Title: **Repeated genetic and adaptive phenotypic divergence across tidal elevation in a foundation plant species**

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Abstract:

Microgeographic genetic divergence can create fine-scale trait variation. When such divergence occurs within foundation species, then it might impact community structure and ecosystem function, and cause other cascading ecological effects. We tested for parallel microgeographic trait and genetic divergence in *Spartina alterniflora*, a foundation species that dominates salt marshes of the US Atlantic and Gulf coasts. *Spartina* is characterized by tall-form (1-2 m) plants at lower tidal elevations and short-form (<0.5 m) plants at higher tidal elevations, yet whether this trait variation reflects plastic and/or genetically differentiated responses to these environmental conditions remains unclear. In the greenhouse, seedlings raised from tall-form plants grew taller than those from short-form plants, indicating a heritable difference in height. When we reciprocally transplanted seedlings back into the field for a growing season, composite fitness (survivorship and seed production) and key plant traits (plant height and biomass allocation) differed interactively across origin and transplant zones in a manner indicative of local adaptation. Further, a survey of single nucleotide polymorphisms revealed repeated, independent genetic differentiation between tall- and short-form *Spartina* at 5 of 6 tested marshes across the native range. The observed parallel, microgeographic genetic differentiation in *Spartina* likely underpins marsh health and functioning, and provides an underappreciated mechanism that might increase capacity of marshes to adapt to rising sea levels.

Introduction

Local adaptation is widespread in nature (Leimu and Fischer 2008, Hereford 2009, Savolainen et al. 2013) and can underpin patterns in the distribution and abundance of organisms (Schoener 2011), generate high genetic variation among populations (Bolnick et al. 2011), and affect the responses of populations to both disturbance and climate change (Valladares et al. 2014). Adaptive phenotypic divergence can occur across spatial scales finer than a species' dispersal range (i.e., microgeographic adaptation; Kawecki and Ebert 2004, Roda et al. 2013, Richardson et al. 2014), a pattern that has been regularly documented within plant species. For instance, plants can adapt to variability in edaphic conditions (e.g., serpentine soils, Kruckeberg 1967, Brady et al. 2005; mine tailings, McNeilly 1968, Antonovics and Bradshaw 1970, Antonovics 2006) and physical factors (e.g., emersion time, Hays 2007; hydrodynamics, Roberson and Coyer 2004; soil moisture, Scotti et al. 2016) over spatial scales that are smaller than the scale of dispersal. Such microgeographic adaptation can evolve repeatedly across independent populations, each of which responds to similar local gradients in the environment, resulting in the evolution of similar traits (i.e., parallel adaptive phenotypic divergence; Rundle et al. 2000, Nosil et al. 2002, Johannesson et al. 2010).

Although many plant species that exhibit microgeographic adaptation are widely distributed, few studies have tested whether this divergence occurs repeatedly across sites (but see Roda et al. 2013, Bertel et al. 2018, Brousseau et al. 2020). Parallel adaptive phenotypic divergence can arise through two main processes in the presence of gene flow: a) initial adaptive divergence occurs once and is followed by colonization of differently adapted forms into distinct habitats at multiple sites, or b) divergence between habitats occurs repeatedly at multiple sites following colonization (Schluter and McPhail 1992, Johannesson et al. 2010, Butlin et al. 2013).

This repeated evolution could follow many non-parallel genetic pathways and still result in similar phenotypic differentiation (Johannesson et al. 2010, Bolnick et al. 2018, Van Belleghem et al. 2018). Thus, similar phenotypes occurring in similar environments across independent populations strongly suggest natural selection, as non-adaptive processes are unlikely to yield the same evolutionary shifts that are correlated with the environment (Conte et al. 2012, Bertel et al 2018, Bolnick et al 2018). However, a recent synthesis of bacteria and fungi experiments illustrated that non-adaptive processes (e.g., mutation) can also contribute to parallel evolution (Bailey et al. 2017). Given that genetic divergence prior to colonization also can give arise to parallelism (Butlin et al. 2013), observations of similar phenotype-environmental associations are not sufficient to infer parallel evolution. Instead, parallel adaptive divergence requires evidence of local adaptation in multiple locations (Bertel et al. 2014) that evolved after demographic separation (Langerhans and DeWitt 2004, Butlin et al. 2013, Savolainen et al. 2013).

One promising scenario for generating parallel adaptive phenotypic divergence arises when species persist across strong environmental gradients that occur repeatedly throughout their distribution (Bailey et al. 2015), such as wetland plant species that span an inundation gradient. Prior studies have documented small-scale genetic or trait divergence in such species (e.g., *Puccinellia*, Gray 1985; *Phragmites*, Gao et al. 2012; *Schoenoplectus*, Sweetman et al. 2013), including examples of genetic differentiation across the land-to-water gradient (e.g., *Salicornia,* Davy et al. 1990; *Phragmites*, Clevering 1999a,b). In addition, there is evidence for both genetic variation among individuals and phenotypic plasticity in the response of wetland species to environmental factors including flooding (Lessmann et al. 1997, Clevering and Hundscheid 1998, Howard and Rafferty 2006), salinity (Hester et al. 1998, Hester et al. 2001, Howard 2010,

Grewell et al. 2016) and nutrient availability (Clevering 1999a,b). For example, *Spartina patens* exhibited adaptive genetic and phenotypic differences across distinct sand dune, swale grassland and marsh microhabitats (Silander 1979, Silander and Antonovics 1979, Silander 1984, Silander 1985). In combination, these studies suggest strong potential for microgeographic adaptation in wetland plants to environmental differences across an inundation gradient, yet there have been few comprehensive tests of this hypothesis (but see Knight and Miller 2004, Lenssen et al. 2004), nor any tests of parallel divergence to inundation across a species' range.

The plant species *Spartina alterniflora* (hereafter, *Spartina*; Poaceae) is arguably the most important foundation species (*sensu* Ellison 2019) in estuaries of the Atlantic and Gulf coasts of the United States. Monocultures of *Spartina* create millions of acres of salt marshes, critical estuarine ecosystems that provide shoreline protection, water purification, carbon sequestration and habitat for economically valuable and ecologically important species (Barbier et al. 2011). Within most marshes, *Spartina* exhibits pronounced variation in stem height over tens of meters across a natural stress gradient in tidal inundation (Pennings and Bertness 2001), which is within the distance that *Spartina* pollen and seeds can disperse (Taylor et al. 2004, Travis et al. 2004, Blum et al. 2007). Tall-form *Spartina* (~1-2m stem height) is found in low elevations along creek banks that experience daily inundation, and short-form *Spartina* (<0.5m) is found at higher elevations that are less consistently flooded (fig. 1a,b; Shea et al. 1975, Valiela et al. 1978). Early experiments provided evidence both for phenotypic plasticity (Shea et al. 1975, Mendelssohn and McKee 1988) and genetic differentiation of plant height (Stalter and Baston 1969, Gallagher et al. 1988) in *Spartina*. For instance, long-term fertilization of field plots shifted plant morphology from short to tall (Valiela et al. 1978, Morris et al. 2002), which was interpreted as supporting environmental control, though nutrient availability could have also

selected for these trait differences. Alternatively, both reciprocal transplant (Stalter and Baston 1969) and common garden (Gallagher et al. 1988) experiments conducted with adult transplants detected consistent morphological differences across the two growth forms over a single growing season, indicating a genetic basis. Yet, these studies could not eliminate the potential confounding effects of irreversible development plasticity often exhibited in adult plants (Callaway et al. 2003). In addition to experiments, population genetic surveys can generate estimates of historical demography and thus provide context to understand the evolution of phenotypic divergence. Previous work using allozyme and AFLP technologies did not detect genetic differentiation between tall- and short-form *Spartina* (Shea et al. 1975, Valiela et al. 1978, Richards et al. 2004, Foust et al. 2016), suggesting that weak to no barriers to gene flow exist among these forms. To date, no analyses have been done with highly variable single nucleotide polymorphisms (SNPs), which are better suited to detect low levels of genetic differentiation.

We combined field and greenhouse experiments of seed-reared individuals with a field survey of genetic differentiation using SNPs to examine genetic and trait differentiation across both biogeographic regions and marsh elevations. By doing so, we evaluated whether *Spartina* exhibits microgeographic adaptation to tidal elevation, as well as examined whether this divergence occurred in parallel in *Spartina* populations across its native North American range. We focused on fitness metrics including survival and reproduction (e.g., flower production and seed set) as well as growth responses (e.g., density, height and biomass). Biomass production and allocation are important components of long-term fitness in perennial clonal plants (Younginger et al. 2017). In addition, stem height can influence pollen dispersal in wind-pollinated grasses such as *Spartina* (Van Boheemen et al. 2019), which may impact fitness.

Given that *Spartina* morphology can influence both abiotic (e.g., sediment accumulation, soil erodibility, light availability; Morris et al. 2002, Seliskar et al. 2002, Bernik et al. 2018) and biotic (e.g., associated species composition; Seliskar et al. 2002) marsh characteristics, determining the degree to which these two *Spartina* phenotypes are genetically determined and reflect evolutionarily-distinct entities has broad implications for predicting how salt marshes will respond to sea level rise (Kirwan et al. 2010), as well as for informing marsh restoration and management efforts (McKay et al. 2005).

Methods

Common garden experiment

We germinated seeds collected in the field from tall- and short-form *Spartina* at three sites in South Carolina separated by at least 3km (Fig. A1; Appendix 1) and grew them in a greenhouse common garden for 11 months to assess trait differences. Although using first generation seedlings cannot completely eliminate maternal effects (Roach and Wulff 1987, Bischoff and Muller-Scharer 2010), because few F1 plants flowered in the subsequent two years of our study, breeding additional generations in the greenhouse was not feasible. Inflorescences containing seeds were collected from the field in October 2014 from 20 reproductive stems per marsh, 10 each from characteristically tall- and short-form *Spartina* plants (N=60 total). We also characterized the surrounding natural marsh by measuring *Spartina* stem density and height, and sediment redox (mV), in the tall and short zones at these three marshes in May 2015 (see Appendix 3 for Methods and Results). Seeds were stored in the dark at 4^{o}C to simulate the natural dormant period (Biber and Caldwell 2008), and then transferred to the Northeastern University Marine Science Center (MSC) greenhouse in January 2015 to stimulate germination

(Appendix 1). In April 2015, we planted 25 individual seedlings from each site (N=3) by zone (N=2) combination in 6" diameter pots (volume = 2.9 L) in a 50:50 sand and potting soil mix. Pots were randomly assigned to one of two water tables in the MSC greenhouse. All pots were irrigated daily with freshwater and submerged in seawater for 8 hours weekly; we note that these conditions more closely mimic the environmental conditions in the short-form *Spartina* zone. We measured survival and density, as well as height and leaf number of the tallest stem in each pot monthly during the growing season (April-October 2015; density measurements began in June and leaf number was not measured in June) and then bi-monthly through the following winter and spring (November 2015-March 2016). At the end of the experiment, we also measured the height of four additional randomly selected live stems to estimate average stem height. We were unable to measure above and belowground biomass, a destructive response variable, at the end of the common garden experiment as many of these individuals were then used in the reciprocal transplant experiment (see below).

As noted above, the use of first-generation seedlings reduces, but does not eliminate, maternal effects. To test for this potential confounding effect, we measured seed size, a commonly-cited trait that can influence germination and early seedling survival and growth (Roach and Wulff 1987, Kalisz 1989). Using field collected seeds that had not germinated by May 2015, we dried tins with 5 seeds for each site x zone combination (N=7-8 per combination) in a drying oven at 60°C for at least 48 hours. Total dry weight was divided by the number of seeds measured to obtain an average dry weight (g) per individual seed. While ungerminated seeds may be a biased subset of the total seed pool, as size could have contributed to why these seeds did not germinate, we have no reason to expect this would vary consistently between tall- and short- form *Spartina*. In addition, we note that we cannot rule out the potential role of

maternal effects that are unrelated to seed weight (e.g., nutrient provisioning, seed architecture, epigenetic changes including DNA methylation; Rossiter 1996, Richards et al. 2017).

Reciprocal transplant experiment

We ran an 8-month factorial reciprocal transplant experiment at the collection sites using greenhouse-reared individuals. We measured a suite of morphological and fitness metrics both during and at the end of the experiment (Appendix 2). In March 2016, we selected 10 genets (i.e., unique genotypes as confirmed by DNA microsatellites developed for *Spartina*, Blum et al. 2004, Sloop et al. 2005) from each site by zone combination in the common garden experiment for our reciprocal transplant experiment (N=60 genets; fig. A1). Six clonal ramets from each genet (i.e., 360 transplants total) were isolated into separate 6" diameter plastic pots in a 50:50 sand:soil mix, with the pot bottom removed and lined with window screen to allow for water transfer and soil interactions in the field. This set-up eliminated the ability of our transplants to directly interact with the surrounding plant neighborhood in the field except for any shading effects, thus isolating the effects of the environment in the absence of neighbor effects. Transplants were irrigated daily with freshwater, fertilized bi-weekly using Miracle Gro water soluble all-purpose plant food (100 mL per pot; NPK at 24-8-16 concentration and trace elements), and submerged in seawater twice weekly for 8 hours for one month prior to transplanting in the field.

In May 2016, we transported all transplants overnight by truck to Charleston, SC, and planted them into the field over the course of two days. We established six transplant locations adjacent to each of the seed collection areas. We planted each genotype (N=10 per origin site x

zone) in each transplant location (N=60 individuals total per transplant location). This design allowed us to control for potential effects of genetic identity on the response variables of interest (Zerebecki et al. 2017). Within each transplant location, we set up two parallel 30-m transects separated by 2-5 m. We randomly assigned and planted 30 transplants flush with the sediment surface at 1 m intervals along each transect (N=5 per origin site x zone per transect; See Fig. A1). We left intact natural stands of *Spartina* surrounding our potted transplants within each transect; thus, in the tall-zone, transplanted seedlings were planted under the canopy of naturally occurring tall-form *Spartina* (See Appendix 3 for additional details of the plant characteristics our field sites).

We measured transplant survival, live stem density, and height of the tallest stem (i.e., maximum stem height) at approximately monthly intervals from June through December 2016. In addition, we quantified 3 metrics of *Spartina* reproductive output throughout the flowering period (September – December 2016, sampled approximately every 2 weeks): proportion of transplants that flowered; total number of flowering stems per pot; average number of seeds per flowering stem. At the end of the experiment, we measured the height of all live stems, harvested the plants, separated the biomass into above- (stems and leaves) versus below-ground (roots and rhizome) portions, and then dried samples at 60ºC for at least 48 hours to calculate total dry biomass as well as examine variation in above- and below-ground allocation.

Population genetics field survey

Logistical constraints limited our common garden and reciprocal transplant experiments to evaluate plants from multiple sites at a single geographic location. We complemented these results with a broader genetic field survey to more completely examine adaptation to zonal

location in the marsh in *Spartina* and to evaluate the generality of our experimental findings. To do so, we generated a SNP survey to assess genetic differentiation at two scales: across biogeographic regions and between tall- and short-forms within marshes (Table A6), including two of the three marshes at which we conducted the experiment. At six marshes across MA (MAS, MAW), RI, SC (SCBI, SCFB), and FL, we collected plants from the tall zone within approximately 2 m of creeks or open water, and from the short zone at least 20 m away. We also collected tissue within the short zone only at one marsh (SCFJ), the tall zone only at two marshes (SC2, SC3) and from unidentified zones at two marshes (NC1, NH1, Table A6). At all locations, ~10 cm^2 of green leaf tissue was collected from 20 plants at least 1 m apart along a linear transect and placed into silica for drying. Given high genotypic diversity within marshes (Richards et al. 2004, Hughes and Lotterhos 2014, Foust et al. 2016), the probability that plants 1 m apart are clonal replicates is low.

To generate genotypes, we entered all sample extracts into a single genomic library by following protocols in Parchman et al. (2012) and used Illumina HiSeq technology to generate 249.2 x 10^6 reads approximately 80-100 basepairs in length. We then created a *de novo* assembly using a subset of these reads with SEQMAN NGNEN, aligned reads from individuals to this assembly using *bwa* ver. 0.7.12 (Burrows-Wheeler Aligner; Li and Durbin 2009) and generated genotype likelihoods with samtools/bcftools 1.3.1 (- mpileup and –call protocols) with the multiallelic calling option (Li 2011). After filtering, we generated genotype likelihoods for 2,735 SNPs across 309 individuals (n=13-21 per zone per marsh; Table A6), with 5.20 ± 0.07 reads (mean ± standard error) per SNP per sample. More details on DNA extraction, library preparation and *in silico* processing and filtering are found in Appendix 4.

Data analysis

Common garden and reciprocal transplant: We included three sites in our study to evaluate the consistency of morphological differences across tidal elevations, and the potential for parallel adaptive phenotypic divergence to zone. Because we were not interested in specific characteristics of each site, we treated origin site and transplant site (when applicable) as independent fixed block effects in our experimental analyses. We did not design our experiment to robustly test all possible interactions between origin site and transplant site.

Morphological responses in the common garden experiment (Appendix 1) were analyzed with a linear model using origin zone and origin site as fixed factors. Further, we also assessed if seed weight, a proxy for maternal investment, was correlated with differences in maximum seedling height (see Results) by conducting a linear regression on final average maximum height and average seed weight from each site x zone combination.

In the reciprocal transplant experiment, we assessed survival over time using a Cox proportional hazard regression with origin zone, transplant zone, and their interaction as fixed effects. Transplants that did not survive were then removed from further analyses. We conducted linear models with fixed effects of origin zone, transplant zone, and their interaction, as well as origin site and transplant site, on all other response variables (e.g., height, density, etc.) at the end of the experiment. Flowering occurrence was analyzed using a generalized linear model with a binomial response. Local adaptation was indicated by a significant two-way interaction of origin and transplant zone with the local vs foreign criteria (i.e., higher fitness of local individuals than foreign) satisfied in post-hoc comparisons (Kawecki and Ebert 2004). Statistical methods and results of morphological metrics measured repeatedly throughout the reciprocal transplant experiment are detailed in Appendix 2.

To assess local adaptation in composite fitness (i.e., survivorship and total seed production), we used the R *aster* package (Geyer et al 2019), following Geyer et al 2007, Shaw et al. 2008, and as implemented in Samis et al. 2016. We modelled survival with a Bernoulli distribution, presence or absence of seed set with a Poisson distribution, and number of seeds produced as a truncated Poisson distribution. We assessed fixed effects of transplant and origin site, origin zone (tall / short), transplant zone (tall / short) and the origin zone by transplant zone interaction using likelihood ratio tests with the *aster* function. For graphical purposes, we conducted univariate analyses of the effect of origin zone separately for tall and short transplant zones, and generated a mean estimate of composite fitness.

All experimental analyses were conducted in R statistical software (version 3.6.3; R Core Team 2018). We used the *lm* function in the *car* package (Fox and Weisberg 2019) for linear models. Tukey's post-hoc tests were performed using the *lsmeans* (Lenth 2016), *pbkrtest* (Halekoh and Højsgaard 2014), and *multcompView* (Graves et al. 2015) packages. Cox proportional hazards regressions were performed and visualized using the *survival* (Therneau 2015), and *survminer* (Kassambara and Kosinski 2018) packages.

Population genetic analysis: We examined genetic differentiation across and within biogeographic realms with several analytical methods. With noted exceptions, all analyses were based on genotype likelihoods to incorporate the uncertainty that arises from errors in sequencing and sampling (Nielsen et al. 2012). We estimated expected (He) and observed heterozygosity (HO) using angsd (Komeliussen et al. 2013). We compared estimated patterns of heterozygosity across latitude using one-way ANOVAs and between tall- and short-zones using a paired t-test.

For the principal components analysis (PCA), we converted the phred-scale genotype likelihoods (from *samtools/bcftools*) per SNP-sample combination into probabilities that summed to 1, and then converted these to a single value that ranges from 0 to 2, where 0, 1 and 2 represent the highest probability of a homozygote, heterozygote, and alternative homozygote, respectively. We assessed differences among samples at two hierarchical spatial scales: across all marshes and between tall-and-short zones within each of six marshes, using the *prcomp* function in R. We used analysis of similarity (ANOSIM) implemented in *vegan* (Okansen et al. 2019), a Bray distance matrix and 1000 permutations to assess differences between tall- and short-zone plants in marsh-specific analyses. We note that we repeated these PC analyses with a subset of loci in which loci within the upper 10% quantile of loadings on PC were removed.

We confirmed these PC analyses using hard-called genotypes as an input. *Spartina alterniflora* is hexaploid derived from an ancient allopolyloidization event (Ainoche et al. 2012). Previous work with *Spartina* microsatellites indicated inheritance patterns of alleles consistent with genome diploidization (Blum et al. 2009) and our genotype-likelihood similarly treated genotypes as diploid. Recently, Clevenger et al. (2015) recommended that RADseq studies of polyploids utilize the approach of Stacks (Catchen et al. 2013). As suggested by Paris et al. (2017) for polyploids, we used a lower M (e.g., M=1; number of mismatches allowed between two alleles of a heterozygote sample) and m=3 (number of identical reads required to initiate a putative allele). After filtering (i.e., r80 = those loci found in 80% of samples or more), we generated 391 variant sites that we analyzed with PCA.

We implemented maximum-likelihood admixture methods to infer ancestry of individuals (*NGSadmix*; Meisner and Albrechtsen 2018) of eight datasets: all marshes, only marshes with both tall- and short-form *Spartina* samples (N=6), and tall- versus short-form for

each of the 6 marshes independently. For the 1st two datasets, genotype likelihoods (from *samtools/bcftools*) were run across an *a priori* range of genetic clusters (k=2-20) and replicated five times. An analysis of delta K (as suggested by Evanno et al. 2005) indicates that best fit k to the dataset with all marshes and with six (6) marshes was k=5 (Fig. A8) and k=4 (Fig. A12), respectively. For the marsh-specific admixture analyses, genotype likelihoods were run across k=1-10. For 5 of 6 marshes, the best fit k was k=2; the best fit k was k=3 when comparing MAWS and MAWT.

To assess how genetic variation is partitioned among populations (i.e., marshes) and among subpopulations (i.e., zones) within marshes, we performed hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) using *pegas* for three subsets of the data: all 12 subpopulations at six marshes in FL, SC, RI, MA; 4 subpopulations at 2 SC marshes; and 4 subpopulations at 2 MA marshes (Appendix 4 for details). We also calculated pairwise F_{ST} among and within marshes using *ngsFST* (Fumagalli et al. 2013) and compared pairwise F_{ST} among populations categorized into three bins of geographic distance: short vs tall within a marsh (<0.2 km), and marshes separated by 0.5-5km and 5-15 km (Appendix 4 for more details).

We conducted a preliminary redundancy analysis (RDA; implemented in R package *vegan*; Oksanen et al. 2019) to identify if there were loci that best correlated with genomic divergence between ecotypes. For comparison purposes, we also isolated the 20 loci that had greatest loadings in the correspondence analysis for each marsh independently, and we compared the identity of these loci across marshes. To generate imputed genotypes for this redundancy analysis (RDA), we first compared the log likelihood (Lnl) of the 3 genotypes of each SNP by individual combination. We called a genotype when its Lnl was greater than 2 times the Lnl of

any other genotype. If no genotype reached that threshold, then no call was made (NA). For each marsh, we imputed missing genotypes by using the most common genotype.

Results

After 11 months in a greenhouse common garden, tall-origin seedlings had ~10% greater maximum height compared to short-origin seedlings, despite similar heights at the start of the experiment (RM ANOVA zone * date: $p = 0.005$, Fig. 1c; Table A1). Average stem height was also greater at the end of the common garden experiment (Appendix 1). Thus, the height difference between tall- and short-form *Spartina* likely has a heritable component.

We did not find a significant relationship between field-collected seed weight – a potential proxy for maternal investment – and plant height in the common garden experiment (Fig. A2a; $F_{1,4} = 0.43$, $p = 0.54$). Further, the weight of ungerminated seeds did not differ among origin zones (global mean = 1.79 mg; $F_{1,43} = 0.007$, $p = 0.93$, Fig. A3). Survival, stem density, and leaf number also did not consistently vary between tall- and short-origin seedlings (see Appendix 1 for details).

At the end of our 8-month reciprocal transplant experiment, there was a significant interaction between origin zone and transplant zone on fitness (composite metric of survivorship and seed production) in the aster analysis (Fig. 2, Table 1; Deviance = 6.60; $p = 0.010$). Short-origin seedlings had higher fitness in the short zone than tall-origin seedlings ($p = 0.005$), whereas tall-origin seedlings tended to have slightly higher fitness in the tall zone than did short-origin seedlings ($p=0.101$; Fig. 2). This marginal effect likely occurred because we found only a single seed-producing stem in the tall transplant zone (i.e., a tall-origin plant from Bowens Island

that flowered in the tall zone at Fort Johnson), but this individual transplant produced a large number of seeds (i.e., 190).

We also detected an interaction between origin zone and transplant zone on plant height ($p = 0.006$; Fig. 3a, Table 1) and the ratio of above-to-belowground biomass ($p = 0.02$, Fig. 3b). In the tall zone, tall-origin transplants were significantly taller (Fig. 3a) and allocated more biomass aboveground (Fig. 3b) than short-origin transplants. In contrast, there were no significant trait differences between tall- and short-origin *Spartina* when transplanted to the short zone.

There were also clear transplant-zone effects on survival, growth, and reproduction when examined independently. For instance, survival of *Spartina* transplants was dramatically higher in the short than tall zone (92% versus 29%, respectively; $p < 0.001$, fig. 3f). In addition, transplants produced more flowering stems in the short than tall zone (18% versus 2%; $p = 0.007$, Fig. 3e; Appendix 2). Similarly, both stem density and total biomass were significantly greater in the short than tall zone (density and total biomass: $p < 0.001$; Fig. 3c,d). Together, these results (Fig. 2, 3) highlight that *Spartina* seedlings had lower fitness in the tall-zone environment compared to the short-zone environment.

Population genomics

Our analysis of 2,735 SNPs from 11 marshes from the Atlantic and Gulf coasts of the United States identified strong biogeographic differentiation that reflects historical separation of *Spartina* across this range. For example, principal components analysis (PCA) detected three geographic groups (Fig. 4b): Gulf of Mexico (FL), southeastern US (SC and NC), and northeastern US (RI, MA, and NH). An admixture analysis indicated that five genetic clusters

(k=5) best explained variation among genotypes; these clusters broke across similar phylogeographic breaks (FL, SC, NC, RI, MA+NH; Fig. A8). These results mirror previous studies using chloroplast and microsatellite markers (Blum et al. 2007).

Expected heterozygosity (H_E) within southern marshes (FL, SC and NC) was greater than within northern marshes of RI, MA, NH when all individuals were considered (average H_E = 0.348 and 0.328, respectively; $F_{1,15} = 16.5$, $p < 0.002$). This pattern also held when short-zone samples were excluded ($F_{1,9} = 6.4$; p=0.032) and tall-zone samples were excluded ($F_{1,9} = 10.6$; p<0.010). There was no significant difference between southern and northern marshes in observed heterozygosity (Ho = 0.313 and 0.302, respectively; $F_{1,15} = 1.34$; p=0.265). At the six marshes in which tall and short zones were collected, paired t-tests detected no significant difference in H_E (t=-0.206; df =5; p=0.845) nor H_O (t=-0.023; df=5; p=0.983).

We detected repeated genetic differentiation between tall- and short-form phenotypes that is consistent with independent evolution in multiple analyses. First, marsh-specific PCAs detected significant differentiation (ANOSIM p<0.001) between tall- and short-form plants at all six marshes for which we had samples from both zones (Fig. 4c). Second, marsh-specific admixture analysis (k=2; Fig. 4c) also detected strong differentiation between tall- and short-form *Spartina* at all but one marsh, St. Teresa in FL, which is a microtidal system with less distinct zonation patterns in *Spartina* height (Zerebecki and Hughes, personal observation). These patterns are consistent whether including all loci (fig. 4) or excluding a subset of loci (i.e., those with an F_{ST} >90% of all loci; Fig. A9, ANOSIM p<0.001). The PCA results are also robust when using a relatively small number of called genotypes (i.e., using STACKS-generated genotypes; Fig. A10) instead of genotype likelihoods (ANOSIM p<0.001 for all comparisons).

Third, an analysis of molecular variance (AMOVA) confirmed significant hierarchical genetic structure among the six marshes from which we had both short- and tall-form samples, as well as between zones within these marshes (Table 2). When all six marshes were included, 28% and 13% of the genetic variance was partitioned among marshes and between zones, respectively; both hierarchical levels were statistically significant. In contrast, an AMOVA with only the two SC marshes (separated by <2 km) indicated no significant genetic variance between marshes (0.3%; p=0.332), but strong genetic structure among zones within marshes (10.3%; p<0.001). An identical pattern was found when analyzing the two MA marshes separated by 1 km: between marsh variance was negligible (1.0%; p=0.331), but among zone variation was significant (21.7%; p<0.001). The level of differentiation (as measured by genome-wide mean F_{ST}) between short- and tall-form plants separated by less than 200m (i.e., within marshes; mean $F_{ST} \pm SE = 0.204 \pm 0.029$) was significantly greater than mean F_{ST} between marshes separated by 650m - 4.5 km (mean $F_{ST} \pm SE = 0.128 \pm 0.019$), and comparable to differentiation among marshes separated by 5 to 15 km (mean $F_{ST} \pm SE = 0.209 \pm 0.006$; $F_{2,16} = 14.939$, p<0.001; Fig. A11).

Finally, the admixture analyses of all 11 marshes (Fig. A8) and the six marshes sampled at tall and short zones (Fig. A12) indicated that genetic clusters become increasingly differentiated by tall- vs short-form samples as the *a priori* number of genetic clusters included increased (i.e., moving from k=5 to k=16). These splits were especially evident at five marshes; three in the northeastern US (MAWS vs MAWT; MASS vs MAST; RIT vs RIS), and the two in the southeastern US where the field experiment was conducted (SCBT vs SCBT and SCFT vs SCFS). If these ecotypes had evolved once and subsequently expanded into all other marshes, we would expect genetic clusters that were specific to only tall- or short-form plants to occur at

multiple marshes. The lack of phenotypic-specific clusters across marshes suggests that this differentiation evolved independently.

Another view of the marsh-specific genomic response to ecotypic divergence comes from an RDA analysis, which detected no loci that consistently differed between ecotypes across 3 or more marshes. There were 6 loci that were shared at two marshes, but the identity of those two marshes differed by locus.

Discussion

Examples of parallel adaptive phenotypic divergence in independent populations across similar environments are particularly fascinating because they imply a repeatability in and predictability to evolution. The extent of parallelism can vary widely among replicated populations (Stuart et al. 2017), as a continuum from parallel (i.e., similar phenotypes) to divergent (i.e. discrete phenotypes; Bolnick et al. 2018, Thompson et al. 2019). However, this too may be predictable depending on both the selective environmental landscape as well as other non-adaptive mechanisms including genetic drift, gene flow, mutation and standing genetic variation (Bailey et al. 2017, Bolnick et al. 2018, Thompson et al. 2019). Our study provides compelling evidence that differences between tall- and short-form *Spartina* in part reflect parallel and independent microgeographic (<200 m) genetic divergence among habitat-associated genotypes and their traits in this widespread salt marsh foundation species. We detected a genetic basis for *Spartina* seedling height in a common greenhouse environment, and we confirmed adaptive divergence in fitness (survival and seed production) in a field reciprocal transplant experiment. We also documented signals of ecotypic divergence in two morphological traits, stem height and biomass allocation, that likely have adaptive value in this system. Shorter stems

and reduced allocation to aboveground biomass of short-origin plants in the tall zone (Fig. 5) both result in a higher likelihood of complete submergence when inundated, as well as shading from neighboring plants. In turn, submergence and/or shading can limit biomass production (Smith 1982), an important component of long-term fitness in perennial plants (Younginger et al. 2017). Finally, we detected repeated genetic divergence between zones at multiple independent marshes across the geographic range of *Spartina*, indicating that our experimental results are likely applicable beyond the specific sites where the experiments were conducted. Our study highlights that species with well-documented patterns of microgeographic phenotypic divergence that occur repeatedly across environmental gradients, like *Spartina*, are ideal systems to test for parallel evolution and its underlying ecological and genetic mechanisms.

The most parsimonious explanation for the maintenance of spatial differentiation in plant height and biomass allocation is that these traits, or traits correlated with them, generate local fitness consequences (i.e., are locally adaptive) and that strong, local post-settlement selection removes the phenotype-environment mismatch in each generation (Schmidt and Rand 2001, Hereford 2009). The observed adaptive phenotypic divergence in *Spartina* (Fig. 2) is likely also reinforced by mechanisms that minimize gene flow and generate the genomic differentiation revealed by our SNP survey. Seed dispersal barriers seem an unlikely mechanism, given the short distances (<200m) relative to documented seed dispersal distances in *Spartina* (Taylor et al. 2004) across which we detected both genetic divergence in traits and differentiation at SNPs. Barriers to pollen dispersal (e.g., differences in flowering time; Crosby et al. 2015, O'Connell et al. 2019) may act as a reproductive barrier to gene flow between zones, as in other systems (Hall and Willis 2006, Richardson et al. 2014). The relative influence of restricted gene flow and

selection on the generation and maintenance of genetic and phenotypic differentiation remains to be tested in this system.

Parallel adaptive phenotypic divergence can arise through either shared genetic changes, alternative genomic routes, or some combination of both (Elmer and Meyer 2011, Soria-Carrasco et al. 2014). The extent of parallelism is typically highest for fitness, then phenotypes, followed by genes and mutations (Bailey et al. 2015, Thompson et al. 2017) due to many-to-one mapping (e.g., many distinct genes or phenotypes yield the same phenotype or function respectively; Bolnick et al. 2018). Our study demonstrates that while phenotypic differentiation is parallel between marshes (i.e., tall and short-form *Spartina* repeatedly evolve), the genomic responses underlying this differentiation do not appear to be parallel, according to multiple lines of evidence. First, differences in *Spartina* genetic structure and composition across biogeographic zones (Fig. 4, Fig. A8) reflect 1000s of generations of regional differentiation (Wares 2002, Blum et al. 2007), and likely generated regional differences in standing genetic variation on which selection has acted. Gene reuse in parallel phenotypic evolution is more likely in recently diverged populations (Conte et al. 2012), given the impact of historical legacy on standing genetic variation and resulting genetic constraints on responses to similar selection (Langerans and DeWitt 2004, Lucek et al. 2013, Thompson et al. 2019). Second, theory and empirical results predict that changes in polygenic traits (e.g., plant height) are likely mediated by different sets of loci between populations (Walsh and Lynch 2018, Bolnick et al. 2018). Third, a preliminary redundancy analysis (RDA) yielded no anonymous markers that strongly and consistently correlated with ecotypic differentiation. Finally, differences among marshes in the particular abiotic variables underlying environmental differences across tidal elevation may yield marsh-specific genomic responses. For example, the soil salinity gradient across tidal elevation is

typically much stronger in southern marshes than northern marshes (Pennings and Bertness 2001), and this spatial variation may alter both environmental selective pressures and adaptive responses across habitats. Cryptic environmental differences between seemingly equivalent habitat-types are reflected in deviations from phenotypic parallelism in lake-stream population pairs of sticklebacks (Stuart et al. 2017) and interestingly, slight variation in the selective landscape may underpin the variability in differentiation among tall- and short- *Spartina* populations (e.g., Atlantic vs. Gulf of Mexico marshes).

Our finding of microgeographic adaptation to tidal elevation in *Spartina* is likely conservative for several reasons. First, our experiment was not ideally designed to detect local adaptation in survivorship, as we used 1-year old seedlings as our field transplants. Thus, we may have missed differences in survivorship that occurred earlier in the life cycle (e.g., seed dormancy, germination, or seedling establishment; Galloway and Fenster 2000, Bischoff et al. 2006). Mortality is known to be highest during early-life history stages of *Spartina* (Metcalfe et al. 1986): 28% of seedlings died even in the optimal environmental conditions of our greenhouse. In addition, the low survivorship of seedlings (<30%) in the tall zone lowered our statistical power to detect origin-zone differences in reproduction in the tall zone, as did the short duration of our experiment relative to the lifespan of this perennial plant. Second, we did not observe any negative consequences for tall-origin *Spartina* in the short zone for individual traits (i.e., no antagonistic pleiotropy; Fig. 3), consistent with prior results that the magnitude of local adaptation is greater when examining composite fitness than individual traits (Hereford 2009). Perhaps, individual trait trade-offs only become evident under other ecological contexts or environmental extremes (Bischoff et al. 2006, Latta et al. 2012). For instance, short-origin plants allocated a greater proportion of resources to belowground biomass and thus may have been

superior competitors to tall-origin plants over longer time frames, particularly in the denser short zone (Appendix 3).

Our study confirms prior research (e.g., Shea et al. 1975, Valiela et al. 1978, Mendelssohn and McKee 1988, Morris et al. 2002) that local environmental conditions contribute to the observed trait variation in *Spartina* across tidal elevation. In particular, there was plasticity in height of tall- and short-origin seedlings when transplanted into the two zones (Fig. 3a). The magnitude of difference in plant height in our common garden experiment (~ 5 cm) was much smaller than that in the field (~ 60 cm; Fig. 1b,c; Appendix 3), because tall-zone seedlings never grew as tall in the greenhouse as we recorded among natural populations (Fig. 1b) or the field experiment (Fig. 3a). This pattern highlights the importance of environmental context to the tall phenotype. Our results reinforce the notion that genetic differentiation and phenotypic plasticity are not mutually exclusive (Richards et al. 2006, Ghalambor et al. 2007), consistent with variation in plasticity among plant populations to light availability (Winn and Evans 1991, Schmitt 1993), water availability (Heschel et al. 2004, Pratt and Mooney 2013) and habitat type (Gray 1985, Thompson et al. 1991).

Indeed, heritable variation in plasticity has been observed within *Spartina* (e.g., Elsey-Qurick et al. 2011, Bernik et al. 2018). So, it should not be surprising that tall- and short-form *Spartina* can vary in their plastic response, and such heritable variation in plasticity may help resolve conflicting prior results. Nitrogen addition experiments have shown an increase in height in short-form *Spartina*, however this increase in productivity was not equivalent to natural tall-form stands (Valiela et al. 1978, Howes et al. 1986). Although this difference may be due to the combination of other abiotic factors typical of the tall-zone environment (e.g., soil waterlogging, sediment oxidation, salinity, etc.; Linthurst and Seneca 1980, Howes et al. 1986, Mendelssohn

and Morris 2002) not concurrently manipulated, an alternative hypothesis is that tall-origin seedlings may either have greater recognition of the environmental cues for the tall zone, or they may have an underlying genetic architecture that more strongly prompts this plastic response (DeWitt et al. 1998, Callaway et al. 2003). Understanding the relative importance of genetic differentiation and plastic responses, and their potential interaction in generating and maintaining these two growth form will be crucial to fully comprehend the ecological consequences of our observed microgeographic genetic divergence.

Foundation species drive ecological functions of entire ecosystems (Ellison 2019); thus, any adaptive phenotypic divergence within them can have far-reaching impacts on community and ecosystem processes (Norberg 2004, Whitham et al. 2006, Hughes et al. 2008, Matthews et al. 2011, Urban et al. 2020). In this system, *Spartina* structural traits such as stem height and biomass allocation are key drivers of marsh sediment accumulation rates (Mendelssohn and Morris 2002, Morris et al. 2002), and thus the genetic- and environmentally-mediated microgeographic divergence of these traits is likely to be critical for the response of marshes to sea level rise (Kirwan and Murray 2007). Our results suggest that with increasing inundation, the ability of tall-form plants to migrate landward may be crucial for stabilizing the retreating marsh edge and preserving marsh function (Fig. 5). We also add to the growing evidence of parallel adaptive phenotypic evolution occurring across a wide range of taxa (e.g., Rundle et al. 2000, Nosil et al. 2002, Roda et al. 2013) and highlight the importance of exploring the commonality of this process in foundation species. Greater understanding of the ecological factors driving and evolutionary processes underlying microgeographic adaptation in foundation species is needed, both to advance eco-evolutionary research and to inform the management and conservation of critical habitat-forming species and their associated ecosystem functions and services.

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Statement of Authorship

RAZ, EES, TCH, and ARH conceptualized and designed the experiment. RAZ, TCH, and ARH performed the common garden. RAZ, EES, TCH, ARH, CG, and CLR conducted the reciprocal transplant experiment. EES, TCH, KLB, and CCN performed the molecular analyses. RAZ and EES analyzed the data and wrote the first draft of the manuscript. All authors contributed substantially to revisions.

Data and Code Accessibility:

Data supporting the results is available on Dryad (https://doi.org/10.5061/dryad.1c59zw3vq). Genetic data is deposited in GenBank BioProject ID PRJNA733197 and SRA ID PRJNA733197.

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Tables

Table 1. *Spartina* responses at the end (December 2016) and aster analysis of composite fitness (survivorship and seed production) throughout the reciprocal transplant experiment. F statistics are for responses analyzed with linear models; X^2 for logistic regression. Transplant zone is location (tall vs short) where planted; origin zone is location (tall vs short) where maternal plant was from. Numerator degrees of freedom = 1 for each zone factor and = 2 for each site factor.

		Transplant Zone		**Origin Zone**		**Transplant x Origin Zone**		**Transplant Site**		**Origin Site**	
Response Variables	Den Df	F	P	F	P	F	P	F	P	F	P
Max. Stem Height	207	1.2	0.27	12.68	**< 0.001**	7.7	**0.006**	1.64	0.2	2.69	0.07
Average Stem Height	207	24.36	**<0.001**	5.92	**0.02**	0.0025	0.96	10.37	**<0.001**	0.93	0.39
Stem Density	207	34.8	**<0.001**	0.14	0.71	1	0.32	41.09	**<0.001**	0.15	0.86
Total Biomass	198	15.27	**<0.001**	1.15	0.29	0.88	0.35	25.52	**<0.001**	3.75	**0.04**
Aboveground Biomass	199	15.1	**<0.001**	2.02	0.16	1.56	0.21	28.82	**<0.001**	6.04	**0.006**
Belowground Biomass	206	16.95	**<0.001**	1.33	0.25	0.46	0.49	19.18	**<0.001**	1.96	0.14
Biomass Allocation	198	0.83	0.36	8.51	**0.004**	5.21	**0.02**	6.55	**0.001**	7.70	**<0.001**
	Den Df	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
Survival	352	174.64	**<0.001**	0	1	26.52	<0.001	0.82	0.66	0.44	0.51
Proportion Flowering	207	7.39	**0.007**	0.31	0.57	1.81	0.18	5.04	0.08	3.5	0.17
		Deviance	*p*	Deviance	*p*	Deviance	*p*	Deviance	*p*	Deviance	*p*
Composite Fitness		40.27	**<0.001**	3.81	0.051	6.60	**0.010**	6.08	**0.048**	12.12	**0.002**

Table 2. Hierarchical analysis of molecular variance (AMOVA) of marshes in which tall- versus short-form *Spartina alterniflora* were sampled and genotyped.

	d.f.	MS	% Var.	*p*	Φ
Six marshes					
Among marshes	5	10066.10	28.1%	0.001	0.282
Between zones within marsh	6	2228.70	12.7%	<0.001	0.177
Error	204	457.95	59.1%		
SC only (2 marshes)					
Among marshes	1	1636.07	0.3%	0.332	0.010
Between zones within marsh	2	1568.81	10.3%	<0.001	0.219
Error	59	558.59	89.4%		
MA only (2 marshes)					
Among marshes	1	2876.44	1.0%	0.331	0.013
Between zones within marsh	2	2661.20	21.7%	<0.001	0.372
Error	75	407.00	77.3%		

Note: Separate AMOVAs were performed for all 12 subpopulations (i.e., tidal zones) at 6 marshes (FL, SC, RI, MA), 4 subpopulations at 2 SC marshes and 4 subpopulations at 2 MA marshes. **Φ** = phi statistic. n=1000 permutations in *pegas::amova* protocol.

Figure Legends

Figure 1. A) Short- and tall-form *Spartina alterniflora* in a salt marsh near Bowens Island, Charleston, SC. A yard (0.91m) stick is shown for comparison. B) Maximum stem height (mean cm ± S.E.) of short- and tall-form plants (red and black, respectively) surveyed at the beginning of the growing season in the field survey ($F_{1,27} = 130.38$, $p < 0.001$; see Appendix 3 for Methods). C) Maximum stem height of plants reared in the greenhouse common garden from tall-zone seeds (black) is significantly greater than maximum stem height of plants reared from short-zone seeds (red; $p < 0.05$ at all timepoints except for day 110; n = 51-75). Photo credit: Erik Sotka. Data underlying figure 1 are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.1c59zw3vq (Zerebecki et al. 2021).

Figure 2. Composite expected fitness (survivorship and seed production) of short-origin transplants (red) and tall-origin transplants (black) grown in either the short or tall transplant zone (x-axis). Local treatments are open markers and foreign treatments are closed markers.

Figure 3. *Spartina* responses during the reciprocal transplant experiment. (A) Maximum stem height, (B) biomass allocation, (C) stem density, (D) total biomass, (E) proportion of plants that flowered and (F) survival curves in tall- and short-zones. In panels A-E, the mean response is split by short-origin ("red") and tall-origin plants ("black") transplanted into either the short or tall zone (x-axis). Error bars represent ± 1 SE. Local and foreign treatments are open and closed markers respectively, and an asterisk denotes significant differences between local and foreign treatments. N = 82-28 per origin x transplant zone combination for responses A-E. In panel F, we

plot the mean and 95% confidence intervals of survival in short- and tall-zones against the number of weeks from the start of experiment (May 2016). N = 180 per transplant zone.

Figure 4. Population genomic analyses of *Spartina alterniflora.* A) Map of the 11 marshes along the Atlantic and Gulf coasts of the United States sampled for population genomic analyses. B) Principal components analysis (PCA) of genotype likelihoods across 2,735 SNPs and 309 individuals. Populations are colored as in (A). C) PCA (left panels) and admixture analyses (k=2; right panels) of genotype likelihoods comparing tall- versus short-zone plants (black and red points, respectively) within six marshes. In admixture plots, k=2 for all comparisons except k=3 for West Marsh, MA. Black and grey shades represent different genetic clusters assigned to each individual.

Figure 5. Summary figure to illustrate the results of our reciprocal transplant experiment. Tall-origin *Spartina* in the tall zone (left) is 60% taller and has 58% greater above to belowground biomass than short-origin *Spartina,* illustrating the genetic constraint on short-origin plants. In contrast, tall-origin and short-origin *Spartina* in the short zone (right) have similar morphology. Adapted from Mudd and Fagherazzi 2016 (Fig 12.2) Image credit: Matt Freedman.

Figure 1

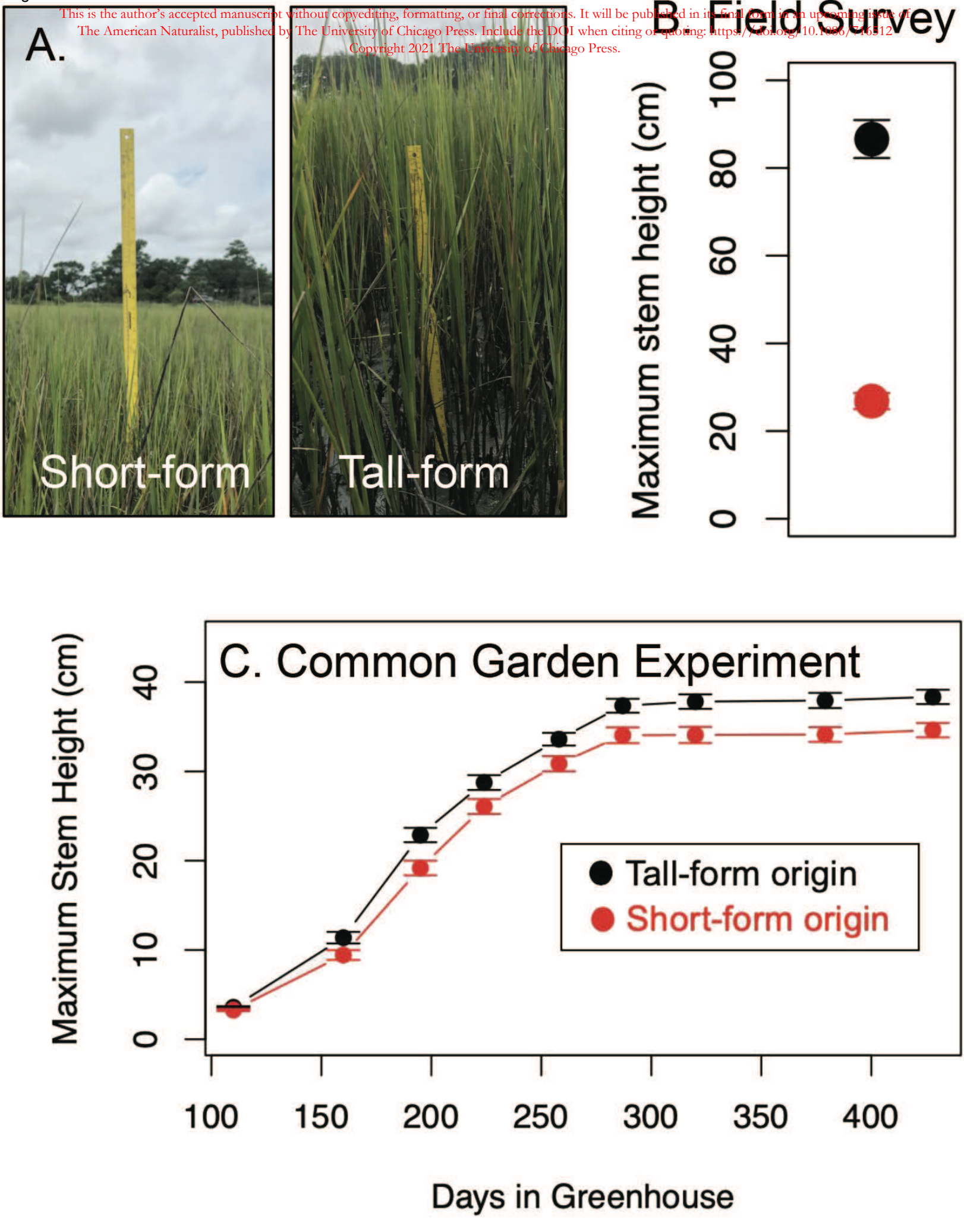

Figure 2

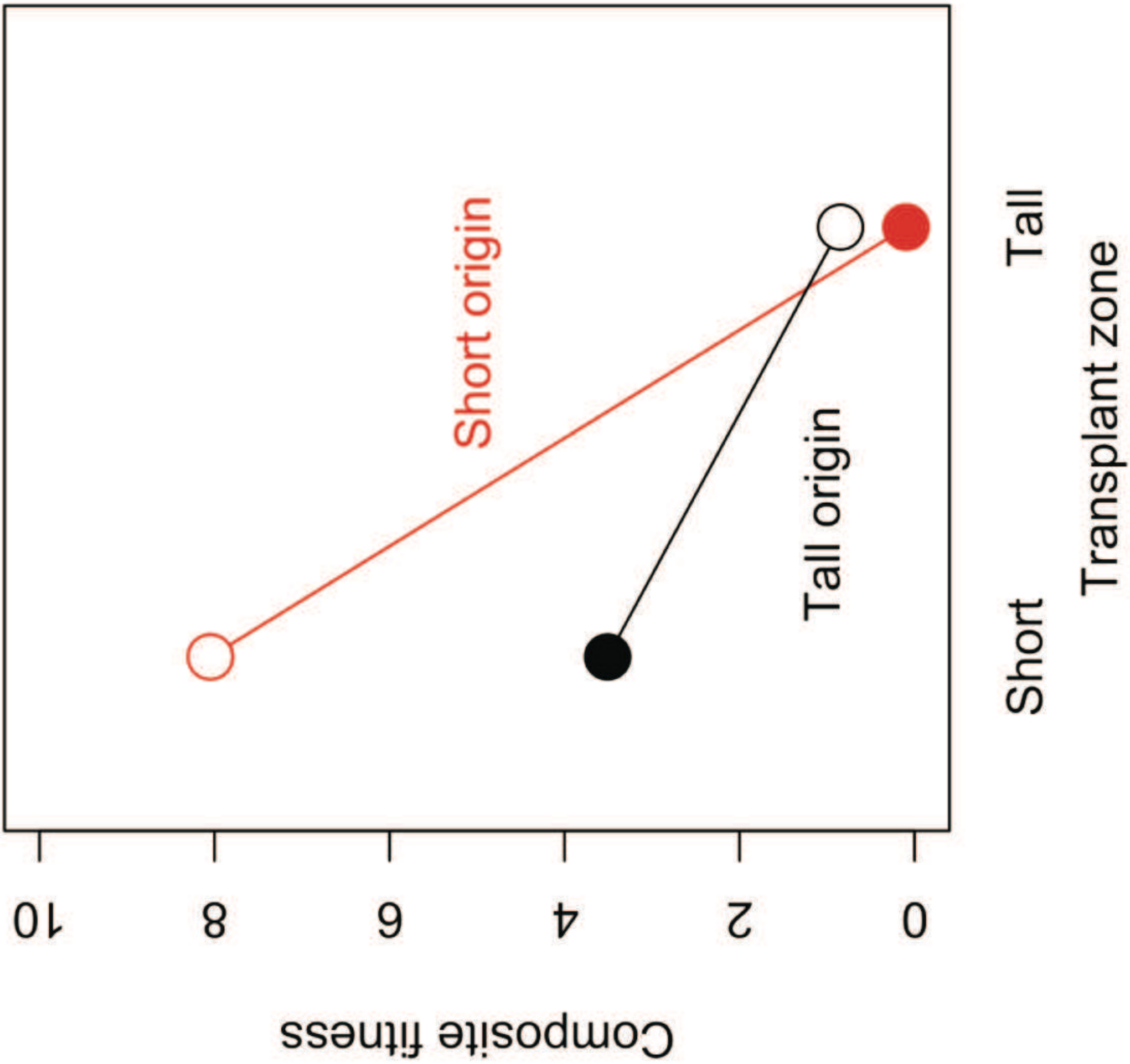

Figure 3

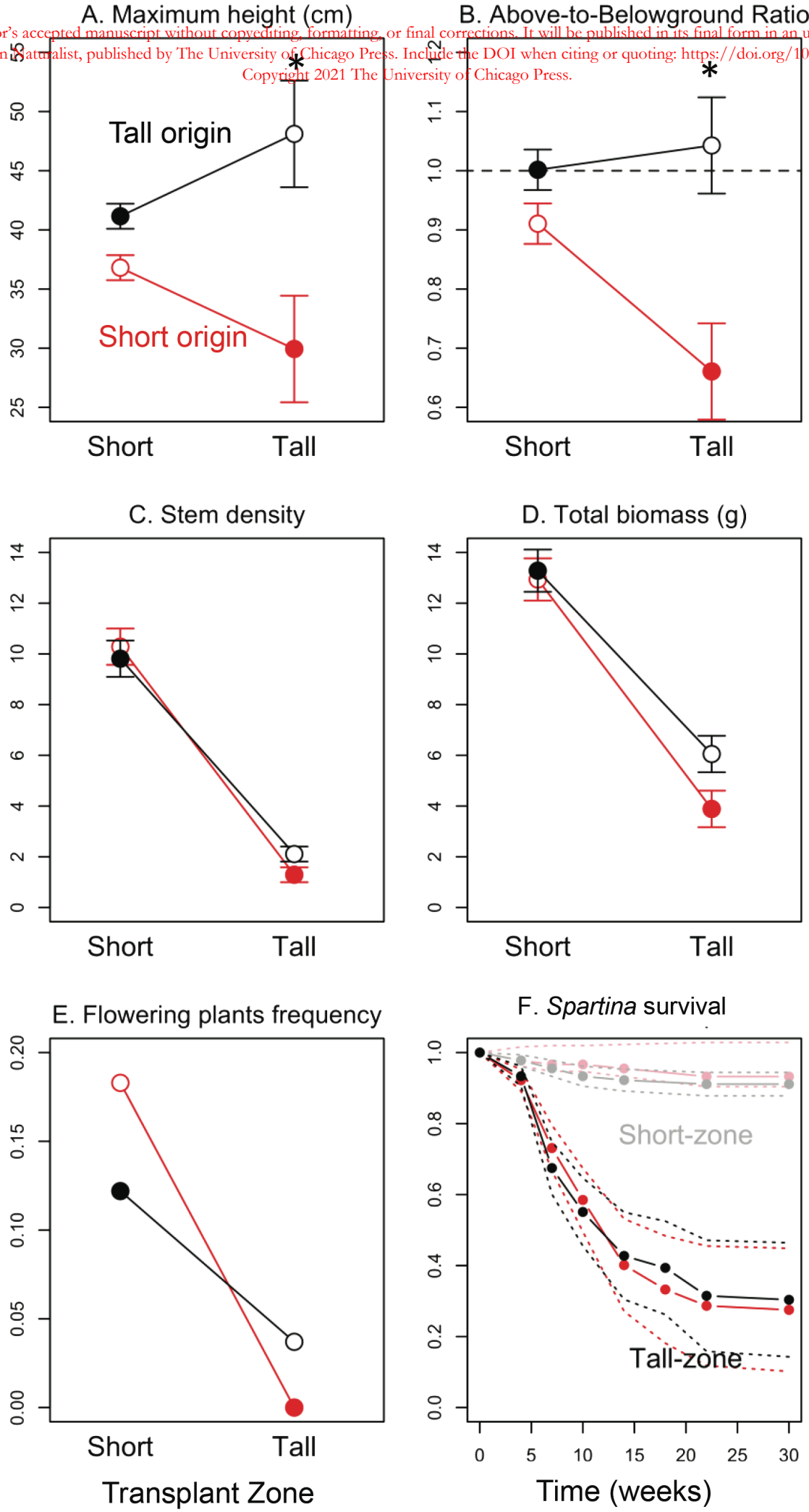

Figure 4

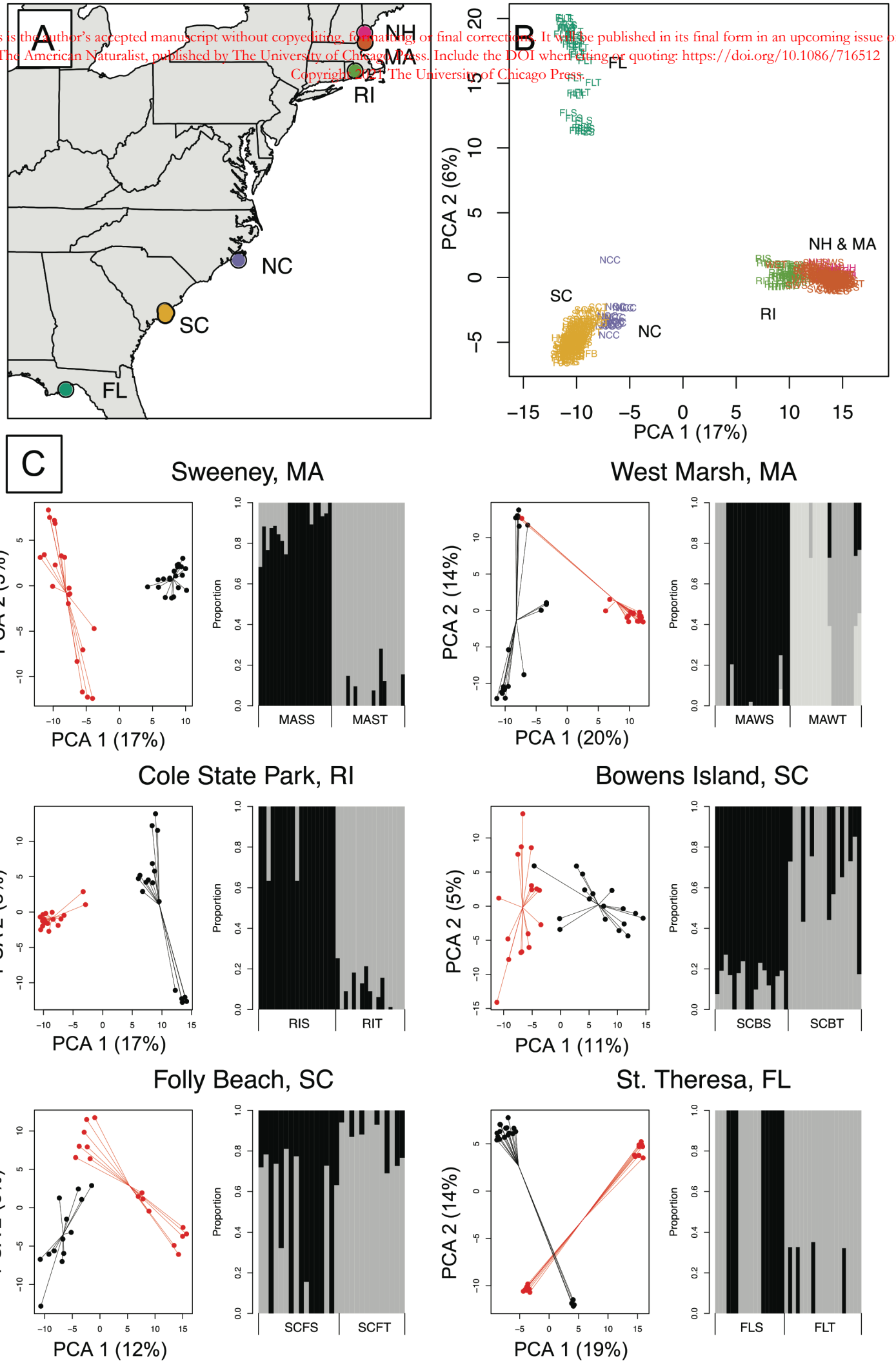

Figure 5

Supplementary Information Text for

Repeated genetic and adaptive phenotypic divergence across tidal elevation in a foundation plant species in *The American Naturalist*

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Appendix 1: Common garden experiment: Additional response variables

Methods

Seed Collection

Seeds were collected in October 2014 from three marshes in Charleston, SC (Folly Beach, Fort Johnson, and Bowens Island; Fig. A1; see Table A6 for latitude and longitude) that had characteristic tall- and short-form *Spartina* zones. We collected seed-bearing flowers from 10 haphazardly selected reproductive *Spartina* plants at least 1 m apart along linear transects within the tall and short zones. We also surveyed the tall and short zones at each site to document natural differences in plant morphology and environmental variation across zones (Appendix 3). Seeds were shipped to the Northeastern University Marine Science Center (MSC) for germination and propagation. We simulated the natural dormant period by combining seeds from all maternal plants of the same site x zone together and submerging the seeds in freshwater in the dark at 4°C for 2.5 months (Biber & Caldwell 2008). In January 2015, we replaced the freshwater and moved the seeds into ambient sunlight in the MSC greenhouse, which was heated to remain above freezing temperatures. Seeds were checked twice weekly for germination. Once individual seeds had germinated, they were planted in 3" pots (volume = 365mL) with commercial potting soil with up to 5 individuals from the same site x zone origin and irrigated with freshwater daily.

Data Analysis

In addition to maximum stem height and seed quality (see main text), we analyzed seedling survival, stem density, and number of leaves on the tallest stem over the course of the common garden experiment, and average stem height at the end of the experiment. We assessed whether survival differed between seedlings from the tall- and short-zones over the course of the experiment using a Cox proportional hazards regression with zone and site as independent fixed effect. Seedlings that did not survive were removed from further analyses. To determine if there

were morphological differences among seedlings across zones, we ran individual repeated measures ANOVAs (hereafter referred to as RM ANOVA) on density, maximum height (presented in main text), and leaf number, with origin zone, and date as independent and interactive fixed factors, origin site as fixed factor and pot (to account for the non-independence of following pots across time) as a random factor. If the RM ANOVA had a significant zone x time effect, we then ran individual linear mixed effect ANOVAs with origin zone and origin site as fixed factor on the response variable of interest for each time point. We also examined average height at the end of the experiment with a similar ANOVA. All experimental analyses were conducted in R statistical software (version 3.1.1) using packages as described in the main text. We also used the *lme4* (Bates et al. 2015) and *lmerTest* (Kuzentsove et al. 2017) packages to determine the Satterthwaite approximation for degrees of freedom and generate F and p-values on RM ANOVAs

Results

Seedling survival was high in the greenhouse common garden, with 72% of the *Spartina* seedlings surviving until the end of the common garden experiment. There was no effect of origin zone on survival (Cox proportional hazard regression: X^2= 1.26, p =0.26).

Spartina stem density increased over time (RM ANOVA date: $F_{1,747}$=401.08, p < 0.001, Table A1) from a mean[SE] of 2.07[0.11] stems at the beginning of the experiment to 18.24[0.71] stems at the end of the experiment (Fig. A2c). There was no independent or interactive effect of zone on stem density (p > 0.29).

Time and zone interacted to influence the number of leaves on the tallest *Spartina* stem (RM ANOVA zone * date: $F_{1,789}$ = 4.25, p < 0.001, Fig. A2b, Tables A1 & A2): tall-zone seedlings had more leaves per stem than short-zone seedlings, but only during July and August.

Average *Spartina* stem height at the end of the experiment was also significantly affected by zone (ANOVA $F_{1,104} = 33.68$, $p < 0.0001$): mean[SE] height of tall-zone seedlings (28.06[0.47] cm) was greater than short-zone seedlings (23.33[0.69] cm).

Appendix 2: Reciprocal transplant experiment: Additional response variables

Methods

Six (6) response variables are presented in the main text (survival, maximum stem height, stem density, total biomass, and biomass allocation, and flowering frequency) from the end of the experiment. We also assessed maximum stem height and stem density monthly over the course of the experiment; additional fecundity responses throughout the experiment; and average stem height and above- and below-ground biomass separately at the end of the experiment.

We first conducted a RM ANOVA on *Spartina* density and maximum stem height over the course of the experiment with origin zone, transplant zone, and date as fixed effects, including all interactions, as well as independent fixed effects of origin site and transplant site. Plot nested in transect (to account for the non-independence of following plots across time) was included as a random effect. Because we found significant interactions with time, we then used individual linear models at each time point with transplant zone, origin zone, and their interaction, along with origin site and transplant site as fixed effects (see main text). We also used this approach to examine the responses only measured at the end of the experiment. One exception was survival at the end of the experiment (Table 1), we instead utilized a similar generalized linear model with a binomial response.

Because we found a significant effect of transplant zone on the proportion of transplants producing flowers (see Results), we tested whether the a) total number of flowers produced, and b) average number of seeds per flowering stem differed among origin zones in the short transplant zone using a linear model with origin zone, origin site and transplant site as fixed factors. Low flowering in the tall transplant zone precluded a combined analysis. We also assessed differences in flower timing between origin zones within the short transplant zone using a Cox proportional hazard regression with both origin site and transplant site also included as

fixed factors. All experimental analyses were conducted in R statistical software (version 3.1.1) with packages as described in the main text. We also used the *lme4* (Bates et al. 2015) and *lmerTest* (Kuzentsove et al. 2017) packages to determine the Satterthwaite approximation for degrees of freedom and generate F and p-values on RM ANOVAs.

Results

Survival in the field varied by transplant zone throughout (Fig. 3F), and at the end of the experiment (Table 1), transplants were more likely to survive in the short zone. As transplant site also had an independent effect ($p < 0.001$), and at one site (Bowens Island) all transplants in the tall zone died by the end of the experiment, we ran survival analyses across zones at each site independently to test if the pattern held across sites and found the pattern of higher survival in the short zone was consistent across sites (Fig. A4).

Spartina maximum stem height varied through time, and the magnitude and direction of this difference varied by origin zone x transplant zone (RM origin zone*transplant zone*date: $F_{6,1453} = 7.35$, $p < 0.001$, Table A3). Initial *Spartina* height did not differ by transplant zone, origin zone, or their interaction ($p > 0.34$; Table A4). After planting in the field, tall origin seedlings were consistently taller than short origin seedlings (Table A4). In addition, both transplant zone and the origin x transplant zone interaction had significant effects on stem height at multiple time points (June, August, September, October; Fig. A5a). *Spartina* stem density also increased over time, and the magnitude of this increase varied across transplant zone (RM transplant zone*date: $F_{1,1765} = 65.02$, $p < 0.0001$, Table A3). Fewer stems were produced in the tall zone than the short zone at all time points after planting ($p < 0.05$, Table A5, Fig. A5b).

At the end of the experiment, average *Spartina* stem height was independently affected by both transplant zone (ANOVA: $F_{1,207} = 24.36$, $p < 0.001$) and origin zone ($F_{1,207} = 5.92$, $p = 0.02$, Fig. A6a): average stem height was greater in both the tall transplant zone and for tall-origin

seedlings. These patterns were consistent with maximum stem height (Fig. 3A). In addition, both above- and below-ground biomass differed by transplant zone, with reduced biomass in the tall zone (aboveground: $F_{1,199} = 15.10$, $p < 0.001$, Fig. A6b; belowground: $F_{1,206} = 16.95$, $p < 0.001$, Fig. A6c). Neither origin zone, nor the transplant x origin zone interaction was not significant for either above- or below-ground biomass (Origin Zone: aboveground: $F_{1,199} = 2.02$, $p = 0.16$; belowground: $F_{1,206} = 1.33$, $p = 0.25$; Origin x Transplant Zone Interaction: aboveground: $F_{1,199} = 1.56$, $p = 0.21$; belowground: $F_{1,206} = 0.46$, $p = 0.49$).

Neither flower timing ($p = 0.46$), the number of flowers per stem (short-origin: mean[SE]= 1.88[0.31] flowers vs. tall-origin: 1.46[0.18]; origin zone: $p = 0.27$), nor number of seeds produced per flowering stem ($p = 0.36$, Fig. A7) were significantly different between origin zones.

Appendix 3: Field survey: Plant morphology and environmental variation across tidal zones in SC

At the same three marshes in Charleston, SC that we collected seeds for our reciprocal transplant experiment (Folly Beach, Fort Johnson, and Bowens Island; Fig. A1), we also characterized the natural tall- and short- *Spartina* zones. In May 2015, we measured *Spartina* stem density, stem height of 5 haphazardly selected plants, and sediment redox (mV) at 5cm depth in 5 haphazardly selected 0.25m^2 quadrats in a ~ 5 x 30 m area adjacent to the location of our 2016 reciprocal transplant experiment. We assessed natural differences in tall- and short-zone *Spartina* for each response variable individually using linear models with marsh zone and marsh site as fixed effects.

Results

Tall-zone vegetative stems were, on average, taller (mean[SE]= 86.6[4.3]cm) than stems in the short zone (26.8[1.8]cm; ANOVA: $F_{1,25} = 194.87$, $p < 0.001$, Fig. 1B). *Spartina* stem density was also lower in the tall zone (mean[SE]: 12.5[0.89] stems) compared to the short zone (21.9[2.7] stems; ANOVA: $F_{1,25} = 12.79$, $p = 0.001$). In addition, the short zone had lower soil oxygen (Redox: -285.7[42] mV) compared to the tall zone (-35.2[44]) in the upper sediment layer (ANOVA: $F_{1,21} = 26.23$, $p < 0.001$). This pattern was consistent with redox in the reciprocal transplant experiment (ANOVA: transplant zone $F_{1,86} = 25.44$, $p < 0.001$): redox potential was more negative in the short zone (mean[SE] = -60.50[20.7] mV) compared to the tall zone (121.30[17.6]) when measured at the end of the experiment.

Appendix 4: Supplemental population genetics analyses

Methods

DNA extraction, library preparation and in silico *processing*

DNA of ~1 cm piece of each sample was extracted using Nucleospin® Plant Kit (Macherey-Nagel, Bethlehem, PA, USA) or E-Z 96® Plant DNA Kit (Omega Bio-Tek, Norcross, GA, USA), and extractions were visualized with a 1.5% agarose gel to confirm presence of high-quality DNA.

To conduct reduced-representation genomic sequencing and genotyping, we prepared one genomic library following protocols in Parchman et al. (2012). We digested genomic DNA with two restriction enzymes, *Eco*RI and *Mse*I, and then ligated adaptors containing 8-10 bp barcodes unique to each sample. The restriction-ligation products were PCR amplified using standard Illumina primers in two independent reactions. Amplicons were pooled and sent to the University of Texas Genomic Sequencing and Analysis Facility. The library was size selected via Blue Pippen Prep (300-450bp) and single-read sequenced (SR 100) on both HiSeq 2000 and HiSeq 4000 Illumina platforms. After filtering out PhiX sequences using bowtie2 (Langmead et al 2009) and those without identifiable barcodes, this yielded 249.2 x 10^6 reads.

We performed *de novo* assembly on a subset of 25 million randomly sampled reads using *SEQMAN NGNEN* software (DNASTAR, Madison, WI, USA) with a 92 minimum match percentage, yielding 697,430 contigs. To help eliminate paralogs within our *de novo* assembly, we assembled our *de novo* to itself using *SEQMAN NGNEN* software (DNASTAR, Madison, WI, USA) with an 83 minimum match percentage and retained only unassembled contigs. All reads were then aligned to the *de novo* assembly using bwa ver. 0.7.12 (Burrows-Wheeler Aligner; Li & Durbin 2009).

Genotype likelihoods on all variant sites were generated with samtools/bcftools 1.3.1 (-mpileup and –call protocols) with the multiallelic calling option (Li 2011). We then used custom *R* (R Core Team 2017) script and the *vcfR* package (Knaus and Grunwald 2017) to subset SNPs

with at least one read in 50% of individuals and a minor allele frequency (MAF) greater than 0.05. We randomly chose a single variable SNP within each of the remaining contigs and included only individuals with a single read in at least 70% of loci. This left us with 2,735 SNPs across 309 individuals (see sample size per population in Table S7), and 5.20 ± 0.07 reads (mean ± standard error) per locus per sample.

To perform the hierarchical analysis of molecular variance (AMOVA) for our three datasets in the main text, genotype likelihoods (i.e., the dataset converted to 0-2 as described in the Methods) were used to generate Euclidean distances among individuals, which was then used in *R::pegas::amova* (Paradis 2010) to assess how genetic variation is partitioned among marshes and among zones within marshes. We also calculated Phi-statistics and the proportion of total variance for each hierarchical level. Each analysis was permuted 1000 times to generate statistical significance. Results were confirmed when performing AMOVAs based on Nei's genetic distance generated in *R::StAMPP* (Pembleton et al 2013; analyses not shown). Further, to calculate pairwise F_{ST} among and within marshes using *ngsFST* (Fumagalli et al. 2013), we used genotype likelihoods (from *samtools/bcftools*) to generate a site frequency spectrum (SFS), allele frequencies, and per-locus F_{ST} with a methods-of-moments estimator. We compared pairwise F_{ST} among populations categorized into bins of geographic distance, as estimated by great-circle distance using *R::geosphere* (Hijmans 2017): short vs tall within a marsh (<0.2 km), and marshes separated by 0.5-5km and 5-15 km. For the latter two categories, we only used comparisons among similar phenotypes (e.g., short-form was not compared to tall-form). Analysis proceeded using a one-way ANOVA and Tukey's HSD in *R*. Because of our sampling design, these distance comparisons could only be made with marshes in SC and New England (MA and RI).

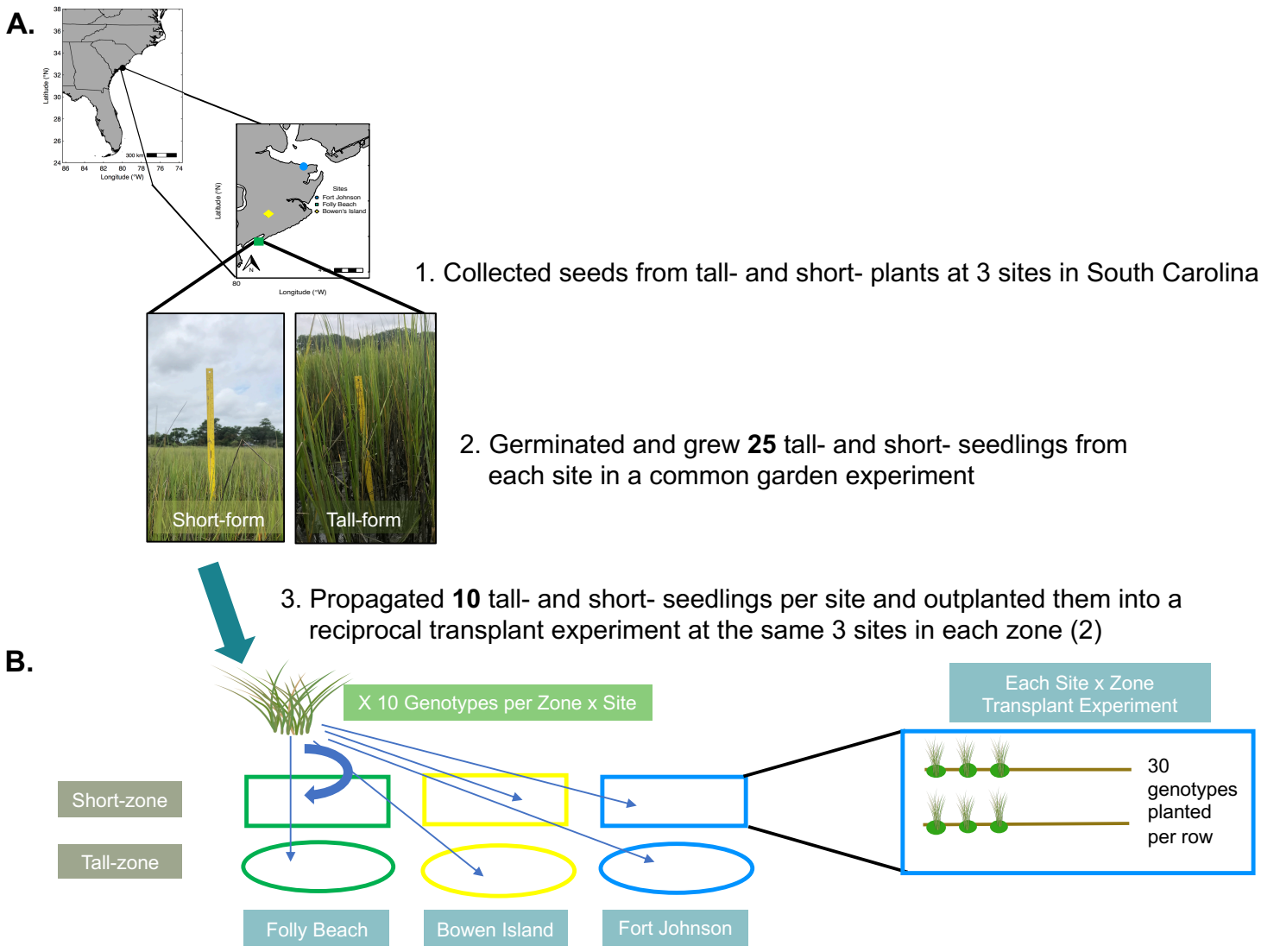

Figure A1. Experimental work-flow and set-up. A. Map of our 3 experimental sites (Bowens Island = yellow diamond, Folly Beach = green square, Fort Johnson = blue circle) in South Carolina where we collected tall and short seeds in fall 2014 and conducted field reciprocal transplant experiment in 2016. Tall zone occurred at 1.19, 0.95, and 1.17m MLLW, while the short zone occurred at 1.73, 1.59, and 1.61m MLLW at each site respectively. B. Set-up of reciprocal transplant experiment. 10 genotypes that originated from each zone x site combination (6) were transplanted into all foreign zone x sites (5) as well as their origin site x zone. At each transplant experiment, there were 2 transects with 30 *Spartina* genotypes planted 1m apart.

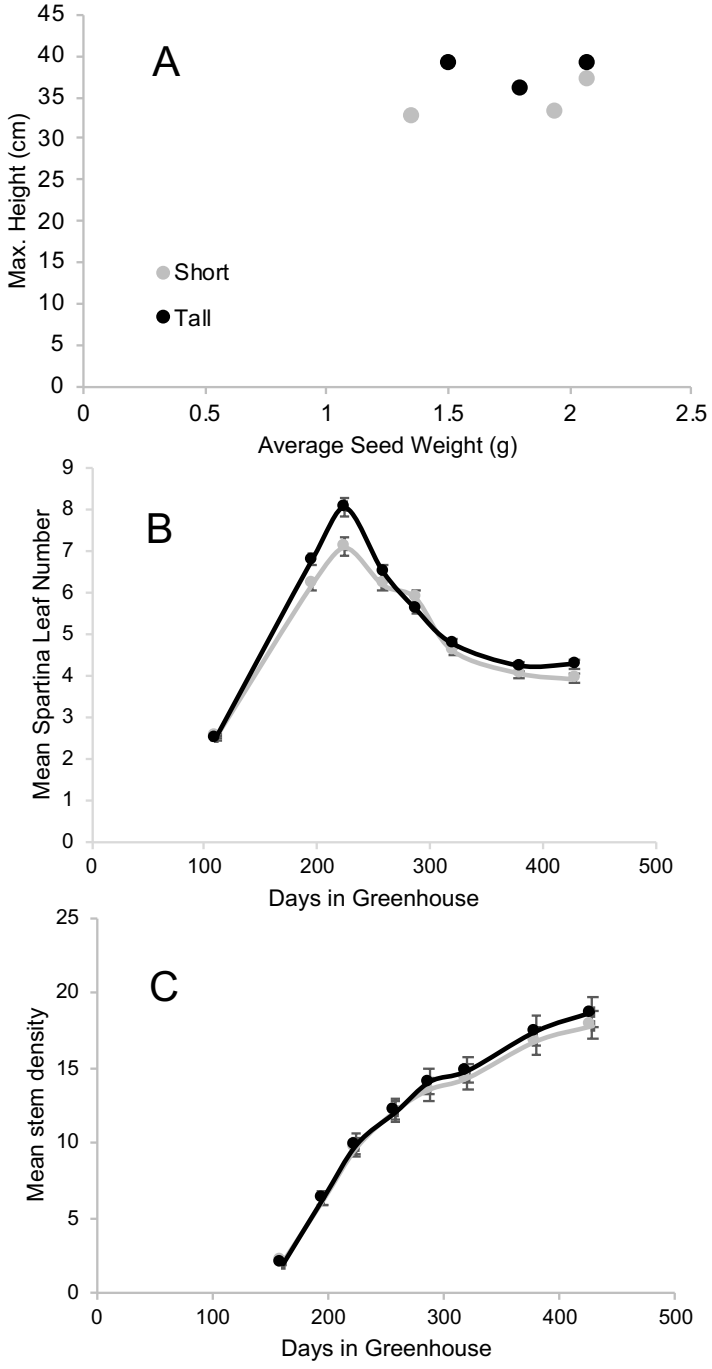

Figure A2. A) Correlation between average seed weight (g) and average maximum stem height (g) of each site x zone at the end of the common garden experiment. Mean ± 1 SE number of leaves of the tallest *Spartina* stem (B) and *Spartina* stem density (C) over the course of our common garden experiment. Tall-origin seedlings are in black, and short-origin seedlings are in light grey.

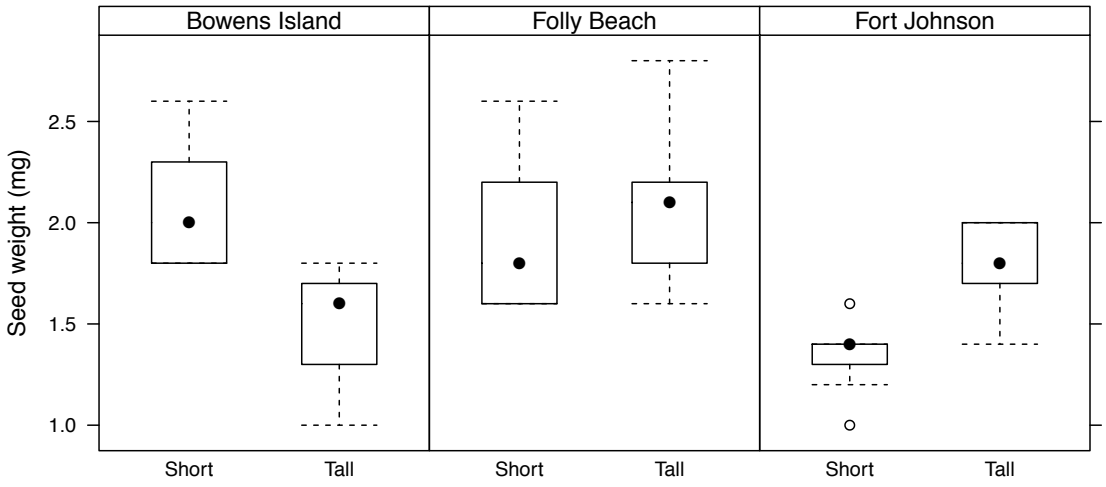

Figure A3. Dry mass of field-collected ungerminated seeds grouped by site of origin (Bowens Island, Folly Beach and Fort Johnson) and origin zone (Short, Tall).

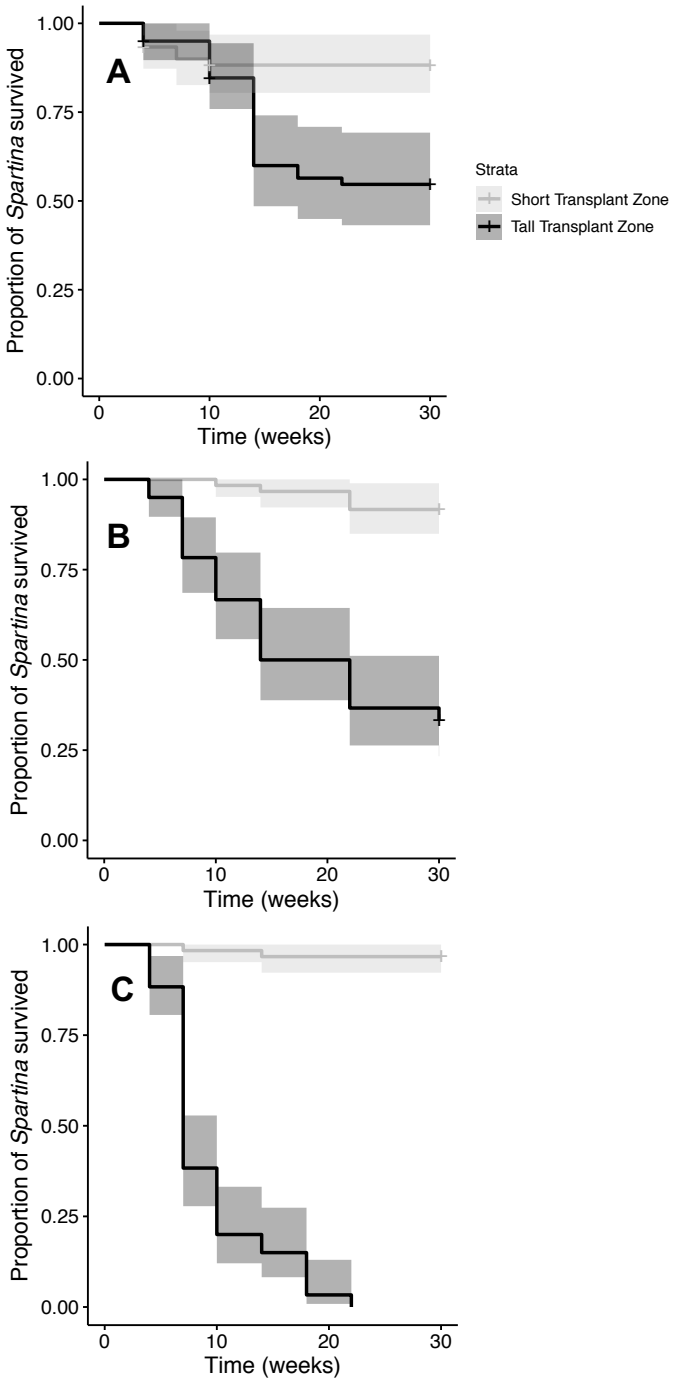

Figure A4. Survival of transplants in field experiment over time (weeks) across sites: A) Fort Johnson, B) Folly Beach, and C) Bowens Island. Solid black lines denote seedlings transplanted in the tall zone, while light grey lines represent transplants in the short zone. Mean ± 95% confidence intervals.

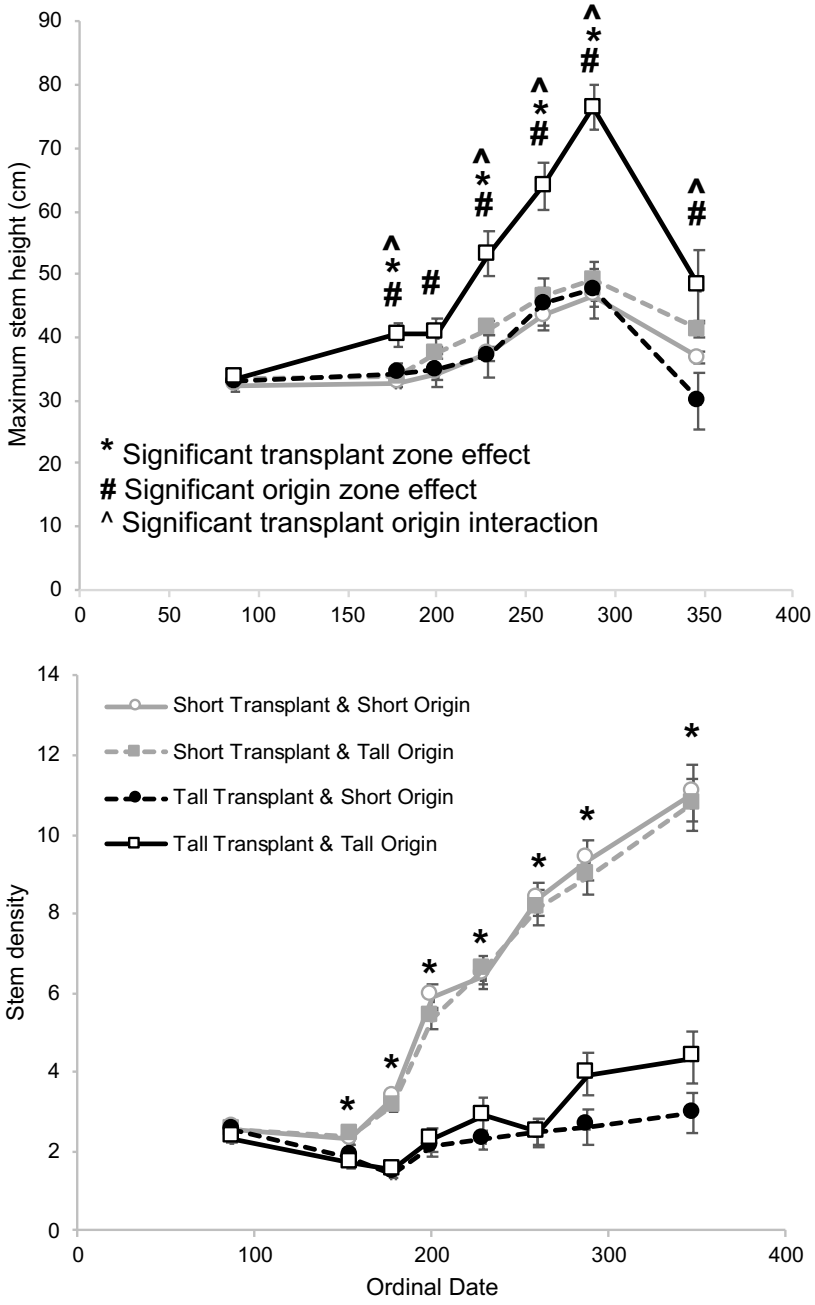

Figure A5. Mean ± 1 SE *Spartina* maximum height (cm; A) and stem density (B) over the course of the reciprocal transplant experiment in 2016. Color indicates transplant zone: light grey = Short transplant zone; black = Tall transplant zone. Shape denotes origin zone: circles = Short-zone origin, squares = Tall-zone origin. Foreign transplants are open markers with solid lines, whereas local transplants are closed markers with dashed lines. Symbols signify significant transplant zone (*), origin zone (#), and transplant x origin zone interaction (^) at each time point.

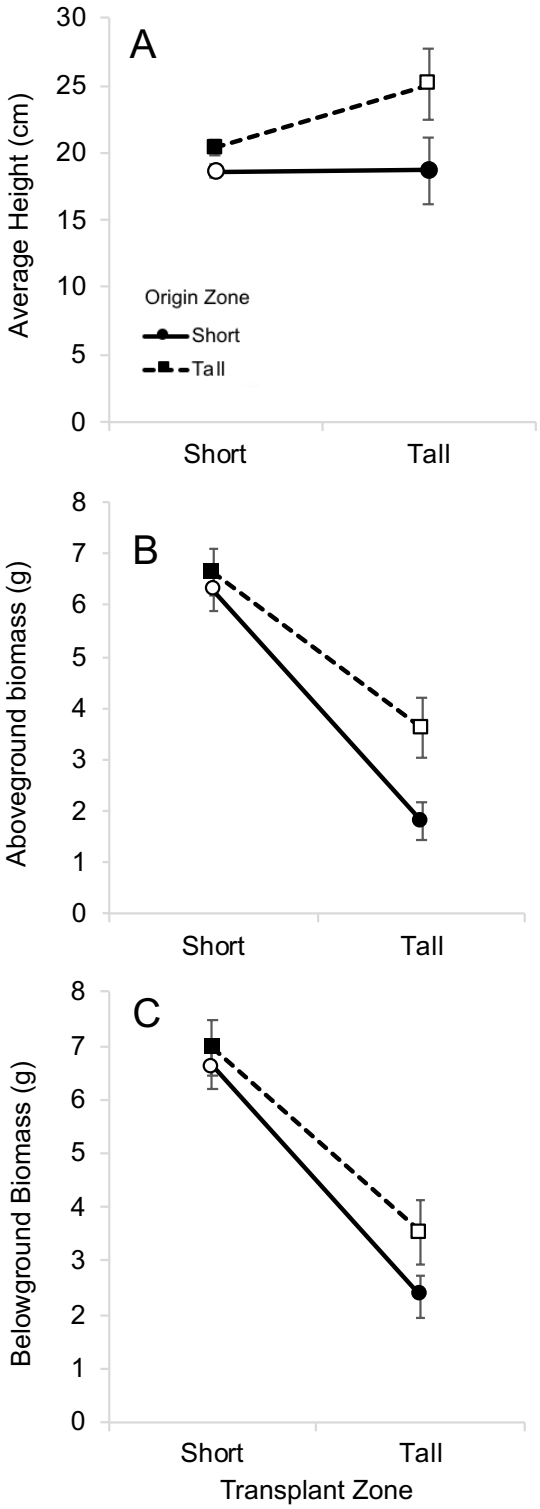

Figure A6. Mean ± 1 SE *Spartina* average stem height (a), aboveground biomass (b), and belowground biomass (c) of short-origin transplants (circles and solid line) and tall-origin transplants (squares and dashed line) grown in either the short or tall transplant zone at the end (December 2016) of the reciprocal transplant experiment. Local transplants are open markers and foreign are closed markers.

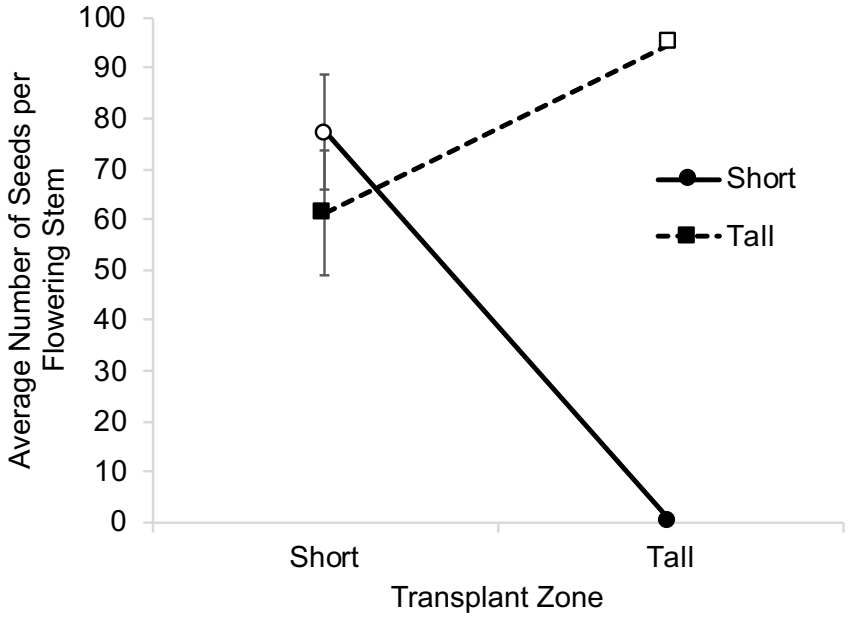

Figure A7. Mean ± 1 SE number of seeds per flowering stem of short-origin transplants (circles and solid line) and tall-origin transplants (squares and dashed line) grown in either the short or tall transplant zone. Local treatments are open markers and foreign treatments are closed markers.

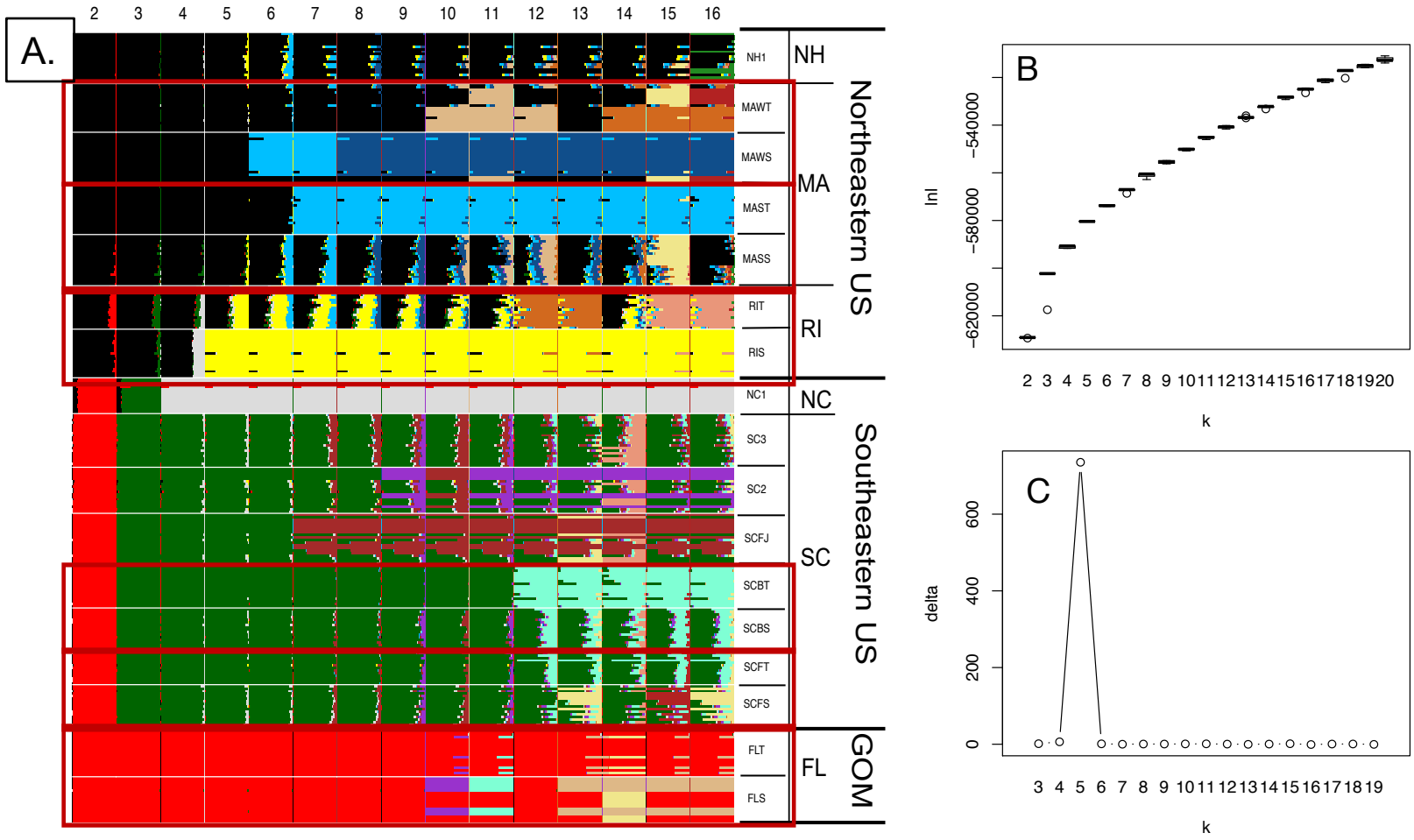

Figure A8. Admixture analysis for all populations. A) Plot of k=2-16 genetic clusters, where colors represent different genetic clusters assigned to each individual. Sites are ordered by region from north to south; within red boxes represent tall (T) and short (S) -zone within one marsh. B). Model fit for ngsadmix analysis – all populations. B) Log probability of data L(k) as a function of k=2-20 across 5 replicate runs. C) Magnitude of delta K (sensu Evanno et al. 2005).

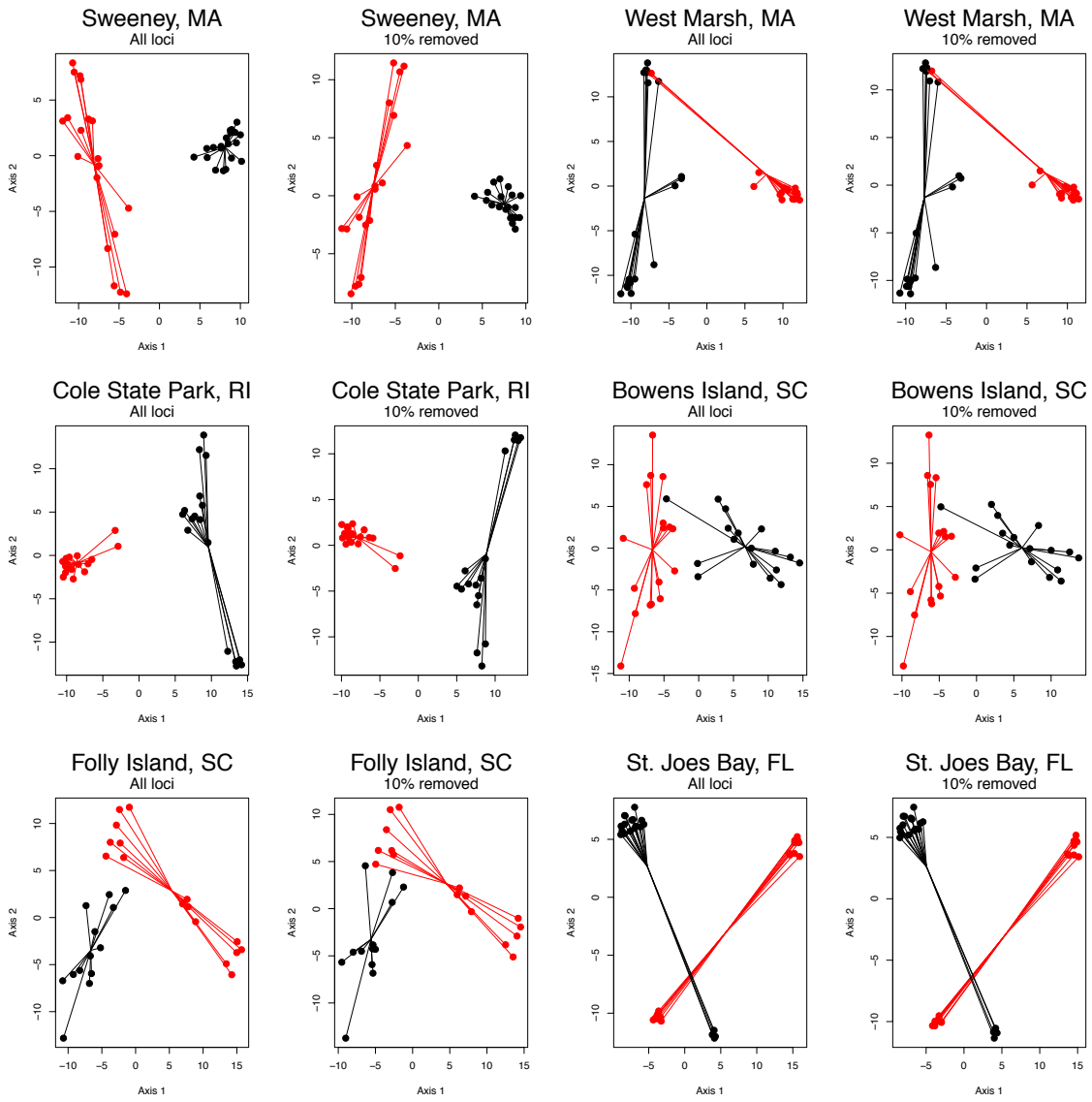

Figure A9. PCA of tall- versus short-zones when including all or excluding a subset (i.e., those within the upper 10%) of F_{ST} loci. Red and black points of the PCA indicate short- and tall-zone plants, respectively.

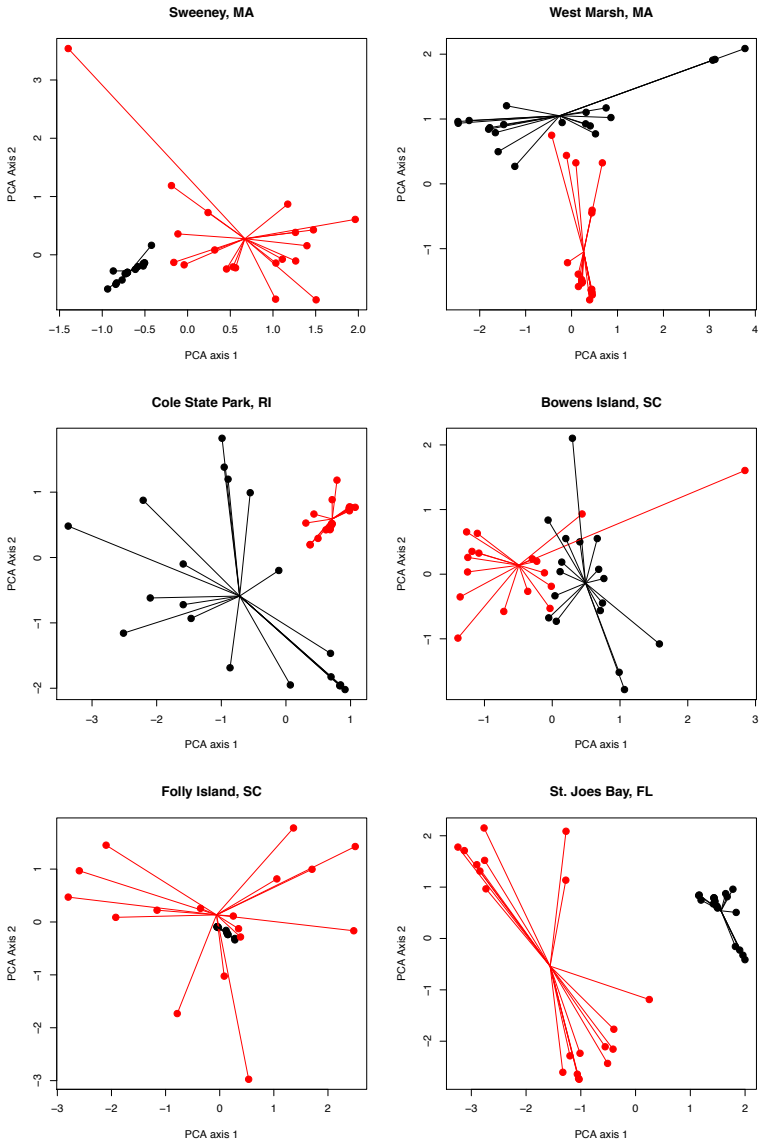

Figure A10. PCA of Stacks-generated hard-called genotypes on 391 variant sites. Red and black points indicate short- and tall-zone plants, respectively.

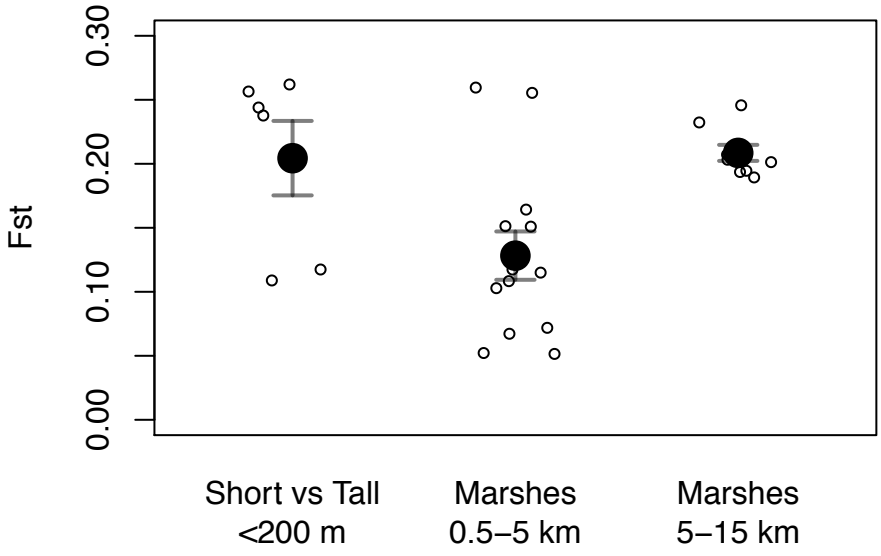

Figure A11. Genetic isolation (mean ± SE of F_{ST}) between short- and tall-zone *Spartina* within a marsh is greater than F_{st} between marshes separated by 0.5 to 5km, but indistinguishable from marshes separated by 5-15 km ($F_{2,16} = 14.939$, $p < 0.001$; Tukey HSD indicates that "Short vs Tall" = "Marshes 5-15 km" > "Marshes 0.5 – 5km").

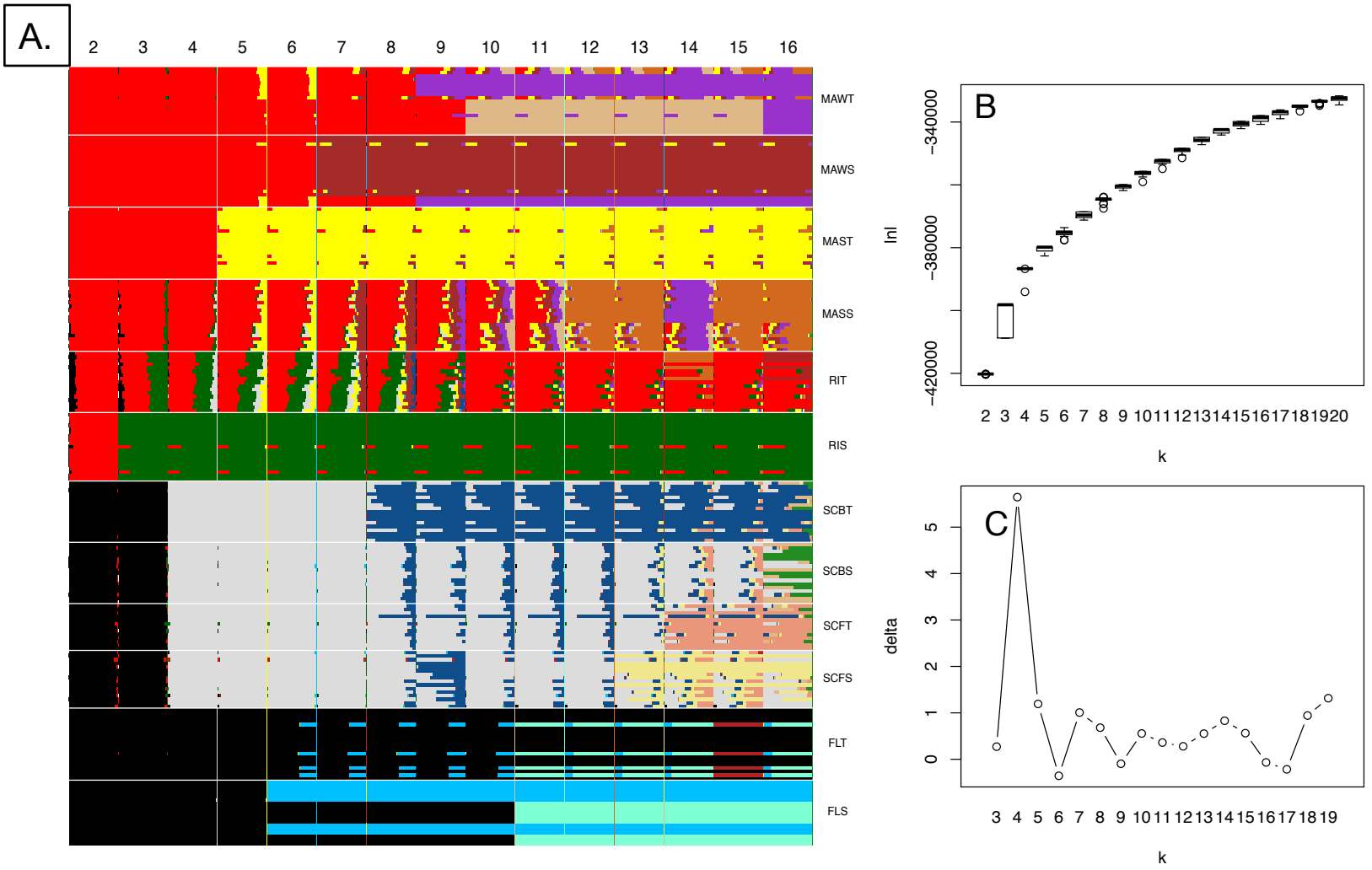

Figure A12. Admixture analysis of only marshes with tall-vs-short comparisons (N = 6). A) Plot of k=2-16 genetic clusters, where colors represent different genetic clusters assigned to each individual. B). Model fit for ngsadmix analysis – all populations. B) Log probability of data L(k) as a function of k across 5 replicate runs for k=2-20. C) Magnitude of delta K (sensu Evanno et al. 2005).

Table A1. Repeated measures analysis of variance (RM ANOVA) of *Spartina alterniflora* maximum stem height, stem density and leaf number over the course of the common garden experiment (April 2015 – March 2016). Model = lm (Response ~ Origin Zone + Origin Site + (1 | pot))

	Max. Height (cm)				**Stem Density**				**Number of Leaves**			
Factor	Num DF	Den DF	F	p-value	Num DF	Den DF	F	p-value	Num DF	Den DF	F	p-value
Origin Zone	1	355	6.12	**0.01**	1	342	1.11	0.29	1	163	6.49	**0.01**
Date	8	886	1356	**< 0.001**	7	747	401.1	**< 0.001**	7	789	342.2	**< 0.001**
Origin Zone * Date	8	885	2.75	**0.005**	7	747	0.35	0.93	7	789	4.25	**< 0.001**
Origin Site	2	301	7.03	**0.001**	2	268	3.89	**0.02**	2	160	3.1	**0.05**

Table A2. Analysis of variance of *Spartina alterniflora* maximum stem height and leaf number for individual dates over the course of the common garden experiment (April 2015 – March 2016). Model = lm (Response ~ Origin Zone + Origin Site, data = individual months); numerator degrees of freedom = 1 for origin zone and = 2 for origin site.

	Maximum stem height					Leaf number				
		Origin Zone		Origin Site			Origin Zone		Origin Site	
Date	**Den DF**	**F**	**P**	**F**	**P**	**Den DF**	**F**	**P**	**F**	**P**
April	146	3.8	0.053	0.37	0.69	146	0.2	0.66	1.33	0.27
June	109	12.91	**0.0005**	1.37	0.26	-	-	-	-	-
July	104	10.23	**0.002**	0.55	0.58	104	7.51	**0.007**	2.83	**0.06**
August	104	5.08	**0.026**	0.44	0.64	104	10.15	**0.002**	1.47	0.23
September	104	5.94	**0.016**	0.72	0.49	104	2.01	0.16	0.79	0.46
October	104	7.79	**0.006**	1.21	0.3	104	1.53	0.22	4.45	**0.02**
November	104	9.31	**0.003**	1.27	0.29	104	0.59	0.44	0.8	0.45
January	104	10.39	**0.002**	1.48	0.23	103	1.04	0.31	1.23	0.3
March	104	11.31	**0.001**	4.15	**0.02**	104	3.85	0.052	0.87	0.42

Table A3. Repeated measures analysis of variance (RM ANOVA) of *Spartina alterniflora* maximum stem height, and stem density over the course of the reciprocal transplant experiment (May – December 2016). Model = lmer (Response ~ Transplant Zone * Origin Zone * Date + Transplant Site + Origin Site + (1|Plot/Transect)). Transplant zone is location (tall vs short) where planted; origin zone is location (tall vs short) where maternal plant was from.

	Max. Height (cm)				**Stem Density**			
Factor	Num DF	Den DF	F	p-value	Num DF	Den DF	F	p-value
Transplant Zone	1	389	10.47	**0.001**	1	446	257.83	**<0.001**
Origin Zone	1	402	58.05	**<0.001**	1	434	0.7	0.4
Date	6	1451	80.31	**<0.001**	7	1765	124.75	**<0.001**
Transplant * Origin Zone	1	396	22.47	**<0.001**	1	437	1.99	0.16
Transplant Zone * Date	6	1451	6.47	**<0.001**	7	1765	65.02	**<0.001**
Origin Zone * Date	6	1453	10.63	**<0.001**	7	1767	0.75	0.63
Transplant * Origin Zone * Date	6	1453	7.35	**<0.001**	7	1767	1.34	0.23
Transplant Site	2	323	0.1	0.9	2	383	9.23	**<0.001**
Origin Site	2	342	7.54	**<0.001**	2	377	1.07	0.34

Table A4. Analysis of variance of *Spartina alterniflora* maximum stem height for each month throughout the reciprocal transplant experiment (May – December 2016). Model = lm (Maximum stem height ~ Transplant Zone * Origin Zone + Transplant Site + Origin Site); data = each individual month). Transplant zone is location (tall vs short) where planted; origin zone is location (tall vs short) where maternal plant was from. Numerator degrees of freedom = 1 for each zone factor and = 2 for each site factor

		Transplant Zone		**Origin Zone**		**Transplant x Origin Zone**		**Transplant Site**		**Origin Site**	
Date	**Den Df**	F	P	F	P	F	P	F	P	F	P
Initial	349	0.12	0.72	0.79	0.37	0.09	0.77	0.04	0.96	9.47	**<0.001**
June	257	10.19	**0.002**	5.87	**0.02**	4.48	**0.04**	0.26	0.77	5.49	**0.005**
July	236	1.22	0.27	8.04	**0.005**	0.93	0.34	1.08	0.34	2.69	0.07
August	213	9.55	**0.002**	17.27	**<0.001**	9.19	**0.002**	0.005	0.99	3.27	**0.04**
September	204	19.86	**<0.001**	10.07	**0.002**	9.67	**0.002**	0.25	0.78	0.69	0.5
October	197	26.51	**<0.001**	12.8	**<0.001**	23.88	**<0.001**	1.83	0.16	4.24	**0.02**
December	207	0.05	0.82	12.81	**<0.001**	7.7	**<0.001**	2.19	0.11	2.65	0.07

Table A5. Analysis of variance of *Spartina alterniflora* stem density for each month throughout the reciprocal transplant experiment (May – December 2016). Model = lm (Stem density ~ Transplant Zone * Origin Zone + Transplant Site) + Origin Site; data = each individual month). Transplant zone is location (tall vs short) where planted; origin zone is location (tall vs short) where maternal plant was from. Numerator degrees of freedom = 1 for each zone factor, and = 2 for each site factor.

		Transplant Zone		**Origin Zone**		**Transplant x Origin Zone**		**Transplant Site**		**Origin Site**	
Date	**Den Df**	F	P	F	P	F	P	F	P	F	P
Initial	349	0.9	0.34	1.11	0.29	0.51	0.48	0.46	0.63	5.37	**0.005**
May	310	28.59	**<0.001**	0.1	0.75	1.31	0.25	1.37	0.26	1.88	0.15
June	259	118.05	**<0.001**	0.32	0.6	1	0.32	3.03	**0.05**	0.86	0.43
July	236	108.84	**<0.001**	0.85	0.36	1.4	0.24	2.74	0.07	0.37	0.69
August	213	86.3	**<0.001**	0.52	0.47	0.4	0.53	2.62	0.07	1.16	0.32
September	204	119.65	**<0.001**	0.13	0.72	0.06	0.81	10.98	**<0.001**	1.08	0.34
October	197	83.92	**<0.001**	0.005	0.95	2.19	0.14	11.86	**<0.001**	0.46	0.63
December	207	76.07	**<0.001**	0.04	0.85	1	0.32	20.49	**<0.001**	0.17	0.84

Table A6. *Spartina* population genetic survey sampling locations. Distance S:T is an estimated great-circle distance between short and tall zones within a single marsh. Ho and H_E are observed and expected heterozygosity, respectively.

Site ID	Site Name	State	Zone	Latitude (ºN)	Longitude (ºW)	Distance S:T (km)	n	Collection Date	H_O	H_E
NH1	Great Bay	NH	unknown	43.11646	-70.85351	–	20	Jul-12	0.264	0.329
MASS	Sweeney	MA	Short	42.72182	-70.84873	0.09	20	Oct-15	0.319	0.334
MAST	Sweeney	MA	Tall	42.72211	-70.84795	–	20	Oct-15	0.322	0.325
MAWS	West	MA	Short	42.73952	-70.84874	0.03	20	Oct-15	0.314	0.320
MAWT	West	MA	Tall	42.73934	-70.84845	–	19	Oct-15	0.305	0.317
RIS	Cole State Park	RI	Short	41.68175	-71.29807	0.02	19	Aug-12	0.303	0.324
RIT	Cole State Park	RI	Tall	41.68153	-71.29826	–	17	Aug-12	0.284	0.344
NC1	Middle Marsh	NC	unknown	34.68933	-76.62108	–	14	Aug-12	0.326	0.327
SCBS	Bowens Island	SC	Short	32.68014	-79.95422	0.10	17	Oct-14	0.333	0.355
SCBT	Bowens Island	SC	Tall	32.68037	-79.95335	–	17	Oct-14	0.319	0.351
SCFS	Folly Beach	SC	Short	32.64429	-79.96561	0.16	16	Oct-14	0.305	0.347
SCFT	Folly Beach	SC	Tall	32.64548	-79.96703	–	13	Oct-14	0.332	0.354
SCFJ	Fort Johnson	SC	Short	32.74887	-79.89853	–	20	Aug-12	0.321	0.351
SC2	Highway 7 Bridge	SC	Tall	32.83056	-79.98524	–	18	Aug-12	0.317	0.352
SC3	Wapoo Cut	SC	Tall	32.76888	-79.95129	–	21	Aug-12	0.306	0.366
FLS	St. Teresa	FL	Short	29.91642	-84.50655	0.05	18	Aug-12	0.276	0.341
FLT	St. Teresa	FL	Tall	29.91657	-84.50607	–	20	Aug-12	0.290	0.336

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