



# Genetic diversity of native and introduced *Phragmites* (common reed) in Wisconsin

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## Abstract

Two subspecies of common reed (*Phragmites australis*; Poaceae) exist in northern North America: the native *P. australis* subsp. *americanus* and the introduced *P. australis* subsp. *australis*. There are numerous native populations in Wisconsin, in addition to the more recently established populations of the introduced subspecies. We studied populations of both *P. australis* subspecies across Wisconsin in order to characterize the genetic diversity of both subspecies and to investigate whether any instances of hybridization could be ascertained in Wisconsin. Using eight microsatellite markers, we found minimal overlap in the alleles that could be recovered from native and introduced plants, and we found no evidence to suggest hybridization between subspecies, even in localities where native and introduced plants grow in close proximity. Overall, we found greater genetic variation in plants of the introduced subspecies relative to the native subspecies, and we observed some geographic patterns of allelic diversity.

**Keywords** Common reed · Genetic diversity · Microsatellites · *Phragmites australis* · Population genetics

## Introduction

Invasive plants disrupt natural ecology in many ways, including by outcompeting native plants and altering trophic relationships (Pyšek et al. 2012; Gallardo et al. 2016). Among the most widespread and ecologically impactful plant invaders in North America is the common reed, *Phragmites australis* (Cav.) Trin. ex Steud. Two subspecies co-occur in northern North America: the native *P. australis* subsp. *americanus* Saltonst., Peterson & Soreng and the introduced *P. australis* subsp. *australis* (Saltonstall et al. 2004). The introduced subspecies has been implicated as a noxious weed in Canada and the USA, and in the USA the expansion of introduced plants has contributed to the decline of the native subspecies (Marks et al. 1994; Saltonstall 2002, 2011; Saltonstall and Stevenson 2007; Park and

Blossey 2008; Catling and Mitrow 2011; Kettenring et al. 2011; Mozdzer and Magonigal 2012). *Phragmites* populations must be identified correctly, because the management objectives are entirely different for each subspecies (i.e., conserving native populations versus controlling or eradicating introduced populations; Marks et al. 1994; Kiviat 2013; Hazelton et al. 2014). Although morphological characters are fairly reliable for distinguishing the two *Phragmites* subspecies (Saltonstall et al. 2004), molecular tools can provide additional confidence in identification (Saltonstall 2002, 2003a, b). Microsatellite markers are arguably the most effective molecular method for characterizing *Phragmites* populations and have been used to investigate genetic diversity at a variety of geographic scales (e.g., Saltonstall 2003b; Kettenring and Mock 2012; Lambertini et al. 2012; Albert et al. 2015; McCormick et al. 2016).

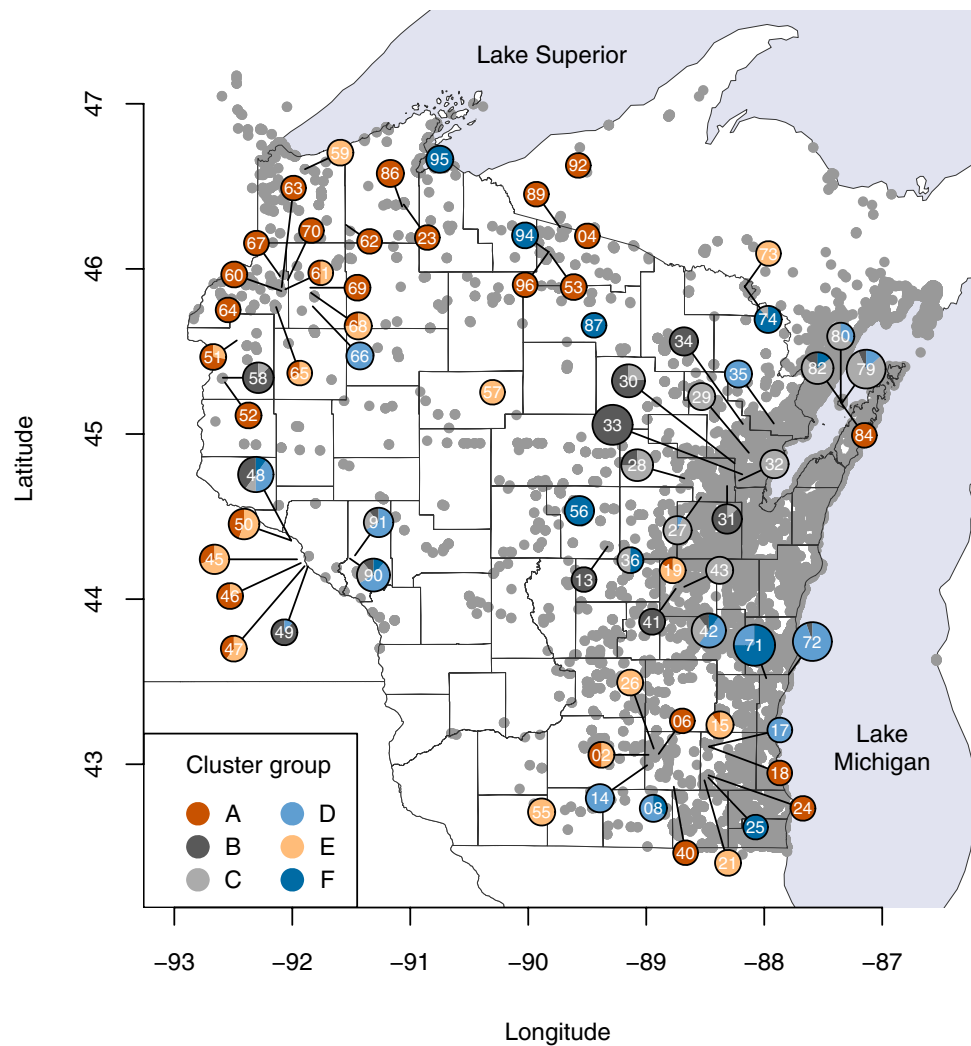
In Wisconsin (USA), *Phragmites* populations occur most commonly near Lake Michigan, where the density of the introduced subspecies also is greatest (Fig. 1; Granberg and Woods 2017). The native subspecies, in contrast, grows rather sparsely in the majority of Wisconsin counties. Molecular methods for studying *Phragmites* have not been applied widely in Wisconsin, and these would be able to confirm morphological identifications, as well as provide information about genetic diversity. Microsatellite markers,

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**Fig. 1** Sampling localities and genetic cluster groups for *Phragmites* plants in Wisconsin. Gray background dots indicate reported *Phragmites* localities (subspecies unspecified). Sampling sites for this study are indicated with site identifiers (see Supplementary Table 1), and circle size is proportional to the number of genetically distinct individuals that were sampled in each locality. Each locality has a pie chart showing the proportion of individuals that were assigned to each of six genetic cluster groups. Cluster groups 'A' and 'E' correspond to the native subspecies, and groups 'B', 'C', 'D', and 'F' represent the introduced subspecies



which are variable among individuals and populations of both subspecies (Saltonstall 2003a, b; Meyerson et al. 2009; Albert et al. 2015; McCormick et al. 2016), offer the potential to estimate the overall genetic diversity of each subspecies and to evaluate geographic patterns of isolation or spread.

Although the two *Phragmites* subspecies are closely related and wind-pollinated (Ishii and Kadono 2002; Lambert et al. 2007; McCormick et al. 2010), there are relatively few examples of hybridization between them. Hybrids have been induced under controlled conditions (Meyerson et al. 2009) and detected in a small number of natural populations (Paul et al. 2010; Saltonstall et al. 2014, 2016; Wu et al. 2015). *Phragmites* hybrids exhibit intermediate morphological traits, thereby confounding their identification using morphological characters (Saltonstall et al. 2014). Moreover, hybrids cannot be verified using molecular data from the uniparentally inherited chloroplast and instead must be confirmed using nuclear DNA data such as microsatellites (Saltonstall et al. 2014). In other plant invasions,

hybridization has been a driving force enabling the spread of non-native taxa and their displacement of native, congeneric taxa (Thompson 1991; Ellstrand and Schierenbeck 2000; Moody and Les 2007). Genetically, hybridization offers the potential to produce novel gene combinations that might increase competitive ability or broaden environmental tolerances (Ellstrand and Schierenbeck 2000; Saltonstall et al. 2014). Hybridization between the two Wisconsin *Phragmites* subspecies could accelerate the rate of invasion, and a molecular survey of populations using microsatellite markers would be able to detect hybrids, if they exist.

*Phragmites* plants have been studied extensively in North America, and many eastern USA populations of the native subspecies have effectively been replaced by adventive populations of the introduced subspecies (Saltonstall 2002). Wisconsin offers a rare opportunity to study an ongoing *Phragmites* invasion, where the introduced populations are relatively few and geographically restricted, whereas the native populations are fairly numerous and widespread. In order to understand more fully the extent of the *Phragmites*

invasion and the current status of the native subspecies in Wisconsin, we sampled populations across the state and analyzed them using microsatellite markers. We set out to ascertain the genetic diversity within and among populations of both subspecies, and to determine whether there was evidence for interspecific hybridization.

## Methods

*Phragmites* samples were obtained from 73 localities in Wisconsin and adjacent states (Fig. 1; Supplementary Table 1). In larger stands, samples were collected from multiple individuals, and these were separated from one another by at least 10 m to reduce the chance of gathering multiple samples from the same genet. The number of samples taken at each locality was roughly proportional to the number of individuals in the stand, up to a maximum of 21 samples. Voucher specimens were deposited in the UW-Whitewater herbarium (UWW). Plants were preliminarily identified on the basis of morphological characters (Saltonstall et al. 2004), and identifications were later confirmed using molecular data from DNA sequencing and microsatellite fragment analysis (see below). Fresh leaf material was kept in a freezer (− 20 °C) or in liquid preservative (Rogstad 1992) until DNA extraction. DNA was extracted using a previously reported protocol (Doyle and Doyle 1987), adjusted to extract from approximately 1 cm<sup>2</sup> of leaf blade tissue using an initial buffer volume of 600 µl, and modified to use pure chloroform (without isoamyl alcohol) at the extraction step and ethanol in place of isopropanol at the DNA precipitation step.

Plastid DNA sequences were obtained for the *trnT-trnL* spacer and *trnL* intron, which have been used previously to identify *Phragmites* subspecies, using the ‘a’, ‘b’, ‘c’, and ‘d’ primers (Taberlet et al. 1991), following methods reported previously (Tippery et al. 2018). We sequenced plastid DNA for a subset of individuals from both subspecies, to compare against morphological identifications and microsatellite results. For the microsatellite regions, we initially attempted to amplify the ten markers that were reported by Saltonstall (2003a, b, using the same primers reported in that study. When necessary, we substituted primers that were developed in a subsequent study (Meyerson et al. 2009). Forward primers were modified by appending an oligonucleotide ‘tail’ that served to incorporate fluorescently labeled oligonucleotides using the M13 (FAM fluorophore) or M13A (HEX fluorophore) sequence (Culley et al. 2013). After initial primer screening, we were able to use eight primer sets to collect data (see “Results”).

Polymerase chain reaction (PCR) was conducted separately with each primer set on each sample. Each 10 µl reaction contained 10 ng DNA, 1 µl buffer, 0.15 mM dNTP,

64 nM tailed forward primer, 200 nM fluorescent tail oligonucleotide, 200 nM reverse primer, and 0.25 units Titanium® *Taq* (Takara Bio Inc., Kusatsu, Shiga, Japan) or AmpliTaq Gold™ polymerase (Thermo Fisher Scientific). PCR reactions were carried out using a Bio-Rad S1000 Thermal Cycler using the following program: 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s, 55 °C/58° 60 °C (depending on the primer used) for 30 s, 68 °C for 30 s (microsatellites) or 60 s (plastid DNA), with a final extension phase at 68 °C for 3 min. Plastid DNA amplicons were cleaned using the ExoSAP-IT enzyme mix (Thermo Fisher Scientific), then Sanger sequenced (Sanger et al. 1977) using the BigDye® v3.1 reaction mix, with reactions run on an ABI 3730xl DNA Analyzer (Applied Biosystems).

Microsatellite reactions were randomized into positions on 96-well plates to increase our ability to detect cross-contamination. In addition, two out of every 16 PCR reactions used deionized water (negative control) and a *Phragmites* DNA standard (positive control), respectively, for their templates. A subset of samples from PCR reactions were run on a 1% agarose gel to confirm amplification. The amplified samples were each diluted 1:10 in water, then 1.0 µl of the dilution was combined with 16 µl of Hi-Di™ formamide (Thermo Fisher Scientific) and 0.08 µl of GeneScan™ 400HD ROX™ or 500 LIZ™ (Thermo Fisher Scientific) size standard in preparation for microsatellite analysis. Fragments were analyzed on an ABI 3730xl DNA Analyzer (Applied Biosystems). Fragment sequences for at least one individual of each subspecies were verified against published sequences for each of the microsatellite markers using Sanger sequencing as described above.

Fragment sizes were scored in Geneious® ver. 6.1.8 (Biomatters Ltd.) or the package *Fragman* ver. 1.0.9 (Covarrubias-Pazaran et al. 2016) in the R statistical environment (R Development Core Team 2019) and exported as allele data for further analysis. *Phragmites* individuals of both subspecies in northern North America are predominantly tetraploid (Clevering and Lissner 1999; Lambertini et al. 2006), and the presumed tetraploidy of samples in our study prevented effective analyses of heterozygosity and allele transmission (Dufresne et al. 2014). Instead, we evaluated the presence/absence of codominant allelic markers (with presence indicating at least one copy of the allele in question) and recorded the number of alleles (i.e., allelic richness) found in each locality. No more than two alleles were recovered per individual, so the allele data were analyzed like diploid genotypes, as has been done previously for *Phragmites* (Saltonstall 2003a, b).

Potential clonal genotypes were removed using the *clone-correct* function of the *poppr* package ver. 2.8.6 in R (Kamvar et al. 2014), with individuals stratified by locality (i.e., only one individual of each distinct multilocus genotype was retained for each locality). Next, individuals were binned

into six cluster groups using the *find.clusters* function of the *adeget* package ver. 2.1.3 in R (Jombart 2008; Jombart et al. 2010). Clone-corrected genotypes were also evaluated using principal components analysis (PCA) via the *dudi.pca* function in the R package *ade4* ver. 1.7.15 (Thioulouse et al. 1997; Dray and Dufour 2007). Correlations were tested using a linear regression analysis (*lm* function), and differences were evaluated using a Welch t-test (*t.test* function; Welch 1947), both in R. When investigating the proportion of presumed clones (i.e., individuals with identical multi-locus genotypes to other individuals at the same locality), localities with  $n = 1$  sampled individual were excluded.

Individuals also were clustered using the *snmf* function in the R package *LEA* (Frichot and François 2015), which assigns individuals to one or more hypothetical ancestral populations using sparse non-negative matrix factorization, similarly to the method used by the program STRU CTURE (Pritchard et al. 2000; Falush et al. 2003). After examining a range of ancestral population values from 1 to 10, the entropy criterion showed a substantial decrease at  $K = 2$  populations, followed by gradual decreases thereafter. Because we were using the analysis primarily to evaluate the distinctness of the two *Phragmites* subspecies, we ran the final analysis with  $K = 2$  populations, with ten repetitions, using the repetition with the lowest cross-entropy value.

## Results

A total of 202 *P. australis* subsp. *americanus* and 346 *P. australis* subsp. *australis* individuals were tested, across 29 counties in Wisconsin and adjacent states (Supplementary Table 1; Fig. 1). DNA sequences for the *trnT-trnL* spacer and *trnL* intron were obtained for six individuals of the introduced subspecies and eight individuals of the native subspecies (GenBank accession numbers MT316423–MT316436). These were identical, respectively, to previously published sequences for each subspecies (Saltonstall 2003a, b). Initial morphological identifications were consistent, in every case, with DNA sequence and microsatellite data.

Of the ten microsatellite primer sets that were attempted, six (*PaGT04*, *PaGT08*, *PaGT11*, *PaGT13*, *PaGT14*, and *PaGT16*) were amplified successfully using the original primer sequences (Saltonstall 2003a, b), and an additional two (*PaGT09* and *PaGT12*) were amplified using modified primers (Meyerson et al. 2009). Nucleotide sequences for each microsatellite marker (i.e., the GT dinucleotide repeat and adjacent sequence) were identical to previously published sequences for each respective subspecies (GenBank accession numbers MT316437–MT316457; Saltonstall 2003b). Two primers (*PaGT21* and *PaGT22*; Saltonstall, 2003b) failed to amplify or failed to produce amplification products that were comparable to those previously reported,

and thus were excluded from further analysis. The ‘tailed’ primer method (Culley et al. 2013) successfully produced PCR products that could be detected by fragment analysis, and the fragments amplified using this method were determined to be 16–18 bp longer than the corresponding alleles from prior studies (Saltonstall 2003a, b; Meyerson et al. 2009; Table 1). Meyerson et al. (2009) were determined to be 20 bp longer (*PaGT09*) and 185 bp longer (*PaGT12*) than those reported by Saltonstall (2003a, b) for the same markers. Missing microsatellite data amounted to 18.5% for *P. australis* subsp. *americanus* (range 5.4–42.9% per marker) and 10.0% for *P. australis* subsp. *australis* (range 4.3–25.1%).

Alleles for both *Phragmites* subspecies in Wisconsin were recovered largely at similar frequencies to what Saltonstall (2003a, b) reported previously (Table 1). In most cases the most abundant allele for a subspecies was the same as what had been observed previously. A notable exception was the *PaGT12* marker for *P. australis* subsp. *americanus*, which included majority alleles 394 and 396 that were not prominent in other studies. Individual marker variation and geographic distributions of allele data are provided as supplementary data. The number of sampled plants per native locality ranged from 1 to 16 ( $\bar{x} = 5.46$ ), exhibiting 1–6 ( $\bar{x} = 1.78$ ) distinct genotypes (Supplementary Table 1). In the introduced subspecies, 1–21 ( $\bar{x} = 10.18$ ) plants were sampled per locality, and 1–15 ( $\bar{x} = 5.21$ ) distinct genotypes were recovered from each locality. Overall, 136 of 202 native individuals (67%) were potentially clones of other individuals collected at the same locality, and for introduced individuals this number was 169 of 346 (49%). Larger stands (i.e., with more individuals sampled) did not have significantly smaller or larger proportions of presumed clones ( $R^2 = 0.044$ ,  $F(1,64) = 2.976$ ,  $p = .089$ ). However, the mean proportion of presumed clones per locality was significantly larger for the native subspecies ( $\bar{x} = 0.86$ ) than it was for the introduced subspecies ( $\bar{x} = 0.58$ ) ( $t(52.11) = 4.801$ ,  $p < 10^{-4}$ ).

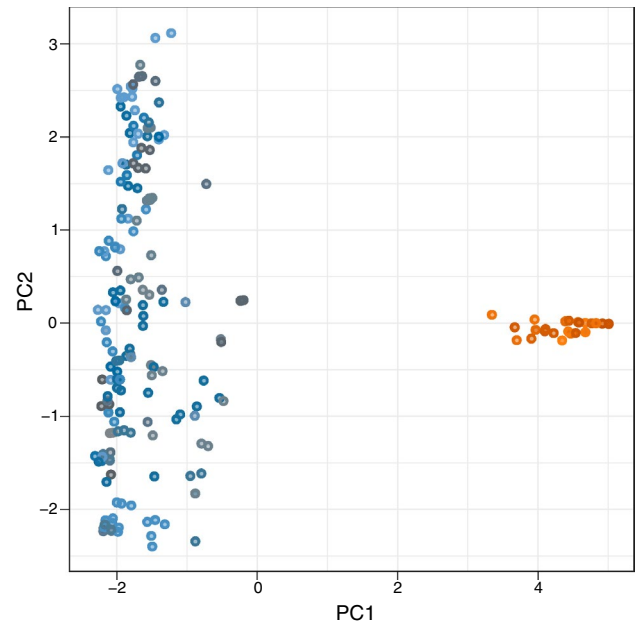
We found the overall genetic diversity to be substantially greater in introduced *Phragmites* plants than in native plants. Sampled native plants had a mean of 2.0 alleles per marker, whereas this value was 3.5 for introduced plants. In comparison, Saltonstall (2003a, b) reported means of 5.9 alleles per marker for the native subspecies and 6.5 for the introduced subspecies, in a study across the native and introduced ranges of *P. australis*. Moreover, two microsatellite markers (*PaGT04*, *PaGT08*) were entirely homozygous in all native stands studied, and another four markers (*PaGT09*, *PaGT11*, *PaGT13*, *PaGT14*) were homozygous in over 90% of native individuals (Table 1). Detailed locality information for genotypes was not available from several other studies, so we could not directly compare our data to another study of geographically proximate populations. Individuals of the native subspecies were assigned via *find.clusters* to cluster



**Table 1** Microsatellite allele frequencies in native and introduced *P. australis* populations. Up to two alleles were recovered from any one individual. Dashes (–) indicate alleles that were not found in any individuals of a given subspecies

Locus	Allele size (This study)	Equivalent allele size (Saltonstall 2003a, b)	Frequency	
			Native	Introduced
<i>PaGT04</i>	284	266	1.000	–
	292	274	–	0.629
	294	276	–	0.738
	302	284	–	0.017
<i>PaGT08</i>	192	176	–	0.955
	194	178	1.000	0.148
<i>PaGT09</i>	228	190	–	0.019
	236	198	–	0.799
	240	202	–	0.201
	244	206	–	0.019
	246	208	–	0.116
	248	210	0.993	–
	250	212	0.028	–
<i>PaGT11</i>	159	142	0.077	0.985
	161	145	0.994	–
	163	147	–	0.785
	165	149	–	0.066
<i>PaGT12</i>	377	174	–	0.066
	379	176	0.112	0.837
	381	178	–	0.085
	383	180	–	0.301
	387	184	–	0.097
	394	191	0.759	–
	396	193	0.259	–
<i>PaGT13</i>	398	195	0.069	–
	226	208	0.040	0.976
	228	210	–	0.282
	236	218	1.000	–
<i>PaGT14</i>	198	181	0.995	–
	200	183	–	0.362
	202	185	0.005	0.006
	204	187	–	0.588
	207	189	–	0.500
	<i>PaGT16</i>	271	255	–
277		261	–	0.731
282		265	1.000	–
307		290	–	0.453
311		294	0.129	–
313		296	–	0.262

groups ‘A’ ( $n = 123$ ) and ‘E’ ( $n = 79$ ), whereas individuals of the introduced subspecies were assigned to clusters ‘B’ ( $n = 95$ ), ‘C’ ( $n = 104$ ), ‘D’ ( $n = 81$ ), and ‘F’ ( $n = 66$ ) (Fig. 1). Each cluster group contained individuals of only one subspecies. The principal components analysis produced two

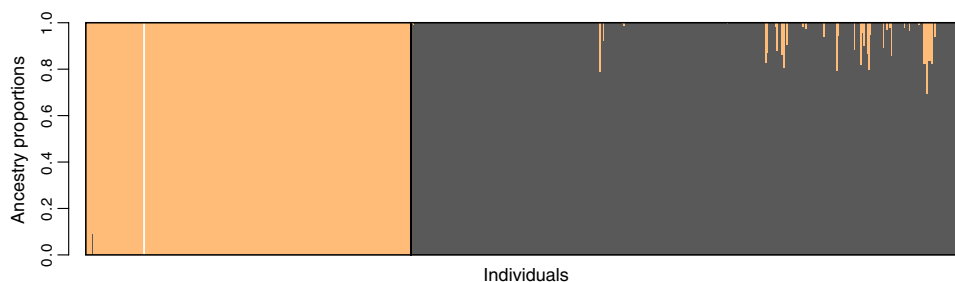


**Fig. 2** Principal components analysis of clone-corrected data for 176 introduced and 66 native *Phragmites australis* individuals, derived using genotype data from eight microsatellite loci. PC1 explained 54% of the variation in the data and PC2 explained 11% of the variation in the data. Points are colored according to subspecies, with the introduced subspecies in shades of gray and blue (left side of image), and the native subspecies in shades of orange (right side of image)

groups, differentiated along the PC1 axis and corresponding to the two subspecies, with no overlap between subspecies groups and no points assigned to an intermediate position (Fig. 2). The *snmf* analysis cleanly divided individuals into two groups according to their subspecies (Fig. 3). Only 20 individuals (all of the introduced subspecies) were assigned to a group with less than 0.90 ancestry proportion.

## Discussion

Introduced *Phragmites* plants in Wisconsin exhibit higher genetic diversity than plants of the native subspecies, consistent with the higher diversity that was found for this subspecies across North America (Saltonstall 2003a, b). Introduced taxa typically undergo a population bottleneck as they migrate to their adventive ranges, because few individuals are introduced and the starting populations are small with substantial inbreeding (Dlugosch and Parker 2008). However, higher genetic diversity could be achieved in a sexually reproducing taxon that was introduced as a large number of genetically diverse individuals, either through a large initial introduction or repeated introductions from a diverse source population (Roman and Darling 2007; Estoup et al. 2016). Saltonstall (2002) reported that introduced *Phragmites* plants in northern North America share the same plastid



**Fig. 3** Plot of ancestry proportions (i.e., assignment of individuals to one of two hypothetical populations) for all sampled individuals. Individuals are grouped by subspecies, with native individuals at the left-hand side and introduced individuals at the right-hand side. Each

individual corresponds to a vertical bar, with colors indicating the proportion of ancestry estimated to be native (orange) or introduced (gray)

haplotype, also found in plants throughout the *P. australis* subsp. *australis* native range. The microsatellite diversity of the introduced subspecies in North America is substantial and suggests that these plants can draw from a broad stock of genetic material as they adapt to novel environments.

In general, a long-established native taxon would be expected to harbor greater variation as a result of accumulating genetic mutations over time and maintaining them through interbreeding among relatively large metapopulations (e.g., Tsai and Manos 2010; Roberts and Hamann 2015). However, Wisconsin habitats are predominantly postglacial, and native *Phragmites* populations would have recolonized most of the state in relatively recent evolutionary time (Curtis 1959). Recolonizing postglacial populations typically exhibit lower genetic diversity than would be found in longer-established populations, resulting from founder effects in relatively recent time (e.g., Comps et al. 2001; Cheddadi et al. 2006). One point of comparison for Wisconsin may be the Driftless region, an area that was not glaciated during the most recent Ice Age (Clayton et al. 2006). In our study, however, native *Phragmites* plants from the Driftless region (i.e., localities 45–47 and 50) had comparable genotypes and were assigned to the same cluster groups as plants in other parts of Wisconsin (Fig. 1; Supplementary Table 1).

*Phragmites* plants are capable of extensive clonal growth using underground rhizomes, and this mechanism of reproduction would be expected to generate large patches of plants that are genetically uniform. We attempted to avoid gathering multiple data points from the same clone by sampling plants that were separated by at least 10 m, however this method could not guarantee that genetically distinct plants (i.e., genets) were sampled. Using the microsatellite marker data, we can estimate the degree to which plants are clonal, by identifying unique genetic signatures, i.e., allelic combinations not found in other plants from the same population. We found a wide range of genetically distinct individuals across the sampled localities, with the introduced subspecies

generally having a higher proportion of genetically distinct individuals (Supplementary Table 1). Sampling a larger number of plants in a stand allows for the most thorough estimation of genetic diversity, and we recovered up to 15 genetically distinct individuals in stands of the introduced subspecies. In contrast, all but two stands of the native subspecies had three or fewer distinct genotypes. A comparable study of *Phragmites* plants in Utah reported several native populations with greater than ten distinct genotypes, and in the introduced subspecies most populations had 15–20 distinct genotypes (Kettenring and Mock 2012). The contrast between the two subspecies also reflects the noted difference in their allelic diversity, because a lower diversity of alleles would limit the number of possible multilocus genotypes.

We detected five alleles (*PaGT08*: 194, *PaGT11*: 159, *PaGT12*: 379, *PaGT13*: 226, and *PaGT14*: 202) that were present in both the native and introduced *Phragmites* subspecies (Table 1), but only two of these were arranged in identical genotypes in both subspecies (*PaGT08*: 194/194, *PaGT13*: 226/226). All but two such alleles (*PaGT11*: 159 and *PaGT14*: 202) were found previously in both subspecies (Saltonstall 2003a, b; Table 1). All individuals that had less than 0.90 ancestry proportion in the *snmf* analysis (Fig. 3) also possessed one or more of the alleles and single-locus genotypes that occur in both subspecies. Ambiguous ancestry assignment may reflect intersubspecific hybridization (e.g., Wu et al. 2015); however, in our study the fact that ambiguously assigned individuals all possessed alleles that were not specific to one subspecies decreases the likelihood of this possibility. Moreover, the genotypes of ambiguously assigned individuals at three markers (*PaGT09*, *PaGT14*, *PaGT16*) consisted entirely of alleles that otherwise are found only in the introduced subspecies.

Intersubspecific hybridization has been demonstrated several times in *Phragmites* using principal components analysis, which depicts hybrids as occupying intermediate positions between otherwise differentiated subspecies (Paul et al. 2010; Saltonstall et al. 2014, 2016). There were

no individuals in our study group that showed patterns that we would interpret as evidence for interspecific hybridization (Fig. 2). Hybridization has been documented in North American *Phragmites* using morphological data (Williams et al. 2019) and microsatellite markers (Paul et al. 2010; Saltonstall et al. 2014, 2016; Wu et al. 2015). Additionally, some hybrid plants also exhibit heteroplasmy that manifests as sequence and length variation in plastid loci (Lambertini 2016). We observed no patterns in Wisconsin *Phragmites* that resembled previously reported genetic data for *Phragmites* intersubspecific hybrids.

In conclusion, the most substantial results of our study were that (1) we found no evidence for hybridization between native and introduced *Phragmites* and (2) genetic variation was substantially greater in introduced plants than in native plants. *Phragmites* plants are capable of both sexual and asexual reproduction, and it will be important for land managers to guard against the potential for hybridization between subspecies. Even without hybridization, the continued sexual reproduction of introduced plants represents a persistent source of genetically variable propagules that may establish as novel aggressive variants of the subspecies. Introduced *Phragmites* plants have persisted in North America for over 100 years, and they have continued to expand their range over that time (Saltonstall 2002). It will be important to remain vigilant to prevent the establishment of new introduced populations in Wisconsin. To expand upon the results we obtained, it would be worthwhile to investigate genetic patterns in *Phragmites* populations over a greater geographic area, potentially to uncover evidence for multiple introductions or ecological specialization. In addition, it would be valuable to increase sampling throughout the state, in order to construct a more complete picture of the genetic diversity in native and introduced *Phragmites*. Collecting more data on the current spread and genetic diversity of *Phragmites* also could provide a useful benchmark for future studies, in the event that introduced *Phragmites* plants continue to expand their range.

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