

Genetic differentiation of common reed (*Phragmites australis*) populations from selected lakes of Pomerania (NW Poland), revealed by electrophoretically detected peroxidase variability

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Abstract: Three populations of common reed (*Phragmites australis* (Cav) Trin. ex Steud.), growing under differing edaphic conditions in lakes near the town of Koszalin (the Pomerania region in north-western Poland), were studied in respect to frequencies of electrophoretically detected allozymes in two peroxidase loci: a monomeric locus with three alleles and a dimeric locus with two alleles. On the basis of genetic distances, similarity between these populations and levels of variation were assessed both within populations ($GST = 0.0376$) and between populations ($DST = 0.0194$). Gene flow between populations was low ($Nm = 6.399$).

Keywords: *Phragmites australis*, population variability, electrophoresis, peroxidases

1. Introduction

Aquatic plants (macrophytes), particularly those that deeply penetrate the bottom of water bodies by means of their rhizomes and roots, are still poorly known, as plant plankton has aroused much more interest so far. Morphological variation of common reed (*Phragmites australis*) has intrigued botanists for a long time, which has resulted in intensive research on Scandinavian populations (Björk 1967). Investigations into morphological variation of common reed were conducted in Polish populations on a smaller scale (Krzakowa *et al.* 2003).

Reports on genetic variability between common reed populations are not numerous and have been published in the last two decades (Kühl & Neuhaus 1993; Zeidler *et al.* 1994; Krzakowa 1996; Koppitz 1999; Pellegrini & Stauber 1999; Krzakowa & Drapikowska 2000; Keller 2000; Krzakowa *et al.* 2008; Drapikowska & Krzakowa 2009). Electrophoretic separation of enzymes and RAPD-PCR for DNA analysis were used in those studies.

The present study is focused on the interpopulation variation in peroxidase loci, which is very informative not only for common reed populations (Krzakowa *et al.* 2008), but also for grasses of different genera, such as *Lolium* (Krzakowa & Mikulski 1997), *Calamagrostis* (Krzakowa *et al.* 2005; Krzakowa & Dunajski 2007), *Stipa* (Krzakowa *et al.* 2006) and *Apera* (Krzakowa & Adamczewski 2007).

2. Material and methods

Plant material was collected from populations located in three lakes of the Koszalin Coast District (near the Baltic Sea), which represent over 1600 lakes situated in the geomorphologically variable West Pomerania. The largest studied lake is Lake Jamno, located in the Karnieszewice Forest District. The lake used to be a marine embayment; now its area is 2246 ha, while depth is up to 3.9 m. It lies within the Słowińskie Coast and is separated from the Baltic Sea by a sandy spit. The lake is characterized by a cryptodepression, i.e. its bottom lies below the sea level.

The other two lakes are situated in the Manowo Forest District. The typical post-glacial Lake Lubiatowo, of 296 ha in area and depth of up to 2.9 m, is a nature reserve. Lake Cewlino, created from a flooded sand pit, is intensively used by local inhabitants for recreation. It could be expected that under such differing water conditions, genetic variation of the common reed populations should distinguish them, although Lakes Lubiatowo and Jamno are linked by the river Dzierżęcinka, which connects them. Thus, completely different edaphic conditions were the reason why these lakes were selected for the study on genetic variability of common reed populations. However, investigations conducted on seedlings cultivated under uniform greenhouse conditions exclude environmental influences.

In each population, panicles from 30 plants were randomly selected along a transect in the winter time. Fortunately, the winter was frosty, so the naturally vernalized seeds, sown under identical greenhouse conditions, germinated very well. Three-leaf seedlings were subjected to electrophoretic analyses. Crude extract was prepared from one leaf of a plant. Chromatographic paper wicks (5 mm × 5 mm, Beckmann 319329) were soaked in the extract and placed in 11% starch gel (Sigma) based on lithium-borate buffer system, pH 8.3. The electrophoresis was conducted at 200 V and 40 mA at 5°C. The gels were then stained with 3-amino-9-ethylcarbazole for 30 min. at room temperature. The data were statistically analysed to calculate allozyme frequencies, observed heterozygosity (H_o), the polymorphism coefficient (P_g) and Wright's fixation index (F). The extent of intrapopulation variability (GST), interpopulation differences (DST) and gene flow (Nm) were determined (Nei 1977). The UPGMA (method of closest neighbourhood) based on gene frequencies (Gregorius 1978) and a dendrite constructed according to Hedrick (1974), permitted us to illustrate genetic distances between the populations.

3. Results and discussion

Electrophoretic separation of peroxidases shows the occurrence of two polymorphic loci (named A and C), migrating toward the cathode. The monomorphic locus B was not included in interpopulation comparisons. The fastest-migrating locus C (monomeric) consisted of three alleles, while the slower migrating locus A (dimeric) showed two allozymes (Krzakowa & Judek 2009).

Every population was characterized in respect to genotype frequencies (Fig. 1). Genetic distances between populations allowed us to construct the dendrite according to Hedrick (1974) (Fig. 2). As it may be seen from Fig. 1, population 3 shows a higher frequency of genotype C1C3 in comparison with populations 2 and 1, where this genotype has not been not found.

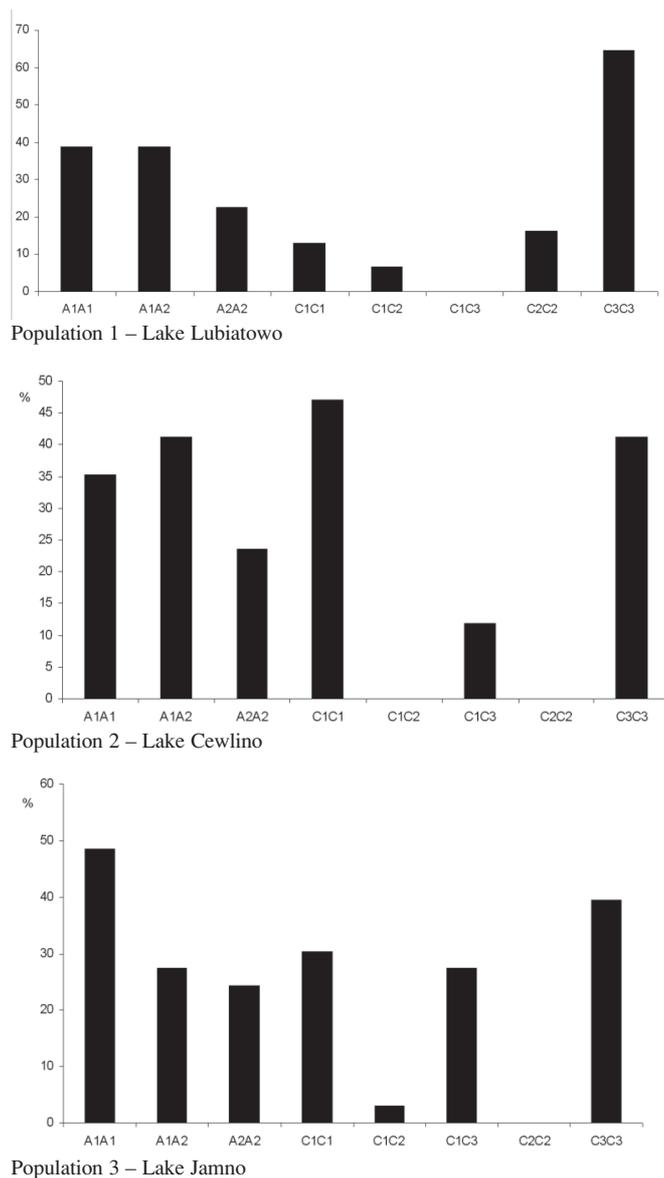


Fig. 1. Frequencies of peroxidase genotypes (loci A and C) in the three populations investigated

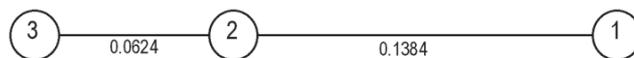


Fig. 2. The dendrite illustrates genetic distances between common reed populations

Explanations: 1 – Lake Lubiatowo, 2 – Lake Cewlino, 3 – Lake Jamno

Populations differed also in allozyme frequencies: allele A1 was the most frequent in population 3, while allele C3 is the most frequent in population 1 (see Table 1).

Table 1. Allele frequencies in the three populations investigated

Loci	Alleles	Populations		
		1	2	3
PX A	1	0.581	0.559	0.621
PX A	2	0.419	0.441	0.379
PX C	1	0.161	0.529	0.455
PX C	2	0.194	0.000	0.015
PX C	3	0.645	0.471	0.530

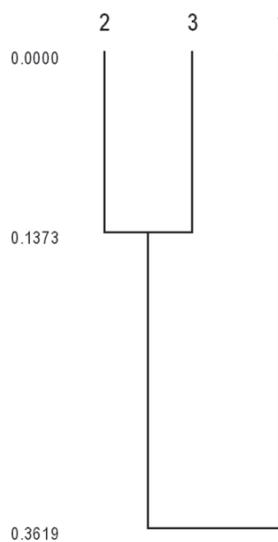


Fig. 3. The shortest genetic distances (UPGMA) between populations in question
 Explanations: 1 – Lake Lubiatowo, 2 – Lake Cewlino, 3 – Lake Jamno

The populations were also compared with respect to genetic distances characterised by dendrograms (Fig. 3), constructed on the basis of allele frequencies according to Gregorius (1978). Similarly as on the dendrite, populations 2 (from Lake Cewlino) and 3 (from Lake Jamno) are similar, while population 1 (of Lake Lubiatowo) is clearly distinct. This is a very interesting result, as population 2 is located only 4.5 km away from population 1, but it proved to be more similar to population 3, located ca. 15 km away. This could exert some influence on the genetic structure of the populations, due to the possible gene flow.

The population most polymorphic in respect to locus C (Table 2) was population 3 from Lake Jamno ($P_g = 0.677$). The lowest values of Wright’s fixation index (F) for locus A show that populations 1 (Lake Lubiatowo) and 2 (Lake Cewlino) remain in the Hardy-Weinberg equilibrium. However, the highest value of the fixation index ($F = 0.876$) for population 1 (Lake Lubiatowo) might suggest some degree of self-fertilization or hybridization of similar genotypes. The level of heterozygosity (H_o) is not high in all of the populations in question.

Table 2. Genetic parameters of *Phragmites australis* population

Locus	Populations	He	Ho	F	Pg
PX A	1	0.4870	0.3871	0.2051	0.6493
PX A	2	0.4931	0.4118	0.1649	0.6505
PX A	3	0.4706	0.2727	0.4205	0.6318
PX C	1	0.5203	0.0645	0.8760	0.5369
PX C	2	0.4983	0.1176	0.7639	0.5952
PX C	3	0.5119	0.3030	0.4081	0.6777

Explanations: H_o – observed heterozygosity, H_e – expected heterozygosity, F – Wright’s fixation index, P_g – genotype polymorphism coefficient

Some gene flow between the populations ($Nm = 6.399$) exists and probably affects the variation of the populations more strongly than genetic drift.

Population 3 differs from the other two also in its larger panicle size, which is probably due to edaphic conditions. Variation in panicle size and shape was the subject of earlier investigations (Björk 1963; Krzakowa *et al.* 2003). So far, no correlation between morphological and genetic variation has been detected (Drapikowska & Krzakowa 2009).

Genetic variation within populations was assessed on the basis of mean values of Nei’s (1973) statistics for single loci (Table 3). Intrapopulation variation ($G_{ST} = 0.0376$), although relatively low, exceeds the values of interpopulation variation (mean $D_{ST} = 0.0194$).

Table 3. Genetic parameters estimating variation within and between examined populations

Locus	HT	HS	DST	GST
PX A	0.4829	0.4816	0.0013	0.0026
PX C	0.5498	0.5123	0.0376	0.0683
Mean	0.5163	0.4969	0.0194	0.0376

Explanations: HT – total genetic diversity, HS – mean diversity, G_{ST} – degree of gene diversity within populations, D_{ST} – degree of gene diversity between populations

The occurrence of heterozygous forms of both peroxidase loci suggests that genes are exchanged in the process of sexual reproduction (Krzakowa & Drapikowska 2000). This contrasts with the belief that reed is propagated primarily by means of transportation of vegetative parts (i.e. roots or stems) mostly by birds that inhabit reed beds, while gene exchange is limited. Moreover, the rapid expansion and spread of *P. australis* in North America is supposed to be due to clones introduced accidentally from Europe, which were more expansive than local ones (Koppitz 1999).

Common reed spreads horizontally by runners. In favourable habitats they can spread in various directions, and both the numbers and density of their ramets increase there. Such penetration is termed “foragin” (Cain 1994; Evans & Cain 1995). Thus genetically identical individuals may react differently during such penetration and consequently show morphological differences

(de Kroon & Hutchings 1995). Nonetheless, results in this study suggest that genetic variation of common reed does not depend on edaphic factors in lakes of various geological origin.

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