Introduction

Oak species (*Quercus* L.) have the highest forest area proportion in Hungary and are therefore main elements of our broadleafed forests not only in economical but also in ecological point of view. Research studies concerning oak species still hold unresolved key questions such as the lack of taxonomic isolation, hybridization and other ecological problems in connection with population dinamics. Answering these questions can facilitate such economically important research fields as the monitoring of climate change impacts on our forests, the better utilization of adaptation potential of the different tree species and consequently the safeguarding of sustainability or just the conscious application of adventages derived from preservin biodiversity.

Aims

The main aim of the study is the fine-scale genetic pattern analysis of a mixed oak stand rich in different oak species comprising the following tasks

- exact classification of all the adult individuals growing on the study plot by the application of a numeric taxonomic method,

- comparative analyses of the different species (in broad and strict sense) groups’ genetic pattern and diversity, the evaluation of genetic relationship between the studied oak species,
- comparative genetic analyses of the juvenile and adult subpopulations found on the study plot,
- study of spatial genetic structure in the stand,
- exploration of hybridization or introgression events by the genetic pattern analyses of each and all of the individuals.

Matterial and method

The study site is a mixed oak forest that lays near to Sopron (Szárhalom forest) and comprises a high diversity of native white oak species in taxonomical and morphological aspects. For the fine-scale genetic pattern analysis of the plot an exhaustive sampling of all the adult individuals and moreover two juvenile clumps growing on the site were done.

The taxonomical classification of the trees was carried out by the application of multivariate classification functions of an exact numeric taxonomic method.

The investigation of genetic relationship between species – regarding the species complexes in broad sense and the species in strict sense as well – was performed by the analyses of three different molecular marker groups that were the followings: (1) four nuclear microsatellite markers, (2) eight izozyme locus, (3) three RAPD primers' combination as a dominant marker type.

Calculating population genetic indices the genetic diversity of the whole study plot was evaluated covering the analyses of genetic relationship between the different oak groups and the adult-juvenile subpopultions. Furthermore, the spatial genetic structure of the stand was defined by Mantel test and the spatial genetic autocorrelation analysis option of Genalex.

The introgression rate in the stand was appraised by the complex estimation of individual genotype informations (K clustering by Structure and Assignment test by Genalex) and other taxonomical details.

Results

The first fine-scale genetic pattern analysis was carried out in case of a mixed oak stand in Hungary that combined a numeric taxonomic classification method and three different molecular marker types.

Based on the numeric taxonomic classification and the representation of individuals on a map, two main white oak groups could be identified – the pedunculate oak (*Quercus robur*) and the pubescent oak complex

(*Quercus pubescens* s.l.) – that dominate in the stand but also segregate in space by their ecological demands. The pubescent oak group appeared in a big variety: *Qu. virgiliana* and *Qu. pubescens* sensu stricto × *Qu. virgiliana* hybrids could also be identified in a high ratio besides *Qu. pubescens* s.str. However, the sessile oak group (*Quercus petraea* sensu lato) appeared only in a very low number, but also *Qu. dalechampii*, *Qu. petraea* s.str. and their hybrids were found. Hybrids between the main oak species could be identified only in minimal ratio in despite of the high morphological diversity of the stand.

A low genetic differentiation could be detected based on all the three molecular marker types according to the former, foreign research results. Nevertheless, the small sessile oak group showed a kind of unic genetic pattern that resembeled rather to the pedunculate oak's pattern after two marker types (microsatellites, RAPD) and on the other hand corresponded to the pubescent group based on the isozyme pattern. Within the pubescent oak complex there were no evidence of genetic differentiation of the two species in strict sense and their hybrids. The further classification of the pubescent oak group is not supported by the genetic data described here.

From the outcome of the genetic pattern analysis of each and all individuals only two genetically diffenet groups turned out to be distinguishable by the *Structure*

K-clustering method. These two clusters well corresponded to the two morphologic groups, the ’*robur’* and the ’*pubescens*’ groups. The ratio of hybrid genotypes between the two clusters were 10%. Sessile oaks had no private genetic pattern and were grouped partly to the cluster of pubescens oaks and also appeared among the hybrid genotypes. Then again, based on the *Assignment Test* made by the program Genalex, sessile oaks were supposed after their genotypes in the pubescent oaks’ group and also in the pedunculate group, in addition in higher proportion in this later species group. The sessile oak complex was presumably affected by introgression from two directions (from both the pubescent and pedunculate oaks) and could be therefore found in a low number in the stand regarding the genetic and morphologic recognition marks.

However not all the individuals with hybrid genotype showed a real transitional morphology and the applied numeric taxonomic method were not able in all cases to demonstrate introgressed forms. It is highly recommended to keep these possabilities in view in case of oak stands on mosaic sites where different oak species are able to grow togethet due to the various microhabitats and the hybridisation could be very frequent.

The proportion of individuals with traces of introgression was almost 20% based on the complex evaluation of genetic pattern, numeric taxonomy and traditional taxonomic characteristics. These trees were located between the parent species zone in most cases.

The ratio of clones was only 9% despite of the long time coppicing in the stand. These clone trees appeared mainly as stump shoots and only in case of two pubescent oak tree pairs were detected higher spatial distance between the clones. These two pairs are rather root suckers. Vegetativ strategy was more diversified and more frequent in the pubescent oaks' groups in general. Genetic diversity analyses without the clone genotypes showed no drastic genetic depression in the stand due to the presence of clones. Coppicing could presumably facilitate the protection of the native stand in this case. During the procedure of designation of *in situ* reservation sites, it is recommended to consider this possibility when the given stand is worthy of protection in the high taxonomical or morphological diversity point of view.

Vegetative strategy could be detected also on the two analysed juvenile clumps where clones appeared probably as an old tree root suckers. The taxonomic status of the two juvenile sapling clumps could also be identified, however they showed a rather distinct genetic pattern from the adult group of the study plot and were originated from trees standing outside the plot. The marginal situation of the two juvenile cohorts also verified the external origin.

A weak spatial genetic structure could be detected concerning the two main oak species groups – pedunculate oak and pubescent oaks – in the stand. At the same time a clear correlation was identified as the structure of the whole stand was analaysed. This phenomenon could be referable also to the high number of introgressed individuals.

Theses

1. In case of Hungarian oak stands that was the first fine-scale genetic pattern analysis that rests on the exhaustive sampling of all the trees standing on the study plot, furthermore applies a numeric taxonomic classification method in order to classify *Quercus robur*, *Qu. petraea* s.str. *Qu. dalechampii*, *Qu. pubescens* s.str., *Qu. virgiliana* species in strict sense and their hybrids, in addition evaluates the genetic diversity and the genetic pattern of individuals with various approaches and the combination of three different molecular marker types.
2. A low genetic differentiation could be detected based on all the three molecular marker types according to the former, foreign research results. Nevertheless, the small sessile oak group showed a kind of unique genetic pattern that resembeled rather to the pedunculate oak's pattern after two marker types (microsatellites, RAPD)

and on the other hand corresponded to the pubescent group based on the isozyme pattern.

3. Within the pubescent oak complex there were no evidence of genetic differentiation of the two species in strict sense and their hybrids

4. The proportion of individuals with traces of introgression was almost 20% (18,3%) based on the complex evaluation of genetic pattern, numeric taxonomy and traditional taxonomic characteristics. These trees were located between the parent species zone in most cases.

5. The sessile oak complex was presumably affected by introgression from two directions (from both the pubescent and pedunculate oaks) and could be therefore found in a low number in the stand regarding the genetic and morphologic recognition marks. This observation is supported by two approaches of individual genotype pattern analysis methods.

6. A clear spatial genetic structure was detected as the whole stand was analaysed. At the same time only a weak correlation could be identified concerning the two main oak species (pedunculate oak and pubescent oaks) groups separately. This phenomenon could be referable also to the high number of introgressed individuals.

7. However not all the individuals with hybrid genotype showed a real transitional morpholog. It is highly recommended to keep these possabilities in view

in case of oak stands on mosaic sites where different oak species are able to grow togethet due to the various microhabitats and the hybridisation could be very frequent. For taxonomic research purpose a combined strategy of various different methods and aspects should be used.

8. Individuals could be identified with their unic genetic fingerprints based on the applied four microsatellite markers and therefore clones owning the same genotype were sorted. Thereby the result of the long-term coppicing could be demonstrated, however it can not be considered too drastic as the rate of clones was only 9% in the stand. These were mainly stump shoots and only in case of two pubescent oak tree pairs were detected higher spatial distance between the clones. These two pairs are rather root suckers. Vegetativ strategy was more diversified and more frequent in the pubescent oaks' groups in general. Clones were detected in the two juvenile cohorts as well, so the vegetative strategy appeared also among the offsprings besides the generative propagation. These clones were presumably originated as root suckers of an adult tree or emerged due to game browsing.

9. Genetic diversity analyses without the clone genotypes showed no drastic genetic depression in the stand due to the presence of clones. Coppicing could presumably facilitate the protection of the native stand in

this case. During the procedure of designation of *in situ* reservation sites, it is recommended to consider this possibility when the given stand is worthy of protection in the high taxonomical or morphological diversity point of view.

10. The taxonomic status of the two juvenile sapling clumps could also be identified, however they showed a rather distinct genetic pattern from the adult group of the study plot and were originated from trees standing outside the plot. The marginal situation of the two juvenile cohorts also verified the external origin.

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