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REVISITING *PHRAGMITES AUSTRALIS* VARIATION IN THE DANUBE DELTA WITH DNA MOLECULAR TECHNIQUES

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Abstract

The Danube delta hosts a very diverse population of *Phragmites australis* that has attracted the interest of generations of scientists. In this paper we focus on the genetic diversity of this population and how such variation is reflected in the morphology, cytology and physiological response of its genotypes. A crucial genetic trait that makes the *P. australis* population in the Danube delta a focal for the study of the evolutionary history of the genus *Phragmites* is its variation in chromosome numbers and ploidy levels co-existing next to each other. We review the literature from 1965 to today and explore the genetic relationships among the Romanian genotypes within the delta population, and within the *Phragmites* genus, on the base of molecular data obtained from chloroplast DNA sequencing and nuclear DNA markers (microsatellites and AFLPs). Even though cell size often increases with ploidy level, cytological variation appears not to be the only factor explaining differences in physiology and size between Romanian "fine" and "giant" common reeds. The presence of tall maternal lineages and their possibility to hybridize provides an alternative explanation to the phenotypic variation pattern observed in this region. A specifically designed study of genetic variation in the delta will reveal the genetic dynamics within this special population and the processes driving the evolution in the genus.

Keywords: *Phragmites australis,* Common reed, Danube delta, morphology, cytology, physiology, genetic diversity, polyploidy, hybridization

1. INTRODUCTION

Much of what we learn from text books about the ecology and diversity of the common reed (*Phragmites australis* Cav. (Trin) ex Steud., formerly *Phragmites communis* L.), is based on studies conducted in the Danube delta in the 1960s by Rudescu, Niculescu and Chivu (1965) and subsequently by Rodewald-Rudescu (1974), and by the cytologists Raicu, Staicu, Stoian and Roman (1970-1980) (Raicu et al., 1972; Gorenflot et al., 1972). The dominance of *P. australis* in the Danube delta (Covaliov et al., 2010) and elsewhere, has captured the interest of generations of scientists, who wanted to identify the reasons for its abundance and ecological success, and ways to exploit the biomass of this productive species (Haslam, 1972; Graneli, 1980; Kresovich et al., 1981; Meyerson, Vogt and Chambers, 2000).

Among the five species of the *Phragmites* genus, *P. australis* is the most widespread and is one of the few species of higher plants that can be regarded as cosmopolitan. *P. australis* is found wherever there is water, along the banks of rivers and lakes, in brackish coastal marshes and can tolerate also long drought periods. It is a perennial grass that propagates both by rhizomes and sexual reproduction. Pollen and seeds are wind dispersed. *P. australis* is cosmopolitan in both hemispheres and is only absent in polar regions. Given its wide distribution and ecological amplitude it is not surprising that *P. australis* shows morphological variation. A variation pattern, however, that is complex to understand and classify. An evident variable trait that has puzzled *Phragmites* scientists is "size", a quantitative character determined by the combination of genetics and the environment.

"Fine" and "giant" reeds are sympatric in the Danube delta and have been thoroughly investigated in the last 50 years. In the 1990s the Eureed project (Brix 1999) established a network of European scientists for the study of various aspects of *Phragmites* ecology, ecophysiology and genetics. Clonal replicates were obtained from *Phragmites* genotypes collected along a boreal-mediterranean and an oceanic-continental climatic gradient in Europe, and grown in common garden experiments throughout Europe (Clevering, 1999, Clevering et al., 1999, Bastlova et al, 2004; Hansen et al., 2007). Many of those clones have been kept in the live *Phragmites* collection at Aarhus University, which contains clones from all over the world and all *Phragmites* species in addition to European clones. The collection includes today about 200 clones and has been analyzed with different DNA markers in several studies at the global (Lambertini et al., 2006; Lambertini et al. 2012a) and at the population level (Lambertini et al., 2008; Lambertini et al. 2012b). In this study we focus on the genetic pattern of 18 Romanian clones in our collection that were collected from the Danube delta and propagated either by rhizome or from seeds. Because of the variation in chromosome number, the information provided by this set of Romanian samples is very valuable for understanding

polyploidization in *Phragmites* and the processes driving its evolution (Lambertini et al., 2012a). We here briefly review the current knowledge on morphological, cytological and physiological variation in Danube delta *Phragmites*, based on available information in the literature as well as unpublished data from the collection at Aarhus University. Given the complex pattern of genetic diversity in the Danube delta, this review highlights several important processes taking place in the Delta that deserve further research.

2. METHODS

We collected information about morphological and cytological diversity in the Danube delta from published literature and completed the genetic variation section with our own data. We also reviewed physiological studies. However, we considered only common garden experiments in order to focus on genetically determined differences and exclude the effect of environmental factors. Haplotypes were defined based on the variation in two cpDNA regions: *trn*T-*trn*L and r*bc*L-*psa*I following Saltonstall (2002) and Lambertini et al. (2012a). The protocols for the amplification and analysis of SSRs (single sequence repeats or microsatellites) and AFLPs (amplified fragment length polymorphism) are described in Saltonstall (2003) and Lambertini et al. (2006). The observations reported from the living *Phragmites* collection at Aarhus University are based on 10 years of monitoring. As the clones have grown under identical conditions for such a long time, morphological differences among clones are genetically determined.

3. RESULTS AND DISCUSSION

3.1 Morphological variation

Phragmites variation in morphology in the Danube delta is impressive and this has been interpreted in very different ways. In the sixties, Rudescu et al. described the phenotypes of 10 ecotypes of *P. communis* in the Danube delta (Rudescu et al, 1965). The ecotypes were defined from 10 biotopes differing in soil conditions, hydrologic regime, salinity and plant communities. *Phragmites* stands growing in flooded conditions were much taller than those growing in dry conditions and temporary flooded wetlands (biotopes 5, 6, 9, 10). However, *Phragmites* growing in brackish lagoons, were not as tall as those growing in freshwater, even though both were flooded (biotopes 7 and 8). Biotopes 9 and 10 were floating mats. Rodewald-Rudescu (1974) realized that further research was needed to understand if the different phenotypes were due to phenotypic plasticity, as evidenced by the experiments of Bjork (1967) in Sweden, or if they were due to genetic differences. An observation supporting genetic differences was that different forms of *Phragmites* were growing next to each other in the delta (Rodewald-Rudescu, 1974).

Taxonomically, the observed variation has been interpreted in radically different ways: from two species, five varieties and two formae, as reported by Rodewald-Rudescu (1974), to one single species when the name was changed from *P. communis* to *P. australis*. The classification at the varietal level is no longer used. However, it describes the variation in size and forms found in the Danube delta:
P. communis var. *gigantissima* (very giant)
P. communis var. *pseudodonax* (8-10 m tall with leaves up to 75 cm long)
P. communis var. *stolonifera* (creeping form typical of the beach)
P. communis var. *flavescens* forma *flavescens* (with yellow-brown inflorescences)
P. communis var. *flavescens* forma *rivularis* (with small leaves and growing on sand and in brackish water)
P. isiaca (5-6 m tall and with a distribution range in the Mediterranean region).

Today, *P. australis* is treated as one single species with two subspecies: *P. australis* ssp. *americanus* in North America and *P. australis* ssp. *altissimus* in the Mediterranean region, and one variety: *P. australis* var. *berlandieri* in the Gulf Coast of the United States. DNA molecular work by Saltonstall (2002 and 2003) and by Lambertini et al. (2006 and 2012a) has shown that there is more variation within *P. australis* than these groups, and that subspecies and varieties that were described before the molecular era can be recognized also genetically (Lambertini et al. 2012a). One such taxon is *P. australis* ssp. *altissimus* (very tall), previously *P. isiaca* or *P. communis* var. *isiaca*. The current known distribution of this subspecies is the Mediterranean region, including Southern Europe, North Africa and the Middle East and the Gulf Coast of the United States, where *P. australis* ssp. *altissimus* occurs as an invasive species (Lambertini et al., 2012b). Not only fine and giant common reeds are present in the Danube delta, but variation is present within both size types. Interestingly, the giant reeds of the Danube delta were classified in three groups (var.

gigantissima, var. *pseudodonax a*nd *P. isiaca*) (Rodewald-Rudescu, 1974), one of which might be *P. australis* ssp. *altissimus* (Lambertini et al., 2012).

3.2 Cytological variation

Raicu et al. (1972) explained the observed morphological differences in the Danube delta as differences in ploidy levels. Giant reeds of var. *gigantissima* were found to be octoploids (2n=8x=96), and fine reeds of var. *flavescens* were tetraploids (2n=4x=48). Morphologically, the two varieties were very different in size but both samples analysed by Raicu et al. were from floating mats, and hence belonging to the same biotope. Var. *gigantissima* was collected from a young floating mat, while var. *flavescens* was collected from an old mat (Raicu et al., 1972). Based on the analysis of the caryotype of these cytotypes, the authors suggested that the octoploid was likely due to recent chromosome doubling (autopolyploidy) of a tetraploid, as two identical sets of chromosomes were recognized, whereas the tetraploid was much older and likely of hybrid origin (allopolyploidy), because chromosomes could not be gathered into groups of four. Another observation made by these authors is that var. *gigantissima* was flowering later than var. *flavescens*. This was attributed to polyploidy as well, as cells with a bigger genome take longer to divide.

Previous work by Tavarnaschi (1948) found heptaploids in the delta (2n=7x). The more recent study by Clevering and Lissner (1999) using flow cytometry identified four ploidy levels, which the authors inferred to 2n=4x, 6x, 8x and 12x. Different ploidy levels co-occur in close proximity of each other in the delta (Hanganu et al., 1999; Pauca-Comanescu et al., 1999). Interestingly, seeds obtained from a single inflorescence (the same mother plant) produced seedlings with different ploidy levels (Pauca-Comanescu et al., 1999). This finding suggested a possible hybrid origin for the hexaploids from crosses between tetra and octoploids, as hexaploids frequently occur in mixed stands with tetra- and octoploids (Pauca-Comanescu et al., 1999). Hexaploid seeds proved to be viable (Pauca-Comanescu et al., 1999). Dodecaploids were found only among the seeds germinated in the greenhouse and not in the field. One such clone is in our collection. Morphologically, tetraploids, hexaploids and octoploids are not readily recognized in the field. However, octoploids are in general taller than tetraploids, with thicker shoots, larger leaves, larger panicles and thicker rhizomes (Pauca-Comanescu, 1999), but exceptions are frequent (giant tetraploids at Tataru 3-West, Hanganu et al., 1999). Hexaploids are not intermediate in size between tetra- and octoploids, but show traits of the tetraploid fine reeds (Hanganu et al., 1999; Pauca-Comanescu et al., 1999). At Comana lake both tetraploids and octoploids show variation in panicle size and flowering times, with the octoploids growing on the land starting flowering one week earlier than the neighboring tetraploids, and octoploids growing in deep water starting flowering 3-4 days after the tetraploids (Pauca-Comanescu et al., 1999) as also observed by Raicu et al. (1972).

In our experimental garden at Aarhus University, some of the octoploids from the Danube delta are among the tallest clones of our living collection at the end of the vegetative season in the autumn, together with South African octoploids (N=6) Mediterranean tetraploids (N=7), North American tetraploids and hexaploids from the Gulf Coast (N=17) and the species *P. mauritianus* (N=3), *P. frutescens* (N=2) and *P. karka* (N=3) (Fig. 1). Octoploids from the Far East and Australia (N=34) are neither taller than most of the tetraploids of the collection nor taller than hexa-, deca- and dodecaploids. This suggests that euploidy *per se* might not be the explanation to differences in size, as shown also by Achenbach et al. (2012). In addition, none of the tall clones set seeds in Denmark, suggesting adaptations to warmer climates.

3.3 Physiological variation

Since stomatal density varies with cell size (Hetherington and Woodward 2003), and since the size and the number of stomata directly affect photosynthesis, polyploidization could potentially aid increased rates of gas-exchange. Hansen et al. (2007) found that guard cell length and stomatal density can be indicators of ploidy level in Romanian *P. australis* clones, longer guard cells and lower stomatal density suggesting higher ploidy levels. However, Saltonstall (2007) found that differences in guard cell size and stomatal densities were indicators of *Phragmites* subspecies and not genome size. Also, Hansen et al. (2007) found no correlation between the light-saturated rates of photosynthesis (P_{max}) and ploidy level of Romanian clones cultivated in Denmark, since P_{max} of octoploids was low compared to tetra- and hexaploids. Moreover, also rubisco activities and chlorophyll concentrations were uncorrelated with ploidy level. Achenbach et al. (2012) compared plant size and physiological traits between European and Asiatic P. *australis* clones in a common garden experiment, each geographic range represented by different ploidy levels. Although the Danube delta octoploid was still among the tallest genotypes, considerable variation in size and physiological

response was observed within each ploidy level and, interestingly, among the clonal replicates of each genotype. A Czech octoploid was the shortest among the clones investigated and an Asiatic tetraploid was comparable in size with the giant octoploid of the Danube delta. Despite its size, the Danube delta octoploid was not superior in photosynthetic activity (Achenbach et al., 2012). Eller and Brix (2012) found significant physiological differences between a tall genotype from Algeria and a short genotype from Denmark grown in a common environment, however both genotypes were tetraploids. Nguyen et al. (2011) attributed common-garden physiological differences among *Phragmites* lineages in the US Gulf Coast to their phylogeographic relationships. It is broadly proved that physiological differences among genotypes are genetically determined (Hansen et al., 2007; Howard et al., 2008; Mozder and Zieman, 2010, Achenbach et al., 2012, Eller and Brix 2012). However, DNA variation and phylogeographic relationships appear more important than ploidy level and cell size to explain this variation.

3.4 Genetic variation

3.4.1 Chloroplast DNA (cpDNA)

While nuclear DNA is a recombination of the parents´ DNA, chloroplast DNA is an exclusive maternal inheritance and can indicate dispersal pathways within species. Six different cpDNA-haplotypes (haplotypes) were found in the Danube delta among the 18 samples in our collection (Table 1; Lambertini et al. 2012a).

Haplotype M is the most frequent in the Danube delta, like in the rest of Europe (Lambertini et al., 2012a) and in North America where it occurs as an invasive species (Saltonstall, 2002). Haplotype M1 is a cp-microsatellite variant of haplotype M, as it differs from haplotype M only in a single repeat at a microsatellite locus in the *trn*T-*trn*L region. Haplotype M1 (or Delta-type, Lambertini et al. 2012b) has the genetic fingerprint of Mediterranean P. *australis* ssp. *altissimus* (Lambertini et al. 2012a) and has been introduced to the Mississippi River delta, where it is the dominant type of *Phragmites* (Hauber et al., 2011; Lambertini et al., 2012b). Both Mediterranean *Phragmites* and the Delta-type in the Mississippi River delta are very tall.

Haplotype AI shares the *trn*T-*trn*L sequences with Haplotype K, from which it differs in a single nucleotide substitution in the *rbc*L-*psa*I region. Haplotype K is frequent in South Africa (Southafrica, Namibia and Botswana). Haplotype AI (or Greeny3-type, Lambertini et al., 2012b) has been introduced to the US Gulf Coast (Lambertini et al., 2012b). The name refers to the special blue-green color of the leaves. The South African genotypes of Haplotype K in our collection are all octoploids and very tall, whereas the Greeny3-type in the Mississippi River delta is not exceptionally tall, but has very broad leaves. Ploidy level for the Greeny3-type in the Mississippi River delta is unknown.

Haplotype L1 is frequent in Northern Europe (Lambertini et al., unpublished data). It shares the *trn*T-*trn*L sequences with *P. mauritianus* (tropical Africa), *P. frutescens* (Mediterranean) and *P. karka* (tropical Asia), but differs from these species in the *rbc*L-*psa*I region.

Haplotype AJ and AK differ from haplotype M in substitutions in the *rbc*L-*psa*I region. Haplotype AK has so far been found only in Romania, whereas haplotype AJ has been found in Romania and Turkey (Lambertini et al., 2012a).

With the exception of those haplotypes of which we have only one genotype, cytological variation occurs within all maternal lineages in the Danube delta (Table 1), indicating that polyploidization has occurred several times. The seedlings with different ploidy levels obtained from seeds of the same panicle (Pauca-Comanescu, 1999; now in our collection) have the same haplotype, confirming that one single genotype can produce seeds with different ploidy levels. This implies that cytological variation is produced locally in the Danube delta by sexual reproduction.

3.4.2 Nuclear DNA

Independently from ploidy level, polyploidization is followed by genomic and chromosomic re-arrangements which re-establish a diploid chromosome set (diploidization), which is the most suitable chromosome set for cell division (Soltis and Soltis, 1993; Otto and Whitton, 2000; Meimberg et al., 2009). One or two alleles are most frequently observed at the nuclear microsatellite loci of *Phragmites* worldwide, indicating that diploidization has occurred in *Phragmites* a very long time ago. Bivalents at meiosis were observed by Raicu et al., (1972), Gorenflot et al. (1979) and Gaudreault et al. (1989). However, individuals showing polysomic variation (more than two alleles) are present in almost every population (Saltonstall, 2003; Fer and Hroudova, 2009, Paul et al., 2011; Hauber et al., 2011; Lambertini et al. 2012b, Kettenring

and Mock, 2012). There are regions, like the Danube delta, in which polysomies are very frequent (Table 1) suggesting recent polyploidization events. Also Raicu et al. (1972) recognized old and new caryotypes in the Danube delta. Other regions in which polysomic variation is frequent are the Mississippi River delta, where different *Phragmites* species and haplotypes co-exist and interbreed (Lambertini et al., 2012b), the Mediterranean region where interspecific hybridization is also suspected to occur (Lambertini et al., 2012a) and Sakhalin Island in North East Russia where another mixed cytotype population (2n=4x, 6x, 8x, 10x), is present.

If we look at the microsatellite pattern of the Romanian siblings we can identify alleles shared among siblings and alleles that are unique to single individuals. As we do not know the mothers´ microsatellite pattern, we cannot distinguish maternally and paternally inherited alleles, nor if the siblings are the result of outcrossing or self-pollination. *Phragmites* is partially self-compatible (Ishii and Kadono, 2002, Lambert and Casagrande, 2007; Kettenring *et al.,* 2011). However, we expect a different pattern of relationships in the case of outcrossing versus selfing. In the case of selfing, siblings would be closely related whereas a more variable pattern of relationships would be observed in the case of outcrossing, as pairwise genetic distances among seedlings vary as a function of the genetic similarities of the parents and the extent of recombination.

Our previous studies show that the Danube delta siblings are genetically more similar to genotypes from other countries than to their own relatives in the delta. This is true for both microsatellite and AFLP alleles (Lambertini et al. 2006; Lambertini et al., 2012a). In addition, the mixed cytotype population at Razim Lake is not isolated within Europe (Lambertini et al. 2012a), nor different in the extent of genetic diversity and allelic composition from 8 populations in the Po Plain (Italy) (Lambertini et al., 2008) suggesting gene flow rather than reproductive isolation caused by selfing (Zohary, 1997). However, several of the genotypes from the Danube delta do not set seeds in Denmark because they start flowering very late in the summer or do not flower at all (Table 1). This might be due to climatic barriers, but also to cytologic/genomic incompatibilities. The siblings are likely the viable F1 progeny of inter-ploidy crosses and might therefore be partially or completely sterile. When seeds are viable, the F1 generation can be dispersed and established in the populations, providing evidence of gene flow, though F1 individuals might be sterile. *Phragmites australis* is a perennial species and isolation patterns can be difficult to detect in a short time frame. Interestingly, the other genotypes which do not set seeds in our collection belong to genetically differentiated groups (Lambertini et al., 2012a).

4. CONCLUSIONS

The Danube delta hosts a very complex population of *Phragmites* genotypes. The cp-DNA sequences indicate that several maternal lineages are sympatric in the region and some of them might not be native to temperate Europe. Haplotype M1 (or Delta-type) is likely originating in the Mediterranean region and the evolutionary story of haplotype AI (Greeny3-type) is linked to that of a population in South Africa (Lambertini et al. 2012a and b). Ploidy level appears not to be the only reason explaining differences in plant size, as both the Mediterranean and South African populations are very tall, irrespective of their ploidy level. The Danube delta shares also several similarities with the *Phragmites* population in the Mississippi River delta. This might be due to natural or human-aided migrations between the two populations, but also to wider ranges in the distribution of certain *Phragmites* lineages.

The polysomic variation found in the Danube delta indicates the presence of non-pairing chromosomes at cell division. This suggests hybridization between very different genomes. We have too few genotypes from the delta to understand which are the genomes involved, though we have evidence that more haplotypes are sympatric. We have also a collection of siblings obtained from panicles collected in the field. We don´t know how frequent these allelic combinations are in the wild population.

A more thorough study of Danube delta *Phragmites* is necessary to understand the genetic dynamics within this complex population. A phenological study can indicate anomalies at cell division, as well as the occurrence of non-native genotypes. Experimental crossings will reveal gene flow barriers and opportunities, and DNA markers will put the puzzle together and explain the history of this very special population. Finally, gene expression studies can clarify the roles of hybridization and polyploidy, i.e. if gene expression levels are (or are not) additive, and how phenotypic plasticity in ecophysiological traits is affected (te Beest et al. 2011). In light of the fragmented information that we could obtain from the DNA of eighteen genotypes in our collection, we are overwhelmed by how far our colleagues could reach forty years ago without molecular tools.

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REFERENCES

Achenbach L, Lambertini C., Brix H. 2012. Phenotypic traits *of Phragmites australis* clones are not related to ploidy level and distribution range. *AoB Plants*, doi: 10.1093/aobpla/pls017.

Bastlová D, Cižková H, Bastl M, Kvet J. 2004. Growth of *Lythrum salicaria and Phragmites australis* plants originating from a wide geographical area: response to nutrient and water supply. *Global Ecology and Biogeography* 13: 259-271.

Bjork S. 1967. Ecological investigations of *Phragmites communis*. Studies in theoretic and applied limnology. *Folia Limnologica Scandinavica*. Lund 1967, CWK Gleerup Lund, Einar Munksgaard Copenhagen, Oslo University Press Oslo.

Brix, H. 1999. The European research project on reed die-back and progression (EUREED). *Limnologica* 29: 5-10.

Covaliov S, Doroftei M, Hanganu J. 2012. Danube Delta biosphere reserve (D.D.B.R.): reed dynamics within actual context. *AES Bioflux* 2: 69-79.

Clevering OA. 1999. Between- and within- population differences in *Phragmites australis*. The effects of nutrients on seedling growth. *Oecologia* 121: 447-457.

Clevering OA, Lissner J. 1999. Taxonomy, chromosome numbers, clonal diversity and population dynamics of *Phragmites australis*. *Aquatic Botany* 64: 185-208.

Clevering OA, Brix H, Lukavská J. 2001. Geographic variation in growth responses in *Phragmites australis*. *Aquatic Botany* 69: 89-108.

Eller FP, Brix H. 2012. Different genotypes of *Phragmites australis* show distinct phenotypic plasticity in response to nutrient availability and temperature. *Aquatic Botany* (in press).

Fér T, Hroudová Z. 2009. Genetic diversity and dispersal of *Phragmites australis* in a small river system. *Aquatic Botany* 90: 165-171.

Gaudreault S, White DA, Hauber DP. 1989. *Phragmites australis*: an analysis of reproductive differences in two adjacent populations in the Mississippi River delta. *American Journal of Botany* 76: 103.

Gorenflot R, Raicu P, Cartier D, Ciobanu I, Stoian V, Staicu S. 1972. Le complexe polyploide du *Phragmites communis* Trin. *Comptes rendus de l'Académie des Sciences*, 274 Serie D: 1501-1504.

Gorenflot R, Sanei-Chariat Panahi M, Liebert J. 1979. Le complexe polyploide du *Phragmites australis* (Cav.) Trin. ex Steud. en Iran. *Rev Cytol Biol Veget – Bot* 2: 67-81.

Graneli W. 1980. Energivass – Rapport Etapp II and III. Institute of Limnology, Lund University, Sweden. Code LUNBDS/(NBLI-3028)/1-37/ 1980, ISSN 0348-0798.

Hanganu J, Mihail G, Coops H. 1999. Responses of ecotypes of *Phragmites australis* to increased seawater influence: a field study in the Danube Delta, Romania. *Aquatic Botany* 64: 351-358.

Hansen DL, Lambertini C, Jampeetong A, Brix H. 2007. Clone-specific differences in *Phragmites australis*: Effects of ploidy level and geographic origin. *Aquatic Botany* 86: 269-279.

Haslam AS, 1972. *Phragmites* communis Trin. (Arundo *Phragmites* L., ? *Phragmites australis* (Cav.) Trin. Ex Steudel). *Journal of Ecology* 60: 585-610.

Hauber DP, Saltonstall K, White DA, Hood CS. 2011. Genetic Variation in the Common Reed, *Phragmites australis*, in the Mississippi River Delta Marshes: Evidence for Multiple Introductions. *Estuaries and Coasts* 34:851–862.

Hetherington A.M., Woodward F.I. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424: 901-908.

Howard R, Travis S, Sikes B. 2008. Rapid growth of a Eurasian haplotype of *Phragmites australis* in a restored brackish marsh in Louisiana, USA. *Biological Invasions* 10, 369-379.

Ishii J, Kadono Y. 2002. Factors influencing seed production of *Phragmites australis*. *Aquatic Botany* 72: 129-141.

Kettenring KM, McCormick MK, Baron HM, Whigham DF. 2011. Mechanisms of *Phragmites australis* invasion: feedbacks among genetic diversity, nutrients, and sexual reproduction. *Journal of Applied Ecology* 48: 1305-1313.

Kettenring KM, Mock KE. 2012. Genetic diversity, reproductive mode, and dispersal differ between the cryptic invader, *Phragmites australis*,and its native conspecific. *Biological invasions* doi: 10.1007/s10530-012-0246-5.

Kresovich S, Wagner CK, Scantland DA, Lawhon WT. 1981. Emergent aquatic plants: biological and economic perspectives. *Biomass* 1:127-144.

Lambert AM, Casagrande RA. 2007. Characteristics of a successful estuarine invader: evidence of self-compatibility in native and non-native lineages of *Phragmites australis*. *Marine Ecology Progress Series* 337: 299-301.

Lambertini C, Gustafsson MHG, Frydenberg J, Lissner J, Speranza M, Brix H. 2006. A phylogeographic study of the cosmopolitan genus *Phragmites* (Poaceae) based on AFLPs. *Plant Systematics and Evolution* 258: 161-182.

Lambertini C, Gustafsson MHG, Frydenberg J, Speranza M, Brix H. 2008. Genetic diversity patterns in *Phragmites australis* at the population, regional and continental scales. *Aquatic Botany* 88: 160-170.

Lambertini C, Sorrell BK, Riis T, Olesen B, Brix H. 2012a. Exploring the borders of European *Phragmites* within a cosmopolitan genus. *AoB Plants,* doi: 10.1093/aobpla/pls020.

Lambertini C, Mendelsshon IA, Gustafsson MGH, Olesen B, Riis T, Sorrell BK, Brix H. 2012b. Tracing the origin of Gulf Coast *Phragmites* (Poaceae) – a story of long distance dispersal and hybridization. *American Journal of Botany* 99, 538-551.

Meimberg H, Rice KJ, Milan NF, Njoku CC, McKay JK. 2009. Multiple origins promote the ecologicial amplitude of allopolyploid *Aegilops* (Poaceae). *American Journal of Botany* 7: 1262-1273.

Meyerson LA, Vogt KA, Chambers RM. 2000. Linking the success of *Phragmites australis* to the decoupling of ecosystem nutrient cycles. In M Weinstein, Kreeger D, eds. *Concepts and controversies of tidal marsh ecology*. Kluwer, 817-834.

Modzer TJ, Zieman JC. 2010. Ecophysiological differences between genetic lineages facilitate the invasion of non-native *Phragmites australis* in North American Atlantic coast wetlands. *Journal of Ecology* 98: 451-458.

Nguyen LX, Lambertini C, Brix H. 2011. Ecophysiological characteristics of different lineages of *Phragmites australis* in the Gulf Coast of North America. Book of abstracts, joint meeting of Society of Wetland Scientists, Wetpol and Wetland Biogeochemistry Symposium. 3-8 July 2011, Prague, Czech Republic. P-86, 450.

Otto SP, Whitton J. 2000. Polyploid evidence and evolution. *Annual Review of Genetics* 34: 401-437.

Pauca-Comanescu M, Clevering OA, Hanganu J, Gridin M. 1999. Phenotypic differences among ploidy levels of *Phragmites australis* growing in Romania. *Aquatic Botany* 64: 223-234.

Raicu P, Staicus S, Stoian V, Roman T. 1972. The *Phragmites australis* Trin. chromosome complement in the Danube delta. *Hydrobiologia* 39: 83-89.

Paul J, Kirk H, Freeland J. 2011. Genetic diversity and differentiation of fragmented reedbeds (*Phragmites australis*) in the United Kingdom. *Hydrobiologia* 665: 107-115.

Rudescu, L., Niculescu, C., Chivu, I.P. 1965. Monografia Stufului din Delta Dunarii, Editura Academiei Republicii.Socialiste, Romania.

Rodewald-Rudescu, L., 1974. Das Schilfrohr. E. Schweizerbartsche Verlagsbuchhandlung, Stuttgart, Germany.

Saltonstall K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the Natural Academy of Science USA* 99: 2445-2449.

Saltonstall K. 2003. Microsatellite variation within and among North American lineages of *Phragmites australis*. *Molecular Ecology* 12: 1689-1702.

Saltonstall K, Glennon K, Burnett A, Hunter RB, Hunter KL. 2007. Comparison of morphological variation indicative of ploidy level in *Phragmites australis* (POACEAE) from Eastern North America. *Rhodora*, 109: 415–429.

Soltis DE, Soltis PS. 1993. Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Sciences* 12, 243-273.

Tarnavaschi IT. 1948. Die Chromosomenzahlen der Antophyten – Flora von Rumaenien mit einem Ausblick auf das Polyploidie-Problem. Bul. Grad. Bot. Cluj.

te Beest M, Le Roux JJ, Richardson DM, Brysting AK, Suda J, Kubesova M, Pysek P. 2011. The more the better? The role of polyploidy in facilitating plant invasions. *Annual Botany*. doi:10.1093/aob/mcr277.

Zohary D. 1997. Speciation patterns in predominantly self-pollinated Mediterranean annuals. *Lagascalia* 19: 283-288.

Figure 1. *Phragmites* collection at Aarhus University. Differences in height are clearly visible from the picture. The tallest genotypes are Danube delta octoploids, Mediterranean tetraploids, South African octoploids, North American tetraploids and hexaploids from the US Gulf Coast, and the species *P. mauritianus*, *P. frutescens* and *P. karka*. As the clones are growing under identical environmental conditions, difference in size can be attributed to genetic differences between clones. Euploidy appears less important than genetic relationships as Australian octoploids are not taller than the majority of the remaining tetraploids that are homogenous in size.

Table 1. Genetic variation of *Phragmites australis* in the Danube delta. Samples are labeled according to our previous studies, so that the same sample reference indicates the same genotype throughout all our studies. Siblings are indicated by the same letter. We refer to Clevering and Lissner (1999) for ploidy levels inferred from flow cytometry. Chromosome counts where done by squash and staining. The number in brackets indicates the number of root tip cells that were isolated and chromosomes could be reliably counted. Chloroplast DNA haplotypes (cpDNA) are defined by the combination of *trn*T-*trn*L and *rbc*L-*psa*I sequences following Saltonstall (2002). All sequences are deposited in GenBank (Saltonstall, 2002; Lambertini et al., 2012a). In the nuclear DNA section (nDNA) we indicated the number of alleles amplified at each microsatellite locus (PaGT4, PaGT9 etc.). Polysomic patterns indicate genomes which did not undergo diploidization. Reproductive stage indicates how far the genotypes cultivated in Denmark can reach in the reproductive cycle. The stage is based on two years of monitoring in the period May-November. "Seeds" indicates that seeds were set but not dispersed at the last visit, whereas "dispersing" indicates that seeds were dispersing. "Flowering" indicates that only inflorescences were set. Missing data are blank.

Sample reference	Collection site	Siblings	Ploidy level inferred from flow cytometry	Chromosome counts	cpDNA			nDNA - polysomies								Reproductive stage in Denmark
					*trn*T-*trn*L	*rbc*L-*psa*I	Haplotype	PaGT4	PaGT8	PaGT9	PaGT11	PaGT14	PaGT16	PaGT21	PaGT22	
Pa81RO	Obretinu-Mare	A	8x		4a	4	M1									
Pa84RO	Oborny	B	6x		4	4	M									dispersing
Pa624RO	Obretinu-Mare	C	8x		4	21b	AJ									flowering
Pa625RO	Obretinu-Mare	D	6x		4	4	M									flowering
Pa643RO	Razim	E	4x		3	21b	AI									flowering
Pa647RO	Razim	F	6x		4	4	M									
Pa650RO	Razim	G	6x		4	4	M									flowering
Pa654RO	Razim	H	8x	89-96 (7)	4	22	AK				4			4	3	dispersing
Pa655RO	Razim	I	4x	42-47 (4)	4	4	M				4			3		dispersing
Pa651RO	Razim	L	6x	64-72 (5)	4	4	M	3		3		3		3		seeds
Pa652RO	Razim	L	8x	90-94 (6)	4	4	M	3								flowering
Pa656RO	Razim	M	6x	71-74 (3)	3	21b	AI				4					flowering
Pa657RO	Razim	M	4x	46-49 (4)	3	21b	AI				4			3		flowering
Pa658RO	Razim	N	6x	59-66 (9)	4	22	AK						3	3		flowering
Pa659RO	Razim	N	8x	95 (1)	4	22	AK	4					3	4		dispersing
Pa660RO	Razim	N	12x	146 (3)	4	22	AK	3			4		3	3	3	not flowering
Pa661RO	Obretinu-Mare	O	8x	72 (3)	5c	4	L1		3	3	3			3		not flowering
Pa662RO	Obretinu-Mare	O	4x	47-52 (3)	5c	4	L1				3			3		dispersing