THÜNEN

German Russian Conference on Forest Genetics - Proceedings - Ahrensburg, 2017 November 21-23

Bernd Degen, Konstantin V. Krutovsky, Mirko Liesebach (eds.)

Thünen Report 62

Bibliografische Information:
Die Deutsche Nationalbibliothek verzeichnet diese Publikationen in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet unter www.dnb.de abrufbar.

Bibliographic information:
The Deutsche Nationalbibliothek (German National Library) lists this publication in the German National Bibliography; detailed bibliographic data is available on the Internet at www.dnb.de

Bereits in dieser Reihe erschienene Bände finden Sie im Internet unter www.thuenen.de

Volumes already published in this series are available on the Internet at www.thuenen.de

Zitationsvorschlag – *Suggested source citation:*
Degen B, Krutovsky KV, Liesebach M (eds) (2018) German Russian Conference on Forest Genetics - Proceedings - Ahrensburg, 2017 November 21-23. Braunschweig: Johann Heinrich von Thünen-Institut, 148 p, Thünen Rep 62, DOI:10.3220/REP1539855736000

THÜNEN

Thünen Report 62

Herausgeber/Redaktionsanschrift – *Editor/address*

Johann Heinrich von Thünen-Institut
Bundesallee 50
38116 Braunschweig
Germany

thuenen-report@thuenen.de
www.thuenen.de

ISSN 2196-2324
ISBN 978-3-86576-189-7
DOI:10.3220/REP1539855736000
urn:nbn:de:gbv:253-201810-dn060232-8

German Russian Conference on Forest Genetics - Proceedings - Ahrensburg, 2017 November 21-23

Bernd Degen, Konstantin V. Krutovsky, Mirko Liesebach (eds.)

Thünen Report 62

Bernd Degen, Mirko Liesebach
Thünen-Institut für Forstgenetik
Sieker Landstr. 2
22927 Großhansdorf
Germany
Telefon: +49 4102 696-0
Fax: +49 4102 696-200
E-Mail: bernd.degen@thuenen.de

Konstantin V. Krutovsky
Georg-August University Göttingen
Abteilung Forstgenetik und Forstpflanzenzüchtung
Büsgenweg 2
37073 Göttingen
Germany

VERANTWORTLICHE AUTOREN:
Bernd Degen, Konstantin V. Krutovsky, Mirko Liesebach (eds.)

Thünen Report 62

Braunschweig/Germany, October 2018

Abstract

Proceedings German Russian Conference on Forest Genetics

The practical applications of forest genetics contribute substantially to sustainable forest management. Genetic data on optimally adapted and productive provenances as well as intensive tree breeding help to produce high quality forest reproductive material. Recent advances in tree genomics and DNA-sequencing techniques provide variable and more efficient tools for application in forestry, such as marker assisted tree breeding, screening for genes responsible for adaptive and economically important traits, and tree improvement. In the last two decades, the application of highly variable gene markers has produced a lot of very useful data on the genetic diversity and genetic differentiation of genetic resources of important forest tree species. The recent advances in the development of gene markers for variable diagnostic purposes enable to improve DNA-tests on wood and forest reproductive material including better control for timber origin, species protection and early detection of dangerous infections and diseases.

The German-Russian MaRussiA project is a good example of the application of the above mentioned techniques. The Project is focused on aspen (*Populus tremula*): https://www.thuenen.de/en/fg/projects/current-projects/marussia-gene-marker-for-aspen-in-russia.

Objectives: One of the main objectives of the joint German-Russian conference was to communicate the current results and experiences obtained in the MaRussiA project to a group of Russian and German experts in this field. We expected to get valuable feedbacks and recommendations for the remaining tasks within the project. Further, we wanted to get an overview on the state of the art of forest genetic research in both countries. Based on this overview and experiences of past and ongoing co-operation projects, we wanted to identify knowledge gaps and to stimulate the future scientific research and cooperation on these fields in Russia and Germany.

Conference venue: The conference was planned and organised by the Thünen Institute of Forest Genetics (Dr. BERND DEGEN, Dr. GEORG VON WÜHLISCH, and Dr. MATTHIAS FLADUNG) together with the George August University of Göttingen (Prof. Dr. KONSTANTIN V. KRUTOVSKY). The conference was held with 25 participants at the "Hotel am Schloss" in Ahrensburg, Germany from 21th to 23th of November, 2017.

Conference structure: General scope sessions:

- Tree Breeding and Provenance Research
- Genome Research
- Population Genetics
- DNA identification methods for wood and forest tree material (for timber tracking, control of forest reproductive material, etc.)
- Discussion on future collaborative projects

Key words: Forest genetics, forest tree breeding, *Populus tremula*, DNA, molecular marker

Zusammenfassung

Deutsch-russische Tagung zu Forstgenetik

Die praktischen Anwendungen der Forstgenetik tragen wesentlich zur nachhaltigen Waldbewirtschaftung bei. Genetische Informationen zu optimal angepassten und produktiven Herkünften sowie intensive Forstpflanzenzüchtung helfen, qualitativ hochwertiges forstliches Vermehrungsgut zu produzieren. Jüngste Fortschritte in der Genomik von Bäumen und bei den DNA-Sequenzierungstechniken liefern variable und effizientere Werkzeuge für die Anwendung in der Forstwirtschaft, wie z. B. markerunterstützte Züchtung, Screening nach Genen, die für adaptive und ökonomisch wichtige Merkmale verantwortlich sind, und zur Verbesserung von Eigenschaften. In den vergangenen zwei Jahrzehnten hat die Anwendung hochvariabler Genmarker eine Menge sehr nützlicher Daten zur genetischen Vielfalt und genetischen Differenzierung genetischer Ressourcen wichtiger Waldbaumarten hervorgebracht. Die jüngsten Fortschritte in der Markerentwicklung für variable diagnostische Zwecke ermöglichen es, DNA-Tests an Holz und forstlichem Vermehrungsgut zu verbessern, einschließlich einer besseren Kontrolle des Holzursprungs, des Artenschutzes und der Früherkennung von gefährlichen Infektionen und Krankheiten.

Das deutsch-russische MaRussiA-Projekt ist ein gutes Beispiel für die Anwendung der oben genannten Methoden. Das Projekt ist vorrangig auf die Zitter-Pappel (*Populus tremula*) ausgerichtet: https://www.thuenen.de/en/fg/projects/current-projects/marussia-gene-marker-for-aspen-in-russia.

Ziele: Eines der Ziele der deutsch-russischen Konferenz bestand darin, die bisher beobachteten Ergebnisse und Erfahrungen des MaRussiA-Projekts einer größeren Gruppe von russischen und deutschen Experten auf diesem Gebiet der Forstgenetik und -pflanzenzüchtung zu vermitteln. Wir erwarteten wertvolle Rückmeldungen und Empfehlungen für die verbleibenden Aufgaben innerhalb des Projekts. Darüber hinaus wollten wir einen Überblick über den Stand der forstgenetischen Forschung in beiden Ländern erhalten. Auf diesem Überblick und den Erfahrungen aus vergangenen und laufenden Kooperationsprojekten beabsichtigten wir, Wissenslücken zu identifizieren und die zukünftige wissenschaftliche Forschung und Kooperation in diesen Bereichen in Russland und Deutschland anzuregen.

Tagungsort: Die Tagung wurde geplant und vom Thünen-Institut für Forstgenetik (Dr. BERND DEGEN, Dr. GEORG VON WÜHLISCH, Dr. MATTHIAS FLADUNG) zusammen mit der George August Universität Göttingen (Prof. Dr. KONSTANTIN V. KRUTOVSKY) ausgerichtet. Die Tagung fand mit 25 Teilnehmern vom 21. bis 23. November 2017 im “Hotel am Schloss” in Ahrensburg, Deutschland statt.

Tagungsgliederung: Allgemeine Themen der Sessions:

- Forstpflanzenzüchtung und Herkunftsforschung
- Genomforschung
- Populationsgenetik
- DNA identification methods for Holz und Pflanzenmaterial (zum Holzherkunftsnachweis, Kontrollen von forstlichem Vermerhungsgut usw.)
- Diskussion über eine Zusammenarbeit in küftigen Projekten

Schlüsselworte: Forstgenetik, Forstpflanzenzüchtung, *Populus tremula*, DNA, molekulare Marker

Participants of the conference in Ahrensburg, Germany from 21st to 23rd of November, 2017

Content

Abstract, Zusammenfassung 1
German Russian Conference on Forest Genetics, Deutsch-russische Tagung zu Forstgenetik

Gruppenbild 3

Content 5

I. Tree Breeding and Biotechnology 7

Improving the productivity, resistance, and adaptability in poplar – Development of genetic markers for aspen ("MaRussiA") 9
MATTHIAS FLADUNG, GEORG VON WÜHLISCH

Aspen clonal sustainability assessment in natural populations by tree-ring based information 17
OXANA CHERNYSHENKO, D. RUMYANTSEV, ANNA SIROTOVA

Testing of mutagenic substances in aspen *in vitro* culture 23
DMITRII A. SHABUNIN

Poplars breeding in temperate climate of Russia 27
ANATOLY TSAREV, R.P. TSAREVA, V.A. TSAREV

Development of research resources for marker-assisted selection of aspen (*Populus tremula* L.) in Russia 35
A.V. ZHIGUNOV, P.S. ULIANICH, M.V. LEBEDEVA, E.K. POTOKINA

The German breeding concept – the base for new seed orchards 41
MIRKO LIESEBACH, VOLKER SCHNECK

From *in vitro* clones to high-quality timber production: the project "Wavy Grain Maple" 49
CORNELIA BÄUCKER, HEIKE LIESEBACH

The mysteries of the origin of curly birch 55
LIDIIA VETCHINNIKOVA, ALEXANDER TITOV

The importance of fuel characteristics of poplars and aspens (*Populus* spp.) from German short rotation plantations and Russian forests 61
MATTHIAS MEYER, KATHRIN GEBAUER, ALWIN JANSSEN, DORIS KRABEL

II. Population Genetics and Gene Conservation 67

Twenty years German-Russian co-operation for genetic diversity in forests 69
BERND DEGEN, HILKE SCHROEDER, CÉLINE BLANC-JOLIVET, YULAI YANBAEV

Nucleotide polymorphism and genetic structure of populations of *Populus* lars in the Urals 73
SVETLANA BORONNIKOVA, T.N. LISINA, N.A. MARTYNENKO

GenMon – Implementation of a genetic monitoring system in European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* [L.] Karst.) populations in Germany 77
JENS SCHMIEDEL, UTE TRÖBER, HEINO WOLF, BARBARA FUSSI, MARCO KUNZ

Population genetic studies of small-leaved forest tree species using molecular markers 83
TATIANA POLIAKOVA, D.V. POLITOV, M.M. BELOKON, YU.S. BELOKON, E.A. MUDRIK, A.V. SHATOKHINA, A.P. EFIMOVA, K.A. SHESTIBRATOV

III. Timber Tracking, Genomics and Adaptation 87

Genetic timber tracking of *Larix* sp. in Eurasia 89
CÉLINE BLANC-JOLIVET, YULAI YANBAEV, BERND DEGEN

Development of mtDNA markers for Siberian conifers and their application in phylogeographic studies 95
VLADIMIR L. SEMERIKOV, SVETLANA A. SEMERIKOVA, K.V. KRUTOVSKY

Genomics for practical forestry: development of genome-wide markers for timber origin identification and other applications 101
YULIYA A. PUTINTSEVA, NATALIAY V. ORESHKOVA, EUGENIYA I. BONDAR, VADIM V. SHAROV, DMITRY A. KUZMIN, STEPAN V. MAKOLOV, KONSTANTIN V. KRUTOVSKY

DNA-marker sets for determination of white oaks (section *Quercus*) in wood products 107
HILKE SCHRÖDER, BIRGIT KERSTEN, YULAI YANBAEV, BERND DEGEN

Introgression of genes between two hybridizing red oak species with different drought tolerance 113
OLIVER GAILING

IV. Excursions 121

Biomass production on the ProLoc-site Trenthorst (Bio26) 123
MIRKO LIESEBACH

Clone test with hybrid aspen (As130) 127
MIRKO LIESEBACH, VOLKER SCHNECK

International beech provenance trial 1993/95 - site Schädtbek (Bu19-1) 131
MIRKO LIESEBACH

V. Annex 139

Programme 141

List of Participants 145

I. Tree Breeding and Biotechnology

Improving the productivity, resistance, and adaptability in poplar – development of genetic markers for aspen ("MaRussiA")

MATTHIAS FLADUNG[1], GEORG VON WÜHLISCH[2]

[1] Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany (matthias.fladung@thuenen.de)

[2] Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany

Abstract

The joint German-Russian co-operation project with the acronym "MaRussiA" focuses on the improvement of poplar in eastern and western Europe (Russia respectively Germany), where the aspen species *Populus tremula* is indigenous and distributed widely. Background and objective of the joint research project is the development of genetic markers for marker-assisted selections (MAS). Such markers would represent major advances in forest tree breeding e.g. for accelerating the breeding process. Different full-sib families were provided by the Russian partners, and those with the highest progeny numbers were chosen for the development of genetic maps. Several hundreds of microsatellite (SSR) markers were tested in the parents for heterozygosity and mapping ability, and 30-40 SSR markers were successfully selected covering all 19 chromosomes. The available full-sib progenies will be tested in field trials to phenotype productivity and resistance characters, e.g. heart rot and drought. Segregating populations will be identified and QTL analyses for the phenotyped traits will be performed. Based on the results obtained, gene markers for a marker-based selection will be developed to intensify poplar / aspen breeding in Russia.

Key words: Microsatellite marker, genetic map, poplar breeding, heart rot disease, drought

"MaRussiA" participating institutions

The "MaRussiA" consortium consists by the Russian partners St. Petersburg Forest Technical University, St. Petersburg (A. ZHIGUNOV, E. POTOKINA, M. VISHNEVSKAYA), Saint-Petersburg Forestry Research Institute, St. Petersburg (D. SHABUNIN), All-Russian Institute of Forest Genetics, Breeding and Biotechnology, Voronezh (A. TSAREV), and Moscow State Forest University, Moscow (OXANA CHERNISHENKO), and the German partner Thuenen-Institute of Forest Genetics, Grosshansdorf (G. VON WUEHLISCH, M. FLADUNG), being the coordinator of this project.

General and specific project aims

The general aim of the German-Russian co-operation "MaRussiA" project is to raise the productivity of the indigenous and widely distributed poplar species aspen (*Populus tremula*) by enhancing tolerance against biotic and abiotic stresses. In economically active target regions of St. Petersburg and Moscow, wood is intensively used and the efficiency of wood production needs to be raised. Large areas of abandoned land can be put into use by producing wood, a renewable resource forecast to be required more intensely in future. Classical tree breeding strategies are establishment and conservation of genetic resources, germplasm exchange, hybridisation, selections and field testing of elite material. However, to intensify and accelerate the breeding process, also modern technologies e.g. development of genetic markers for clone identification and marker-assisted selections (MAS) need to be considered (SCHROEDER and FLADUNG 2010, FLADUNG and GEBHARDT 2010).

Focus of any breeding activity is on the end product, the wood produced and its quality, which are to be investigated. Within this joint co-operation project, poplar and aspen reproductive materials for cross breeding by a jointly developed factorial crossing scheme are to be exchanged. The produced progenies will be shared among the partners for further testing in field trials and in the lab. In the field, productivity and resistance characters, e.g. heart rot and drought, are phenotyped by the Russian partners in the segregating populations, and QTLs will be identified. The German partner will test as many segregating microsatellite (simple sequence repeats, SSR) and single nucleotide polymorphism (SNP) markers as possible in the same progeny populations. Identified QTLs will be mapped on the respective genetic maps and association studies with SSR- and/or SNP-markers will be performed. An already available, with SSR- and SNP-markers highly saturated *P. tremula × P. tremuloides* map (Brauna11 × Tur141; PAKULL et al. 2009, 2011) will serve as reference map. SSR- and/or SNP-markers linked to QTLs will be used to screen already available *P. tremula* and *P. tremuloides* total genomic sequences to identify putative candidate genes.

WP 1 Crossings and establishment of F1 populations

In 2015, leaf material from 20 different aspen clones, used for crossings, was provided by Russian partner (A. TSAREV [PETROZAVODSK and VORONEZH, Russia]) (TSAREV et al. 2016, 2018) (Table 1). The progenies were established and planted in the field. In Table 2, five male and five female individuals with the numbers of the respective offspring are shown. From these, progenies having different mothers (05-02, 15-04, 25-05), but the same father (here: 08-02) with 62, 61 and 120 individuals, respectively, were selected for further molecular analyses.

Table 1: Leaf material from 20 different aspen clones (kindly provided by A. Tsarev [Petrozavodsk and Voronezh, Russia]) collected in the Central Chernozem region (Russia). In addition to the clone name (Index), the gender, the local collection and origin, and the "form of life" are given. In red: gender information not provided, newly determined.

Number	Index	Gender	Local of collection	Origin	Life form
Parents of hybrids-frozen and alcohol transport, collected in May 2015					
1	07-02	male	Semiluksky tremuletum	Voronezh	tree
2	07-04	male	-"-	-"-	-"-
3	25-05	female	-"-	-"-	-"-
4	05-02	female	-"-	Latvia	-"-
5	06-04	female	-"-	Latvia	-"-
6	15-04	female	-"-	Voronezh	-"-
7	08-02	male	-"-	-"-	-"-
8	22-05	female	-"-	-"-	-"-
9	23-03	male	-"-	Saval (Voronezh)	-"-
10	20-04	male	-"-	Oboyan (Kursky)	-"-
Leaves collected in natural forests and tremuletum and put in envelops in July 2015					
11	W-1	male	Voronezh suburb	Voronezh	tree
12 (15)	17-05	male	Semiluksky tremuletum	Gubkino (Belgorod)	-"-
13 (16)	02-01	female - male	-"-	Voronezh	-"-
14 (17)	15-01	female	-"-	-"-	-"-
15 (18)	18-04	male	-"-	Valuyky (Belgorod)	-"-
16	W-2	female	Voronezh suburb	Voronezh	little tree
17	W-3	male	-"-	-"-	tree
18	W-4	male	-"-	-"-	little tree
19	W-5	female	-"-	-"-	root shoot
20	W-6	"supermale"	-"-	-"-	-"-

Table 2: Already available progenies of five female and five male parents. Parents of the grey tagged progenies, namely 05-02, 15-04, 25-05 (female) and 08-02 (male), were selected for microsatellite analyses.

♀	♂ 07-02	07-04	08-02	20-04	23-03	∑
05-02	24	-	62	66	5	157
06-04	8	1	7	1	-	17
15-04	2	6	61	2	48	119
22-05	1	-	-	-	34	35
25-05	34	1	120	15	32	202
∑	69	8	250	84	119	530

WP 2 Development and use of molecular markers

The microsatellite markers tested were either selected from (*i*) *Populus*-database http://www.ornl.gov/sci/ipgc/ssr_resource.htm; YIN et al. 2009) or (*ii*) developed in own work (BRÜGMANN and FLADUNG 2013, SCHRÖDER and FLADUNG 2010). All selected microsatellite markers were tested in the parental clones first for amplificability in PCR reactions and later for polymorphism on ALF DNA

Automated Sequencer (Amersham Pharmacia Biotech, Buckinghamshire, UK). Overall, about 270 microsatellite markers turned out to be useful. Second, about 60 microsatellite markers could successfully be transferred to the ABI3730 Capillary sequencer (Applied Biosystems, Foster City, CA, USA; SCHROEDER et al. 2017). In different sets, four to five microsatellites could be combined and analysed simultaneously in one run ("multiplex analysis"). The aim is to amplify one to four microsatellite markers for each of the 19 poplar chromosomes.

Gender determination in different poplar clones

As first molecular investigation, gender determination was carried out in plant material from Table 1 according to PAKULL et al. (2015). The results are shown in Figure 1. With exception of sample 02-01, which is declared as female in the sample delivery protocol but clearly revealing the male-specific band in the PCR (yellow in Figure 1), the gender of all other genotypes is confirmed. However, the Russian side confirmed that sample 02-01 is actually female. Further molecular analyses of 02-01 have shown that this genotype is not a pure *P. tremula* but an unknown species hybrid. As the PAKULL et al. (2015) procedure for gender determination is exclusively in aspen (*P. tremula* and *P. tremuloides*) as well as their hybrids, the presence of an unknown species hybrid could explain the discrepancy between Russian declarations (female) and molecular analysis (male).

For six clones (W1 to W6), gender was newly determined (red in Table 1) as no respective information was given in the sample delivery protocol. Three clones turned out to be male (W1, W3, W4) and two female (W2, W5). For clone W6, a "supermale" status could be determined as no female allele could be PCR-amplified.

In 2016, the Russian partners provided leaf material from two additional progenies, namely 10-03 × 08-02 with 127 individuals and 18-02 × 08-02 with 90 individuals. Also for these parental individuals, all available microsatellite markers were tested for being polymorphic in the respective parental individuals. In addition, all F_1 individuals were included in molecular analyses.

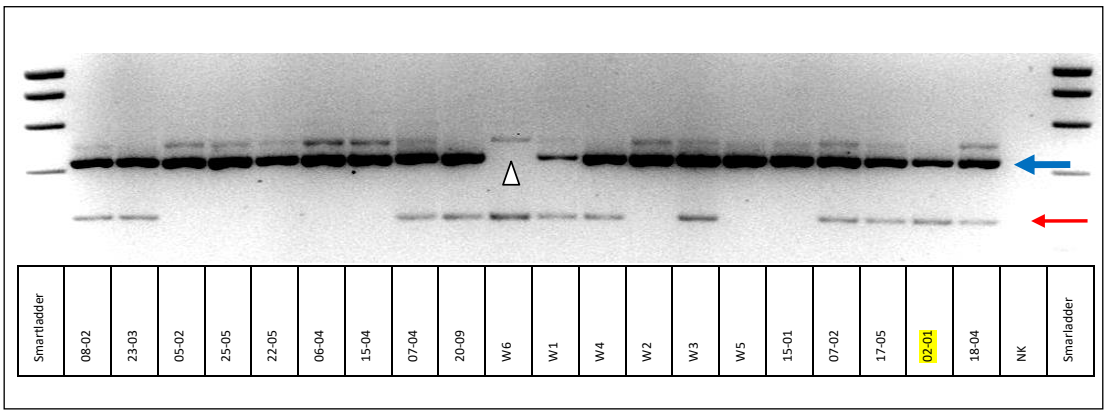

Figure 1: Gender check for the genotypes listed in Table 1. The band indicated by the red arrow is formed in the PCR only by male genotypes. The yellow-labelled clone 02-01 shows the male-specific band, but is declared as female in Table 1. The black triangle in W6 indicates the missing female band (blue arrow), thus this clone is possibly a "supermale".

Analysis of 05-02, 15-04, 25-05 (female) and 08-02 (male)

The three female parents, namely 05-02, 15-04, 25-05, as well as the one male genotype 08-02 were intensively characterized by microsatellite markers (Table 3). In total, 32 microsatellite markers qualified to be applicable for further molecular studies. All microsatellite markers were also tested in *P. tremula* Brauna11 (female) and *P. tremuloides* Tur141 (male) being parents of a progeny of about 120 F_1-individuals. The genetic map of this offspring has already been published (PAKULL et al. 2009).

Table 3: Extract from the results table of the molecular analysis of the Russian parents 05-02, 15-04, 25-05 (female), and 08-02 (male), compared to Brauna11 and Tur141 (PAKULL et al. 2009), by a total of more than 120 microsatellites (primer). Green and yellow-green tagged primer: applicable, red tagged primer: not applicable.

Primer	LG	Motif	08-02 ♂		05-02 ♀		15-04 ♀		25-05 ♀		Tur141		Brauna 11	
GCPM1108-1	7	TC	162	198	196		198	202	194	198	198		198	202
GCPM124	1	CAC	197	212	206		206		197	206	210		196	
GCPM134-2	3	ATTT	225	239	227		233		227		224	228	240	244
GCPM1809	4	TTA	186		180	183	183		180	183	183	186	183	186
GCPM1831	6	AG	223	227	223		223	227	225	227	223	225	223	
GCPM2081	14	TC	204	206	194	198	202	206	202	204	204		194	
GCPM2167	13	AG	180	184	178	202	178	200	178	200	184		180	182
GCPM2768	2	GA	206	220	206	210	206	208	204	206	196	200	194	206
GCPM3261	16	AAT	190	193	187	193	187	196	190	196	193	196	187	196
GCPM3362	8	CTT	218		221		218	224	218	221	209	212	212	221
ORPM1031	1		146	158	152	170	143	146	146		131	146	146	188
ORPM190	6	[TG]7*	202		202	216	216		216		202		202	218
ORPM202	8	[TAA]5	193		193	196	193	196	196		196		193	196
ORPM30	3	[TC]9	222	232	222		236	238	230	236	230	236	230	
ORPM489	18	[TC]12	172	186	172	180	172	176	176		166		176	190
PMGC108	12	CTT	340	346	340		343	346	346		328	340	340	346
PMGC2020	4	GA	148	150	148	152	144	150	144	146	150	152	136	150
PMGC2088	2	GA	180	184	180	184	186		186	192	172	176	182	192
PMGC2419	10	GA	111	117	111	117	125	131	117	125	101	105	125	131
PMGC2531	11	GA	126	150	140	146	126	148	126		130	138	136	
PMGC2550	1	GA	150	156	150	156	156	162	156	162	150		156	162
PMGC2607	8	GA	143	161	143	153	143	169	153	169	161		143	
PMGC2658	13	GA	149	303	149		231		149		245		253	303
PMGC2826	4	GA	238	252	216	236	236	242	240	242	246	252	226	242
PMGC2880	18		174	0	202		176		174	194	170	198	202	
PMGC420	14	GA	101		101	105	101		103	105				
PMGC433	19	GA	197	207	197	201	197	209	197		205		197	
PMGC648	17	GA	184	208	194	198	198		196		188	214	198	226
PTR2	9	TGG	231		228	234	228		228		228	234	228	241
PTR5	11		268		268		268	278	268		270		262	268
PTR7	12		240		242	264	266		242	266	242		286	
WPMS05	12	GT	293	303	303		293		295		277	303	281	295
WPMS14	5	CGT	234	249	249		231	240	240	249	228	231	234	249
WPMS20	13	TTCTGG	245		245		239	245	221	245				

Analysis of families 10-03 (female) and 08-02 (male), and 18-02 (female) and 08-02 (male)

The results of the analyses for parents 10-03 (female) and 08-02 (male) as well as for some of the progeny F_1- individuals by testing more than 120 microsatellite markers are shown in Table 4. In total, 21 microsatellite markers revealed polymorphic in both parent genotypes. These markers were used to check whether all individuals actually belong to this progeny. In total, 5 individuals had to be excluded who had allelic combinations at 5 to 14 loci that could not be derived from both parents (Table 4).

For the second progeny (18-02 [female] and 08-02 [male]), 28 microsatellites revealed polymorphic in the two parent genotypes (data not shown). These markers were also used to check for individuals actually not belonging to this progeny. In this progeny, no individual had to be excluded on the basis of a non-derivable allele combination.

Table 4: Extract from the results table of the molecular analysis of the Russian parents 10-03 (female) and 08-02 (male), by a total of more than 120 microsatellites (primer). Green and yellow-green tagged primer: applicable, red tagged primer: not applicable. The yellow fields in the progeny individuals indicate allele combinations that cannot be derived from both parents.

Primer	LG	Motif	08-02 ♂		10-03_C ♀		01-01		01-02		01-112		01-113		01-114		01-115		01-116		01-117		01-118		01-119	
GCPM1108-1	7	TC	162	198	162	198	198		162		198		198		198		162	198	162	198	162		198		198	202
GCPM124	1	CAC	197	212	197	212	197	212	197		212		207	212	197	212	212		197		197		197	212	197	212
GCPM134-2	3	ATTT	225	239	227	239	239		225	239	225	239	227		239		227	239	225	239	239		225	227	227	
GCPM1831	6	AG	223	227	221	231	227	231	227	231	221	227	221	225	221	233	221	227	223	231	221	223	221	223	221	231
GCPM2081	14	TC	204	206	196	204	204		196	206	204	206	202	204	198	204	196	204	204		196	204	204		196	204
GCPM2167	13	AG	180	184	178	184	180	184	184		178	180	178		178		178	184	180	184	178	184	178	180	184	
GCPM2768	2	GA	206	220	206	220	206	220	206	220	220		206	220	206	212	206	220	220		206		206	220	206	
GCPM3261	16	AAT	190	193	190	193	190	193	190	193	193		190	193	187	190	190	193	190		193		190	193	190	193
ORPM1031	1		146	158	143	146	143	146	143	158	143	158	143		143	146	143	146	146		143	146	143	158	143	146
ORPM30	3	[TC]9	222	232	222	238	222	232	222	238	222	232	222	238	222		232	238	222		222		222		230	238
ORPM489	18	[TC]12	172	186	180	196	180	186	172	196	172	180	176	180	170	196	172	196	186	196	172	196	172	180	166	180
PMGC2020	4	GA	148	150	136	152	136	150	150	152	148	152	136	146	136	144	148	152	148	152	148	152	136	150	136	150
PMGC2088	2	GA	180	184	180	184	180	184	180	184	180	184	184		180	184	180	184	184		180	184	180	184	180	184
PMGC2419	10	GA	111	117	125		111	125	111	125	117	125	95	125	125		111	125	117	125	117	125	117	125	105	125
PMGC2531	11	GA	126	150	136	142	126	142	136	150	136	150	136		142		142	150	126	142	136	150	142	150	126	1136
PMGC2550	1	GA	150	156	150	162	150	156	150	156	150	156	162		150	164	156	162	150		150	162	150	162	150	
PMGC2658	13	GA	149	303	303		303		149		303		231	303	303		149	303	303		149		303		258	305
PMGC2826	4	GA	238	252	234	246	234	252	234	238	238	246	242	246	216	246	234	238	234	252	246	252	246	252	216	234
PMGC433	19	GA	197	207	197	201	197	207	197		197	207	197		197	205	197		197		197		197		197	201
PTR2	9	TGG	231		228	240	228	231	228	231	231	240	231	240	228		228	231	228	231	231	240	231	240	228	
PTR7	12		242		242	286	242	286	242		240	286	240	270	254	286	240		240		240	286	240	286	240	286

WP3 *in vitro* propagation

Buds of selected aspen genotypes were successfully transferred to tissue culture for further propagation, including five *P. tremula* "davidiana" clones that are considered to be resistant to heart rot. The cultures were treated with three different mutagenic substances. The resulting callus tissues were sub-cultured and shoots could be regenerated. A total of 140 plants were obtained. The exact number of plants for the rooting experiments will be evaluated in the spring of 2018. These plants will be transferred to the field and studies on growth, lignin content and fibre length will be performed. Finally, resistance tests against heart rot will be conducted.

Acknowledgements

This project was financially supported by the Federal Ministry of Food and Agriculture (BMEL) via the "Bundesanstalt für Landwirtschaft und Ernährung" (BLE). We would like to thank the technical assistants MANUELA WILL and KATRIN GROPPE for excellent lab work. We thank all Russian partners for material supply and kind cooperation.

References

BRUEGMANN T, FLADUNG M (2013): Potentials and limitations of the cross-species transfer of nuclear microsatellite marker in six species belonging to three sections of the genus *Populus* L. Tree Genetics & Genomes 9: 1413-1421.

FLADUNG M, GEBHARDT K (2010): Mit Smart-Breeding-Methoden neue Wege in der Forstpflanzenzüchtung gehen. Forst- und Holz 65: 37-40.

PAKULL B, GROPPE K, MECUCCI F, GAUDET M, SABATTI M, FLADUNG M (2011): Genetic mapping of linkage group XIX and identification of sex-linked SSR markers in a *Populus tremula* L. x *P. tremuloides* Michx. cross. Can J Forest Res 41: 245-253.

PAKULL B, KERSTEN B, LUENEBURG J, FLADUNG M (2015): A simple PCR-based marker to determine sex in aspen. Plant Biology 17: 256–261.

PAKULL B, GROPPE K, MEYER M, MARKUSSEN T, FLADUNG M (2009): Genetic linkage mapping in aspen (*Populus tremula* L. and *P. tremuloides* Michx.). Tree Genetics & Genomes 5: 505-515.

SCHROEDER H, FLADUNG M (2010): SSR and SNP markers for the identification of clones, hybrids and species within the genus *Populus*. Silvae Genetica 59: 257-262.

SCHROEDER H, KERSTEN B, FLADUNG M (2017): Development of Multiplexed Marker Sets to Identify the Most Relevant Poplar Species for Breeding. Forests 8: 492.

TSAREV A, WÜHLISCH G VON, TSAREVA R (2016): Hybridization of poplars in the central Chernozem region of russia. Silvae Genetica 65: 1-10.

TSAREV A, TSAREVA R, TSAREV V, FLADUNG M, VON WÜHLISCH G (2018): Aspen hybridization: Parents' compatibility and seedlings' growth. Silvae Genetica 67: 12-19.

YIN TM, ZHANG XY, GUNTER LE, LI SX, WULLSCHLEGER SD, HUANG MR, TUSKAN GA (2009): Microsatellite primer resource for *Populus* developed from the mapped sequence scaffolds of the Nisqually-1 genome. New Phytologist 181: 498-503.

Aspen clonal sustainability assessment in natural populations by tree-ring based information

O. CHERNYSHENKO[1], D. RUMYANTSEV[2], ANNA SIROTOVA[3]

[1] Mytischi department of Bauman State Technical University (National Research University), 141005, Moscow Region, Mytischi-5, st. 1-Institutskaya, 1 MF MGTU, Forest engineering Faculty, Russia (tchernychenko@mgul.ac.ru)

[2] Mytischi department of Bauman State Technical University (National Research University), 141005, Moscow Region, Mytisci-5, st. 1-Institutskaya, 1, MF MGTU, Forest engineering Faculty, Russia (dendro15@list.ru)

[3] Mytischi department of Bauman State Technical University (National Research University), 141005, Moscow Region, Mytisci-5, st. 1-Institutskaya, 1, MF MGTU, Russia (annasirotova@gmail.com)

Abstract

Dendrochronological method allows to estimate the productivity of woody plants for the entire period of the tree life and select trees for breeding with exceptional forest properties. The aim of our study is to assess the stability and viability of aspen clones in the natural populations located on the Russian plain in Russia. The article presents the results of the survey of natural aspen stands in Molokcha botanical and entomological reserve and Central forest reserve in 2016. For both aspen stands there were identified years adversely affecting aspen tree growth. There were selected tree groups stable and unstable to wood core rot damaged in the *Populus tremula* stands. The influence of drought on tree growth was analyzed. Polymorphism of aspen populations in terms of resistance to extreme environmental factors was investigated. Studies of tree growth with the help of dendrochronological methods enabled us to identify factors limiting the success of their growth within and outside the natural range.

Key words: natural aspen stands, dendrochronological information, resistance

Introduction

The top forestry task is the creation of new highly productive forest plantations. It is necessary to have a high-quality planting material of wood plants to create fast growing forest plantations. Dendrochronological method allows to estimate the productivity of woody plants for the entire period of the tree life and select trees for breeding with the exceptional forest properties (MATVEEV and RUMYANTSEV 2013). This information allows us to analyze patterns of the tree growth, its stability and viability *in vivo* under different extreme influences. Tree ring data help to identify the woody plant clones with a high radial growth rate.

Aspen plantations with the specified technical wood properties are widely used for sustainable forest management in many countries. The aim of our study is to assess the stability and viability of aspen clones in natural populations reserves, located on the Russian Plain, Russia. The results of the survey of aspen stands in Molokcha botanical and entomological reserve and Central state forest reserve in 2016 are given below.

Material and methods

State Natural Reserve “Molokcha botanical and entomological reserve” with the territory of 325.1 is located in the Moscow region. During reconnaissance survey of the territory of the reserve, we selected the aspen stand with trees strongly and weakly damaged by the wood core rot. The permanent plot was

set in the stand, and for each tree we measured the circumference, height and geographic coordinates. The average diameter of the trees was 38 cm, the average height 22 m, average age 75 years. Sample plot had the following geobotanical characteristics. Stand composition: 7 Aspen 3 Spruce + Grey alder + Birch. Undergrowth composition: *Populus tremula*, *Picea abies*, *Acer platanoides*. Understory composition: *Alnus incana, Lonicera xylosteum*, *Euonymus verrucosus*, *Sorbus aucuparia*, *Corylus avellane*. Forest live cover composition: ferns, *Lamium galeobdolon*, *Pulmonaria obscura*, *Galium odoratum*, *Lathyrus sylvestris*.

The state central forest reserve was established in 1931. It covers an area of 21,348 ha and is located in the Tver region. The stand selected for the study had the following geobotanical characteristics. Stand composition: 7 Aspen 3 Spruce + Grey alder + Birch. Undergrowth composition: *Picea abies*, *Acer platanoides*, *Tilia cordata*. Understory composition: *Sorbus aucuparia*, *Corylus avellane*, *Rubus*. Forest live cover composition: ferns, *Oxalis acetosella*, *Vaccinium myrtillus*, *Lamium galeobdolon*, *Stellaria holostea*, mosses. The average height of the stand was 35 m, average diameter of accounted trees 55 cm, with an average age of 150 years.

In each of two selected stands cores from 20 accounted trees were sampled at a height of 1.3 m with the help of PRESSLER's borer along two perpendicular radii. We took 2 test cores from each accounted tree. The tree rings were measured by using Lintab with accuracy 0.01 mm and after that cross dated by using computer program TSAP Win. We conducted Graphical and statistical analysis of data using spreadsheet MicrosoftExcel and a software package STATISTICA 6.0.

Results and discussion

The influence of various factors on the growth of aspen trees in two populations allows to determine the time series analysis of radial growth. The width of annual tree rings depends on many factors: weather conditions of the growing season, age, phytocenotic interactions, edaphic and orographic factors and others (RUMYANTSEV 2010).

Molokcha botanical and entomological reserve

The individual tree ring chronologies for each model tree on plot were averaged and the average chronology was used to estimate the influence of various factors on aspen tree radial growth. The data showed that there are two tendencies in variability of radial growth. On the one hand, the width of tree rings gradually decreases with age. It relates to the fact that crown of tree moves up the height of the trunk of the growing tree, and the amount of auxins decreases in the cambium zone at the place of coring. On the other hand, weather conditions change from year to year, and this affects the fluctuations in the width of annual rings. The graph of the average chronology for investigated forest stand is presented in Figure 1.

The graph shows that the most unfavorable conditions for the growth of aspen were observed in 1955, 1965, 1974, 1982, 1993, 2000, and 2012. Abnormally narrow growth rings were formed during these years. In the formation of abnormally narrow rings there is some periodicity which is on average nearly 11 years that can be attributed to the 11 year cycle of solar activity (this opinion is shared by most researchers (SOLOMINA et al. 2017).

To study the effect of rot impact on the growth formation of aspen trees all the model trees were divided into two groups. The first group includes trees that were not affected by rot. Initially, they were selected from the grounds by the absence of the fruiting bodies of aspen polypore on the trunks, and these data were confirmed by the results of the analysis of the cores. The trees strongly damaged by wood rot were identified by the presence of more than 10 fruiting bodies of polypore on the trunks and were assigned to the second group. A larger width of annual rings was recorded in the group of trees without rot compared to the group of trees affected by rot. The tendency to fall and increase of the growth increment is

observed in the same years for two groups. The difference in width of annual rings, and consequently in growth rate, became apparent in recent years only, after about 1992 (Figure 2). The development of rot in the tree trunks did not reduce significantly the amount of growth in this stand.

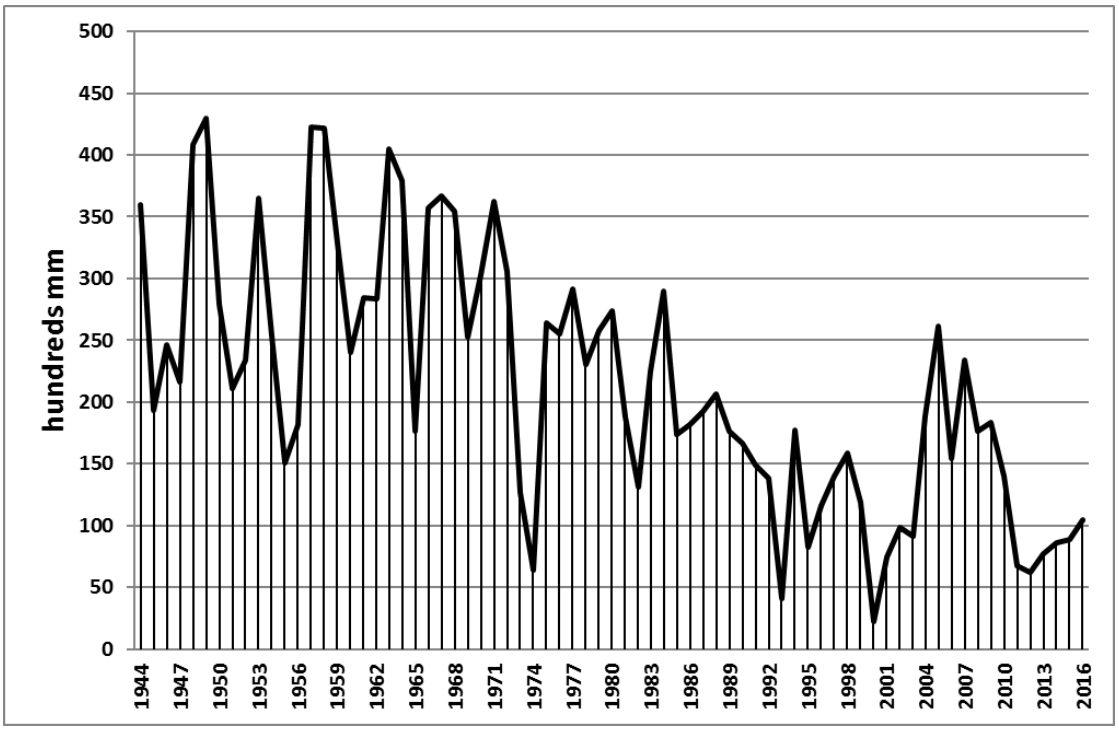

Figure 1: Dynamics of width of annual rings for years in Molokcha reserve.

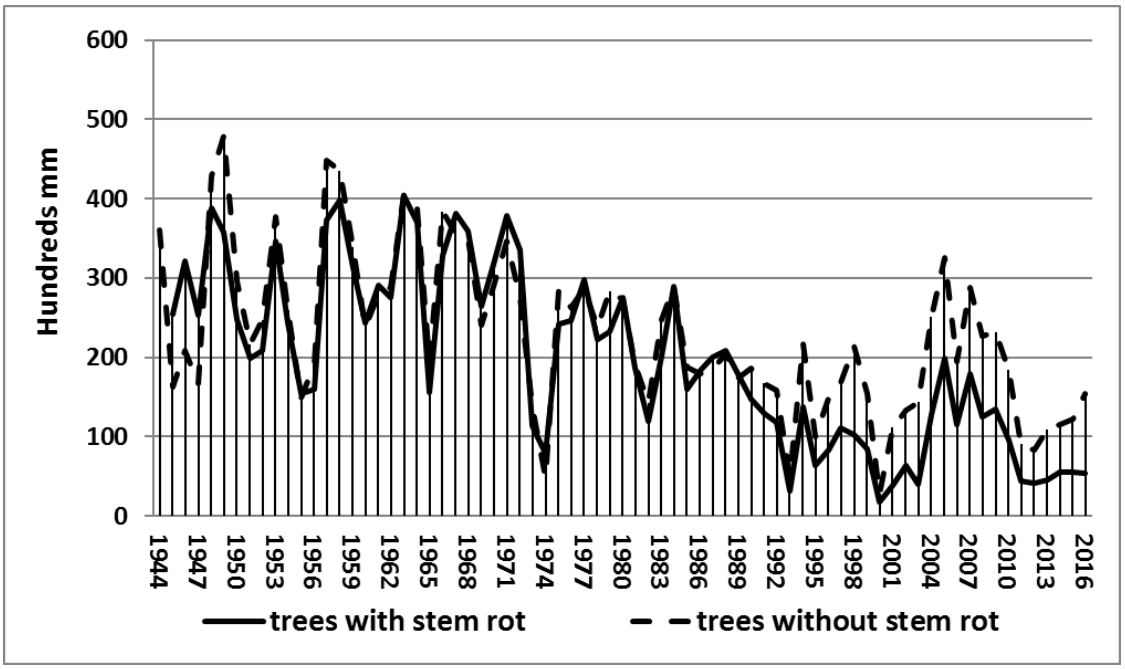

Figure 2: Dynamics of average radial growth in healthy and diseased trees in Molokcha reserve.

We conducted a dendroclimatic analysis for all trees to assess their drought tolerance and to identify individuals that are resistant to drought. The dendroclimatic analysis by the correlation method gave information about weather factors, which are significant for tree ring forming and limit the width of tree rings. For investigated length of time interval the coefficients more than 0.23 are significant (at the confidence level of 0.05). For trees with clear rot disease the significant values of correlation coefficients were discovered for June temperature of previous year (-0.41); for July temperature of previous year (-0.31); for June precipitation of previous year (0.28). For healthy trees the significant values of correlation coefficients were discovered for June temperature of previous year (-0.39); for July temperature of previous year (-0.29); for June precipitation of previous year (0.34). Therefore, the spectrum of significant values of correlation coefficients is equal for two investigated groups of trees. As follows from the results of calculation of correlation coefficients between climatic data and radial growth indices the drought at the beginning of growing season previous to ring forming year is the main factor, which leads to forming thin tree rings.

Central State Forest Reserve

On the basis of individual chronologies of tree ring width the average chronology for all model trees from the plot was calculated. The graph in Figure 3 demonstrates features which are characteristic for most of the tree-ring chronologies. First of all, there is the tendency for regular reduction of tree ring width with age which is clearly seen. This trend is quite well approximated by linear regression function, the graph of which is shown in Figure 3 as a dotted line. The difference of ring width values in this model is very significant: from 4 mm at the starting period of growth to 1.3 mm at the final periods of growth within investigated time interval.

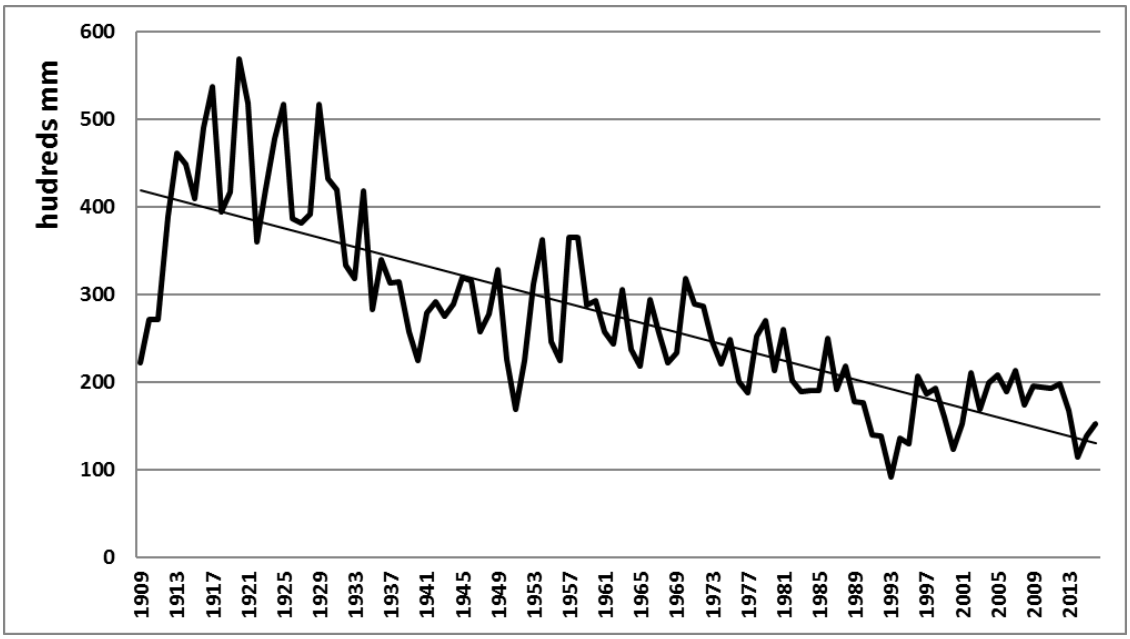

Figure 3: Dynamics of width of annual rings for years in Central State Forest Reserve.

The correlation dendroclimatic analysis was made for chronologies of this object. The results are that the significant values of correlation coefficients were established for April temperatures of the previous year (-0.43) and the April temperatures within the calendar year of ring forming (-0.32). The drought impact on the cambial activity was not found for this object. It can be accounted for the fact that the investigated

aspen stand is situated on the bank of a forest stream and even in the years with drought the aspen trees do not suffer from water shortage. This object is not suitable in the future for investigation of aspen population polymorphism by drought resistance.

The next aim of our investigation was the comparison of tree ring width values for the groups of healthy trees and the groups of ill trees (trees with a great stem rot). The trees which had no signs of wood rot on cores from the height of 1.3 m were grouped as healthy trees. The trees, which had the stem rot nearly or more than 50% of the investigated stem radius were grouped as ill trees. The results of tree-ring chronology comparison for two groups are presented in Figure 4.

The trees with a great stem rot have smaller values of tree ring width than the trees without stem rot and this tendency is more clearly expressed at the final stage of investigated time interval.

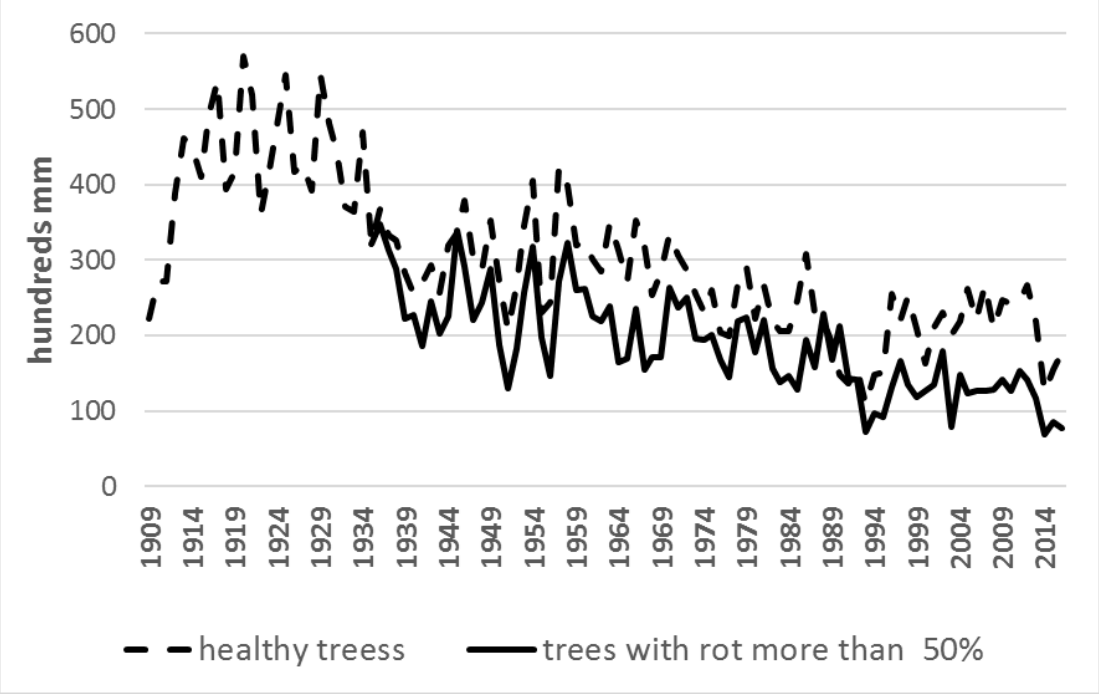

Figure 4: Dynamics of average radial growth in healthy and diseased trees in Central State Forest Reserve.

Conclusions

Fluctuations of the tree-ring width may be the indicator of gene complex, which determines the organism resistance to the extreme environment factors impact. Comparing groups of trees with different phenotype in equal growing conditions may help to identify the organisms with increased resistance. Dendrochronological information helps to evaluate the gene pool for aspen trees in natural populations and select valuable stands or trees with definite forestry features. The selected trees may be used for future breeding, the plantation organization and also for hybridization experiments. The investigation of tree growth by dendrochronological methods allows to detect objectively the factors limiting the cambial activity both within the natural area and outside it. One of the main conclusions is that YABLOKOV's hypothesis (YABLOCOV 1949, 1962) about the link between rot resistance of aspen trees and hereditary specified tempo of radial growth was proved by our investigations.

Acknowledgements

The research during 2015-2017 was a part of the MaRussiA project supported by the Federal Ministry of Food and Agriculture of Germany.

Literature

CHERNYSHENKO O, RUMYANTSEV D, SARAPKINA E (2016) Methods for diagnosis of aspen sustainability, International poplar commission: Poplars and Other Fast-Growing Trees - Renewable Resources for Future Green Economies, 25th Session Berlin, Germany, 13- 16 September 2016: P.80.

MATVEEV SM, RUMYANTSEV DE (2013) Dendrochronology, VSAFE, Voronezh, Russia: 139 p. (ISBN 978-5-7994-0535-9)

RUMYANTSEV DE (2010) History and methodology of forest dendrochronology. Moscow State University of Forest, Russia: 109 p. (ISBN 978-5-8135-0503-4)

SOLOMINA O et al. (2017) Droughts of the East European Plant according to hydrometeorological and tree-ring data, Spb.: Nestor-Historia, Moscow, Russia: 360 p. (ISBN 978-5-4469-1126-4)

YABLOCOV AS (1949) Growing and breeding of health aspen, Goslesbumizdat, Moscow, Leningrad, USSR: 276 p.

YABLOCOV AS (1962) Forest tree species selection, Moscow, Selkhozidat, USSR: 463p.

Testing of mutagenic substances in aspen *in vitro* culture

DMITRII A. SHABUNIN

Research division of Selection, Reproduction and Chemical Forest Care, Saint-Petersburg Forestry Research Institute, Institutsky 21, 194021 Saint-Petersburg, Russia (ds1512@mail.ru)

Abstract

For experiments on mutagenic effects, the culture of aspen (*Populus tremula* L.) microshoots was used. As a mutagen, 1,4-bis-diazo-acetyl-butane was used. Mutations were detected by fingerprinting using the ISSR PCR primers. The following plant indicators were also investigated: growth, libriform fiber length, and lignin content in the wood. A conclusion is drawn on the possibility of obtaining aspen mutants under *in vitro* conditions.

Key words: Mutant, *in vitro*, Aspen, ISSR primers

Introduction

In the last century, chemicals that cause a large number of mutations were discovered. These substances were called supermutagens. In the 1960s and a little later, works with the substances were carried out very actively, but forest plants were not involved in these works. The reason was that trees have a long life cycle.

In vitro culture allows quickly and in large quantities to obtain offspring of trees. *In vitro* conditions make plant tissues more susceptible to mutagenic effects. Thus, we decided to use mutagens in aspen culture under *in vitro* conditions.

Material and methods

For experiments on mutagenic effects, the culture of aspen (*Populus tremula* L.) microshoots originating from the Kostroma region (Russia) was used. The design of the experiment was as follows. Pieces of plants were placed in a flask on the surface of the nutrient medium. Then, a mutagen solution was added. The flask was aseptically closed and cultivating for several months under 24-hour illumination in the laboratory. The LP medium (QUOIRIN and LEPOIVRE 1977) was used as a base nutrient medium. The medium also contained phytohormones: 0,2 mg/l α-naphthylacetic acid (NAA) and 0,02 mg/l 6-benzylaminopurine (BAP). This caused callus growth and formation of new shoots. Cultivation was carried out until the nutrient medium was completely depleted, and all explants were died.

As a mutagen, 1,4-bis-diazo-acetyl-butane (DAB) was used. The water solution of mutagen was preparing immediately before use. The concentration was 1 mg/l. The use of larger concentrations is also possible, but this causes vitrification of shoots.

Emerged shoots were transplanted to a phytohormone-free nutrient medium for rooting. Then rooted plants were removed from the flasks and their adaptation was carried out in a micro greenhouse. Then, the plants were planted in a greenhouse and grew during one growing season there.

To assess the mutagenic effect, several traits have been measured: genetic, plant growth, fiber lengths and lignin content studies.

Genetic study

The mutation can be detected by comparing with each other the genomes of cloned plants which were subjected to mutagen treatment. The fastest and most effective method of genome studying in order to identify differences is fingerprinting.

DNA was extracted by CTAB method. PCR was performed as described in ZHIGUNOV et al. (2017). We compared the DNA electrophoretic profiles generated with different ISSR primers: HB12, M2, M10, M12C, M13T (ZHIGUNOV et al. 2017).

Mutations were detected if deviations in the distribution of the bands were observed in the electrophoretic profiles (Table 1).

Table 1: The results of detection of changes in the electrophoretic profile of PCR products with different primers

Plant #	Primer				
	HB12	M10	M2	M12C	M13T
M.100	unchanged	unchanged	unchanged	unchanged	unchanged
M.101	unchanged	**mutation**	unchanged	unchanged	**mutation**
M.104	unchanged	**mutation**	unchanged	unchanged	unchanged
M.118	unchanged	**mutation**	unchanged	unchanged	unchanged
M.125	–	–	–	unchanged	unchanged
M.138	unchanged	unchanged	unchanged	unchanged	unchanged
M.142	unchanged	unchanged	unchanged	unchanged	unchanged
M.015	unchanged	unchanged	unchanged	unchanged	unchanged
M.161	unchanged	**mutation**	unchanged	unchanged	unchanged
M.180	unchanged	**mutation**	unchanged	unchanged	**mutation**
M.183	unchanged	unchanged	unchanged	unchanged	unchanged
M.184	unchanged	unchanged	unchanged	unchanged	unchanged
M.186	unchanged	unchanged	unchanged	unchanged	unchanged
M.200	unchanged	unchanged	unchanged	unchanged	unchanged
M.201	unchanged	**mutation**	unchanged	unchanged	unchanged
M.214	unchanged	unchanged	unchanged	unchanged	unchanged
M.218	unchanged	unchanged	unchanged	unchanged	unchanged
M.226	unchanged	unchanged	unchanged	unchanged	unchanged
M.228	unchanged	unchanged	unchanged	unchanged	unchanged
M.235	unchanged	unchanged	unchanged	unchanged	unchanged
M.241	unchanged	unchanged	unchanged	unchanged	unchanged
M.245	unchanged	**mutation**	unchanged	unchanged	unchanged
M.251	unchanged	unchanged	unchanged	unchanged	unchanged
M.258	unchanged	unchanged	unchanged	unchanged	unchanged
M.265	unchanged	**mutation**	unchanged	unchanged	unchanged
M.268	unchanged	unchanged	unchanged	unchanged	unchanged
M.027	unchanged	unchanged	unchanged	unchanged	unchanged
M.271	**mutation**	unchanged	unchanged	unchanged	unchanged
M.271	unchanged	unchanged	unchanged	unchanged	unchanged
M.272	unchanged	**mutation**	unchanged	unchanged	**mutation**
M.280	unchanged	unchanged	unchanged	unchanged	unchanged
M.290	unchanged	unchanged	unchanged	unchanged	unchanged

Table 1 (continued)

M.297	unchanged	**mutation**	unchanged	unchanged	**mutation**
M.298	unchanged	unchanged	unchanged	unchanged	unchanged
M.299	unchanged	unchanged	unchanged	unchanged	unchanged
M.003	unchanged	unchanged	unchanged	unchanged	unchanged
M.300	unchanged	unchanged	unchanged	unchanged	unchanged
M.301	unchanged	unchanged	unchanged	unchanged	unchanged
M.301	unchanged	unchanged	unchanged	unchanged	unchanged
M.305	unchanged	unchanged	unchanged	unchanged	unchanged
M.316	unchanged	unchanged	unchanged	unchanged	unchanged
M.335	unchanged	unchanged	unchanged	unchanged	unchanged
M.336	unchanged	–	unchanged	unchanged	–
M.339	unchanged	unchanged	unchanged	unchanged	unchanged
M.035	unchanged	unchanged	unchanged	unchanged	unchanged
M.368	unchanged	unchanged	unchanged	unchanged	unchanged
M.369	unchanged	unchanged	unchanged	unchanged	unchanged
M.370	unchanged	unchanged	unchanged	unchanged	unchanged
M.371	unchanged	unchanged	unchanged	unchanged	unchanged
M.373	unchanged	unchanged	unchanged	unchanged	unchanged
M.374	unchanged	unchanged	unchanged	unchanged	unchanged
M.376	unchanged	unchanged	unchanged	unchanged	unchanged
M.038	unchanged	unchanged	unchanged	unchanged	unchanged
M.040	unchanged	**mutation**	unchanged	unchanged	unchanged
M.047	unchanged	unchanged	unchanged	unchanged	unchanged
M.055	unchanged	unchanged	unchanged	unchanged	unchanged
M.060	**mutation**	unchanged	unchanged	unchanged	unchanged
M.061	unchanged	unchanged	unchanged	unchanged	unchanged
M.063/r.56	unchanged	unchanged	unchanged	unchanged	unchanged
M.064/r.18	unchanged	unchanged	unchanged	unchanged	unchanged
M.065/r.22	unchanged	unchanged	unchanged	unchanged	unchanged
M.077	unchanged	unchanged	unchanged	unchanged	unchanged
M.077	unchanged	unchanged	unchanged	unchanged	unchanged
M.078	unchanged	unchanged	unchanged	unchanged	unchanged
M.008	unchanged	unchanged	unchanged	unchanged	unchanged
M.084	unchanged	unchanged	unchanged	unchanged	unchanged
M.085	unchanged	unchanged	unchanged	unchanged	unchanged
M.086	unchanged	unchanged	unchanged	unchanged	unchanged
M.092	unchanged	unchanged	unchanged	unchanged	unchanged
M.290	unchanged	unchanged	unchanged	unchanged	unchanged
untreated	unchanged	unchanged	unchanged	unchanged	unchanged

Notes:
- unchanged – normal distribution of PCR products in the electrophoretic profile;
- **mutation** – there are changes in the distribution of PCR products in the electrophoretic profile
- «–» – no PCR products
- untreated – plant obtained from material which was not treated with mutagen

13 mutant individuals were identified (Table 1), which is 18.8 % of the total number of individuals examined. There are certain patterns in the occurrence of mutations. It can be seen that there are primers

that often detect abnormalities (for example primer M10). There are primers that do not detect abnormalities at all (for example primer M2). Also, it is possible to trace the patterns along the horizontal. All individuals which have the abnormalities detected with primer M13T have the abnormalities detected also with primer M10.

Considering that we examined only a very small part of the genome, we can assume that in fact the number of mutations is much greater.

Clearly, most of the mutations are not in the genes. Cells with altered vital genes usually die quickly. But, genes that regulate quantitative indicators, such as the growth, the size of parts of the plant, can have even significant mutations without causing the death of the plant.

Study of plant growth

Aspen individuals obtained from a material subjected to the mutagen impact were grown in a greenhouse during one growing season. At the end of the growing season, the height of the plants was measured. The histogram shows the distribution of plants in height (Figure 1). Here we can see both retarded plants and plants with very high growth rates. The best plant in height is twice the average and more than 13 times higher than the smallest plant. The smallest plant M.47 is 9.5 cm in height. It shows a tendency to bushiness. The largest plant M.90 is 130.1 cm in height. It is not very typical picture for cloned plants. Clonal plantations have a growth feature. There may be lagging trees, and there are no outstanding trees. The upper limit is very smooth (level). The observed distribution (Figure 1) is more characteristic of the seed progeny.

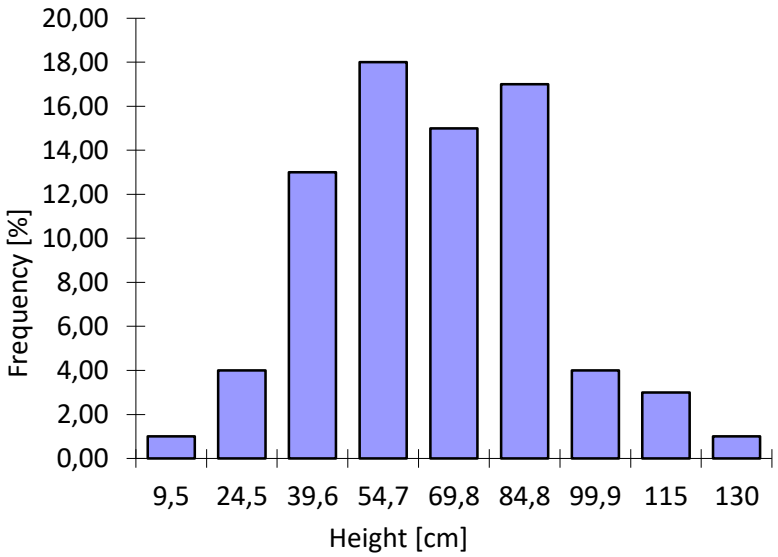

Figure 1: Distribution of aspen plants in height

In the forest the natural aspen clones differ well in autumn according to the color of the leaves. In this connection, it is interesting to note that the largest plant M.90 differs phenologically from the other plants by an earlier entry into the phase of autumnal leaves coloring. It became red, while all other plants remained green. We can assume that this is another phenotypic sign of genetic differences.

Study of libriform fiber length

We also measured the length of libriform cells of wood. But the number of measurements was small because we had to use the lateral branches only. The obtained data (Table 2) indicate different mean lengths of fibers. This does not contradict the assumption of a mutagenic effect.

Table 2: Length of the libriform fibers in plants derived from biological material subjected to mutagenic impact

Plant #	Length of fibers [μm]
M.009	346 ± 6
M.032	350 ± 5
M.036	470 ± 10
M.062	379 ± 7
M.065	372 ± 7
M.089	411 ± 6
M.093	367 ± 10
M.108	259 ± 12
untreated (1)	301 ± 6
untreated (2)	347 ± 14
untreated (3)	290 ± 11

Study of lignin content

The last indicator we examined is the lignin content in the wood. The lignin was determined by the phenolic method described by FUCHS (1926). The lignin content in the samples turned out to be different (Figure 2). The obtained data fit well into the curve, perhaps with the exception of one sample. A wide range of values supports the assumption of a mutagenic effect.

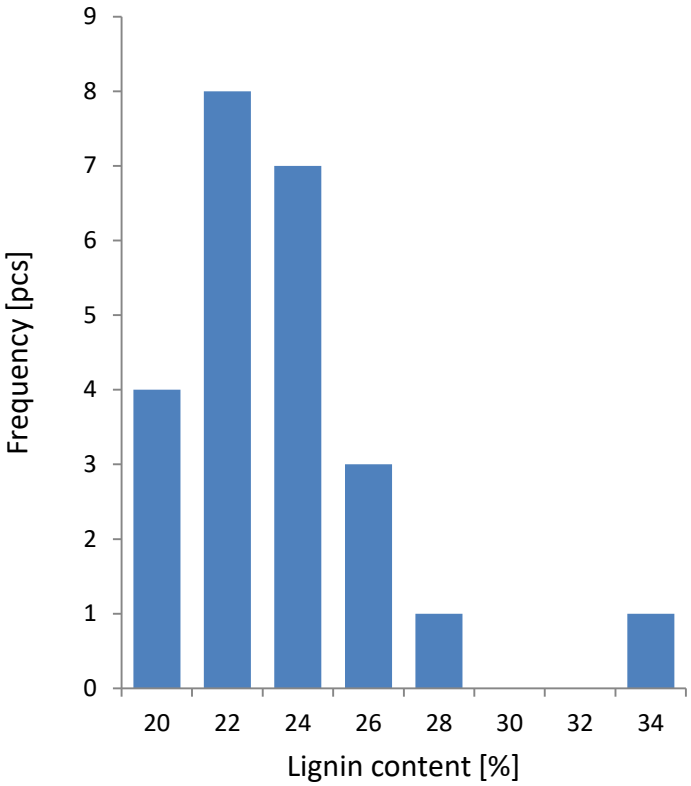

Figure 2: Lignin content in the wood of aspen plants

The plants with low lignin content (M.3, M.9, M.36, M.64) and the plants with increased lignin content (M.81, M.86, M.90, M.91, M.99) are of interest. Clones with a reduced lignin content may be of interest for the pulp and paper industry. Clones with a high lignin content can have high strength wood.

Conclusions

The obtained data indicate the possibility of obtaining mutant aspen plants under in vitro conditions. For the manifestation of mutations, it is necessary to develop methods for obtaining haploids.

Acknowledgements

The research within the MaRussiA project was supported by the Ministry of Food and Agriculture of Germany.

References

FUCHS W (1926) Die Chemie des Lignins. Verlag von Julius Springer, Berlin: 328 pp.

QUOIRIN M, LEPOIVRE P (1977) Etude de milieux adaptes aux cultures in vitro de *Prunus*. Acta Horticulturae 78: 437–442.

ZHIGUNOV AV, SHABUNIN DA, BUTENKO OY, LEBEDEVA MV (2017) Identification of elite aspen clones in the experimental plantation in the North-West of Russia // Proceedings of the Saint-Petersburg Forestry Research Institute №4: 39–47.

Poplar breeding in the temperate climate of Russia

ANATOLY P. TSAREV, R.P. TSAREVA, V.A. TSAREV

All-Russian Research Institute of Forest Genetics, Breeding and Biotechnology, Lomonosov str., 105, 394087, Voronezh, Russia (anatolytsa@gmail.com)

Abstract

Results are presented of breeding different poplar species in the Central Chernozem Region of Russia. White and black poplars as well as aspen selected in natural forests, as well as introduced ones were hybridized. The different progenies were field tested in different soil-climatic zones (forest steppe, steppe and semidesert). The standing wood volume of the best natural stands of white poplars reached 709–1,513 m^3/ha at age 64–95 years, the black poplar had 414–623 m^3/ha at age 70 years. In aspen stands individuals free of heart rot symptoms were selected. Field testing on chernozem soil showed that best cultivars were *'Regenerata-79' and'Regenerata-78'*whose wood volume at age of 25 years was 976-1,151 m^3/ha. Some tested hybrids were approved and registered by the Russian Federation Test Variety Commission as varieties: '*Veduga', 'Bolide',* '*Steppe Lada' and 'Breeze'*.

Key words: breeding, poplars, aspen, selection, field testing, hybridization, wood stock, heart rot

Introduction

Russia is a country of abundant forest resources. According to the last official forest inventory data [State Forest Register 2014] it has 795 257.2 thousand ha of forest-covered land, including 770 627.4 thousand ha forest-covered land belonging to the State-Forest Fund with 79 888.85 million m^3 growing stock. However, not all of them are available for use. This can be explained by several reasons:

1) Uneven distribution of forests in the European and in the Asian parts of the country. Thus, 73 % of the population lives in the European part, but it has only 20 % of the forests. And 27 % of the population lives in the Asian part, but it has 80 % of the forests.
2) Only 55 % of the forested area is available for industrial use. The rest of the lands are the waterlogged, with low quality sites. There are only few land and waterways for logging operations and transportation of the timber materials. Also, they are situated far from the timber industry enterprises and other consumers.
3) In the last years the volume of timber-harvesting in Russia has decreased considerably by 2-2.5 times compared to the former USSR. The reason being that the majority of the accessible and economically profitable forest areas have already been used heavily. High expenditures are required for long-term reforestation and reclamation operations.
4) The cost of timber has risen considerably because of its distant transports.

Therefore, wood resources are in short supply in some regions of the country. This deficit can be reduced by afforestation, particularly by cultivation of fast growing tree species, e.g. poplars, aspens, willows etc. But it is economically unreasonable to be using low-productive and unproven planting-material in new plantations.

To achieve more successful results high quality planting-material obtained by forest tree breeders should be employed. In Russia many forest researchers were engaged in poplars breeding, e.g. KUNITSKY 1988, BOGDANOV 1934-1965, ALBENSKY, 1935-1965, YABLOKOV 1935-1965, VERESIN 1953-1992, OZOLIN 1955-1990, IVANNIKOV 1956-1992, STAROVA 1955-2005, BESSCHETNOV 1957-1990, KONOVALOV 1959-1980 and others.

In this manuscript, the poplars breeding results in partially-wooded steppe, steppe and half-desert areas of European part of Russia are presented.

Material and methods

The studies of intensive poplar trees selection were carried out in the forests of the Voronezh region. The latitude is 51-52º of North and 39-42º of East.

To establish gene pool collections in the Central Research Institute of Forest Genetics and Breeding (now All-Russian Research Institute of Forest Genetics, Breeding and Biotechnology) about 300 clones and varieties of poplar were introduced and selected in Voronezh region. Series of the field experimental sites in various soil-climatic zones of the country were laid out for testing and reproduction.

Subsequently, some hybridization cycles were carried out in the Central Chernozem Region and lines of perspective hybrids and varieties of poplars were obtained. These poplar breeding activities are summarized below.

Results and discussion

Selection

Selection of the best forms in natural stands was carry out in some poplar stands of Voronezh region. There are selected in:

a) *Populus tremula* L.
- Phenological forms (early - and late leafing),
- Morphological (color and fracture of the bark, lift height rough peel),
- Forms associated with sex (male and female),
- The best forms (plus stands and trees).

b) *Populus alba* L.
- The best forms (plus stands and trees).

c) *Populus nigra* L.
- The best forms (plus stands and trees).
- Excrescence occurrence (“cap”) forms.

Main directions of selection:
- Growth performance (height, diameter, stock, biomass, average and current increments).
- Resistance to heart rot caused by fungus *Phellinus tremulae* (*Fomes igniarius*).
- The quality of stems and wood (straightness of the trunks, the technical properties of wood, the content and quality of pulp).

The best white poplar stands at the age of 64-95 years and black poplar stands at the age of 70 years were selected in central forest-steppe. Average height of selected white poplar stands was 28-42 m, average diameter – 47-82 cm, wood stock – 709-1 513 m^3/ha and average increment – 11.1-15.9 m^3/ha per year. Such indices for black poplar stands varied from 28 to 30 m, 51-52 cm, 414-623 m^3/ha, 5.2-8.9 m^3/ha per year. The lower indices of black poplar stands are related to excrescence forms. Average growing stocks of natural white and black poplars stands in the same age and conditions varied from 195 to 295 m^3/ha. It can be shown that best (plus) stands excel the ordinary natural stands in the same geographical

environments in productivity by 2-3 times. They can reach larger diameters and are of great practical interest (VERESIN et al. 1974, TSAREV 2013a).

By aspen form diversity study in central forest-steppe A.P. TSAREV selected some aspen plus trees and four aspen plus stands which were resistant to heart rot. In the age of 43-46 and forest location of C_2-E_3 the part of trees with fruiting bodies of heart rot in these stands varied from 0 to 6 %. While at the same time in the control ordinary natural stands this part was 21.3-91.1 % (TSAREV 2013b).

Field testing

During many years of research, an experimental base of field testing poplars was created by the authors (Table 1).

These tests in Semiluksky District of Voronezh region were used to select some best cultivars. Test sites were established at forest soil type D_2 (fresh chernozem soil). Each tree was planted within 5 × 4 m space. Some selected euramerican hybrids (old varieties: *'Brabantica-175', 'Gelrica', , 'Robusta', 'Sacrau-59', 'Serotina', 'Vernirubens'*, a. o) showed following growth parameters: trees at age 25 years had height 28.7-32.8 m, diameter 32.3-46.1 cm, trunk volume 0.94-2.09 m^3, wood stock 414-824 m^3/ha. The *'Regenerata-79' and'Regenerata-78'* poplars were best of euramerican hybrids which in that environment locality and age were 33.6-34.4 m high, diameter 48.0-49.1 cm, trunk volume 2.22-2.50 m^3, wood stock 976-1,151 m^3/ha (TSAREV 2013a).

Table 1: Experimental field poplar test sites

Type of site	Number of sites	Area [*ha*]
Regeneration plantations	15	8.8
Collections of clones and cultivars	4	8.4
Collections of newly created hybrids	5	5.5
Mini-rotation plantations	4	1.7
Test plantations in different Russian regions	22	70. 9
Total	50	95.3

Soil-climatic zones	Number of objects	Area [*ha*]
Forest - steppe	36	63.1
Steppe	8	12.6
Semi desert (North Caspian region)	6	19.6
Total	50	95.3

Hybridization

In USSR & RF there were many programs of poplar hybridization. The largest programs of hybridization in Russia were:

1) A.M. BEREZIN in Ufa (more than 80 different crossing variants, received more than 80 thousand hybrid seedlings, of which 18 best trees were selected).
2) P.L. BOGDANOV's crossings in Leningrad (160 crossings, 75 of which were successful, 2,500 hybrid seedlings were grown, 32 trees were selected, 4 of which were recommended for cultivation and use).

3) Hybridization in the Central Chernozem Region, where 736 crossing variants were generated, 54,150 hybrid seedlings were obtained including 10,000 by M.M. VERESIN, 1,650 – by A.P. TSAREV, 2,500 – by V.P. PETRUKHNOV and 40,000 – by R.P. TSAREVA. Overall, more than 240 of the best trees were selected. Some of those hybrids had registered as variety.

Two of best hybrids were received by Prof. M.M. VERESIN: *'Voronezh Giant'* ***(Es-38),*** which was obtained from crossing *P. deltoides × P. balsamifera*. The trees of its clone at age 39 years in the Semiluksky District had a height of 33 m, diameter 56.7 cm, stem volume 3.2 m^3.

Other hybrid poplar *'Veresin-1'* was obtained by M.M. VERESIN from crossing *P. alba × P. tremula*. The age of the trees is 63 years. Some trees in collection 1 (Voronezh) had the height of 40 m, diameter of 69.7 cm, stem volume of 5.95 m^3 (TSAREV et al. 2017).

The best hybrids of A. P. Tsarev and R.P. TSAREVA had fast growth and hardiness. Some of them were registered of the Russian Federation Test Variety Commission as varieties (Tsarev e. a., 2017). Among them are the following:

1. Variety '*Bolide*' obtained by A.P. TSAREV from crossing *P. alba × P. bolleana*. It is a winter-hardy hybrid with pyramidal crown. The height of mother tree of clone was 26 m, diameter 32.2 cm, stem volume 0.83 m^3 at the age of 38 years. The variety had received state registration (patent and certificate).
2. Variety '*Veduga*' obtained by A.P. TSAREV also from crossing *P. alba × P. bolleana.* It is a winter-hardy hybrid with half pyramidal crown. The height of mother tree of clone was 24.7 m, diameter 42.4 cm, stem volume 1.36 m^3 at the age of 38 years. The variety had received state registration (patent and certificate).
3. Variety '*Steppe Lada*' obtained by A.P. TSAREV from crossing *P. deltoides × 'Pyramidalno-osokorevy Kamyshinsky'*. It is a winter-hardy hybrid with spread crown. The height of mother tree of clone was 25 m, diameter 47.8 cm, stem volume 1.75 m^3 at the age of 38 years. The variety had received state registration (patent and certificate).
4. Variety '*Breeze*' obtained by R.P. TSAREVA as a half-sib from *P.× 'Pioneer'* selected by academician A.S. YABLOKOV. Variety '*Breeze*' is a winter-hardy male hybrid with spread crown. The height of mother tree of clone was 26 m, diameter 43 cm, stem volume 1.47 m^3 at the age of 32 years. The variety had received state registration (patent and certificate).
5. Poplar '*Surprise*' received by R.P. TSAREVA as a half-sib of '*I-455*' poplar. The age of mother tree of clone is 32 years, height 21.5 m, diameter 43.5 cm, and stem volume 1.25 m^3. It is frost resistant in Voronezh region. The clone is male (state registration is in implementation).

The next stage of hybridization was factorial crossbreeding initiated in the 2015-2016 years by support of MaRussiA project (Table 2).

Table 2: Scheme of aspen factorial crossings

♀	♂ 07-02	08-02	32-05	45-03	48-02	Free pollination
02-01	×	×	×	×	×	×
10-03	×	×	×	×	×	×
18-02	×	×	×	×	×	×
23-05	×	×	×	×	×	×
45-01	×	×	×	×	×	×

In these experiments were received more than 1.5 thousand aspen hybrids. They will be planted on the three experimental poplar field testing sites. One of these field tests will be established at an experimental site with the chernozem soil. Second test will be planted on the sandy-loam soil. Third field test is planning also on the sandy-loam soil but in different time of planting. Design of these hybrid collections of aspens and poplars is full randomized blocks (COCHRAN and COX 1957). The time of plant of aspen hybrids seedlings in first two experiments was autumn, the third experiment is planned in spring. The stem cuttings of poplars will be planted on these sites in spring. So, the planting will be done in different soil and in different time. It will allow investigating the ecological stability and adaptability of new aspen hybrids and poplar cultivars (WRICKE 1965, EBERHART and RUSSEL 1966, TSAREV 1985).

Conclusions

- The results show that in natural stands of the temperate zone of the European part of Russia valuable trees and stands have remained. They can be used for conservation and increasing the valuable gene pool.
- Besides naturally growing perspective poplars in the region of exploration several hybridization cycles were carried out which can result in some groups of economically valuable poplar hybrids.
- Testing of poplar clones and hybrids introduced and collected in Russia allow recommending some of them in the various types of stands.
- For more precise assessment of ecological stability and adaptation of perspective poplar genotypes it is necessary to test them in various local environments.
- Various sorts and clones are showing during their ontogenesis not only positive properties but also their defects. Therefore, it is necessary to regularly evaluate the breeding stock.
- A new hybridization cycle was undertaken in Central Chernozem Region of Russia in the frame of the German-Russian project “MaRussiA”. During its realization a complex of new aspen hybrids was created and is now being tested in field trials.

Acknowledgements

The authors thank the administration of the All-Russian Institute of Forest Genetics, Breeding and Biotechnology for facilitating the work of the hybridization from cutting branches to growing seedlings and the Federal Ministry of Food and Agriculture of Germany for the financial support of the research conducted in the framework of the “MaRussiA” project (grant 68706, BLE). Also, we thank Dr. GEORG VON WUEHLISH for supporting our work and help with the preparing of the manuscript.

References

COCHRAN WG, COX GM (1957) Experimental designs, second edition. John Wiley& Sons, Inc. London, Sydney. Library of Congress Catalog Card Number: 57-5908. Printed in the United States of America: 617 pp

EBERHART SA, RUSSEL W.A. (1966) Stability parameters for comparing varieties. *Crop science* 6 (1): 36-40.

STATE FOREST REGISTER-2013 (2014) Statistical reference book. Moscow: Russia Forest Information Organisation: 690 p. (In Russian).

TSAREV AP (1985) Cultivarology of poplar. Publishing house of Voronezh University. Voronezh, Russia: 152 pp. (In Russian).

TSAREV AP (2013a) Programs of Forest Tree Breeding (In Russia and abroad). Moscow State Forest University Edition: 552 p. (ISBN 978-5-8135-0592-8) (In Russian).

TSAREV AP (2013b) Growth and breeding of aspen in Russia. *Silvae Genetica* 62 (4-5): 153-160.

TSAREV AP, POGIBA SP, LAUR NV (2014) Breeding of Forest and Decorative Tree Plants. Moscow: Moscow State Forest University Edition: 552 p. (ISBN 978-5-8135-0605-5) (In Russian).

TSAREV AP, WUEHLISH G VON, TSAREVA RP (2017) Hybridization of Poplars in the Central Chernozem Region of Russia. Published Online: 2017-06-28 | doi: https://doi.org/10.1515/sg-2016-0011. Edited by the Thünen Institute of Forest Genetics, Germany. De Gruyter Open:. 10 p.

VERESIN MM, TSAREV AP, SIVOLAPOV AI (1974) High-quality stands of poplars and treelike willows in Hopersky State Reserve. In: Environment Conservation Society, Council of Voronezh region (eds) *Natural Resources of the Voronezh Area and Their Protection*. Publishing house of Voronezh University, Voronezh, Russia: 65-67. (In Russian).

WRICKE G (1965) Die Erfassung der Wechselwirkung zwischen Genotyp und Umwelt bei quantitativen Eigenschaften. *Zeitschrift für Pflanzenzüchtung* 53 (3): 266-300, (4): 301-343.

Development of research resources for marker-assisted selection of aspen (*Populus tremula* L.) in Russia

A.V. ZHIGUNOV[1], P.S. ULIANICH[1,2], M.V. LEBEDEVA[1], E.K. POTOKINA[1,2]

[1] Saint Petersburg State Forest Technical University, Institutskiy per. 5, 194021 St. Petersburg, Russia (a.zhigunov@bk.ru)

[2] Vavilov Institute of Plant Genetic Resources (VIR), Bolshaya Morskaya 42-44, 190000 St. Petersburg, Russia (e.potokina@vir.nw.ru)

Abstract

An F1 mapping population derived from an intraspecific cross of two *P. tremula* genotypes was developed to investigate the genetic control of valuable variation of traits in aspen under different environmental conditions. A high-density genetic map of *P. tremula* was established using the RADseq approach. The developed research resources are expected to provide a basis for marker-assisted selection of aspen in Russia.

Key words: Aspen, full siblings, linkage map, ecological testing.

Introduction

Populus L. species are perennial woody plants, reaching the generative phase at 6-10 years of age. The time period required for the development of F2 hybrid populations is often extended for several decades, which seriously complicates the breeding process. There are also some difficulties in assessing young woody hybrid plants for economically important traits. Nevertheless, marker-assisted selection (MAS) could significantly improve the breeding process in poplar, predicting the beneficial potential of some seedlings based on the analysis of their genotypes. For the MAS application in forest genetics, knowledge of the key genes underlying economically valuable traits are required. To identify the genes, a tool is essential—a genetic map, which**,** in the case of wood cross-pollinated species, such as poplar and aspen, can be established on the basis of F1 population genotyping.

The restriction-site associated DNA sequencing (RADseq) method makes it possible to provide thousands of sequenced markers across many individuals for any organism at reasonable costs (DAVEY et al. 2010). Thus, RADseq could be successfully employed for genotyping purposes of hybrid populations of *Populus* species, which would allow further establishing a linkage map. Recently, the RADseq approach was employed to identify 2545 SNPs for linkage mapping of F1 progenies derived from an interspecific cross of North American *P. deltoides* and *P. simonii*, a native tree species widely distributed in northern China (TONG et al. 2016).

Once the linkage map has been constructed, it becomes possible to proceed to environmental testing and phenotyping of the F1 hybrid populations to map QTLs underlying the key morphological and physiological traits. QTLs affecting rust resistance (NEWCOMBE et al. 1996, JORGE et al. 2005), growth rates (BRADSHAW and STETTLER 1995, WULLSCHLEGER et al. 2005), phenology traits (FREWEN et al. 2000) were mapped for various *Populus* species. In Russia, such QTL mapping studies were initiated only recently in the frame of the MaRussiA project.

The aims of the present study are 1) to perform an intraspecific cross between parental genotypes of *P. tremula* (European aspen) displaying different phenotypic traits performance, 2) to obtain F1 hybrid seeds and grow the progeny seedlings, 3) to establish experimental hybrid aspen plantations in different geographic regions of Russia using the obtained full-sibling progenies, 4) to construct a linkage map of the

F1 aspen population, 5) to initiate the QTL mapping studies in aspen in Russia using the developed research resources.

Material and methods

The information of parental aspen genotypes and procedure of crossing were described previously (ZHIGUNOV et al. 2017b). Briefly, the male and female parental trees had comparable ages (30 years old) and grew in the same location in the St. Petersburg area. Inhabiting the same environment, both parents had similar dendrometry records, except for phenological traits and winter hardiness. Ten- and six-day delays were recorded for the female tree compared to the male tree for generative bud flush and vegetative bud flush, respectively. As a consequence, serious frost damage of twigs was registered for the male parent, whereas no frost damage was recorded for the female tree. Controlled crossing was performed in April 2016 in a greenhouse of St. Petersburg State Forest Technical University (SPSFTU).

For genotyping purposes DNA was extracted from dried leaves collected from parental trees and each of the one-year-old progeny seedlings. RADseq libraries were constructed for the two parental genotypes and their 122 progenies. DNA fragments were sequenced as 150 base pair reads on an Illumina HiSeq2500 at the University of Southern California Genome and Cytometry Core. A RADseq linkage map was constructed using the pseudo-testcross mapping strategy (GRATTAPAGLIA and SEDEROFF 1994), where a mapping population is developed by hybridizing two unrelated highly heterozygous parents to produce a set of F1 progeny (SUN et al. 2017).

Results and discussion

As a result of the controlled crossing experiment**,** 1100 containerized F1 hybrid aspen seedlings were obtained (Figure 1). Next, the F1 population was divided into three parts. The first group of seedlings was planted on the experimental field of SPSFTU in the St. Petersburg area (Druzhnaya Gorka). This region is characterized by an Atlantic-continental climate with a relatively mild winter and a mildly warm summer; the amount of precipitation per year is 600-700 mm. The second group of seedlings was planted in a field nursery in Surgut (West Siberia) for frost resistance testing. This environment features a continental subarctic climate with long, cold winters (Figure 2). The third part of seedlings was used to create an experimental plantation in arid conditions of the forest-steppe (Voronezh Region) for drought resistance tests.

Figure 1: The F1 hybrid aspen seedlings in an SPSFTU greenhouse ready for the field (September, 2016)

Figure 2: Establishing the F1 hybrid aspen plantations in September, 2016 in the St. Petersburg area (left) and Surgut area (right)

To construct a linkage map, the F1 population of 122 aspen seedlings planted in Druzhnaya Gorka was employed. To perform the RADseq procedure**,** the Illumina HiSeq2500 sequencer was used, as a result**,** 204.108 million reads were obtained for 122 F1 progenies (Zhigunov et al. 2017a, b). 82% of the reads were perfectly mapped to the reference genome developed earlier for *P. trichocarpa* (Tuskan et al., 2006) (Figure 3). For the aspen linkage map construction 2055 SNPs were employed. Each of the resulting paternal and maternal linkage maps contained 19 linkage groups spanning 3090.56 cM and 3054.9 cM of the genome, respectively.

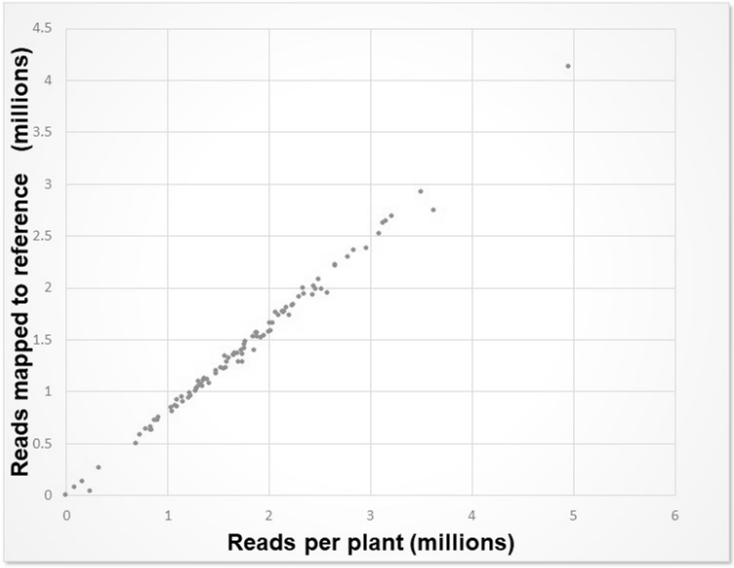

Figure 3: The scatterplot showing percentage of reads produced by Illumina HiSeq2500 for each of 122 F1 offspring (dots) that were successfully mapped on the reference genome of *P. trichocarpa*.

The developed F1 mapping population and the linkage map established for the intraspecific aspen cross allowed us to proceed with phenotypic traits assessment of the segregating aspen plantations growing in different environmental conditions. At present, detailed information on frost resistance and bud flushing variation is available for the F1 population maintained in St. Petersburg area. Here, the main threat of climatic conditions for overwintering perennials is often winter thaws followed by a return to sufficiently cold temperatures injuring plants.

In St. Petersburg area the F1 full siblings were planted in September, 2016 (Figure 2). In May, 2017 the plants were assessed for their ability to survive the winter 2016-2017 and their bud flushing date. Winter survival varied in the population significantly. 38.2% of the plants showed no visible signs of frost damage, whereas 36.3% did not survive the winter (Figure 4). The remaining 25.5% showed different winter survival ability (Table 1).

Figure 4: Winter survival of the F1 aspen progenies planted in the St. Petersburg area, left to right: the stem is completely damaged; just the upper part of the stem is damaged and regrowth from the root collar was observed; no visible sign of the stem damage.

Table 1: Winter survival of the F1 aspen progenies of in the field of Druzhnaya Gorka (St. Petersburg area) after the winter period of 2016-2017

No visible sign of stem damage	Just the upper part of the stem is damaged	The stem is completely damaged, regrowth from the root collar was observed	The stem completely damaged, no regrowth from the root collar was observed
38.2 %	7.3 %	18.2 %	36.3 %

The segregation of bud flushing in the F1 hybrid aspen population was less obvious. 90 % of the plants showed nearly the same bud-flushing date (between 21.05.2017 and 27.05.2017). 9 % of the plants showed their bud-flushing one week later and 1 % - two weeks later. In September, 2017 the plants of the F1 population finished growing and formed buds within the same period of time. As a result, the annual increment of the plants in the F1 population varied from 6 to 12 cm. The significant variation of autumn senescence in the hybrid aspen population was recorded by monitoring chlorophyll degradation in trees growing in the St. Petersburg plantation in September, 2017. Trees with red, yellow and brown leaf colours segregated as 1:1:1.

Conclusions

The developed research resources that combine plantations of full siblings of *P. tremula* established in three ecologically different regions and the high-density linkage map developed for the European aspen could provide a basis for marker-assisted selection of aspen in Russia.

Acknowledgements

This work was supported by the Ministry of Food and Agriculture of Germany (MaRussiA project 2015–2017).

References

BRADSHAW HD, STETTLER RF (1995) Molecular genetics of growth and development in populus. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics 139 (2): 963-973.

DAVEY JW, BLAXTER ML (2010) RADSeq: next-generation population genetics. Briefings in functional genomics 9 (5-6): 416-423.

FREWEN BE, CHEN TH, HOWE GT, DAVIS J, ROHDE A, BOERJAN W, BRADSHAW HD (2000) Quantitative trait loci and candidate gene mapping of bud set and bud flush in Populus. Genetics 154 (2): 837-845.

GRATTAPAGLIA D, SEDEROFF R (1994) Genetic linkage maps of Eucalyptus grandis and Eucalyptus urophylla using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137 (4): 1121-1137.

JORGE V, DOWKIW A, FAIVRE RAMPANT P, BASTIEN C (2005) Genetic architecture of qualitative and quantitative Melampsora larici-populina leaf rust resistance in hybrid poplar: genetic mapping and QTL detection. New Phytologist 167 (1): 113-127.

Newcombe G, Bradshaw Jr. HD (1996) Quantitative trait loci conferring resistance in hybrid poplar to Septoria populicola, the cause of leaf spot. Canadian Journal of Forest Research 26 (11): 1943-1950.

SUN C, NIU Y, YE X, DONG J, HU W, ZENG Q, LU M (2017) Construction of a high-density linkage map and mapping of sex determination and growth-related loci in the mandarin fish (Siniperca chuatsi). BMC genomics 18 (1): 446.

TONG C, LI H, WANG Y, LI X, OU J, WANG D, ZHANG B (2016) Construction of high-density linkage maps of Populus deltoids × P. simonii using restriction-site associated DNA sequencing. PLoS One 11(3), e0150692.

TUSKAN GA, DIFAZIO S, JANSSON S, BOHLMANN J, GRIGORIEV I, HELLSTEN U, SCHEIN J (2006) The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). Science 313(5793): 1596-1604.

WULLSCHLEGER SD, YIN TM, DIFAZIO SP, TSCHAPLINSKI TJ, GUNTER LE, DAVIS MF, TUSKAN GA (2005) Phenotypic variation in growth and biomass distribution for two advanced-generation pedigrees of hybrid poplar. Canadian Journal of Forest Research 35 (8): 1779-1789.

ZHIGUNOV AV, ULIANICH PS, LEBEDEVA MV, POTOKINA EK (2017a) Development and ecological trials of two aspen/poplar mapping populations. Genetics of Populations: Progress and Perspectives. Vash Format. Moscow, 99-101. [Proceedings of the International Scientific Conference, April, Moscow (Russia): 17-19, 2017]

ZHIGUNOV AV, ULIANICH PS, LEBEDEVA MV, CHANG PL, NUZHDIN SV, POTOKINA EK (2017b) Development of F1 hybrid population and the high-density linkage map for European aspen (*Populus tremula* L.) using RADseq technology. BMC plant biology 17 (1): 180.

The German forest tree breeding concept – the base for new seed orchards

MIRKO LIESEBACH[1], VOLKER SCHNECK[2]

[1] Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany (mirko.liesebach@thuenen.de)

[2] Thünen Institute of Forest Genetics, Eberswalder Chaussee 3a, 15377 Waldsieversdorf, Germany (volker.schneck@thuenen.de)

Abstract

Climate change is one of the key challenges of the 21st century. The environmentally friendly, CO2-saving and resource-saving raw material wood is of particular importance in efforts to counteract the undesirable consequences of this development. With the search for CO2-neutral energy resources and rising energy prices, the demand for wood is steadily increasing. However, the demand for wood is increasing not only in the energy sector (heating sector, power generation, biofuels), but also in the material-mechanical and chemical sectors, high growth rates are forecast. Forest tree breeding can contribute to the solution.

Key words: forest tree breeding, seed orchard, Douglas-fir, Scots pine

Current forest situation in Germany

Germany is covered by 10.9 Mio ha of forests. The percentage is 32 %. About half of the forest area is public owned (4 % Federal Government, 29 % state, and 19 % municipal). The remaining 48 % are private owned.

The last German federal forest inventory 2012 (BMEL 2016) showed that 43.4 % of the forest area are covered by broadleaves and 54.2 % are occupied by conifers. Since the previous inventory (BMELV 2005) the area of broadleaves has increased (+2.8 %) and the percentage of conifers has decreased (-2.8 %) (BMEL 2016). The highest losses are observed for Norway spruce followed by Scots pine. The area of silver fir and Douglas-fir increased during the last 10 years.

Regarding the age pyramid of forests there is a lack in the age classes 1 (age up to 20) and 2 (21-40 years for the conifers between the two inventories. The reason is the change in manner of forest regeneration. Since the 1980th the percentage of planting and seeding on one hand to natural regeneration on the other site has changed to the opposite. The percentage of natural regeneration was 80 % in 2002 and 85 % in 2012.

Planting is restricted nowadays on afforestation, if natural regeneration is missing, when a change in tree species is planned, to introduce additional species, after external events (e.g. storm, fire), and in some cases to change the provenance to increase growth / quality.

Due to implement the National Strategy of Biodiversity (BMU 2007) natural forest development without any use is planned on 5 % of the forest area in Germany until 2020, and 10 % of the public forests, respectively. By 2020, an increase in raw wood demand for material and energy use of up to 20 million m³ is expected (MANTAU 2012). For these reasons, it is necessary to develop a precautionary strategy to meet future challenges. An important starting point is forest tree breeding with the provision of high quality, high performance and resistant seed and seedlings.

Potential of forest tree breeding

The potential of forest tree breeding is demonstrated by conservative model calculations. The models are based on the following fix assumptions:

For afforestation on one third of the area the current choice of the provenance is right. On one third of the area the present stand will be substituted by a better growing provenance of the category "selected" and an increase in yield of 5 % is supposed. On the remaining third of the afforestation area the present stand will be replaced by a forest reproductive material of the category "tested" with an assumption of an increase in yield of 10 %. For conifers a mean increase of 14.24 m³/ha*year was assumed, and for broadleaves of 9.70 m³/ha*year.

In 4 scenarios three variable assumptions were used (Table 1):

(1) The annual regeneration area is (1.a) calculated as the mean of the last 20 years or (1.b) 1 % of the forest area. These are 64,000 ha/year and 103,200 ha/year, respectively.

(2) The proportion of the planting area in comparison to the natural regeneration is (2.a) 50 % and (2.b) 70 %. This means that on 50 % and 30 %, respectively, the forests will still be regenerated naturally.

(3) The species proportion of broadleaves to conifers is (3.a) 50:50 and (3.b) 40:60.

Table 1: Combination of variable assumption in the four calculated scenarios

Scenario	Regeneration area	Planting proportion of the regeneration area	Species proportion: broadleaves : conifers
Scenario 1	mean of last 20 years (64,000 ha/year)	50 %	50 : 50
Scenario 2	mean of last 20 years (64,000 ha/year)	70 %	40 : 60
Scenario 3	1 % of forest area (103,200 ha/year)	50 %	40 : 60
Scenario 4	1 % of forest area (103,200 ha/year)	70 %	40 : 60

The models show that after 40 years between 0.77 million m³ (scenario I) and 1.78 million m³ (scenario IV) wood can be available annually. This sustainable wood supply is only (mid- to) long-term possible when the process has started once.

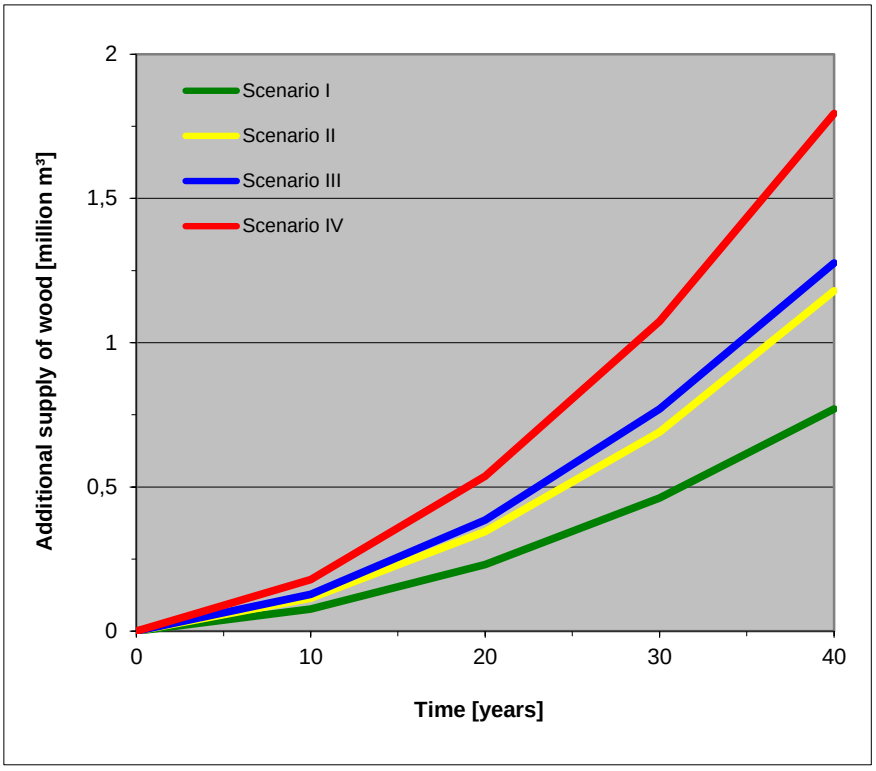

Figure 1: Addition mean annual wood supply in the 4 scenarios after 40 years.

Political development

Since the beginning of this decade there are new activities by the federal government to promote the need of forest tree breeding.

In the ***Forest Strategy 2020*** ("Waldstrategie 2020" [BMELV 2011]) contains in the chapter silviculture solutions which are connected with forest tree breeding. The following approaches are suitable ways of achieving a close to nature and environmentally compatible increase in forest productivity:

- Creation of diverse, stable and high yield mixed forests
- Planting of site-adapted species of trees with a high level of resistance and growth rate
- Use of high quality, site-adapted, resistant and high yield forest plants

As a milestone to achieve the "Climate protection plan 2050" the ***Charter for Wood 2.0*** ("Charta für Holz 2.0" [BMEL 2017]) was adopted. The Charta puts its focus on a sustainable wood supply, which is addressed for forest tree breeding in the chapter forests and wood as resources within the field of actions. Important statements are:

- Cultivating productive tree species
- Securing the supply of softwood
- Forest tree selection/forest genetics
- Alternative sources of raw materials (e.g. short-rotation-coppices [SRC], agro-forestry)

Forest tree breeding strategy

In Berlin the 3rd meeting on the Forest Strategy 2020 of the BMELV in April 2010 and the BMELV workshop on forest tree breeding organized by the Thünen Institute of Forest Genetics and the Agency for Renewable Resources (FNR) in November 2011 gave a good overview on the current state of work, the enormous potential, but also to the clear need for Germany to catch up in this area compared to other

countries (report in LIESEBACH 2011). An important objective of breeding is to provide reproductive material that is adaptable and powerful enough to meet the expected changes in the performance of all forest functions. The workshop has clearly clarified that forest tree breeding in Germany can only meet these great requirements of the future and deliver visible results, if all remaining federal and state breeding facilities cooperate even more closely by sharing the tasks and financing not only in the short term but also in the medium to long term for a period of at least 15 years.

Based on comparable strategies abroad and in view of the available capacities, forest tree breeding will have to focus on a few tree species in the future in Germany. Therefore, the more important is the right selection of these species. Due to the long generation times of the trees, forest tree breeding programs are only successful after decades. However, because it is possible to build on already existing results, clear successes can be expected after about 15 years. New programs would take significantly longer periods of time. Experience has shown that within this approximately 15-years period, a significantly increased growth and value performance can be expected through breeding. When choosing the tree species, different aspects are important. On the one hand, it should be tree species that can be expected to show significant breeding progress in the planned period, on the other hand, the future orientation of silviculture in view of climate change must be taken into consideration and the expected demand for products and services of the forest included.

Finally this process leads to a „Breeding strategy“ for 6 tree species or species groups (Table 2), which was published in November 2013 (LIESEBACH et al. 2013). There are much more species of interest in breeding. The 6 species were chosen as model species because for each a different approach is followed.

The intensity of breeding will vary depending on the species (Table 2). The range extends from the testing of progenies of stands (e.g. oak) to the installation of new high-performance seed plantations (e.g. sycamore maple) up to controlled crossings (e.g. larch). The achievable improvement in volume yield after 15 years of breeding work averages of 10 %. In the longer term, a value increase of at least 20 % is to be expected.

Table 2: Future breeding priorities the 6 focus tree species or tree species groups (LIESEBACH et al. 2013)

Species	Reason	Breeding priorities			
		Stands	Seed orchards	Parents of families	Clones
Douglas-fir	growth potential	x	x	(x)	-
Larches	breeding experience, growth potential	(x)	x	x	(x)
Sycamore maple	expected growth potential	(x)	x	-	-
Norway spruce	main species, breeding experience	-	x	-	(x)
Scots pine	main species, breeding experience	-	x	-	-
Oaks	valuable main broadleaves	x	(x)	-	-

The **Douglas-fir** (*Pseudotsuga menziesii* [Mirb.] Franco.) is particularly important due to its growth performance and its ability to replace Norway spruce in some locations. Breeding focuses on the testing of progenies and the establishment of seed orchards. Furthermore, family crossings are conceivable.

Larches (*Larix decidua* Mill., *L.×eurolepis* Henri) are promising because of their growth performance and the extensive breeding advance. Here, the establishment of seed plantations and the approval of parents of families of promising hybrids are in the foreground.

The **sycamore maple** (*Acer pseudoplatanus* L.), which has not yet undergone breeding, is of particular importance both in the mountains and in the lowlands and as a tree in mixed stands. In the foreground are seed orchards, whereby also increases can be expected due to the testing of progenies.

Norway spruce (*Picea abies* [L.] Karst.) and **Scots pine** (*Pinus sylvestris* L.) are the most common tree species in German forestry and are expected to remain so for the foreseeable future. Here, among other things, the establishment of seed orchards is in the foreground.

In the case of **oaks** (*Quercus petraea* [Matt.] Liebl., *Q. robur* L.), breeding activities will focus mainly on the testing of progenies with the aim of the approval of forest reproductive material of the category "tested".

Beside these six species breeding within the Genus *Populus* was financed in the FastWOOD projects by the BMEL through the FNR with the focus on SRC. These projects started in 2008 and will end between 2017 and 2019 (e.g. BORSCHEL et al. 2012; LIESEBACH 2015). During that time more than 1,000 new crosses were done, over 60 progeny trials were established and new material in the category “tested” was approved by analysing existing trials (LIESEBACH 2013). BMEL and BMUB are funding a project within the Waldklimafonds (2014-2017) to develop a management concept for seed stand with beech to increase the quality of the seed (EUSEMANN et al. 2017a, b). Recently a project on ash breeding started on the background of ash die back.

Implementation of the breeding strategy

In 2014 the implementation of the breeding strategy started with the joint project FitForClim (2014-2018) (MEIßNER et al. 2015, JANßEN et al. 2017). Within this joint project all breeding organizations work together. The bigger ones coordinate the work within the six species: Norway spruce and oaks (Northwest German Forest Research Institute [NW-FVA]), sycamore maple (Bavarian office for Forest Seeding and Planting [ASP]), larches (Public Enterprise Sachsenforst [SBS]), and Douglas-fir and Scots pine (Thünen Institute of Forest Genetics).

The aims of the project FitForClim are to delineate deployment zones (approx. 3 per species), to select plus trees (between 500 and 800 per species), vegetative propagation of the plus trees, growing plants to establish progeny trials, genetic characterisation of the plus trees, analyses of mating system of Douglas-fir, approval of forest reproductive material in the categories “*qualified*” and “*tested*”, and investigation on frost hardiness. The delineation of deployment zones takes future climate scenarios into consideration. The majority of the plus trees are selected in progeny trails where a direct comparison with other progenies is given.

The vegetative propagated plus trees will be saved in archives. An archive (“*breeding population*”) contains the approx. 200-300 genotypes of a deployment zone. This is done in the joint project AdaptForClim (2017-2019) (WOLF et al. 2017). Further work packages in this project are the development of a concept for the structure of seed orchards to maintain genetic sufficient diversity, and of a concept on additional provenance trials to realize the breeding strategy, the reorganization of clonal trials with Norway spruce into stands to harvest seeds, physiological investigation on the grafts of the plus trees, and the establishment of several progeny test (e.g. with Douglas-fir progenies from seed orchards, with oak progenies and single tree progenies of Scots pine). The clone archives are the base to compile new seed orchards (“*production population*”) in a further step. Both projects FitForCLim and AdaptForClim are funded by BMEL and BMUB within the Waldklimafonds.

The breeding strategy for Douglas fir is shown in Figure 2 and for Scots pine in Figure 3 which are coordinated by the Thünen Institute of Forest Genetics.

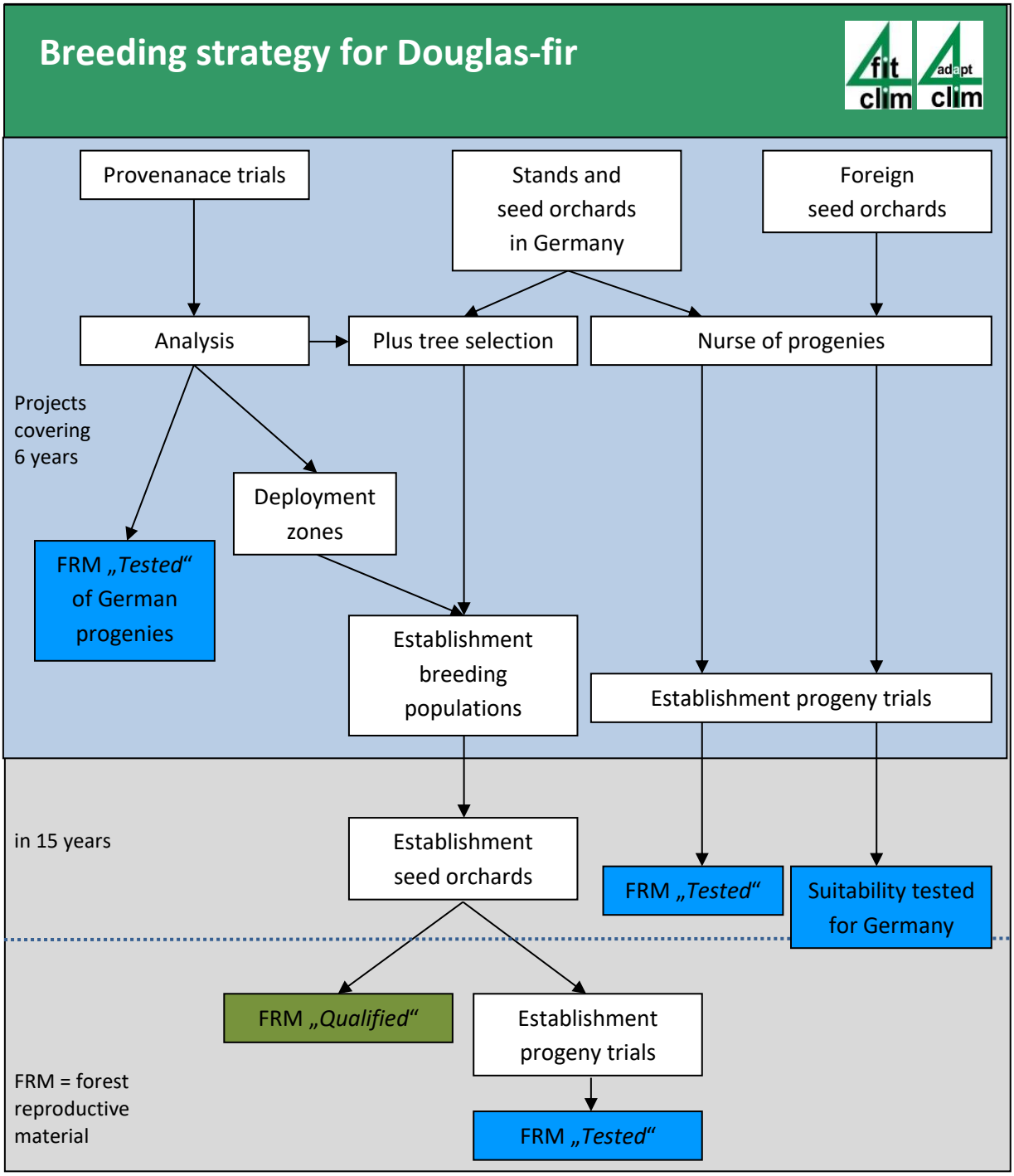

Figure 2: Breeding strategy for Douglas-fir. The blue shaded part is financed by the projects FitForClim and AdaptForClim.

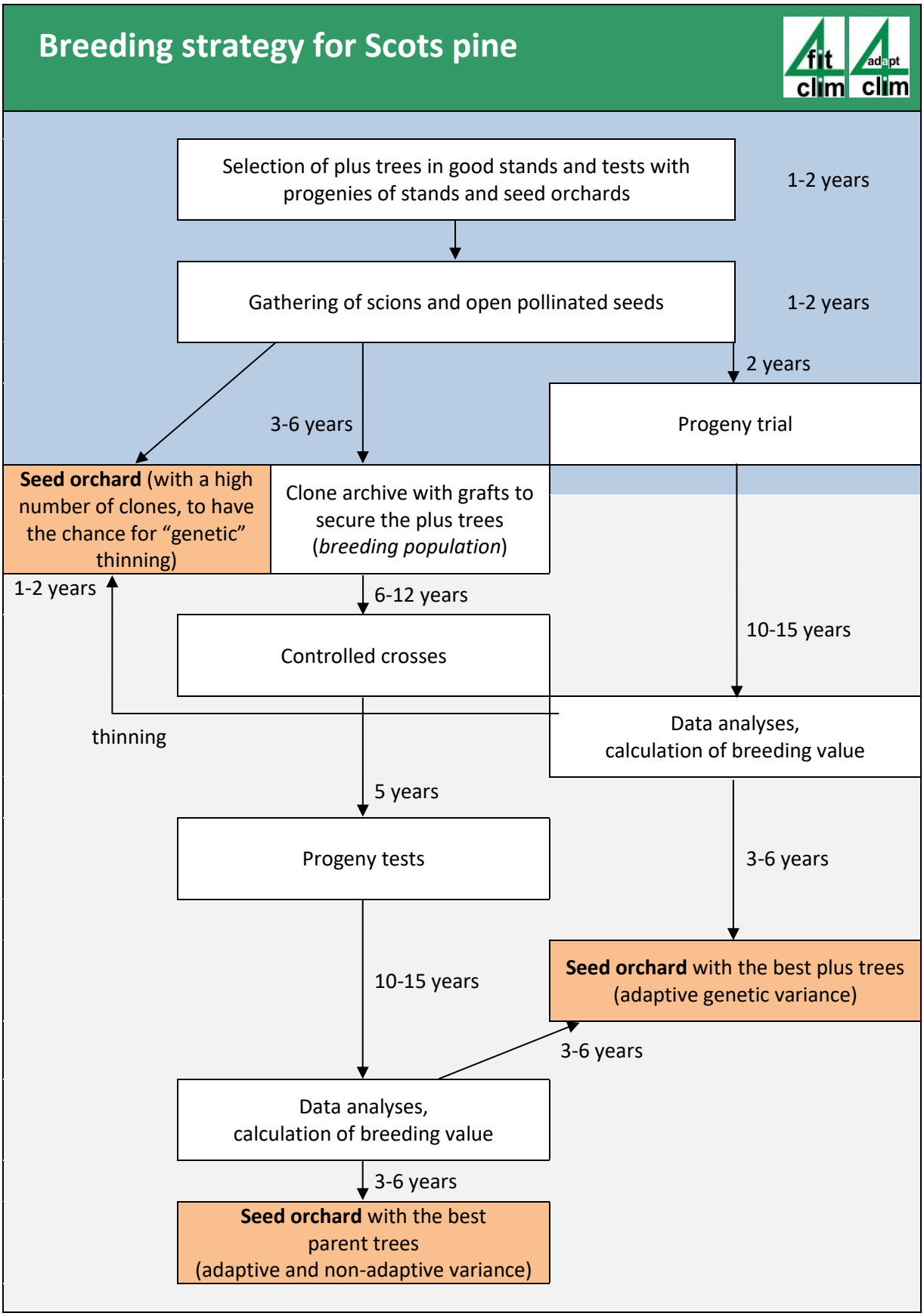

Figure 3: Breeding strategy (positive selection of individuals with progeny tests) for Scots pine. The blue shaded part is financed by the projects FitForClim and AdaptForClim.

Acknowledgements

This projects FitForClim and AdaptForClim are financially supported by the Federal Ministry of Food and Agriculture (BMEL) and by the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) and via the "Bundesanstalt für Landwirtschaft und Ernährung" (BLE) within the "Waldklimafonds".

References

BMELV [Federal Ministry of Food, Agriculture and Consumer Protection] (2005) Die zweite Bundeswaldinventur – BWI^2. Der Inventurbericht. BMELV, Berlin: pp. 231.

BMELV [Federal Ministry of Food, Agriculture and Consumer Protection] (2011) Forestry Strategy 2020. Sustainable Forest Management – An Opportunity and a Challenge for Society. BMELV, Bonn: pp. 36.

BMEL [Federal Ministry of Food and Agriculture] (2016) Ergebnisse der Bundeswaldinventur 2012. BMEL, Berlin: pp. 280.

BMEL [Federal Ministry of Food and Agriculture] (2017) Mitigation climate change. Creating value. Utilising resources efficiently. - Charter for Wood 2.0. BMEL, Bonn: pp. 60.

BMU [Federal Ministry for the Environment, Nature Conservation and Nuclear Safety] (2007): National Strategy on Biological Diversity. BMU, Berlin: pp. 180.

BORSCHEL M, FEY-WAGNER C, JANßEN A (Red.) (2012) Züchtung und Ertragsleistung schnellwachsender Baumarten im Kurzumtrieb. Erkenntnisse aus drei Jahren FastWOOD, ProLoc und Weidenzüchtung. Beiträge aus der NW-FVA, Band 8: pp. 430.

EUSEMANN P, PREUSS A, LIESEBACH M, LIESEBACH H (2017a) Optimierte Saatgutqualität durch einzelbaumweise Beerntung - eine Untersuchung an Buche (*Fagus sylvatica* L.). Forstarchiv 88 (1): 17-23.

EUSEMANN P, LIESEBACH M, LIESEBACH H (2017b) Mit Drohnen Ernteaussichten in Saatgutbeständen erkunden. AFZ Wald 72 (10): 28-30.

JANßEN A, KONNERT M, LIESEBACH M, MEIßNER M, WOLF H (2017) Waldklimafonds unterstützt Forstpflanzenzüchtung. AFZ-DerWald 72 (10): 15-16.

LIESEBACH M (2011) Keine schnellen Erfolge, aber ungenutzte Potenziale. Forstpflanzenzüchtung - ein Instrument zur Minderung der Versorgungslücke beim heimischen Holzangebot? HZBl. 137 (49): 1244-1246.

LIESEBACH M (2013) Aspen-Züchtung: neues Vermehrungsgut. 2 Familieneltern und 4 Klone zugelassen. Gülzower Fachgespräche 45: 432.

LIESEBACH M (Hrsg.) (2015) FastWOOD II: Züchtung schnellwachsender Baumarten für die Produktion nachwachsender Rohstoffe im Kurzumtrieb – Erkenntnisse aus 6 Jahren FastWOOD. Thünen Report 26: pp. 210.

LIESEBACH M, DEGEN B, GROTEHUSMANN H, JANSSEN A, KONNERT M, RAU H-M, SCHIRMER R, SCHNECK D, SCHNECK V, STEINER W, WOLF H (2013) Strategie zur mittel- und langfristigen Versorgung mit hochwertigem forstlichem Vermehrungsgut durch Züchtung in Deutschland. Thünen Report 7: pp. 81.

MANTAU U (2012): Holzrohstoffbilanz Deutschland, Entwicklungen und Szenarien des Holzaufkommens und der Holzverwendung 1987 bis 2015. Hamburg: pp. 65.

MEIßNER M, JANßEN A, KONNERT M, LIESEBACH M, WOLF H (2015) FitForClim – das Züchtungsprojekt. AFZ-Der Wald 70 (11): 24-26.

WOLF H, JANßEN A, KONNERT M, LIESEBACH M (2017) Mehrleistung und Qualitätsverbesserung durch Züchtung. AFZ-DerWald 72 (10): 12-14.

From *in vitro* clones to high-quality timber production: the Project "Wavy Grain Maple"

CORNELIA BÄUCKER[1], HEIKE LIESEBACH[2]

[1] Thünen Institut of Forest Genetics, Eberswalder Chaussee 3a, 15377 Waldsieversdorf, Germany (cornelia.baeucker@thuenen.de)

[2] Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany (heike.liesebach@thuenen.de)

Abstract

Wavy grain maple is a rare growth variation of sycamore maple (*Acer pseudoplatanus* L.) with wood fibres undulating in the tree rings. Due to its attractive timber, it belongs to the most expensive hardwoods in Central Europe. It has never been produced systematically for commercial use, although wavy grain maple has a great popularity and is in demand. In 2016, therefore, a national joint project started to explore possibilities for its commercial use, and to investigate the causes for the wavy grain structure in sycamore maple. Here, we report about first results achieved by one of the five project partners, the Thünen Institute. We give an overview on selected clones and their propagation by tissue culture, as well as the establishment of planned field trials. Furthermore, we present a method for the clone identification of sycamore maple using highly variable nuclear microsatellite markers.

Key words: wavy grain, figured wood, fiddleback maple, *Acer pseudoplatanus,* commercial use

Introduction

In wood, the normal orientation of longitudinal fibres is parallel to the longitudinal tree axis, which is called straight grain (BEALS and DAVIS 1977). Beside this, various deviations from this parallel growth pattern are known, one of which is wavy grain. Here, the wood fibres undulate in the tree rings creating a "washboard" effect in the split radial section of the log (RICHTER 2015). The result of such a wavy growth is timber with a longitudinal radial surface exhibiting a series of alternately bright and dark stripes shading into one another and giving an optical illusion of waves (BEALS and DAVIS 1977). The surface of standing trees with wavy grain shows no visible external symptoms, because the growth phenomenon is restricted primarily to the radial plane of wood (BEALS and DAVIS 1977).

Wavy grain is mentioned to occur in many hardwood species (e.g. CONRAD 1988). According to BEALS and DAVIS (1977), a well-developed wavy grain structure over the entire tree stem is rare in trees of most species, but can be found in the genera maple (*Acer*), ash (*Fraxinus*), birch (*Betula*), and walnut (*Juglans*). Within populations, the percentage of individuals with wavy grain may vary from location to location. For populations of sycamore maple, different frequencies of occurrence of wavy grain figure have been reported ranging between 1 – 7 % (e.g. ROHR and HANUS 1987, CONRAD 1988, KRAJNC et al. 2015).

Sycamore maple (*Acer pseudoplatanus* L.) exhibiting wavy grain is known under different names, for example wavy grain maple or wavy grain sycamore. In German, it is mainly referred as "Riegelahorn", in Russian as "явор с волнистой структурой". Another term is also fiddleback maple, because this quality of wood is extensively used for backs of string instruments since the sixteenth century (e.g. BEALS and DAVIS 1977, CONRAD 1988).

Still today, there is a strong demand for wavy grain maple in the musical instrument making. Moreover, the timber is used as veneer and has some importance for exclusive furniture industry (NAUJOKS et al. 2013). Therefore, maple logs with a regular, well-developed wavy grain are always sold for very high prices at auction sales, so-called submissions. In Germany, such logs regularly achieve prices over

10,000.00 Euro, e.g. 12,100.00 Euro at the submission Waging am See in 2017. According to KRAJNC et al. (2015), wavy grain maple achieves significantly higher prices than any other wood in Slovenia. Thus, wavy grain maple belongs to the most expensive hardwoods in Central Europe.

Although wavy grain maple has such a great popularity and is in demand, it has never been produced systematically for commercial use, because research concerning wavy grain maple has not been continuously promoted in many cases (KRABEL and WOLF 2013). In Germany, however, activities to preserve valuable sycamore material led to the micropropagation of a few wavy grain maple clones (EWALD and NAUJOKS 2015), as well as the establishment of seed orchards and progeny trails (KRABEL and WOLF 2013). Nevertheless, research into the reliable production of nursery trees of wavy grain maple on a larger scale with the aim to establish commercial plantations is lacking. Moreover, the factors causing wavy grain in sycamore maple are still unclear. Since there is accumulating evidence that figure in other tree species can be genetically inheritable, such as in curly birch (KÄRKKÄINEN et al. 2017) and curly poplar (FAN et al. 2013), the growth phenomenon may also have a genetic basis in wavy grain maple.

In order to explore possibilities for the commercial use of wavy grain maple, and also to investigate the causes for its wavy growth, a national joint project is funded by the Landwirtschaftliche Rentenbank (Federal Ministry of Food and Agriculture). In the frame of this project, methods for the identification, propagation and commercial use of wavy grain maple will be developed (https://www.thuenen.de/en/fg/projects/current-projects/maple-trees/). The project partners are: RLP AgroScience, Thünen Institute of Forest Genetics, Northwest German Forest Research Institute (NW-FVA), Institute for Plant Cultivation in Solkau, and the Reinhold Hummel GmbH & Co KG in Stuttgart.

Within the project, the Thünen Institute and the NW-FVA work closely together. Their tasks include the search for valuable material of wavy grain maple and its genetic conservation with methods of *in vitro* culturing and grafting. In this context, the clone collection of both Institutes will be enlarged, the *in vitro* cultivation of wavy grain maple will be improved, and clone material is exchanged among all project partners.

Beside this, a fingerprint method for the genetic identification of sycamore maple clones will be established by the Thünen Institute. Based on this method, the identification of *in vitro* propagated clone material can be ensured. Furthermore, the establishment of field trails testing tissue culture propagated material is included in the joint project, because the commercial use of clonal propagated material of sycamore as a forest tree species needs an official approval procedure. This implies the evaluation of field testing results according to the regulations on forest reproductive material (FRM) in Germany.

In the following, we report about first results of the work package of the Thünen Institute.

Material and methods

Search for material of wavy grain maple

The Thünen Institute and the NW-FVA are responsible to find new material of wavy grain maple in different regions of the German area. In the winter of 2015/2016 and 2016/2017, the institutes contacted the organisers of submissions, and asked for sycamore maple logs with wavy pattern. In case, logs exhibiting clear evidence of wavy grain were offered for sale, efforts were made to find the original tree stump and the remains of the tree crown outside in the forest. For a few logs, the corresponding stumps and also remains of the crowns could be clearly identified with the help of district foresters or private forest owners. Scions from the tree crown were harvested only if their belonging to the felled wavy grain maple tree was undoubtedly determined. If possible, the tree stumps were protected to facilitate the development of stump sprouts.

In vitro cultivation of wavy grain maple

Vegetative winter buds from scions of the tree crown were used as starting material for the establishment of tissue cultures of wavy grain maple in many cases. If existing, we also used vegetative buds from stump sprouts or grafted plants. The buds were surface-disinfected for 20 minutes with 0.4 % "FINK - Antisept P" followed by another disinfection step with 0.05 % silver nitrate for five minutes. After three rinses with autoclaved water, buds were prepared under a binocular microscope as described by EWALD and NAUJOKS (2015). For vegetative propagation of the wavy grain clones, we also followed the method published by EWALD and NAUJOKS (2015).

Planned field trails

To investigate the long-term growth behaviour and the manifestation of the wavy grain structure in the clone material selected and propagated by tissue culture, two field trials are planned by the Thünen Institute. One of those trails will be located in Saxony, district Großröhrsdorf near Dresden. We will test as many as possible clones originating from material of wavy grain maple trees and two approved seed standards of *A. pseudoplatanus* as controls.

Genetic characterization of the wavy grain clones

Genetic markers are a very important tool for the identification of clones in tree breeding, as morphological traits are not sufficient for that purpose. To genotype material of sycamore maple, we selected 12 nuclear microsatellite markers, which were known from the literature. The markers were used in three different sets of multiplex PCR (Table 1) and analysed using DNA fragment length analyses of PCR-amplified repetitive DNA sequences (Beckman Coulter CEQ-8000 Genetic Analysis System).

Table 1: Overview of the 12 nuclear microsatellite markers selected for genetic characterization of *A. pseudoplatanus* and their use in three different sets of multiplex PCR

Set	Marker	Fluorescent dye	Size range published	Motif	Reference
Set 1	MAP12	Cyanine 5	142-178	$(GT)_7$	PANDEY et al. (2004)
	MAP33	BMN-6	146-182	$(GT)_{18}$	PANDEY et al. (2004)
	Aop122	DY-751	185-199	$(CT)_{11}$	SEGARRA-MORAGUES et al. (2008)
	MAP40	Cyanine 5	238-246	$(GT)_6$	PANDEY et al. (2004)
Set2	MAP9	BMN-6	96-110	$(GA)_8$	PANDEY et al. (2004)
	Am118	Cyanine 5	140-190	$(CT)_{16}$	KIKUCHI and SHIBATA (2008)
	SM21A	DY-751	179-243	$(GAT)_{14}$	GRAIGNIC et al. (2013)
	SM60	BMN-6	231-237	$(AAC)_6$	GRAIGNIC et al. (2013)
	SM29	Cyanine 5	281-301	$(CTT)_{10}$	GRAIGNIC et al. (2013)
Set3	Aop116	Cyanine 5	109-139	$(GA)_{16}$	SEGARRA-MORAGUES et al. (2008)
	Aop943	DY-751	142-164	$(GA)_8$	SEGARRA-MORAGUES et al. (2008)
	MAP2	BMN-6	144-198	$(GT)_{23}$	PANDEY et al. (2004)

Results and discussion

Selected clones and their propagation by tissue culture

During the first half of the project, material from eight wavy grain maple trees was established using methods of tissue culture (Table 2). Together with *in vitro* clone material, which had already been cultivated by the Thünen Institute before the project started and clones received from the NW-FVA, the Thünen collection was extended to a number of 27 *in vitro* clones in total. From these *in vitro* clones, 17 might be regarded as stable clones, while the others are still in the establishment phase, which is characterized by a very small shoot elongation.

The difficulties in shoot multiplication of wavy grain maple are often related to the maturity of the material used (Ewald and Naujoks 2015). The rejuvenation process, therefore, may be considered as the most important aspect for the success of *in vitro* cultures of sycamore. In this context, rejuvenation may be induced by regular cutting of grafted plants (EWALD and NAUJOKS 2015), or continuous subculturing and regeneration of shoots in tissue cultures of wavy grain maple. Another possibility may result from using a juvenile starting material, such as buds from stump sprouts. For the clone "Poldi", for example, our attempts to establish a tissue culture arising from buds of scions of the tree crown failed, but we successfully established material from stump sprouts using the same method (i.e. preparation of buds). Therefore, the physiological status of material from stump sprouts may be more suitable for *in vitro* cultivation of *A. pseudoplatanus* compared to material harvested from the tree crown. The time period, furthermore, in which scions of the crown of felled wavy grain maple trees are influenced by changing weather conditions and fungal attack outside in the field, may also be of significant importance for the success of *in vitro* cultures originating from those material.

Table 2: Overview of wavy grain maple clones, which were selected or re-established during the first half of the project by the Thünen Institute.

Clone	Submission	Price per cubic metres in Euro	Material used for *in vitro* culture
Bonn	2001, Bonn	4,000.00	buds from grafted plant
Haini	2017, Erfurt-Egstedt	3,740.00	buds from tree crown scions
Isen	2017, Waging am See	7,160.00	buds from tree crown scions
Pfull	2003, Bad Waldsee	5,768.00	buds from grafted plant
Poldi	2016, Waging am See	2,290.00	buds from stump sprouts
Rhön	2016, Sailershausen	2,741.00	buds from tree crown scions
Schussi	2012, Bad Schussenried	2,730.00	buds from stump sprouts
Staig	2001, Bad Waldsee	6,952.00	buds from grafted plant

Planned field trails

Within the first year of the project, *in vitro* material of eight clones, which originated from buds of wavy grain maple trees, was successfully propagated by tissue culture in larger quantities. In 2017, therefore, we already produced *in vitro* plants from these well-performing clones (Figure 1). In addition, control plants of two approved seed standards were also grown. For 2018, we expect to produce *in vitro* plants of several more clones cultivated from material of wavy grain maple (ca. seven clones), and to test them on the field trails planned.

Figure 1: Produced *in vitro* plants of sycamore clones, which originated from buds of wavy grain maple, in the tree nursery of the Thünen Institute in Waldsieversdorf.

Genetic characterization of wavy grain clones and planned parentage analysis

A method for genotyping and clone identification of wavy grain maple was established using highly variable nuclear microsatellite markers. Because *A. pseudoplatanus* is a tetraploid tree species, a maximum rate of four different alleles was detected within a single gene locus. As expected, we found extended size ranges of alleles for some loci in comparison to the size ranges reported in the literature, e.g. loci of MAP12, MAP40, Aop122, Aop943, and SM60.

The developed microsatellite analysis will further be used to conduct a parentage analysis, since microsatellite markers are optimal for clone identification, as well as for parentage and sibship analysis. As parent generation of sycamore maple, we use 21 genotypes including seven clones with wavy grain from the seed orchard in Reinhardshagen, which was planted from 1959 to 1964 by the NW-FVA. Regarding the offspring generation, we will analyse individuals of two progeny testings, which were established with open-pollinated seeds from Reinhardshagen in 1986.

Conclusions

Within the project, valuable material of wavy grain maple was selected during submissions or re-established from grafted plants using methods of tissue culture. As starting material for the establishment of *in vitro* cultures of wavy grain maple, dormant buds from stump sprouts seem to be more suitable than vegetative buds from scions of tree crowns and grafts, respectively. In future, material from stump sprouts will be preferred. Therefore, the protection of tree stumps of felled wavy grain maple is highly recommended to avoid browsing of stump sprouts by animals. A method of tissue culture propagation of sycamore maple was successfully applied, and nursery plants of wavy grain maple clones were produced in larger quantities to test their growth performance and the manifestation of wavy grain on field trails. A reliable genotyping method with nuclear microsatellite markers was established and can be used for routine genotyping.

Acknowledgements

This project is financially supported by the German Federal Ministry of Food and Agriculture (via Landwirtschaftliche Rentenbank). We thank D. EWALD and G. NAUJOKS for initiating the project, and for their research into the micropropagation of wavy grain maple. Indispensable technical assistance by P. KNAUER is also gratefully acknowledged. Moreover, we thank M. QUAMBUSCH and A. MEIER-DINKEL at the NW-FVA for the exchange of information and *in vitro* material of wavy grain maple. Furthermore, we thank the following people for their help with scion material: C. ZAISER, L. KLÖFFER, R. WARMUTH, D. FRITZLAR,

and R. HABERLAND. Finally, we thank A. ZEIBIG from Sachsenforst for organising one of the sites for field testing.

References

BEALS HO, DAVIS TC (1977) Figure in wood: an illustrated review. Alabama Agricultural Experiment Station, Bulletin 486, Auburn University, Auburn, United States of America: 79 pp. (ISBN B0006WK30Y)

CONRAD J (1988) Seltene, abnorme Holzstrukturen und -farben bei Laubhölzern – Erkennungsmerkmale, wirtschaftliche Bedeutung, Entstehungstheorien. Die Holzzucht 42: 1-9.

EWALD D, NAUJOKS G (2015) Vegetative propagation of wavy grain *Acer pseudoplatanus* and confirmation of wavy grain in wood of vegetatively propagated trees: a first evaluation. Dendrobiology 74: 135-142.

FAN Y, RUPERT K, WIEDENHOEFT AC, WOESTE K, LEXER C, MEILAN R (2013): Figured grain in aspen is heritable and not affected by graft-transmissible signals. Trees 27: 973-983.

GRAIGNIC N, TREMBLAY F, BERGERON Y (2013) Development of polymorphic nuclear microsatellite markers in sugar maple (*Acer saccharum* Marsh.) using cross-species transfer and SSR-enriched shotgun pyrosequencing. Conservation Genetics Resources 5: 845-848.

KÄRKKÄINEN K, VIHERÄ-AARNIO A, VAKKARI P, HAGQVIST R, NIEMINEN K (2017) Simple inheritance of a complex trait: figured wood in curly birch is caused by one semi-dominant and lethal Mendelian factor? Canadian Journal of Forest Research 47 (7): 991-995.

KIKUCHI S, SHIBATA M (2008) Development of polymorphic microsatellite markers in *Acer mono* Maxim. Molecular Ecology Resources 8: 339-341.

KRABEL D, WOLF H (2013) Sycamore Maple (*Acer pseudoplatanus* L.). In: L.E. Pâques (Ed.), *Forest Tree Breeding in Europe: Managing Forest Ecosystems,* Chapter 8, 373-402, Springer, Dordrecht, DE: 527 pp. (ISBN 978-94-007-6145-2)

KRAJNC L, ČUFAR K, BRUS R (2015) Characteristics and geographical distribution of fiddleback figure in wood of *Acer pseudoplatanus* L. in Slovenia. Drvna Industrija 66 (3): 213-220.

NAUJOKS G, EWALD D, MEIER-DINKEL A, WALLBRAUN M (2013) Endlich hinter Schloss und Riegel: Stand und Perspektiven der Forschung beim Riegelahorn. AFZ Wald 68 (5): 10-12.

PANDEY M, GAILING O, FISCHER D, HATTEMER HH, FINKELDEY R (2004) Characterization of microsatellite markers in sycamore (*Acer pseudoplatanus* L.). Molecular Ecology Notes 4: 253-255.

RICHTER C (2015) *Wood characteristics: description, causes, prevention, impact on use and technological adaptation*, Springer International Publishing, Switzerland: 222 pp. (ISBN 978-3-319-07421-4)

ROHR R, HANUS D (1987) Vegetative propagation of wavy grain sycamore maple. Canadian Journal of Forest Research 17: 418-420.

SEGARRA-MORAGUES JG, GLEISER G, GONZÁLEZ-CANDELAS F (2008) Isolation and characterization of microsatellite loci in *Acer opalus* (Aceraceae), a sexually-polymorphic tree, through an enriched genomic library. Conservation Genetics 9: 1059-1062.

The mysteries of the origin of the curly birch

LIDIA VETCHINNIKOVA[1], ALEXANDER TITOV[2]

[1] Forest Research Institute of Karelian Research Centre Russian Academy of Sciences, Petrozavodsk, Russia (vetchin@krc.karelia.ru)

[2] Institute of Biology of Karelian Research Centre Russian Academy of Sciences, Petrozavodsk, Russia (titov@krc.karelia.ru)

Abstract

The biological features of the curly birch, noted for its figured grain, and its distribution range, which is limited to continental northwestern Europe, are outlined. The settings and conditions under which the curly birch can potentially appear are considered. Having analyzed own and published data, we hypothesize that the curly birch diverged as a separate form of birch during so-called Little Ice Age, and its geography ensued from a peculiar evolutionary pathway in the genus *Betula* L., which is associated with secondary introgression zones in Fennoscandia, where unusual haplotypes could appear through interspecies hybridization. The factors and conditions, as well the molecular-genetic processes assumed to have caused the emergence of the curly birch are described.

Key words: *Betula pendula* Roth var. *carelica* (Mercklin) Hämet-Ahti, origin, phylogeny, introgression, phylogeography, continental northwestern Europe

Why is there so much interest in the curly birch and what is so unique about it?

The curly birch *Betula pendula* Roth var. *carelica* (Mercklin) Hämet-Ahti (also known as Karelian birch, mazur birch, etc.) features unique figured wood that looks a lot like marble, and is also very hard and heavy (930 kg/m^3 raw weight). That is why curly birch timber has for over 500 years been widely used to make souvenirs, furniture and wood patterns, and specialists name it among the most expensive veneers (SCHOLZ 1960). Owing to its singular ornamental qualities, the curly birch is valued highly in the global market, and in contrast to other timbers, the pricing is per kilo rather than per cubic meter.

The curly birch grows naturally in Northern, Eastern and occasionally Central Europe. Because of decades of active logging (often uncontrolled), its resources have declined severely, and in some areas its very existence is threatened.

Since the first decades of the 20th century, scientists from several countries have been specifically studying the biological features of the birch. There were weighty reasons for this interest, and it was not only the valuable timber. *Firstly*, the curly birch does not form forests. Instead, it grows as separate trees or in clumps, and its range is discontinuous. *Secondly*, it features high polymorphism and high variation among individual trees in grain pattern (from barely noticeable waviness to intense figure) and growth forms (from shrub-like to high-stemmed, sometimes multi-stemmed). *Thirdly*, the curly birch is of interest as an object by studying which one can find answers regarding the causes and mechanisms of wood figure formation, and identify patterns in the variation and inheritance of this unique quality.

How and why could the curly birch appear?

The genus *Betula* L. has an extensive distribution range and is highly polymorphic. Its most common members in continental northwestern Europe are the silver birch *Betula pendula* Roth, downy birch *B. pubescens* Ehrh., and dwarf birch *B. nana* L. This is where the curly birch appeared and its range took

shape. The biological characteristics of the curly birch and its intraspecies diversity have apparently enabled it to settle firmly in this territory, where the natural and climatic conditions facilitated not only its origin but also its persistence.

It is common knowledge that large-scale climatic fluctuations usually entail changes in the structure of biological communities, including the emergence of some and the vanishing of other species. It is therefore highly likely that the curly birch emerged as a separate form through an evolution associated with climatic upheavals, namely those happening during so-called Little Ice Age (roughly between 1300 and 1850 C.E.) in continental northwestern Europe. It began with a prolonged decline in solar activity (known as Spörer Minimum), and then in the temperature, which continued for several centuries. Specialists argue that over the past two millennia the Little Ice Age has been the coldest period in terms of mean annual temperatures (EDDY 1976, LOCKWOOD 2010, DELAYGUE and BARD 2011). The most probable reason for this phenomenon is thought to be the changes triggered by complex interactions of the atmosphere and the ocean in the North Atlantic region, which is one of the most climatically unstable regions globally.

That said, external conditions and factors can only activate and/or promote the expression and fixation of certain advantages in some genotypes that had supposedly appeared in the genus *Betula* L. through various genetic processes. As to the curly birch, we should rather speak about introgression, although an important contribution of mutations and recombination to the infra- and interspecies variation in the genus *Betula*, with its vast distribution across the northern hemisphere, cannot be completely ruled out. It is however unlikely that similar mutations or recombination could simultaneously appear and be fixed in the progeny of plants growing so far apart and under rather different natural and climatic conditions.

The fact that the curly birch is confined solely to continental northwestern Europe is probably a result of a specific pathway in the evolution of birches. Equally important for the emergence of the curly birch is that there are areas of secondary introgression and the feasibility of natural hybridization between closely related birch species or varieties (i.e., at present the downy birch *Betula pubescens*, silver birch *B. pendula*; in the past the dwarf birch *B. nana*, shrubby birch *B. humilis* and, possibly, some extinct species). The role of introgression in Fennoscandia is to be highlighted, since there hardly remain any 'pure' populations in the territory, and the hybridization could involve not only two, but even several closely related birch species (TSVELEV 2002, PALMÉ et al. 2003, 2004, SCHENK et al. 2008).

These processes were facilitated not only by the cohabitation of different birch species, but also by the absence of phenological isolation between them in some years. The ratio of 'figured' and 'straight-grained' curly birch plants is significantly influenced by panmixia, as well as the genetic characteristics of the male pollinator's pollen (VETCHINNIKOVA et al. 2013, VETCHINNIKOVA and TITOV 2017). Note that introgressive hybridization is observed only in the zones where the distribution ranges or ecological niches of closely related species overlap and where there is free space available for the hybrids to disperse to, for instance those that were then appearing as a result of slash-and-burn agriculture (GROMTSEV 2008), and, as mentioned above, if the species reproductive isolation is breached and the timing of flowering coincides. Natural hybridization between species is impossible if at least one of these factors is missing. Thus, the probability of hybridization between the silver and the downy birch decreases sharply eastwards (NATHO 1959, MIGALINA et al. 2009), since the climate becomes more continental and the two species therefore diverge more significantly not only in the timing of flowering but also in the choice of habitats.

What molecular-genetic processes could stand behind the origin of the curly birch?

The major advances in molecular phylogeography have opened up new opportunities for reconstructing the dispersal pathways of individual plant species in the Late Glacial period, with some applications already (PALMÉ et al. 2003, 2004, MALIOUCHENKO et al. 2007, JADWISZCZAK 2012). For example, two main groups of haplotypes, A and C, have been detected in all birch species growing in Europe. Given their wide distribution,

these haplotypes are supposed to be the most ancient (WATTERSON and GUESS 1977) and were most probably present in the common ancestors of the investigated birch species (JÄRVINEN et al. 2004) (Figure 1). Haplotype A is prevalent in the populations of the downy, silver, and dwarf birches growing in the west and northwest of Europe (PALMÉ et al. 2003, 2004), and haplotype C is prevalent mainly in the east and southeast (MALIOUCHENKO et al. 2007, JADWISZCZAK 2012). Nuclear DNA tests confirmed there existed two ancestral populations, roughly divided into the European and the Asian groups, with secondary introgression zones in Finland (SALOJÄRVI et al.2017). The geographical distribution of the haplotypes demonstrates that different species growing in a sympatric population are more similar to one another than individuals of the same species in allopatric populations; most likely, this is the result of their hybridization (PALMÉ et al. 2004). The rare haplotype H, more frequently found in the downy and dwarf birches, probably appeared quite recently through hybridization and spread locally, since it has not yet been encountered anywhere outside Fennoscandia (Figure 1). Haplotype T can be assumed to have participated in the origin of the curly birch, since there was no development in the areas lacking the mentioned haplotypes (southern and eastern parts of the range of the genus *Betula*) or in areas where the climate has become more continental (eastern part of the range the genus *Betula*). These factors have shaped the specific geographic confinement of the curly birch, whose range is limited to continental northwestern Europe. It is very unlikely that the curly birch can be found on islands in this macroregion, since a majority of the birches growing, for instance, in Ireland are polyploids (SALOJÄRVI et al. 2017).

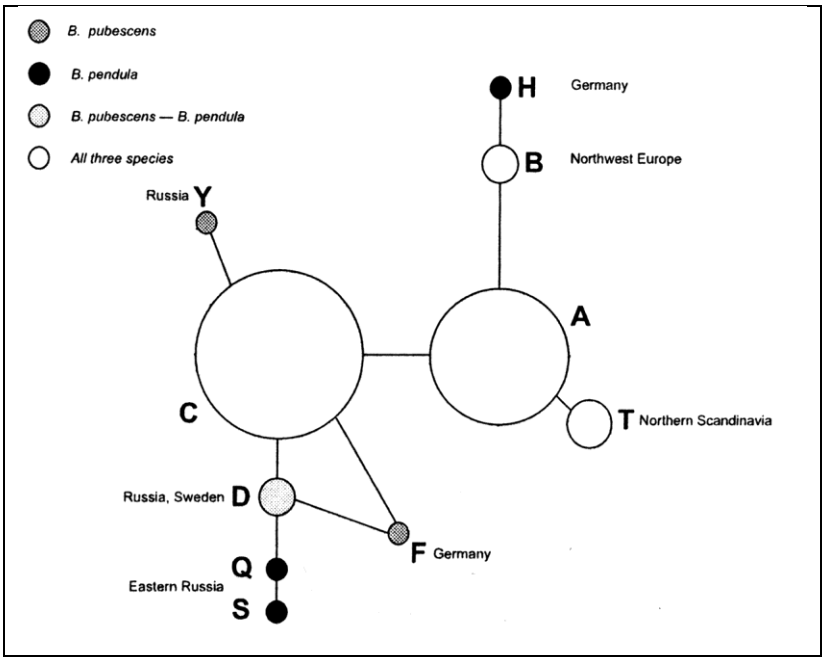

Figure 1: Frequency of occurrence of individual chloroplast DNA haplotypes in different species of birch from different countries in the European part of the distribution range. Circle diameters are roughly proportionate to haplotype frequency (after: PALMÉ et al. 2004).

Genome-related adaptations in the silver birch growing under various natural-climatic conditions in boreal forests of Eurasia were studied by sequencing the nuclear genome. In was found, in particular, that in the course of its evolution the silver birch underwent whole-genome and tandem duplications. Changes in ploidy levels promoted the number of transcription factors regulating the plants' growth and development, whereas tandem duplicates were overrepresented by environmental responses (SALOJÄRVI et al. 2017). Furthermore, these studies for the first time identified the light response genes *PHYC* and *FRS10*, whose activity correlates with latitude and longitude, and which enhance the plants' tolerance of

temperature and moisture fluctuations. It is not unlikely that the origin of the curly birch was initiated exactly by a change in light conditions after a reduction in solar activity (Spörer Minimum, and then Mounder Minimum and Dalton Minimum) observed during the Little Ice Age, which necessitated a quest for vacant niches with adequate illuminance for normal growth and development. This is indirectly evidenced by the numerous observations showing that stunted curly birch plants shaded by the closing canopy of the neighboring straight-grained birch trees or other accompanying species would gradually die back and fall out of the stand. Another indirect evidence is the ability of the curly birch to grow on rocky soils and in other sites poorly suited for other tree species but more open and better lit.

According to TSVELEV (2002), the very speciation of the downy birch is a result of a more ancient hybridization, which partly explains its higher polymorphism compared to the silver birch. It was long believed that the downy birch is an autotetraploid formed by a duplication of the chromosome set of the silver birch. Later on, however, it was established that the downy birch is not an autotetraploid but an allotetraploid carrying two different diploid genomes, only one of which can correspond to the silver birch. The allotetrapoidy of the downy birch, or the presence of the silver birch genome ($2n$ = 28) in its genotype ($2n$ = 56) makes hybridization between the birch species with different ploidy levels 'easier'. Opinions concerning the source of the second genome in the tetraploid downy birch differ. Some authors propose the dwarf birch *Betula nana* (BARANOV 2003, ANAMTHAWAT-JÓNSSON et al. 2010, WANG et al. 2014) while others favor the shrubby birch *B. humilis* (WALTERS 1968) or a possibly extinct diploid (HOWLAND et al. 1995). A comparative analysis of a number of diploid species (Figure 2) based on allele distribution showed that another possible diploid ancestor of the tetraploid *Betula pubescens*, alongside *B. pendula*, is a source species closely related to *B. lenta* (SALOJÄRVI et al. 2017). *Betula nana* was more closely related to *B. pubescens* and *B. pendula* than to *B. humilis* (JÄRVINEN et al. 2004).

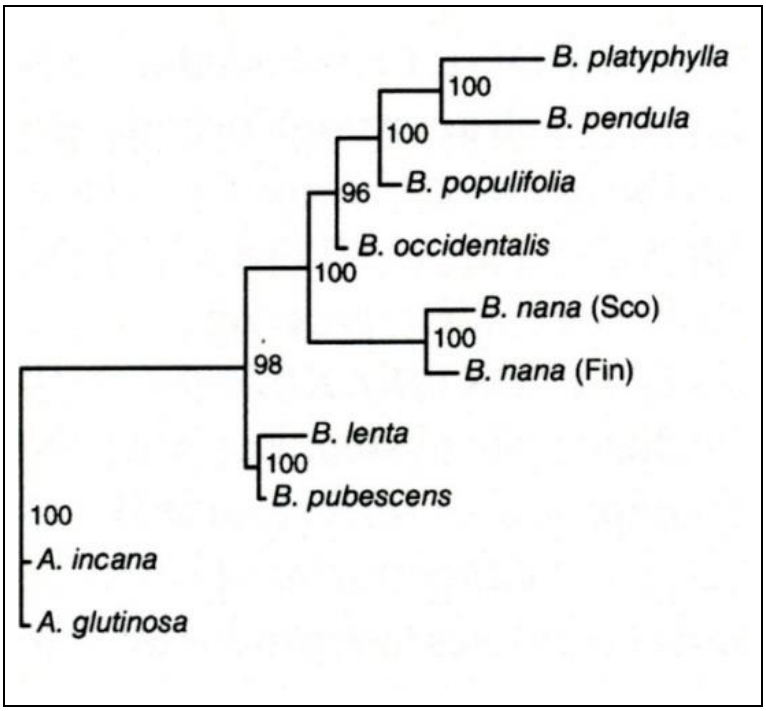

Figure 2: Dendrogram of interspecies genetic similarity between members of the family *Betulaceae* (after: SALOJÄRVI et al. 2017)

Finally, the hypothesis about the role of interspecies hybridization in the origin of the curly birch is further supported by the data on the degree of genetic differentiation between various birch species (BARANOV 2003), as well as the results of molecular certification of curly birch clones (MATVEEVA et al. 2008).

Conclusions

Having analyzed and summarized own and published data we formulated a hypothesis of the ecological-genetic origin of the curly birch (Vetchinnikova et al., 2013; Vetchinnikova, Titov, 2017), postulating that its emergence was probabilistic in nature, associated with the natural-climatic conditions in the northwestern part of continental Europe, which facilitated the appearance and persistence of this unique hybrid genotype, and with the genetic characteristics of the pollen produced by the male pollinator. The main factors that made the emergence of the curly birch possible were the existence of secondary introgression zones in the region and the feasibility of natural hybridization between closely related birch species or varieties (downy, silver and dwarf birches at present and possibly also other species in the past), and the conditions that facilitated the process were their cohabitation in the European part of their ranges and the absence of phenological isolation between them in some years. We believe it was owing to the genetic variation promoted by crossings that the curly birch came to being. At the same time, its emergence and/or settlement in areas lacking the relevant birch genotypes (southern and eastern parts of the range of the genus *Betula*) or where the climate has become more continental (eastern part of the range of the genus *Betula*) was impossible. As a result, the distribution range of the curly birch is limited and discontinuous, confined to the Baltic region only.

A remark to be made is that at present there is hardly a chance that the curly birch can form through hybridization, since not only the climate has changed significantly over the at least 500 years of its existence but even the genetic structure of its populations has altered substantially, probably losing the putative ancestral genotypes. Nonetheless, all the studied species, although belonging to different sections of the genus *Betula*, have preserved high genetic affinity, which suggests they have very probably descended from a common ancestor.

Acknowledgements

This work was supported by the Federal Budget for a state assignment to the Karelian Research Centre Russian Academy of Sciences.

References

ANAMTHAWAT-JÓNSSON K, THORSSON ET, TEMSCH EM, GREILHUBER J (2010) Icelandic Birch Polyploids – the case of a perfect fit in genome size. Journal of Botany 2010 (2): 1-9. http://dx.doi.org/10.1155/2010/347254.

BARANOV OYU (2003) The population-genetic structure of representatives of the genus Betula L. in Byelorussia, and its application in forest selection activities. Avtoref. Dissertation. Gomel' (In Russ).

DELAYGUE G, BARD E (2011) An Antarctic view of Beryllium-10 and solar activity for the past millennium. Clim. Dyn. 36 (11): 2201-2218. http://dx.doi.org/10.1007/s00382-010-0795-1.

EDDY JA (1976) The Maunder Minimum. Science 192 (4245):1189-1202.

HOWLAND BDE, OLIVER RP, DAVY AJ (1995) Morphological and molecular variation in natural populations of *Betula.* New Phytol. 130: 117-124.

JADWISZCZAK KA (2012) What can molecular markers tell us about the glacial and postglacial histories of European birches? Silva Fenn. 46 (5): 733-745. http://dx.doi.org/10.14214/sf.923.

JÄRVINEN P, PALMÉ A, MORALES LO, LÄNNENPÄÄ M, KEINÄNEN M, SOPANEN T, LASCOUX M (2004) Phylogenetic relationships of *Betula* species (*Betulaceae*) based on nuclear ADH and chloroplast matK sequences. Am. J. Bot. 91 (11): 1834-1845. http://dx.doi.org/10.3732/ajb.91.11.1834.

GROMTSEV AN (2008) The Fundamentals of the Landscape Ecology of European Boreal Forests of Russia. Petrozavodsk: KarRC RAS. (In Russ).

LOCKWOOD M, HARRISON RG, WOOLLINGS T, SOLANKI SK (2010) Are cold winters in Europe associated with low solar activity? Environ. Res. Lett. 2: 021001. http://dx.doi.org/10.1088/1748-9326/5/2/024001

MALIOUCHENKO O, PALMÉ AE, BUONAMICI A, VENDRAMIN GG, LASCOUX M (2007) Comperative phylogeography and population structure of European *Betula* species, with particular focus on *B. pendula* and *B. pubescens.* J. of Biogeogr. 34:1601-1610. DOI: 10.1111/j.1365-2699.2007.01729.x.

MATVEEVA TV, MASHKINA OS, ISAKOV YUN, LUTOVA LA (2008) Molecular passportization of clones of karelian birch using PCR with semi-specific primers. Jekologicheskaja genetika 6 (3): 18-23 (In Russ).

MIGALINA SV, IVANOVA LA, MAKHNEV AK (2009) Russ J Plant Physiol. 6: 948-953. (In Russ).

NATHO G (1959) Variationsbreite und Bestradbildung bei mitteleuropäischen Birkensippen. Feddes Repert. Spec. nov. regni veget. 61 (3): 211-273.

PALMÉ AE, SU Q, PALSSON S, LASCOUX M (2004) Extensive sharing of chloroplast haplotypes among European birches indicates hybridization among *Betula pendula*, *B. pubescens* and *B. nana*. Mol. Ecol. 13: 167-178. DOI: 10.1046/j.1365-294X.2003.02034.x.

PALMÉ AE, SU Q, RAUTENBERG A, MANNI F, LASCOUX M (2003) Postglacial recolonization and cpDNA variation of silver birch, *Betula pendula.* Mol. Ecol. 12: 201-212. DOI: 10.1046/j.1365-294X.2003.01724.x.

SALOJÄRVI J, SMOLANDER OP, NIEMINEN K, RAJARAMAN S, ... VETCHINNIKOVA L. ... et al. (2017) Genome sequencing and population genomic analyses provide insights into the adaptive landscape of Silver Birch. Nature Genetics 49 (6): 904-912. Doi:10.1038/ng.3862.

SCHENK MF, THIENPONT CN, KOOPMAN WJM, GILISSEN JWJ, SMULDERS MJM (2008) Phylogenetic relationships in *Betula* (Betulaceae) based on AFLP markers. *Tree Genet. Genomes* 4: 911-924. DOI: 10.1007/s11295-008-0162-0.

SCHOLZ E (1960) Die vegetative Vermehrung der Braunmaserbirke. Forst und Jagdz. Sondern. Forstl. Samenplantagen 2: 52-55.

TSVELEV NN (2002) On the genera *Betula* L. and *Alnus* Mill (*Betulaceae*) in Eastern Europe. Novosti sistematiki vysshikh rasteniy 34: 47-73 (In Russ).

VETCHINNIKOVA LV, TITOV AF, KUZNETSOVA TYU (2013) Curly birch: biological characteristics, resource dynamics, and reproduction. Petrozavodsk: KarRC RAS (In Russ).

VETCHINNIKOVA L, TITOV A. (2017). The origin of the Karelian birch: An ecogenetic hypothesis // Russian Journal of Genetics: Applied Research 7 (6): 665-677.

WALTERS SM (1968) *Betula* L. in Britain. Proc. bot. Soc. Br. Isl. 7 (2): 179-180.

WANG N, BORRELL JS, BODLES WJA, KUTTAPITIYA A, NICHOLS RA, BUGGS RJA (2014) Molecular footprints of the Holocene retreat of dwarf birch in Britain. Mol Ecol. 23 (11): 2771-2782. DOI: 10.1111/mec.12768.

WATTERSON GA, GUESS HA (1977) Is the most frequent allele the oldest? Theor. Popul. Biol. 11: 141-160.

The importance of fuel characteristics of poplars and aspens (*Populus* spp.) from German short rotation plantations and Russian forests

MATTHIAS MEYER[1], KATHRIN GEBAUER[2], ALWIN JANSSEN[3], DORIS KRABEL[1]

[1] TU Dresden, Faculty of Environmental Sciences, Department of Forest Sciences, Research Group for Molecular Tree Physiology, Pienner Str. 7, D-01737 Tharandt

[2] TU Dresden, Faculty of Mechanical Science and Engineering, Institute of Process Engineering and Environmental Technology, Chair of Energy Process Engineering, George-Bähr-Straße 3b, D - 01069 Dresden (kathrin.gebauer@tu-dresden.de)

[3] Bavarian Office for Forest Seeding and Planting (ASP), Forstamtsplatz 1, D-83317 Teisendorf (alwin.janssen@asp.bayern.de)

Abstract

Both Russia and the EU currently have large unused agricultural land resources that can sustainably be used for natural forest succession, for short rotation coppices (SRC) or for other dendromass supply. Poplars and aspens (*Populus* spp.) are among the most important fast growing tree species used in SRC or in forests of the northern hemisphere. Even if priority will be given in the future to material utilization of wood, dendromass (wood and/or bark biomasses) will remain an extraordinary important source of bio-fuel. Systematic samples from clonal hybrid poplar field trials on contrasting sites and from mature aspen stems infested by the aspen tinder fungus *Phellinus tremulae* were investigated. With this, the importance of the gross calorific value (GCV), of the ash content (% ash) and of the nitrogen content (% N) for fuel characterisation of dendromass was assessed. So far, the results indicate that GCV site variability is not very important for industrial utilization as compared with the over-all GCV in *Populus* dendromass. In contrast, % ash or % N or both can become important for bark utilization. Even with the observed high ash content of > 2 % in decayed aspen heartwood, Russian aspen resources which are affected by the stem rot provide an opportunity for sustainable dendromass supply.

Key words: SRC, calorific value, ash content, nitrogen content, dendromass, *Phellinus tremulae*

Introduction

Europe has made political decisions towards a bio-based economy to promote the transition off of the fossil-based industrial consumer society. In Germany, in addition, legislation was created that provides the roadmap for the so called 'Energiewende' (Energy Transition) away from nuclear and fossil-based energies. Both transition processes in the consume goods and in energy supply will likely result in a remarkable increase of the dendromass demand (= woody biomass = wood and/or bark biomass). A future wood supply gap was predicted for Europe (Mantau et al. 2010). Classic wood-based industries, wood-based bio-refineries as well as rather regional wood-based bio-energy supply systems will compete for the dendromass resources on the world market. Additional wood resources must be created, e.g. dedicated agricultural tree crops with fast growing poplars or other species, forest plantations on open land, or reforestation areas. With this, the pressure on existing natural forests must be reduced to help enabling future life on earth. At the end of all material utilization cascades, the thermal processing of disintegrated dendromass (chips, particles, fibres, pellets etc.) or of waste wood products will remain a key step in all circular wood utilization chains. Heat and power, bio-fuels, ash (for instance as fertilizer) or pyrolytic gases and oils will likely be the target products. Therefore, even if sustainable material utilization would be (legally) prioritized in the future, the fuel characteristics and pyrolytic behaviour will remain very important.

In accordance with the agricultural legislation, poplars or willows can be used as an agricultural biomass crop in the European Union (short rotation coppice, SRC). This had opened up a rather new chain for bio-energy supply some 20 years ago. Agro-industrial dendromass utilization is however still rarely implemented and lacks the exploitation of the full potential for material utilization. More traditionally, poplars have been used extensively as landscape and urban trees (e.g. along roads or waterways) or in planted forests in all European countries and in the former USSR. Nowadays, the lightweight poplar wood is the target raw material for important traditional and rather innovative industrial applications, e.g. veneer production for packaging, furniture or wood composites, for specifically bio-economical, industrial value chains (e.g. project dendromass4europe.eu) or for local bio-energy supply chains.

In Russia, the global framework conditions open up comparable opportunities for bio-energy supply chains. Unlike the European aspen, the Russian resources in aspen or mixed aspen forests are huge (en: trembling or common aspen; de: Aspe, Espe, Zitterpappel; ru: осина обыкновенная, тополь дрожащий; *Populus tremula* L.). But large aspen and poplar utilization is not yet well established. The importance of aspen for the forest species composition has been growing for many decades in the USSR and Russia. Two reasons account for this. Aspen wood was used less intensively than pine or spruce wood; and it is a distinct pioneer species exhibiting the ability to occupy open land or clear cuts extraordinarily fast. The aspen stem taper and quality of healthy wood is often excellent in Russian stands, much better than in Central and West Europe. However, the Russian aspen forests suffer from an aspen trunk rot (ru: сердцевинная гниль осины), caused by the false aspen tinder fungus (de: Aspen-Feuerschwamm; ru: ложный осиновый трутовик; *Phellinus tremulae* (Bond.) Bond. et Boris.). Often almost all old stems in a forest stand are affected and their financial wood value diminishes (cf. TSAREV 2013, YABLOKOV 1963). But unfortunately, the infected trees' viability is normally not affected and the old trees reproduce very well both from seeds and from root suckers. Likely, new bio-energy or bio-economy utilization pathways for the infested Russian aspen wood could promote a more intensive aspen utilization and removal from forests. This could in turn result in an improved forest composition with more conifer species in the future forest stands.

The present work introduces preliminary fuel characterization results for German SRC hybrid poplar samples and compares them with results for aspen (*P. tremula*) which was affected by the trunk rot caused by *Ph. tremulae*. Two major questions were focused:

- How relevant is the site effect on the gross calorific value (GCV), on the ash content (% ash) and on the nitrogen content (% N; can result in NO_x emissions)?
- Does the fuel quality of rotten aspen heartwood from northern forests allow efficient and sustainable thermal dendromass utilization?

Material and methods

In general, only wood discs were sampled. The German samples were kindly provided by project partners of the consortium FastWOOD (www.fastwood.org). These wood discs were taken at stem basis in three field trials which contrasted in their growth conditions. The trials were established for testing of newly bred clones. They can be ranked according to relative yield height as follows: Lehmbach (field trial with highest average yield, South Germany, Bavaria); Wallstawe ('medium' or 'good', North Germany, Saxony-Anhalt); Thammenhain (ranked last, Eastern Germany, Saxony). At each field trial, six trees per clone were sampled evenly distributed over the diameter spectrum of the respective clone in the field trial. For internally defined laboratory routines, only the part of the wood disc west of a north-south middle axis on the disc were taken for investigating fuel characteristics (Figure 1). All six specimens were carefully debarked with a chisel and a scalpel. The bark of the six trees and the wood were mixed separately in order to obtain one mixed wood sample and one mixed bark sample per clone and per field trial. For the present data, six clones were selected and their measurement results for each of the field trials were used for showing mean values or respective non-parametric data plots. This 6-clone subsample consisted of the

three not (yet) certified clones NW 7-234 L; NW 7-344 S; NW 7-91 R and of the three standard clones NW 7-728 Z (Hybride 275); NW 7-729 A (Max 1); NW 7-843 L (Robusta), cf. GROTEHUSMANN et al. (2015). All the trees were three years old. The six hybrid poplar clones belong to the black and/or balsam poplar sections (*Populus* spp., sectiones *Aigeiros*, *Tacamahaca*).

In contrast, the samples from northern East Europe were stem discs sampled on five different stems in an industrial plant. The stems derive from mature aspen trees (*P. tremula*, sectio *Populus*) infested with *Ph. tremulae* (Figure 1). Fuel characterization of aspen was carried out separately for bark, for healthy sapwood and for decayed heartwood samples.

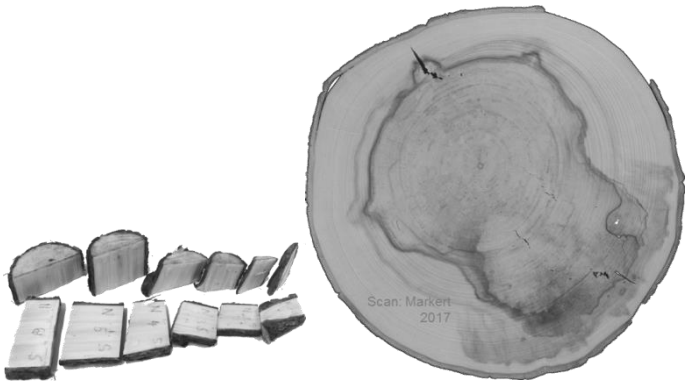

Figure 1: Left: Systematic 6-tree sample, evenly distributed over the diameter spectrum of a reference clone (hybrid poplar, *Populus* spp.), from a field trial of the project FastWOOD. Upper specimens are the parts of the wood discs which were oriented to the west and which were added to the present investigated mixed samples per clone / field trial. Right: Wood disc (d ≈ 25 cm) of a mature aspen stem (*Populus tremula*) from northern East Europe showing the symptoms of a long-lasting aspen tinder fungus (*Phellinus tremulae*) infestation, a distinct heart rot.

The treatment for measuring the traits of fuel characteristics was equal for all samples. The sample material was ground into a powder with an ultra-centrifugal mill (Retsch, ZM100, 0.25 mm particle diameter). The gross calorific value (GCV) was measured according to the standard DIN 51900-1:2000-04 using a bomb calorimeter (IKA C6000). The determination of the ash content (% ash) was carried out thermo-gravimetrically according to the standard ASTM E1131-98 or with a muffle furnace according to DIN EN ISO 18122:2015. Nitrogen content (% N) was derived using an elementary analyser Vario EL (Elementaranalysesysteme GmbH) in accordance with DIN EN ISO 16948: 2015.

Results and discussion

The gross calorific values (GCV) ranged from 17.88 MJ/kg for decayed aspen heartwood to 19.67 MJ/kg for aspen bark. This range is small as compared with the over-all mean value over all investigated wood or bark and hybrid poplar or aspen samples of 18.97 MJ/kg (Figure 2, Table 1). Most important differences of the GCV were observed between wood and bark (Figure 2). Hybrid poplars from SRC had a lower and the mature aspen a higher GCV in bark as compared with the respective wood samples. The difference between the GCV of hybrid poplar wood or bark samples from the three contrasting German field trials was small (range for wood: 19.44 to 19.58 MJ/kg; range for bark: 18.59 to 18.85 MJ/kg; Table 1). As expected, a lower GCV was observed for decayed aspen heartwood as compared with the respective healthy sapwood (17.88 vs. 18.53 MJ/kg). This difference illustrates a heart-rot related decrease of GCV by less than 4 % caused by the aspen tinder fungus (*Phellinus tremulae*). As a consequence, both the site effect in German SRC trials on GCV and the effect of wood decay caused by the aspen tinder fungus on

GCV do not need to be considered as relevant in terms of industrial dendromass utilization for bio-energy supply.

The ash content (% ash) and nitrogen content (% N) both show much higher variability than GCV, mainly between bark and wood samples (Table 1). Mean % ash ranged from 4.26 % in hybrid poplar bark from the field trial Thammenhain to 8.11 % in the bark of the mature aspens. Interestingly, the respective hybrid poplar wood samples from the field trial Thammenhain had the highest and the mature aspens the lowest mean % ash of all healthy wood samples (0.95 and 0.44 %). The mean % ash in hybrid poplars was shown to range from 0.68 to 0.95 % in wood and from 4.26 to 6.63 % between the three German SRC field trials. For decayed aspen heartwood, the mean % ash was 2.13 % which is approximately the fivefold higher % ash as compared with the healthy sapwood.

Mean % N was approximately five- to tenfold higher in bark as compared with wood samples. Both in bark and wood, the aspens which originate from northern East European forests had generally lower % N than the hybrid poplars from German SRC. Mean % N of the hybrid poplar bark samples ranged from 0.73 to 1.23 % and of the respective wood from 0.12 to 0.22 %. The higher mean % N was observed for the two field trials Thammenhain and Wallstawe that were used for classic agricultural cropping prior to SRC planting.

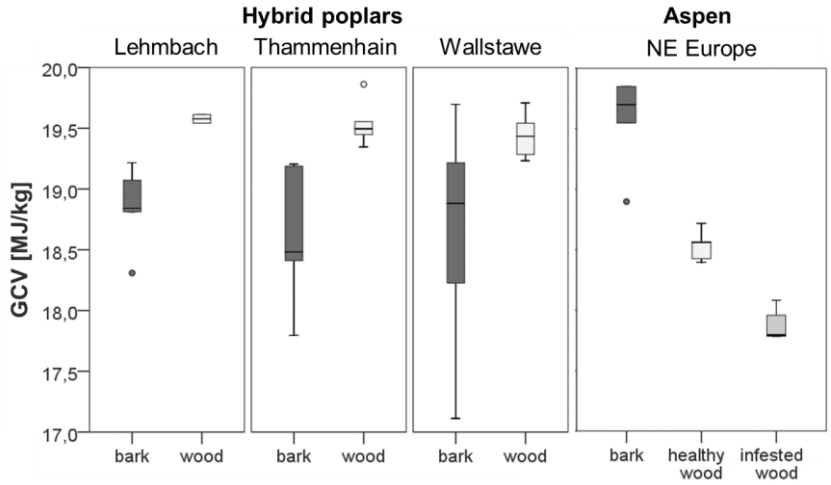

Figure 2: Comparison of gross calorific values (GCV, in MJ/kg, medians and quartiles) between bark and wood of hybrid poplars from short rotation coppices in three FastWOOD field trials (tree age 3, sampled at stem basis) and between bark, healthy sapwood and decayed heartwood of mature aspens from northern East Europe. (n = 6 for poplars, n = 5 for aspens) (MARKERT 2017, WACHSMUTH 2015).

The standard DIN EN ISO 17225-4 prescribes a maximum % ash of 3.0 % and a maximum % N of 1.0 % for high-quality woodchips. The present results generally show values for bark samples that tend to exceed the % ash threshold. However, in common fuel woodchips, the percentage of bark is much smaller than that of wood. Only in young stems from SRC with clones that have very high % ash, the ash content might become relevant for the quality of produced woodchips. This holds true for % N as well. The investigated wood samples from mature aspens revealed no excess over any of the mentioned thresholds even though the decayed wood had a significantly higher mean % ash of 2.13 %. The bark is relatively rich in ashes but the bark percentage in dendromass derived from mature aspen stems is very small. From the present data, it is however not clear whether the high % ash of decayed wood was due to the microbial decay by the aspen tinder fungus *Ph. tremulae* or due to generally higher % ash of heart wood. Unfortunately, the present study did not yet include healthy heartwood. Irrespective of this lack of explanation, decayed aspen wood seems to be potentially useful for bio-energy utilization because its GCV is only slightly

smaller than that of the respective healthy sapwood, its % ash is higher but it does not exceed the standard threshold and because its % N is smaller than in healthy sapwood.

Table 1: Comparison of gross calorific mean values (GCV) in MJ/kg ± standard deviation (±SD), mean contents of ashes and mean contents of nitrogen (% ash; % N; as % of water-free (wf) matter ±SD) between hybrid poplars from short rotation coppices in three German field trials (tree age 3, sampled at stem basis) and between bark, healthy sapwood and decayed heartwood of mature aspens from northern East Europe (n = 6 for poplars, n = 5 for aspens) (MARKERT 2017, WACHSMUTH 2015)

	Hybrid poplars Lehmbach		Hybrid poplars Thammenhain		Hybrid poplars Wallstawe		Aspen Northern East Europe		
	bark	wood	bark	wood	bark	wood	bark	healthy wood	decayed wood
GCV (wf) [MJ/kg]	18.85 ± 0.35	19.58 ± 0.05	18.59 ± 0.53	19.53 ± 0.18	18.67 ± 0.9	19.44 ± 0.17	19.67 ± 0.53	18.53 ± 0.13	17.88 ± 0.14
% ash (wf)	6.63 ± 0.27	0.74 ± 0.06	4.26 ± 1.54	0.95 ± 0.22	5.35 ± 0.62	0.68 ± 0.05	8.11 ± 1.6	0.44 ± 0.08	2.13 ± 0.67
% N (wf)	0.73 ± 0.1	0.12 ± 0.03	1.23 ± 0.13	0.2 ± 0.07	1.08 ± 0.18	0.22 ± 0.07	0.53 ± 0.14	0.06 ± 0.07	0.04 ± 0.04

Conclusions

The present results suggest that the site-specific differences of the gross calorific values are not important in terms of industrial bio-energy production using poplar dendromass. Different soil or site conditions result in differences in the ash and nitrogen contents of bark that are relevant at least for the utilization of dendromass with a very high bark percentage.

So far, the results indicate that fuel characteristics of decayed Russian aspen heartwood allow sustainable bio-energy production with raw aspen wood produced in northern forests.

Acknowledgements

The project FastWOOD (II and III) has received funding from the FNR e.V. / BMEL (FKz.: 220-027-11; 220-170-07) and the participation of the first author at the German-Russian Symposium on Forest Genetics was kindly supported by the Thünen Institute for Forest Genetics; both are gratefully acknowledged. We thank the BSc. students JOHANNA WACHSMUTH and MATTHIAS MARKERT for their substantial support in the laboratory.

References

GROTEHUSMANN H, JANSSEN A, HAIKALI A, HARTMANN K-U, HÜLLER W, KAROPKA M, SCHILDBACH M, SCHIRMER R, SCHUPPELIUS T, TÖPFNER K (2015) Pappelsortenprüfungen im Projekt FastWOOD [Poplar variety test in the project FastWOOD]. Forstarchiv 86 (3): 67-79.

MANTAU U, SAAL U, PRINS K, STEIERER F, LINDNER M, VERKERK H, EGGERS J, LEEK N, OLDENBURGER J, ASIKAINEN A, ANTTILA P (2010) EUwood - Real potential for changes in growth and use of EU forests. Final report of the research project EUwood. University of Hamburg, Centre of Wood Science, Hamburg: 126 pp.

MARKERT M (2017) Vergleich des Brennstoffcharakters des von Stammfäule betroffenen und nicht betroffenen Aspenholzes (*Populus tremula* L.) mit dessen Rinde. BSc thesis. German language. TU Dresden, Department of Forest Sciences, Tharandt: 45 pp.

TSAREV AP (2013) Growth and breeding of aspen in Russia. Silvae Genetica 62 (4-5): 153-160.

WACHSMUTH J (2015) Sortenunterschiede des biogenen Brennstoffes aus Rinde von jungen Plantagen-Pappeln (*Populus* spp.) unter Berücksichtigung ausgewählter deutscher Standorte. BSc thesis. German language. HTW Dresden University of Applied Sciences, Dresden: 64 pp.

YABLOKOV AS (1963) Growth and breeding of healthy aspen. Russian language. Goslyesbumisdat, Moscow, Russia: 442 pp. [Яблоков А.С. *Воспитание и разведение здоровой осины*. М. Гослесбумиздат, 442 с.]

II. Population Genetics and Gene Conservation

Twenty years German-Russian co-operation for genetic diversity in forests

BERND DEGEN[1], HILKE SCHROEDER[1], CÉLINE BLANC-JOLIVET[1], YULAI YANBAEV[2]

[1] Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany (bernd.degen@thuenen.de, hilke.schroeder@thuenen.de, celine.blanc-jolivet@thuenen.de)

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

Abstract

Since 1994 there is a close cooperation among the forest genetic groups in Grosshansdorf (Germany) and Ufa (Russia). In the beginning of the common research, questions linked to genetic impacts air pollution on forests have been addressed. Then comparative studies on genetic variation and genetic differentiation of trees (oak, pine, larch) and insects (green oak leaf roller) have been conducted. A study of an isolated small pedunculate oak population on the south-eastern edge of the species distribution in the South-Urals brought insights on the extend of long distance pollen flow and a surprisingly high level of genetic diversity of its pest (green oak leaf roller). In the recent years, the work on large scale genetic structure of larch and different white oak species has been a focus. These results find their application for tests on tree species and geographic origin on wood samples and have the purpose to secure the trade of legally harvested timber.

Key words: Genetic diversity, genetic differentiation, green leaf roller, oak, pine, timber tracking

Research topics

Impact of air pollution

In the period from 1980 to 1995, a lot of research has been done on the impact of air pollution on forest ecosystems. One aspect studied was the influence of air pollution on the genetic composition of tree populations and genetic differences among tolerant and sensitive trees (SCHOLZ et al. 1989). In Germany, specific focus has been given to beech, spruce and pine (BERGMANN and SCHOLZ 1987, GEBUREK et al. 1987, IPSEN et al. 1998, MUELLER-STARCK 1985). The main result was – based on allozymes studies – that there was a significant risk in losing genetic diversity due to air pollution and another general finding was that the tolerant trees had higher levels of heterozygosity. Thus, for example, genetic inventories were made in six old beech stands in Germany exposed to long-term air pollution stress, identifying genotypes by paired comparison between tolerant and sensitive trees (MUELLER-STARCK and HATTEMER 1989). In this study, genetic comparisons were also made on germinating seed samples, and on young plants surviving under different stress conditions in the field. All the comparisons gave statistically significant differences for the genetic composition, especially those between germinating seed and surviving young plants. The results revealed losses of genetic variation in the group of sensitive individuals, and confirmed the importance of a high degree of heterozygosity and great genetic diversity for the survival ability of beech populations.

Using isozymes of five polymorphic loci the frequency of mutations and mutation-like events in populations of Scots pine (*Pinus sylvestris* L.) in South Urals (Russia) were studied (BAKHTIYAROVA et al. 1995). The frequency of rare electrophoretic variants of isozymes was shown to be significantly higher in two populations growing under industrial air pollution conditions (p=5.0 x 10(-3) and p=5.2 x10(-3)) than that of the control (1.6 x 10(-4)).

Green oak leaf roller (Tortrix viridana)

A geographically isolated population of an herbivorous insect (*Tortrix viridana*, Lepidoptera: Tortricidae) in the Bashkir Transural region and five further populations were investigated for genetic variation using eight microsatellite markers (SCHROEDER et al. 2010). The sample size per population was between 48 and 62 individuals. The genetic variation was higher within the isolated population than within populations in the centre of the distribution area. No bottleneck effects were discovered during analyses that could have formatted the gene pool of this population. Balancing or directed selection toward preservation of specific alleles or higher fitness of heterozygous individuals could be an explanation for the unexpected high genetic diversity within this small and isolated population.

In another study, a total of 401 individuals of the green oak leaf roller from four stands in North Rhine-Westphalia (Western Germany) were examined. In three of four populations, the AFLP markers revealed clearly spatial genetic structure up to 40 m, which can be explained by the mating behaviour within this species (SCHROEDER and DEGEN 2008).

Genetic diversity and differentiation of oaks

The large scale genetic structure of European white oaks was studied for chloroplast gene markers with samples from more than 2600 populations (PETIT et al. 2002). The observed pattern is the result of the re-colonisation process from different refugia after the last glacial period. The group in Grosshansdorf contributed to this work samples and results for Germany and the Benelux countries (KOENIG et al. 2002). Oak chloroplast DNA (cpDNA) variation was studied in a grid-based inventory in western Central Europe, including Belgium, The Netherlands, Luxembourg, Germany, the Czech Republic, and the northern parts of Upper and Lower Austria. A total of 2155 trees representing 426 populations of *Quercus robur L*. and *Q. petraea* (Matt.) Liebl. were screened for polymorphism in up to four PCR-amplified cpDNA fragments. Eleven haplotypes belonging to four lineages were detected; these lineages were formerly restricted to glacial refugia in the Iberian Peninsula, the Apennine Peninsula and the Balkan Peninsula (Figure 1). The haplotypes originating from the Apennines are particularly well represented in the study region, but there is also a significant contribution from the other refugia, which explain the high overall level of cpDNA diversity. The strong human impact in western Central Europe during the past centuries, which has resulted in the clearance of most forests, was followed by reforestation, sometimes involving seed transfers. Despite this strong human impact, broad geographic patterns of lineages and haplotypes could still be detected.

We studied also material collected in Russia. This material could be clearly genetically separated from the genetic composition in Central Europe. The extent and spatial pattern of genetic variation at polymorphic allozyme loci in a population of pedunculate oak *Quercus robur* from the Bashkir Transural region was investigated using autocorrelation analysis (REDKINA et al. 2008). In the stand examined, statistically significant local concentration of most of the alleles in two-dimensional space was identified. Conservation strategies for this small population located outside of the western border of the species range, in the mountain-steppe habitat, and characterized by specific gene pool, were suggested.

To detect and avoid illegal logging of valuable tree species, identification methods for the origin of timber are necessary. In a German-Russian-US American cooperation project we used next-generation sequencing to identify chloroplast genome regions that differentiate the origin of white oaks from the three continents; Asia, Europe, and North America (SCHROEDER et al. 2016). For details on this see paper of SCHROEDER et al. in these proceedings.

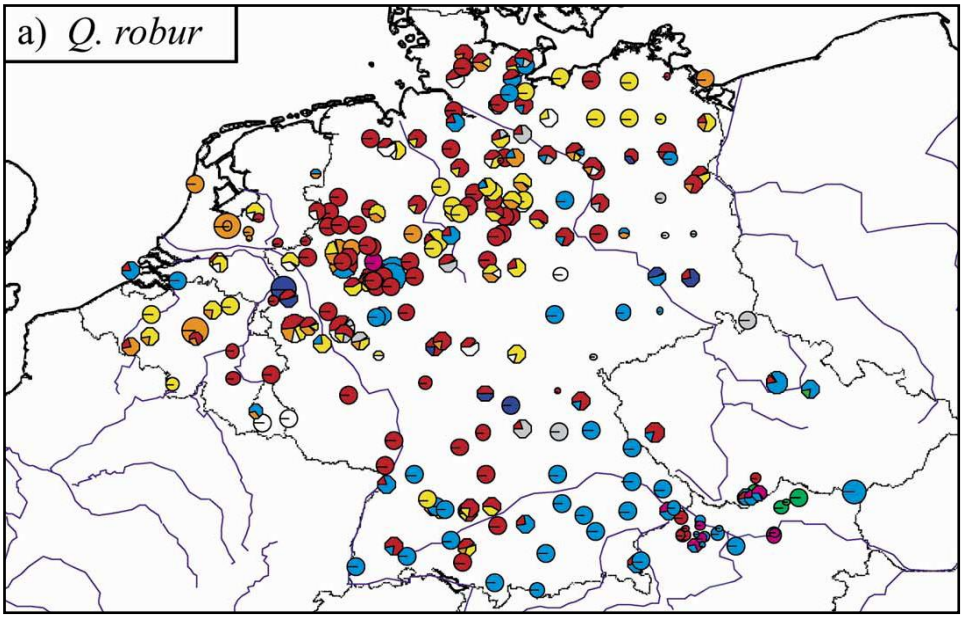

Figure 1: Distribution of chloroplast haplotypes of *Quercus robur* in Central Europe (from KOENIG et al. 2002)

Gene flow

In another German-Russian cooperation study we analysed long-distance pollen-mediated gene flow into an isolated relict stand (Figure 2) consisting of seven individuals of *Quercus robur* based on a total sample of 177 trees and 9 microsatellite loci (BUSCHBOM et al. 2011). We showed that pollen-mediated gene flow across more than 80 km in this wind-pollinated tree species contributed at least 35% of all successful pollinations in the investigated isolated and small oak stand at the eastern limit of the species' distribution. The observed pollen immigration shaped the genetic diversity of acorn progenies in the stand and might explain the comparably high genetic diversity in the persisting adult population.

Figure 2: Isolated pedunculate oak populations studied in the south-east Ural region.

Larix

More than 2000 larch samples have been collected in a transect from Western Europe to Japan. Using RAD sequencing, a large set of SNPs has been developed and the material has been genotyped on more than 250 SNPs. The results enable us to differentiate among six larch species and to check the geographic origin. For more details see the paper of Blanc-Jolivet et al. in these proceedings.

Outlook

Our future cooperation will continue to work on large scale genetic structure of spruce, pine, birch and aspen, with focus in the Western part of Russia. Further, we intend to intensify the technology transfer to the genetic lab of the Bashkiria state university in Ufa.

References

BAKHTIYAROVA RM, STAROVA NV, YANBAEV YA (1995) Genetic changes in populations of Scots pine growing under industrial air pollution conditions. Silvae Genet. 44(4): 157-160.

BERGMANN F, SCHOLZ F (1987) The impact of air-pollution on the genetic structure of Norway spruce. Silvae Genet. 36(2): 80-83.

BUSCHBOM J, YANBAEV Y, DEGEN B (2011) Efficient Long-Distance Gene Flow into an Isolated Relict Oak Stand. J. Hered. 102(4): 464-472.

GEBUREK T, SCHOLZ F, KNABE W, VORNWEG A (1987) Genetic studies by isozyme gene loci on tolerance and sensitivity in an air polluted pinus sylvestris field trial. Silvae Genet. 36(2): 49-52.

IPSEN A, KASTEN B, SCHOLZ F, ZIEGENHAGEN B (1998) Studying allelic diversity and stress response of PEPC (phosphoenolpyruvate carboxylase) in Norway spruce (*Picea abies*). Chemosphere 36(4-5): 825-828.

KOENIG AO, ZIEGENHAGEN B, VAN DAM BC, CSAIKL UM, COART E, DEGEN B, BURG K, DE VRIES SMG, PETIT RJ (2002) Chloroplast DNA variation of oaks in western Central Europe and genetic consequences of human influences. For. Ecol. Manage. 156(1-3): 147-166.

MUELLER-STARCK G (1985) Genetic differences between tolerant an sensitive beeches (*Fagus sylvatica*) in an environmentally stressed adult forest stand Silvae Genet. 34(6): 241-247.

MUELLER-STARCK G, HATTEMER HH (1989) Genetic effects of environmental stress on old stands and young growth of beech (*Fagus sylvatica*) Genetische Auswirkungen von Umweltstress auf Altbestande und Jungwuchs der Buche (*Fagus sylvatica* L.). Forstarchiv 60(1): 17-22.

PETIT RJ, CSAIKL UM, BORDACS S, BURG K, COART E, COTTRELL J, VAN DAM B, DEANS JD, DUMOLIN-LAPEGUE S, FINESCHI S, FINKELDEY R, GILLIES A, GLAZ I, GOICOECHEA PG, JENSEN JS, KONIG AO, LOWE AJ, MADSEN SF, MATYAS G, MUNRO RC, OLALDE M, PEMONGE MH, POPESCU F, SLADE D, TABBENER H, TAURCHINI D, DE VRIES SGM, ZIEGENHAGEN B, KREMER A (2002) Chloroplast DNA variation in European white oaks - Phylogeography and patterns of diversity based on data from over 2600 populations. For. Ecol. Manage. 156(1-3): 5-26.

REDKINA NN, MULLAGULOV RY, YANBAEV YA, DEGEN B (2008) Fine spatial structure of allozyme genotypes in isolated population of pedunculate oak *Quercus robur* L. (Fagaceae). Russ. J. Genet. 44(8): 997-999.

SCHOLZ F, GREGORIUS H-R, RUDIN D (1989) Genetic Effects of Air Pollutants in Forest Tree Populations: Proceedings of the Joint Meeting of the IUFRO Working Parties Genetic Aspects of Air Pollution Population and Ecological Genetics Biochemical Genetics held in Großhansdorf, August 3–7, 1987. Springer Science & Business Media

SCHROEDER H, CRONN R, YANBAEV Y, JENNINGS T, MADER M, DEGEN B, KERSTEN B (2016) Development of Molecular Markers for Determining Continental Origin of Wood from White Oaks (*Quercus* L. sect. *Quercus*). PLoS One 11(6): 15.

SCHROEDER H, DEGEN B (2008) Genetic structure of the green oak leaf roller (Tortrix viridana L.) and one of its hosts, Quercus robur L. For. Ecol. Manage. 256(6): 1270-1279.

SCHROEDER H, YANBAEV Y, DEGEN B (2010) A Very Small and Isolated Population of the Green Oak Leaf Roller, *Tortrix viridana* L., with High Genetic Diversity-How Does This Work? J. Hered. 101(6): 780-783.

Nucleotide polymorphism and genetic structure of populations of *Populus* in the Ural

S.V. BORONNIKOVA, T.N. LISINA, N.A. MARTYNENKO

Department of Botany and Genetics of Plants, Perm State University, Bukirev St., 15, Perm, Russia (SVBoronnikova@yandex.ru)

Abstract

We studied nucleotide polymorphism of 14 genes involved in formation of the cell wall or cell differentiation (*PopLTP1*, *PopFLA*, *PtrCOBL4*, *PopCHT*), resistance to water stress and dehydration (*Erecta*, *PopDhn1*), the biosynthesis of lignin (*Ptr4CL1*, *CAM1*, *F5H1*, *C4H1*, *GH3-5*) and resistance to pathogens (*PIN-T*, *TR-INH2*, *PatRelProt*, *OX-RED*, *RIB-PROT*) in seven populations of *Populus tremula* L. in Perm Krai. In the sequences of 5 genes of lignin biosynthesis 29 SNPs were revealed, whereof only 4 (13.8 %) of them were non-synonymous SNPs that led to the replacement of the amino acids. The highest frequency of SNPs in the genome of *P. tremula* were identified in introns (1 SNP per 51 nucleotide), and lowest in exons (1 SNP per 217 nucleotides). The average frequency of SNPs in the genome of *P. tremula* was 1 SNP per 92 nucleotides, which was higher by 13.0 % than in genome of *P. trichocarpa* and by 21.7 % than in genome of *P. nigra*. Analysis of genetic structure of populations of two poplar species in the Urals shows that *P. tremula* populations in the Middle and Southern Urals are differentiated more (G_{ST}=0.550) than *P. nigra* (G_{ST}=0.239). On the basis of molecular genetic analysis the method of molecular-genetic identification of woody plant species populations is developed (BORONNIKOVA and BOBOSHINA 2012).

Key words: molecular markers, genome, polymorphism, SNP, genetic structure, method of molecular-genetic identification, *Populus tremula* L., *P. nigra*

Introduction

Genetic structure of populations determines the adaptive capacity and sustainability of natural populations, therefore, development of a strategy for the conservation and sustainable use of forest resources requires a deep knowledge of their genetic diversity at the population level. The successful solution of problems of population genetics of many species depends on the knowledge of the different elements of the genome and polymorphism. The genus *Populus* L. became a model native woody deciduous plants due to the relatively small genome and huge adaptive capabilities (Douglas, 2017). Representatives of this genus have a wide geographic distribution and are among the fastest growing woody plants in the temperate zone of the Northern hemisphere (JANSSON et al. 2010). The North American species of poplar *Populus trichocarpa* Torr. et A. Gray was the first woody plant species, whose genome was completely sequenced (TUSKAN et al. 2006). Russia, including the Urals, has unique genetic resources of the genus *Populus*, but the genetic diversity of populations of species of poplar, especially at the nucleotide level has not been studied. The aim of the research is to study the nucleotide polymorphism and the genetic structure of populations of two species of poplar (*P. nigra and P. tremula*) in the Middle and Southern Urals.

Material and methods

As an objects of research 7 populations of trembling poplar or aspen (*Populus tremula* L.) and 4 populations of black poplar or poplar (*Populus nigra* L.) located in the Middle Urals (Perm Krai) and in

South Ural (Bashkortostan) were selected. In National Institute of Agricultural Research (INRA, France; Unité de Recherches en Génomique végétale,) a strategy of study the nucleotide diversity of the species of the genus *Populus* was developed (GAUDET et al. 2008), 27 candidate genes involved in the processes of formation of the cell wall, cell differentiation, biosynthesis of lignin, resistance to pathogens were selected. For sequencing reaction the Big Dye® Terminator v3.1 Cycle Sequencing Kit («Applied Biosystems», USA) was used together with a single reverse primer from the pair of primers that were used for the PCR amplification. Purification of the sequencing reaction products from the unreacted labeled nucleotides were carried out using a set of BigDye® XTerminator™ Purification Kit («Applied Biosystems», USA). Sequencing of the synthesized sequences was performed in a PCR lab of Department of Botany and Genetics of Plants, Perm State University (Russia) in a 24-capillary Genetic Analyzer 3500xL («Applied Biosystems», USA). Primer design was carried out with the program Primer 3 on the basis of the genome *P. trichocarpa* in INRA (France). Sequenced DNA sequences were compared with available sequences in the genetic databases through a system of automatic on-line BLAST alignment. Multiple sequence alignment was carried out in MultAlin (CORPET 1988).

The genetic diversity of populations of two species of poplar also was studied using ISSR-markers polymorphism (Inter-Simple Sequence Repeat, Zietkiewicz et al., 1994). Amplification of DNA samples was also carried out in thermocycler Gene Amp PCR System 9700 («Applied Biosystems», USA). The detection of amplification products was done by using electrophoresis in 2% agarose gel in 1x TBE buffer. It was colored with ethidium bromide and photographed in passing ultraviolet light system gel documentation Gel Doc XR («Bio-Rad», USA). For the analysis of population genetic structure, following parameters were used (NEI 1975): the expected proportion of heterozygous genotypes (H_T) in the whole population; the expected proportion of heterozygous genotypes in the subpopulation (H_S); the proportion of interpopulation genetic diversity in general, diversity or increased differentiation of populations (G_{ST}). Cluster data evaluation was made using the program STRUCTURE 2.3.4 (FALUSH et al. 2003).

Results

With each of the 36 pairs of primers developed for 27 poplar candidates genes in INRA (France) PCR was performed using the genomic DNA of *P. tremula* from the Perm region (Russia). 16 pairs of primers were informative for 14 genes of *P. tremula* (SVETLAKOVA 2012). These genes can be divided into 4 groups: the formation of cell walls and cellular differentiation (*PopLTP1*, *PopFLA*, *PtrCOBL4*, *PopCHT*), resistance to water stress and dehydration (*Erecta*, *PopDhn1*), the biosynthesis of lignin (*Ptr4CL1*, *CAM1*, *F5H1*, *C4H1*, *GH3*-5) and resistance to pathogens (*PIN-T*, *TR-INH2*, *PatRelProt*, *OX-RED*, *RIB-PROT*). To analyze the frequency and location of SNPs we sequenced 5 genes involved in the biosynthesis of lignin in 7 populations of *P. tremula*. 29 SNPs were detected in these 5 loci of *P. tremula*. The most conservative was the *C4H1* gene. The non-synonymous SNPs were 13.8% of all SNPs. In the fourth population SNPs were discovered, which were not observed in other populations: the SNP A/G at position 272 in the *GH3-5* gene with a frequency of 0.500 and SNP C/T at position 85 in the *GH5-3* gene with a frequency of 0.375 (Table 1).

The study of the genetic structure of 7 populations of *P. tremula* based on polymorphism of ISSR markers showed (SVETLAKOVA et al. 2013) that the proportion of heterozygous genotypes in the general population (H_T) of *P. tremula* was 0.281, and the expected proportion of heterozygous genotypes in the subpopulation (H_S) – 0.127, therefore, the coefficient of differentiation of populations (G_{ST}) of the studied populations of *P. tremula* was 0.550.

Cluster analysis of molecular-genetic data of 7 populations of *P. tremula* using the program STRUCTURE 2,3,4 confirmed that these 7 populations are separate genetic populations (Figure 1).

Table 1: SNPs in sequences of 5 genes of *P. tremula*

Loci	Primer sequences (5'-3')	Amount of SNPs in general	The number of synonymous substitutions	The number of non-synonymous substitutions
C4H1	F-AATCTCTTTCAGTACTCCTTTGG R-GCAGCCTTCTCTCTTTAACC	1	1	0
Ptr4CL1	F- AACTCACCATCTCTCCCTCT R- CCTCCAGATTTTATCATCCTC	6	5	1
CAM1	F- GTGGATGCTGACAAGGACAA R- TCCACTTACAGAAATTTAAGAGAGG	4	3	1
F5H1	F- AACATCCATAGGCACCAAC R- CAGGGAATGAAATCAGACA	10	10	0
GH3-5	F- ACCCATCATCATTACCTCAA R- TTGAATGCCCAAGTTCTG	8	6	2
Bcero:		29	25	4

Notice: C4H1 – trans-cinnamate 4-monooxygenase, Ptr4CL1 – 4-Coumarate: CoA ligase, CAM1 – trans-caffeoyl-coa 3-o-methyltransferase, F5H1 – flavonoid 3'-hydroxylase, GH3-5 – GH3 family protein

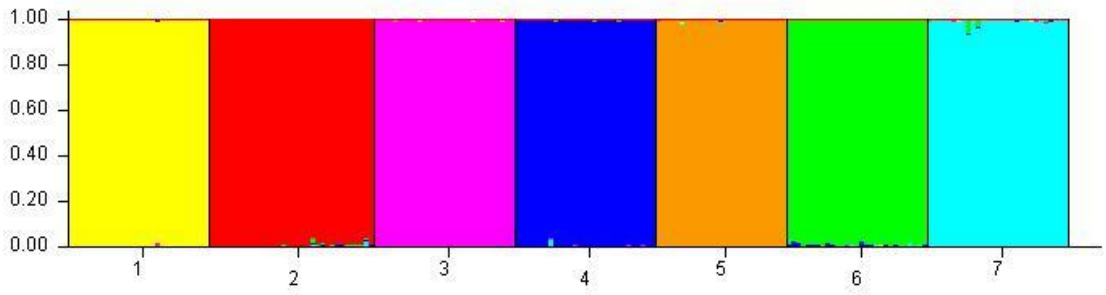

Figure 1: The genotypes distribution of studied populations of *P. tremula* from Ural using the program STRUCTURE 2,3,4. On the vertical axis – the proportion of allele frequency of the corresponding cluster, on the horizontal axis– the number of populations; colors with different shading indicated population *P. tremula.*

The analysis of genetic structure of 4 populations of *P. nigra* on the Middle and Southern Ural showed that the expected proportion of heterozygous genotypes in the total sample (H_T) was 0.291, and the expected proportion of heterozygous genotypes (H_S) in subpopulations smaller than in *P. tremula* and *P. nigra* was 0.221. The coefficient of differentiation of populations (G_{ST}) of *P. nigra* is below and equal 0.239.

Discussion

From 36 pairs of primers developed for 27 poplar candidate genes (GAUDET et al. 2008), 16 pairs for 14 genes turned out to be effective for *P. tremula*. In 5 genes sequenced in Perm populations of *P. tremula* 56 SNPs were determined. It was found that the most variable in populations of *P. tremula* from Perm Krai are genes of resistance to pathogens (1.62 SNPs per 100 nucleotide positions), and the most conservative are genes of cell wall formation and cell differentiation (0.75 SNPs per 100 nucleotide positions). In the genome of *P. tremula* the highest frequency of SNPs was in introns (1 SNP per 51 nucleotides) and the lowest – in exons (1 SNP per 217 nucleotides). Multiple alignment showed that for the other species of this genus (*P. tremuloides, P. trichocarpa* and *P. tomentosa*) the *C4H1* gene was also quite conservative.

Comparing the nucleotide polymorphism with the similar indicators of the two other species of the genus *Populus* (*P. nigra* - 1 SNP per 112 nucleotides, *P. trichocarpa* - 1 SNP per 104 nucleotides), *P. tremula* in the Perm region revealed a higher level of nucleotide variability – 1 SNP per 92 nucleotides (SVETLAKOVA et al. 2014).

Studied populations of *P. tremula* from the Middle and Southern Urals differentiated in the greater degree (G_{ST} = 0.550) than *P. nigra* (G_{ST} = 0.239).

On the basis of molecular-genetic analysis recommendations for conservation and usage of populations of two species of poplar in the Urals were given. Also, using the developed method of molecular-genetic identification of populations of woody plant species based on the polymorphism of two types of molecular markers (BORONNIKOVA and BOBOSHINA 2012) the selection of populations and trees was made for the conservation of genetic resources of poplars to the extent of their intra - and interpopulation genetic variation.

References

BORONNIKOVA SV, BOBOSHINA IV (2012) Method of molecular genetic identification of populations of tree species [Sposob molekulyarno-geneticheskoy identifikatsii populyatsyy drevesnikh vidov rasteniyj] Patent RF, no. 2012119341. (In Russ.).

BRUNNER AM, BUSOV VB, STRAUSS SH (2004) Poplar genome sequence: functional genomics in an ecologically dominant plant species. Trends in Plant Science 9 (1): 49-56.

CORPET F (1988) Multiple sequence alignment with hierarchical clustering. Nucleic acids research 16 (22): 10881-10890.

DOUGLAS CJ (2017) *Populus* as a Model Tree. In: GROOVER A and CRONK Q (eds.) Comparative and Evolutionary Genomics of Angiosperm Trees 21: 61-84. Springer International Publishing AG, Cham, Switzerland: 366 pp. (ISBN 978-3-319-49327-5)

FALUSH D, STEPHENS M, PRITCHARD JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164(4): 1567-1587.

GAUDET M, JORGE V, PAOLUCCI I (2008) Genetic linkage maps of *Populus nigra* L. including AFLPs, SSRs, SNPs, and sex trait. Tree Genetics and Genomes 4: 25-36.

JANSSON S, BHALERAO SH, GROOVER AT (2010) Preface. In: S. Jansson, S.H. Bhalerao; A.T. Groover (eds.), Genetics and Genomics of Populus 8, 5-7, Springer, New York, USA: 381 pp. (ISBN 978-1-4419-1540-5)

NEI M (1975) Molecular population genetics and evolution, North Holland publishing company, Amsterdam, Netherlands: 278 pp. (ISBN 0 7204 7141 9)

SVETLAKOVA TN (2012) Molecular genetic analysis and condition assessment of the gene pools of *Populus tremula* L. populations in the Permskiy Krai. Synopsis of Thesis for a Candidate's Degree [Molekulyarno-geneticheskiy analiz i otsenka sostoyaniya genofondov populyatsyy Populus tremula L. v Permskom krae. Avtoreferat dissertatsii kandidata biologicheskikh nauk]. Printing house of PSU, Perm, Russia, 20 pp. (In Russ.).

SVETLAKOVA TN, BOBOSHINA IV, BORONNIKOVA SV et al. (2013) An ecological and genetic analysis of population structure Populus tremula L. in Perm region. Russian Journal of Genetics: applied researches 3 (5): 382-387.

SVETLAKOVA TN, BORONNIKOVA SV, YANBAEV YA (2014) Genetic diversity and differentiation in Ural populations of the aspen, *Populus tremula L.*, as revealed by inter-simply sequence repeat (ISSR) markers. Silvae Genetica 63 (1-2): 39-41.

TUSKAN GA, DIFAZIO S, JANSSON S et al. (2006) The genome of black cottonwood, Populus trichocarpa (Torr. and Gray). Science 313: 1596-1604.

ZIETKIEWICZ E, RAFALSKI A, LABUDA D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20 (2): 176-183.

GenMon - Implementation of a Genetic Monitoring System in European Beech (*Fagus sylvatica* L.) and Norway Spruce (*Picea abies* [L.] Karst.) Populations in Germany

JENS SCHMIEDEL[1], UTE TRÖBER[1], HEINO WOLF[1], BARBARA FUSSI[2], MARCO KUNZ[2]

[1] Staatsbetrieb Sachsenforst, Kompetenzzentrum für Wald und Forstwirtschaft, Bonnewitzer Str. 34, 01796 Pirna, Germany (jens.schmiedel@smul.sachsen.de, ute.troeber@smul.sachsen.de, heineo.wolf@smul.sachsen.de)

[2]Bayerisches Amt für forstliche Saat- und Pflanzenzucht, Forstamtsplatz 1, 83317 Teisendorf, Germany (barbara.fussy@asp.bayern.de, marco.kunz@asp.bayern.de)

Abstract

The reduction of the loss of genetic diversity and the protection of the sustainability of forest ecosystems and all their habitats and services are objectives of high priority for forest management. Furthermore, long-term adaptability of forest stands to a changing environment must be ensured through adapted forest management. A population's adaptability is determined by genetic variation. However, established environmental monitoring systems consider biodiversity only on the species level to evaluate state and development of a forest stand. Aspects regarding the genetic system on a fundamental level have not been integrated so far, although the genetic system creates the basis of reactivity and adaptability of forest tree populations to cope with changing environmental influences. In Germany, a concept for a genetic monitoring scheme in forest tree species was elaborated, which aspires to delineate the genetic system in its entirety on the groundwork of criteria, indicators and verifiers. The implementation of the concept to the structures of German state forest institutions is the main objective of the ongoing project GenMon, which establishes a net of monitoring plots for European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) to survey the intactness of the genetic system in observed populations.

Introduction

It is a task of today's and future generations to reduce the ongoing loss of biodiversity at global, national and local levels (GRAUDAL et al. 2014, MONASTERSKY 2014). Furthermore, the adaptability of forest ecosystems to changing environment must be ensured. The adaptability of trees to environmental factors such as climate change or outbreaks of pests and diseases depends fundamentally on their genetic variation. Therefore, the conservation and monitoring of biodiversity on all levels (species, population and genetic, CBD 1992) is essentially (GREGORIUS and DEGEN 2007). In order to allow a proper prediction of the adaptability of trees and to stop the erosion of genetic diversity, the existing monitoring has to be supplemented with a monitoring system on population level (KONNERT et al. 2011, TRÖBER et al. 2011, FUSSI et al. 2016).

Genetic variation with its inherent amplitude of allelic and genotypic structures determines adaptability and capability of forest ecosystems. It forms the fundamental basis for a sustainable development of forests and its biological diversity (GREGORIUS and DEGEN 2007). Currently, the increasing temperatures resulting from climate change may cause a directional selection within populations and result in altering genotype frequencies as evolutionary reaction (JUMP et al. 2006). It implicates a movement of efforts on national and international levels to conserve genetic diversity in situ and ex situ. In this context numerous efforts, organizations and collaborating activities like IUFRO, EUFORGEN and the CBD-process should be mentioned.

Via various pilot studies there are certain attempts in Germany. The “Concept for Conservation of Forest Genetic Resources” was published 1987 and altered in 2000 and 2010. The current version of PAUL et al. (2010) describes the necessity of an implementation of genetic monitoring in forest ecosystems. As mentioned before, genetic aspects are still lacking in environmental monitoring programs that have been established in the last 20 years. With well-considered criteria and indicators, information from genetic monitoring can serve as an early warning and controlling system, because changes in the stand structures, vitality and natural regeneration can be observed earlier than in the natural stand (KONNERT and HOSIUS 2010). From this point of view the implementation of a genetic monitoring system is overdue.

The genetic system of populations comprises all mechanisms and processes resulting in the generation, modification and maintenance of genetic variation as well as in its transfer to the following generations. The German “Concept for Genetic Monitoring in Forests” as published by the BLAG-GROUP OF EXPERTS "GENETISCHES MONITORING" (2004) is based on indicators proposed by (NAMKOONG et al. 1996) for population genetic processes concerning the following points:

- level of genetic variation,
- directional change in gene or genotypic frequencies,
- changes in mating system processes and
- gene migration between populations.

Gene frequencies and different genetic parameters based on gene frequencies, like genetic diversity, number of polymorphic loci, mean number of alleles per locus can be applied efficiently as measures for assessing the level of genetic variation. The outcrossing rate, the number of effective pollen donors, but also the quality of seed reflected by the proportion of empty seed or by germination capacity supply valuable information about the mating system. Gene migration is verified by pollen and seed dispersal distance as well as by presence or absence of family structures within a tree population.

The determination of indicators by repeated research at different times can reveal the dynamics and conditions of the processes of the genetic system (GREGORIUS and DEGEN 2007). To evaluate different methodical aspects, pilot studies have been performed for two tree species with a different mating behavior – wild cherry (*Prunus avium* L.*,* DEGEN et al. 2008) and European beech (*Fagus sylvatica* L., MAURER et al. 2008) – resulting in a precise conception for the implementation of a genetic monitoring for forest tree species (BLAG-GROUP OF EXPERTS "GENETISCHES MONITORING" 2008).

Implementation of a genetic monitoring system

Participating institutions and choice of trees

Within the framework of the project GENMON, there are several institutions cooperating (Table 1). Presently, a net of monitoring plots for *Fagus sylvatica* L. and *Picea abies* (L.) Karst. has been established throughout Germany to evaluate the intactness of the genetic system. Both species represent about 40 % of the forest-covered area in Germany (European beech: 15.4 %, Norway spruce: 25.4 %; THÜNEN-INSTITUT 2012). They are very important tree species from the ecological and economical point of view respectively. Facing climate change, Norway spruce is considered to be potentially endangered in some regions. This expectation is caused by the fact that only a small proportion of the current distribution is formed by autochthonous, naturally regenerated populations. Spruce proveniences have been translocated over long distances and planted within and beyond the native distribution area very often on non-suitable sites, resulting in disturbed adaptation patterns. On the other hand, European beech is native all over Germany and promises to be a stable part of forest ecosystems during climate change. Both species serve as models for the implementation of a genetic monitoring in forest tree populations in Germany and presumably form a template for the introduction of suitable programmes for other forest tree species. First results will be available in summer 2019.

Table 1: Collaborating Institutions and their tasks (S: Sampling procedure, G: Genetic analyses, Q: Testing Germination Capacity of seeds, A: Analysis and evaluation of collected data)

Institution	Tasks
Bayerisches Amt für forstliche Saat- und Pflanzenzucht (ASP)	Coordination, S, G, Q
Forstliche Versuchs- und Forschungsanstalt Freiburg (FVA)	S
Landeskompetenzzentrum Forst Eberswalde	S
Landesforst Mecklenburg-Vorpommern	S
Nordwestdeutsche Forstliche Versuchsanstalt Göttingen (NW-FVA)	S, G
Forschungsanstalt für Waldökologie und Forstwirtschaft Trippstadt	S
Staatsbetrieb Sachsenforst (SBS), Kompetenzzentrum Wald und Forstwirtschaft	S, G
Thüringen Forst, Forstliches Forschungs- und Kompetenzzentrum Gotha (FFK)	S
ISOGEN am Institut für Forstgenetik der Universität Göttingen	S, G, Q
Thünen-Institut für Forstgenetik	A

Monitoring plots and sampling procedure

The establishment of the plots and the sampling procedure have already been used and characterized in pilot studies (KONNERT et al. 2011, TRÖBER et al. 2011). To ensure comparability of data, the installation of plots, sampling procedure and genetic analyses have to be similar for each investigated stand.

For European beech 14 and for Norway spruce 10 monitoring plots have been established throughout Germany (Figure 1). Every plot covers a total area of 4 hectares, with a fenced core section with 50x50 meters placed in the middle, surrounded by the intensive section, both together covering 100x100 meters (Figure 2). Within core and intensive section, each adult tree has to be sampled.

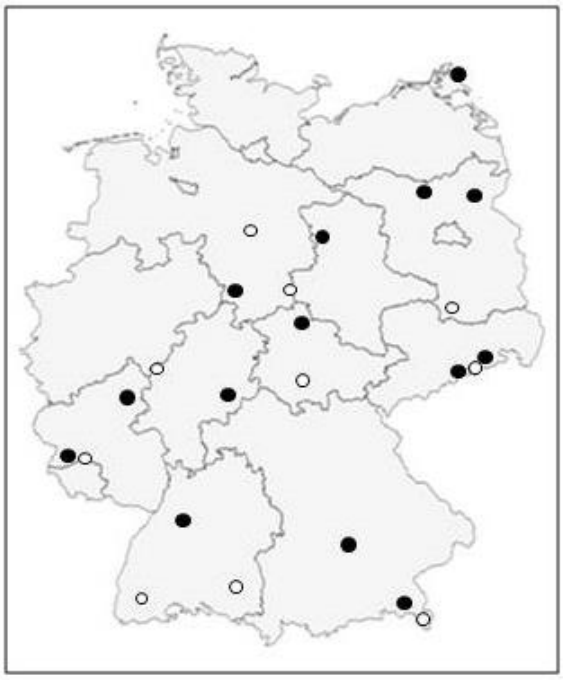

Figure 1: Location of monitoring plots (black dots: European beech, white dots: Norway spruce)

In case the number of trees in the intensive zone is insufficient, the extensive section serves to provide additional individuals up to 250 trees. Furthermore, 20 dominant trees in the intensive section (including core section) have to be chosen as seed trees. They are observed intensively to evaluate flowering phenology, leaf flushing, degree of fructification and vitality. Furthermore, at least 20 seeds per seed tree are harvested directly from the crown. Natural regeneration is characterized by sampling of 200 young plants distributed throughout the monitoring plot and of 4 clusters with 50 individuals each. The clusters enable conclusions about family structures as well as seed and pollen dispersal while the regular distributed samples provide information about the genetic diversity throughout the plot. All samples (at least 1050 per monitoring plot) are utilized for genetic analyses. A seed sample is collected from the entire plot area of 4 ha to investigate seed quality parameters. The sampling procedure is summarized in Table 2.

Table 2: Survey on the sampling procedure

Development state	Intensive section including core section	Extensive section
Genetic studies		
Adult trees	All individuals present	Additional individuals up to 250
Natural regeneration	200 young plants representative for overall plot	
	4 clusters of natural regenerations comprising 50 individuals each	
Seeds	Single tree collections of seeds from 20 adult trees	
Qualitiy structure of seeds		
Seeds	Seed mixture from entire stand area	

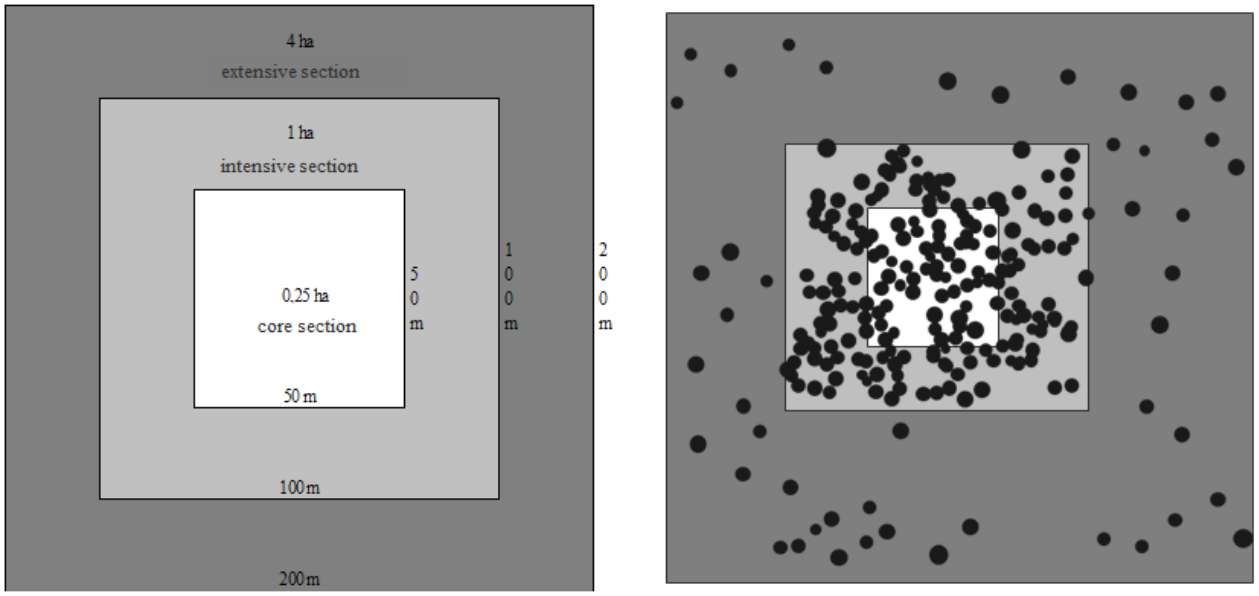

Figure 2: Schematic design of monitoring plot with core, intensive and extensive section (left) and distribution of investigated adult trees in the monitoring plot Weicholdswald (Saxony, European Beech)

Outlook

The collected data will be secured in a database. They will provide information about the intactness of the genetic system in the investigated populations and will enable estimations about the state of genetic diversity in European beech and Norway spruce for large parts of German forests. Beside information about genetic structures in a certain monitoring plot, it will be possible to prove whether there are genetic differences between different plots or between different development stages in the same plot. With appropriate software, i.e. GDA-NT (DEGEN 2008), GSED (GILLET 2008) or SGS (DEGEN et al. 2001) spatial structures and family structures will be visible and allow a deductive reasoning about pollen and seed distribution. In connection with phenotypic characteristics it may be possible to connect certain molecular markers with phenotypic attributes.

The implementation of genetic monitoring presented has also the character of a role model, pioneering application of equivalent methods for other tree species or initiating an international network of monitoring plots. It will hopefully be regarded as useful tool in the section of environmental monitoring and considered in certain programs.

For the moment, the results will represent a “snapshot” of the current condition of the investigated populations. To display the dynamics of the genetic system and its processes over time it is necessary to repeat certain investigations periodically. The “Guidance of implementation of a genetic monitoring for standforming tree species” BLAG-GROUP OF EXPERTS "GENETISCHES MONITORING" 2008) schedules seed sampling every 4 years (if fructification is sufficient) and actualizing the list of adult trees and new sampling of natural generation every 10 years. The re-estimation/re-examination of the selected indicators and verifiers allows a review of the suitability of genetic monitoring as an early warning system for ecosystem changes. It will presumably reveal reactions of the genetic system to influences of a changing environment and enlighten our understanding about mechanisms of evolutionary reactions of tree populations.

Acknowledgements

The project is sponsered by the Federal Ministry of Food and Agriculture and the Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety on the basis of a decision of the German Parliament. Special thanks to all former and current members of the BLAG-group of experts “Genetisches Monitoring” for their commitment and efforts to establish a genetic monitoring for forest tree species.

References

BLAG-GROUP OF EXPERTS "GENETISCHES MONITORING" (2004): Konzept zum genetischen Monitoring für Waldbaumarten in der Bundesrepublik Deutschland. https://blag-fgr.genres.de/genetisches-monitoring/konzept-zum-genetischen-monitoring/, checked on 7/8/2018.

BLAG-GROUP OF EXPERTS "GENETISCHES MONITORING" (2008): Anleitung zur Durchführung des genetischen Monitorings für bestandesbildende Baumarten. https://blag-fgr.genres.de/genetisches-monitoring/ anleitung-zum-genetischen-monitoring/, updated on 1/8/2018.

CBD (Ed.) (1992): Convention on biological diversity. United Nations Conference on Environment and Development, UNCED. Rio de Janeiro.

DEGEN B, PETIT R, KREMER A (2001) *SGS—Spatial Genetic Software.* A computer program for analysis of spatial genetic and phenotypic structures of individuals and populations. Journal of Heredity 92 (5): 447–448.

DEGEN B, JOLIVET C, LIESEBACH H, HOELTKEN A, DOUNAVI A, FLADUNG M, NAUJOKS G, SCHNECK V, STAUBER T, STEINER W, KÖHL M (2008) Erfassung genetischer Strukturen wichtiger Waldbaumarten, Teillos 2: Erfassung der genetischen Struktur der Vogelkirsche (*Prunus avium*) als Grundlage für ein genetisches Monitoring wichtiger Waldbaumarten in Deutschland. Endbericht BLE-Projekt-Nr. 05/BE003/2: pp. 56.

DEGEN B (2008) GDA_NT 2.0. Genetic data analysis and numerical tests. Available from bernd.degen @thuenen.de

EUSEMANN P, PREUß A, LIESEBACH M, LIESEBACH H (2017) Optimized crop quality by single-tree harvesting – A study on European beech (*Fagus sylvatica L.*). Forstarchiv 88: 17-23. DOI: 10.4432/0300-4112-88-17.

FUSSI B, WESTERGREN M, ARAVANOPOULOS F, BAIER R, KAVALIAUSKAS D, FINZGAR D (2016) Forest genetic monitoring. An overview of concepts and definitions. Environmental monitoring and assessment 188 (8): p. 493. DOI: 10.1007/s10661-016-5489-7.

GILLET EM (2008) Computer program GSED–Genetic Structures from Elelctrophoretic Data. http://www. uni-forst. gwdg. de/forst/fg/index. htm. Version.

GRAUDAL L, ARAVANOPOULOS F, BENNADJI Z, CHANGTRAGOON S, FADY B, KJÆR ED et al. (2014) Global to local genetic diversity indicators of evolutionary potential in tree species within and outside forests. Forest Ecology and Management 333: 35–51. DOI: 10.1016/j.foreco.2014.05.002.

GREGORIUS H-R, DEGEN B (2007) Monitoring genetischer Ressourcen – Prinzipien und Methoden. In Bundesanstalt für Landwirtschaft und Ernährung (ed): Monitoring und Indikatoren der Agrobiodiversität. Tagungsband eines Symposiums am 7. und 8. November 2006 in Königswinter. With assistance of Frank Begemann, Stefan Schröder, Karl-Otto Wenkel, Hans-Joachim Weigel: IBV: 39–65.

JUMP AS, HUNT JM, MARTÍNEZ-IZQUIERDO JA, PEÑUELAS J (2006) Natural selection and climate change. Temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. Molecular ecology 15 (11): 3469-3480. DOI: 10.1111/j.1365-294X.2006.03027.x.

KONNERT M, HOSIUS B (2010) Contribution of forest genetics for a sustainable forest management. Forstarchiv 81 (4): 170–174.

KONNERT M, MAURER WD, DEGEN B, KÄTZEL R (2011) Genetic monitoring in forests-early warning and controlling system for ecosystemic changes. iForest-Biogeosciences and Forestry 4 (2): p. 77.

MAURER WD, CREMER E, DEGEN B, KONNERT M, TRÖBER U (2008) Erfassung genetischer Strukturen wichtiger Waldbaumarten, Teillos 1: Erfassung der genetischen Struktur der Rotbuche (*Fagus sylvatica*) als Grundlage für ein genetisches Monitoring wichtiger Waldbaumarten in Deutschland. Endbericht BLE-Projekt-Nr. 05/BE003/1: pp. 81.

MONASTERSKY R (2014) Life-a status report. Nature 516 (7530): p. 158.

NAMKOONG G, BOYLE TJB, GREGORIUS H-R, JOLY H, SAVOLAINEN O, RATNAM W, YOUNG A (1996) Testing Criteria and Indicators for assessing the sustainability of forest management. Genetic criteria and indicators.

PAUL M, HINRICHS T, JANßEN A, SCHMITT HP, SOPPA B, STEPHAN BR, DÖRFLINGER H (2010) Concept for the conservation and sustainable utilization of forest genetic resources in the federal republic of Germany. Pirna. https://www.genres.de//fileadmin/SITE_GENRES/content/Dokumente/Downloads/Konzept/Forst genressourcen-2010.pdf.

THÜNEN-INSTITUT (2012) Dritte Bundeswaldinventur - Ergebnisdatenbank. https://bwi.info/, updated on 1/8/2018.

TRÖBER U, MAURER WD, KONNERT M, DEGEN B, CREMER E (2011) Implementation of a genetic Monitoring System ion European Beech (*Fagus sylvatica*) Populations. First results of a Pilot Study. In WAGNER S, FAHLVIK N, FISCHER H (eds.): Proceedings. The 9th IUFRO International Beech Symposium organized by IURFO working party 1.01.07 "Ecology and Silviculture of Beech", 12-17 September. Dresden/Göttingen, Germany: 55–57.

Population genetic research of small-leaved forest tree species using molecular markers

T.A. POLIAKOVA[1], D.V. POLITOV[1], M.M. BELOKON[1], YU.S. BELOKON[1], E.A. MUDRIK[1], A.V. SHATOKHINA[1], A.P. EFIMOVA[2], K.A. SHESTIBRATOV[3]

[1] Vavilov Institute of General Genetics Russian Academy of Sciences, Moscow, Russian Federation

[2] Institute of Biological Problems of Cryolithozone Siberian Branch Russian Academy of Sciences, Yakutsk, Russian Federation

[3] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences, Puschino Branch, Puschino, Moscow region, Russian Federation

Abstract

In this report, some examples of population-genetic research of small-leaved forest tree species (*Populus tremula*, aspen; *Betula* spp*.,* birch; *Salix* spp*.,* willow) using molecular markers are given. Panels of 13-14 SSR loci developed for individual identification of aspen, willow and birch were applied for the clonal structure analysis. Molecular genetic markers proved to be suitable for confirmation of hybrid nature of *Salix* × *zhataica* from Yakutia were detected.

Key words: *Populus tremula*, aspen, birch, willow, clonality, molecular identification, *Salix × zhataica*, hybrid

Introduction

Identification of individual genotypes of forest tree species is useful for control of artificial propagation of elite clones, analysis of clonal structure of natural stands and plantations, detection of natural and artificial hybrid and other practically oriented applications. In this report we present our population genetic studies based on molecular identification of genotypes and clones of small-leaved forest tree species (*Populus tremula*, aspen; *Betula pendula, B. pubescens,* birch; *Salix* spp*.,* willow) including analysis of clonality in natural populations of aspen *Populus tremula,* karyological analysis of trees and clones; development of test systems for molecular genetic marking for identification of elite genotypes of small-leaved forest tree species/clones; identification of species and interspecific hybrids of willow and birch in natural populations. In this extended summary we give several examples on these problems.

Material and methods

Material and methods of molecular (by means of nuclear SSR loci) identification of aspen clones and samples from natural populations are described elsewhere (POLITOV et al. 2015, 2016, 2017) as well as the methods for the study of variability in the ITS region of rDNA (POLYAKOVA et al. 2016).

Results and discussion

Molecular marking of genotypes and elite clones of aspen/birch/willow and development of genetic test systems for identification has the following research design. First of all, we formulated principles of development of test systems of molecular marking/identification, searched and analysed available publications on the subject. Comparative analysis of the properties of classes of markers allowed us to

choose specific markers for each subject and task. After choice of candidate genetic markers for molecular testing we tested selected reliable, polymorphic and informative markers.

Test system for aspen ("Aspen-M") based on the analysis of 14 SSR loci has been developed. The dependence of the probability of random coincidence of genotypes (PI) by the used microsatellite loci was shown. PCR analysis of selected set of microsatellite loci allowed the reliable identification the individual genotypes of aspen. The probability of a random match not more than $10^{-5} – 10^{-13}$ or one match on a few trillions comparisons for unrelated genotypes. Eight elite clones of aspen were reproduced in replicates of multilocus genotypes and differed both from each other and from any of the investigated trees from natural populations.

In course of microsatellite analysis of clonality and individual heterozygosity in natural populations of aspen *Populus tremula* a highly heterozygous rampant clone was revealed. A panel of 14 SSR loci developed for individual identification of aspen was applied for the clonal structure analysis in four natural aspen stands: Moscow and Voronezh oblasts, the Mari-El Republic, and the Republic of Tatarstan. In the first three localities, all the trees had different genotypes, but in the stand from Sabinsky Forestry, Tatarstan, all of the examined 29 trees were represented by a single genotype. The ancestral tree carrier of this genotype which was the most heterozygous (0.929) among all studied Aspen individuals obviously has spread over a large territory during several cutting and reproduction cycles, currently occupying the area of 2.2 ha. This highly vigorous clone showed a diploid number of chromosomes (2n=38) (POLITOV et al. 2015, 2016, 2017).

Panels of 14 nuclear SSR loci developed for individual identification of willow and 13 SSR loci for individual identification of birch were applied for the clonal structure analysis. Preliminary tender documents for the test systems "Aspen-M", "Birch-M", "Willow-M" were made. The instructions for use of test systems including research workflow are prepared (Patent RU 2630662).

To verify the natural hybrid *Salix* × *zhataica* Efimova, Shurduk et Ahti and its parental species (EFIMOVA et al. 2009) – *S. brachypoda* (Trautv. et C. A. Mey.) Kom. and *S. pyrolifolia* Ledeb., polymorphism of molecular genetic markers such as nuclear microsatellite loci, nucleotide sequences of ITS region and intron 2 of gene C4H2 of cyp73 gene family, coding for cinnamate 4-hydroxylase of the lignin biosynthesis pathway, were analyzed and their effectiveness was shown. Out of the total number of 14 tested loci, 3 microsatellite loci (*Sa54B*, *SB24*, *SB233*) were selected, which make it possible to clearly distinguish representatives of these willow species. According to the results of factor analysis of genotype distribution hybrid individuals occupy an intermediate position between species *S. brachypoda* and *S. pyrolifolia*. Moreover, some samples are most similar to one of the parent species, which suggests the possibility of backcrosses *S.* × *zhataica* with *S. brachypoda*. In ITS region of this species we detected 2 single-nucleotide transitions, indicating the borrowing of a hybrid of the specific nucleotides from both parental species (EFIMOVA et al. 2018). The variability of intron 2 of gene C4H2 showed both common and specific bands for these willow species. All studied molecular genetic markers proved to be suitable for confirmation of hybrid nature of *S.* × *zhataica*.

Conclusions

Panels of 13-14 nrSSR loci were developed for individual identification of aspen, willow and birch and applied for the clonal structure analysis. Highly vigorous clone found in Sabinsky Forestry, Tatarstan, occupied an area of 2.2 ha and had diploid number of chromosomes (2n=38). Molecular genetic markers proved to be suitable for confirmation of hybrid nature of *Salix* × *zhataica* from Yakutia were detected.

Acknowledgements

Development of test systems of molecular identification of small-leaved forest tree species was performed under the Agreement No. 14.607.21.0044 under the project «Cell selection and micropropagation of elite planting material for establishment of fast-growing genetically marked forest plantations» (coordinator K.A. SHESTIBRATOV). Study of willow species in Yakutia this research is supported by the Russian Foundation for Basic Research (Project № 15-44-05103, PI A. EFIMOVA). This publication was also supported by themes of state assignment 0112-2016-0002, 0112-2015-0045 (PI D. POLITOV) and 0112-2015-0040 (Co-PI D. POLITOV).

References

EFIMOVA AP, SHURDUK I.F, AHTI T (2009) New interspecific hybrids of the genus *Salix* (Salicaceae) from Yakutia. Botanicheskij zhurnal 94 (1): 83-89.

EFIMOVA et al. 2018, (in press)

Patent RU 2630662. (2017) Molecular marking method based on microsatellite loci, intended for identification of willow genotypes // POLITOV DV, BELOKON MM, BELOKON YS, SHATOKHINA AV, SHESTIBRATOV KA. Patent RU. Bulletin no. 26.

POLITOV DV, BELOKON MM, BELOKON YS, POLYAKOVA TA, SHATOKHINA AV, MUDRIK EA, AZAROVA AB, FILIPPOV MV, SHESTIBRATOV KA (2015) Application of microsatellite loci for molecular identification of elite genotypes, analysis of clonality, and genetic diversity in aspen *Populus tremula* L. (Salicaceae). International Journal of Plant Genomics: 11 pp. (doi: 10.1155/2015/261518).

POLITOV DV, BELOKON MM, BELOKON YS, POLYAKOVA TA, SHATOKHINA AV, MUDRIK EA, KHANOV NA, SHESTIBRATOV KA (2016) Microsatellite analysis of clonality and individual heterozygosity in natural populations of aspen *Populus tremula* L.: Identification of highly heterozygous clone. Russian Journal of Genetics 52 (6): 636-639.

POLITOV DV, BELOKON YS, SHATOKHINA AV, BELOKON MM, KHANOV NA, MUDRIK EA, POLYAKOVA TA, AZAROVA AB SHESTIBRATOV KA (2017) Molecular identification and kariological analysis of a ramnant aspen *Populus tremula* L. (Salicaceae) clone. International Journal of Plant Genomics: 8 pp. (doi: 10.1155/2017/5636314).

POLYAKOVA TA, EFIMOVA AP, SHATOKHINA AV (2016) Molecular-genetic researches on *Salix* L. and *Spiraea* L. of Yakutia on the basis of sequencing data of ITS rDNA. International Journal of Applied and Fundamental Research 12: 965-968.

III. Timber Tracking, Genomics and Adaptation

Genetic timber tracking of *Larix* sp. in Eurasia

CÉLINE BLANC-JOLIVET[1], YULAI YANBAEV[2], BERND DEGEN[1]

[1] Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany (celine.blanc-jolivet@thuenen.de, bernd.degen@thuenen.de)

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

Abstract

We sampled *Larix sp.* individuals over Eurasia and conducted genotyping at four chloroplast microsatellite and 253 newly developed SNP loci. Both marker types showed a very clear geographical pattern corresponding in Russia to different *Larix* species. The best SNP loci were selected to develop next sets of 76 and 13 loci for respectively cost-effective MassARRAY and SNaPShot genotyping. Genetic assignment tests provided theoretical success rates between 73 and 90% according to the marker set used. An analysis of timber samples showed varying amplification success rates, but demonstrated the feasibility of larch timber tracking with molecular markers at the regional level. We further discussed statistical improvements needed for forensic applications.

Key words: *Larix* sp., illegal logging, genetic timber tracking, genetic assignment, chloroplast microsatellites, Single Nucleotide Polymorphism

Introduction

With the entry in force of new laws for timber importation (EU Timber Regulation (EUTR), the US Lacey Act and the Australia Illegal Logging Prohibition Act prohibit), reliable information on the species and geographical origin are required to avoid placing on the market illegally harvested timber. The trade of larch timber (*Larix* sp.) exhibits a significant importance in the European market, and is harvested in natural forests of Europe and Russia. However, several *Larix* species occur in Eurasia, all showing contrasting geographical ranges (ARAKI et al. 2008, KHATAB et al. 2008, ORESHKOVA et al. 2013, POLEZHAEVA et al. 2010, SEMERIKOV and LASCOUX 1999, SEMERIKOV and LASCOUX 2003, SEMERIKOV et al. 2013). Since the anatomical timber identification of *Larix* species is not possible, other timber tracking methods are necessary to control the species declaration in the frame of timber market regulations. We present here the genetic variation of *Larix sp*. within Eurasia with chloroplastic microsatellite (cpSSRs) and newly developped SNP markers and show how this genetic variation can be used for the control of species and geographical origin.

Material and methods

Fresh cambium, buds or needles were collected from *Larix* sp trees ranging from the French Alps to the eastern cost if Russia, and a from a provenance trial located in Germany. DNA was extracted according to DUMOLIN et al. (1995). One *Larix decidua* and one *Larix gmelinii* tree were sampled from the collection at the Thünen Institute in Grosshansdorf and were used for RAD sequencing to detect putative SNP loci (RADseq, Baird et al. 2008, SLAVOV et al. 2014). 2094 individuals were genotyped at four cpSSRs (SEMERIKOV et al. 2013) on a capillary sequencer (Figure 1) and 1885 individuals were genotyped at 253 SNP loci (249 nuclear, 1 chloroplast and 3 mitochondrial) derived from the RADseq analysis and from MOSCA *et al.* (2012) on a MassARRAY platform (Figure 2). Cluster analysis was conducted to detect genetic groups (cpSSRs: SAMOVA (DUPANLOUP et al. (2002)), SNPs: STRUCTURE (PRITCHARD et al. 2000)). Since genotyping

of SNPs is more robust on timber material, we adressed the success rate of genetic assignment methods on the SNP dataset based on the results of the grouping of individuals according to the cluster analysis with self-assignment tests together with exclusion probability calculation (CORNUET et al. 1999, DEGEN et al. 2017, RANNALA and MOUNTAIN 1997). Most samples from West Russia were not included due to timing reasons.

Figure 1: Frequency of most common haplotypes observed in *Larix* sp. samples using four cpSSRs. The white colour in the pie charts represents the frequency of rare haplotypes.

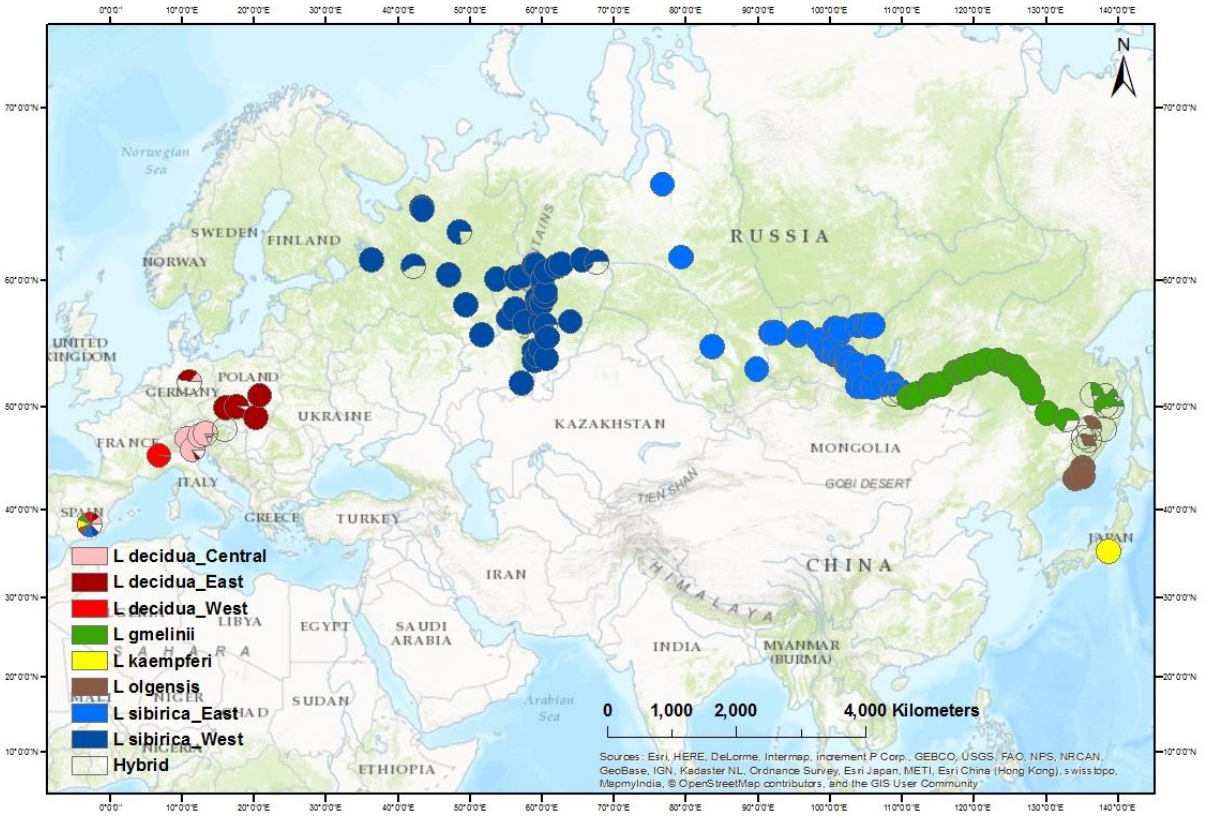

Figure 2: Frequency of the genetic clusters and admixed individuals ("Hybrids") in the sampled populations together with the corresponding *Larix* species.

We compared analysis methods and the complete set of loci with a reduced set of 76 loci optimised for informativeness. Finally, to adress the feasilbility to cost-effectively trace the origin of larch timber, we

genotyped three *L. decidua* timber samples with known origin and four blind timber samples at the reduced set of 76 loci. To adress genotyping errors and decrease in DNA quality of timber, we included in the sampling needles or cambium from the logged tree and repeated each timber samples four times. Finally, we developped and optimised a SNaPShot Assay with highly informative 13 loci based on the primers used for MassARRAY analysis.

Amplification rate of timber samples ranged from 58 to 100 %, but genotyping errors among replicated samples were observed in 0 to 18 loci (Table 2). Most genotyping errors in timber samples were due to allele dropout at heterozygous loci. All blind samples were successfully assigned to their true population of origin, while two timber samples from Germany would have been misassigned to a population in Austria, albeit not very far from the true population. This result was not only due to the low amplification rate of the sample RP1, but also on the weakness of the reference data for genetic assignment at the population level. These results therefore suggest that genetic tracking should be done at a regional and not at a population level.

The SNaPShot assay was successfully tested on reference material from all *Larix sp.* regions of origin (Figure 3). Based on the reference data obtained with the MassARRAY genotyping, this set of 13 loci correctly assigned 90% of the samples to the most likely group, but here no exclusion probabilities were calculated. We nevertheless do not expect the assignment success rate to be substantially lower than at the other set of loci. The selection of loci showing strong differentiation among groups can therefore contribute to the development of cost-effective methods for timber tracking without strong decrease in statistical power.

Table 1: Assignment success rates of the genetic assignment based on the Bayesian criteria (Rannala and Mountain, 1997) and on the nearest-neighbour approach (DEGEN et al. 2017)

	Bayesian criteria		Nearest-neighbour	
	All loci	Selected loci	All loci	Selected loci
Number of individuals	775	775	914	913
Assignment success (%)	76.0	83.6	78.3	73.8

Table 2: Amplification rates and genotyping errors of timber samples together with genetic assignment results based on the Bayesian criteria (RANNALA and MOUNTAIN 1997) obtained from the genotyping of 76 loci

Sample	Origin	Amplification success timber/fresh material [%]	Number of loci with mismatch	Assigned to	True Origin
RP1	Germany	58	18	Austria	Germany
RP2	Germany	100	1	Austria	Germany
M1	France	75	17	France	France
1	?	100	0	Germany	Germany
2	?	100	0	Germany	Germany
3	?	100	4	Germany	Germany
4	?	75	15	France	France

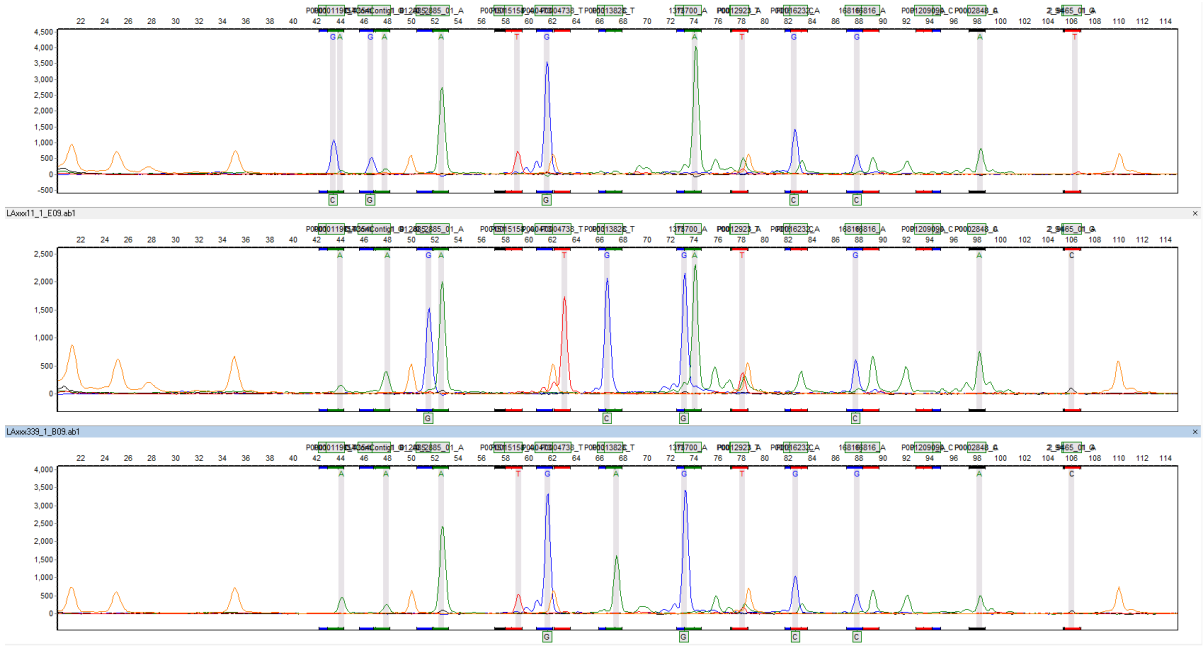

Figure 3: SNaPShot analysis at a combination of 13 loci.

Conclusions

The genotyping at newly developed SNP loci was very effective for cost-effective timber tracking purposes. However, average success rates are lower than 90%, which still hinders the use of this method for forensic timber identification. Further work is therefore needed. Besides the improvement of the marker set which would require further NGS sequencing of individuals from regions where success rates of our method is low, estimation of error rates might in some cases still forensically validate the presently described method. The calculation of likelihood ratios allows estimating the probability that the assignment test returned an erroneous result, and rely on the choice of a likelihood ratio threshold which minimise the probability or type 1 and type 2 errors. A preliminary analysis showed that likely ratio tests would be very helpful, but with limited interests to distinguish among *L. gmelinii* and *L. olgensis*.

Acknowledgements

We would like to thank VIVIAN KUHLENKAMP and OLCA CAKMAK for their work in the laboratory. Timber samples for the blind testing were provided by BARBARA FUSSI (Germany) and PASCAL TRÉZALET (France). The Northwest German Forest Research Institute (AXEL NOLTENSMEIER) supported us with the sampling of trees from a European Larch provenance trial. The SNP genotyping was conducted at the Genomic and Sequencing Facility of Bordeaux.

References

ARAKI NHT, KHATAB IA, HEMAMALI KKGU, INOMATA N, WANG X-R, SZMIDT AE (2008) Phylogeography of *Larix sukaczewii* Dyl. and *Larix sibirica* L. inferred from nucleotide variation of nuclear genes. Tree Genet. Genomes 4: 611-623.

BAIRD NA, ETTER PD, ATWOOD TS, CURREY MC, SHIVER AL, LEWIS ZA et al. (2008) Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers. Plos One 3: 10.

CORNUET JM, PIRY S, LUIKART G, ESTOUP A, SOLIGNAC M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153: 1989-2000.

DEGEN B, BLANC-JOLIVET C, STIERAND K, GILLET E (2017) A nearest neighbour approach by genetic distance to the assignment of individuals to geographic origin. Forensic Sci. Int.-Gen. 27: 132-141.

DUMOLIN S, DEMESURE B, PETIT RJ (1995) Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method. Theor. Appl. Genet. 91: 1253-1256.

DUPANLOUP I, SCHNEIDER S, EXCOFFIER L (2002) A simulated annealing approach to define the genetic structure of populations. Molecular Ecology 11(12): 2571-81.

KHATAB IA, ISHIYAMA H, INOMATA N, WANG X-R, SZMIDT AE (2008) Phylogeography of Eurasian Larix species inferred from nucleotide variation in two nuclear genes. Genes Genet. Sys. 83: 55-66.

MOSCA E, ECKERT AJ, LIECHTY JD, WEGRZYN JL, LA PORTA N, VENDRAMIN GG et al. (2012) Contrasting patterns of nucleotide diversity for four conifers of Alpine European forests. Evol. Appl. 5: 762-775.

ORESHKOVA NV, BELOKON MM, JAMIYANSUREN S (2013) Genetic diversity, population structure, and differentiation of Siberian larch, Gmelin larch, and Cajander larch on SSR-marker data. Russ. J. Genet. 49: 178-186.

POLEZHAEVA MA, LASCOUX M, SEMERIKOV VL (2010) Cytoplasmic DNA variation and biogeography of *Larix* Mill. in Northeast Asia. Mol. Ecol. 19, 1239-1252.

PRITCHARD JK, STEPHENS M, DONNELLY P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

RANNALA B, MOUNTAIN JL (1997) Detecting immigration by using multilocus genotypes. P. Natl. Acad. Sci. USA 94: 9197-9201.

SEMERIKOV VL, LASCOUX M (1999*)* Genetic relationship among Eurasian and American *Larix* species based on allozymes. Heredity 83: 62-70.

SEMERIKOV VL, LASCOUX M (2003) Nuclear and cytoplasmic variation within and between Eurasian *Larix* (*Pinaceae*) species. Am. J. Bot. 90: 1113-1123.

SEMERIKOV VL, SEMERIKOVA SA, POLEZHAEVA MA, KOSINTSEV PA, LASCOUX M (2013) Southern montane populations did not contribute to the recolonization of West Siberian Plain by Siberian larch (*Larix sibirica*): a range-wide analysis of cytoplasmic markers. Mol. Ecol. 22: 4958-4971.

SLAVOV GT, NIPPER R, ROBSON P, FARRAR K, ALLISON GG, BOSCH M, et al. (2014) Genome-wide association studies and prediction of 17 traits related to phenology, biomass and cell wall composition in the energy grass Miscanthus sinensis. New Phytol. 201: 1227-1239.

Development of mtDNA markers for Siberian conifers and their application in phylogeographic studies

VLADIMIR L. SEMERIKOV[1], SVETLANA A. SEMERIKOVA[2], KONSTANTIN V. KRUTOVSKY[3,4,5,6]

[1] Laboratory of Plant Molecular Ecology, Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russia (semerikov@ipae.uran.ru)

[2] Laboratory of Plant Molecular Ecology, Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, Ekaterinburg, Russia (s.a.semerikova@ipae.uran.ru)

[3] Department of Forest Genetics and Forest Tree Breeding, Georg-August University of Göttingen, Göttingen, Germany (konstantin.krutovsky@forst.uni-goettingen.de)

[4] Laboratory of Forest Genomics, Genome Research and Education Center, Siberian Federal University, Krasnoyarsk, Russia (kkrutovsky@gmail.com)

[5] Laboratory of Population Genetics, N. I. Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia (kkrutovsky@gmail.com)

[6] Department of Ecosystem Science and Management, Texas A&M University, College Station, TX 77843-2138, USA (k-krutovsky@tamu.edu)

Abstract

The results of a phylogeographic study of three conifer species of Northern Eurasia (*Larix sibirica, Abies sibirica* and *Pinus sylvestris*) based on mitochondrial DNA markers newly developed by re-sequencing of mitochondrial genome fragments are presented. The geographic distribution of the mtDNA haplotypes (mitotypes) was similar in Siberian larch and Siberian fir and indicated the migration from several refugia in southern Siberia and the Urals. The western Scots pine populations located west of 38 °E sharply differed from the eastern ones, which indicates the post-glacial colonization of these parts of the range from different sources – supposedly from European and Ural refugia, respectively.

Key words: Phylogeography, Pleistocene, refugia, mitochondrial DNA, genetic markers, *Larix sibirica, Abies sibirica, Pinus sylvestris*

Introduction

The distribution, magnitude and structure of genetic variation carry traces of past demographic processes, such as contractions and expansions of populations, migrations and hybrid contacts. Species of forest biota reduce their areas during the glacial epochs, surviving in isolated refugia, and again dispersing when climate improve. Analysis of genetic variation allows to infer information about these events. Unlike the relatively thermophilic species of European broadleaf forests, the taiga trees of Northern Eurasia in the glacial phases often did not disappear completely in the middle latitudes, surviving in supposed refugia in Central Europe (WILLIS and VAN ANDEL 2004) and Western Siberia (BINNEY et al. 2009). It was interesting to study the evolutionary history of Siberian conifers in connection with the Pleistocene glaciations since it has not been studied enough. Siberian larch (*Larix sibirica*) and Siberian fir (*Abies sibirica*) are the key components of the boreal taiga in Northern Eurasia with largely overlapping ranges. They are distributed from Baikal Lake region to the north of European Russia, including the mountains of the south of Siberia, the West Siberian Plain and the Urals, which points at their common colonization history. At the same time, the ecological properties of these species are rather different. Contrary to the larch, which is the most drought- and frost- resistant tree, the fir is very sensitive to drought and avoids permafrost. The floristics of taiga forests in Siberia and the Urals definitely indicate the southern Siberian origin of the modern populations of Siberian and Ural conifers, which is a common feature of the biogeography of

these species. In opposite of them, another boreal tree Scots pine *Pinus sylvestris* has even wider distribution in the North Eurasia, but likely has European, not Siberian, origin and secondary distribution in the North Asia.

For biogeographical studies of wind-pollinated trees with extensive areas and populations, genetic markers with maternal inheritance are especially useful because they are transmitted with seeds and have a reduced ability to disperse, unlike markers transmitted with pollen. Consequently, traces of historical events, such as range contraction, isolation and migration, are better preserved in the genetic structure of populations identified with such markers. In the Pinaceae family the mitochondrial DNA has maternal inheritance, and chloroplast - paternal. Because of this, mitochondrial DNA markers were widely used to study the phylogeography of conifer species. However, the number of available "universal" mitochondrial DNA markers is limited, and for many species it is necessary to develop specific markers, which can be done by several methods. One of them is based on detection among RAPD polymorphic fragments those that have a mitochondrial origin. In particular, PCR based mtDNA markers for Siberian larch were derived from the RAPD fragments which variation within population sample was associated with the variation detected by the Southern blot hybridization with mitochondrial probes (SEMERIKOV et al. 2006). Another method is based on searching for polymorphic sites in the PCR amplified and then re-sequenced mitochondrial genome fragments using a panel of individuals of different geographic origins or different taxonomic assignment. However, this requires knowledge of nucleotide sequences of mitochondrial DNA of sufficient length, which are usually absent for non-model species. They, in turn, can be obtained by examining the non-coding flanking regions of known mitochondrial genes (for example using inverse PCR), or by sequencing the mitochondrial genome with NGS.

In this review, we present the results of studies of the phylogeography of Siberian fir, Siberian larch (SEMERIKOV et al. 2013) and Scots pine in the eastern part of its range (SEMERIKOV et al. 2018) employing species-specific mtDNA markers.

Material and methods

Population samples and genetic markers used

In the phylogeographic study of Siberian larch (SEMERIKOV et al. 2013) we used four mtDNA markers - one based on the "universal" primers, two - on the fragments isolated from RAPD and one - on the re-sequencing of the flanking region of the *Atp-A* gene. One hundred sixteen range-wide collected populations were analyzed.

To developed species-specific mtDNA markers for Siberian fir we used the Sanger re-sequencing of the contigs assembled from the paired-end sequencing reads of the Siberian fir genome generated using the Illumina HiSeq 2000. The mitochondrial contigs were selected by mapping them to the homologous mitochondrial genome sequences of Norway spruce and loblolly pine, and then were used to design PCR primers. Then, some amplicons were sequenced in a small panel of trees representing different geographic regions. The single nucleotide polymorphic sites (SNPs) were genotyped in population samples using the Single Strain Conformation Polymorphism (SSCP) method. In total, the developed markers were genotyped in 8-24 individual trees per each of 45 populations used previously in earlier studies of allozyme, chloroplast microsatellite and AFLP variation of Siberian fir (SEMERIKOVA and SEMERIKOV 2006, 2007, 2011).

To study mtDNA diversity in the eastern part of the *P. sylvestris* range (SEMERIKOV et al. 2018) three mitochondrial DNA markers were used: *nad7* (NAYDENOV et al. 2007, PYHÄJÄRVI et al. 2008, VIDYAKIN et al. 2012, BUCHOVSKA et al. 2013), *coxI* (SEMERIKOV et al. 2015) and a new *syn31* marker recently designed based on the whole genome sequencing approach as described above. DNA samples isolated from two to 24 individual trees per population were genotyped in 90 populations. Among them, 36 populations were used previously (VIDYAKIN et al. 2012, SEMERIKOV et al. 2014), and 52 were newly collected.

Statistical analysis of the data

The mitotype network for each species was constructed using NETWORK 5.0.0.1 and Median Joining method (BANDELT et al. 1999).

Hierarchical analysis of molecular variation (AMOVA) within populations, among populations within groups and between groups was performed using the Arlequin v.3.5 software (EXCOFFIER et al. 2005). Genetic differentiation parameters, such as G_{ST} (NEI 1987) based only on the frequencies of mitotypes and N_{ST} taking into account the genetic distance between mitotypes (Pons and Petit 1996) were calculated and compared to each other using the PermutCpSSR v.1.0 software (BURBAN et al. 1999). The clustering (grouping) of populations was done using the SAMOVA program (DUPANLOUP et al. 2002), which uses both the genotypic data and the geographic location of populations. The algorithm of the program is aimed at finding a clustering of geographically adjacent populations into the *K* groups, in which the differentiation of groups (F_{CT}) would be the greatest.

Results

In total, 22 mitotypes were identified in Siberian larch populations (SEMERIKOV et al. 2013). Geographical distribution of mitotypes was heterogeneous. Based on the geographical distribution of the mitotypes and their genetic relationships four major groups were inferred in the SAMOVA analysis: 1) western part of the Siberian larch range (*L. sukaczewii*) - west of the Ob River (V), 2) the Central West Siberian Plain (VI), 3) around and west of the Baikal Lake (I, III) and 4) the Altai and Tien Shan Mountains (IV). In addition to these four major groups the SAMOVA analysis also identified a minor group located near the border between *L. sukaczewii* and *L. sibirica* (II) and two smaller subgroups within the West Siberian Plain (VI, VIII).

The total length of the resequenced regions of Siberian fir was 47000 bp. Four variable loci were identified: A65, A37-1, A167 and A126-2. All loci were diallelic SNPs. Genotyping of these loci in 45 populations of Siberian fir revealed three mitotypes different by 1-4 SNPs. Most of the studied populations contained only one mitotype. The mitotype 2 was fixed in the Baikal region, in north-east of the Eastern Sayan, in the Middle and Lower Yenisei, in most of the West Siberia and in the Northern and Subpolar Urals. The mitotype 1 was fixed or dominant in the Western Sayan, Kuznetsk Alatau and Altai. In the Middle and Southern Urals, in the west of the West Siberia Plain and in European Russia both these mitotypes had a mosaic structure and sometimes co-occurred within the same populations. The mitotype 3 was fixed or present as an admixture to mitotype 1 in the southernmost populations of the Altai and Kuznetsk Alatau and completely absent in more northern populations.

Analysis of the mitochondrial DNA variation in Scots pine samples collected from Slovakia in the west to northeast Mongolia revealed seven mitotypes. The presence of cycles on the mitotype network indicates the likely recombinant origin of some of them. The distribution of mitotypes divided the area into two sharply different regions approximately along the White Sea-Volga river line. These regions had almost no shared mitotypes, except mitotype 2, common in the western group of populations, and also present in the eastern group, in a limited area around the Southern Urals. The most frequent in the eastern group, mitotype 1 was also found in populations of the western group, but only close to the zone of contact with the populations of the eastern group. The western group was significantly richer in the number of mitotypes.

Discussion

Several relatively homogeneous groups of populations of *L. sibirica* were identified, presumably associated with the post-glacial recolonization from individual refugia, in which the species was preserved

during unfavorable periods of the Pleistocene. Such refugia were probably located in the Altai, Sayans, Baikal area and the Urals. The frequencies of haplotypes in the north of Western and Central Siberia, in contrast to the mountain regions of southern Siberia, were more homogeneous and suggest a possible recent colonization of the north of Siberia from a single source, presumably located in the northern foothills of the Sayan Mountains.

The distribution of the haplotype frequencies in the range of Siberian fir resembled those of Siberian larch. Distinct regions, especially in the south of Siberia, were sharply different with each other in composition of the haplotypes, which, like the Siberian larch, indicates their occurrence due to recolonization from different sources. The populations of the southern Altai and Sayan marked by distribution of mitotype 3 differed sharply from the more northern populations, which demonstrates their non-participation in the colonization of the northern regions. At the same time, there were significant differences between the phylogeography of mitochondrial DNA of Siberian larch and fir: unlike Siberian larch, which recolonization of the north of Siberia probably occurred with the participation of the populations from the northern foothills of Sayans, the recolonization of the north of Siberia and the Northern Urals by Siberian fir was due to migrations from the Baikal region. While the Siberian larch in Ural (considered by some taxonomists as a subspecies or a closely related species *L. sukaczewii*) had an endemic mitotype fixed throughout the Urals, both of the most common mitotypes of Siberian fir are distributed in mosaic pattern in the Urals, which probably indicates the repeated migrations of Siberian fir from South Siberia to the Urals, whereas migration of Siberian larch likely was single and more ancient.

The colonization zone of Siberian fir associated with the Baikal region extends along the Yenisei and covers the north of Western Siberia and the Northern Urals. The genetic proximity of fir in the Northern Urals and Baikal was also suggested in the earlier studies based on the nuclear markers (SEMERIKOVA and SEMERIKOV 2006, 2011).

Comparison of the results of phylogeographic studies of *L. sibirica* and *A. sibirica* indicates common features inherent to the phylogeography of these species, which is probably due to physical geography of Northern Eurasia and past climate fluctuations affecting forest species in a similar way, as well as due to presence of a common source of distribution of these species - Southern Siberia. Unlike Siberian species, boreal species in Europe have a different colonization history and other phylogeographic structure.

Phylogeography of Scots pine was investigated earlier with mitochondrial DNA markers. NAYDENOV et al. (2007) and PYHAYARVI et al. (2008) used the universal PCR primers for the *nad7* and *nad1* markers, which were variable in the western part of the range. It was shown that Scots pine spread during the post-glacial period from several refugia. Moreover, the north of Europe was populated by trees from a refugium located in the central part of Europe. Unfortunately, the variation of the used markers was limited, and the mtDNA structure was not described in the eastern part of the range. In the recent Scots pine study (SEMERIKOV et al. 2018), we used an increased set of mtDNA markers that allowed us to investigate the phylogeographic structure of Scots pine in the eastern part of the range in more detail.

The distribution of haplotypes divided the area into two sharply different regions approximately along the White Sea-Volga river line. These regions had almost no common haplotypes. This differentiation indicates a long standing division into the western and eastern groups. The western group was significantly richer in the number of haplotypes, which proves that the eastern group is secondary to the western group, and that the pine originally came to Siberia from Central Europe.

The homogeneity of the haplotype frequencies in the western group is probably a result of the post-glacial dispersion of pine from one refugium, possibly located in the Carpathians.

With regard to the eastern group, one can also assume a recent colonization, but there could be several refugia. At least one of them was in the Urals, as indicated by the distribution of the haplotype 2 around the South Urals.

Conclusions

Species-specific mtDNA markers were developed for Scots pine, Siberian fir and Siberian larch. The geographic structure of the mtDNA variation revealed using them indicates the post-glacial recolonization of the ranges of these species from isolated refugia located in southern Siberia and the Urals in the case of *L. sibirica* and *A. sibirica* and in central Europe and the Urals in the case of *P. sylvestris*.

References

BANDELT HJ, FORSTER P, ROHL A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16: 37-48.

BINNEY HA, WILLIS KJ, EDWARDS ME, BHAGWAT SA, ANDERSON PM, ANDREEV AA, BLAAUW M, DAMBLON F, HAESAERTS P, KIENAST F, KREMENETSKI KV, KRIVONOGOV SK, LOZHKIN AV, MACDONALD GM, NOVENKO EY, OKSANEN P, SAPELKO TV, VALIRANTA M, VAZHENINA L (2009) The distribution of late-Quaternary woody taxa in northern Eurasia: evidence from a new macrofossil database. Quaternary Science Review 28: 2445-2464.

BUCHOVSKA J, DANUSEVICIUS D, BANIULIS D, STANYS V, SIKSNIANIENE JB, KAVALIAUSKAS D (2013) The Location of the Northern Glacial Refugium of Scots Pine Based on Mitochondrial DNA Markers. Baltic Forestry 19: 2-12.

BURBAN C, PETIT RJ, CARCREFF E, JACTEL H (1999) Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc. (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. Molecular Ecology 8: 1593-1602.

DUPANLOUP I, SCHNEIDER S, EXCOFFIER L (2002) A simulated annealing approach to define the genetic structure of populations. Molecular Ecology 11: 2571-2581.

EXCOFFIER L, LAVAL G, SCHNEIDER S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1: 47-50.

NAYDENOV K, SENNEVILLE S, BEAULIEU J, TREMBLAY F, BOUSQUET J (2007) Glacial vicariance in Eurasia: mitochondrial DNA evidence from Scots pine for a complex heritage involving genetically distinct refugia at mid-northern latitudes and in Asia Minor. BMC Evolutionary Biology 7: 233.

NEI M (1987) Molecular evolutionary genetics. New York: Columbia University Press: 512 pp.

PONS O, PETIT RJ (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144: 1237-1245.

PYHAJARVI T, SALMELA MJ, SAVOLAINEN O (2008) Colonization routes of Pinus sylvestris inferred from distribution of mitochondrial DNA variation. Tree Genetics & Genomes 4: 247-254.

SEMERIKOV VL, PUTINTSEVA YA, ORESHKOVA NV, SEMERIKOVA SA, KRUTOVSKY KV (2015) Development of new mitochondrial DNA markers in Scots pine (*Pinus sylvestris* L.) for population and phylogeographic studies. Russian Journal of Genetics 51: 1199-1203.

SEMERIKOV VL, SEMERIKOVA SA, DYMSHAKOVA OS, ZATSEPINA KG, TARAKANOV VV, TIKHONOVA IV, EKART AK, VIDYAKIN AI, JAMIYANSUREN S, ROGOVTSEV RV, KALCHENKO LI (2014) Microsatellite loci polymorphism of chloroplast DNA of Scots pine (*Pinus sylvestris* L.) in Asia and eastern Europe. Russian Journal of Genetics 50: 577-585.

SEMERIKOV VL, SEMERIKOVA SA, POLEZHAEVA MA, KOSINTSEV PA, LASCOUX M (2013) Southern montane populations did not contribute to the recolonization of West Siberian Plain by Siberian larch (*Larix sibirica*): a range-wide analysis of cytoplasmic markers. Molecular Ecology 22 (19): 4958-4971.

SEMERIKOV VL, SEMERIKOVA SA, PUTINTSEVA YA, TARAKANOV VV, TIKHONOVA IV, VIDYAKIN AI, ORESHKOVA NV, KRUTOVSKY KV (2018) Colonization history of Scots pine in Eastern Europe and North Asia based on mitochondrial DNA variation. Tree Genetics and Genomes 14: 8.

SEMERIKOV VL, VENDRAMIN GG, SEBASTINIANI F, LASCOUX M (2006) RAPD-derived, PCR-based mitochondrial markers for *Larix* species and their usefulness in phylogeny. Conservation Genetics 7: 621–625.

SEMERIKOVA SA, SEMERIKOV VL (2006) Genetic variation and population differentiation in Siberian fir *Abies sibirica* Ledeb. inferred from allozyme markers. Russian Journal of Genetics 42 (6): 636-644.

SEMERIKOVA SA, SEMERIKOV VL (2007) The diversity of chloroplast microsatellite loci in Siberian fir (*Abies sibirica* Ledeb.) and two Far East fir species *A. nephrolepis* (Trautv.) Maxim. and *A. sachalinensis* Fr. Schmidt. Russian Journal of Genetics 43 (12): 1373-1381.

SEMERIKOVA SA, SEMERIKOV VL (2011) Genetic variability of Siberian fir *Abies sibirica* Ledeb. inferred from AFLP markers. Russian Journal of Genetics 47 (2): 241-246.

VIDYAKIN AI, SEMERIKOV VL, POLEZHAEVA MA, DYMSHAKOVA OS (2012) Spread of mitochondrial DNA haplotypes in population of Scots pine (*Pinus sylvestris* L.) in northern European Russia. Russian Journal of Genetics 48: 1267-1271.

WILLIS KJ, VAN ANDEL TH (2004) Trees or no trees? The environments of central and eastern Europe during the Last Glaciation. Quaternary Science Review 23: 2369-2387.

Genomics for practical forestry: development of genome-wide markers for timber origin identification and other applications

YULIYA A. PUTINTSEVA[1], NATALIAY V. ORESHKOVA[1,2], EUGENIYA I. BONDAR[1], VADIM V. SHAROV[1], DMITRY A. KUZMIN[1], STEPAN V. MAKOLOV[1], KONSTANTIN V. KRUTOVSKY[1,3,4,5]

[1] Laboratory of Forest Genomics, Genome Research and Education Center, Siberian Federal University, Prospect Svobodny 79, Krasnoyarsk 660041, Russia (yaputintseva@mail.ru)

[2] Laboratory of Forest Genetics and Selection, V. N. Sukachev Institute of Forest, Federal Research Center «Krasnoyarsk Science Center of the Siberian Branch of Russian Academy of Sciences », Akademgorodok 50/28, Krasnoyarsk 660036, Russia (oreshkova@ksc.krasn.ru)

[3] Department of Forest Genetics and Forest Tree Breeding, Georg-August University of Göttingen, Büsgenweg 2, 37073 Göttingen, Germany (konstantin.krutovsky@forst.uni-goettingen.de)

[4] Laboratory of Population Genetics, N. I. Vavilov Institute of General Genetics of Russian Academy of Sciences, Gubkina Str. 3, Moscow 119991, Russia (kkrutovsky@gmail.com)

[5] Department of Ecosystem Science and Management, Texas A&M University, 305 Horticulture and Forest Science Building, College Station, TX 77843-2138, USA (k-krutovsky@tamu.edu)

Abstract

The forest genetics, tree improvement and protection can greatly benefit from complete genome sequence data made recently available for several major conifer species. They allow to identify and annotate genes, other functional elements (sRNA, transcription factors, regulatory elements, etc.) and genetic networks that control adaptation and disease resistance. They can be used to develop highly informative genetic markers that can be used in population genetic studies to create database of barcodes for individual populations to fight illegal timber harvest and trade. They are very much needed for development of genome-wide genetic markers for association studies for linking genetic variation (SNPs, alleles, haplotypes, and genotypes) with environmental factors, adaptive traits and phenotypes for better understanding genetic control of agronomically and economically important traits. They can be also used to develop genome-wide genetic markers for genomic-assisted selection to breed for better adapted, stress resistant and climate change resilient trees with desirable quality ecological and economic traits. Finally, whole genome sequences allow to integrate proteomics, transcriptomics and metabolomics and provide reference genomes for resequencing. In this brief summary we would like to present one of many practical applications of genetics and genomics in forestry– development of highly polymorphic and informative molecular genetic markers for several very important boreal forest species in Eurasia, Siberian larch (*Larix sibirica* Ledeb.), Siberian stone pine (*Pinus sibirica* Du Tour) and Scots pine (*Pinus sylvestris* L.), based on the whole genome data obtained in the "Genomics of the Key Boreal Forest Conifer Species and Their Major Phytopathogens in the Russian Federation" project funded by the Government of the Russian Federation (grant no. 14.Y26.31.0004), which is also result of the productive international collaboration between Russian and German scientists.

Key words: genetic diversity, genome, *Larix sibirica*, microsatellite markers, NGS, *Pinus sibirica*, *Pinus sylvestris*, Siberian larch, Siberian stone pine, Scots pine, whole genome sequencing

Introduction

The whole genome sequence data are the foundation for subsequent studies of evolutionary, biochemical and physiological processes in the sequenced organisms. Deep knowledge of the genome structure including the fine exon-intron gene structure, repeated sequences and intergenic sites help us better

understand the mechanisms of gene regulation and expression, as well as the genome evolution. The whole genomic data become more available recently, including conifer species, and are widely used now to develop new DNA markers, such as single nucleotide polymorphisms (SNPs) and microsatellite loci or simple sequence repeats (SSRs) that can be used in population genetic analysis and for solving practical forestry problems, for example, to identify the origin of wood and planting material, for certification and identification of clones.

The development of molecular genetic markers for the main forest-forming tree species are extremely important and needed for solving problems of forestry, reforestation and afforestation. To solve these problems, estimates of the level of genetic variability, data on the population structure and differentiation, and effective methods of genetic identification of the wood and plant material origin are required.

Among the available genetic markers, nuclear microsatellite loci can be used to address these problems and are most fully meet requirements for reliable and convenient genetic markers. They are characterized by high specificity, reproducibility, codominance, multiple alleles, high heterozygosity and, moreover, do not require sophisticated equipment for analysis.

For example, Siberian larch (*Larix sibirica* Ledeb.) is one of the main forest-forming conifer species in Siberia, such species-specific markers have not been developed till recently. Siberian larch grows in the forest zone of the east and northeast of the European part of Russia, the Urals, Western and Eastern Siberia. Its area stretches from tundra (71 °N latitude) on the north to the southern latitudes of Altai and Sayan (46 °N) on the south. On the territory of the Russian Federation, larch forests occupy 263 million hectares, about 40 % of the forest area of the country (769.8 million hectares). Previously, markers based on nuclear microsatellite loci developed for other species of this genus were used to analyze the population-genetic variation of *L. sibirica* (KHASA et al. 2000, ISODA and WATANABE 2006, CHEN et al. 2009). With the help of these markers, genetic diversity and differentiation were studied in several populations of this species (ORESHKOVA and BELOKON 2012, ORESHKOVA et al. 2013). However, a small number of markers was used in these studies due to poor PCR amplification and the presence of a large number of "null alleles" for many non-species-specific markers.

Siberian stone pine, *Pinus sibirica* Du Tour and Scots pine (*Pinus sylvestris* L.) are also among the most economically and environmentally important forest-forming species of conifers in Eurasia. To study these forests a large number of highly polymorphic molecular genetic markers, such as microsatellite loci, are also required that were unavailable for Siberian stone pine till recently.

Prior to the new high-throughput next generation sequencing (NGS) methods, discovery of microsatellite loci and development of microsatellite markers were very time consuming and laborious. The recently developed draft assemblies of the Siberian larch, Siberian stone pine and Scots pine genomes sequenced using the NGS methods in the Laboratory of Forest Genomics of the Siberian Federal University (KRUTOVSKY et al. 2014, ORESHKOVA et al. 2015, SADOVSKY et al. 2016), it has become possible to develop species-specific microsatellite primers for these species.

Material and methods

The draft genome assemblies presented in Table 1 allowed us to identify a large number of microsatellite loci in the Siberian larch and Siberian stone pine genomes and to develop species-specific PCR primers for their amplification and genotyping. The primers were designed using contigs containing short simple sequence tandem repeats.

To develop new highly informative microsatellite genetic markers for Siberian larch and Siberian stone pine using their whole genome assemblies a computer search for microsatellite loci with high repetitive simple motifs was done in the genomic DNA sequences, oligonucleotide primers were developed,

synthesized and tested for the selected loci. A preliminary estimate of allelic diversity was made on two test samples of a Siberian larch population collected in the Republic of Khakassia (Russia) and several Siberian stone pine populations (BELOKON et al. 2016, ORESHKOVA *et al*. 2017).

The most promising markers were selected, and multiplex genotyping panels were designed for Siberian larch and tested for fragment analysis using the ABI 3130xl GeneticAnalyzer with capillary electrophoresis (ORESHKOVA et al. 2017).

Table 1: Whole-genome sequencing data used to develop microsatellite markers in Siberian larch (*Larix sibirica*) and Siberian stone pine (*Pinus sibirica*) and mitochondrial markers in Scots pine (*Pinus sylvestris*)

Genome assembly	Total number of sequence reads [mln]	N50 [bp]	Longest, [bp]	Total assembly length [Gbp]
		Larix sibirica		
Contigs	12.4	1074	128642	7.99
Scaffolds	11.33	6443	354326	12.34
		Pinus sibirica		
Contigs	10.75	948	105599	7.01
Scaffolds	9.45	6920	110935	13.56
		Pinus sylvestris		
Contigs	15.22	488	75010	6.748
Scaffolds	14.79	654	105091	7.807

The sequencing of the Siberian larch genome was done with 93X coverage using the Illumina HiSeq 2000 platform. To select high quality reads and to remove adapter dimers the raw reads were filtered using MUSKET (LIU et al. 2013) and Trimmomatic (BOLGER et al. 2014). A draft assembly was generated using the CLC Assembly Cell assembler (https://www.qiagen-bioinformatics.com). The obtained assembly contained 12.4 million contigs with a total length of ~8 Gbp. This assembly was searched for contigs containing microsatellite loci using the GMATo program (WANG et al. 2013). The preliminary analysis showed that microsatellite loci with tri-, tetra- and pentanucleotide motifs were much less variable in larch than the loci with dinucleotide motifs. Therefore, from all microsatellite loci found, only loci with dinucleotide motifs repeated at least 20 times were selected for the PCR primer design. Primers for the selected microsatellite loci were designed using the WebSat online service (MARTINS et al. 2009). As a result, 59 primers pairs were designed and tested. Needle samples collected from 100 individual Siberian larch trees in 2014 in two populations (50 trees per population) in the Republic of Khakassia were used in this study (ORESHKOVA et al. 2017). The one population is located in the Shirinsky District of Khakassia near the Shira-Berenjak highway (larch forest with pine on a gentle slope), another – near the Efremkino Village (larch ona steep slope and at its foot).

Similar search for microsatellite loci were done using the Siberian stone pine 32X genome coverage assembly (BELOKON et al. 2016). The designed primers were first tested on DNA samples of four P. sibirica trees to select successful primers that generate amplification product and to optimize the PCR conditions. The selected primers were then tested on eight specimens from the same population in order to detect polymorphisms. Variability of the loci that were monomorphic in this sample was tested further in nine individuals from nine geographically distant populations representing different regions of the Siberian stone pine area. The final testing of the polymorphic loci was performed using 10-12 specimens per each of several populations.

To develop mitochondrial DNA markers in Scots pine contigs from its partial genome assembly were mapped to the mitochondrial DNA (mtDNA) contigs of Norway spruce (*Picea abies* (L.) Karst.) and loblolly pine (*Pinus taeda* L.) to identify homologous mitochondrial fragments of Scots pine. Then, they were resequenced in a sample of the Scots pine trees of European, Siberian, Mongolian and Caucasian origin in order to develop mtDNA markers. Flanking non-coding regions of some mitochondrial genes were also investigated and resequenced (SEMERIKOV et al. 2015).

Results and discussion

Larix sibirica SSRs

Among 59 primer pairs selected in the first test 20 produced no product, 12 had non-specific amplification and 27 stably amplified supposedly a single-locus PCR product that could be well-genotyped on gels. After the first selection, the forward primer in each of the 27 pairs was labelled either by "blue" (FAM) or "green" (HEX) fluorescent dyes for further testing on the ABI PRISM 3730 sequencer. The labelled oligonucleotide primers were synthesized by Sigma (Germany). The trial PCR multiplexes consisting of two or three primer pairs were made taking into account the size of the PCR fragments. Multiplexing was done at the PCR reaction stage by combining two or three different primer pairs in the same PCR reaction and adjusting the total volume by reducing the water portion accordingly. The obtained PCR amplification product was necessarily diluted 50–100 times before electrophoresis. The testing of polymorphic loci at this stage was carried out using 8–16 samples from each of the two populations. After this testing on a capillary sequencer, additional 9 pairs of primers had to be excluded due to poor or nonspecific amplification, and supposedly a large number of null alleles.

Pinus sibirica SSRs

Based on the testing of primers for 70 microsatellite loci with tri-, tetra- or pentanucleotide repeats, 18 most promising, reliable and polymorphic loci were selected that can be used further as molecular genetic markers in population genetic studies of Siberian stone pine (BELOKON et al. 2016).

Pinus sylvestris mitochondrial DNA markers

Five SNPs and a single minisatellite locus were identified (SEMERIKOV et al. 2015). Caucasian samples differed from the rest by three SNPs. Two SNPs have been linked to an early described marker in the first intron of the *nad7* gene, and all together revealed three haplotypes in European populations. No variable SNPs were found in the Siberian and Mongolian populations. The minisatellite locus contained 41 alleles across European, Siberian, and Mongolian populations, but, this locus demonstrated a weak population differentiation (F_{ST} = 0.058), probably due to its high mutation rate.

These new markers were further used in the Scots pine population and phylogeographic studies (SEMERIKOV et al. 2018). Three mitochondrial DNA markers were genotyped in 90 populations of Scots pine located from Eastern Europe to Eastern Siberia. The geographic distribution of seven mitotypes demonstrated the split between western and eastern populations approximately along the 38th meridian. Genetic diversity in the western part was significantly higher than in the eastern one. Five mitotypes were western- and one eastern-specific. One mitotype was common in both regions, but in the eastern part it occurred only in the South Urals and adjacent areas. The geographic structure in the mitotype distribution supports a hypothesis of post-glacial recolonization of the studied territory from the European and Ural refugia.

Conclusions

The whole genome sequencing data provided rich material for developing highly polymorphic molecular genetic markers that were efficiently used for genotyping of natural and artificial populations of Siberian stone pine, Siberian larch and Scots pine, Newly developed markers will allow us obtaining reliable quantitative estimates of the parameters of their genetic structure, such as within and between population allelic and genetic diversity, genetic subdivision and differentiation at different hierarchical levels, inbreeding, gene flow, etc.

Acknowledgements

The study was done as part of the project "Genomics of the Key Boreal Forest Conifer Species and Their Major Phytopathogens in the Russian Federation" funded by the Government of the Russian Federation (grant no. 14.Y26.31.0004).

References

BELOKON MM, POLITOV DV, MUDRIK EA, POLYAKOVA TA, SHATOKHINA AV, BELOKON YUS, ORESHKOVA NV, PUTINTSEVA YUA, SHAROV VV, KUZMIN DA, KRUTOVSKY KV (2016) Development of Microsatellite Genetic Markers in Siberian Stone Pine (*Pinus sibirica* Du Tour) Based on the *De Novo* Whole Genome Sequencing. Russian Journal of Genetics 52 (12): 1284–1292.

BOLGER AM, LOHSE M, USADEL B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30 (15): 2114–2120. doi: 10.1093/bioinformatics/btu17

CHEN C, LIEWLAKSANEEYANAWIN C, FUNDA T, KENAWY A, NEWTON CH, EL-KASSABY YA (2009) Development and characterization of microsatellite loci in western larch (*Larix occidentalis* Nutt.). Mol. Ecol. Resour. 9 (3): 843-845. doi: 10.1111/j.1755-0998.2008.02289.x

ISODA K, WATANABE A (2006) Isolation and characterization of microsatellite loci from *Larix kaempferi.* Mol. Ecol. Notes 6 (3): 664-666. doi: 10.1111/j.1471-8286.2006.01291.x

KHASA DP, NEWTON CH, RAHMAN MH, JAQUISH B, DANCIK BP (2000) Isolation, characterization, and inheritance of microsatellite loci in alpine larch and western larch. Genome 43 (3): 439-448. doi 10.1139/g99-131

KRUTOVSKY KV, ORESHKOVA NV, PUTINTSEVA YUA, IBE AA, DEICH KO, SHILKINA EA (2014) Preliminary results of *de novo* whole genome sequencing of the Siberian Larch (*Larix sibirica* Ledeb.) and the Siberian Stone Pine (*Pinus sibirica* Du Tour). Siberian Journal of Forest Science 1 (4): 79–83 (in Russian with abstract in English).

LIU Y1, SCHRÖDER J, SCHMIDT B (2013) Musket: a multistage *k*-mer spectrum-based error corrector for Illumina sequence data. Bioinformatics 29 (3): 308-315. doi: 10.1093/bioinformatics/bts690.

MARTINS WS, LUCAS DCS, NEVES KFS, BERTIOLI DJ (2009) WebSat - a web software for microsatellite marker development. Bioinformation 3 (6): 282–283.

ORESHKOVA NV, PUTINTSEVA YUA, SHAROV VV, KUZMIN DA, KRUTOVSKY KV (2017) Development of Microsatellite Genetic Markers in Siberian larch (*Larix sibirica* Ledeb.) Based on the *De Novo* Whole Genome Sequencing. Russian Journal of Genetics 53 (11): 1194–1199; doi: 10.1134/S1022795417110096

ORESHKOVA NV, BELOKON MM (2012) Assessment of the genetic variation of Siberian larch use microsatellite markers. Vestnik MSGL - Lesnoy Vestnik 84 (1): 118-122, in Russian (*Орешкова Н.В., Белоконь М.М.* Оценка генетической изменчивости лиственницы сибирской с использованием микросателлитных маркеров // Вестник МГУЛ - Лесной вестник. 2012. Т. 84. № 1. С. 118-122.)

ORESHKOVA NV, BELOKON MM, JAMIYANSUREN S (2013) Genetic Diversity, Population Structure, and Differentiation of Siberian Larch, Gmelin Larch, and Cajander Larch on SSR-Marker Data. Russian Journal of Genetics 49 (2): 178–186. doi: 10.1134/S1022795412120095

ORESHKOVA NV, PUTINTSEVA YUA, KUZMIN DA, SHAROV VV, BIRYUKOV VV, MAKOLOV SV, DEICH KO, IBE AA, SHILKINA EA, KRUTOVSKY KV (2015) Genome sequencing and assembly of Siberian larch (*Larix sibirica* Ledeb.) and Siberian pine (*Pinus sibirica* Du Tour) and preliminary transcriptome data. Proceedings of the 4th International Conference on Conservation of Forest Genetic Resources in Siberia. Barnaul, Russia, 24–29 August, 2015, pp. 127–128.

SADOVSKY MG, PUTINTSEVA YUA, BIRUKOV VV, NOVIKOVA S, KRUTOVSKY KV (2016) *De novo* assembly and cluster analysis of Siberian larch transcriptome and genome. Lecture Notes in Bioinformatics 9656: 455-464.

SEMERIKOV VL, PUTINTSEVA YUA, ORESHKOVA NV, SEMERIKOVA SA, KRUTOVSKY KV (2015) Development of new mitochondrial DNA markers in Scots pine (*Pinus sylvestris* L.) for population and phylogeographic studies. Russian Journal of Genetics 51 (12): 1199–1203.

SEMERIKOV VL, SEMERIKOVA SA, PUTINTSEVA YA, TARAKANOV VV, TIKHONOVA IV, VIDYAKIN AI, ORESHKOVA NV, KRUTOVSKY KV (2018) Colonization history of Scots pine in Eastern Europe and North Asia based on mitochondrial DNA variation. Tree Genetics and Genomes 14: 8 https://doi.org/10.1007/s11295-017-1222-0

WANG X, LU P, LUO Z (2013) GMATo: A novel tool for the identification and analysis of microsatellites in large genomes. Bioinformation 9 (10): 541–544.

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).

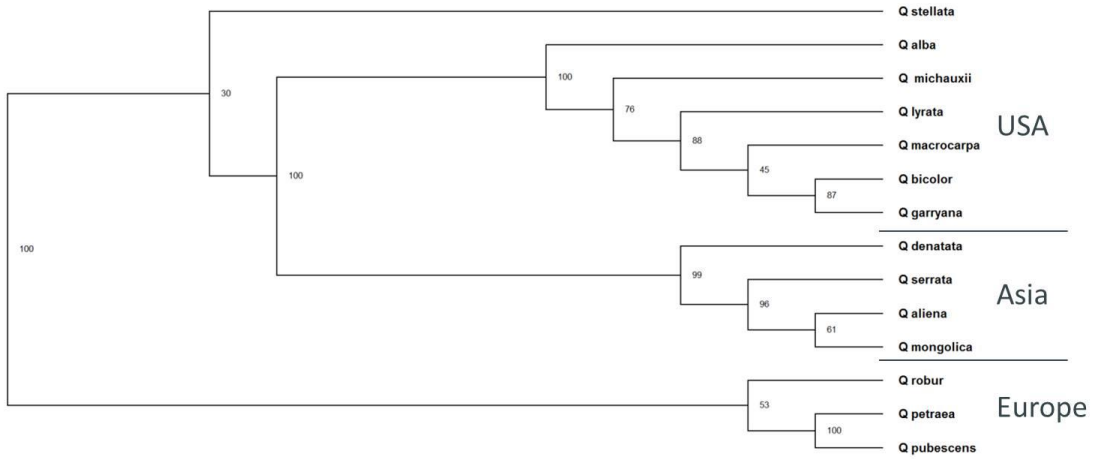

Figure 1: Dendrogram differentiating the white oak species within the three continents. The dendrogram is created using the program GDA_NT (Degen 2008) and PAST (Hammer *et al*. 2001). The numbers at the branches are boots trapping values.

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

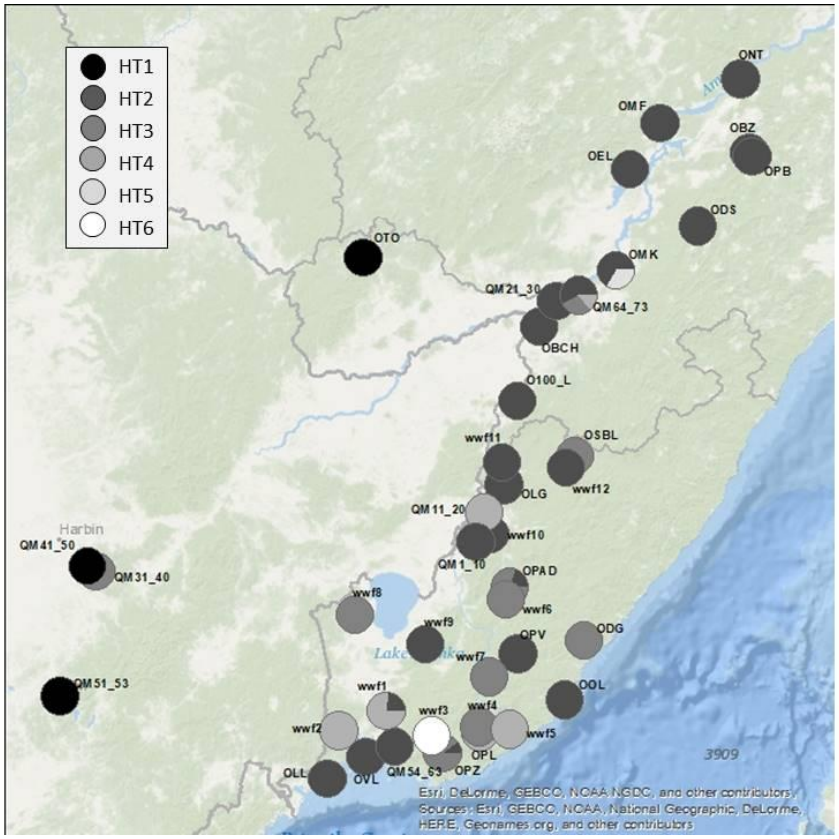

Figure 2: Distribution of the six haplotypes within 40 populations of *Q. mongolica* in Far East Russia based on mitochondrial and chloroplast markers.

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.

Acknowledgements

This project has been financially supported by German Federal Ministry of Food and Agriculture (BMEL), the Deutsche Bundesstiftung Umwelt (DBU) and the USDA Forest Service International Programs Office.

We thank collaborators and institutions that provided samples of North American oaks, including the Morton Arboretum, Lisle, IL (ANDREW HIPP, MARLENE HAHN), North Carolina State University, Raleigh, NC (PAUL MANOS), and the USDA Forest Service (RICHARD CRONN, TARA JENNINGS, PAUL BERRANG, ANDY BOWER, BRITTON FLASH, STEVEN FORRY, BEN GOMBASH, MARK TWERY). Furthermore, we thank MARK DASENKO, MATTHEW PETERSON, CHRIS SULLIVAN (Oregon State University Center for Genome Research and Biocomputing) for assistance with sequencing, and BRAD KINDER, ALEX MOAD, and SHELLEY GARDNER (US Forest Service) for project and planning assistance. Finally, we thank the Botanical Gardens of Duesseldorf, Bayreuth, Kiel, Goettingen, Giessen, Bonn, Bochum, Frankfurt, Ulm, Osnabrueck, and the Forest Botanical Gardens Tharandt and Eberswalde in Germany that provided us with material of different oak species. We also like to thank VIVIAN KUHLENKAMP, LAURA SCHULZ, SUSANNE JELKMANN and ANN-CHRISTINE BERGMANN for technical assistance.

References

CASSENS D (2007) White oak. In: Hardwood lumber and veneer series. Purdue Extension, FNR-292-W.53.

DEGEN B (2008) GDA_NT 2.0: Genetic data analysis and numerical tests. [last change 17.11.2015]. Available from bernd.degen@thuenen.de

DORMONTT EE, BONER M, BRAUN B, BREULMANN G, DEGEN B, ESPINOZA E, *et al*. (2015) Forensic timber identification: It's time to integrate disciplines to combat illegal logging. Biological Conservation 191: 790-798.

GONCALVES PFM, OLIVEIRA-MARQUES AR, MATSUMOTO TE, MIYAKI CY (2015) DNA Barcoding identifies illegal parrot trade. Journal of Heredity 106: 560-564.

HAMMER Ø, HARPER DAT, RYAN PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4 (1): 9pp.

HANDY SM, PARKS MB, DEEDS JR, LISTON A, DE JAGER LS, LUCCIOLI S et al. (2011) Use of the chloroplast gene *ycf1* for the genetic differentiation of pine nuts obtained from consumers experiencing dysgeusia. Journal of Agriculture and Food Chemistry 59: 10995-11002.

HOLLINGSWORTH PM, FORREST LL, SPOUGE JL (2009) A DNA barcode for landplants. Proceedings of the National Academy of Science USA 106: 12794-12797.

LEROY SAG, ARPE K (2007) Glacial refugia for summer-green trees in Europe and south-west Asia as proposed by ECHAM3 time-slice athmospheric model simulation. Journal of Biogeography 34: 2115-2128.

OKAURA T, QUANG ND, UBUKATA M, HARADA K (2007) Phylogeographic structure and late Quaternary population history of the Japanese oak *Quercus mongolica* var. *crispula* and related species revealed by chloroplast DNA variation. Genes & Genetic Systems 82: 465-477.

PAPPALARDO AM, FERRITO V (2015) DNA barcoding species identification unveils mislabeling of processed flatfish products in southern Italy markets. Fishery Research 164: 153-158.

SCHROEDER H, CRONN R, YANBAEV Y, JENNINGS T, MADER M, DEGEN B, KERSTEN B (2016) Development of easy-to-use molecular markers for differentiation within the genus *Quercus.* PLoS One 11 (6), e0158221, DOI:10.1371/journal.pone.0158221.

US DEPARTMENT OF JUSTICE (2015) Statement of facts, United States of American v. Lumber Liquidators. Case document 2:15-cr-00126-RAJ-LRL. Available online: http://www.justice.gov/opa/file/787141/download

Introgression of genes between two hybridizing red oak species with different drought tolerance

OLIVER GAILING[1,2]

[1] Department of Forest Genetics and Forest Tree Breeding, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany (ogailing@gwdg.de)

[2] School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Drive, Houghton, Michigan 49931-1295, USA

Abstract

Hybridizing species that maintain different local adaptations despite interspecific gene flow are models for the discovery of genes involved in reproductive isolation and adaptive divergence of species. *Quercus rubra* L. and *Quercus ellipsoidalis* E.J. Hill are two interfertile red oak species with different adaptations to drought which hybridize in contact zones in intermediate environments. Among 36 genic microsatellites (EST-SSRs) and 8 nuclear microsatellites (nSSRs), one EST-SSR was highly differentiated between species from different regions both in sympatric and parapatric stands. The outlier locus was annotated as a CONSTANS-like gene, and high species differentiation was found to be due to variation in a trinucleotide microsatellite which codes for a poly-glutamine (Q) repeat. The characteristic *Q. ellipsoidalis* allele has five poly-Q repeats (Q5) and the characteristic *Q. rubra* allele has six poly-Q repeats (Q6). Despite symmetric gene flow, in the two sympatric populations introgression of allele Q5 into *Q. rubra* was significantly higher than introgression of Q6 into *Q. ellipsoidalis*. Furthermore, allele copy number of outlier allele Q5 (characteristic for *Q. ellipsoidalis*) in *Q. rubra* and of outlier allele Q6 (characteristic for *Q. rubra*) in *Q. ellipsoidalis* showed as weak but significant association with soil characteristics. This pattern suggests that introgression of outlier alleles between species is affected by environmental selection and that adaptive introgression of genes could be an important mechanism for the adaptation to changing and stressful environmental conditions.

Key words: *Quercus rubra*, *Quercus ellipsoidalis*, CONSTANS-like, hybridization

Introduction

Introgression of adaptive traits and genes between hybridizing species can be a mechanism to increase genetic variation for the rapid adaptation to changing and stressful environments (ARNOLD 2004, ARNOLD 2006, SOLTIS and SOLTIS 2009, ARNOLD 2016). Hybridization seems to be common for many plant species, but the role of hybridization in adaptive evolution has been critically discussed (SCHEMSKE 2000). To assess the importance of hybridization for the maintenance of a species´ adaptive potential under changing environmental conditions, the frequency of hybridization and introgression between species and the fitness of different hybrid genotypes have to be evaluated. Related oak species of the same taxonomic section are known to frequently hybridize in contact zones and are a model to study species coherence in the face of interspecific gene flow (CURTU et al. 2007, 2009, GOICOECHEA et al. 2012, GOICOECHEA et al. 2015). Genome-wide marker screenings of hybridizing oak species with different local adaptations can help to identify genes with high interspecific genetic differentiation as signature of divergent selection (outlier genes) (SCOTTI-SAINTAGNE et al. 2004, GOICOECHEA et al. 2012, LIND-RIEHL et al. 2014, GOICOECHEA et al. 2015). Once these genes are identified, the frequency and direction of outlier allele introgression can be estimated by calculating the frequency of the rare outlier alleles in parapatric and sympatric populations in each species.

Here, a summary of already published results on hybridizing North American red oak species is presented. The analyses focussed on the interfertile species *Quercus rubra* L. and *Quercus ellipsoidalis* E.J. Hill which represent the most drought averse and the most drought tolerant of red oak species in their northern distribution range (ABRAMS 1988, 1990, BURNS and HONKALA 1990, NIXON 1997). Specifically, we will provide indirect evidence for interspecific gene flow based on genetic differentiation analyses at nuclear and chloroplast DNA markers (LIND and GAILING 2013, ZHANG et al. 2015), and direct evidence for contemporary interspecific gene flow using paternity analyses in sympatric stands (KHODWEKAR and GAILING 2017). Finally, the results of outlier screens in two sympatric and four parapatric interspecific population pairs are presented (LIND-RIEHL et al. 2014). One genic marker with high interspecific differentiation as signature of strong divergent selection was further analysed in sympatric populations where both species and putative hybrids growth next to each other on intermediate soils (KHODWEKAR and GAILING 2017). The gene was annotated as CONSTANS-like and both species were differentiated for the number of poly-glutamine (Q) repeats (LIND-RIEHL and GAILING 2017). We compared the frequency of the typical *Q. ellipsoidalis* allele (Q = 5, Q5) in *Q. rubra* and the frequency of the typical *Q. rubra* allele (Q = 6, Q6) in *Q. ellipsoidalis* to estimate the level and direction of introgression of these alleles between species (KHODWEKAR and GAILING 2017). Finally, potential future population level and experimental analyses are discussed for the identification of outlier loci across the genome and to test the fitness of genotypes at outlier genes in each species.

Material and methods

Parapatric and sympatric population pairs of *Q. rubra* and *Q. ellipsoidalis* were collected at the northern distribution range of both species where they are the only interfertile red oak species within gene flow distance. Parapatric *Q. rubra* / *Q. ellipsoidalis* populations were adjacent to each other (3.2 km to 8.8 km apart) but not overlapping (LIND-RIEHL et al. 2014). Sympatric populations were mixed *Q. rubra* and *Q. ellipsoidalis* populations where distribution ranges of both species overlapped in a narrow contact zone (KHODWEKAR and GAILING 2017). In total, four parapatric and two sympatric population pairs were included in the analyses. Initial outlier screens were performed in all parapatric population pairs for genetically identified species using different programs and methods (LIND-RIEHL et al. 2014). Finally, selected markers including one outlier under strong divergent selection in parapatric population pairs were analysed in the two sympatric population pairs (KHODWEKAR and GAILING 2017). Genetic assignment analyses in both sympatric and parapatric populations were performed to estimate the frequency and direction of interspecific gene flow. Likewise, the frequency of the outlier allele characteristic for *Q. ellipsoidalis* (allele Q5) in *Q. rubra* and of the outlier allele characteristic for *Q. rubra* (allele Q6) in *Q. ellipsoidalis* was calculated as an estimate of outlier allele introgression between species (KHODWEKAR and GAILING 2017).

Additionally, genetic differentiation at nuclear SSRs and chloroplast SSRs was analysed in populations of the interfertile species *Q. rubra*, *Q. ellipsoidalis* and *Quercus velutina* Lam in the Great Lakes region (OWUSU et al. 2015, ZHANG et al. 2015, SULLIVAN et al. 2016). Analyses of genetic differentiation patterns and genetic structure analyses were performed to provide evidence for interspecific gene flow. Finally, levels of contemporary gene flow were estimated in sympatric populations using paternity analyses (KHODWEKAR and GAILING 2017).

Results and Discusion

Adaptive species differentiation

Quercus rubra and *Q. ellipsoidalis* are two hybridizing species with different drought adaptations and soil preferences. *Quercus ellipsoidalis* is more deeply rooted, has smaller and more deeply dissected leaves and is slower growing than *Q. rubra*. Furthermore, *Q. ellipsoidalis* showed a later bud burst and leaf senescence and a lower survival rate in a seedling common garden trial (GAILING 2013). Deep roots facilitate the maintenance of relatively high predawn water potentials and effective water transport

during drought (ABRAMS 1990). *Quercus ellipsoidalis* grows on dry sandy outwash plains while *Quercus rubra* is more common on soils with a higher organic matter content and water holding capacity (BURNS and HONKALA 1990, NIXON 1997). However, both species can occur in close proximity to each other, and on intermediate soil types the distribution of both species overlaps and members of both species can grow next to each other (KHODWEKAR and GAILING 2017).

Evidence for recurrent gene flow between species

Leaf morphometric and genetic differentiation analyses at isozyme makers suggested that *Q. rubra* and *Q. ellipsoidalis* form hybrids in sympatric stands (HOKANSON et al. 1993, JENSEN et al. 1993). Here, we present our more recent results on genetic structure and gene flow analyses at biparentally inherited microsatellites and on interspecific genetic differentiation at uniparentally (maternally) inherited chloroplast microsatellites. Low genetic differentiation between species, the occurrence of morphologically and genetically intermediate types and estimates of contemporary gene flow using paternity analyses provide evidence for recurrent interspecific gene flow between species.

For example, interspecific genetic differentiation measured as F_{ST} at 21 randomly selected microsatellite markers (nuclear SSRs, nSSRs) was 6.7% and 7.1% for sympatric populations from two geographic regions (Baraga Plains, Escanaba) (KHODWEKAR and GAILING 2017). For neighboring (parapatric) populations from the Baraga Plains region, interspecific differentiation at 16 microsatellites (LIND and GAILING 2013) (10 microsatellites were overlapping with KHODWEKAR and GAILING 2017) ranged from 6.0% to 7.0% in the same order of magnitude than interspecific differentiation in the sympatric population from the same region (Khodwekar and Gailing 2017; Lind-Riehl and Gailing 2017). Interspecific differentiation for these two red oak species is lower than intraspecific differentiation in other genera such as *Populus* (GAILING 2014). However, for *Q. rubra* and *Q. ellipsoidalis* genetic differentiation was still consistently lower for intra- than for interspecific population pairs, and populations always grouped by species and not by region at nuclear microsatellites. For example, genetic differentiation between populations from different regions was 1.2 % for *Q. rubra* and 2.7 % for *Q. ellipsoidalis* (LIND-RIEHL et al. 2014, KHODWEKAR and GAILING 2017). Likewise, genetic differentiation at maternally inherited chloroplast markers among the three interfertile red oak species *Q. rubra*, *Q. ellipsoidalis* and *Q. velutina* was very low (G_{ST} = 0.023, R_{ST} = 0.016) while genetic differentiation among geographic regions within species was high for all three species (*Q. velutina*, G_{ST} = 0.5771; *Q. ellipsoidalis*, G_{ST} = 0.240, R_{ST} = 0.301; *Q. rubra*, G_{ST} = 0.206, R_{ST} = 0.253) (ZHANG et al. 2015). Low interspecific differentiation at biparentally inherited nuclear and at maternally (via the seed) inherited chloroplast markers, high genetic differentiation among regions within species at chloroplast markers but not at nuclear markers and a sharing of locally restricted chloroplast haplotypes among species suggest recurrent gene flow among these closely related red oak species.

Likewise, based on genetic assignment analyses at SSRs (excluding outlier loci) in parapatric *Q. rurba* / *Q. ellipsoidalis* population pairs, putative F_1 hybrids and introgressive forms were identified, their frequency ranging from 6 % to 33 % depending on the population (LIND-RIEHL and GAILING 2017). In the sympatric population from the Baraga Plains the frequency of hybrids and introgressive forms was slightly higher (16.7 %) than in the parapatric populations from the same region (12.0 %) (KHODWEKAR and GAILING 2017). Also, the location of genetically admixed individuals close to both parental species supports recent hybridization in a mixed *Q. velutina*, *Q. rubra* and *Q. ellipsoidalis* stand in the contact zones between species (OWUSU et al. 2015). Finally, paternity analyses using seeds collected from 15 *Q. ellipsoidalis* seed parents confirmed contemporary interspecific gene flow in sympatric stands. The percentage of seeds derived from interspecific gene flow ranged from 0 % to 25 % with an average of 6.7 % of all seeds being pollinated by a *Q. rubra* pollen parent (KHODWEKAR and GAILING 2017). MORAN et al. (2012) estimated hybridization rates of > 20 % among morphological species based on paternity analyses in a sympatric *Q. rubra*, *Q. velutina* and *Q. coccinea* stand suggesting that gene flow among members of the American red oaks (section *Lobatae*) can be frequent in sympatric stands.

Outlier analyses

Given the low genetic differentiation between *Q. ellipsoidalis* and *Q. rubra* populations and gene flow between both species in contact zones, we searched for gene loci that resisted the homogenizing effect of gene flow showing elevated levels of interspecific differentiation as signature of divergent selection. For this purpose, four parapatric and two sympatric interspecific population pairs were screened at genic EST-SSRs and at nSSRs. The initial outlier screen at 36 EST-SSRs and 8 nSSRs in four parapatric population pairs revealed one outlier EST-SSR, FIR013, with very high interspecific differentiation across all interspecific population pairs from different regions (F_{ST} = 0.38, 0.64, 0.79, 0.83) (LIND-RIEHL et al. 2014) (Figure 1). Likewise, in the two sympatric population pairs FIR013 was identified as outlier under strong divergent selection (F_{ST} = 0.64, 0.63) (KHODWEKAR and GAILING 2017), supplementary material S12, S13). The EST, in which the SSR was located, was annotated as a CONSTANS-like gene (LIND-RIEHL et al. 2014). The trinucleotide microsatellite codes for a poly-glutamine (Q) repeat. The *Q. ellipsoidalis* allele was characterized by the lack of one Q-repeat (Q = 5, Q5 vs. Q =6, Q6) (LIND-RIEHL and GAILING 2017).

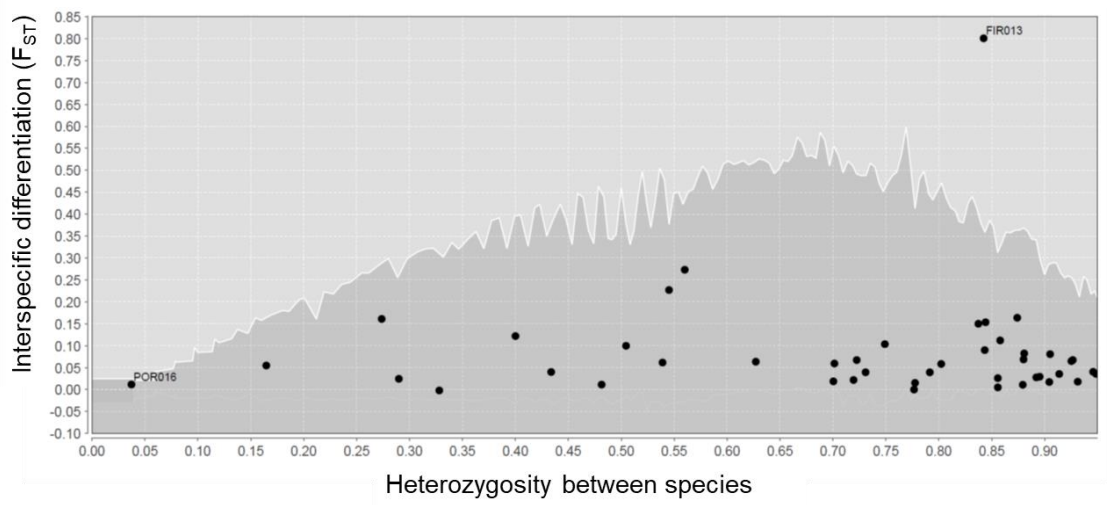

Figure 1: Outlier screen in the Baraga Plains populations in Lositan (ANTAO et al. 2008). Data from LIND-RIEHL et al. (2014).

While interspecific gene flow was estimated to be symmetric between species, the frequency of the *Q. ellipsoidalis* allele Q5 in *Q. rubra* was significantly higher than the frequency of the *Q. rubra* allele Q6 in *Q. ellipsoidalis* in sympatric populations suggesting asymmetric introgression of outlier alleles driven by environmental selection (KHODWEKAR and GAILING 2017). Finally, a weak but significant association of outlier allele copy number with soil quality in both *Q. rubra* and *Q. ellipsoidalis* was in accordance with this interpretation. Thus, the COL gene or closely linked genes are likely to play an important role in local adaptation and reproductive isolation between species, and the introgression of these alleles between species may facilitate the rapid adaptation of both species to changing and stressful environments.

A role of COL genes in local adaptation and reproductive isolation is also suggested by results of association analyses in other species. Thus, COL genes were associated with flowering time and growth in *Medicago sativa* L. and poplar (HERRMANN et al. 2010, HSU et al. 2012). In *Quercus petraea* the same COL gene as identified as outlier between *Q. rubra* and *Q. ellipsoidalis* was associated with vegetative bud burst in a common garden experiment (ALBERTO et al. 2013). Also, COL-1 and COL-2 were associated with vegetative bud burst in a common garden trial of *Fagus sylvatica* L. (MULLER et al. 2015).

Finally, variation in a poly-glutamic acid (E) repeat in COL2 of poplar was associated with growth cessation suggesting a role of this gene in phenology and growth (MA et al. 2010).

Quercus ellipsoidalis shows a later bud burst, later growth cessation, lower growth and survival rate than *Q. rubra* in a common garden trial (GAILING 2013). To evaluate the role of poly (Q) variation in COL in reproductive isolation and adaptive divergence between species, the association of outlier alleles with phenological and drought related traits should be assessed under drought and control conditions. However, these experiment are impeded by the relatively low frequency of the Q5 allele in *Q. rubra* and of the Q6 allele in *Q. ellipsoidalis* (see below).

While the role of poly-Q variation in circadian clock genes is largely unknown in plants, variation in poly-Q repeat number showed association with phenology and signatures of selection in various animal species (HAERTY and GOLDING 2010). In plants, poly-Q variation in the pleiotropic flowering time protein and abscisic acid receptor (FCA) was associated with morphological change and likely contributed to rapid adaptation to environmental change in endemic Hawaiian mint taxa (LINDQVIST et al. 2007).

Based on our results and reports in the literature we consider COL in oaks as an important gene for adaptive divergence and reproductive isolation in oaks. Furthermore, we have evidence for environment-dependent introgression of COL outlier alleles between species *Q. rubra* and *Q. ellipsoidalis*.

In future studies we plan to assess the genome-wide patterns of adaptive divergence between hybridizing oak species and experimentally test associations of outlier alleles with adaptive trait variation. In the following, potential future analyses are briefly summarized.

Comparative outlier screens

Several gene markers have been identified as outliers between hybridizing oak species with different adaptations to drought for different species pairs of the same section and of different sections. For example, nSSRs QrZag87 and QrZag112 were found as outliers within section *Quercus* between *Quercus pyrenaica* Willd. and *Q. faginea* Lam. (GOICOECHEA et al. 2015) and between *Q. robur* and *Q. petraea* (GOICOECHEA et al. 2012). The nuclear SSR QrZag112 was also identified as outlier between *Q. alnifolia* Poech and *Q. coccifera* L. from the Ilex group (NEOPHYTOU et al. 2011). Likewise, EST-SSR GOT021 was identified as outlier between *Q. rubra* and *Q. ellipsoidalis* (section *Lobatae*) in two population pairs as well as between *Q. pyrenaica* and *Q. faginea* (section *Quercus*) (GOICOECHEA et al. 2015). Identification of the same outlier alleles between species with different adaptations to drought across oak sections, which are reproductively isolated, suggests parallel adaptive evolution in oaks. In order to identify genes under divergent selection in different oak sections, we plan genome-wide outlier screens using the same methods and gene probes for oak species with different adaptations to drought both in European white oaks (section *Quercus*) and in North American red oaks (section *Lobatae*). Finally, we can map the outliers back to the *Q. robur* genome sequence (PLOMION et al. 2016) to identify the genes that underlie outlier genomic regions.

Association analyses

Association of outlier genes such as COL with adaptive trait variation can be done in common gardens of individual species if both species are polymorphic for the outlier alleles. Seeds for these experiments could be collected in hybrid zones between both species to maximize the representation of rare outlier alleles. Loci that are nearly fixed on alternative alleles in different species are more difficult to analyse since homozygotes for a heterospecific outlier allele are rare (e.g. for COL, Q5 in *Q. rubra*, Q6 in *Q. ellipsoidalis*). In this case seeds could be collected from individual seed parents (*Q. rubra* and *Q. ellipsoidalis*) that are heterozygous for the outlier alleles (Q5/Q6). Specifically, growth, phenology and drought related traits should be assessed for different genotypes at outlier genes such as COL (Q5/Q5, Q5/Q6, Q6/Q6) for both species.

Additionally, transcript levels could be assessed for different genotypes, for example prior to vegetative bud burst and growth cessation and under different water stress. Functional testing, for example of poly-Q variation in COL could be done by transformation of *Arabidopsis* plants with Q5 and Q6 COL alleles of both *Q. rubra* and *Q. ellipsoidalis*.

Conclusions

Signatures of divergent selection and asymmetric introgression of outlier alleles at a CONSTANS-like gene between two red oak species with different adaptations to drought suggests an important role of this gene in adaptive divergence and reproductive isolation between species. Since the level of outlier allele introgression was dependent on the environment and associated with soil characteristics, adaptive introgression of outlier alleles at COL may be an important mechanism to augment genetic variation within species for a rapid adaptation to changing environmental conditions. Common garden trials in control and drought-stressed environments are needed to analyse the association of outlier alleles with survival and fitness related traits. Genome-wide outlier screens between interfertile species with different drought adaptations in distinct taxonomic groups (e.g. within section *Quercus* and section *Lobatae*) can reveal the genetic architecture of species divergence and parallel evolution driven by natural selection in different oak sections.

Acknowledgements

Funding for the research works presented here was provided by the McIntire Stennis fund (USDA-NIFA # 1001471), the National Science Foundation Plant Genome Research Program (TRPGRA1 IOS-1025974), the Hanes Trust and the Huron Mountain Wildlife Foundation. Additional funding was provided by the Life Science and Technology Institute (LSTI) and Ecosystem Science Center (ESC) at Michigan Technological University. Finally, I would like to thank the organizers of the German-Russian conference on forest genetics in Ahrensburg.

References

ABRAMS MD (1988) Comparative water relationships of 3 successional hard wood species in central Wisconsin. Tree Physiology 4: 263-273.

ABRAMS MD (1990) Adaptations and responses to drought in *Quercus* species of North America. Tree Physiology 7: 227-238.

ALBERTO FJ, DERORY J, BOURY C, FRIGERIO JM, ZIMMERMANN NE, KREMER A (2013) Imprints of natural selection along environmental gradients in phenology-related genes of *Quercus petraea*. Genetics 195: 495-512.

ANTAO T, LOPES A, LOPES RJ, BEJA-PEREIRA A, LUIKART G (2008) LOSITAN: A workbench to detect molecular adaptation based on a F_{ST} outlier method. BMC Bioinformatics 9: 323.

ARNOLD ML (2004) Transfer and origin of adaptations through natural hybridization: Were Anderson and Stebbins right? Plant Cell 16: 562-570.

ARNOLD ML (2006) Evolution through genetic exchange. Oxford University Press, Oxford, United Kingdom: 252 pp.

ARNOLD ML (2016) Divergence with genetic exchange. Oxford University Press, Oxford, United Kingdom: 251 pp.

BURNS RM, HONKALA BH (1990) Silvics of North America, Washington, DC: 877 pp.

CURTU AL, GAILING O , FINKELDEY R (2007) Evidence for hybridization and introgression within a species-rich oak (*Quercus* spp.) community. BMC Evolutionary Biology 7: 218.

CURTU AL, GAILING O, FINKELDEY R (2009) Patterns of contemporary hybridization inferred from paternity analysis in a four-oak-species forest. BMC Evolutionary Biology 9: 284.

GAILING O (2013) Differences in growth, survival and phenology in *Quercus rubra* and *Q. ellipsoidalis* seedlings. Dendrobiology 70: 71-79.

GAILING O (2014) Identification of genes under divergent selection in interfertile, but ecologically divergent oaks. Bulletin of the Transilvania University of Brasov 7: 1-10.

GOICOECHEA PG, HERRAN A, DURAND J, BODÉNÈS C, PLOMION C, KREMER A (2015) A linkage disequilibrium perspective on the genetic mosaic of speciation in two hybridizing Mediterranean white oaks. Heredity 114: 373-386.

GOICOECHEA PG, PETIT RJ, KREMER A (2012) Detecting the footprints of divergent selection in oaks with linked markers. Heredity 109: 361-371.

HAERTY W, GOLDING GB (2010) Low-complexity sequences and single amino acid repeats: not just "junk'' peptide sequences. Genome 53: 753-762.

HERRMANN D, BARRE P, SANTONI S, JULIER B (2010) Association of a CONSTANS-LIKE gene to flowering and height in autotetraploid alfalfa. Theor. Appl. Genet. 121: 865-876.

HOKANSON SC, ISEBRANDS JG, JENSEN RJ , HANCOCK JF (1993) Isozyme variation in oaks of the apostle islands in Wisconsin - genetic structure and levels of inbreeding in *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). Amer. J. Bot. 80: 1349-1357.

HSU CY, ADAMS JP, NO K, LIANG HY, MEILAN R, PECHANOVA O *et al.* (2012) Overexpression of Constans homologs CO1 and CO2 fails to alter normal reproductive onset and fall bud set in woody perennial poplar. PLoS One 7, e45448.

JENSEN RJ, HOKANSON SC, ISEBRANDS JG, HANCOCK JF (1993) Morphometric variation in oaks of the apostle islands in Wisconsin - evidence of hybridization between *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). Amer. J. Bot. 80: 1358-1366.

KHODWEKAR S, GAILING O (2017) Evidence for environment-dependent introgression of adaptive genes between two red oak species with different drought adaptations. Amer. J. Bot. 104: 1088-1098.

LIND-RIEHL J, GAILING O (2017) Adaptive variation and introgression of a CONSTANS-like gene in North American red oaks. Forests 8: 3.

LIND-RIEHL JF, SULLIVAN AR, GAILING O (2014) Evidence for selection on a CONSTANS-like gene between two red oak species. Ann. Bot. 113: 967-975.

LIND J, GAILING O (2013) Genetic structure of *Quercus rubra* L. and *Q. ellipsoidalis* E. J. Hill populations at gene-based EST-SSR and nuclear SSR markers. Tree Genet. Genomes 9: 707-722.

LINDQVIST C, LAAKKONEN L, ALBERT VA (2007) Polyglutamine variation in a flowering time protein correlates with island age in a Hawaiian plant radiation. Bmc Evolutionary Biology 7: 13.

MA XF, HALL D, ST ONGE KR, JANSSON S, INGVARSSON PK (2010) Genetic differentiation, clinal variation and phenotypic associations with growth cessation across the *Populus tremula* photoperiodic pathway. Genetics 186: 1033-1044.

MORAN EV, WILLIS J, CLARK JS (2012) Genetic evidence for hybridization in red oaks (*Quercus* sect. *Lobatae*, Fagaceae). Amer. J. Bot. 99: 92-100.

MULLER M, SEIFERT S, FINKELDEY R (2015) A candidate gene-based association study reveals SNPs significantly associated with bud burst in European beech (*Fagus sylvatica* L.). Tree Genet. Genomes 11: 13.

NEOPHYTOU C, DOUNAVI A, FINK S, ARAVANOPOULOS FA (2011) Interfertile oaks in an island environment: I. High nuclear genetic differentiation and high degree of chloroplast DNA sharing between *Q. alnifolia* and *Q. coccifera* in Cyprus. A multipopulation study. European Journal of Forest Research 130: 543-555.

NIXON KC (1997) Fagaceae. In: Flora of North America. Volume 3. Oxford University Press, New York.

Owusu SA, Sullivan AR, Weber JA, Hipp AL, Gailing O (2015) Taxonomic relationships and gene flow in four North American *Quercus* species. Syst. Bot. 40: 510-521.

PLOMION C, AURY J-M, AMSELEM J, ALAEITABAR T, BARBE V, BELSER C et al. (2016) Decoding the oak genome: public release of sequence data, assembly, annotation and publication strategies. *Mol. Ecol. Resour.* 16: 254-265.

SCHEMSKE DW (2000) Understanding the origin of species. Evolution 54: 1069-1073.

SCOTTI-SAINTAGNE C, MARIETTE S, PORTH I, GOICOECHEA PG, BARRENECHE T, BODÉNÈS C et al. (2004) Genome scanning of interspecific differentiation between two closely related oak species (*Quercus robur* L. and *Q. petraea* (Matt.) Liebl.). Genetics 168: 1615-1626.

SOLTIS PS, SOLTIS DE (2009) The role of hybridization in plant ppeciation. In: Annual Review of Plant Biology. Annual Reviews Palo Alto: 561-588 pp.

SULLIVAN AR, OWUSU SA, WEBER JA, HIPP AL, GAILING O (2016) Hybridization and divergent selection in multispecies oak communities. Botanical Journal of the Linnean Society 181: 99-114.

ZHANG R, HIPP AL, GAILING O (2015) Sharing of chloroplast haplotypes among red oak species suggests interspecific gene flow between neighboring populations. Botany 93: 691-700.

IV. Excursions

Biomass production on the ProLoc-site Trenthorst (Bio26)

MIRKO LIESEBACH

Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany (mirko.liesebach@thuenen.de)

Abstract

The idea to assess clone-site interactions for poplar and willow on agricultural land could be realized in the ProLoc project. One site is managed in a 3-year rotation period by the Thünen Institute. So far three harvests have been made. Biomass production increased from harvest to harvest. The 2 willows clones have a higher biomass production than the 3 poplar clones tested.

Key words: *Populus, Salix,* clone, SRC

Background

To assess clone-site interactions for growing poplar and willow clones on agricultural land, 38 study sites were established throughout Germany (Figure 1). Soil parameters, yield and vitality data were recorded. Thus in this project, for the first time, a universal experimental design was used for the establishment of trial sites across as many regions as possible in Germany, which were then analyzed jointly (HOFMANN et al. 2012, STIEHM et al. 2015). Correlations between particular site variables with total yield were estimated and tested for causality. Empirical statistics was used to develop algorithms with the parameters variety/clone, soil and climate (AMTHAUER GALLARDO 2014). One site is operated by the Thünen Institute.

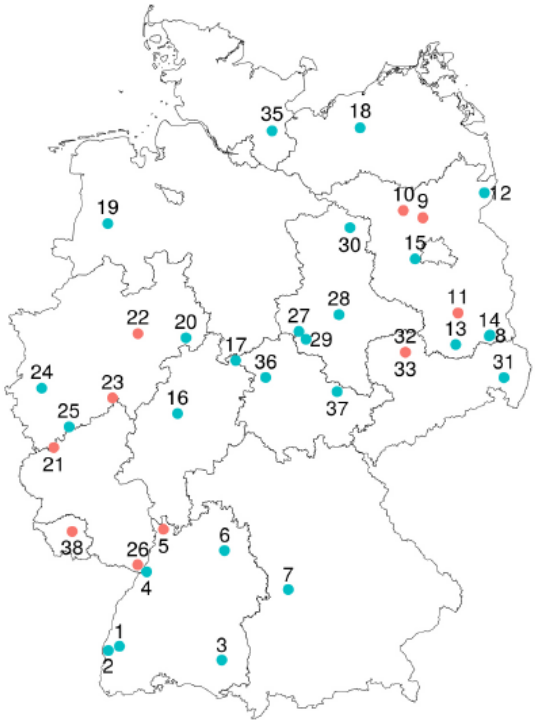

Figure 1: Trial sites of the ProLoc poject (35= Trenthorst / Schleswig-Holstein)

Material and methods

In total 5 variants, two willow varieties (Inger, Tordis) and three well known poplar clones (Max 1, Hybrid 275, AF2) were planted as unrooted cuttings (20 cm) at a spacing of 1.8 m x 0.5 m. The site Trenthorst / Schleswig-Holstein (Bio26; 53° 47' N, 10° 31' E, 37 m a.s.l.) was established in spring 2008. A plot was established with 5 rows and 20 plants each (100 plants per plot). This results in a plot size of 9 m * 10 m (90 m²). The variants were planted in a randomized one factorial block design at each site with 4 replications (Figure 2).

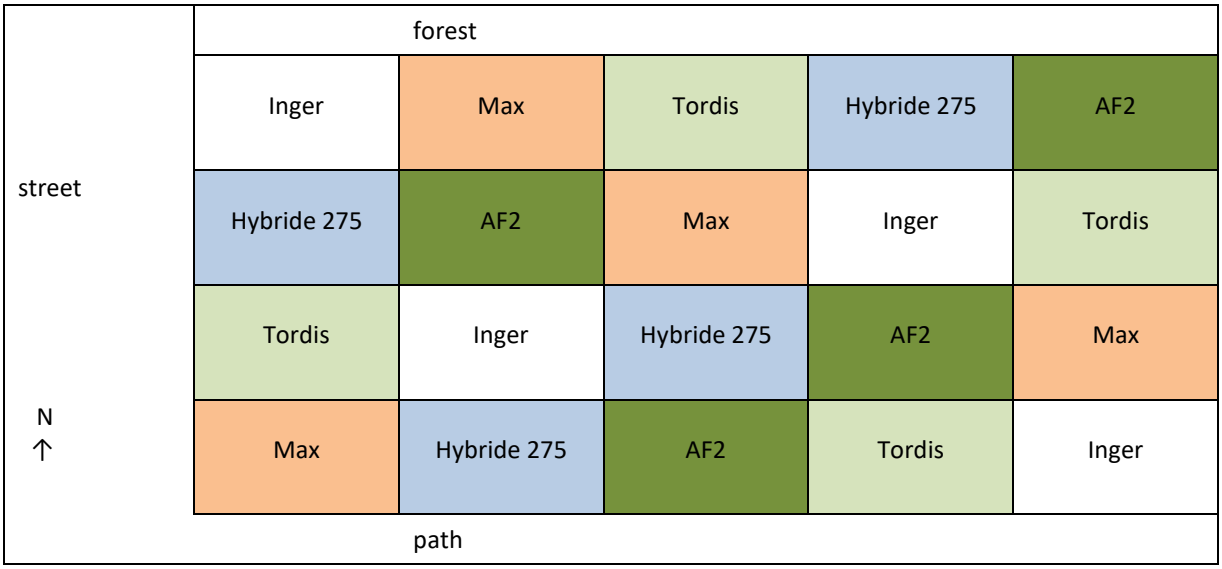

Figure 2: Lay-out of the ProLoc-site in Trenthorst (Bio26).

The site was managed in a 3-year rotation period. So far three harvests have been made. To calculate the above-ground woody biomass which was grown in the first 3-year rotation circle a sample of 24 trees per plot was harvested and weighted. The sample for the second 3 year rotation circle was reduced to 16 trees per plot. The biomass for the third circle was determined by harvesting and weighting the complete biomass of a plot. In each of the 3 harvests a sample was taken to determine the water content. Therefore, these samples were dried in an oven by 104 °C until weight was constant.

Results

The willow clones are producing more biomass (metric tons absolutely dried matter) than the poplar clones within 3 rotation periods. The biomass production on the site Trenthorst increases from rotation circle to the next rotation circle.

The total biomass production in the first rotation period was very low. An average of 3.2 t d.m. ha^{-1} was calculated over the 5 clones. The biomass production varied between 0.9 t d.m./ha for the poplar clone 'Hybride 275' and 5.7 t d.m. ha^{-1} for the willow clone 'Inger'. Differences between clones are significant.

During the second rotation period the biomass yield increased. On average a biomass of 29.7 t d.m. ha^{-1} was harvested. Between the clones the biomass production varied between 17.7 t d.m. ha^{-1} for the poplar clone 'Hybride 275' and 38.4 t d.m. ha^{-1} for the willow clone 'Inger'. Differences between clones were significant.

In the third rotation period the biomass production increased again. On average 37.8 t d.m. ha^{-1} biomass was produced. The willow clone 'Tordis' (51.9 t d.m. ha^{-1}) had the best performance. The lowest biomass production had the poplar clone 'AF2' (25.5 t d.m. ha^{-1}). The willow clones were significant better in biomass production than the poplar clones.

The total biomass production per rotation period is given in Table 1. The mean annual increment per clone and rotation period is shown Figure 3.

Table 1: Above-ground woody biomass production [t d.m. ha^{-1}] per clone and rotation circle

Genus	Clone	1. rotation	2. rotation	3. rotation
Poplar	Max 1	4.5	32.7	31.5
	Hybride 275	0.9	17.7	31.2
	AF2	2.4	22.8	25.5
Willow	Inger	5.7	38.4	48.9
	Tordis	2.7	36.9	51.9

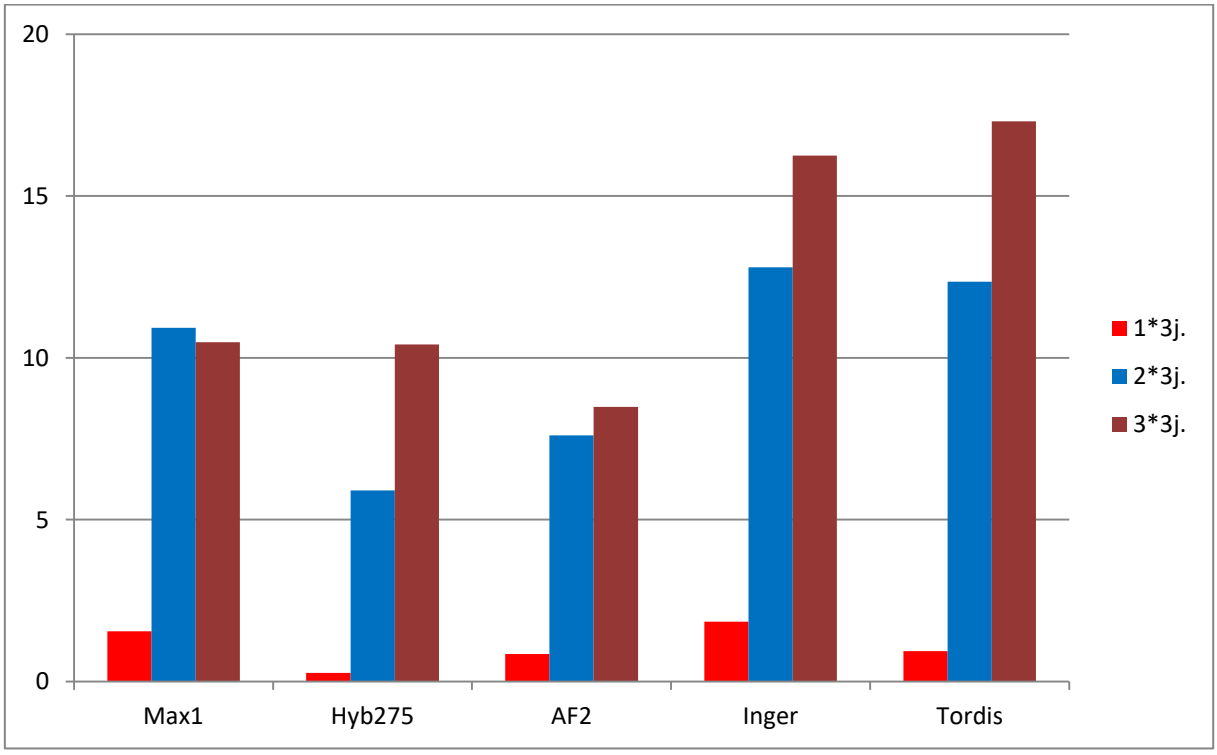

Figure 3: Mean annual increment [t d.m. ha^{-1} a^{-1}] per clone and rotation circle.

The mean annual biomass production of the first and second rotation period is listed for all sites of the ProLoc project in Table 2. The annual increment is very low in the first rotation circle on the site Trenthorst (VFL-Nr. 35) compared to other ProLoc sites. While after the second rotation the annual increment is at average.

Table 2: Mean annual biomass production [t d.m. ha^{-1} a^{-1}] in two 3-year rotation periods (Source: STIEHM et al. 2017) (1. Rotation= '10, 2. Rotation= '13)

VFL-Nr.	Max 1 '10	Max 1 '13	Hyb. 275 '10	Hyb. 275 '13	AF2 '10	AF2 '13	Inger '10	Inger '13	Tordis '10	Tordis '13
1	7.67	13.61	7.42	16.84	6.33	6.85	8.29	11.53	8.99	11.64
2	7.32	10.76	3.29	8.63	6.57	6.67	1.65	3.88	1.98	6.75
3	10.00	10.80	5.64	9.51	8.36	7.50	7.90	12.12	7.20	21.67
4	5.46	7.83	4.41	9.90	4.52	4.19	2.76	3.50	2.26	1.20
6	3.77	12.03	0.64	6.53	3.84	9.01	2.76	8.72	0.93	5.52
7	0.72	5.47	1.62	7.87	2.65	6.66	2.38	5.97	2.25	6.93
8	0.44	1.10	0.45	1.56	0.48	1.25	0.29	0.27	0.13	0.18
9	5.89	-	2.92	-	4.27	-	2.83	-	2.08	-
12	1.96	3.00	1.97	3.42	0.87	1.49	1.91	2.17	1.72	2.66
13	4.63	7.30	3.87	7.40	3.58	3.61	3.43	3.23	4.75	5.53
14	0.17	1.66	0.25	1.72	0.21	2.15	0.18	0.54	0.08	0.46
15	4.51	11.51	3.37	12.56	6.23	13.53	3.80	16.40	3.92	17.10
16	3.25	13.13	1.11	12.08	4.83	12.46	4.72	15.35	4.70	18.63
17	10.09	12.65	10.21	13.74	8.76	11.84	9.13	13.81	9.97	12.89
18	1.39	9.03	0.55	5.77	0.77	5.45	1.39	6.89	0.75	6.21
19	6.43	11.99	1.15	6.89	7.26	11.57	9.23	16.32	9.14	16.23
20	2.06	9.70	1.46	11.29	1.48	7.56	1.82	12.50	1.96	12.47
24	7.06	6.17	3.70	4.96	7.02	6.24	9.49	7.71	9.43	7.46
25	9.61	14.05	9.07	16.48	9.25	19.11	6.95	10.94	8.01	11.76
27	1.02	5.31	0.94	5.60	0.62	0.87	1.46	1.91	1.12	1.20
28	10.14	11.89	8.06	10.91	11.05	11.21	10.60	7.85	9.99	8.21
29	8.41	13.84	7.49	15.02	6.90	11.25	8.28	9.47	8.08	11.80
30	0.37	2.25	0.50	1.40	1.77	4.60	0.84	2.20	0.41	0.96
31	7.36	11.68	8.88	13.01	6.61	5.09	8.08	12.14	9.10	12.73
35	1.55	10.93	0.26	5.90	0.85	7.60	1.85	12.80	0.94	12.35
36	4.92	10.36	2.45	5.48	7.12	10.56	7.04	9.01	6.73	12.95
37	7.39	15.47	4.77	11.87	5.82	14.08	6.71	12.01	7.33	12.82

Acknowledgements

The joint project "Detection of clone-site interactions in poplar and willow on agricultural sites in short rotation (ProLoc)" was funded by the Federal Ministry of Food and Agriculture (BMEL) through the Agency for Renewable Resources e.V. (FNR). The author gratefully acknowledges STEFAN JENCSIK doing the assessments and measurements conscientiously. For providing the test site I thank the Thünen Institute of Organic Farming, and for doing the third harvest the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB).

References

AMTHAUER GALLARDO D (2014): Standortbasierte Ertragsmodellierung von Pappel- und Weidenklonen in Kurzumtriebsplantagen. TH Dresden, Fakultät Umweltwissenschaften. Dissertation. XVII + 201 pp.

HOFMANN M, AMTHAUER GALLARDO D, SIEBERT C (2012): Verbundvorhaben ProLoc: Klon-Standort-Wechselwirkungen bei Pappel und Weide auf landwirtschaftlichen Standorten in kurzen Umtriebszeiten. Beitr. aus der NW-FVA, Band 8: 9-20.

STIEHM C, HOFMANN M, JANßEN A, KURTH W, NAGEL J (2015): Ergebnisse des ProLoc Verbundvorhabens Projektphase II – Implementierung eines Wachstumssimulators basierend auf der Modellierung der Klon-Standort-Wechselwirkung bei Pappel und Weide im Kurzumtrieb. Mitt. Ges. Pflanzenbauwiss. 27: 39-40.

STIEHM C, HOFMANN M, SCHMIDT C, JANßEN A (2017): Erfassung von Klon-Standort-Wechselwirkungen bei Pappel und Weide auf landwirtschaftlichen Standorten in kurzen Umtrieben - ProLoc (Ertrags – Programm – Locus) – Projektphase II: 97 pp.

Clone test with hybrid aspen (As130)

MIRKO LIESEBACH[1], VOLKER SCHNECK[2]

[1] Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany (mirko.liesebach@thuenen.de)

[2] Thünen Institute of Forest Genetics, Eberswalder Chaussee 3a, 22927 Waldsieversdorf, Germany (volker.schneck@thuenen.de)

Abstract

In Trenthorst / Schleswig-Holstein a test site was established mainly with hybrid-apsen clones of the clone mixture ‘Großhansdorf’ to approve the best clones in 2012. Preliminary results at age 5 were given. Yet there are differences between the clones. Survival and growth traits (height and dbh) are not correlated.

Key words: *Populus × wettsteinii*, growth, field test

Introduction

In the 1980s, the clone mixture 'Großhansdorf' was approved with 14 clones of the hybrid aspen (*Populus × wettsteinii, P. tremula × P. tremuloides*). This mixture could only be distributed if all 14 clones are included. However, the ability for vegetative propagation was very different for the single clones. Therefore, the mixture was never commercially available.

About 15 years ago, interest in hybrid aspen clones reappeared. This was taken as an opportunity for testing the clones which could be propagated vegetative easily and in high numbers with the aim to approve the best performing single clones.

Material and methods

Eight of the 14 clones of the mixture ‘Großhansdorf’ could be propagated in numbers so that they could be included in a clone test. The experiment was supplemented by 3 more clones of hybrid aspen, including the triploid clone 'Astria'. In addition, a grey poplar (*P.* × canescens) was included in the test. The hybrid aspen clones were selected in progeny trials of the institute. The clones of the test and their crossing parents are listed in Table 1.

The plants for the experiment were propagated by tissue culture in 2007 and were cultivated for further years in the nursery of the institute. In 2012, the site in Trenthorst (As130), Schleswig-Holstein (53° 47’ N, 10° 31’ E, 37 m a.s.l.), could be planted with cut back plants on 5-year old roots (0/5). The trial layout was 3 rows with 4 plants (12 plants per plot, spacing 2 m x 2 m). The plot size is 6 m * 8 m (48 m²). Each clone is replicated up to 6 times.

Survival was assessed and height and diameter (dbh) growth were measured in the field.

Table 1: Clones of hybrid-aspen and their parents (* clones of the mixture 'Großhansdorf')

Species	No.	Klon name	Crossing
P. × wettsteinii (P. tremula × P. tremuloides)	1	Astria	
	2*	Esch 5	Brauna 11 × Turesson 141
	3*	Se 1	Brauna 11 × Turesson 141
	4*	L 176 / Se 3	Brauna 11 × Turesson 141
	5*	Wä 1	Großdubrau 1 × Maple
	6*	Wä 13	Großdubrau 1 × Maple
	7*	L 191 / Wä 14	Großdubrau 1 × Maple
	8*	Kh 73	W 3 × Clone 13
	9*	Kh 83	W 3 × Clone 13
	10	Ihl 174/9	W 18 × Clone 13
	11	Ihl 174/10	W 18 × Clone 13
Populus × canescens *(P. tremula × P. alba)*	12	Ca 2.75	

Results

Two years after establishing the trial site the **survival** rate was 68 % over all 12 clones. Two years later in autumn 2016 survival was at the same level (67 %). The best survival rates had the clones Ca 2.75 (*P. × canescens*) (95 %) and Wä 13 (90 %). The lowest survival could be observed for clone Kh 83 (13 %) followed by Se 1 (36 %) (Figure 1).

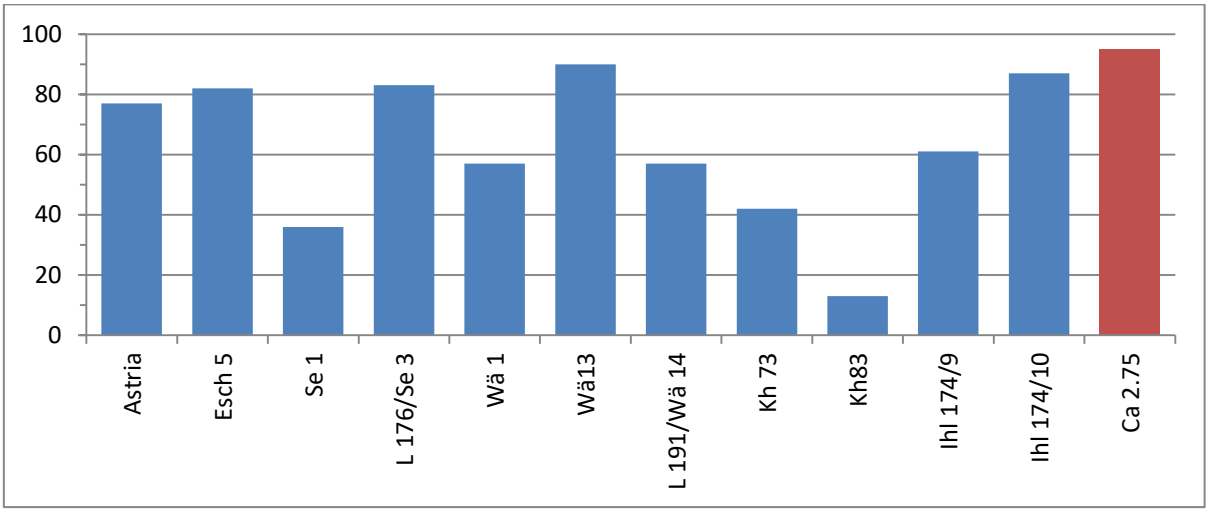

Figure 1: Survival [%] at age 5 in the field (autumn 2016).

The mean **height** overall all 12 clones was 1.6 m in 2013, two years later (2015) it was 4.5 m and 7.9 m in 2016. The variation between the clones at age 5 in the field (2016) is shown in Figure 2. At age 4 and 5 the hybrid aspen clones (Wä 1: 8.5 m; Wä 13: 8.7 m; L 191/Wä 14: 9.4 m, and Ihl 174/10: 8.3 m) have a significant better height growth than the *P. × canescens* clone Ca 2.75 (6.1 m).

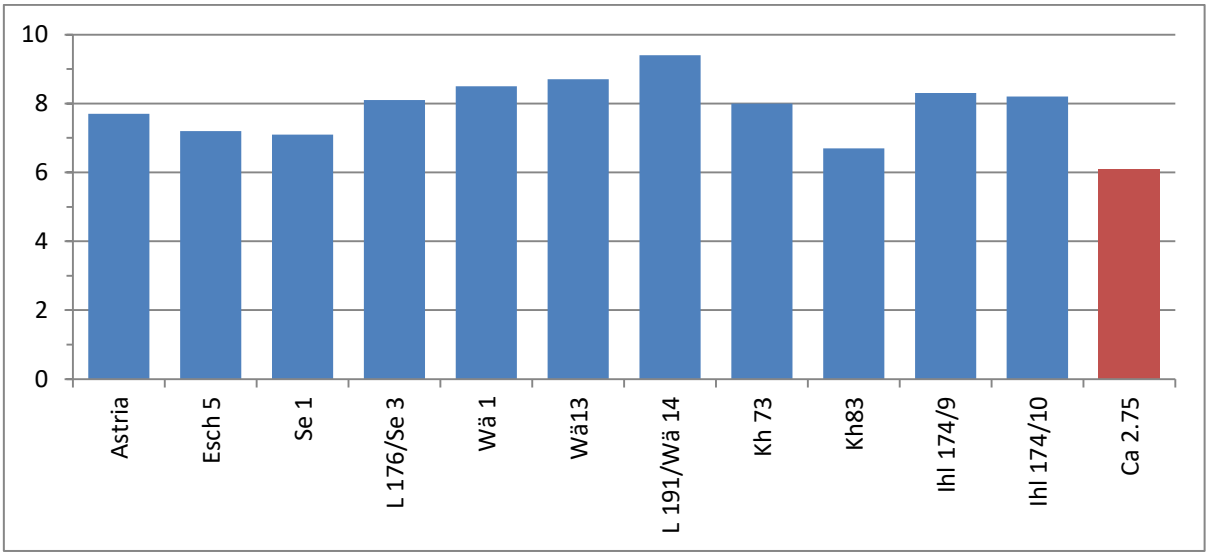

Figure 2: Mean height [m]; at age 5 in the field (autumn 2016).

The mean **dbh** overall all 12 clones was 2.9 cm in 2015, and a year later (2016) it was 4.9 cm. The variation between the clones is given in Figure 3. The mean dbh varies between 4.6 cm and 6.5 cm. However, differences between the clones were not significantly.

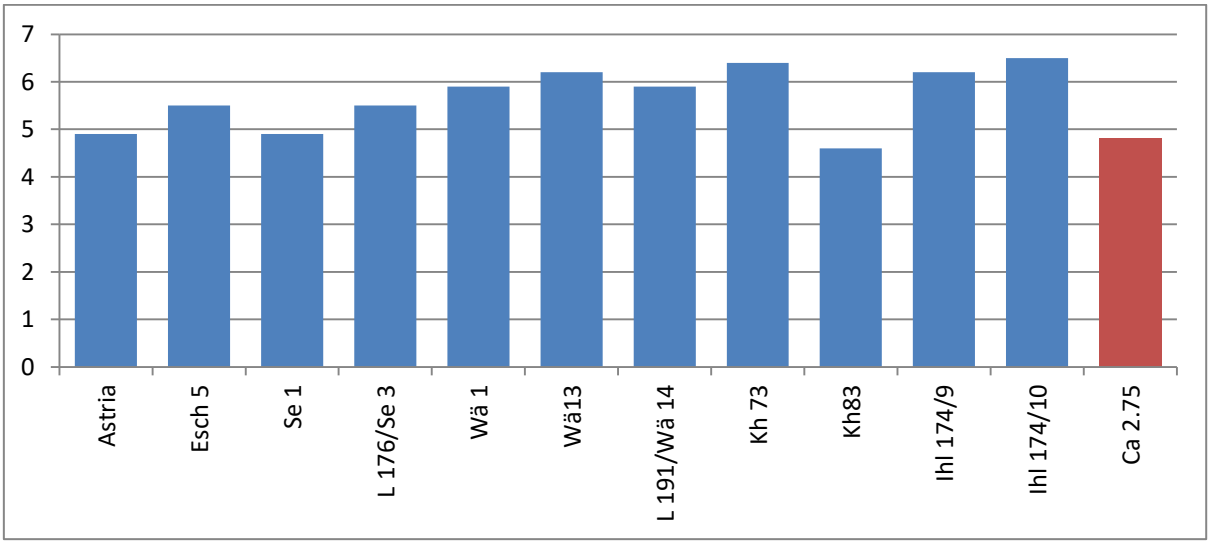

Figure 3: Mean dbh [cm] at age 5 in the field (autumn 2016).

Correlation between traits

The survival rates between the 3 assessments are correlated. Survival is also correlated with the height at age 2 in the field (2013). There is no correlation between survival and the growth traits (height and diameter) at age 4 (2015) and 5 (2016). Height at age 2 (2013) and height 5 (2016) are correlated with the diameter growth at age 4 (2015) and 5 (2016). Height at age 4 (2015) is additionally correlated with height at age 5 (2016). The diameter at age 4 (2015) is correlated with those of age 5 (2016). The results are summarized in Table 2.

Table 2: PEARSON's correlation coefficient for survival (S..), and height (H..) and dbh (D..), respectively. (* significant $\alpha < 0.05$)

Trait	S13 (age 2)	S15 (age 4)	S16 (age 5)	H13 (age 2)	H15 (age 4)	H16 (age 5)	D15 (age 4)	D16 (age 5)
S13 (age 2)	-							
S15 (age 4)	0.998*	-						
S16 (age 5)	0.998*	0.999*	-					
H13 (age 2)	0.654*	0.648*	0.656*	-				
H15 (age 4)	0.901	0.062	0.079	0.561	-			
H16 (age 5)	0.101	0.086	0.098	0.517	0.890*	-		
D15 (age 4)	0.231	0.202	0.221	0.653*	0.949*	0.752*	-	
D16 (age 5)	0.248	0.219	0.235	0.668*	0.941*	0.755*	0.991*	-

Conclusions

The site is still young. Yet there are differences between the clones. Clones with a high survival rate are distinguished by a well height and diameter growth performance. The results will be compared with those from a parallel site in Poland.

Acknowledgments

The joint project FastWOOD was funded by the Federal Ministry of Food and Agriculture (BMEL) through the Agency for Renewable Resources e.V. (FNR). We gratefully acknowledge MANFRED RADIES, STEFAN JENCSIK and JÜRGEN BEIERMEISTER doing the assessments and measurements conscientiously. For providing the test site we thank the Thünen Institute of Organic Farming.

International beech provenance trial 1993/95 - site Schädtbek (Bu19-1)

MIRKO LIESEBACH

Thünen Institute of Forest Genetics, Sieker Landstraße 2, 22927 Großhansdorf, Germany (mirko.liesebach@thuenen.de)

Abstract

In 1995 an international beech provenance trial was established at 27 sites in Europe. One of the sites is located in Schädtbek / Schleswig-Holstein. At this site 100 provenances are in test. Growth traits were measured and survival and stem form assessed at several times. There is variation between the provenances for all traits. No correlation could be detected between growth and stem form.

Key words: *Fagus sylvatica*, field test, growth, stem form

Background

European beech (*Fagus sylvatica* L.) is a major forest tree species in western and central Europe and covers roughly 12 million ha of forest land. The natural distribution range is shown in Figure 1. The area is increasing due to changes in the forest management currently. Beech is of interest not only for economic but also for ecological reasons. Beech is a species of high silvicultural value with many positive attributes which act to stabilize forest ecosystems.

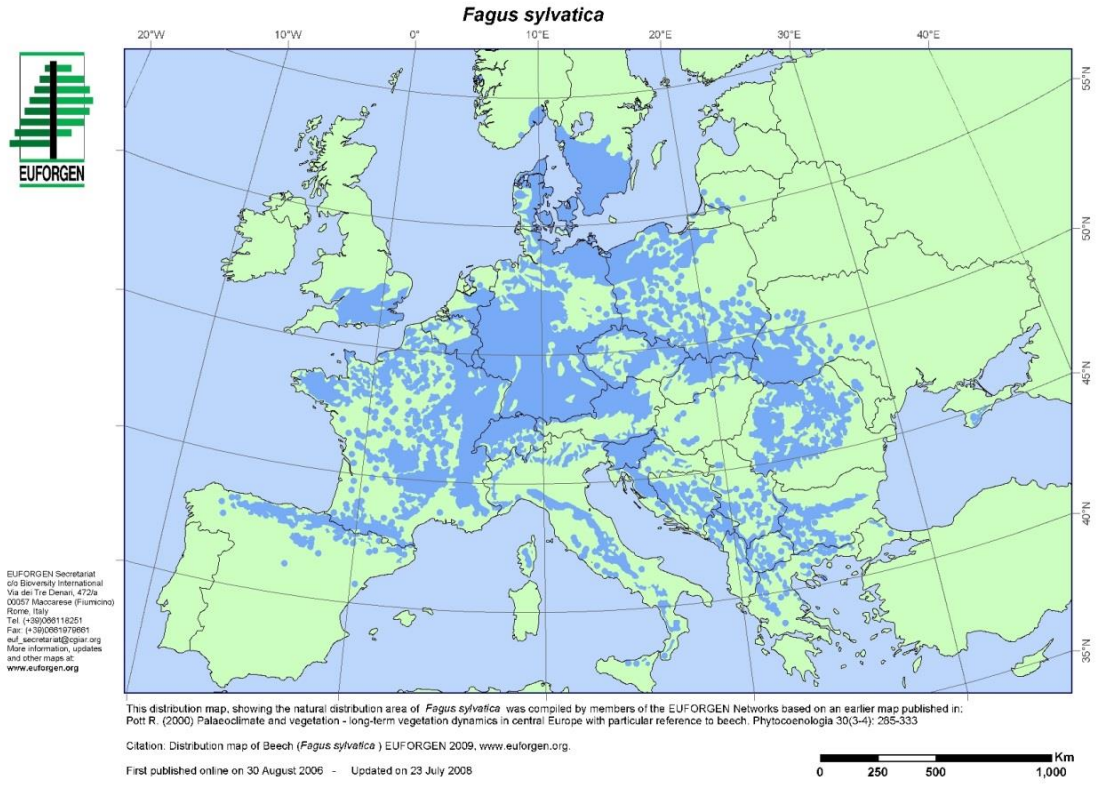

Figure 1: Natural distribution range of *Fagus sylvatica* - European beech (Source: EUFORGEN 2009).

To determine the extent of genetic variation in European beech (*Fagus sylvatica* L.) and to evaluate its genetic resources, it is necessary to know how different populations of the species are able to cope with different environments. Thus, adaptiveness and adaptability of beech populations are to be estimated. This is accomplished by growing a set of provenances in field trials located in the different regions inhabited by the species.

The institute initiated an international beech network of 6 trail series which were established since the mid-1980th (VON WÜHLISCH et al. 1998, LIESEBACH 2015). The 6 series comprises together 75 sites which include altogether 465 provenances. The trials contain between 14 and 158 provenances and are located in altogether 23 European countries. On the field trials, traits which best reflect adaptedness and adaptiveness are being assessed.

International beech trial series 1993/95

The seed samples for the 1993/95 series delivered to the institute differed strongly in many respects: cleanliness, means and duration of transport, collection method, pre-treatments, etc. Generally, seed samples from distant places which had a longer journey were in worse condition than samples from nearby places. The seed were stored and stratified at the Thünen Institute in Großhansdorf.

Seeding was done at the nursery of the institute at Großhansdorf and in a state owned nurserie. The plants were lined out after one year, and transplanted for a further year. All plants were lifted after two years and stored in cooling containers in the institute. During winter plants were prepared for shipment to the trial sites. Planting of the 1+1 seedlings was organized by each joint partner institute. The trial lay-out was designed at the Thünen Institute for all sites.

The series comprised 23 trials in 17 countries and testing a total number of 158 provenances (Figure 2). During the first years a nursery trial existed as well.

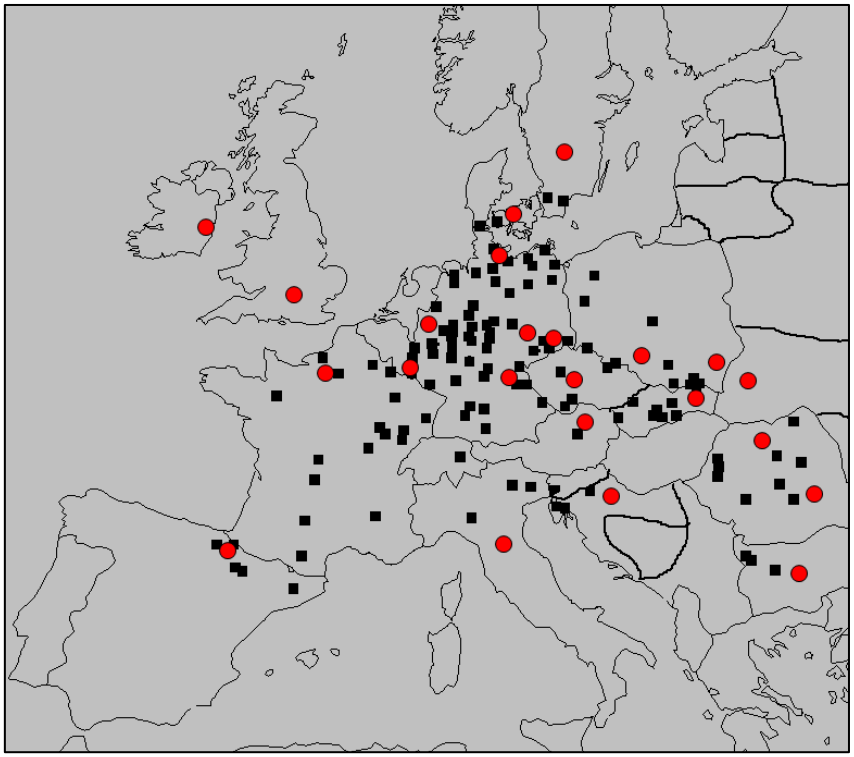

Figure 2: Provenances and trial sites of the International beech trial series 1993/95 (red dot = trial site, black square = origin of provenance).

The lay-out of the trials is based on a randomized complete block design with three replications. Planting was done in rows with a space of 2 m x 1 m. Each plot was laid out with 50 plants), resulting in a plot size of 10 m x 10 m. Thus a trial with 33 provenances occupies about 1 ha. Plots are considered large enough to maintain the trials for 60 years. There are no bordering rows between the plots. Usually two rows were planted around the trials.

The trials are laid out and planned to serve multiple purposes, and the objectives of the different beech provenance trials include:

1. Tree improvement
   - Testing suitability of provenances for different sites
   - Selection of basic material
   - Setting up recommendations for trade and use of provenances at national and international level
2. Gene conservation
   - Assessment of genetic and phenotypic variation
   - Development of conservation strategies
   - Evaluation methods and ecodistances
3. Evolution biology
   - Adaptiveness, adaptability
   - Research on the impacts of global climate change

Site Schädtbek (Bu19-1)

The field trail at "Schädtbek" is located near Kiel in Schleswig-Holstein (54° 18' N, 10° 18' E, 40 m a.s.l.) The size of the site is 3 ha (exclusively boarder rows). The field trail includes 100 provenances which were planted in April 1995 (April 10th-13th and 24th-28th). A list of the provenances is given in Table 1.

Together with the planting of beech a mixture of herbs (trefoil [*Trifolium* sp.], lupine [*Lupinus* sp.], fodder radish [*Raphanus* sp.], rape [*Brassica napus*], California blue bell [*Phacelia campanularia*], common buckwheat [*Fagopyrum esculentum*], oat [*Avena sativa*], rye [Secale cereale], mallow [*Malva sylvestris*], and others) was sown. There were two reasons for this step: to repress the rising weed and to give the young beech plants shadow. The summer 1995 was very dry and only about 40-50 % of the sown herbs germinated. Most of the site was covered by natural generated camomile (*Tripleurospermum inodorum* and *Matricaria chamomilla*).

During the years 1995 and 1996 the climatic conditions were not the best to establish a field trial in Northern Germany. Both years were very dry, however, only a few plants died.

In September 1995 several beech plants lost their leaves totally and had a second flushing.

The winter 1995/96 was long and cold. O n average the year 1996 was not so warm as 1995. In 1996 there were about 100 mm less rain and 200 hours less sunshine than in the year 1995.

In spring 1996 (May 7th and 8th) there were several nights with a weak late frost, but all plants survived.

The beech is still growing between the sown herbs and the weed. In 1996 natural generated dock (*Rumex* sp.) had a large portion. The weed was not cut.

During the winter (1995/96 and 1996/97) poison against mice was distributed over the whole trial site. Damages could be avoided.

Table 1: List of provenances tested at the site Schädtbeck (Bu19-1) German Bundesländer: SH= Schleswig-Holstein, MV= Mecklenburg-West Pomerania, NI= Lower Saxony, BB= Brandenburg, He= Hesse, NW= North Rhine-Westfalia; TH= Thuringia, SN= Saxony, RP= Rhineland-Palatinate, BW= Baden-Württemberg, BY= Bavaria)

No	Provenance	Country
2	Limitaciones	ES
5	Anguiano	ES
7	F.D des Corbières occid.	FR
8	F.D de Crécy	FR
9	F.D de Fougères	FR
10	F.D d'Halatte	FR
11	F.D des Charmettes	FR
12	F.D des Colettes	FR
13	F.D de Planoise	FR
14	F.D de Lagast	FR
18	F.D de Ligny en Barrois	FR
20	F.D de Verrières du G.	FR
23	F.D de Villafans	FR
24	Fyn	DK
25	Grasten	DK
26	Glorup	DK
27	Skäralid	SE
28	Ryssberget	SE
29	Lensahn	DE SH
30	Farchau (Standard)	DE SH
32	Malchin I'92	DE MV
36	Osterholz-Scharmbeck	DE NI
37	Deister	DE NI
38	Harsefeld	DE NI
39	Seelzerthurm	DE NI
40	Bovenden	DE NI
43	Busschewald	DE NI
44	Oderhaus	DE NI
46	Gransee, Abt. 3082a1	DE BB
48	Monschau, Abt. 38A	DE NW
49	Schleiden, Abt. 403A	DE NW
51	Eitorf 1502/262a	DE NW
52	Eitorf 1502/209a/b	DE NW
53	Steinfurt	DE NW
54	Schmallenberg	DE NW
55	Glindfeld Vilden, Abt. 19	DE NW
58	Wünnenberg Glashütte, Abt. 15b	DE NW
59	Wünnenberg Hirse, Abt. 8b	DE NW
61	Neuenheerse, Abt. 175	DE NW
66	Dillenburg	DE HE
67	Hadamar	DE HE
68	Jesberg	DE HE
69	Büdingen Abt. 762 (Standard)	DE HE
70	Büdingen Abt. 763 (Standard)	DE HE
71	Sinntal Abt. 410 (Standard)	DE HE
72	Sinntal Abt. 411 (Standard)	DE HE
73	Sinntal Abt. 414 A (Standard)	DE HE
74	Schlüchtern	DE HE
75	Spangenberg, Rfö. Kaltenbach	DE HE
76	Bad Salzungen	DE TH
77	Eisenach	DE TH
80	Ebeleben	DE TH
83	Heinzebank	DE SN
84	Tharandt (Pferdestall)	DE SN
87	Osburg	DE RP
88	Morbach	DE RP
89	Hermeskeil	DE RP
90	Kirchheimbolanden	DE RP
91	Elmstein-Süd, Rfö. Wolfsgrube XIV 1a	DE RP
92	Elmstein-Süd, Appenthal. XIV Buch.	DE RP
93	Montabaur	DE RP
94	Ettenheim	DE BW
95	Münsingen Brente	DE BW
97	Herrenberg	DE BW
98	Giengen I, Abt. 16 (Standard)	DE BW
99	Ehingen	DE BW
100	Ebrach	DE BY
101	Kaufbeuren	DE BY
102	Vohenstrauß	DE BY
103	Vohenstrauß, Rfö. Waishaus	DE BY
104	Zwiesel	DE BY
108	Veneto	IT
109	Neuberg-Mürzsteg	AT
110	Kladská	CZ
111	Ceský Krumlov	CZ
114	Krynica	PL
115	Stary Sacz	PL
116	Bnerko	PL
117	Ladek Zdroj	PL
118	Henryków	PL
120	Brzeziny	PL
124	Zamutov	SK
126	SLP Poruba	SK
127	Ubla	SK
129	Smolenice	SK
130	Trenc in	SK
132	Muran	SK
135	Medzilaborce-Koskovce	SK
136	Idrija	SL
137	Postojna	SL
138	Rogaska Slatina	SL
139	Opatija	CR
141	Svaljava Polana	UA
142	Tura Polana	UA
144	Rachiv	UA
145	Beliu-Arad	RO

No	Provenance	Country
46	Beius-Bihor	RO
150	Sovata (25)	RO
158	Ribaritza	BG
161	Fläming	DE ST

Up to now the trial is not thinned. Losses are due to competition. Only upcoming natural regeneration of willow (*Salix caprea*), maple (*Acer pseudoplatanus*) and cherry (*Prunus avium*) were removed.

The following traits were assessed or measured at several ages in the field: Survival was assessed at age 3, 5, 10, 15 and 20. Height growth were measured at age 3, 5, 10 and 15, and diameter (dbh) growth at age 15 and 20. Stem form was recorded using a 4 step scale (1= good to 4= poor).

Results

On the site Schädtbek only 2 % of the plants died during the first vegetation period or had not took root. In 24 provenances all 150 plants are still alive. These are 2 provenances from France (no. 11, 13), 1 from Sweden (no. 28), 15 from Germany (no. 29, 38, 39, 43, 44, 48, 61, 66, 68, 71, 77, 83, 94, 97, 100), 4 from Slovakia (no. 127, 130, 132, 135), 1 from Slovenia (no. 137), and 1 from the Ukraine (no. 141). The highest plant losses are registered in 5 provenances from Germany (no. 51 [4.6 %], 95 [4.0 %], 40, 84 and 99 [each 3.3 %]). In total, all provenances have a survival rate which is higher than 95 %.

Further results at age 10 are presented by LIESEBACH et al (2011).

The development of the survival over all provenances is listed in Table 2. At age 20 the survival rate is 61 %. Losses can be mainly explained by natural competition between the beech trees. At age 15 the mean height of the trial was 5.3 m (Table 2).

Table 2: Development of survival rate and mean height and dbh of beech at the site Schädtbek

Trait	Age 3	Age 5	Age 10	Age 15	Age 20
Survival [%]	98 %	86 %	76 %	69 %	61 %
Height	0.33 m	1.1 m	2.9 m	5.3 m	
Dbh				6.5 cm	9.2 cm
Stem form*					2.6

*1= good to 4= poor

At age 15 the mean dbh of the trial was 6.5 cm (Table 2). The dbh increased to age 20 up to 9.2 cm (table 2).The mean diameter of the provenances varied between 7.0 cm (No. 138: Rogaska Slatina / Slovenia) and 11,2 cm (No. 130: Trenc in / Slovakia) (Figure 3).

An average of 2.6 is calculated over all provenances for the stem form (Table 2) which is not the best. Figure 4 shows the mean stem form for each of the 100 provenances, which varies between 2.2 (No. 74: Schlüchtern / Germany, 104: Zwiesel / Germany, and 109: Neuberg-Mürzsteg / Austria) and 3.1 (No. 14: F.D de Lagast / France). About one third of the provenances (32) have a mean stem form of less than 2.6 (dark green bars in Figure 4). The stem form of only 5 provenance is higher than 2.9. The frequency for each provenance is shown in Figure 5.

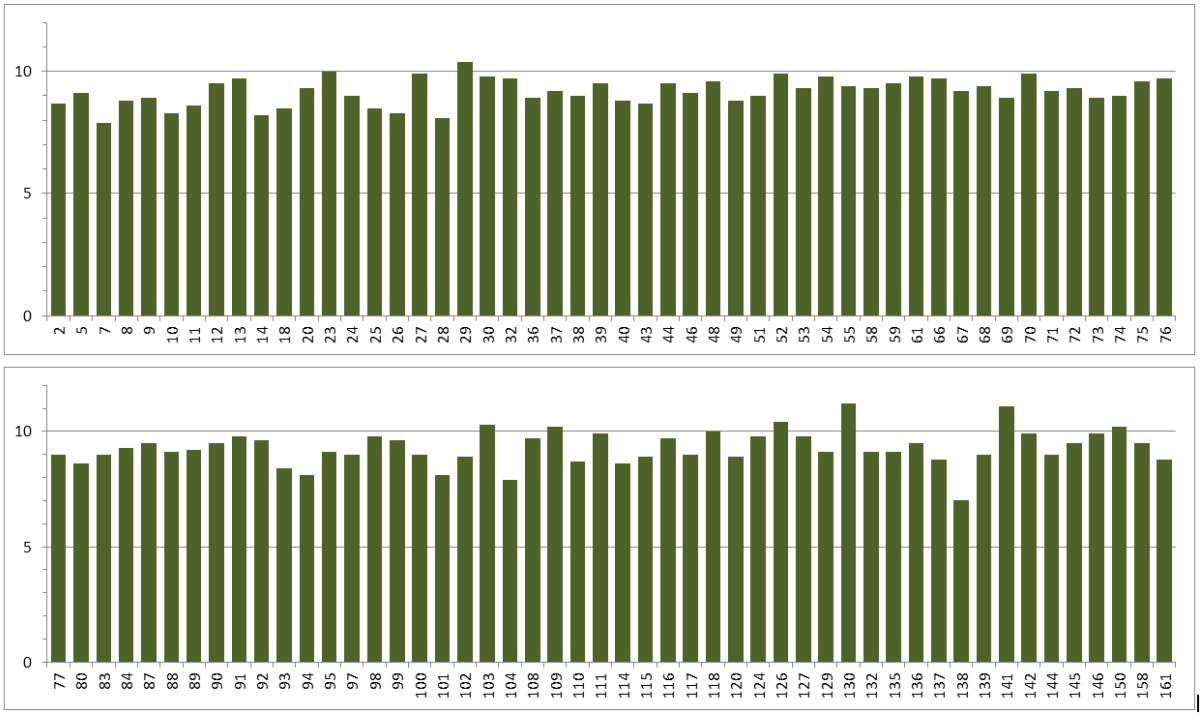

Figure 3: Mean dbh at age 20 of the 100 provenances tested at the site Schädtbek (Bu19-1).

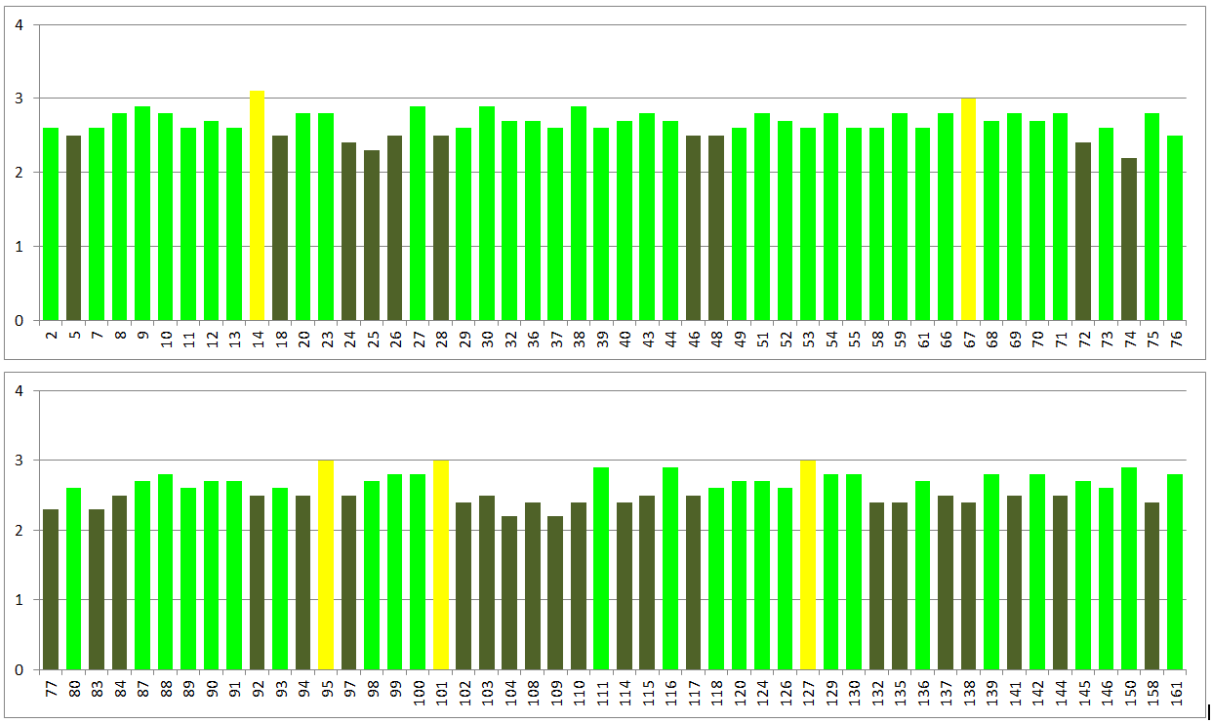

Figure 4: Mean stem form at age 20 of the 100 provenances tested at the site Schädtbek (Bu19-1).

Figure 5: Frequency of stem form (4 step scale: good [dark green] to red [poor]) at age 20 of the 100 provenances tested at the site Schädtbek (Bu19-1).

Correlation between traits

At all ages height and diameter growth are correlated (Table 3). However, there is no correlation between the mean stem form and the growth traits height and dbh. The results of the correlation analysis are summarized in Table 3.

Table 3: PEARSON's correlation coefficient for the growth traits height (H..) and dbh (D..), and stem form respectively. (*significant $\alpha < 0.05$)

Trait age	Height 5	Height 10	Height 15	Dbh 15	Dbh 20	Form 20
Height 5	-					
Height 10	0.90494*	-				
Height 15	0.58069*	0.68881*	-			
Dbh 15	0.70594*	0.80749*	0.63085*	-		
Dbh 20	0.63085*	0.48717*	0.37263*	0.78120*	-	
Form 20	-0.01073	-0.04759	-0.04165	0.10056	0.16546	-

Conclusions

Due to the high number of provenances originated over whole Europe it is not surprising that is variation between the provenances regarding growth and stem form. However, the variation is lower than expected compared with other forest tree species. Of specific interest is the result that growth and stem form are not correlated.

Acknowledgements

The trail series was initialized by HANS-J. MUHS (the former head of the Institute), GEORG VON WÜHLISCH and MIRKO LIESEBACH. We gratefully acknowledge the technicians and the staff of the nursery doing the assessments and measurements conscientiously. RALF BOETTCHER, Forest district Ostholstein, Bundesforsten Trave is also thanked for the care of the site.

References

LIESEBACH M, SCHÜLER S, WOLF H (2011): Klima-Wachstums-Beziehungen von Rotbuchen-Herkünften im Vergleich. Mitteilungen aus der Forschungsanstalt für Waldökologie und Forstwirtschaft Rheinland-Pfalz. 69/11: 79-91.

LIESEBACH M (2015): The international network of European beech trials – the base to study intra-specific variation. In: Dendroflora of Central Europe – utilization of knowledge in research, education and practice. Zvolen, Technicka Univerzita vo Zvolene: 169-187.

VON WÜHLISCH G, LIESEBACH M, MUHS H-J, STEPHAN BR (1998): A network of international beech provenance trials. In J Turok, A Kremer, S de Vries (eds.): First EUFORGEN Meeting on Social Broadleaves. Bordeaux, France, 23-25 October 1997. International Plant Genetic Resources Institute, Rome, 1998: 164-172.

Annex

Program for the German-Russian conference on forest genetics

21/11/2017-23/11/2017

Day / Time	Name, Institution	Presentation title
21.11.2017		
9:00	**BERND DEGEN**, Thünen Institute of Forest Genetics, Großhansdorf	Welcome notes
9:15	**KONSTANTIN KRUTOVSKY**, University of Göttingen, Göttingen	Welcome notes
Session I: Tree breeding / Moderator: MIRKO LIESEBACH		
9:30	**GEORG VON WÜHLISCH**, Thünen Institute of Forest Genetics Grosshansdorf	The MaRussia project - Improving the Productivity, Resistance, and Adaptability in Poplar – Development of Genetic Markers for Aspen
10:00	**OXANA CHERNYSHENKO**, Moscow State Forest University, Moscow	Choice of resistant and fast-growing aspen forms using dendrochronological information from nature reserves
10:30	**DMITRII SHABUNIN**, St. Petersburg Forestry Research Institute, St. Petersburg	Testing of mutagenic substances in aspen *in vitro* culture
11:00	**Coffee break**	
11:30	**ANATOLY TSAREV**, All-Russian Research Institute of Forest Genetics, Voronezh	Poplars breeding in temperate climate of Russia
12:00	**ANATOLY ZHIGUNOV**, St Petersburg State Forest Technical University, St Petersburg	Development of F1 hybrid population and the high-density linkage map for European aspen (*P. tremula* L.) using RADseq technology
12:30	**INNA VARIVODINA**, Research Institute of Forest Genetics, Breeding and Biotechnology, Voronezh	Forest Biotechnology Center: scientific results and development perspectives
13:00	**Discussion**	
13:30	**Lunch**	
Session II: Tree breeding & Biotechnology / Moderator: BERND DEGEN		
14:30	**MIRKO LIESEBACH**, Thünen-Institute of Forest Genetics, Großhansdorf	The German breeding concept – the base for new seed orchards
15:00	**CORNELIA BÄUCKER**, Thünen-Institute of Forest Genetics, Waldsieversdorf	From in vitro clones to high-quality timber production – the Project “Wavy Grain Maple”
15:30	**Coffee break**	
16:00	**LIDIIA VETCHINNIKOVA**, Forest Research Institute Karelian Research Center, Russian Academy of Sciences, Petrozavodsk	The origin of Curly (Karelian) birch: molecular and genetic aspects

Day / Time	Name, Institution	Presentation title
16:30	**MATTHIAS MEYER**, University, Dresden, Dresden	The importance of fuel characteristics of Populus in short rotation plantation
17:00	**Discussion**	
18:00	**Dinner**	
22.11.2017		
Session III Population Genetics & Gene conservation / Moderator: KONSTANTIN KRUTOVSKY		
9:00	**BERND DEGEN**, Thünen-Institute of Forest Genetics, Großhansdorf	Twenty years of German-Russian co-operation on genetic diversity in forests
9:30	**YULAI YANBAEV**, Bashkirian State University, Ufa	Sampling techniques and sampling strategy for large scale genetic studies
10:00	**SVETLANA BORONNIKOVA**, Perm State University, Perm	Nucleotide polymorphism and genetic diversity in poplars in the Urals
11:30	**Coffee break**	
12:00	**JENS SCHMIEDEL**, Sachsenforst, Graupa	Genetic monitoring in German forests –GenMon project
12:30	**TATIANA POLIAKOVA**, Russian Centre of Forest Health, Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow	Population genetic studies of small-leaved forest tree species using molecular markers
13:00	**Discussion**	
13:30	**Lunch**	
Session IV Timber tracking / Genomics / Adaptation / Moderator: BERND DEGEN		
14:30	**CÉLINE BLANC-JOLIVET**, Thünen-Institute of Forest Genetics, Großhansdorf	Genetic timber tracking of *Larix* sp. in Eurasia
15:00	**SVETLANA SEMERIKOVA**, Institute of Plant and Animal Ecology, Ural Branch of Russian Academy of Sciences, Ekaterinburg	Development of mtDNA markers for Siberian conifers and their application in phylogeographic studies
15:30	**KONSTANTIN KRUTOVSKY**, University of Göttingen, Göttingen	Genomics for practical forestry: development of genome-wide markers for timber origin identification and other applications
16:00	**Coffee break**	
16:30	**HILKE SCHRÖDER**, Thünen-Institute of Forest Genetics, Großhansdorf	DNA-Markersets zur Bestimmung von Weißeichen (Sektion *Quercus*) in Holzprodukten
17:00	**OLIVER GAILING**, University of Göttingen, Göttingen	Introgression of genes between two hybridizing red oak species with different drought tolerance
17:30	**DMITRY POLITOV,** Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow	Population genetic studies of forest tree species: spatio-temporal differentiation and adaptedness

Day / Time	Name, Institution	Presentation title
18:00	**Discussion & Conclusion**	
19:00	**Lunch**	
23.11.2017		
	Excursion (MIRKO LIESEBACH)	
9:00	**Excursion (9:00 – 17:00)**	

List of Participants

Dr. CORNELIA BÄUCKER
Thünen Institute of Forest Genetics
Eberswalder Chaussee 3 a
15377 Waldsieversdorf
Germany
cornelia.baeucker@thuenen.de

Dr. CÉLINE BLANC-JOLIVET
Thünen Institute of Forest Genetics
Sieker Landstr. 2
22927 Grosshansdorf
Germany
celine.blanc-jolivet@thuenen.de

Prof. SVETLANA BORONNIKOVA
Department of Botany and Plant Genetics,
Faculty of Biology
Perm State University
Perm
Russia
SVBoronnikova@yandex.ru

Dr. OXANA CHERYSHENKO
Moscow State Forest University
Moscow
Russia
oxana-foret@yandex.ru

Dr. BERND DEGEN
Thünen Institute of Forest Genetics
Sieker Landstr. 2
22927 Grosshansdorf
Germanybernd.degen@thuenen.de

Dr. VLADIMIR DMITRIEV
Department of Science, Education and International Cooperation
Federal Forestry Agency
Moscow
Russia

Prof. Dr. OLIVER GAILING
University of Göttingen
Forest Genetics and Tree Breeding
Büsgen-Institut
Büsgenweg 2
37077 Göttingen
Germany
ogailin@gwdg.de

Dr. ALEXANDER GRADEL
University of Göttingen
Department of Silviculture and Forest Ecology of the Temperate Zones
Büsgenweg 1
37077 Göttingen
Germany
alexander.gradel@forst.uni-goettingen.de

SERGEJ KASCHURO
Thünen Institute of Wood Research
Leuschnerstr. 91
21031 Hamburg
Germany
sergej.kaschuro@thuenen.de

Dr. BIRGIT KERSTEN
Thünen Institute of Forest Genetics
Sieker Landstr. 2
22927 Grosshansdorf
Germany
birgit.kersten@thuenen.de

Prof. Dr. KONSTANTIN V. KRUTOVSKY
University of Göttingen
Forest Genetics and Tree Breeding
Büsgen-Institut
Büsgenweg 2, 37077 Göttingen
Germany
konstantin.krutovsky@forst.uni-goettingen.de

Dr. MIRKO LIESEBACH
Thünen Institute of Forest Genetics
Sieker Landstr. 2
22927 Grosshansdorf
Germany
mirko.liesebach@thuenen.de

Dr. MATTHIAS MEYER
Technische Universität Dresden
Pienner Str. 7
01737 Tharandt
Germany
matthias.meyer@tu-dresden.de

Dr. NIELS MÜLLER
Thünen Institute of Forest Genetics
Sieker Landstr. 2
22927 Grosshansdorf
Germany
niels.mueller@thuenen.de

Dr. TATIANA A. POLIAKOVA
Department for Monitoring of Forest Genetic Resources
FSI Russian Centre of Forest Health
Nadsonovskaya 13, Pushkino
Moscow region, 141207
Russia
tat-polyakova@yandex.ru

Dr. DMITRY POLITOV
Laboratory of Population Genetics
N.I. Vavilov Institute of General Genetics
Russian Academy of Sciences
119991 Moscow
Russia
dmitri_p@inbox.ru

JENS SCHMIEDEL
Staatsbetrieb Sachsenforst
Kompetenzzentrum Wald und Forstwirtschaft, Referat Forstgenetik/Forstpflanzenzüchtung
Bonnewitzer Str. 34
01796 Pirna
Germany
jens.schmiedel@smul.sachsen.de

Dr. HILKE SCHRÖDER
Thünen Institute of Forest Genetics
Sieker Landstr. 2
22927 Grosshansdorf
Germany
hilke.schroeder@thuenen.de

Dr. SVETLANA SEMERIKOVA
Laboratory Molecular Ecology of Plants
Institute of Plant and Animal Ecology
Ural Branch of Russian Academy of Science
8 Marta Str., 202
620144 Ekaterinburg
Russia
s.a.semerikova@ipae.uran.ru

DIMITRI SHABUNIN
St. Petersburg Forestry Research Institute
St. Petersburg
Russia
ds1512@mail.ru

Dr. KONSTANTIN SHESTIBRATOV
Forest Biotechnology
M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry
Russian Academy of Sciences, Pushchino 142290, Moscow Region
Russia

ANATOLY TSAREV
All-Russian Research Institute of Forest Genetics
Voronezh
Russia
anatolytsa@gmail.com

Pavel Ulianich
St. Petersburg State Forestry University
St. Petersburg
Russia
goblinkmv@gmail.com

INNA VARIVODINA
Research Institute of Forest Genetics, Breeding and Biotechnology
Voronezh
Russia
biotechcenter@lesgen.vrn.ru

Dr. LIDIIA VETCHINNIKOVA
Laboratory for Forest Biotechnology
Forest Research Institute
Karelian Research Centre of Russian Academy of Sciences
11 Pushkinskaya St.
185910 Petrozavodsk Karelia
Russia
vetchin@mail.ru

Dr. GEORG VON WÜHLISCH
Thünen Institute of Forest Genetics
Sieker Landstr. 2
22927 Grosshansdorf
Germany

Prof. YULAY YANBAEV
Bashkirian State University
Z. Validi Str.-32
450076 Ufa
Russia
Yanbaev_UA@mail.ru

ANATOLY ZHIGUNOV
St Petersburg State Forest Technical University
St Petersburg
Russia
a.zhigunov@bk.ru

Thünen Report

Bereits in dieser Reihe erschienene Hefte – *Volumes already published in this series*

1 - 39	siehe http://www.thuenen.de/de/infothek/publikationen/thuenen-report/
40	Frank Offermann, Martin Banse, Claus Deblitz, Alexander Gocht, Aida Gonzalez-Mellado, Peter Kreins, Sandra Marquardt, Bernhard Osterburg, Janine Pelikan, Claus Rösemann, Petra Salamon, Jürn Sanders **Thünen-Baseline 2015 – 2025: Agrarökonomische Projektionen für Deutschland**
41	Stefan Kundolf, Patrick Küpper, Anne Margarian und Christian Wandinger **Koordination, Lernen und Innovation zur Entwicklung peripherer ländlicher Regionen** **Phase II der Begleitforschung zum Modellvorhaben *LandZukunft***
42	Sebastian Rüter, Frank Werner, Nicklas Forsell, Christopher Prins, Estelle Vial, Anne-Laure Levet **ClimWood2030 'Climate benefits of material substitution by forest biomass and harvested wood products: Perspective 2030' Final Report**
43	Nicole Wellbrock, Andreas Bolte, Heinz Flessa (eds) **Dynamik und räumliche Muster forstlicher Standorte in Deutschland – Ergebnisse der Boden-zustandserhebung im Wald 2006 bis 2008**
44	Walter Dirksmeyer, Michael Schulte und Ludwig Theuvsen (eds) **Aktuelle Forschung in der Gartenbauökonomie – Nachhaltigkeit und Regionalität – Chancen und Heraus-forderungen für den Gartenbau** – Tagungsband zum 2. Symposium für Ökonomie im Gartenbau
45	Mirko Liesebach (ed) **Forstgenetik und Naturschutz – 5. Tagung der Sektion Forstgenetik/Forstpflanzenzüchtung am 15./16. Juni 2016 in Chorin – Tagungsband**
46	Claus Rösemann, Hans-Dieter Haenel, Ulrich Dämmgen, Annette Freibauer, Ulrike Döring, Sebastian Wulf, Brigitte Eurich-Menden, Helmut Döhler, Carsten Schreiner, Bernhard Osterburg **Calculations of gaseous and particulate emissions from German agriculture 1990 - 2015** **Berechnung von gas- und partikelförmigen Emissionen aus der deutschen Landwirtschaft 1990 – 2015**
47	Niko Sähn, Stefan Reiser, Reinhold Hanel und Ulfert Focken **Verfügbarkeit umweltrelevanter Daten zur deutschen Süßwasseraquakultur**
48	Markus Ehrmann **Modellgestützte Analyse von Einkommens- und Umweltwirkungen auf Basis von Testbetriebsdaten**
49	Mirko Liesebach, Wolfgang Ahrenhövel, Alwin Janßen, Manuel Karopka, Hans-Martin Rau, Bernd Rose, Randolf Schirmer, Dagmar Schneck, Volker Schneck, Wilfried Steiner, Silvio Schüler, Heino Wolf **Planung, Anlage und Betreuung von Versuchsflächen der Forstpflanzenzüchtung** **Handbuch für die Versuchsanstellung**
50	Tobias Mettenberger **Jugendliche Zukunftsorientierungen in ländlichen Mittelstädten** Zur Rolle des alltäglichen (sozial-)räumlichen Kontexts beim Übergang von der Hauptschule in den weiteren Ausbildungsweg
51	Stefan Neumeier **Modellvorhaben chance.natur – Endbericht der Begleitforschung –**

52	Andreas Tietz **Überregional aktive Kapitaleigentümer in ostdeutschen Agrarunternehmen: Entwicklungen bis 2017**
53	Peter Mehl (ed) **Aufnahme und Integration von Geflüchteten in ländliche Räume:** **Spezifika und (Forschungs-)herausforderungen** Beiträge und Ergebnisse eines Workshops am 6. und 7. März 2017 in Braunschweig
54	G. Rahmann, C. Andres, A.K. Yadav, R. Ardakani, H.B. Babalad, N. Devakumar, S.L. Goel, V. Olowe, N. Ravisankar, J.P. Saini, G. Soto, H. Willer **Innovative Research for Organic 3.0 - Volume 1** Proceedings of the Scientific Track at the Organic World Congress 2017 November 9-11 in Delhi, India
54	G. Rahmann, C. Andres, A.K. Yadav, R. Ardakani, H.B. Babalad, N. Devakumar, S.L. Goel, V. Olowe, N. Ravisankar, J.P. Saini, G. Soto, H. Willer **Innovative Research for Organic 3.0 - Volume 2** Proceedings of the Scientific Track at the Organic World Congress 2017 November 9-11 in Delhi, India
55	Anne Margarian unter Mitarbeit von Matthias Lankau und Alena Lilje **Strategien kleiner und mittlerer Betriebe in angespannten Arbeitsmarktlagen** Eine Untersuchung am Beispiel der niedersächsischen Ernährungswirtschaft
56	Frank Offermann, Martin Banse, Florian Freund, Marlen Haß, Peter Kreins, Verena Laquai, Bernhard Osterburg, Janine Pelikan, Claus Rösemann, Petra Salamon **Thünen-Baseline 2017 – 2027: Agrarökonomische Projektionen für Deutschland**
57	Hans-Dieter Haenel, Claus Rösemann, Ulrich Dämmgen, Ulrike Döring, Sebastian Wulf, Brigitte Eurich-Menden, Annette Freibauer, Helmut Döhler, Carsten Schreiner, Bernhard Osterburg **Calculations of gaseous and particulate emissions from German agriculture 1990 - 2016** **Berechnung von gas- und partikelförmigen Emissionen aus der deutschen Landwirtschaft 1990 – 2016**
58	Anja-Kristina Techen **Reduzierung von landwirtschaftlichen Stickstoffeinträgen in Gewässer: die Wirksamkeit von Beratung am Beispiel der hessischen WRRL-Beratung**
59	Katja Oehmichen, Susann Klatt, Kristin Gerber, Heino Polley, Steffi Röhling, Karsten Dunger **Die alternativen WEHAM-Szenarien: Holzpräferenz, Naturschutzpräferenz und Trendfortschreibung** **Szenarienentwicklung, Ergebnisse und Analyse**
60	Anne Margarian **Strukturwandel in der Wissensökonomie: Eine Analyse von Branchen-, Lage- und Regionseffekten in Deutschland**
61	Meike Hellmich **Nachhaltiges Landmanagement vor dem Hintergrund des Klimawandels als Aufgabe der räumlichen Planung - Eine Evaluation im planerischen Mehrebenensystem an den Beispielen der Altmark und des Landkreises Lüchow-Dannenbergs -**
62	Bernd Degen, Konstantin V. Krutovsky, Mirko Liesebach (eds.) **German Russian Conference on Forest Genetics - Proceedings - Ahrensburg, 2017 November 21-23**

THÜNEN

Thünen Report 62

Herausgeber/Redaktionsanschrift

Johann Heinrich von Thünen-Institut
Bundesallee 50
38116 Braunschweig
Germany

www.thuenen.de