*. (eds de Kroon, H. & van Groenendael, J.). Backhuys Publishers, The Netherlands, pp. 109–136.

McKee, K.L., Mendelssohn, I.A. & Materne, M.D. (2004). Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? *Global Ecol. Biogeog.*, 13, 65–73.

Paetkau, D. & Strobeck, C. (1994). Microsatellite analysis of genetic variation in black bear populations. *Mol. Ecol.*, 3, 489–495.

Pennings, S.C. & Callaway, R.M. (2000). The advantages of clonal integration under different ecological conditions: a community-wide test. *Ecology*, 81, 709–716.

Pielou, E.C. (1969). *An Introduction to Mathematical Ecology*. Wiley-Interscience, New York, NY.

Pomeroy L.R. & Wiegert, R.G. (1981). *The Ecology of a Salt Marsh*. Springer-Verlag, New York, NY.

Proffitt, C.E., Travis, S.E. & Edwards, K.R. (2003). Genotype and elevation influence *Spartina alterniflora* colonization and growth in a created salt marsh. *Ecol. Appl.*, 13, 180–192.

Richards, C.L. (2004). *Evolution in Closely Adjacent Salt Marsh Environments*. PhD Thesis, University of Georgia, Athens, GA.

Richards, C.L, Pennings, S.C. & Donovan, L.A. (2004). Habitat range and phenotypic variation in salt marsh plants. *Plant Ecol.*, in press.

Salzman, A.G. (1985). Habitat selection in a clonal plant. *Science*, 228, 603–604.

Sanchez, J.M., SanLeon, D.G. & Izco, J. (2001). Primary colonization of mudflat estuaries by *Spartina maritima* (Curtis) Fernald in Northwest Spain: vegetation structure and sediment accretion. *Aquat. Bot.*, 69, 15–25.

Schmidt, P.S. & Rand, D.M. (1999). Intertidal microhabitat and selection at MPI: interlocus contrasts in the northern acorn barnacle, *Semibalanus balanoides*. *Evolution*, 53, 135–146.

Schweitzer, J.A., Bailey, J.K., Rehill, B.J., Mertinsen, G.D., Hart, S.C., Lindroth, R.L. *et al.* (2004). Genetically based trait in a dominant tree affects ecosystem processes. *Ecol. Lett.*, 7, 127–134.

Shea, M.L., Warren, R.S. and Neiring, W.A. (1975). Biochemical and transplantation studies of the growth form of *Spartina alterniflora* on Connecticut salt marshes. *Ecology*, 56, 461–466.

Shumway, S.W. (1995). Physiological integration among clonal ramets during invasion of disturbance patches in a New England salt marsh. *Ann. Bot. Lond.*, 76, 225–233.

Shumway, S.W. & Bertness, M.D. (1992). Salt stress limitation of seedling recruitment in a salt marsh plant community. *Oecologia*, 92, 490–497.

Silander, J.A. (1979). Microevolution and clone structure in *Spartina patens*. *Science*, 203, 658–660.

Silander, J.A. (1984). The genetic basis of the ecological amplitude of *Spartina patens*. III. Allozyme variation. *Bot. Gaz.*, 145, 569–577.

Silander, J.A. (1985a). The genetic basis of the ecological amplitude of *Spartina patens*. II. Variance and correlation analysis. *Evolution*, 39, 1034–1052.

Silander, J.A. (1985b). Microevolution in clonal plants. In: *Population Biology and Evolution of Clonal Organisms* (eds Jackson, J.B.C., Buss, L.W. & Cook, R.E.). Yale University Press, New Haren, CT, USA, pp. 107–152.

Soltis, D.E., Haufler, C.H., Darrow, D.C. & Gastony, G.J. (1983). Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.*, 73, 9–27.

Steufer, J.F., de Kroon, H. & During, H. J. (1996). Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. *Funct. Ecol.*, 10, 328–334.

Stiling, P. (1994). Coastal insect herbivore populations are strongly influenced by environmental variation. *Ecol. Entomol.*, 19, 39–44.

Turkington, R. & Harper, J.L. (1979). The growth, distribution and neighbor relationships of *Trifolium repens* in a permanent pasture. IV. Fine-scale biotic differentiation. *J. Ecol.*, 67, 245–254.

Ungar, I.A. (1987). Population ecology of halophyte seeds. *Bot. Rev.*, 53, 301–334.

Valiela, I., Teal, J.M. & Deuser, W.G. (1978). The nature of growth forms in the salt marsh grass *Spartina alterniflora*. *Am. Nat.*, 112, 461–470.

Wendel, J.F. & Parks, C.R. (1982). Genetic control of isozyme variation in *Camellia japonica* L. (Theaceae). *J. Hered.*, 73, 197–204.

Whitham, T.G., Young, W.P., Martinsen, G.D., Gehring, C.A., Schweitzer, J.A., Shuster, S.M. *et al.* (2003). Community and ecosystem genetics: a consequence of the extended phenotype. *Ecology*, 84, 559–573.

Editor, Thomas R. Meagher
Manuscript received 7 June 2004
First decision made 14 July 2004
Manuscript accepted 4 August 2004