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Genetic diversity of riverine reed stands indicating the water regime of the habitat

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ABSTRACT

Although genetic diversity of reed stands developing in habitats with unchanged water levels have been frequently investigated, little is known about reed propagation along rivers, where fluctuating water levels may provide various conditions for germination and seedling development.

The present paper evaluates genetic diversity of reed stands grown in (i) an oxbow with significant water level fluctuations related to the flood events in the main river; (ii) a separated oxbow which has lost connection to the main channel; and (iii) artificially controlled running water with low water level fluctuation. At each site, reed stands were investigated along transects parallel to the shore and multilocus phenotypes were determined by microsatellite analysis.

The results demonstrated that genetic diversity of riverine reed stands can indicate the water regime of the habitat. Reed colonizes mostly by vegetative propagation where regular inundation can impede germination and seedling development and, therefore, genetic diversity is low in the whole stand. If a former oxbow becomes practically a lake, the clonal diversity and colonization processes are similar to those observed in regular lakes; clone number decreases toward the open water. When reed forms floating mats (i.e. the effect of water level fluctuation is excluded), generative reproduction prevails in the entire stand, resulting in high genetic diversity even at the open water edge of the stand.

1. Introduction

Lakeshores and riverbanks are frequently populated by extensive stands of reed (*Phragmites australis* (Cav.) Trin. ex Steud.) which play important and complex roles in aquatic ecosystems. Among others, reed stands provide food and habitat for other organisms and take part in the purification of water and protection of the shore (see the review by Kiviat, 2013 and the literature cited therein). These roles depend, however, on the stability of stands and their ability to adapt to site conditions. The latter is related to genetic diversity since, as Neuhaus et al. (1993) emphasized, 'the higher the number of clones in a stand, the better is their ability to adapt to unfavorable conditions'. According to the hypothesis of Koppitz et al. (1997) and Koppitz and Kühl (2000), reed is established in three main stages, namely (i) generative propagation ('Settlement'); (ii) expansion of new, suitable clones and selection of the best adapted ones ('Propagation and Establishment') and (iii) competition between reed clones leading to the selection of a few but well adapted clones with narrow ecological tolerance ('Stationary stage').

Effects of water depth on the above stages and reed establishment in lakes were demonstrated by Engloner et al. (2010) and Engloner and Major (2011) who disclosed how genetic diversity changes when stands develop along static water depth gradients. According to their results, genetic diversity – regardless the health status of the stands – decreases toward the open water. At the lakeshore where water level never rises above the soil surface, seedling establishment maintains high clonal diversity. In deep water, however, germination is impossible and, if clone settlement from rhizome fragments drifted from other areas is also limited, the presence of reed clones is the outcome of vegetative spreading from the lakeshore. During vegetative spreading, the majority of clones (with low competitive ability) remain at higher elevations and only the most successful clones reach the deepest water. It was also demonstrated that clone size parallel to the shore is considerably larger at the waterside than at the landward edge of the stand and genets successively replace each other in the deepest water, while they form a mixed arrangement at the lakeshore.

In contrast, little is known about how reed propagates in the hydrologically different and diverse riverine habitats (for instance, main channels, side arms and oxbows with or without open inflows,

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inside or outside the flood protection dams, etc.), where different water regimes may provide various circumstances for reed propagation. It is unclear, whether stands developing in different riverine habitats have specific genetic diversity relating to the water regime.

To answer the above questions, the present paper presents the first detailed information on the genetic diversity of riverine reeds.

2. Materials and methods

2.1. Study sites

Along the Hungarian section of the Danube river, three habitats were selected: (i) an oxbow with significant water level fluctuations related to the flood events in the main river – Nyéki Holt Duna; (ii) a separated oxbow outside the flood protection dams – Riha-tó; and (iii) an artificially controlled running water with low water level fluctuation – Soroksári Duna.

Nyéki Holt Duna is an oxbow in the Gemenc floodplain and is located at 1479 rkm on the right bank of the Danube, entirely within the dam system of the river. The total amplitude of water level fluctuation reaches 9 m. The inundation of the floodplain starts at 87.5 m above the Baltic which corresponds to 650 cm on the water-gauge in the main river-bed at Baja (Mátrai et al., 2011). The second site, Riha-tó lies on the left bank of the Danube at Mohács (1447 rkm). It has completely lost the connection to the main river channel and became a lake supported by inland inundation. Finally, Soroksári Duna is the second largest side arm of the Danube in Hungary, located between 1642 and 1586 rkms and enclosed by sluices at its both ends. The water level fluctuation in this side arm is about 20–60 cm (Vadadi-Fülöp et al., 2007).

To provide comparability of clonal patterns of the selected riverine stands and the previously investigated lakeside reeds; sampling, genetic investigations and data analysis were carried out exactly in the same way as in Engloner et al. (2010) and Engloner and Major (2011).

2.2. Sampling

At each habitat, a 300–500 m long sector of reed stands was investigated along three transects parallel to the shore; Transect I was taken along the landward edge, Transect II was positioned in the middle of the stand and Transect III at the open water edge. At the time of sampling (July 2013), water levels were below the soil surface along Transects I of all study sites, while at the lowest elevations water depths were around 60, 125 and 65 cm at Nyéki Holt Duna, Riha tó and Soroksári Duna, respectively.

Distances between sampling points along the transects ranged from 25 to 35 m and the total number of samples collected at the Nyéki Holt Duna, Riha tó and Soroksári Duna were 44, 35 and 43, respectively. Where large patches of species other than *P. australis* occurred inside the investigated stands, the number of samples decreased. That was the situation, for instance, in the Riha-tó, where impassable patches of willow occupied the middle of the reed stand.

2.3. Genetic investigations

At all sampling points, strongly growing shoot tips were collected for microsatellite analysis. Genomic DNA from the 122 culm tip samples was isolated using the CTAB protocol described by Doyle and Doyle (1987). Following Saltonstall (2003), four microsatellite primer pairs: PaGT14, PaGT16, PaGT21 and PaGT22 (Table 1) were selected. The forward primers were 5′-labeled with 6-FAM, PET, VIC or NED. The reverse primers were PIG-tailed (Brownstein et al., 1996) for increasing the accuracy of the identification of the allelic sizes. PCR amplifications were carried out after Saltonstall (2003). Fragment length polymorphism of the alleles was detected by capillary electrophoresis on an ABI 3130 Genetic Analyser and evaluated by Peak Scanner (ABI).

2.4. Data analysis

Clonal composition of the investigated stands was determined on the basis of multilocus phenotypes. Binary character matrices were evaluated by hierarchical clustering [group average method (UPGMA) with the complement of simple matching coefficient] using the SYN-TAX 2000 package (Podani, 2001). Ramets were considered to belong to the same clone if they were uniform at all investigated microsatellite loci. The proportion of the distinguishable variations (PD) was calculated for the entire stand and the separate transects as PD = G/N, where G is the number of identified clones/genets and N is sample size (Ellstrand and Roose, 1987).

Table 1
Microsatellite primer pair sequences and calculated characteristics of alleles and clones.

Locus	5′ → 3′ sequence	Label	N_A	S (bp)	MN_A	N_P	N_{Ho}
PaGT14	F: GTTGCAGCAAGTATTTGG R: CAAGCATTCTAGTAGTAGC	VIC	10	182–195	4	20	17
PaGT16	F: ACCAATCAGTCAGACTAGCC R: GTTCTCATGTTGGAGAAGCC	PET	9	225–292	2	15	12
PaGT21	F: GCTACTCAACAGGTATACGG R: ATTGAGGATTGAGGTGGTGG	6-FAM	12	172–248	5	37	31
PaGT22	F: GTTGAGTGCCTGGTGTATTCG R: AAGCTTCTGTCATGGAACCG	NED	16	182–211	2	39	31
Total			47			111	

N_A: number of alleles detected at the locus, S: size range of the alleles in base pairs, MN_A: maximum number of alleles detected in a genotype, N_P: number of the observed phenotypes/clones, N_{Ho}: number of the observed partial heterozygous clones.

Table 2
The numbers of samples (N) and genets (G) and the PD values in the three investigated reed stands.

	Nyéki Holt Duna				Riha-tó				Soroksári Duna			
	I	II	III	Total	I	II	III	Total	I	II	III	Total
N	15	14	15	44	15	6	14	35	15	13	15	43
G	7	10	4	14	14	5	10	25	13	11	12	31
PD	0.467	0.714	0.267	0.318	0.933	0.833	0.714	0.714	0.867	0.846	0.800	0.721

Transects are I: landward edge; II: middle of the stand and III: open water edge.