Clone-specific differences in *Phragmites australis*: Effects of ploidy level and geographic origin

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**Abstract**

*Phragmites australis* (Cav.) Trin. ex Steud. is virtually cosmopolitan and shows substantial variation in euploidy level and morphology. The aim of this study was to assess clone-specific differences in morphological, anatomical, physiological and biochemical traits of *P. australis* as affected by the geographic origin, the euploidy level (4x, 6x, 8x and 12x), and to assess differences between native and introduced clones in North America. Growth, morphology, photosynthetic characteristics, photosynthetic pigments and enzymes were measured on 11 geographically distinct clones propagated in a common environment in Denmark. Any differences between the measured parameters were caused by genetic differences between clones.

Overall, the largest differences between clones were found in ontogeny, shoot morphology and leaf anatomy. The North Swedish clone was adapted to short growing seasons and sprouted very early in the spring but senesced early in July. In contrast, clones from southern regions were adapted to warmer and longer growing seasons and failed to complete the whole growth-cycle in Denmark. Some clones from oceanic habitats with climatic conditions that do not differ much from conditions at the Danish growth site did flower in the common environment.

The octoploid genotype in general had larger dimensions of leaves, taller and thicker shoots and larger cell sizes than did the hexaploid and tetraploid clones. The dodecaploid clone was neither bigger than the octoploid, nor significantly different from tetraploid and hexaploid clones in most of the morphological characters observed. Stomatal density decreased with increasing ploidy level, while length of guard cells increased. Tetraploid clones generally had morphometric dimensions, similar to hexaploids. Hence, polyploidy did not always result in an increase in plant size, probably because the number of cell divisions during development is reduced.

Four North American clones were included in the study. The clone from the Atlantic Coast and the supposed invasive European clone resembled each other. The Gulf Coast clone differed from the rest of the clones in having leaf characters resembling *Phragmites mauritianus* Kunth. Thus, morphological characters are not unmistakable parameters that can be used to discriminate between introduced and native clones.

The physiological and biochemical processes also differed between clones, but these processes showed considerable phenotypic plasticity and were therefore very difficult to evaluate conclusively.

It is concluded that *P. australis* is a species with very high genetic variability which is augmented by its cosmopolitan distribution, clonal growth form and the large variation in chromosome numbers. It is therefore not surprising that large genetically determined differences in ontogeny, shoot morphology and leaf anatomy occur between clones.

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**Keywords:** Common reed; Exotic; Genotypic variation; Invasion; Phenotypic plasticity; Photosynthesis; *Phragmites australis*; Polyploidy; Wetland

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**1. Introduction**

The common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) is a perennial grass with perhaps the largest geographical distribution of any flowering plant in the World (Brix, 1999; Clevering and Lissner, 1999). *P. australis* is a clonal plant with annual stems that develop from a system of rhizomes which also function in the vegetative spreading of the plant. The species has a wide ecological amplitude and is very variable in morphology and shows plastic responses to growth conditions (Hanganu et al., 1999; Kühl et al., 1999; Lessmann et al., 2001). Significant morphological differences have been found both between different populations of *P. australis* and between different clones within the same population, irrespective of site conditions (Björk, 1967; Clevering, 1999; Rolletschek et al., 1999; Pauca-Comanescu et al., 1999; Clevering et al., 2001). Part of the
Clone-specific variability in *Phragmites australis* can be attributable to differences in chromosome number. A euploid range of 3x, 4x, 6x, 7x, 8x, 10x, 11x and 12x (with x = 12) has been found for this species, with tetraploids (2n = 48) and octoploids (2n = 96) being the most frequently observed (Clevering and Lissner, 1999). Shoots of octoploids are generally longer and thicker and have larger leaves than those of tetraploids. Octoploid reeds sometimes are referred to as ‘giant’ reeds and tetraploid reeds as ‘fine’ reeds (Hanganu et al., 1999; Pauca-Comanescu et al., 1999; Clevering et al., 2001). This relationship between euploidy level and morphology is common because the most immediate and universal effect of polyploidy is an increase in cell size. However, polyploidy does not always lead to an overall increase in the plant size, since a common effect of polyploidy is also a reduction in the number of cell divisions during development (Stebbins, 1971).

It has been suggested that genetic variation among populations from different geographic regions has arisen as a result of growth in different climatic environments (Clevering et al., 2001). Along a latitudinal gradient gradual change occur in the relative day length, amount of solar radiation and air temperature. Also, differences occur between oceanic and continental habitats. In oceanic habitats, differences between summer and winter are generally smaller than in continental habitats. *Phragmites australis* populations originating from different geographic regions along a latitudinal gradient from Northern Sweden to Spain differ in time of cessation of growth, shoot morphology and biomass allocation (Clevering et al., 2001). Grown under the same environmental conditions *P. australis* originating from higher latitudes started to grow earlier than southern populations, but finished growth early in the season. The southern populations failed to complete the whole growth-cycle before the first frost and did not develop mature seeds. *Phragmites australis* is considered native to North America; however, over the last 200 years an aggressive expansion has occurred in coastal wetland communities where it has out-competed other native species of the wetland communities. The invasion is taking place particularly along the Atlantic Coast and in the Mississippi delta region of the Gulf of Mexico (Chambers et al., 1999). This invasion can be attributable to the effective vegetative spread of *P. australis*, colonizing habitats it is unable to invade directly with seeds or rhizomes (Amsberry et al., 2000). Studies by Saltonstall (2002) have however, documented that a non-native type of *P. australis* is responsible for the observed spread. Based on genetic studies of *P. australis* populations from all over the world, 11 haplotypes were found to be unique to North America and were therefore considered to be native to this part of the continent. Two haplotypes (I and M) had a wide distribution on multiple continents, and haplotype M is the most common type in North America today. Haplotype I was distributed along the Gulf Coast. Comparing herbarium samples collected before 1910 with modern samples revealed that haplotype M has spread along the Atlantic Coast and westward displacing native types and expanding to regions previously not known to have *P. australis* (Saltonstall, 2002).

Genetic analyses have shown that this introduced clone is more closely related to European clones than to native North American groups (Saltonstall, 2003), and it is therefore considered to be introduced from European populations.

The aim of the present study was to assess clone-specific differences in morphological, anatomical, physiological and biochemical parameters of *P. australis* in order to identify and quantify differences in traits that can be associated with the

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### Table 1

Designation of *Phragmites australis* clones used in the study and information about their origin, ploidy level, colour of stem, time of flowering and leaf sheaths during senescence

<table>
<thead>
<tr>
<th>Clone</th>
<th>Sample label</th>
<th>Origin</th>
<th>Group</th>
<th>Ploidy level</th>
<th>Colour of stem</th>
<th>Time of flowering</th>
<th>Leaf sheaths during senescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO4x</td>
<td>Pa 657 RO</td>
<td>Romania, Lake Razim</td>
<td>4x</td>
<td>Green, green and red to brown at stem base</td>
<td>–</td>
<td>Lower sheaths fall off</td>
<td></td>
</tr>
<tr>
<td>RO6x</td>
<td>Pa 650 RO</td>
<td>Romania, Lake Razim</td>
<td>6x</td>
<td>Green, red to brown at stem base.</td>
<td>–</td>
<td>Stay on stem</td>
<td></td>
</tr>
<tr>
<td>RO8x</td>
<td>Pa 661 RO</td>
<td>Romania, Lake Obretin</td>
<td>8x</td>
<td>Green.</td>
<td>–</td>
<td>Stay on stem</td>
<td></td>
</tr>
<tr>
<td>RO12x</td>
<td>Pa 660 RO</td>
<td>Romania, Lake Razim</td>
<td>12x</td>
<td>Green, red to brown at stem base</td>
<td>–</td>
<td>Lower sheaths fall off</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>Pa 637 SE</td>
<td>Sweden, Gammelstaden</td>
<td>4x</td>
<td>Green</td>
<td>–</td>
<td>Stay on stem</td>
<td></td>
</tr>
<tr>
<td>IL</td>
<td>Pa 90 IL</td>
<td>Israel, Yerokham, Negev Highlands</td>
<td>4x</td>
<td>Green</td>
<td>–</td>
<td>Stay on stem</td>
<td></td>
</tr>
<tr>
<td>ES-MW</td>
<td>Pa 72 ES</td>
<td>Spain, Gallocanta N United States, Utah, Midwest</td>
<td>4x</td>
<td>Green</td>
<td>Middle of August</td>
<td>Stay on stem</td>
<td></td>
</tr>
<tr>
<td>US-MW</td>
<td>Pa 111 US</td>
<td>United States, Utah, Green River</td>
<td>4x</td>
<td>Green and red to brown.</td>
<td>–</td>
<td>Lower sheaths fall off</td>
<td></td>
</tr>
<tr>
<td>US-AC</td>
<td>Pa 190 US</td>
<td>United States, NY, Buffalo, Orchard Park Atlantic Coast</td>
<td>4x</td>
<td>Red to brown at stem base</td>
<td>–</td>
<td>Lower sheaths fall off</td>
<td></td>
</tr>
<tr>
<td>US-GC</td>
<td>Pa 100 US</td>
<td>United States, Alabama, Mobile Gulf Coast</td>
<td>?</td>
<td>Green and red to brown at stem base</td>
<td>–</td>
<td>Lower sheaths fall off</td>
<td></td>
</tr>
<tr>
<td>US-INTR</td>
<td>Pa 193 US</td>
<td>United States, Virginia, Upshur Creek Introduced</td>
<td>4x</td>
<td>Green, green and red to brown at stem base</td>
<td>Middle of September</td>
<td>Stay on stem</td>
<td></td>
</tr>
</tbody>
</table>

* Sample labels as presented by Lambertini et al. (2006).

* Group refers to the four American groups (see text).
different growth dynamics of the species in various environments. How are morphology and growth affected by (i) the geographic origin of the clone, (ii) the euploidy level of the clones; and (iii) what are the differences between an introduced non-native and three native North American clones?

2. Materials and methods

2.1. Plant material

The clones used in this study (Table 1) were chosen from a large collection of live *P. australis* clones that are kept in a common environment plot at Aarhus University, Denmark (56°13′N; 10°07′E). The choice of clones was based on their geographic origin and their genetic relationships as reported by Saltonstall (2003) and Lamberti et al. (2006). The clones have been kept in the common environment for at least 5 years prior to the experiment. To study differences between ploidy levels, four Romanian clones from the same area in Romania with ploidy levels of 4x, 6x, 8x and 12x (Clevering and Lissner, 1999) were chosen. To study differences between geographic regions of origin clones from North Sweden, Spain, Israel, Romania and North America were chosen. Three North American clones were believed to be native genotypes and belong to the Midwest (US-MW), the Atlantic Coast (US-AC) and the Gulf Coast (US-GC) groups, whereas a fourth clone was believed to be an introduced clone (US-INTR) of European origin according to the genetic investigations of Saltonstall (2003) and Lamberti et al. (2006).

2.2. Experimental set-up

At the end of March 2004 rhizome blocks of approximately 15 cm × 15 cm of the selected clones were transplanted into 65 l plastic tanks (diameter 60 cm, height 30 cm) containing about 45 l river sand. The tanks were dug into the ground to minimize temperature fluctuations. Three replicates were prepared of each clone and placed in three rows in an experimental plot with a distance of about 2.5 m between individual tanks. After transplanting the substrates were kept waterlogged by an automatic watering system. From May 5, the plants were fertilized weekly with 100 ml of nutrient solution containing 10 g of macronutrients: Tot-N: 19.3%, NO₃-N: 11.9%, NH₄-N: 7.4%, P: 2.3%, K: 15.4%, Mg: 3.0% (Pioner NPK Makro 19-2-15 + Mg, Brøstø, Lyngby, Denmark) and 1% of a micronutrient solution (Pioner Makro with iron, Brøstø).

2.3. Environmental conditions

Ambient climatic conditions were measured at a nearby weather station and showed that the average air temperatures in June, July and August were 13.1 ± 1.8, 14.6 ± 1.6 and 17.7 ± 2.8 °C, respectively. The first frost occurred in mid-November. The monthly precipitation during May until August was higher than normal (80–92 mm/month). Soil temperature was measured at 12 cm depth every 2–4 weeks throughout the study and varied between 12.5 ± 0.3 °C in May to 21.4 ± 0.6 °C in August. In November, the soil temperatures dropped as low as 1.0 ± 0.3 °C.

2.4. Seasonal development and shoot morphology

Number of shoots per tank, shoot length and number of leaves per shoot were measured every 2–4 weeks during the growing season. Shoot length and number of leaves were measured on 10 tagged shoots per replicate. Shoot length was measured from the shoot base to the apex of the youngest leaf. The number of leaves per shoot included all fully developed leaves.

When shoot length did not increase further it was assumed that the clones had reached their maximum biomass. For the Swedish clone maximum shoot length was reached by the end of June whereas the rest of the clones continued growing until the end of October. At maximum biomass the three tallest shoots per replicate were harvested and their morphological traits (growth form, stem colour, stem texture, colour of leaves, various characteristics of inflorescences and leaf sheaths) were noted. Leaves were excised immediately after harvesting and kept in plastic bags with moist paper towels to avoid leaf rolling until arrival at the laboratory. Leaves were weighed and photocopied, and total leaf area of each shoot calculated using the area/weight ratio of the photocopies. Shoot length, basal diameter, number of nodes, number of live and dead leaves (dead defined as leaves with more than 50% of the blade dead or discoloured), and length and width (cm) of the largest leaf were measured for each shoots. Basal diameter was measured with a calliper between the two lowest nodes. Afterwards shoots were fractionated into stem, leaf blades and leaf sheaths. All were dried at 105 °C to constant weight and dry weight (g) was determined.

Subsequent to harvesting three shoots per tank were tagged to monitor leaf senescence. The numbers of live and dead leaves were counted weekly or biweekly until all leaves were dead or discoloured.

2.5. CO₂-exchange

Photosynthesis of the third or fourth youngest leaf was measured at maximum biomass with an ADC LCA-4 infrared gas analyzer equipped with a Leaf Microclimate Control System (ADC BioScientific Ltd., UK). The PLC-4 leaf chamber was air-conditioned at 20 °C and placed on a tripod to ensure stability during readings. The leaf chamber was supplied with atmospheric air drawn from a height of 5 m above ground at 300 ml min⁻¹ using an external air pump. Light was supplied from a white halogen source (Portable Light Unit, type PLU-002, ADC BioScientific Ltd.) at a photosynthetic photon flux density (PPFD) of approximately 2000 μmol m⁻² s⁻¹.

Light response curves were prepared by measuring net CO₂ assimilation at a range of light intensities. Neutral filters were placed between the light source and leaf chamber to regulate irradiance level. The measurements began at the highest light intensity (2000 μmol m⁻² s⁻¹) and were step-wise lowered to approximately 1000, 160, 100, 80, 50 and 8 μmol m⁻² s⁻¹. At
the three lowest light intensities rate of air supply was lowered to 200 ml min$^{-1}$. Apparent quantum efficiency ($\phi_i$), dark respiration ($R_d$) and light compensation point ($L_c$) were estimated from the linear part (PPFD: 0–100 $\mu$mol m$^{-2}$ s$^{-1}$) of the light response curves using linear regression analysis. Maximum rate of photosynthesis ($P_{\text{max}}$) was calculated as the average of five readings at the highest light intensity (PPFD 2000 $\mu$mol m$^{-2}$ s$^{-1}$).

Photosynthesis was determined by measuring light-saturated net CO$_2$-assimilation at normal atmospheric (21%) and reduced (2%) O$_2$-pressure. An O$_2$-pressure of 2% was obtained by mixing atmospheric air and N$_2$-gas using a gas mixer (ADC BioScientific Ltd.). Normal atmospheric O$_2$-pressure was obtained by only letting atmospheric air through the gas mixer. CO$_2$ was supplied from a pressurized gas tank and controlled at 380 vpm by the microclimate control system. The CO$_2$-assimilation rate was first determined at 21% O$_2$ and afterwards at 2% O$_2$. Light response curves and photosynthesis were measured on two to three leaves per replicate.

2.6. Anatomical measurements

Stomatal density and length of guard cells were determined on the third or fourth youngest leaf. Three leaves per replicate were harvested in September, fixed in 70% ethanol for several days and then hydrated by placing them in 70, 50, 25 and 0% ethanol sequentially, for at least 2 h in each solution. The leaves were cleared by Fuchsia-KOH for 5 days at 60°C, then rinsed in water and dehydrated by placing the leaves in water, then in 70, 96 and 99% ethanol, sequentially. Finally, leaves were embedded in Euparal. Stomatal density and length of guard cells on both upper (adaxial) and lower (abaxial) side of leaves were determined under a 40x light transmission-microscope. Stomatal density (SD) was calculated as number of stomata per unit area of one leaf surface. Stomatal index (SI) was calculated as number of stomata per unit leaf area divided by the number of epidermal cells plus guard cells per unit leaf area multiplied by 100 (Willmer and Fricker, 1996).

2.7. Biochemical analyses

Concentrations of chlorophyll$_{a+b}$, and total carotenoids in the third youngest leaves were analyzed after extraction with 96% ethanol and spectrometry according to Wellburn and Lichtenwalner (1984). Dry weight and surface area of leaves were determined before analysis and concentrations of pigments were expressed in $\mu$g cm$^{-2}$ leaf.

One of the third youngest leaves per replicate was harvested for analysis of non-activated and total rubisco and PEPcase activity. The harvesting took place in the light and the entire leaves were frozen in liquid nitrogen immediately after excision. The frozen plant material was ground in a mortar containing liquid nitrogen. Approximately one-half laboratory spoon of homogenate was transferred to a chilled mortar and ground in 5 ml extraction buffer containing 50 mM Bicine (pH 8), 0.1 mM EDTA-Na$_2$, 10 mM MgCl$_2$, 5 mM DTT, 10 mM isoascorbate and 2% (w/v) PVP. Non-activated rubisco and PEPcase were determined in an assay solution consisting of 50 mM Bicine (pH 8), 0.1 mM EDTA-Na$_2$, 10 mM MgCl$_2$, 5 mM DTT, 17.85 mM NaH$_4$CO$_3$ and 50 $\mu$l RuDP or PEP, respectively. The reaction was initiated by adding ground extract and was stopped after 60 s with 6 M HCl. Total rubisco and PEPase activities were determined in an assay solution similar to the above minus RuDP or PEP, initially. After adding ground extract vials were incubated for 5 min before activation with 5 mM RuDP or 5 mM PEP. The reaction was stopped after 60 s with 6 M HCl. Assay reactions were carried out at 20°C in a total volume of 500 $\mu$l using 20 ml vials. Samples were dried at 55°C for 2 days and afterwards re-dissolved in two drops of 6 M KOH and 1.2 ml ultra-filtered water. The amount of radioactive decay energy was measured by a scintillation counter (Liquid Scintillation Analyzer, Tri-CARB 2100 TR, Packard, Meriden, USA).

The concentration of chlorophyll$_{a+b}$ in the extract was analyzed using 96% ethanol ($\mu$g chl ml$^{-1}$ ground extract). The activity of rubisco and PEPcase was expressed in $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$.

2.8. Statistics

Morphological, physiological and biochemical data were analyzed by one-way analysis of variance (ANOVA) using the software Statgraphics Plus 4.1 (Manugistics, Inc., MD, USA). Data were tested for normal distribution and variance homogeneity using Cochran’s C-test. If necessary, data were transformed to ensure homogeneity of variance. Tukey Honestly Significant Differences (HSD) was used to identify differences between clones at the 5% significance level. Factor analysis was used to reduce the 24 measured variables to 5 lumped factors which are independent linear combinations of the measured variables. The reduction in number of variables was needed in order to be able to use a principal component analysis to investigate interrelationships between clones. The measured variables were grouped based on similarity and so that only one factor had an eigenvalue greater than one. The resulting five lumped factors were (i) a morphology factor containing most of the morphological parameters (total dry weight of shoots, length and width of the largest leaf, the shoot length, the shoot diameter and the total leaf area), (ii) a shoot density and shoot morphology factor containing the number of shoots per tank, the number of nodes per shoot and the number of leaves per shoot, (iii) a photosynthesis factor containing $P_{\text{max}}$, light compensation point and dark respiration, (iv) a pigment and photosynthesis factor containing chlorophyll$_{a+b}$, total carotenoids, photosynthesis and quantum yield, and (v) a stomata factor containing the stomatal densities, the stomatal index and the length of guard cells on the abaxial and adaxial sides of the leaves. A rotated principal component analysis was conducted using these five lumped factors as independent variables. Two new factors were identified with eigenvalues greater than one which together accounted for 70% of the variability in the original data.
3. Results

3.1. Seasonal development and shoot morphology

The clones all started to grow in late April. Among the Romanian clones plants with the lowest chromosome numbers (RO4x and RO6x) developed significantly higher shoot densities than RO8x and RO12x (Table 2). Shoot length showed continuous variation between ploidy levels, with RO8x being the tallest, followed by RO4x and eventually by RO12x and RO6x. Number of leaves per shoot differed little between the ploidy levels. Comparing clones from different geographic regions it is evident that RO8x, RO12x, IL, SE, US-GC, US-MW and US-INTR have relatively low shoot densities compared to RO4s, RO6s, ES and US-AC. The Swedish clone (SE) had relatively high shoot densities, but low final shoot heights. The clone grew fast in the early spring but ceased growth by the end of June and started to senesce over summer (Fig. 1b). The non-native North American clone (US-INTR) did develop higher shoot densities than the Midwest and Gulf Coast clones, but lower shoot densities than the Atlantic Coast clone. Shoot height of the US-INTR clone was slightly lower than that of the Midwest and US-AC clone and higher than that of the Atlantic Coast clone.

Leaves of the US-INTR clone started to grow later than the rest of the clones (end of April) and did not produce leaves until mid-May. Hereafter, the production of leaves increased rapidly (Fig. 1c).

Only a few of the clones produced inflorescences (Table 1). Inflorescences of the North American clones were fully developed. The Spanish clone (ES) started producing panicles from mid-August and had 47 ± 16 (mean ± S.D., n = 3) panicles at harvest. Many of the inflorescences were not fully developed at the end of the growing season. The Atlantic Coast clone (US-AC) produced inflorescences from the beginning of September, and the non-native North American clone (US-INTR) started to produce inflorescences about 2 weeks later. At harvest the number of inflorescences of US-AC and US-INTR were 16 ± 11 and 3.5 ± 0.7 (mean ± S.D., n = 3), respectively. No inflorescences of the North American clones were fully developed by the end of the growing season.

Senescence varied between clones, with the most striking difference being (SE) that started to senesce in July, and the rest of the clones, which started to senesce in October (Fig. 1). Concurrent with the first night frost in mid-November the senescence of all clones accelerated. However, leaves of the Gulf Coast and US-INTR clones stayed green for about 1 week later than the rest of the clones.

Growth form of all clones was vertical except for the Gulf Coast clone, which had both vertical and horizontal growth (up to 4 m long runners with many lateral shoots). The Gulf Coast clone also differed from other clones by having stiff leaf tips and closely positioned leaves. Stem colour varied from green

![Fig. 1. Number of leaves on tagged shoots of Phragmites australis clones during the growing season and during senescence in the autumn. (A) Romanian clones (RO) with ploidy levels of 4x, 6x, 8x and 12x; (B) Swedish (SE), Israeli (IL), Spanish (ES) clones and (C) North American clones, US-MW: Midwest; US-AC: Atlantic Coast; US-GC: Gulf Coast; and US-INTR: introduced. Mean ± S.D. (n = 3).](image-url)
for RO8x, SE, IL and ES clones to different degrees of red or brown for the other clones (Fig. 2). Texture of all stems was smooth, and all clones had dark green leaves. From the end of June dark spots were observed on leaves of IL, US-MW and US-GC. Leaf sheaths of RO6x, RO8x, SE, ES, IL and US-INTR stayed on the stem during senescence. For the rest of the clones lower leaf sheaths fell off. Inflorescences of the Spanish and Atlantic Coast clone were dense, while the non-native US clone had more sparse inflorescences (Fig. 3).

Most parameters varied 3-fold between clones, and although it was generally true that clones with high shoot densities had shorter and thinner shoots, this was not always the case (Table 2). The octoploid Romanian clone (RO8x) had significantly greater basal culm diameter, greater total leaf area, higher length of the biggest leaf and dry weight of leaf and leaf sheaths than the Romanian clones with lower ploidy level.
Table 3
Stomatal density, stomatal index and length of guard cells of the upper (adaxial) and lower (abaxial) leaf surface of the Phragmites australis clones (n = 3)

<table>
<thead>
<tr>
<th>Clone</th>
<th>Upper leaf blade</th>
<th>Lower leaf blade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomatal density (mm⁻²)</td>
<td>Stomatal index</td>
</tr>
<tr>
<td>RO4x</td>
<td>779 ± 75abc</td>
<td>14.1 ± 1.8bc</td>
</tr>
<tr>
<td>RO6x</td>
<td>661 ± 30abc</td>
<td>14.8 ± 1.4a</td>
</tr>
<tr>
<td>RO8x</td>
<td>483 ± 33bc</td>
<td>12.4 ± 0.7bc</td>
</tr>
<tr>
<td>RO12x</td>
<td>278 ± 47ab</td>
<td>11.1 ± 0.7bc</td>
</tr>
<tr>
<td>SE</td>
<td>254 ± 83a</td>
<td>9.5 ± 1.0a</td>
</tr>
<tr>
<td>IL</td>
<td>607 ± 60cd</td>
<td>13.1 ± 1.7bc</td>
</tr>
<tr>
<td>ES</td>
<td>615 ± 106cd</td>
<td>12.5 ± 1.8bc</td>
</tr>
<tr>
<td>US-MW</td>
<td>257 ± 31c</td>
<td>10.6 ± 1.3bc</td>
</tr>
<tr>
<td>US-AC</td>
<td>842 ± 131c</td>
<td>13.6 ± 1.4bc</td>
</tr>
<tr>
<td>US-GC</td>
<td>311 ± 54ab</td>
<td>11.3 ± 1.4bc</td>
</tr>
<tr>
<td>US-INTR</td>
<td>660 ± 83abc</td>
<td>12.3 ± 1.0abc</td>
</tr>
</tbody>
</table>

Means ± S.D. are shown. Different letters within columns indicate significant differences (*P < 0.05*) between clones.

RO4x and RO6x (Table 2; Fig. 4). For these characters the dodecaploid clone (RO12x) had dimensions between RO8x and RO4x/6x. Concerning shoot length, RO8x was significantly taller than RO6x and RO12x, while RO4x was between these two ranges. RO8x had significantly more nodes than RO12x, while RO4x and RO6x showed intermediate values. RO8x had a significantly wider biggest leaf than RO4x, and RO12x and RO6x had intermediate values. Among the North American clones, US-INTR differed from the rest by having a significantly higher number of live leaves at greater maximum biomass. The Gulf Coast clone (US-GC) had a significantly lower number of nodes than US-INTR, a significantly smaller biomass. The Gulf Coast clone (US-GC) had a significantly taller than RO6, RO4, RO12 dodecaploid clone (RO12x) had dimensions between RO8 and RO4/6x.

3.2. Stomata

The stomatal density was higher on the abaxial leaf side than on the adaxial side for all clones (Table 3), but the length of guard cells on the abaxial and adaxial side did not differ. As expected, the stomatal density and stomatal index of Romanian clones decreased, and length of guard cells increased, with increasing ploidy level. For the rest of the clones SE, US-MW and US-GC had significantly fewer, but larger stomata in the lower side of leaf blade compared to RO4x, IL, ES, US-AC and US-INTR.

3.3. Photosynthesis and photorespiration

Although shoots of RO8x had large shoot/leaf dimensions, Pmax was low compared to the rest of the Romanian clones (Table 4). The apparent quantum yield (φi), dark respiration (RD), light compensation point (Ic) and photorespiration (Rp) did not differ significantly between the different Romanian ploidy levels. Some differences in photosynthetic parameters between the other clones were observed. The Swedish clone had the highest Pmax value, but significantly higher only than US-MW clone, and the Spanish clone had the lowest Ic. The non-native North American clone (US-INTR) had a low Ic, significantly lower than the native US-GC clone and a low Rp, significantly lower than the native US-MW clone.

3.4. Rubisco and PEPcase activities and pigment concentrations

Comparing the Romanian ploidy levels, no significant differences were observed in enzymes activities and pigment concentrations.

Table 4
Photosynthetic parameters of Phragmites australis clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>φi (mol mol⁻¹)</th>
<th>RD (µmol m⁻² s⁻¹)</th>
<th>Ic (µmol m⁻² s⁻¹)</th>
<th>Pmax (µmol m⁻² s⁻¹)</th>
<th>Rp (µmol m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO4x</td>
<td>0.04 ± 0.01</td>
<td>1.58 ± 0.69</td>
<td>34.2 ± 9.9abc</td>
<td>17.3 ± 0.9bc</td>
<td>2.9 ± 1.8bcd</td>
</tr>
<tr>
<td>RO6x</td>
<td>0.04 ± 0.01</td>
<td>1.74 ± 0.65</td>
<td>42.8 ± 14.9abc</td>
<td>17.1 ± 2.4bc</td>
<td>2.3 ± 0.6bcd</td>
</tr>
<tr>
<td>RO8x</td>
<td>0.04 ± 0.01</td>
<td>1.99 ± 1.06</td>
<td>46.8 ± 15.7abc</td>
<td>11.9 ± 4.3bc</td>
<td>1.7 ± 0.9abc</td>
</tr>
<tr>
<td>RO12x</td>
<td>0.04 ± 0.00</td>
<td>1.48 ± 0.97</td>
<td>36.3 ± 18.6abc</td>
<td>15.1 ± 2.3b</td>
<td>2.6 ± 0.7bde</td>
</tr>
<tr>
<td>SE</td>
<td>0.03 ± 0.00</td>
<td>0.96 ± 0.21</td>
<td>29.3 ± 5.7a</td>
<td>23.3 ± 1.1</td>
<td>2.2 ± 0.8bde</td>
</tr>
<tr>
<td>IL</td>
<td>0.04 ± 0.00</td>
<td>1.36 ± 0.45</td>
<td>32.6 ± 6.8abc</td>
<td>21.8 ± 0.7bc</td>
<td>5.9 ± 0.3ad</td>
</tr>
<tr>
<td>ES</td>
<td>0.04 ± 0.00</td>
<td>0.67 ± 0.07</td>
<td>17.3 ± 0.2a</td>
<td>18.5 ± 0.7bc</td>
<td>2.9 ± 0.2bcd</td>
</tr>
<tr>
<td>US-MW</td>
<td>0.04 ± 0.01</td>
<td>2.01 ± 0.82</td>
<td>52.8 ± 7.8abc</td>
<td>15.3 ± 3.9b</td>
<td>3.9 ± 0.8cd</td>
</tr>
<tr>
<td>US-AC</td>
<td>0.04 ± 0.00</td>
<td>1.37 ± 0.24</td>
<td>35.0 ± 6.4abc</td>
<td>17.9 ± 1.4a</td>
<td>1.3 ± 0.8ab</td>
</tr>
<tr>
<td>US-GC</td>
<td>0.03 ± 0.01</td>
<td>1.89 ± 0.62</td>
<td>60.6 ± 11.8bc</td>
<td>18.8 ± 2.6bc</td>
<td>1.6 ± 0.5abc</td>
</tr>
<tr>
<td>US-INTR</td>
<td>0.03 ± 0.00</td>
<td>0.81 ± 0.23</td>
<td>26.0 ± 5.4ab</td>
<td>17.2 ± 2.0bc</td>
<td>0.7 ± 0.2a</td>
</tr>
</tbody>
</table>

φi: apparent quantum efficiency; RD: dark respiration rate; Ic: light compensation point; Pmax: maximum light-saturated rate of photosynthesis; Rp: photorespiration. Measurements were carried out at maximum biomass before the plants started to senesce. Means ± S.D. (n = 3) are shown. Different letters within columns indicate significant differences (*P < 0.05*) between clones.
Table 5
Activity of non-activated and total rubisco and non-activated and total PEPcase (μmol C m⁻² s⁻¹) and concentrations of chlorophylla,b and total carotenoids (μg cm⁻² leaf) in leaves of the different Phragmites australis clones

<table>
<thead>
<tr>
<th>Clone</th>
<th>Non-activated rubisco (μmol C m⁻² s⁻¹)</th>
<th>Total rubisco (μmol C m⁻² s⁻¹)</th>
<th>Non-activated PEPcase (μmol C m⁻² s⁻¹)</th>
<th>Total PEPcase (μmol C m⁻² s⁻¹)</th>
<th>Chlorophylla,b (μg cm⁻² leaf)</th>
<th>Total carotenoids (μg cm⁻² leaf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO4x</td>
<td>23.2 ± 7.6abc</td>
<td>27.3 ± 8.2ab</td>
<td>0.65 ± 0.31a</td>
<td>1.29 ± 1.25ab</td>
<td>37.6 ± 7.1bc</td>
<td>6.6 ± 2.0bcd</td>
</tr>
<tr>
<td>RO6x</td>
<td>12.2 ± 4.2a</td>
<td>15.6 ± 4.3a</td>
<td>0.22 ± 0.23a</td>
<td>0.24 ± 0.18a</td>
<td>21.8 ± 5.9b</td>
<td>3.9 ± 1.6bc</td>
</tr>
<tr>
<td>RO8x</td>
<td>17.7 ± 2.6abc</td>
<td>23.3 ± 1.5ab</td>
<td>1.96 ± 1.50a</td>
<td>3.63 ± 3.75ab</td>
<td>28.9 ± 1.4b</td>
<td>5.3 ± 0.7bc</td>
</tr>
<tr>
<td>RO12x</td>
<td>18.4 ± 2.9bc</td>
<td>23.3 ± 1.5ab</td>
<td>2.95 ± 1.88a</td>
<td>4.16 ± 2.62ab</td>
<td>33.4 ± 2.7b</td>
<td>6.6 ± 0.4bcd</td>
</tr>
<tr>
<td>SE</td>
<td>19.5 ± 3.7abc</td>
<td>30.5 ± 8.2ab</td>
<td>2.89 ± 4.29a</td>
<td>2.30 ± 1.54ab</td>
<td>24.4 ± 7.7b</td>
<td>1.3 ± 0.8a</td>
</tr>
<tr>
<td>IL</td>
<td>26.0 ± 7.1bc</td>
<td>33.2 ± 9.3ab</td>
<td>1.07 ± 1.39a</td>
<td>0.91 ± 0.90ab</td>
<td>41.1 ± 5.3b</td>
<td>8.1 ± 1.3cd</td>
</tr>
<tr>
<td>ES</td>
<td>12.8 ± 1.2a</td>
<td>16.3 ± 3.8a</td>
<td>3.08 ± 4.13a</td>
<td>2.30 ± 1.54ab</td>
<td>35.6 ± 7.4b</td>
<td>7.6 ± 1.2cd</td>
</tr>
<tr>
<td>US-MW</td>
<td>30.1 ± 2.8c</td>
<td>33.3 ± 2.0b</td>
<td>1.74 ± 0.43a</td>
<td>4.67 ± 2.11b</td>
<td>36.9 ± 6.3b</td>
<td>8.1 ± 0.6cd</td>
</tr>
<tr>
<td>US-AC</td>
<td>14.1 ± 4.5ab</td>
<td>23.9 ± 7.1ab</td>
<td>1.73 ± 2.42a</td>
<td>0.35 ± 0.02ab</td>
<td>30.3 ± 4.8b</td>
<td>9.1 ± 2.5d</td>
</tr>
<tr>
<td>US-GC</td>
<td>26.3 ± 3.0bc</td>
<td>31.1 ± 2.4ab</td>
<td>4.83 ± 5.62a</td>
<td>3.44 ± 2.48ab</td>
<td>32.9 ± 5.6b</td>
<td>6.7 ± 0.3bcd</td>
</tr>
<tr>
<td>US-INTR</td>
<td>19.1 ± 2.3abc</td>
<td>21.3 ± 3.7ab</td>
<td>3.56 ± 2.41a</td>
<td>8.39 ± 7.84ab</td>
<td>27.8 ± 2.1ab</td>
<td>6.8 ± 0.4bcd</td>
</tr>
</tbody>
</table>

Means ± S.D. (n = 3) are shown. Different letters (a–d) within columns indicate significant differences (P < 0.05) between clones.

Concentrations, but RO4x had nearly 2-fold higher activity of non-activated and total rubisco and concentrations of pigments than RO6x (Table 5). Concerning the other clones, US-MW clone had the highest activities of non-activated, total rubisco and total PEPcase, significantly higher only than ES (non-activated and total rubisco). The average values of the other clones showed high standard deviations. The contents of pigments were high in IL (chlorophylla,b) and US-AC (total carotenoids) compared to the Swedish clone which had the lowest content.

3.5. Grouping of clones

The rotated principal component analysis (Fig. 5) identified two major factors with eigenvalues of 1.96 and 1.56, respectively, that together accounted for 70% of the variability in the original data. The first principal component (Factor 1) with the largest variation (39.2) had high loadings for the morphology-related factor (0.89), photosynthesis-related factor (0.77) and pigment-related factor (0.68). The second principal component (Factor 2) accounted for 31% of the variation and had high loadings for the stomata-related factor (0.92) and shoot density- and shoot morphology-related factor (0.86). The Swedish clone (SE) that has small and thin shoots with few nodes and small leaves is located in the lower left corner of the plot whereas the clones with large, tall and thick shoots with many leaves and low densities of stomata have lower Factor 2 scores whereas clones that have few leaves and more stomata have higher Factor 2 scores. Similarity between clones is evident from the plot, with the Swedish clone very different from the other clones. Among the Romanian clones, the giant octoploid clone separates out. RO4x and RO6x resemble each other and RO12x separates out because of its low shoot density and its tall and thick culms. US-AC and US-INTR, and US-MW and US-GC separate widely along the Factor 2 axis.

4. Discussion

For this study, plants were grown under similar environmental conditions in terms of soil, water, nutrition and climate, and the clones had been grown at the same common environments. However, physiological processes, for instance photosynthesis, show considerable phenotypic plasticity and display nearly immediate response to the prevailing environmental conditions, as opposed to the morphological parameters and growth traits. These are more conservative and...
do not change as much as a response to changes in the environment. In the present study, the clones displayed large variability in ontogeny, shoot morphology and leaf anatomy, and only minor differences between clones in photosynthetic parameters and leaf enzymes and pigments content. This is in agreement with Lessmann et al. (2001) who found that physiological processes show an immediate acclimation to new environments, while growth and reproduction, which are the products of the physiological and biochemical processes, respond more slowly to environmental conditions (Callaghan et al., 1992).

4.1. Effects of euploidy level on morphology and physiology

This paper is the first to present morphological, physiological and biochemical characters of a dodecaploid Romanian *P. australis* clone and to compare it with co-occurring ploidy levels. Only shoots of the octoploid clone RO8x showed characters typical of giant reeds (Pauca-Comanescu et al., 1999). Particularly RO8x had larger shoots, larger leaves and lower shoot densities than the clones with lower ploidy levels (RO4x and RO6x). Previous studies have reported similar morphological differences between tetraploid and octoploid clones (Hanganu et al., 1999; Pauca-Comanescu et al., 1999; Clevering et al., 2001), but dodecaploid clones were not included in their studies. In natural Romanian *P. australis* stands, octoploid clones predominate in deeper water (Hanganu et al., 1999; Pauca-Comanescu et al., 1999), related to their higher vegetative vigour (Pauca-Comanescu et al., 1999). The distribution of dodecaploid clones in relation to environmental conditions is unfortunately not known. The dodecaploid clone did have larger cells than the lower ploidy levels, but the overall dimensions of the shoots (basal diameter and number of nodes, leaf area, size of the biggest leaf, dry weight of leaf and leaf sheaths) were not different either from those of the tetra- and hexaploid clones or from the octoploid. Shoot length was instead significantly shorter than that of the octoploid clone. Hence, polyploidy does not always result in an increase in plant size. This is probably because the number of cell divisions during development is likely reduced (Stebbins, 1971). The tetra- and hexaploid clones did not differ much in leaf dimensions, shoot diameter, number of nodes and seasonal development of shoots, which is in agreement with Björk (1967) who did not find any distinct morphological differences between Swedish tetra- and hexaploids.

The fact that 6x and 12x clones are neither bigger nor perform significantly better than lower ploidy levels could perhaps be associated with the polyploidization process. Tetraploid *Phragmites* are considered to be allotetraploids because of differences in chromosome morphology, the strict bivalent formation at meiosis and disomic (two loci with two alleles), instead of tetrasomic (one locus with four alleles), inheritance (Raicu et al., 1972; Hauber et al., 1991). Hexaploid *Phragmites* are considered to be hybrids between tetra- and octoploids because of meiotic aberrations and reduced pollen fertility (Björk, 1967; Gorenflo et al., 1972, 1990; Lenoir et al., 1975). The polyploidization process of the dodecaploid *Phragmites* is not known. Imperfect cell divisions in hexaploids can probably explain their reduced vegetative vigour compared to the octoploids. *P. australis* hexaploids are probably hybrids between 4x and 8x and can be compared with hybrid triploids of other species (Dykjová and Pazourková, 1979; Clevering and Lissner, 1999), and there are many cases of triploids in nature (including animals) which are neither bigger (Kerby et al., 2002) nor more competitive (Clifton-Brown and Lewandowski, 2002) than parental diploids.

The studied physiological processes did not differ between ploidy levels. The hexaploid Romanian clone had nearly 2-fold lower activities of rubisco and concentrations of chlorophyll$_a$ and carotenoids than the tetraploid clone. These differences did, however, not influence the photosynthetic gas exchange parameters significantly and might reveal acclimation.

4.2. Effect of geographical origin

Clones from different geographic regions clearly differed in ontogeny and other growth parameters when grown in a common environment resulting from genetic differentiation in response to their original environmental conditions. The Swedish clone grew before the other clones but ceased very early in the season without producing inflorescences. Only the Spanish clone and US Atlantic Coast and US Introduced clones produced inflorescences, and most flowers did not develop fully before the first frost in November. Such differences in ontogeny and timing of flowering are common for plants growing in different climatic regions and were also found by Clevering et al. (2001) who studied European *P. australis* clones, and by McNaughton (1966) who studied North American *Typha* species. Generally, plants from higher latitudes are genetically adapted to shorter growing seasons than plants from lower latitudes. Thus, the Romanian, Israeli, US Midwest and Gulf Coast clones did not have enough time to complete their life cycles in the common environment climatic conditions. The Spanish, US Atlantic Coast and US Introduced clones originate from more oceanic habitats with climatic conditions that do not differ so much from conditions at the Danish growth site. These clones therefore are probably better adapted to climatic conditions at the common environment, and might also be the reason why these clones were able to produce inflorescences. The dark spots on the leaves of the Israeli, US Midwest and Gulf Coast clones observed in early July could have been caused by the low temperatures and abundant precipitation in June. The origin of these clones are subtropical or dry regions, hence the clones might be more sensitive to adverse environmental conditions than the clones from colder and more humid areas.

The Swedish clone had small shoot dimensions which is consistent with the general observation that shoots of plants from higher latitudes are smaller than shoots of plants from lower latitudes. This appears to be a common characteristic among plants and has been demonstrated for a number of other plant species, for example Carex aquatilis (Chapin and Chapin, 1981), Verbascum thapsus (Reinartz, 1984) and Arabidopsis thaliana (Li et al., 1998).
Shoots of the Israeli clones had significantly higher shoot length and dimensions of the biggest leaf than the European tetraploids and North American clones. The large leaves were not caused by large cell sizes, as the length of guard cells did not deviate from that of the other clones. Genetic AFLP analyses have shown that the Israeli clone, even though it is tetraploid, is genetically closely related to the Australian group of octoploids (Lambertini et al., 2006). The Israeli clone therefore seems to resemble the Australian *P. australis* both genetically and in morphology.

The US Gulf Coast clone differed from the rest of the clones in having horizontal growth and shoots with closely positioned leaves and stiff leaf blade tips. The leaf characters resemble those of *Phragmites mauritianus* Kunth (Scholz and Böhling, 2000). Recent genetic studies have shown that the Gulf Coast clones are more related to *P. mauritianus* than to *P. australis* (Lambertini et al., 2006). Hence, it is not surprising that this clone differs significantly, particularly in morphological parameters. The ploidy level of this clone is not known.

### 4.3. Differences between introduced and native North American clones

The aggressive expansion of *P. australis* that has occurred in North America has been attributed to the rapid spreading of introduced exotic clones genetically different from the native clones (Saltonstall, 2002). As the introduced clones out-compete the native clones, the growth dynamics of the clones must be different. In the present study, the introduced clone had relatively high shoot densities and low dark respiration rates and light compensation points which indicate that the introduced clone is a better competitor for light. This agrees with the results by Lynch and Saltonstall (2002) concerning the higher competitiveness of introduced exotic genetic strains compared to the native genotypes. However, the native clone from the US Atlantic Coast shares several morphological characters with the introduced genotypes, as also reported by Blossey (2002). In the present study, the grouping of the North American clones places the US-AC clone next to the US-INTR clone in the factor plot, and only the Atlantic Coast and the introduced clones produced inflorescences. So, the two clones had several traits that differed from those of the other North American clones. There are several data in the present study that indicate that the US-AC and the US-INTR clones are very similar, and it cannot be excluded that they both belong to the introduced population. Further genetic studies are needed to clarify this point.

### 5. Conclusion

*P. australis* is a cosmopolitan species and its geographic distribution extends from cold temperate regions to tropics. Furthermore, many different ploidy levels occur with tetraploid (4x) and octoploid (8x) being most common. It is therefore not surprising that large genetically determined differences in ontogeny, shoot morphology and leaf anatomy and morphology between the clones occur, and that these differences are sustained when the plants are grown in a common environment. Physiological and biochemical processes also differ between clones, but these processes show considerable phenotypic plasticity and are therefore very difficult to evaluate. High ploidy level usually results in plants with larger dimensions (large leaves, tall shoots, large cell sizes, etc.) but this is not always the case. Hence, it is impossible to identify the ploidy-levels of *P. australis* by simple visual inspection and measurements of morphological parameters: Romanian hexaploids and dodecaploids cannot be distinguished from Romanian tetraploids, and some of the tetraploid clones have morphological characteristics that resemble octoploids. It can thus be concluded, that *P. australis* is a wetland species with high genetic variability which is augmented by its cosmopolitan distribution, its clonal growth form and the large variation in chromosome numbers. When different clones are grown in a common environment the physiological and biochemical processes differ between clones, but because of the large phenotypic plasticity in these processes the functional importance needs further study.

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### References


