Diversity and Distributions, (Diversity Distrib.) (2016) 22, 1301–1313

BIODIVERSITY RESEARCH

The influence of habitat disturbance on genetic structure and reproductive strategies within stands of native and non-native *Phragmites australis* (common reed)

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ABSTRACT

Aim A surprising finding of genetic studies of *Phragmites australis* is that native populations, whether in Europe or North America, are characterized by lower genetic diversity than non-native populations. What is not clear is whether higher diversity within invasive stands results from disturbance facilitating sexual reproduction or whether individuals with higher diversity have greater invasion potential.

Location Upper Midwest, United States, North America.

Methods To answer this question, we investigated genetic structure of native and non-native *P. australis* stands at multiple spatial scales and under differing levels of anthropogenic disturbance. Our goals were to assess the influence of habitat disturbance on genetic structure and determine whether patterns differed in native versus non-native stands. We also screened for hybrid genotypes, which have not been reported from this region.

Results When controlling for disturbance, native and non-native *P. australis* exhibited relatively similar within-site genetic diversity and degrees of clonal growth, suggesting that their spread is comparably reliant on clonal versus sexual dispersal. In all cases, discrete clumps of *P. australis* were composed of multiple, closely related genotypes, indicating seed heads as the key dispersal units. The genetic structure of *P. australis* stands did not differ with disturbance; however, only non-native *P. australis* was found in highly disturbed anthropogenic habitats. Native *P. australis* stands exhibited lower genetic diversity overall, lower sexual reproduction in less disturbed sites and greater isolation by distance and differentiation among sites, which is consistent with its longer residence time in this region. We found no evidence of hybrid genotypes, despite native and non-native stands sometimes co-occurring.

Main conclusions Our results suggest that invasion by non-native *P. australis* is sporadic, associated with disturbance and involves closely related individuals, likely derived from a single seed head. The ability of the non-native lineage to exploit a variety of anthropogenic habitats helps to explain its invasiveness.

Keywords
clonal growth, dispersal, disturbance, invasion, microsatellite, wetland.

INTRODUCTION

Phragmites australis (Cav.) Trin. ex Steud. (common reed) is a tall, perennial wetland grass found on every continent except Antarctica. *Phragmites australis* was historically a minor component of North American wetlands, and native populations in both North America and Europe have experienced declines (van der Putten, 1997; Saltonstall, 2011).

DOI: 10.1111/ddi.12492
http://wileyonlinelibrary.com/journal/ddi

A Journal of Conservation Biogeography
Diversity and Distributions

However, there have been multiple introductions to North America of European genotypes, which have expanded rapidly throughout the continent (Saltonstall, 2002; Lambertini *et al.*, 2012a; Meyerson & Cronin, 2013). Impacts of non-native *P. australis* in North American wetlands include changes to habitat structure and food webs, reduced plant diversity, altered ecosystem functioning and decreased support for invertebrates, fishes and birds (Benoit & Askins, 1999; Chambers *et al.*, 1999; Windham & Lathrop, 1999; Able & Hagan, 2000; Gratton & Denno, 2006).

Global studies of *P. australis* genetics have identified five major geographic lineages: European, Mediterranean and African, Asian and Australian, North American, and South American (Saltonstall, 2003a; Hauber *et al.*, 2011; Lambertini *et al.*, 2012b). Non-native *P. australis* invading the northern United States and Canada (haplotype M) is European in origin and appears to have arrived from the United Kingdom (Saltonstall, 2002; Plut *et al.*, 2011). Hybridization between geographic lineages has been verified in a greenhouse experiment (Meyerson *et al.*, 2010), and spontaneous hybrids have been found in the field (Chu *et al.*, 2011; Lambertini *et al.*, 2012a). Nonetheless, hybridization between European and North American strains is uncommon (Paul *et al.*, 2010; Hauber *et al.*, 2011; Kettenring & Mock, 2012; Saltonstall *et al.*, 2014; Wu *et al.*, 2015), despite these genotypes frequently co-occurring (Meyerson *et al.*, 2010; Taddeo & De Blois, 2012). Based on molecular and morphological data, the North American lineage is now recognized as a distinct subspecies, *P. australis* ss *P. australis* (Saltonstall *et al.*, 2004).

It had long been thought that *P. australis* spread was via asexual propagules being carried along waterways, rivers and ditches (Clevering & Lissner, 1999). However, recent molecular studies indicate that colonization of new sites by the invasive haplotype is driven by sexual reproduction (seed dispersal) (Belzile *et al.*, 2010; McCormick *et al.*, 2010a,b; Hauber *et al.*, 2011; Kettenring & Mock, 2012; Hurry *et al.*, 2013). Observed relationships between genetic and geographic distance in *P. australis* are weak (Guo *et al.*, 2003; Lambertini *et al.*, 2008; Fer & Hroudova, 2009; McCormick *et al.*, 2010b; Plut *et al.*, 2011), suggesting that colonization may be sporadic rather than following a steady progression. This pattern has also been reported for other aquatic plant species Les, 1991; Barrett *et al.*, 1993; Santamaria, 2002).

Recent studies show that, while seed recruitment may be the predominant means by which new areas are colonized, seedling survival can be very low (Albert *et al.*, 2015). Consequently, it is thought that once a new site is colonized, formation of large, dense stands is through asexual expansion (Alvarez *et al.*, 2005; Albert *et al.*, 2015). This is supported by studies of European populations, where clonal growth strongly influences the composition of sites and of individual stands within sites, with some very large stands comprising a single clone (Neuhaus *et al.*, 1993; Koppitz *et al.*, 1997; Kuhl *et al.*, 1999; Curn *et al.*, 2007; Křiváčková-Suchá *et al.*, 2007; Lambertini *et al.*, 2008). This dynamic has also been observed in the North American subspecies (Kettenring & Mock, 2012). Thus native populations of *P. australis*, whether European or North American, are characterized by clonal dominance and low genetic diversity.

In contrast, where the European haplotype has invaded North America, high genetic diversity is observed at fine spatial scales, with discrete stands that may outwardly appear to be a single clone often comprising multiple genotypes (Keller, 2000; Kettenring *et al.*, 2010; McCormick *et al.*, 2010b; Kettenring & Mock, 2012). This higher genetic diversity in non-native stands could arise from temporal dynamics. Over time, intraspecific competition and drift can lead to loss of genetic diversity and greater differentiation among subpopulations, especially as less fit genotypes are displaced by superior competitors (Curn *et al.*, 2007; Křiváčková-Suchá *et al.*, 2007). Such a process would be centuries further along in native than in invading populations. Alternatively, habitat characteristics could contribute to differences in genetic structure. Disturbed sites, which are highly susceptible to invasion in general, are likely to have more bare soil available, facilitating establishment by seed and thereby increasing genetic diversity (Zedler & Kercher, 2004; Albert *et al.*, 2015; Kettenring *et al.*, 2015). Higher genetic diversity, in turn, is associated with higher production of viable seed (Kettenring *et al.*, 2011). Thus, there could be a positive feedback wherein disturbance facilitates sexual reproduction, leading to higher genetic diversity, further promoting spread by seed.

We compared landscape genetic structure of native and non-native *P. australis* populations at multiple spatial scales across the greater Chicago/Central USA Plains ecological region of the Midwestern United States (CEC, 1997). The persistence of native *P. australis* and recent, rapid spread of the non-native haplotype in this area (Saltonstall, 2002; Larkin, 2012; Price *et al.*, 2014) makes it an ideal location to investigate differences between expanding, invasive stands and remnant, native stands. In addition, improved understanding of *P. australis* invasion dynamics in this region, where there has been relatively little research, is needed to support prevention and control efforts. Finally, this landscape contains *P. australis* stands that span a broad gradient of disturbance, from relatively intact, natural wetlands to highly disturbed anthropogenic habitats like stormwater retention basins. This enables genetic structure of *P. australis* subpopulations to be investigated in relation to disturbance.

Our genetic analyses addressed three main questions. First, what is the distribution of native versus non-native *P. australis* within the Chicago region? Second, how does population genetic structure and diversity compare between invasive and native populations? And third, does genetic structure differ as a function of disturbance? In addition, because we sampled many stands and native and non-native lineages were often in close proximity, we looked for evidence of hybrid genotypes, which have not been reported from this region.

Given the recent, aggressive spread of non-native *P. australis* across North America, we expected both the invasive

haplotype and native subspecies to be well-represented in the Chicago region. As the invasive haplotype appears to reproduce sexually at higher rates, we hypothesized that genetic diversity would be higher in invasive than in native populations. Because of the relatively recent arrival of non-native *P. australis* in the region, we expected there to be less genetic differentiation among non-native stands than native stands. Finally, as seed recruitment increases with disturbance, we expected genetic diversity to be higher in non-native *P. australis* stands occurring in disturbed areas.

METHODS

Study sites

In spring and summer of 2010 and 2012, we visited Lake Michigan coastal and interior wetlands in Cook, DuPage, Kane and McHenry counties in Illinois and in Lake and Porter counties in Indiana to identify stands of *P. australis* – both through consultation with wetland managers and opportunistically as we drove through the area. We defined a 'site' as a single wetland, water body, ditch or other area of contiguous habitat in which *P. australis* was found. If the distribution of *P. australis* was patchy within a site, we further divided the site into multiple stands, that is disjunct, discrete areas of *P. australis* culms. There were also cases where multiple, distinct sites occurred within larger reserves, for example county forest preserves. For each stand, we recorded geographic coordinates using a handheld GPS and haphazardly sampled *P. australis* leaves from different ramets scattered throughout its extent (n = 3–12, depending on stand area). Each site was characterized as belonging to one of three disturbance categories: highly disturbed areas such as roadsides, drainage ditches or detention basins ('anthropogenic', n = 12); degraded natural wetlands ('disturbed', n = 11); and higher quality reference wetlands ('reference', n = 13). The latter two categories were designated in consultation with site managers. In total, we sampled 511 ramets from 94 stands found within 36 sites across 26 reserves (Table 1).

Molecular markers

Leaves were stored in a freezer at −20 °C until DNA could be extracted. DNA was extracted using Qiagen DNeasy Kits (Qiagen Inc., Valencia, CA, USA). Two samples were selected from each stand to test for native and non-native chloroplast DNA using the PCR-RFLP technique for *P. australis* (Saltonstall, 2003b). Nine microsatellite regions were amplified from leaf tissue with primers developed by Saltonstall (2003a) and Meyerson *et al.* (2010). Forward primers were modified using an M13 extension (CACGACGTTGTAAAACGAC), which matched the sequence of the fluorescently tagged label (WellRed D2, D3 or D4, Sigma-Proligo, St. Louis, MO, USA). PCRs were conducted in two phases, starting with a 10-μL reaction mixture containing 15 ng template DNA, 0.2 μM of reverse and modified forward primer and 10 μL PCR MasterMix 2x (Promega, Madison, WI, USA: 50 units mL^{-1} Taq DNA polymerase in a proprietary reaction buffer, pH 8.5, 400 μM of each dNTP, and 3 mM $MgCl_2$). The PCR was run at 94 °C for 3 min, then 13 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 1 min and final extension of 72 °C for 10 min. After PCR was completed, 5 μL of label mix (0.2 μM of labelled primer, 5 μL PCR MasterMix 2x) was added to each reaction and then returned to the PCR machine and run at 94 °C for 3 min, then 27 times at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and 72 °C for 10 min. Genotypes were scored using a CEQ 8000 Genetic Analysis System and CEQ FRAGMENT ANALYSIS software (Beckman Coulter, Fullerton, CA, USA).

Data analysis

We identified clones using the ALLELEMATCH package in R 3.1.2 (Galpern *et al.*, 2012; R Development Core Team, 2014). Allelematch identifies similarities between samples using a metric based on Hamming distance (Hamming, 1950), while allowing for potential misidentification associated with modest scoring error and missing information at some loci. As this matching requires diploid data, primers that produced > 3 peaks were excluded. Putative clones were then re-examined manually with polyploid markers included to determine whether additional unique genotypes could be identified. Clones considered unique based on missing data at more than two loci, or because of variability at a single microsatellite marker (two alleles), were considered to have limited support and were reassigned to the most similar clone. This approach errs on the side of estimating a lower number of unique clones and higher degree of clonal spread. Identified clones were assigned as native or non-native based on PCR-RFLP (Saltonstall, 2003b).

Clonal diversity was calculated as the proportion of individuals representing new multilocus genotypes (MLG) as described in Arnaud-Haond *et al.* (2005): $(G - 1)/(N - 1)$, where G is the number of MLG and N is the sample size. This value is the probability of sampling a different genet with each newly sampled ramet (Brzosko *et al.* 2002), with lower values indicating a higher proportion of clonal reproduction (Pielou 1969; Diggle *et al.* 1998; Fant *et al.* 2008). KINGROUP (Konovalov *et al.* 2004) was used to determine the likelihood that clones were related ('half sibs'), and therefore more likely the result of seed produced from a single colonization event, or unrelated and arising from multiple colonization events. Generalized linear models were performed in R to test for differences in measures of diversity (alleles per loci, MLG per stand, genet-to-ramet ratio, number of sib families) based on site, lineage and disturbance categories.

The Bayesian clustering analysis software STRUCTURE v2.2 (Pritchard *et al.*, 2000; Falush *et al.*, 2007) was used to visualize genotypic subdivision among clones (number of genetic

Table 1 *Phragmites australis* sites and stands sampled and their genetic diversity.

Location* (Code)	No. stands	No. samples	Lineage	$K = 1$† (%)	$K = 2$† (%)	No. MLG	Purely clonal stands (%)‡	Alleles per loci (mean)§	Clonal ratio (mean)§	No. sib groups (mean)§
Anthropogenic										
Buffalo Grove (BG)	2	12	Non-native	1	99	3	50	2.33	0.10	1.0
Costco ditch (CD)	1	4	Non-native	1	99	3	0	2.56	0.67	1.0
Deerfield (D)	3	15	Non-native	2	98	6	67	3.33	0.25	1.0
Dundee ditch (DD)	1	5	Non-native	6	94	2	0	1.89	0.25	1.0
Eggers Grove (EG)	1	7	Non-native	6	94	7	0	2.33	1.00	2.0
Indian Ridge 1 (IRM)	2	8	Non-native	22	78	8	0	2.60	1.00	2.0
Ind. Dunes 1 (ID)	1	5	Non-native	5	95	3	0	1.75	0.50	1.0
Kraft ditch (KD)	1	5	Non-native	1	99	1	100	1.50	0.00	N/A
NU lakefill (NU)	15	45	Non-native	5	95	11	60	2.56	0.20	1.0
Otter Creek (OC)	2	12	Non-native	1	99	8	0	1.89	0.60	2.0
Poplar Creek (PC)	2	10	Non-native	2	98	2	100	2.00	0.00	N/A
NU waterworks (N)	5	29	Non-native	1	99	13	20	2.89	0.32	1.0
Disturbed										
Indian Ridge 2 (IRM)	3	8	Native	92	8	4	0	1.55	0.83	1.5
Spring Bluff (SB)	1	6	Native	60	40	3	0	1.67	0.40	1.0
Burnidge 1 (B)	1	6	Non-native	57	43	1	100	1.55	0.00	1.0
Calumet Prairie 1 (CP)	3	16	Non-native	3	97	4	67	1.44	0.05	1.0
Indian Ridge 3 (IRM)	3	9	Non-native	23	77	9	25	3.00	1.00	1.3
Ind. Dunes 2 (ID)	2	11	Non-native	1	99	9	0	3.10	0.77	3.0
Lake in Hills (LITH)	4	20	Non-native	1	99	8	25	3.00	0.25	1.0
Long Grove (LG)	2	13	Non-native	3	97	11	0	3.00	0.82	1.5
Miller Meadow (MM)	2	10	Non-native	2	98	7	0	2.67	0.63	1.5
Rutland (R)	1	6	Non-native	1	99	2	0	1.78	0.20	1.0
Willow Pond (WP)	2	9	Non-native	2	98	7	0	2.78	0.75	1.5
Reference										
Burnham 1 (B)	1	4	Native	100	0	1	100	1.00	0.00	N/A
Calumet Prairie 2 (CP)	4	39	Native	91	9	4	25	3.00	0.12	1.0
Dick Young 1 (DY)	1	9	Native	40	60	3	0	2.33	0.25	1.0
Illinois Beach 1 (IB)	7	35	Native	99	1	14	29	2.56	0.25	1.0
Ind. Dunes 3 (ID)	2	10	Native	88	12	7	0	2.22	0.63	1.5
West Chi. Prairie (WCP)	4	28	Native	99	1	9	50	1.44	0.17	1.0
Burnham 2 (BH)	1	4	Non-native	1	99	1	100	1.44	0.00	N/A
Burnidge 2 (B)	1	9	Non-native	15	85	3	0	1.88	0.22	1.0
Dick Young 2 (DY)	3	28	Non-native	9	91	12	0	3.89	0.38	1.7
Illinois Beach 2 (IB)	5	48	Non-native	6	94	13	40	4.11	0.24	1.3
Ind. Dunes 4 (ID)	2	9	Non-native	4	96	7	0	2.70	0.75	1.0
Pratts Wayne (PW)	2	12	Non-native	1	99	3	50	1.67	0.18	1.0
Warrenville Gr. (WG)	1	5	Non-native	1	100	2	0	1.56	0.25	1.0

*Multiple sites within a larger reserve are indicated by sequential numbers. A total of 39 sites were sampled across 26 reserves.
†Probability of belonging in respective STRUCTURE clusters.
‡Stands with only a single genotype.
§Summary statistics at site level.

clusters, K). The data were first converted to binary presence–absence data by alleles. STRUCTURE tests for the presence of genetic groupings, without a priori assignment, by systematically grouping individuals into incrementally increasing clusters (K), and identifies the groupings with the least possible disequilibrium using a Markov Chain Monte Carlo method. We carried out 20 independent runs per K using a burn-in of 10^5 and collected data for 10^5 iterations for $K = 1$ through $K = 30$. The minimum value of K that can best explain the data was determined using the rate of change in the log-likelihood probability of data between corresponding K values (ΔK) (Evanno *et al.*, 2005). This identifies the uppermost level of genetic structure, corresponding to taxa delineated by Saltonstall *et al.* (2004). We then repeated the analysis in a hierarchical manner as described in Coulon *et al.* (2008) to identify potential structure within these taxa. To examine the distribution of genetic diversity within and among populations, analysis of molecular variance (AMOVA) was performed in GENALEX (Peakall and Smouse 2006). This was repeated using the groupings identified by

STRUCTURE analysis, allowing us to compare distribution of genetic diversity among taxa, sites and stands within sites.

We also used spatial genetic and phylogenetic analyses to examine landscape genetic structure. We constructed separate genotype phylogenies for native and exotic *P. australis*, with each unique MLG represented as a phylogenetic tip. To do this, microsatellite alleles for each genotype were converted to allelic frequencies and pairwise genetic distances were calculated between genotypes using the 'char2genet' and 'dist.genet' functions, respectively, in the ADE4 package of R (Dray & Dufour, 2007). Genetic distances were calculated using Reynold's distance, which treats gene frequency changes as arising only through genetic drift and does not require assumptions of constant and equal population sizes (Reynolds *et al.*, 1983). Pairwise genetic distances were the basis for phylogeny construction using the 'bionjs' function in the R package APE (Paradis, 2012). A single non-native genotype was used as an outgroup to root the native tree and vice versa. Bootstrap support was evaluated using the 'boot.phylo' function in ape, with 1000 bootstrap replicates.

We used two approaches to test for spatial phylogenetic structure in native and non-native populations. First, we employed the method of Hardy & Senterre (2007) to partition genetic diversity into alpha (within-site) and beta (among-site) components using two metrics: (1) I_{ST} reflects community differentiation among sites based on taxonomic identity (in this case, genotype), in a manner analogous to the population genetic coefficients F_{ST} and G_{ST}. Phylogenetic distance between genotypes (relatedness) is not factored in to this measure. (2) In contrast, Π_{ST} is a measure of community phylogenetic distinctiveness between sites. For both measures, values > 0 would indicate that genotypes occurring within sites are more similar than genotypes occurring across sites. These values were calculated separately for native and non-native *P. australis* using the SPACODIR package in R (Eastman *et al.*, 2011). A permutation test was used to determine whether spatial phylogenetic structure indicated by Π_{ST} was significant relative to a tip-shuffling null model.

This partitioning method tests for differences within and among populations but is not spatially explicit. Next, we tested for isolation by distance using Mantel tests of correlations between spatial and phylogenetic distances among subpopulations. A matrix quantifying pairwise spatial distance among all patches was created with the 'dist' function in R using UTM coordinate data. Pairwise phylogenetic distance among patches was calculated using the 'comdist' function in the R package PICANTE (Kembel *et al.*, 2010). We then performed a two-tailed test for correlations between these spatial and phylogenetic matrices using the 'mantel.test' function in the R package VEGAN (Oksanen *et al.*, 2013). We also repeated this test using a non-phylogenetic approach, with the 'vegdist' function in VEGAN used to calculate pairwise similarity in genotypic composition among patches without accounting for relatedness.

RESULTS

Identification and distribution of genotypes

All microsatellite markers produced a maximum of two peaks, except PaGT11 and PaGt12, which produced three peaks in some non-native MLG. The optimal criterion of dissimilarity identified by allelematch was a mismatch of two alleles; hence, all genotypes that were distinct by greater than two alleles were identified as unique clones. With seven primers, this allows a mismatch of ~14%, providing a modest estimate of the number of unique clones and thus a higher estimate of clonality. Using the seven di-allelic markers, we identified 242 unique MLG from 511 samples. Following re-examination of data from two markers that occasionally produced three alleles and after accounting for missing data or variation attributed to single loci, this was reduced to 232 unique MLG.

Of the 232 MLG identified, 41 were native and 191 were non-native. Of the 26 reserves sampled, two had only native *P. australis*, 17 had only non-native and six had both (Table 1). At the eight sites where native genotypes were found, two were disturbed and six were reference sites; native *P. australis* was not found in any of the anthropogenic sites. The 24 reserves that had non-native genotypes comprised 31 sites, of which 12 were anthropogenic, nine were disturbed and seven were reference sites. In the six reserves that included both lineages, they sometimes grew in close proximity but were never found within the same stand.

Using one representative of each MLG per population, STRUCTURE showed data could best be explained by two groupings ($K = 2$) (Fig. 1). When examining assignment of each MLG, we found that 198 of 232 MLG could be matched with ≥85% confidence to a cluster. Of these, 31 were native and assigned to one cluster and 167 were non-native and assigned to the other cluster. Thus, STRUCTURE was able to correctly classify these genotypes by lineage without any *a priori* assignment. The 34 MLG that did not show strong assignment to a grouping comprised both native ($n = 10$) and non-native ($n = 24$) lineages.

Ambiguous STRUCTURE assignments for 34 of the MLG (well-mixed bands in Fig. 1) could indicate hybrid genotypes; hence, we used the native and non-native allele frequencies reported by Saltonstall (2003a) to look for evidence of F1 genotypes. The 31 native and 169 non-native MLG showing strong assignment to groupings all had allele frequencies very similar to those expected based on Saltonstall (2003a). Those with ambiguous assignments did not exhibit intermediate gene frequency between native and non-native, as would be expected for F1 hybrids. Instead, they had higher frequencies of rarer alleles, which is likely what led to their ambiguous assignment. This was confirmed using the marker PaGT4, which was the only locus to produce alleles that were lineage specific and supported by RFLP-PCR analysis. No individuals were heterozygous at this locus, which would have been indicative of F1 hybrids.

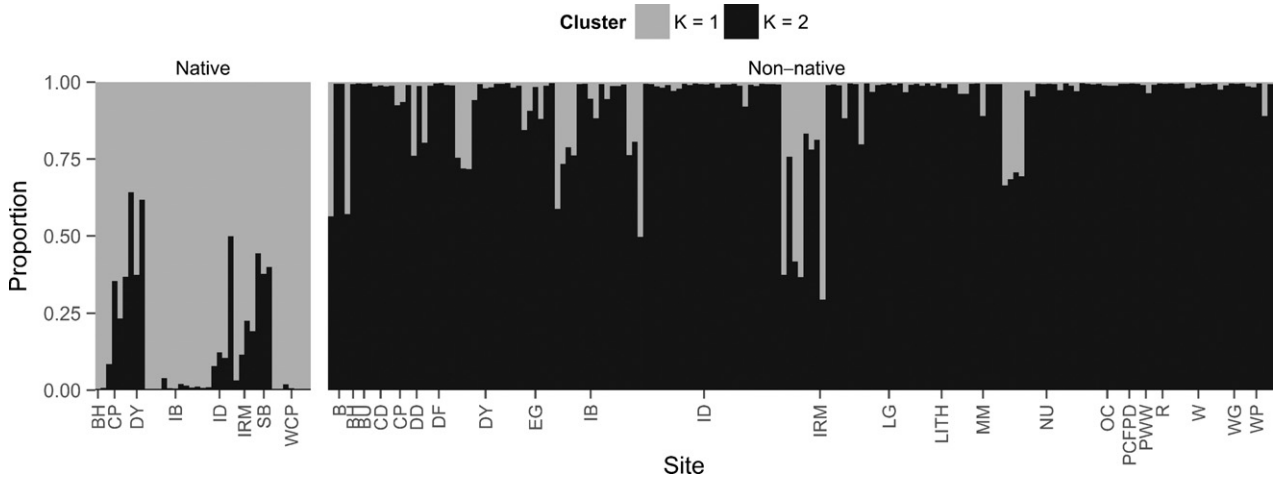

Figure 1 STRUCTURE groupings for native and non-native *P. australis* genotypes. Each vertical band represents a unique MLG (native: $n = 31$, non-native: $n = 167$). Shading indicates strength of assignment to each cluster, with native and non-native samples generally aligned with $K = 1$ and $K = 2$, respectively. See Table 1 for site names.

A majority of MLG were encountered only once, with less than half (99 of 232) represented by more than one ramet. Of the MLG that were identified more than once, 77% occurred within the same stand, 8% were found in different stands within the same site and 15% were found at multiple sites. This pattern was reflected in the distribution of all MLG within a site, as most stands comprised multiple unique MLG. This suggests that most stands arose from sexual recruitment rather than spread of clonal propagules.

Genetic diversity

Despite the relatively high number of MLG per site, the average genetic diversity (alleles per loci) was low (Table 2), compared to region-wide pooled averages of 4.9 for natives MLG and 6.8 for exotic MLG. This suggests that most sites contained a small subset of the total regional genetic diversity. This was supported by AMOVA, which indicated that 44% of genetic diversity occurred within sites and 56% of genetic diversity was found between sites. This indicates large genetic differences between sites and low diversity within sites.

The maximum number of sibling groups found within a site was three (Table 1) and mean values across lineages and site types were < 1.5 (Table 2), indicating that most sites were established by two or fewer independent colonization events. For 15 sites (4 native, 11 non-native), all unique MLG appeared to be full siblings. This suggests that these sites were either colonized by full sibs derived from a single dispersal event (e.g. multiple seeds from a single inflorescence) or sibling descendants from seed produced by an initial colonizer. Even for sites that had more than one sibling group, generally just one MLG could not be assigned as a full sibling.

When data were pooled across sites, there were significantly fewer alleles per loci in the native MLG (4.9 alleles per marker) than the non-native MLG (6.8 alleles per marker) ($F_{1,69} = 14.9$, $P = 0.0003$). However, there were no significant differences between native and non-native genotypes for any other measures of genetic diversity (MLG per stand, maximum number of MLG per stand, per cent of stands with multiple MLG, genet-to-ramet ratio or number of sibling families).

Comparison of genetic parameters between native and non-native stands as a function of disturbance categories could only be performed for disturbed and reference sites. A significant interaction ($F_{1,40} = 5.92$, $P = 0.02$) of lineage and disturbance on alleles per loci indicated that native stands

Table 2 Summary of *P. australis* genetic diversity by site type and lineage.

Site type	Lineage	No. sites	No. stands	No. samples	Total MLG	Mean MLG (±SE)*	Purely clonal stands (%)*	Alleles per loci (mean)*	Clonal ratio (mean)*	No. sib groups (mean)*
Anthropogenic	Non-native	12	36	157	67	5.58 ± 1.12	33	2.30	0.41	1.30
Disturbed	Native	2	4	14	7	3.5 ± 0.5	0	1.61	0.62	1.25
	Non-native	9	20	100	58	6.44 ± 1.13	24	2.48	0.50	1.43
Reference	Native	6	19	125	38	6.33 ± 1.93	34	2.09	0.24	1.10
	Non-native	7	15	115	41	5.86 ± 1.86	27	2.46	0.29	1.17

*Summary statistics at site level.

had higher diversity in reference sites, but non-native stands did not differ by disturbance level. The number of MLG per stand did not significantly differ between disturbed and reference sites ($P = 0.39$) but was higher in non-native than native stands ($P = 0.045$). The proportion of clonal growth was higher in disturbed sites than reference sites ($P = 0.01$), but did not differ by lineage ($P = 0.49$).

Comparisons of anthropogenic sites with other disturbance categories were limited to the non-native haplotype. There were no significant differences in the number of MLG per stand as a function of disturbance category ($P = 0.30$), but there were fewer alleles per loci in anthropogenic sites ($P = 0.01$), and lower clonal diversity in reference sites ($P = 0.01$). In pairwise comparisons of native and non-native stands at the locations in which they co-occurred (Table 1), only alleles per loci significantly differed ($P = 0.01$).

The taxonomic distinction between native and non-native genotypes was supported by STRUCTURE; thus, we performed a hierarchical analysis to test for further genetic differentiation within lineages. For native MLG, the modal minimum value was $K = 4$; for non-native MLG, there were no strong peaks but likelihoods stabilized at $K = 2$. Although the groupings differed by locations, there was no obvious geographic pattern to the groupings. The eight sites with the native subspecies were divided into four genetic groups, two of the groups contained three sites each, with one composed of Burnham, Calumet Prairie and West Chicago Prairie, and the other comprising Indiana Dunes, Indian Ridge Marsh and Spring Bluff. The remaining two genetic groups split into Illinois Beach State Park and Dick Young, with both being identified as unique. The sites with non-native MLG split into two broad groups, with 10 sites predominately aligning with one genetic group (Buffalo Grove, Burnham, Deerfield, Indian Ridge Marsh, Indiana Dunes, Lake in the Hills, Long Grove, Miller Meadow, Popular Creek FPD, Willow Pond) and eight sites aligning predominantly with the other group (Costco Ditch, Dundee Ditch, Northwestern, Otter Creek, Pratts Wayne Woods, Rutland, Warrenville Grove, Waterworks), while five groups were intermediate between the two (Burnidge, Calumet Prairie, Dick Young, Eggers Grove, Illinois Beach State Park). There were no clear relationships between genetic clusters and disturbance categories.

Spatial genetic structure

Native and non-native lineages showed different patterns with respect to spatial genetic differentiation. Based only on composition of microsatellite alleles, native sites were highly differentiated from each other, as were non-native sites, regardless of disturbance category (I_{ST} results, Fig. 2). However, when relatedness was factored in using phylogenetic analysis, native sites showed greater differentiation from each other than non-native sites, although both were more differentiated from each other than predicted under a null model (Π_{ST} results, Fig. 2).

The observed pattern of greater differentiation among native sites and higher sensitivity of phylogenetic tests was reinforced by Mantel tests. Using comparisons based only on composition of alleles, there were significant correlations between genetic and spatial distance for both native and non-native *P. australis* ($P = 0.001$ for both). In contrast, when relatedness was factored in, isolation by distance was highly significant for native *P. australis* ($P = 0.001$) but not for non-native ($P = 0.50$).

DISCUSSION

We analysed genetic structure and diversity of *P. australis* stands distributed throughout the Chicago region, where both native and non-native lineages are well-represented. However, we encountered substantially more non-native than native stands, and only non-native *P. australis* was found in highly disturbed anthropogenic habitats. Native and non-native populations differed in terms of their overall neutral genetic variation. Native stands showed greater isolation by distance and genetic differentiation among sites. However, native and non-native *P. australis* were relatively similar in terms of within-site genetic diversity and degree of clonal growth, suggesting that their spread is comparably reliant on clonal versus sexual dispersal. Despite native and non-native stands often co-occurring at close distances – and increasing reports of hybrid genotypes in the literature (Saltonstall *et al.*, 2014; Wu *et al.*, 2015) – we found no evidence of hybrid *P. australis* in our genetic analyses of 511 ramets.

Distribution

In this study, non-native *P. australis* was more frequently encountered in terms of number of individuals, number of sites and types of habitats. Given that Saltonstall (2002) found no evidence of the non-native lineage within the Midwest prior to 1910, the predominance of non-native *P. australis* within the Chicago region today highlights its ability to rapidly expand following introduction. The ubiquity of non-native *P. australis* in this landscape appears due in part to its ability to colonize novel anthropogenic habitats, such as roadside ditches and detention basins, where the native subspecies was not observed. The non-native lineage was also the predominant form found in disturbed wetlands, reinforcing the role of disturbance in *P. australis* invasion (Silliman & Bertness, 2004; Price *et al.*, 2014; Kettenring *et al.*, 2015) – although the non-native haplotype also showed the ability to colonize relatively high-quality reference wetlands. In contrast, the native subspecies was largely confined to reference wetlands. This dominance by non-native *P. australis* of novel and disturbed habitats – even with native source populations nearby that could potentially compete for those locations – is consistent with non-native *P. australis*' physiological advantages at exploiting resources (Holdredge *et al.*, 2010; Mozdzer *et al.*, 2013).

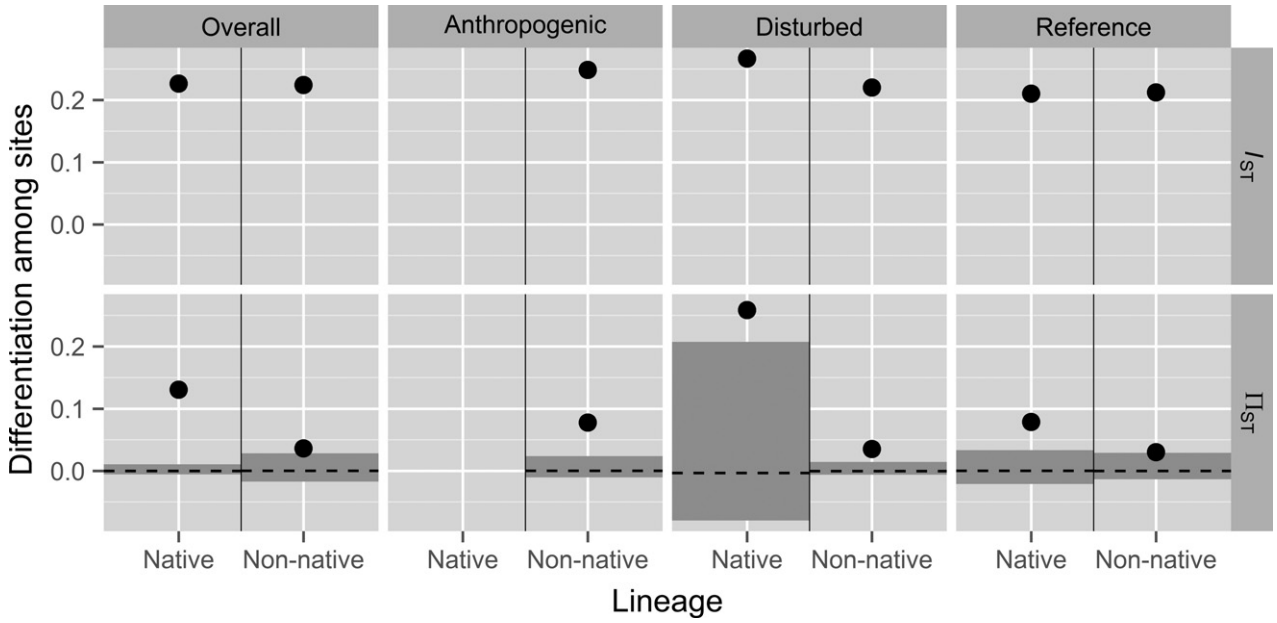

Figure 2 Partitioning of genetic diversity into within- versus among-site components for *P. australis* overall and sites of different disturbance categories. I_{ST} is based on overlap in composition of microsatellite alleles. Black symbols represent observed values, which indicate that genotypes occurring within sites were more similar than genotypes occurring across sites, that is sites were genetically differentiated from each other. Π_{ST} is based on relatedness inferred using a microsatellite phylogeny. Circles indicate observed values, grey bands and dashed lines represent the 95% confidence interval and mean, respectively, of a tip-shuffling null model. In all cases, sites were significantly differentiated from each other, but the magnitude of genetic differentiation among sites was greater in the native lineage.

Genetic structure and diversity

Differences in genetic structure between the lineages mirror their contrasting natural history. Populations of the native subspecies showed strong differentiation, with STRUCTURE identifying many populations as unique (> 95% association with one *K* grouping), indicating that either the sites were colonized by different founders or the sites differentiated over time due to genetic drift. Greater differentiation has also been shown for the European lineage within its native range (Paul *et al.*, 2011). This suggests that differentiation arises over long time periods due to limited gene flow between populations. In contrast, the relative lack of differentiation among non-native populations is consistent with relatively recent and rapid invasion within the Midwest (Saltonstall, 2002; Larkin, 2012). Interestingly, STRUCTURE divided the non-native haplotype into two groupings with limited overlap. This is somewhat surprising given this lineage's short residence time in the Chicago region and may be indicative of introductions from multiple source populations, for example, by different pathways of spread. A similar result was reported for the Mississippi Delta region by Hauber *et al.* (2011): most sites were occupied by only one strain and there was little introgression. In our case, the distribution of the two strains shows no obvious geographic or habitat pattern, suggesting that this phenomenon is stochastic rather than arising from differential adaptation or partitioning of the landscape through competition among genotypes.

The importance of sexual reproduction in plant species that are capable of clonal spread is often underappreciated (Davis *et al.* 1999; Widen *et al.* 1994), as was previously the case for *P. australis* in North America (Kettenring & Whigham, 2009). The paucity of genotypes shared between sites shows that seed dispersal, rather than clonal spread, was the dominant mechanism by which new sites were colonized. High relatedness within sites and high differentiation among sites further suggest that colonization is rare and new seeds are derived from few individuals. The spatial distribution of clones within sites was similar to other clonally reproducing species (Davis *et al.*, 1999; Ellstrand and Roose, 1987; Hamrick and Godt, 1989; Widen *et al.*, 1994; Brzosko *et al.*, 2002; Diggle *et al.*, 1998) , with most stands being unique and typically composed of 2–3 unique genotypes, with few large monomorphic clumps. Our data mirror reports from other regions suggesting that the formation of new stands within a site is predominantly by seed rather than vegetative propagules. By contrast, the limited number of genotypes within stands suggests that subsequent expansion of these stands is likely driven by clonal growth along with limited sexual reproduction (Keller, 2000; Albert *et al.*, 2015).

Role of disturbance

The rapid spread of the non-native haplotype within this landscape could be due to adaptation to disturbance-associated environmental conditions, higher propagule pressure

or a combination of both factors. The rapid expansion of the non-native lineage in the region is likely at least partly due to its ability to exploit novel habitats, increasing opportunities for diffusion across the landscape. Given that non-native *P. australis* is highly abundant along major highways in the Chicago region, its movement and connectivity across the landscape is facilitated by the frequently disturbed habitat that roads provide, which has been identified as a major means of *P. australis* invasion within North America (Lelong *et al.*, 2007).

In addition, disturbance has been identified as an important driver of seed recruitment and genetic diversity in *P. australis* (Lambertini *et al.*, 2008; Kettenring *et al.*, 2010; McCormick *et al.*, 2010a; Tulbure & Johnston, 2010). We found that native and non-native populations growing in comparable habitats comprised similar numbers of clones and magnitudes of clonal growth. This suggests that, although the non-native haplotype may have had higher neutral allelic diversity, it was likely habitat characteristics and stand ages that influenced within-site genetic structure, rather than any innate genetic diversity. This is consistent with studies of European *P. australis* in its native range, where stands in newer habitats have been shown to have higher genetic diversity than those in older habitats (Curn *et al.*, 2007). Hence, we propose that, despite native source populations responding similarly to disturbance and being locally common throughout the region, the predominance of non-native stands likely reflects their greater tolerance of anthropogenic disturbance, higher fecundity and greater landscape connectivity due to the frequency of anthropogenic habitats, rather than absolute differences in genetic diversity per se.

Colonization of sites

An interesting pattern in our data was that, while there were multiple genotypes within stands, these genotypes tended to be genetically similar, suggesting that stands arose from seed produced by a few closely related individuals. Multiple factors could cause this pattern of relatedness, including repeated colonization from the same maternal seed source, initial colonization by multiple siblings, as with an inflorescence being the dispersal unit, or internal reseeding within a stand following an initial colonization event. We cannot distinguish among these mechanisms. However, repeated colonization from single maternal sources seems unlikely to have occurred independently across many sites; consequently, we feel the other two scenarios are more probable. Reseeding within a site from an initial colonization event also seems less probable given temporal limitations. For internal reseeding to drive this pattern would require a seed to germinate, establish and grow to maturity, and then produce viable seed. The resulting seedlings would then need to displace already established plants that are larger and capable of clonal expansion. In contrast, initial colonization by multiple siblings would require only a single colonization event and would be less dependent on outcomes of competition over multiple generations. This would be most likely with an inflorescence (seed head) being the dispersal unit rather than multiple individual seeds. In *P. australis*, inflorescences can persist on plants for long periods of time, before breaking off of senesced stems following disturbances such as wind or mowing (Fig. 3). These seed heads can then disperse via wind or water movement or anthropogenic activity to colonize new locations or germinate in situ (Fig. 3b) (Kettenring *et al.*, 2010).

The abundance of non-native *P. australis* in the Chicago region poses a growing threat to regional wetlands. The non-native haplotype is able to exploit anthropogenic and disturbed habitats, which are a major component of land cover in this highly urbanized region, allowing it to percolate through the landscape (sensu With 2002, Cons Bio: http://www.k-state.edu/withlab/publications/With2002.pdf) and spread to habitats of greater conservation value. As disturbed habitats are often adjacent to high-quality wetlands, rapid management responses to new infestations – even where they occur in roadside ditches or other anthropogenic habitats – are recommended. Our results suggest that invasion of sites by non-native *P. australis* is sporadic, associated with disturbance and involves closely related individuals, likely derived

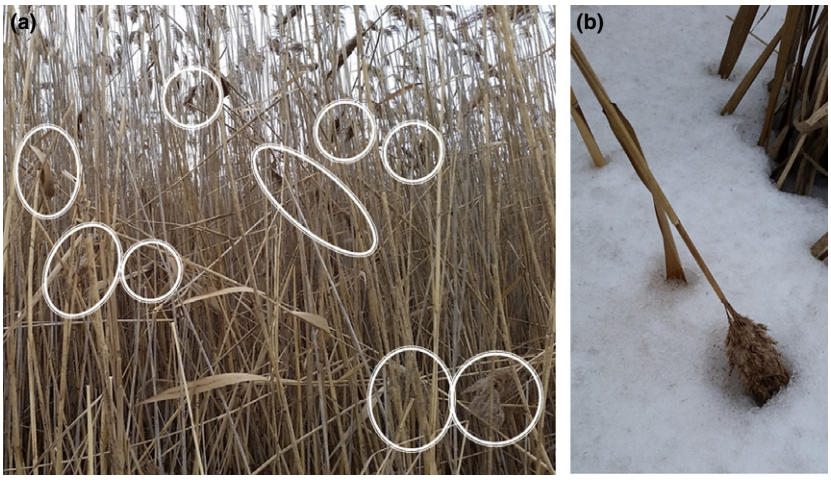

Figure 3 A stand of non-native *P. australis* in winter. (a) Collapsed inflorescences are noted with ellipses. (b) On the edge of this stand, a seed head penetrated into the snow (foreground), which could give rise to new genets expanding the size of the patch, especially as cold-moist stratification increases germination rates in non-native *P. australis* (Kettenring & Whigham, 2009). Colour figure can be viewed at wileyonlinelibrary.com

from single seed heads. Control is more feasible when stands are smaller, and elimination of new stands can help reduce propagule pressure within the landscape. Management actions that focus on eliminating seed heads to limit dispersal, even in cases where the stands themselves cannot be eradicated, may be a practical means of containing further spread.

ACKNOWLEDGEMENTS

This research was supported by funding from Illinois-Indiana Sea Grant and the Graduate Program in Plant Biology and Conservation. For identification of sites and sampling permissions, we thank the City of Chicago Department of the Environment, Forest Preserve District of Cook County, Forest Preserve District of DuPage County, Forest Preserve District of Kane County, Illinois Department of Natural Resources, Illinois Nature Preserves Commission, Indiana Department of Natural Resources, Indiana Dunes National Lakeshore, Lake County Forest Preserve District and the United States Army Corps of Engineers. Adewale Adeoba, Jeb Boyer, Cat Collins, David Ford, Thomas Graan, Clément Kouyoumdjian, Laura Steger and Dara Wise assisted with field sampling and/or genetic analyses. Emily Lewis and two referees provided valuable comments on earlier drafts of this manuscript.

REFERENCES

Able, K.W. & Hagan, S.M. (2000) Effects of common reed (*Phragmites australis*) invasion on marsh surface macrofauna: response of fishes and decapod crustaceans. *Estuaries*, **23**, 633–646.

Albert, A., Brisson, J., Belzile, F., Turgeon, J. & Lavoie, C. (2015) Strategies for a successful plant invasion: the reproduction of *Phragmites australis* in north-eastern North America. *Journal of Ecology*, **103**, 1529–1537.

Alvarez, M.G., Tron, F. & Mauchamp, A. (2005) Sexual versus asexual colonization by *Phragmites australis*: 25-year reed dynamics in a mediterranean marsh, Southern France. *Wetlands*, **25**, 639–647.

Arnaud-Haond, A., Alberto, F., Teixeira, S., Procaccini, G., Serrao, E.A. & Duarte, C.M. (2005) Assessing genetic diversity in clonal organisms: Low diversity or low resolution? Combining power and cost efficiency in selecting material. *Journal of Heredity*, **96**, 434–440.

Barrett, S.C.H., Eckert, C.G. & Husband, B.C. (1993) Evolutionary processes in aquatic plant populations. *Aquatic Botany*, **44**, 105–145.

Belzile, F., Labbe, J., LeBlanc, M.C. & Lavoie, C. (2010) Seeds contribute strongly to the spread of the invasive genotype of the common reed (*Phragmites australis*). *Biological Invasions*, **12**, 2243–2250.

Benoit, L.K. & Askins, R.A. (1999) Impact of the spread of *Phragmites* on the distribution of birds in Connecticut tidal marshes. *Wetlands*, **19**, 194–208.

Brzosko, E., Wroblewska, A. & Ratkiewicz, M. (2002) Spatial genetic structure and clonal diversity of island populations of lady's slipper (*Cypripedium calceolus*) from the Biebrza National Park (northeast Poland). *Molecular Ecology*, **11**, 2499–2509.

CEC (1997) *Ecological Regions of North America: Toward a Common Perspective*. Commission for Environmental Cooperation, Montréal, QC, Canada.

Chambers, R.M., Meyerson, L.A. & Saltonstall, K. (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic Botany*, **64**, 261–273.

Chu, H., Cho, W.K., Jo, Y., Kim, W.I., Rim, Y. & Kim, J.Y. (2011) Identification of natural hybrids in Korean *Phragmites* using haplotype and genotype analyses. *Plant Systematics and Evolution*, **293**, 247–253.

Clevering, O.A. & Lissner, J. (1999) Taxonomy, chromosome numbers, clonal diversity and population dynamics of *Phragmites australis*. *Aquatic Botany*, **64**, 185–208.

Coulon, A., Fitzpatrick, J.W., Bowman, R., Stith, B.M., Makarewich, C.A., Stenzler, L.M. & Lovette, I.J. (2008) Congruent population structure inferred from dispersal behavior and intensive genetic surveys of the threatened Florida scrub-jay (*Aphelocoma coerulescens*). *Molecular Ecology*, **17**, 1685–1701.

Curn, V., Kubatova, B., Vavrova, P., Krivackova-Sucha, O. & Cizkova, H. (2007) Phenotypic and genotypic variation of *Phragmites australis*: comparison of populations in two human-made lakes of different age and history. *Aquatic Botany*, **86**, 321–330.

Davis, J.L., Childers, D.L. & Kuhn, D.N. (1999) Clonal variation in a Florida Bay *Thalassia testudinum* meadow: molecular genetic assessment of population structure. *Marine Ecology Progress Series*, **186**, 127–136.

Diggle, P.K., Lower, S. & Ranker, T.A. (1998) Clonal diversity in alpine populations of *Polygonum viviparum* (Polygonaceae). *International Journal of Plant Sciences*, **159**, 606–615.

Dray, S. & Dufour, A.-B. (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software*, **22**, 1–20.

Eastman, J.M., Paine, C.E.T. & Hardy, O.J. (2011) spacodiR: structuring of phylogenetic diversity in ecological communities. *Bioinformatics*, **27**, 2437–2438.

Ellstrand, N.C. & Roose, M.L. (1987) Patterns of genotypic diversity in clonal plant species. *American Journal of Botany*, **74**, 123–131.

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Falush, D., Stephens, M. & Pritchard, J.K. (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, **7**, 574–578.

Fant, J.B., Holmstrom, R.M., Sirkin, E., Etterson, J.R. &, Masi, S. (2008) Genetic structure of threatened native

populations and propagules used for restoration in a clonal species *Ammophila breviligulata* (American beachgrass). *Restoration Ecology*, **16**, 594–603.

Fer, T. & Hroudova, Z. (2009) Genetic diversity and dispersal of *Phragmites australis* in a small river system. *Aquatic Botany*, **90**, 165–171.

Galpern, P., Manseau, M., Hettinga, P., Smith, K. & Wilson, P. (2012) Allelematch: an R package for identifying unique multilocus genotypes where genotyping error and missing data may be present. *Molecular Ecology Resources*, **12**, 771–778.

Gratton, C. & Denno, R.F. (2006) Arthropod food web restoration following removal of an invasive wetland plant. *Ecological Applications*, **16**, 622–631.

Guo, W.H., Wang, R.Q., Zhou, S.L., Zhang, S.P. & Zhang, Z.G. (2003) Genetic diversity and clonal structure of *Phragmites australis* in the Yellow River delta of China. *Biochemical Systematics and Ecology*, **31**, 1093–1109.

Hamming, R.W. (1950) Error detecting and error correcting codes. *Bell System Technical Journal*, **29**, 147–160.

Hamrick, J.L. & Godt, M.J.W. (1989) Allozyme diversity in plant species. In: *Plant Population Genetics, Breeding, and Genetic Resources* (eds. Brown, A,H.D., Kahler, A.L. & Weir, B.S.), pp. 43–44. Sinauer Associates, Massachusetts.

Hardy, O.J. & Senterre, B. (2007) Characterizing the phylogenetic structure of communities by an additive partitioning of phylogenetic diversity. *Journal of Ecology*, **95**, 493–506.

Hauber, D.P., Saltonstall, K., White, D.A. & Hood, C.S. (2011) Genetic variation in the common reed, *Phragmites australis*, in the Mississippi River delta marshes: evidence for multiple introductions. *Estuaries and Coasts*, **34**, 851–862.

Holdredge, C., Bertness, M.D., Von Wettberg, E. & Silliman, B.R. (2010) Nutrient enrichment enhances hidden differences in phenotype to drive a cryptic plant invasion. *Oikos*, **119**, 1776–1784.

Hurry, C.R., James, E.A. & Thompson, R.M. (2013) Connectivity, genetic structure and stress response of *Phragmites australis*: issues for restoration in a salinising wetland system. *Aquatic Botany*, **104**, 138–146.

Keller, B.E.M. (2000) Genetic variation among and within populations of *Phragmites australis* in the Charles River watershed. *Aquatic Botany*, **66**, 195–208.

Kembel, S.W., Cowan, P.D., Helmus, M.R., Cornwell, W.K., Morlon, H., Ackerly, D.D., Blomberg, S.P. & Webb, C.O. (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463–1464.

Kettenring, K.M. & Mock, K.E. (2012) Genetic diversity, reproductive mode, and dispersal differ between the cryptic invader, *Phragmites australis*, and its native conspecific. *Biological Invasions*, **14**, 2489–2504.

Kettenring, K.M. & Whigham, D.F. (2009) Seed viability and seed dormancy of non-native *Phragmites australis* in suburbanized and forested watersheds of the Chesapeake Bay, USA. *Aquatic Botany*, **91**, 199–204.

Kettenring, K.M., McCormick, M.K., Baron, H.M. & Whigham, D.F. (2010) *Phragmites australis* (Common Reed) invasion in the Rhode River subestuary of the Chesapeake Bay: disentangling the effects of foliar nutrients, genetic diversity, patch size, and seed viability. *Estuaries and Coasts*, **33**, 118–126.

Kettenring, K.M., McCormick, M.K., Baron, H.M. & Whigham, D.F. (2011) Mechanisms of *Phragmites australis* invasion: feedbacks among genetic diversity, nutrients, and sexual reproduction. *Journal of Applied Ecology*, **48**, 1305–1313.

Kettenring, K.M., Whigham, D.F., Hazelton, E.L.G., Gallagher, S.K. & Weiner, H.M. (2015) Biotic resistance, disturbance, and mode of colonization impact the invasion of a widespread, introduced wetland grass. *Ecological Applications*, **25**, 466–480.

Konovalov, D.A., Manning, C. & Henshaw, M.T. (2004) kingroup: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Molecular Ecology Notes*, **4**, 779–782.

Koppitz, H., Kuhl, H., Hesse, K. & Kohl, J.G. (1997) Some aspects of the importance of genetic diversity in *Phragmites australis* (Cav.) Trin. ex Steudel for the development of reed stands. *Botanica Acta*, **110**, 217–223.

Křiváčková-Suchá, O., Vávřová, P., Čížková, H., Čurn, V. & Kubátová, B. (2007) Phenotypic and genotypic variation of *Phragmites australis*: a comparative study of clones originating from two populations of different age. *Aquatic Botany*, **86**, 361–368.

Kuhl, H., Koppitz, H., Rolletschek, H. & Kohl, J.G. (1999) Clone specific differences in a *Phragmites australis* stand I. Morphology, genetics and site description. *Aquatic Botany*, **64**, 235–246.

Lambertini, C., Gustafsson, M.H.G., Frydenberg, J., Speranza, M. & Brix, H. (2008) Genetic diversity patterns in *Phragmites australis* at the population, regional and continental scales. *Aquatic Botany*, **88**, 160–170.

Lambertini, C., Mendelssohn, I.A., Gustafsson, M.H.G., Olesen, B., Riis, T., Sorrell, B.K. & Brix, H. (2012a) Tracing the origin of Gulf Coast *Phragmites* (Poaceae): a story of long-distance dispersal and hybridization. *American Journal of Botany*, **99**, 538–551.

Lambertini, C., Sorrell, B.K., Riis, T., Olesen, B. & Brix, H. (2012b) Exploring the borders of European *Phragmites* within a cosmopolitan genus. *AoB Plants*; 2012:pls020. doi:10.1093/aobpla/pls020.

Larkin, D.J. (2012) Lengths and correlates of lag phases in upper-Midwest plant invasions. *Biological Invasions*, **14**, 827–838.

Lelong, B., Lavoie, C., Jodoin, Y. & Belzile, F. (2007) Expansion pathways of the exotic common reed (*Phragmites australis*): a historical and genetic analysis. *Diversity and Distributions*, **13**, 430–437.

Les, D.H., Garvin, D.K. & Wimpee, C.F. (1991) Molecular evolutionary history of ancient aquatic angiosperms. *Proceedings of the National Academy of Sciences USA*, **88**, 10119–10123.

McCormick, M.K., Kettenring, K.M., Baron, H.M. & Whigham, D.F. (2010a) Extent and reproductive mechanisms of *Phragmites australis* spread in brackish wetlands in Chesapeake Bay, Maryland (USA). *Wetlands*, **30**, 67–74.

McCormick, M.K., Kettenring, K.M., Baron, H.M. & Whigham, D.F. (2010b) Spread of invasive *Phragmites australis* in estuaries with differing degrees of development: genetic patterns, Allee effects and interpretation. *Journal of Ecology*, **98**, 1369–1378.

Meyerson, L.A. & Cronin, J.T. (2013) Evidence for multiple introductions of *Phragmites australis* to North America: detection of a new non-native haplotype. *Biological Invasions*, **15**, 2605–2608.

Meyerson, L.A., Viola, D.V. & Brown, R.N. (2010) Hybridization of invasive *Phragmites australis* with a native subspecies in North America. *Biological Invasions*, **12**, 103–111.

Mozdzer, T.J., Brisson, J. & Hazelton, E.L.G. (2013) Physiological ecology and functional traits of North American native and Eurasian introduced *Phragmites australis* lineages. *AoB Plants*, **5**, 5: plt048 doi: 10.1093/aobpla/plt048.

Neuhaus, D., Kuhl, H., Kohl, J.G., Dorfel, P. & Borner, T. (1993) Investigation on the genetic diversity of *Phragmites* stands using genomic fingerprinting. *Aquatic Botany*, **45**, 357–364.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.G., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2013) *vegan: Community Ecology Package*. R package version 2.0-9. https://github.com/vegandevs/vegan/

Paradis, E. (2012) *Analysis of Phylogenetics and Evolution with R*. Springer, New York.

Paul, J., Vachon, N., Garroway, C.J. & Freeland, J.R. (2010) Molecular data provide strong evidence of natural hybridization between native and introduced lineages of *Phragmites australis* in North America. *Biological Invasions*, **12**, 2967–2973.

Paul, J., Kirk, H. & Freeland, J. (2011) Genetic diversity and differentiation of fragmented reedbeds (*Phragmites australis*) in the United Kingdom. *Hydrobiologia*, **665**, 107–115.

Peakall, R. & Smouse, P.E. (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pielou, E.C. (1969) *An introduction to mathematical ecology*. Wiley-Interscience, New York.

Plut, K., Paul, J., Ciotir, C., Major, M. & Freeland, J.R. (2011) Origin of non-native *Phragmites australis* in North America, a common wetland invader. *Fundamental and Applied Limnology*, **179**, 121–129.

Price, A.L., Fant, J.B. & Larkin, D.J. (2014) Ecology of native vs. introduced *Phragmites australis* (common reed) in Chicago-area wetlands. *Wetlands*, **34**, 369–377.

Pritchard, J.K., Stephens, M. & Donnely, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945.

van der Putten, W.H. (1997) Die-back of *Phragmites australis* in European wetlands: an overview of the European Research Programme on Reed Die-back and Progression (1993–1994). *Aquatic Botany*, **59**, 263–275.

R Development Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Reynolds, J., Weir, B.S. & Cockerham, C.C. (1983) Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, **105**, 767–779.

Saltonstall, K. (2002) Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences USA*, **99**, 2445–2449.

Saltonstall, K. (2003a) Microsatellite variation within and among North American lineages of *Phragmites australis*. *Molecular Ecology*, **12**, 1689–1702.

Saltonstall, K. (2003b) A rapid method for identifying the origin of North American *Phragmites* populations using RFLP analysis. *Wetlands*, **23**, 1043–1047.

Saltonstall, K. (2011) Remnant native *Phragmites australis* maintains genetic diversity despite multiple threats. *Conservation Genetics*, **12**, 1027–1033.

Saltonstall, K., Peterson, P. & Soreng, R.J. (2004) Recognition of *Phragmites australis* subsp. *americanus* (Poaceae: Arundinoideae) in North America: evidence from morphological and genetic analyses. *SIDA*, **21**, 683–692.

Saltonstall, K., Castillo, H.E. & Blossey, B. (2014) Confirmed field hybridization of native and introduced *Phragmites australis* (Poaceae) in North America. *American Journal of Botany*, **101**, 211–215.

Santamaria, L. (2002) Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecologica – International Journal of Ecology*, **23**, 137–154.

Silliman, B.R. & Bertness, M.D. (2004) Shoreline development drives invasion of *Phragmites australis* and the loss of plant diversity on New England salt marshes. *Conservation Biology*, **18**, 1424–1434.

Taddeo, S. & De Blois, S. (2012) Coexistence of introduced and native common reed (*Phragmites australis*) in freshwater wetlands. *Ecoscience*, **19**, 99–105.

Tulbure, M.G. & Johnston, C.A. (2010) Environmental conditions promoting non-native *Phragmites australis* expansion in Great Lakes coastal wetlands. *Wetlands*, **30**, 577–587.

Widen, B., Cronberg, N. & Widen, M. (1994) Genotypic diversity, molecular markers, and spatial distribution of genets in clonal plants: a literature survey. *Folia Geobotanica*, **29**, 245–263.

Windham, L. & Lathrop, R.G. (1999) Effects of *Phragmites australis* (common reed) invasion on aboveground biomass and soil properties in brackish tidal marsh of the Mullica River, New Jersey. *Estuaries*, **22**, 927–935.

Wu, C.A., Murray, L.A. & Heffernan, K.E. (2015) Evidence for natural hybridization between native and introduced lineages of *Phragmites australis* in the Chesapeake Bay watershed. *American Journal of Botany*, **102**, 805–812.

Zedler, J.B. & Kercher, S. (2004) Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. *Critical Reviews in Plant Sciences*, **23**, 431–452.

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Editor: Bethany Bradley