The Role of Genetic Factors in the Differential Invasion Success of Two *Spartina* Species in China

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Biological invasions have become one of the greatest threats to global biodiversity and ecosystem conservation. Most previous studies have revealed how successful invasive species adapt to new environments and climate change through phenotypic and genetic evolution. Some researchers suggested that understanding unsuccessful or less successful biological invasions might be important for understanding the relationships between invasion adaptability and climate factors. We compared the sexual reproduction ability, genetic diversity, and gene × environment interaction in two intentionally introduced alien species in China (*Spartina anglica* and *Spartina alterniflora*) based on restriction site-associated DNA (RAD) sequencing. After more than 50 years, the distribution of *S. alterniflora* has rapidly expanded, while *S. anglica* has experienced extreme dieback. A total of 212,939 single nucleotide polymorphisms (SNPs) for the two *Spartina* species were used for analysis. The multilocus genotype (MLG) analysis revealed that clonal reproduction was the prevalent mode of reproduction in both species, indicating that a change in the mode of reproduction was not the key factor enabling successful invasion by *Spartina*. All genetic diversity indicators (H_e, H_o, π) in *S. alterniflora* populations were at least two times higher than those in *S. anglica* populations, respectively ($p < 0.001$). Furthermore, the population genetic structure and stronger patterns of climate-associated loci provided support for rapid adaptive evolution in the populations of *S. alterniflora* in China. Altogether, our results highlight the importance of genetic diversity and local adaptation, which were driven by multiple source populations, in increasing the invasiveness of *S. alterniflora*.

Keywords: *Spartina alterniflora*, biological invasions, RAD, genetic paradox, environmental adaptation

INTRODUCTION

Biological invasions have become one of the greatest threats to global biodiversity and ecosystem conservation (Clavero and García-Berthou, 2005; Simberloff et al., 2013; Early et al., 2016). Understanding the mechanisms of invasion would contribute to methodologies for its prevention and control. In recent years, many researchers have explored invasion mechanisms by examining the genetic backgrounds of invasive alien species, especially the links between genetic diversity, bottlenecks, founder events, adaptive evolution, and invasion success (Dlugosch and Parker, 2008; Prentis et al., 2008; Estoup et al., 2016). Although the genetic diversity of invasive

populations may be reduced due to founder events and genetic bottlenecks, most successful invasive species can overcome these negative effects through multiple introductions, phenotypic plasticity, asexual reproduction and hybridization. For instance, *Ambrosia artemisiifolia* invaded European, Australian and Chinese areas with high levels of genetic diversity due to multiple introductions (Genton et al., 2005; Gaudeul et al., 2011; van Boheemen et al., 2017; Li et al., 2019b). Phenotypic plasticity rather than genetic adaptation helps *Alternanthera philoxeroides* (Geng et al., 2007; Roman and Darling, 2007) and *Spartina densiflora* (Castillo et al., 2018) colonize a wide range of habitats. Some populations of *Eichhornia crassipes* in China can successfully invade without genetic variation via clonal reproduction in the introduced range (Ren et al., 2005; Roman and Darling, 2007). *Spartina anglica* successfully invaded the British Isles (Raybould et al., 1991) through interspecies hybridization. Therefore, disadvantages associated with founder events might be overstated (Estoup et al., 2016), and genetic diversity does not necessarily predict invasion success (Roman and Darling, 2007).

Some researchers hypothesized that understanding unsuccessful or less successful biological invasions is also important for determining the role of genetic diversity and bottlenecks in invasion success (Fridley et al., 2007; Roman and Darling, 2007; Zenni and Nuñez, 2013). Comparing genetic and environmental characteristics between successful and unsuccessful populations can also help reveal mechanisms producing fitness variations in different environments, which are valuable in studies of genotype-by-environment interactions in the introduced range (Lee, 2002). Wellband et al. (2017) showed that allelic diversity reduction was associated with lower invasion success by comparing highly successful and less successful invasive species of gobies and tunicates. Following this study, the authors used functional genetic markers to test the link between genetic selection and the invasion success of two pairs of invasive goby species. They proposed that increased evolutionary potential in invaded ranges may be associated with invasion success (Wellband et al., 2018). However, studies on unsuccessful or less successful invasions of different biological groups remain limited.

Spartina anglica and *Spartina alterniflora* are perennial grasses native to British estuaries and the Atlantic and Gulf Coast estuaries of North America, respectively. *S. anglica* originated by natural chromosome doubling of an infertile hybrid (*S. ×townsendii*) between the European native species *S. maritima* and the alien species *S. alterniflora* (Groves and Groves, 1880). The new fertile dodecaploid species has rapidly expanded in salt marshes in several European countries (Gray and Raybould, 1991). As *S. anglica* and *S. alterniflora* are "ecological engineers," their histories of introduction in China were recorded in detail. While *S. anglica* populations expanded rapidly on the European continent, they experienced dieback in coastal China. The species was introduced to China from England in 1963 (Chung, 1993), with planting sites as far as Dongow, Liaoning, in the north, and Hepu, Guangxi, in the south and a total planting area of almost 36,000 ha in 1981. *S. alterniflora* was introduced to China later from North Carolina, Georgia, and Florida of North America in

1979 (Chung, 1993). However, after more than 50 years, there were only three sites with *S. anglica* in Jiangsu and Zhejiang in 2000, with the area shrunken to 50 ha, whereas the area of *S. alterniflora* had expanded to 112,000 ha, as reported by An et al. (2007).

Most studies have proposed that the coexistence of various intraspecific hybrids, and mixtures of the three ecotypes sampled from their native ranges is the main reason for the wide spread of *S. alterniflora* (Xia et al., 2015), even though the genetic diversity of populations in China was lower than that in native populations (Deng et al., 2007; Xia et al., 2015; Bernik et al., 2016). Such genetic admixture within or between species in the invaded range may allow them to adapt to local conditions via increases in heterozygote frequency and the production of novel genotypes (Ellstrand and Schierenbeck, 2006; Schierenbeck and Ellstrand, 2008; Rius and Darling, 2014). On the basis of chloroplast sequence analysis and reciprocal transplant experiments, Qiao et al. (2019) reported that genetic admixture has facilitated the evolution of super competitive genotypes of *S. alterniflora* in China and that the super competitive genotypes could overcome the negative correlation between plant height and shoot regeneration in the invasive range. In addition, some studies have indicated that a lack of nitrogen in soil (Li et al., 2007), high plant density (Li et al., 2009), and unsuitable soil texture (Liu et al., 2016a) can cause dieback in *S. anglica*. One important reason for the failure of *S. anglica* in China is weak sexual propagation, while *S. alterniflora* had a strong capacity for both sexual and asexual reproduction (Chung, 1985; Xu and Zhuo, 1985; Xiao et al., 2011). Li et al. (2008) studied the breeding system of the species in one field site and a greenhouse, and the results implied that protogyny, poor pollen quality, and abnormal pollen grains and pollen tubes were the main causes of low seed production in *S. anglica* in coastal China. However, no studies have compared the genetic characteristics and genotype-by-environment interactions of the successful invader *S. alterniflora* and the less successful invader *S. anglica*.

Moreover, the distribution areas of *S. anglica* have become unclear in recent years. As reported by An et al. (2007), there were no populations of *Spartina* in Liaoning. Based on geographical information systems (GISs), global positioning systems (GPSs), and on-site investigations in 2007, Zuo et al. (2012) found 1 ha of *S. anglica* in Liaoning, 10 ha in Hebei, 3 ha in Shandong, 1 ha in Jiangsu, and 1 ha in Guangdong. The authors found that populations of *S. alterniflora* were also distributed in these provinces and occupied larger areas than *S. anglica*. Zhang et al. (2017) also reported one population of *S. alterniflora* in Liaoning but no *S. anglica*. In 2019, we found and identified *S. anglica* in Dandong and Huludao cities of Liaoning Province. We chose four populations of *S. alterniflora*, which are distributed from north to south in China, for comparison with the two populations of *S. anglica*. We used restriction site-associated DNA (RAD) sequencing (Baird et al., 2008) to improve the resolution to detect finer-scale spatial patterns of genetic structure and diversity. This sequencing method allows concurrent single nucleotide polymorphism (SNP) identification and genotyping via high-throughput sequencing of flanking regions of restriction enzyme digestion sites dispersed throughout the genome. Here, we

addressed three main questions: (1) Did low sexual reproduction cause the dieback of *S. anglica* in coastal China? (2) Does the successful invasive species *S. alterniflora* have higher genetic diversity than the less successful invasive species *S. anglica*? (3) Do the highly successful invasive species *S. alterniflora* and less successful invasive species *S. anglica* differ in their levels of genetic adaptation? We also expected to provide evidence to clarify the role of genetic diversity in the colonization or invasion failure of species.

MATERIALS AND METHODS

Sample Collection and Species Confirmation

We collected leaf samples from 88 individuals of *Spartina* from six sites (14–15 leaf samples per site) along the coastline of China in October 2018 (**Supplementary Figure 1**). From north to south, the six populations were distributed in Dandong (DD), Huludao (HLD), Tanggu (TG), Cixi (CX), Quanzhou (QZ), and Fangchenggang (FCG). Information on the numbers of individuals and locations of populations is listed in **Table 1**. Within each population, the distance between each pair of sampled individuals was approximately 10 m. We also obtained three pieces of soil samples from each site, and the depth of the soil samples was 0–25 cm.

According to the manufacturer's instructions, genomic DNA was extracted from the leaves using the EasyPure Plant Genomic DNA Kit (Beijing TransGen Biotech Co., Ltd., Beijing, China). Based on the published nrITS sequences of 145 species of *Spartina* available from GenBank,[1] nrITS (KM010334 of *S. anglica* and KM010330 of *S. alterniflora*) was used to identify *S. anglica* and *S. alterniflora* (Peterson et al., 2014). We confirmed that the HLD and DD populations were *S. anglica* and the other four populations were all *S. alterniflora* through multiple sequence alignment and neighbor joining (NJ) tree construction with 1,000 bootstrap by MAFFT v. 7 (Katoh et al., 2019; **Supplementary Figure 2**).

Sequencing and Genotyping

All RAD libraries were created using 200 ng of genomic DNA from samples of *Spartina*. This DNA was double-digested with *Eco*RI and *Mse* l (New England Biolab, Beverly, MA, United States) and ligated to two-end adapters, allowing the resulting amplified fragments to bind to Illumina flow cells and uniquely identify the individual. All libraries with insert sizes of 300–500 bp were isolated by gel extraction and sequenced on the Illumina XTen platform using 150 nt paired-end reads (Jierui Biotech, Guangzhou, China). A total of ∼260 GB of data was obtained from Illumina sequencing of 88 samples from six populations. The data for each sample were split and filtered using the *process_radtags* module of Stacks 2.3e (Catchen et al., 2011). We removed reads with low quality, missing the restriction site or linked with incorrect barcodes. We combined each end of the retained reads from each sample. Here, we set two as

[1]www.ncbi.nlm.nih.gov/genbank

the minimum depth of coverage (m) required to create a stack and three as the maximum distance (in nucleotides) allowed between stacks (M) within an individual. We then used the *cstacks* module in Stacks to build the catalogs for all these individuals, with two as the maximum number of mismatches allowed between individuals (n). We finally used the *cstacks, ustacks,* and *populations* modules in Stacks to obtain SNPs. We obtained the loci using the following limits in the *populations* module: 0.01 as the minor allele frequency, 75% as the percentage of individuals sharing the locus within the population, and appearance of the locus in all populations. Linkage disequilibrium (LD) between each pair was tested using VCFTOOLS v.0.1.15 (Danecek et al., 2011), and one marker was excluded from each pair with $R2 > 0.8$. The LD-excluded VCF data were further divided into *S. anglica* and *S. alterniflora* subsets for subsequent analysis by Tassel 5 (Bradbury et al., 2007), and the three datasets were converted into file formats necessary for analyses using PLINK v.1.9 (Purcell et al., 2007) and PGDSPIDER 2.0.9.0 (Lischer and Excoffier, 2012).

Environmental Data

To test for correlations between genetic and environmental variables of *Spartina* in China, we required environmental data at the sampling locations. We extracted the standard nineteen bioclimatic variables (**Supplementary Table 1**) from the WORLDCLIM dataset (Version 2; period 1970–2000) (Hijmans et al., 2005) for the set of georeferenced locations of available localities using DIVA-GIS software (Hijmans et al., 2004). The total organic nitrogen (TN) and total phosphorus (TP) of the soil samples were determined using the Kjeldahl method and the molybdenum-antimony antispectrophotometric method at Bowu Company, Beijing. Soil electrical conductivity (EC) was detected by a New Digital DDS-307 Conductivity-Salinity Meter Tester in a mixture of 1:5 soil:distilled water as an index of soil salinity (S). The average TN, TP, and S values of three samples and the standard nineteen bioclimatic variables at each site were all used as environmental variables (**Supplementary Table 1**).

Genetic Diversity and Clone Detection

Genetic diversity indices including the percentage of polymorphic loci (*%poly*), average nucleotide diversity (π), average observed heterozygosity per locus (*Ho*), and average expected heterozygosity per locus (*He*) were calculated using *populations* in Stacks version 2.3 based on the raw dataset (Catchen et al., 2011). Then, we used analysis of variation (ANOVA) to test for a difference in genetic diversity between *S. anglica* and *S. alterniflora* using the R 4.1.2 function "aov" (Chambers et al., 2017). The Tukey's multiple comparisons test (TukeyHSD) was used for multiple comparisons (Yandell, 2017).

We referred to several analysis results to evaluate the reproductive systems of six populations. First, we calculated the average Wright's inbreeding coefficient (F_{IS}) using *populations* in Stacks version 2.3. The inbreeding coefficient (F_{IS}) was often used to quantify the partial asexual reproduction population (Stoeckel and Masson, 2014). Negative F_{IS} values may reflect excess heterozygosity associated with reproduction via clonality (Pantoja et al., 2017).

TABLE 1 | Location information, number of sampled individuals, and genotypic diversity in populations of *Spartina anglica* and *Spartina alterniflora*.

Species	Pop	Collection location	Lon. (°E)	Lat. (°N)	N	G	G/N	H	λ	E	I_A	r_d
Spartina anglica	DD	Dandong, Liaoning	124.209°	39.872°	15	15	1.000	2.708	0.933	1.000	7.132	0.001
	HLD	Huludao, Liaoning	120.962°	40.799°	15	6	0.400	1.173	0.533	0.512	22.653*	0.005*
	Total	-	-	-	30	21	0.700	2.634	0.867	0.503	32.076*	0.005*
Spartina alterniflora	TG	Tanggu, Tianjin	117.713°	38.958°	14	14	1.000	2.639	0.929	1.000	140.806	0.009
	CX	Cixi, Zhejiang	121.263°	30.369°	15	13	0.867	2.523	0.916	0.945	75.422*	0.006*
	QZ	Quanzhou, Fujian	118.667°	24.922°	14	4	0.286	0.895	0.459	0.586	3077.454*	0.337*
	FCG	Fangchenggang, Guangxi	108.213°	21.510°	15	1	0.070	0.000	0.000	NA	147.187*	0.037*
	Total	-	-	-	58	32	0.552	2.891	0.892	0.484	833.676*	0.044*

*N is the number of samples, G is the number of MLGs, G/N is the number of MLGs divided by the number of individuals analyzed, H is the Shannon–Wiener index of diversity, λ is Simpson's complement index of genotypic diversity, E is evenness, I_A is the index of association, r_d is the standardized index of association, and * indicates significant p-values (p = 0.001, permutations = 1,000).*

Second, the genetic clones of *S. anglica* and *S. alterniflora* subsets were calculated using the package "poppr v. 2.9.3" based on the putatively neutral dataset (Kamvar et al., 2014, 2015), which can identify multilocus genotypes (MLGs) in clonal, partially clonal, and/or sexual reproduction populations. Clonal membership to the same genet can be inferred through a shared MLG among multiple individuals within a population. The true number of MLGs was determined by the "mlg.filter" function with a Nei's distance (Nei, 1978) threshold determined using the cutoff_predictor tool, which finds a gap in the distance distribution. We investigated additional evidence of clonal reproduction through the "poppr" function (Kamvar et al., 2014, 2015). Since this function cannot work with a large dataset, we used two stricter datasets that restricted data analysis to one random SNP per locus (8,314 SNPs for *S. anglica* and 21,029 SNPs for *S. alterniflora*) to calculate the index of association (I_A), the standardized index of association (r_d), and their p-values (**Supplementary Table 2**). We also ensured that the MLGs obtained from strict datasets were consistent with the MLGs obtained from non-strict datasets. The I_A index determines individual's recombination, which indicates panmixia, using the ratio of observed-to-expected variance in the number of loci (Agapow and Burt, 2001). The r_d index is a modification of I_A that removes sample size bias (Agapow and Burt, 2001). Clone correction was allowed, and the p-values of I_A and r_d were obtained with 999 permutations. We also constructed an unweighted pair group method with arithmetic mean (UPGMA) tree of all individuals, and minimum spanning networks (MSNs) of each MLG based on distance matrices.

We used "poppr" to calculate the clonal diversity of each population, including the Shannon–Wiener index (H) (Shannon, 1948), Simpson's index (λ) (Simpson, 1949), and evenness (E). The parameter G/N was also estimated, and while it could be biased, it is frequently used (Saleh et al., 2012; Pantoja et al., 2017; Tsujimoto et al., 2020). G represents the number of MLGs and N is the number of samples.

Population Structure

To infer the genetic structure of *Spartina* populations, we used ADMIXTURE version 1.3.0 (Alexander et al., 2009; Zhou et al., 2011) to estimate individual ancestries with the combined datasets of the two species and subsets of each species. We designated K = 1 to K = 9 as ancestral modes, and the most likely number of K given the populations was estimated according to the lowest cross-validation error (Alexander et al., 2009). We created an output file for the most likely number of K and visualized the results in the R environment. Discriminant analysis of principal components (DAPC) (Jombart et al., 2010) was performed using the R package "adegenet." This method is more appropriate than others for the analysis of invasive species because it does not make any assumptions about migration-drift equilibrium (Jombart et al., 2010). The number of clusters was assessed for both species and their combined dataset, ranging from 1 to 10. The optimal number of clusters was identified based on the Bayesian information criterion (BIC), as suggested by Jombart et al. (2010). We used the function *find.*clusters to transform the original data into principal components (PCs), retaining 100 PCs in the analysis. Discriminant analysis was performed using 50 PCs (>90% of variance explained), and one, four or five eigenvalues were retained and examined. The optimal number of PCs to be retained in the final analysis was identified by the *optim.a.score* function with twenty independent runs.

Moreover, a redundancy analysis (RDA) within the "vegan" package (v.2.5–7) (Oksanen et al., 2021) in R 4.1.2 was used to assess whether environmental variables influenced the genetic differentiation of *S. alterniflora*. A principal component analysis (PCA) was performed by TASSEL 5 (Glaubitz et al., 2014). Variance inflation factors (VIF) were excluded through the "vif.cca" function of "vegan" package (Oksanen et al., 2021). The important environmental variables were chosen by the "forward.sel" function of the "adespatial" package (Dray et al., 2018). Then we used the "rdacca.hp" package to estimate the percentage contribution of explained variation to each or the total important environmental variables among the genetic groups, and the significance of the RDA results was tested with a global permutation (999 permutations) (Lai et al., 2022).

Outlier Detection

The outlier loci were detected using two population differentiation (PD) analysis methods and two environment

association (EA) analyses. The first PD method was implemented in ARLEQUIN (Excoffier et al., 2005), which identifies outliers by comparing genetic diversity and differentiation between populations. Both infinite island and hierarchical demographic models of this approach were used to estimate outliers, and we only chose the outliers identified by both models. The second PD method we used was in the "PCAdapt" package in R 4.1.2, which detects outlier loci based on principal component analysis (PCA). This approach overcomes the limitation of the traditional "outlier test" approach, which assumes an unrealistic island model of migration between populations. SNP with *qvalue* less than 0.01 was considered as an outlier (Capblancq et al., 2018).

For EA analysis, BAYENV identified outliers based on a correlation matrix of loci using SNPs, and 22 environmental variables and two geographical variables (longitude and latitude). A batch file estimated the BayesFactor with 100,000 MCMC steps. Regarding interpretation (Jeffreys, 1961), a BayesFactor more than 10 indicated strong supportive evidence for an association between an environmental parameter and a locus (Cullingham et al., 2014). Second, we used latent factor mixed models (LFMMs) implemented in the LEA package in R 4.1.2 (Frichot and François, 2015) to detect outliers correlated with climatic variables. The implementation of the LFMM is based on least-squares estimates (Caye et al., 2019). The prcomp function determines the number of latent factors (K). A fitted model was then used to perform association testing based on the ridge_lfmm function after ridge estimation. Finally, any obtained p-values were transformed into *qvalues*. The *qvalues* less than a cutoff of 0.01 were indicative of candidate loci. A Benjamini and Hochberg (1995) false discovery rate (FDR) correction of 5% was applied to p-values using the *qvalue* package in R (Storey et al., 2015). Binomial tests were used to assess differences in the number of outliers in the two species obtained by the four methods.

Single Nucleotide Polymorphism Annotation

To gain insights into the potential adaptive significance of outlier loci, we obtained the flanking sequence of outlier loci identified by at least two methods from the RAD library. Functional annotation was performed using the online Basic Local Alignment Search Tool (BLAST) database. We searched the Genbank database using BLASTn with >60% sequence similarity. If the sequence was related to a protein, we further searched for its molecular function from UniProt[2] and identified its related functions in plants according to literature. Then, we calculated correlations between allele frequencies of outlier loci and climate variables to identify putative genes associated with local adaptation.

RESULTS

Genome Sequencing

A total of 1680,866,670 paired-end (PE) reads (read length = 150 bp, 252.13 Gbp) were obtained from the ddRAD

[2]https://www.uniprot.org/

TABLE 2 | Diversity indices of each sampled population of *Spartina anglica* and *Spartina alterniflora*.

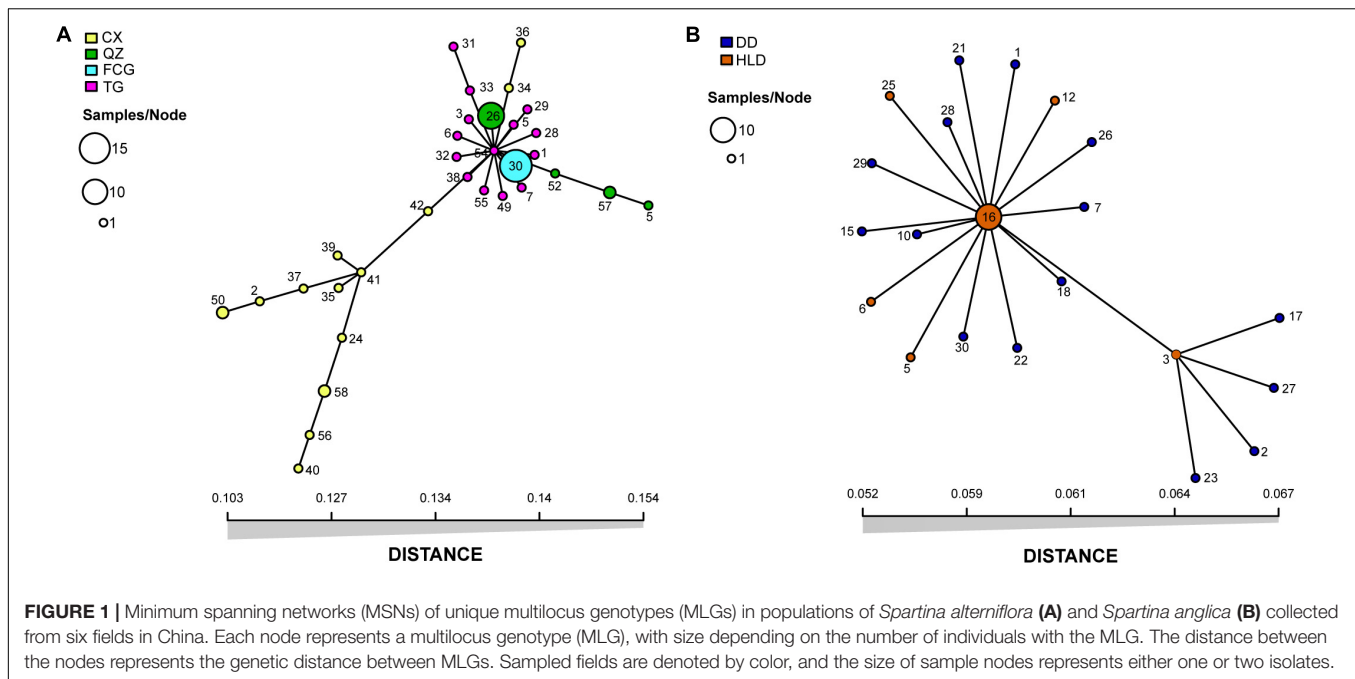| Species	Pop	%poly	π	H_O	H_e	F_{IS}	P_A
Spartina anglica	DD	0.484	0.035	0.042	0.034	−0.010	0.049
	HLD	0.474	0.035	0.052	0.034	−0.031	0.049
Spartina alterniflora	TG	2.143	0.151	0.121	0.145	0.090	0.220
	CX	1.555	0.120	0.110	0.115	0.029	0.073
	QZ	1.179	0.096	0.113	0.093	−0.020	0.057
	FCG	0.667	0.068	0.114	0.066	−0.089	0.049

%poly is the percentage of polymorphic loci, π is average nucleotide diversity, H_O is observed heterozygosity, H_e is expected heterozygosity, F_{IS} is Wright's inbreeding coefficient, and P_A is the frequency of private alleles.

library constructed for 30 individuals of *S. anglica* and 58 individuals of *S. alterniflora*. We obtained a total of 1674,211,878 reads (read length = 135 bp, 226.02 Gbp) after demultiplexing and removing reads of low quality. The number of reads for the samples varied from 3,632,316 to 52,525,600, with the average number of reads per sample being 19,025,135. Our analysis with ustacks showed that the number of ddRAD tags recovered from these samples varied from 248,767 to 1,385,706. Using cstacks, we obtained 4,664,866 loci from the 30 *S. anglica* and 58 *S. alterniflora* samples. After the initial filtering steps, 212,939 SNPs were retained, followed by removing sites showing linkage disequilibrium, and 169,522 SNPs were retained for all samples. Based on the dataset with 169,522 SNPs, a total of 90,671 SNPs were identified in the *S. alterniflora* subset, and 10,542 SNPs were identified in the *S. anglica* subset, with a minimum allele frequency of 0.01 (**Supplementary Table 2**).

Mode of Reproduction

Using the clonal threshold of 0.124 identified by *cutoff_predictor*, we found 32 MLGs among 58 individuals of *S. alterniflora* (**Supplementary Figure 3B**), while 21 MLGs among 30 individuals of *S. anglica* were found with a threshold of 0.163 (**Table 1** and **Supplementary Figure 3D**). **Table 1** also shows genotypic (MLG) diversity calculated separately for each population. The FCG population of *S. alterniflora* had the lowest genotypic diversity, with a single MLG identified among 15 sampled individuals (G:N = 0.067). Other populations with low genotypic diversity were QZ (*S. alterniflora*) and HLD (*S. anglica*), which had G:N ratios of 28.6 and 40%, respectively. In contrast, populations DD (*S. anglica*) and TG (*S. alterniflora*) had G:N ratios of 100% (**Table 1**).

Both indices of association, I_A and r_d, demonstrated that the population of genotypes was significantly different ($p = 0.001$) from the expected products of panmixia for populations HLD (*S. anglica*), CX, QZ, and FCG (*S. alterniflora*) (**Table 1** and **Supplementary Figure 4**), indicating clonal reproduction as the prevalent mode of reproduction at these sites. The p-values of I_A and r_d were not significant ($p = 1$) only for populations DD (*S. anglica*) and TG (*S. alterniflora*) (**Table 1** and **Supplementary Figure 4**), indicating that the null hypothesis of sexual reproduction could not be rejected for these populations. The inbreeding coefficients (F_{IS}) of all *S. anglica* populations (DD and HLD) and the QZ and FCG populations of *S. alterniflora*

FIGURE 1 | Minimum spanning networks (MSNs) of unique multilocus genotypes (MLGs) in populations of *Spartina alterniflora* **(A)** and *Spartina anglica* **(B)** collected from six fields in China. Each node represents a multilocus genotype (MLG), with size depending on the number of individuals with the MLG. The distance between the nodes represents the genetic distance between MLGs. Sampled fields are denoted by color, and the size of sample nodes represents either one or two isolates.

were negative, showing a clear trend of heterozygote excess (**Table 2**). In addition, the minimum spanning networks (MSNs) based on Euclidean distances of the clone-corrected datasets showed no sign of reticulation (**Figure 1**). MLG 54 at the center of the *S. alterniflora* network consisted of only one individual from the TG population, and MLGs 26 and 30, which consisted of 10 individuals from the TG population and all individuals from the FCG population, were near the center (**Figure 1A**). Ten individuals of HLD belonging to MLG 16 were at the center of the *S. anglica* network, and other MLGs were found in only one individual (**Figure 1B**).

Genetic Diversity

There was higher genetic diversity in *S. alterniflora* populations than in *S. anglica* populations in terms of nucleotide diversity (π, 0.035 vs. 0.068-0.151, $p < 0.001$), observed heterozygosity (*Ho*, 0.042–0.052 vs. 0.110–0.121, $p < 0.001$) and expected heterozygosity (*He*, 0.034 vs. 0.145-0.066, $p < 0.001$) (**Table 2** and **Figures 2A–C**). TukeyHSD also revealed significant differences among the six populations ($p < 0.001$), except *He* and π between HLD and DD ($p = 0.976$, 0.988), *Ho* between FCG and QZ ($p = 0.699$, **Figures 2D–F** and **Supplementary Table 3**). The TG population had the highest frequency of private alleles (21.98%), while the DD and FCG populations had the lowest frequencies (4.85 and 4.91%, respectively) (**Table 2**).

Genetic Structure

Five genetic clusters ($K = 5$) were identified using ADMIXTURE based on the lowest cross-validation error (**Supplementary Figure 5**). The two *Spartina* species were clearly separated (**Figure 3**). All populations of *S. alterniflora* were divided into four clusters, and only four individuals of QZ belonged to the red cluster (**Figures 3A,B**). The two populations of

S. anglica did not separate from each other (**Figures 3A,B**). DAPC performed on SNP datasets showed clear genetic structure in *S. alterniflora* populations but not in *S. anglica* (**Figure 3C** and **Supplementary Figures 5B–D**). All four populations of *S. alterniflora* were also separated from each other, and individuals of the FCG population were most distanced from other populations' individuals (**Figure 3D** and **Supplementary Figure 5C**). The two populations of *S. anglica* were not separated based on the subdataset (**Supplementary Figure 5**). A UPGMA tree showed that individuals of *S. alterniflora* and *S. anglica* were divided into two major clusters with high bootstrap support. Individuals of four populations of *S. alterniflora* also separated into four subclusters with 100% bootstrap support (**Figure 3E**). The genetic distances among *S. anglica* individuals did not show clear separation (**Figure 3E**).

Three important environmental variables were kept through forward selection in the RDA analysis, including the minimum temperature of the coldest month (BIO6), the mean temperature of the wettest quarter (BIO8), and the precipitation of the warmest quarter (BIO18), which were significantly associated with genetic variation among populations of *S. alterniflora* ($p = 0.001$). The total explained variation was 0.784, and the precipitation of the warmest quarter (BIO18; 38.58% explained variation, $p = 0.001$) was the most important predictor (**Supplementary Figure 6** and **Supplementary Table 4**).

Identification of Outliers and Analysis of Their Clines

A total of 7,868 and 238 outliers were identified in *S. alterniflora* and *S. anglica*, respectively, using four methods (**Supplementary Figure 7**). For the *S. alterniflora* subset, the two PD methods identified 4,791 outliers (4,145 from PCAdapt and 1,005 from
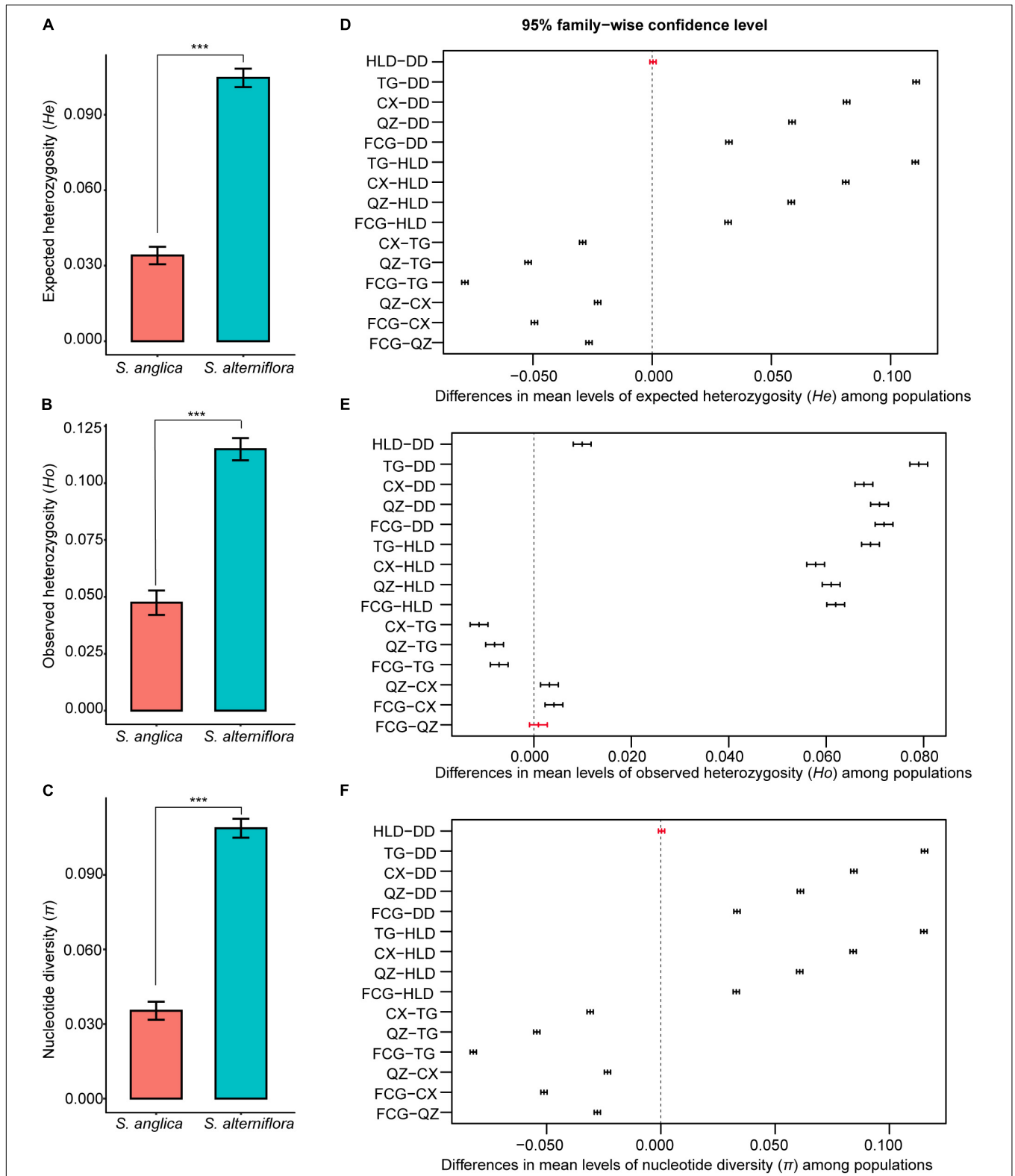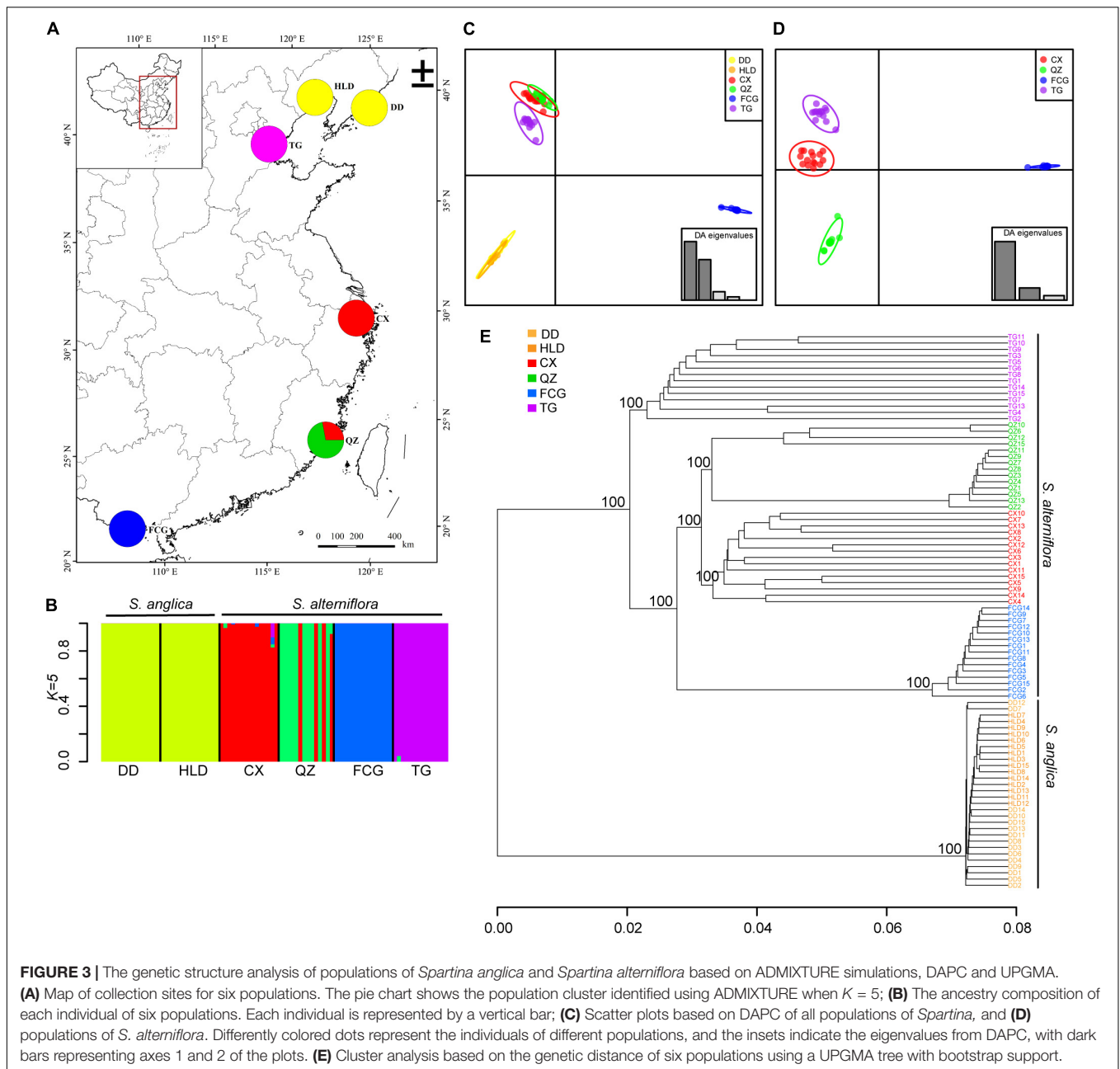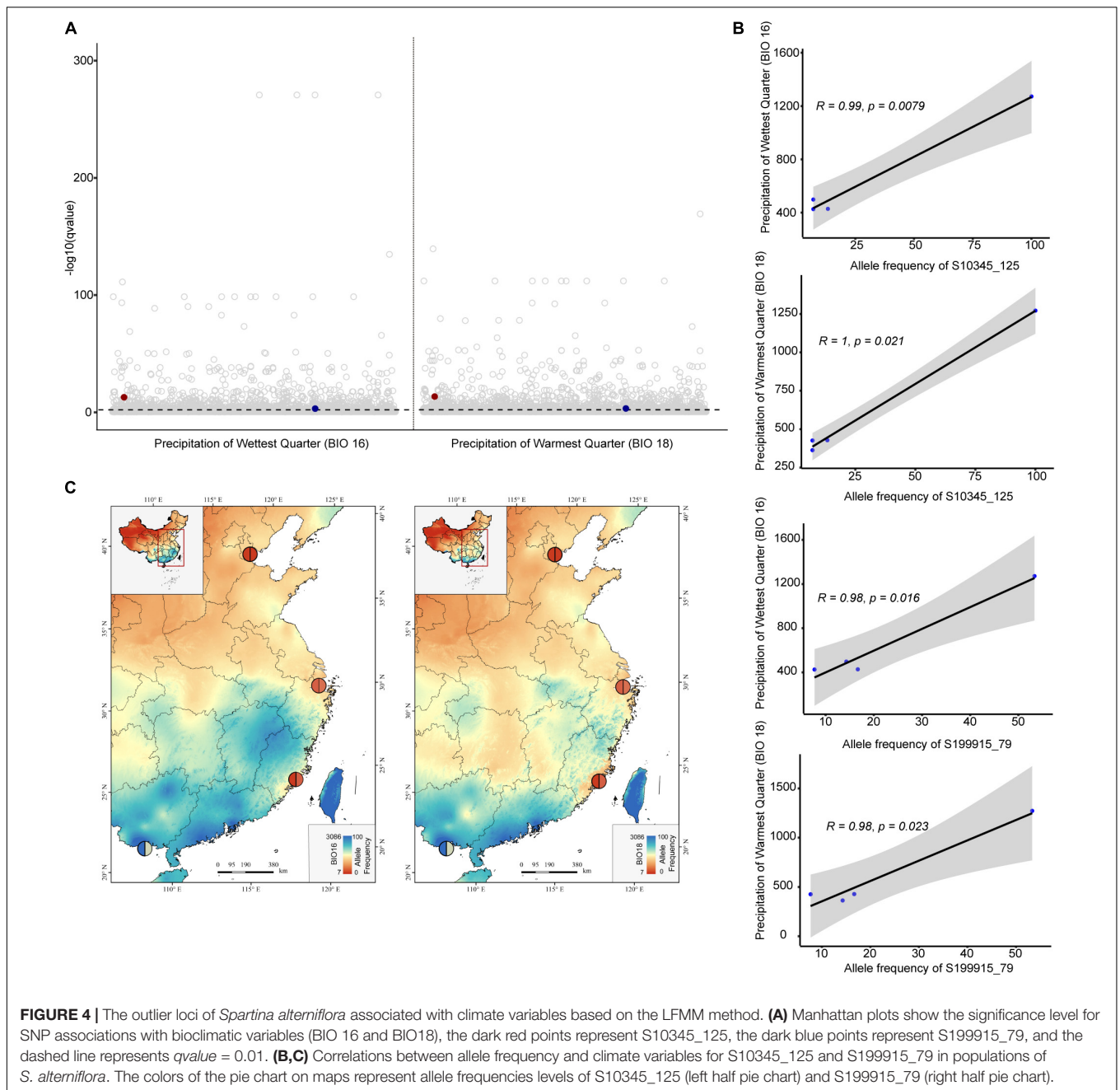
FIGURE 2 | Comparison of expected heterozygosity (*He*), observed heterozygosity (*Ho*), and nucleotide diversity (π) between *Spartina anglica* and *S. alterniflora* **(A–C)** by the one-way ANOVA analysis, and among their populations **(D–F)** by Tukey's multiple comparisons test. ***In panels **(A–C)** indicates $p < 0.001$. In panels **(D–F)**, the bars represent differences in mean levels of genetic diversity indicators (*He*, *Ho*, π) between populations, p-values of black bars were less than 0.001, while those of red bars were more than 0.05.

FIGURE 3 | The genetic structure analysis of populations of *Spartina anglica* and *Spartina alterniflora* based on ADMIXTURE simulations, DAPC and UPGMA.
(A) Map of collection sites for six populations. The pie chart shows the population cluster identified using ADMIXTURE when $K = 5$; **(B)** The ancestry composition of each individual of six populations. Each individual is represented by a vertical bar; **(C)** Scatter plots based on DAPC of all populations of *Spartina*, and **(D)** populations of *S. alterniflora*. Differently colored dots represent the individuals of different populations, and the insets indicate the eigenvalues from DAPC, with dark bars representing axes 1 and 2 of the plots. **(E)** Cluster analysis based on the genetic distance of six populations using a UPGMA tree with bootstrap support.

ARLEQUIN), and the two EA methods identified 3,619 outliers (889 from BAYENV and 2,769 from LFMM). Only one outlier was identified by all four methods, 91 outliers were identified by three methods, and 848 outliers were identified by two methods (**Supplementary Figure 7**). For the *S. anglica* subset, the two PD methods identified 148 outliers (177 from PCAdapt and 93 from ARLEQUIN), and the two EA methods identified 31 outliers (12 from BAYENV and 19 from LFMM). None of the outliers were identified by all four methods, and only three outliers were identified by three methods; 60 outliers were identified by two methods (**Supplementary Figure 7**). The proportion of outliers obtained by the four methods was statistically

different between the two species based on binomial tests (for PCAdapt, BAYENV and LFMM, $p < 0.001$; for ARLEQUIN, $p < 0.05$).

Functional annotation was not successful for any outlier loci of *S. anglica*. Fifteen loci obtained from the *S. alterniflora* subset were annotated (**Supplementary Table 5**). All of them were identified by at least two methods (11 from ARLEQUIN, 13 from PCAdapt, five from LFMM, and one from BAYENV). Two-thirds of the annotated outlier loci (10 outlier loci) were obtained with the two PD methods, while only one was obtained with the two EA methods. We identified 12 adjacent genes with a range of functions that were associated

FIGURE 4 | The outlier loci of *Spartina alterniflora* associated with climate variables based on the LFMM method. **(A)** Manhattan plots show the significance level for SNP associations with bioclimatic variables (BIO 16 and BIO18), the dark red points represent S10345_125, the dark blue points represent S199915_79, and the dashed line represents *qvalue* = 0.01. **(B,C)** Correlations between allele frequency and climate variables for S10345_125 and S199915_79 in populations of *S. alterniflora*. The colors of the pie chart on maps represent allele frequencies levels of S10345_125 (left half pie chart) and S199915_79 (right half pie chart).

with plant growth and development, and four genes were associated with abiotic stress and pathogen attack. Based on the LFMM method, two (S10345, S199915) and four (S10345, S107946, S125087, S190589) genes identified were associated with precipitation (BIO11, 12, 13, 16, 18) and temperature (BIO1, 3, 4, 6, 7, 8, 9), respectively (**Supplementary Table 6**). The locus S199915 was identified by both EA methods, and was associated with precipitation and the TN content by the LFMM method, while it was associated with precipitation and temperature by BAYENV method. However, we only found strong associations between precipitation (Bio13, Bio16, and Bio18) and allele frequencies of two SNPs (S10345_125

and S199915_79), respectively ($p < 0.05$, **Figure 4** and **Supplementary Figure 8**).

DISCUSSION

Our study confirmed that there are two species of *Spartina* along the coast of China. We compared the genetic backgrounds of the two species that were intentionally introduced to China decades ago. We used genome wide SNPs to analyze differences in modes of reproduction, genetic diversity, and gene × environment correlations between the two species in an effort to explain the

reasons behind their differential invasion success. These analyses point to that high genetic diversity and genetic adaptation in response to the environment might promote *S. alterniflora* spreading on the coast of China.

Both High Clonality and Sexual Reproduction Were Revealed in the Two *Spartina* Species

Strong evidence of high clonal reproduction, such as a low number of genotypes, significant I_A and r_d ($p < 0.001$), and negative F_{IS}, was found in populations of both *S. alterniflora* (FCG and QZ) and *S. anglica* (HLD). No reticulation of the minimum spanning networks (**Figure 1**) also provided an evidence of clonal spread (Maurice et al., 2019). However, sexual reproduction cannot be ignored in either species; population DD of *S. anglica* and population TG of *S. alterniflora* showed an extremely high G:N ratio. Although a slightly negative F_{IS} (-0.010) was found in population DD of *S. anglica*, we still suspected that sexual reproduction rather than clonality was the main mode of reproduction in this population in consideration of the high number of genotypes, non-significant I_A and r_d, and lack of a large excess of heterozygotes. Negative F_{IS} was usually interpreted as a signal of clonal reproduction in previous studies (Balloux et al., 2003; Halkett et al., 2005), while it was recently considered unreliable for evaluating reproduction. Both non-negative and negative F_{IS} values can be frequently found in partially clonal populations, and the dynamics of F_{IS} in (partially) clonal populations might be impacted by genetic drift (Reichel et al., 2016). Therefore, we focused more on the number of genotypes and the indices of association (I_A and r_d) when evaluating the mode of reproduction. Inconsistencies in F_{IS}, I_A, and r_d were also found in population CX, which might be a partially clonal population with significant indices of association but a non-negative F_{IS} (0.029). The results of reproduction analysis based on molecular genetic information in our study did not support the finding of previous studies that *S. anglica* mainly relies on asexual reproduction for population regeneration (Li et al., 2008). This may be due to population DD of *S. anglica* being located at a northern latitude (N 39.87°) with a lower annual mean temperature (9.3°C) and maximum temperature (28.88°C), while the population site in the study of Li et al. (2008) was located along the coast of the Yellow Sea in China (N 33.7°), with a higher annual mean temperature (15.0–15.6°C) and maximum temperature (39.0°C). The native distribution of *S. anglica* is in cool regions (the first record was in Lymington, Hampshire, England) (Gray and Raybould, 1991), and high temperatures might limit its seed production through less effective pollination or pollen germination (Li et al., 2008). Lower genotype diversities were found in populations FCG and QZ (latitudes below N 25°) than in TG and CX (latitudes above N 30°) (**Table 1**). This result was consistent with those of previous studies showing that sexual reproduction differed among populations of *S. alterniflora* in China and seed production decreased at low latitudes (Liu et al., 2016b).

High clonality is common in some notorious invasive alien species (Liu et al., 2006), such as *Pueraria montana* var. *lobata*

(Bentley and Mauricio, 2016), *Eichhornia crassipes* (Barrett et al., 2008), *Pistia stratiotes,* and *Eichhornia crassipes* (Wang et al., 2016). In addition, most of these species are also capable of sexual propagation (Eckert, 2002) and plastic in their two methods of reproduction (Liu et al., 2006). Although low sexual reproduction may limit the local adaptation of alien species during an invasion due to little opportunity for genetic recombination, clonal reproduction can allow alien species to quickly establish populations regardless of whether the founder group sizes are small (Barrett et al., 2008). Our study revealed that the two *Spartina* species adopted both clonal and sexual reproduction strategies and might adjust seed production across latitudes. Therefore, the method of reproduction was not considered a major constraint on the dieback of *S. anglica* in China, even if this species may experience a reduced ability to produce seeds in low-latitude regions.

The Effect of High Genetic Diversity on Invasion Cannot Be Ignored

The newly introduced species suffered from bottlenecks, followed by reduced fitness and evolutionary potential, yet they often become invasive, and this dilemma was called the genetic paradox of invasion (Roman and Darling, 2007; Estoup et al., 2016). The genetic paradox of invasions has been thoroughly discussed by researchers in recent decades (Frankham, 2005; Stapley et al., 2015; Estoup et al., 2016; Schrieber and Lachmuth, 2017), and its importance among genetic mechanisms of invasions may be overestimated (Estoup et al., 2016). Many invaders do not exhibit the genetic paradox phenomenon because they have no loss of genetic diversity and no significant adaptive challenge in the invaded area compared to their native areas (Bossdorf et al., 2005; Roman and Darling, 2007). Some studies have already found that a negative founder effect with low genetic diversity can be overcome by high phenotypic variability, which can help *Spartina* species establish well in new environments (Castillo et al., 2018; Li et al., 2019a). In this study, the average nucleotide diversity (π) and observed heterozygosity (*Ho*) of each population reflected lower genetic diversity of the failed invader *S. anglica* (π: 0.035, *Ho*: 0.042–0.052) relative to the successful invader *S. alterniflora*. (π: 0.068–0.151, *Ho*: 0.110–0.121). Even though population FCG had extremely low genotype diversity, its genetic diversity was significantly higher than that in populations of *S. anglica* (*p < 0.001*, **Figures 2D–F** and **Supplementary Table 3**). This also suggests that differences in reproduction cannot explain the difference in genetic diversity observed here for *S. anglica* and *S. alterniflora*. Both species were intentionally introduced into China only once; the difference was that *S. anglica* came from a single source, while *S. alterniflora* came from multiple sources (Chung, 1993). The diverse genetic backgrounds resulting from multiple sources of *S. alterniflora* provided an opportunity for interbreeding and genetic recombination through sexual reproduction, whereas this was not possible for *S. anglica* with a single genetic source. Most previous studies also revealed that outcrossing of plants from the locations of origin (North Carolina, Georgia, and Florida in the US) of *S. alterniflora* generated a genetic admixture, and genetic variation remained

high compared to those in the native populations (Bernik et al., 2016; Qiao et al., 2019; Xia et al., 2020). Moreover, only the TG and CX populations had higher expected heterozygosity (*He*, 0.145 and 0.115) than observed heterozygosity (*Ho*, 0.121 and 0.110), which may have been a reflection of earlier population bottlenecks (Rengefors et al., 2021), whereas, other populations did not show evidence of bottlenecks. Therefore, neither of the two *Spartina* species in China showed the genetic paradox of invasion. The low genetic diversity limiting population expansion of *S. anglica* might be due to inbreeding depression and loss of adaptive potential (Wellband et al., 2017, 2018). Significant differences in the levels of genetic diversity of successful and unsuccessful invasive species were consistent with those in a study of four aquatic invasive species (gobies and oysters) (Wellband et al., 2017). We also agree with the conclusion that the limitation of invasions via genetic diversity is species specific (Wellband et al., 2017).

Unlike most previous studies of *S. alterniflora* in China (Xia et al., 2015; Bernik et al., 2016; Qiao et al., 2019), ours revealed high genetic differentiation and low genetic admixture of populations based on structure analysis and DAPC, while the two populations of *S. anglica* in China could not be separated from each other. The findings of the current study do not support the previous findings of Wellband et al. (2017), who demonstrated greater divergence among populations of less-successful invasive species than among highly successful invasive species due to less gene flow among populations of the former. Population genetic structure in *S. alterniflora* in China was also found in the study of Xia et al. (2020), and they speculated that genetic divergence was mainly due to the low natural gene flow among populations. However, we believe that the existing genetic divergence is more likely a product of rapid adaptation, Qiao et al. (2019) proved in their study. The significant association between environmental variables and genetic structure in this study also suggests that temperature (BIO6, 8) and precipitation (BIO18) are important drivers of genetic variation within *S. alterniflora* (**Supplementary Figure 6**). Therefore, high genetic variation promoted rapid genetic differentiation under environmental selection pressures is the possible explanation for why *S. alterniflora* in our study showed population genetic structure. Another reason is that RADseq technique afforded numerous SNPs with much more genetic information than other markers.

Genomic Signature Provides Support for Local Adaptation of *Spartina alterniflora* in China

Rapid local adaptation of invasive alien species usually includes two aspects: phenotypic plasticity and genetic evolution (Wellband and Heath, 2017; VanWallendael et al., 2018; Cao et al., 2021). Early studies on rapid adaptive evolution were mostly based on phenotypic changes between generations through common garden experiments or reciprocal transplant experiments (Turner et al., 2014; Ochocki and Miller, 2017). Recently, large genome-wide variation provided a more thorough understanding of the relationship between adaptive evolution and climate at the gene level (Zenni et al., 2014; Bay et al.,

2018; Wellband et al., 2018). Based on the genomic scan, the performance of outlier test methods has been evaluated and used to provide evidence for local adaptive evolution in several studies (Nielsen et al., 2018; Gallego-Garcia et al., 2019; Cao et al., 2021). For example, Bay et al. (2018) identified the genomic regions which adapted across contemporary climate gradients in populations of yellow warbler (*Setophaga petechia*) by LFMM. To reveal signatures of adaptation within two montane bumble bee species, Jackson et al. (2020) detect outliers through several EA methods, including LFMM, Bayenv2, and RDA. All of these methods could lead to false positives and negatives, and it is hard to identify which outlier loci are false positives (Lotterhos and Whitlock, 2014), We try to avoid missing true positive outliers and adopt a cautious screening attitude to avoid false positives, the same as previous studies did (Cullingham et al., 2014; Wellband et al., 2018; Cao et al., 2021).

Although cross-validation with multiple methods may increase the confidence of true positive outliers (Cullingham et al., 2014), we still consider the outlier loci, which can be successfully annotated and have significant correlations with environment variables, to be more reliable. As significant larger number of outliers in populations of *S. alterniflora* than in *S. anglica*, some of which were successfully annotated, provided evidence for rapid local adaptation in this study. It supports hypothesis of previous studies that rapid adaptive evolution is a key mechanism driving successful invasion of *S. alterniflora* along the coast of China through reciprocal transplant experiments (Qiao et al., 2019). Previous authors speculated that genetic admixture might promote the formation of super invasive genotypes with high competitive abilities (Qiao et al., 2019). We also cannot ignore the genetic admixture in our study; even though we chose four large geographically distant populations, genetic admixture was still found in population QZ. Therefore, a single introduction but with multiple sources could promote the adaptation of *S. alterniflora* to different environments in China even with the short time lag of invasion (Facon et al., 2006). Rapid adaptive evolution can promote phenotypic trait shifts to increase the fitness of alien species when they face new environments (Suarez and Tsutsui, 2008; Molina-Montenegro et al., 2018).

Previous studies revealed high phenotypic variation in *S. alterniflora* in China and proved the occurrence of latitudinal clines in vegetative and sexual traits of populations, especially a linear increase in seed set, which was the result of rapid evolution, through garden experiments at multiple latitudes (Liu et al., 2017, 2020). Moreover, some studies have also proven that seed traits are critical for dispersal syndromes and mechanisms allowing plants to cope with environmental stress (Baskin and Baskin, 1998; Moles et al., 2005), and adaptive evolution in an invasive plant can enhance the probability of seedling survival by increasing the thickness of the seed coat (Molina-Montenegro et al., 2018). Interestingly, the function of locus S50498 (**Supplementary Table 5**) in our study was related to grain size and shape in rice (Huang et al., 2017). In addition, we also found that locus S135470 (**Supplementary Table 5**) might play a crucial role in organelle biogenesis and seedling establishment (Rigas et al., 2009).

We further revealed two SNPs of locus S10345 and S199915 were strongly associated with precipitation variables. The highest allele frequencies at these SNPs both occurred in the Fangchengang city of Guangxi province, areas of the low latitude and high rainfall (**Figure 4**). The SNP on locus S10345 was related to diacylglycerol kinase 2 that belongs to the diacylglycerol kinase family (DGKs). DGKs phosphorylate diacylglycerol (DAG) to produce phosphatidic acid (PA), which is required for plant development and responses to both biotic and abiotic stress (Tan et al., 2018; Kue Foka et al., 2020), such as drought (Li et al., 2015), cold (Ruelland et al., 2002), and pathogen attack (Zhang and Xiao, 2015). The SNP on locus S199915 was the H + - pyrophosphatase gene, which is related to vacuolar membrane solute transport (Graus et al., 2018). Previous studies also proved up-regulation of this gene can enhance resistance to salt and drought stress in plants (Park et al., 2005; Brini et al., 2007). Nevertheless, there are clear limitations in this study to further confirm the role of these two genes in the adaptation of *S. alterniflora* to the environment, and we believe that these loci are worthy of further research and validation.

To conclude, we explored aspects of invasiveness from an evolutionary perspective through a genomic comparison of *S. alterniflora* and *S. anglica*, which have been introduced to China for several decades. Our results suggest that low sexual reproduction was not the reason for the dieback of *S. anglica* in coastal China. This further implies that the high genetic diversity caused by multiple introduction sources of *S. alterniflora* in China plays a role in the success of invasion. Moreover, comparisons of invading species and non-invasive related species might be more helpful for understanding the genetic paradox. Due to the limitations of the sampling strategy in our study (we only found two populations of *S. anglica* in China), we cannot directly demonstrate that rapid adaptive evolution was one of the main factors which promoted the success of *S. alterniflora* invasion. However, the evidence of the correlation between gene and environment of *S. alterniflora* revealed in this study might provide genetic support for previous research conclusion. Additionally, annotated outlier loci are worthwhile of further study in consideration that the functional protein-coding genes obtained from four populations of *S. alterniflora* might be critical for understanding processes influencing the range expansion of invasive species.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.909429/full#supplementary-material

REFERENCES

Agapow, P. M., and Burt, A. (2001). Indices of multilocus linkage disequilibrium. *Mol. Ecol. Notes* 1, 101–102. doi: 10.1046/j.1471-8278.2000.00014.x

Alexander, D. H., Novembre, J., and Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19, 1655–1664. doi: 10.1101/gr.094052.109

An, S., Gu, B., Zhou, C., Wang, Z., Deng, Z., Zhi, Y., et al. (2007). Spartina invasion in China: implications for invasive species management and future research. *Weed Res.* 47, 183–191. doi: 10.1111/j.1365-3180.2007.00559.x

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., et al. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS One* 3:e3376. doi: 10.1371/journal.pone.0003376

Balloux, F., Lehmann, L., and de Meeûs, T. (2003). The population genetics of clonal and partially clonal diploids. *Genetics* 164, 1635–1644. doi: 10.1093/genetics/164.4.1635

Barrett, S. C., Colautti, R. I., and Eckert, C. G. (2008). Plant reproductive systems and evolution during biological invasion. *Mol. Ecol.* 17, 373–383. doi: 10.1111/j.1365-294X.2007.03503.x

Baskin, C. C., and Baskin, J. M. (1998). *Seeds: Ecology, Biogeography, And, Evolution Of Dormancy And Germination*. Elsevier: Amsterdam, doi: 10.1016/B978-0-12-080260-9.X5000-3

Bay, R. A., Harrigan, R. J., Underwood, V. L., Gibbs, H. L., Smith, T. B., and Ruegg, K. (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science* 359, 83–86. doi: 10.1126/science.aan4380

Bentley, K. E., and Mauricio, R. (2016). High degree of clonal reproduction and lack of large-scale geographic patterning mark the introduced range of the invasive

vine, kudzu (*Pueraria montana* var. lobata), in North America. *Am. J. Bot.* 103, 1499–1507. doi: 10.3732/ajb.1500434

Bernik, B. M., Li, H., and Blum, M. J. (2016). Genetic variation of *Spartina alterniflora* intentionally introduced to China. *Biol. Invasions* 18, 1485–1498. doi: 10.1007/s10530-016-1096-3

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., and Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144, 1–11. doi: 10.1007/s00442-005-0070-z

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., and Buckler, E. S. (2007). Tassel: software for association mapping of complex traits in diverse samples. *Bioinformatics* 23, 2633–2635. doi: 10.1093/bioinformatics/btm308

Brini, F., Hanin, M., Mezghani, I., Berkowitz, G. A., and Masmoudi, K. (2007). Overexpression of wheat Na$^+$/H$^+$ antiporter TNHX1 and H$^+$-pyrophosphatase TVP1 improve salt-and drought-stress tolerance in *Arabidopsis thaliana* plants. *J. Exp. Bot.* 58, 301–308. doi: 10.1093/jxb/erl251

Cao, L. J., Li, B. Y., Chen, J. C., Zhu, J. Y., Hoffmann, A. A., and Wei, S. J. (2021). Local climate adaptation and gene flow in the native range of two co-occurring fruit moths with contrasting invasiveness. *Mol. Ecol.* 30, 4204–4219. doi: 10.1111/mec.16055

Capblancq, T., Luu, K., Blum, M. G., and Bazin, E. (2018). Evaluation of redundancy analysis to identify signatures of local adaptation. *Mol. Ecol. Resour.* 18, 1223–1233. doi: 10.1111/1755-0998.12906

Castillo, J. M., Gallego-Tévar, B., Figueroa, E., Grewell, B. J., Vallet, D., Rousseau, H., et al. (2018). Low genetic diversity contrasts with high phenotypic variability in heptaploid *Spartina densiflora* populations invading the Pacific coast of North America. *Ecol. Evol* 8, 4992–5007. doi: 10.1002/ece3.4063

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., and Postlethwait, J. H. (2011). Stacks: building and genotyping loci de novo from short-read sequences. *G3-Genes. Genom. Genet.* 1, 171–182. doi: 10.1534/g3.111.000240

Caye, K., Jumentier, B., Lepeule, J., and François, O. (2019). LFMM 2: fast and accurate inference of gene-environment associations in genome-wide studies. *Mol. Biol. Evol.* 36, 852–860. doi: 10.1093/molbev/msz008

Chambers, J. M., Freeny, A. E., and Heiberger, R. M. (2017). "Analysis of variance," in *Statistical Models*, ed Edn, ed. T. J. Hastie (Milton Park: Routledge), 145–193.

Chung, C. (1985). *Brief History Of Spartina Anglica And Research Work Abroad*. Nanjing: Nanjing University Press, 30.

Chung, C.-H. (1993). Thirty years of ecological engineering with *Spartina plantations* in China. *Ecol. Eng.* 2, 261–289. doi: 10.1016/0925-8574(93)90019-C

Clavero, M., and García-Berthou, E. (2005). Invasive species are a leading cause of animal extinctions. *Trends Ecol. Evol.* 20:110. doi: 10.1016/j.tree.2005.01.003

Cullingham, C. I., Cooke, J. E., and Coltman, D. W. (2014). Cross-species outlier detection reveals different evolutionary pressures between sister species. *New Phytol.* 204, 215–229. doi: 10.1111/nph.12896

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., et al. (2011). The variant call format and VCFtools. *Bioinformatics* 27, 2156–2158. doi: 10.1093/bioinformatics/btr330

Deng, Z., An, S., Zhou, C., Wang, Z., Zhi, Y., Wang, Y., et al. (2007). Genetic structure and habitat selection of the tall form *Spartina alterniflora* loisel. in China. *Hydrobiologia* 583, 195–204. doi: 10.1007/s10750-006-0529-x

Dlugosch, K. M., and Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.* 17, 431–449. doi: 10.1111/j.1365-294X.2007.03538.x

Dray, S., Blanchet, G., Borcard, D., Guenard, G., Jombart, T., Larocque, G., et al. (2018). *Package 'Adespatial'*. Available online at https://cran.microsoft.com/web/packages/adespatial/adespatial.pdf (accessed April 10, 2022).

Early, R., Bradley, B. A., Dukes, J. S., Lawler, J. J., Olden, J. D., Blumenthal, D. M., et al. (2016). Global threats from invasive alien species in the twenty-first century and national response capacities. *Nat. Commun.* 7, 1–9. doi: 10.1038/ncomms12485

Eckert, C. G. (2002). "The loss of sex in clonal plants," in *Ecology and Evolutionary Biology Of Clonal Plants, Dordrecht*, edts Edn, eds J. F. Stuefer, B. Erschbamer, H. Huber, and J. I. Suzuki (New York:NY: Springer Press), 279–298. doi: 10.1007/978-94-017-1345-0_15

Ellstrand, N. C., and Schierenbeck, K. A. (2006). Hybridization as a stimulus for the evolution of invasiveness in plants. *Euphytica* 148, 35–46. doi: 10.1007/s10681-006-5939-3

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., and Facon, B. (2016). Is there a genetic paradox of biological invasion? *Annu. Rev. Ecol. Evol. Syst.* 47, 51–72. doi: 10.1146/annurev-ecolsys-121415-032116

Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinf.* 1, 47–50.

Facon, B., Genton, B. J., Shykoff, J., Jarne, P., Estoup, A., and David, P. (2006). A general eco-evolutionary framework for understanding bioinvasions. *Trends Ecol. Evol.* 21, 130–135. doi: 10.1016/j.tree.2005.10.012

Frankham, R. (2005). Resolving the genetic paradox in invasive species. *Heredity (Edinb)* 94:385. doi: 10.1038/sj.hdy.6800634

Frichot, E., and François, O. (2015). LEA: An R package for landscape and ecological association studies. *Methods Ecol. Evol.* 6, 925–929. doi: 10.1111/2041-210X.12382

Fridley, J. D., Stachowicz, J., Naeem, S., Sax, D., Seabloom, E., Smith, M., et al. (2007). The invasion paradox: reconciling pattern and process in species invasions. *Ecology* 88, 3–17. doi: 10.1890/0012-9658(2007)88[3:tiprpa]2.0.co;2

Gallego-Garcia, N., Forero-Medina, G., Vargas-Ramirez, M., Caballero, S., and Shaffer, H. B. (2019). Landscape genomic signatures indicate reduced gene flow and forest-associated adaptive divergence in an endangered neotropical turtle. *Mol. Ecol.* 28, 2757–2771. doi: 10.1111/mec.15112

Gaudeul, M., Giraud, T., Kiss, L., and Shykoff, J. A. (2011). Nuclear and chloroplast microsatellites show multiple introductions in the worldwide invasion history of common ragweed, Ambrosia artemisiifolia. *PLoS One* 6:e17658. doi: 10.1371/journal.pone.0017658

Geng, Y.-P., Pan, X.-Y., Xu, C.-Y., Zhang, W.-J., Li, B., Chen, J.-K., et al. (2007). Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. *Biol. Invasions* 9, 245–256. doi: 10.1007/s10530-006-9029-1

Genton, B. J., Shykoff, J. A., and Giraud, T. (2005). High genetic diversity in French invasive populations of common ragweed, Ambrosia artemisiifolia, as a result of multiple sources of introduction. *Mol. Ecol.* 14, 4275–4285. doi: 10.1111/j.1365-294X.2005.02750.x

Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., et al. (2014). TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. *PLoS One* 9:e90346. doi: 10.1371/journal.pone.0090346

Graus, D., Konrad, K. R., Bemm, F., Patir Nebioglu, M. G., Lorey, C., Duscha, K., et al. (2018). High V-PPase activity is beneficial under high salt loads, but detrimental without salinity. *New Phytol.* 219, 1421–1432. doi: 10.1111/nph.15280

Gray, M., and Raybould, A. (1991). "A century of evolution in Spartina anglica," in *Advances in Ecological Research, Elsevier*, eds A. MacFayden and E. D. Ford (Cambridge: Academic Press), 1–62. doi: 10.1016/s0065-2504(08)60096-3

Groves, H., and Groves, J. (1880). Spartina townsendii nobis. *Rep. Bot. Soc. Exch. Club Br. Id.* 1, 37.

Halkett, F., Simon, J.-C., and Balloux, F. (2005). Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecol. Evol.* 20, 194–201. doi: 10.1111/nph.15280

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., and Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25, 1965–1978. doi: 10.1016/j.tree.2005.01.001

Hijmans, R. J., Guarino, L., Bussink, C., Mathur, P., Cruz, M., Barrentes, I., et al. (2004). *Diva-gis: Vsn*. Available online at http://diva-gis.org (Accessed 6 December 2019)

Huang, K., Wang, D., Duan, P., Zhang, B., Xu, R., Li, N., et al. (2017). Wide and thick grain 1, which encodes an otubain-like protease with deubiquitination activity, influences grain size and shape in rice. *Plant J.* 91, 849–860. doi: 10.1111/tpj.13613

Jackson, J. M., Pimsler, M. L., Oyen, K. J., Strange, J. P., Dillon, M. E., and Lozier, J. D. (2020). Local adaptation across a complex bioclimatic landscape in two montane bumble bee species. *Mol. Ecol.* 29, 920–939. doi: 10.1111/mec.15376

Jeffreys, H. (1961). *The Theory Of Probability*. New York:NY: Oxford University Press.

Jombart, T., Devillard, S., and Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet.* 11:94. doi: 10.1186/1471-2156-11-94

Kamvar, Z. N., Brooks, J. C., and Grünwald, N. J. (2015). Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Front. Genet.* 6:208. doi: 10.3389/fgene.2015.00208

Kamvar, Z. N., Tabima, J. F., and Grünwald, N. J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *Peer. J.* 2:e281. doi: 10.7717/peerj.281

Katoh, K., Rozewicki, J., and Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* 20, 1160–1166. doi: 10.1093/bib/bbx108

Kue Foka, I. C., Ketehouli, T., Zhou, Y., Li, X.-W., Wang, F.-W., and Li, H. (2020). The emerging roles of diacylglycerol kinase (DGK) in plant stress tolerance, growth, and development. *Agronomy* 10:1375. doi: 10.3390/agronomy10091375

Lai, J., Zou, Y., Zhang, J., and Peres-Neto, P. R. (2022). Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca. hp R package. *Methods Ecol. Evol.* 13, 782–788. doi: 10.1111/2041-210X.13800

Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends Ecol. Evol.* 17, 386–391. doi: 10.1016/S0169-5347(02)02554-5

Li, F., van Kleunen, M., Li, J., Liu, X., Gao, K., Zhu, J., et al. (2019b). Patterns of genetic variation reflect multiple introductions and pre-admixture sources of common ragweed (*Ambrosia artemisiifolia*) in China. *Biol. Invasions* 21, 2191–2209. doi: 10.1007/s10530-019-01966-2

Li, F.-F., Gong, L., Li, J.-S., Liu, X.-Y., and Zhao, C.-Y. (2019a). Low genetic differentiation yet high phenotypic variation in the invasive populations of *Spartina alterniflora* in Guangxi China. *PLoS One* 14:e0222646. doi: 10.1371/journal.pone.0222646

Li, H., An, S., Zhi, Y., Yan, C., Zhao, L., Zhou, C., et al. (2008). Protogynous, pollen limitation and low seed production reasoned for the dieback of Spartina anglica in coastal China. *Plant Sci.* 174, 299–309. doi: 10.1016/j.plantsci.2007.11.015

Li, H., Zhi, Y., An, S., Zhao, L., Zhou, C., Deng, Z., et al. (2009). Density-dependent effects on the dieback of exotic species *Spartina anglica* in coastal China. *Ecol. Eng.* 35, 544–552. doi: 10.1016/j.ecoleng.2008.03.001

Li, H., Zhi, Y., Zhao, L., An, S., Deng, Z., Zhou, C., et al. (2007). Eco-physiological responses of the declining population *Spartina anglica* to N and P fertilizer addition. *Sheng. Xuebao* 27, 2725–2732. doi: 10.1016/s1872-2032(07)60060-2

Li, Y., Tan, Y., Shao, Y., Li, M., and Ma, F. (2015). Comprehensive genomic analysis and expression profiling of diacylglycerol kinase gene family in *Malus prunifolia* (Willd.) Borkh. *Gene* 561, 225–234. doi: 10.1016/j.gene.2015.02.029

Lischer, H. E., and Excoffier, L. (2012). PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. *Bioinformatics* 28, 298–299. doi: 10.1093/bioinformatics/btr642

Liu, J., Dong, M., Miao, S. L., Li, Z. Y., Song, M. H., and Wang, R. Q. (2006). Invasive alien plants in China: role of clonality and geographical origin. *Biol. Inv.* 8, 1461–1470. doi: 10.1007/s10530-005-5838-x

Liu, L., An, S., Zhi, Y., Zhang, M., and Li, H. (2016a). Effects of different sediment type and burial depth on growth traits and biomass accumulation of *Spartina anglica*. *Shengwu Duoyangxing* 24, 1279–1287. doi: 10.17520/biods.2016024

Liu, W., Maung-Douglass, K., Strong, D. R., Pennings, S. C., and Zhang, Y. (2016b). Geographical variation in vegetative growth and sexual reproduction of the invasive *Spartina alterniflora* in China. *J. Ecol.* 104, 173–181. doi: 10.1111/1365-2745.12487

Liu, W., Strong, D. R., Pennings, S. C., and Zhang, Y. (2017). Provenance-by-environment interaction of reproductive traits in the invasion of *Spartina alterniflora* in China. *Ecology* 98, 1591–1599. doi: 10.1002/ecy

Liu, W., Zhang, Y., Chen, X., Maung-Douglass, K., Strong, D. R., and Pennings, S. C. (2020). Contrasting plant adaptation strategies to latitude in the native and invasive range of *Spartina alterniflora*. *New Phytol.* 226, 623–634. doi: 10.1111/nph.16371

Lotterhos, K. E., and Whitlock, M. C. (2014). Evaluation of demographic history and neutral parameterization on the performance of FST outlier tests. *Mol. Ecol.* 23, 2178–2192. doi: 10.1111/mec.12725

Maurice, S., Montes, M. S., Nielsen, B. J., Bodker, L., Martin, M. D., Jonck, C. G., et al. (2019). Population genomics of an outbreak of the potato late blight pathogen, *Phytophthora* infestans, reveals both clonality and high genotypic diversity. *Mol. Plant Pathol.* 20, 1134–1146. doi: 10.1111/mpp.12819

Moles, A. T., Ackerly, D. D., Webb, C. O., Tweddle, J. C., Dickie, J. B., Pitman, A. J., et al. (2005). Factors that shape seed mass evolution. *Proc. Natl. Acad. Sci.* 102, 10540–10544. doi: 10.1073/pnas.0501473102

Molina-Montenegro, M. A., Acuna-Rodriguez, I. S., Flores, T. S. M., Hereme, R., Lafon, A., Atala, C., et al. (2018). Is the success of plant invasions the result of rapid adaptive evolution in seed traits? Evidence from a latitudinal rainfall gradient. *Front. Plant Sci.* 9:208. doi: 10.3389/fpls.2018.00208

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583–590. doi: 10.1093/genetics/89.3.583

Nielsen, E. S., Henriques, R., Toonen, R. J., Knapp, I. S. S., Guo, B., and Von Der Heyden, S. (2018). Complex signatures of genomic variation of two non-model marine species in a homogeneous environment. *BMC Genomics* 19:347. doi: 10.1186/s12864-018-4721-y

Ochocki, B. M., and Miller, T. E. X. (2017). Rapid evolution of dispersal ability makes biological invasions faster and more variable. *Nat. Commun.* 8, 1–8. doi: 10.1038/ncomms14315

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., et al. (2021). *Vegan: Community Ecology Package. R package version 2.5-7. 2020.*Available online at https://CRAN.R-project.org/package=vegan. (accessed 10 February 2022).

Pantoja, P. O., Simón-Porcar, V. I., Puzey, J. R., and Vallejo-Marín, M. (2017). Genetic variation and clonal diversity in introduced populations of *Mimulus guttatus* assessed by genotyping at 62 single nucleotide polymorphism loci. *Plant Ecol. Divers.* 10, 5–15. doi: 10.1080/17550874.2017.1287785

Park, S., Li, J., Pittman, J. K., Berkowitz, G. A., Yang, H., Undurraga, S., et al. (2005). Up-regulation of a H^+-pyrophosphatase (H^+-PPase) as a strategy to engineer drought-resistant crop plants. *Proc. Natl. Acad. Sci.* 102, 18830–18835. doi: 10.1073/pnas.0509512102

Peterson, P. M., Romaschenko, K., Arrieta, Y. H., and Saarela, J. M. (2014). A molecular phylogeny and new subgeneric classification of *Sporobolus* (poaceae: chloridoideae: sporobolinae). *Taxon* 63, 1212–1243. doi: 10.12705/636.19

Prentis, P. J., Wilson, J. R., Dormontt, E. E., Richardson, D. M., and Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends Plant Sci.* 13, 288–294. doi: 10.1016/j.tplants.2008.03.004

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., et al. (2007). Plink: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575. doi: 10.1086/519795

Qiao, H., Liu, W., Zhang, Y., Zhang, Y. Y., and Li, Q. Q. (2019). Genetic admixture accelerates invasion via provisioning rapid adaptive evolution. *Mol. Ecol.* 28, 4012–4027. doi: 10.1111/mec.15192

Raybould, A., Gray, A., Lawrence, M., and Marshall, D. (1991). The evolution of *Spartina anglica* CE Hubbard (Gramineae): origin and genetic variability. *Biol. J. Linn. Soc. Lond.* 43, 111–126. doi: 10.1111/j.1095-8312.1991.tb00588.x

Reichel, K., Masson, J.-P., Malrieu, F., Arnaud-Haond, S., and Stoeckel, S. (2016). Rare sex or out of reach equilibrium? *BMC Genet.* 17:76. doi: 10.1186/s12863-016-0388-z

Ren, M. X., Zhang, Q. G., and Zhang, D. Y. (2005). Random amplified polymorphic DNA markers reveal low genetic variation and a single dominant genotype in Eichhornia crassipes populations throughout China. *Weed Res.* 45, 236–244. doi: 10.1111/j.1365-3180.2005.00445.x

Rengefors, K., Gollnisch, R., Sassenhagen, I., Svensson, M., Lebret, K., Čertnerová, D., et al. (2021). Genome-wide SNP markers reveal population structure and dispersal direction of an expanding nuisance algal bloom species. *Mol. Ecol.* 30, 912–925. doi: 10.1111/mec.15787

Rigas, S., Daras, G., Laxa, M., Marathias, N., Fasseas, C., Sweetlove, L. J., et al. (2009). Role of Lon1 protease in post-germinative growth and maintenance of mitochondrial function in Arabidopsis thaliana. *New Phytol.* 181, 588–600. doi: 10.1111/j.1469-8137.2008.02701.x

Rius, M., and Darling, J. A. (2014). How important is intraspecific genetic admixture to the success of colonising populations? *Trends Ecol. Evol.* 29, 233–242. doi: 10.1016/j.tree.2014.02.003

Roman, J., and Darling, J. A. (2007). Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecol. Evol.* 22, 454–464. doi: 10.1016/j.tree.2007.07.002

Ruelland, E., Cantrel, C., Gawer, M., Kader, J.-C., and Zachowski, A. (2002). Activation of phospholipases C and D is an early response to a cold exposure in Arabidopsis suspension cells. *Plant Physiol.* 130, 999–1007. doi: 10.1104/pp.006080

Saleh, D., Xu, P., Shen, Y., Li, C., Adreit, H., Milazzo, J., et al. (2012). Sex at the origin: an asian population of the rice blast fungus *Magnaporthe oryzae* reproduces sexually. *Mol. Ecol.* 21, 1330–1344. doi: 10.1111/j.1365-294X.2012.05469.x

Schierenbeck, K. A., and Ellstrand, N. C. (2008). Hybridization and the evolution of invasiveness in plants and other organisms. *Biol. Invasions* 11, 1093–1105. doi: 10.1007/s10530-008-9388-x

Schrieber, K., and Lachmuth, S. (2017). The genetic paradox of invasions revisited: the potential role of inbreeding x environment interactions in invasion success. *Biol. Rev. Camb. Philos. Soc.* 92, 939–952. doi: 10.1111/brv.12263

Shannon, C. E. (1948). A mathematical theory of communication. *Bell Syst. Tech. J.* 27, 379–423. doi: 10.1002/j.1538-7305.1948.tb01338.x

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., et al. (2013). Impacts of biological invasions: what's what and the way forward. *Trends Ecol. Evol.* 28, 58–66. doi: 10.1016/j.tree.2012.07.013

Simpson, E. (1949). Medición de la diversidad. *Nature* 163, 688–688.

Stapley, J., Santure, A. W., and Dennis, S. R. (2015). Transposable elements as agents of rapid adaptation may explain the genetic paradox of invasive species. *Mol. Ecol.* 24, 2241–2252. doi: 10.1111/mec.13089

Stoeckel, S., and Masson, J. P. (2014). The exact distributions of F(IS) under partial asexuality in small finite populations with mutation. *PLoS One* 9:e85228. doi: 10.1371/journal.pone.0085228

Storey, J. D., Bass, A. J., Dabney, A., and Robinson, D. (2015). *qvalue: Q-Value Estimation For False Discovery Rate Control. R package version 2.6. 0.*Available online at: github. com/jdstorey/qvalue. (accessed April 14, 2019).

Suarez, A. V., and Tsutsui, N. D. (2008). The evolutionary consequences of biological invasions. *Mol. Ecol.* 17, 351–360. doi: 10.1111/j.1365-294X.2007.03456.x

Tan, W.-J., Yang, Y.-C., Zhou, Y., Huang, L.-P., Xu, L., Chen, Q.-F., et al. (2018). Diacylglycerol acyltransferase and diacylglycerol kinase modulate triacylglycerol and phosphatidic acid production in the plant response to freezing stress. *Plant Physiol.* 177, 1303–1318. doi: 10.1104/pp.18.00402

Tsujimoto, M., Araki, K. S., Honjo, M. N., Yasugi, M., Nagano, A. J., Akama, S., et al. (2020). Genet assignment and population structure analysis in a clonal forest-floor herb. *AOB Plants* 12:lz080. doi: 10.1093/aobpla/plz080

Turner, K. G., Hufbauer, R. A., and Rieseberg, L. H. (2014). Rapid evolution of an invasive weed. *New Phytol.* 202, 309–321. doi: 10.1111/nph.12634

van Boheemen, L. A., Lombaert, E., Nurkowski, K. A., Gauffre, B., Rieseberg, L. H., and Hodgins, K. A. (2017). Multiple introductions, admixture and bridgehead invasion characterize the introduction history of ambrosia artemisiifolia in Europe and Australia. *Mol. Ecol.* 26, 5421–5434. doi: 10.1111/mec.14293

VanWallendael, A., Hamann, E., and Franks, S. J. (2018). Evidence for plasticity, but not local adaptation, in invasive *Japanese knotweed* (*Reynoutria japonica*) in North America. *Ecol. Evol.* 32, 395–410. doi: 10.1007/s10682-018-9942-7

Wang, P., Alpert, P., and Yu, F. H. (2016). Clonal integration increases relative competitive ability in an invasive aquatic plant. *Am. J. Bot.* 103, 2079–2086. doi: 10.3732/ajb.1600160

Wellband, K. W., and Heath, D. D. (2017). Plasticity in gene transcription explains the differential performance of two invasive fish species. *Evol. Appl.* 10, 563–576. doi: 10.1111/eva.12463

Wellband, K. W., Pettitt-Wade, H., Fisk, A. T., and Heath, D. D. (2017). Differential invasion success in aquatic invasive species: the role of within- and among-population genetic diversity. *Biol. Inv.* 19, 2609–2621. doi: 10.1007/s10530-017-1471-8

Wellband, K. W., Pettitt-Wade, H., Fisk, A. T., and Heath, D. D. (2018). Standing genetic diversity and selection at functional gene loci are associated with differential invasion success in two non-native fish species. *Mol. Ecol.* 27, 1572–1585. doi: 10.1111/mec.14557

Xia, L., Geng, Q., and An, S. (2020). Rapid genetic divergence of an invasive Species, *Spartina alterniflora*, in China. *Front. Genet.* 11:284. doi: 10.3389/fgene.2020.00284

Xia, L., Zhao, H., Yang, W., and An, S. (2015). Genetic diversity, ecotype hybrid, and mixture of invasive *Spartina alterniflora* Loisel in Coastal China. *CLEAN–Soil Air Water* 43, 1672–1681. doi: 10.1002/clen.201300882

Xiao, Y., Tang, J., Qing, H., Zhou, C., and An, S. (2011). Effects of salinity and clonal integration on growth and sexual reproduction of the invasive grass *Spartina alterniflora*. *Flora Morphol. Distrib. Funct. Ecol. Plants.* 206, 736–741. doi: 10.1016/j.flora.2010.12.003

Xu, G. W., and Zhuo, R. Z. (1985). *Preliminary Studies Of Introduced Spartina alterniflora Loisel in China (I),"* In Research Advances in Spartina: Achievements of Past 22 Years. Nanjing: Journal of Nanjing University, 212–215.

Yandell, B. S. (2017). *Practical Data Analysis for Designed Experiments*. New York:NY: Routledge Press.

Zenni, R. D., and Nuñez, M. A. (2013). The elephant in the room: the role of failed invasions in understanding invasion biology. *Oikos* 122, 801–815. doi: 10.1111/j.1600-0706.2012.00254.x

Zenni, R. D., Bailey, J. K., and Simberloff, D. (2014). Rapid evolution and range expansion of an invasive plant are driven by provenance-environment interactions. *Ecol. Lett.* 17, 727–735. doi: 10.1111/ele.12278

Zhang, D., Hu, Y., Liu, M., Chang, Y., Yan, X., Bu, R., et al. (2017). Introduction and spread of an exotic plant, *spartina alterniflora*, along coastal marshes of China. *Wetlands* 37, 1181–1193. doi: 10.1007/s13157-017-0950-0

Zhang, Q., and Xiao, S. (2015). Lipids in salicylic acid-mediated defense in plants: focusing on the roles of phosphatidic acid and phosphatidylinositol 4-phosphate. *Front. Plant Sci.* 6:387. doi: 10.3389/fpls.2015.00387

Zhou, H., Alexander, D., and Lange, K. (2011). A quasi-newton acceleration for high-dimensional optimization algorithms. *Stat. Comput.* 21, 261–273. doi: 10.1007/s11222-009-9166-3

Zuo, P., Zhao, S., Liu, C. A., Wang, C., and Liang, Y. (2012). Distribution of *Spartina* spp. along China's coast. *Ecol. Eng.* 40, 160–166. doi: 10.1016/j.ecoleng.2011.12.014