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EVOLUTION OF A NEW ECOTYPE OF *SPARTINA ALTERNIFLORA* (POACEAE) IN SAN FRANCISCO BAY, CALIFORNIA, USA[1]

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We report the discovery and spread of a dwarf ecotype of *Spartina alterniflora* in San Francisco Bay. Relative to typical *S. alterniflora*, this dwarf ecotype has one-fifth the tiller height (~21 cm), tenfold the tiller density (~4000 tillers/m^2), and is restricted to growth in the upper intertidal zone. Chromosome counts of the dwarfs are identical to typical smooth cordgrass ($2n = 62$), and smooth cordgrass-specific random amplified DNA markers confirm the species identity of the dwarf. Field-collected clonal fragments of the dwarf grown for 2 yr under high-nutrient conditions maintained the dwarf syndrome, as did plants grown from the seed of a dwarf. The dwarf condition is not caused by endophytic fungi. The first dwarf smooth cordgrass patch was discovered in 1991, and by 1996 five separate dwarf patches had appeared within 200 m of the original. Since 1991, total area covered by the dwarf ecotype has increased sixfold to 140 m^2. The ecological range of the dwarf smooth cordgrass ecotype is similar to that of *S. patens*, a competitor on the Atlantic coast. We suggest that the absence of *S. patens* from most of San Francisco Bay has allowed the dwarf ecotype of smooth cordgrass to survive and spread.

Key words: competition; dwarf; founding population; invasion; Poaceae; smooth cordgrasss; *Spartina*; random amplified polymorphic DNA.

An ecotype is a subpopulation characterized by genetically based differences in morphology, physiology or life history in comparison with another conspecific subpopulation. These genetic differences are generally attributed to natural selection in differing local environments (Clausen and Hiesey, 1958; Hiesey and Milner, 1965). Ecotypic differentiation is common within plant species and has been reported between subpopulations separated by as little as a few metres (Blits and Gallagher, 1991; Masuda and Washitani, 1992). Recent theory indicates that changes in fitness functions, often associated with population introductions into a new environments, can lead to peak shifts in a theoretical fitness landscape, resulting in substantial morphological evolution (Whitlock, 1997). Earlier theories also predicted rapid genetic differentiation of small, founding populations in association with genetic drift (Wright, 1931; Carson and Templeton, 1984). Thus, several theories have suggested that new ecotypes can quickly evolve in small, introduced populations.

Here we report the discovery and spread of a distinctly dwarf ecotype of introduced smooth cordgrass, *Spartina alterniflora* Loisel., in San Francisco Bay, California, USA. This dwarf ecotype has evolved since the introduction of *S. alterniflora* to San Francisco Bay from Maryland in the 1970s (Daehler and Strong, 1994), and as far as we know, a similar dwarf ecotype has not been reported from within the native range of *S. alterniflora*. The new ecotype differs ecologically from typical or ''wild-type'' *S. alterniflora* and also differs significantly from the previously reported ''short-form'' *S. alterniflora* (Gallagher et al., 1988).

MATERIALS AND METHODS

Field survey—A single homogeneous patch of the dwarf ecotype was first noticed covering 25 m^2 near Point San Bruno in San Francisco Bay during July 1991. In order to determine the total distribution of the dwarf ecotype, we thoroughly searched the area surrounding Point San Bruno in July of each year from 1994 through 1996. In addition, in 1995 and 1996 we surveyed other sites in San Francisco Bay that had been invaded by *S. alterniflora* (Hayward Marsh, Coyote Hills Slough). A map identifying these locations has been published in Callaway and Josselyn (1992). In August 1996, measurements of stem height and stem density were taken in the field on five patches of the dwarf ecotype and five adjacent patches (within 1 m) of wild-type *S. alterniflora.*

Greenhouse growth studies—Four replicate clonal fragments were dug from each of five separate patches of the dwarf ecotype and five wild-type *S. alterniflora* patches at San Bruno in April 1994. The fragments were propagated in a greenhouse at Bodega Bay, California for 2 yr in 2.8-L pots, in a mixture of 50% Bodega Bay intertidal mud and 50% vermiculite (by volume). During the summer, plants were fertilized once per week with 50 mL of a solution of 5% Plantex 20-20-20 (dissolved in water). High-nutrient common growing conditions were used to determine whether the short stature of the dwarf plants in the field was due to nutrient limitation. Under these high-nutrient greenhouse conditions *S. alterniflora* grows vigorously (Daehler, personal observations). Stem heights and stem diameters of the dwarf ecotype and wild-type *S. alterniflora* were compared after 2 yr in the common environment.

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TABLE 1. Species-specific RAPD markers used to confirm species identity of the dwarf ecotype.

Marker[a]	Specific to	Present in dwarf ecotype[b]
OP-$B10_{280}$	*S. alterniflora*	Yes
OP-$B7_{650}$	*S. alterniflora*	Yes
OP-$C10_{470}$	*S. alterniflora*	Yes
OP-$C12_{1050}$	*S. alterniflora*	Yes
OP-$D11_{575}$	*S. alterniflora*	Yes
OP-$A2_{575}$	*S. foliosa*	No
OP-$A17_{725}$	*S. foliosa*	No
OP-$B7_{800}$	*S. foliosa*	No
OP-$D5_{575}$	*S. foliosa*	No

[a] Letters indicate Operon primer kit and subscript numbers indicate size of the marker fragment in base pairs. Primer sequence information can be obtained from the Operon Technologies (web site, http://www.operon.com).

[b] DNA from five separate patches of dwarf ecotype were tested. Known *S. alterniflora* and *S. foliosa* specimens were run as controls.

Field experiments—To compare the growth range potential of the dwarf ecotype and wild-type *S. alterniflora,* field transplants were made along an intertidal gradient ranging from +0.6 to +1.7 m above mean low water (MLW). In June 1996, seven common gardens were established along the tidal gradient, with each garden consisting of five replicates of the dwarf ecotype containing at least ten live stems each (dug directly from the field) and two replicates of wild-type *S. alterniflora.* Survival of the transplants along the tidal gradient was recorded in December 1996. Plants classified as dead in December 1996 were confirmed to be dead in spring 1997. To examine competitive interactions between the dwarf ecotype and wild type, we recorded lateral spread at points where the two ecotypes came into direct contact. In June 1996, wires were buried in the mud, tracing the border between patches of the two ecotypes (seven contact zones in total, each averaging ~1 m in length). In September 1996, new growth of each ecotype at these borders was assessed by counting the number of new tillers of each ecotype that had spread into the patch of the opposing ecotype.

Heritability of dwarf growth characters—Three open-pollinated seeds of the dwarf ecotype and ten seeds from neighboring wild-type *S. alterniflora* were stored over winter in 50% seawater at 5°C (Daehler and Strong, 1994) and germinated in spring 1992. These seedlings were grown in a greenhouse at Bodega Bay for three summers, after which time tillers were measured.

Chromosome counts—Root tips of rapidly growing plants from two patches of the dwarf ecotype were collected in the late morning and pretreated in a saturated aqueous solution of alpha bromo napthalene for 22 h at 4°C. The root tips were then fixed in 3:1 ethanol : glacial acetic acid, hydrolyzed for 10 min in 5 mol/L hydrochloric acid, and placed into 70% ethanol. Meristems were dissected out in 2% aceto-orcein, squashed, and examined with a Zeiss Universal microscope at 1000X.

Confirmation of species using DNA markers—We used species-specific DNA markers to confirm that the patches of the dwarf ecotype were *S. alterniflora.* We also wanted to confirm that the patches of dwarf ecotype were not hybrids between *S. alterniflora* and the native *S. foliosa* (Daehler and Strong, 1997). Five patches of the dwarf ecotype were screened for the presence of *S. alternflora*-specific and *S. foloisa*-specific DNA markers. Additional species-specific markers that have been identified since publication of Daehler and Strong (1997) were used in this study (see Table 1).

DNA extraction—Genomic DNA was extracted from 100 mg of fresh leaf tissue by grinding in liquid nitrogen and incubating the homogenate at 50°C in 1000 μL of Guidet's (1994) extraction buffer. After 1–2 h, the proteinase K was denatured by heating, and samples were centrifuged at 8000 × *g* for 10 min; 500 μL of supernatant was withdrawn and combined with 50 μL of 2 mol/L sodium acetate and 500 μL isopropanol. The solution was placed at −20°C for at least 1 h and then centrifuged as above. The pellet was recovered and suspended in 100 μL Tris-EDTA buffer; 5 μg of RNAase were added and the sample was incubated at 37°C for 20 min. The DNA was precipitated by adding 10 μL of 2 mol/L sodium acetate and 250 μL 100% ethanol and chilling at −20°C for at least 1 h. The pellet was recovered and resuspended as above. Centrifugation was repeated as above; 90 μL of supernatant were withdrawn and mixed with 200 μL Tris-EDTA buffer.

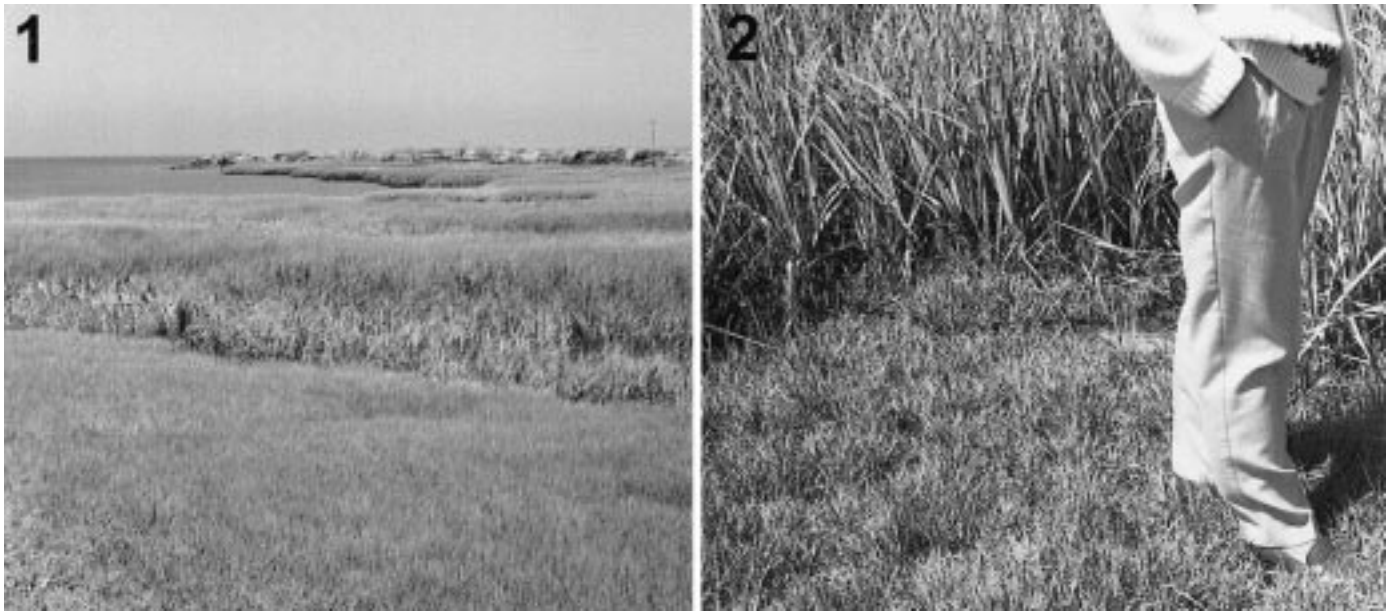
Amplification—Polymerase chain reaction was performed with a Perkins Elmer 9600 thermocycler (Norwalk, Connecticut) using the following protocol: 1 cycle, 94°C (1.5 min), 42°C (30 sec), 72°C (65 sec); 44 cycles, 94°C (15 sec), 42°C (30 sec), 72°C (65 sec); 1 cycle 72°C (4 min). Reaction volumes of 15 μL contained 10% by volume $MgCl_2$-free 10× reaction buffer A (Promega, Madison, Wisconsin), 0.6 units Taq polymerase (Promega, Madison, Wisconsin), 360 picounits primer (Operon Technologies, Alameda, California), 3 mmol/L $MgCl_2$, 200 μmol/L each dATP, dCTP, dGTP,and dTTP (Promega, Madison, Wisconsin), and 30 ng genomic DNA. All reactions were repeated at least twice to confirm consistency. Following electrophoresis on 1.5% agarose gels, DNA was stained with ethidium bromide and visualized under UV light.

Assaying for endophyte infection—To test the hypothesis that endophytic fungi were responsible for the dwarfism (Fisher and Holton, 1957), we assayed the leaves of the dwarf and wild-type *S. alterniflora* using the culture techniques of Carroll and Carroll (1978). Each sample comprised two pieces from the same leaf, each 3 cm in length, cultured on sterile water agar in separate 150-mm petri plates. Of the dwarf, we tested four patches, two replicate leaves from each. Of the wild-type *S. alterniflora,* we cultured leaves from three clones growing near the dwarfs.

RESULTS AND DISCUSSION

The dwarf ecotype is S. alterniflora—Species-specific RAPD markers confirmed that all five of the dwarf ecotype patches assayed were pure *S. alterniflora,* not native *S. foliosa* or hybrids. Hybrids contain *S. foliosa* markers (Daehler and Strong, 1997), while the dwarf ecotype does not. The vegetative and reproductive morphologies of the dwarf ecotype do not match any other described *Spartina* species described by Mobberly (1956). Instead, the dwarf morphology is consistent with a miniaturized form of *S. alterniflora.*

Morphology of the dwarf ecotype—The most striking characteristic of the dwarf ecotype is its small size and extremely high tiller density, relative to the wild-type *S. alterniflora* (Figs. 1–2). These differences were maintained after 2 yr of growth in common greenhouse environment under high-nutrient conditions (Table 2). In addition, progeny grown from open-pollinated seed also maintained the dwarf morphology, demonstrating heritability of the dwarf condition (Table 2). These seed progeny were likely selfed because the cross-pollination rates in San Francisco Bay were low (Daehler, 1998). A ''short-form'' of *S. alterniflora* has been previously reported from the species' native range, and there has been considerable controversy over whether this ''short-form'' is environmentally induced or has a genetic basis (Val-



Figs. 1–2. Dawrf ecotype of *Spartina alterniflora* invading San Francisco Bay, California. **1.** Original founding dwarf patch (foreground) spreading into typical *S. alterniflora* behind. **2.** Closer view contrasting the ankle-high dwarf ecotype (foreground) and typical *S. alterniflora* behind.

iela, Teal, and Deuser, 1978; Gallagher et al., 1988). Some controversy remains because heritability studies based on seed progeny have never been published. Nevertheless, the dwarf ecotype we describe here differs substantially from the ''short form'' *S. alterniflora* found commonly on the Atlantic and Gulf coasts. Stem density of the dwarf ecotype is over five times higher than in the ''short form'' *S. alterniflora* (4000 vs. 700 tillers/m^2), and mean stem diameter is less than half that of the ''short form'' (1.7 vs. 3.7 mm) (Gallagher et al., 1988). Endophytic fungi can sometimes cause dwarfism in grasses (Fisher and Holton, 1957), but they were not detected in any of our plants. Chromosome counts of the dwarf and wild type were identical (Table 1). In many cereal crops, dwarf genetic mutants have been identified that may be similar to the dwarf ecotype of *S. alterniflora.* In most of these cases, dwarfism is due to mutations that either reduced synthesis of gibberellins, or mutations involving the gibberellin response pathway (Devi et al., 1994; Evans, Blundell, and King, 1995; Ogawa et al., 1996).

TABLE 2. Comparison of the dwarf ecotype and neighboring wild-type *Spartina alterniflora*.[a]

Trait	Dwarf	Wild type
Chromosomes (two dwarfs assayed)	$2n = 62$	$2n = 62$
Mean tiller density (no./m^2)[b]	3986 ± 300	380 ± 120
Tiller diameter at base (mm)[c]	1.7 ± 0.1	10.2 ± 0.5
Tiller height (cm)[c]	21 ± 6	106 ± 7
Inflorescence length (cm)[b]	5.5 ± 1	22.5 ± 3
Lower limit in intertidal (cm)[b,d]	+60	+35
Upper limit in intertidal (cm)[b,d]	>+168	>+168
Spread under competition from opposing ecotype (no. new tillers/m)[b]	14 ± 5	3 ± 3
Progeny height[e]	18.3 ± 6	101 ± 10

[a] = five plants of each type, ± 1 SE, and measurements taken in September unless otherwise noted.
[b] Measurements taken from field.
[c] Following growth for 2 yr in a common high nutrient greenhouse environment.
[d] Height above mean low water (MLW).
[e] Average of three seed-grown plants, measurements taken in August of 3rd yr.

Ecological properties of the dwarf ecotype—Field transplants revealed that the wild-type *S. alterniflora* survives and grows farther down an intertidal gradient than the dwarf ecotype (Table 2). The dwarf ecotype died in the two lowest gardens, while all wild-type transplants survived, even in the lowest garden. The lower intertidal limit for wild-type *S. alterniflora* cited in Table 2 is taken from a previous transplant study conducted at the same site (Daehler and Strong, 1996). Because stems of the dwarf are inundated much sooner on the rising tide than wild-type *S. alterniflora,* the longer submergence times of the dwarf could account for its restriction to the high intertidal.

When grown in the absence of competition, both ecotypes survived equally well in the upper intertidal (Table 2). However, where the dwarf ecotype and wild type came into contact in the upper intertidal, the dwarf was more successful at spreading (Table 2). The extremely high tiller density of the dwarf ecotype appears to prevent the wild-type *S. alterniflora* from invading. Patches of the dwarf ecotype have remained homogeneous and have continued to spread, despite coming into contact with neighboring wild-type plants. The much lower stem density in the wild-type *S. alterniflora* allows more light to reach the mud surface and apparently allows shoots of the dwarf to spread into established wild-type patches, especially in the spring when both ecotypes are more similar in height.

Distribution of the dwarf ecotype—Since discovery of the original dwarf patch in 1991, we have observed the development of four new patches, all within 200 m of

the original. The new patches probably established from seeds because we did not observe erosion surrounding the original patch that would have led to vegetative fragmentation. All dwarf patches show very similar RAPD profiles, suggesting, but not proving that the original patch was the source of the new patches. The total area covered by the dwarf ecotype has grown from 25 m^2 in 1991 to 140 m^2 in 1996. Given the abundant open mud habitat and the dwarf ecotype's high growth rate and high competitive ability, the dwarf ecotype will probably continue to spread in San Francisco Bay. On the Atlantic and Gulf coasts of North America, *S. patens*, a much shorter species is competitively dominant over *S. alterniflora* in the upper intertidal (Bertness, 1991). We speculate that the absence of *S. patens* in San Francisco Bay has contributed to the success of the dwarf *S. alterniflora* ecotype.

Conclusions—A similar dwarf form was reported for the hybrid species *Spartina anglica* during its invasion of mudflats in Britain (Chater and Jones, 1951) and New Zealand (Bascand, 1970). In contrast to the vigorous seedling growth we observed in the dwarf *S. alterniflora* ecotype, seeds of the *S. anglica* dwarf plants could not be germinated (Chater and Jones, 1951), limiting its potential for spread. Chater and Jones (1951) suggested that *S. anglica* dwarfs resulted from disintegration of a formerly stable hybrid polyploid. Since *S. alterniflora* is not a hybrid and the dwarf ecotypes is of the normal ploidy level, a new explanation for the dwarf ecotypes of both species is suggested based on consistencies between these cases. In both cases, invasion of new geographic regions led to the appearance and spread of a new ecotype. Natural variants that would be selected against in the native habitat may survive and flourish under novel selective regimes associated with invasions, particularly when an invader spreads over a heterogeneous habitat while experiencing little interspecific competition from native species.

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