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Genetic diversity and dispersal of *Phragmites australis* in a small river system

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ABSTRACT

Even though the reed, *Phragmites australis*, is an extensively studied wetland species, little is known about reproduction and dispersal modes within and among reed populations at the scale of small river systems. Using microsatellite analysis of 189 individuals from three adjacent river catchments in the Czech Republic, we elucidated the role of the river corridors in the dispersal of *P. australis*. Using Bayesian clustering of individuals, we found that 19% of clusters were distributed only along one river, which implied dispersal by water (or by wind) along river corridors, whereas 38% of clusters were widely distributed and were likely the product of wind long-distance dispersal among rivers. Intensive exchange of propagules among river systems is further demonstrated by only 6% of total variance being attributed to the variance among rivers in the AMOVA-analysis. Spatial autocorrelation analysis revealed a decreasing pattern up to 5–10 km and no clear pattern over longer distances. This gives an evidence for pollen and seed dispersal at short distances (up to 1 km), whereas most likely only seed dispersal at longer distances up to 10 km. We found five multilocus genotypes distributed in two different populations. The distances between populations with the same genotype ranged from 0.5 to 10.8 km. This can be interpreted as long-distance vegetative dispersal.

1. Introduction

Phragmites australis is one of the most extensively studied wetland species in the world. Besides the comprehensive monograph by Rodewald-Rudescu (1974) numerous studies have been published dealing with plant morphology and development (Haslam, 1969a,b,c, 1970a), ecology and plant communities (Björk, 1967; Haslam, 1970b; Borhidi, 1970), production ecology (e.g., Dykyjová and Hradecká, 1976; Fiala, 1976; Květ et al., 1998), ecophysiology (Gloser, 1977; Rychnovská, 1978; Armstrong et al., 1999), die-back in European lakes (Van den Putten, 1997), karyology and phenotypic differentiation of cytotypes (Gorenflot et al., 1972; Dykyjová and Pazourková, 1979; Paucă-Comanescu et al., 1999), various aspects of clonality (Hradecká and Květ, 1973; Hara et al., 1993; Rolletschek et al., 1999), and some of these also included studies of genetic variation. Several ploidy levels were found in *P. australis*: $2n = 24, 48, 72, 84$ (Clevering and Lissner, 1999), but within the plants from freshwater habitats in the Czech Republic, only tetraploids $2n = 48$ have been reported so far (Pazourková, 1973). Only in one saline locality (Nesyt pond) octoploid plants have been observed (Clevering and Lissner, 1999).

The first studies of genetic variation focused on the development of reed populations and growth response of the plants to habitat changes (Neuhaus et al., 1993; Koppitz et al., 1997). Recent studies focused on the genetic variation within *P. australis* populations worldwide (Koppitz, 1999), global phylogeography of the genus *Phragmites* (Lambertini et al., 2006), clone-specific differences (Hansen et al., 2007), genetic variation in a watershed (Keller, 2000) and the comparison of clones from different habitats and of different ages (Čurn et al., 2007; Křiváčková-Suchá et al., 2007; Lambertini et al., 2008).

Nonetheless, little is known about prevailing reproduction and dispersal modes within and among reed populations and how the dispersal processes could affect distribution of the species. Dispersal is one of the most important factors influencing spatial distribution of genetic variation (Ouborg et al., 1999). The role of dispersal (by water or by wind) and the effect of different types of propagules (seeds or vegetative propagules) on species distribution can be evaluated based on the analysis and interpretation of the spatial distribution of genetic variation among *P. australis* populations. *P. australis* reproduces vegetatively by rhizomes and generatively. Seed reproduction is rarely successful under natural conditions (Rodewald-Rudescu, 1974) and clonal spread prevails, possibly also by rhizome fragments by water drift (Luther, 1951; Haslam, 1972). Seed dispersal by wind is facilitated by the presence of pappus, and seed dispersal by running water has also

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been reported (Boedeltje et al., 2004); seeds can float for several days (Morton and Hogg, 1989). However, seed reproduction within established populations is limited by the litter mat (Haslam, 1970a, 1971; Szczepańska, 1971). Although the reproduction mechanism of *P. australis* is well known, little is known about (1) the degree of vegetative dispersal among populations, (2) the importance of wind and water dispersal, (3) the distances over which dispersal takes place, and (4) the dispersal among different streams in the riverine landscape.

Rivers serve as dispersal corridors for aquatic plants (Johansson et al., 1996; Nilsson et al., 1991) since they form migration routes for propagules. Whether the majority of propagules are dispersed along rivers or if among-stream dispersal takes place as well can be best detected using molecular markers. Two basic approaches may be used when analyzing molecular marker data, either direct or indirect (Sork et al., 1999). Direct methods (including assignment tests and parentage analysis) address contemporary gene flow in the landscape, but assume complete genotyping of the parent pool. In contrast, widely used indirect methods summarize historical gene flow and enable detection of localities possibly connected by dispersal in the past. The latter approach is possible to use when studying dispersal within and among rivers and their tributaries (Fér and Hroudová, 2008). Furthermore, highly polymorphic molecular markers (AFLPs or microsatellites) can be used to distinguish between generative and vegetative dispersal. The spatial pattern of genetic variation may then help to elucidate the role of the rivers in dispersal of *P. australis*.

The aim of this study is to explain the type of colonization by reeds in the riverine landscape, using, as an example, the mid-size river catchments of the small tributaries of the Labe River in the Polabí lowland (Central and East Bohemia; Czech Republic) using microsatellite markers. *P. australis* is frequently distributed along these rivers, which provided a satisfactory source of material for sampling (Rydlo, 1990, 1991). In addition, the expansion of the range of this species has been observed recently in changing landscapes (e.g., abandoned meadows along small streams), and elucidation of the spreading mechanisms of the reed may contribute to understanding how the range is expanded at regional scales.

Due to the complicated definition of individuals and local populations in this clonal plant species (unclear clonal origin of ramets, boundaries between 'populations' are hardly to be defined), we do not aim to study the processes at the population scale. Analysis and interpretation of genetic variation within and among rivers may provide answers to the following questions:

1. What is the proportion of generative and vegetative dispersal in relation to the distance among sampling sites? Can we find some evidence for clonal long distance dispersal?
2. Are the reed propagules dispersed only along rivers (by water), or is the spatial distribution of genetic variation substantially affected by seed dispersal among rivers and their tributaries (by wind or by animals)?
3. Is there an indication of isolation by distance as a result of restricted seed dispersal among adjacent populations?

2. Methods

2.1. Sample collection

The most of samples of *P. australis* were collected from catchments of the Cidlina and the Mrlina rivers, several of them also from the adjacent part of the Labe, the Jizera, and the Ohře Rivers; all the rivers are tributaries of the Labe River (coordinates of sampling sites see Table 1). Due to the complicated definition of a single population in *P. australis*, we collected individuals at so-called sampling sites. These were defined as one to several stands of *P. australis* growing along both sides of the river segment several 100 m long. At each sampling site we collected one to six individuals. In total, we collected 189 samples from 78 sampling sites (Table 1). Of these, 129 samples were from the Cidlina River, 28 from the Mrlina River, 30 from the Labe River, 1 from the Jizera River, and 1 from the Ohře River. A small portion of a young leaf was collected from each selected individual and dried using silica gel.

2.2. DNA extraction and microsatellite analysis

Total genomic DNA was obtained from silica gel-dried material using the CTAB extraction method (Doyle and Doyle, 1987). The DNA concentration was measured photometrically and adjusted to a final concentration of 5 ng μL^{-1}. Eight microsatellite loci (PaGT4, PaGT8, PaGT9, PaGT11, PaGT12, PaGT13, PaGT14, and PaGT16) were amplified using primers by Saltonstall (2003). The PCR reactions were done in a total volume of 20 μL containing 15 ng of DNA, 0.5 U of RedTaq Polymerase (Sigma–Aldrich, St. Louis, MO, U.S.A.), 6.25 pmol of each forward and reverse primer, 0.2 mM dNTPs, and 2 μL of 10× buffer for RedTaq (Sigma–Aldrich). Forward primers were fluorescence-labeled and all eight loci were amplified in four PCR reactions using a multiplex PCR approach (Table 2). PCR reactions 1a and 1b, and 2a and 2b, respectively, were pooled, precipitated using ethanol and sodium acetate and mixed with GeneScan ROX-500 (Applied Biosystems, Foster City, CA, U.S.A.) size standard. The final visualization was done using the ABI 3100 Avant automated sequencer. Because of different labeling of PCR products within the same bp size range it was possible to recognize alleles of different loci (primers).

Table 1
Seventy-eight sampling sites of *Phragmites australis* in the catchments of the Cidlina, the Mrlina, the Labe, the Ohře and the Jizera rivers (Czech Republic). Locality code, latitude, longitude, number of sampled individuals and clustering of individual(s) from the particular sampling site to the 31 Bayesian clusters defined using BAPS 3.2 are given. Numbers in parentheses indicate weak support for clustering of individual(s) to the particular Bayesian group.

Code	Latitude (N)	Longitude (E)	Sampled individuals	Description	Bayesian cluster(s)
Cidlina River catchment					
1	50.4715283	15.3787395	6	Pond to the W from Železnice	6, 28
2	50.4332943	15.3379571	1	Porák brook, W from Jičín	26
3	50.3964606	15.3796620	1	Cidlina river, between Vtíněves and Malá Strana	4
4	50.3542601	15.4245431	1	Cidlina river	(23)
5	50.3551206	15.4202931	1	Cidlina river, E from Žeretice	4
6	50.3315156	15.4294531	1	Cidlina river, pond in Vysoké Veselí	3
7	50.3113612	15.4364035	1	Cidlina river, Hrobičany	30
8	50.2591217	15.5049495	2	Cidlina river, Sloupno	26
9	50.2402842	15.5015548	1	Cidlina river, Metličany	5
10	50.1977500	15.5040928	4	Cidlina river, between Mlékosrby and Zachrašťany	21, 22, 30