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Population structure of *Miscanthus sacchariflorus* reveals two major polyploidization events, tetraploid-mediated unidirectional introgression from diploid *M. sinensis*, and diversity centred around the Yellow Sea

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• **Background and Aims** *Miscanthus*, a C_4 perennial grass native to East Asia, is a promising biomass crop. *Miscanthus sacchariflorus* has a broad geographic range, is used to produce paper in China and is one of the parents (along with *Miscanthus sinensis*) of the important biomass species *Miscanthus* × *giganteus*. The largest study of *M. sacchariflorus* population genetics to date is reported here.
• **Methods** Collections included 764 individuals across East Asia. Samples were genotyped with 34 605 single nucleotide polymorphisms (SNPs) derived from restriction site-associated DNA sequencing (RAD-seq) and ten plastid microsatellites, and were subjected to ploidy analysis by flow cytometry.
• **Key Results** Six major genetic groups within *M. sacchariflorus* were identified using SNP data: three diploid groups, comprising Yangtze (*M. sacchariflorus* ssp. *lutarioriparius*), N China and Korea/NE China/Russia; and three tetraploid groups, comprising N China/Korea/Russia, S Japan and N Japan. *Miscanthus sacchariflorus* ssp. *lutarioriparius* was derived from the N China group, with a substantial bottleneck. Japanese and mainland tetraploids originated from independent polyploidization events. Hybrids between diploid *M. sacchariflorus* and *M. sinensis* were identified in Korea, but without introgression into either parent species. In contrast, tetraploid *M. sacchariflorus* in southern Japan and Korea exhibited substantial hybridization and introgression with local diploid *M. sinensis*.
• **Conclusions** Genetic data indicated that the land now under the Yellow Sea was a centre of diversity for *M. sacchariflorus* during the last glacial maximum, followed by a series of migrations as the climate became warmer and wetter. Overall, *M. sacchariflorus* has greater genetic diversity than *M. sinensis*, suggesting that breeding and selection within *M. sacchariflorus* will be important for the development of improved *M.* × *giganteus.* Ornamental *M. sacchariflorus* genotypes in Europe and North America represent a very narrow portion of the species' genetic diversity, and thus do not well represent the species as a whole.

Key words: *Miscanthus sacchariflorus*, *Miscanthus* × *giganteus*, *Miscanthus sinensis*, *Miscanthus sacchariflorus* ssp. *lutarioriparius*, restriction site-associated DNA sequencing (RAD-seq), population genetics, polyploidy, plastid haplotype, hybridization, bioenergy.

INTRODUCTION

Miscanthus is a genus of C_4 perennial grasses in the Andropogoneae tribe that are obligately allogamous and have wind-dispersed seeds. The genus is native to East Asia (especially eastern areas with relatively high precipitation) and Oceania, ranging from tropical to cold temperate climates (Clifton-Brown *et al.*, 2008; Sacks *et al.*, 2013). *Miscanthus* × *giganteus*, which is a hybrid between *Miscanthus sacchariflorus* and *Miscanthus sinensis*, has recently attracted considerable attention as a feedstock crop for bioenergy and bioproducts

(Hodkinson *et al.*, 2002; Clifton-Brown *et al.*, 2008; Dwiyanti *et al.*, 2013*b*; Sacks *et al.*, 2013). However, nearly all *M.* × *giganteus* that is currently grown commercially is a single triploid clone, '1993-1780' (named after the type specimen at Kew Royal Botanic Gardens Herbarium; it is also commonly referred to as *M.* × *giganteus* 'Illinois' in North America; Hodkinson and Renvoize, 2001; Głowacka *et al.*, 2015), which was collected in southern Japan and imported to Denmark in the 1930s (Nielsen, 1990; Linde-Laursen, 1993). Though biomass yields of *M.* × *giganteus* '1993-1780' can exceed those of maize and switchgrass in temperate mid-latitude environments (Heaton *et al.*, 2008; Dohleman and Long, 2009; Somerville *et al.*, 2010), this clone can suffer stand losses during the first winter after planting in the central Midwest USA and locations with similarly cold or colder climates (Greef *et al.*, 1997; Clifton-Brown and Lewandowski, 2000; Farrell *et al.*, 2006; Clark *et al.*, 2016). Yet, in the US southern coastal plain, *M.* × *giganteus* '1993-1780' typically flowers too early to achieve the high yields obtained in the Midwest. Thus, breeding efforts are needed to generate improved *M.* × *giganteus* cultivars, and these efforts will depend on knowledge of its parent species' genetic diversity, population structure and adaptation.

Miscanthus sacchariflorus is native to a broad geographic area in China, Korea, Japan and eastern Russia, from 28 to 50 °N and from sea level to approx. 2000 m elevation (Hirayoshi *et al.*, 1957; Lee, 1964; Clifton-Brown *et al.*, 2008; Sun *et al.*, 2010; Sacks *et al.*, 2013). The geographic ranges of *M. sacchariflorus* and *M. sinensis* largely overlap, although *M. sacchariflorus* can be found further north and *M. sinensis* can be found further south. Especially noteworthy is that *M. sacchariflorus* probably has the greatest winter hardiness among all the Saccharinae, with populations from northern China and eastern Russia adapted to an average annual minimum air temperature of –40.0 °C (USDA hardiness zone 3; Clark *et al.*, 2016). *Miscanthus sacchariflorus* also differs from other *Miscanthus* species in its spreading habit due to long rhizomes and in its preference for wet soil in damp meadows or near the edges of lakes, rivers and streams (Dwiyanti *et al.*, 2013*b*; Sacks *et al.*, 2013). *Miscanthus sacchariflorus* ssp. *lutarioriparius*, which grows along the southern edge of the species' range near the Yangtze River, has especially tall (3–7 m) and thick (10–20 mm) stems that are harvested to produce paper locally on an industrial scale (Chen and Renvoize, 2005; Sacks *et al.*, 2013); it is adapted to USDA hardiness zones 8 and 9 (average annual minimum temperature of –12.2 to –1.2 °C). *Miscanthus sacchariflorus* ssp. *lutarioriparius* is sometimes designated as a separate species, although recent taxonomic evaluation and molecular results favour the subspecies status (Chen and Renvoize, 2005; Sun *et al.*, 2010; Głowacka *et al.*, 2015); a detailed study of its relationship with other populations of *M. sacchariflorus* has not been conducted previously.

Diploid ($2x = 38$) and tetraploid ($4x = 76$) forms of *M. sacchariflorus* are both common (Hirayoshi *et al.*, 1957; Rayburn *et al.*, 2009; Sun *et al.*, 2010; Moon *et al.*, 2013; Chae *et al.*, 2014), in contrast to *M. sinensis*, which is nearly always diploid (Clark *et al.*, 2014). However, neither differences in the geographic distribution between diploid and tetraploid *M. sacchariflorus*, nor the evolutionary relationships between these populations, have been fully described. Diploid *M. sacchariflorus* has been documented in China, Korea and eastern Russia (Li *et al.*, 2013; Moon *et al.*, 2013; Clark *et al.*, 2016), but is apparently absent from Japan (Hirayoshi *et al.*, 1957; Clark *et al.*, 2015). Tetraploid *M. sacchariflorus* is common in Japan (Hirayoshi *et al.*, 1957) and Korea (Moon *et al.*, 2013) but less frequent in China (Li *et al.*, 2013) and Russia (Clark *et al.*, 2016). Although early cytogenetic studies suggested an allopolyploid origin for the tetraploids (Adati, 1959; Adati and Shiotani, 1962), recent molecular and cytogenetic studies support an autopolyploid origin from diploid *M. sacchariflorus* (Takahashi and Shibata, 2002; Dwiyanti *et al.*, 2013*a*; Clark *et al.*, 2015).

Previous population genetic studies of *M. sacchariflorus* have sampled from limited areas of the species native range, and no region-wide studies have yet been conducted. Yan *et al.* (2016) used microsatellite markers to study 644 *M. sacchariflorus* ssp. *lutarioriparius* individuals from 25 populations along or near the Yangtze River from 28.9 to 32.5 °N and 111.7 to 120.2 °E, finding high genetic diversity, low clonality and frequent migration among populations, with some anthropogenic influences on population structure. Yook *et al.* (2014) genotyped 22 *M. sacchariflorus* accessions using 31 microsatellite markers, and were able to distinguish Korean and non-Korean individuals, but did not find population structure at a finer scale. Using restriction site-associated DNA sequencing (RAD-seq), single nucleotide polymorphisms (SNPs) and plastid microsatellites, our research group has published several population genetic studies of *Miscanthus* that included two tetraploid *M. sacchariflorus* individuals from South Korea, two tetraploid and six diploid *M. sacchariflorus* from China and 11 diploid *M.* × *giganteus* from China (Clark *et al.*, 2014), 78 *M. sacchariflorus* and *M.* × *giganteus* (tetraploids and triploids) from Japan (Clark *et al.*, 2015) and 157 diploid and three tetraploid *M. sacchariflorus* from Russia (Clark *et al.*, 2016). Our previous studies of *Miscanthus* in China, Korea and Japan lacked sufficient sample size to identify population structure within *M. sacchariflorus*. Population structure among *M. sacchariflorus* in Russia was limited to a weak signal of isolation by distance, as well as tetraploid individuals being genetically differentiated from diploids (Clark *et al.*, 2016). The absence of a region-wide assessment of *M. sacchariflorus* genetic diversity and population structure has been a critical obstacle to using this germplasm efficiently for crop improvement; removing this barrier is a major goal of this study.

Although current knowledge of population structure in *M. sacchariflorus* is limited, hybridization with *M. sinensis* is a well-established phenomenon. Here we use the term *M.* × *giganteus* to refer to hybrids of any ploidy that have at least 20 % of their ancestry each from *M. sacchariflorus* and *M. sinensis*; this cut-off is arbitrary but includes first-generation hybrids (F_1) as well as first-generation backcrosses (BC_1) of all ploidies. Jiang *et al.* (2013) demonstrated that *M. purpurascens*, endemic to China, was a diploid *M.* × *giganteus* with equal genetic contributions from *M. sinensis* and *M. sacchariflorus*. However, there is no evidence that *M. purpurascens* introgresses into either parent species beyond the BC_1 generation (Jiang *et al.*, 2013; Clark *et al.*, 2014). In Korea, Yook *et al.* (2014) identified natural *M.* × *giganteus* based on morphological data and microsatellite markers, although ploidy and the proportion of ancestry from the two parental species were not assessed. A separate study identified a triploid individual in Korea that

was putatively *M.* × *giganteus* based on flow cytometry and chromosome counts (Moon *et al.*, 2013). Perhaps owing to the Japanese origin of the high-yielding biomass cultivar *M.* × *giganteus* '1993-1780', Japan is the region where *Miscanthus* hybridization has been most thoroughly studied. Both triploid and tetraploid *M.* × *giganteus* have been identified in southern Japan by flow cytometry, chromosome counting, internal transcribed sequence (ITS) sequencing, intron-flanking PCR markers, microsatellites and high-density SNP markers (Hirayoshi *et al.*, 1957; Adati, 1958; Nishiwaki *et al.*, 2011; Dwiyanti *et al.*, 2013*a*; Clark *et al.*, 2015; Tamura *et al.*, 2016). Variation for agronomic traits such as biomass yield, plant height and spreading habit has been observed among triploid Japanese *M.* × *giganteus* accessions (Uwatoko *et al.*, 2016). Unlike the diploid hybrids found in China, tetraploid *M.* × *giganteus* in Japan appears to intermate readily with *M. sacchariflorus*, resulting in substantial introgression of *M. sinensis* ancestry into tetraploid *M. sacchariflorus* (Clark *et al.*, 2015). Based on chloroplast data, diploid, triploid and tetraploid *M.* ×*giganteus* can have either *M. sinensis* or *M. sacchariflorus* as the female parent (Jiang *et al.*, 2013; Clark *et al.*, 2014, 2015). Though interspecific hybridization and cross-ploidy introgression have been well documented in Japan, it is not known if similar processes occur in China and Korea where diploid *M. sinensis* and tetraploid *M. sacchariflorus* also grow sympatrically.

In this study, we characterized population structure and its interaction with ploidy for a large collection of *M. sacchariflorus* and *M.* × *giganteus* from China, South Korea, Japan and Russia ranging from 28.6 to 49.3 °N and from 104.5 to 145.2 °E. Our objectives were (1) to better delineate the geographic range and frequency of tetraploid *M. sacchariflorus* in mainland Asia; (2) to identify distinct genetic clusters and centres of diversity for *M. sacchariflorus*, and establish their relationship to each other in terms of ancestry and gene flow; (3) to quantify the amount of hybridization with *M. sinensis* and identify geographic regions in which hybridization is frequent; and (4) to compare *M. sacchariflorus* (data from the current study) with *M. sinensis* (data from prior studies) for degree of population structure, genetic diversity and locations of centres of diversity. Using high-density SNP markers obtained from RAD-seq, we provide an in-depth assessment of *M. sacchariflorus* population genetics throughout most of the species' native range.

MATERIALS AND METHODS

Plant material, DNA extraction and genotyping

Plant material consisted primarily of *M. sacchariflorus* collected in the wild across East Asia, including Russia, China, South Korea and Japan (Table 1; Fig. 1). Collection sites were targeted based on herbarium records as well as the tendency of *M. sacchariflorus* to grow near water. With the goal of broad regional sampling, between one and five individuals were sampled at each collection site. In total, 764 individual *Miscanthus* genotypes were studied, comprising 722 *M. sacchariflorus*, 37 *M.* × *giganteus* and five *M. sinensis* (Tables 1 and 2; Fig. 1; Supplementary Data S1). Of the individuals analysed in the current study, 255 were included in previous studies that examined population structure and genetic relationships at smaller geographic scales (Clark *et al.*, 2014, 2015, 2016; Głowacka *et al.*, 2015). For the new individuals, rhizomes were collected in the wild (primarily in 2014; Supplementary Data S1) and propagated in greenhouses. DNA was extracted from lyophilized, ground leaf tissue using a CTAB (cetyltrimethylammonium bromide) method with minor modifications (Fulton *et al.*, 1995; Clark *et al.*, 2016). All individuals were genotyped at ten plastid microsatellites (de Cesare *et al.*, 2010; Jiang *et al.*, 2012) as previously described (Clark *et al.*, 2014). Size separation of the PCR products was accomplished by capillary electrophoresis on a 3730 × l DNA Analyzer (Applied Biosystems, Foster City, CA, USA) with the GeneScan 500 LIZ size standard at the Roy J. Carver Biotechnology Center at the University of Illinois. RAD-seq using *Pst*I and *Msp*I with size selection from 200 to 500 bp was performed as previously described (Clark *et al.*, 2014). A total of seven new RAD-seq libraries with 95 samples each

Table 1. *Summary of provenance and ploidy for* Miscanthus *spp. individuals included in the study*

Country of origin	Total no. of individuals	No. of individuals from previous publications	Diploid	Triploid	Tetraploid	Not determined
			M. sacchariflorus			
Russia	150	150*	147	0	3	0
South Korea	106	1†	27	1	78	0
China	262	8†,‡	206	2	52	2
Japan	194	82‡,§	0	0	184	10
Unknown	10	8†,‡	7	0	3	0
Total	722	249	387	3	320	12
			M. × *giganteus*			
South Korea	24	0	9	5	10	0
Japan	13	5§	0	4	9	0
Total	37	5	9	9	19	0
			M. sinensis			
China	3	0	3	0	0	0
Japan	1	0	0	0	0	1
Unknown	1	1‡	1	0	0	0
Total	5	1	4	0	0	1

*Clark *et al.* (2016); †Clark *et al.* (2014); ‡Głowacka *et al.* (2015); §Clark *et al.* (2015).

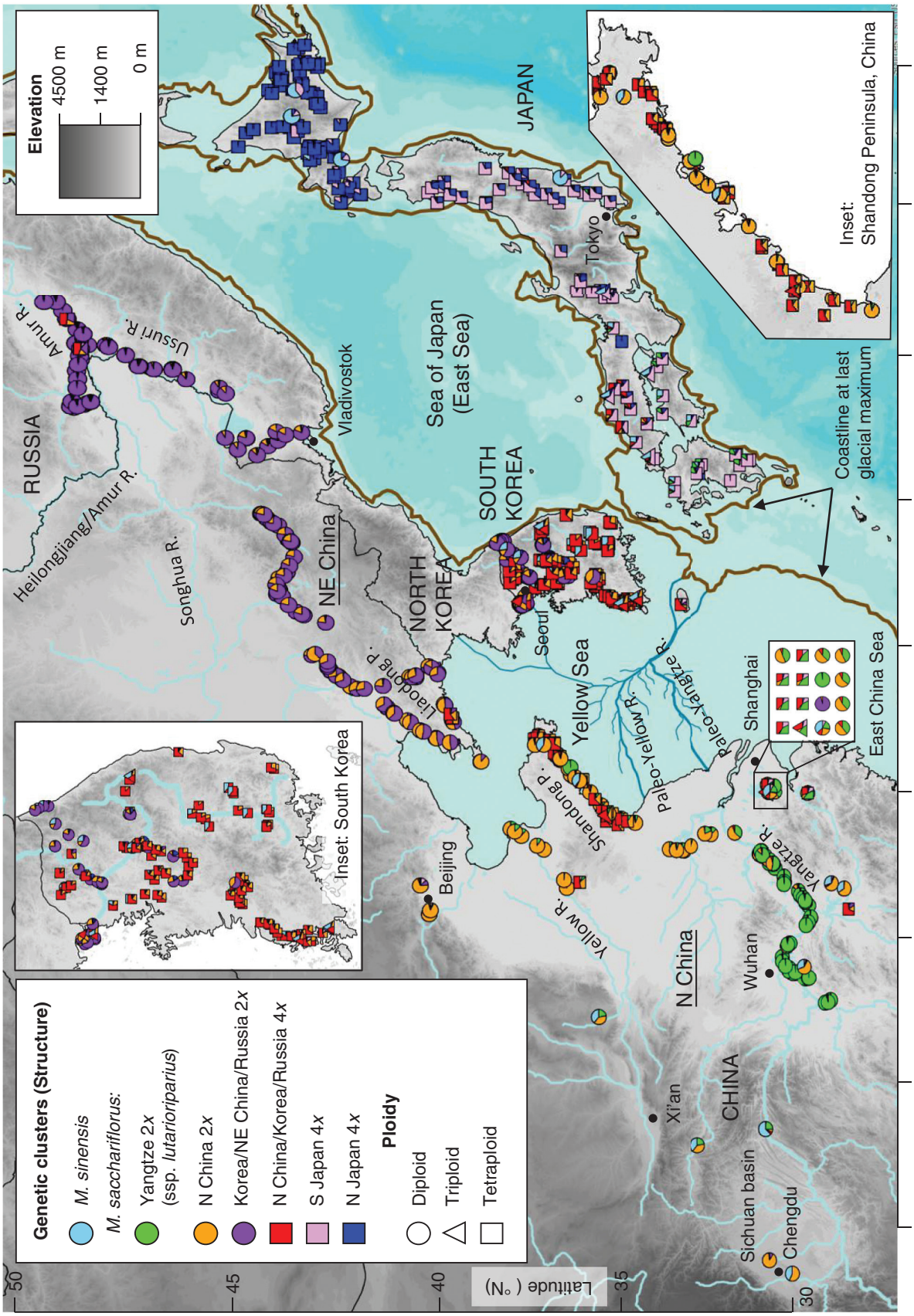


FIG. 1. Map of *M. sacchariflorus and M.* × *giganteus* individuals indicating collection sites, ploidy and genetic relationships. Each accession is represented by a pie chart showing ancestry (Q) among seven genetic clusters as determined by analysis with the software Structure using 34 605 RAD-seq SNPs. All individuals from the current study with known collection site and ploidy are plotted, including 699 *M. sacchariflorus* and 38 *M.* × *giganteus*. Fourteen diploid *M.* × *giganteus* found in China and Japan in previous studies (Clark *et al.*, 2014, 2015) are also included. Elevation is shown as a grey scale. The coastline at the last glacial maximum is indicated by a brown line drawn according to Lee *et al.* (2008). Paleo rivers on the land that is now under the Yellow Sea are drawn according to Song *et al.* (2016) and Yoo *et al.* (2016). High-resolution maps of Structure results are available in Supplementary Data S3.

Table 2. *Genetic diversity within* Miscanthus sacchariflorus *and* M. × giganteus *collected in eastern Asia*

DAPC group	No. of individuals	SNP diversity (*D*)	Mean extrapolated allelic richness*	Number of SNPs with minor allele frequency >0.05	N_P	$N_{P\ unique}$	Plastid Gini–Simpson index	Plastid Gini–Simpson index excluding Msi haplotypes
Yangtze diploids (ssp. *lutarioriparius*)	67	0.115	1.71	11 377	17	5	0.69 ± 0.06	0.69 ± 0.06
N China diploids	64	0.139	1.82	15 087	18	3	0.85 ± 0.03	0.85 ± 0.03
Korea/NE China/Russia diploids	256	0.141	1.89	14 778	20	9	0.85 ± 0.01	0.85 ± 0.01
N China/Korea/Russia tetraploids	139	0.133	1.94	14 648	19	7	0.79 ± 0.03	0.79 ± 0.03
S Japan tetraploids	120	0.139	1.89	15 509	20	6	0.75 ± 0.04	0.74 ± 0.04
N Japan tetraploids	76	0.134	1.80	14 249	9	2	0.46 ± 0.07	0.45 ± 0.07
Mxg (2*x* × 2*x*)	7	0.123	1.82	12 616	5	0	0.86 ± 0.10	0.86 ± 0.10
Mxg (4*x* × 2*x*)	30	0.187	1.86	18 897	9	0	0.77 ± 0.06	0.53 ± 0.13

Groupings are based on discriminant analysis of principal components (DAPC) for 34 605 RAD-seq SNPs (Fig. 2).

Msi, *M. sinensis*; Mxg, *M.* × *giganteus*; *D*, diversity, as calculated from expected heterozygosity of RAD-seq SNPs (allele frequencies were estimated from read count data using the R package polyfreqs for tetraploid and triploid groups, and directly from genotypes called with UNEAK for diploid groups; standard error of $D = 0.001$ for each group); N_P, number of plastid haplotypes; $N_{P\ unique}$, number of plastid haplotypes unique to each group.

*Standard error of 0.001 or 0.002 for each group for allelic richness.

were generated for the study, with data available on the NCBI Sequence Read Archive, accession SRP087645. RAD-seq data from our previous studies of *M. sacchariflorus* and *M.* × *giganteus* are available under accessions SRP026347, SRP048207 and SRP063572, and were also included in the current analysis.

Ploidy analysis

Flow cytometry, for determining DNA content and inferring ploidy, was performed using previously described protocols (Clark *et al.*, 2015, 2016). In brief, leaves were co-chopped in buffer with *Sorghum bicolor* (1.74 pg/2C) or *M. sinensis* (5.38 pg/2C) as an internal standard, then stained with propidium iodide or 4',6-diamidino-2-phenylindole (DAPI), before analysis on an LSR II Flow Cytometry Analyzer (BD Biosciences, San Jose, CA, USA) at Zheijiang University or at the Roy J. Carver Biotechnology Center at the University of Illinois or on a Partec PA II Flow Cytometer (Sysmex Partec, Görlitz, Germany) at Aarhus University, respectively. Expected genome sizes were 4.4 pg/2C for diploid *M. sacchariflorus*, 8.5 pg/2C for tetraploid *M. sacchariflorus* and 6.9 pg/2C for triploid *M.* × *giganteus* (Rayburn *et al.*, 2009; Li *et al.*, 2013; Chae *et al.*, 2014). Out of 764 individuals included in the population structure analysis, ploidy was inferred from genome sizes determined by flow cytometry for 694 individuals with available leaf tissue. Out of the remaining 70 individuals, we were able to infer ploidy of 57 based on heterozygosity of RAD-seq data (Supplementary Data Fig. S1), leaving only 13 individuals with undetermined ploidy.

RAD-seq data analysis

The UNEAK pipeline (Lu *et al.*, 2013) was used to identify SNPs from RAD-seq data and to genotype individuals. Tags with fewer than five reads were excluded from tag pair identification. After filtering for SNPs with <50 % missing data, a minimum minor allele frequency of 0.01 and no apparent heterozygous genotypes in any of three doubled haploid *M. sinensis* accessions (Głowacka *et al.* 2012), 34 605 SNPs remained (Supplementary Data S2). In *M. sinensis*, we previously found improved resolution of population structure by using these filtering parameters as compared with more stringent parameters (Clark *et al.*, 2014), which we confirmed in *M. sacchariflorus* (Supplementary Data Fig. S2).

Structure 2.3.4 (Falush *et al.* 2003), discriminant analysis of principal components (DAPC) implemented in the R package adegenet (Jombart *et al.*, 2010) and TESS3 implemented in the tess3r R package (Caye *et al.*, 2016) were used to assess population structure, hybridization and major groupings of *M. sacchariflorus*. Structure was run with 50 000 Markov chain Monte Carlo (MCMC) reps after a 10 000 rep burn-in using the admixture model with correlated allele frequencies. A preliminary Structure run at K = 1 was performed to infer lambda (the parameter for determining allele frequency priors), which was estimated at 0.4686. This value of lambda was then fixed, and six Structure runs each at K = 1–10 were performed. The Evanno method (Evanno *et al.*, 2005) was used to explore possible values of K (Supplementary Data Fig. S3). Principal components analysis for DAPC was performed using the 'glPca' function of adegenet, with parameters set so that each marker would be centred and scaled, and all principal components would be retained. The 'find.clusters' function was then used to make initial groupings, with 1000 randomly chosen centroids to ensure convergence, and all principal components included. The Bayesian information criterion was used as a guide for selection of the number of clusters (Supplementary Data Fig. S4). DAPC was then performed with the 'dapc' function using the groupings from 'find.clusters', the first 250 principal components and all discriminant axes for K = 2–10. TESS3 was run in six replicates at K = 1–10, with the optimal replicate at each K being selected by the software. The cross-validation score at each value of K was plotted in order to help

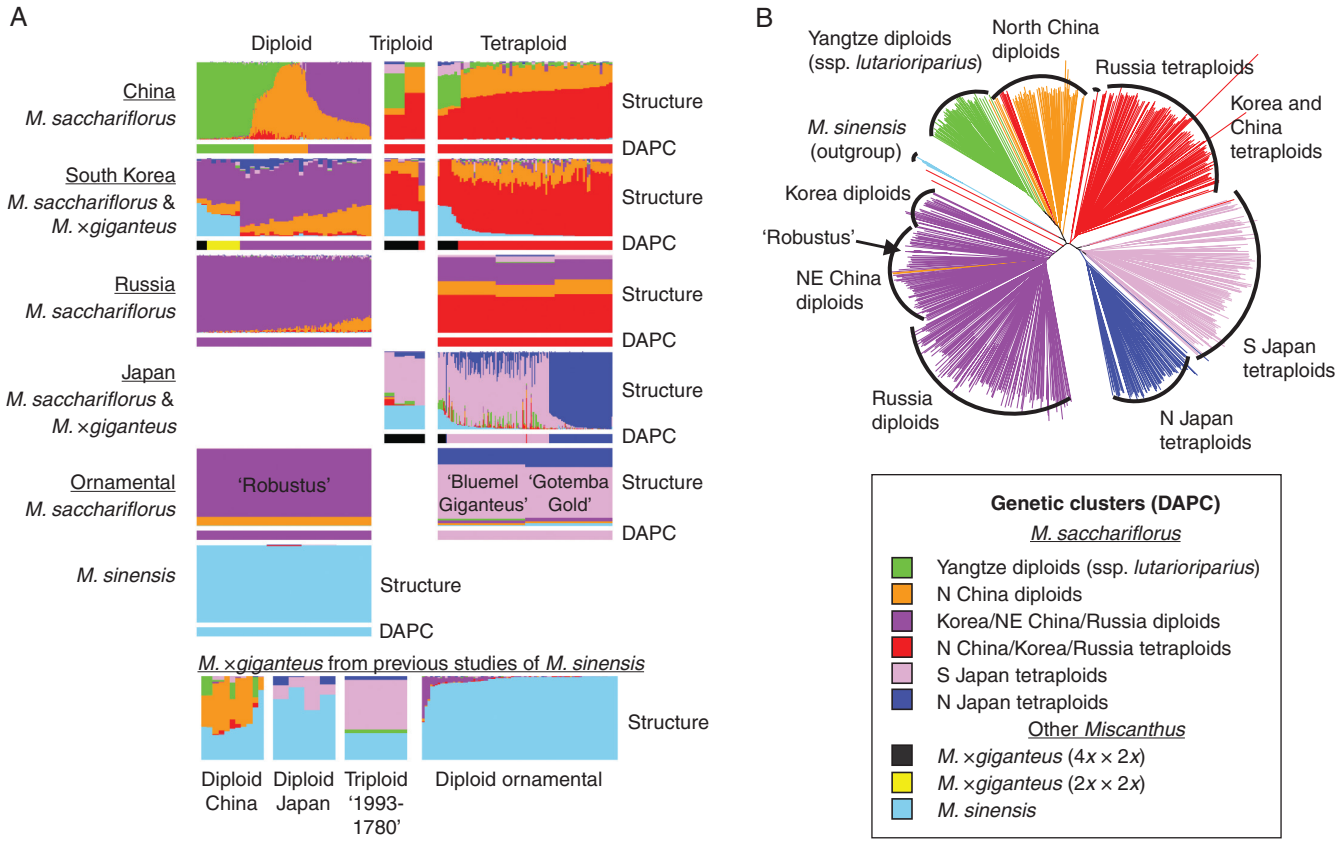


FIG. 2. Individual-based analysis of population structure of *Miscanthus sacchariflorus* and *M.* × *giganteus* using RAD-seq SNP data. Individuals that had ≥20 % *M. sinensis* admixed with *M. sacchariflorus* were considered to be *M.* × *giganteus*. (A) Bar charts showing Q values from Structure analysis (top of each set by provenance) or assignment to groups based on discriminant analysis of principal components (DAPC, bottom of each set) using 34 605 RAD-seq SNPs. Shown are nine DAPC groups, comprising six *M. sacchariflorus*, one *M. sinensis* and two *M.* ×*giganteus*; in the Structure analysis, seven groups are shown with colour as for DAPC groupings except that the *M.* × *giganteus* individuals are represented as an admixture of *M. sinensis* and *M. sacchariflorus*. All 764 individuals from the current study are plotted. The bottom portion includes an additional 93 individuals from previous studies (Clark *et al.*, 2014, 2015). 'Ornamental' indicates cultivars available from the horticultural nursery trade in North America. (B) Neighbor–Joining tree of 722 *Miscanthus sacchariflorus* individuals using 31 743 RAD-seq SNPs. Five *M. sinensis* individuals were included as an outgroup. Branches are coloured based on DAPC groupings (A). To reduce the signal of hybridization, 37 early generation hybrids were excluded, as were 2862 SNPs that were highly differentiated between *M. sinensis* and *M. sacchariflorus*.

identify the optimal K value (Supplementary Data Fig. S5). The K value that was ultimately chosen for further analysis was the lowest K that resulted in consistency between groups identified by Structure, DAPC and TESS3, and was also biologically and geographically meaningful (Supplementary Data Fig. S6).

Allele frequencies within DAPC groups were estimated directly from the sampled genotypes for the diploid groups, and with the R package polyfreqs (Blischak *et al.*, 2016) for the tetraploid and triploid groups. SNP diversity (*D*) was estimated as the expected heterozygosity (probability of drawing two different alleles from the population) at each SNP, averaged across all SNPs (Nei, 1973). Allelic richness was estimated using the extrapolation method of Foulley and Ollivier (2006). Pairwise Jost's *D* (Jost, 2008) among DAPC groups was estimated from genotypes and allele frequencies using a custom R function (available at doi:10.5281/zenodo.58614). Pairwise Jost's *D* values were then used as a distance matrix for the calculation of a Neighbor–Joining tree among populations using the R package ape (Paradis *et al.*, 2004). The software TreeMix (Pickrell and Pritchard, 2012) was used to infer evolutionary relationships and gene flow among the groups identified by DAPC, including one group for *M. sinensis*. The number of migration edges selected was the highest number at which each edge connected a unique pair of tree edges.

A second Neighbor–Joining tree was calculated to show relationships among individuals. To reduce the confounding effect of hybridization on the calculation of a Neighbor–Joining tree between *Miscanthus* individuals, individuals in *M.* × *giganteus* DAPC groups were excluded, as were SNPs that were highly differentiated between *M. sinensis* and *M. sacchariflorus* according to Structure results, leaving 731 individuals and 31 743 SNPs, respectively. Manhattan distances were calculated in R between each pair of individual genotypes; the distance between two genotypes homozygous for different alleles was 2, the distance between a homozygous and heterozygous genotype was 1 and the distance between identical genotypes was 0; these distances were then summed across all SNPs and scaled by the proportion of non-missing data (Black, 2006). Manhattan distances were used for calculating the Neighbor–Joining tree using the R package ape (Paradis *et al.*, 2004). This same genetic distance matrix was used for conducting Mantel tests using the R package ade4 (Chessel *et al.*, 2004) utilizing geographic distances calculated with the R package geosphere (Hijmans, 2017).

To test hypotheses about the history of population divergence and admixture, scenarios were tested using DIYABC 2.1.0 (Cornuet *et al.*, 2014). The origins of tetraploid populations and the relationships among diploid populations were examined separately. In both cases, six scenarios with uniform prior probabilities were tested using a total of 600 000 simulations. Posterior probabilities of scenarios were estimated using the logistic regression approach.

Plastid haplotype analysis

Each unique combination of amplicon sizes across ten microsatellite loci was considered to be one plastid haplotype. Individuals with any missing data were excluded from plastid analysis, leaving 759 individuals. In our previous studies of *Miscanthus* (Clark *et al.*, 2014, 2015, 2016), distances between haplotypes were calculated simply as the number of markers at which alleles differed (i.e. with ten microsatellites, distances ranged from zero to ten). However, due to the large number of closely related haplotypes in this study, greater resolution was needed so distances between haplotypes were calculated as the sum of differences in amplicon size across all ten loci. A plastid haplotype network was then calculated using a modified source code from pegas (Paradis, 2010). Pairwise Jost's *D* using plastid haplotype frequencies among the DAPC groups identified from RAD-seq SNPs was estimated using the R package mmod (Winter, 2012) then used for generating a Neighbor–Joining tree among populations using the R package ape (Paradis *et al.*, 2004).

All data sets and scripts used for both SNP and plastid analysis are available at the Illinois Data Bank (https://doi.org/10.13012/B2IDB-0170190_V3).

RESULTS

Ploidy types and their geographic distribution

The distribution of ploidy types among species and among geographies within species was not uniform. Among all the *M. sacchariflorus* and *M.* × *giganteus* studied, we identified 396 diploids, 12 triploids and 339 tetraploids; ploidy was not determined for 12 individuals (Table 1). Four out of the five *M. sinensis* included in the study were diploid, as expected (Clark *et al.*, 2014), and ploidy was not determined for one individual. From mainland Asia, 380 diploid, three triploid and 133 tetraploid *M. sacchariflorus* individuals were observed (Table 1). Among the mainland Asian *M.* × *giganteus*, we observed nine diploids, five triploids and ten tetraploids (Table 1). In China, tetraploid *M. sacchariflorus* individuals were found primarily in coastal regions, especially on the Shandong Peninsula, Liaodong Peninsula and near Taihu Lake (near Shanghai), whereas tetraploids were common throughout South Korea (Fig. 1). In Russia, we found only three *M. sacchariflorus* tetraploids, which were collected near the Amur River. Diploid *M. sacchariflorus* predominated in inland regions of China and Russia. In South Korea, *M. sacchariflorus* diploids were frequently found in the north but were uncommon south of 36.5 °N. Three triploid *M. sacchariflorus* were collected at Taihu Lake, the Shandong Peninsula and in north-western South Korea, all regions where both diploid and tetraploid *M. sacchariflorus* were common. We also identified five triploid *M.* × *giganteus* from geographically diverse areas of South Korea, which was consistent with previous reports (Moon *et al.*, 2013). No triploid *M.* × *giganteus* individuals were found in China.

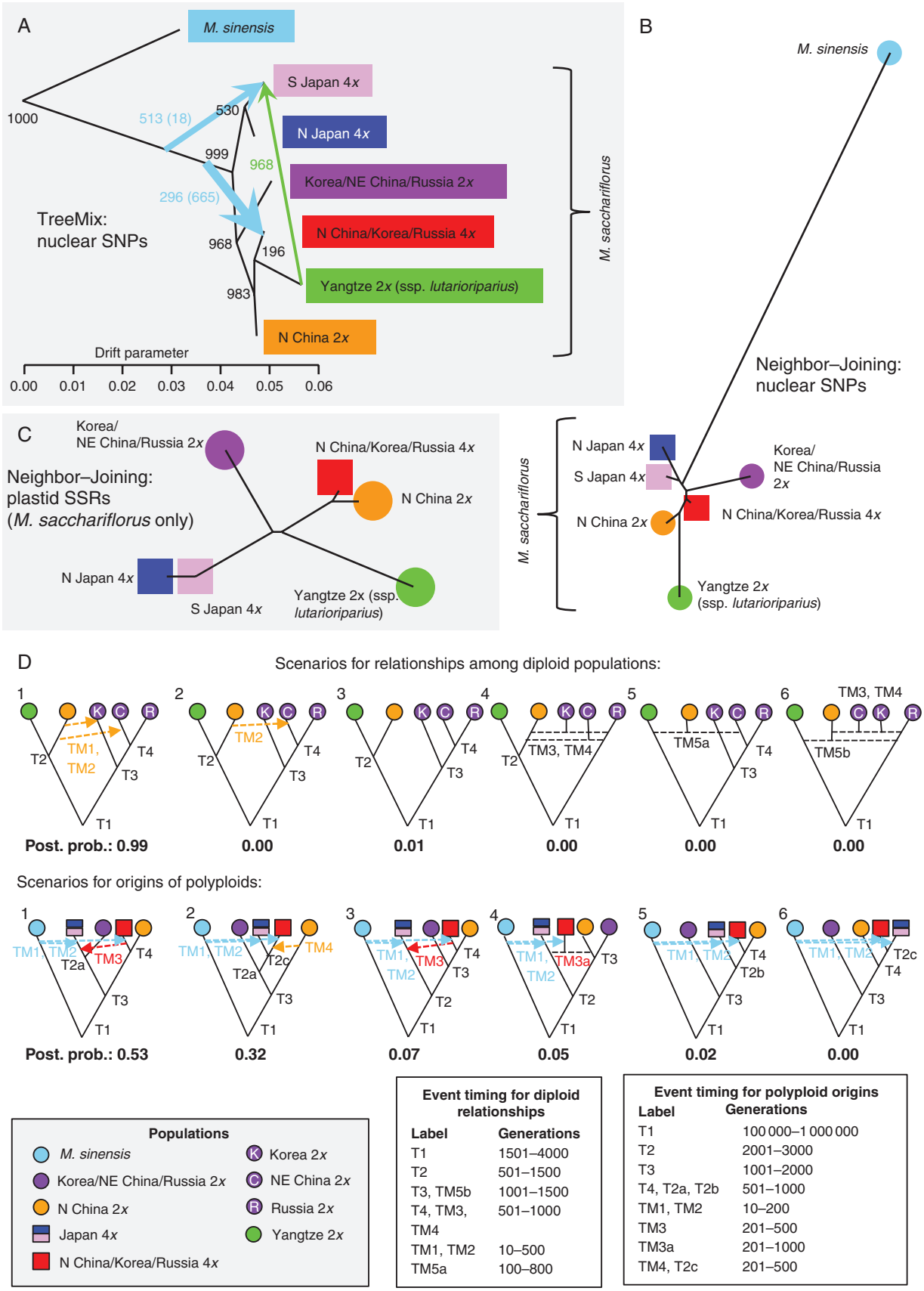
All 184 Japanese *M. sacchariflorus* individuals that we tested were tetraploid (Table 1; Fig. 1). In addition to three triploid Japanese *M.* × *giganteus* that we identified previously (Clark *et al.*, 2015), one new triploid *M.* × *giganteus* from the Japanese island of Shikoku was identified (JM2014-S-4). To the best of our knowledge, this is the first discovery of triploid *M.* × *giganteus* on Shikoku Island.

Population structure inferred from RAD-Seq SNPs

We identified seven genetic groups via Structure and TESS3 (K = 7; six *M. sacchariflorus* and one *M. sinensis*) and nine groups via DAPC (K = 9; six *M. sacchariflorus*, one *M. sinensis* and two *M.* × *giganteus*); given that Structure and TESS3 can detect hybrids between groups as an admixture but DAPC cannot, this represented a consistent result among all three analyses. Cluster assignments of individuals for all values of K tested are shown in Supplementary Data Fig. S6. Out of the 34 605 SNPs used in analysis, 34 564 had call rates above zero in all six *M. sacchariflorus* genetic groups. Cluster assignments of each individual for the selected values of K are also provided in Supplementary Data S1. Structure results at K = 7 are mapped in Fig. 1, with higher resolution maps provided in Supplementary Data S3.

Three of the *M. sacchariflorus* groups were primarily diploid, including Yangtze (ssp. *lutarioriparius*), N China and Korea/NE China/Russia (Figs 1 and 2A; green, orange and purple, respectively). The other three *M. sacchariflorus* groups were primarily tetraploid, including N China/Korea/Russia, S Japan and N Japan (Figs 1 and 2A; red, pink and dark blue, respectively). One *M.* × *giganteus* DAPC group consisted only of diploids from Korea (yellow), whereas the other consisted primarily of triploids and tetraploids from Korea and Japan, plus two diploids from Korea (black) (Fig. 2A; Supplementary Data S1). Structure results indicated that all diploid *M.* × *giganteus* were derived from hybridization between *M. sinensis* (diploid; sky blue) and diploid *M. sacchariflorus*, whereas all triploid and tetraploid *M.* × *giganteus* were derived from hybridization between *M. sinensis* (diploid) and tetraploid *M. sacchariflorus*; in all cases, the *M. sacchariflorus* parent originated from the same geographic region as where the *M.* × *giganteus* genotype was collected (Fig. 2A). The three triploid *M. sacchariflorus* from China and Korea appeared to be the result of hybridization between diploid and tetraploid *M. sacchariflorus* (Figs 1 and 2A).

The divergence of *M. sinensis* and *M. sacchariflorus*, as indicated by TreeMix analysis, was followed chronologically by the Japan–mainland split within *M. sacchariflorus*, then by the split of the Korea/NE China/Russia diploids from the other mainland groups (Fig. 3A). Divergence of N China diploids, Yangtze diploids and N China/Korea/Russia *M. sacchariflorus* tetraploids occurred approximately simultaneously based on TreeMix. In the Neighbor–Joining tree of the consolidated groups (Fig. 3B), *M. sinensis* branched off the tree between the Japan and mainland *M. sacchariflorus* groups, indicating that the most basal division of *M. sacchariflorus* was Japan


A
M. sinensis
S Japan 4x
N Japan 4x
Korea/NE China/Russia 2x
N China/Korea/Russia 4x
Yangtze 2x (ssp. lutarioriparius)
N China 2x
1000
513 (18)
530
999
968
296 (665)
968
196
983
TreeMix:
nuclear SNPs
M. sacchariflorus
Drift parameter
0.00 0.01 0.02 0.03 0.04 0.05 0.06
B
M. sinensis
Neighbor–Joining:
nuclear SNPs
N Japan 4x
S Japan 4x
N China 2x
Korea/
NE China/Russia
2x
N China/Korea/Russia 4x
Yangtze 2x
(ssp. lutarioriparius)
M. sacchariflorus
C
Korea/
NE China/Russia 2x
N China/Korea/Russia 4x
N China 2x
Neighbor–Joining:
plastid SSRs
(M. sacchariflorus only)
N Japan 4x
S Japan 4x
Yangtze 2x (ssp.
lutarioriparius)
D
Scenarios for relationships among diploid populations:
1
2
3
4
5
6
TM3, TM4
T1
T2
T3
T4
TM1,
TM2
TM5a
TM5b
Post. prob.: 0.99
0.00
0.01
0.00
0.00
0.00
Scenarios for origins of polyploids:
TM3
TM3a
T2a
T2b
T2c
TM4
Post. prob.: 0.53
0.32
0.07
0.05
0.02
0.00
Populations
M. sinensis
Korea/NE China/Russia 2x
N China 2x
Japan 4x
N China/Korea/Russia 4x
Korea 2x
NE China 2x
Russia 2x
Yangtze 2x
Event timing for diploid relationships
Label Generations
T1 1501–4000
T2 501–1500
T3, TM5b 1001–1500
T4, TM3, TM4 501–1000
TM1, TM2 10–500
TM5a 100–800
Event timing for polyploid origins
Label Generations
T1 100 000–1 000 000
T2 2001–3000
T3 1001–2000
T4, T2a, T2b 501–1000
TM1, TM2 10–200
TM3 201–500
TM3a 201–1000
TM4, T2c 201–500

vs. mainland, consistent with the TreeMix results. Moreover, in TreeMix, the clade containing all mainland Asian *M. sacchariflorus* was found in 968 out of 1000 bootstrap replicates, strongly supporting an early Japan–mainland split (Fig. 3A).

Population sub-structure of *M. sacchariflorus* was further elucidated via Neighbor–Joining trees and estimates of genetic differentiation (Figs 2B and 3B, C; Table 3). Neighbor–Joining trees indicated that the tetraploid S Japan group was basal to the tetraploid N Japan group (Figs 2B and 3C) which, in combination with S Japan being the group least differentiated from N Japan (Table 3), suggested that N Japan was derived from S Japan. Similarly, the diploid N China group was basal to the diploid Yangtze group (Fig. 2B) and the Yangtze diploids were most closely related to the N China diploids (Table 3), suggesting that the Yangtze diploids were derived from the N China diploids. Within the Korea/NE China/Russia diploid group, Korea was closest to the centre of the tree, followed by NE China, then Russia as the most derived group (Fig. 2B). Eight individuals from the N China/Korea/Russia tetraploid DAPC group also appeared in the N China clade of the Neighbor–Joining tree; these were tetraploid and triploid hybrids with the Yangtze diploid group (Fig. 2). Moreover, the two most closely related *M. sacchariflorus* groups were the N China diploids and N China/Korea/Russia tetraploids, suggesting recent derivation of the latter group from the former (Table 3). Although the two *M. sacchariflorus* Japan groups were more closely related to the N China/Korea/Russia tetraploids than to any other mainland group (Table 3), the Neighbor–Joining trees positioned the Japanese *M. sacchariflorus* as approximately equally related to all mainland groups (Figs 2B and 3B). Jost's *D* statistic from SNP data indicated that S Japan tetraploids and N China/Korea/Russia tetraploids were the *M. sacchariflorus* groups least diverged from *M. sinensis*, whereas Yangtze diploids were the most diverged from *M. sinensis* (Table 3). Mantel tests indicated significant isolation by distance within all *M. sacchariflorus* groups except for N Japan tetraploids and Yangtze diploids (Supplementary Data Fig. S7).

High intraspecific admixture within and across ploidies of *M. sacchariflorus* was indicated by the Structure results (Figs 1 and 2A). The observed admixture was typically consistent with geographical proximity of groups, and probably reflects some combination of isolation by distance and true admixture. For example, tetraploid *M. sacchariflorus* from S Japan (pink) frequently had some ancestry from the adjacent tetraploid populations in northern Japan (blue) or South Korea (red). Similarly, a mostly continuous gradient of admixture was observed among diploid *M. sacchariflorus* in mainland Asia from the Yangtze River to north China and from north-east China to Russia (Figs 1 and 2A; green to orange to purple). Other admixtures suggested gene flow over long distances and across ploidy barriers; for example, ancestry from the Yangtze diploid (ssp. *lutarioriparius*; green) group was found not only in nearby tetraploids in China, but also in tetraploids in S Japan. TreeMix analysis also identified gene flow from the Yangtze diploid (ssp. *lutarioriparius*) group to the S Japan tetraploids (Fig 3A). The rare tetraploids in Russia that were primarily part of the N China/Korea/Russia tetraploid group appeared to be admixed with diploids from Korea/NE China/Russia (purple). Korean diploids (purple) had some ancestry from the Japan tetraploids, and notably it appeared to be more from N Japan (blue) than from S Japan (pink).

Hybridization between tetraploid *M. sacchariflorus* and diploid *M. sinensis* resulted in considerable introgression of *M. sinensis* DNA into *M. sacchariflorus* in both Korea and southern Japan, but curiously evidence of such cross-ploidy introgression was absent from China, as indicated in the Structure results (Fig. 2A). TreeMix indicated gene flow from ancestral *M. sacchariflorus* to the S Japan and N China/Korea/Russia tetraploid *M. sacchariflorus* groups (Fig. 3A); given that it is biologically impossible for an ancestral and extant population to interbreed, this is likely to be an artefact of TreeMix not being designed for interspecies comparisons, but rather represents interspecific gene flow from *M. sinensis* to the tetraploid groups, as identified by Structure (Fig. 2A). In contrast to the tetraploids, diploid *M.* × *giganteus* hybrids were observed in Korea, but without introgression. No *M.* × *giganteus* hybrids were identified in China; however, diploid hybrids have been identified in previous studies (Jiang *et al.*, 2013; Clark *et al.*, 2014; Figs 1 and 2A) but probably were not collected for this study because they morphologically resemble *M. sinensis*. The *M.* × *giganteus* DAPC group that consisted of triploids and tetraploids from Korea and Japan had substantially greater SNP diversity than all the other groups studied (Table 2), probably the result of combining genomes that had evolved in isolation over considerable time.

The SNP diversity was high and similar among five of the six *M. sacchariflorus* DAPC groups. However, the Yangtze diploid (ssp. *lutarioriparius*) group had approx. 10–20 % lower SNP diversity based on three different estimates [expected heterozygosity (*D*), allelic richness and number of SNPs with a minor allele frequency >0.05; Table 2]. Among the tetraploid *M. sacchariflorus* groups, S Japan had slightly higher SNP diversity than N Japan or N China/Korea/Russia (Table 2). The low relative diversity estimates for the Yangtze diploid group and for the N Japan group (Table 2) are consistent with genetic bottlenecks associated with sub-groups that formed via migration from larger initial groups, as indicated in the Neighbor–Joining trees (Figs 2B and 3C).

Model testing with DIYABC was performed to test hypotheses about the relationships among populations based on the results of Structure, Neighbor–Joining and TreeMix analyses

FIG. 3. Relationships among genetic groups of *Miscanthus sacchariflorus* based on RAD-seq SNPs and plastid microsatellites. Groups were identified using discriminant analysis of principal components (DAPC; Fig. 2A). (A) Relationships among six *M. sacchariflorus* groups, and one *M. sinensis* outgroup, as determined by the software TreeMix based on 34 605 RAD-seq SNPs and 727 individuals. The black tree indicates population divergence. Coloured arrows indicate subsequent migration, colour coded by population of origin. Arrow width signifies the magnitude of migration. The numbers of bootstrap replicates out of 1000 supporting each clade or migration event are indicated. Numbers in parentheses indicate the number of replicates in which there was a migration event from *M. sinensis* to the indicated population. (B) Neighbor–Joining tree calculated from pairwise Jost's *D* statistics using allele frequencies at 34 605 RAD-seq SNPs across 727 individuals. (C) Neighbor–Joining tree calculated from pairwise Jost's *D* statistics using frequencies of plastid haplotypes across ten microsatellite markers, across 712 *M. sacchariflorus* individuals with complete plastid data (*M. sinensis* is not shown due to the lack of shared haplotypes with *M sacchariflorus*). (D) Scenarios tested by DIYABC and their estimated posterior probabilities. Arrows indicate migration events, and dashed horizontal lines indicate admixture events. Model constraints upon divergence (T) and migration times (TM) are indicated in terms of number of generations before the present.

TABLE 3. *Differentiation among* Miscanthus sacchariflorus *genetic groups using Jost's* D *statistic*

	M. sinensis	Yangtze diploids (ssp. *lutarioriparius*)	N China diploids	Korea/NE China/ Russia diploids	N China/Korea/Russia tetraploids	S Japan tetraploids
Nuclear SNPs*						
Yangtze diploids (ssp. *lutarioriparius*)	0.1533 ± 0.0020					
N China diploids	0.1392 ± 0.0019	0.0267 ± 0.0004				
Korea/NE China/Russia diploids	0.1452 ± 0.0020	0.0477 ± 0.0007	0.0241 ± 0.0004			
N China/Korea/Russia tetraploids	0.1294 ± 0.0019	0.0290 ± 0.0005	0.0082 ± 0.0002	0.0220 ± 0.0003		
S Japan tetraploids	0.1288 ± 0.0018	0.0316 ± 0.0005	0.0186 ± 0.0003	0.0258 ± 0.0004	0.0110 ± 0.0002	
N Japan tetraploids	0.1368 ± 0.0019	0.0419 ± 0.0006	0.0250 ± 0.0004	0.0277 ± 0.0004	0.0171 ± 0.0003	0.0131 ± 0.0002
Plastid haplotypes†						
N China diploids		0.79				
Korea/NE China/Russia diploids		0.91	0.64			
N China/Korea/and Russia tetraploids		0.81	0.15	0.62		
S Japan tetraploids		0.89	0.72	0.64	0.53	
N Japan tetraploids		0.92	0.83	0.77	0.68	0.1

A colour scale is used to highlight similarities between groups, with green being most similar and red being least similar. Groups are the same as those in Table 2, plus an outgroup including five *M. sinensis* individuals.

*Mean ± s.e. values of Jost's *D* are shown across 34 605 RAD-seq SNP loci using 727 individuals.

†Jost's *D* estimated using plastid haplotype frequencies identified using ten microsatellite markers and 712 individuals. Standard errors are not presented due to the entire plastid genome being treated as one marker.

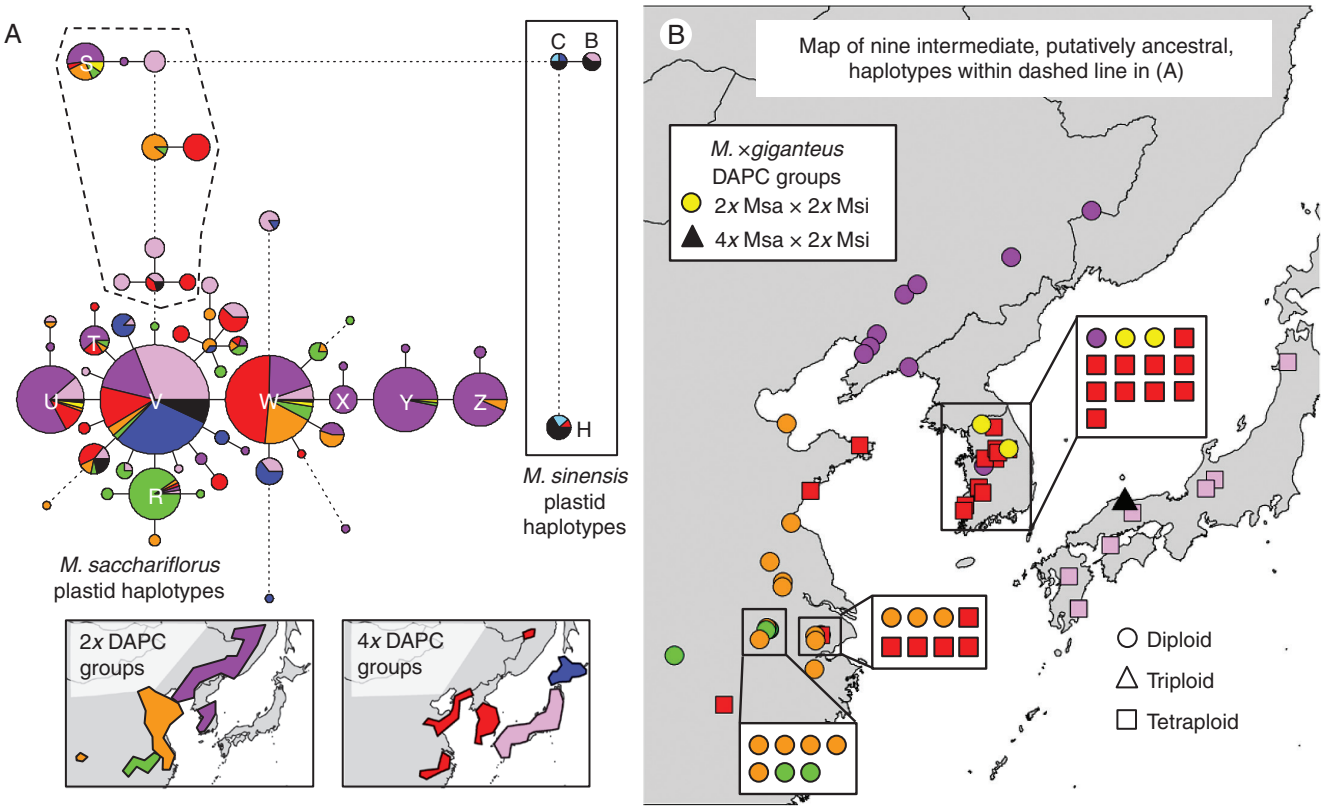


FIG. 4. Plastid haplotypes for 759 *Miscanthus* spp. individuals using ten microsatellite markers. Colours from Fig. 2 indicate discriminant analysis of principal components (DAPC) groups identified with nuclear SNPs, with six *M. sacchariflorus*, one *M. sinensis* (light blue) and two *M.* × *giganteus* (yellow and black) shown; geographical distributions of *M. sacchariflorus* groups are shown in the inset. (A) Haplotype network. Each circle or pie chart represents one haplotype, and the area is proportional to the number of individuals with the haplotype. Letters indicate haplotypes referred to in the text. Solid lines connecting circles and pie charts indicate single mutational steps (one nucleotide difference in amplicon size at one microsatellite marker). Dotted lines indicate multiple mutational steps, proportional in number to the length of the line. Nine haplotypes surrounded by a dashed line are intermediate between common *M. sacchariflorus* and common *M. sinensis* haplotypes. (B) Map of individuals possessing any of the nine intermediate haplotypes surrounded by the dashed line in (A). Shapes indicate ploidy.

(Fig. 3D). Of six scenarios that tested relationships among the diploid *M. sacchariflorus* populations, the most probable (0.99) involved all extant populations radiating from a common ancestor, with subsequent gene flow from N China to Korea and NE China. Hypotheses involving independent refugia in the north and south of mainland Asia, followed by admixture to produce extant diploid populations surrounding the Yellow Sea, were not supported. Of six scenarios that tested the origins of the *M. sacchariflorus* polyploids, the most probable (0.53) supported multiple polyploidization events, with the Japan tetraploids being most closely related to the Korea/NE China/Russia diploids, and the N China/Korea/Russia tetraploids being most closely related to the N China diploids. Among scenarios that tested the hypothesis of a single *M. sacchariflorus* polyploidization event, the most probable (0.32) involved all tetraploids being derived from the Korea/NE China/Russia diploid group, with subsequent gene flow from the N China diploids to the N China/Korea/Russia tetraploids.

Plastid haplotype analysis

Among all *M. sacchariflorus* and *M. sinensis* entries studied, 56 unique plastid haplotypes were identified. Eight (S–Z) were identified previously in a study of Russian *M. sacchariflorus* (Clark *et al.*, 2016). The most common *M. sacchariflorus* haplotype (V) was widely distributed across the sampling range, and the second most common haplotype (W) was common on the mainland but rare in Japan (Fig. 4). Several common haplotypes (U, X, Y and Z) were found primarily in the diploid Korea/NE China/Russia DAPC group, and one (R) was found primarily in the Yangtze diploid DAPC group. Eight newly observed *M. sacchariflorus* haplotypes plus the previously observed haplotype S (dashed line, Fig. 4A) were intermediate between the most common *M. sacchariflorus* and *M. sinensis* haplotypes; these haplotypes could be found throughout most of the sampling range in China, Korea and southern Japan, but were notably absent from Hokkaido (N Japan) and Russia (except for one individual near the Chinese border; Fig. 4B). Geographic distributions of common haplotypes are shown in Supplementary Data Fig. S8.

Three plastid haplotypes (B, C and H) that were previously found to be common in *M. sinensis* (Clark *et al.*, 2014, 2015) were observed but infrequent in *M. sacchariflorus* (Fig. 4A). The *M. sinensis* haplotype C was found in *M. sacchariflorus* from N Japan and *M.* × *giganteus* in S Japan and Korea, haplotype B was found in *M. sacchariflorus* and *M.* × *giganteus* from S Japan, and haplotype H was found in *M. sacchariflorus* and *M.* × *giganteus* from Korea. Haplotypes B, C and H were common in *M. sinensis* from these same regions (Clark *et al.*, 2014, 2015), supporting the hypothesis that sympatric hybridization between *M. sinensis* and *M. sacchariflorus* was common in Japan and Korea. These three haplotypes are highly differentiated from all other *M. sacchariflorus* haplotypes (Fig. 4A), and therefore the alternative hypothesis that they were found in *M. sacchariflorus* due to shared ancestry with *M. sinensis* is unlikely. *Miscanthus* × *giganteus* individuals from Japan and Korea also possessed haplotypes typical of *M. sacchariflorus*, indicating that hybridization occurred in both directions but perhaps with preference for *M. sacchariflorus* as the female parent, which may be due to *M. sacchariflorus* being more common at our sampling sites. Out of 30 individuals in the triploid and tetraploid ($4x \times 2x$) *M.* × *giganteus* DAPC group, only 11 had plastid haplotypes from *M. sinensis*. Among the seven individuals in the Korean diploid ($2x \times 2x$) *M.* × *giganteus* DAPC group, all had haplotypes characteristic of *M. sacchariflorus*.

Assessment of plastid haplotype diversity indicated a strong bottleneck in the N Japan DAPC group, and a weaker bottleneck in the Yangtze diploid DAPC group (Table 2). Plastid haplotype diversity was highest among the N China diploids and Korea/NE China/Russia diploids (Table 2). Pairwise Jost's *D* using plastid haplotype frequencies between pairs of DAPC groups indicated a very close relationship between the N China/Korea/Russia tetraploids and the N China diploids, as well as between N Japan and S Japan (Table 3). Although the smallest Jost's *D* between Japan and mainland Asia was between S Japan and the N China/Korea/Russia tetraploid group (Table 3), a Neighbor–Joining tree constructed from the Jost's *D* matrix indicated that Japan was similarly distant from all mainland groups, and thus not derived from the mainland tetraploids (Fig. 3C).

DISCUSSION

Centre of radiation for M. sacchariflorus

Multiple lines of evidence indicate that current populations of *M. sacchariflorus* were derived from an ancestral population that refuged during the last glacial maximum (LGM) on land that is now under the Yellow Sea and East China Sea (Figs 1 and 5). Perhaps most compelling is the observation that the four most genetically diverse extant *M. sacchariflorus* groups currently inhabit land surrounding these seas, whereas derived sub-groups are found more distant, indicating a radiation from this region. For example, the N China diploid group is found on the western edge of the Yellow and East China Seas, and was the parent population of the substantially less genetically diverse Yangtze group found further south (Figs. 1, 3B and 5; Tables 2 and 3). To the east, the S Japan tetraploid group gave rise to the less diverse N Japan tetraploids (Figs 2B and 5; Table 2). Similarly, the basal clade of the Korea/NE China/Russia diploid group in the RAD-seq SNP Neighbor–Joining tree was from Korea, followed by NE China, then Russia (i.e. from south to north; Fig. 2B). Additionally, the N China/Korea/Russia tetraploid group is common in coastal areas adjacent to both the western and eastern sides of the Yellow Sea, especially on the Shandong Peninsula in the west and on the Korean Peninsula in the east, but is infrequent in the interior of the continent (Fig. 1A), suggesting that it arose on a land mass between the Shandong and Korean Peninsulas. Moreover, none of the *M. sacchariflorus* groups surrounding the Yellow and East China Seas had substantially greater genetic diversity than the rest (Table 2), suggesting that they each similarly represent the diversity of the ancestral population.

Model testing with DIYABC strongly supported a scenario with a single refugium for all diploid populations, as opposed to multiple refugia followed by admixture (Fig. 3D). In both the TreeMix (Fig. 3A) and Neighbor–Joining (Fig. 3B,C) analyses, Japanese and mainland Asian *M. sacchariflorus* appeared to be derived from an extinct common ancestor, with Japan vs. mainland Asia being the first split, although DIYABC results suggest

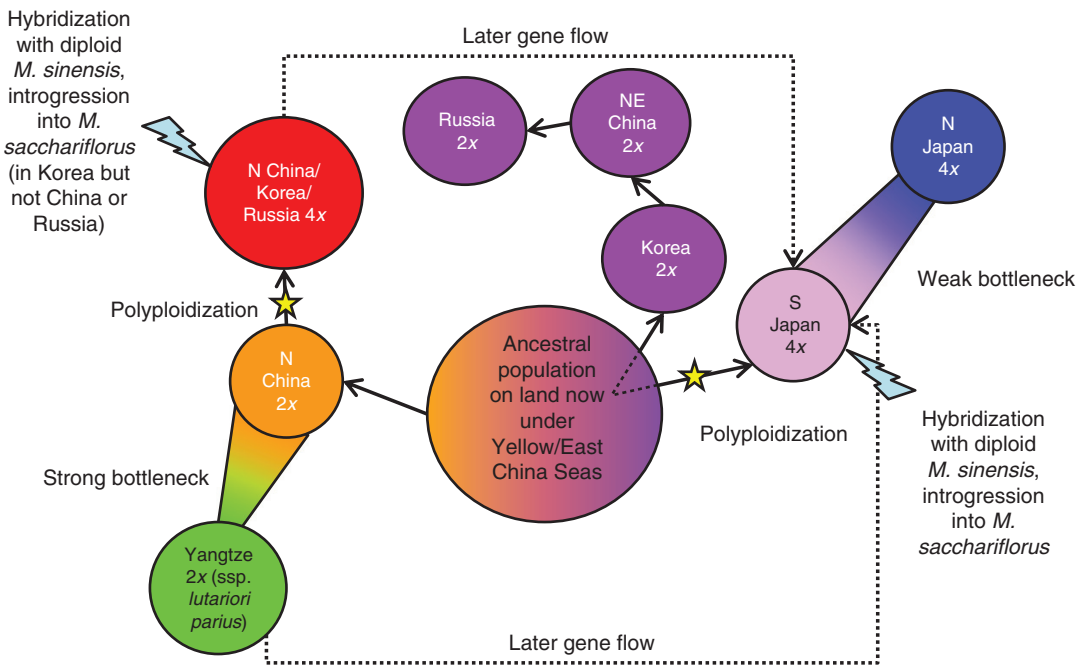


FIG. 5. Model depicting relationships among *Miscanthus sacchariflorus* populations based on RAD-seq SNP and plastid microsatellite data. Colours are based on discriminant analysis of principal components (DAPC) in Fig. 1.

that an ancestor of both the Japanese *M. sacchariflorus* (tetraploids) and the diploid Korea/NE China/Russia group diverged from all other *M. sacchariflorus* before Japan became a distinct population (Fig. 3D).

Plastid haplotypes that were intermediate between common *M. sacchariflorus* and *M. sinensis* haplotypes, and therefore putatively ancestral, were absent from N Japan and Russia, the regions of our sampling range that are furthest from the Yellow Sea and East China Sea (Fig. 4B). Consistent with the RAD-seq SNP data, the N China diploid and Korea/NE China/Russia diploid DAPC groups had similarly high plastid diversity (Table 2), but did not share all of their plastid haplotypes (Fig. 4A), suggesting that they were independently derived from a nearby centre of diversity.

During the LGM, the climate of East Asia was too cold and dry to support populations of *M. sacchariflorus* throughout much of its current range, but the environment in the southern Yellow Sea Basin would have been favourable. Contemporary populations of *M. sacchariflorus* are found primarily in open riparian areas of climatic regions that predominantly support cool temperate forests and have average annual low temperatures warmer than –40 °C (USDA hardiness zone ≥3). In the northern portion of *M. sacchariflorus*' current range in north-east China, eastern Russia and northern Japan, vegetation during the LGM was steppe–tundra (Adams and Faure, 1997; Ray and Adams, 2001) and thus unsuitably cold and dry for this species. However, during the LGM, a steppe-dominated ecosystem covered a wide area from the present day Korean Peninsula in the east to the mountains surrounding the Sichuan Basin in the west, including all of the Yellow Sea Basin and the north-western edge of the East China Sea Basin (Adams and Faure, 1997; Ray and Adams, 2001). Moreover, the two largest rivers in East Asia, the Yellow River and the Yangtze River, flowed over the southern portion of the Yellow Sea Basin during the LGM (Ryu *et al.*, 2008; Song *et al.*, 2016; Yoo *et al.*, 2016). Song *et al.* (2016) reported that the main paleo-channel of the Yellow River was at approx. 35 °N, south of the present day Shandong Peninsula (in contrast to its present position north of the Shandong Peninsula) and flowed to the east and south-east; whereas the paleo-channel of the Yangtze River entered the Yellow Sea Basin approx. 2 °N north of its current location, flowed from east to north-east where it merged with the Yellow River at approx. 35 °N and 124.5 °E, and ultimately formed a large delta near present day Jeju Island (Fig. 1). During the LGM, average flow rates of the Yellow River (460 $m^3 s^{-1}$) and Yangtze River (730 $m^3 s^{-1}$) were approx. six and 41 times less than current flow rates, respectively (Song *et al.*, 2016); however, the flow rate of the merged paleo-Yellow and Yangtze River was similar to that of the present day Ussuri River in eastern Russia, which currently supports large populations of *M. sacchariflorus* (Clark *et al.*, 2016). Thus the climate and hydrology of the southern Yellow Sea Basin during the LGM would have been a conducive refugium for *M. sacchariflorus*, and this is consistent with inferences from our population genetics analyses. Additional sampling of mud cores from the southern Yellow Sea to enable analysis of phytoliths and pollen deposited during the LGM would help test the hypothesis that this region was key to the survival and subsequent expansion of *M. sacchariflorus* during the last ice age.

Origins of M. sacchariflorus *ssp.* lutarioriparius

Our genetic data support the conclusion that *M. sacchariflorus* ssp. *lutarioriparius* is a subspecies of *M. sacchariflorus*, and not a separate species. *Miscanthus sacchariflorus*

ssp. *lutarioriparius*, which has been described as *Triarrhena lutarioriparia* (Liu, 1997; Liu *et al.*, 2001; Liu and Yu, 2004) or *M. lutarioriparius* (Chen and Renvoize, 2005, 2006), is reported to be endemic to the Yangtze River watershed, has tall (3–7 m) and thick (15–30 mm) stems, yields about 30 Mg ha^{-1} of dry biomass, and is used to manufacture paper on a commercial scale in China. Subspecies status of *lutarioriparius* was the conclusion of Sun *et al.* (2010) based on trait comparisons and of Chen *et al.* (2007) based on ITS nuclear ribosomal DNA sequence data. The Yangtze diploid DAPC group in the current study corresponded to *M. sacchariflorus* ssp. *lutarioriparius*, given its geographical location and great height (Supplementary Data S1). Our population genetic analyses clearly indicated that the Yangtze diploid group was derived from the N China diploid *M. sacchariflorus* group via a substantial genetic bottleneck (Figs 2B, 3 and 5; Tables 2 and 3). Moreover, all plastid haplotypes found in the Yangtze diploid group were also found in or closely related to plastid haplotypes found in other *M. sacchariflorus* DAPC groups (Fig. 4A). If *M. sacchariflorus* ssp. *lutarioriparius* had truly been a different species, then we would have expected the Neighbor–Joining and TreeMix results to show it forming a basal branch separate from *M. sacchariflorus*, before the Japan–mainland split, but this was not observed.

Previous studies have found that *M. sacchariflorus* ssp. *lutarioriparius* included both diploid and tetraploid individuals, with the latter observed primarily along the most eastern portion of the Yangtze River and around nearby Lakes Taihu and Hung-tse (Li *et al.*, 2013; Sheng *et al.*, 2016). In contrast, our DAPC analysis identified only diploid individuals in the Yangtze group. However, our Structure analysis identified seven tetraploid individuals that had 41–45 % of their ancestry from the Yangtze diploid group (in some cases, this was the single greatest genetic contribution to the admixture), with most of the remainder being from the N China/Korea/Russia tetraploid group (Fig. 2A; Supplementary Data S1). We collected six of these tetraploids on the shores of Lake Taihu along with a triploid individual of similar ancestry, and the seventh was found 131 km south (Fig. 1; Supplementary Data S1 and S3). It is likely that the tetraploid *M. sacchariflorus* ssp. *lutarioriparius* identified by Li *et al.* (2013) and Sheng *et al.* (2016) were similar to those that we collected near Lake Taihu given that all three studies sampled there, although we did not have any samples from Lake Hung-tse to compare with the previous studies. Notably, our assessment of admixture indicated a small genetic contribution of *M. sacchariflorus* ssp. *lutarioriparius* to S Japan *M. sacchariflorus* tetraploids from Kyushu and Shikoku Islands (Figs 1 and 2A), though it is unknown if the most direct source of *lutarioriparius* was diploid or tetraploid. Further study of the eastern tetraploid population of *M. sacchariflorus* ssp. *lutarioriparius* and its gene flow with other *M. sacchariflorus* is warranted given the breeding potential of *M. sacchariflorus* ssp. *lutarioriparius* for high biomass yield.

Origins of tetraploid M. sacchariflorus *and subsequent gene flow*

Our results confirm and add resolution to the known geographic range of tetraploid *M. sacchariflorus*. In Japan, we found *M. sacchariflorus* to be exclusively tetraploid, consistent with previous surveys of ploidy (Hirayoshi *et al.*, 1957; Clark *et al.*, 2015). In a previous study, we found rare diploid *M. sinensis* × *M. sacchariflorus* hybrids in Japan (Clark *et al.*, 2015), suggesting that diploid *M. sacchariflorus* might exist in Japan. New genetic analysis of these hybrid individuals in the context of the current study indicates that the *M. sacchariflorus* component of their ancestry corresponds to the S Japan and N Japan tetraploid populations (Figs 1 and 2A), suggesting that gene flow may occur from tetraploid *M. sacchariflorus* to diploid *M. sinensis*, perhaps by rare haploid gametes produced by triploid *M.* × *giganteus*.

In mainland Asia, we found both diploid and tetraploid *M. sacchariflorus*. No mainland region was exclusively tetraploid, although tetraploids were concentrated in the Korean and Shandong Peninsulas. Tetraploids were found as far north as the Amur River, 1400 km away from the nearest mainland Asia tetraploids, which were on the Liaodong Peninsula. The Amur River tetraploids might be a relict population, indicating that tetraploids were once much more widespread north of the Liaodong Peninsula or, alternatively, they might be recent anthropogenic introductions. The only tetraploids found south of the Shandong Peninsula were the hybrids with *M. sacchariflorus* ssp. *lutarioriparius* mentioned above.

Our genetic results are most consistent with the hypothesis that the Japanese and mainland Asian populations of tetraploid *M. sacchariflorus* originated from distinct polyploidization events (Fig. 5). In Neighbor–Joining and TreeMix analyses (Fig. 3), the mainland tetraploids (N China/Korea/Russia tetraploids) appeared to be closely related to the N China and Yangtze diploid groups, suggesting that the mainland tetraploids were derived from a common ancestor of these diploid groups. Derivation of the mainland tetraploids exclusively from the Yangtze diploid group is unlikely given that the genetic diversity of the mainland tetraploids is higher than that of the Yangtze diploids. The Japan tetraploids (S Japan and N Japan), on the other hand, were similarly related to all extant diploid groups that we identified, suggesting that they were derived either from the common ancestor of all diploid *M. sacchariflorus* or from a diploid population that once existed in Japan but has become extinct. The DIYABC results indicated that the Japan tetraploids and the Korea/NE China/Russia diploids instead shared a common ancestor that had already diverged from all other *M. sacchariflorus* (Fig. 3D), and in this scenario the ancestor was probably located in Korea. Notably, the Korean diploids have apparent admixture from Japan according to Structure (Figs 1 and 2A), which could in reality reflect shared ancestry. Some combination of the two hypotheses is possible, for example if there was a gradient of isolation by distance in the ancestral Yellow Sea population, and the eastern segment of the population gave rise to both the Korean diploids and Japanese tetraploids (Fig. 5). After the LGM, coastal regions in the east warmed sooner than more interior areas to the west (Winkler and Wang, 1993), and this warming probably drove the initial migration and differentiation of populations out of the Yellow Sea basin. It should be noted that the Japan tetraploids were more closely related to the mainland tetraploids than to any diploid group; however, our analysis suggests that this similarity is due to gene flow between the tetraploid groups and hybridization with *M. sinensis* in both groups, as opposed to shared ancestry.

Structure results indicated that all tetraploid *M. sacchariflorus* arose by autopolyploidization (doubling of the diploid *M. sacchariflorus* genome) rather than allopolyploidization (hybridization with *M. sinensis* followed by genome doubling). It should also be noted that we did not observe any tetraploid *M.* × *giganteus* derived from diploid *M. sacchariflorus*, indicating that allotetraploid formation via hybridization of diploid *M. sinensis* and diploid *M. sacchariflorus* is rare to non-existent. However, polyploidy in *M. sacchariflorus* appears to have facilitated considerable introgression from *M. sinensis*. In Korea and S Japan, where both triploid and tetraploid *M.* × *giganteus* were found, we observed a gradient of introgression of *M. sinensis* into tetraploid *M. sacchariflorus* (Fig. 2A), similar to what we previously observed in Japan (Clark *et al.*, 2015). Intriguingly, however, no hybridization or introgression from *M. sinensis* was observed among tetraploid *M. sacchariflorus* in China or Russia; we do not know what has caused this notable difference in cross-ploidy interspecific hybridization and introgression. Interestingly, we also identified many diploid *M.* × *giganteus* in Korea, but did not observe any introgression of *M. sinensis* into diploid *M. sacchariflorus*. Therefore, it appears that there exists a barrier to introgression of *M. sinensis* into diploid *M. sacchariflorus* that is broken by polyploidy, allowing introgression of *M. sinensis* into tetraploid *M. sacchariflorus*. Moreover, polyploidy has broken the barrier to introgression independently in both Korea and Japan.

It is worthwhile to consider what mechanisms might permit the generation and normal development of interspecific F_1 hybrids from a diploid species crossed with diploid or tetraploid forms of another species, but allow introgression only or highly preferentially into the tetraploid form. Sterility of diploid F_1 *M.* × *giganteus* is an unlikely mechanism, because we have observed more than a dozen such populations in crossing experiments, and the progeny were typically fertile (unpublished data); moreover, many ornamental cultivars that are predominantly *M. sinensis* in genetic composition have a substantial portion of their genome introgressed from *M. sacchariflorus* (Clark *et al.*, 2014, 2015). Lack of fitness in the diploid F_1 is also unlikely to be the cause, given that we have observed many diploid *M.* × *giganteus* F_1 hybrids to be vigorous and high yielding in field trials (Dong *et al.*, 2018). Perhaps then, a decrease in fitness in subsequent generations (F_2 or backcross) may occur to a greater extent for the interspecific diploids than for the tetraploids. For any given backcross generation, the overall proportion of alleles from *M. sinensis* would be the same regardless of whether the *M. sacchariflorus* was diploid or tetraploid, but tetraploid introgressed *M. sacchariflorus* would have *M. sinensis* alleles distributed across a greater number of loci than would a diploid introgressed *M. sacchariflorus*. Moreover, most loci with an *M. sinensis* allele would have a 1:3 ratio of *M. sinensis* alleles to *M. sacchariflorus* alleles in an introgressed tetraploid, whereas any locus with an *M. sinensis* allele would necessarily have a 1:1 ratio of *M. sinensis* to *M. sacchariflorus* alleles in a diploid. These differences in distribution of *M. sinensis* alleles could affect fitness via dominance and epistasis effects. Additionally, polyploidy in plants is associated with an increased rate of meiotic recombination (Melamed-Bessudo *et al.*, 2016), which could facilitate introgression by breaking linkage between deleterious alleles and neutral or advantageous alleles.

Intraspecific cross-ploidy gene flow primarily from diploid to tetraploid forms of *M. sacchariflorus* was also indicated by the Structure and TreeMix results. We identified six tetraploid individuals in China that appeared to have half of their ancestry from the Yangtze diploid group, and all three tetraploid individuals from Russia had approximately half of their ancestry from the Korea/NE China/Russia and N China diploid groups (Figs 1 and 2A). Both TreeMix and Structure also identified gene flow from the Yangtze diploid group into the S Japan tetraploid group (Figs 1, 2A, 3A and 5). Given that *M. sacchariflorus* ssp. *lutarioriparius* (Yangtze diploids) are exceptionally tall and high yielding, ancestry from this group may have contributed to the high yield potential of the biomass cultivar *M.* × *giganteus* '1993-1780', which had approx. 4 % ancestry from *M. sacchariflorus* ssp. *lutarioriparius* (Fig. 2A). Also, diploid *M. sacchariflorus* in Korea notably appeared to have some ancestry from the N Japan tetraploids (Figs 1 and 2A). The N Japan group, which is more geographically isolated and less impacted by admixture and hybridization than the S Japan group, may more closely resemble an ancient population that existed in S Japan and in the area that is now the Yellow Sea. Thus, the Korea diploids might not truly be admixed with N Japan, but may appear that way because they are also closely related to the ancestral population from that area (Figs 2B and 5).

Provenance of cultivated Miscanthus

By taking into account both historical records and new genomic data from our study, we determined that the ornamental and much-studied *M. sacchariflorus* cultivar 'Robustus' was probably derived from a wild population along the southern Amur River between the Songhua and Zeya Rivers, or on the lower (northern) reaches of the Songhua River, in either case near the border of Russia and China. In the summers of 1855 and 1856, Carl Maximowicz, Conservator of the Imperial Botanical Garden at St. Petersburg, became the first Western botanist to collect *M. sacchariflorus*, which he named *Imperata* (*Triarrhena*) *sacchariflora*. By his own account, Maximowicz observed this species and collected specimens along the southern Amur River between the mouths of the Songhua River and Zeya River (near the city of Blagoveshchensk), and along the lower (northern) reaches of the Ussuri River (Maximowicz, 1859). Maximowicz returned to the Amur Region in 1859 and was able to travel about 270 km up the Songhua River in China before returning to Russia (Ravenstein, 1861; Stapf, 1891; Bretschneider, 1898). By 1862, the *M. sacchariflorus* that Maximowicz introduced from the Amur region to St. Petersburg was found to be winter-hardy there, and it was promoted as a beautiful ornamental as well as a valuable forage crop for cattle (Regel, 1862). *Miscanthus sacchariflorus* seeds and divisions were subsequently distributed from St. Petersburg to other botanical gardens in Europe (Regel, 1862). Karl Foerster, an influential horticulturalist with a nursery in Potsdam, Germany and who led the mid-twentieth century revival of using perennial grasses in Western gardens (Foerster, 1988; Darke, 1994, 2007), obtained germplasm of *M. sacchariflorus* from a botanical garden (M. Deuter, pers. comm. from K. Naeser, E. Pagels, R. Simon and H. Hagemann). Foerster selected the 'Robustus' cultivar in around 1950 (Simon, 1952),

which like all ornamental *Miscanthus* was propagated vegetatively for distribution. Genomic data from our current study indicated that 'Robustus' is closely related to populations of *M. sacchariflorus* found along the southern Amur and northern Songhua Rivers, which is consistent with the locations from which Maximowicz reported collecting, and less related to populations from the Ussuri River (Figs 2B and 6). The analysis presented in Fig. 6 also indicates that 'Robustus' could have originated from the upper (southern) Songhua River, but we know of no historical botanical introductions from this area of China to Europe. Thus, 'Robustus' was probably derived from the material Maximowicz introduced from the north-western portion of his collection expedition.

In contrast to the diploid 'Robustus', the ornamental tetraploid *M. sacchariflorus* cultivars 'Gotemba Gold' and 'Bluemel Giganteus' were both in the S Japan group, with Structure results similar to individuals from the Kanto region of Japan (Fig. 2A). 'Gotemba Gold', a variegated cultivar also known as 'Gotemba', originated from Kenji Watanabe's nursery in 20th century Japan (Darke, 2007) and is most probably named after the city of Gotemba in the Kanto region. The biomass cultivar *M.* × *giganteus* '1993-1780' had ancestry matching *M. sacchariflorus* from southern Japan (Fig. 2A), consistent with its origins as a wild collection from that region (Greef *et al.* 1997).

We previously determined that about half of ornamental *Miscanthus* cultivars sold as *M. sinensis* in North America and Europe were actually interspecific hybrids with *M. sacchariflorus* ancestry introgressed into a central or southern Japan *M. sinensis* genetic background (about BC_2, typically; Clark *et al.*, 2014, 2015). The results presented here indicate that the *M. sacchariflorus* ancestry of these ornamental cultivars came from the Korea/NE China/Russia diploid *M. sacchariflorus* group (Fig. 2A). Given the different geographic origins of the *M. sinensis* and *M. sacchariflorus* portions of the genomes of these ornamentals, this introgression almost certainly did not occur in nature, but instead was likely to be the result of European breeding efforts for early flowering and winter hardiness (Darke, 1994; Clark *et al.*, 2014). Results from our current study indicated that the ornamental *M.* × *giganteus* 'Purpurascens' was also derived from the Korea/NE China/Russia diploid *M. sacchariflorus* group, consistent with our previous hypothesis that 'Purpurascens' was the source of the *M. sacchariflorus* ancestry in ornamental *M. sinensis* (Clark *et al.*, 2015). 'Purpurascens' in cultivation is itself likely to be a naturally occurring hybrid given that its *M. sinensis* ancestry also came from Korea, NE China or Russia (Clark *et al.*, 2015, 2016).

Differences in genetic diversity and population structure between M. sinensis *and* M. sacchariflorus

Higher genetic diversity and weaker population structure were observed for *M. sacchariflorus* in comparison with *M. sinensis*, which suggests differences in their demographic history. We previously hypothesized, based on a comparison between population genetics of *M. sacchariflorus* in Russia with *M. sinensis* across East Asia, that *M. sacchariflorus* underwent less of a genetic bottleneck during the LGM than *M. sinensis* (Clark *et al.*, 2016), and our new results also support this hypothesis. Genetic diversity, measured as the number of SNPs identified with a minor allele frequency >0.05, ranged from 11 337 to 15 509 among DAPC groups in *M. sacchariflorus* (Table 2) but was only 6942–9262 in *M. sinensis* (Clark *et al.*, 2016). Similarly, pairwise Jost's *D* for the six *M. sacchariflorus* genetic groups ranged from only 0.008 to 0.048 (Table 3), whereas for six *M. sinensis* genetic groups that we identified previously it ranged from 0.019 to 0.076 (Clark *et al.*, 2014), indicating about half as much within-species differentiation for *M. sacchariflorus* compared with *M. sinensis*.

Differences between the plastid haplotype networks of *M. sacchariflorus* and *M. sinensis* were also consistent with our hypothesis of a greater genetic bottleneck for *M. sinensis* during the LGM. In contrast to *M. sinensis*, in which the most common haplotypes consisted of two groups separated by multiple mutational steps (Clark *et al.*, 2014), the most common haplotypes in *M. sacchariflorus* formed a continuous network of single mutational steps (Fig. 4A). This indicates that extinction of haplotypes due to genetic drift has occurred less frequently in *M. sacchariflorus* than in *M. sinensis*, which was probably due to a greater effective population size over time. Moreover, the most common haplotype in *M. sacchariflorus* (V) was distributed very broadly across East Asia, whereas the three most common haplotypes in *M. sinensis* (B, D and H; Clark *et al.*, 2014) all had distinctly restricted geographies, indicating large changes in haplotype frequency due to founder effects as

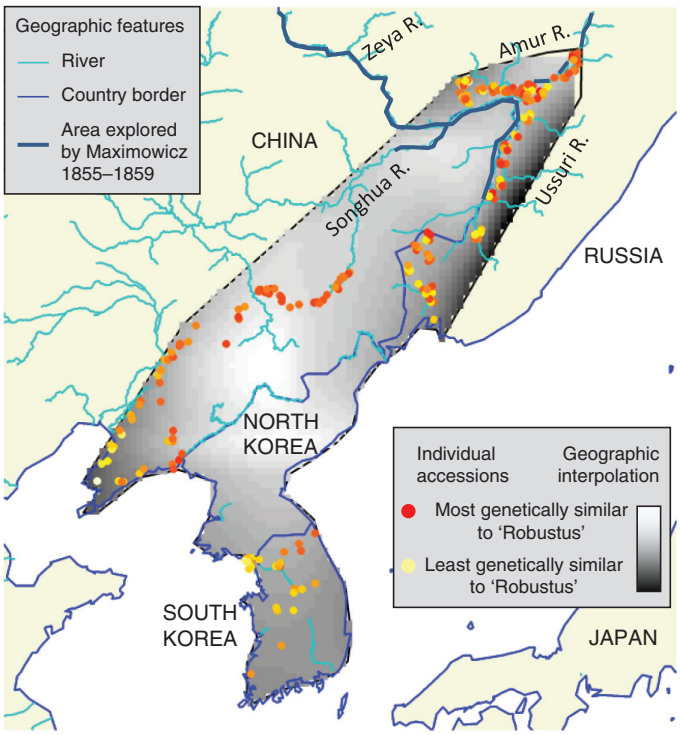


FIG. 6. Genetic relationship between the ornamental *Miscanthus sacchariflorus* 'Robustus' and wild populations of *M. sacchariflorus* in eastern Russia, north-east China and Korea, based on 34 605 RAD-seq SNPs. A total of 249 individuals in the Korea/NE China/Russia diploid group with known provenance are plotted. The thick blue line in the upper right indicates river segments known to have been explored in the mid to late 1850s by botanist Carl Maximowicz, who collected and brought back to St. Petersburg seed and/or rhizomes of *M. sacchariflorus* from which we believe 'Robustus' is derived (Maximowicz, 1859; Simon, 1952).

M. sinensis colonized Asia. Lastly, we found rare haplotypes in *M. sacchariflorus* that potentially belonged to the common ancestor of *M. sinensis* and *M. sacchariflorus*, or at least an ancient population of *M. sacchariflorus*, given that they were intermediate between the common *M. sinensis* haplotypes and common *M. sacchariflorus* haplotypes (Fig. 4A). Such ancestral haplotypes appear to have become extinct in *M. sinensis* (Clark *et al.*, 2014, 2015).

Conclusions

Here we provide the most extensive analysis of *M. sacchariflorus* population structure to date, revealing high genetic diversity, geographical differentiation of six genetic groups, multiple origins of polyploidy and a history of introgression from diploid *M. sinensis* into tetraploid *M. sacchariflorus* in Japan and Korea but not in China or Russia (Fig. 5). Over half of the SNP markers identified in this study (18 555 out of 34 605) had not been identified in any of our previous studies of *Miscanthus* (Supplementary Data S2), highlighting the importance of broad sampling for capturing the diversity of this genus. These results provide important context for physiological and agronomic studies of *M. sacchariflorus*.

Given the great genetic diversity and population structure in *M. sacchariflorus*, we caution against using any single genotype to draw conclusions about the species as a whole. Because the ornamental cultivar 'Robustus' has been one of only two *M. sacchariflorus* clones readily available in North America and Europe, considerable research and breeding have been conducted on this genotype, including development of the 'Amuri' clones that were released by Tinplant (Deuter and Abraham, 2004; Anonymous, 2006; Pude, 2011; Supplementary Data S4). However, 'Robustus' is exceptionally early flowering and adapted to higher latitudes and colder winter temperatures than many other *M. sacchariflorus* because it originated near the north-eastern edge of the species' range (Figs 2B and 6). For example, if studies were to include representatives of the Yangtze group (ssp. *lutarioriparius*) or the S Japan group, we would expect very different conclusions about yield potential, height, flowering time and winter-hardiness than from studies that include only 'Robustus' or its progeny. Thus, for studies that wish to make broad conclusions about *M. sacchariflorus* as a species, it would be advisable to include multiple representatives from each of the six genetic groups that we identified.

To develop new triploid *M.* × *giganteus* biomass cultivars, plant breeders should especially note that China and Korea offer tetraploid germplasm that is genetically distinct from the Japanese tetraploid germplasm that has predominantly been used for breeding thus far. The two tetraploid groups of *M. sacchariflorus* should be evaluated for differences in yield potential and stress tolerance, and used in controlled crosses with *M. sinensis* to test for heterosis and their potential to improve yield of *M.* × *giganteus*. For experiments seeking to examine the consequences of polyploidy in *M. sacchariflorus*, we recommend comparing the diploids and tetraploids from the Shandong Peninsula, since these possess the closest relationship between diploid and tetraploid *M. sacchariflorus* populations, and hybridization with *M. sinensis* is rare or non-existent in that region (Figs 1, 2A and 5).

Because of the greater amount of *M. sinensis* germplasm imported to North America and Europe during the last century and a half in comparison with all other *Miscanthus* species, a disproportionate amount of *Miscanthus* research to date has focused on *M. sinensis* (Hodkinson *et al.*, 2015). However, an understanding of the biology and genetic diversity of *M. sacchariflorus* will be equally important, if not more so, for breeding improved *M.* × *giganteus* cultivars. The tetraploid *M. sacchariflorus* parent not only contributes two-thirds of the genetic material of triploid *M.* × *giganteus* (Rayburn *et al.*, 2009), but is also thought to make a large contribution to its high yield via chilling-tolerant photosynthesis, which effectively extends its growing season (Głowacka *et al.*, 2014). Given that *M. sacchariflorus* also has substantially more genetic diversity than *M. sinensis*, we expect that selection within *M. sacchariflorus* will provide the greatest opportunity to make genetic gains for breeding improved *M.* × *giganteus*.

SUPPLEMENTARY DATA

Data S1: list of all accessions in the study, including collection locations, plastid haplotypes, and DAPC, Structure and TESS3 results. Data S2: list of SNP markers used in the study, including tag sequences and allele frequencies within genetic groups. Data S3: GeoTIFF images showing Structure Q values on high-resolution maps. Data S4: flyer from Tinplant describing the *Miscanthus* × *giganteus* 'Amuri' clones. Figure S1: heterozygosity vs. missing data rate, by individual, across 34 605 RAD-seq SNPs. Figure S2: comparison of population structure identified with different SNP filtering criteria. Figure S3: Delta K values calculated using the method of Evanno *et al.* (2005) across six Structure runs each at K = 1–10. Figure S4: Bayesian information criterion for selection of the optimal number of genetic groups using 'find.clusters' in adegenet. Figure S5: cross-validation score for selection of the optimal number of groups by TESS3. Figure S6: barplots indicating clustering result of 768 *Miscanthus* individuals using 34 605 SNP markers and the TESS3, Structure and DAPC methods. Figure S7: genetic vs. geographic distance in *Miscanthus sacchariflorus*. Figure S8: geographic distributions of common plastid haplotypes.

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