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THE GENETICAL STRUCTURE OF *POPULUS EUPHRATICA* AND *ALHAGI SPARSIFOLIA* STANDS IN THE TAKLIMAKAN DESERT

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1 INTRODUCTION

The population ecology of the most important plant species in the foreland of the Qira Oasis at the southern rim of the Tarim Basin (Hotan Province, Chinese Xinjiang Uygur Autonomous Region) was studied by Chinese and German researchers within the joint research project on the development of sustainable management strategies to combat desertification. It was found that the successful generative propagation of all species is very rare, with the exception of *Tamarix ramosissima* Ledeb. (Bruelheide, in prep.). In the experiment of experimental flooding that was carried out from 1999 to 2000 (Thomas et al. 2000), only *T. ramosissima* and *Calligonum caput-medusae* Schrenk produced some seedlings in the artificially flooded plots. However, no seedling survived the following year. During all field trips for preparing the vegetation map (see Bruelheide and Jandt, this volume) only very few seedlings of *Populus euphratica* Oliv. were found in the bed of the Qira River and only in the period after flooding in summer. No seedling of *Alhagi sparsifolia* Shap. was ever found in the field. In contrast, *T. ramosissima* was the only species that seemed to have no problems to regenerate generatively. At certain places in the Qira River bed after flooding, *Tamarix* produced up to 3000 seedlings per square meter. Although seedling mortality is high, many of the seedlings survive and produce new cohorts of plants on the various floodplain levels in the river in every summer.

The difficulty to regenerate from seeds seems to be compensated for by a pronounced ability to grow clonally. By digging out, individual plants, in particular of *Alhagi sparsifolia* and *Populus euphratica*, were observed to produce long subterranean shoots that could cover several ten meters. Unfortunately, digging out root and shoot systems of individuals did not turn out to be an appropriate method to detect the size of clones. Although it was principally feasible to dig out shoots, the work was very laborious. As some excavations exposed underground systems of some ten meters (in the case of *P. euphratica* up to a maximum of 44 m; Runge and Zhang, personal communication), the digging method proved not to be practicable to test whether a particular tree was the clonal ramet of the neighbour tree.

It is the objective of this contribution to present the first results of a molecular fingerprinting technique that allows to detect ramets of the same genet, i.e. to detect the clonal parts produced by the same generatively established plant, and to distinguish between genets, i.e. between generatively established plants, recognizable by different genotypes.

2 METHODS

The study on the extent of clones was carried out at the PFU plot, one of the fenced experimental plots at the *Populus euphratica* site, north of Qira (Fig. 1). Leaf samples were taken of all *P. euphratica* trees within the 10 m x 10 m plot that had been marked for a study on sand accumulation and for studies in population ecology. The position of all trees (n = 40) in this 100-m^2 plot, all of which were sampled for fingerprinting analysis, had been mapped accurately. Additional trees at the same site were sampled along transects extending for 100 m in 8 directions. For comparison, other sites were included, separated by approximately 8 km (PEG, Fig. 1). A similar sampling design was employed for *Alhagi sparsifolia* at the *Alhagi* site (Fig. 1), with the exception that only 21 individuals in the center of the 10 m x 10 m plot were sampled.

The sampled leaves (*Populus*) or shoots (*Alhagi*) were put in bags and dried on Silica. DNA was extracted from the dried samples by grinding the tissue in buffer using the DNA isolation kit of Invitek. DNA fingerprinting was performed with AFLP (amplified fragment length polymorphism; Vos et al. 1995). The AFLP technique involves restriction digestion of genomic DNA with a subsequent amplification by polymerase chain reaction (PCR) with a subset of the produced fragments. Figure 2 shows a schematic diagram of the main steps of the procedure.

In the first step, DNA is digested into numerous fragments with *Eco*RI and *Mse*I restriction enzymes. *Mse*I is a frequent cutter at all sites characterized by a sequence of 4 specific nucleotides (4-cutter) producing fragments with the sticky end T/TAA. *Eco*RI is a 6-cutter with G/AATTC ends. The second step is to ligate the fragments to double-stranded adaptors, oligonucleotides with known nucleotide sequence. The adaptors bind specifically at the produced fragment ends. The third step is called preamplification. The fragments are amplified using primers that are complementary to the adaptor and restriction site sequences, but, in addition, have 1 additional randomly chosen nucleotide added to the 3' end extending into the unknown part of the genome beyond the restriction enzyme site. The result is a selective amplification of only 1/4 of the fragments for each restriction site, reducing the number of fragments to 1/16. This reduction is necessary to select medium sized fragments among the thousands of fragments produced by the digestion. The fourth and last step is the main amplification. The number of fragments is further reduced to 1/256 using primers as in the preamplification but with 3 additional selective nucleotides. The amplified fragments are then run out and visualized on acrylamide gels on an automated sequencer (Licor). The size of fragments (number of base pairs, bp) is assessed using DNA standards of known length.

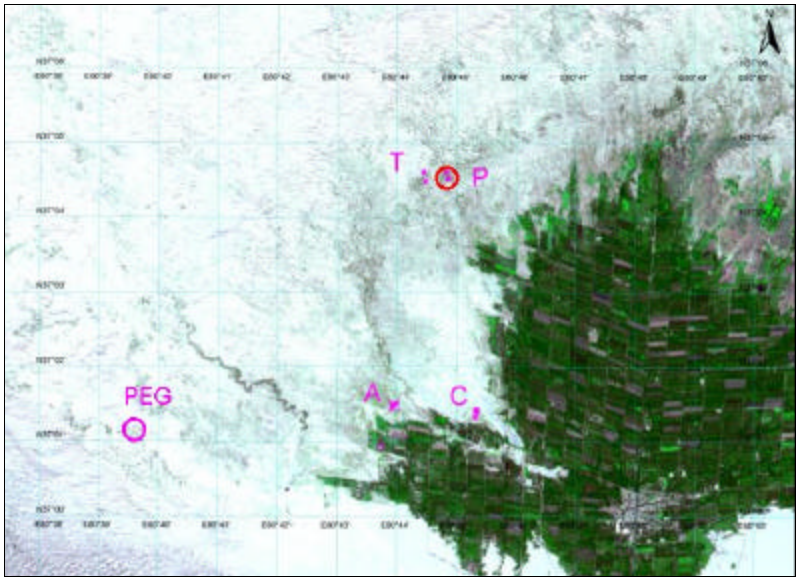

Fig. 1: Study sites where the genetical analyses of *Populus euphratica* trees in the western and northwestern sector of the Qira Oasis foreland were performed (A = *Alhagi sparsifolia* site, P = *Populus euphratica* site, PEG = *Populus* elevational gradient site).

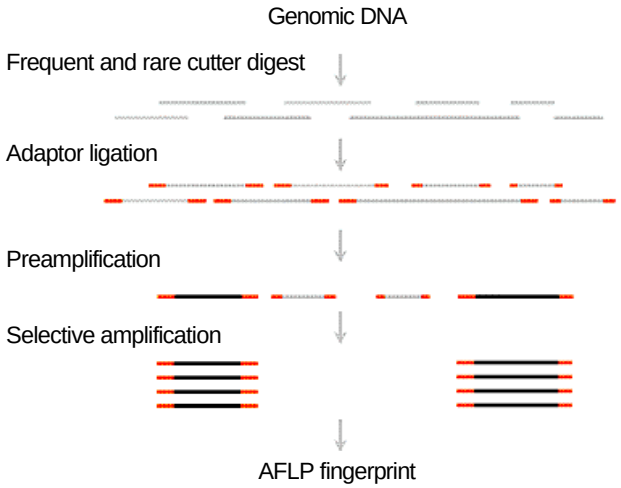

Fig. 2: Scheme of the AFLP (amplified fragment length polymorphism) technique used for DNA fingerprinting of *Populus euphratica*, adapted from www.keygene.com/html/aflp.htm. 1. Digestion of DNA with *Eco*RI and *Mse*I restriction enzymes. 2. The ends of fragments are ligated to adaptors, DNA strands of a length of 10 bp, marked in red in the graph. 3. Preamplification with primers complementary to the adaptor and restriction site sequences with 1 additional selective nucleotide, resulting in a reduction of fragment number to 1/16. 4. Main amplification with primers as in the preamplification but with 3 additional selective nucleotides, resulting in a further reduction of fragment number to 1/256.

Since AFLP produces fragments from the whole genome, differences in genotypes that have alternative alleles will be apparent in the variation in fragment length caused by the presence or absence of restriction sites or by insertions and deletions between restriction sites. Small genetic differences will be apparent even in a single primer combination by at least some fragments of different length. By using primers with different sets of selective nucleotides, the genetic identity or differences between two samples can be assessed with a very high reliability (Ritland and Ritland 2000).

At present, only 21 samples out of a total of 130 *Populus* samples have been analyzed and 8 samples out of a total of 160 *Alhagi* samples. Figures 3 to 5 show the position of all *Populus* samples that have been analyzed up to now.

3 RESULTS

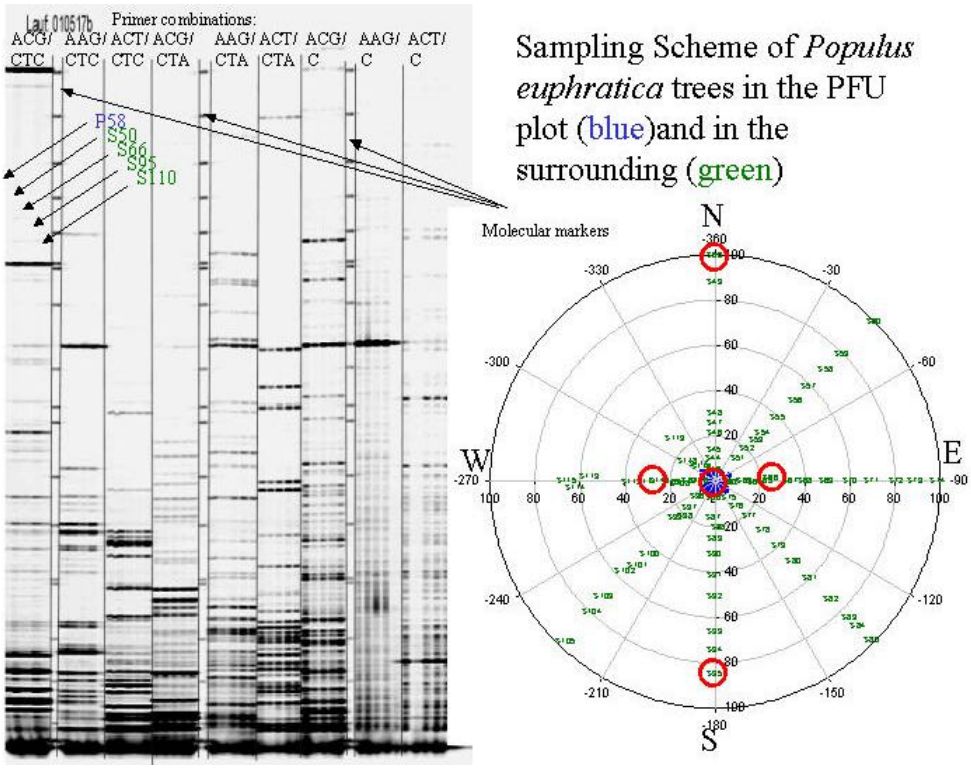

Fig. 3: Sampling design and AFLP fingerprinting results using 9 different primer combinations. On the right: Position of all sampled *Populus euphratica* trees. Blue colours in the center indicate all sampled trees within the PFU plot, which had a size of 10 m x 10 m. Green colours indicate trees that were sampled on transects in 8 directions over a distance of 100 m each. The red circles indicate the 5 individual trees analyzed. On the left: DNA fragments on acrylamid gel of the five selected *Populus* individuals and the 9 primer combinations. Fragment length does not vary, regardless of which primer combination was used.

The results of nine different primer combinations for 5 selected samples are shown in Figure 3. Irrespective of primer combination, the five individuals exhibit the same pattern of fragment sizes. Consequently, they are genetically identical. This implies, that the northernmost tree no. 50 belongs to the same genet as the southernmost tree no. 95, although they are separated by 185 m.

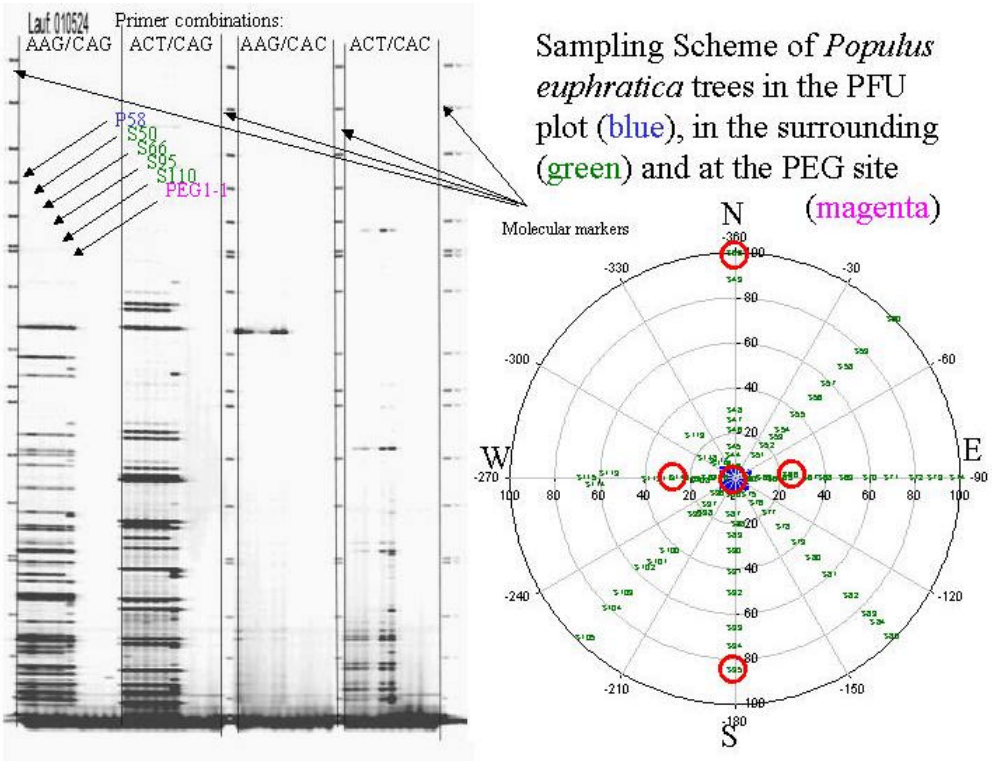

Fig. 4: Sampling design and AFLP fingerprinting results using 4 different primer combinations. On the right: Position of all sampled *Populus euphratica* trees, as in Figure 3. In addition, a sample of a *P. euphratica* tree, separated by approximately 8 km, marked in magenta (PEG1-1) is included as a reference in the analysis. This tree was sampled at the *Populus* elevational gradient site in the old Qira River bed west of the oasis (see Fig. 1). On the left: DNA fragments on acrylamid gel of the six selected *Populus* individuals and the 4 primer combinations. While all individuals at the *Populus* site (marked in blue and green colours) exhibit the same fragment lenght, the pattern of reference sample PEG1-1 is different.

The capability of the AFLP method to distinguish between genotypes is evident when a reference sample is included that is separated by a large geographical distance, and thus, is most probably not belonging to the same clone. In Figure 4 sample PEG1-1 from the old Qira Valley was included. While the fragment length of the trees sampled at the PFU plot is identical (as in Figure 3), the pattern is different for tree PEG1-1. Although this sample shares numerous fragment lengths with

the PFU samples, some bands differ. The conclusion is that the genetic diversity between trees at the PFU and the PEG site is comparably low, but that the PEG tree does not belong to the PFU genet.

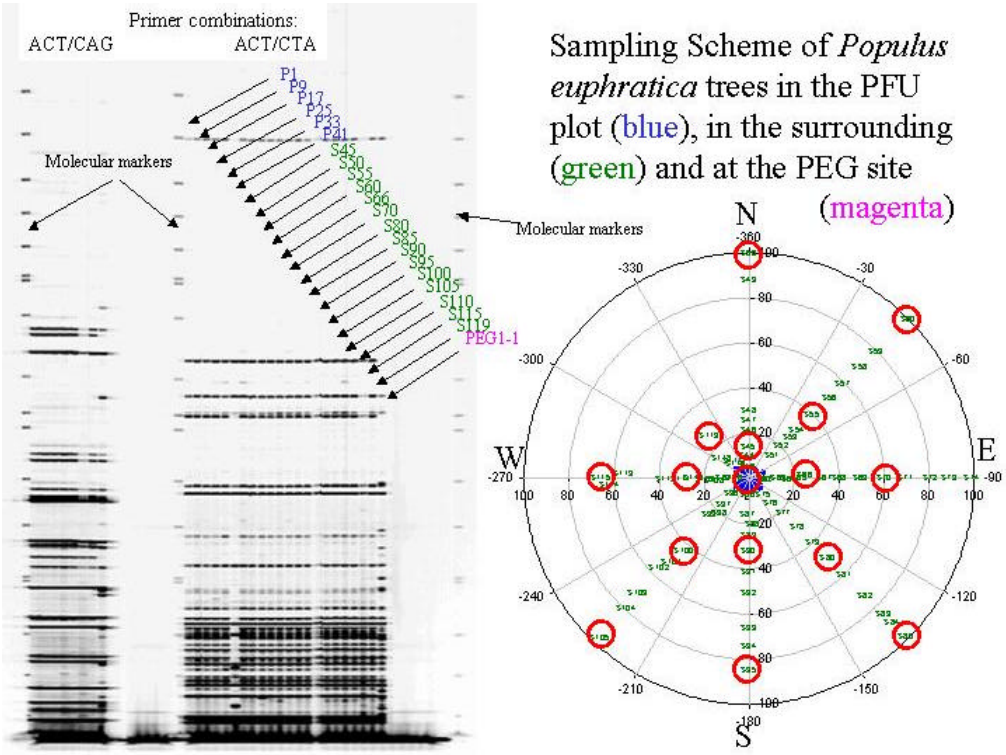

Fig. 5: Sampling design and AFLP fingerprinting results with the ACT/CTA primer combination. On the right: Position of all sampled *Populus euphratica* trees. Blue colours in the center indicate all sampled trees within the PFU plot, which had a size of 10 m x 10 m. Green colours indicate trees that were sampled on transects in 8 directions over a distance of 100 m each. The red circles indicate the 21 individual trees analyzed. In addition, a sample of a *P. euphratica* tree, separated by approximately 8 km, marked in magenta (PEG1-1) is included in the analysis. This tree was sampled at the *Populus* elevational gradient site in the old Qira River bed west of the oasis (see Fig. 1). On the left: DNA fragments on polyacrylamid gel of the 21 selected *Populus* individuals for the ACT/CTA primer combination. As in Figures 4 and 5 fragment length does not vary between individual trees, except for PEG1-1 and the particular tree P41 within the PFU plot.

In Figure 5 more samples of the PFU site have been included. The banding patterns is comparable to Figure 4, with differences between the PFU samples and the PEG1-1 sample. An exception is tree P41, sampled from the center of the PFU plot. This tree exhibits deviating fragment lengths and, thus probably does not belong to the same genet as all other PFU samples. However, artefacts

cannot be ruled out since the analysis with P41 has not been repeated yet with other primer combinations. It can also not be excluded that the sample was contaminated with foreign DNA, e.g., of pathogens living on the sampled leaf.

As far as the *Alhagi sparsifolia* samples have been analyzed, they reveal a smaller clone size. Although samples from the center of the circular sampling site that were separated by distance of 5 m were genetically identical, samples separated by 100 m belonged to different genets.

4 DISCUSSION

The conclusion of the AFLP analyses is that *Populus euphratica* grows in large clones that have a radius of at least 100 m, while the clone size of *Alhagi sparsifolia* is larger than 5 m but smaller than 100 m. This is an indication that clone size is highly species-specific, even under comparable environmental conditions.

A clone diameter of 100 m in the case of *P. euphratica* approximately corresponds to a clone size of 4 ha. However, the spatial extent might be much larger since no samples beyond this circle had been sampled. It is possible that *P. euphratica* attains clone sizes of similar magnitude as the so far largest organism on earth, a record hold by another *Populus* species, *P. tremuloides* in North America. Grant (1993) describes a *P. tremuloides* clone of 42.9 ha from the Wasatch Mountains in Utah. One genet consists of 47 000 ramets that physiognomically do not differ from distinct trees. Other authors even mention clone sizes of *P. tremuloides* of 81 ha (Kemperman and Barnes 1976). The clone size in a *P. tremuloides* stand is primarily a function of clone age and the frequency and degree of disturbance since seedling establishment. It has been suggested that the *P. tremuloides* clone in Utah is more than 1 million years old. This might also be the case for *P. euphratica* forests in the Taklimakan Desert.

The AFLP technique does not distinguish between clones that have grown in situ and clonal trees that were planted by forestry. There is still the vague possibility that the stand at the PFU site had been planted with cuttings that had been taken from the same genet. However, this suspicion is not supported by the information obtained from the local authorities, according to which the *Populus euphratica* stand in that region grew up naturally after a long-lasting inundation event. In any case, *P. euphratica* has the capability to spread vegetatively by horizontal growth of subterranean shoots as was confirmed by several attempts to dig out root systems.

The fingerprinting results support the field observations and the results from the regeneration experiments. They confirm that the successful establishment of *P. euphratica* from seeds is an extremely rare event under natural conditions. Without doubt the prevailing form of natural regeneration is vegetative spreading. Most probably a single seedling had established hundreds or thousands years ago and then grew up to the whole forest found today. However, there is still the possibility that within this clone new genets might establish themselves later. If not being an artefact, establishment of another seedling would be the explanation of the different genotype of the single tree P41 within the homogenous *Populus* clone at this site.

The conclusion of these results for the management of *Populus euphratica* in the periphery of the Qira Oasis, as well as for all remaining *P. euphratica* forests in the Tarim Basin, is that the preservation of poplar forests should be a prior objective. Once a *P. euphratica* forest has been destroyed by cutting, grazing or decline of the ground water table, it cannot be replaced by artificial means. This is mainly due to the fact that present poplar forests grow far away from sites where generative regeneration would be possible today. These regeneration sites are river beds like the floodplain of the Qira River. Once established, the forest grows up and is continuously covered by drifting sand while the river continuously shifts its bed away from the initial regeneration site. Exactly this was demonstrated for the Qira River by the comparison of aerial photographs. In the last 50 years, the main Qira River has shifted its bed significantly northward and developed a new furcation with a large new bed in 1986 (Bruelheide and Jandt, this volume). Consequently, suitable regeneration conditions are no longer given at the site where the forest grows today. Therefore, planting of new trees at sites where poplar forests have been destroyed might be not successful.

Apart from these practical considerations the preservation of *Populus euphratica* should be seen in its own right. The *P. euphratica* clones of today have probably been existing in the Taklimakan Desert long before man arrived. It is a challenge for the people in Xinjiang to ensure the survival of one of the largest organisms in the world.

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