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Population genetic structure of N. American and European *Phalaris arundinacea* L. as inferred from inter-simple sequence repeat markers

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ORIGINAL PAPER

Population genetic structure of N. American and European *Phalaris arundinacea* L. as inferred from inter-simple sequence repeat markers

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Abstract *Phalaris arundinacea* L. (reed canary-grass) has become one of the most aggressive invaders of North American wetlands. *P. arundinacea* is native to temperate N. America, Europe, and Asia, but repeated introductions of European genotypes to N. America, recent range expansions, and the planting of forage and ornamental cultivars complicate the resolution of its demographic history. Molecular tools can help to unravel the demographic and invasion history of populations of invasive species. In this study, inter-simple sequence repeat markers were used to analyze the population genetic structure of European and N. American populations of reed canary grass as well as forage and ornamental cultivars. We found that *P. arundinacea* harbors a high amount of genetic diversity with most of the diversity located within, as opposed to among, populations. Cluster analyses suggested that current populations are admixtures of two formerly distinct genetic groups.

Keywords ISSRs · *Phalaris arundinacea* · STRUCTURE · Population structure · AMOVA

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Introduction

Phalaris arundinacea L. (reed canary grass) is a perennial, rhizomatous herb native to temperate North America, Europe, and Asia (Merigliano and Lesica 1998; Galatowitsch et al. 1999; Casler et al. 2009). As a member of the Poaceae, *P. arundinacea* is wind pollinated and this highly self-incompatible species reproduces primarily through outcrossing (Weimarck 1968). Although most commonly considered a wetland plant, *P. arundinacea* is highly tolerant of both flooding and drought (Alway 1931). Under favorable conditions, *P. arundinacea* establishes rapidly and spreads vegetatively to form dense monospecific stands (Barnes 1999; Miller and Zedler 2003). *P. arundinacea* also produces significant amounts of alkaloids (Cheeke 1995), thereby reducing its palatability to herbivores relative to other plants (Marten et al. 1976). These life history traits have allowed *P. arundinacea* to become an aggressive invader of N. American wetlands, reducing the diversity of native

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species (Henderson 1991; Miller and Zedler 2003). During the twentieth century, *P. arundinacea* greatly increased its range in N. America, becoming an invasive weed in many wetlands (Galatowitsch et al. 1999).

The complex demographic history of *P. arundinacea* in N. America may have played a role in its success as an invader. European genotypes were introduced and intentionally planted in many locations as a forage crop (Galatowitsch et al. 1999). This widespread planting likely resulted in hybridization and the lack of a founder effect in the introduced populations as well as the mixing of alleles from disparate areas of Europe within individual N. American populations (Lavergne and Molofsky 2007).

Life history traits and demographic history, including human-mediated introduction and dispersal, can influence the molecular signatures of diversity and differentiation. Self-incompatible, wind-pollinated plants, such as *P. arundinacea*, tend to have higher within-population diversity and lower levels of differentiation among populations (Hamrick 1989). Likewise, human-mediated dispersal of propagules among existing populations contributes to interpopulation gene flow, which lowers the among-population differentiation. On the other hand, founder effects resulting from human-mediated introduction of one or a few genotypes into a new habitat can produce populations with very low diversity if there is little interpopulation gene flow. In the case of *P. arundinacea*, high levels of within-population diversity, and the lack of a founder effect are expected due to the self incompatibility system and extensive human-mediated dispersal.

Neutral genetic markers have become an important tool for examining demographic histories of introduced species (Ward and Jasieniuk 2009). Inter simple sequence repeats (ISSRs) are dominant markers located between microsatellite sites in the genome (Zietkiewicz et al. 1994). ISSRs are often highly polymorphic at the species level, require no prior sequence information, and are more reproducible than RAPD markers (McGregor et al. 2000). These characteristics make ISSRs ideal for studying population structure in a non-model species such as *P. arundinacea*.

The currently prevailing hypothesis is that invasiveness in N. American populations is due to hybridization of European genotypes introduced for forage (Lavergne and Molofsky 2007). The role of selection for agronomically important traits, however, has been called into question (Jakubowski et al. 2011). If the Lavergne and Molofsky (2007) hypothesis were correct, the invasive N. American populations, particularly the hybrid forage and ornamental cultivars, would be more genetically diverse than the European populations. We tested this hypothesis and characterized genetic diversity and differentiation in European and N. American invasive populations and cultivars (forage and ornamental). The relatedness among populations was determined using a Bayesian clustering analysis. Since all N. American populations were invasive and collected over a wide geographic area, it was assumed that there would be a moderate level of among-population differentiation due to local adaptation. Since *P. arundinacea* has such a large range, a secondary objective was to examine the effect of geographic scale on the analyses of population diversity and differentiation.

Materials and methods

Genotypes

P. arundinacea populations were collected from N. America and Europe with a focus on the American Midwest region (comprising the US states of Iowa, Minnesota, Missouri, Nebraska, North Dakota, and Wisconsin). The American Midwest region was a focus because there are numerous wild populations in the region as well as seed production programs for *P. arundinacea* located in northern Minnesota. *A* total of 386 *P. arundinacea* genotypes from 52 populations were sampled. Plants were contributed by multiple collectors (Supplemental Table 1) and maintained at the University of Minnesota (UM) or the University of Wisconsin, Madison (UW). Sampled plants included five forage and six ornamental cultivars as well as individuals collected from wild populations in N. America (37) and Europe (9) (Fig. 1; Supplemental Table 1). Wild populations were defined as patches of *P. arundinacea* separated from other patches by at least 1 km. In all analyses, each of the five forage cultivars was treated as a separate population, each plant representing one genotype within the population, while all six ornamental genotypes were grouped together as one population.

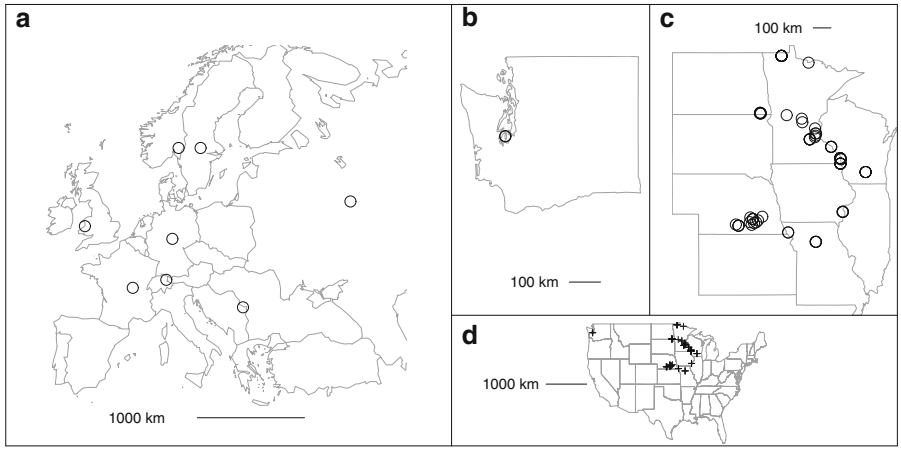

Fig. 1 Map of European and North American *P. arundinacea* population sampling locations (*circles*), from which one or more individuals were sampled, corresponding to the analysis of molecular variance (AMOVA) and STRUCTURE analyses populations. **a** The European sampling locations, **b** shows the location of the Washington sampling location, **c** locations of the Midwest collection locations, **d** the relative locations of all N. American collection sites

DNA extraction and molecular markers

For plants maintained at the University of Minnesota, a modified cetyltrimethyl ammonium bromide (CTAB) protocol was used for DNA extraction (Doyle and Doyle 1987). Complete details on the DNA extraction and PCR protocol are included in the supplementary materials. The resuspended DNA was quantified in a Qbit fluorometer (Invitrogen Corporation, Carlsbad, CA) using the Qbit dsDNA BR assay kit (Invitrogen Corporation, Carlsbad, CA). Working solutions were made by diluting the resuspended DNA in TE buffer to 12.5 ng/mL For the plants maintained at the UW, total genomic DNA was extracted using the method of Štorchová et al. (2000). All conditions for the subsequent molecular work, including polymerase chain reactions (PCR) and imaging, was identical for DNA from plants grown at the UM and UW.

An initial screen of was performed on 100 primers from the University of British Columbia (UBC 800-899). Twenty randomly genotypes were used for the screen at an annealing temperature of 55 °C. Three primers (UBC 810—$[GA]_8T$, 881—$G_3[TGGGG]_2TG$, and 890—$VHV[GT]_7$) were chosen for ISSR analysis on the basis of the presence of clear, repeatable, and polymorphic markers. The three primers yielded 90 usable markers.

Polymerase chain reactions were performed in an Applied Biosystems 2700 Thermal Cycler (Life Technologies Corporation, Carlsbad, CA). The PCR products were separated for visualization on 1.5 % agarose gels containing ethidium bromide at 70 V for 4.5 h. Photographs of the gels (Supplemental Fig. 1) were taken with UV transillumination using an Alpha-Innotech FluorChem 8900 imaging cabinet (Protein Simple, Santa Clara, CA). Distinct and reproducible bands were scored as present (1) or absent (0) and the molecular weights (in base pairs) were estimated using Gel Analyzer (Lazar 2010).

Genetic diversity and differentiation measures and geographic scales

Genetic diversity refers to the total amount of genetic variation present within a species or population (Toro and Caballero 2005) whereas population differentiation refers to the degree to which populations are genetically distinct from one another. To assess genetic diversity, the percentage of polymorphic genetic loci (PPL) and Nei's average genetic diversity (H_e) were calculated. To examine the partitioning of genetic diversity among and within populations, analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was performed using the package 'ade4' (Dray and Dufour 2007) in R (R Development Core Team 2010). The Φ statistics generated by AMOVA were used to assess population genetic differentiation.

To examine the effect of geographic scale on the analyses, we used the framework of Ward (2006), arranging the populations into four geographic scales

(Global, Continental, Regional, and Local). The Global Scale consisted of all genotypes from N. America and Europe (Eu); the Continental Scale consisted of all N. American genotypes; the Regional Scale included all genotypes collected from the US states of Iowa (IA), Minnesota (MN), Missouri (MO), Nebraska (NE), North Dakota (ND), and Wisconsin (WI); the Local Scale consisted of genotypes from 14 populations in the Rainwater Basin area of NE (An area of Southcentral Nebraska that consists of a series of wetlands surrounding the Platte River, Table 1; Fig. 1). Within each geographical scale, as required by AMOVA, populations were grouped into regions (henceforth referred to as AMOVA regions, Table 1). In the Global Scale, the AMOVA regions consisted of N. American and European populations. In the Continental Scale, the AMOVA regions consisted of populations from Washington, the upper Midwest, the lower Midwest, the Northern Midwest, and the ornamental cultivars. In the Regional Scale the AMOVA regions were the upper, lower, and Northern Midwest populations. For the Local Scale, populations were not grouped into regions.

Table 1 Geographic scales, number of populations, number of individuals, and AMOVA regions of *P. arundinacea* analyzed for ISSRs

Geographic scale	Number of populations	Number of individuals	AMOVA regions (no. populations, no. individuals)
Global	76	386	North America (68, 354)
			Europe (8, 32)
Continental	68	354	Washington (2, 10)
			Lower Midwest (23, 80)
			Upper Midwest (35, 174)
			Northern Midwest (7, 83)
			Ornamental (1,7)
Regional	65	337	Lower Midwest (23, 80)
			Upper Midwest (35, 174)
			Northern Midwest (7, 83)
Local	14	47	N/A

The genotypes were arranged into four geographically nested hierarchies (Geographic Scales) of regions and populations. Region and population designations in each scale correspond to factors by the same name in the AMOVA. Populations in scale 4, the most geographically restricted scale, were not grouped into regions

The percentage of polymorphic genetic loci was calculated for all genetic loci for the Continental Scale AMOVA regions plus the European genotypes. Populations with fewer than five sampled individuals were excluded from the analysis (Tables 3, 4). The null allele frequencies were calculated using a Bayesian estimator,

$$\widehat{q} = \frac{B(m + a + 0.5, n - m + b)}{B(m + 0.5, n - m + b)} \quad (1)$$

from Zhivotovsky (1999) where $\widehat{q}$ is the allele frequency, B() is the beta function, m is the number of individuals that do not have a band at the given locus, n is the number of individuals in the sample, and a and b are the parameters of the beta distribution. A uniform prior distribution was assumed with $a = 0.5$ and $b = 1$. For each of the Continental Scale AMOVA regions plus the European genotypes, Nei's average gene diversities ($\widehat{H}_e$) were calculated as

$$\widehat{H}_e = \frac{1}{L}\sum_{i=1}^{L}[2\widehat{q}_{ri}(1 - \widehat{q}_{ri}) + 2Var(\widehat{q}_{ri})] \quad (2)$$

(Lynch and Milligan 1994) where $\widehat{q}_{ri}$ is the Bayesian estimated null allele frequency at locus i in region r, and L is the number of marker loci (Table 2).

For Φ, (analogous to Fisher's F-statistics) the H_o tested = there is no genetic difference among all populations ($\Phi_{ST} = 0$) or populations within regions ($\Phi_{CT} = 0$), or among regions ($\Phi_{SC} = 0$) (Excoffier et al. 1992). To determine significance values for Φ, a null distribution was calculated based on 999 permutations of the binary data matrix. The analyses for the Global, Continental, and Regional Scales partitioned variance at three levels (among regions, among populations, and within populations), variance for the Local Scale was partitioned into two levels (among and within populations). For all scales, populations correspond to the collection sites in Figs. 1 and 2.

Cluster analyses

To estimate the number of genetically distinct groups in *P. arundinacea*, and to test if geographic origin of populations (e.g. N. America vs. Europe) corresponded

Table 2 Percentage of polymorphic loci (PPL), Nei's genetic diversity (H_e) and number of sampled genotypes (n) of *P. arundinacea* by region for geographic scales 1 and 2 and the ornamental and forage cultivars

	Scale 1		Scale 2				Forage cultivars	Ornamental cultivars	All
	EU	North America	Midwest-Lower	Midwest-North	Midwest-Upper	West-WA			
PPL	92	100	100	88	100	48	97	60	100
H_e	0.24	0.22	0.22	0.27	0.22	0.21	0.22	0.28	0.22
n.	32	347	79	15	174	10	69	7	386

Regions included in Scale 1 were the EU (European Union), North America, while regions included from scale 2 were Midwest-Lower, Midwest-North, Midwest-Upper, ornamental cultivars, West-Washington (WA). Forage cultivars, grouped in the Midwest-North region for AMOVA, were also treated separately for the PPL and H_e analyses. The percent of polymorphic loci was calculated by dividing the number of loci that were polymorphic in each region by the total number of loci (90). Nei's genetic diversity was calculated from the Bayesian estimator for the null allele frequency in Zhivotovsky (1999)

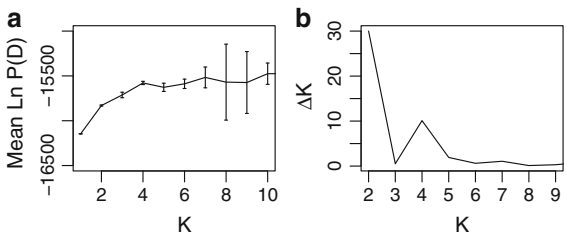

Fig. 2 Line graphs from the admixture model of STRUCTURE of Ln P(D) (a measure of the natural logarithm of the posterior probability, P of the data, D) and ΔK for sampled *P. arundinacea* populations (P), where K is the hypothesized number of populations. **a** Mean value of the statistic Ln P(D) produced by STRUCTURE at each value of K, **b** ΔK

to cluster assignments, all genotypes were analyzed in the Bayesian clustering algorithm STRUCTURE v.2.3.2 (Pritchard et al. 2000; Falush et al. 2007). All STRUCTURE runs used the following settings: dominant marker data mode, correlated alleles assumed, 500,000 burn-in repetitions, 500,000 MCMC repetitions. To infer the numbers of genetically distinct populations, five replicate simulations were run with at each value of K from one to 10. To find the most likely value of K, LnP(D), a measure of the natural logarithm of the posterior probability (P) of the data (D), and Δ(K), an empirical statistic based on the second-order rate of change and the variance of LnP(D) (Evanno et al. 2005), were calculated using Structure Harvester (Earl and von Holdt 2011).

The software package CLUMPP (Jakobsson and Rosenberg 2007) was used to combine the STRUCTURE group-membership output data for each population from the five replicates for the molecular data for K = 2. To illustrate the STRUCTURE results geographically, pie charts showing the proportional group membership coefficients (the average of the individual group membership coefficients within the population) for each population were overlain on maps of the sampling locations. The map data was adapted from package 'maps' (Brownrigg and Minka 2012) in R (R Development Core Team 2010).

A neighbor-joining tree was constructed to visualize the relatedness of each of the populations. Nei's genetic distance among populations was calculated using the function 'dist.genet' in package 'ade4' (Dray and Dufour 2007) in R (R Development Core Team 2010). The neighbor-joining dendrogram was constructed using the functions 'nj' and 'plot.phylo' in package 'ape' (Paradis et al. 2004) in R (R Development Core Team 2010).

Results

Diversity and differentiation measures

Each of the 90 marker loci were polymorphic when considering the entire set of genotypes (Tables 3, column "All"). There was a high level of polymorphism for all subdivisions at the Global and Continental Scales; for example, in the Global Scale, the percentage of polymorphic loci of the Eu genotypes was 92 %, while all loci (100 %) were polymorphic in N. America. For the Continental Scale, loci ranged from 48 % (West—WA, n = 10) to 100 % (upper and lower Midwest regions, n = 174 and 80, respectively) polymorphic. Considering populations that were represented by at least two samples (Table 3),

Table 3 Populations, percent polymorphic loci (PPL), Nei's genetic diversity (H_e) and number of individuals (n) of *P. arundinacea* analyzed from the inter simple sequence repeat (ISSR) data

Population	PPL	H_e	n
FSU	48	0.30	4
Germany	57	0.29	5
IA Port Louisa	88	0.26	14
MN Bend	38	0.32	3
MN Falcon Heights	63	0.22	12
MN Fort Snelling	69	0.25	10
MN McGregor	71	0.25	9
MN Rainy River	24	0.36	2
MN River Slough	42	0.32	3
MN Roseau Ma	76	0.25	11
MN Roseau Pa	77	0.21	22
MN Roseau Ri	79	0.24	15
MN Roseau Va	70	0.23	12
MN Roseau Ve	64	0.25	9
MN Roseau Ditch	83	0.25	13
MN Roseville	81	0.27	10
MN Saint Cloud	79	0.25	14
MN UMR L	83	0.25	13
MN UMR Millers	68	0.25	8
MN UMR Running	53	0.25	6
MN UMR Walters	79	0.23	12
MN UMR Whalen	34	0.25	5
MN Voyager	50	0.35	3
MN Winona	79	0.26	14
MO Squaw Creek	66	0.24	8
MO Swan Lake	73	0.26	10
Montenegro	43	0.32	3
ND Tewaukon 1	79	0.25	16
ND Tewaukon 2	78	0.27	10
NE RWB County Line	30	0.30	3
NE RWB Funk	27	0.26	4
NE RWB G	77	0.27	9
NE RWB Harvard	33	0.29	3
NE RWB Lange	50	0.33	3
NE RWB Massie	36	0.31	3
NE RWB N	69	0.28	7
NE RWB Nelson	44	0.24	6
NE RWB Springer	28	0.34	2
NE RWB Wilkins	38	0.26	5
Norway	38	0.32	3
Ornamental cultivars	60	0.26	7
Sweden	59	0.28	6
Switzerland	68	0.26	7
WA Lakebay	48	0.19	10
Wales	43	0.32	3
WI La Crosse	78	0.24	13
WI Madison	80	0.24	12

Populations represented by only one sample genotype were excluded from the analysis

% polymorphic loci ranged from 24 % (n = 2, MN Rainy River) to 88 % (n = 14, IA Port Louisa). The % polymorphic loci was generally higher for larger populations. Nei's genetic diversity (H_e) ranged from 0.20 (Washington region) to 0.28 (ornamental cultivars) for the Continental Scale (Tables 3, 4). The overall H_e for all samples was intermediate at $H_e = 0.22$ (Table 2). At the population level, H_e ranged from $H_e = 0.19$ (WA Lakebay, n = 10) to $H_e = 0.36$ (MN Rainy River, n = 2).

An AMOVA revealed that the vast majority of the total genetic variance was within populations. For example in the Global Scale, 85 % of the total genetic variance was found to occur within populations (Table 4). The within population proportion of variance was similar for all geographic scales (85, 90, 90.0, and 87 % respectively; Table 4). The proportion of among-region variance was low with the variance not exceeding 6 % at any scale (Table 4). The small among-region variance component, along with the low, but significant, Φ_{CT} values (0.015–0.057; Table 4) imply that regions are only slightly differentiated from one another. The among-population variance components were low and consistent at all scales, ranging from 8 to 13 % (Regional and Local Scales, Table 4) of the total. The Φ_{ST} values ranged from 0.099 to 0.149 (Table 4) indicating a moderate amount of population differentiation.

Cluster analyses

In the STRUCTURE analysis, the slopes of the LnP(D) plots rise quickly to K = 2 with continued increases to K = 4, followed by leveling off and accompanied by an increase in variance (Fig. 2a). A decrease in slope and increase in variance of LnP(D) is diagnostic for the true value of K. The ΔK plot shows a large peak at K = 2 with a secondary peak at K = 4

Table 4 Analysis of molecular variance (AMOVA) results for sources (between regions, between populations, within populations) for geographic scales 1–4: degrees of freedom (df), sums of squares (SS), mean squares (MS), genetic variance estimates (Est. Var.), proportion of variance (% Var.) attributed to the different levels in the spatial hierarchy of *P. arundinacea*

Source	Df	SS	MS	Est. var.	% Var.	Φ	*P*
Global Scale							
Among regions	1	55	55	0.6	6	$\Phi_{CT} = 0.057$	<0.001
Among populations	74	1,522	21	2	9	$\Phi_{SC} = 0.097$	<0.001
Within populations	310	3,607	12	12	85	$\Phi_{ST} = 0.149$	<0.001
Total	385	5,184	13	14	100		
Continental Scale							
Among regions	4	148	37	0.2	2	$\Phi_{CT} = 0.018$	<0.001
Among Populations	63	1,239	20	2	9	$\Phi_{SC} = 0.088$	<0.001
Within populations	286	3,305	12	12	90	$\Phi_{ST} = 0.104$	<0.001
Total	353	4,692	13	13	100		
Regional Scale							
Among regions	2	92	46	0.2	1	$\Phi_{CT} = 0.015$	<0.001
Among Populations	62	1,228	20	2	8	$\Phi_{SC} = 0.086$	<0.001
Within populations	272	3,189	12	12	90	$\Phi_{ST} = 0.099$	<0.001
Total	336	4,509	13	14	100		
Local Scale							
Among Populations	13	206	16	2	13	$\Phi_{SC} = 0.134$	<0.001
Within populations	33	355	11	11	87		
Total	46	560	12	12	100		

Phi (Φ) statistics are analogous to Wright's F-statistics

P values are based on 999 permutations

(Fig. 2b) indicating that there are two genetically distinct clusters. The secondary ΔK peak at $K = 4$ may indicate further population substructure.

In the STRUCTURE simulations for $K = 2$, plotting the proportional group membership coefficients (ranging from 0 to 1) for each *P. arundinacea* population in the Global Scale (all genotypes, Fig. 3), the Midwest populations (Fig. 3a) were mixtures of group one (gray, Fig. 3b) and group two (white). European populations were predominantly assigned to group one (gray, Fig. 3b), while the Washington population was predominantly group two (white, Fig. 3d), although care should be taken in the interpretation because there was only one Washington population included in the analysis. Most N. American populations, except for WA Lakebay, were not well segregated into either group. Several populations within NE (Fig. 3d) had higher proportions of group two than group one. Only a few such populations occurred in the Midwest (Fig. 3a).

Consistent with the low proportion of among-region variance in the AMOVA, the grouping of populations on the neighbor-joining tree is only weakly correlated with geographical distance (Fig. 4). Approximately half (5/9) of the European populations cluster together on the dendrogram, as do the forage cultivars. However, there are several examples of geographically unrelated populations belonging to the same terminal group, for example MN Voyager and Sweden 2.

Discussion

As expected, the percent polymorphic loci and Nei's genetic diversity measures (Table 2) indicate that there is a high level of molecular diversity within *P. arundinacea* and at all geographic scales the majority of the diversity is within populations (Table 4). As noted by Ward (2006), high levels of gene flow lead to populations that harbor most of the species' genetic diversity. In this case the lack of genetic structuring among regions at any of the geographic scales analyzed is consistent with the history of multiple

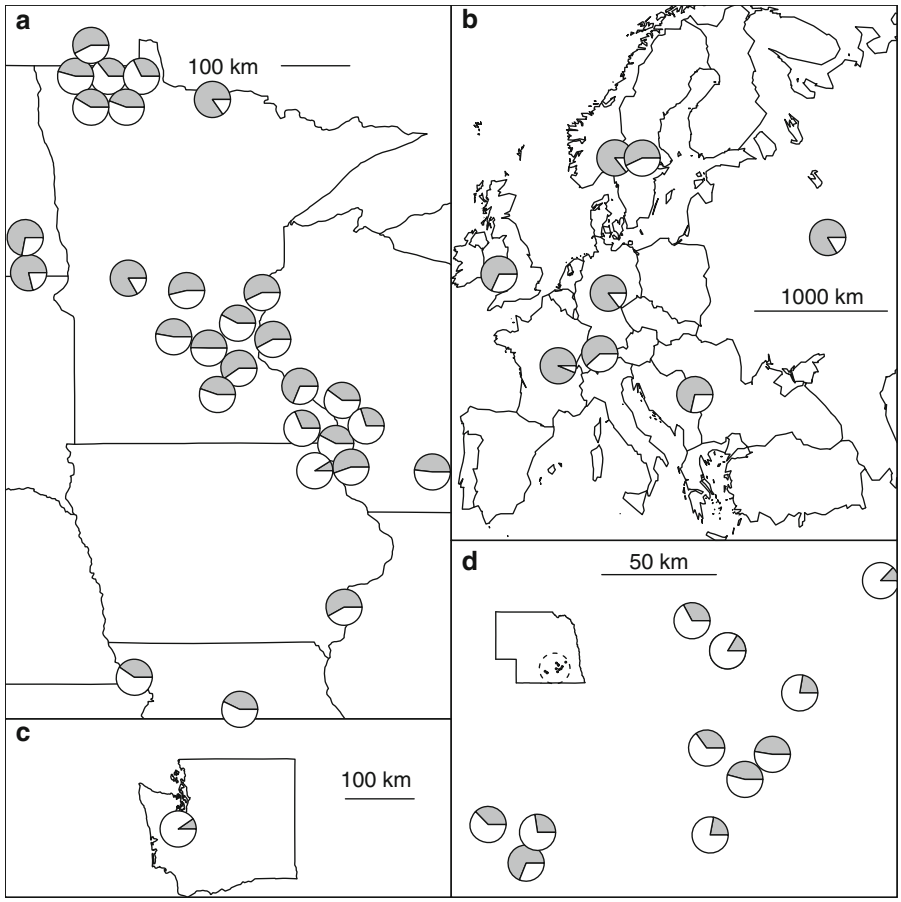

Fig. 3 Group membership coefficients, ranging from 0 to 1, for each *P. arundinacea* population in scale 1 (all genotypes) for K = 2. **a** Midwest (MW), **b** Europe (Eu), **c** State of Washington (WA), and **d** State of Nebraska (NE). The proportional coefficients (averaged across all individuals within the population) for each population are superimposed over the collection sites on the maps. Group 1 is represented in *gray*, group 2 in *white*

introductions, human-mediated dispersal, and self incompatibility.

The genetic differentiation partitioning observed in *P. arundinacea* contrasts with invasive plants having different life history traits and invasion histories. For example, the self-compatible invasive herb *Alliaria petiolaria* has most of its variability among populations within continents (Meekins et al. 2001) and the self-compatible tropical tree *Miconia calvescens* has most of its genetic variance between the invasive and native regions (Hardesty et al. 2012). Like *P. arundinacea*, the self-incompatible invasive herb *Linaria vulgaris* had most of its genetic variance partitioned at the population level (Ward et al. 2008). However, unlike *P. arundinacea*, *L. vulgaris* still had a large proportion of among population variance (29.1 %) at the interpopulation level (Ward et al. 2008). The distinctive partitioning of genetic variance, with an extremely high within-population component (Tables 3, 4) observed in *P. arundinacea* highlights the importance of human-mediated dispersal in explaining its observed pattern of genetic diversity.

Although the majority of the total variance was found within populations, there was also a moderate and statistically significant amount of population differentiation (Φ_{ST} = 0.099–0.149). This population differentiation may be due, in part, to small founder effects. However, given the amount of human-mediated dispersal, a large contribution from founder effects seems unlikely.

The grass species with perhaps the most similar invasion history in N. America is *Phragmites australis*

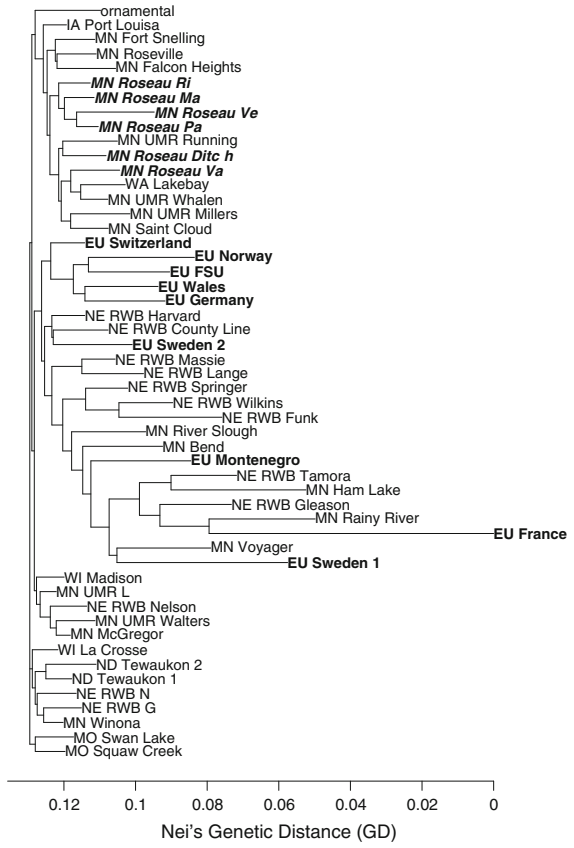

Fig. 4 The neighbor-joining tree based on Nei's genetic distance for *P. arundinacea* populations. European populations are shown in **bold** and forage cultivars are in ***bold italic***

because there were native N. American populations prior to the introduction of non-native genotypes (Saltonstall 2002). Like *P. arundinacea, P. australis* may be self-incompatible (Ishii and Kadono 2002). Given these similarities, it is not surprising that a similar partitioning of molecular variance was found in invasive populations of *P. australis*, although native populations had a different pattern (Kettenring and Mock 2012). While hybridization between native and invasive *P. australis* genotypes is possible (Meyerson et al. 2010), the extent to which such hybridization occurs in the wild is presently unknown (Meyerson et al. 2010).

Hybridization is often associated with invasiveness in plants (Prentis et al. 2008). Lavergne and Molofsky (2007) found that alleles unique to disparate regions of Europe co-occurred in invasive populations of *P. arundinacea* in N. America, supporting a scenario of intraspecific hybridization. We did not find large differences between the genetic diversity (H_e, Table 2) of forage and native European populations compared with native N. American populations as would be expected if the invasive populations are hybrids. It should be noted, however, that our sampling scheme in Europe was limited so these results should be interpreted with care.

The degree of hybridization between native N. American and introduced genotypes in invasive N. American populations of *P. arundinacea* is unknown. A major obstacle in this and other studies that aim to understand invasiveness in N. American *P. arundinacea* populations in terms of hybridization has been the lack of benchmark N. American native populations. Recently Jakubowski et al. (2013) discovered distinct, native N. American genotypes using herbarium specimens collected from 1875 to 1996. Given this new information, identification of extant native N. American populations is now possible and future studies should aim to infer hybridization by sampling from known native N. American populations to compare against native European populations, forage cultivars, and invasive N. American populations. Hybrid individuals should occupy either intermediate positions to those of their parents on a plot of a principal coordinates (Beismann 1997) or, if they are introgressive hybrids, they could be a new species (Haghighi and Ascher 1988; Anderson 1949; Rieseberg et al. 1990).

Understanding the degree of hybridization between native and introduced genotypes in N. America will be key in fully describing the invasion process of *P. arundinacea.* The pattern of high relative within-population genetic diversity is consistent with a scenario of admixture between different lineages, however, the lack of reference native N. American populations prevents us from concluding that it is the reason for invasiveness. Now that it is possible to classify native N. American populations, a determination of the degree of hybridization is theoretically plausible. Patterns of diversity of neutral genetic markers, however, cannot fully explain the mechanisms behind the invasion process. Future studies will need to compare physiological, reproductive, competition, and other phenotypic traits among the various native, introduced, and hybrid genotypes to understand how *P. arundinacea* has become one of the most aggressive plant invaders in N. America.

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