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

Justyna Nowakowska, Catherine Bastien, Brigitte Musch

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Analysis of microsatellites in Scots pine
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Justyna Nowakowska[1], Catherine Bastien[2], Brigitte Musch[2]

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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
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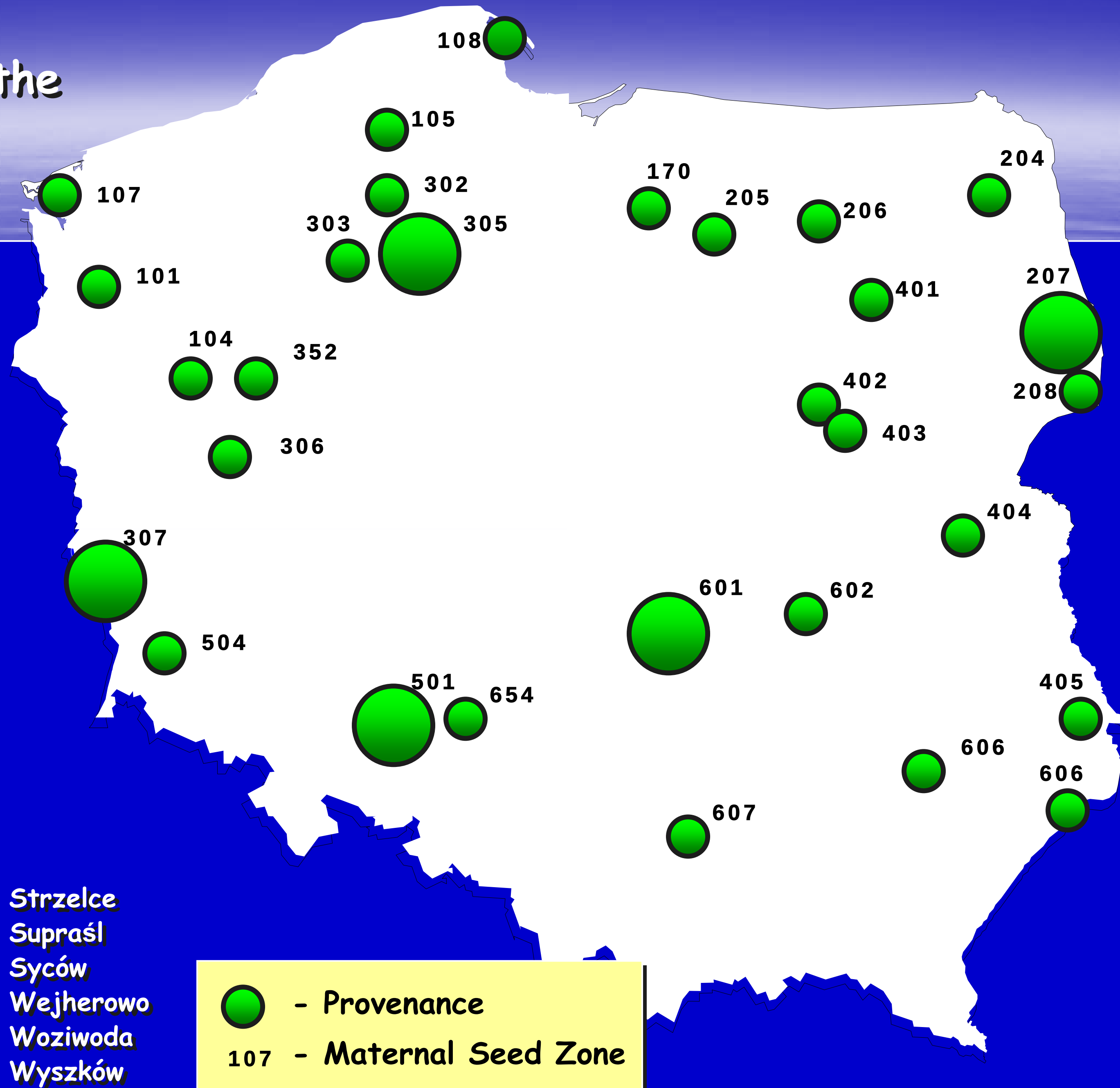
501. Syców
108. Wejherowo
305. Woziwoda
402. Wyszków



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

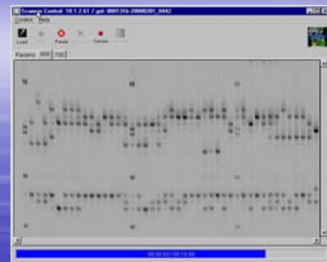
- Microsatellite loci and primers designed for their amplification

No	Locus	Repeat Length (bp)	Primer Sequence 5' – 3'	Tm °C	Labelling
1	SPAC 7.14 *P. sylvestris*	$(TG)_{17}(AG)_{21}$ 209 bp	F: TTCGTAGGACTAAAATGTGTG R: CAAAGTGGATTTTGACCG	55	B, 6 Fam
2	SPAC 11.6 *P. sylvestris*	$(CA)_{29}(TA)_{7}$ 165 bp	F: TTACAGCGGTTGGTAAATG R: CTTCACAGGACTGATGTTCA	55↘50	B, 6 Fam
3	SPAC 12.5 *P. sylvestris*	$(GT)_{20}(GA)_{10}$ 155 bp	F: CTTCTTCACTAGTTTCCTTTGG R: TTGGTTATAGGCATAGATTGC	54	B, Ned
4	Rptest11 *P. pinaster*	$(ATC)_{7}$ 213 bp	F: AGGATGCCTATGATATGCGC R: AACCATAACAAAAGCGGTCG	56	B, 6 Fam
5	SsrPtctg4363 *P. pinaster*	$(AT)_{10}$ 100 bp	F: TAATAATTCAAGCCACCCCG R: AGCAGGCTAATAACAACACGC	60↘50	Hex
6	SsrPtctg7824 *P. pinaster*	$(AT)_{12}$ 501 bp	F: TGACCTGTCTTGTGAGACGC R: TTTTGAAACAGATTGCAGCC	60↘50	Hex

Soranzo *et al.* 1998

General scheme of microsatellite loci analysis

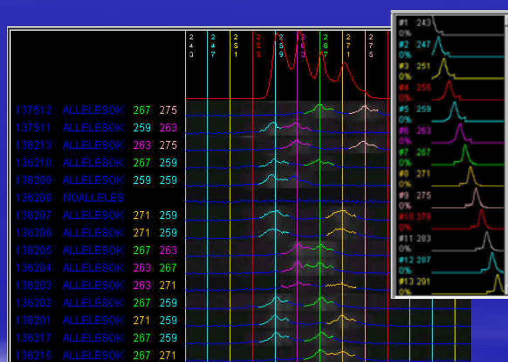
PCR Amplification



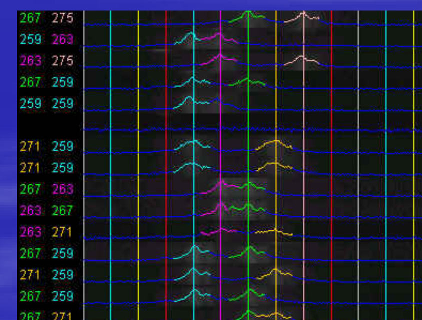
Electrophoresis

Load on HITACHI Abi-Prism® 3100 Genetic Analyzer

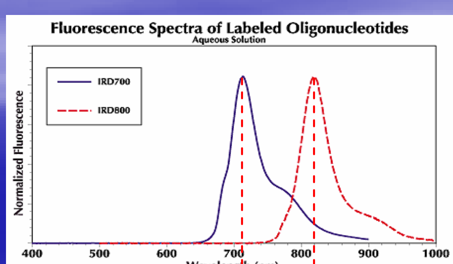
Abi-Prism® GeneScan® 3.7 software



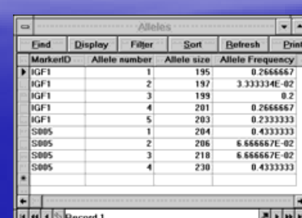
Genotyping



Loci Location



Visual Inspection



MarkerID	Allele number	Allele size	Allele Frequency
IGF1	1	195	0.2666667
IGF1	2	197	3.333334E-02
IGF1	3	199	0.2
IGF1	4	201	0.2666667
IGF1	5	203	0.2333333
S005	1	204	0.4333333
S005	2	206	6.666667E-02
S005	3	218	6.666667E-02
S005	4	230	0.4333333

Text Output

S-Plus

Statistical Analysis

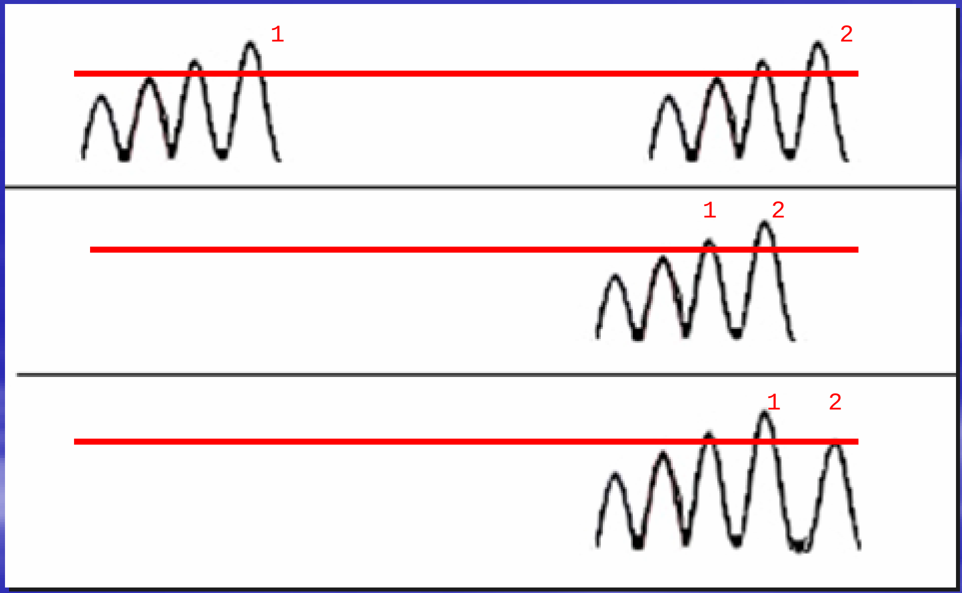
Material and Methods

- Microsatellite alleles taken from the sequencer

Well Separated Heterozygote

Homozygote

2 bp separated Heterozygote

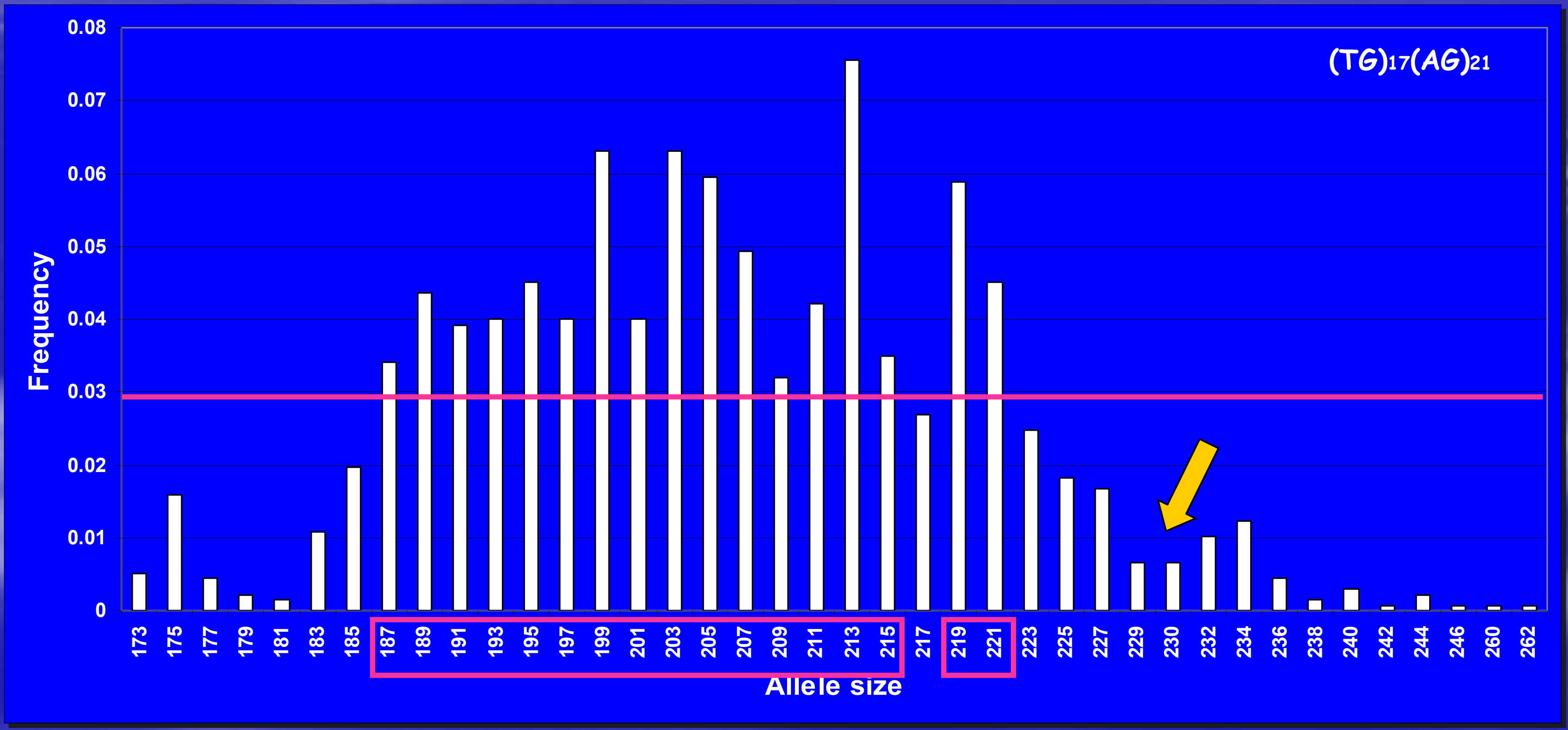


Every peak was examined for appropriate threshold scoring

Results

Allele frequency distribution:

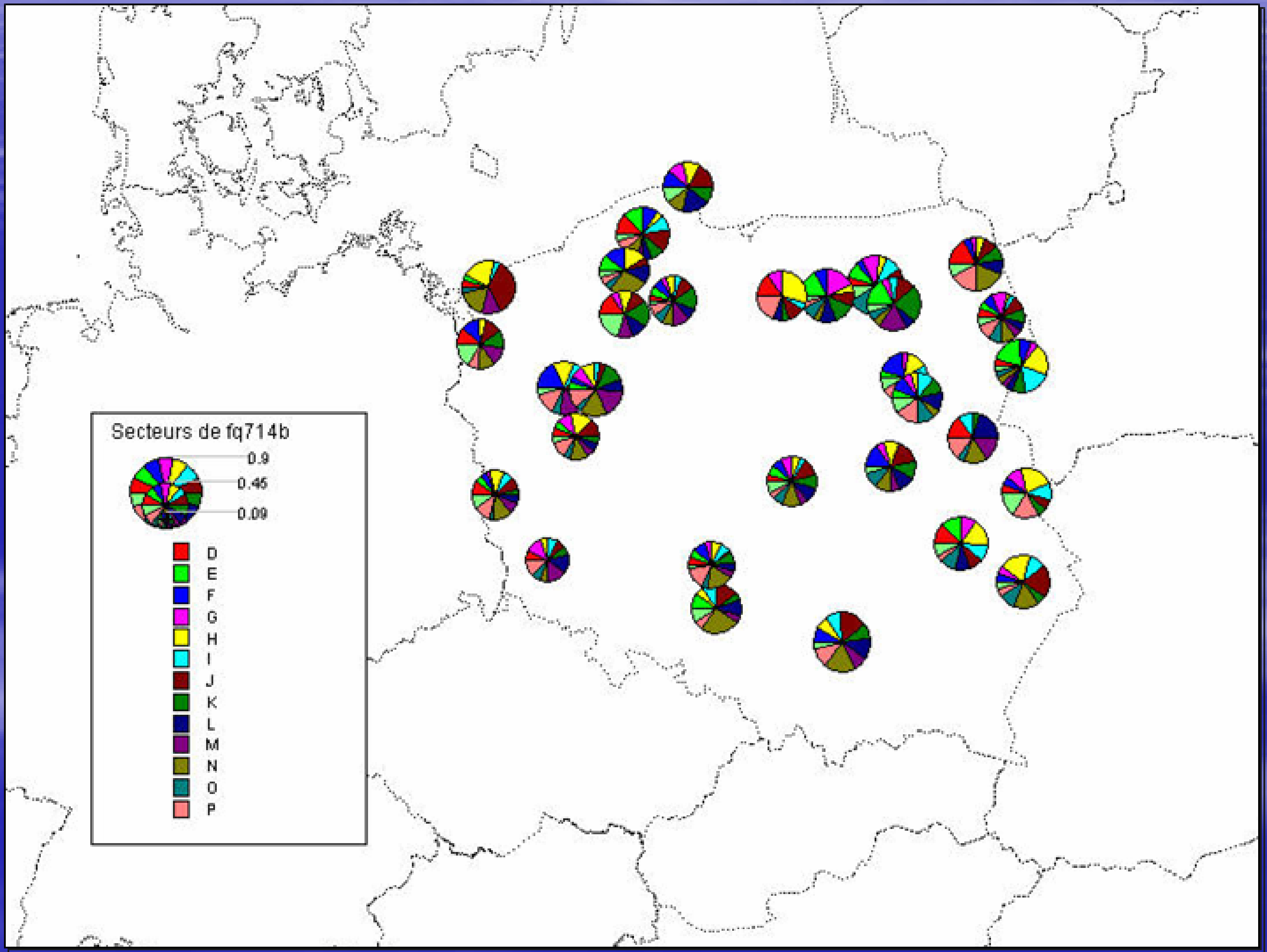
SPAC 7.14 microsatellite loci (N=1378, 40 alleles)



Allele frequency > 0.03

Results

Geographical distribution of the most frequent alleles of 7.14 locus

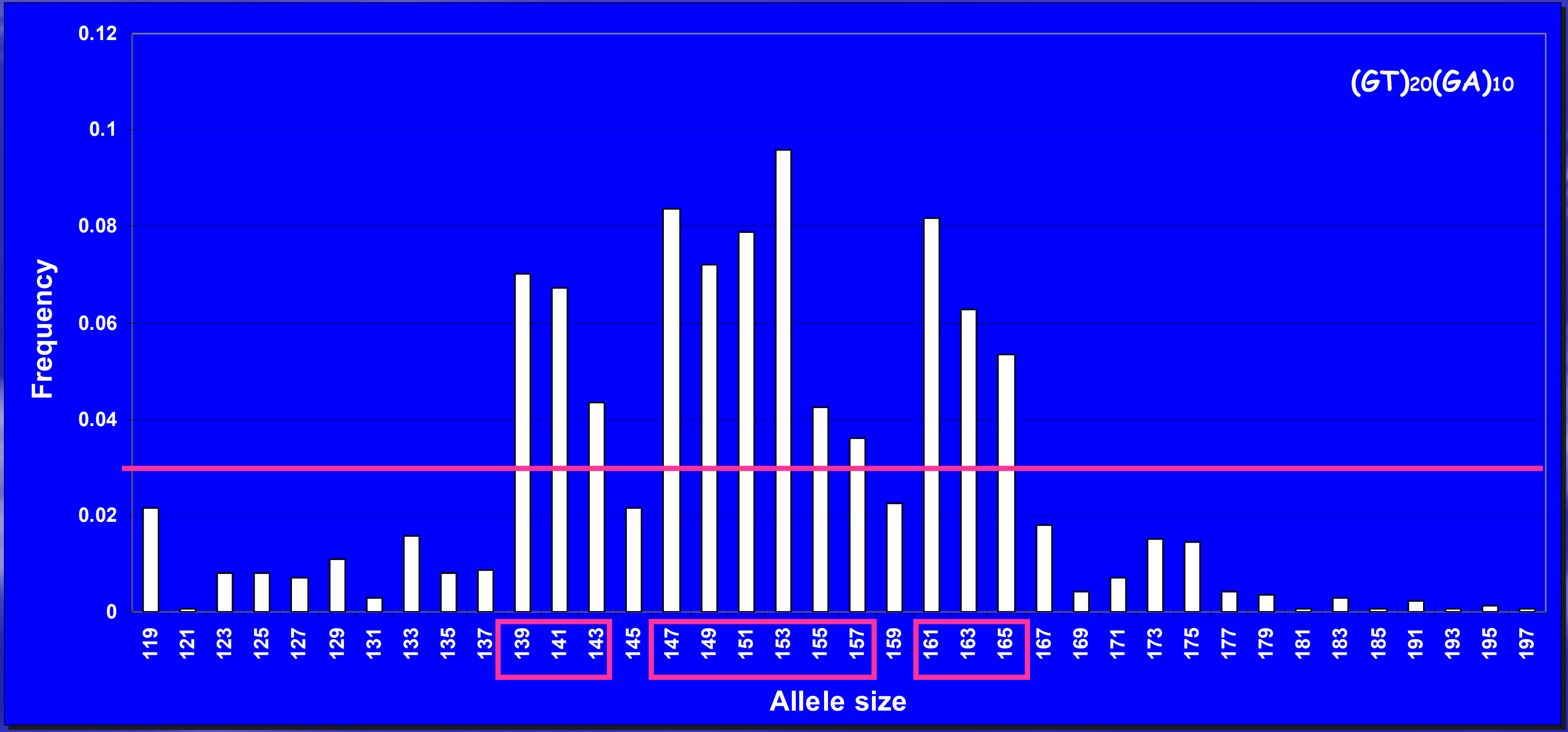


MapInfo Professional™ 4.1

Results

Allele frequency distribution:

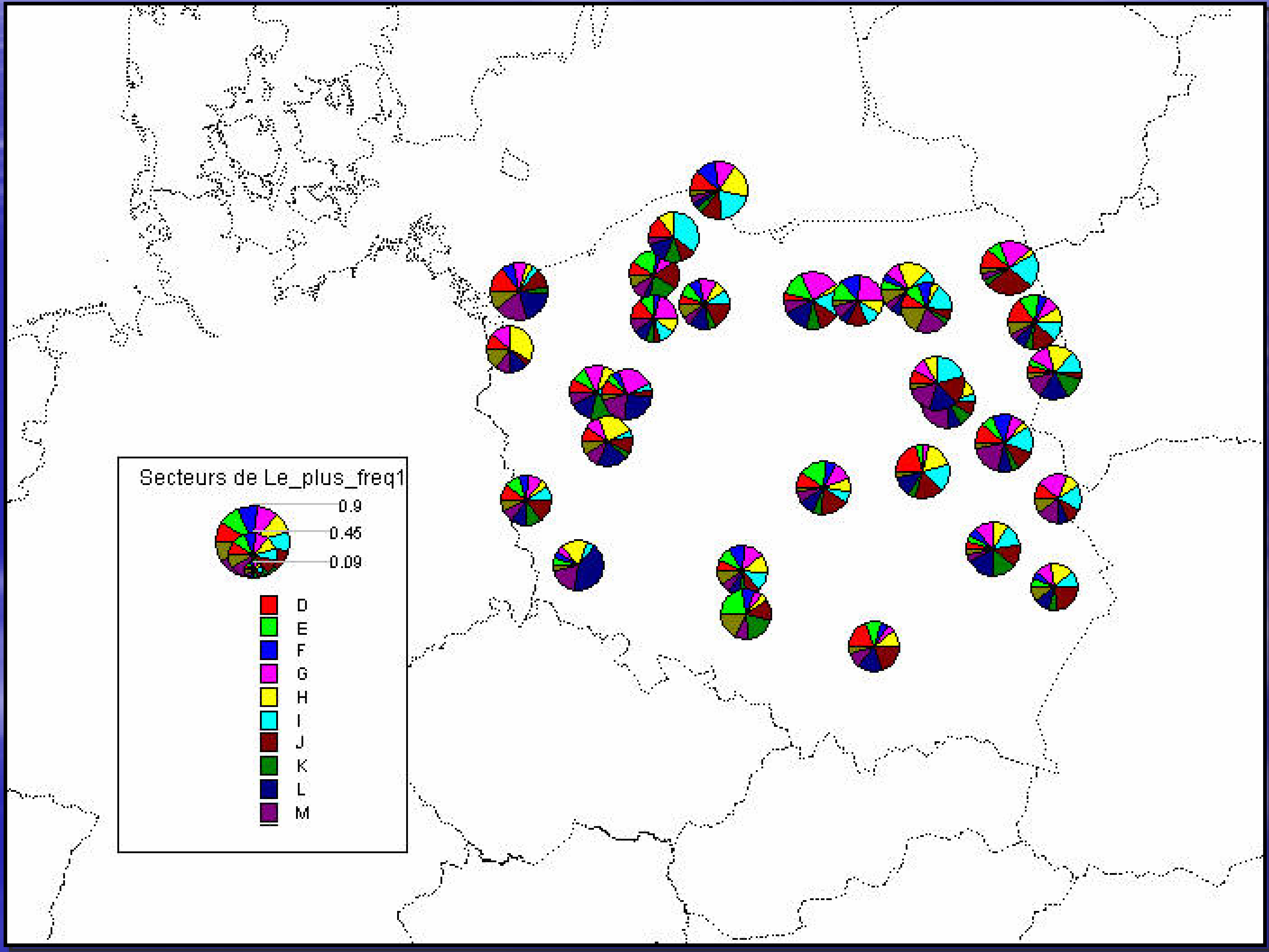
SPAC 12.5 microsatellite loci (N=1385, 38 alleles)



Allele frequency > 0.04

Results

Geographical distribution of the most frequent alleles of 12.5 locus



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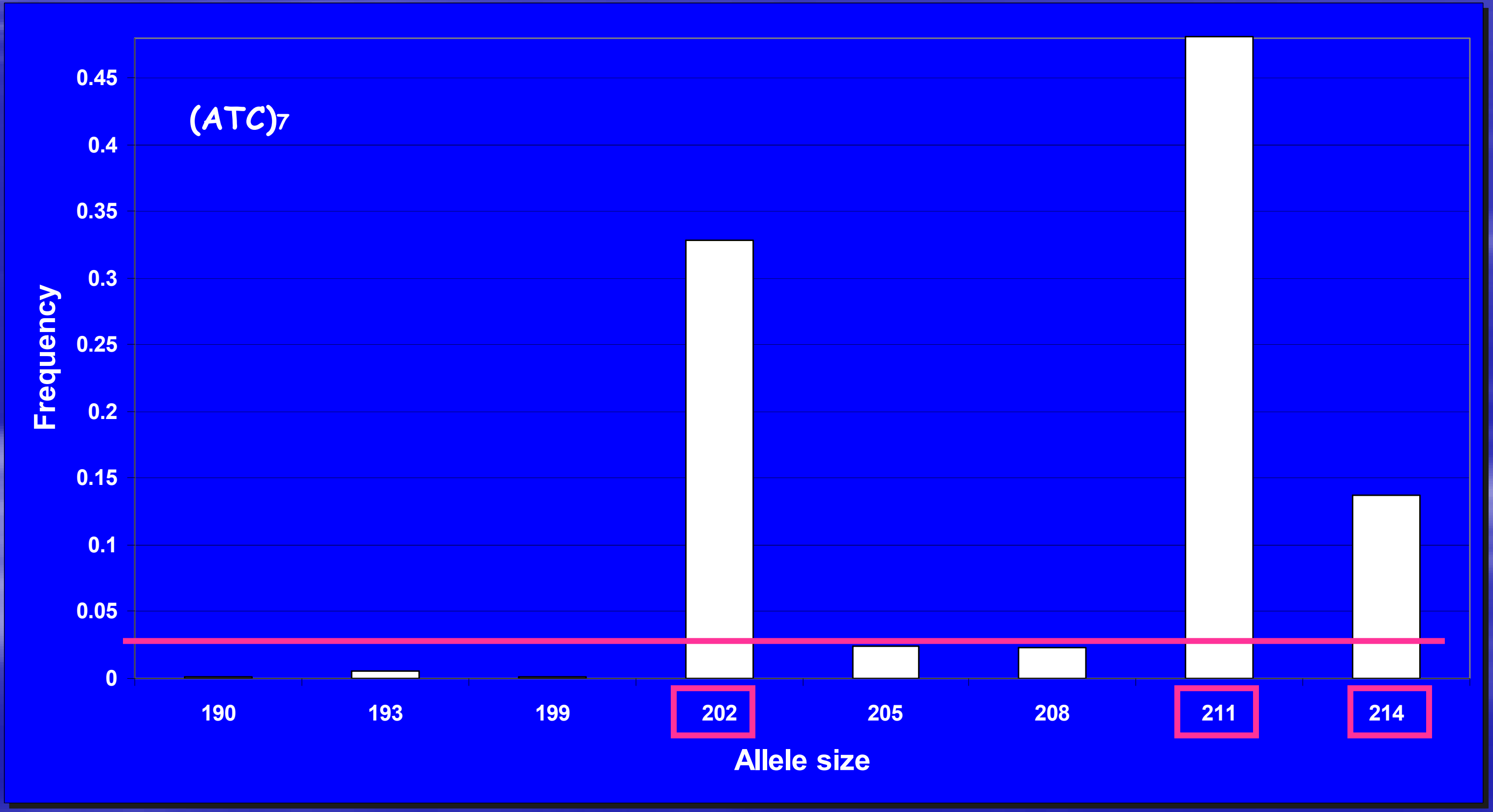
Results

Allele frequency distribution:

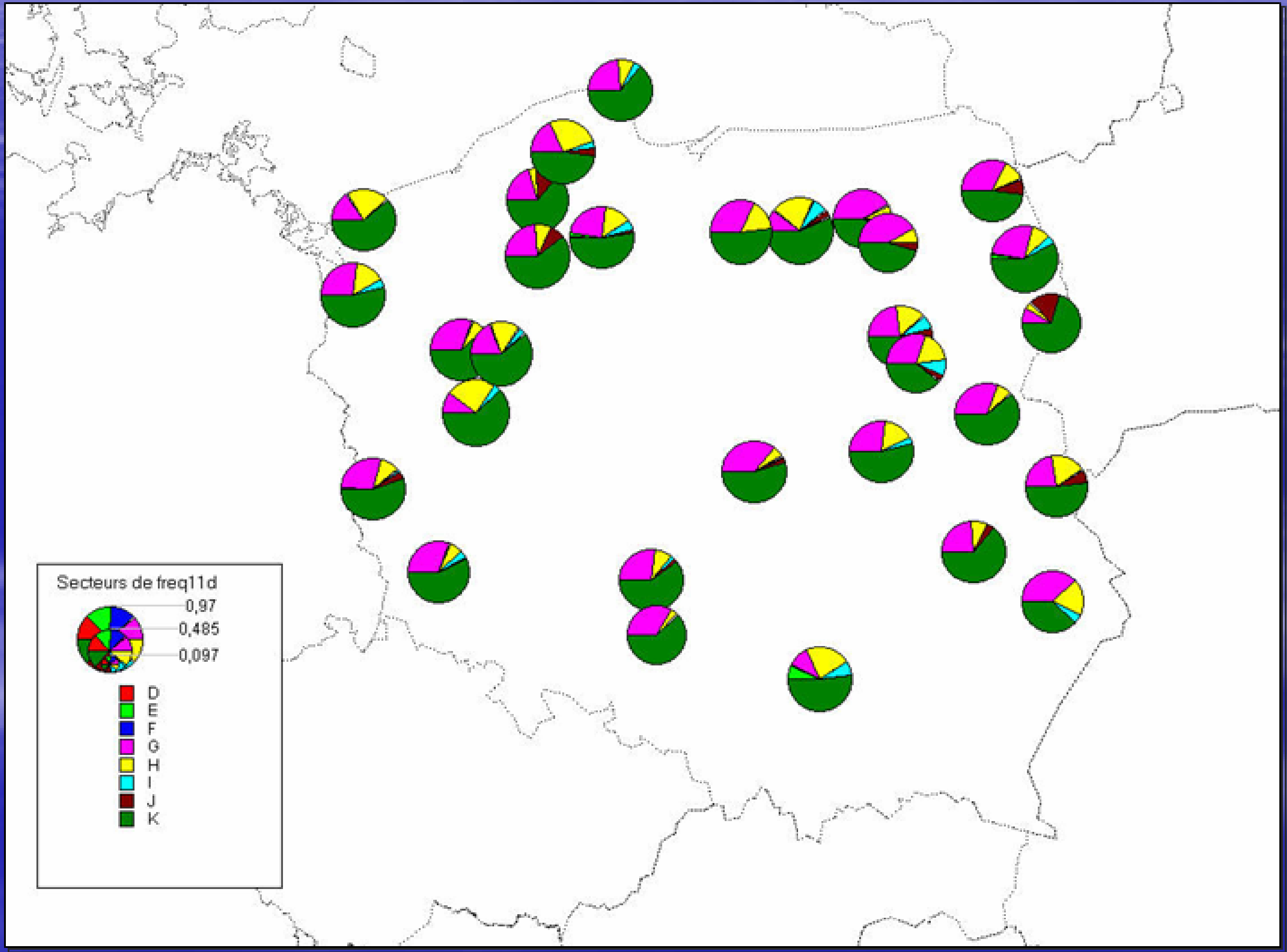
Rptest11 microsatellite loci (N=1398, 8 alleles)



Allele frequency > 0.03

Results

Geographical distribution of the most frequent alleles of Rptest11 locus



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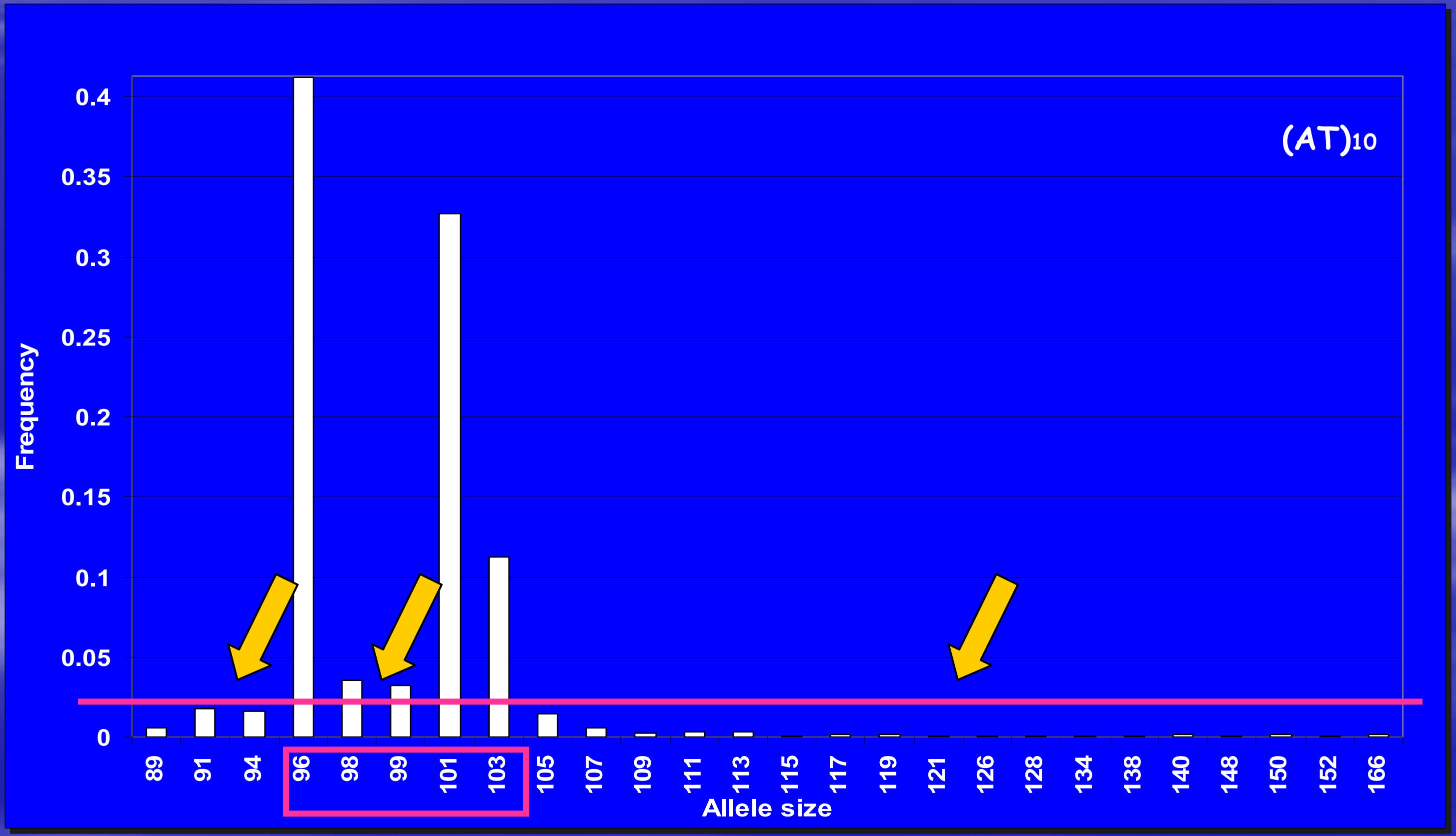
Results

Allele frequency distribution:

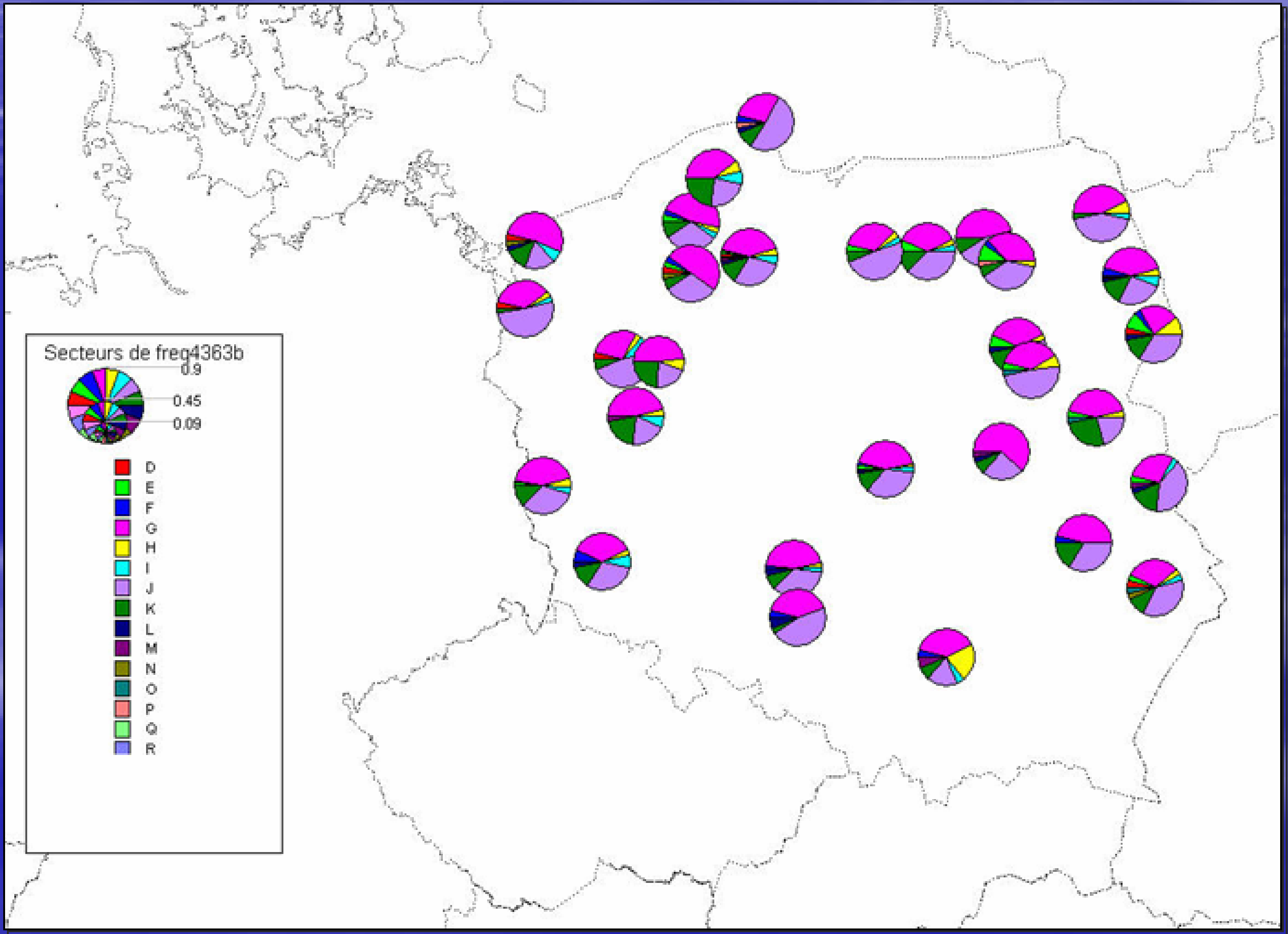
SsrPt-ctg4363 microsatellite loci (N=1385, 26 alleles)



Allele frequency > 0.03

Results

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



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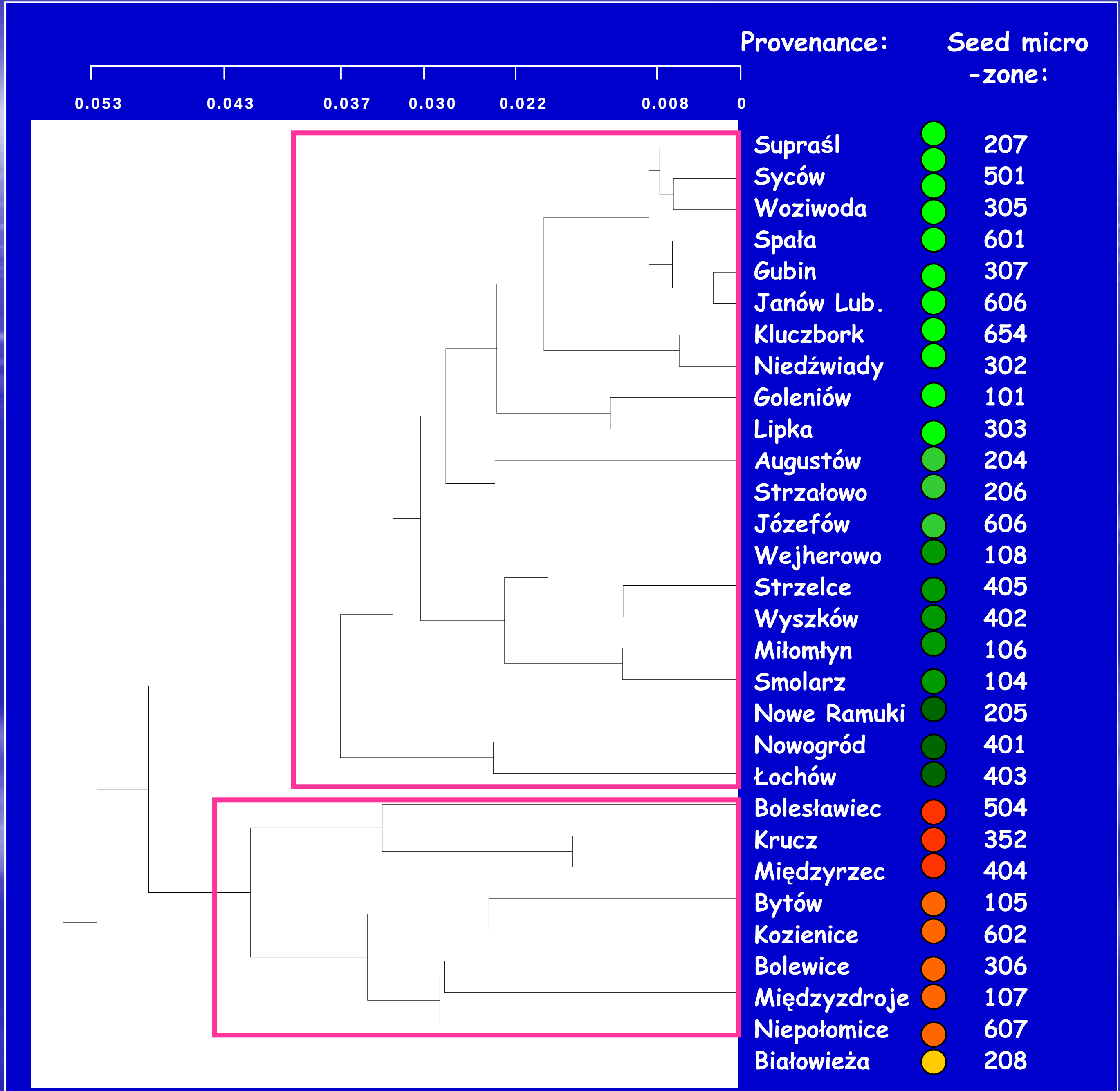
Neighbor-joining tree of Scots pine provenances in Poland based on microsatellite data



POPGENE v 1.32.

Geographical distribution of the Scots pine linkage groups in Poland

204. Augustów
208. Białowieża
504. Bolesławiec
306. Bolewice
105. Bytów
101. Goleniów
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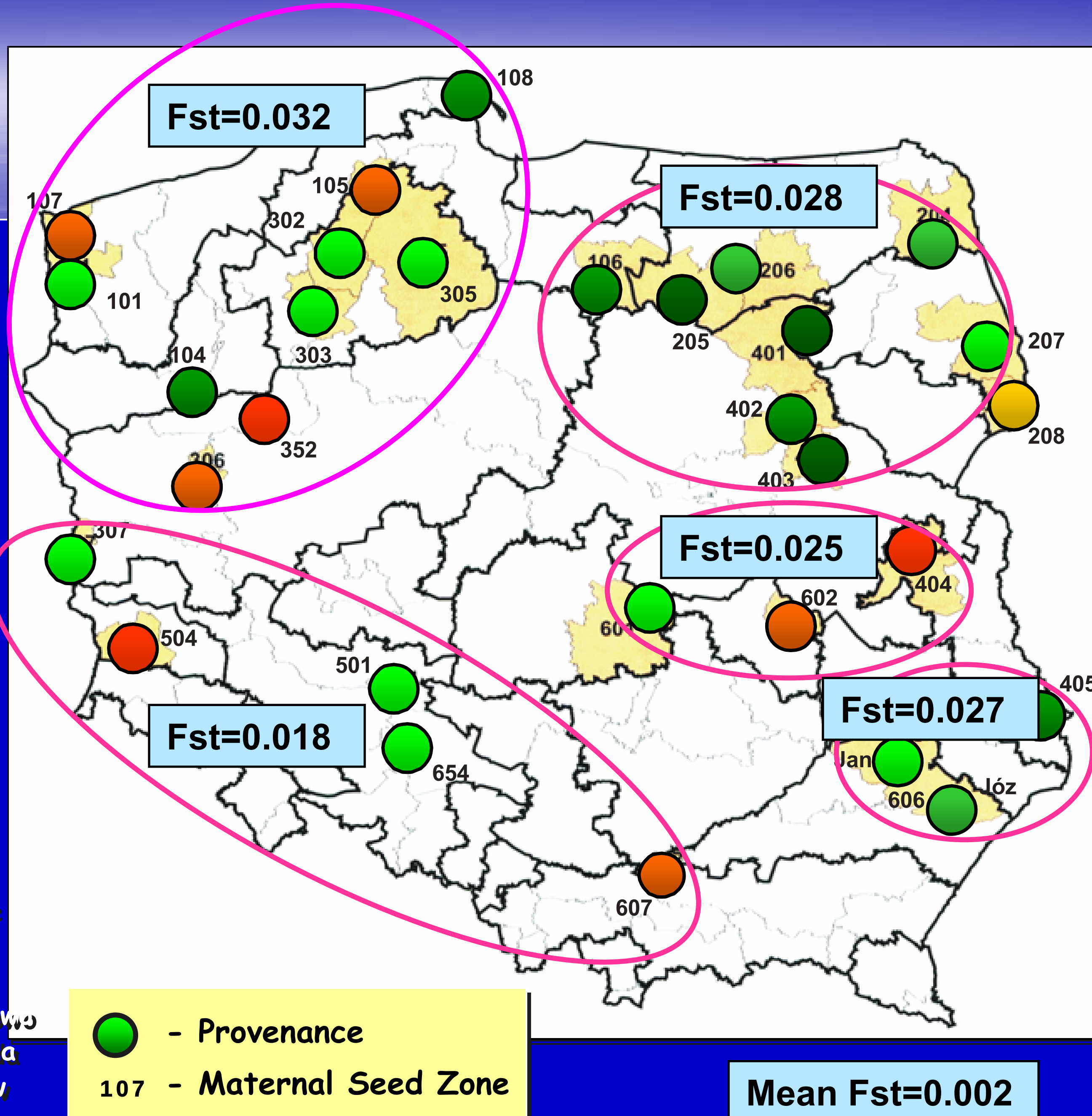
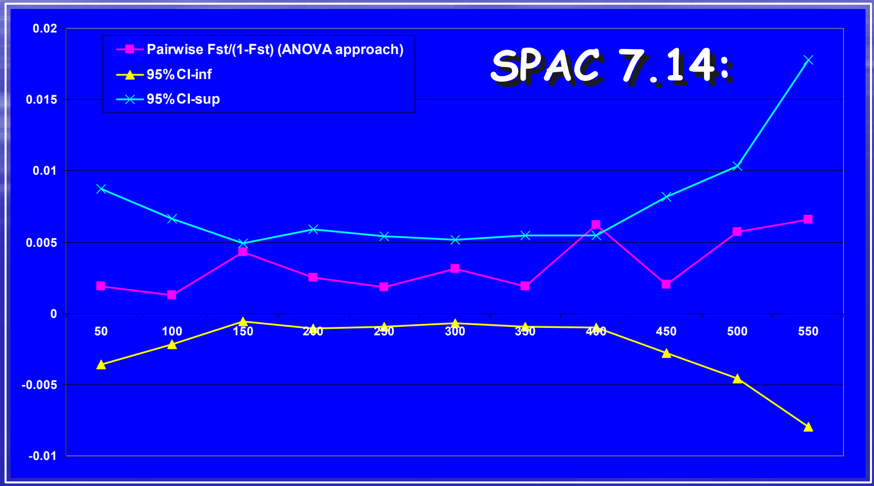
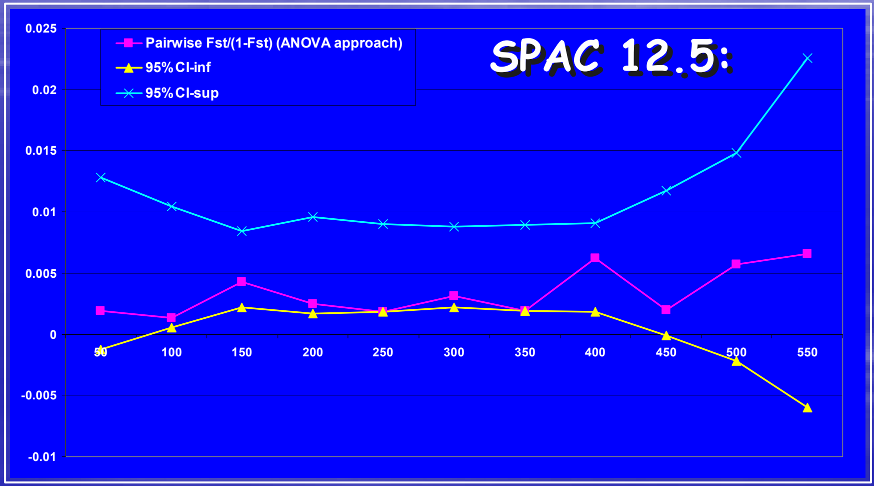
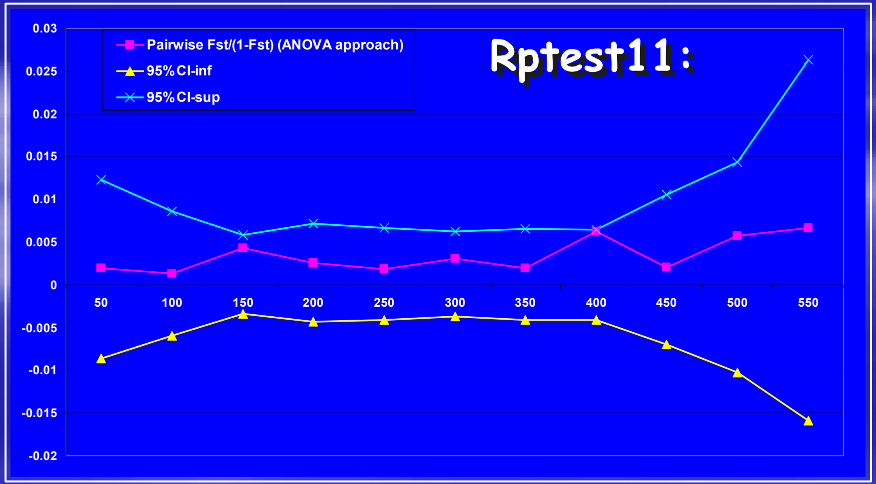


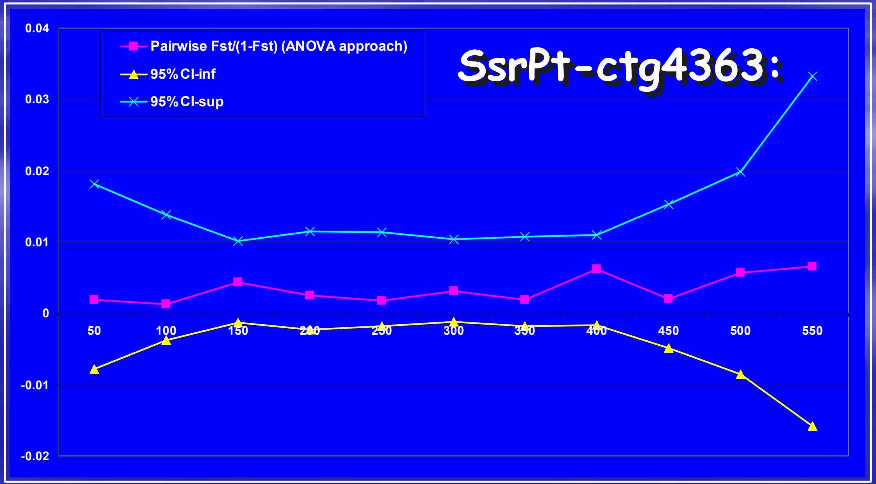
Results

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 Fst=0.002)
- no significant differences between Fst 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.