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Provenance-by-environment interaction of reproductive traits in the invasion of *Spartina alterniflora* in China

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Abstract. Ecological invasions are facilitated by pre-adaptation and phenotypic plasticity, upon which evolution can act. The rapid invasion of the intertidal grass *Spartina alterniflora* in China during the last 36 yr is a test case for the roles of these mechanisms. A previous study of *S. alterniflora* in China found strong latitudinal clines in vegetative and sexual traits and concluded that most of this variation was due to phenotypic plasticity. Recent observations suggested provenance-by-environment interactions, and we employed common gardens at multiple latitudes as a test of this idea. Phenotypically, field plant height, which correlates strongly with biomass and other indices of vegetative performance in this species, showed a hump-shaped relationship across 10 sites, covering 19° of latitude; field seed set increased linearly with latitude. To assess the role of plasticity vs. genetic differentiation in these patterns, we grew plants from the ten field sites in three common gardens at low (20.9° N), mid (28.3° N), and high (38.0° N) latitudes to maturity, at 18 months. Plant height varied among common gardens, with the tallest plants at mid latitude, mirroring the field pattern, consistent with the previous study. Within the gardens, latitude of origin also affected plant height. Seed set varied among the gardens, with the greatest values at high latitudes, again mirroring the field pattern and indicating substantial plasticity. Evidence of evolution was found as increasing seed set with latitude among provenances within common gardens. However, the effect differed among common gardens, with the greatest slope in the high-latitude garden, lower slope in the mid-latitude garden, and no relationship in the low-latitude garden, indicating a provenance-by-environment interaction. The number of surviving plants also suggested a provenance-by-environment interaction; no relationship with latitude among provenances in the two southern gardens and increasing survival with latitude in the northern garden. Field seedling density was ~200-fold greater at high than at low latitude sites. The profuse seed germination and recruitment in the north could have created high selection intensity resulting in evolution of reproductive traits at high latitudes with the result that the mechanisms of the invasion differ with latitude.

Key words: biological invasion; genetic differentiation; latitudinal cline; pre-adaptation; provenance-by-environment interaction; recruitment; sexual reproduction; Spartina alterniflora.

Introduction

Biological invasions are facilitated by phenotypic plasticity and pre-adaptation. The former provides adaptation of the species to the range of conditions in its new environment (Richards et al. 2006, Davidson et al. 2011) and the latter is an incidental match between the environments of the origin and destination (Dlugosch and Parker 2007, Jenkins and Keller 2011). Additionally, new genotypes of the invader unique to the new environment created by mutation, intraspecific hybridization (Geiger et al. 2011), and interspecific hybridization (Strong and Ayres 2013) can contribute to colonization and spread.

Disentangling these mechanisms is central to understanding invasions (Colautti and Barrett 2013). Provenance-by-environment interactions are a window into such understanding. These interactions can exist within the structure of introductions and the new environment and serve to drive invasions (Zenni et al. 2014*a*, *b*), and they can develop subsequently to an introduction as the product of evolution in the new environment without a role in promoting the invasion at the outset. While a great deal of attention has been directed toward species spreading into novel environments, a simpler case is the spread of introduced species into an environment that is similar to the native environment, which represents a simple kind of pre-adaptation. Phenotypic plasticity of the introduced entity can add layers of complexity to the pre-adaptation and play a substantial role in invasion without evolutionary change (Dlugosch and Parker 2007). Thus, there is a multidimensional spectrum of null hypotheses about

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pre-adaptation and phenotypic plasticity (Cano et al. 2008, Zenni et al. 2014*a*, Li et al. 2016) for invasions in the absence of or preceding evolutionary change.

We examined these issues working with a recent and dramatic biological invasion. *Spartina alterniflora* is a long-lived clonal plant that was introduced into China in 1979. It is an intertidal native of low energy shores on the Atlantic and Gulf Coasts of North America. In China, it spread rapidly by deliberate planting and natural dispersal to range over 19° (20° - 39° N) of north latitude in less than four decades (An et al. 2007, Zuo et al. 2012, Liu et al. 2016). China now has the largest *Spartina* invasion ever known (Strong and Ayres 2013). Conditions in the new estuarine habitat on the shores of the Bohai Sea, Yellow Sea, East China Sea, and South China Sea are virtually the same as those in the Western Atlantic Ocean where *S. alterniflora* evolved; it is pre-adapted to China where no native vascular plant occupies most of this habitat (Murray et al. 2014, Cui et al. 2016).

Phenotypes of *S. alterniflora* vary considerably over its native range (~27° - 45° N) in North America; this variation correlates with wide variation in abiotic conditions such as annual temperature, annual growing degree days, total precipitation, tide range, soil organic content, and water content (Turner 1976, Kirwan et al. 2009, McCall and Pennings 2012, Crosby et al. 2017). Provenances vary in stem height and diameter, leaf size, flowering time, palatability to herbivores, and other traits. Many of these differences appear to be genetically based because they persist in common-garden experiments (Seneca 1974, Somers and Grant 1981, Seliskar et al. 2002, Salgado and Pennings 2005, Crosby et al. 2015). Molecular analyses show considerable genetic structure in *S. alterniflora* (Hughes 2014) with variation across the native range (Blum et al. 2007). A key vegetative trait of *S. alterniflora* is plant height (Hughes 2014), because it correlates highly with a full suite of other variables related to vegetative growth.

In China, phenotypic plasticity in the vegetative traits of stature, foliage, and biomass is displayed in this species as in the native range, facilitating exploitation of similarly variable habitats on the two continents (Strong and Ayres 2013, Shang et al. 2015, Liu et al. 2016). Microsatellite and chloroplast variation occurs in Chinese *S. alterniflora* in relation to latitude (Guo et al. 2015, Bernik et al. 2016).

A previous study of *S. alterniflora* in China found distinct clines in plant height, shoot density, and sexual reproduction across latitude (Liu et al. 2016). Most of this geographic variation disappeared in a common garden experiment, and only a slight tendency for latitudinal variation in seed set remained; it weakly suggested evolution of this trait. Liu et al. (2016) inferred that the lion's share of variation was due to phenotypic plasticity. The study was performed at low latitude, in a subtropical common garden (23.9° N).

Motivated by new observations of phenotypic differences across latitude, such as profuse seed germination and recruitment at the northern end of the invasion as well as the hint of seed set differentiation (Liu et al. 2016), we explored the possibility that provenance-by-environment interactions (Zenni et al. 2014*a*) had occurred during the invasion; they would be manifested as provenance performance changing with latitude. Our thinking was that the prodigious seed germination and recruitment in the north could have created high selection intensity resulting in evolution of reproductive traits at high latitudes. To this end, using three common gardens, at low, medium, and high latitudes, we studied plants from 10 sites (provenances) that represented the latitudinal range of *S. alterniflora* in China. This set-up allowed us to measure phenotypic plasticity, genetic differentiation, and provenance-by-environment interactions in the traits of this plant. Specifically, we tested the null hypotheses that (1) traits would not vary by provenance in the field, (2) any observed variation by provenance in the field would disappear in common gardens (no genetic differentiation), and (3) any observed variation among provenances that persisted in common gardens would be expressed similarly in all common gardens (no provenance-by-environment interactions). Genetic differentiation was revealed as variation among the provenances within common gardens, and provenance-by-environment interactions as different latitudinal patterns of the provenances across the three common gardens. Phenotypic plasticity in the absence of genetic differentiation was revealed as variation among the common gardens independent of variation within the common gardens.

Materials and Methods

Study species

Spartina alterniflora Loisel., commonly known as smooth cordgrass, is a perennial grass, grows 1–3 m tall, and flowers annually (Mobberley 1953). It has smooth flowers and panicles. The panicle is usually 10–30 cm long and composed of 5–30 spikes alternately arranged and appressed to a main axis with 10–50 sessile spikelets along one side of the axis of each spike (Liu et al. 2016). *Spartina alterniflora* reproduces by two main routes: clonal reproduction by the formation of underground rhizomes and sexual reproduction by flowers to form seeds (Daehler and Strong 1994, Trilla et al. 2009). It was introduced to China from native populations in North Carolina, Georgia, and Florida (Xu and Zhuo 1985). Although there was no reference to a breeding program in China, it is reasonable to infer that people disseminated the seed from the most vigorous plants (Strong and Ayres 2013).

Field survey

To document patterns of latitudinal phenotypic variation, we sampled 10 field sites (provenances). At each site, we made 10 collections of data and seeds in September and October of 2012 and 2013 (Liu et al. 2016). The field sites spanned almost the entire geographic range of *S. alterniflora* in China (Fig. 1). The collections at each field site were at least 30 m apart. For each collection, we sampled a 0.5 × 0.5 m quadrat and measured the height of the three tallest *S. alterniflora* shoots in the quadrat. We also collected 10 inflorescences from each quadrat, distinguishing filled seeds from unfilled seeds (Daehler and Strong 1994, Ayres et al. 2008). For the 2014 multiple common gardens experiment, the filled seeds from each plot were collected in September–October 2013.

Multiple common garden experiments

To assess the role of plasticity and potential genetic differentiation among field sites, we grew the seed of the 10 collections from each field site until maturity at 18 months in three common gardens, at three latitudes: low (20.9° N), mid (28.3° N), and high (38.0° N; Fig. 1). Common gardens were established in the field in the spring of 2014, within the elevational range occupied by *S. alterniflora* in the upper intertidal at each site. Aggressive economic development of coastal wetlands in China has led to intensive reclamation of the shoreline, altered vegetation, and rapid succession to upland vegetation in what were previously high marshes (Murray et al. 2014, Cui et al. 2016). Therefore *S. alterniflora* is now mainly restricted to low marshes, where it dominates the upper part of the remaining intertidal zone. Common garden plantings were located more than 10 m from existing *S. alterniflora* clones. The three common gardens were similar in porewater salinity during the growing season (21–26 PSU). Average temperature, degree days, precipitation, tidal range, soil water content, carbon and nitrogen content, and soil type varied across latitude (Appendix S1: Table S1a and S1b).

Each common garden consisted of 10 plots spaced at least 5 m apart. Each plot consisted of 10 PVC tubes

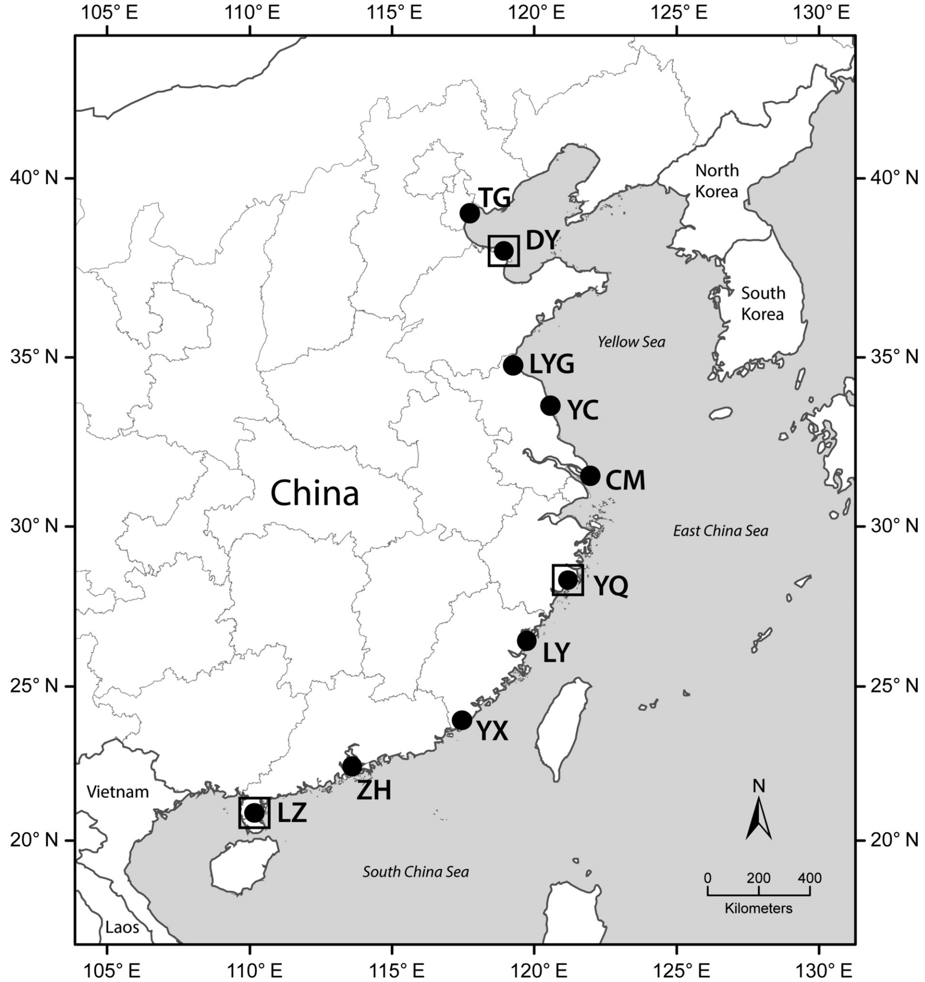

FIG. 1. Map of field sites. Ten survey sites are indicated with circles; three common garden sites are indicated with squares. Sites were TG, Tanggu (39.1° N); DY, Dongying (38.0° N); LYG, Lanyungang (34.8° N); YC, Yancheng (33.6° N); CM, Chongming (31.5° N); YQ, Yueqing (28.3° N); LY, Luoyuan (26.5° N); YX, Yunxiao (23.9° N); ZH, Zhuhai (22.4° N); LZ, Leizhou (20.9° N).

(16 cm in diameter and 33 cm deep, with the top flush with the soil surface), which were grouped into three rows and four columns, separated by ~1 cm. Each provenance was assigned to one of the 10 pipes in the plot. This gave 100 pipes in each common garden.

In April 2014, we placed 10 seeds from a single seed family into each PVC pipe such that each plot contained seeds from all of the 10 latitudinal field sites. The locations of source sites varied haphazardly within each plot. To protect seeds from removal by the tide, we pressed them gently into the surficial sediment to ~1 cm depth. One month later, after seeds had germinated, we thinned the seedlings to one per pipe. We surrounded each plot with a 1.5 m high mesh and PVC fence barrier to reduce grazing from rodents and crabs.

Eighteen months after planting, after two growing seasons, when all plants had reached maturity, we counted the number of surviving collections out of the original 10 collections from each field site of origin, and measured the height of the three tallest shoots in each pipe. When fewer than three shoots were present, we measured all the shoots. We also collected all the mature inflorescences from each pipe before the seeds had shattered, avoiding inflorescences that showed any herbivory. For each inflorescence (1–3 per pipe), we counted the number of spikelets (seeds), distinguishing filled from unfilled seeds; viable seeds are filled (Daehler and Strong 1994). We also measured the number of spikes per inflorescence, the number of seeds per inflorescence, the mass of the tallest stem, the number of ramets per pipe, the proportion of ramets flowering, stem diameter, and aboveground biomass as in Liu et al. (2016).

We sampled the density of seedlings in the field at five of the study sites: Leizhou (20.9° N), Yunxiao (23.9° N), Yueqing (28.3° N), Lianyungang (34.8° N), and Dongying (38.0° N), in March–May of 2016, starting from low latitudes and moving progressively to higher latitudes as the warm season developed and seedlings appeared. At each site, we counted all seedlings within twenty 0.5 × 0.5 m quadrats that were placed haphazardly within the mudflat adjacent to stands of *S. alterniflora*.

Statistical analysis

Field data on plant height and seed set for each of the 10 seed families at each field site (provenance) were averaged across the 2 yr to yield a single data point for each site. We used linear and quadratic regression to analyze the relationships between plant traits (height and seed set) and latitude of origin. To test for plasticity in plant traits (plant height, seed set) among the multiple common gardens, we used one-way ANOVAs (linear models) with Tukey-Kramer HSD contrasts. To explore genetic differentiation within common gardens, we also used linear and quadratic regressions to analyze the relationships between plant traits (height, seed set and survival from germination to harvest at 18 months) and latitude of origin. Provenance-by-environment interactions of plant height, seed set, and survival (whether or not seed families survived to 18 months) were tested by ANCOVA (mixed model of analysis of covariance with random effect of seed family). For seedling survival, data were averaged across the surviving seed families (n = 3–10) from each site within each common garden to give a single value per site per common garden. Data on seed set was square-root (x + 1) transformed to achieve normality of errors and homogeneity of variance. However, we applied a nonparametric statistical test, Kruskal-Wallis (KW), to analyze survival. The effects of latitude of origin on seedling density were examined using natural logarithm regression analysis. All analyses were performed with R statistical software (R Development Core Team 2015). The analyses are described in detail in the Statistical Appendix (Appendix S2: Table S1a, S1b, and S1c).

RESULTS

Over the 10 field sites, plant height showed a hump-shaped relationship with latitude, with the tallest plants at mid latitudes (Fig. 2a). In the three common gardens, plant height (averaged across all provenances) was also greatest in the mid-latitude garden (Fig. 2b, Table 1a). Plant height varied with latitude of origin overall

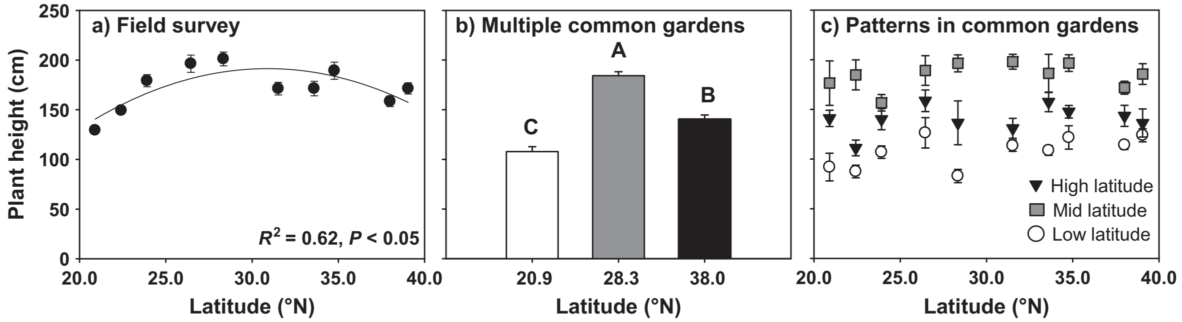

FIG. 2. Height of *Spartina alterniflora*: (a) 10 field sites (provenances) in relation to latitude, (b) overall average for the 10 field sites grown in three common gardens (different letters indicate $P < 0.05$; ANOVA with Tukey-Kramer HSD), (c) averages of each of the ten collections grown from each site of origin in each of the three common gardens. No trend lines are shown because we found no relationships between plant height and latitude of origin for any of the three individual common gardens. Values are means and SE; error bars are not shown if smaller than symbols.

TABLE 1. Common garden results: (a) plant height, (b) seed set, and (c) survival.

Source	df	*F*	*P*
a) Height			
Garden sites	2,2	184.55	**<0.0001**
Latitude^2	1,9	5.52	**0.02**
Garden sites × latitude^2	2,18	0.86	0.65
b) Seed set			
Garden sites	2,2	71.23	**<0.0001**
Latitude	1,9	12.27	**0.0005**
Garden sites × latitude	2,18	11.01	**0.004**
c) Survival			
Garden sites	2,2	8.48	**0.01**
Latitude	1,9	35.81	**<0.0001**
Garden sites × latitude	2,18	2.44	0.30

Note: Each variable was analyzed with ANCOVA (mixed models), with common garden sites, latitude (or latitude^2), and garden sites × latitude as fixed factors, and seed family as a random effect. Significant *P* values ($P<0.05$) are in bold.

(Table 1a), but not within any of the three individual gardens (Fig. 2c). Mean plant height in the common gardens was similar to that in the natural stands in the field (Fig. 2a, b).

Seed set (proportion of seeds that were filled) increased sharply with latitude over the ten field sites in nature, ranging from <10% at low latitudes to >80% at high latitudes (Fig. 3a). Seed set (averaged across all provenances) in the three common gardens also increased from low to high latitudes (Fig. 3b, Table 1b). In contrast to plant height, seed set was strongly affected by provenance. Plants originating from high latitudes set more seed (Fig. 3c, Table 1b). This pattern, however, differed among common gardens, with the greatest slope in the high-latitude garden, a lower slope in the mid-latitude garden and no relationship in the low-latitude garden, indicating a provenance-by-environment interaction (Table 1b). Overall, seed set in the common gardens was somewhat lower than in the field, ranging up to ~60% vs. >80% in the natural stands in the field (Fig. 3a, b).

We studied survival of germinating seeds until 18 months in the common gardens. Overall survival, without regard to provenance, did not vary among common gardens (Fig. 4a). Survival increased with latitude among sites without regard for particular common gardens (Fig. 4b, Table 1c), a pattern that suggests evolution of traits affecting survival in relation to latitude. Comparing gardens, there was no significant relationship between survival and latitude for the two most southern gardens, but survival increased with latitude among sites in the northern garden (Fig. 4b). This significant interaction between common garden and latitude of the site of origin suggests a provenance-by-environment interaction for survival. It is consistent with the provenance-by-environment interaction in seed set, with the greatest influence in the north. Several other traits (number of spikes per inflorescence, number of seeds per inflorescence, mass of the tallest stem, the number of ramets per pipe, the proportion of ramets flowering, and aboveground biomass) varied among common gardens, but did not vary with latitude of origin (data not shown). Stem diameter did not vary with either common garden or latitude of origin (data not shown).

At low latitudes in the field, seedlings were so rare as to be absent from most sampling quadrats, but at high latitudes they were very dense (>200 seedlings/m^2; Fig. 5). We saw this pattern during our studies across latitudes in 2012–2015 (W. Liu and Y. Zhang, *personal observations*), and measured it in 2016.

DISCUSSION

We found that there was a significant, positive relationship between latitude of origin and seed set that was observed in both the field survey and the two higher latitude common gardens (Fig. 3a, c), indicating that genotypes from high latitudes set more viable seed than genotypes from low latitudes. These data indicate that a clear latitudinal cline in sexual reproduction of *Spartina alterniflora* has developed since its introduction

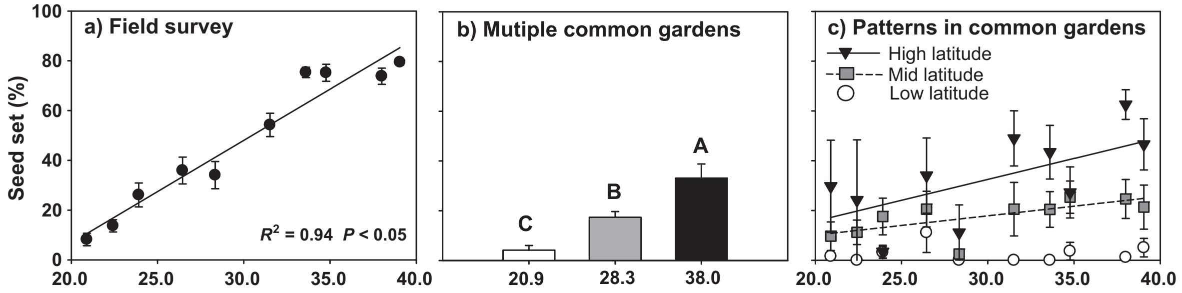

FIG. 3. Seed set of *Spartina alterniflora*: (a) 10 field sites (provenances) in relation to latitude, (b) overall average for the 10 field sites grown in three common gardens (different letters indicate $P < 0.05$; ANOVA with Tukey-Kramer HSD), (c) averages of each of the 10 collections grown from each site of origin in each of the three common gardens. Trend lines indicate relationships between seed set and latitude of origin for the high- and mid-latitude common gardens (high-latitude, $R^2 = 0.45$, $P < 0.05$; mid-latitude, $R^2 = 0.42$, $P < 0.05$). Values are means and SE; error bars are not shown if smaller than symbols.

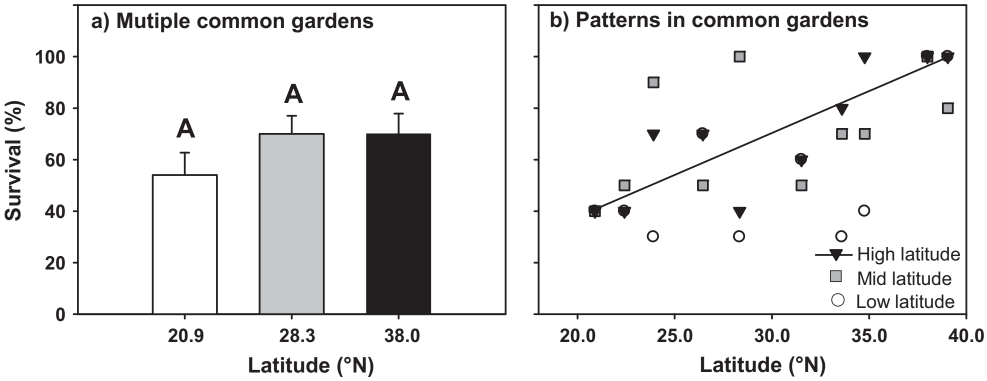

FIG. 4. Survival of *Spartina alterniflora* from germination to harvest at 18 months: (a) overall average for the 10 field sites grown in three common gardens (different letters indicate $P < 0.05$; ANOVA with Tukey-Kramer HSD), (b) survival from each site of origin in each of the three common gardens. Trend lines indicate relationships between survival per site and latitude of origin for the high-latitude common garden ($R^2 = 0.71$, $P < 0.05$). Values are means and SE.

to China 36 yr ago. Moreover, plants from high latitudes also had higher seedling survival in the high-latitude common garden. Because clines in both reproductive traits were observed in a common garden, these differences are likely genetically based. The field increase in seedling densities with latitude is consistent with the observed higher seed set and survival at high latitudes (Table 1b, c). In contrast to seed set, we found no evidence for genetic based variation in plant height along latitude in the field or in common gardens (Fig. 2a, c), which indicates great plasticity in vegetative growth (Fig. 2b).

Spartina alterniflora was pre-adapted to its new habitat on the Chinese coast when it was introduced in 1979 (Xu and Zhuo 1985, An et al. 2007). In its native range on the East and Gulf Coasts of North America, this plant occupies vast swaths of the intertidal zone between mean sea level and mean high water in low-energy habitats (McKee and Patrick 1988, Morris et al. 2002). The geographic ranges of Chinese and native North American *S. alterniflora* overlap broadly in latitude and have similar climates. Moreover, although a number of native species in China overlap with the upper intertidal distribution of *S. alterniflora* (Li et al. 2009, Zhang et al. 2012), no native vascular plant grows in the lower part of *S. alterniflora*'s elevational niche. Thus, when *S. alterniflora* arrived in China, it was presented with abundant, empty suitable habitat, allowing rapid and unhindered spread.

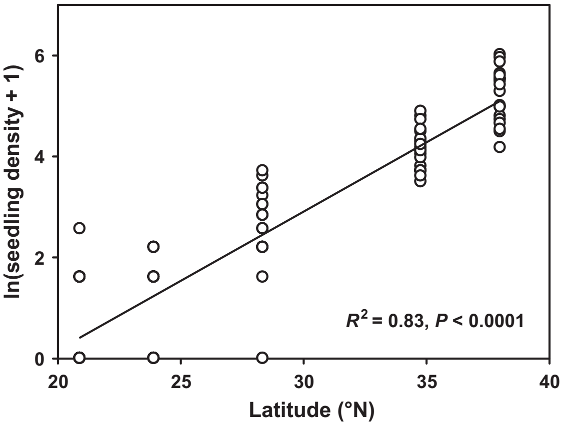

FIG. 5. Seedling density in the field in relation to latitude in March–May of 2016.

Although *S. alterniflora* has spread over ~19° of latitude in China (An et al. 2007, Zuo et al. 2012, Liu et al. 2016), the introduced plants came from three locations in the southern part of the native range: North Carolina, Georgia, and Florida (Xu and Zhuo 1985), that represent only 7° of latitude. The high degree of pre-existing phenotypic plasticity characteristic of this species (Richards et al. 2005) facilitated its spread (Liu et al. 2016).

While previous work found distinct clines in plant height, shoot density, and sexual reproduction across latitude in China, most of this geographic variation disappeared in a common garden; the tendency for latitudinal variation in seed set over latitude within the common garden was weak, but suggestive of evolutionary differentiation in this trait (Liu et al. 2016). The inference was that most of the variation was due to phenotypic plasticity. The single common garden in this 2016 study was subtropical, 23.9° N.

The results in the current manuscript build upon the previous work by clearly showing that *S. alterniflora* has evolved a latitudinal cline in sexual reproduction since its introduction to China 36 yr ago. Plants from high latitudes set more seed and have higher seedling survival, consistent with higher seedling densities in the field at high latitudes. Moreover, these latitudinal clines were most strongly expressed in common gardens located at high latitudes, a provenance-by-environment interaction.

Results from the common gardens were consistent with both field data and previous studies, lending confidence to our interpretations. The hump-shaped pattern of shoot height across the three common gardens was consistent with field patterns, and the actual values of shoot height were very similar to those observed in the field. The increasing seed set with latitude in the two northern gardens was consistent with field patterns. *S. alterniflora* is wind pollinated (Davis et al. 2004) and outcrossing (Sloop et al. 2009), so the lower seed set in the common gardens than in the field was perhaps a result of their greater distance to pollen sources (Dennis et al. 2011). For seed set, Liu et al. (2016) worked at a single garden at a site between our low and mid-latitude gardens and observed levels of seed set intermediate to those reported here from this pair of gardens.

Our results suggest that the causes and consequences of *S. alterniflora* spread in China differ with latitude. In the far north, plants set more seed, seedling survival is higher, and seedlings are much more abundant in the field (Deng et al. 2009), consistent with the spread being affected by sexual reproduction. In contrast, at low latitudes, plants set much less seed, seedling survival is lower, and seedlings are not as abundant in the field (Li et al. 2014). As a result, although new clones do originate from sexual reproduction at the south end of the range, there are few seedlings. Where *S. alterniflora* was introduced to the West Coast of North America, differences among invasion sites in seed set and clonal expansion also occurred; and in San Francisco Bay, sexual reproduction and self fertilization evolved as introduced lines hybridized with native *Spartina foliosa* (Sloop et al. 2009, Strong and Ayres 2013, Bernik et al. 2016).

Sexual reproduction of *S. alterniflora* has not been much studied in the native range (Fang et al. 2004), and we lack information on variation in sexual reproduction with latitude in the native range. High seedling densities are not often observed in the native range of *S. alterniflora* (D. R. Strong and S. C. Pennings, *personal observations*), and dense clonal stands usually occupy most of the habitat. Seedlings are observed after disturbances such as storms that destroy *S. alterniflora* and when plants colonize new habitat such as developing sandbars (D. R. Strong and S. C. Pennings, *personal observations*). Thus, the current evolution of profuse sexual reproduction at high latitudes in the invasive range may be a transitory phenomenon that will disappear once *S. alterniflora* spreads to fill the intertidal habitat in China.

The rapid spread and impressive performance of *S. alterniflora* in China was likely due to pre-adaptation and phenotypic plasticity followed by rapid evolution. *S. alterniflora* was pre-adapted to conditions in China, which overlap broadly with climate and edaphic conditions in the native range in the United States. *S. alterniflora* occupies a wide range of conditions in the native range, and matches its phenotype to these conditions through a high degree of phenotypic plasticity (Turner 1976, Kirwan et al. 2009, McCall and Pennings 2012, Crosby et al. 2017). As a result, it was able to flourish and spread across a wide range of conditions in China. We previously documented striking variation in *S. alterniflora* traits across latitude in China (Liu et al. 2016). This variation was largely a plastic response to variation in temperature, growing degree days, tidal range and soil nitrogen content across the introduced range, with many of these phenotype–environment correlations matching those seen in the native range (Liu et al. 2016). Here we have built on that result by showing that reproductive traits exhibit both plastic and genetically based variation across latitude, with sexual reproduction far more important at high than low latitudes. In its native range, *S. alterniflora* exhibits genetically based morphological variation across latitude that underlies considerable phenotypic plasticity (Seneca 1974, Somers and Grant 1981, Seliskar et al. 2002, Salgado and Pennings 2005, Crosby et al. 2015). We suggest that plants in the introduced range in China are starting to evolve similar genetic clines across latitude. Some of this evolution is likely due to variation across latitude in the mechanisms by which *S. alterniflora* is spreading, with sexual reproduction far more important at high than low latitudes. Why sexual reproduction is favored at high vs. low latitudes will require more work to elucidate, but it is possible that the high temperatures and increased consumer pressure (Li et al. 2014) typical of low latitudes are unfavorable for seedling survival.

Our work points out the importance of studying introduced species across the entire geographic range of the introduction. If we had worked only at a single high- or low-latitude site, we would have come to opposite conclusions about the importance of sexual reproduction in the spread of this exotic species. Moreover, the general importance of phenotypic plasticity and evolutionary change in facilitating the invasion was only apparent at the geographic scale.

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LITERATURE CITED

An, S. Q., B. H. Gu, C. F. Zhou, Z. S. Wang, Z. F. Deng, Y. B. Zhi, H. L. Li, L. Chen, D. H. Yu, and Y. H. Liu. 2007. *Spartina* invasion in China: implications for invasive species management and future research. Weed Research 47: 183–191.

Ayres, D. R., K. Zaremba, C.M. Sloop, and D.R. Strong. 2008. Sexual reproduction of cordgrass hybrids (Spartina foliosa x alterniflora) invading tidal marshes in San Francisco Bay. Diversity and Distributions 141:187–195.

Bernik, B. M., H. S. Li, and M. J. Blum. 2016. Genetic variation of *Spartina alterniflora* intentionally introduced to China. Biological Invasions 18:1485–1498.

Blum, M. J., K. J. Bando, M. Katz, and D. R. Strong. 2007. Geographic structure, genetic diversity and source tracking of *Spartina alterniflora*. Journal of Biogeography 34:2055–2069.

Cano, L., J. Escarré, I. Fleck, J. M. Blanco-Moreno, and F. X. Sans. 2008. Increased fitness and plasticity of an invasive species in its introduced range: a study using *Senecio pterophorus*. Journal of Ecology 96:468–476.

Colautti, R. I., and S. C. H. Barrett. 2013. Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342:364–366.

Crosby, S. C., A. Angermeyer, J. M. Adler, M. D. Bertness, L. A. Deegan, N. Sibinga, and H. M. Leslie. 2017. *Spartina alterniflora* biomass allocation and temperature: implications for salt marsh persistence with sea-level rise. Estuaries and Coasts 40:213–223.

Crosby, S. C., M. Ivens-Duran, M. D. Bertness, E. Davey, L. A. Deegan, and H. M. Leslie. 2015. Flowering and biomass allocation in US Atlantic coast *Spartina alterniflora*. American Journal of Botany 102:669–676.

Cui, B. S., Q. He, B. H. Gu, J. H. Bai, and X. H. Liu. 2016. China's coastal wetlands: understanding environmental changes and human impacts for management and conservation. Wetlands 36:1–9.

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

Davidson, A. M., M. Jennions, and A. B. Nicotra. 2011. Do invasive species show higher phenotypic plasticity than native species and if so, is it adaptive? A meta-analysis. Ecology Letters 14:419–431.

Davis, H. G., C. M. Taylor, J. G. Lambrinos, and D. R. Strong. 2004. Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*). Proceedings of the National Academy of Sciences USA 101:13804–13807.

Deng, Z. F., Z. W. Deng, S. Q. An, Z. S. Wang, Y. H. Liu, Y. Ouyang, C. F. Zhou, Y. B. Zhi, and H. L. Li. 2009. Habitat choice and seed–seedling conflict of *Spartina alterniflora* on the coast of China. Hydrobiologia 630:287–297.

Dennis, B., J. C. Civille, and D. R. Strong. 2011. Lateral spread of invasive *Spartina alterniflora* in uncrowded environments. Biological Invasions 13:401–411.

Dlugosch, K. M., and I. M. Parker. 2007. Molecular and quantitative trait variation across the native range of the invasive species *Hypericum canariense*: evidence for ancient patterns of colonization via pre-adaptation? Molecular Ecology 16: 4269–4283.

Fang, X. B., P. K. Subudhi, B. C. Venuto, and S. A. Harrison. 2004. Mode of pollination, pollen germination, and seed set in smooth cordgrass (*Spartina alterniflora*, Poaceae). International Journal of Plant Sciences 165:395–401.

Geiger, J. H., P. D. Pratt, G. S. Wheeler, and D. A. Williams. 2011. Hybrid vigor for the invasive exotic Brazilian peppertree (*Schinus terebinthifolius* Raddi., Anacardiaceae) in Florida. International Journal of Plant Sciences 172:655–663.

Guo, W., S. Qiao, Y. Wang, S. Shi, F. Tan, and Y. Huang. 2015. Genetic diversity, population structure, and genetic relatedness of native and non-native populations of *Spartina alterniflora* (Poaceae, Chloridoideae). Hydrobiologia 745:313–327.

Hughes, A. R. 2014. Genotypic diversity and trait variance interact to affect marsh plant performance. Journal of Ecology 102:651–658.

Jenkins, C., and S. R. Keller. 2011. A phylogenetic comparative study of preadaptation for invasiveness in the genus *Silene* (Caryophyllaceae). Biological Invasions 13:1471–1486.

Kirwan, M. L., G. R. Guntenspergen, and J. T. Morris. 2009. Latitudinal trends in *Spartina alterniflora* productivity and the response of coastal marshes to global change. Global Change Biology 15:1982–1989.

Li, J. M., L. Du, W. B. Guan, F. H. Yu, and M. van Kleunen. 2016. Latitudinal and longitudinal clines of phenotypic plasticity in the invasive herb *Solidago canadensis* in China. Oecologia 182:755–764.

Li, Z. J., W. Q. Wang, and Y. H. Zhang. 2014. Recruitment and herbivory affect spread of invasive *Spartina alterniflora* in China. Ecology 95:1972–1980.

Li, B., et al. 2009. *Spartina alterniflora* invasions in the Yangtze River estuary, China: an overview of current status and ecosystem effects. Ecological Engineering 35:511–520.

Liu, W. W., K. Maung-Douglass, D. R. Strong, S. C. Pennings, and Y. H. Zhang. 2016. Geographical variation in vegetative growth and sexual reproduction of the invasive *Spartina alterniflora* in China. Journal of Ecology 104:173–181.

McCall, B. D., and S. C. Pennings. 2012. Geographical variation in salt marsh structure and function. Oecologia 170:777–787.

Mckee, K. L., and W. H. Patrick. 1988. The relationship of smooth cordgrass (*Spartina alterniflora*) to tidal datums: a review. Estuaries 11:143–151.

Mobberley, D. G. 1953. Taxonomy and distribution of the genus *Spartina*. PhD dissertation, Iowa State College, Ames, Iowa, USA.

Morris, J. T., P. V. Sundareshwar, C. T. Nietch, B. Kjerfve, and D. R. Cahoon. 2002. Responses of coastal wetlands to rising sea level. Ecology 83:2869–2877.

Murray, N. J., R. S. Clemens, S. R. Phinn, H. P. Possingham, and R. A. Fuller. 2014. Tracking the rapid loss of tidal wetlands in the Yellow Sea. Frontiers in Ecology and the Environment 12:267–272.

R Development Core Team. 2015. R: a language and environment for statistical computing, version 3.3.2. R Project for Statistical Computing, Vienna, Austria. www.r-project.org

Richards, C. L., O. Bossdorf, N. Z. Muth, J. Gurevitch, and M. Pigliucci. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9:981–993.

Richards, C. L., S. C. Pennings, and L. A. Donovan. 2005. Habitat range and phenotypic variation in salt marsh plants. Plant Ecology 176:263–273.

Salgado, C. S., and S. C. Pennings. 2005. Latitudinal variation in palatability of salt-marsh plants: are differences constitutive? Ecology 86:1571–1579.

Seliskar, D. M., J. L. Gallagher, D. M. Burdick, and L. A. Mutz. 2002. The regulation of ecosystem functions by ecotypic variation in the dominant plant: a *Spartina alterniflora* salt-marsh case study. Journal of Ecology 90:1–11.

Seneca, E. D. 1974. Germination and seedling response of Atlantic and Gulf coasts populations of *Spartina alterniflora*. American Journal of Botany 61:947–956.

Shang, L., S. Y. Qiu, J. X. Huang, and B. Li. 2015. Invasion of *Spartina alterniflora* in China is greatly facilitated by increased growth and clonality: a comparative study of native and introduced populations. Biological Invasions 17:1327–1339.

Sloop, C. M., D. R. Ayres, and D. R. Strong. 2009. The rapid evolution of self-fertility in *Spartina* hybrids (*Spartina alterniflora* ×

foliosa) invading San Francisco Bay, CA. Biological Invasions 11:1131–1144.

Somers, G. F., and D. Grant. 1981. Influence of seed source upon phenology of flowering of *Spartina alterniflora* Loisel. and the likelihood of cross pollination. American Journal of Botany 68:6–9.

Strong, D. R., and D. R. Ayres. 2013. Ecological and evolutionary misadventures of *Spartina*. Annual Review of Ecology, Evolution, and Systematics 44:389–410.

Trilla, G. G., P. Kandus, V. Negrin, R. Vicari, and J. Marcovecchio. 2009. Tiller dynamic and production on a SW Atlantic *Spartina alterniflora* marsh. Estuarine, Coastal and Shelf Science 85:126–133.

Turner, R. E. 1976. Geographic variations in salt marsh macrophyte production: a review. Contributions in Marine Science 20:47–68.

Xu, G. W., and R. Z. Zhuo. 1985. Preliminary studies of introduced *Spartina alterniflora* Loisel in China [In Chinese with English abstract.]. Journal of Nanjing University (Natural Science) 40:212–225.

Zenni, R. D., J. K. Bailey, and D. Simberloff. 2014*a*. Rapid evolution and range expansion of an invasive plant are driven by provenance-environment interactions. Ecology Letters 17: 727–735.

Zenni, R. D., J.-B. Lamy, L. J. Lamarque, and A. J. Porté. 2014*b*. Adaptive evolution and phenotypic plasticity during naturalization and spread of invasive species: implications for tree invasion biology. Biological Invasions 16: 635–644.

Zhang, Y. H., G. M. Huang, W. Q. Wang, L. Z. Chen, and G. H. Lin. 2012. Interactions between mangroves and exotic *Spartina* in an anthropogenically disturbed estuary in southern China. Ecology 93:588–597.

Zuo, P., S. H. Zhao, C. A. Liu, C. H. Wang, and Y. B. Liang. 2012. Distribution of *Spartina* spp. along China's coast. Ecological Engineering 40:160–166.

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