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Biological Invasions

ISSN 1387-3547
Volume 15
Number 12

Biol Invasions (2013) 15:2605-2608
DOI 10.1007/s10530-013-0491-2

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INVASION NOTE

Evidence for multiple introductions of *Phragmites australis* to North America: detection of a new non-native haplotype

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Received: 27 July 2012 / Accepted: 12 May 2013 / Published online: 17 May 2013

Abstract We found a new non-native haplotype of *Phragmites australis* in North America that provides convincing evidence for multiple introductions of this highly invasive reed from Europe. Prior to our detection of this new non-native haplotype, invasion of North America by this reed grass was thought to be limited to a single cp-DNA haplotype–haplotype M. However, we found two sites colonized by haplotype L1 in Quebec, Canada, a haplotype native to northern Europe, Great Britain and Romania. Because the invasion of North America by *P. australis* is ongoing, and because there is evidence for intra- and interspecific hybridization and increased fecundity resulting from outcrossing, more attention should be paid to genetic differences and associated vigor of populations of introduced *Phragmites* across North America.

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Keywords Common reed · Haplotype M · Cp-DNA · Morphology · Homoplasy

Introduction

Multiple introductions of invasive species increase invader success through increased propagule pressure, genetic diversity (Simberloff 2009, Schierenbeck and Ellstrand 2009, Lockwood et al. 2005, Ellstrand and Schierenbeck 2000), and potential adaptation to local conditions (Roman 2006). *P. australis* is globally ubiquitous and economically important in many regions of the world in both its native and introduced ranges. An invasive strain of *P. australis* (haplotype M) was introduced from Europe to North America and first appears in herbarium records there approximately 200 years ago (Saltonstall 2002). Haplotype M has spread aggressively throughout the North American east coast, the Gulf coast, the Great Lakes region (Chambers et al. 1999, Saltonstall 2002; Lambertini et al. 2012a; Howard et al. 2008) and more recently the southwestern U.S. (Meyerson et al. 2010a).

The cosmopolitan range of this species, the extensive trade and transport between Europe and North America over the last several centuries, and the relatively high allelic diversity of the introduced *Phragmites* previously identified in North America suggests that introduction of non-native *P. australis* to

North America has occurred multiple times (Saltonstall et al. 2010; Kirk et al. 2011; Plut et al. 2011; Chambers et al. 2012). However, conclusive evidence for multiple introductions of *P. australis* from Europe has been lacking, and only one introduced haplotype (M) was previously identified as colonizing North America outside of the Gulf Coast (but see Saltonstall 2003). We found a new haplotype of *P. australis* (haplotype L1), not previously reported in North America, at two locations in Quebec, Canada. This haplotype is native to Europe and the detection of this second non-native *P. australis* haplotype in North America, in addition to non-native haplotype M, provides strong evidence for multiple introductions and even greater genetic diversity of the European *P. australis* to North America.

The detection of this new haplotype (L1) is important for several reasons. First, outside of the Gulf Coast region (see Hauber et al. 2011; Lambertini et al. 2012a), haplotype M has long been thought to be the only invasive haplotype of *P. australis* present in North America. This was surprising because there are 14 haplotypes of *P. australis* in Europe, yet only a single type (haplotype M) was thought to have become invasive in North America. Second, *Phragmites* researchers have often overlooked the substantial genetic and morphological variation at the population level of introduced *P. australis* and frequently present research results as applicable to all introduced populations of *P. australis* in North America. This tendency has obscured our understanding of this invader thus far. Recent research has begun to identify some of the differences between introduced populations of *P. australis* (Meyerson et al. 2010a; McCormick et al. 2010), including frequency and direction of hybridization across lineages and *Phragmites* species (Lambertini et al. 2012a; Meyerson et al. 2012; Meyerson et al. 2010a) and vulnerability to herbivory across haplotypes (Cronin and Meyerson, unpublished data). Third, recent evidence for hybridization (Lambertini et al. 2012a; Meyerson et al. 2012, 2010a) and outcrossing in *P. australis* (McCormick et al. 2010) make it likely that new genetic admixtures of *P. australis* are present in North America that do not exist in the home range of this species. These new genotypes may be better adapted as invaders and are potentially more resistant to top down pressures from herbivores. Finally, distinguishing native haplotypes of *P. australis* in North America from the two introduced haplotypes (M and L1) may require revisions in the available morphological key (e.g., Swearingen and Saltonstall 2010) and necessitate cp-DNA sequencing to determine the correct introduced haplotype.

Leaf tissue sampling, DNA extraction, and sequencing

Leaf tissue of *P. australis* was collected from two sites near the Saint Lawrence River in Quebec, Canada: La Pocatière (47.37756, –70.05212) and Rivière-du-Loup, Quebec, Canada (47.85374, 69.55374). DNA was extracted from one stem from each stand using the Qiagen DNeasy® plant extraction kit and two non-coding chloroplast DNA (cp-DNA) regions were sequenced using primers (trn-T trn-L and rbc-L psaI) designed by Saltonstall (2002) to obtain a cp-DNA haplotype. For the trn-T trn-L primer, template DNA (1ul) was added to a mastermix of 9.6 ul dda H_2O, 3.0 ul 5 × HF buffer, 0.3 ul 10 mM dNTPs, 0.75 ul 10 uM trnT, 0.75ul 10 uM trnL, 0.15 ul Phusion. For the rbcL-psaI primer, 1 ul of template was added to 10.9 ul dda H2O, 1.5 ul 10x Tpol reaction buffer, 0.3 ul 10 mM dNTPs, 0.75 ul 10 uM rbcL, 0.75 ul 10 uM psal and 0.22 ul taq. PCR conditions were 94 °C for 3 min, 94 °C for 30 s, 52 °C for 30 s, 40 cycles of 72 °C for 60 s, followed by 72 °C for 5 min. PCR products were sequenced at the University of Rhode Island on an ABI 3130xl genetic analyzer Sequences were aligned using BioEdit 7.1.3.0 (Hall 1999) and compared in GenBank with known *Phragmites* haplotypes.

Results

We found two stands of introduced *P. australis* Haplotype L1 (NCBI accession no. KF005041) from sequenced cp-DNA from leaf tissue collected in Quebec, Canada. Lambertini et al. 2012b found the identical L1 sequences in Belgium, Finland, and Romania and called it L1 (trnT-trnL cp-microsatellite variant of haplotype 5 + rbcL-psaI haplotype 4) following Saltonstall's (2002) classification system (trnT-trnL NCBI no. AYO16328, haplotype 5 and rbcL-Psal NCBI no. AY016335, haplotype 4). Lambertini et al. 2012a also found an additional cp-microsatellite variant (L2) in Sweden. All of these samples are missing the indel that defines Haplotype 4 (trnT-trnL) (Saltonstall 2002). The origin of haplotype L deposited in GenBank in GenBank by Saltonstall (2002) has not been published. Our samples from La Pocatière and Rivière-du-Loup also match the trnT-trnL sequences (haplotype M2) found in Great Britain and deposited to GenBank by Vachon and Freeland (2011, trnT-trnL NCBI accession no GQ468794—haplotype M2) and Freeland and Vachon (2012, trnT-trnL NCBI accession no JQ409553, rbcL-psaI NCBI accession no JQ409548), but these also correspond to haplotype L1 following the Saltonstall classification (2002) as pointed out by Lambertini et al. 2012b. The two stands of Haplotype L1 that we sampled were in a larger landscape matrix with other *Phragmites* patches that we identified as Haplotype M based on sequenced cp-DNA.

Conclusions

Our results provide support for earlier studies (Saltonstall et al. 2010; Kirk et al. 2011; Plut et al. 2011) that suggested multiple introductions of *P. australis* have occurred in North America and provide convincing additional evidence for the introduction of at least two European haplotypes (M and L1) in North America. Previously, Saltonstall (2003) reported a single sample in eastern Washington State, USA that she identified as haplotype L noting that this haplotype is the second most common type in Europe (Saltonstall 2002). However, she did not analyze cp-microsatellite variants so it is possible that this sample is also L1. She also suggested that this sample could represent an additional introduction of a non-native genetic type to North America or could have resulted from a mutation in haplotype M (i.e., a result of homoplasy) that did not spread beyond the population where the mutation occurred. While it is not possible to rule out that the L1 haplotype that we identified at the two sites in Quebec are a result of homoplasy (see Vachon and Freeland 2011, Saltonstall and Lambertini 2012; Freeland and Vachon 2012 for the full debate), the genetic pattern of the invasive type in North America suggests multiple introductions (Saltonstall et al. 2010; Kirk et al. 2011; Plut et al. 2011; Meyerson et al. 2012; Chambers et al. 2012).

Whether or not haplotype L1 is as invasive as haplotype M is yet to be determined, as is the extent of its present distribution in North America. Finer scale sampling and experimental research are needed to address these gaps. However, in Quebec, the L1 and M introduced haplotypes co-occur and may be interbreeding thereby muddying the assumed link between invasive traits and a particular introduced haplotype. Finally, because haplotype L1 is has a native distribution in Great Britain, Northern Europe and in Romania, it is likely that the source of the North American L1 populations is in Europe (Lambertini et al. 2012b).

There may be additional evidence of other *P. australis* haplotype introductions to North America. For example, Lambertini et al. (2012a) recently reported both interspecific and intraspecific hybridization of in the Gulf Coast of the U.S., which suggests that multiple introductions have also taken place in that region. Given the extensive and long term nature of the *P. australis* invasion in North America, it is likely that additional evidence for multiple introductions from Europe and elsewhere will emerge. The invasion of North America by *P. australis* is ongoing (Meyerson et al. 2010b) and new evidence for interbreeding and hybridization within the genus in North America is emerging rapidly (Lambertini et al. 2012a; Paul et al. 2010; Meyerson et al. 2012). Closer attention by researchers to the ecological and genetic differences of introduced *Phragmites* populations is needed to better understand this invasion and appropriately tailor management actions.

Acknowledgments We thank Jacques Brisson, Sylvie de Blois and Claude Lavoie for their assistance in locating field sites. We thank Carla Lambertini for sharing her data and for helpful comments on this manuscript. We thank the reviewer, Steve Keller and Fred Meyerson for comments on this manuscript. Funding was provided to LAM and JTC by the US National Science Foundation DEB Awards 1049914 and 1050084. Additional funding to LAM was provided by the Fulbright Commission of the United States and the Czech Republic, and the University of Rhode Island College of Environment and Life Sciences Agricultural Experiment Station Project RI00H-332, 311000-6044.

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