Large scale project on genetic timber verification

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Coordination

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Background

Illegal logging is one of the chief causes of worldwide deforestation and, by releasing greenhouse-relevant gasses, contributes to climate change. Moreover, trade with illegal timber and wood products creates market disadvantages for products from sustainable forestry. The OECD assesses global damages through illegal timber at approx. € 15 billion per year. According to estimates, approx. 50 % of timber exports from the Amazon Basin, Central Africa, Southeast Asia and the Russian Federation originate from illegal logging.
Now several legal instruments have been established against illegal logging and the related trade (EU timber trade regulation with its national implementation in Germany (HSiG), the Laecy Act in the USA, Prohibition Bill in Australia). Moreover many tree species are protected by the CITES inter-national species protection convention.

But there is a lack of practicable control mechanisms to check if claims on tree species and geographic origin are correct. Such methods are the fundamental prerequisites for efficient import controls or corresponding origin testing by public authorities, industry and the trade. DNA testing methods have the fundamental advantage compared to other tracking methods that they cannot be manipulated because they screen an essential part of all living beings on earth – the DNA. Differences of the genetic code among tree species can be used as gene markers to check claims on the botanical species. Trees in natural forest but also in plantations have differences in their genetic composition. This spatial genetic variation can be used to check claims on the geographic origin. And finally unique DNA-fingerprints can be screened to secure the chain of custody of high value timber species.

In the last years the Thünen-Institute of Forest Genetics has invested a lot of efforts to develop and apply the methods necessary for efficient DNA verification techniques. Among the most important outcomes so far belong a genetic reference data base to check the country of origin of mahogany in Latin America (Degen et al. 2013); a pilot study demonstrating genetic tests to check claims on the forest concession of origin of Sapelli in Cameroon (Jolivet & Degen 2012) and the development of a set of DNA barcodes to distinguish different tree species within the meliaceae family (Höltken et al. 2012).

Within the work on DNA timber tracking project the Thünen-Institute has established a large network of partners in many countries. This includes for example 14 partners in Africa, Europe and Asia in frame of the ongoing ITTO-project "*Development and implementation of a species identification and timber tracking system in Africa with DNA fingerprints and stable* *isotopes”*. Other very useful co-operations exist in this field with several countries in Latin America especially Brazil, Bolivia and Peru and with Russia, China, Singapore and South Korea. Recently a research alliance on genetic timber tracking methods has been signed between the Thünen Institute and the University of Adelaide in Australia.

The technology transfer is always an important element of the co-operation with developing countries. So the Thünen-Institute is giving training to students and scientists from timber producing countries and is setting up and supporting three genetic reference labs in Africa (Kumasi in Ghana, Libreville in Gabon, Nairobi in Kenya).

Objectives

In the next 39 months we want to develop genetic reference data to check the country of origin for timber of 14 high priority species in Africa (7 species) and in Latin America (7 species). This will be realised in frame of bilateral projects among the Thünen-Institute and institutions timber producing countries and with institutes that contributes lab work and training. A preference will be given to institutes to which already co-operation projects exist. As in other projects the technology transfer and the capacity building will be an important element of these bi-lateral co-operation projects. The goal is to support with equipment and training one reference lab in Africa and one reference lab in Latin-America.

This work covers part of the regions and tree species identified as priority by the Global Timber Tracking Network (GTTN). The genetic reference data will be implemented into the data base of the GTTN.

Structure / workpackages

The Thünen –Institute will be responsible for the genetic screening and the co-ordination of the overall initiatives as a framework of bi-lateral contracts. The framework has 4 different work packages (WP):

- WP Sampling
- WP Genetic Screening
- WP Technology Transfer
- WP Co-ordination

WP sampling

We plan three different sampling campaigns:

a) The first sampling operations are focused on the fast sampling of a very limited number of individuals per species. The objective of this sampling is to get high quantity and quality of DNA for the gene marker development using Next Generation DNA Sequencing technologies.
b) For each species 40 locations within its natural distribution range will be sampled. At each place cambium or leaves of 20-30 individual trees will be collected. Thus we expect to collect 800-1200 individual trees per species as reference samples. We expect that there is strong overlapping of the distribution range of species. Thus we assume to find 50% of all target species from a region at each sampling point.
c) Finally 20 wood samples per species will be collected for blind tests in order to check the quality of the genetic reference data and applicability of the gene markers in timber.

In a few former project not particularly aiming on timber tracking a lot of samples of target tree species have been collected. Part of this samples might be still accessible and with sufficient DNA quality. The project aims to enrich the planned new sampling with these existing samples. For this a special budget line is planned in order to enable an attractive involvement of the institutions / persons who are holding these samples.

In consultation with the partners in Africa and Latin America we have generated the following short list tree species (tab. 1a,b). We did not include tree species for which genetic reference data have been already developed or for which large scale reference data are on the way of development in ongoing projects. The tree species in each region are ranked according to the priority.

Botanical Name	**Local Name**	**Countries of importance**							
		Cameroon	**Congo**	**DRC**	**Gabon**	**Ivory Coast**	**Ghana**	**Liberia**	**Nigeria**
Central Africa									
Entandrophragma utile	Sipo	X	X	X	X	X	X	X	
Aucoumea klainea	Okoumé		X	X	X				
Cylicodiscus gabonensis	Okan	X	X	X	X		X		
Pterocarpus soyauxii	Padouk	X	X	X	X				
Lophira alata	Azobé	X	X					X	X
West Africa									
Nauclea diderrichii	Bilinga	X	X	X	X	X		X	X
Khaya anthotheka	Acajou/khaya			X	X	X	X	X	X
Khaya ivorensis	Mahogany	X	X		X	X	X		X

Table 1a: List priority species for the development of genetic reference data in Africa

Botanical name	**Local name**	**Family**	**Occurance of the species**			
			Brazil	**Bolivia**	**Peru**	**French Guiana**
Carapa guianensis (Aubl.)	Andiroba	Meliaceae	X		X	X
Cedrela fissilis Vell	Cedro	Meliaceae	X	X	X	
Cedrela odorata L.	Cedro-cheiroso	Meliaceae	X	X	X	X
Dinizia excelsa Ducke	Angelim Vermelho	Leguminosae	X			X
Hymenaea courbaril L.	Jatobá	Fabaceae	X	X	X	X
Jacaranda copaia (Aubl.) D. Don	Pará-pará	Bignoniaceae	X	X	X	X
Manilkara huberi (Ducke) Chevalier	Maçaranduba	Sapotaceae	X			X
Simarouba amara Aubl	Marupá	Simaroubaceae	X	X	X	X
Tabebuia serratifolia (Vahl.) Nich.)	Ipê-amarelo	Bignoniaceae	X		X	X
Virola surinamensis (Rol. Ex Rottb.) Warb	Cumala	Myristicaceae	X		X	X
Dipteryx odorata	Cumaru, Shihuahuaco	Fabaceae	X		X	X

Table 1b: List priority species for the development of genetic reference data in Latin America

WP Genetic screening

The genetic screening will be done centralised at the Thünen-Institutes. Part of the preparation steps (e.g. DNA extraction, gene marker development) might be done in partner's lab. Based on the experiences in former projects and the day to day work experience of the Thünen-Centre of Excellence on Timber origin we plan 4 steps.

a) Using next generation DNA sequencing techniques to identify for each species more than 1000 SNPs (Single Nucleotide Polymorphism). For this we have good experiences with the Rad-Sequencing (Pujola et al 2014) and „Skimming" (Besnard et al 2014). The SNPs are the best markers to be applied for the degraded DNA of timber. The University of Adelaide will add SNPs developed using a new approach called "CROPS".
b) Selection of optimal SNPs and prae-screening of several hundred SNPs at 95 individuals using the Sequenome genotyping platform (Mc Kernan et al. 2002).

c) Selection of a final set of 120-240 SNPs and genetic screening of all 800-1200 individuals per species on these markers => development of the genetic reference data base
d) Development of simple methods for the screening of the top 20 highly informative SNPs in labs in timber producer countries (selection of 20 “golden” or “silver” gene markers). Suitable screening methods for this are PCR/RFLP methods (Cullings 1992) or the SnapShot approach using DNA-Sequencers. Partners’ labs will be enabled due to training and support with additional equipment to use these gene markers.

WP Technology transfer

Here we plan to reinforce the labs of FORIG in Kumasi in Ghana in Africa and the genetic lab of the Peruvian Amazon Research Institute in Iquitos (AIIP) in Peru. The support includes additional equipment and training of scientists and technicians in western labs, training courses at the reference labs and common ring tests for standardisation of the methods. Also scientists from other partner’s labs will be included in this training.

Literature

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