Hybridization, polyploidy and clonality influence geographic patterns of diversity and salt tolerance in the model halophyte seashore paspalum (*Paspalum vaginatum*)

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Abstract

Seashore paspalum (*Paspalum vaginatum* Swartz) is a halophytic turfgrass and emerging genomic model system for the study of salt tolerance in cereals and other grasses. Despite recent interest and an increase in available tools, little is known about the diversity present in wild populations of *P. vaginatum* and its close relative *P. distichum*. Variation in ploidy, clonal propagation, hybridization, and subgenome composition appear to occur in the wild and may interact to influence geographic patterns of adaptation, particularly in response to environmental salinity levels. Using 218 accessions representing >170 wild collections from throughout the coastal southern United States plus existing USDA germplasm, we employed genotyping-by-sequencing, cpDNA sequencing and flow cytometry to identify genetic differentiation and ploidy variation. Within *P. vaginatum*, there are two morphologically distinct ecotypes: the fine-textured ecotype is diploid and appears to reproduce in the wild both sexually and by clonal propagation; in contrast, the coarse-textured ecotype consists largely of clonally-propagating triploid and diploid genotypes. The coarse-textured ecotype appears to be derived from hybridization between fine-textured *P. vaginatum* and an unidentified *Paspalum* species. These clonally propagating hybrid genotypes are more broadly distributed than clonal fine-textured genotypes and may represent a transition to a more generalist adaptive strategy. The triploid genotypes vary in whether they carry one or two copies of the *P. vaginatum* subgenome, indicating multiple evolutionary origins. This variation in subgenome composition shows associations with local ocean salinity levels across the sampled populations and may play a role in local adaptation.

Introduction

A hallmark of angiosperm diversification has been the ability of plant species to adapt to an extremely wide range of environments characterized by different biotic and abiotic stresses. Among abiotic stresses, the physiological stress of growing in high salt environments is particularly challenging for plants. High levels of salt change the osmotic environment, leading to dehydration; in addition, both Na+ and Cl- ions are toxic if they accumulate in cells. Nonetheless, a small number of terrestrial plant species (ca. 2%) have managed to colonize and even thrive in saline ecosystems such as beaches, salt marshes, and estuaries (Glenn, Brown, & Blumwald, 1999). These plants, collectively called halophytes, have become important models for the study of salt tolerance mechanisms and evolution (Cheeseman, 2013).

The term "salt tolerance" is often used as though it is a binary characteristic; a plant is either tolerant or not. However, salt tolerance in halophytes is likely to be quantitative, with tolerance tuned to match the local environment (Cheeseman, 2013). Saline environments are often characterized by broad gradients of environmental salt concentration, from relatively low concentrations up to (and occasionally even beyond) the concentration of seawater. Such gradients are common where fresh and sea water mix, with areas of lower

salinity extending along the coastline and far offshore from the mouths of major rivers (Fournier, Reager, Dzwonkowski, & Vazquez-Cuervo, 2019). This variation offers extensive opportunities for local salinity adaptation within widely distributed halophytic species; however, whether such local adaptation occurs and the forces that influence it are largely unknown. As described below, three common characteristics of halophytic species that could shape patterns of local salinity adaptation are clonal propagation, interspecific hybridization and ploidy variation.

Clonal propagation occurs widely in halophytes and has important implications for geographic patterns of diversity and local salinity adaptation (Bricker, Calladine, Virnstein, & Waycott, 2018; Jefferies & Rudmik, 1991; Róis et al., 2015). Depending on the dispersal capacity of clonal propagules and the frequency at which novel genotypes arise (e.g., through sexual reproduction or somatic mutation), one of two reproductive strategies might be expected. If new genotypes arise often but have limited clonal dispersal, a "frozen niche" strategy might be favored, whereby multiple genetically distinct clonal populations occur, each restricted to the small geographical area to which it is locally adapted (Róis et al., 2015; Vrijenhoek, 1979). Alternatively, if the production of new genotypes is infrequent and/or dispersal potential is high, a "general-purpose genotype" strategy might instead be favored, whereby a few generalist genotypes are present in multiple environments across a wide geographic range (Baker, 1965; Bricker, Calladine, Virnstein, & Waycott, 2018; Coughlan, Han, Stefanović, & Dickinson, 2017). The underlying conditions that lead genotypes to follow one pattern or another remain poorly understood (Vrijenhoek & Parker, 2009).

In contrast to clonal propagation, interspecific hybridization can provide access to new ecological niches by introducing novel genetic variation into a population and/or increasing heterosis (Rieseberg, Archer, & Wayne, 1999; Soltis & Soltis, 2009). Adaptation to saline environments has been shown to be influenced by natural hybridization in several wild systems. Specifically, hybrids derived from two halophytic species may show a relative increase or decrease in tolerance relative to their parents (Gallego-Tévar, Curado, Grewell, Figueroa, & Castillo, 2018; Lee, Ayres, Pakenham-Walsh, & Strong, 2016). It can also produce a novel halophyte from two non-halophytic parental species (Edelist et al., 2009; Welch & Rieseberg, 2002). The relative roles of the genomic contributions from different parental species for phenotypic and niche differentiation in hybrids remains an open question (Bar-Zvi, Lupo, Levy, & Barkai, 2017). High-throughput genomic techniques can help in identifying hybrid genotypes, their genomic composition, and the relative importance of their parental genomes for salinity adaptation.

Polyploidy (i.e. whole-genome duplication) may also play a role in salinity adaptation. Studies in non-halophytes have suggested that inducing autopolyploidy in a diploid species (i.e. doubling its genome) can increase salt tolerance (Chao et al., 2013; Wu, Lin, Jiao, & Li, 2019). However, in the wild most halophytic polyploids are not autopolyploids but rather allopolyploids — having arisen through interspecific hybridization where each parental species contributes its entire genome (Ainouche, Baumel, Salmon, & Yannic, 2004). In such cases the effect of the genome duplication is confounded with the potential effects of interspecific hybridization described above (Fort et al. 2016). Systems where repeated natural hybridization events have resulted in a mix of diploid and polyploid genotypes could potentially be used to untangle these effects. If such a group also varied with respect to the relative numbers of genome copies received from each parental species, whole-genome dosage effects on fitness and other phenotypes could also be examined (Betto-Colliard, Hofmann, Sermier, Perrin, & Stöck, 2018; Harvey, Fjelldal, Solberg, Hansen, & Glover, 2017; Tan et al. 2016).

The halophytic grass seashore paspalum (*Paspalum vaginatum*Swartz*)* provides an advantageous system for addressing how clonality, hybridization and polyploidy interact to shape patterns of genotypic variation across salinity gradients. First, it has a worldwide distribution across habitats of varying salinity (Duncan & Carrow, 2000). Second, it shows quantitative variation in salt tolerance, up to and including sea water concentrations (Lee, Duncan, & Carrow, 2004; Lee, Carrow, & Duncan, 2004, 2005; Lee, Carrow, Duncan, Eiteman, & Rieger, 2008), although the genetic basis and geographical distribution of this variation is almost completely unknown. Third, it spreads vegetatively by stolons and is capable of clonal propagation under cultivation, suggesting that dispersal of clonal propagules may affect geographical patterns of genotypic variation in the wild. Fourth, it has been proposed to undergo hybridization with a closely related non-salt

tolerant species (*P. distichum* L.) (Eudy, Bahri, Harrison, Raymer, & Devos, 2017), which could contribute to natural phenotypic variation in salt tolerance. Finally, although it is believed to be predominantly diploid (2n = 2x =20) (Duncan and Carrow, 2000; Eudy, Bahri, Harrison, Raymer, & Devos, 2017), both diploid and triploid cytotypes have been reported (Eudy, Bahri, Harrison, Raymer, & Devos, 2017), which suggests that ploidy level and subgenome composition might also contribute to population structure and local salinity adaptation.

In addition to these life history features, *Paspalum vaginatum*also provides the benefits of a genome-enabled model system with experimental tractability and economic value that facilitate applied studies. It has a manageably-sized ~600 Mb diploid genome which has recently been assembled, allowing for genomic analyses (Eudy, Bahri, Harrison, Raymer, & Devos, 2017; Qi et al., 2019). It is closely related to several economically important grass species in the subfamily Panicoideae, including maize, sorghum and sugarcane (Morrone et al., 2012), which could facilitate the transfer of salt tolerance mechanisms to major cereal crops. It is also economically important in its own right as a turfgrass for salt-affected soils. As such, it has resources and germplasm available through breeding programs (Duncan & Carrow, 2000).

One current limitation of publicly available (USDA) *P. vaginatum*germplasm is its narrow genetic variability due to genotypic redundancy among accessions (Eudy, Bahri, Harrison, Raymer & Devos, 2017) and collection biases towards plants with favorable turfgrass qualities (Duncan & Carrow, 2000). Additionally, due to the decades-long gap between the collection and genotyping of these USDA samples, it is unclear whether the high degree of genotypic redundancy in the germplasm collection represents natural clonal propagation in the wild or post-collection cross-contamination of accessions, which can easily occur as the aggressive stolons typical of the species invade neighboring pots (Eudy, Bahri, Harrison, Raymer, & Devos, 2017). Genotyping of newly collected material is thus required to quantify the actual levels of clonal propagation in the wild and geographical patterns of genotypic diversity.

In this study, we undertook the largest collection of living wild specimens of *P. vaginatum* and *P. distichum* to date and analyzed them together with available USDA germplasm to investigate the nature of genotypic distributions across salinity gradients in the North American species range. Specifically, we asked the following questions: 1) Do wild *P. vaginatum* populations represent many distinct genotypes or replicates of a few geographically widespread clones? 2) To the extent that clonal propagation occurs in the wild, do geographical and environmental distributions suggest a localized "frozen niche" reproductive strategy, a widespread "general purpose genotype" strategy, or no discernible pattern? 3) To what extent does interspecific hybridization and/or ploidy variation in this system influence patterns of genetic differentiation and diversity in wild populations? 4) How do clonal structure, ploidy variation and subgenome composition vary across geographical gradients in salinity? Our findings provide insight into the roles of genome composition and clonal propagation in local salinity adaptation in *P. vaginatum* , and lay the necessary groundwork for future investigations into the genetic basis of salt tolerance in this genomic model halophytic grass species.

Methods

Study system

Paspalum vaginatum is distributed throughout tropical and subtropical saline ecosystems worldwide (Duncan & Carrow, 2000). It is characterized by two common ecotypes that differ in shoot and leaf morphology, referred to as the 'coarse-textured' and 'fine-textured' ecotypes (Fig. 1B; Duncan & Carrow, 2000). The fine-textured ecotype is more economically important because its prostrate growth form and smaller leaves makes it valuable for use as a turfgrass; the coarse-textured ecotype has also seen limited use as a dune stabilizer. Within the US, the coarse-textured ecotype is typically found on the Gulf Coast and Florida, while fine-textured plants are found on the Atlantic coast from North Carolina to Georgia (Duncan & Carrow, 2000). Due to the focus on collecting samples for turf breeding programs, the USDA *P. vaginatum* germplasm collection is composed almost entirely of fine-textured accessions (USDA-ARS, 2015).

Paspalum vaginatum and its closely related non-halophytic sister species *P. distichum* together comprise the Disticha clade, which is distantly related to the other ca. 300 species in the genus (Burson, 1981; Scataglini,

Zuloaga, Giussani, Denham, & Morrone, 2014). The two species are morphologically very similar and often confused (Bor & Guest, 1968; Brummitt, 1983), although they are distinguishable based on cpDNA and nuclear sequences (Scataglini, Zuloaga, Giussani, Denham, & Morrone, 2014). Unlike *P. vaginatum* , *P. distichum* is believed to be predominantly hexaploid (2n = 60), with tetraploid (2n = 40), pentaploid (2n = 50) and hyperpentaploid (2n = 52, 54, 57, and 58) cytotypes occurring occasionally in the wild Echarte, Clausen, & Sala, 1992). A putative diploid *P. distichum* accession has also been reported (Eudy, Bahri, Harrison, Raymer, & Devos, 2017).

Sampling

A set of 146 coarse-textured *P. vaginatum* , 18 fine-textured*P. vaginatum* and 10 *P. distichum* plant samples was collected along the Atlantic and Gulf coasts of the southeastern United States, from North Carolina to Texas, during June and July of 2016 and 2017 (Fig. 1A; Table S1). Collection habitats included beaches, salt marshes, brackish estuaries and freshwater rivers and lakes. To reduce the chance of repeatedly collecting representatives of the same clone, localities were separated by at least 1.5 km. One to three samples were collected in each location. When multiple samples were collected from a single locality, they were separated by at least 100 m to minimize replicated sampling of a single clone. Samples were harvested as stolons which were wrapped in a damp paper towel and then placed in a plastic bag for no longer than one week before planting. Stolons were propagated in the Donald Danforth Plant Science Center greenhouses. In addition to the wild-collected material, a set of 40 samples, representing 35*P. vaginatum* (two of which are coarse-textured) and five *P. distichum* , were obtained from the USDA National Genetic Resources Program. Additionally, dried tissue for DNA extraction, representing four *P. vaginatum* accessions and one *P. distichum*accession, were acquired from Uruguay from Dr. Pablo Speranza (Universidad de la República de Uruguay).

DNA extraction and genotyping-by-sequencing

Genomic DNA was extracted using the CTAB protocol of Brock, Dönmez, Beilstein, & Olsen (2018), which was modified from Webb & Knapp (1990). Genotyping-by-sequencing (GBS) libraries were prepared following Poland & Rife (2012), using a double digest of *Pst* I and*Msp* I. Samples were sequenced on three lanes of the Illumina HiSeq 2500 platform with 1x100 reads at the Roy J. Carver Biotechnology Center at the University of Illinois Champaign-Urbana.

Bioinformatics

Read processing and variant calling were performed using the Fast-GBS pipeline with default parameters (Torkamaneh, Laroche, Bastien, Abed, & Belzile, 2017), which demultiplexes with sabre v1.000 (Joshi, 2011), trims with cutadapt v1.14 (Martin, 2011), maps with BWA MEM v0.7.12-r1039 (Li & Durbin, 2009) and calls variants with Platypus v0.8.1 (Rimmer et al., 2014). This produced a large SNP dataset which was then further filtered with plink v1.90b4.6 (Purcell et al. 2007). Sites were removed if there were missing calls for more than 10% of the samples or had less than 0.01 minor allele frequency. Individuals that were missing calls at 50% or more sites were also removed.

To identify clonal replicates among collected plants, all samples were included in a principal component analysis (PCA) performed with plink (Purcell et al., 2007); within each visually identified genotype cluster in the PCA, a pairwise genetic distance matrix was constructed. To produce a group-specific similarity threshold to identify identical genotypes, we then created a histogram of distances and heuristically chose a threshold that separated the first and second peaks (see Fig. S1). Refined variant datasets were then created with duplicate genotypes removed; for each set of duplicate genotypes, only the accession with the highest coverage was retained.

To prevent biases due to multiple collections of the same genotype, minor allele frequency and missing data filters were repeated in plink for the dataset consisting of unique genotypes only. An additional PCA was then performed on this dataset. To assess population structure within diploid *P. vaginatum* , ADMIXTURE v1.3.0 (Alexander, Novembre, & Lange, 2009) analysis was performed on a further reduced dataset comprising coarse- and fine-textured diploids, with assumed population numbers ranging from K = 2-6. Cross validation

error estimation was used to determine the optimal K.

Geographic and environmental dispersion of clonal genotypes.

For genotypes with multiple clones collected from the wild, we calculated pairwise geographic distance between collections within each clonal genotype based on GPS coordinates of each collection with the*distHaversine* function in R v3.4.1 (Hijmans, Williams, & Vennes, 2019; R Core Team, 2013). To measure pairwise environmental distance, we calculated Euclidian distance for environmental variables in three dimensions with the *dist* function in R, as follows: coordinates for the first and second dimension were obtained as the first and second principal components in a PCA of the 19 WorldClim BioClim variables (Fick & Hijmans, 2017) for each of our collection locations. Principal components were calculated using the *prcomp* function in R with scaled unit variance. The values of the first two PCs explained 50.9% and 22.7% of the variance respectively. For the third coordinate, we downloaded the average sea surface salinity dataset for the years 2005-2017 from the World Ocean Atlas (Zweng et al., 2019) and identified the salinity of the ocean water nearest to each collection site (excluding one sample collected $>$ 40 km from the coast). All three coordinates where then centered and scaled with the*scale* function in R before calculating distance.

Chloroplast gene sequencing

To look for differences in maternal subgenome between *P. distichum* and *P. vaginatum* and to confirm that the coarse-textured ecotypes are part of the Disticha clade (see Results), we sequenced the intron of the *rpl16* chloroplast ribosomal genes of 16 accessions (3 *P. distichum* , 13 *P. vaginatum*) using the F71 and R1661 primers described in Giussani et al. (2009). The*P. vaginatum* samples included 3 fine-textured and 10 coarse-textured ecotypes. Direct Sanger sequencing of purified PCR products was performed at the University of Wisconsin Biotechnology Center using an ABI 3730xl DNA Analyzer. Sequences were aligned against the *P. vaginatum rpl16* sequence (accession KF853027; Scataglini, Zuloaga, Giussani, Denham, & Morrone, 2014) and compared using BioEdit (Hall, 1999).

Ploidy estimation with flow cytometry

In order to assess genome size variation and ploidy, fresh leaf tissue was collected from one accession of each unique genotype, except for fine-textured samples where only two of the 24 unique genotypes were tested. Tissue was chopped with a razor blade in 1 mL cold LB01 (Doležel, Binarová, & Lucretti, 1989) along with maize line B73 tissue, which was included as an internal standard. Nuclei were stained with propidium iodide, and samples were run on an Accuri C6 flow cytometer. Genome sizes were then calculated by taking the ratio of sample DNA to B73 DNA. These calculations were then divided by the value calculated for PI 509022 (the *P. vaginatum* reference genome accession, 0.259 relative to B73) to determine relative genome size of each accession compared to PI 509022.

Ploidy estimates with genome-wide heterozygosity

For GBS-genotyped samples that vary in ploidy, measures of genome-wide heterozygosity can potentially be used as a complement to flow cytometry for identifying ploidy variation (Gompert & Mock, 2017). This approach is based on the rationale that if all reads are mapped to a diploid reference genome, any subgenome differences in polyploids will be recorded as 'heterozygous' loci. At increasing ploidy levels, each additional subgenome will introduce more differences and increase the number of heterozygous locus calls. This approach can also be used to identify diploid hybrids, which are identifiable as showing a quantum increase in heterozygosity with no associated increase in genome size based on flow cytometry. We employed these strategies to assess ploidy variation in the *Paspalum* samples, focusing on genotypically unique accessions with the highest sequence coverage. Genome-wide heterozygosity was calculated as the individual inbreeding coefficient (F) with the het function in plink (Purcell et al., 2007).

Subgenome comparisons

Heterozygous genotype calls were also used to infer subgenome identity using a custom python script (https://github.com/david-goad/paspalum-hybridization/het_venn.py). For this analysis we used the da-

taset comprising all unique genotypes after removal of sites with <0.05 heterozygosity. We calculated the number of loci that were heterozygous in each of our three morphological groups (the two *P. vaginatum* ecotypes and *P. distichum,*excluding three putatively admixed accessions). We then compared the number of loci with heterozygous calls unique to each group to those that were shared between groups. Following the logic that 'heterozygous' loci reflect subgenome differences within individuals, we expected that subgenomes shared between groups would be reflected as shared 'heterozygous' loci. In the case of allopolyploid and diploid hybrid groups (*P. distichum* and coarse-textured *P. vaginatum* ; see Results), high numbers of shared loci would indicate sharing of a subgenome that is not present in the diploid fine-textured *P. vaginatum* samples.

To investigate the number of subgenome copies received from each parent in triploid coarse-textured genotypes, we used a custom python script (https://github.com/david-goad/paspalum-hybridization/triploid_comp.py) that counts reads at heterozygous loci in a SAM file (Fig. S2). Based on the distribution of the relative number of reference vs non-reference reads at each locus, the number of subgenome copies can be inferred (Delomas, 2019; Gompert & Mock, 2017). We chose the highest coverage individual from each of the three putative triploid genotypes and the diploid genotype with the greatest number of genotypically-identical accessions (to serve as a 1:1 control). In cases where a locus was called as heterozygous in all four of the genotypes, we counted the number of each read type covering the position in the same file based on their CIGAR string (the column of the SAM file which identifies the position of variants in the read). To remove the effect of low-quality reads (often manifested as singletons) we only compared the two most common read types. Additionally, sites were excluded if neither of the two most common reads was identical to the reference genome (to ensure unambiguous assignment to a subgenome) or if their combined count was less than 20 (to ensure adequate depth). The number of reference and alternate reads at each locus was then visualized as a scatter plot for the raw counts and a histogram for the percent of reads matching the reference genome. The diploid genome should return a distribution of loci centered on a 1:1 read count ratio (50% reference genome reads, 50% alternate reads). Triploids should return distributions that are centered on either 1:2 (33.3% reference genome, 66.7% alternate) or 2:1 (66.7% reference genome, 33.3% alternate) depending on the subgenome composition (e.g. ABB or AAB respectively, where the reference genome is A).

Correlations with ocean salinity levels

To test for associations between genotypic variation and local ocean salinity within *P. vaginatum,* we used the local ocean salinity measurements from the World Ocean Atlas dataset (Zweng et al., 2019) to perform two statistical comparisons. First, we used a two-tailed t-test to test for differences in local ocean salinity between diploids and triploids. Then we used a fixed-effects linear model to test the effects of copy number of both the *P. vaginatum* subgenome (N_{Pv}) and the other unidentified subgenome (N_{other}). We compared the models with an interaction (salinity ~ N_{Pv} + N_{other}+ N_{Pv}:N_{other}) and without it (salinity ~ N_{Pv} + N_{other}). We chose the model without an interaction because it had a lower AIC (200.27 vs. 201.09) and because the interaction term was not significant when it was included.

Results

Identification of clonal replicates

The filtered GBS dataset was characterized by 127,282 SNPs in 218 accessions. Principal component analysis (PCA) and application of genetic similarity threshold criteria to identify clonal replicates (see Methods; Supporting Information, Fig. S1) revealed a large number of genotypically identical accessions (Supporting Information, Table S1). For the coarse-textured *P. vaginatum* ecotype, only 12 unique genotypes were detected among the 148 accessions, with just three widely-distributed genotypes accounting for >85% of the accessions. This indicates that extensive clonal propagation occurs in wild populations of this ecotype. Somewhat greater genotypic diversity was found in the fine-textured ecotype, but extensive clonality was still present; 24 unique genotypes were identified in 55 accessions. Four out of eight genotypes in the wild-collected fine-textured accessions were represented by multiple clonal replicates. For *P. distichum* , there were four unique genotypes in the collection of 15 accessions, two of which were unique to our wild collections. The

final SNP dataset for all genotypically unique samples across both species, including wild-collected and USDA samples, totaled 40 genotypes (Table 1). Together these results suggest that clonal propagation occurs extensively in wild *P. vaginatum* and *P. distichum*populations.

Geographic patterns of clonal propagation

Having detected extensive clonality among the wild-collected *P. vaginatum* samples, we next assessed the distribution of geographic and environmental distances between clones of the same genotype in our wild collections. Specifically, we investigated the extent to which they showed patterns consistent with a "frozen-niche" strategy (multiple geographically and environmentally restricted clones) or a "general-purpose genotype" strategy (a few broadly distributed clones). For the fine-textured ecotype, the four genotypes with multiple clones in the wild tended to have relatively small geographic ranges; two of the four had genotypes restricted to a single location, while the two found in multiple locations had a maximum range of 126 km (Table S1; Fig. 1; Fig. 2A). Consistent with their narrow geographical distributions, assessments of pairwise environmental distances among locations (based on local climatic and salinity conditions; see Methods) indicated a low level of environmental dispersion (Fig. 2B). These patterns are generally consistent with a "frozen-niche" reproductive strategy for fine-textured *P. vaginatum* .

In contrast to the fine-textured ecotype, genotypes of the coarse-textured ecotype showed patterns more consistent with a "general purpose genotype" strategy. Not only did a handful of unique genotypes make up the majority of wild accessions (Table 1), but these genotypes showed broad distributions with respect to both geography and environmental variables. With the exception of one genotype that was restricted to a 1 km area (coarse_06), the narrowest geographical range of any coarse-textured clonal genotype was 750 km (coarse_04), and the three most common genotypes (coarse_01, coarse_02 and coarse_03) all had ranges >1400 km (Fig. 1; Fig. 2A). Pairwise environmental distance measures were similarly higher for the coarse-textured ecotype compared to fine-textured accessions (Fig. 2B); this indicates that clonal propagules of these widely dispersed genotypes are not specializing on particular environments in the locations where they occur.

Genetic differences between genotypes and species

Following the identification and characterization of clonal variation, subsequent analyses focused on a reduced dataset composed of one high-coverage accession per genotype. Removal of the lower coverage samples in each clonal group allowed more sites to pass the missing data filter, resulting in an increase to 177,954 variant sites for this highest-quality dataset. A PCA on the set of 40 unique genotypes revealed the same groupings as in the total dataset (Fig. 3, Fig. S3). For both PCAs, most accessions fell into three distinct groups of tightly clustered accessions, corresponding to *P. distichum* and the two ecotypes of *P. vaginatum.* For the reduced-sample dataset, PC 1 explained 31.6% of the variance and separated *P. distichum*from *P. vaginatum* ; PC 2 explained 20.2% of the variance and primarily separated the coarse- and fine-textured ecotypes of *P. vaginatum* .

In addition to the three main PCA clusters, two coarse-textured *P. vaginatum* genotypes were placed as intermediate between the fine- and coarse-textured accessions along PC2 (Fig. 3, Fig. S3). These two genotypes corresponded to GRIN accession PI 612771 and two of the wild-collected accessions which were clones of one genotype (coarse_05, Table S1). The intermediate placement of these genotypes suggests possible admixture between the coarse-textured and fine-textured ecotypes. To test this hypothesis, we created a reduced SNP dataset containing only unique diploid *P. vaginatum* genotypes (33 accessions and 117,218 polymorphic sites). ADMIXTURE analysis shows that the genetically intermediate coarse-textured individuals indeed have genetic compositions consistent with admixture between coarse- and fine-textured ecotypes (Fig. S4). This result suggests that there is occasional gene flow between the two *P. vaginatum* ecotypes.

SNP variation in the *rpl16* intron of the chloroplast genome confirmed the three major groups identified by the GBS data. In the 1.15 kb aligned sequence, *P. distichum* differed from both ecotypes of*P. vaginatum* by a total of 6 SNPs and one 10-bp indel, with one additional SNP segregating within *P. distichum* (Table S2). Within*P. vaginatum* , accessions of the fine-textured ecotype carried two unique SNPs that distinguished

them from the other two groups. These results are consistent with the current taxonomy recognizing *P. vaginatum* and *P. distichum* as two distinct species (Brummitt, 1983). In addition, the pattern of SNP variation within *P. vaginatum* indicates that the fine-textured ecotype is characterized by unique, derived variants.

Ploidy variation and hybridization

Using flow cytometry to estimate genome size on a subset of our samples, we identified extensive ploidy variation associated with the three genetically differentiated groups. Within *P. vaginatum* , the fine-textured ecotype had an average estimated genome size consistent with diploidy (1.01, when 1.0 is designated as the size of the diploid*P. vaginatum* reference genome) (Table 1). In contrast, the relative genome size estimates for coarse-textured samples were consistent with a mix of diploids (1.04) and triploids (1.56). For*Paspalum distichum* , samples had higher average relative genome size estimates overall, consistent with predictions that this species is predominantly hexaploid and pentaploid (Echarte, Clausen, & Sala, 1992). Unlike *P. vaginatum* samples, *P. distichum* genome size estimates did not form obvious multiples of the *P. vaginatum*reference genome. Instead, the difference in genome size between putative pentaploids and hexaploids (2.25 and 2.69, respectively) suggests a *P. distichum* basal genome size that is ˜11% smaller than *P. vaginatum* . When *P. distichum* relative genome sizes were recalculated assuming a basal genome size of 0.89 instead of 1.0 as the point of reference, the values closely match numerical expectations for pentaploid and hexaploid genomes (2.53 and 3.03, respectively) (Table 1). Together these results suggest that *P. vaginatum* is composed primarily of diploid and triploid genotypes, that *P. distichum* is largely composed of pentaploid and hexaploid genotypes, and that the basal genome size of the latter species is likely ˜11% smaller than the*P. vaginatum* reference genome.

By using genome-wide heterozygosity calls from the SNP dataset (F-values generated by plink) in conjunction with the flow cytometry results above, we were able to estimate ploidy for all genotypes (see Gompert & Mock, 2017). Specifically, individuals containing multiple subgenomes (e.g. allopolyploids) are expected to show an increase in heterozygosity with each additional subgenome. The highest genome-wide heterozygosity values (corresponding to more negative F-values) occurred in *P. distichum* and were consistent with a combination of allohexaploid and allopentaploid genotypes (average F = -1.323 and -1.241 respectively) (Table 1). Conversely, the fine-textured cluster of *P. vaginatum*(composed entirely of diploids) had the lowest genome-wide heterozygosity calls (average F = 0.541). For the coarse-textured*P. vaginatum* group, which flow cytometry data indicated to be a combination of diploids and triploids, F-values were consistent with a slight increase in heterozygosity in putative triploids relative to diploids (average F = -0.520 and -0.279 respectively). Unexpectedly, there was a large difference in F-value between diploid coarse- and fine-textured accessions (-0.278 and 0.541); the greater heterozygosity in the coarse-textured genotypes, despite a lack of difference in genome size, is potentially consistent with hybridization in the origin of the diploid coarse-textured ecotype. Collectively these findings support the inference that *P. vaginatum* not only falls into two genetically distinct subgroups according to ecotype, but that the coarse-textured ecotype can be further subdivided by ploidy variation.

Subgenome composition of allopolyploids and hybrids

Because allopolyploid genotypes were identified in both coarse-textured*P. vaginatum* and in *P. distichum* , we examined levels of heterozygous SNP sharing between these groups to assess whether the additional subgenomes in polyploid *P. vaginatum* might be shared with *P. distichum* . Patterns of heterozygous locus sharing indicate that this is not the case. Whereas 28,881 heterozygous loci were unique to coarse-textured individuals and 33,585 heterozygous loci were unique to *P. distichum* , only 3713 loci were shared between species (Fig. S5). These patterns are consistent with PCA results (Fig. 3, Fig. S3) in indicating that *P. distichum* is no more closely related to coarse-textured *P. vaginatum* genotypes (regardless of ploidy) than to fine-textured genotypes. Thus, the triploid coarse-textured *P. vaginatum* genotypes appear to carry a distinct subgenome from *P. distichum* from an unknown source.

Heterozygous locus calls further indicated that in addition to ploidy variation, there were also subgenome

composition differences within the coarse-textured *P. vaginatum* group. By counting the number of reference versus alternate reads at heterozygous loci, we could estimate the number of haploid subgenomes within a genotype that are from*P. vaginatum* or some other source (see Methods). Accession DG076, a representative diploid coarse-textured genotype (coarse_03; Table S1), followed the expected pattern of a 1:1 ratio of reference to alternate reads (50% reference; Fig. 4A; Fig. S6A). Among the three triploid genotypes, one of them (coarse_01, represented by accession DG117) showed a 1:2 ratio (33.3% reference reads; Fig. 4B; Fig. S6B), while the other two (coarse_02 and coarse_04, represented by accessions DG 107 and DG120, respectively) showed 2:1 ratios (66.6% reference reads; Fig. 4 C-D; Fig. S6 C-D). This difference among triploid genotypes indicates that they do not have the same subgenome composition. More specifically, one genotype appears to carry one copy of the fine-textured *P. vaginatum* reference genome and two subgenome copies from an unknown source, whereas the other two genotypes appear to have the reverse pattern. Our use of the same set of heterozygous loci in all four of the tested coarse-textured genotypes allows us to infer that the same two subgenomes are present in all of them, but that the number of copies of each subgenome differs.

Correlations with ocean salinity

To investigate the potential roles of ploidy and subgenome composition in adaptation to salt tolerance, we compared the surface salinity of the ocean nearest to the collection location of each *P. vaginatum*sample. Salinity levels did not differ significantly between the locations of diploid and triploid individuals (P = 0.5328, Fig. 5); thus, ploidy variation alone does not obviously contribute to adaptive variation in salinity tolerance. In contrast, a linear model that incorporated both the number of *P. vaginatum* subgenomes (one vs. two, independent of ploidy) and the number of the unidentified subgenomes (zero, one, or two) revealed that having two *P. vaginatum* subgenomes was strongly associated with higher local ocean salinity (P = 7.6e-11), while having an additional copy of the unknown genome was weakly associated with decreased salinity (P = 0.033, Fig. 5). This finding suggests that the relative proportion of subgenomes from the halophytic species has a detectable effect on salinity adaptation. It should be noted, however, that we could not entirely remove the effect of ploidy from this analysis because after accounting for the number of *P. vaginatum* subgenomes, a change in the copy number of the other subgenome necessarily changes ploidy as well (2:0 to 2:1 and 1:1 to 1:2).

Discussion

Paspalum vaginatum is a promising new genomic model system for the study of salt tolerance in cereals and other plant species. Our population genomic analyses, using new wild samples collected widely throughout the southern US, and without a bias toward turf-like (fine-textured) ecotypes, has allowed us to make several key insights into the genetic diversity, population structure and genomic composition of this species, with broader implications for mechanisms of salinity adaption in widespread species. Our findings include the following: 1) Clonal propagation occurs extensively in both *P. vaginatum*ecotypes, as well as in the non-halophytic sister species *P. distichum* (Table 1; Table S1); 2) Clones of the coarse-textured ecotype are more widely distributed than the fine-textured ecotype, both geographically and with respect to the environment (Fig. 1; Fig. 2), suggesting a "general purpose genotype" adaptive strategy for the former and a "frozen niche" strategy for the latter; 3) The system is characterized by extensive ploidy variation, including diploids and allotriploids that vary in their subgenome composition (Table 1; Fig. S5, S6); and 4) Increasing number of *P. vaginatum* subgenomes within a genotype are associated with occurrence in more saline environments (Fig. 5), suggesting a direct effect of the halophytic species' genome on salinity adaptation. Collectively these findings suggest that both clonal propagation and hybridization-associated ploidy variation play key roles in the ability of this species to grow widely across environments of varying salinity.

Differing patterns of clonal propagation

For species that commonly reproduce clonally, there are two proposed strategies that can explain geographic distributions of individual clonal genotypes. The "frozen niche" strategy is characterized by multiple genotypes that are each adapted to a specific geographically localized environment (Róis et al., 2015; Vrijenhoek

1979). Conversely, the "general purpose genotype" strategy is characterized by a few genotypes that are widely distributed across a range of environments (Baker, 1965; Bricker, Calladine, Virnstein, & Waycott, 2018; Coughlan, Han, Stefanović, & Dickinson, 2017). The occurrence of interspecific hybridization could be an important factor in determining clonal propagation strategy. Hybrids, which are often obligately asexual, are a common model system to investigate patterns of clonal distribution; however, opportunities to investigate differential clonal reproduction strategies between a hybrid and its parental species are rare as the parental species are often obligately sexual (Coughlan, Han, Stefanović, & Dickinson, 2017). With our collection of clones from both the hybrid coarse-textured *P. vaginatum* and one of its parent species, fine-textured *P. vaginatum,* we were able to explicitly test for and confirm differences in the geographic and environmental ranges of clonal genotypes.

Hybridization is expected to yield genotypes with increased phenotypic plasticity, as they can access alleles from both parental species (Shimizu-Inatsugi et al., 2017). Since high plasticity genotypes should have increased ability to survive in multiple environments, hybrid genotypes may thus be more likely to follow the "general-purpose genotypes" strategy than either parental species (Baker, 1965; Coughlan, Han, Stefanović, & Dickinson, 2017; Lynch, 1984). Our findings are consistent with this prediction in that the hybrid coarse-textured ecotypes follow a "general-purpose genotype" pattern while fine-textured plants follow a "frozen niche" pattern. This indicates that hybridization may have induced a transition in clonal propagation strategy in *P. vaginatum.* Furthermore, within the hybrid coarse-textured genotypes, allotriploidy may provide an additional boost to plasticity relative to diploids as the two most common and widespread genotypes are both triploids (Fig. 1). This is consistent with the assumption that allopolyploids have access to even more potentially adaptive alleles than diploid hybrids.

Differences in the rate of sexual reproduction may also influence clonal propagation strategy. Recombination during sexual reproduction can produce novel clonally propagating genotypes. Clones of the most fit genotype in a given location will then dominate it, resulting in a "frozen-niche" pattern (Vrijenhoek & Parker, 2009). Consistent with this proposed strategy, fine-textured *P. vaginatum* genotypes are known to be capable of sexual reproduction in addition to vegetative clonal propagation (Duncan & Carrow 2000). Sexual reproduction of coarse-textured ecotypes has not been tested; however, the low number of unique genotypes indicate that it is likely rare (although the presence of admixed individuals suggests that it is possible) (Fig. 3, Fig. S3, Fig. S4). This lack of post-hybridization recombination could make it difficult for coarse-textured plants to specialize in a given location. Additionally, clonal propagation can serve to maintain plasticity by preventing the loss of heterozygosity in hybrids. Both of these factors could explain the "general-purpose genotype" pattern seen in coarse-textured clones.

Subgenome composition of hybrids and allopolyploids drives genetic differentiation between groups

Interspecific hybridization and gene flow can introduce alleles into a population, which can potentially result in adaptation to novel environmental conditions. Previous work identified apparent admixture between salt-tolerant *P. vaginatum* and non-tolerant *P. distichum* , which could produce genotypes adapted to intermediate salinity levels (Eudy, Bahri, Harrison, Raymer, & Devos, 2017). We found that the two ecotypes of *P. vaginatum* and *P. distichum* formed distinct genetic groups, with no signatures of gene flow between *P. vaginatum* and *P. distichum* (Fig. 3, Fig. S3), although we did identify limited gene flow between the coarse- and fine-textured ecotypes of *P. vaginatum* (Fig. S4). It is possible that ploidy differences between *P. distichum* (5x and 6x) and *P. vaginatum* (2x and 3x) represent a sufficient reproductive barrier to prevent gene flow.

While we did not find evidence for interspecific hybridization in the wild between *P. distichum* and *P. vaginatum* , both the coarse-textured and *P. distichum* groups appear to have been formed from interspecific hybridization between fine-textured *P. vaginatum* and other *Paspalum* species. Interestingly, coarse-textured *P. vaginatum* and *P. distichum* do not appear to share the same non-*P. vaginatum* subgenome (Fig. S5). This difference in subgenome composition is thus likely the result of hybridization between *P. vaginatum* and at least two different*Paspalum* species.

Niche differentiation between these two hybrid groups could be due to the genomic contribution of these non-*P. vaginatum* parents. To our knowledge, no *Paspalum* species is as salt-tolerant as*P. vaginatum* ; therefore, it is likely that the non-*P. vaginatum* parental species of both groups are not halophytic. Despite this, coarse-textured *P. vaginatum* largely maintained its high salt tolerance while *P. distichum* shows adaptations for freshwater environments. Hypotheses for why this might have occurred include lower expression of the *P. vaginatum* subgenome in*P. distichum* relative to coarse-textured *P. vaginatum* , differences in salt tolerance between the non-*P. vaginatum*parents, and post-hybridization selection for freshwater adaptations in*P. distichum* .

Subgenome composition but not ploidy is associated with local ocean salinity

Studies in other systems indicate that an increase in ploidy tends to be associated with increased salt tolerance both within and between closely related species (Chao et al., 2013; Wu et al., 2019; Yang et al., 2014). However, when ploidy is considered alone, there is no detectable difference in local ocean salinity between diploid and triploid *P. vaginatum* (Fig. 5). Instead, copy number of the *P. vaginatum*subgenome may be the driving factor. Gene dosage effects can have a dramatic influence on phenotypes and increased copy number of salt tolerance genes has been implicated in the evolution of salt tolerance in halophytes (Oh, Dassanayake, Bohnert, & Cheeseman, 2012). Variation in gene copy number can also occur on a whole genome level in populations of hybrids that vary in the number of subgenomes they received from each parental species. Other studies have investigated reciprocal allotriploid hybrids (e.g. 1:2 vs 2:1), similar to those we describe here, which are often more similar to the parent from which they received two copies (Betto-Colliard, Hofmann, Sermier, Perrin, & Stöck, 2018; Harvey, Fjelldal, Solberg, Hansen, & Glover, 2017; Tan et al., 2016; Yao, Gray, Auger, & Birchler, 2013).

Our results support the hypothesis that the other unknown subgenome present in the coarse-textured ecotype is not as adapted for saline environments as is *P. vaginatum* (Fig 5). Plants with two copies of the *P. vaginatum* subgenome (fine-textured, and 2:1 triploids) were found in locations where the local ocean salinity was on average ~15% higher than those with only one copy (1:1 diploids and 1:2 triploids). The effect of increasing counts of the unknown subgenome was weaker and in the opposite direction (slightly less ocean salinity with each copy). We cannot say for certain whether this weaker effect is due to increased ploidy or subgenome dosage effects, or both. If it is a ploidy effect then it is the opposite of predictions that polyploids should be more salt tolerant.

The large effect of the number of *P. vaginatum* subgenomes is consistent with our finding that coarse-textured hybrids are likely "general-purpose genotypes" because their local ocean salinity is not only lower, but also more variable. Furthermore, unlike most other studies of reciprocal triploids, we can rule out maternal subgenome effects because all of the coarse-textured hybrids carry the same cpDNA haplotype, implying the same maternal parent species for each cross.

Although the trend we observe between *P. vaginatum* subgenome copy number and local salinity is striking, environmental associations are necessarily correlational in nature and must be confirmed with controlled salt tolerance assays and transcriptomics. It is also possible that unknown demographic or introduction history could contribute to these patterns. Additionally, our sea surface salinity dataset does not have the resolution to identify the microhabitat variation likely experienced by plants in the wild (for example, in an estuary with a steep salinity gradient). While higher resolution salinity data exist for some coastal areas, temporal and methodological variation between datasets make them unsuited for comparison across the geographic scale of this study.

Implications for plant breeding

Most of our knowledge of the genetic and physiological underpinnings of salt tolerance comes from crop species and plant model species that are generally not salt tolerant, with limited studies of highly salt-tolerant plant species. Additionally, studies on the genetic basis of salt tolerance in halophytes have been limited to a small number of species, few of which are grasses (Fan, 2020; Mishra & Tanna, 2017). This is problematic because soil salinity affects agricultural fields worldwide, and breeding for increased tolerance in

cereal crops, which are all grasses, has been slow. Understanding the mechanisms and genes involved in salt tolerance in halophytic grass species may allow us to transfer that knowledge to develop more salt tolerant cereals. Our finding that salt tolerance in the wild may be associated with whole-genome dosage effects indicates that a single gene approach may be insufficient and that changes to copy number or expression of multiple interacting genes and pathways may be the key to improving crop salt tolerance.

Breeding programs for seashore paspalum turfgrass have been hampered by the lack of genetic diversity in publicly available germplasm, as confirmed in this study. The apparent presence of natural crosses between fine and coarse-textured ecotypes points to the possibility of introgression of useful traits from coarse-textured populations. This could provide a valuable source of genetic diversity in turf breeding programs for the genetically depauperate, but more economically relevant, fine-textured ecotypes. Future work to identify these traits and genetic markers associated with them in the coarse-textured ecotypes will be required.

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Data Accessibility

Demultiplex and trimmed reads will be submitted to NCBI SRA before publication. The *rpl16* cpDNA sequences are available on GenBank (accessions MT361650- MT361665). SNP datasets and snapshots of code used in the project will be submitted to dryad. Future development of scripts will occur at https://github.com/david-goad/paspalum-hybridization/.

Author Contributions

DMG collected plants, performed lab work, analyzed data, generated figures, and drafted the manuscript. KMO aligned cpDNA sequences. All authors contributed to designing the study, data analysis and interpretation, writing grant proposals and writing the manuscript.

Supporting Information

Additional supporting information may be found in the on-line version of this article.

Table S1 Information for all samples including collection location, taxonomic and genotype labels, and data used to infer ploidy

Table S2 . Polymorphic sites in the intron of the chloroplast gene *rpl16* . Positions are based on alignment to GenBank accession KF853027 (Scataglini et al. 2014).

Figure S1 Histogram of pairwise genetic distances between samples within (**A**) fine-textured *P. vaginatum* , (**B**) Coarse-textured *P. vaginatum* , and (**C**)*P. distichum* . Dotted red line indicates distance threshold for identifying clonal genotypes.

Figure S2 (**A**) The basic workflow of triploid_comp.py. (**B**) An illustration of how triploid_comp.py counts reads at a locus for a hypothetical triploid genotype. Because GBS reads start at the same location due to the restriction cut site, reads representing each allele at a locus are expected to be identical to each other. Therefore, reads not identical to the most common two read types are considered to be sequencing errors and ignored (i.e. not included in calculating ratios or percentages of reference reads). Due to the ˜2:1 ratio of reference reads this hypothetical example could represent a triploid with two haploid copies of *the P. vaginatum* subgenome and one from the unknown progenitor; however, a single locus could have a skewed ratio due to chance so it is necessary to look at the trend genome wide to make a definitive assessment.

Figure S3 Principal component analysis (PCA) showing the genetic separation of all sampled *Paspalum* subgroup Disticha accessions into three well defined groups as well as three admixed individuals (two of which are clonal and overlap). The color of each individual indicates its genome-wide heterozygosity rate (reported as the individual inbreeding coefficient F). Darker shades of blue indicate lower heterozygosity.

Figure S4 ADMIXTURE plots showing population assignment of 33 unique diploid *P. vaginatum* genotypes at K=2 (**A**) and K=3 (**B**). Putative fine-coarse admixed individuals are indicated with stars. (**C**) Cross-validation error for K = 1-7.

Figure S5 Number of SNP markers where heterozygous calls are unique to given group or shared between groups. The relatively small number of loci that are heterozygous in both coarse-textured *P. vaginatum* and *P. distichum* but not fine-texture *P.vaginatu* m (light blue region) indicates that *P. distichum*and coarse-textured *P. vaginatum* likely do not share a non-*P. vaginatum* subgenome.

Figure S6 The raw counts of the two most common reads (where one is identical to the reference and the other is alternate) covering heterozygous SNPs in four coarse-textured *P. vaginatum* samples representing the most common diploid hybrid genotype (coarse_03, represented by DG076) (**A**) and the three triploid genotypes: coarse_01(accession DG117) (**B**) , coarse_02 (accession DG107) (**C**) and coarse_04 (accession DG120) (**D**) . Red lines indicate expected ratio of reference to alternate reads for diploids at 1:1 and reciprocal triploids at 1:2 and 2:1. A bias toward either 1:2 or 2:1 in the triploids reflects having one or two haploid copies of the*P* . *vaginatum* subgenome respectively.

Tables and Figures

Table 1 Inferred clonality and ploidy variation for sampled*Paspalum* accessions. Relative genome size estimates were calculated assuming a diploid genome size of 2x = 1.0 for the *P. vaginatum* reference genome (accession PI 509022); values in brackets indicate relative genome size estimates for *P. distichum* assuming a basal genome size that is 11% smaller than the *P. vaginatum*reference genome.

Species	Ecotype	Esti-mated ploidy	No. accessions sampled	No. unique geno-types	Relati
P. vaginatum					
	Fine-textured	2x	55	24	1.01 (2
	Coarse-textured	2x§	32	9	1.04 (9
		3x	116	3	1.56 (3
P. distichum		5x	9	2	2.25 [2.
		6x	6	2	2.69 [3.

+ N = Number of accessions measured with three replicates per accession

++ F = individual inbreeding coefficient (Purcell et al. 2007); more negative values indicate higher genome-wide heterozygosity.

§. Includes three putative coarse/fine-textured admixed samples (see Fig. 2 and Table S1).

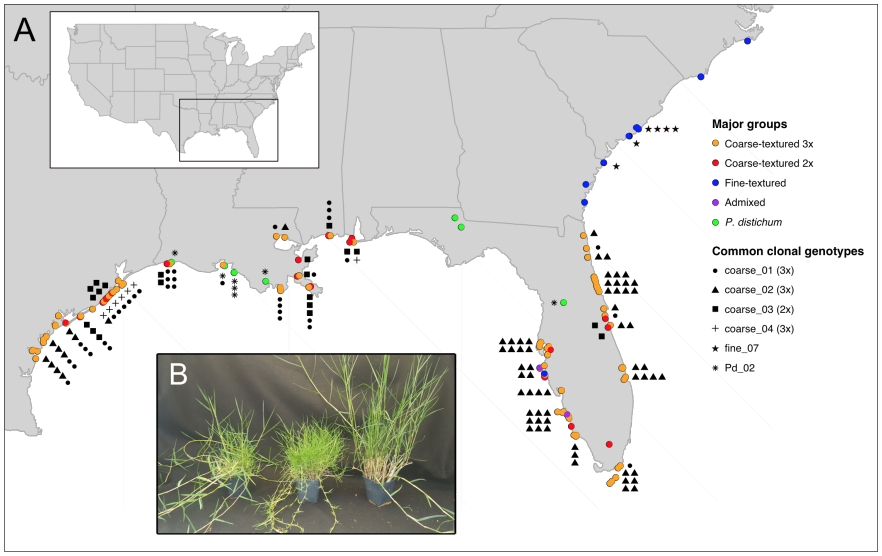

Figure 1 (**A**) Map of US collections of *Paspalum*group Disticha accessions in the southeastern United States. Colors indicate major genetic subgroups and ploidy variants. Shapes indicate geographical locations of the most common clonal genotypes (detected in 6 or more accessions). (**B**) From left to right: *P. distichum,* fine-textured *P. vaginatum* and coarse-textured

P. vaginatum.

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`image2.emf` available at https://authorea.com/users/351065/articles/475745-hybridization-polyploidy-and-clonality-influence-geographic-patterns-of-diversity-and-salt-tolerance-in-the-model-halophyte-seashore-paspalum-paspalum-vaginatum

Figure 2 Distributions of pairwise geographic (**A**) and environmental (**B**) distances between collection locations of clones from each clonally propagating *P. vaginatum* genotype. A single dot indicates that there is no variation in pairwise distance for that genotype. Unique genotype IDs indicate whether a genotype is coarse-textured or fine-textured. The number of each genotype collected in the wild (with GPS coordinates) is indicated by n. Color indicates ploidy level.

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Figure 3 Principal component analysis (PCA) of *Paspalum*group Disticha accessions based on a single sample from each clonal group. Three well defined groups are formed as well as two putatively admixed individuals. The color of each individual indicates its genome-wide heterozygosity rate (reported as the individual inbreeding coefficient F). Darker shades of blue indicate lower heterozygosity.

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Figure 4 Distribution of the percent of reads that are identical to the *P. vaginatum* reference genome at heterozygous loci in four coarse-textured *P. vaginatum* samples representing the most common diploid hybrid genotype (coarse_03, represented by DG076) (**A**) and the three triploid genotypes: coarse_01 (accession DG117) (**B)** , coarse_02 (accession DG107) (**C**) and coarse_04 (accession DG120) (**D)** . Bars in the top right corner indicate the number of haploid subgenomes from *P. vaginatum* (black) or an unidentified progenitor species (grey).

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Figure 5 Local ocean salinity in practical salinity units (PSU) for each of our wild *P. vaginatum* collections based on subgenome composition. Black and grey bars represent copy number of haploid subgenomes from *P. vaginatum* (N_{Pv}) and the unknown progenitor species (N_{other}) respectively. Boxes indicate mean and standard error. Color indicates ploidy.