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Use of DNA-Fingerprints to Control the Origin of Forest Reproductive Material

By B. DEGEN[1),*], A. HÖLTKEN[2)] and M. ROGGE[3)]

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Abstract

Well-adapted, high quality reproductive material is key to the success of forest plantations. Consequently in many countries the collection and trade of forest reproductive material is regulated. Paper documents are usually the only evidence for the origin of forest reproductive material. Certification schemes already established in Germany use genetic inventories to compare reference samples collected at different steps of the chain-of-custody. A new approach using DNA-fingerprints efficiently controls the origin of seed sources without these multiple reference samples. Only a sample of adult trees within the seed stands is needed. The control is directly made for each suspicious plant or a group of suspicious plants by use of multilocus genotype assignment. We made a field test with samples of adults and seedling from 5 registered seed stands of *Quercus robur* in Western Germany. Eight highly variable nuclear microsatellites were used to genotype each individual. We found in total 255 different alleles at all loci in the adult populations. The observed levels of genetic variation ($A_e = 9.18$), genetic differentiation (delta = 0.187) and population fixation ($F_{ST} = 0.01$) were slightly higher than results

[1)] Johann Heinrich von Thunen Institut, Institut für Forstgenetik, Sieker Landstrasse 2, D-22927, Grosshansdorf, Germany.

[2)] Plant Genetic Diagnostics GmbH, Sieker Landstrasse 2, D-22927, Grosshansdorf, Germany. E-Mail: hoeltken@holz.uni-hamburg.de

[3)] Landesbetrieb Wald und Holz Nordrhein-Westfalen, Obereimer 13, D-59821 Arnsberg, Germany. E-Mail: martin.rogge@wald-und-holz.nrw.de

[*)] Corresponding author: BERND DEGEN. Johann-Heinrich-von-Thunen-Institut, Institut für Forstgenetik, Sieker Landstrasse 2, D-22927, Grosshansdorf, Germany. E-Mail: bernd.degen@vti.bund.de

of similar studies. Individual and group assignment tests were performed with the Bayesian multi-locus approach. The proportion of correctly assigned seedlings was 65% for individuals with completely scored genotypes. In all 5 cases the groups of seedlings were assigned to the correct seed stand and an additional sample of seedlings from another stand could be successfully excluded with a probability test. The conclusion of the field study is that a large scale application of this new approach to control of the origin of forest reproductive material is feasible.

Key words: Certification, forest reproductive material, genotype assignment, nSSRs, seed, tracing origin, *Quercus robur*.

Introduction

Well-adapted, high quality reproductive material is key to the success of forest plantations. Thus the collection and trade of forest reproductive material is regulated in many countries by laws, regulations and certification to promote the use of reproductive material of known origin and genetic quality for consumer protection, and the use of material well adapted to a given region. More economic, efficient methods of identifying reproductive material are needed to comply with these regulations. These regulations include the Organisation for Economic Co-operation and Development's (OECD) *Scheme for the Certification of Forest Reproductive Material* at the international level, the Council Directive 1999/105/EC for the European Union, and within Germany, the Act on Forest Reproductive Material (FoVG), its executive regulations and the technical standards of the German Programme for the Endorsement of Forest Certification Schemes (PEFC).

In Germany forest seeds are mainly collected in registered stands of the category "selected" or "tested", and to a lesser degree in seed orchards. Paper documents are usually the only proof for material origin. Documents have been repeatedly falsified, resulting in the use of unsuitable seed sources for a given area, and reduced growth, poor form, increased mortality, or increased vulnerability to disease in forest plantations in Germany and elsewhere (KONNERT and RUETZ, 2006). Thus there is an urgent need for an efficient control system using traits that cannot be manipulated. In the last years two certifications systems have been successfully established in Germany (BEHM and KONNERT, 2000; KONNERT, 2006). These systems use reference samples of forest reproductive material taken at different steps along the chain of custody. Gene markers are used to check the similarity between suspicious probes and reference samples. The systems work well, but they are time-consuming, involving many reference samples, and the statistical power of these approaches has never been tested.

In this paper we test a new approach using DNA-fingerprinting that efficiently controls the origin of seed source without multiple reference samples along the chain of custody. Only a sample of adult trees within the seed stands is needed. Multilocus genotype assignment of individuals or groups of suspicious plants will be used to control the origin of the material. This approach has already successfully been used to assign the geographic origin of plants and animals (HONJO et al., 2008; ODGEN, 2008; WASSER et al., 2004, 2007). We performed a field-study using DNA-fingerprints based on nuclear microsatellites to check the origin of forest reproductive material in five sessile oak seed stands in Western Germany.

Material and Methods

Plant material

For the field study five seed tree stands of the pedunculate oak, *Quercus robur*, 8 to 240 km apart were selected in North Rhine-Westphalia, Germany (NWR) (*Fig. 1*, *Table 1*). In each stand cambium from 56 to 89 adult trees was collected. A punch was cleaned with ethanol and then hammered into the bark of the target tree until stopped by the wood. The resultant bark plug had the cambial zone on the inner surface. These were kept on ice until further treatment in the lab. For further information on this method see (COLPAERT et al., 2005). Between 27 and 34 seedlings were also collected randomly within each stand.

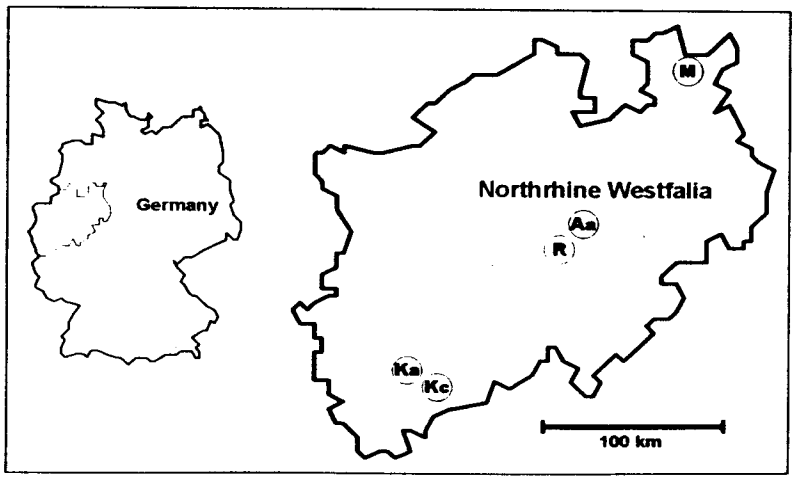

Figure 1. – Locations of the studied seed stands of *Quercus robur* (sessile oak) in North Rhine-Westphalia (Germany); Mindener Wald (M), Rumbeck (R), Arnsberg (Aa), Kottenforst (Ka), Kottenforst (Kc).