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## Hybridization of common reed in North America? The answer is blowing in the wind

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## ABSTRACT

**Background and aims:** We review evidence for hybridization of *P. australis* in North America and the implications for the persistence of native *Phragmites australis* ssp. *americanus* populations in North America. We also highlight the need for an updated classification system, which takes *P. australis* intraspecific variation and hybridization into account.

**Methodology:** We reviewed available published, in press, and in preparation literature to assess the likelihood of hybridization and interbreeding in genotypes of *Phragmites australis* present in North America.

**Principal results:** Experimental results demonstrate that hybridization among introduced and native haplotypes is possible within the genus *Phragmites*, yet evidence that hybridization has naturally occurred is only starting to emerge. The lag in identifying hybridization in *Phragmites* in North America may be related to under-sampling in some parts of North America and to a lack of molecular tools that provide the capability to recognize hybrids.

**Conclusions:** Our understanding of the gene flow within and between species in the genus *Phragmites* is moving at a fast pace, especially on the east and Gulf coasts of North America. More attention should also be focused on the Great Lakes region, the southwestern and the west coast of the U.S. where sympatry has created opportunities for hybridization. Where hybridizations have been detected, there is currently no published data on how hybridization affects plant vigor, morphology, invasiveness, or conservation of the genetic integrity of the North American native subspecies. We conclude that detection of more hybridization is highly likely and that there is a need to develop new markers for the different *Phragmites* species and lineages to fill current knowledge gaps. Finally, we suggest that the classification

system for *P. australis* should be updated and published to help clarify the nomenclature.

## INTRODUCTION

As an ecologically and economically globally important species, *Phragmites australis* has been of significant interest to researchers for decades (e.g., Harris and Marshall 1960; Haslam 1969; Hauber *et al.* 1991; van der Putten 1997; Chambers *et al.* 1999; Meyerson *et al.* 1999; Brix 1999; Orson 1999). Because of its global distribution, its ability to thrive in a wide range of environmental conditions (Meyerson *et al.* 2000a,b), sexual and clonal reproductive strategies (Brisson *et al.* 2010; Saltonstall *et al.* 2010) and high genetic diversity within the species (Lambertini *et al.* 2012a; McCormick *et al.* 2010a,b; Saltonstall 2011), *Phragmites* is increasingly used as a model species in a variety of ecological and genetic research. The identification of three distinct lineages of *P. australis* in North America (i.e., North American native, Introduced, and Gulf Coast) and the development of species-specific chloroplast and nuclear markers, catalyzed research on the ecology, evolution, and success of different *P. australis* haplotypes (Saltonstall 2002, 2003). The current genetic knowledge of *Phragmites* worldwide is largely based on this original set of markers.

One area of particular interest for ecology and evolution is whether genotypes of this cosmopolitan grass are able to disperse across continents and interbreed within *P. australis* as well as hybridize across species within the genus *Phragmites*. It has been speculated that hybridization in *Phragmites* could potentially result in offspring with even greater vigor than the highly invasive genotypes that are currently expanding across North America and that pollen swamping or outbreeding depression could hasten the decline of North American native populations (Meyerson *et al.* 2010a). *Phragmites australis* is self-compatible (e.g. Ishii and Kadono 2002) but Kettenring *et al.* (2011) clearly demonstrated that in the

Chesapeake Bay *P. australis* needs to outcross in order to produce significant amounts of viable seed. This need for outcross pollen would seem to greatly increase the likelihood of hybridization, especially in newly invaded areas where within-species pollen may not be available but where pollen from related species (or subspecies) might be abundant. Despite evidence that native and introduced populations can interbreed under controlled conditions (Meyerson *et al.* 2010a), no convincing data have been published that demonstrate wild hybrids resulting from crosses of the North American native and introduced *Phragmites* (Saltonstall 2011). Recently, however, conclusive evidence for hybridization between the introduced and the more distantly related Gulf Coast lineage has been confirmed using different molecular markers (Lambertini *et al.* 2012a) and that suggests that detection of interbreeding between the native and introduced lineages and native and Gulf Coast lineages is only a matter of time.

In this paper we review evidence for hybridization of *P. australis* in North America and the implications for the persistence of native *Phragmites* populations. We also highlight the need for an updated classification system that takes *P. australis* intraspecific variation and hybrids into account and the need for new molecular markers to facilitate hybrid identification.

## **OVERVIEW OF THE DIFFERENT LINEAGES PRESENT IN NORTH AMERICA**

A growing body of published literature in the last decade describes the ecology and genetics of both the native and introduced (haplotype M) lineages of *P. australis* in North America, particularly on the Atlantic coast. Fewer papers have focused on the Gulf Coast type I and the invasion of type M to the Gulf Coast (Howard *et al.* 2008;

Hauber *et al.* 2011; Lambertini *et al.* 2012a) and only two publications have described the additional haplotypes that have recently been found in the Gulf Coast (Hauber *et al.* 2011; Lambertini *et al.* 2012a). The literature describing *Phragmites* in the western U.S. is growing, particularly in the southwest where Haplotype M is sympatric with the native lineage and with Haplotype I (e.g., Saltonstall 2002; Meyerson *et al.* 2010b; Kulmatiski *et al.* 2010). However, there has been very little published on *Phragmites* on the Pacific Coast of North America which is colonized by both the North American native and Eurasian introduced haplotypes (Saltonstall 2002). Below, we briefly describe each of the identified lineages present in North America (summarized in Table 1) and then discuss the evidence for hybridization in some of these lineages and the likelihood that it is occurring in others.

### **Geographic distribution of *Phragmites* genotypes in North America**

**North American Lineage:** North American native *P. australis* haplotypes are distributed throughout Northern Quebec to North Carolina and west to the Great Lakes, the Pacific northwest of the U.S. and southern British Columbia and the southwestern United States (Table 1). Native haplotypes of *P. australis* do not occur south of North Carolina on the east coast or Gulf Coast of the U.S. The native haplotypes appear very closely related to each other (Saltonstall 2002, Lambertini *et al.* 2006; Vachon and Freeland 2011, Lambertini *et al.* 2012a; Saltonstall and Lambertini 2012) and are considered one single lineage in this review though their origin is still unknown. Their closest relative appears to be Haplotype Q, distributed in Asia and Australia (Saltonstall 2002; Chu 2011; Saltonstall and Lambertini 2012). Lambertini *et al.* (2006) detected a weak nuclear relationship with *P. japonicus* in the Far East. However, this relationship was not evident in Lambertini *et al.* (2012a)



where North American native *P. australis* ssp. *americanus* appeared to have evolved from within *P. australis*. Another relationship recently detected is with *P. mauritanus* in Zambia (Lambertini *et al.* 2012a) which shares a mutation in the trnT-trnL region with the native North American lineage. *Phragmites* diversity in Asia and Africa has been so far underrepresented in phylogeographic studies at the global scale (Saltonstall 2002; Lambertini *et al.* 2006; Lambertini *et al.* 2012a). Collection and analysis of more samples from these continents promise to disclose the origin of the genus (Lambertini *et al.*, 2006) and the history of the North American lineage.

Eleven *P. australis* haplotypes considered native to North America were first identified by Saltonstall in 2002 and since that time five additional native haplotypes have been added. Meadows and Saltonstall (2007) added haplotypes AB and AC and Vachon and Freeland (2011) added haplotypes E2, E3 and E4. However, of these, only E4 is identified as a new haplotype based on Saltonstall's classification system which does not consider cp-microsatellite variants (Saltonstall 2002).

Specifically, Vachon and Freeland (2011) submitted two identical trn-T trn-L sequences they identified as E1 and E2 but these sequences are a cp-microsatellite variant of haplotype AB (Meadows and Saltonstall, 2007) following Saltonstall (2002). Similarly, haplotype E3 (Vachon and Freeland 2011; Freeland and Vachon 2012) corresponds to a cp-microsatellite variant of haplotype E again following Saltonstall (2002). Haplotype E4 (Vachon and Freeland 2011; Freeland and Vachon 2012) is a new haplotype which would be given a new letter in the classification Saltonstall initiated (Saltonstall 2002). Adding more complexity, there is yet another haplotype E4 that was deposited in GenBank by Chu *et al.* (2011) that was found in

South Korea. In GenBank it is identified as *P. australis* but is thought to be *P. japonicus*, a haplotype closely related to haplotype AM (Lambertini et al 2012). The implications of these examples for *Phragmites* classification are discussed in the concluding section.

**Euroasiatic Lineage:** Until relatively recently, it was believed that there was only a single type of introduced *P. australis* from Eurasia introduced to North America, haplotype M. This haplotype has been detected throughout North America, overlaps the range of native *P. australis* (described above) and extends into the Gulf Coast of the U.S where it is known as a “short form” of *P. australis* (Hauber et al., 2012) or the EU-type (Lambertini et al., 2012a). However, more recently, a cp-microsatellite variant of haplotype M, described as haplotype M1 or the Delta-type (Hauber et al., 2011; Lambertini et al., 2012) has been detected in the Mississippi Delta and Gulf Coast (described below in *Gulf Coast Lineages*) raising the possibility that some populations have been misidentified as type M. M1 (differs from haplotype M in the number of repeats in one microsatellite in the trnT-trnL region (Hauber et al. 2011, NCBI accession no. JF271678). It is therefore very closely related to Haplotype M and is thought to originate from the Mediterranean region, extending throughout North Africa, the Middle East and Southern Europe (Lambertini *et al.* 2012). Another introduction to North America of haplotype L (most likely from Europe) was found in Quebec, Canada providing conclusive evidence of multiple introductions of *P. australis* to North America (Meyerson and Cronin in prep.).

**Gulf Coast Lineages:** Similar to the evolving understanding on the Euroasiatic lineage, *Phragmites* researchers had evidence for only one other lineage colonizing

the Gulf Coast of the United States - haplotype I. Haplotype I was also detected in the southwestern U.S. (Meyerson *et al.* 2010b). However, multiple other haplotypes (Table 1) were recently found in the Mississippi Delta and surrounding marshes and one sample of M1 was found also in Florida, which makes the story of *Phragmites* in North America more complicated and suggests additional opportunities to detect interbreeding.

**Haplotype I:** As with the Eurasian haplotypes (M, M1), haplotype I also exhibits cp-microsatellite variation. Gulf Coast *Phragmites* is one such cp-variant (also called the “Land type”, Lambertini *et al.* 2012a, NCBI accession no. HQ664450) and was detected along the Gulf Coast of the US from Texas to Florida and in the Mississippi River Delta. This haplotype is shared with a population of *P. australis* in South America (Ecuador, Peru) and with the species *P. mauritanus* in Uganda and Burkina Faso (Lambertini *et al.* 2012a). Nuclear alleles indicate a hybrid origin for both the Gulf Coast and the South American populations from a cross between the two species *P. mauritanus* and *P. australis*. As the current distribution ranges of these species overlap only in tropical Africa, an African origin has been suggested (Lambertini *et al.* 2012a). However, given the similarities between the Gulf Coast and South American populations and their long establishment in the Americas, a different earlier distribution range of *P. mauritanus* could also entail an autochthonous American origin. With the data available, it is not possible to distinguish between an old accidental introduction and the radiation of *Phragmites* species (Lambertini *et al.* 2012a).

**European-related Haplotypes:** Three other recently detected haplotypes of *P. australis* are named for the special blue-green color of their leaves: Greeny 1 (haplotype M), Greeny 2 (haplotype AD), and Greeny 3 (haplotype AI). Haplotype AI differs from haplotype K (Saltonstall, 2002) in one single substitution in the *rbcl-psal* region (Lambertini *et al.* 2012a, NCBI accession no. HQ664451; Table 1). Although the three Greeny genotypes have three distinct haplotypes, they share the same European nuclear alleles (alleles 195 and 197 at locus PaGT 22, which are distinctive in this group and are shared, along with many more alleles, among the European and NA introduced genotypes). Given the high nuclear similarities among the three Greeny types, the most plausible explanation for their origin is somewhere in Europe. All three haplotypes (M, AD and AI) have, in fact, also been found in Europe (Lambertini *et al.* 2012b). However, the Greeny2 haplotype (AD) is closely related to the native North American haplotypes, whereas the best candidate for the origin of Greeny3 is the South African population of *P. australis* with haplotype K (Lambertini *et al.* 2012a). This suggests that the three Greeny types may also have been previously introduced to Europe as well and this possibility further clouds an identification of the historical introduction pathways.

## HYBRIDIZATION OF PHRAGMITES IN NORTH AMERICA

Does *Phragmites* hybridize in the wild in North America? The answer is probably yes but thus far the conclusive evidence is limited to the Gulf Region of the United States (Figure1, Table 1). An interspecific hybrid between the tropical African species *P. mauritanus* and *P. australis* became established long ago in South America and on the Gulf Coast of United States. The hybrid is the “Land-type”, previously described as *Phragmites australis* var. *berlandieri*. Being an interspecific hybrid, the specific

epithet *australis* does not appear appropriate any longer and should be dropped and renamed when the variation within haplotype I, including its hybrids, is further resolved and better understood.

The recent introductions of the European-related haplotypes of *P. australis* (M, M1 AD and AI) to the Mississippi River Delta have brought the hybrid in sympatry with its paternal species *P. australis*. Hybridization in the Gulf Coast appears to be due to back-crossing of the *P. mauritanus* x *P. australis* hybrid (haplotype I) with *P. australis* haplotypes (M, M1, AD and AI) (Fig. 1). Given the high similarities in nuclear markers among haplotypes M, AD and AI and their sympatry in Europe, it has not been possible to assign haplotype to the European alleles that introgressed into Land-type *Phragmites*. For this reason, in Figure 1, the dotted line refers to high nuclear similarities among haplotypes, which likely imply extensive gene flow. In this case evidence against gene flow should be provided to exclude interbreeding.

Another interesting case suggesting gene exchange is given by the Greeny 2 genotypes of haplotype AD. Haplotype AD shares a mutation in the trnT-trnL region that appears exclusive to the native North American haplotypes, and shares the nuclear alleles with the Euroasiatic genotypes of haplotype M (Lambertini *et al.*, 2012a). Further investigations of this group could reveal another history of hybridization.

### **Why hasn't hybridization been detected previously?**

Since 2002, multiple papers have reported the failure to detect intra or interspecific breeding in the genus *Phragmites* (e.g., Saltonstall 2002, 2011; Meyerson *et al.*,

2010a,b) in the wild, despite evidence that it can occur (Meyerson *et al.* 2010a). Paul *et al.* (2010) detected possible hybrids in Canadian populations where native and introduced lineages are sympatric, but recombining alleles, providing evidence of interbreeding between the two lineages, have not been found. Recent work by Chu *et al.* (2011) and Lambertini *et al.* (2012a) has identified an explanation for this failure. Chu and colleagues detected hybrids between *P. japonicus* and *P. australis* in the sequences of the PhaHKT1 gene (high-affinity K<sup>+</sup> transporter gene). Lambertini *et al.* detected two hybridization events between *P. mauritanus* and *P. australis*, one where *P. mauritanus* is the seed parent (in the Gulf Coast and South America) and one where *P. australis* is the seed parent (in Senegal), in nuclear DNA fragments amplified by the grass-waxy gene primers. Introgression in *P. australis* in the Gulf Coast was recognized by distinguishing ancestral alleles, shared with the native populations, from newly evolved alleles, shared among haplotypes in the Gulf Coast areas but absent in the native populations and therefore likely acquired by gene flow (Lambertini *et al.* 2012a). Lambertini *et al.*'s approach, involving a large geographic and taxonomic sampling and the integration of several DNA sources, showed that microsatellite data alone may fail to detect hybridization.

The reason for this failure may be due to our reliance on the original set of microsatellite primers specifically developed by Saltonstall (2003) to study variation in the nuclear DNA of *P. australis* in North America. These markers were designed based on variation in the Euroasiatic introduced haplotype M (Saltonstall 2011) and therefore may not be optimally transferrable across species (Barbara *et al.* 2007) and across *Phragmites* haplotypes. Meyerson *et al.* (2010a) produced hybrids with native chloroplast but detected alleles from the Euroasiatic lineage using the

microsatellite primers but the same microsatellites did not detect native alleles when the hybrid had a chloroplast from the Euroasiatic lineage. Microsatellites specifically designed for the maternal and paternal lineages should optimally be combined to detect hybrids (Symonds *et al.* 2010). However, this will only increase support for hybridization hypotheses and will not provide compelling evidence, at least until a sufficiently wide part of the genome can be screened for hybridization. Other approaches, like the aforementioned PhaHKT1 gene or the grass-waxy primers, may work but more markers need to be developed to detect *Phragmites* hybrids. Until then, AFLPs appear to be a simple and low cost solution (Lambertini *et al.* 2006, 2008, 2012a, Kettenring and Mock, 2012) to evaluate hybridization on a case by case basis in combination with microsatellites or other nuclear markers. Technical advances to the protocol introduced by Vos *et al.* (1995) have presented new opportunities for data analysis (Bensch and Åkesson 2005; Meudt and Clarke 2007), among which are adaptations for the study of hybrids (Vela *et al.* 2011).

Another reason that microsatellites have failed to detect hybrids may be that polysomic variation (samples with more than two alleles at a microsatellite locus) has thus far been largely disregarded. Microsatellite software programs are mostly designed for diploid organisms, so three or more co-dominant alleles cannot be analyzed in two-entry matrices. Binary matrices are an alternative for the analysis of polysomic markers and a few programs for tetraploids have been developed (AUTOTET, Thrall and Young, 2000; TETRA, Liao *et al.* 2008; TETRASAT, Markwith *et al.* 2006); ATETRA, van Puyvelde *et al.* 2010) and for polyploids with different ploidy levels (PopDist, Guldbbrandsten *et al.* 2000). Given the different ways of handling heterozygotes, calculation of  $F_{st}$  statistics are determined according to

ploidy level and this should be taken into account when interpreting the results (van Tienderen and Meirmans, 2012). While difficult to analyze, polysomic variation may in fact provide evidence of hybridization. Polysomies reflect genomes of recent polyploid origin (which might include F1 hybrids and allopolyploids) that have not yet undergone diploidization (Otto and Whitton 2000) and/or that have somatic instability in chromosome number (Li *et al* 2010). An excellent review on polyploidy, hybridization, and invasion was recently published by te Beest *et al* (2012).

## **INTERBREEDING BETWEEN EUROPEAN AND NORTH AMERICAN *P.***

### ***AUSTRALIS***

Meyerson *et al.* (2010a) showed that no phenological or genetic barriers existed between the North American native and European (M) lineages when the populations were hand crossed. The recent work by Lambertini *et al.* (2012a) and the earlier evidence provided by Meyerson *et al.* (2010a) make the likelihood of conclusive evidence of wild hybrids of the North American and European lineages a near certainty. Saltonstall (2011) showed that despite multiple threats, the genetic diversity in extant populations of native *P. australis* in eastern North America is being maintained. However, it would be worthwhile to reanalyze these populations for evidence of gene flow using different molecular approaches.

## **CONCLUSIONS AND FORWARD LOOK**

Our understanding of the gene flow within and between species in the genus *Phragmites* is moving at a fast pace. The new approaches that have confirmed *Phragmites* hybridization in the Gulf Coast represent significant progress and promise to provide insights for *Phragmites* gene flow throughout North America.



While the east coast of North America is likely to be a focal point for research because of the extensive sympatry of North American native and Eurasian introduced *P. australis*, the Great Lakes region, the southwest and west coast deserve more attention. Furthermore, we do not yet have data on how hybridization will affect vigor, morphology and invasiveness of the introduced types or alter conservation strategies for the native *Phragmites* lineage but these clearly warrant additional investigations as highlighted by Schierenbeck and Ellstrand in their 2009 review of hybridization and invasion. In addition, there is a need to develop new markers for the different *Phragmites* species and lineages.

The lack of a published standardized classification system has resulted in a confused nomenclature. Several sequences are deposited in GenBank that are identified using letters that should indicate haplotype but do not follow the classification system implemented by Saltonstall (2002) and therefore are misleading and can be misinterpreted. In addition, often only one of the two sequences needed to identify *Phragmites* haplotypes is deposited (e.g., either trn-T or rbc-L) and no indication of the haplotype of the other sequence is provided in GenBank or in publications. Therefore, haplotypes already deposited in GenBank should be revised as needed and meta-data, such as information on the sample collection site, would be helpful.

Furthermore, *Phragmites* researchers must reach consensus on whether the microsatellite variations in the trnT-trnL and rbcL-psal regions that are frequently detected constitute new haplotypes (requiring a new labels) or whether the cp-microsatellite variants simply represent intra-haplotype variation. In the latter case,

these variants should also be consistently coded. Finally, developing an accessible common published classification system would greatly increase the understanding of *Phragmites* distribution and phylogeography worldwide. While Saltonstall and Lambertini (in prep.) have begun to examine this issue, contributions from the wider research community would make this effort more robust.

A revision of the taxonomic and systematic classification of *Phragmites* is also needed, but also needed are morphological characters and nuclear markers to describe and identify *Phragmites* hybrids. It is especially relevant to further investigate DNA variation within haplotypes, particularly within haplotype I which was recently shown to liberally hybridize (Lambertini et al. 2012a,b). These missing pieces of the puzzle are critical to ascertain the most appropriate classification system for species that readily interbreed and cannot be classified into separate species based on biological species concept (i.e., reproductive barriers, Mayr 1942).

The genus *Phragmites* is an excellent model system for studying ecology, evolution and species invasions and is particularly interesting from the perspective of inter- and intraspecific hybridization and reverse evolution. Dogged pursuit by researchers to solve the issues raised in this paper will yield insights and opportunities for future studies.

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### **CONTRIBUTIONS BY THE AUTHORS**

Meyerson and Lambertini contributed the greatest effort to early drafts of the manuscript but all authors contributed to the various revisions. All authors have seen and agreed to the submitted manuscript.

### **CONFLICTS OF INTEREST**

There are no conflicts of interest that we are aware of.

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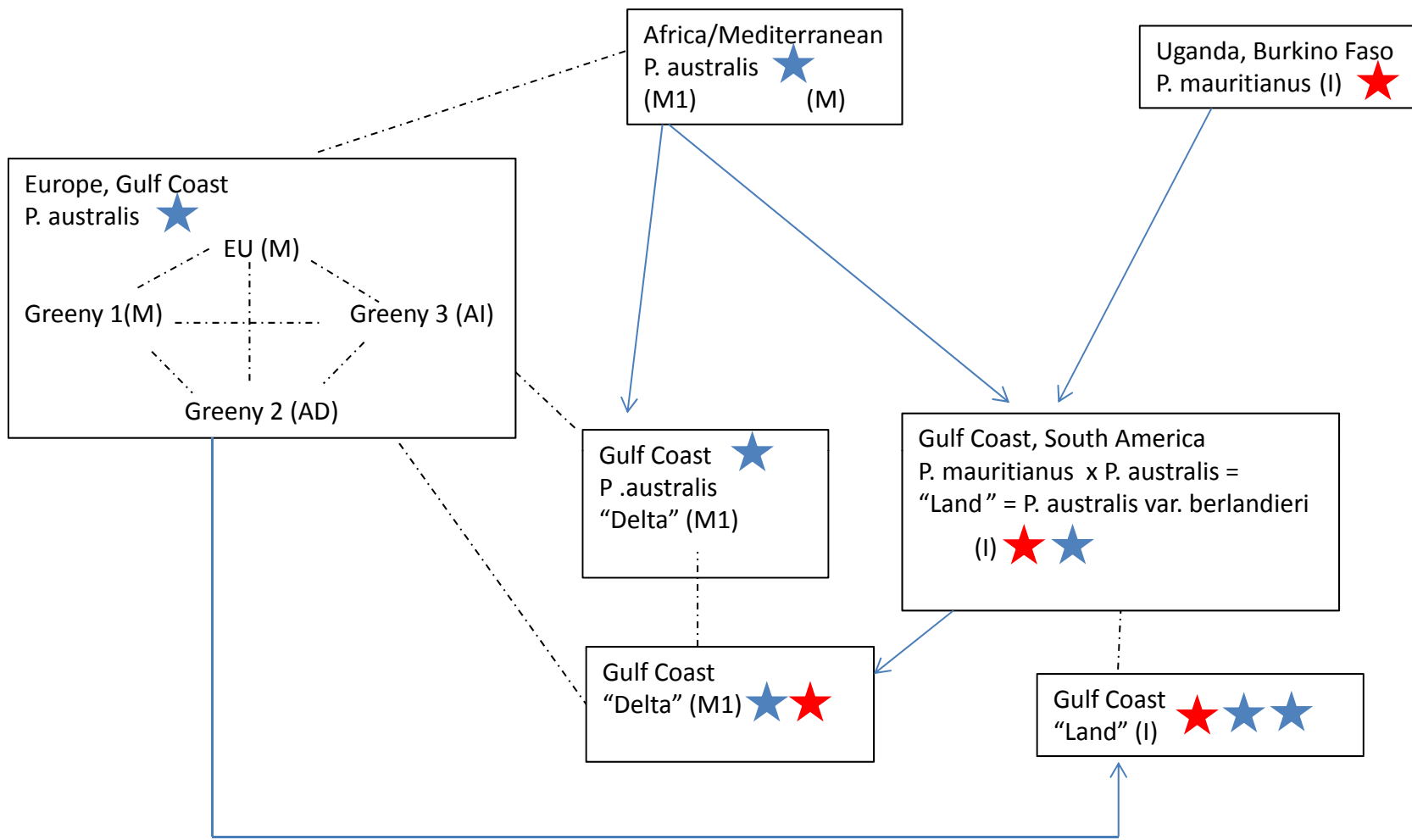
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**Figure 1. Hybridization of *Phragmites* in the Gulf Coast of the United States.**

The stars represent *P. mauritianus* (red) and *P. australis* (blue) nuclear alleles and indicate how they are recombined in the hybrids. Arrows indicate parent-offspring relationships and gene flow direction detected. Dashed lines refer to high nuclear similarities among lineages which likely imply extensive gene flow. In each box *Phragmites* species, haplotype and geographic location of populations involved in gene flow are indicated.



**Table 1 Identified types of *Phragmites australis* in North America.**

This table summarizes the origins and ranges of different haplotypes identified in the North American native, introduced and Gulf Coast lineages. Note, however, that some North American haplotypes are common and widespread, such as E, while others are relative rare and geographically localized, such as AB. The three “Greeny” *Phragmites* types have also been found in Europe but they may have originated elsewhere and also been introduced to Europe relatively recently. Question marks indicate “origin” is likely still under investigation. <sup>(1)</sup>Saltonstall 2002, <sup>(2)</sup> Meadows and Saltonstall 2007, <sup>(3)</sup> Hauber *et al.* 2011, <sup>(4)</sup> Lambertini *et al.* 2012, <sup>(5)</sup> Meyerson and Cronin in prep. Morphology of the different lineages is detailed in Swearington and Saltonstall 2010.

Common designation	Haplotype	Origin	North America Range
North American native	(A-H, S, Z, AA, AB, AC, E1/E2, E3, E4) <sup>1,2</sup>	North America	Widely distributed
North American introduced	M <sup>1</sup> , L <sup>5</sup>	Eurasia	Widely distributed
New European-related introductions to the Gulf Coast	M1 (Delta) <sup>3,4</sup>	Mediterranean Region (South Europe, North Africa, Middle East)	Mississippi “birdfoot” Delta, sporadically in Terrebonne Bay, LA and Grand Isle State Park, LA. Two samples in Florida
	Greeny 1 (M) <sup>4</sup>	Europe?	Atlantic Coast, Great Lakes, Mississippi “birdfoot” Delta
	Greeny 2 (AD) <sup>3,4</sup>	North America? Or Europe?	Mississippi “birdfoot” Delta
	Greeny 3 (AI) <sup>4</sup>	South Africa? Or Europe?	Mississippi “birdfoot” Delta
North American Gulf Coast	Land (I) <sup>4</sup>	South America (Colombia, Ecuador, Peru) or Africa (Uganda Burkina Faso, Senegal)	Gulf Coast Texas to Florida, South West (California).