

**PH.D. DISSERTATION THESIS**

**GENETIC DIVERSITY OF THE REED (*PHRAGMITES AUSTRALIS*)  
STUDIED BY PCR-RAPD METHOD**

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## 1. Introduction

The common reed (*Phragmites australis* (Cav.) Trin ex Steud) is a grass that can reach a height of several meters, and belongs to the Poaceae family. The spring growth of the new shoots is supported not only by photosynthesis but also by the mobilisation of the stored non structural carbohydrates from the rhizomes. This way a tall, dense reed stand develops early in the year leaving less light for other species and eventually resulting in the monodominance of the reed in most of its associations.

Water is a basic element for plants, but only 1-2 per cent of the vascular plants are aquatic because flooded soils are anaerobic. The reed is one of the few species which has developed a specific ventilation system supplying oxygen to the rhizosphere (Armstrong and Armstrong 1991, Brix et al. 1992, Armstrong et al. 1966, Beckett et al. 2001.). Since the reed has unlimited water supply, it gets enough nutrients from the submersed soil, and takes up carbon dioxide from the air, it unites the advantages of the aquatic and terrestrial ways of life. However, its sexual propagation remained terrestrial to such an extent that its seeds do not germinate under the water, and the seedlings do not tolerate longer submergence (Hürlimann, 1951, Haslam 1971, Weisner et al. 1993, Coops et al. 1959) and on surfaces covered by water it propagates exclusively by rhizomes. In established stands the recruitment from seeds also has a low frequency on land because the seeds usually can not contact the soil due to the litter coverage, and the light penetrating a closed stand is not enough to support the development of seedlings.

The reed is distributed on both hemispheres from the tropics up to the 70<sup>0</sup> latitude and from the sea level up to the Tibetan plateau. It prefers shallow waters or wetlands on the shores of rivers, lakes and canals but it also grows well on dry land, if the roots reach water. The developed individuals are rather resistant to climatic and soil conditions and tolerate a significant salinity too (Rosewald-Rudescu 1974, Clevering and Kissner 1999). It is a basic question whether this wide distribution of the species in very different habitats is allowed by the ecological plasticity of the clones, or by the multitude of specialist clones?

Detailed studies into the clonal structure of the reed populations were enabled by molecular techniques. The early RAPD studies demonstrated large monoclonal stands (Kühl and Neuhaus 1993, Neuhaus et al 1993). Further studies detected several hundred meter long clones on the water edge of some stands, while much shorter clones were observed on the landward sides. In some shallow waters significant clonal diversity was detected.

These observations led to an interesting hypothesis (Kühl and Neuhaus 1993, Koppitz et al. 1977), distinguishing three phases in the establishment of reed populations. In the first one a population develops from seeds on dry land, which adapts easily to the changes of the environmental conditions because of its high clonal diversity. In the second phase the rhizomes, starting from the land population, penetrate deeper and deeper into the water, where strong competition decreases the clonal diversity and accordingly the adaptability of the population. For the third phase only a single clone remains which is the most adapted to the environmental conditions, but due to the lost diversity this stabilised monoclonal stand can not adapt to new external changes. It was also suggested that decreased clonal diversity contributed to the European reed die-back (Kühl and Neuhaus 1993, Koppitz and Kühl 2000). This hypothesis however is based on studies of relatively few lakes, and even in these lakes the age of the reeds is not known. The narrow ecological tolerance of the reed clones is also questionable.

The clonal diversity of reeds from the same lake was smaller than among reeds from different waters. The analysis of single clones collected from reed populations of different European countries seemed to indicate an increasing genetic distance by increasing geographical distance, but not unequivocally, since Hungarian and Romanian samples clustered together with the North-European ones (Koppitz, 1999).

## **2. Objectives**

1. Reed colonies were dieing back in Balaton. Therefore our first target was to get a picture about the clonal diversity of reeds of Balaton so that we know whether its decrease could have contributed to die-back.

2. Since reeds of Balaton are quite old and we still found it to be polyclonal, and they did not know the age of those colonies on which the theory was based, our second aim was to check the supposition about monoclonal development by studying the clonal diversity of colonies with known age (Lake Bóden, Osterseen, Lake Fertő, Kis-Balaton).
3. In former studies they gave only that one or more clones were found in certain colonies or we could come to know the distance of sampling as well but it was around 50 metres in general. Since using a 15 m sampling distance in Balaton we found that the most of clones are less than that, therefore we introduced the frequent sampling to get the real picture about the size of clones.
4. In case of overseas studies, they emphasized clonal diversity. We also aimed to know the size of gene diversity, what is the genetic distance between certain clones within a colony, or what kind of differences appear between colonies from other lakes in this respect.
5. For understanding the expansion of reeds, it is essential to know how the genetic distance changes with geographic distance. Eureed program did not give you an unambiguous answer. To answer this question, we compare the geographic distance with the genetic distance of the 12 populations studied by the same method and taken from 5 lakes, and then we extend the analyses by studying different samples taken from 21 Central-European lakes.  
Using our results and more recent literature, we try to form a picture how reed spreads out over long distances, how it takes root and how could the diversity of a colony depend on the way it was formed.
6. Before opening the Sió sluice in 1863, the water level of Balaton was so high that the present area of reeds was covered with 1.5 m deep water everywhere. However, after opening the sluice-gates, an extremely strong and long-lasting drought came and as a consequence the water decreased by 1.5 m compared to its present level which gave a good alternative to multiply from seed and take root on the dry lake bottom. Then these areas were covered with water again and the waterfront of those colonies are continuously under water ever since.

### 3. Materials and methods

#### 2.1. The long term water level changes of the lakes

Lake Constance is a subalpine lake on the border of Germany, Switzerland and Austria with a surface area of 536 km<sup>2</sup>, a maximum depth of 254 m and a mean depth of 90 m. It consists of the larger Obersee and of the smaller Untersee. The Rhine enters the Obersee and drains the Untersee. The Überlingersee is part of the Obersee. Lake Constance has a very long history of research. Water level readings are recorded since 1917 (Ostendorp et al. 2007). The lakeward side of the reed stands of Lake Constance originates from seeds that germinated at least two hundred years ago, but probably even much earlier. The landward side of the reeds standing in shallow water is probably much younger.

The Osterseen consists of 20 small lakes, connected by the Osterseen-Ach. Their total area is 225 ha, the average depth 9 m. The Osterseen-Ach has a catchment of 5,7 km<sup>2</sup> and empties finally into the Starnberger-see. The lakes were formed in basins excavated by ice at the end of the Würm glaciation. There is no long-term data set on their water level but it can be assumed to be stable because the lakes are feed mainly by ground water (Melzer, 1976). The reeds are probably several hundred years old.

Lake Fertő is a large soda lake on the Austro-Hungarian border, with a surface area of 309 km<sup>2</sup>, a maximum depth of 1,8 m and an average depth of 0,7 m. Most of its area is covered by reeds. It is the second largest reed wetland in Europe after the Danube delta. The earliest documents on the water level of this lake date back to the 17<sup>th</sup> century (Bendefy, 1973, Dobesch and Neuwirth, 1979). Nearly the whole lake dried up twice in the 17<sup>th</sup> and once in the 18<sup>th</sup> century according to these historic documents. The largest drought was in the 19<sup>th</sup> century, and the lake dried out totally between 1866 and 1870. The estimated age of these reed stands therefore is 140 years.

Lake Balaton in Hungary is the largest lake in Central Europe with a surface area of 594 km<sup>2</sup> (Entz and Sebestyén 1942, Herodek et al. 1988). The maximum depth of the lake is 11 m, its average depth 3,3 m. The lake consists of four basins. The Zala river empties into the first one, and the Sió-canal drains the water from the fourth basin into the River Danube. From the 240 km long shoreline 110 km is covered by reeds

with a total area of 12 km<sup>2</sup>. Most of the reed stands are on the northern shore where they penetrate the lake to a depth of 1,5 m. On the lotic, sandy southern shore the reed can not grow in water deeper than 1 m. The reeds in Lake Balaton seem therefore to be 140 years old.

The Kis-Balaton is a basin above the mouth of the Zala river. It was once a bay of Lake Balaton holding open water. At the present part of it is dry land, another part is covered by shallow water. 20 km<sup>2</sup> of its 52 km<sup>2</sup> area are covered by reed. The reed here was the youngest in this study.

## 2.2. Sampling stations and sampling strategies

In Lake Constance we had a sampling station on the shore of the Reichenau peninsula on Untersee, and another on the shore of the Überlinger-see. In Osterseen one station was on the shore of the larger, oligo-mesotrophic Ostersee, another on the shore of the smaller, eutrophic Waschsee. On Lake Fertő we had three stations in the National Park, near the villages of Fertőrákos, Hidegség and Hegykő. In Lake Balaton two stations were in the bays of the northern shore (Kerekedi-bay, Bozsai-bay) and one on the southern shore (Balatonmárfürdő). On Kis-Balaton the reed around the small open water area of Zalavári-víz was studied (Fig.1.) The characteristics of the stations are shown by Table 1. At each station 10 samples were collected from the lakeward and another 10 from the landward sides. Sampling distances were 20 m in case of Lake Constance, Osterseen and Neusiedler see, and 15 m in Lake Balaton and in Kis-Balaton.

To obtain more accurate information on the size of the clones samples were also collected with an interval of 5m in the Bozsai-bay of Lake Balaton and in the reed wetlands around the Zalavári-víz in Kis Balaton, and a 40m x 40m area was sampled in a 4m x 4m grid in the reed stand at Alsóörs in Lake Balaton.

In addition to the 11 populations listed above, samples were collected in the summer of 2006 from the Tatai-tó (N:47°38'12", E:18°20'16"), Tamási fish pond (N:46°36'56", E:18°16'40"), Vadása-tó (N:46°56'04", E:16°18'37"), Velencei-tó (N:47°14'11", E:18°38'46") an alkaline pond in the Hortobágy area in Hungary

(N:47°32'49", E:21°08'58"), the Szolyva reed swamp in Ukraine (N:48°31'47", E:22°46'34"), the Transylvanian Medve-tó (N:46°36'22", E:25°05'25") and Csíkrákosi-tó (46°27'14", E:25°45'53"), and from a tetraploid stand (N:44°51'16", E:29°36'24"), and an octoploid stand (N:44°56'38", E:29°43'37") of the Danube delta in Romania. Only one clone was analysed from each of these stations.

The tip of the shoot was cut on the field, wrapped in wet paper, and transferred to the laboratory. About 0,1 mg of undried meristem was prepared from each shoot, and stored at -80 °C.

### 2.3. RAPD analysis

DNA was extracted by Qiagen Plant Mini Kit from the plant material according to the protocol.

Reactions were carried out in volumes of 25 uL, consisting of 1,5 mM of MgCl<sub>2</sub>, 0,75 mM of dNTP, 0,4 mM of primer, 10 ng of genomic DNA, 0,8 units of Taq DNA polymerase and 10x Taq DNA polymerase buffer. PCR amplifications were performed in a PCR Applied Biosystems 2720 Thermocycler. Five primers were selected for amplification. In addition to the (GACA)<sub>4</sub>, M13 and (GATA)<sub>4</sub> applied in earlier investigations (Koppitz, 1999) the Operon B01 (GTTTCGCTCC) and Operon B11 (GTAGACCCGT) were also used. In the case of the first three primers the thermal program described by Koppitz (1999) was adopted. The thermal cycler was programmed for 40 cycles of 1 min at 94 °C, 1 min at 36 °C, 2,5 min at 72 °C, 2,5 min at 72 °C, followed by a termination step of 10 min at 72 °C, and cooling to 4 °C for the B01 and B11 primers. All samples were amplified twice with all the five primers, and in each series a standard sample was used to control the reproducibility.

Bands were resolved by electrophoresis in a 1,8 per cent agarose gel in 1x TBE (Tris-ADTA-borate) buffer and stained with ethidium bromide. A 126 bp ladder helped the identification of the bands. The fingerprints were photographed in UV light. The bands were scored visually. Their presence or absence was indicated by 1 or 0 respectively in a binary character matrix.



## 2.4. Data analysis

Amplified products were scored for the presence (1) or absence (0) of homologous bands, and a matrix of the different genotypes was assembled. 43 reliable and variable markers were used for further analysis. The genetic similarity and genetic distance between genotypes and between populations (Nei 1972), and the gene diversity (Nei 1973) and Shannon diversity (Lewontin, 1972) within the populations were calculated using Popgene version 1.32 (Yeh et al. 1997).

Samples with similarity coefficients higher than 0,95 were assumed to belong to the same clone during the construction of clone maps.

The UPGMS dendrogram based on the similarity coefficients of the populations was drawn by the PhyloDraw program.

The normalized Mantel statistic ( $r_M$ ) (Mantel 1967) was calculated between the genetic and geographic distance matrices. The significance of  $r_M$  was tested by a permutation test (10000 iteration) to determine whether there is a relationship between the genetic composition and the geographic distribution of the individuals or populations.

## 3. Results

### 3.1. The clonal diversity of the reed populations

At the Untersee sampling station on Lake Constance (Fig.3.) six of the ten collected samples belonged to the same clone on the lakeward side of the reed stand. This clone was intruded at two places by other clones so the terminal members of the large clone are 160 m distant from each other. The clone may be even larger as it might continue beyond the boundaries of the studied stretch. Although this is a large clone in a very old reed stand, the population can be described as polyclonal since the ten samples belong to five different clones. Eight clones in ten samples, i.e. a higher diversity was found on the landward side. The difference between the two sides was smaller on Überlinger see: four clones were detected on the lakeward side, the largest containing four samples, and five clones on the landward side.

In Osterseen (Fig.3), on the lakeward side of the stand of Grosser Ostersee one clone was detected which covered four samples and six clones holding one sample; on the landward side one clone with an area of four samples, two clones with two samples and two clones with one sample were found, i.e. seven clones were detected on the water side and only five on the shore side. This is the only stand with a smaller diversity on the landward side. In Waschsee six clones were identified on the water edge and eight clones on the landward side.

In Lake Fertő (Fig.4) on the lakeward side of the reed stand at Fertőrákos one clone consisted of three samples, and the ten samples belonged to eight different clones. On the landward side all the ten samples had different genotypes. At Hidegség one clone was found to hold four samples, two clones held two samples and two covered only one sample, i.e. altogether five clones were found on the water side, while on the landward side all samples differed. At Hegykő there were two clones with two samples on the water edge and another with three samples on the landward side, i.e. eight clones were detected in both zones.

On Lake Balaton both in the Kerekedi-bay and at Máriafürdő three samples belonged to the same clone on the lakeward side, i.e. the ten samples formed seven clones while all samples differed on the landward side. In the Bozsai-bay a long clone stretching along the area covered by five samples was identified on the lakeward side, so the ten samples in intervals of 15 meters belonged to six clones, while eight clones were found on the landward side.

In the Kis-Balaton wetland (Fig. 5) at the sampling station of Zalavári-víz all samples collected in intervals of 15 meters belonged to different clones on both sides of the reed stand.

## 1.2. Clonal diversity by dense sampling

The number of detected clones on a given stretch and the probability that two neighbouring samples belong to the same clone are functions of sampling density. In order to obtain a better estimate of the effective clone sizes the 150 m long stretches on the shore of the Bozsai-öböl and Zalavári-víz were sampled with 5 m distances. In the

calculations described in the previous section only the results of every third sample were used corresponding to a sampling distance of 15 m for the sake of comparability. The results the dense sampling (Fig.6) on the lakeward edge of the reed stand in the Bozsai-bay show a 75 m long clone containing fifteen samples, a 20 m long one containing four samples, two 10 m long clones with two samples, and seven 5 m long clones each with one sample. On the landward side there is one 25 m long clone, two 15 m, two 10 m and fifteen 5 m long clones. The 30 samples showed 11 different genotypes on the lakeward, and 20 different genotypes on the landward side. In the Kis-Balaton 17 and 23 clones were identified on the lakeward and landward sides respectively. On the lakeward side three clones contained four samples, five clones two samples and nine clones one sample. On the landward side six clones contained two samples while the others only one. On the lakeward side three clones were intruded by others. The distance between the two members of the same clone was 35m in one case.

According to these results the diversity is smaller on the lakeward than on the landward side. In order to study the clonal diversity in the interior of the reed stand a 40 m x 40 m area was sampled according to a 4 m x 4 m grid at Alsóörs on the Northern shore of Lake Balaton. There was no reed at 5 points, so 94 samples were collected and they belonged to 74 different clones (Fig.7). One clone contained 5 samples, two 3 samples, ten 2 samples and the remaining 64 clones only one sample. The largest clone was longer than 30m, but the major part of the reed stand consisted of clones with diameters under 4 m. The number of detected clones could be further increased by an even denser sampling grid.

### 1.3. Gene diversities and genetic distances within populations

The samples that belong to the same clone were regarded as belonging to the same unit when calculating the gene diversities, Shannon diversities and genetic distances (Fig.8).

Both the clonal diversity and the gene diversity were much lower in Lake Constance and in Osterseen than in the Hungarian Lakes. The gene diversity was 0,15-0,16 in the four stands in Germany, 0,22-0,28 in Lake Fertő, 0,24-0,29 in Lake Balaton

and 0,48 in Kis-Balaton. The Shannon diversity showed a very similar trend. It was in the range of 0,22-0,26 in the German reed stands, 0,32-0,41 in Lake Fertő, 0,36-0,43 in Lake Balaton and 0,48 in Kis-Balaton.

The average of the genetic distances calculated in pairs for all clones in the same population were 0,28-0,22 in the German lakes, 0,26-0,37 in Lake Fertő, 0,32-0,38 in Lake Balaton and 0,47 in Kis-Balaton. The maximum genetic distances within populations were 0,33-0,47 in the German lakes, 0,49-0,72 in Lake Fertő, 0,72-0,89 in Lake Balaton and 0,93 in Kis-Balaton.

Both the gene diversities and the genetic distances within population were lowest in the German lakes with little differences among the populations of Lake Constance and the Osterseen. The genetic diversities and distances of the reed stand of Lake Fertő and Lake Balaton were similar to each other, while the highest values were found again in the reed stand of Kis-Balaton.

### 3.3. Comparison of the genetic and geographic distances of the populations

The three stands in Lake Fertő are the most similar according to the UPGMA dendrogram based on the similarity coefficient of the populations (Fig.9). They are soon joined by the reeds of Kis-Balaton. In Lake Balaton first the stands of the Kerekedi-bay and Máriafürdő associate, then they join to the Fertő-Kis-Balaton cluster. The stand of Bozsai-bay is attached to them somewhat later. The two populations in the Osterseen differ from each other approximately to the same extent as the stand of Máriafürdő from that of the Kerekedi bay. The Osterseen then join the Hungarian shallow lakes and not Lake Constance which would geographically be much closer. The two populations of Lake Constance have a low similarity, and join the other lakes at very low values.

Both genetic and geographic distances were calculated in pairs for the 11 populations. The distance between the three stands in Lake Fertő is under 10km, and their genetic distance is only 0,02-0,03. The Kis-Balaton is in a distance of 115-125 km from the stands of Lake Fertő, but the genetic distances are only 0,07-0,08, while the Kis-Balaton-Máriafürdő distance is only 18 km, but their genetic distance is 0,18. The three stands in Lake Balaton are 106-129 km away from the three ones in Lake Fertő, and their genetic distances are in the range of 0,23-0,29. The reeds of the Osterseen are

402-412 km from those of the Lake Fertő, and their genetic distances are between 0,23-0,29. These are indeed larger geographic and genetic distances than those between the populations of Lake Balaton and Lake Fertő. But although the geographic distance between the populations of the Osterseen and of the Lake Constance is only 159-168 km, their genetic distances are 0,33-0,52. The genetic distance between the two populations of Lake Constance is 0,26, and they are genetically far away from the populations of the other studied lakes. The Untersee is genetically still the closest to the Waschsee, but its genetic distance of 0,44-0,52 from the populations of the other lakes is surprisingly high. The genetic distance of the population of the Überlinger-see from those of the other lakes varies in the range of 0,34-0,53. According to the Mantel test the geographic and genetic distances are related ( $r=0,729$ ,  $p=0,0001$ ), but this can be a result of the high difference between the genetic character of the populations in the two German lakes and the populations in the Hungarian waters. The different character can be a result of the geographic distance or other causes like the isolation of the subalpine lakes, or the different ages of their reed stands. The rather low gene diversities within population in Lake Constance and in the Osterseen seem to support to the latter causes.

When single samples was chosen randomly from each of the 11 populations above, and one clone from 10 other reed stands in Hungary, Ukraine, and Romania were analyzed and genetic and geographic distances of the 21 samples obtained this way were calculated in pairs, the smallest genetic distance between two samples was 0,21, and the average distance between the pairs was 0,50. No correlation was shown by plotting the genetic distances vs. the geographic distance (Fig. 10), neither is any correlation indicated by the Mantel test ( $r=0,104$ ,  $p=0,129$ ).

## **4. Discussion**

### **4.1. The factors influencing clonal diversity**

Vegetative propagation is frequent in the plant kingdom. Most clonal species form highly diverse starting populations from seedlings, but further propagation from seeds is rare in established stands. In such populations the clonal diversity might be

expected to decrease with time, but usually even old stands have considerable diversities and monoclonal populations are exceptional (Ellstrand and Rose 1987). According to the model of Bengtsson (2003) a population started by a number of sexually derived propagules may retain its genotypic variation for a very long period of time even if it reproduces almost exclusively asexually later. An established reed population reproduces almost exclusively asexually on land, and exclusively asexually in water.

We found a higher diversity on the landward side of the stands than on the lakeward side, since some recruitment from seedlings is possible on the shore side from time to time. In the deep water the clonal diversity was highest in the youngest stand in Kis-Balaton, where the largest clone was only 15 m long, and the lowest in the oldest stand in Lake Constance, where a clone longer than 160 m was found. The ages of the reed populations in Lake Fertő and Lake Balaton were estimated equally to 140 years, and they showed similar diversities. These diversities are high and even the old stands are polyclonal, so the decrease of the clonal diversity must be slow, and there seems to be no sharp competition among the clones. A strong reed die-back occurred in Lake Balaton despite the high genetic diversity which suggests that the decreased genetic diversity has no major role in this process.

Recently the reed populations of two small lakes with known ages were compared (Curn et al., 2007). The Opatovický fish-pond was created between 1510 and 1514, the Hlamky sand pit was excavated between 1970 and 1994. In the fish pond 9, in the sand pit 7 stands were studied. The stands were represented by 200 m long stretches in the fish pond and 100 m long stretches in the sand pit. In all stands 5 samples were taken on the water side. In the fishpond 5 stands proved to be monoclonal from 9, and in the sand pit 4 stands from 7 were monoclonal. The monoclonal stands of the fishpond could have resulted from a selection process where during five hundred years all the other genotypes were outcompeted by the most adapted one, but the forty years in the sand pit were hardly enough for this. It can be assumed that in certain places the starting population on the shore was formed by a very small number of seedlings, and this led to the monoclonal stands in the water of the sand pit; but it is also possible that the stands started from single rhizomes drifting to the shores which were otherwise free

of reeds. However, this propagation mechanism was also possible in the old fishpond, so it seems questionable if monoclonal stands could have been formed in the fish pond and other lakes in the same way as in the young reeds of the sand pit, and how some of the five hundred year old stands of the fish pond remained polyclonal?

We suggest that the establishment of a particular reed stand plays an important role beside its age in determining the clonal structure. As a first attempt perhaps three different ways could be distinguished in the establishment of water reed populations. In the first one, rhizomes drift to the shore where monoclonal stands develop from them without any competition by vegetative growth. In the second way, the reed propagates first from seeds on the land, then rhizomes from the established land reed stand invade the lake deeper and deeper. The clones first compete to occupy the areas that are still open and later to supplant each other from the areas already occupied. The diversity of the reed stand in the water may also depend on the variability of the starting stand on the land, which is again the function of the prevailing founder effects. Thus, the competition in the water may start with more or less participants. The third scenario defines the probable formation of reed stands in shallow water: Here highly diverse populations are established from seeds on the temporarily dry lake bottoms, which become inundated later. The established populations can penetrate deeper waters of the lake later. If the starting population of the water reed is rich in genotypes, they probably have many clones with similar ecological tolerances. This may have helped to preserve the high diversity of the 140 years old stands of Lake Balaton and Lake Fertő.

In shallow lakes part of the sediment of the reed stands becomes dry during long droughts, which may support high diversity, but in closed stands the recruitment from seeds is low, so the mortality of the clones due to competition or to other reasons seems to be very low too.

#### 4.2. Gene diversity of the populations and their geographic and genetic distances

Nybom and Bartish (2000) found the average gene diversity of the wind pollinated plants to be 0,29 by collecting the RAPD results of 41 plant species, which is much higher than the average gene diversity of insect pollinated species. This average

was 0,24 for long living perennial plants, which is the double of the average gene diversity of annual plants. The reed is a long living wind pollinated plant. The gene diversities of the populations of reed in Lake Balaton and Lake Fertő are very similar to the values given by Nybom and Bartish (2000), despite its clonal nature and the fact that it had no sexual recruitment in the past 140 years because it was continually standing in water. The value of 0,48 for Kis-Balaton is exceptionally high. Perhaps this youngest stand is most similar to the land populations of the reed. and the starting populations in Lake Fertő and Lake Balaton once had similar values. It would be important to study the gene diversity of reed populations growing on permanently dry land. The gene diversities of the reed populations of Lake Constance and of the Osterseen are low compared to the other wind pollinated plants, probably due to inbreeding, genetic drift and natural selection, and indicate the old age and isolation of the stands.

The high gene diversity in the stands of the three Hungarian lakes and their genetic proximity to each other, refer to an intensive wind induced gene flow in this relatively large area. Contrarily the gene diversities of the populations of Lake Constance and of the Osterseen are low compared to the other wind pollinated plants indicating the isolation of the populations. This is also shown by their large genetic distances which are much higher than explained by the geographic distance of the two lakes. The genetic distance between the Hungarian and German lakes is probably not only caused by the geographic distance, but also by other isolating factors such as geographic relief and wind conditions. The large difference between the ages of the populations should also be considered.

The correlation between the genetic and geographic distances have been studied using different strategies, where the limiting factor is the amount of samples that can be investigated. In the early studies a single clone was taken from a great number of lakes in a large area (Koppitz, 1999). Some increase of the genetic distance with the geographic distance was shown, but the correlation was not obvious. Recently (Lambertini et al., 2006) the rich reed stands of the Po valley were studied, and the many clones sampled here were compared in pairs to single clones taken from many reed stands in a large European area. The genetic distance did not increase with the



geographic distance within a radius of 500 km, showed only a 10 per cent increase between 500 and 1500 km, and above this another 10 per cent increase was demonstrated. This implies that the reed stands on a large part of Europe form a single metapopulation. A study into the genetic diversity and dispersal of *Phragmites australis* in a small river system indicated a pollen and seed dispersal at short distances, an intensive seed dispersal up to 10 km and a reduced but existing gene flow mainly by seeds over longer distances (Fer and Hroudova, 2009).

When we selected single clones from 21 stands in an area extending from lake Constance to the Danube delta and calculated the genetic and geographic distances in pairs, these values did not correlate, indicating a large general genetic mixing. However, when populations were characterised by several clones, the results showed a very intensive gene flow among the populations of the Hungarian waters, while the two lake systems in Germany proved to be isolated. It seems therefore that there is an intensive gene flow in a very large area, but some isolated areas exist. The question is worth further research of whole populations long distances from each other. The gene flow is mainly by wind transport of pollen and seeds, but the dispersal of seeds and rhizomes by water or human activity also contributes locally. Relatively few results are available on the sexual reproduction of the reed (Haslam, 1973, McKee and Richards, 1966, Ishii and Kadono, 2002). The seed set is variable and low, but there are several thousands of flowers in a panicle and about one hundred stems on a square meter. This way even if only a part of the stems flowers and only a part of the flowers set seed, a mature reed stand still has a huge spreading potential. If the ground is disturbed and the vegetation removed (e.g. for road construction), or if the bottom of a water body runs dry, the reed appears very rapidly. This was observed on Lake Balaton on the sandbanks that were uncovered during the drought of 2000-2003. North-America was invaded by the M haplotype reed introduced from Europe (Saltonstall, 2002) within a century, indicating how intensive the spread of the reed dominant in Europe can be. Perhaps the common reed belongs to those plants that produce small seeds for long distance dispersal, and solve the local distribution through vegetative propagation (Eriksson, 1992).

## **5. Conclusions**

There is no sexual recruitment in reed populations standing in water, and the clonal diversity was lower on the open water edge than on the landward side of the stands (corresponding to our expectations, and in the oldest population lower than in the youngest. However, even the water side of the oldest population proved to be polyclonal, indicating no strong competition of the different genotypes in established populations. High clonal diversity characterised the old populations in shallow lakes. Reed populations are established in very different ways, influencing their clonal structure to a large extent. The starting diversity can be very high in population formed from seeds on the temporarily dry bottom of shallow lakes which is inundated later. Lower diversities result from the invasion of submerged areas by rhizomes of terrestrial populations. Monoclonal stands can also develop from rhizomes drifted to the shore without any competition.

No correlation was found between genetic and geographic distances by calculating pairwise differences of 21 reed clones collected from different Central European stands. When populations were characterised by several clones, the populations of the Hungarian lakes proved to be very close to each other while those in subalpine lakes were distant from them and also from each other. Most of the reeds seem to belong to a metapopulation in this large area, but there are some places that are more isolated.

The specific ecophysiological features, the high clonal diversity, the broad ecological plasticity of the clones and the long distance dispersal of the seeds together may enable the exceptionally large distribution of the species.

## **6. Recent scientific results**

1. All the studied reed colonies of Balaton was found to be polyclonal, therefore the die-back could not be caused by the decrease of diversity.
2. The age of Boden reeds is more than 200 years. The reeds of Ostersee are also very old. Reeds of lake Fertő and Balaton are about 140-150 years old on the water front. The youngest colonies are in Kis-Balaton. On a stretch of 150-200 m, we took 10 samples

3. On the waterfront, the 30 samples taken every 5 m belonged to 13 clones at Alsóörs, 11 at Bozsa, 17 at Zalavár. On the landward side, the 30 samples belonged to 30 clones at Alsóörs, 20 at Bozsa and 23 at Zalavár. In the middle of the Alsóör colony, 95 single reeds taken in accordance with a 4 m x 4 m grid on a 40 m x 40 m area showed 74 different clones. Therefore the diameter of clones growing on the larger part of the land was less than 4 m. The size of ramets is surely smaller than that.
4. Genetic diversity changed between 0.15-0.16 in the 4 German colonies, and 0.22-0.36 in the 6 Hungarian ones. Shannon diversity showed a very similar tendency too. The average genetic distance within the same colony was 0.18-0.22 in German lakes, and 0.26-0.51 in Hungarian lakes. The lower figures of Ostersee and lake Bóden refer to the ageing and isolation of the colonies, while the higher figures of Hungarian reeds make an intense and long term genetic streaming likely.
5. Colonies of Kis-Balaton, Balaton and Lake Fertő are genetically very close to each other but the reeds of Kis-Balaton are genetically closer to the colony of Lake Fertő than to the geographically much closer Balaton colonies. The reeds of Ostersee are genetically also closer to the Fertő colonies than to Boden ones, which are geographically much further. The main roles in genetic distance of reeds can be isolation and the time of formations, which could differ in several hundreds of years. From Lake Bóden to the Danube Delta, we studied 21 single reed taken from different colonies of 5 countries, compared their genetic distance with their geographical distance. Genetic distance did not grow by geographic one. It refers to a large meta-population with a strong genetic streaming within 1600 km.
6. Reed belongs to those plants of which small seeds help the long term spreading while the developed populations spread in a vegetative way. The clonal structure of colonies

can basically depend on the way of formation. A monoclonal colony can easily develop from a bunch of rhizome drifted on the shore. If the reed multiplies from seed on the shore and its rhizomes get gradually deeper in the lake, the development of an averagely diverse colony is expectable. However, in our shallow waters, a highly varied colony can develop from seeds fallen on temporarily dried-up lake bottoms, which is flooded subsequently again.

## **Publications in dissertation's subject**

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