# Certification systems for reproductive material of landscape shrubs and trees in Germany and potential possibilities for verification of their source

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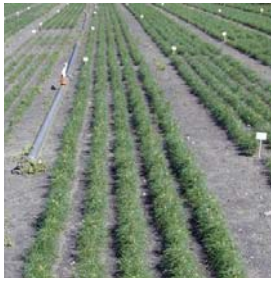
**International Conference of European Seed Kilns, Bernkastel-Kues 04 - 07 June 2013**

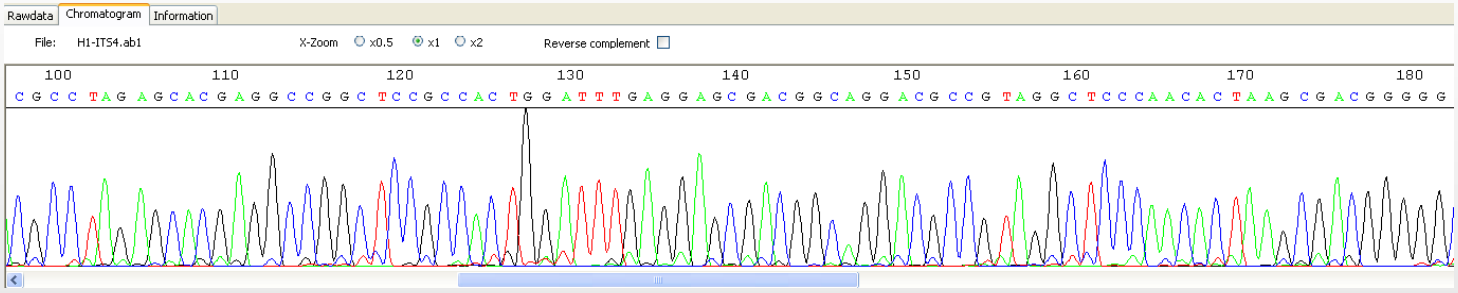
# Thünen Institute of Forest Genetics
*Großhansdorf and Waldsieversdorf*

Federal Ministry
of Food, Agriculture
and Consumer Protection

- Provenance and breeding research
- Pathogen resistance research
- Ecological genetics
- Genome research

http://www.ti.bund.de/



# Outline

- Current certification systems for forest and landscape reproductive materials in Germany – An overview
- Biological basics of genetic markers
- Application of markers for verification of source
- Outlook

# Certification systems in Germany

Federal Nature Conservation Act 2010

Guidelines 2012
developed by a task group and the Federal Ministry for the Environment


6 regions of provenances–
scaled by different environmental conditions

Quellen: Bundesamt für Naturschutz (BfN), 2012; verändert nach Schmidt und Krause (1997)

# Certification systems in Germany

Federal Nature Conservation Act 2010

Guidelines 2012
developed by a task group and the Federal Ministry for the Environment


6 regions of provenances–
scaled by different environmental conditions

1 Norddeutsches Tiefland

2 Mittel- und Ostdeutsches Tief- und Hügelland

3 Südostdeutsches Hügel- und Bergland

4 Westdeutsches Bergland und Oberrheingraben

5 Schwarzwald, Württembergisch-Fränkisches Hügelland und Schwäbisch-Fränkische Alb

6 Alpen und Alpenvorland

Quellen: Bundesamt für Naturschutz (BfN), 2012; verändert nach Schmidt und Krause (1997)

# Certification systems in Germany

| | | Field of action |
|---|---|---|
| ZüF | | Germany |
| FfV | | Germany |
| ZgG | | Germany |
| RAL - GZ | | Germany |
| eab | | Bavaria |
| pro agro | | Brandenburg |
| EZG | | Baden-Württemberg |
| EsB | | Northern lowlands |

# Certification systems in Germany

| | | Field of action | Forest/ Landscape | Since |
|---|---|---|---|---|
| ZüF | | Germany | Forest | 2002 |
| FfV | | Germany | Forest | 2006 |
| | | | Landscape | 2011 |
| ZgG | | Germany | Landscape | 2011 |
| RAL - GZ | | Germany | Landscape | 2011 |
| eab | | Bavaria | Landscape | 2001 |
| pro agro | | Brandenburg | Landscape | 2002 |
| EZG | | Baden-Württemberg | Landscape | 2006 |
| EsB | | Northern lowlands | Landscape | 2007 |

# Certification systems in Germany

| | | Field of action | Forest/ Landscape | Since | Documen-tation | Operating control | Plausi-bility |
|---|---|---|---|---|---|---|---|
| ZüF | | Germany | Forest | 2002 | + | + | + |
| FfV | | Germany | Forest | 2006 | + | + | + |
| | | | Landscape | 2011 | + | + | |
| ZgG | | Germany | Landscape | 2011 | + | + | (+) |
| RAL - GZ | | Germany | Landscape | 2011 | + | + | + |
| eab | | Bavaria | Landscape | 2001 | + | + | + |
| pro agro | | Brandenburg | Landscape | 2002 | + | + | |
| EZG | | Baden-Württemberg | Landscape | 2006 | + | + | + |
| EsB | | Northern lowlands | Landscape | 2007 | + | + | |

# Certification systems in Germany

| | | Field of action | Forest/ Landscape | Since | Documen-tation | Operating control | Plausi-bility | Retain samples | Genetic markers |
|---|---|---|---|---|---|---|---|---|---|
| ZüF | | Germany | Forest | 2002 | + | + | + | + | + |
| FfV | | Germany | Forest | 2006 | + | + | + | + | + |
| | | | Landscape | 2011 | + | + | | + | |
| ZgG | | Germany | Landscape | 2011 | + | + | (+) | | |
| RAL - GZ | | Germany | Landscape | 2011 | + | + | + | | |
| eab | | Bavaria | Landscape | 2001 | + | + | + | | |
| pro agro | | Brandenburg | Landscape | 2002 | + | + | | | |
| EZG | | Baden-Württemberg | Landscape | 2006 | + | + | + | | |
| EsB | | Northern lowlands | Landscape | 2007 | + | + | | | |

# Certification systems in Germany

## Current process

The task group is discussing the minimum standards for certification

## Members

- Federal Ministry for the Environment, Nature Conservation an Nuclear Safety,
- Federal Ministry of Food, Agriculture and Consumer Protection,
- Federal Ministry of Transport, Building and Urban Development,
- Federal and state working group for forest genetic resources,
- Inter-trade organisations (nurseries)
- ...

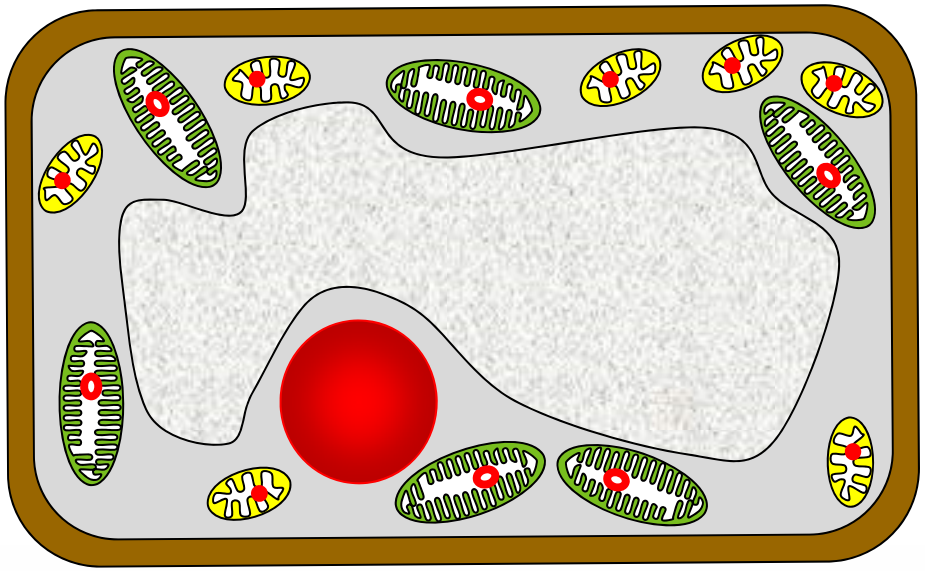
- How we can carry out genetic analyses?

- How can genetic analyses accomplish the verification of sources of reproductive material?

# Genetic markers

## Basics

Scheme of a plant cell



| Inheritance mode | Angiosperms | Gymnosperms |
|---|---|---|
| Nucleus | ♀ ♂ | ♀ ♂ |
| Mitochondria | ♀ | ♀ |
| Chloroplasts | ♀ | ♂ |

# Genetic markers

**What is a genetic marker?**

- Localisation at a certain position in genome (DNA, protein as gene product)
- Reproducible characterisation
- Independently from ontogenetic stage and environment
- Exhibits  variation

**Typical variation patterns**

Nuclear markers
Large variation within populations, individual identification possible

Example „**Nuclear microsatellites**“

Organelle markers
lower variation, but rather geographic differentiation

Example „**cpDNA haplotypes**“

# Genetic markers

## Step by step

- Suitable sample materials: leaves, buds, seeds, root tips, cambium, …
- Extraction of total DNA: incl. nucleus, organelles, bacteria, fungi, …
- Amplification of selected DNA fragments by PCR (Polymerase Chain Reaction) with specific primers
- Detection of fragments by electrophoresis: capillary DNA sequencer, slab gels

# Genetic markers

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- Amplification of selected DNA fragments by PCR (Polymerase Chain Reaction) with **specific primers**
- Detection of fragments by electrophoresis: capillary DNA sequencer, slab gels

→ **for each species and for each marker**

# Genetic markers

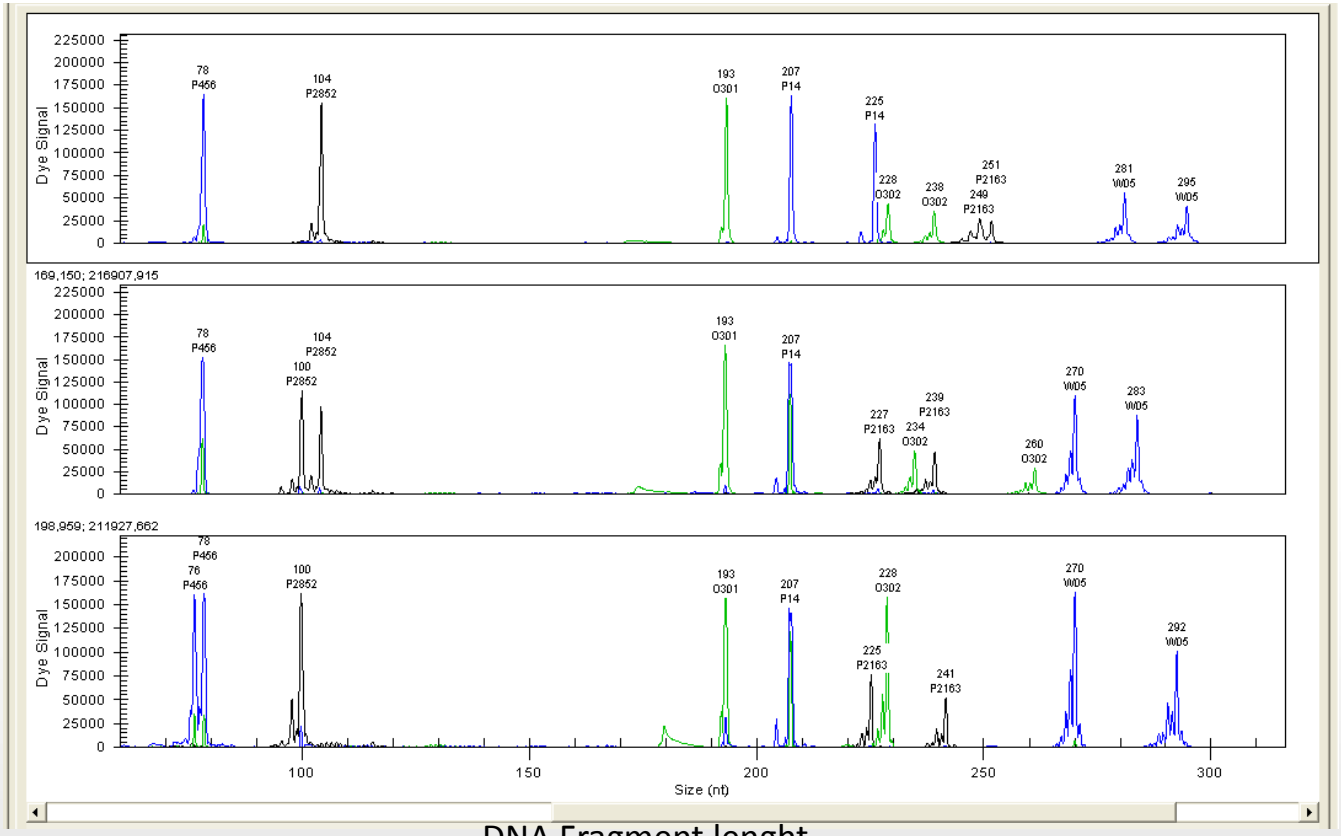
Microsatellite markers:

Variation of DNA fragment lenght caused by different numbers of repeats of short sequences

...ACCATGCCCTAAAGAAGAAGAAGAAGAAGAAAAGTATTGTGTATAGGTCA...
...ACCATGCCCTAAAGAAGAAGAAGAAGAAGAAGAAGAAGAAAAGTATTGTGTATAGGTCA...
...ACCATGCCCTAAAGAAGAAGAAGAAGAAGAAGAAAAGTATTGTGTATAGGTCA...
...ACCATGCCCTAAAGAAGAAGAAGAAGAAGAAGAAAAGTATTGTGTATAGGTCA...
...ACCATGCCCTAAAGAAGAAGAAGAAAAGTATTGTGTATAGGTCA...
...ACCATGCCCTAAAGAAGAAGAAGAAAAGTATTGTGTATAGGTCA...

# Genetic markers

„Genetic fingerprint“ of 3 black poplars (*Populus nigra*) at 7 microsatellite markers



# Genetic markers

Number of possible combinations for individual genotypes

| Number of markers | Number of alleles per marker | Number of possible combinations (Genotypes) |
|---|---|---|
| 1 | 2 (A, B) | 3 (AA, AB, BB) |

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| 1 | 3 (A, B, C) | 6 (AA, AB, AC, BB, BC, CC) |

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|---|---|---|
| 1 | 2 (A, B) | 3 (AA, AB, BB) |
| 1 | 3 (A, B, C) | 6 (AA, AB, AC, BB, BC, CC) |
| 1 | 4 | 10 |
| 1 | 5 | 15 |
| 1 | 10 | 55 |
| 1 | 15 | 120 |

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| 1 | 4 | 10 |
| 1 | 5 | 15 |
| 1 | 10 | 55 |
| 1 | 15 | 120 |
| 2 | 5 | 225 |
| 2 | 10 | 3 025 |
| 5 | 5 | 759 375 |
| 5 | 10 | $5{,}03 * 10^{9}$ |
| 10 | 5 | $5{,}77 * 10^{11}$ |
| 10 | 10 | $2{,}53 * 10^{17}$ |
| 15 | 5 | $4{,}38 * 10^{17}$ |

# Application of markers for verification of sources

Three cases of application of markers for verification of sources

- Identity of clones
- Descent of a population
- Assignment to large-scale regions

# Application of markers for verification of sources

1. Identity of clones

Comparison of a sample with a reference

Exclusion of identity,

if differences were detected

Confirmation of identity,

if no differences were detected

+

if the probability of identy by chance is small enough

# Application of markers for verification of sources

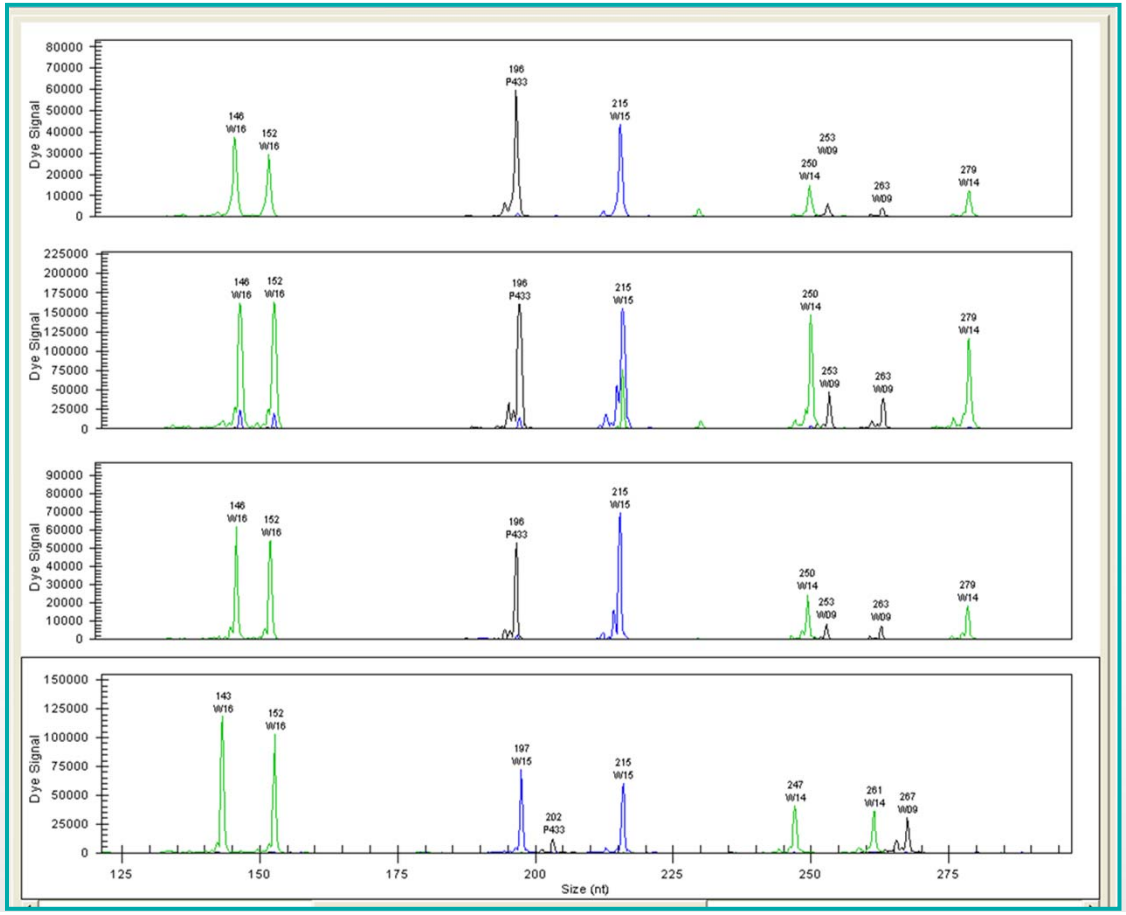
1. Identity of clones - Example

Donor plant

Cutting 1
identical

Cutting 2
identical

Cutting 3
**different**



# Application of markers for verification of sources

## 2. Descent of a population

### With retain samples

Comparison of seed or plant lots with retain samples,
application of exclusion principle

### Without retain samples

Comparison of seed or plant lots with the parental population:

Possible for defined populations or seed orchards by exclusion principle

**No assignment to a region of occurrence without retain sample or without known parental population!!**

# Application of markers for verification of sources

## 2. Descent of a population - Example

The descent of seeds from a certain tree can be excluded for individual seeds carrying genotypes without maternal alleles.

| Sample | Marker005 | Marker410 |
|---|---|---|
| Seed tree | 106 - 126 | 124 - 134 |
| Seed 1 | 106 - 126 | 134 - 134 |
| Seed 2 | 106 - 126 | 134 - 134 |
| Seed 3 | 106 - 126 | 124 - 132 |
| Seed 4 | 106 - 118 | 130 - 132 |
| Seed 5 | 106 - 106 | 134 - 134 |
| Seed 6 | 126 - 126 | 130 - 132 |
| Seed 7 | 110 - 126 | 132 - 132 |
| Seed 8 | 110 - 110 | 130 - 132 |

No maternal alleles

Konnert M. 2005. Erfolge (und Grenzen) bei dem Herkunftsnachweis mittels Isoenzym- und DNA-Analysen. In: 26. Tagung der Arbeitsgemeinschaft Forstgenetik und Forstpflanzenzüchtung 2005 in Fuldatal. Hann. Münden, HESSEN-FORST, 49-57.

# Application of markers for verification of sources

## 2. Descent of a population - Example

The descent of seeds from a certain tree can be excluded for individual seeds carrying genotypes without maternal alleles.

| Sample | Marker005 | Marker410 | Result |
|---|---|---|---|
| Seed tree | 106 - 126 | 124 - 134 | |
| Seed 1 | 106 - 126 | 134 - 134 | Ancestry possible |
| Seed 2 | 106 - 126 | 134 - 134 | Ancestry possible |
| Seed 3 | 106 - 126 | 124 - 132 | Ancestry possible |
| Seed 4 | 106 - 118 | 130 - 132 | Ancestry excluded |
| Seed 5 | 106 - 106 | 134 - 134 | Ancestry possible |
| Seed 6 | 126 - 126 | 130 - 132 | Ancestry excluded |
| Seed 7 | 110 - 126 | 132 - 132 | Ancestry excluded |
| Seed 8 | 110 - 110 | 130 - 132 | Ancestry excluded |

# Application of markers for verification of sources

## 3. Assignment to large-scale regions

Geographic patterns

- can be observed regarding colonisation routes after ice age starting from different glacial refugia
- can result from restricted gene flow among populations (isolation by distance)

Precondition: → Genetic inventory

# Application of markers for verification of sources

## 3. Assignment to large-scale regions

Geographic pattern of cpDNA haplotypes (PCR-RFLPs)

Example *Frangula alnus*

78 populations,
322 individuals

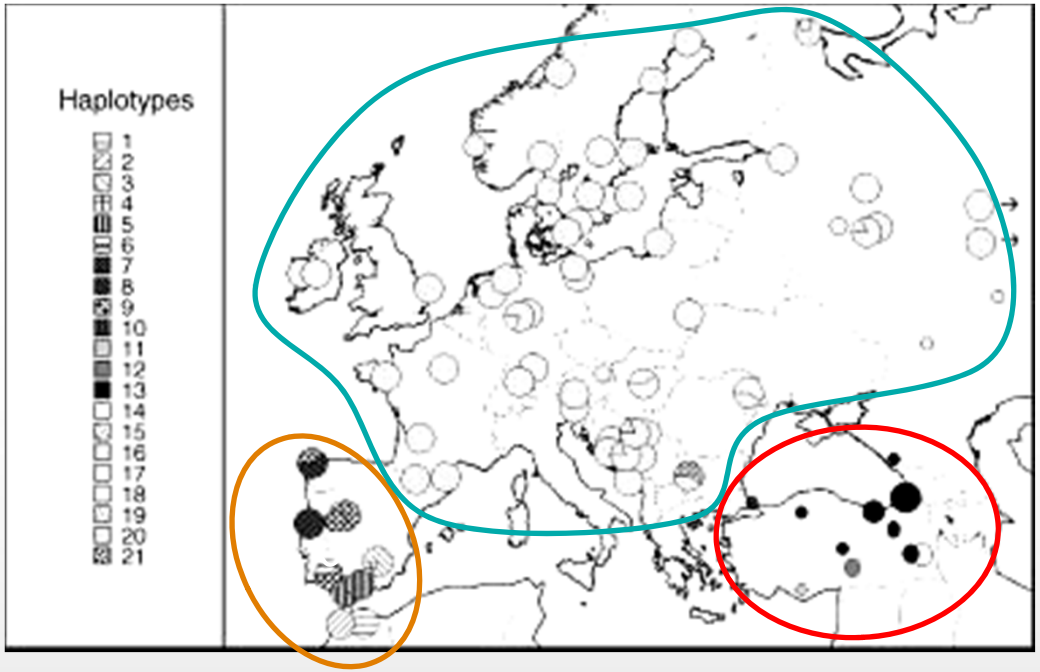



Fig. 3 Geographical distribution of *Frangula alnus chloroplast* haplotypes. Circle sizes are proportional to the number of analysed samples per population (*n = 1–5).*

Hampe A, Arroyo J, Jordano P, Petit RJ. 2003. Rangewide phylogeography of a bird-dispersed Eurasian shrub: contrasting Mediterranean and temperate glacial refugia. *Molecular Ecology, 12: 3415-3426.*

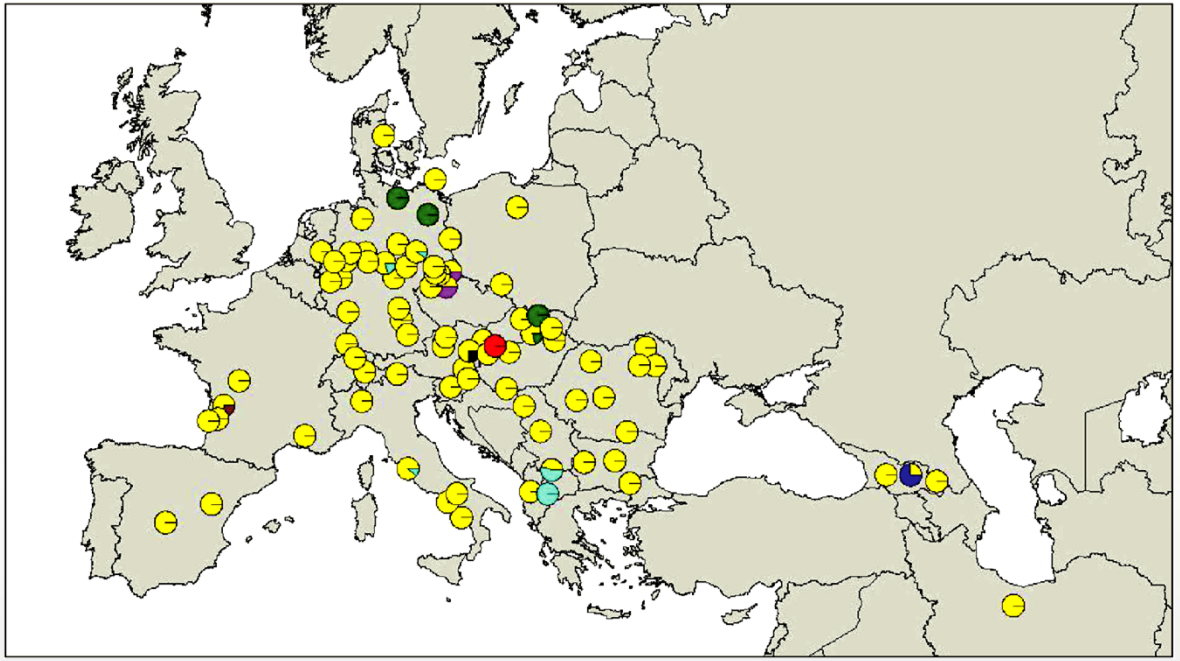
# Application of markers for verification of sources

## 3. Assignment to large-scale regions

Geographic pattern of cpDNA haplotypes (PCR-RFLPs)

Example *Cornus sanguinea*

86 populations,
673 individuals



Liesebach & Götz, Silvae Genetica (2008) 57: 291-300

*Figure 4. – Geographic distribution of eight chloroplast haplotypes identified in Cornus sanguinea (haplotype A = yellow, B = red, C = pink, D = dark blue, E = light blue, F = green, G = brown, H = black).*

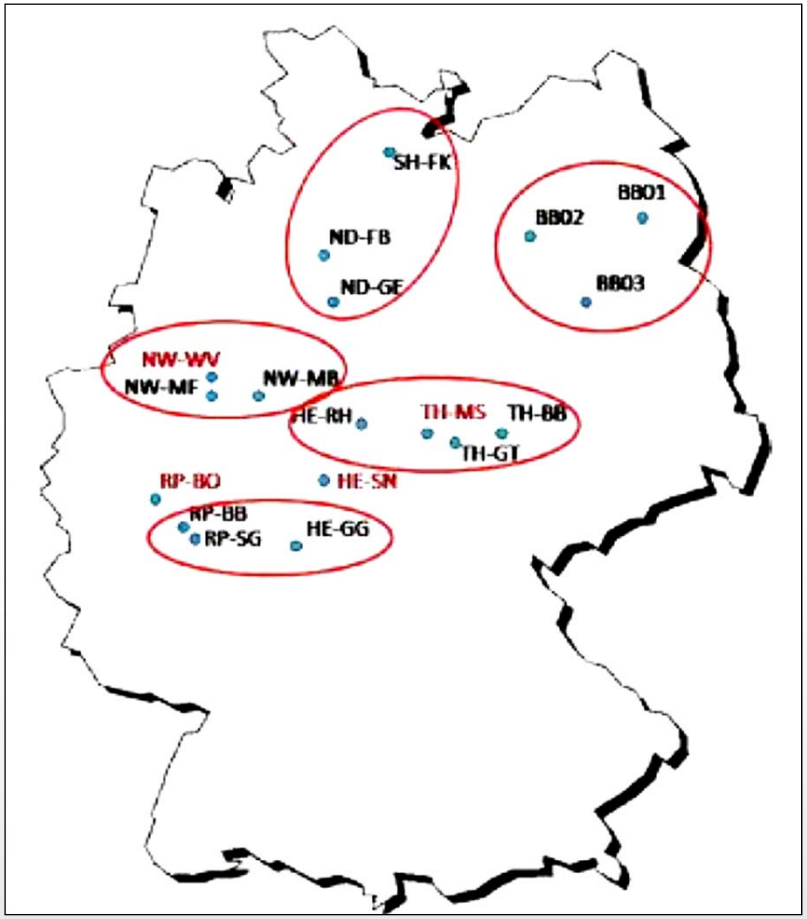
# Application of markers for verification of sources

## 3. Assignment to large-scale regions

Geographic pattern derived from AFLPs, isozymes and cpDNA-SSR haplotypes

Example *Corylus avellana*

20 populations,
948 individuals




Leinemann L, Hosius B, Steiner W. 2012. Hasel: Genetische Analysen stützen ausgewiesene Vorkommensgebiete - Ergebnisse aus der genetischen Inventur der Haselnuss. *TASPO 47, 1*

# Outlook

## Nuclear and chloroplast markers available

| Species name | Deutscher Name |
|---|---|
| *Acer pseudoplatanus* | Spitz-Ahorn |
| *Betula pendula* | Sand-Birke |
| *Betula pubescens* | Moor-Birke |
| *Castanea sativa* | Ess-Kastanie |
| *Corylus avellana* | Gewöhnliche Hasel |
| *Cytisus scoparius* | Besen-Ginster |
| *Fagus sylvatica* | Rot-Buche |
| *Frangula alnus* | Faulbaum |
| *Fraxinus excelsior* | Gewöhnliche Esche |
| *Ligustrum vulgare* | Liguster |
| *Populus alba* | Silber-Pappel |
| *Populus tremula* | Zitter-Pappel |
| *Prunus avium* | Vogel-Kirsche |
| *Quercus petraea* | Trauben-Eiche |
| *Quercus robur* | Stiel-Eiche |
| *Ulmus glabra* | Berg-Ulme |
| *Ulmus minor* | Feld-Ulme |

## Markers not yet available

| Species name | Deutscher Name |
|---|---|
| *Alnus incana* | Grau-Erle |
| *Crataegus laevigata* | Zweigriffliger Weißdorn |
| *Euonymus europaea* | Pfaffenhütchen |
| *Lonicera nigra* | Schwarze Heckenkirsche |
| *Lonicera xylosteum* | Rote Heckenkirsche |
| *Rhamnus cathartica* | Kreuzdorn |
| *Rosa canina* | Hunds-Rose |
| *Rosa majalis* | Zimt-Rose |
| *Sambucus racemosa* | Trauben-Holunder |
| *Sorbus aucuparia* | Eberesche |
| *Tilia platyphyllos* | Sommer-Linde |
| *Viburnum lantana* | Wolliger Schneeball |
| *Viburnum opulus* | Gemeiner Schneeball |

Grey: forest tree species under regulations for forest reproductive materials


Thank you for your attention