Use of DNA-fingerprints for timber tracking

Bernd Degen[a], Céline Jolivet[b], Aki Höltken[b,c]

a) vTI- Institut für Forstgenetik, Grosshansdorf, Germany

b) University of Hamburg, Germany

c) Plant Genetic Diagnostics GmBH

Content

1. Introduction

2. Projects and results

- Controlling the country of origin for mahogany (*Swietenia macrophylla*)

- DNA-Barcoding to distinguish tree species in the family meliaceae

- Timber tracking of Sapelli (*Entandrophragma cylindricum*) and Iroko (*Milicia excelsa*) on the level of a forest concession in Cameroon

1. Introduction - question

Need for control on different scales:

A) Logging of protected species (CITES) => control of species identity

B) Logging in countries with ban of exports or limitations in timber export volume => large scale control of geographic origin (countries)

C) Logging in protected areas or outside of certified forests => small scale control of geographic origin (landscape, forest concessions)

1. Introduction - methods

Requirements for control of timber origin:

Control of traits that cannot be manipulated

Method also applicable for processed timber

Spatial resolution of the control high enough

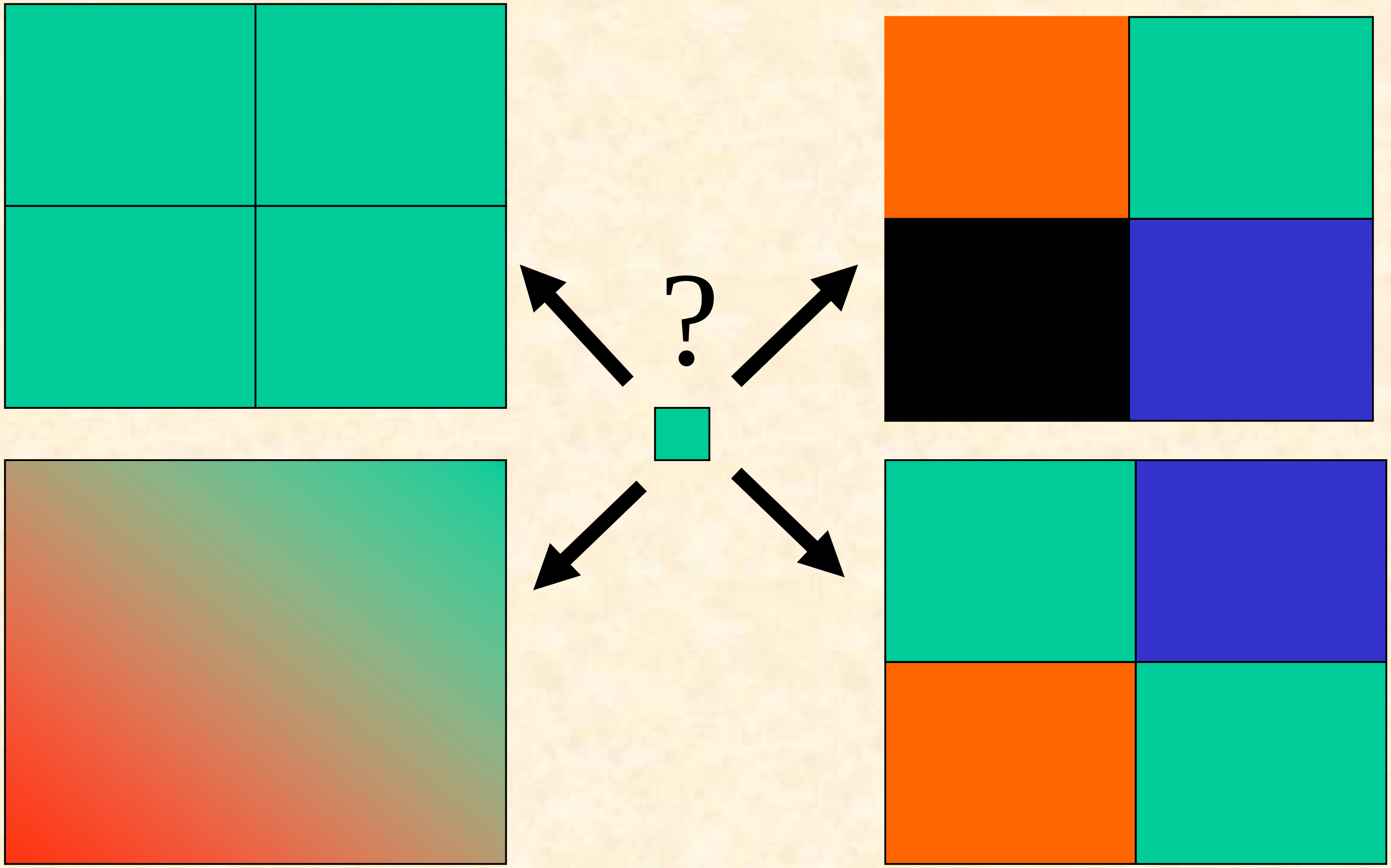
Rapid control

Low costs

Degen et al. 2010

1. Introduction - methods

Spatial pattern is the basis for any control!

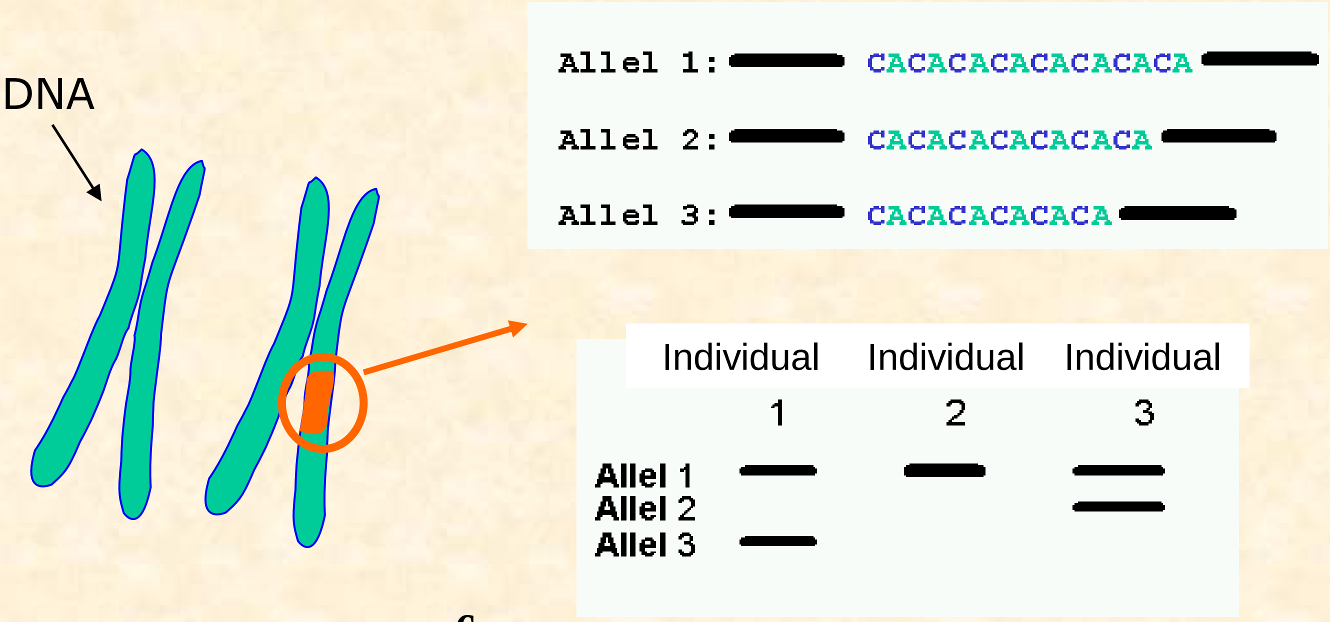


Degen et al. 2010

1. Introduction - methods

Genetic fingerprinting

A microsatellite consists of a specific sequence of DNA bases or nucleotides which contains mono, di, tri, or tetra tandem repeats



Allel 1: —— CACACACACACACACA ——

Allel 2: —— CACACACACACACA ——

Allel 3: —— CACACACACACA ——

	Individual 1	Individual 2	Individual 3
Allel 1	—	—	—
Allel 2			—
Allel 3	—		

Degen et al. 2010

1. Introduction - methods

Causes for spatial genetic pattern of tree populations

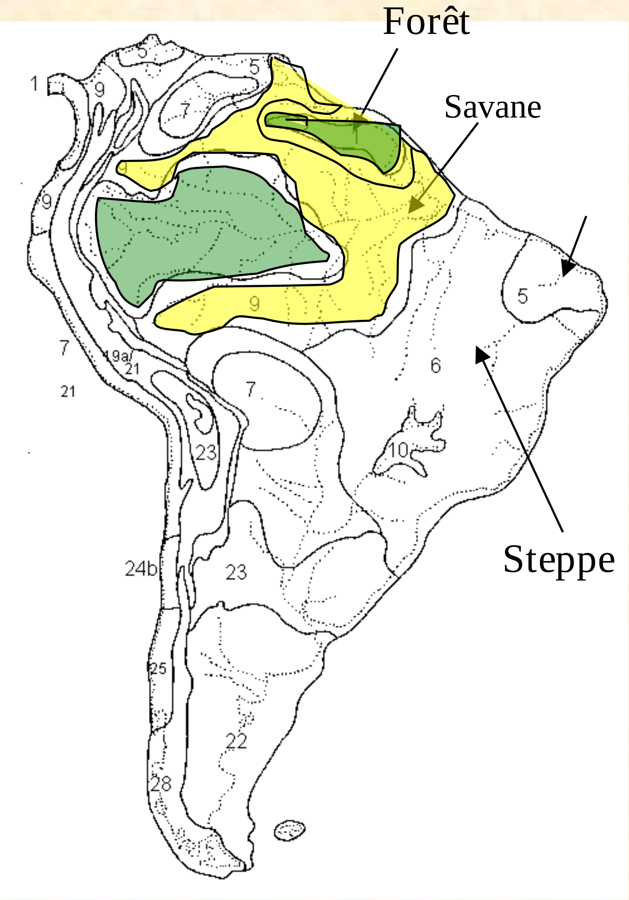
Extinction and recolonisation (glacial periods, natural desasters)

Spatial limited pollen and seed dispersal

Genetic selection along gradients

Artificial regeneration from different seed sources

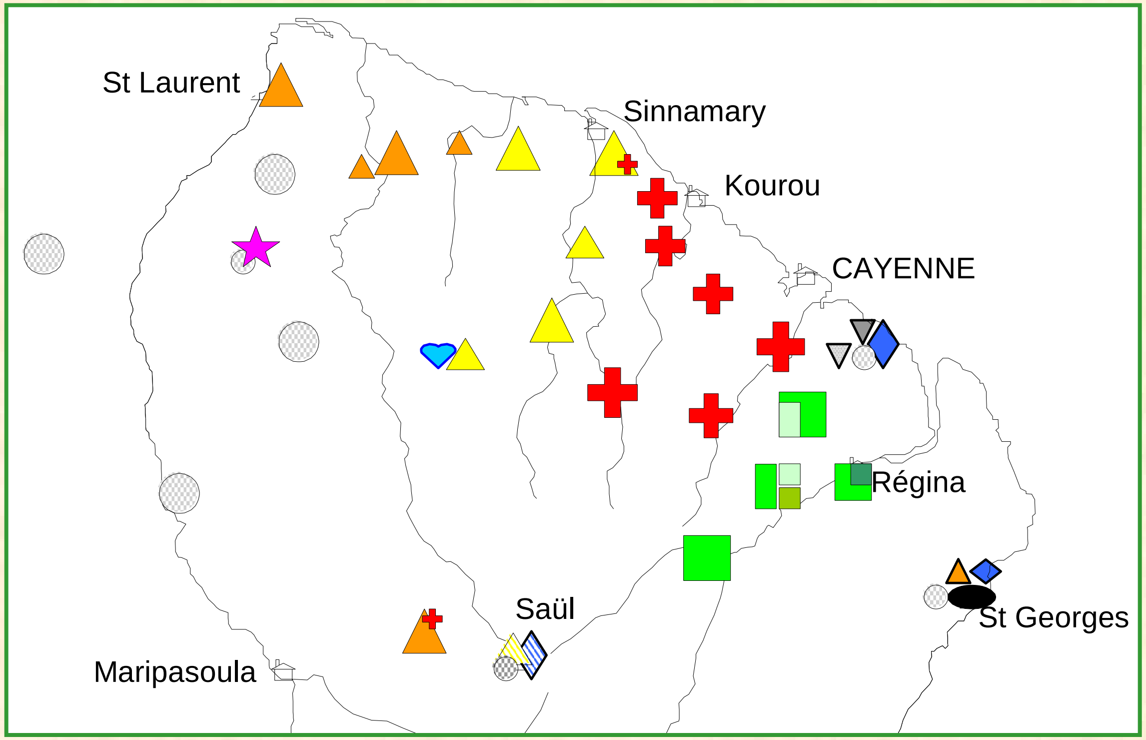
The Amazon during the last glacial period



Degen et al. 2010

1. Introduction - methods

Spatial genetic pattern at chloroplast gene marker of the tropical tree species *Vouacapoua america* in French Guiana



Dutech et al. (2003) Evolution 57: 2753-2764.

1. Introduction - methods

General approach

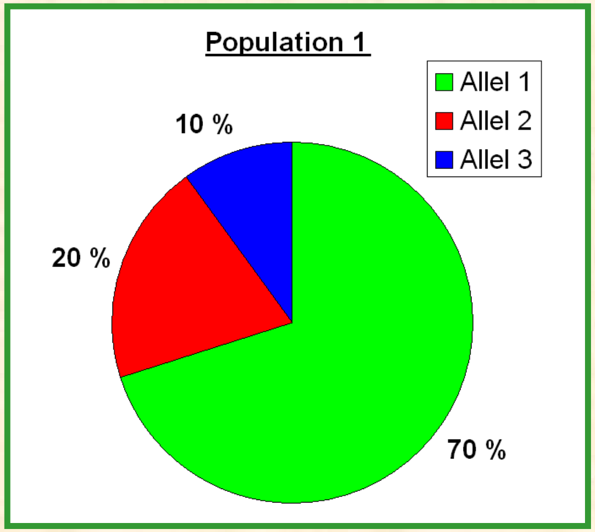
1. **Sampling of individuals in the declared location of origin and in other alternative places => good sampling design**

2. **Sampling of the traded wood => good sampling design**

3. **Genotyping (nSSRs, SNPs, Sequence Variation) => best gene markers**

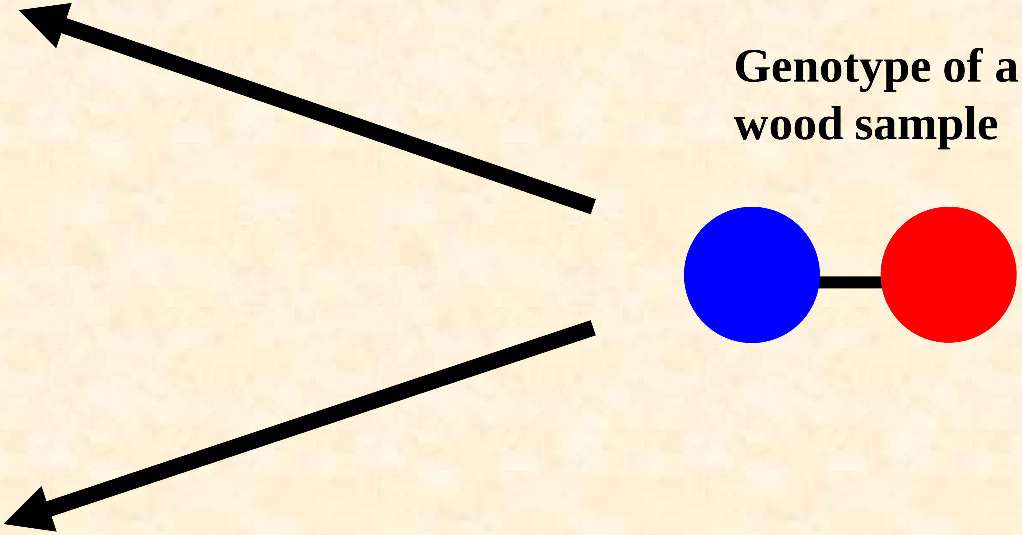
4. **Statistical analysis => most reliable method**

1. Introduction - methods

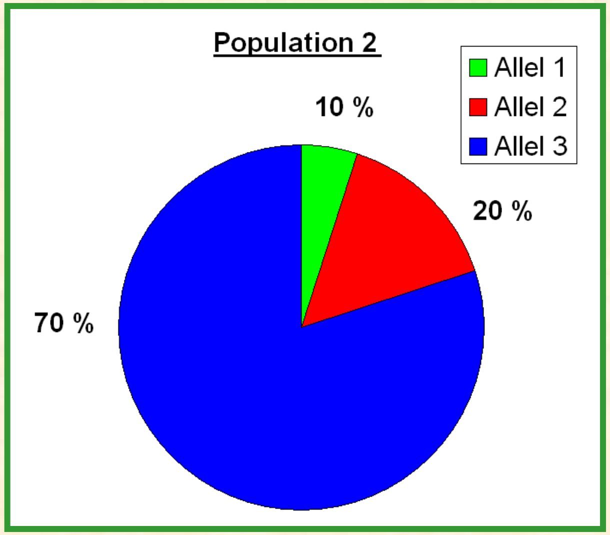


Prob = 0.04

Genotype of a
wood sample



Prob = 0.28

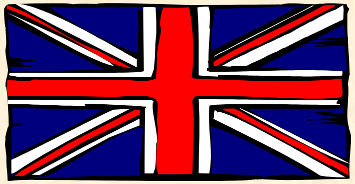


Degen et al. 2010

2. Projects and results - Mahogany

Partners

Bernd Degen, vTI, Germany (co-ordination)



Stephen Cavers, CEH, UK

Carlos Navarro, CATIE , Costa Rica

Sheila Ward, University Puerto Rico, USA

Alexandre Sebbenn, Sao Paulo Forest Institute, Brazil

Maristerra Lemmes, Inpa Manaus, Brazil

Rogerio Gribel, Botanical Garden Rio de Janeiro, Brazil

Budget

vTI

EU-INCO

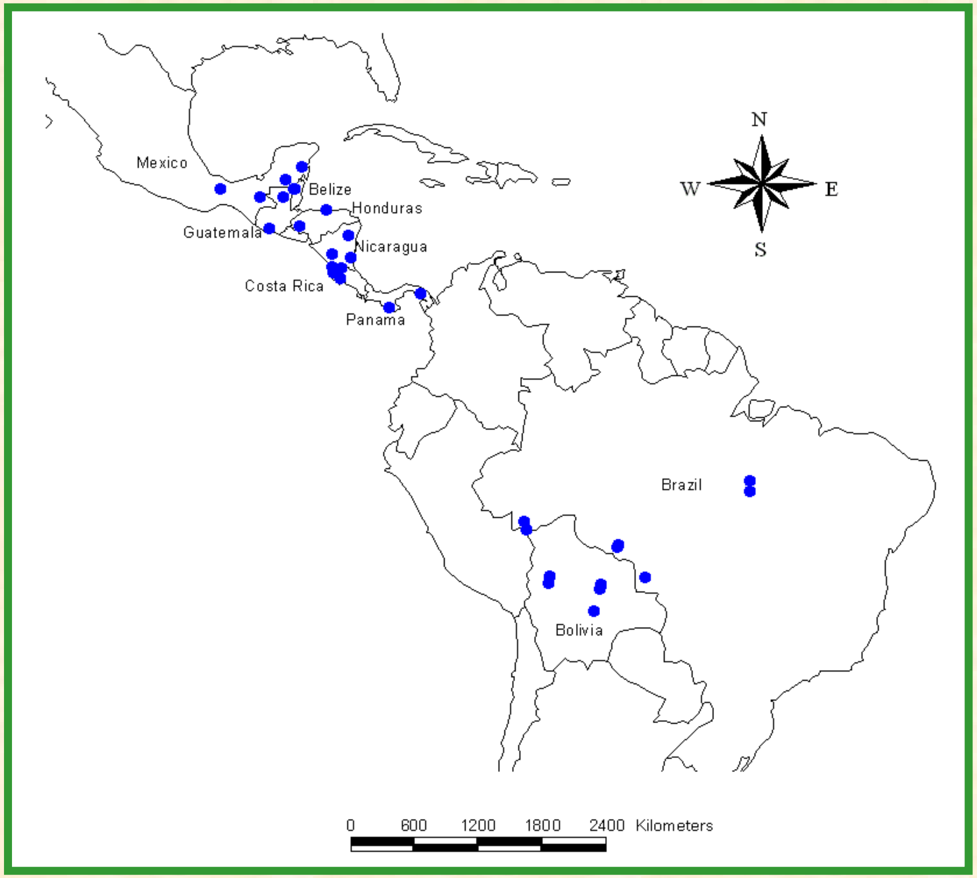
CNPq (Brazil)

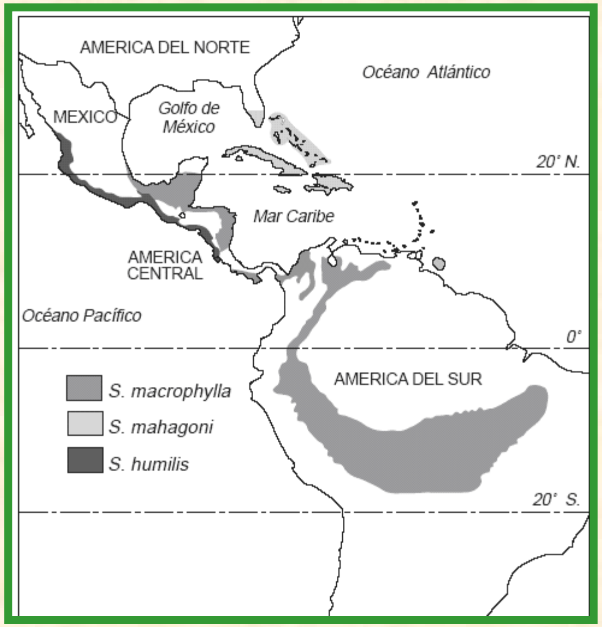
2. Projects and results - Mahogany

Sampling

33 populations with 24-400 individuals

2038 trees sampled





Genetic variation

7 nuclear microsatellites

More than 200 alleles identified

In each population between 44 – 106 alleles

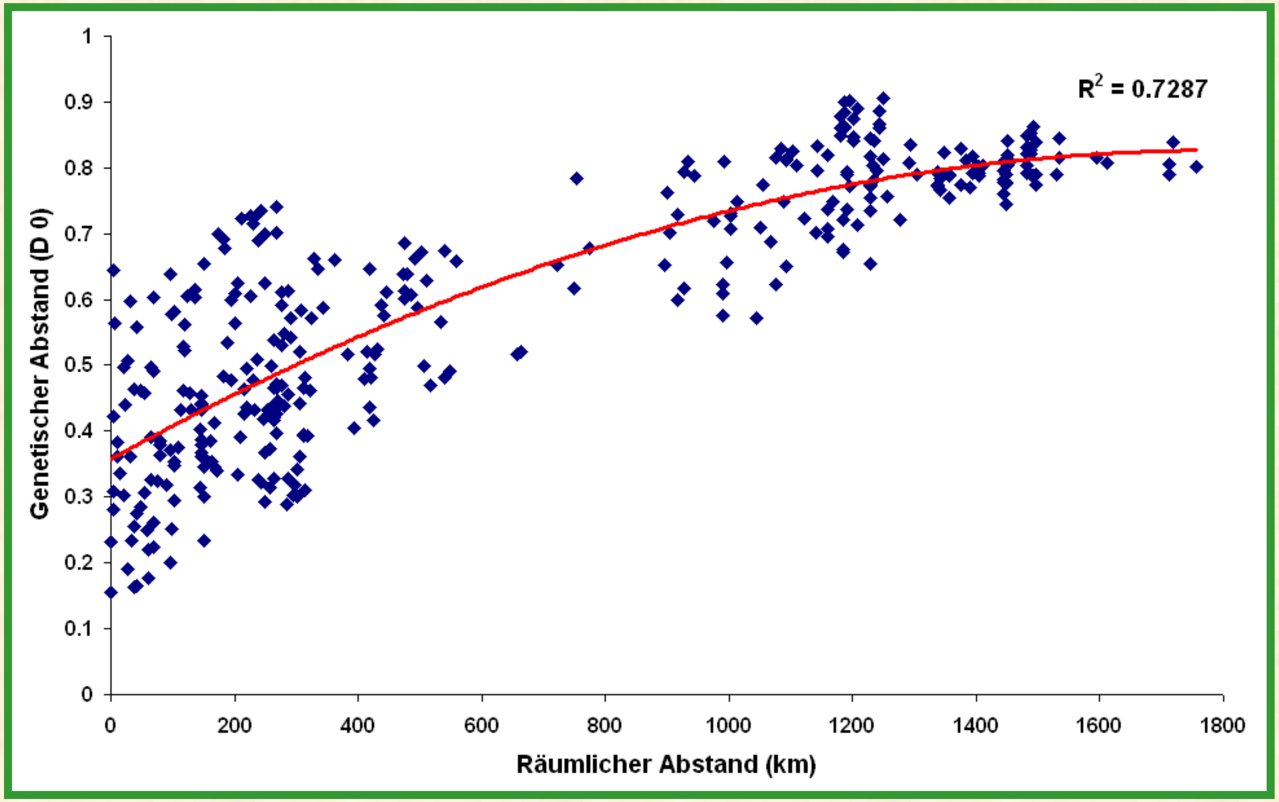
Degen et al. 2010

2. Projects and results - Mahogany

Genetic differentiation

Correlation between spatial and genetic distances

Strong genetic differences among populations (delta = 0,53; F_{ST} = 0,20; $F_{ST\_Hedrick}$= 0,66)

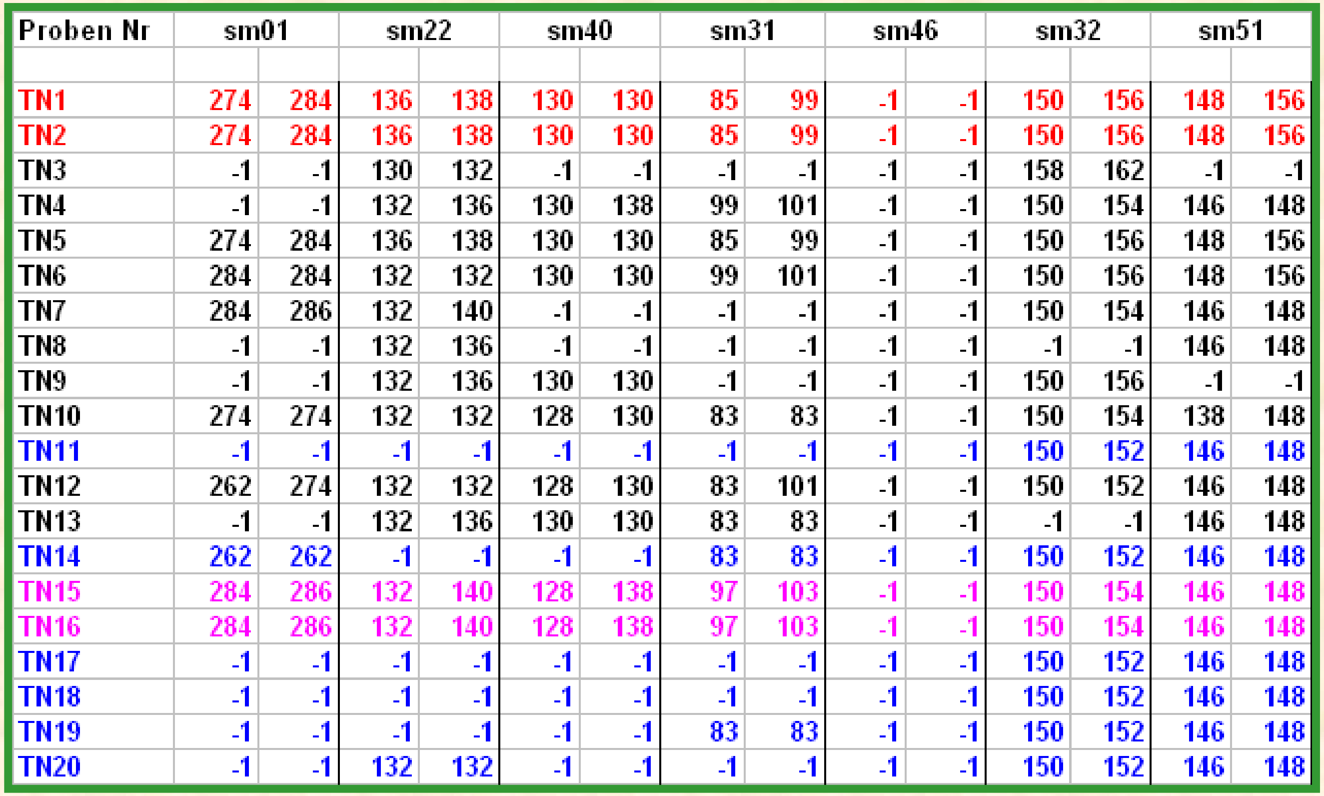


Degen et al. 2010

2. Projects and results - Mahogany

Practical test with 20 mahogany wood samples of the timber trader Nagel + 11 wood samples sent by Alexandre Sebbenn

TN
THEODOR NAGEL
TIMBERTRADE · SAWMILLS
SINCE 1837

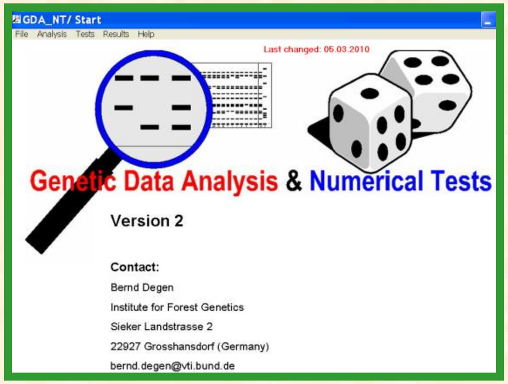


Proben Nr	sm01		sm22		sm40		sm31		sm46		sm32		sm51	
TN1	274	284	136	138	130	130	85	99	-1	-1	150	156	148	156
TN2	274	284	136	138	130	130	85	99	-1	-1	150	156	148	156
TN3	-1	-1	130	132	-1	-1	-1	-1	-1	-1	158	162	-1	-1
TN4	-1	-1	132	136	130	138	99	101	-1	-1	150	154	146	148
TN5	274	284	136	138	130	130	85	99	-1	-1	150	156	148	156
TN6	284	284	132	132	130	130	99	101	-1	-1	150	156	148	156
TN7	284	286	132	140	-1	-1	-1	-1	-1	-1	150	154	146	148
TN8	-1	-1	132	136	-1	-1	-1	-1	-1	-1	-1	-1	146	148
TN9	-1	-1	132	136	130	130	-1	-1	-1	-1	150	156	-1	-1
TN10	274	274	132	132	128	130	83	83	-1	-1	150	154	138	148
TN11	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	150	152	146	148
TN12	262	274	132	132	128	130	83	101	-1	-1	150	152	146	148
TN13	-1	-1	132	136	130	130	83	83	-1	-1	-1	-1	146	148
TN14	262	262	-1	-1	-1	-1	83	83	-1	-1	150	152	146	148
TN15	284	286	132	140	128	138	97	103	-1	-1	150	154	146	148
TN16	284	286	132	140	128	138	97	103	-1	-1	150	154	146	148
TN17	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	150	152	146	148
TN18	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	150	152	146	148
TN19	-1	-1	-1	-1	-1	-1	83	83	-1	-1	150	152	146	148
TN20	-1	-1	132	132	-1	-1	-1	-1	-1	-1	150	152	146	148

Genotypes of the 20 wood samples from the company Theodor Nagel

Degen et al. 2010

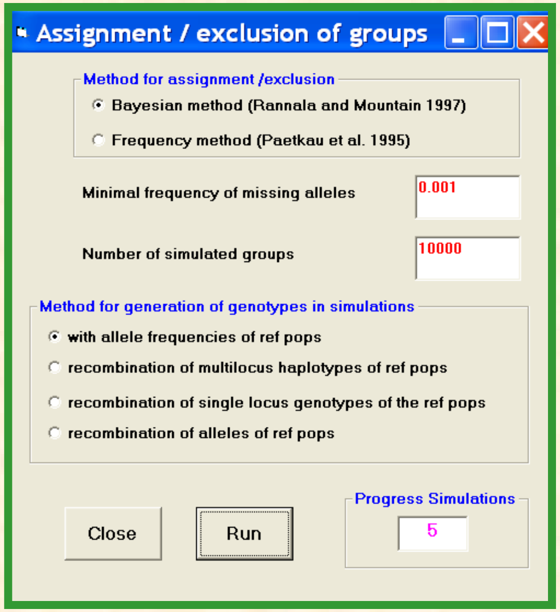
2. Projects and results - Mahogany



Genetic Data Analysis & Numerical Tests

Version 2

Contact:
Bernd Degen
Institute for Forest Genetics
Sieker Landstrasse 2
22927 Grosshansdorf (Germany)
bernd.degen@vti.bund.de



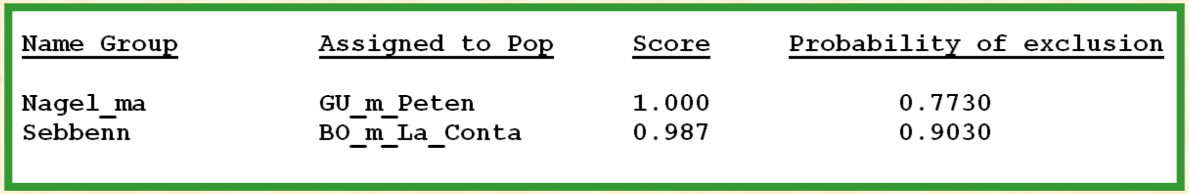
Method for assignment /exclusion
- ● Bayesian method (Rannala and Mountain 1997)
- ○ Frequency method (Paetkau et al. 1995)

Minimal frequency of missing alleles: 0.001

Number of simulated groups: 10000

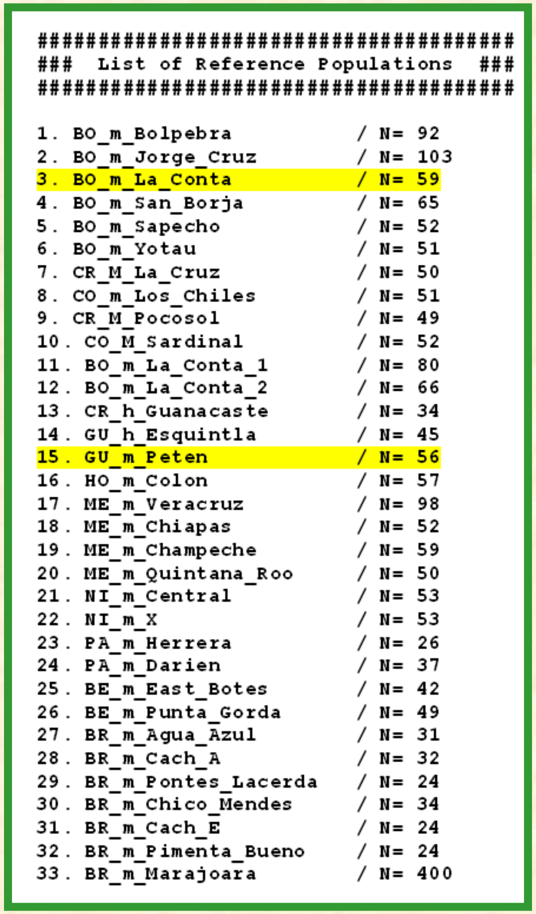
Method for generation of genotypes in simulations
- ● with allele frequencies of ref pops
- ○ recombination of multilocus haplotypes of ref pops
- ○ recombination of single locus genotypes of the ref pops
- ○ recombination of alleles of ref pops

Progress Simulations: 5



```
Name Group          Assigned to Pop     Score     Probability of exclusion

Nagel_ma            GU_m_Peten          1.000          0.7730
Sebbenn             BO_m_La_Conta       0.987          0.9030
```



```
########################################
###  List of Reference Populations  ###
########################################

1. BO_m_Bolpebra             / N= 92
2. BO_m_Jorge_Cruz           / N= 103
3. BO_m_La_Conta             / N= 59
4. BO_m_San_Borja            / N= 65
5. BO_m_Sapecho              / N= 52
6. BO_m_Yotau                / N= 51
7. CR_M_La_Cruz              / N= 50
8. CO_m_Los_Chiles           / N= 51
9. CR_M_Pocosol              / N= 49
10. CO_M_Sardinal            / N= 52
11. BO_m_La_Conta_1          / N= 80
12. BO_m_La_Conta_2          / N= 66
13. CR_h_Guanacaste          / N= 34
14. GU_h_Esquintla           / N= 45
15. GU_m_Peten               / N= 56
16. HO_m_Colon               / N= 57
17. ME_m_Veracruz            / N= 98
18. ME_m_Chiapas             / N= 52
19. ME_m_Champeche           / N= 59
20. ME_m_Quintana_Roo        / N= 50
21. NI_m_Central             / N= 53
22. NI_m_X                   / N= 53
23. PA_m_Herrera             / N= 26
24. PA_m_Darien              / N= 37
25. BE_m_East_Botes          / N= 42
26. BE_m_Punta_Gorda         / N= 49
27. BR_m_Agua_Azul           / N= 31
28. BR_m_Cach_A              / N= 32
29. BR_m_Pontes_Lacerda      / N= 24
30. BR_m_Chico_Mendes        / N= 34
31. BR_m_Cach_E              / N= 24
32. BR_m_Pimenta_Bueno       / N= 24
33. BR_m_Marajoara           / N= 400
```

Degen et al. 2010

2. Projects and results - Mahogany

Degen et al. 2010

3. Outlook

- Create a network of involved institutions, ongoing projects => go for large international projects (ITTO)
- Further development and standardisation of methods (sample strategies, DNA-extraction from wood, gene markers (SNPs), additional isotopes)
- Merging efforts for systematic sampling and inventories with genetic and isotopic fingerprints => creation of reference data
- Setup and maintenance of free accessible data banks with reference data
- Technology transfer to private sector
- Setup of co-ordination office at Bioversity International