

## 1. Introduction

The objective of my research was the following:

1. Elaborating taxonomical methods for the investigation of the variation in the pubescent oak.
2. Giving an overview of the distribution and habitats of pubescent oak in Hungary.
3. Investigating the variation of pubescent oak in Hungary. Research on the variation along geographical and ecological gradients.
4. Examining the potential reasons of the variation.
5. Elaborating suggestions for the forestry and nature conservation management of the species based on the results of the previous points.

The list of the completed investigations is the following:

1. Preparation of the distribution map of the pubescent oak in Hungary based on the data of scientific publications, material of herbaria and the Data bank of the National Forest Inventory Service.
2. Analysis of micromorphological features (stomata and trichome types) by means of scanning electron microscopy (SEM) and stereo microscopy.
3. Analysis of macromorphological features by multivariate methods (principal component analysis, hierarchical clustering, Fourier analysis combined by discriminant analysis).
4. Chemotaxonomy: Research of variation in flavonoides by overpressured layer chromatography (OPLC).
5. Analysis of cpDNA variation. As a part of the international program on the cpDNA haplotype mapping of the European oak species I completed tasks in the Hungarian subproject.

The hybridization of *Q. pubescens* and a *Q. robur* is questioned by several Western-European researchers, at the same time the process plays an important role in the domestic forest-steppe associations not only in the literature but also according to my former research. To prove the hypothesis I completed micro- and macromorphological and chemotaxonomical analysis.

## 2. Results of the micromorphological investigations

### 2.1. Examination of trichome types by stereo microscopy.

Trichomes of leaves and shoots were examined of 77 stands representing the distribution and the habitats of pubescent oak in Hungary from a total of 586 *Quercus pubescens* trees. The most important results are as follows.

- The micromorphological variation of *Q. pubescens* populations in Hungary is similar to the populations in the mediterranean region.
- The introgressive hybridization between *Q. pubescens* and *Q. petraea* in the Hungarian Mountain Range is much higher than formerly presumed (figure 1).

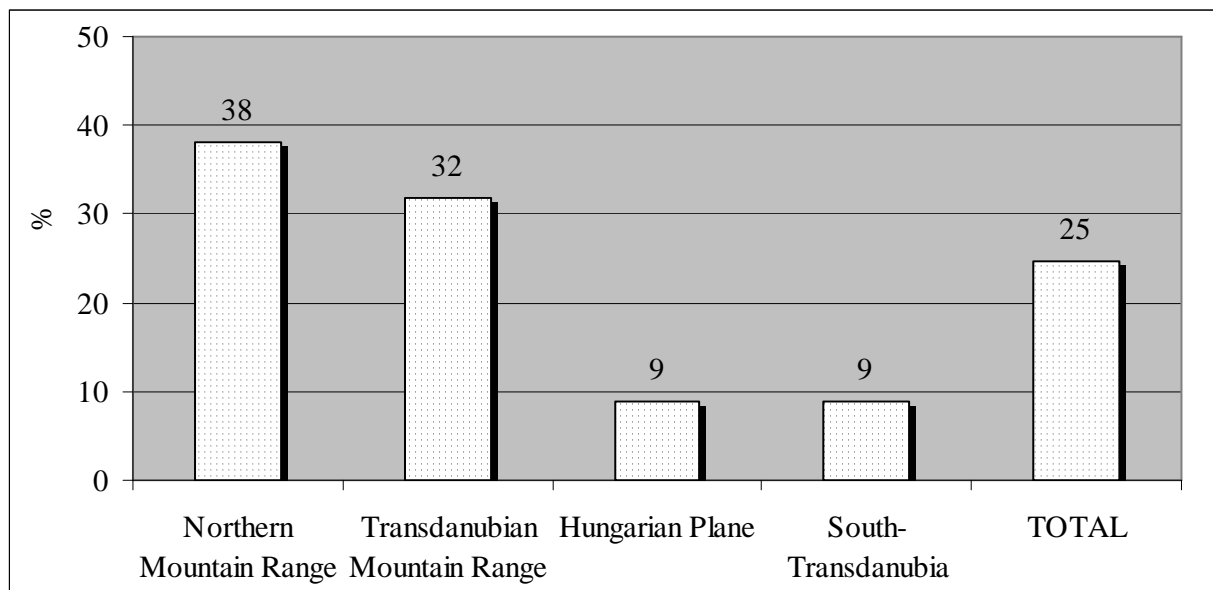


Figure 1: Frequency of samples with stellate trichomes on the abaxial surface of the leaf lamina (*petrae*-type) in the flora-regions of Hungary

- In the South-Transdanubian populations the proportion of samples with stellate trichomes is much below the national average. This can be explained by the fact that the frequency of submediterranean precipitation type increases southwards.
- The proportion of samples with stellate trichomes is low in the samples from the Great Hungarian Plain, as well. At the same time, the variety of pilosity-types is the highest in this region. This can be explained by the absence of *Q. petraea* in most of these stands, while transition forms between *Q. pubescens* and *Q. robur* appear in the populations.
- The introgressive hybridization contributes to the remarkable variety of *Q. pubescens*. In the Hungarian Mountain Range the rate of the transition forms between *Q. pubescens* and *Q. petraea* is significantly higher on soil related to non-calcareous stone than on soil related to calcareous stone (figure 2). We can establish that the extremely dry and acidic sites are favourable for hybridization, consequently that the introgression between the species plays an important role in their ecological adaptation.

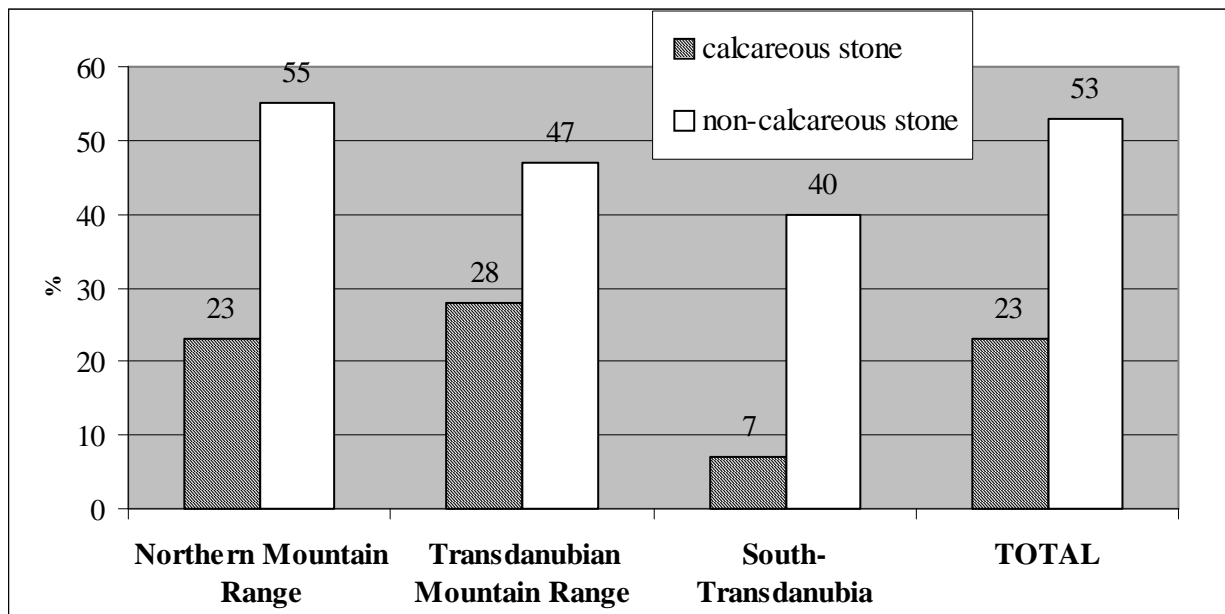


Figure 2: Frequency of samples with stellate trichomes on the abaxial surface of the leaf lamina (*petrae*-type) on calcareous and non-calcareous sites in the hill regions of Hungary

## 2.2 Analysing micromorphological features by means of scanning electron microscope (SEM) observation

- On the basis of SEM investigations on the same sample trees in different times we can establish that the features of stomata change in the vegetation period. We should be critical about former descriptions of the stomata-types of *Q. pubescens*, *Q. robur* and *Q. petraea*. To decide whether the stomatal rim is covered by wax scales we have to collect samples in different times.
- The observation of trichome types and stomata types together seems to be useful for the purpose of detecting the hybrids of *Q. pubescens* and the closely related oak species (*Q. petraea* and *Q. robur*). Trees occur having both intermediate micro- and macromorphological features in the sympatric populations of *Q. pubescens* WILLD. and *Q. robur* L. on the Great Hungarian Plain and on its fringes.

## 3. Results of macromorphological investigations

3.1. Investigations on the differentiation of the two supposed species, *Qu. virgiliana* Ten. and *Qu. pubescens* s. str., by means of multivariate methods (principal components analysis, hierarchical clustering).

Ten macromorphological features of the leaves and fruits of 97 sample trees were analysed.

- Present research could not prove the differentiation of the two pubescent oak species.

3.2. Investigation on the role of introgressive hybridisation between *Q. pubescens* and *Q. robur* by means of Fourier analysis and discriminant analysis. A total of 125 samples were collected in sympatric populations of *Q. pubescens* and *Q. robur* on the Great Hungarian Plain and on its fringes. The Fourier analysis of every leaf resulted in a set of 60 numbers, which characterise the shape of the different leaves. We compared these 125 sets by means of discriminant analysis. We completed the *a priori* grouping of the objects using the determination key of trichome-types developed by AAS.

- The analysis confirmed the role of introgressive hybridization between *Q. pubescens* and *Q. robur*.

#### 4. Results of the chemotaxonomical investigations

Variation in flavonoides was examined by overpressured layer chromatography (OPLC) in the leaves collected from 38 sample trees of a sympatric population of *Q. pubescens* and *Q. robur*.

- There is no qualitative difference in the flavonoid composition of the two species, only a quantitative one.
- There is a strict correlation between the result of the chemotaxonomical investigations and the *a priori* grouping of trichome-types. (figure 3.)

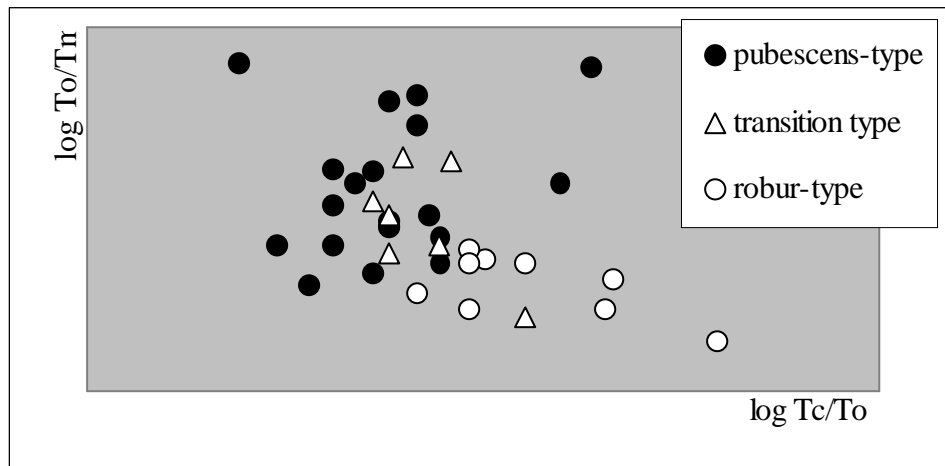


Figure 3: Result of the OPLC.

$T_o$ ,  $T_m$ ,  $T_c$  signify the areas under the peaks O, M and C of the densitogram

- The analysis confirmed the role of introgressive hybridization between *Q. pubescens* and *Q. robur*.
- The method proved to be fast, easy-to-reproduce and very economical.

#### 5. Analysis of cpDNA variation

In the framework of the international program on the cpDNA haplotype-mapping of the European oak species the most important results relating to *Quercus pubescens* are as follows:

- The domestic *Quercus pubescens* samples belong to 6 cpDNA haplotypes.
- The present domestic oak species recolonised Hungary from the refugia in the Balkans and the Italian peninsula.

#### 6. Practical utilization of the results

- It was found that the introgression between the species plays an important role in their ecological adaptation. Based on these findings the only acceptable method in the close-to-nature forest management is natural regeneration.
- Measures have to be taken to save the ecotypes of the *Quercus pubescens* in the Great Hungarian Plain with special attention to the transition forms between *Quercus pubescens* and *Quercus robur*.

## 7. Relevant publications

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