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Genetic diversity patterns in *Phragmites australis* at the population, regional and continental scales

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Abstract

Genetic diversity, population structure and interrelationships were investigated in eight populations of the common reed, *Phragmites australis*, in the Po Plain, Italy, by means of amplified fragments length polymorphisms (AFLPs) and random amplified polymorphic DNAs (RAPDs). Patterns of genetic diversity were analysed in relation to size, age and degree of human impact in the wetlands and compared with that of a distant population in Romania. Genetic distances between Po Plain clones and geographically distant clones were measured to determine the geographical extent of the gene pool.

Nearly all populations studied are polyclonal and little correlation was found between genetic diversity and size, age and degree of human impact on the wetlands. One large (86 ha) monoclonal stand occurred in an old wetland with rather stable environmental conditions over a long time period, whereas polyclonal stands were younger and characterized by disturbance. On the interpopulation level it was not possible to differentiate between Po Plain populations and the Romanian population, indicating that a very extensive gene pool exists in Europe, to which both Po Plain and Romanian populations belong. There is however a certain degree of genetic structure among the populations that is not correlated with geographic distance, but is most likely related to *P. australis* colonization dynamics. A significant ''stepwise'' increase in average genetic distances was observed between clones >500 and >1500 km distant suggesting some kind of genetic pattern on a very large scale. Based on these results, *P. australis* populations in Europe could be considered members of a single meta-population.

Keywords: *Phragmites australis*; AFLP; RAPD; Genetic diversity; Population structure; Genetic relationship; Genetic distance; Meta-population; Common reed; Wetland

1. Introduction

Phragmites australis (Cav.) Trin ex Steud., the common reed, is a perennial emergent aquatic plant with a nearly worldwide distribution. Throughout most of its range, it typically forms closed, monodominant stands in the littoral zone of lakes, along rivers and in marshes of various kinds (Brix, 1999; Brix and Cizkova, 2001). The annual stems develop from an underground perennial rhizome system, which is responsible for the rapid vegetative expansion of the species. Sexual reproduction occurs but is pollen limited and affected by partial self-incompatibility (Ishii and Kadono, 2002).

Varying degrees of clonal diversity have been found in *P. australis* populations, ranging from monoclonal to polyclonal (Neuhaus et al., 1993; Koppitz et al., 1997). The colonization of a wetland by *P. australis* typically begins on the shores where numerous seeds germinate. Colonization then continues with the vegetative expansion of the seedlings, and as spaces between plants are progressively filled in, the clones start to compete for space. This process may result in a complex spatial distribution, where different clones intermingle. Clonal diversity is still high at the ''propagation and establishment stage'', while it decreases during the subsequent ''stationary stage'', in which a small number of clones well adapted to the local environmental conditions prevails (Koppitz and Kühl, 2000). Low clonal diversity and monoclonal populations could be the result of such a selection process and be an indication that stands have grown under stable conditions for a long time (Watkinson and Powell, 1993). Seedling recruitment is

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generally considered to occur only during ''windows of opportunity'' such as after a physical disturbance (Eriksson, 1997; Clevering, 1999). Favourable conditions for seed germination and seedling growth have been observed to occur after clearing of existing vegetation on bare riverbanks and after episodic drawdowns (Weisner et al., 1993). Seedling establishment is not very frequent in mature *P. australis* populations (Barrett et al., 1993). This and the clonal growth described above limit opportunities for gene flow within populations of *P. australis* and decrease genetic diversity.

Little is known about the extent and mechanics of gene flow via seeds and pollen between populations of *P. australis*. New wetlands are often rapidly colonized by *P. australis* even in areas far from reproducing stands. Long-distance gene flow may thus be an important factor in shaping genetic variation patterns.

In the Po Plain, northern Italy, numerous *P. australis*-dominated wetlands occur that are connected by a complex network of natural rivers and channels with regulated flow. Some are very old and represent the remnants of the extensive reed-dominated marshes that once covered the Po floodplain. Today most of the plain is drained and intensively cultivated. In the 1990s a number of wetlands were restored in compliance with European Community policies, which provided financing for transformation of croplands into wetlands. Today these wetlands are primarily maintained as hunting reserves. The management, mostly in the form of harvesting, may affect the genetic structure and diversity of *P. australis* populations as it indirectly creates opportunities for the seedling establishment, modifies the clonal architecture of populations, and affects the mating and dispersal opportunities (Charpentier, 2002). We expect to find high clonal variability in this area as a consequence of intra- and interpopulation gene flow. We also hypothesize that stands will consist of a mosaic of clones due to dispersal of rhizomes in connection with the harvest.

In a recent phylogeographic study of *Phragmites*, based on 238 collections spanning most of the vast distribution area, Lambertini et al. (2006) identified a large, fairly well supported, but poorly resolved ''core group'' within *P. australis*. Most European clones, including the ones from the Po Plain, belong to this group. The aims of the present study were (i) to assess the genetic diversity within and between *P. australis* populations in the Po Plain area and relate the diversity to wetland size, age and degree of human impact, and (ii) to compare the variation patterns in the Po Plain with geographically distant populations.

2. Materials and methods

2.1. Genetic variation at the population scale

Eight *P. australis* populations were sampled in the Po Plain, each representing a distinct wetland differing in size, age as well as past and present uses (Table 1). The geographic distance between the two most distant populations is 25 km. In each wetland a total of 15 specimens (shoots) were collected from 10 to 15 *P. australis*-dominated patches of vegetation, visibly separated from other such patches. In seven out of eight populations, two or three individuals were collected in at least one patch. The random amplified polymorphic DNA (RAPD) technique was used to identify genotypes and detect the presence of individual clones in populations. Four to seven different clones from each population, as identified by the RAPD analyses, were subsequently amplified for fragments length polymorphism (AFLP). In the Bentivoglia population, which appeared to be monoclonal based on RAPDs, two additional specimens were collected at a distance >100 m from the previously sampled patches and AFLP fingerprinted in order to get an idea of the limits of this apparently very extensive clone. In total 46 clones from eight Po Plain populations were analysed by AFLP.

Information about the history and management of the wetlands was acquired from owners and employees of the wetlands and from ''Bonifica Renana'', the board in charge of water regulation and distribution in the Po Plain area.

2.2. Genetic variation at the regional scale

The genetic variation pattern found among Po Plain populations was compared with that of the mixed cytotype population of Lake Razim in the Danube Delta, Romania (Clevering and Lissner, 1999). The eleven samples from Lake Razim have different ploidy levels (4*x*, 6*x*, 8*x* and 12*x*) and some of the specimens are known to be very closely related, as they originated from seeds of the same inflorescence in the greenhouses of the Netherlands Institute of Ecology in Heteren (Clevering, personal communication, 1999). Specimens 654RO and 655RO are from the same inflorescence, and 658RO, 659RO and 660RO from a panicle of another clone (Table 2). The mother panicles were collected along a 500 m transect on the land-side of the shore of Lake Razim (Clevering, 1999).

2.3. Genetic variation at a continental scale

The genetic distances of the Po Plain clones to increasingly more distant *P. australis* clones were evaluated by comparison with clones from Europe and adjacent parts of Africa and Asia. All belong in the *P. australis* ''core group'' retrieved in the phylogeographic study by Lambertini et al. (2006), based on the same AFLP markers. The R Package version R 3.02 (Legendre and Vaudor, 1991) was used to calculate distances of the European clones to the Boscosa wetland of the Po Plain. The European clones were divided into five groups according to their geographical distance from the Po Plain (Table 2). Group 1 included clones at a km range between 25 and 500 km, group 2 clones 500–1000 km away, group 3 clones 1000–1500 km away, and group 4 clones from 1500 to 2000 km distance. Group 5 included clones more than 2000 km away (maximum 2530 km). For each group, minimum, maximum and average genetic distances with Po Plain clones were calculated.

2.4. RAPD

Genomic DNA was extracted from dry leaves with 2× CTAB buffer (Rogers and Bendich, 1985) and Proteinase K.