

Assessment of Eurasian *Phragmites australis* Haplotype M Cryptic Invasion in the Minnesota and Mississippi River Valleys of Minnesota

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Introduction

Phragmites australis (Cav.) Trin. ex Steud. is an aquatic grass species found in wetlands throughout North America. The cryptic invasion of these environments by the introduced Eurasian *Phragmites australis* haplotype M over the past two centuries has recently been reported (Saltonstall, 2003).

In recent decades, the aggressive expansion of clonal *Phragmites australis* populations in many Minnesota wetlands has been noted (Lynch). Wetland scientists have often speculated that these invasive populations may represent non-native strains that are morphologically indistinguishable from the native type, now recognized as *Phragmites australis* subsp. *americanus* (Lynch).

In this study, we use RFLP analysis to investigate the extent of Eurasian *Phragmites australis* haplotype M establishment in the Minnesota and Mississippi River Valley corridors of Minnesota, and within the drainage way of Interstate Highway 94 in central Minnesota.

Materials and Methods

Collection

• *Phragmites* populations were identified at intervals of ten to twenty miles along:

- Minnesota River Valley from Big Stone Lake (Big Stone, Co.) to the confluence of the Minnesota and Mississippi Rivers (Ramsey Co.)
- Mississippi River corridor from its headwaters (Beltrami Co.) to the Iowa state line (Houston Co.)
- I94 drainage ditch system between Minneapolis (Hennepin Co.) and Albany (Stearns Co.)



Figure 1. *Phragmites* sampling locations in Minnesota (2007-2008)

• In each sample population, leaves from shoots were collected at 10m intervals along transects through the stand.

Materials and Methods

Haplotype Determination

Chloroplast DNA (cpDNA) extraction and purification for each specimen was performed using MoBio Labs Ultraclean™ Plant DNA extraction kit and protocols.

The non-coding cpDNA regions *trnL* (primers *trnL(UAA)5'* "b" – *trnLbR*) and *rbcL* (primers *rbcL – rbcL3R*) were amplified by PCR using previously established methods (Saltonstall (2), 2003).

RFLP Analysis

• Amplified *trnL* and *rbcL* cpDNA was digested with the restriction endonucleases *RsaI* and *HhaI*, respectively, and electrophoresed on a 3% TAE gels prior to staining.

• *trnL* amplicons of native *Phragmites* specimens are cut by *RsaI* (235bp band) – Figure 2. Analogous amplicons from non-natives are not cut, leaving a band at ~350bp.

• In contrast, the *rbcL* region 380bp cpDNA amplicons from Eurasian haplotypes are cleaved by *HhaI*, while amplicons from natives are not (Figure 3).

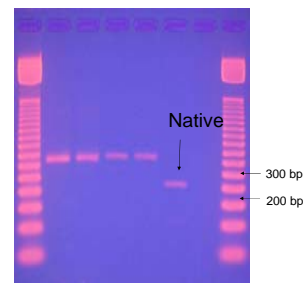


Figure 2. TAE (3%) gel of amplified *trnL* region cpDNA cut with *RsaI*. From left to right: Lanes (1 and 8) DNA marker 50 bp; (2-5) Eurasian haplotype M samples; (6) native haplotype specimen, (7) open.

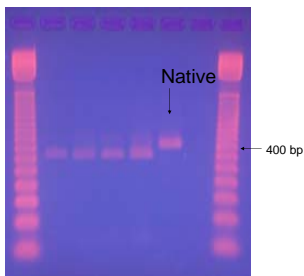


Figure 3. TAE (3%) gel of amplified *rbcL* region cpDNA cut with *HsaI*. From left to right: Lanes (1 and 8) DNA marker 50 bp; (2-5) Eurasian haplotype M samples; (6) native haplotype specimen, (7) open.

Results

Phragmites specimen collection sites and haplotype data is shown in Table 1.

Table 1. *Phragmites* stand haplotypes in sample transects of three corridors.

Corridor	Transect Name	No. of Samples	No. of Native Specimens (%)	No. of Eurasian (M) Specimens (%)
Minn	Ortonville/Big Stone Lk	11	11 (100)	0 (0)
Minn	Lac Qui Parle WMA	10	10 (100)	0 (0)
Minn	Morton/RWF	10	10 (100)	0 (0)
Minn	St. Peter	11	11 (100)	0 (0)
Minn	Belle Plaine	12	12 (100)	0 (0)
Minn	Jordan/LS NWR	12	12 (100)	0 (0)
Minn	Shakopee	7	7 (100)	0 (0)
Minn	Savage/Savage Fen SNA	12	12 (100)	0 (0)
Miss	Headwaters/Itasca SP	4	4 (100)	0 (0)
Miss	Grant Creek	3	3 (100)	0 (0)
Miss	Lake Bemidji	3	3 (100)	0 (0)
Miss	Cass Lake/Pike Bay	5	5 (100)	0 (0)
Miss	Winnibigoshish/Bena	4	4 (100)	0 (0)
Miss	Ball Club Lake/Hwy 2	12	12 (100)	0 (0)
Miss	Grand Rapids/US 169	4	4 (100)	0 (0)
Miss	Libby Lake/Aitkin	4	4 (100)	0 (0)
Miss	Mission Creek/CR 3	3	3 (100)	0 (0)
Miss	Belle Prairie	3	3 (100)	0 (0)
Miss	Little Falls/US 10	3	3 (100)	0 (0)
Miss	Sartell/MN 15 Bridge	4	4 (100)	0 (0)
Miss	Clearwater/Rice Lake	5	Pending	Pending
Miss	Hasty/Silver Creek	5	Pending	Pending
Miss	Prairie Island/Ravenna	6	6 (100)*	0 (0)*
Miss	Weaver Bottoms	7	7 (100)*	0 (0)*
Miss	Winona	6	6 (100)*	0 (0)*
Miss	Lamille/US61	5	5 (100)*	0 (0)*
Miss	Hokah/Root River	8	8 (100)*	0 (0)*
I94	Brooklyn Park/Mpls	6	6 (100)	0 (0)
I94	Maple Grove So.	6	4 (100)	0 (0)
I94	Maple Grove	4	4 (100)	0 (0)
I94	Maple Grove No. (Brockton)	6	0 (0)	6 (100)
I94	Rogers	7	0 (0)	7 (100)
I94	St. Michael	5	0 (0)	5 (100)
I94	St. Augusta	4	4 (100)	0 (0)
I94	St. Joseph	7	7 (100)	0 (0)
I94	Albany	7	7 (100)	0 (0)
Total		231	213 (92.3)	18 (7.7)

*Initial findings in these transects that still require final confirmation. "Pending" specimens are those for which molecular analysis is currently underway.



Conclusions

• Robust stands of the Eurasian haplotype (M) of *Phragmites australis* exist in Minnesota, but appear restricted to highly disturbed drainage ways. We identified several populations of this haplotype in Hennepin County, Minnesota. **Currently, these Eurasian populations have only been found in the Interstate Highway 94 corridor within Hennepin County.**

• Both Eurasian (M) and native haplotype stands appear to be clonal in nature. Furthermore, **no mixed stands** have been discovered in Minnesota to date.

• **Eurasian haplotype M *Phragmites* was not found** during the comprehensive survey of the both the **Minnesota and Mississippi River corridors**. These data suggest that Eurasian *Phragmites* has not yet invaded native *Phragmites* stands in these waterway.

• **Further study** is required to determine why haplotype M has been restricted to the I94 corridor, despite numerous drainage connections to the two river systems in this survey.

Literature Cited

Saltonstall, K. 2003. 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. 2003 (2). A rapid method for identifying the origin of North American *Phragmites* populations using RFLP analysis. *Wetlands* 23(4):1043-1047.

Lynch, E.A. and K. Saltonstall. 2003. Paleocological and genetic analysis provide evidence for recent colonization of native *Phragmites australis* populations in a Lake Superior wetland. *Wetlands* 22:637-646.

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