

# Analysis of *Phragmites australis* haplotypes at the ORWRP based on morphological characteristics

Daniel F. Fink and Li Zhang

*Schiermeier Olentangy River Wetland Research Park, Environmental Science Graduate Program and School of Environment and Natural Resources, The Ohio State University*

## Introduction

*Phragmites australis* (Cav.), or common reed, is a wetland plant species found in every U.S. state. It can grow up to 6 m high in dense stands and is long-lived. *Phragmites* is capable of reproduction by seeds, but primarily does so asexually by means of rhizomes. Recent research has now shown that native and introduced genotypes of this species currently exist in North America. The species is invasive particularly in the eastern states along the Atlantic Coast and increasingly across much of the Midwest and in parts of the Pacific Northwest.

*P. australis* is a clonal grass species (*poaceae*) with woody hollow culms which can grow up to six meters in height. Leaves are lanceolate, often 20-40 cm long and 1-4 cm wide. Flowers develop by mid summer and are arranged in tawny spikelets with many tufts of silky hair. *P. australis* is wind-pollinated but self-incompatible. Seed set is highly variable and occurs through fall and winter and may be important in colonization of new areas. Germination occurs in spring on exposed moist soils. Vegetative spread by below-ground rhizomes can result in dense clones with up to 200 stems m<sup>2</sup>.

## History of *Phragmites* in the United States

Peat core analyses suggest that *Phragmites australis* has been an uncommon member of mixed tidal wetland plant communities in North America for at least 3000 years (Niering et al., 1977; Orson et al., 1987). In the 1800s *P. australis* was described as rare or uncommon (Torrey, 1843; Willis, 1874; MaCain, 1883; Dame and Collins, 1888; all as reported in Saltonstall, 2002). Beginning in the 19th century, *P. australis* began invading fresh and brackish wetlands in North America replacing mixed wetland plant communities with near monocultures of *P. australis*. This change in plant community structure resulted in changes to ecosystem processes and their associated impacts on native wildlife (Marks et al., 1994; Meyerson et al., 2000). In North America, the rapid population expansion of *P. australis* has typically been blamed on changes in land use patterns, hydrologic regimes, increased disturbances, urbanization and eutrophication (Marks et al., 1994). However, these very same factors are thought to cause declines of *P. australis* in Europe (van der Putten, 1997), so clearly more study is needed. Alternatively, it has been suggested that the invasiveness of *P. australis* is attributable to introduction of more aggressive European genotypes (Metzler and

Rosza, 1987; Tucker, 1990; Besitka, 1996) but until recently there has been little data to support this hypothesis.

## Recent developments in discerning haplotypes

The recent development of molecular markers identifying native and non-native populations of *Phragmites* in North America now makes it possible to look for morphological characters potentially distinguishing these lineages (Blossey, 2002—www.invasiveplants.net). These distinguishing morphological characteristics were developed from observations of populations in New York, Maryland, Maine, Indiana, Wisconsin, Minnesota, Virginia, Arizona, Washington, Louisiana, and numerous herbariums.

These traits are based on examinations of native and introduced clones but are not definitive as the various North American haplotypes vary not only geographically in their morphological characters but also over the course of seasons (and especially through the winter). It should be further noted that specimens observed in the field are the result of the genotype and its interactions with the biotic and abiotic environment, which can introduce additional variability. For example, the same genotype grown in the moister Northeast will look quite different in the arid West.

A total of 27 *P. australis* haplotypes have been identified of which 11 (A-H, S, Z, AA) are native to North America (Saltonstall, 2001b). Types AA, F, Z and S are known historically from the Northeast; types E, G, and H are found throughout the Midwest, and types A-D are found in the South and Intermountain West. Two of the invasive haplotypes show worldwide distribution (I and M) with M as the most common type in North America, Europe and Asia. In North America, Type I is found primarily along the Gulf Coast (Saltonstall, 2001b).

While herbarium samples from prior to 1910 show a varied distribution of native haplotypes across North America, modern populations show a decline in among-population variation (now mostly the I and M haplotype) in the traditional range and a range expansion of the M haplotype (Saltonstall, 2001b). Type M (which is most closely related to other European types) has entirely replaced native types in New England and has expanded to the southeast where no historic *P. australis* populations are known to have occurred. Type M is also becoming prevalent in the Midwest. It is likely that the introduction of type M material has occurred sometime in the early part of the 19th century. Today the genetic structure of North American populations strongly resembles those in Europe.

## Methods

### Site Description

Sampling was done in Experimental Wetland 2 (W2) and the created oxbow wetland (billabong) at the Schiermeier Olentangy River Wetland Research Park, Columbus, Ohio (Fig. 1). W2 is a created 1-ha emergent freshwater marsh and the billabong is a 2.8-ha created freshwater marsh. Both receive water from the adjacent Olentangy River; W2 via pumps and the billabong via natural floods (except April 2005- March 2006 where the billabong received pumped inflow water). Sampling was not conducted in experimental Wetland 1 (W1) because *P. australis* was not observed growing in that wetland. After construction in 1994, W2 was left unplanted. After construction in 1996, the billabong was planted in May 1997 with: *Cephalanthus occidentalis*, *Sagittaria latifolia*, *Equisetum* sp., *Zizania aquatica*, *Iris versicolor*, *Spartina pectinata*, *Lobelia cardinalis*, *Saururus cernuus*, *Juncus effusus*, *Asclepias incarnata*, *Pontederia cordata*, *Scirpus cyperinus*, *Sparganium eurycarpum*, *Alisma plantago-aquatica*, *Scirpus americanus*, *Scirpus fluviatilis*, *Acorus calamus*, *Potamogeton pectinatus*, *Polygonum* spp., and *Schoenoplectus tabernaemontani*. Furthermore, native prairie grass and marsh seeds were broadcasted around the perimeter and adjacent upland zones of the excavated basin area.

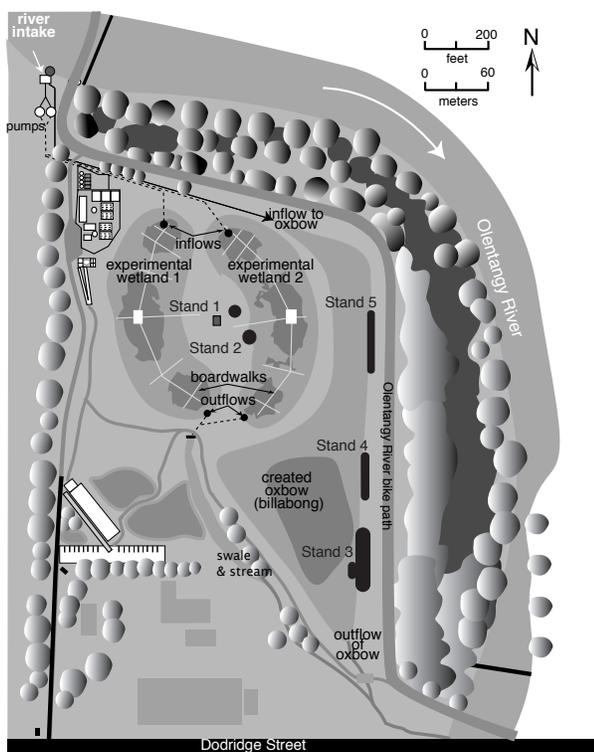


Figure 1. Site map of the ORWRP indicating the locations of *Phragmites australis* stands (see Appendix)..

### Phenotype analysis

Sampling was done in the autumn of 2005 after the end of the growing season and in the late spring, early summer of 2006 following the emergence of inflorescence.

Stands of *P. australis* growing on the western edge of the middle of wetland two and along the eastern edge of the billabong were sampled to determine if they were native or non-native phenotypes (Fig. 1). Five random individuals from each stand were selected and their morphological characteristics were examined and compared against what is common for native and non-native phenotypes (Table 1). A full description of the traits examined follows.

### Morphological characteristics

In general, native populations tend to have a lower stem density, and produce a reddish-purple color on their stems and ligules in spring and summer that is not present in non-native populations (Table 1). The side of the stems exposed to the sun will show the brightest coloration. In autumn the reddish color fades somewhat into a chestnut and in the winter the stems turn lighter further to chestnut brown and may appear somewhat gray. Stems of native genotypes are smooth and shiny as if polished, particularly in the winter, while stems of introduced genotypes are dull, and are rough and ribbed. The ridges on the stems on invasive phenotypes are visible with the naked eye once leaf sheath has been removed. The leaf sheath wraps around the stem almost entirely. It is important to remove the leaf sheath when checking for stem morphology or texture.

The leaf sheaths of native phenotypes either fall off by themselves or are very easily removed. In the introduced genotypes, leaf sheaths may fall off at the base of the stem (which is also occasionally reddish or brownish) but are somewhat difficult to remove on the upper nodes. To check, stems were held close to an internode and subjected to a twisting motion to determine how easily the sheath was removed. In instances where native and introduced clones grow in close vicinity of each other, differences in stem density and stem toughness can become obvious on windy days. Introduced genotypes remain sturdy and erect and move very little while native genotypes easily bend and sway in the wind. Stems of introduced genotypes are often almost straight while stems of native genotypes often grow crooked.

In the fall and winter, differences in the density of inflorescences are also diagnostic. Introduced genotypes have much larger and denser inflorescences. Native genotypes may senesce earlier than introduced genotypes (this is a common phenomenon in introduced species which often show extended growing periods). In addition, an unidentified stem fungus attacks native genotypes with dark spots often clustered around internodes while introduced genotypes remain fungus free.

Rhizomes were excavated using a shovel and were cleaned of soil in field. Native haplotypes have round rhizomes that are yellow with diameters of less than 15mm.

Rhizomes of introduced haplotypes, particularly when freshly excavated and rinsed, are white (they darken over time) and compressed (flattened). Although there are some rhizomes with diameters <15 mm, most rhizome diameters in introduced genotypes are larger than 15 mm (measure in the center of an internode and use largest diameter).

### Genotype analysis

Sampling was done in the autumn of 2005 after the end of the growing season.

Growing conditions of the stand were determined: (flooded, intermittently flooded, rarely flooded) and the dimensions and location (latitude and longitude of the perimeter) of the patches were measured using a GPS. The density of *P. australis* in the stand was recorded as sparse-medium-dense.

Five stems from the previous years growing season were cut at the base of the shoot as far down as possible. Each stem was folded individually and placed into a separate plastic bag. Samples were then shipped to Cornell University, Ithica, NY for genotype and phenotype analysis.

## Results and Discussion

### Phenotype

In the two stands in W2, 7 of the 16 morphological features examined were inconclusive in determining haplotype (Table 1). Some of the leaf sheaths fell off at senescence while others did not, the stem color in the autumn was a greyish tan, stem texture was rough in stand 1 but smooth in stand 2, fungal spots were present in Au 05 but absent in Sp/Su 06, stem density was low in stand 1 but high in stand 2, inflorescence was in August, and the flood frequency was not telling. Three of the features examined indicated native haplotypes. All plants sampled had easy to remove leaf sheaths with the simple twisting motion described above, relatively straight stems, and mostly flexible stems. Six of the 16 features strongly indicated non-native phenotypes. Stem color in the spring and summer was tan, inflorescences were relatively dense, senescence was late in the season, leaf color was dark green, rhizomes were ovoid, and the rhizomes were greater than 15 cm in diameter.

Table 1. Comparison of morphological features of stands of *Phragmites australis* at the Schiermeier Olentangy River Wetland Research Park and observed differences between native and introduced haplotypes. (\*)Indicates that a test was not performed. (SW) indicates 'somewhat'.

Trait	Reference		Experimental				
	Native	Introduced	Stand 1	Stand 2	Stand 3	Stand 4	Stand 5
Leaf sheaths							
Senescence	Fall off stem (F)	Stay on stem (S)	S	F	S	F	S
Removability	Easy (ER)	Hard (HR)	ER	ER	ER	ER	ER
Stem color at base							
Sp/Su	Red to Chestnut (RC)	Tan (T)	T	T	T	T	T
Au/Wi	Light chestnut (LC) -	Tan (T)	LC to T	LC to T	LC to T	LC to T	LC to T
Stem							
texture	Smooth/shiny (SS)	Rough/dull (RD)	SS	SS	RD	RD	RD
fungal spots	Present (P)	Absent (A)					
Sp/Su (2006)			A	A	A	A	A
Su/Au (2005)			P	P	P	P	P
Flexibility	Flexible (F)	Rigid (RG)	SW-RG	F	SW-RG	SW-RG	SW-RG
Straightness	Straight (S)	Crooked (C)	S	S	SW-S	SW-S	S
Density	Low (L)	High (H)	L	H	L-M	L	L
Inflorescence							
Timing	July-Aug (early)	Aug-Sept (late)	Aug	Aug	Aug	Aug	Aug
Density	Sparse (Sp)	Dense (Dn)	Sp	Dn	Dn	Dn	Dn
Senescence	Early	Late	Late	Late	Late	Late	Late
Leaf color	Yellow-green (YG)	Dark green-gray (DG)	DG	DG	DG	DG	DG
Rhizome							
Density	Low	High	*	*	*	*	*
Diameter	<15 cm	>15 cm	<15 cm	~15 cm	~15 cm	>15 cm	>15 cm
Shape	Round to slightly compressed (R)	Oval to highly compressed (O)	R-O	O	O	O	O
Clonal expansion	Slow	Rapid	*	*	*	*	*
Flood frequency	Intermittent flooded (IF)	Flooded to Rarely flooded (F-RF)	RF	IF	IF - RF	RF	IF

The observation that Stand 2 has a high stem density while Stand 1 does not is important. Stand 2 had the highest stem density of any sampled and is also the youngest stand, being in its first year of presence. It is also the only stand sampled that was not competing with previously established macrophyte or forested communities. The observation that Stand 2 had smooth stems may also be a feature of the youth of the stand as more mature plants seem to produce rougher stems.

In the three stands in the billabong, 6 of the 16 morphological features examined were inconclusive in determining haplotype (Table 1). Some of the leaf sheaths fell off at senescence while others did not, the stem color in the autumn was a greyish tan, stems were only somewhat flexible, fungal spots were present in Au 05 but absent in Sp/Su 06, inflorescence was in August, and the flood frequency was not telling. Three of the features examined indicated native haplotypes. All plants sampled had easy to remove with the simple twisting motion described above, all stands had relatively straight stems, and all stands had relatively low stem density. Seven of the 16 features strongly indicated non-native phenotypes. Stem color in the spring and summer was tan, stem texture was rough in all stands, inflorescences were relatively dense, senescence was late in the season, leaf color was dark green, rhizomes were ovoid and compressed, and the rhizomes were greater than 15 cm in diameter.

The features examined in both W2 and the billabong suggests that the *P. australis* haplotype present at the ORWRP is a non-native type. The high number of ambiguous results is not surprising, given the large degree of natural variability within the plant species and its ability to vary phenotypic expression with changing microenvironments. Further, these stands are not mature and are not all in the same microhabitat. Only Stand 1 is older than 5 years, and it is in the driest habitat zone of all the stands. Every stand except for Stand 2 and a portion of Stand 3 are in competition with already established macrophyte communities, which may account for variability in stem counts and measures of general plant health (e.g. fungal spots, stem rigidity).

It is important to note that the mid- and end-of- growing season sampling strongly indicates non-native types. As these morphological features are the ones presented by an actively growing plant, they are more telling than the post-growing season characteristics.

## Genotype

We are still waiting on results from Bernd Blossey's lab at the Cornell University School of Natural Resources.

## Acknowledgments

Many thanks to Chris Anderson for his assistance in collecting field data. We also thank Bernd Blossey and his lab in advance for future results regarding the genetic testing of *P. australis* haplotypes.

## References

- Besitka, M.A.R. 1996. An ecological and historical study of *Phragmites australis* along the Atlantic Coast. M.Sc. thesis. Drexel University, Philadelphia, PA.
- Dame, L. L. & Collins, F. S. (1888) Flora of Middlesex County, MA. Middlesex Institute, Malden, MA.
- Goman, M. & Wells, L. (2000) Quat. Res. 54, 206–217.
- Graves, C. B., Eames, E. H., Bissell, C. H., Andrews, L., Harger, E. B. & Weatherby, C. A. (1910) Bulletin of the Connecticut Geological and Natural History Survey No. 14 (Case, Lockwood: Brainard, Hartford, CT).
- MacCain, J. (1883) Catalogue of Canadian Plants: Part 1, Polypetalae. Dawson Brothers, Montreal.
- Marks, M., Lapin, B., and Randall, J. 1994. *Phragmites australis* (*P. communis*): Threats, management, and monitoring. Natural Areas Journal 14: 285-294.
- Metzler, K., and R. Rosza, R. 1987. Additional notes on the tidal wetlands of the Connecticut River. Newsletter of the Connecticut Botanical Society 15: 1-6.
- Meyerson, L. A., K. Saltonstall, L. Windham, E. Kiviat, & S. Findlay. 2000. A comparison of *Phragmites australis* in freshwater and brackish marsh environments in North America. Wetlands Ecology and Management 8: 89-103.
- Niering, W. A., R. S. Warren, and C. Weymuth. 1977. Our dynamic tidal marshes: Vegetation changes as revealed by peat analysis. Connecticut Arboretum Bulletin # 22.
- Orson, R. A., R.S. Warren, & W.A. Niering. 1987. Development of a tidal marsh in a New England river valley. Estuaries 10: 20-27.
- Saltonstall, K. 2002. Kryptic invasion by non-native genotypes of the common reed, *Phragmites australis*, into North America. Proceedings of the National Academy of Sciences of the United States of America 99: 2445-2449.
- Tewksbury, L. T., R.A. Casagrande, B. Blossey, M. Schwarzlaender, & P. Haefliger. 2002. Potential for biological control of *Phragmites australis* in North America. Biological Control 23: 191-212.
- Torrey, J. (1843) Flora of the State of New York (Carroll and Cook, Albany).
- Tucker, G. C. 1990. The genera of Arundinoideae (Gramineae) in the southeastern United States. J. Arnold Arboretum 71: 14-171.
- van der Putten, W. 1997. Die-back of *Phragmites australis* in European wetlands: an overview of the European research program on reed die-back and progression (1993-1994). Aquatic Botany 59: 263-275.
- Willis, O. R. (1874) Catalogue of Plants Growing Without Cultivation in the State of New Jersey. J. W. Schermerhorn, New York.

Appendix A  
*Phragmites* Patch GPS Coordinates at ORWRP (see Figure 1)

Site	Northing			Westing		
	Degrees	Minutes	Seconds	Degrees	Minutes	Seconds
1	40	1	5.20	83	1	2.68
	40	1	5.27	83	1	2.48
	40	1	5.30	83	1	2.44
	40	1	5.30	83	1	2.32
	40	1	5.40	83	1	2.46
	40	1	5.42	83	1	2.50
	40	1	5.33	83	1	2.52
	40	1	5.20	83	1	2.46
	40	1	4.84	83	1	2.38
2	40	1	5.68	83	1	2.24
	40	1	5.74	83	1	2.16
	40	1	5.90	83	1	2.14
	40	1	6.13	83	1	2.29
	40	1	6.18	83	1	2.37
	40	1	6.01	83	1	2.38
	40	1	5.78	83	1	2.36
	40	1	5.68	83	1	2.34
3	40	1	6.34	83	1	2.21
	40	1	6.56	83	1	2.25
	40	1	6.61	83	1	2.32
	40	1	6.52	83	1	2.29
4	40	1	9.45	83	1	1.76
	40	1	9.68	83	1	1.69
	40	1	9.73	83	1	1.87
	40	1	9.73	83	1	2.14
	40	1	9.41	83	1	1.78
5	40	1	10.03	83	1	1.91
	40	1	10.08	83	1	1.74
	40	1	10.11	83	1	1.69
	40	1	10.30	83	1	1.74
	40	1	10.44	83	1	1.73
	40	1	10.42	83	1	2.28
	40	1	10.31	83	1	2.28
	40	1	10.04	83	1	1.89

