DNA-marker sets for determination of white oaks (section Quercus) in wood products

HILKE SCHROEDER[1], BIRGIT KERSTEN[1], YULAI YANBAEV[2], BERND DEGEN[1]

[1] Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhandorf, Germany (hilke.schroeder@thuenen.de, birgit.kersten@thuenen.de, bernd.degen@thuenen.de)

[2] Bashkir State University, Z. Validi str.-32, 450076, Ufa, Russia (yanbaev_ua@mail.ru)

Abstract

For the detection and thereafter the avoiding of illegal logging of valuable tree species, the development of identification methods for the origin of timber is necessary. Here we present the results of marker development for the differentiation of species and origin within the section white oaks (Quercus) based on extensive next generation sequencing. One marker set allows the identification of the continental origin (USA, Europe, Asia) using a multiplex of six chloroplast markers. An extended set of 179 markers analysing regions from the chloroplast, mitochondrial and nuclear genomes, is available to differentiate between the species within these continents. The third set presented here, based on five chloroplast markers, is usable for the differentiation of populations within the Mongolian oak (*Quercus mongolica*). With these marker sets, we provide the wood trading market with instruments to comply with the U.S. and European laws that require timber companies to avoid the trade of illegally harvested timber.

Key words: Oaks, molecular markers, timber, illegal logging

Introduction

Illegal logging is a serious issue for tropical and non-tropical forests all over the world and causes destruction not only the forests itself but also of whole habitats, thus endangering a lot of different species. White oaks (sect. Quercus, *Quercus* spp., Fagaceae) are relevant examples for illegal logging of temperate zone trees. The validation of taxonomic and geographic sources of related timber products is very challenging for importers and regulatory agencies. A significant percentage of the hardwood flooring and furniture traded in Europe and the USA is made of oak timber, and oak is one of the most important hardwoods in terms of logs and lumber exports from these continents. Five white oak species are the most important trade woods, the European species *Quercus robur* L. and *Q. petraea* (Mattuschka) Liebl., the CITES Appendix III-protected Asian *Q. mongolica* Fisch. Ex Ledeb., and the two North American oaks *Q. alba* L. and *Q. macrocarpa* Michx. (CASSENS 2007). Increases in illegal logging activities for white oaks have been documented, especially in the Russian Far East region (http://eia-global.org/news-media/liquidating-the-forests). The trading of illegally harvested wood is worldwide banned by the U.S. Lacey Act amendment of 2008, the European Union Timber Regulation of 2010, and the Australian Illegal Logging Prohibition Act (2012). Violation of these regulations can be punished with fines, confiscation of wood, and additional payments, e.g., as recently demonstrated with improperly documented shipments of white oak flooring in the United States (US DEPARTMENT OF JUSTICE 2015). According to these laws, timber companies are obliged to declare the species name and geographic origin of traded timber aiming at the reduction of the risk that traded timber originated from illegal logging (DORMONTT et al. 2015).

The increased awareness of the problem “illegal logging” has raised the necessity for methods providing precise species identification and geographic origin verification. Although, wood anatomical methods are widely used for the identification of tree genera (DORMONTT et al. 2015), these methods are limited for

species identification and they cannot discriminate white oak species, nor identify geographic origin of trees generally.

Over the last decade, worldwide programs have been established using the potential of DNA-based methods for identifying organisms (Barcode of Life www.barcodeoflife.org, HOLLINGSWORTH et al. 2009). DNA barcoding has already proven to be appropriate for revealing illegal trading (e.g. GONCALVES et al. 2015, PAPPALARDO and FERRITO 2015), and it is increasingly used to identify plant species in commercial trade (HANDY et al. 2011). For oaks, cost-efficient and easy-to-use marker sets for the identification of the continental origin of species within the section white oak (Quercus) are available (SCHROEDER et al. 2016) and will be shortly summarize here. More in detail, the marker sets developed to identify populations within the mongolian oak (*Q. mongolica*) will be presented here.

Material and methods

The used plant reference material, the detailed description of the next generation sequencing methods and analyses as well as the SNP and InDel detection from the chloroplast genome is given in SCHROEDER et al. (2016). Additionally, we used a RAD sequencing of two *Q. robur* individuals for detection of variations within the nuclear genome. The RAD sequencing has been performed using a restriction enzyme with an eight base pair recognition sequence.

Especially for the search of variation in mongolian oak, we used the same methods as described there. For validation of the SNPs and InDels, we used the MassArray technique at first with a smaller number of individuals (200) and a high number of SNPs/InDels (120). From these first results, a set of five markers has been extracted and validated using 40 populations with three to 10 individuals per population.

Results and discussion

Reference material

For the development of markers and especially for the validation of the marker sets, a high number of individuals per region/population were necessary. In total, we dispose of more than 4500 specimens of eight American, three European, and five Asian white oak species, in cooperation with partners from the US, Russia and Europe (Table 1).

Table 1: Overview of the reference material available at the Thünen Institute of Forest Genetics

Species	origin	[N] populations	[N] Individuals per population	[N] total
Q. mongolica	East-Russia, China, Korea	53	3 - 37	1567
Q. dentata	Russia, Korea	2	6 - 10	16
Q. *acutissima* Q. *serrata* Q. *aliena*	Korea	3	5	15
Q. robur	Germany, Russia, France, Bashkiria, Latvia, Poland, Ukraine, Belarus, Finland, Hungary, Switzerland	90	4 - 40	2400
Q. petraea	Germany, Russia, Ukraine	17	5 - 32	364
Q. pubescens	Germany, Poland, France, Croatia, Russia, Ukraine	27	10 - 58	542
Q. macrocarpa	Bot. Garden, USA	7	1 - 4	26
Q. alba	Bot. Garden, USA	7	1 - 5	26
Q. bicolor	Bot. Garden, USA	4	1 - 2	13
Q. garryana	Bot. Garden, USA	2	1 - 3	5
Q. lyrata	Bot. Garden, USA	4	1	6
Q. stellata	Bot. Garden, USA	6	1 - 2	10
Q. michauxii	Bot. Garden, USA	4	1 - 2	7
Q. prinoides	USA	4	1 - 3	7

Marker sets

We developed a set of six chloroplast markers (five Indels and one SNP) to differentiate white oaks from the three continents USA, Europe and Asia. The marker set can be used as a multiplex on a Genetic Analyser; or a combination of two markers (one SNP and one InDel) can also be visualized on a polyacrylamide gel. Details of the markers and their usability are given in Schroeder *et al*. (2016).

An advanced marker set of 179 SNPs both from the nuclear and the chloroplast genome analysed with the MassArray technique allows the differentiation of the species within the continents (Figure 1). The dendrogram shows the differentiation of the continents into three big clusters. The species within the continents cluster together but can all be differentiated from each other (Figure 1).

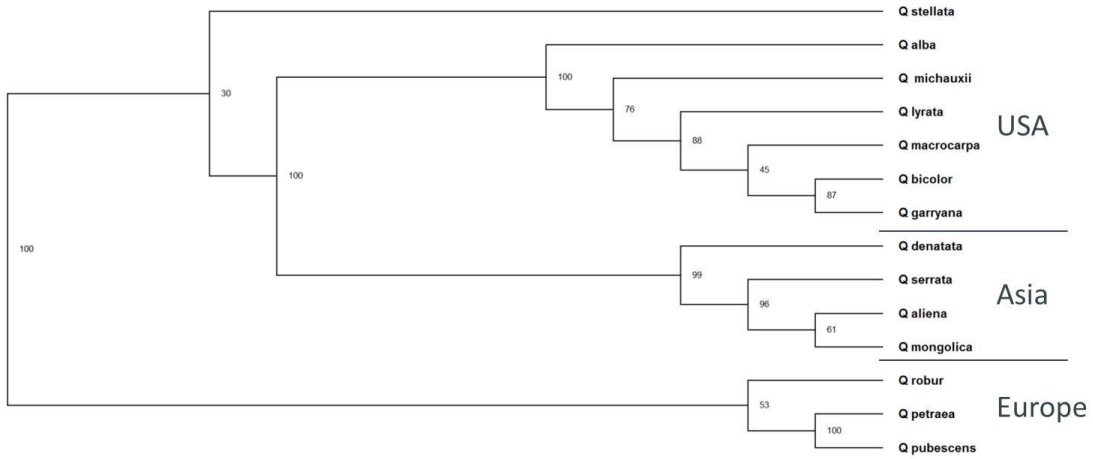

Figure 1: Dendrogram differentiating the white oak species within the three continents. The dendrogram is created using the program GDA_NT (Degen 2008) and PAST (Hammer *et al*. 2001). The numbers at the branches are boots trapping values.

The MassArray analysis of the specimens of Mongolian oak (*Q. mongolica*) led to six different haplotypes. Five markers have been combined in a set that can be analysed by PCR-RFLPs and visualized on a Genetic Analyser to identify these six haplotypes (Table 2).

The haplotypes differ in their distribution (Figure 2). Although, HT2 is the most common haplotype, it cannot be found in the Western populations. The rarer haplotype HT1 only occurs in the Western populations but not in the Eastern ones. Haplotype HT4 exists more often in the Southern populations and cannot be found in the Far North. The distribution area of haplotype HT3 is more scattered; HT3 occurs more often in the South. The last two haplotypes, HT 5 and HT6, are private haplotypes each existing in only one population (Figure 2).

Table 2: Combination of five markers to be used in a multiplex on a Genetic Analyser for differentiation of *Q. mongolica* populations within Asia

	Name of the markers				
Haplotypes	4067	32153	60606	91100	43824
HT 1	97 / 96	177	87 / 77	71 / 114	125
HT 2	193	71/106	164	184	125
HT 3	193	177	87/77	71/114	125
HT 4	193	177	87/77	184	125
HT 5	193	71/106	164	184	63/62
HT 6	193	71/106	87/77	184	125

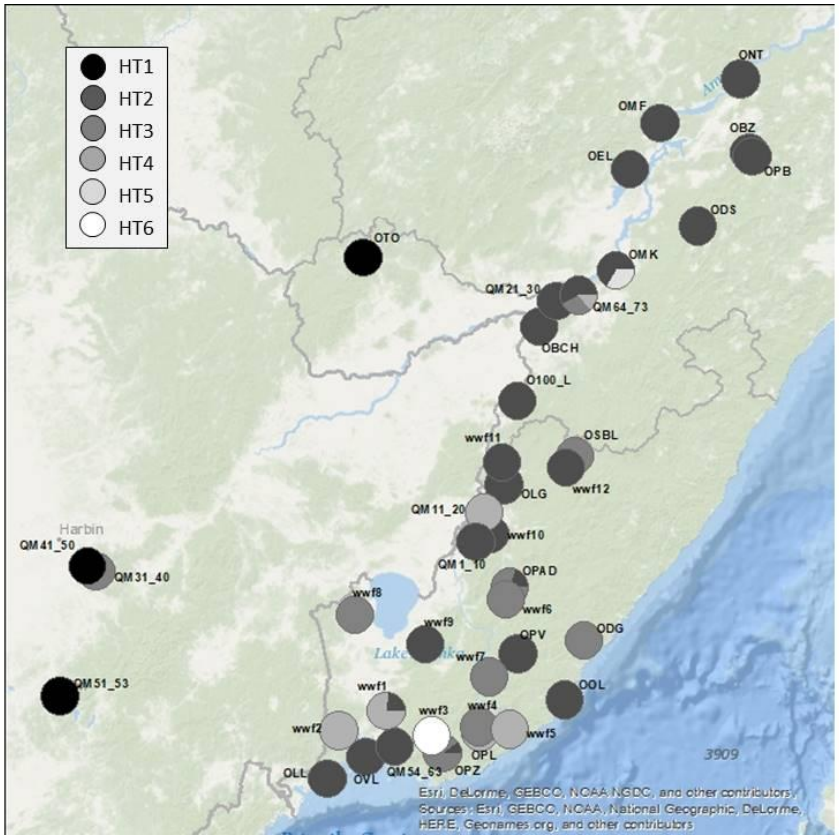

Figure 2: Distribution of the six haplotypes within 40 populations of *Q. mongolica* in Far East Russia based on mitochondrial and chloroplast markers.

Compared to European and American *Quercus* species, the variation within *Q. mongolica* is remarkable lower. Though, using the same methods for all species, much less SNPs and InDels within the chloroplast and mitochondrial genome have been found for the Mongolian oak compared to *Q. robur* (data not shown). And even within *Q. mongolica* there are regional differences; e.g., a study of *Q. mongolica* in 33 populations in Japan revealed a much higher number of haplotypes (OKAURA et al. 2007) than we found in Far East Russia (Figure 2). This difference may be due to the refuge and recolonization after the last ice age. Japan has been a refuge back then, which is often accompanied with high genetic variations (LEROY and ARPE 2007). Probably, only a few number of haplotypes arrived during the recolonization in the Far East Russia. This could be an explanation for the low genetic variation found within *Q. mongolica* in Far East Russia.

Conclusions

Overall, the presented marker sets should give commercial vendors of white oak wood the possibility to exercise 'due diligence' when placing timber on the European market. Should questions emerge on the correct declaration of wood products, the public authorities are also able to control timber imports in accordance with the European and American laws using these marker sets.

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