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Short Communication

Genetic differentiation and phylogenetic relationships among six *Abies* species from European and Turkish areas

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The phylogenetic relationships of the *Abies* species in European and Turkish regions have frequently been an object of controversial conclusions. Therefore, we compared the genetic structures of 21 populations belonging to six *Abies* species which are native to central, eastern and south-eastern European regions and different areas in the Turkey (Asia minor). We used the allele frequency distribution of five isozyme gene loci already showing a high discriminatory power to assess the genetic differentiation among the *Abies* populations. The UPGMA-dendrogram based on genetic distances showed a clear discrimination between the Turkish *Abies* species and *A.alba* from Europe. The particular allele frequency distributions at the isozyme loci PGI-A and 6PGDH-A confirmed a great genetic dissimilarity between *A. alba* and the Turkish *Abies* species. These results contradict the phylogenetic relationships of the *Abies* species postulated in other studies.

Key Words: Genetic divergence, isozyme gene markers, biogeography, *Abies*species

In contrast to other forest tree species (e.g. *Norway spruce*), *Abies alba* has not been artificially regenerated in most cases, thus preserving a great deal of their native genetic structures. As a consequence, the genetic composition and diversity of most present-day fir populations are the result of their distribution during the last glaciation and their postglacial expansion to the present natural areas (Bergmann 1991). Hence, comparisons between the genetic structure of *Abies* populations within or between different species may uncover reliable data on genetic similarities, differentiation and probably phylogenetic relationships within the *genus Abies* occurring in Europe and Asia Minor.

Since the phylogenetic relationships of the *Abies* species in these regions have frequently been an object of controversial conclusions (Fady and Conkle 1993, Scaltsoyiannes et al. 1999, Ziegenhagen et al. 2005, Liepelt et al. 2012), it appears worthwhile to compare the genetic structures of *Abies* populations belonging to six species which are native to central, eastern and south-eastern European mountains and different mountainous regions in the Turkey. In contrast to other studies, we used specific isozyme gene markers of such enzyme systems, which have already indicated a high discriminating power among populations of silver fir (Konnert and Bergmann 1995).

The objectives of the present paper are to show the genetic similarities among populations of the same *Abies* species and the genetic differentiation among populations of different *Abies* species. The latter data were used to evaluate some phylogenetic relationships, especially

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between European and Turkish *Abies* species.

Materials and Methods

The plant material used in this study consisted of 10 *Abies alba* populations from central, eastern and south-eastern Europe, each of two populations of *Abies bornmülleriana*, *Abiesequi-trojani* and *Abies cilicica* from different regions of the Turkey. Four populations of *Abies nordmanniana* are located in Russia (1), Georgia (2) and Turkey (1). Furthermore, one population of the so-called interspecific hybrid *Abies borisii-regis* belongs to the Slovjanska mountains in Bulgaria. The origin of the tree samples and the type and sample size of the test material are listed in Table 1.

Extracts from single seed endosperms (=macrogametophytes) or dormant buds were subjected to horizontal starch-gel electrophoresis. After electro-phoretic separation the gel slabs were stained for the enzyme systems (gene loci in brackets) phosphoglucose isomerase(PGI-A, PGI-B), menandione reductase (MNR-A) and 6phosphogluconate dehydrogenase (6PGDH-A, 6PGDH-B). The experimental methods and the genetic analyses were already published in an earlier study on *Abies alba* (Hussendörfer *et al.* 1995).

Statistical methods

Data analysis was carried out using the program GSED Version 3.0 beta, (Gillet 1994). The dendrogram, illustrating the genetic distances between populations is based on the software NTSYS, Version 2.01, Applied Biostatistics.

The genetic distance between all pairs of populations was based on the allele frequencies of the five isozyme gene loci. We used the genetic distance d_0 (Gregorius, 1974) which is based on a simple and better interpretableformula:

$$d_0(P,P') = \frac{1}{2}\sum_{k=1}^{n}\left|p_k - p'_k\right|$$

The arithmetic mean of d_0-values over single loci was calculated to get the gene pool distance which was used for the calculation of the UPGMA-dendrogram (Sneath and Sokal, 1973).

For simplification of the figures the genetic data of the four populations of *Abies nordmanniana* and each of two populations of *A. bornmülleriana* and *A. equi-trojani* have been pooled. Similarly the three populations of *A. alba* from Bulgaria were also combined since the provenance localities possess similar environmental conditions and a short geographical distance.

Results and Discussion

A reliable assessment of the genetic differentiation of a species, genus or family requires the utilization of those gene markers which exhibit geographically variable allele frequencies and/or possess area-specific allelic variants. Both properties can be attributed to the five isozyme loci examined in 21populations belonging to six *Abies* species (see Table 1). In this study, two data sets based on allele frequencies were used to arrive at the above given objections. One was the measure of genetic distance between all population pairs and the construction of a UPGMA-dendrogram based on gene pool distance values. The other refers to the distribution of particular area-specific allelic variants at two isozyme loci which were traced across the European and Turkish range of the *Abies* genus.

The clustering analysis (depicted as dendrogram in Fig. 1) revealed patterns which were not in agreement with results in earlier studies (Fady and Conkle 1993, Scaltsoyiannes et al. 1999) of this genus, which will be explained later. With the exception of the species *A. cilicica* all other populations clustered into two major groups, of which one includes only populations of *A. alba*, whereas the second major group further divided into two subgroups. One of these subgroups

comprises all other Turkish *Abies* species, whereas the other subgroup contains *A. borisii-regis* and *A. alba* from Bulgaria. This subgrouping corresponds exactly to a transition zone (Bergmann and Gagov, 2001) between European and Turkish *Abies* species where area-specific alleles still occur in low frequencies. The pooled populations of *A. cilicica* clustered greatly from the two major groups indicating a nearly complete genetic differentiation from the other Turkish *Abies* species. For instance, the *A. cilicica* populations possess the allele PGI-B_1 with frequencies of 80% or 90%, whereas in all other fir species from Turkey PGI-B_1 reached only 8-10% (data not shown). By far more genetic difference occurs at the isozyme locus MNR-A, since only *A. cilicica* possesses the allelic variant A_4 in moderate frequencies (20 – 60%), which indicated a total isolation of this fir species (see also Scaltsoyiannes et al. 1999).

Table 1: Origin, test material and sample size of the analyzed populations

Location	Origin	Test Material	Size of Sample
		A. nordmanniana	
Apcheronsk	Russia	bulk seed lot	100 seeds
Ambrolauri	Georgia	bulk seed lot	100 seeds
Bordjomi	Georgia	bulk seed lot	100 seeds
Savsat	Turkey	bulk seed lot	100 seeds
		A. bornmülleriana	
Ulu-dag	Turkey	dormant buds	42 trees
Bolukökez	Turkey	bulk seed lot	100 seeds
		A. equi-trojani	
Kazdagi	Turkey	dormant buds	69 trees
Endremit	Turkey	bulk seed lot	100 seeds
		A. cilicica	
Antalya	Turkey	bulk seed lot	20 trees
Maras	Turkey	bulk seed lot	20 trees
		A. borisii-regis	
Slovjanka Mts.	Bulgaria	single tree seed lots	52 trees
		A. alba	
Ribarica	Bulgaria	single tree seed lots	55 trees
Jundula	Bulgaria	single tree seed lots	29 trees
Jenda	Bulgaria	single tree seed lots	79 trees
Toplita	Romania	bulk seed lot	100 seeds
Sinai	Romania	bulk seed lot	100 seeds
Postojna	Slovenia	dormant buds	86 trees
Siegsdorf	Germany	single tree seed lots	30 trees
Lichte	Germany	dormant buds	50 saplings
Nagold	Germany	single tree seed lots	20 trees
Ottenhöfen	Germany	single tree seed lots	30 trees

Supporting evidence for the genetic dissimilarity between European populations of *A. alba* and Turkish populations of *A. nordmanniana, A. bornmülleriana* and *A. equi-trojani* resulted from the distribution of area-specific allelic variants. For instance, the allele A_1 of the locus PGI-A occurs in all populations of the Turkish fir species with considerable frequencies (60 – 90%), but could not be

detected in nearly all *A. alba* populations (Fig. 2). Only the Bulgarian populations of *A. borisii-regis* and *A. alba* contain this allele in lower frequencies (15 – 25 %), which indicated some gene flow between *Abies* populations of Asia minor and Europe and a relatively narrow transition zone which is represented by the Bulgarian populations (Fig. 2). A similar genetic differentiation has been observed at this isozyme locus by Fady and Conkle (1993), even though the allele numbering was different.

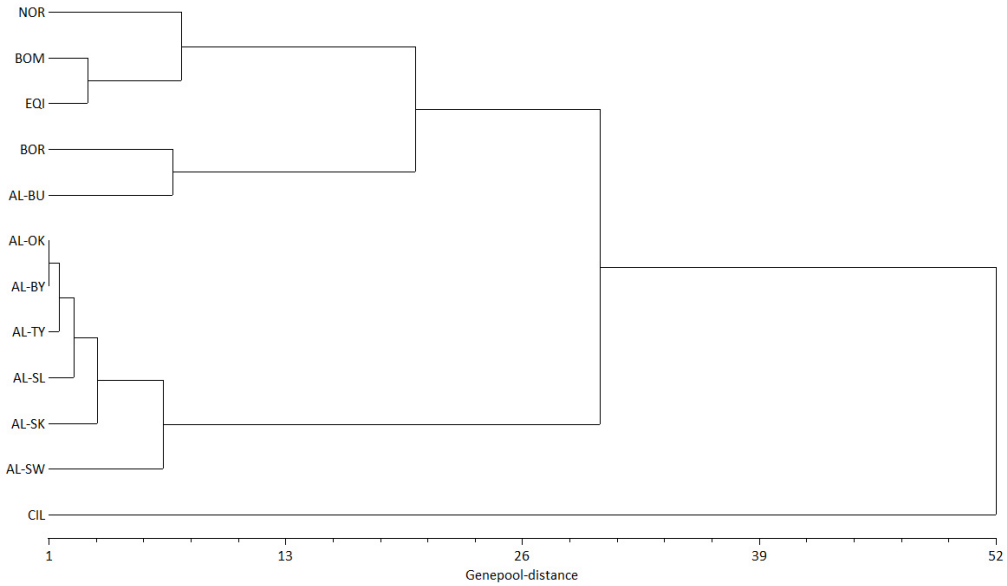

Figure 1: UPGMA-dendrogram of d_0 genetic distances between 11 populations of 6 *Abies* species (*A. nordmanniana*= NOR, *A. bornmülleriana* = BOM, *A. equitrojani* = EQI, *A. cilicica* = CIL, *A.borisiregis* = BOR, *A. alba* = AL-)**

Figure 2: Distribution of the allele frequencies at PGI-A across European and Turkish areas. The black region of the pie diagrams represents the proportion of allele A_1, the white region represents the allele A_2

On the other hand, the area-specific allelic variant A_2 of the locus 6PGDH-A occurs in all populations of *A. alba* with intermediate frequencies (20 – 50%), but could not be observed in the populations of Turkish *Abies* species (based on the size of the tree samples, frequencies lower than 1% were not detectable) (Fig. 3). Again, the Bulgarian populations of *A. alba* and *A. borisii-regis* were found to exhibit the lowest frequencies of this allele A_2, which is typical for a so-called transition zone. Although we presented the genetic data for only 10 European *Abies alba* populations in this study, the allozyme (allelic isozyme) patterns at PGI-A and 6PGDH-A conform closely to patterns in much more populations of this *Abies* species from central, eastern and southeastern European regions (Konnert and Bergmann 1995, Longauer et al. 2003).

Figure 3: Distribution of the allele frequencies at 6PGDH-A across European and Turkish areas. The black region of the pie diagrams represents the proportion of allele A_2, the white region represents allele A_3

With respect to the frequency differences of PGI-A_1 and 6PGDH-A_2 between *Abies alba* in Europe and the four *Abies* species in the Turkey, it is unlikely that either stochastic events or natural selection could result in such large genetic differentiation, if all *Abies* species originated from one ancient mega population (Fady et al. 1992). Rather, this allozyme differentiation may have occurred during and after the last glaciation indicating a complete separation of these two species groups in glacial refugia and postglacial migration routes. This conclusion is not in agreement with the phylogenetic hypothesis of Scaltsoyiannes et al. (1999), who postulated a close relationship between *A. alba*, *A. bornmülleriana* and *A. nordmanniana*. Interestingly, the results of Ziegenhagen et al. (2005) using a mitochondrial DNA marker are more concordant with our data, since they found two haplotypes which occurred only in *Abies alba* and one other haplotype which shared the three *Abies* species from northern Turkey and *A. cephalonica*. The most recent study of Liepelt et al. (2012) employed cpDNA markers with high and low mutation rates in order to distinguish between interspecific genetic contact before speciation and after

speciation. Applying several statistical methods they concluded that in most cases no or only minimal genetic contact had occurred between the different Mediterranean *Abies* species after speciation of this genus, which supports the phylogenetic interpretation of our allozyme patterns based on specific isozyme loci.

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