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ABSTRACT—*Phragmites australis* (common reed) is known to have occurred along the Platte River historically, but recent rapid increases in both distribution and density have begun to impact habitat for migrating sandhill cranes and nesting piping plovers and least terns. Invasiveness in *Phragmites* has been associated with the incursion of a European genotype (haplotype M) in other areas; determining the genotype of *Phragmites* along the central Platte River has implications for proper management of the river system. In 2008 we sampled *Phragmites* patches along the central Platte River from Lexington to Chapman, NE, stratified by bridge segments, to determine the current distribution of haplotype E (native) and haplotype M genotypes. In addition, we did a retrospective analysis of historical *Phragmites* collections from the central Platte watershed (1902–2006) at the Bessey Herbarium. Fresh tissue from the 2008 survey and dried tissue from the herbarium specimens were classified as haplotype M or E using the restriction fragment length polymorphism procedure. The European haplotype was predominant in the 2008 samples: only 14 *Phragmites* shoots were identified as native haplotype E; 224 were non-native haplotype M. The retrospective analysis revealed primarily native haplotype individuals. Only collections made in Lancaster County, near Lincoln, NE, were haplotype M, and the earliest of these was collected in 1973.

Key Words: braided river, common reed, Great Plains, invasive plants, *Phragmites australis*, Platte River, RFLP

INTRODUCTION

The central Platte River (Nebraska, USA) is a large, braided river with origins in the Rocky Mountains. The river provides important roosting habitat for hundreds of thousands of staging sandhill cranes (*Grus canadensis*) and nesting habitat for federally endangered least terns (*Sterna antillarum*) and threatened piping plovers (*Charadrius melodus*) (Sidle et al. 1992; Kirsch and Sidle 1999; Kinzel et al. 2006). Dams upstream of the central Platte River—here defined as the reach between Lexington, NE, and Chapman, NE—regulate flows far below historic records, especially during spring runoff periods (Eschner et al. 1981). Reduced flows have allowed encroachment by woody species, which have subsequently narrowed channels (Johnson 1994). Likewise, the absence of flooding is thought to have removed a major source of sandbar creation, reducing breeding habitat for terns and plovers (Sidle et al. 1992).

Recently (within the last 12–15 years), land managers have noted the rapid expansion of *Phragmites australis* (hereafter, *Phragmites*) throughout the active channel (i.e., the portion of the river influenced by flowing water) of the central Platte River. The expansion has resulted in vegetation of the bare sandbars required by nesting terns and plovers, and substantial armoring of the river banks, which contributes to channelization (Tal et al. 2004). Records at the University of Nebraska Bessey Herbarium

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indicate that *Phragmites* has been collected at sites within the central Platte River watershed since 1887. The reasons for the current rapid expansion are unknown. Elsewhere, low water levels, increased salinity, eutrophication, and the arrival of an exotic genotype have been considered possible triggers for rapid expansion of *Phragmites* (Galatowitsch et al., 1999; Hudon et al. 2005; Vasquez et al. 2005; Lelong et al. 2007).

Saltonstall (2003a) found both a native haplotype of *Phragmites* (haplotype E; now recognized as a subspecies, *P. australis* subsp. *americanus* Saltonstall, P.M. Peterson and Soreng) and the European haplotype (haplotype M; known as *P. australis* [Cav.] Trin. ex Streud.) in six samples collected in 1998 at various locations within the Platte River basin of Nebraska. Because haplotype M has been found to be invasive in freshwater wetlands (T'Ulbure et al. 2007), determining which haplotype is present in the central Platte River is key to developing appropriate management plans. If haplotype M is the dominant form in the central Platte River, managers may take more aggressive action against it than if the native haplotype is dominant. In addition, knowledge of the time course of the arrival of haplotype M may suggest reasons for its spread. Objectives of our study were (1) to assess the current distribution of the native and European haplotypes of *Phragmites* in active channels of the central Platte River and (2) to determine haplotypes of herbarium specimens representing historic collections from the Platte River basin.

METHODS

We collected samples of *Phragmites australis* leaf tissue during the summer of 2008. Our study area extended west from Chapman to Lexington, NE (151 km) and was divided into segments based on bridge crossings near towns (Fig. 1). We sampled at 5–13 random locations within each segment (Table 1); differences in number of locations sampled reflect ownership and access limitations. We collected leaf tissue from three individual shoots at each location (hereafter referred to as a patch, which we define as a discrete group of *Phragmites* shoots, clearly separated from any other patch by open water, bare ground, or other vegetation); only one set of three samples was taken from any given patch. One shoot was sampled from the edge of the patch nearest the center of the channel, the second from the middle of the patch. The third leaf was collected from any shoot that in the opinion of the collector appeared different from the rest of the patch. Although certain morphological characteristics such as ligule length (Saltonstall et al. 2004) and internode coloration (Catling et al. 2007) would have been visible during our early summer collections, we did not base our selection on them. In this way we hoped not to miss one haplotype that might have been present within a larger clone of a differing haplotype. All patches were within the active channel. Sample locations were recorded on a handheld global positioning system unit. Samples were kept chilled in the field and were shipped on ice to the lab at the University of Minnesota where they were frozen until analysis. In all, 238 shoots were sampled.

Tissue from dried herbarium specimens was used for the retrospective analysis. Tissue samples were removed from the collection at the Bessey Herbarium at the University of Nebraska and shipped to the University of Minnesota for analysis. Collection dates ranged from 1902 to 2006; locations were all within the watershed of the central Platte River (Fig. 1).

The protocol for restriction fragment length polymorphism (RFLP) was based on the method developed by Saltonstall (2003b), which distinguishes native from non-native genotypes of *Phragmites australis*. A 0.16 cm disk was punched from frozen or dried leaf tissue and placed in a 96-well plate; the paper punch was sterilized in 70% ethanol in between samples and allowed to dry prior to punching the next sample to avoid contamination between samples. We used the REDExtract-N-Amp Plant PCR kit (Sigma-Aldrich) to extract DNA from frozen leaf tissue and amplify our regions of interest. One hundred milliliters of extraction solution was added to each leaf tissue disk and heated to 95°C for 10 minutes. One hundred milliliters of dilution solution was then added to each well. Two noncoding chloroplast regions were amplified using the polymerase chain reaction (PCR). One region is a portion of the intergenic spacer between the *trn*T (UGU) and *trn*L (UAA) using the amplification primer pairs *trn*L (UAA) 5′ "b" (5′ TCTACCGATTTCGCCATATC)—*trn*LbR (5′ GGAGAAGATAGAATCATAGC) (Taberlet et al. 1991; Saltonstall 2002). The other region is a portion of the intergenic spacer between *rbc*L and *psal* using primer pairs *rbc*L (5′ TGTACAAGCTCGTAACGAAGG)—*rbc*L3R (5′ GATTTGTCAAGTCTCATGATCGT) (Saltonstall 2001; Saltonstall 2003b). PCR reactions consisted of 2 µl leaf disk extract, which included DNA, 10 µl REDExtract-N-Amp PCR ready mix, 2 µl each of the forward and reverse primers, 2 µl deionized and autoclaved water, and 1 µl each of the extraction and dilution solutions in a total volume of 20 µl. The thermal cycling profile was 2 minutes at 94°C, followed by 35

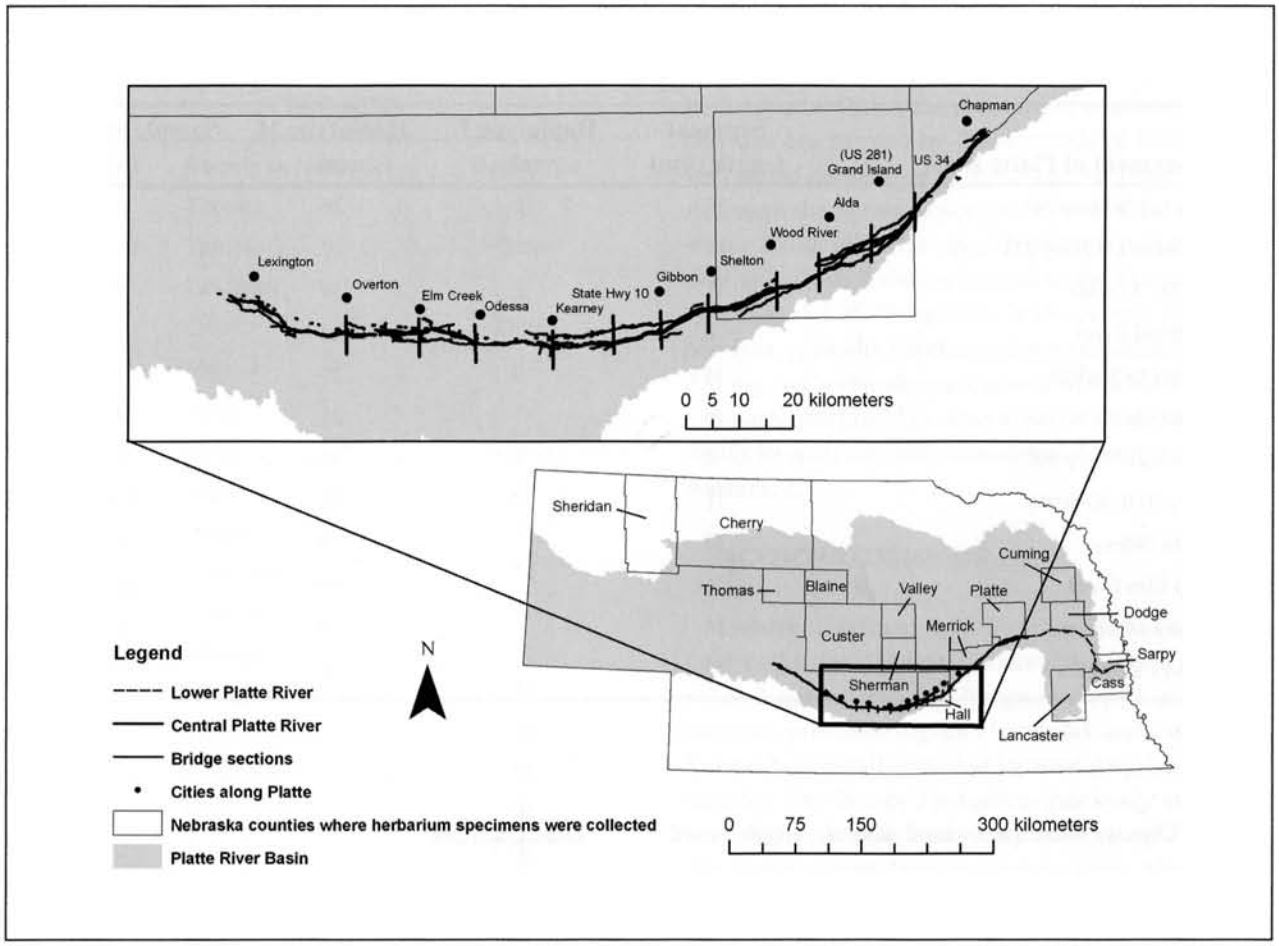

Figure 1. Map of study area in Nebraska, showing bridge sections for sampling *Phragmites* on the Platte River. Bridges are indicated by vertical lines crossing the river. The shaded area indicates the Platte River drainage basin. Identified counties are those from which herbarium specimens we analyzed were collected.

cycles of 94°C for 45 seconds, 52°C at 45 seconds, and 72°C for 1 minute 30 seconds, followed by an extension of 72°C for 1 minute 30 seconds. After the 35 cycles were completed, a final extension step at 72°C was run for 2 minutes. Ten milliliters of PCR product was digested using the restriction enzyme R*sa*I for the *trn*Lb region and the HhaI for the *rbc*L region following the manufacturer's directions. Restriction fragments were electrophoresed in ethidium bromide stained 3% TAE agarose gels and visualized with UV light.

The HhaI restriction enzyme generates a 104 bp fragment that is unique to haplotype M (and other European haplotypes). The R*sa*I restriction enzyme generates a 282 bp fragment in native haplotypes but not in European haplotypes. Verified specimens of haplotype M and a native haplotype were analyzed as controls for the assay.

The extraction technique described above failed to extract DNA from some of the dried tissue from herbarium specimens. For these, we extracted DNA from 50 mg of ground air-dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen). DNA extracts were then diluted to a concentration of 5 ng DNA per 1 µl solution. AmpliTaq Gold DNA Polymerase (Applied Biosystems) was used to amplify the same regions of interest. PCR reactions consisted of 8 µl DNA extract, 4 µl buffer, 4 µl each of the forward and reverse primers, 4 µl dNTPs, 0.4 µl Taq polymerase, 17.6 µl deionized and autoclaved water, and 4 µl magnesium chloride in a total volume of 40 µl. The thermal cycling profile was 2 minutes at 94°C, followed by 35 cycles of 92°C for 30 seconds, 57°C for 15 seconds, and 72°C for 30 seconds. After the 35 cycles were completed, a final extension step at 72°C was run for 5

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