

# Genetic variation and hybridization in a species-rich oak (*Quercus* spp.) community in Romania

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# Outline

- Introduction
  - Hybridization in oaks
  - Objectives
- Material
  - Bejan Forest and sampling
- Diversity and differentiation
  - Hybrid origin of adults
- Extant gene flow
  - Acorns from selected seed trees
- Conclusions



*Quercus robur*; Thomé (1885)

# Introduction

- Oaks are models to study gene flow and hybridization (Petit et al., 2004)
- Main species studied in Europe:
  - mainly *Q. petraea* and *Q. robur*
    - Controlled pollination: asymmetrical success (e.g. Aas, 1991)
      - *Q. robur* ♀ × *Q. petraea* ♂ preferred
    - Differentiation patterns (e.g. Scotti-Saintagne et al., 2004)
    - Paternity assignment (e.g. Streiff et al., 1999; Jensen et al., 2009)
  - other two-species cases:
    - *Q. petraea* and *Q. pyrenaica* (Valbuena-Carabaña et al., 2005)
    - *Q. petraea* and *Q. pubescens* (Salvini et al., 2009)
    - *Q. ilex* and *Q. suber* (Burgarella et al., 2009)



# Introduction

- Two-species comparisons suggest hybridization
  - Genetic similarity of closely related oaks
    - Nuclear markers
      - but: outlier loci (Scotti-Saintagne et al., 2004)
    - cpDNA
      - lack of local differentiation (Petit et al., 2004)
  - Controlled pollination: asymmetrical success (e.g. Aas, 1991)
    - *Q. robur* ♀ × *Q. petraea* ♂ preferred
  - Gene flow assessment
    - Paternity assessment supports asymmetric gene flow



## Objectives and hypotheses

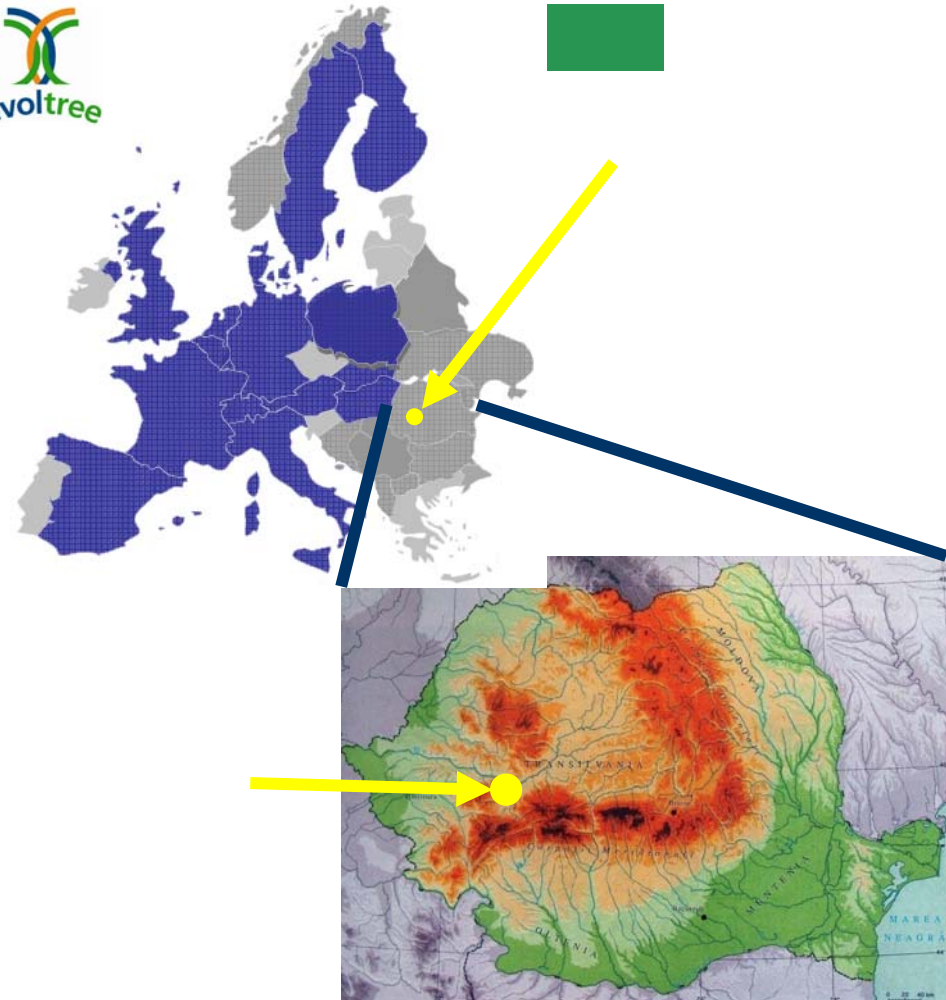
- Differentiation patterns and hybridization in complex communities (>2 species)?
- Hypotheses
  - Low differentiation among closely related taxa
    - Nuclear markers
    - cpDNA haplotypes
  - Outlier loci
    - Strong differentiation of single species
  - Hybridization among all taxa
    - Dominance of introgressive types



# Plot location: The Bejan forest in Romania



- Natural forest
  - no indication for planting/seed transfer
- Section Quercus
  - *Q. robur*
    - Nutrient-rich, wet soils
  - *Q. petraea*
    - Well-drained, more acid soils
  - *Q. pubescens*
    - Xerophile
  - *Q. frainetto*
    - Xerophile-mesophile
- Section Cerris
  - *Q. cerris*
    - Xerophile



Bejan Forest (45°51'N, 22°53'E),  
Carpathian mountains in west-central  
Romania (Curtu, 2006)

# Morphological assessment

## Leaf morphology (Kremer et al., 2002)

- A *Q. robur*
- B *Q. petraea*
- C *Q. pubescens*
- D *Q. frainetto*
- E *Q. cerris*

## Pubescence assessment (Aas, 1998)

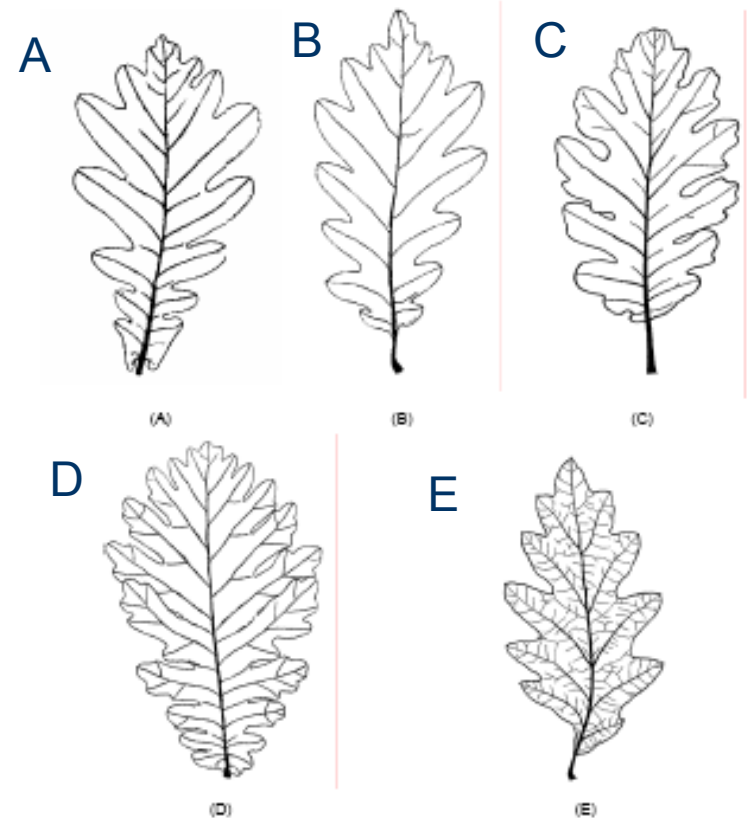


Fig. 1-1 Typical leaf shape for: (A) *Q. robur* (B) *Q. petraea* (C) *Q. pubescens* (D) *Q. frainetto* (E) *Q. cerris* - after SCHWARZ (1936)

# Morphological assessment

- *Q. cerris* clearly distinct
- Section *Quercus* s.s.
  - Unambiguously assigned
    - Q. robur*: 65
    - Q. petraea*: 65
    - Q. pubescens*: 73
    - Q. frainetto*: 50
  - Intermediates: 16 (6.3%)

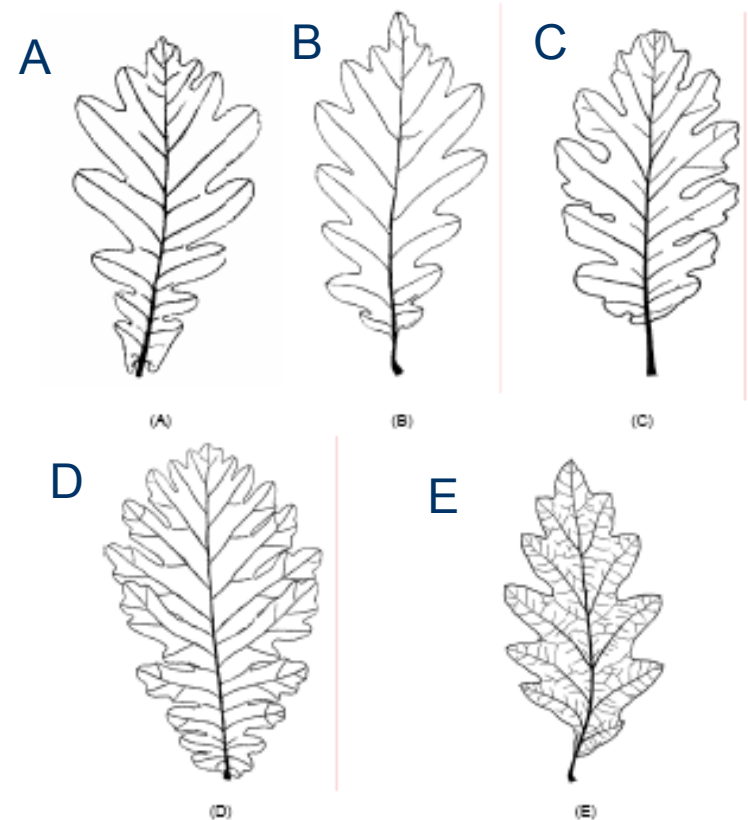
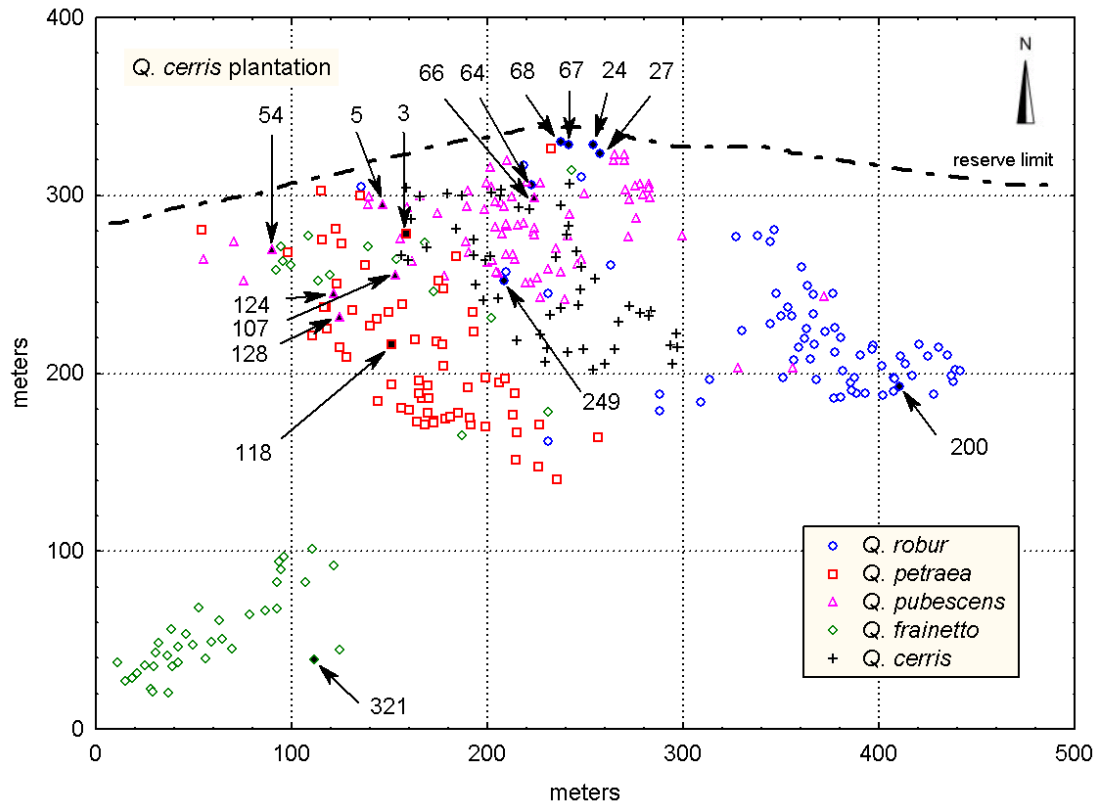


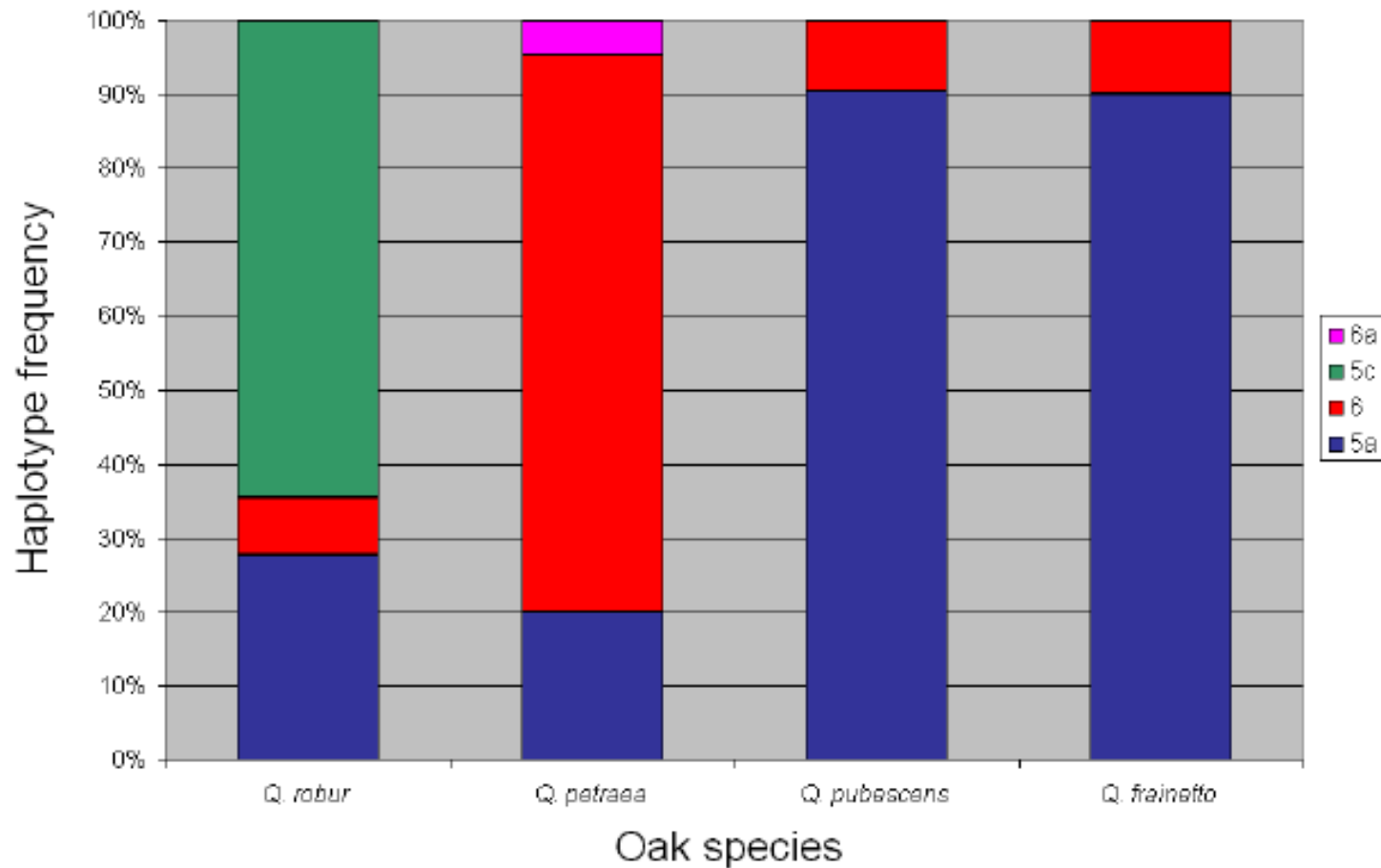
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# Map of sampled trees: morphological assignment



Morphologically intermediate trees are indicated with numbers and arrows

# Chloroplast haplotypes



$F_{ST}=0.533$

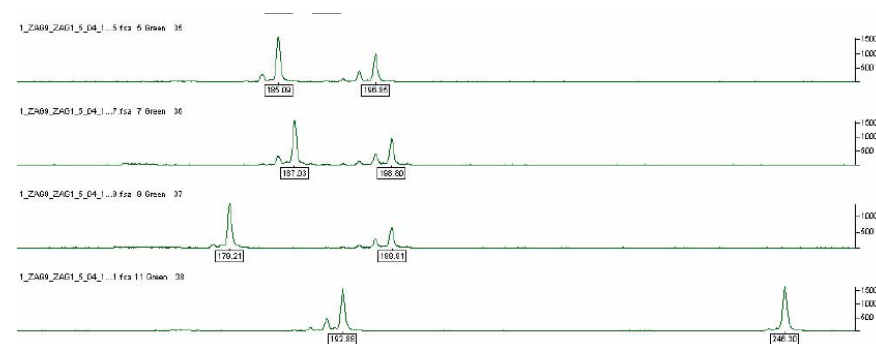
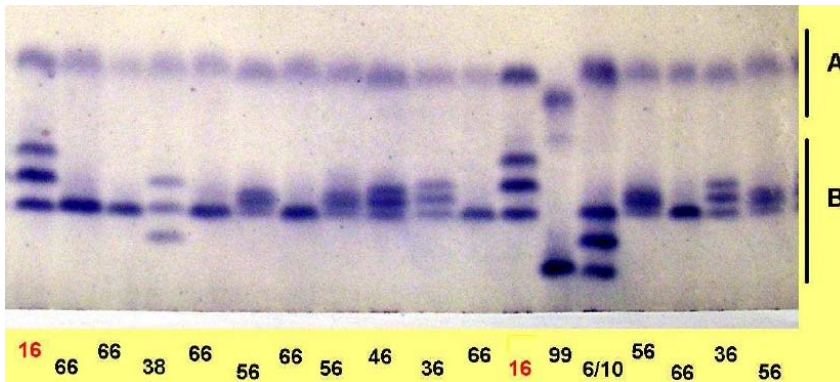
# Nuclear markers

- 7 isozyme loci

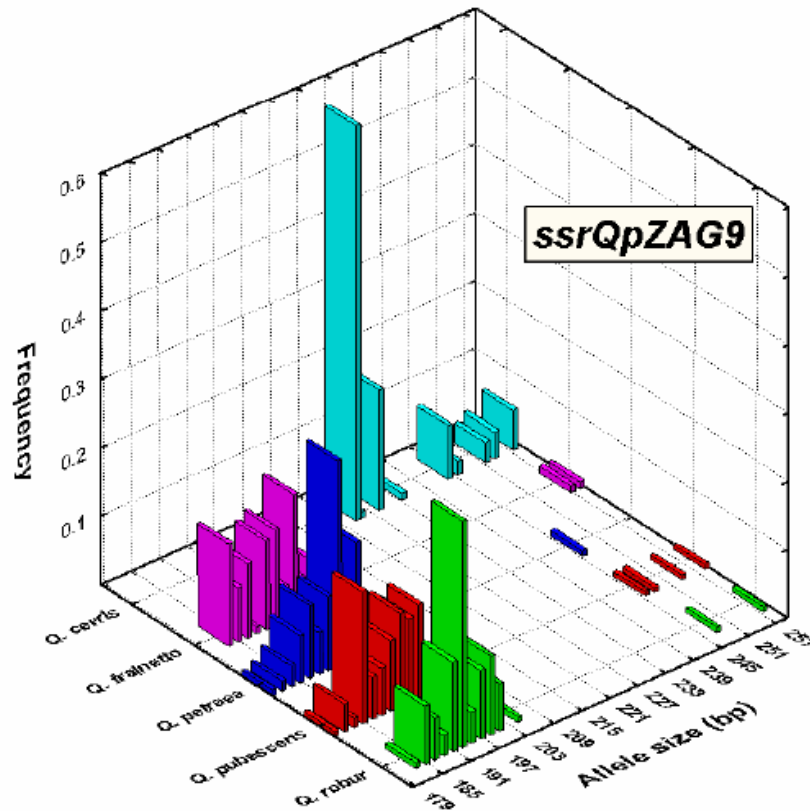
- *Aap-A*
- *Aat-B*
- *Acp-C*
- *IDH-B*
- *MNR-A*
- *6PGDH-B*
- *Pgi-B*

- 6 nSSRs

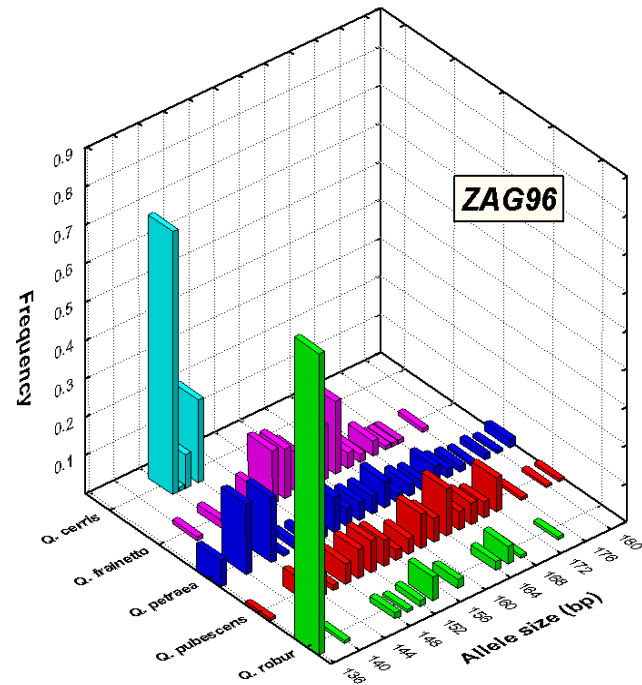
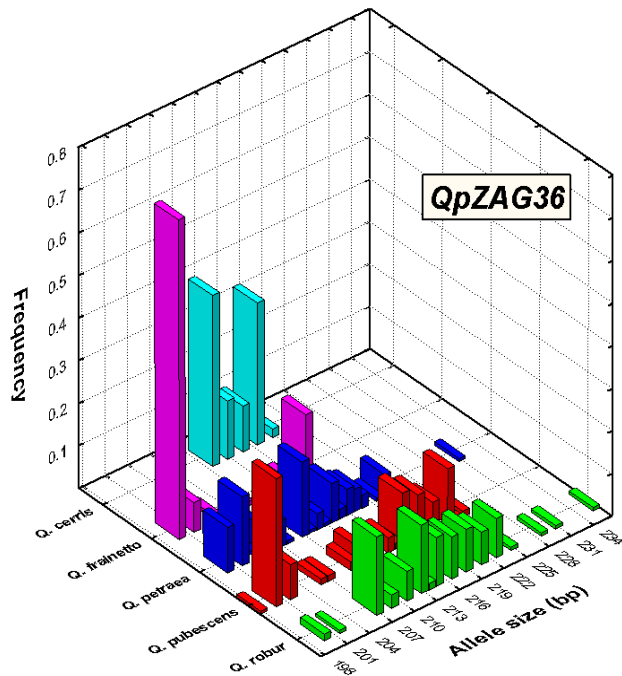
- ZAG 1/5
- ZAG 9
- ZAG 36
- ZAG 96
- ZAG 104
- MSQ 13



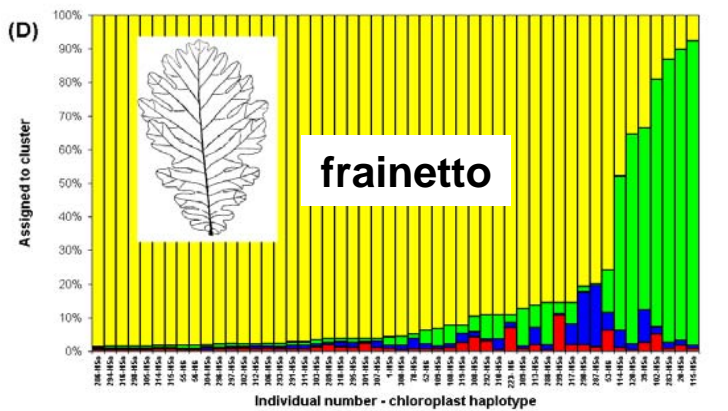
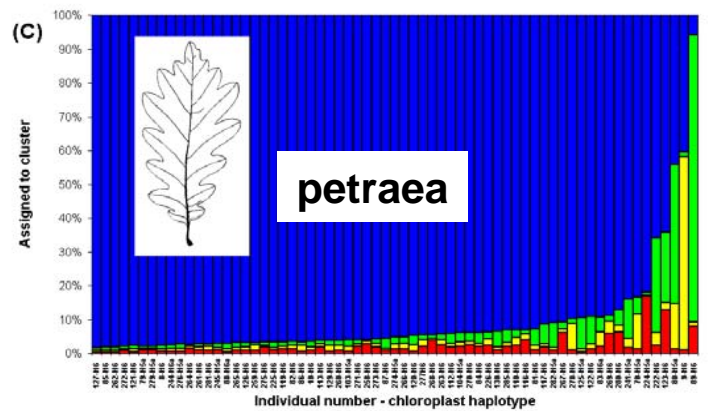
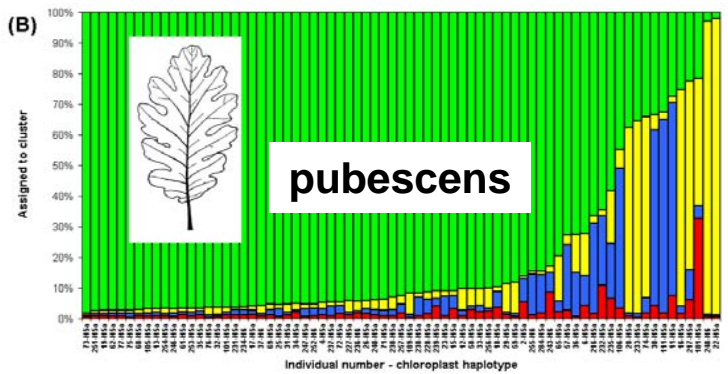
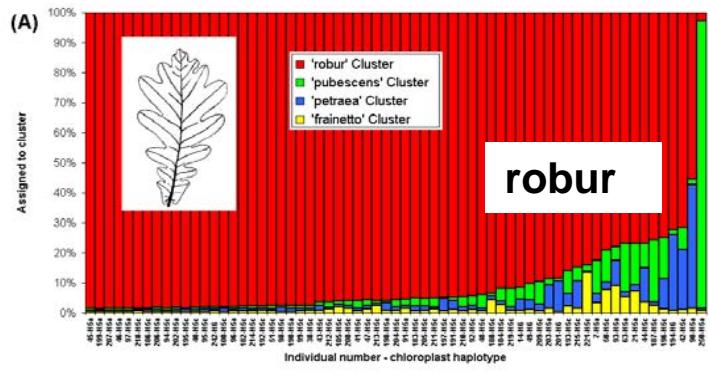
# Low differentiation at SSRs: ZAG9



# Strong differentiation of particular species at SSRs



# Genetic assignment



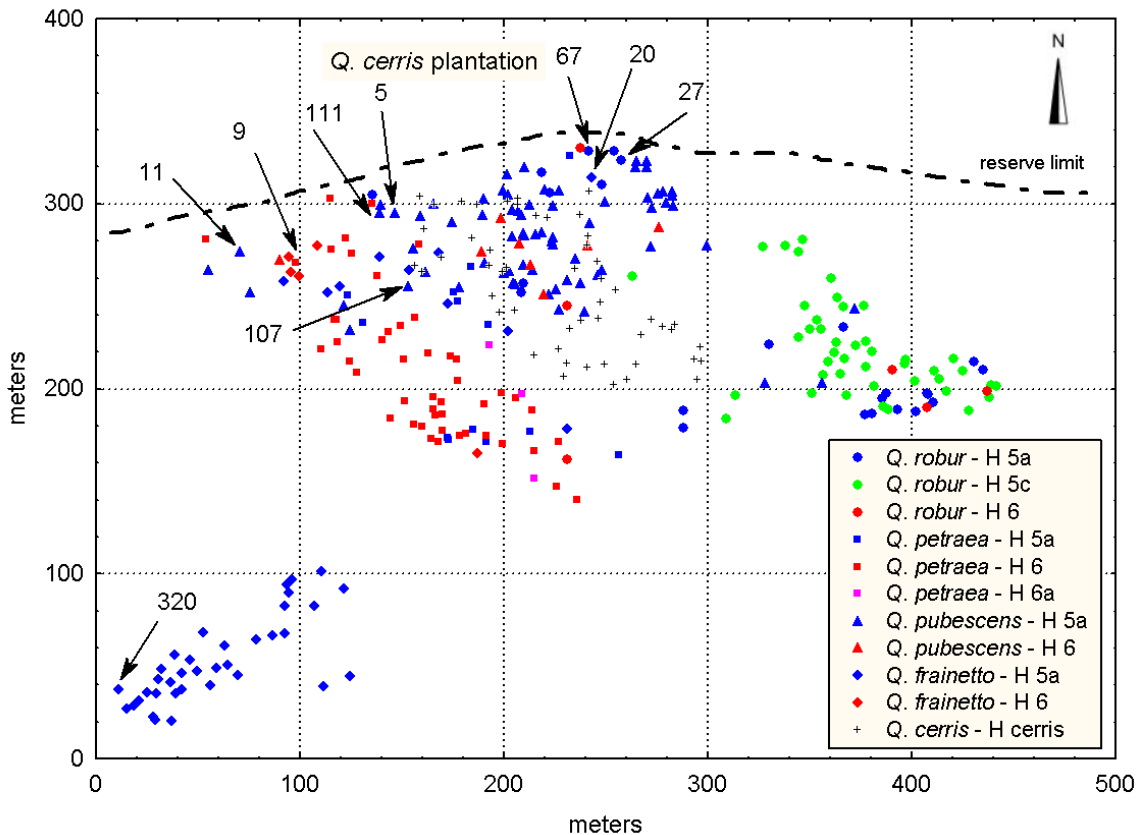
## Putative first-generation hybrids

No.	morph. species	putative F <sub>1</sub> hybrid
67*	( <i>Q. robur</i> )	<i>Q. robur</i> x <i>Q. pubescens</i>
5*	( <i>Q. pubescens</i> )	<i>Q. pubescens</i> x <i>Q. robur</i>
27*	( <i>Q. pubescens</i> )	<i>Q. pubescens</i> x <i>Q. robur</i>
11	<i>Q. pubescens</i>	<i>Q. pubescens</i> x <i>Q. petraea</i>
111	<i>Q. pubescens</i>	<i>Q. pubescens</i> x <i>Q. petraea</i>
107*	( <i>Q. pubescens</i> )	<i>Q. pubescens</i> x <i>Q. frainetto</i>
9	<i>Q. petraea</i>	<i>Q. petraea</i> x <i>Q. frainetto</i>
20	<i>Q. frainetto</i>	<i>Q. frainetto</i> x <i>Q. pubescens</i>
320	<i>Q. frainetto</i>	<i>Q. frainetto</i> x <i>Q. pubescens</i>

\* morphological intermediates according to multivariate analysis



## Location and cpDNA haplotype of putative hybrids



Putative hybrids mainly in 'intermediate' habitats and locations with overlapping distribution of 'pure' species

## Assignment of trees

- ‚Pure‘ species: 74.1% (191/259)
  - Genetic assignment corresponding to morphological assignment
- F1-Hybrids: 3.5% (9/259)
  - Only 4 morphologically intermediate trees
  - All species likely to be parental species of F1-Hybrids
  - *Q. pubescens* is parental species in 8 out of 9 cases
- Introgressive forms: 22.8% (59/259)
  - Lowest: *Q. robur* and *Q. frainetto*
  - Highest: *Q. pubescens* and *Q. frainetto*

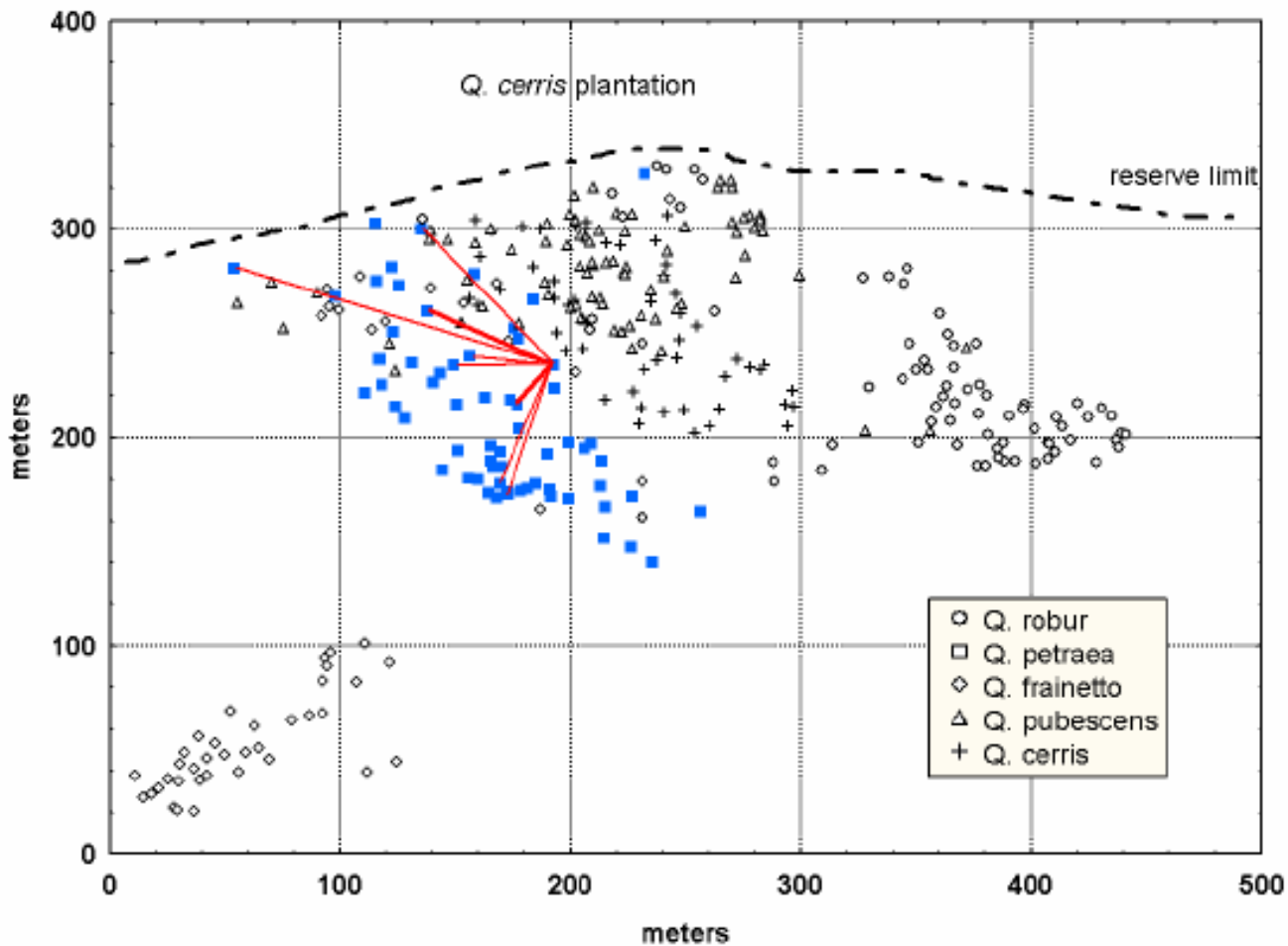


## Extant gene flow

- 320 acorns from eight seed trees
  - Sampled in 2005
- Genotyped at 6 SSRs
  - Cumulative exclusion probability: 0.999955
- Paternity assignment
  - FAMOZ (Gerber et al., 2003)

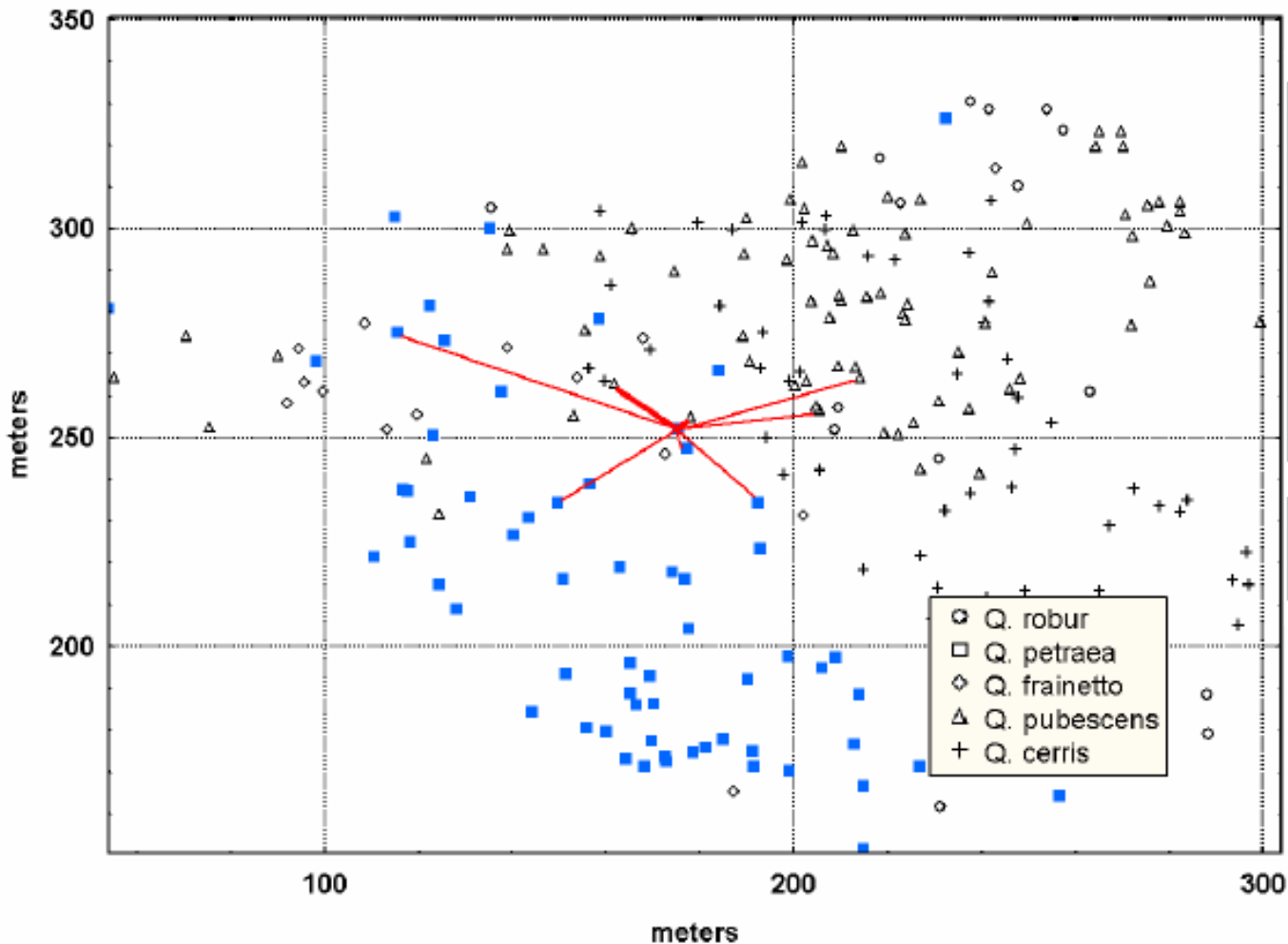


## Seed tree 245 – *Q. petraea*



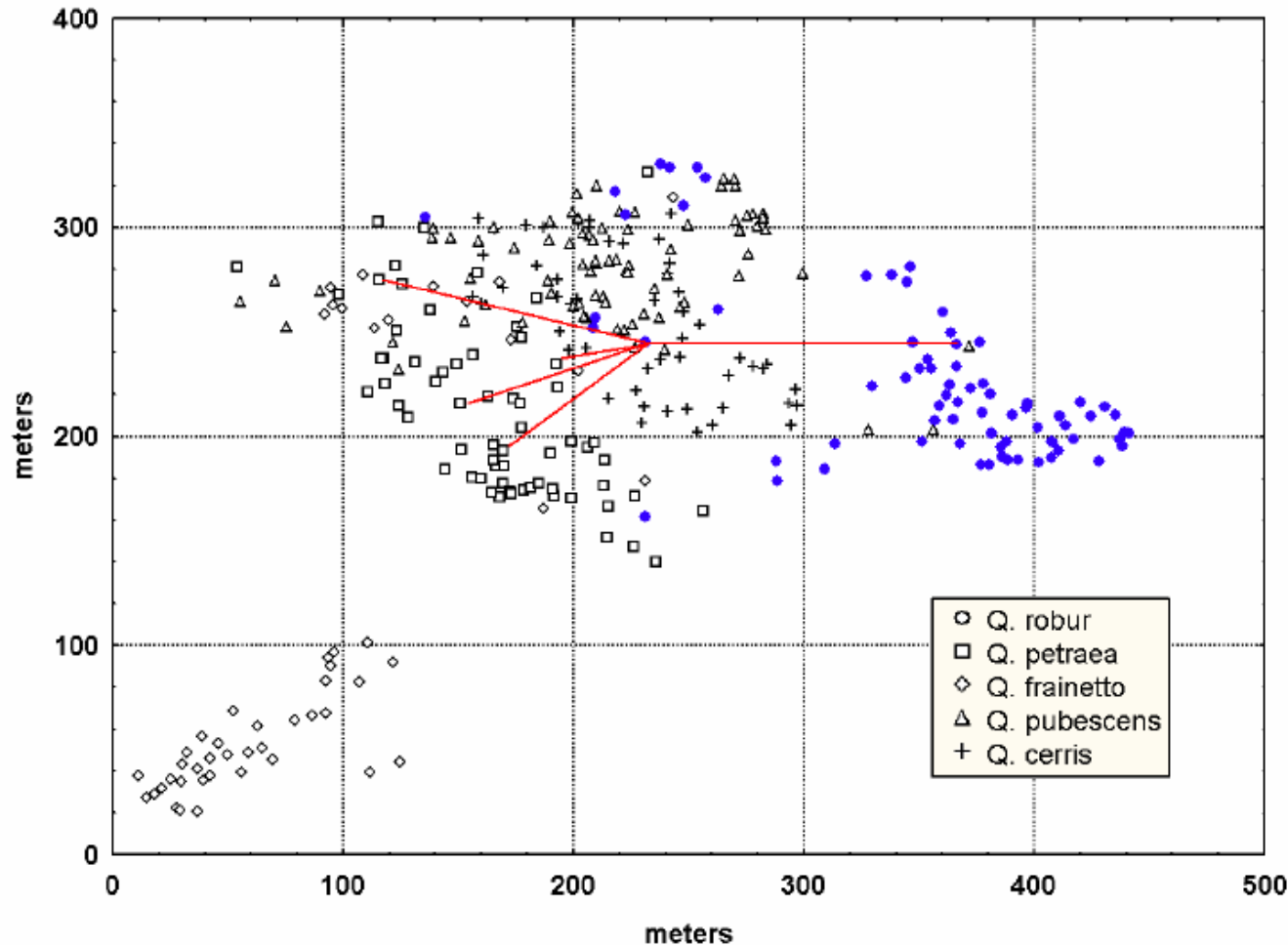
No.	Tree	Sp.	Dist. (m)
1	10	pet	146.2
2	82	pet	24.1
1	110	pet	87.1
1	119	pet	42.9
1	120	pet	36.2
1	268	pet	61.3
1	274	pet	64.1
2	281	pet	60.8
38	-	Mean	65.3
48	8	SD	38.0
<i>Min. outside:</i>			79.20%
<i>Q. petraea:</i>			100.00%

# Seed tree 104 – *Q. petraea*



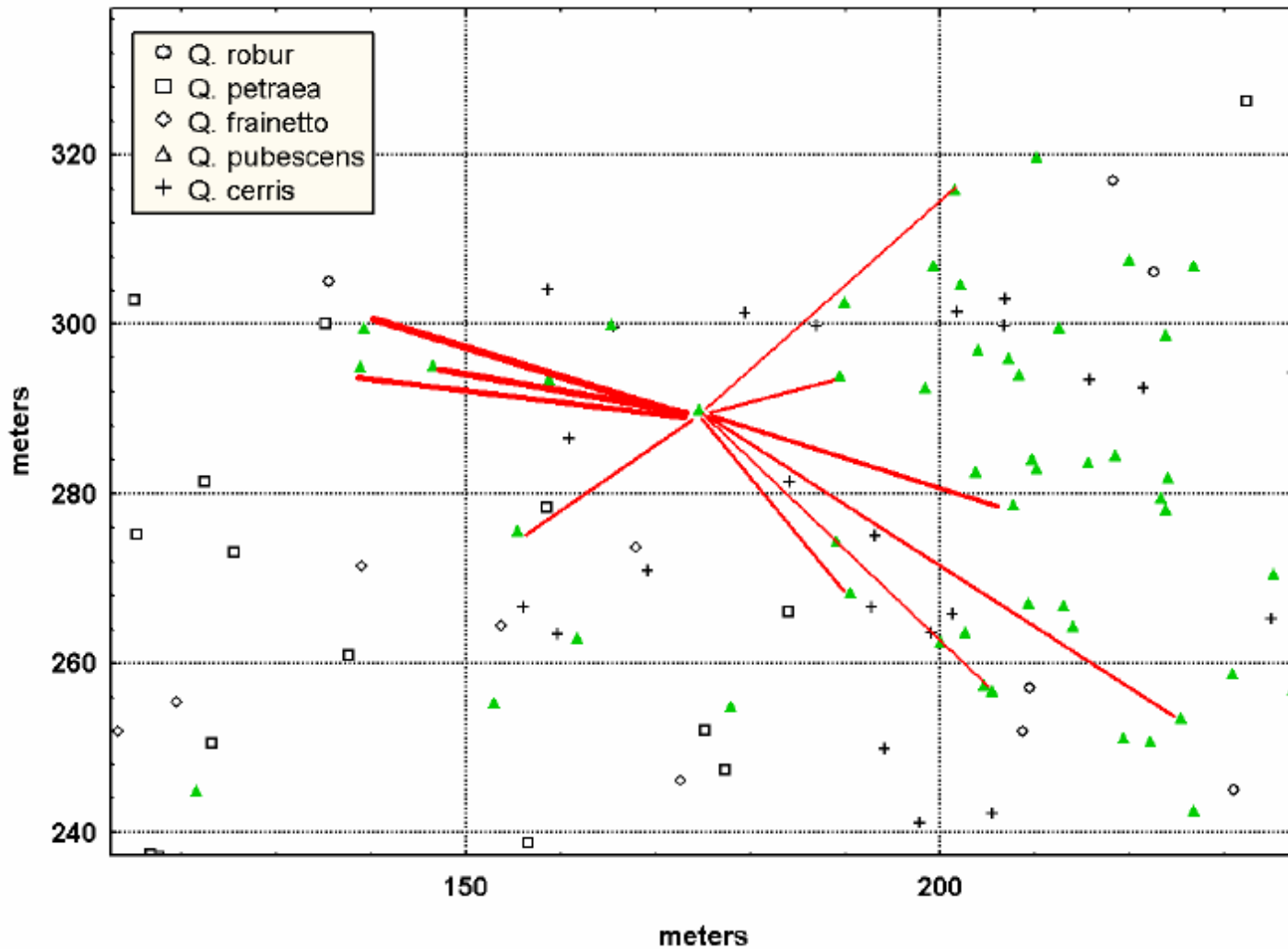
No.	Tree	Sp.	Dist. (m)
1	71	pub	30.6
1	103	pet	5.1
5	105	pub	3.9
5	106	pub	17.3
2*	106/33/29	pub	
1	113	pet	64.2
1	119	pet	31.2
1	237	pub	40.6
1	245	pet	24.6
9	-	Mean	27.2
27	8	SD	19.7
<i>Min. outside:</i>			33.30%
<i>Q. pubescens:</i>			77.80%

# Seed tree 14 – *Q. robur*



No.	Tree	Sp.	Dist. (m)
1	95	rob	135.4
1	113	pet	119.6
1	118*	pet	85.2
1	245	pet	40.0
1	265	pet	80.5
33	-	Mean	92.2
38	5	SD	37.2
<i>Min. outside:</i>			86.80%
<i>Q. robur:</i>			20.00%

# Seed tree 251 - *Q. pubescens*



No.	Tree	Sp.	Dist. (m)
2	2	pub	23.9
7	5 *	pub (!)	28.6
10	6 *	pub	36.6
1	6/235	pub	
1	58	pub	37.5
1	71	pub	45.4
2	75	pub	15.3
4	111*	pub	36.1
2	243	pub	21.2
2	247	pub	62.5
3	255*	pub	34.9
16	-	Mean	34.2
51	10	SD	13.4
<i>Min. outside:</i>			31.40%
<i>Q. pubescens:</i>			100.00%

## Extant gene flow and hybridization

- F<sub>1</sub>–hybrids and introgression (advanced generation hybrids)
  - *Q. petraea* x *Q. pubescens*
  - *Q. frainetto* x *Q. pubescens*
  - *Q. robur* x *Q. petraea*
- Introgression
  - *Q. pubescens* x *Q. robur*
  - *Q. pubescens* x *Q. petraea*
- All species of section *Quercus* involved in hybridization events



## Evidence for selection against hybrids

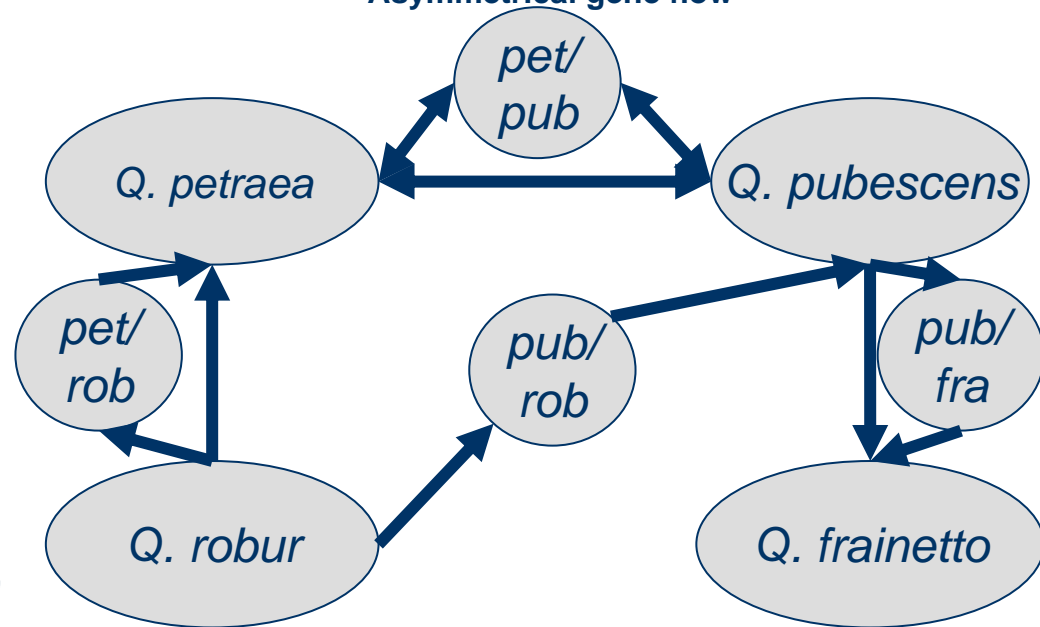
Generation	F <sub>1</sub>	Introgressive forms	Total	Sample size	Percentage (%)
Adult trees	9	59	65	259	25.1
Offspring	10	63	73	162	45.1



# Conclusion: maintenance of adaptive differentiation among species

- Hybridization and introgression confirmed
  - Different affinity for hybridization among species?
- Species identity maintained by
  - Asymmetrical gene flow
  - Selection against (most) hybrids and intermediates; ‘outbreeding depression’
- Maintenance of different adaptive properties of species
- Enhancement of adaptive potential by (rare) hybridization

Asymmetrical gene flow



Selection against hybrids

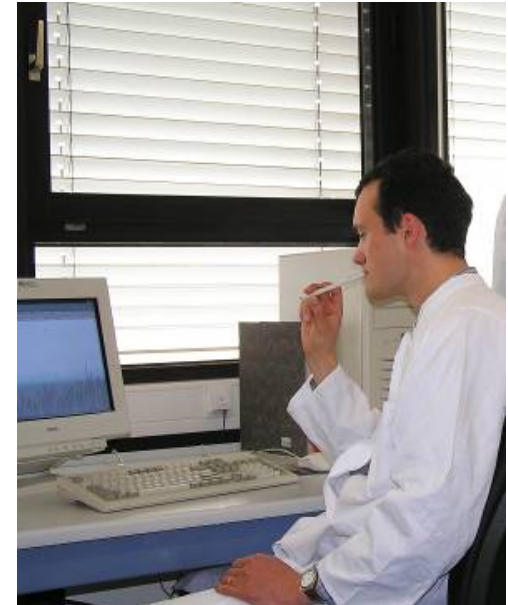
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## Our literature on oaks in the Bejan forest

- Curtu, A.L., Gailing, O., Finkeldey, R. (2007). Evidence for hybridization and introgression within a species-rich oak (*Quercus* spp.) community. *BMC Evolutionary Biology* **7**: 218
- Curtu, A., Gailing, O., Leinemann, L., Finkeldey, R. (2007). Genetic variation and differentiation within a natural community of five oak species (*Quercus* spp.). *Plant Biology* **9**: 116-126
- Curtu, A.-L., Finkeldey, R., Gailing, O. (2004). Comparative sequencing of a microsatellite locus reveals size homoplasy within and between European oak species (*Quercus* spp.). *Plant Molecular Biology Reporter* **22**: 339-346
- Curtu, A.-L., Gailing, O., Finkeldey, R. (submitted) Patterns of contemporary hybridization inferred from paternity analysis in a four-oak-species forest

...and <http://www.uni-goettingen.de/en/82738.html> for related work of the group

Thank you for your attention!



A. Lucian Curtu

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