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Genetic variability of polish Scots pine (*Pinus sylvestris* L.) provenances assessed with microsatellite markers

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HAL Authorization



Workshop on
„New approaches on forest tree genetics“
Analysis of microsatellites in Scots pine
Sękocin, 24-27 August 2004

Genetic variability of Polish Scots pine provenances assessed with microsatellite markers

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Introduction

- Scots pine (*Pinus sylvestris* L.) is one of the most important coniferous species in Poland, which covers 68 % of the total forest area and serves as a main wood resource for industry
- Only few investigations have been done on genetic marker identification among native Scots pine provenances in Poland



Introduction



- Advantages of microsatellite sequences :
 - short repeats of 1-6 bp,
 - uniformly distributed over entire genome,
 - present in high proportion in conifers,
 - highly polymorphic DNA markers,
 - discrete loci and co-dominant alleles,
 - observed mutation rates from 10^{-3} to 10^{-6} .



Aims



- Analysis of **polymorphic** nuclear microsatellite fragments within Scots pine
- Genetic structure of **30 natural populations** in Poland based on chosen microsatellite markers
- Identification of the phylogenetic relationships among analyzed provenances
- Geographical structure of gene diversity



Material and Methods

Plant material:

Total of **700 samples** (needles) were harvested from randomly spaced adult Scots pine (90-173 year-old) trees:

- from thirty different seed micro-zones in Poland
- according to the register of FRI and the General

Directorate of the State Forests in Poland

Localization of the Scots pine provenances:

- 204. Augustów
- 208. Białowieża
- 504. Bolesławiec
- 306. Bolewice
- 105. Bytów
- 101. Goleniów
- 307. Gubin
- 606. Janów Lubelski
- 606. Józefów
- 654. Kluczbork
- 602. Kozienice
- 352. Krucz
- 303. Lipka
- 403. Łochów
- 404. Międzyrzec
- 107. Międzyzdroje
- 106. Miłomłyn
- 302. Niedźwiady
- 607. Niepołomice
- 205. Nowe Ramuki
- 401. Nowogród
- 104. Smolarz
- 601. Spała
- 206. Strzałowo
- 405. Strzelce
- 207. Supraśl
- 501. Syców
- 108. Wejherowo
- 305. Woziwoda
- 402. Wyszaków



 - Provenance
 107 - Maternal Seed Zone



Material and Methods

- **Microsatellite sequences of forest tree species:**
 - <http://www.ebi.ac.uk>
 - <http://www.ncbi.nlm.nih.gov>
 - <http://www.blackwell-synergy.com>

- **For Scots pine:**
 - **SPAC 3.7, SPAC 7.14, SPAC 11.4, SPAC 11.5, SPAC 11.6, SPAC 11.8 and SPAC 12.5.**

Soranzo N., Provan J., Powell W. 1998. Characterization of microsatellite loci in *Pinus sylvestris* L. *Mol. Ecol.* 7: 1260-1261

Material and Methods

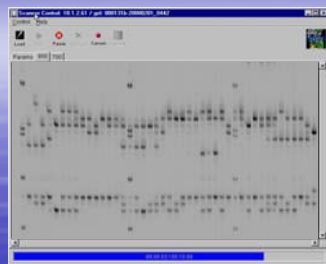
- Microsatellite loci and primers designed for their amplification

No	Locus	Repeat Length (bp)	Primer Sequence 5' - 3'	T _m °C	Labelling
1	SPAC 7.14 <i>P. sylvestris</i>	(TG) ₁₇ (AG) ₂₁ 209 bp	F: TTCGTAGGACTAAAAATGTGTG R: CAAAGTGGATTTTGACCG	55	B, 6 Fam
2	SPAC 11.6 <i>P. sylvestris</i>	(CA) ₂₉ (TA) ₇ 165 bp	F: TTACAGCGGTTGGTAAATG R: CTTACAGGACTGATGTTCA	55↘50	B, 6 Fam
3	SPAC 12.5 <i>P. sylvestris</i>	(GT) ₂₀ (GA) ₁₀ 155 bp	F: CTTCTTCACTAGTTTCCTTTGG R: TTGGTTATAGGCATAGATTGC	54	B, Ned
4	Rptest11 <i>P. pinaster</i>	(ATC) ₇ 213 bp	F: AGGATGCCTATGATATGCCG R: AACCATAACAAAAGCGGTCG	56	B, 6 Fam
5	SsrPtctg4363 <i>P. pinaster</i>	(AT) ₁₀ 100 bp	F: TAATAATTCAAGCCACCCCG R: AGCAGGCTAATAACAACACGC	60↘50	Hex
6	SsrPtctg7824 <i>P. pinaster</i>	(AT) ₁₂ 501 bp	F: TGACCTGTCTTGTGAGACGC R: TTTTGAAACAGATTGCAGCC	60↘50	Hex

General scheme of microsatellite loci analysis



PCR Amplification



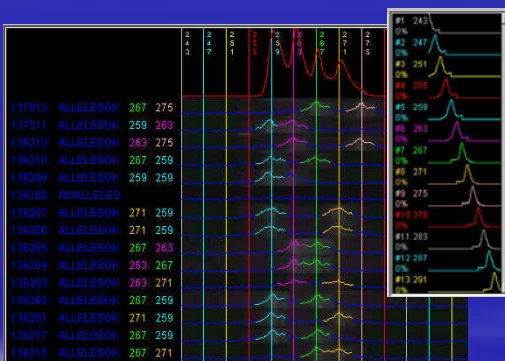
Electrophoresis



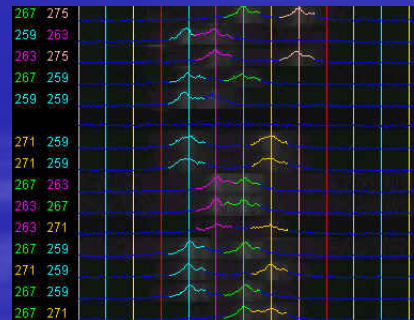
Load on HITACHI
Abi-Prism® 3100
Genetic Analyzer



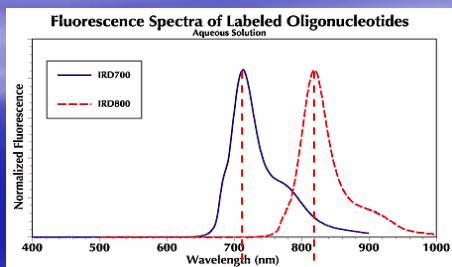
Abi-Prism®
GeneScan® 3.7
software



Genotyping



Loci Location



Visual Inspection

MarkerID	Allele number	Allele size	Allele Frequency
RGF1	1	195	0.756557
RGF1	2	197	3.333334E-02
RGF1	3	199	0.2
RGF1	4	201	0.266667
RGF1	5	203	0.233333
SR95	1	204	0.433333
SR95	2	206	6.666667E-02
SR95	3	210	6.666667E-02
SR95	4	230	0.433333

Text Output

Statistical Analysis Results:

- Length of post variables = 8 bytes
- LINKAGE (P) 11 1170 3-PPOINT AUTOCORR. DATA
- CHANCE OF LINKAGE = 1, 2, 3
- THETA0 = 0.500 0.100
- PERIOD0 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD1 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD2 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD3 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD4 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD5 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD6 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD7 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD8 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD9 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD10 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD11 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD12 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD13 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD14 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD15 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD16 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD17 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD18 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD19 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD20 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD21 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD22 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD23 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD24 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD25 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD26 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD27 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD28 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD29 = 1.0M 1.0M 1.0M 10 LINE
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- PERIOD31 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD32 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD33 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD34 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD35 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD36 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD37 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD38 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD39 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD40 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD41 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD42 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD43 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD44 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD45 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD46 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD47 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD48 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD49 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD50 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD51 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD52 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD53 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD54 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD55 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD56 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD57 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD58 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD59 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD60 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD61 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD62 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD63 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD64 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD65 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD66 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD67 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD68 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD69 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD70 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD71 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD72 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD73 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD74 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD75 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD76 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD77 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD78 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD79 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD80 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD81 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD82 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD83 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD84 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD85 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD86 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD87 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD88 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD89 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD90 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD91 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD92 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD93 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD94 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD95 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD96 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD97 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD98 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD99 = 1.0M 1.0M 1.0M 10 LINE
- PERIOD100 = 1.0M 1.0M 1.0M 10 LINE

S-Plus

Statistical Analysis

Material and Methods

- Microsatellite alleles taken from the sequencer

Well Separated
Heterozygote



Homozygote



2 bp separated
Heterozygote

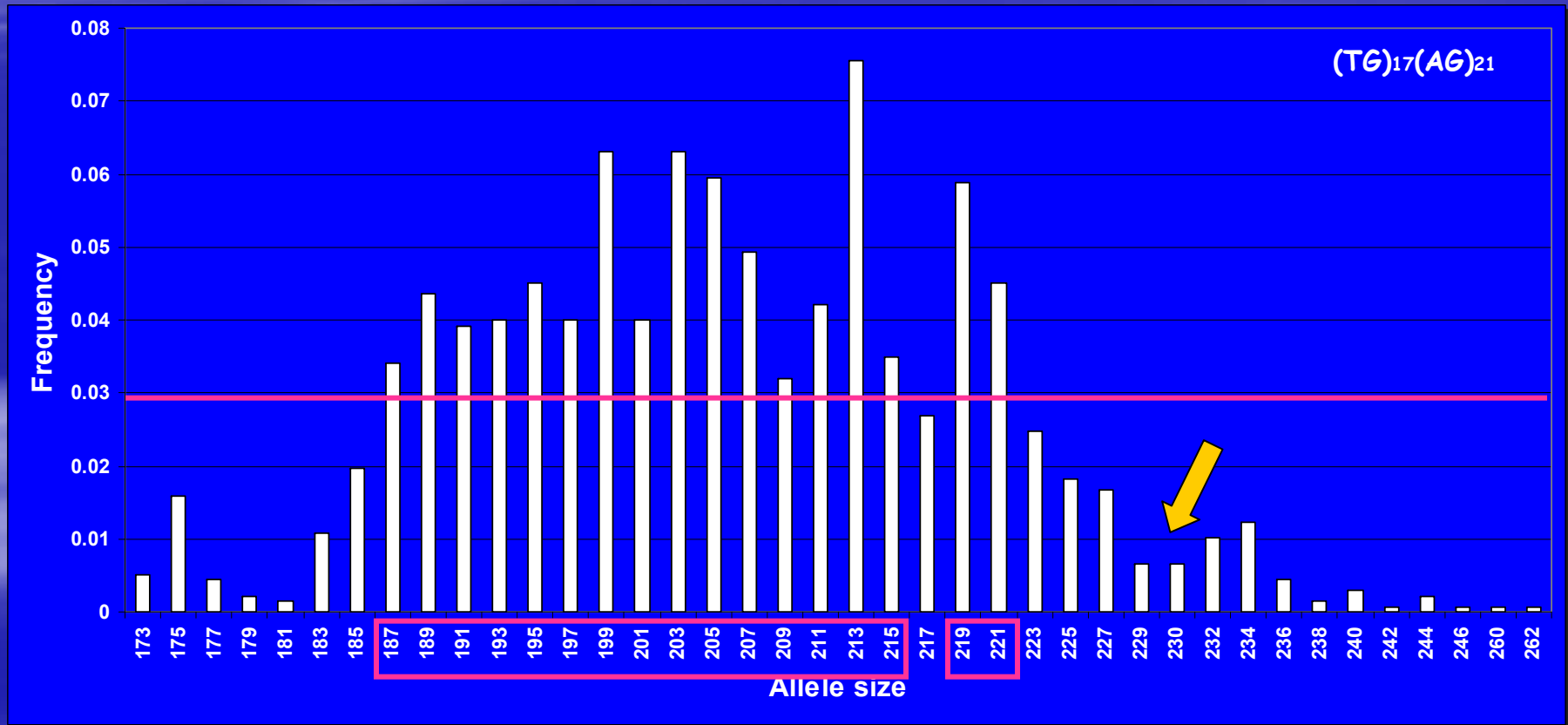


Every peak was examined for appropriate threshold scoring

Results

Allele frequency distribution:

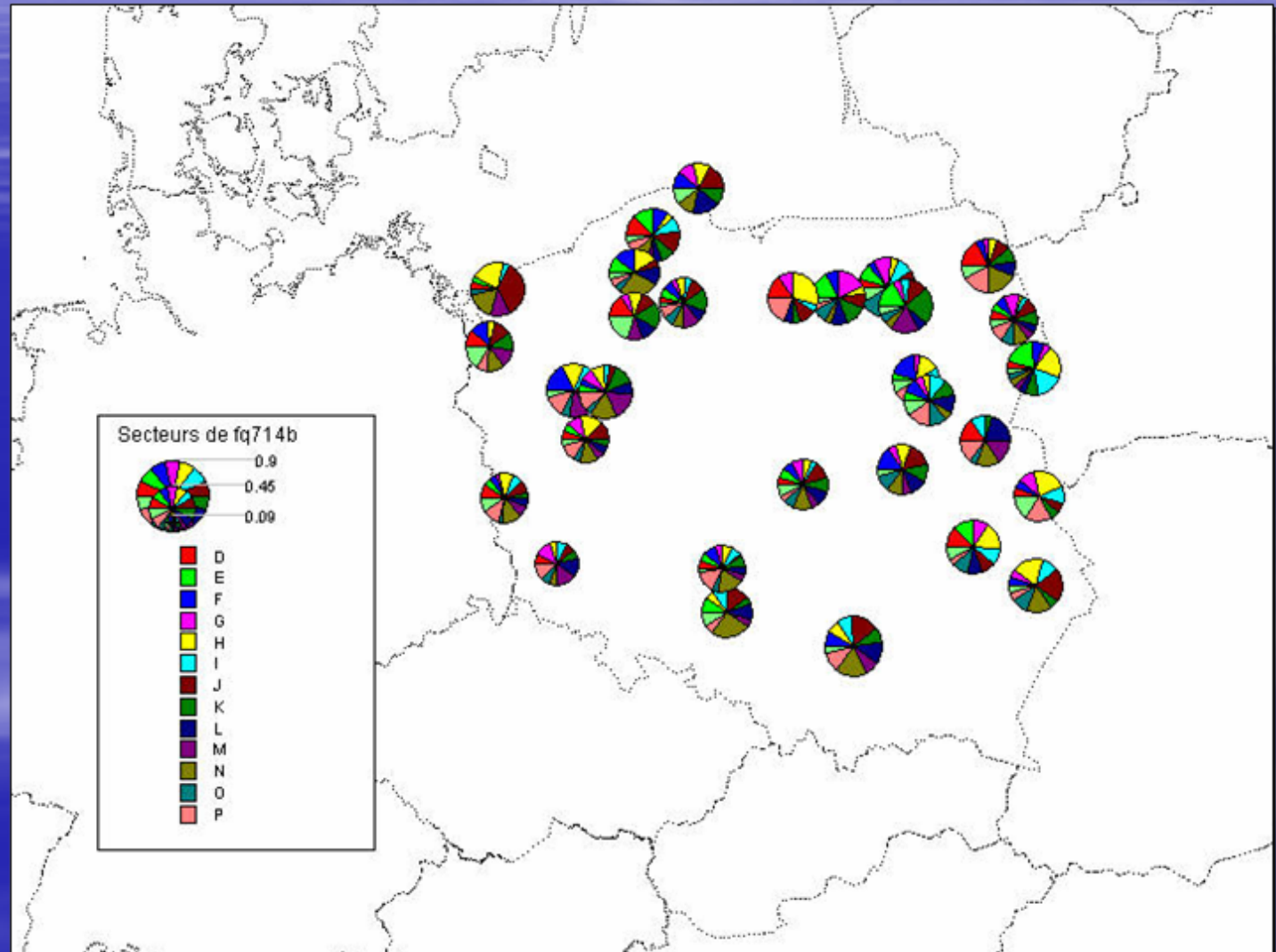
SPAC 7.14 microsatellite loci (N=1378, 40 alleles)



Allele frequency > 0.03

Results

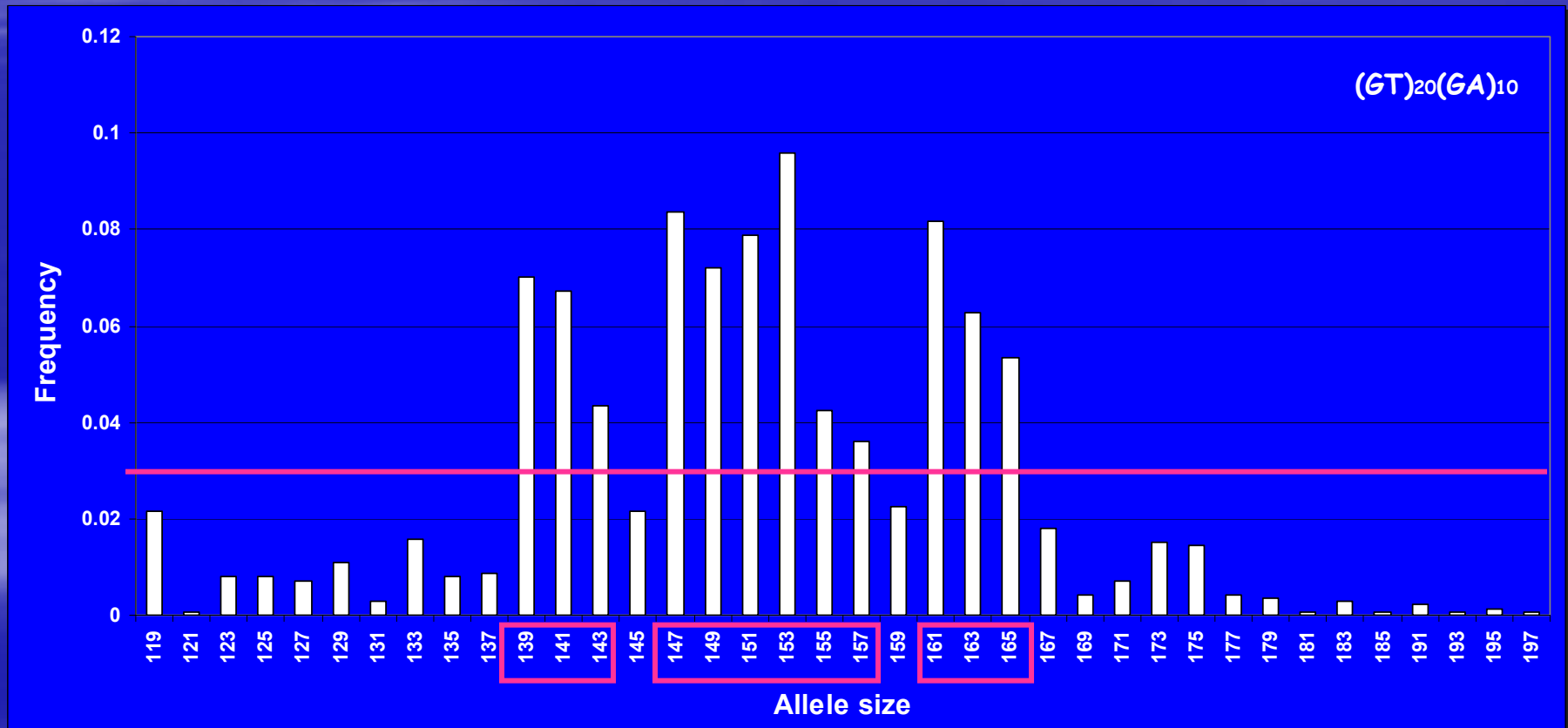
Geographical
distribution
of the most
frequent
alleles of
7.14 locus



Results

Allele frequency distribution:

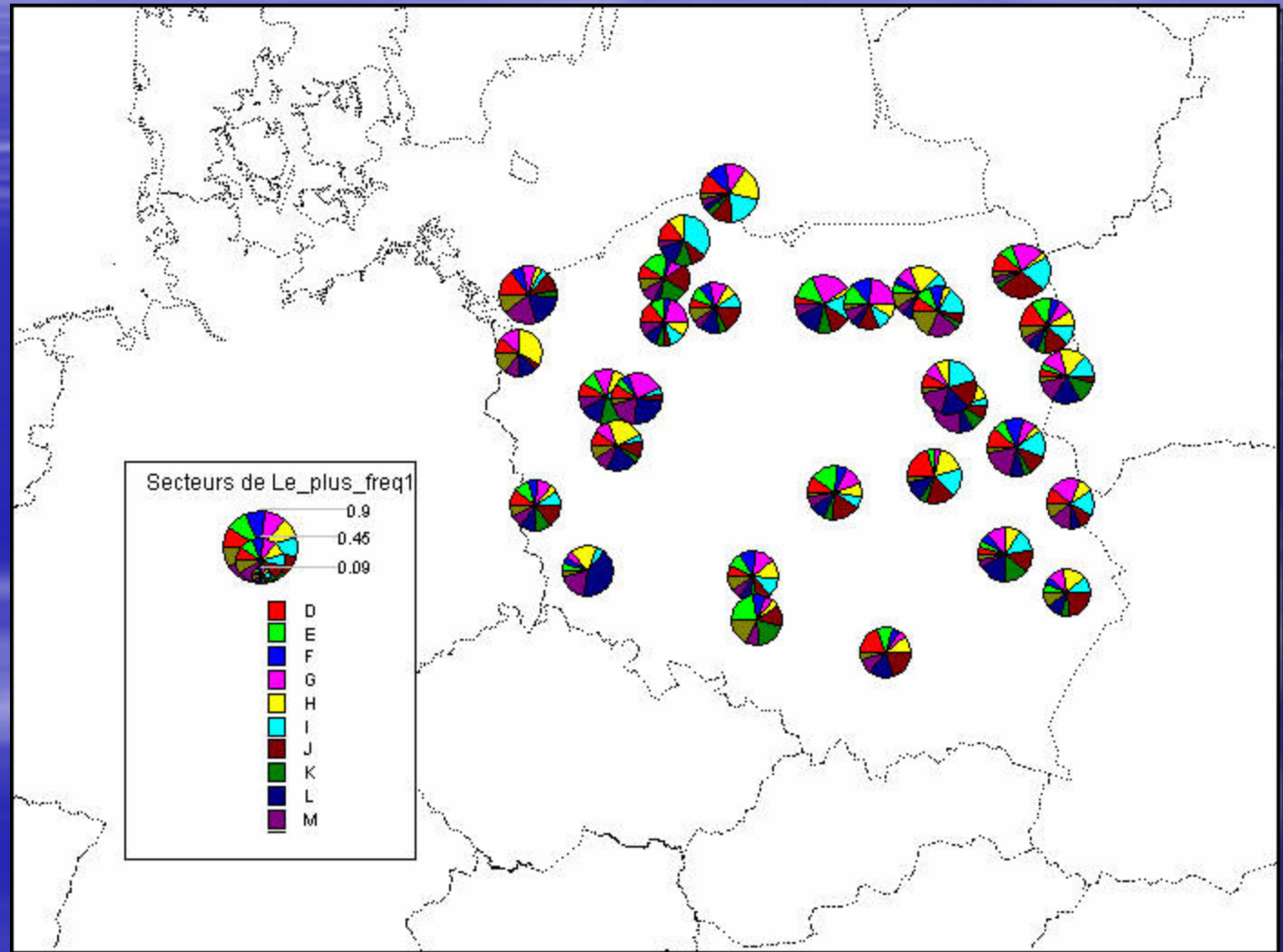
SPAC 12.5 microsatellite loci (N=1385, 38 alleles)



Allele frequency > 0.04

Results

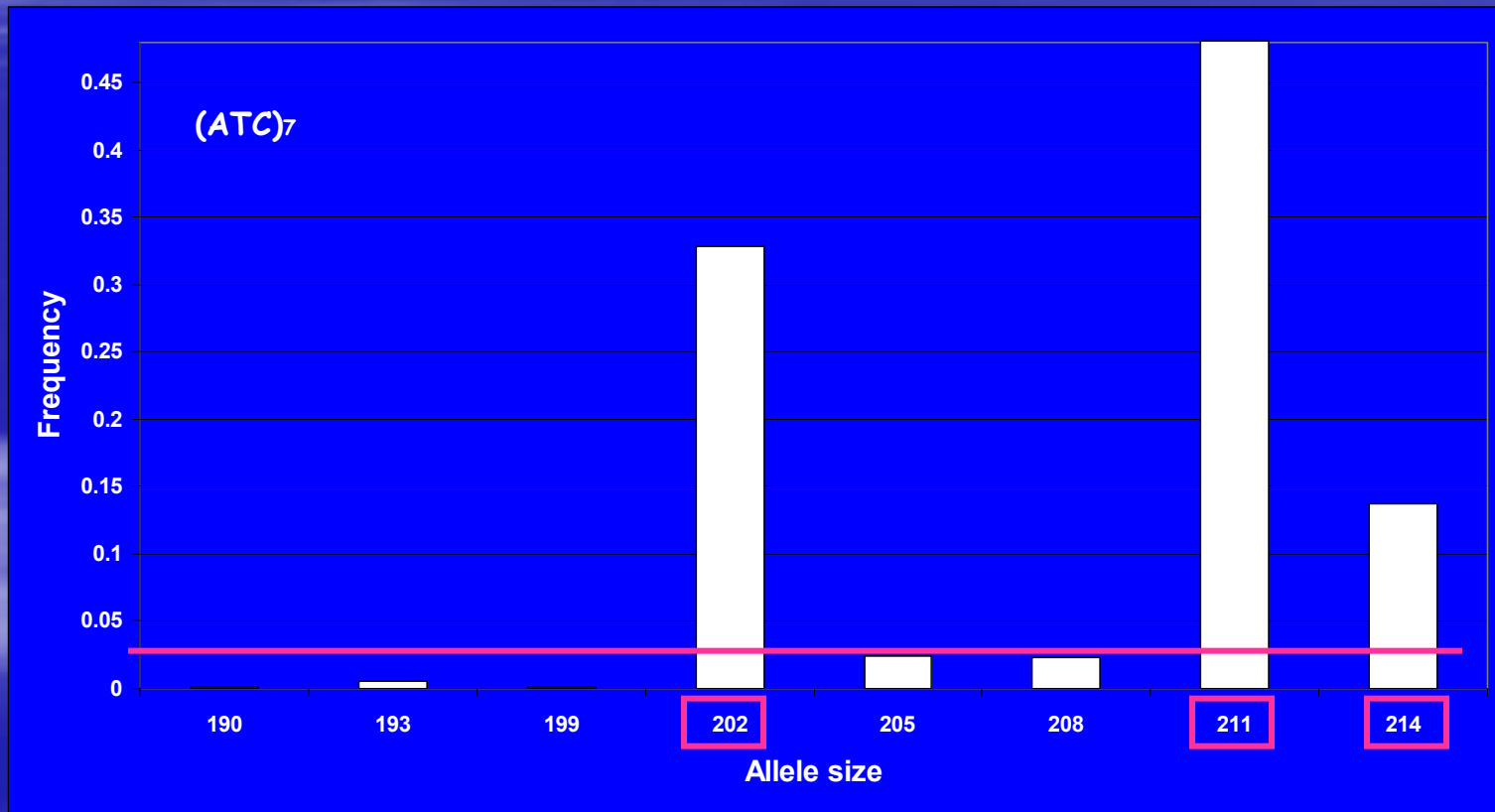
Geographical
distribution
of the most
frequent
alleles of
12.5 locus



Results

Allele frequency distribution:

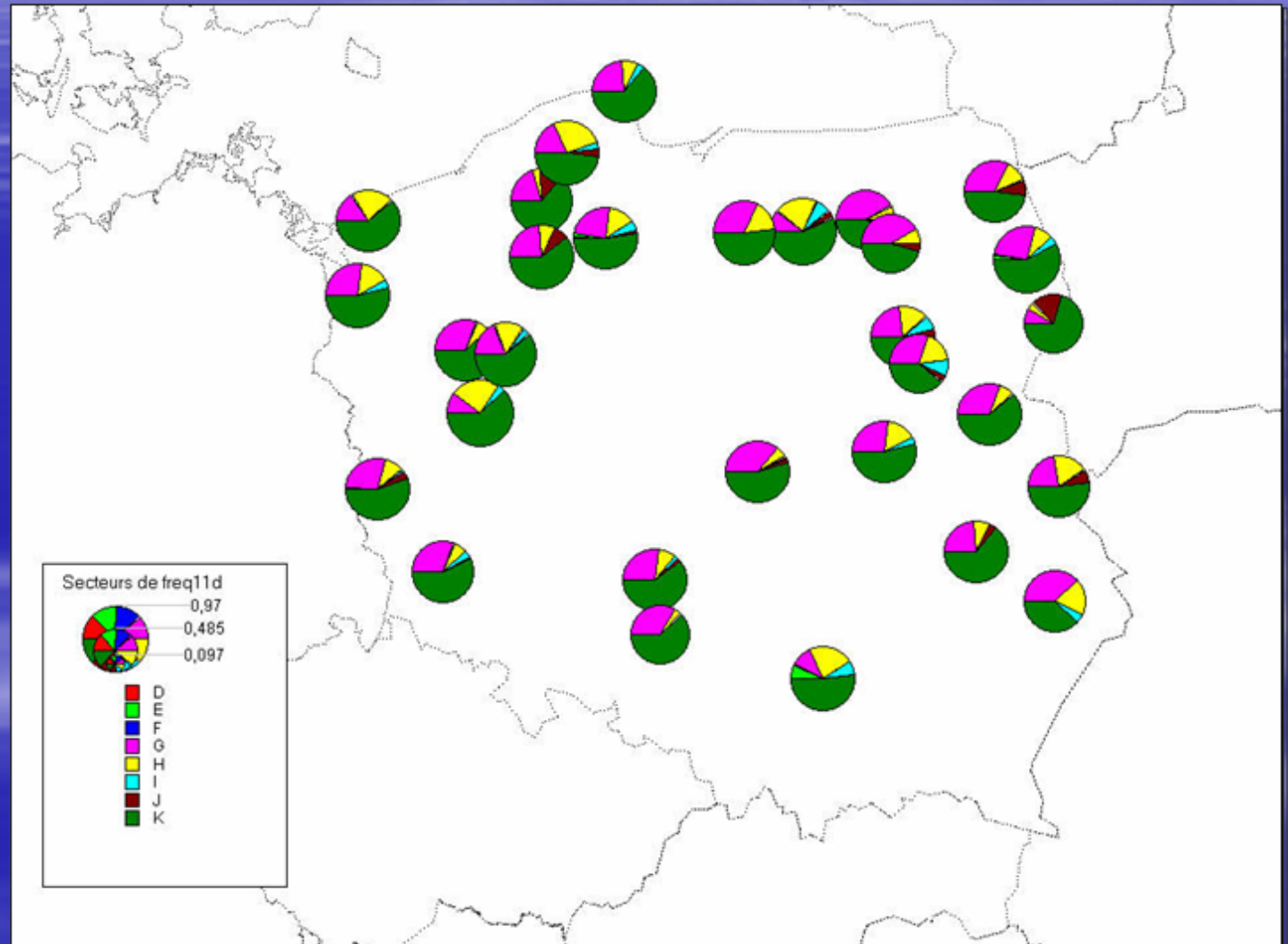
Rptest11 microsatellite loci (N=1398, 8 alleles)



 Allele frequency > 0.03

Results

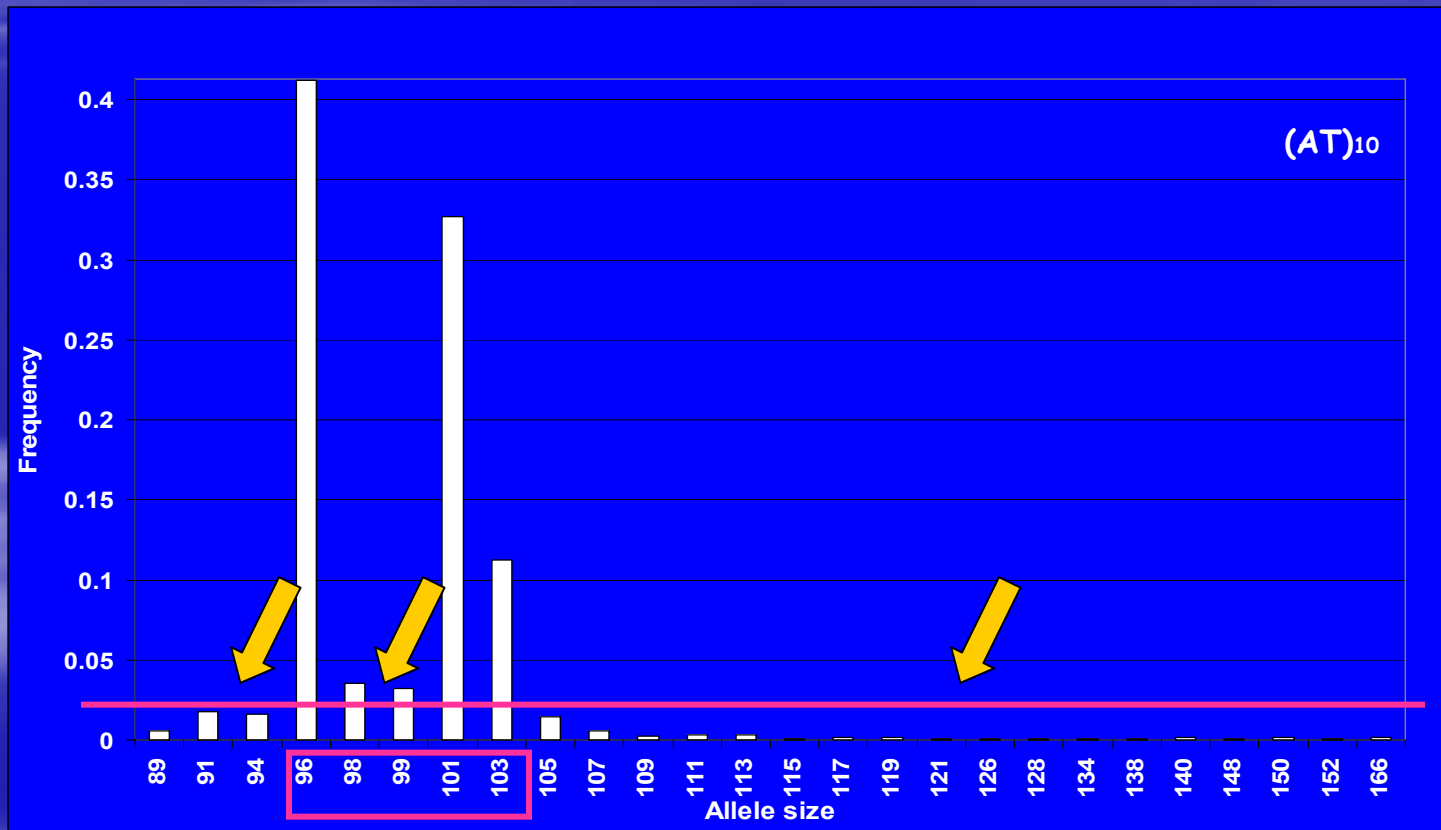
Geographical
distribution
of the most
frequent
alleles of
Rptest11
locus



Results

Allele frequency distribution:

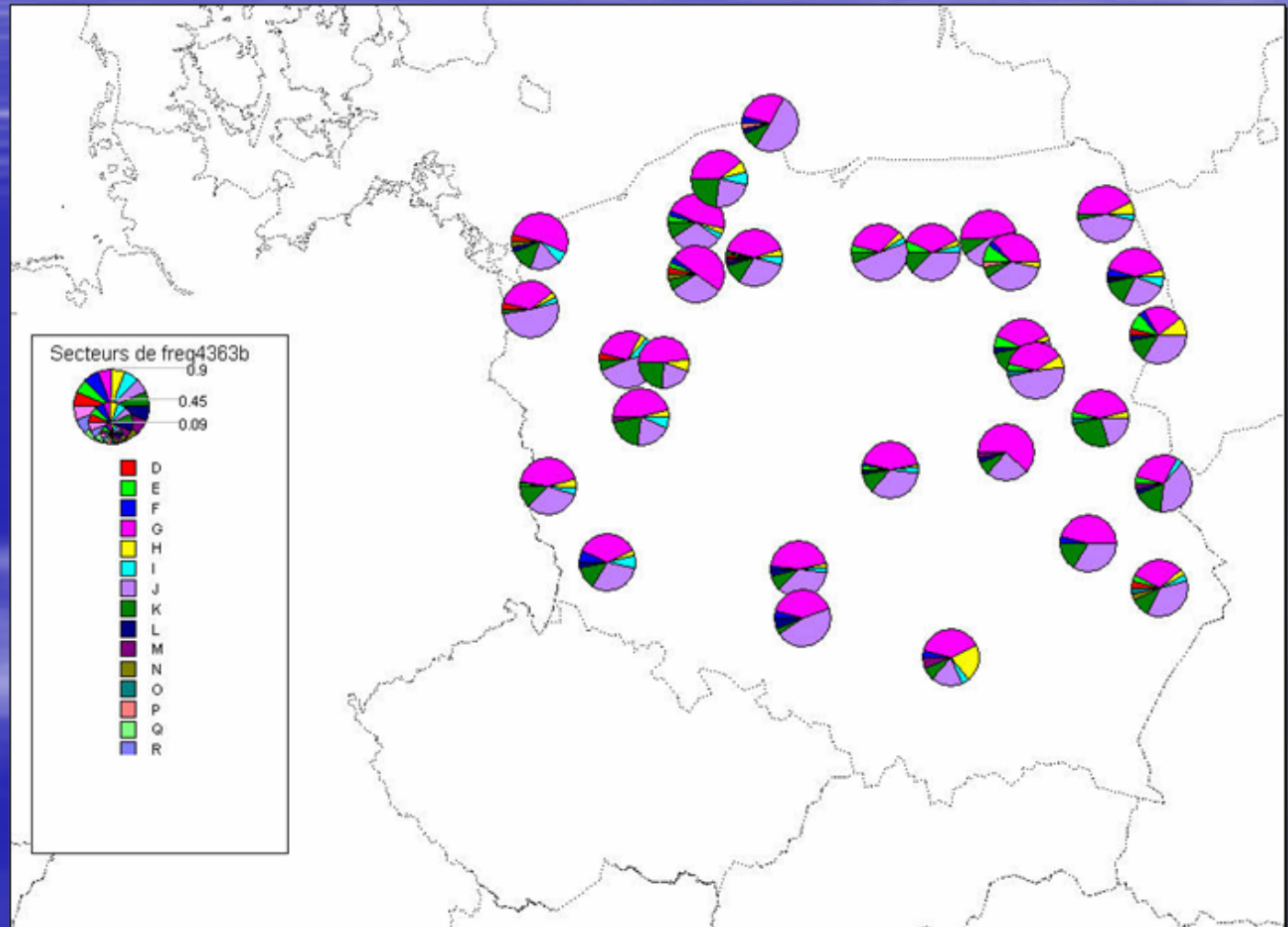
SsrPt-ctg4363 microsatellite loci (N=1385, 26 alleles)



 Allele frequency > 0.03

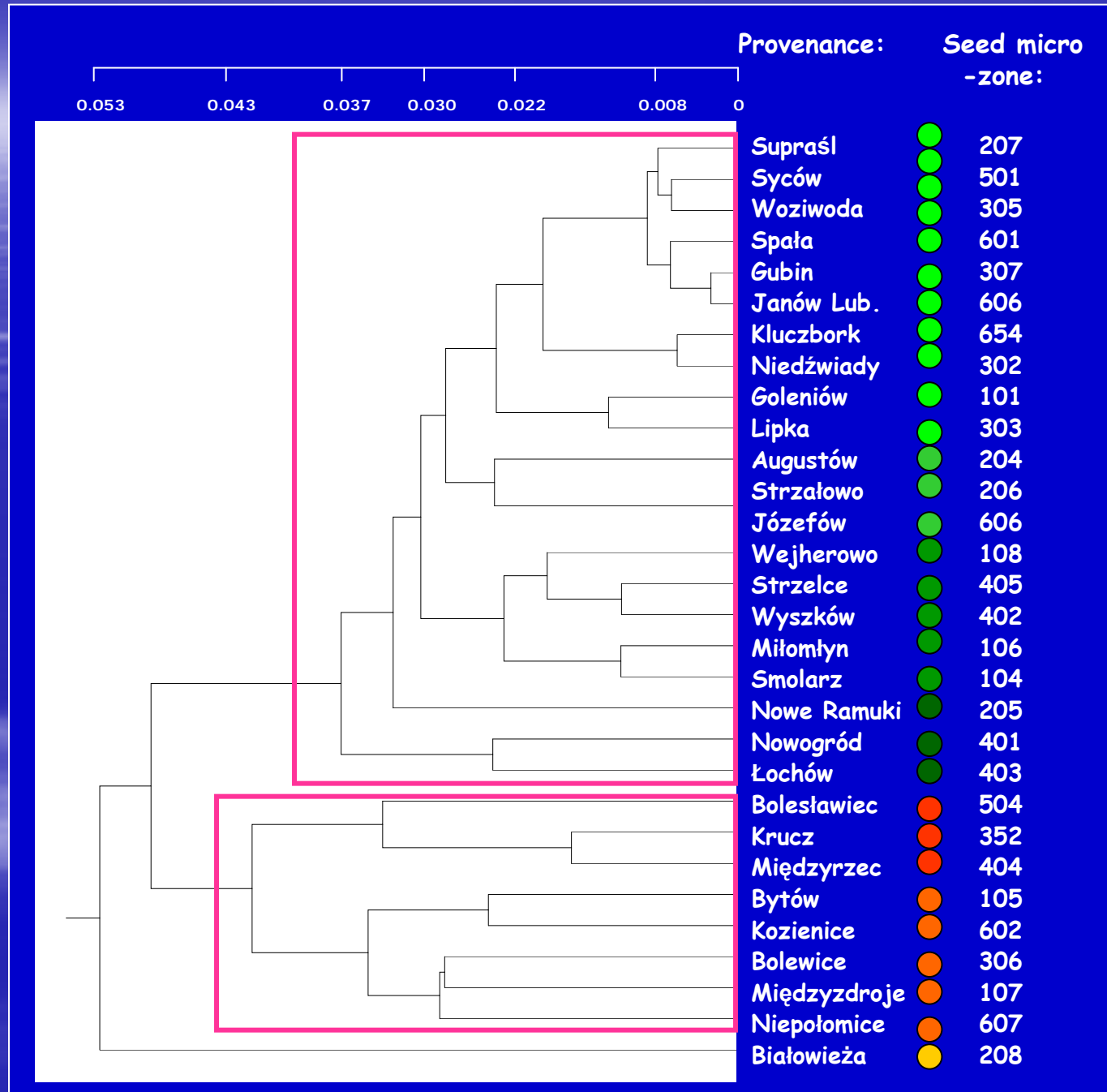
Results

Geographical distribution of the most frequent alleles of SsrPt-ctg4363 locus



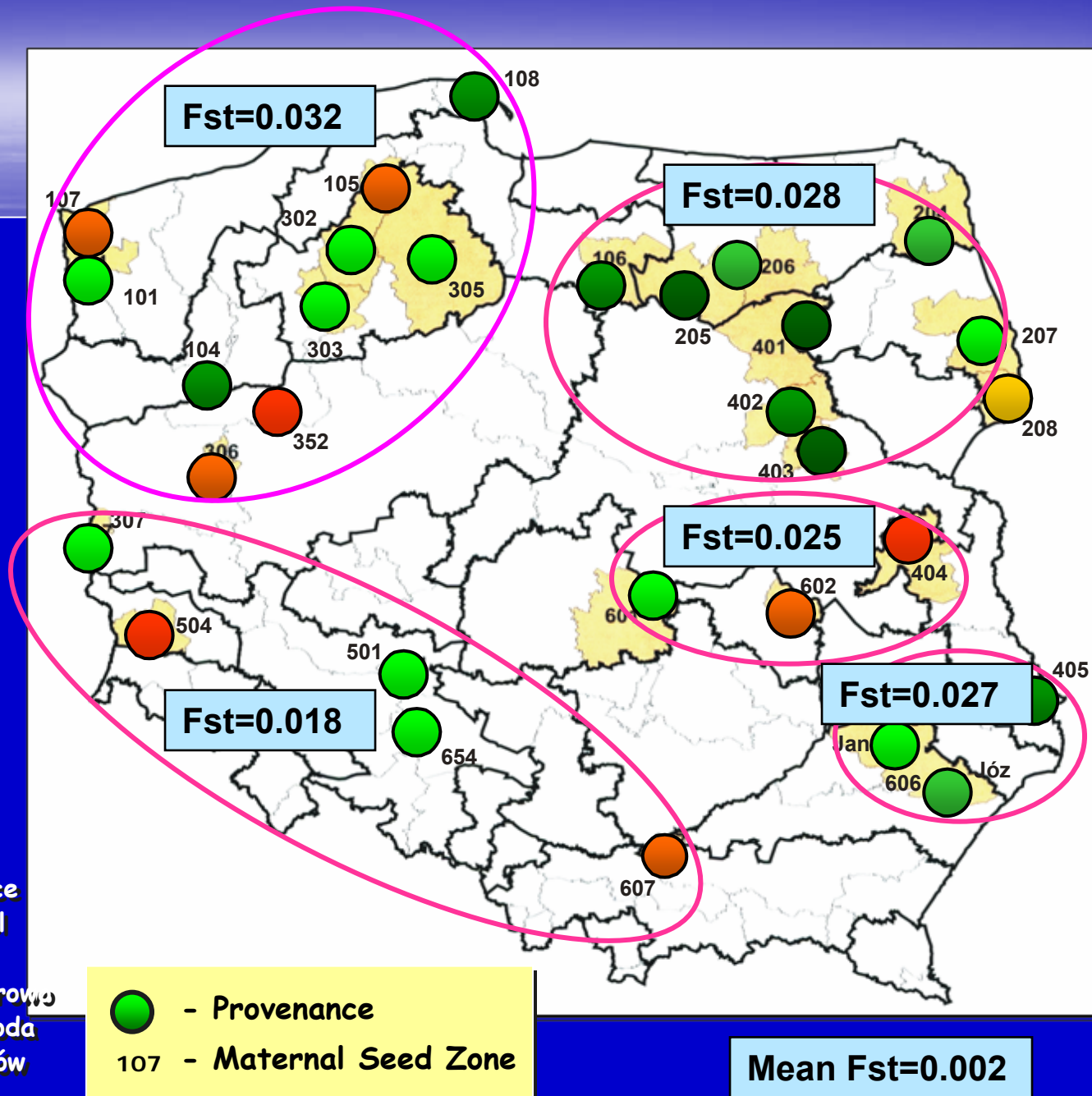


Neighbor-joining tree of Scots pine provenances in Poland based on microsatellite data



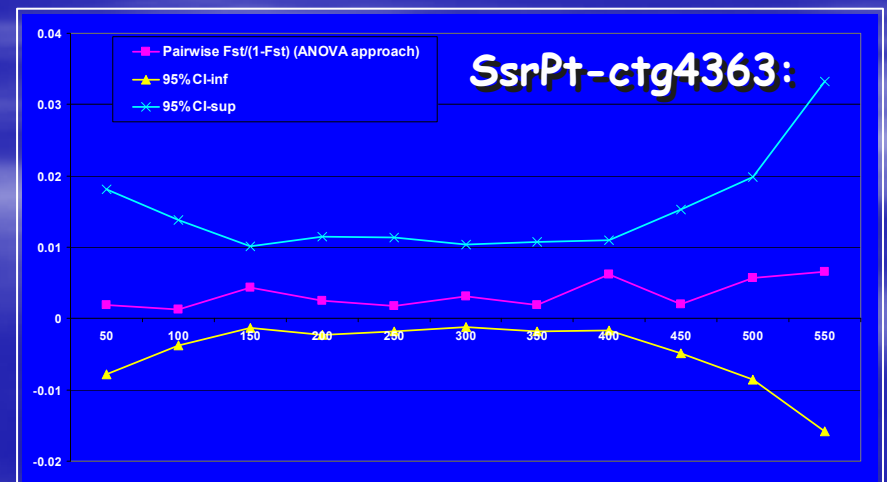
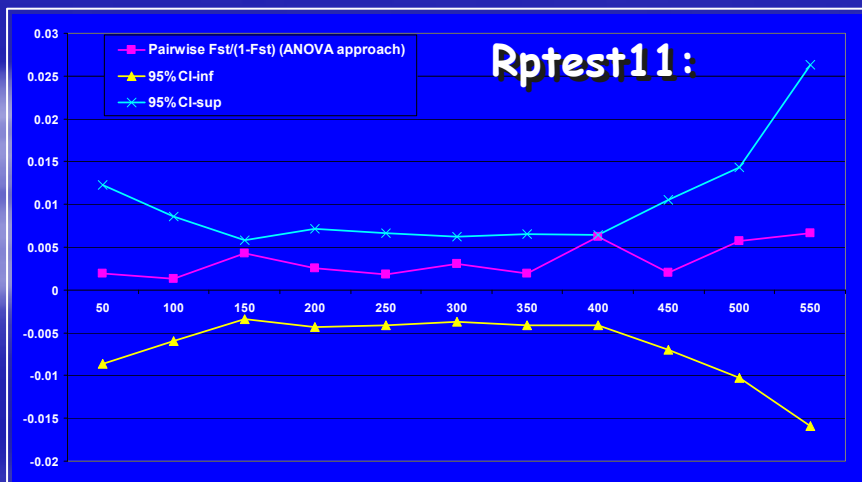
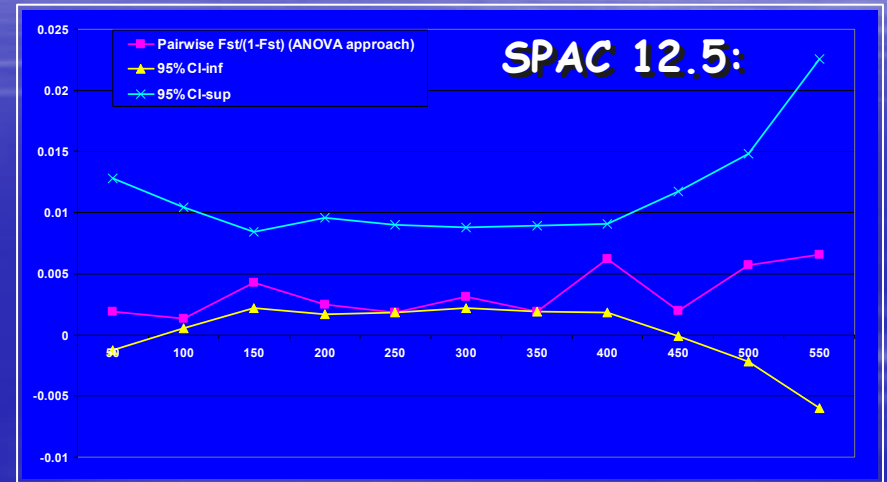
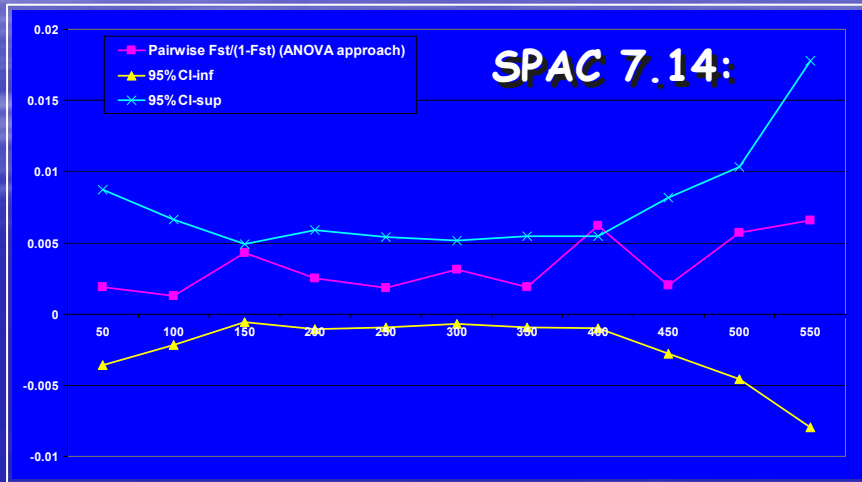
Geographical distribution of the Scots pine linkage groups in Poland

- 204. Augustów
- 208. Białowieża
- 504. Bolesławiec
- 306. Bolewice
- 105. Bytów
- 101. Goleniów
- 307. Gubin
- 606. Janów Lubelski
- 606. Józefów
- 654. Kluczbork
- 602. Kozienice
- 352. Krucz
- 303. Lipka
- 403. Łochów
- 404. Międzyrzec
- 107. Międzyzdroje
- 106. Miłomłyn
- 302. Niedźwiady
- 607. Niepołomice
- 205. Nowe Ramuki
- 401. Nowogród
- 104. Smolarz
- 601. Spała
- 206. Strzałowo
- 405. Strzelce
- 207. Supraśl
- 501. Syców
- 108. Wejherowo
- 305. Woziwoda
- 402. Wyszków



Results

Spatial correlations of gene dispersal



Pairwise Fst/(1-Fst) ANOVA approach



Conclusions

- There is possible to transfer microsatellite markers from *P. pinaster* to *P. sylvestris*
- **SPAC 7.14** was the most polymorphic locus
- **Rptest11** was the less polymorphic one
- Slippage effect occurred in loci developed in *P. sylvestris* (SPAC 7.14) as well as in locus transferred from *P. pinaster* (SsrPt-ctg4363)

Conclusions

- Amplification of alleles of non expected size may be due to:
 - polymerase slippage for large fragment size (229-230 bp for **SPAC 7.14**)
 - some fluorochrome e.g. Ned (**SsrPt-ctg4363**) are more likely to nonspecific amplification (Delmotte *et al.* 2001)
 - composition of microsatellite loci (two motif repeats, **SPAC 7.14**) may lead to nonstandard length of alleles



Conclusions

In Poland, nuclear microsatellite analysis revealed:

- Similar diversity pattern of studied Scots pine provenances in Poland (mean $F_{st}=0.002$)
- no significant differences between F_{st} value in different regions of Scots pine provenances



Conclusions

- Relative **homogeneity** of Scots pine provenances (no spatial correlation observed between gene diversity and geographical localization)
- Little inter-population variation in genetic diversity is often observed for:
 - species with **continuous distribution**
 - and **long-distance pollen** and seed dispersal



Conclusions

The present distribution of the genetic diversity level may also

reflect:

- Large transfer of the seed material in the past among all Scots pine provenances in Poland
- **Białowieża Primeval Forest** (East of Poland), is supposed to be less influenced by human activity during ages



Acknowledgments

- This work represents a part of genetic diversity research on conifer species financed by **Polish General Directorate of the State Forests** and **French Ministry** (DSUR-NGE-4C1-701 Project).
- Special thanks are addressed to Dr Sylvie Oddou (INRA Avignon, France) for valuable discussions and to Mrs. Bénédicte LeGuerroué and Mrs. Vanina Benoit - (INRA Orleans, France) for help in the laboratory task.