Development and use of molecular markers for characterisation of Forest Genetic Resources and for Breeding : a case study, Black poplar (Populus nigra L.)

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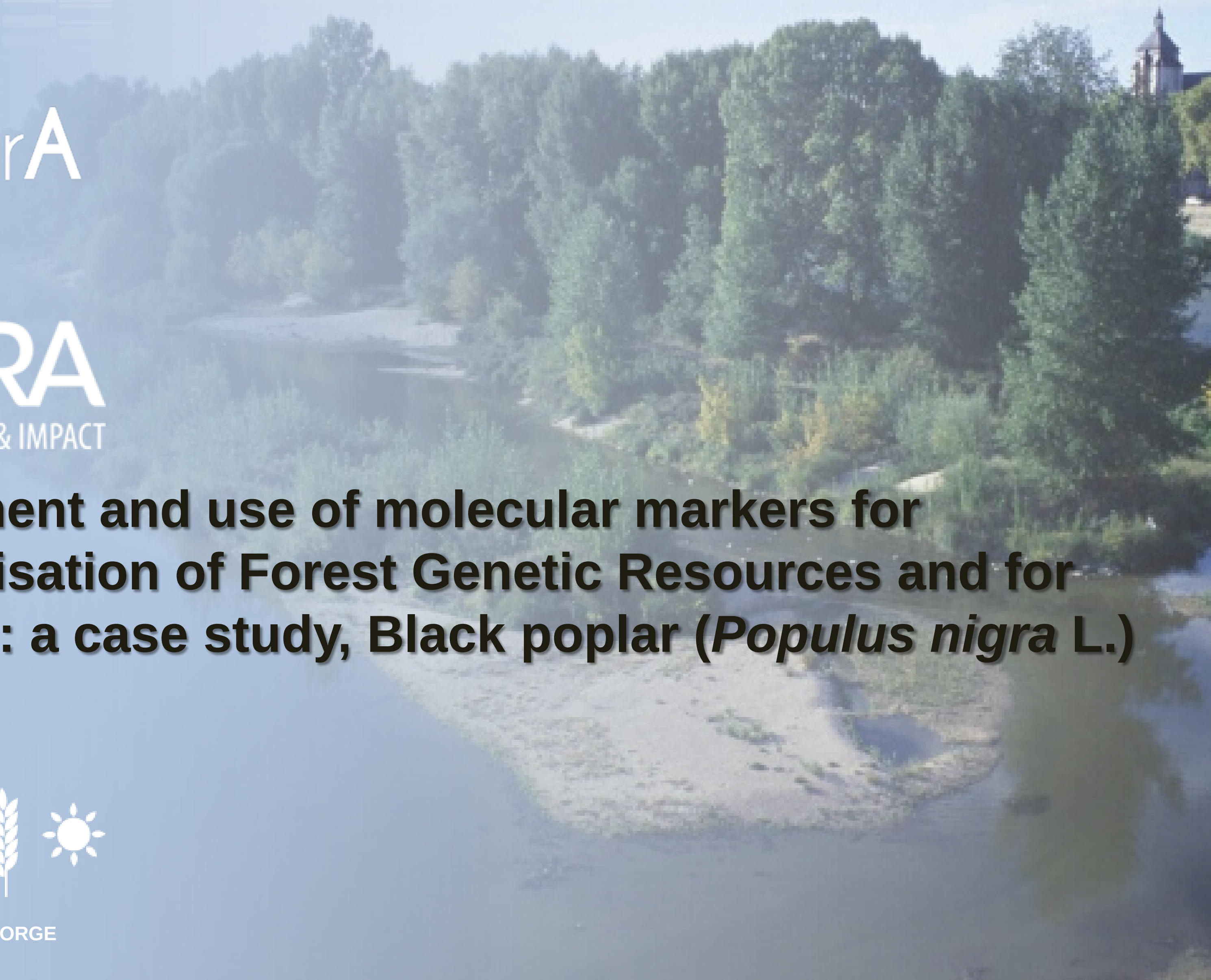


Development and use of molecular markers for characterisation of Forest Genetic Resources and for Breeding : a case study, Black poplar (*Populus nigra* L.)

Véronique JORGE

SUMMARY

1. *Populus nigra*: biology, interest
2. Molecular marker development and genotyping
3. Characterization of Genetic resources
    a. Structuration of diversity
    b. Clonality
    c. Gene flow
4. Breeding

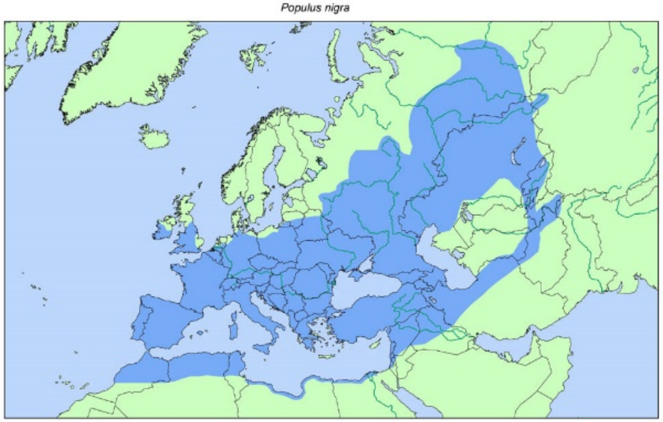
_01

Populus nigra

A key species

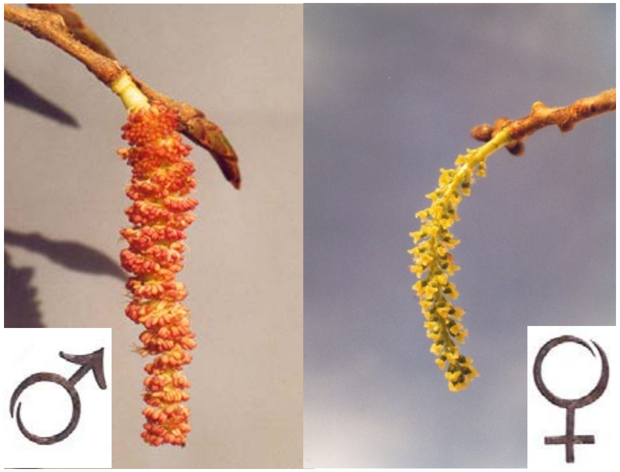
Black poplar (*Populus nigra* L.)

Major component of riparian forests in Europe



Pl.305. Peuplier noir. Populus nigra L.

Wind pollinated dioecious species



Black poplar (*Populus nigra* L.)

Threats

- **Agriculture**
- **Confinement of rivers, dams regulation (no flood)…**
- **Gene flow from cultivated poplars**

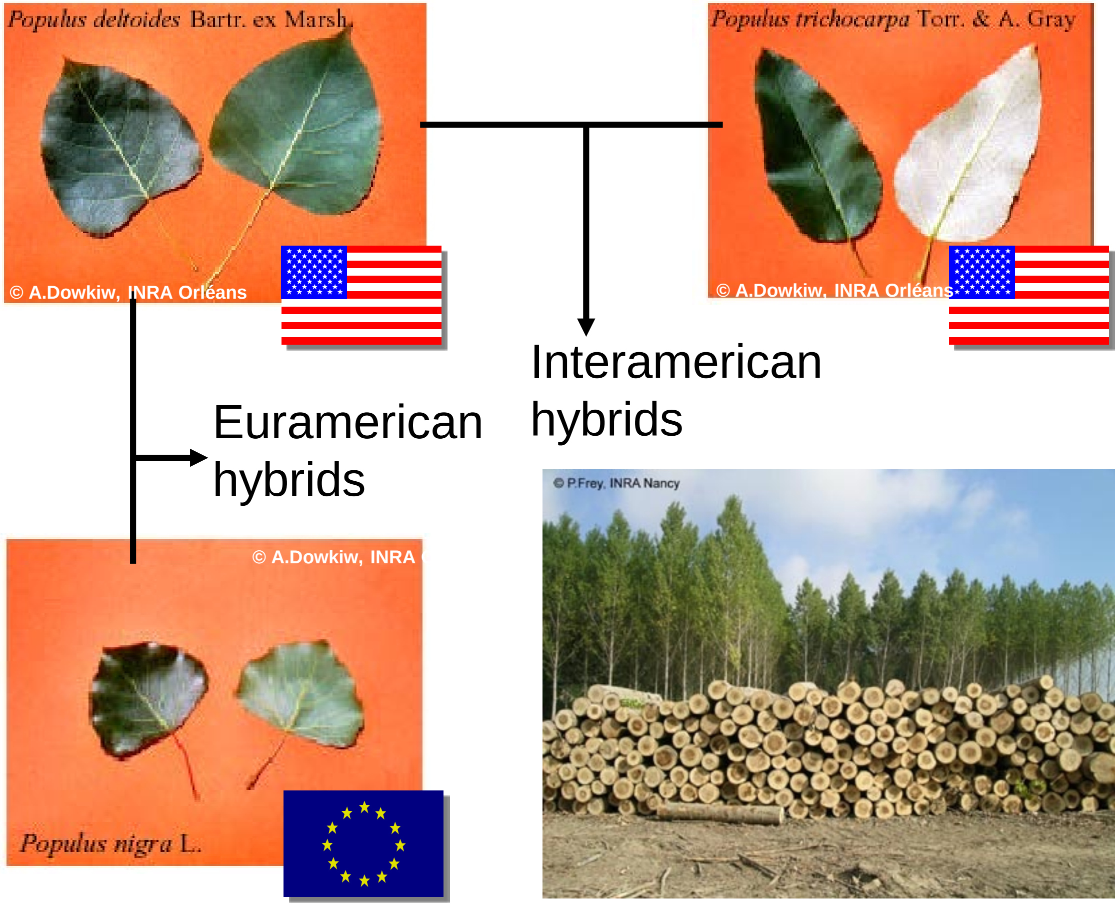
Loire River at Orléans

Villerest dam (France)

© P.Frey, INRA Nancy

Black poplar (*Populus nigra* L.)

Use for breeding interspecific cultivated hybrids



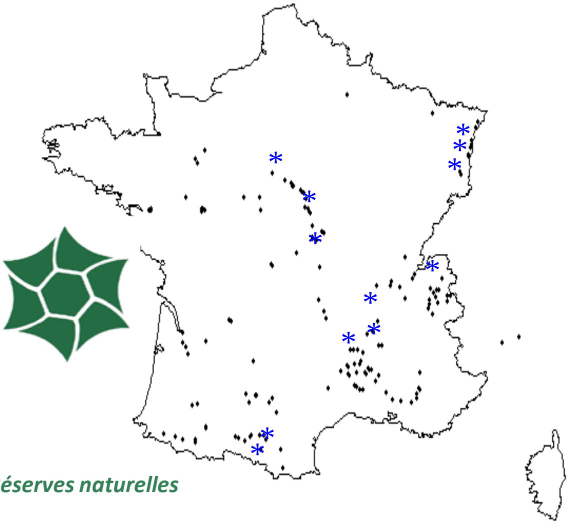
Interamerican hybrids

Euramerican hybrids

Black poplar (*Populus nigra* L.)

Conservation program at national and European level

Ex situ and *in situ* conservation



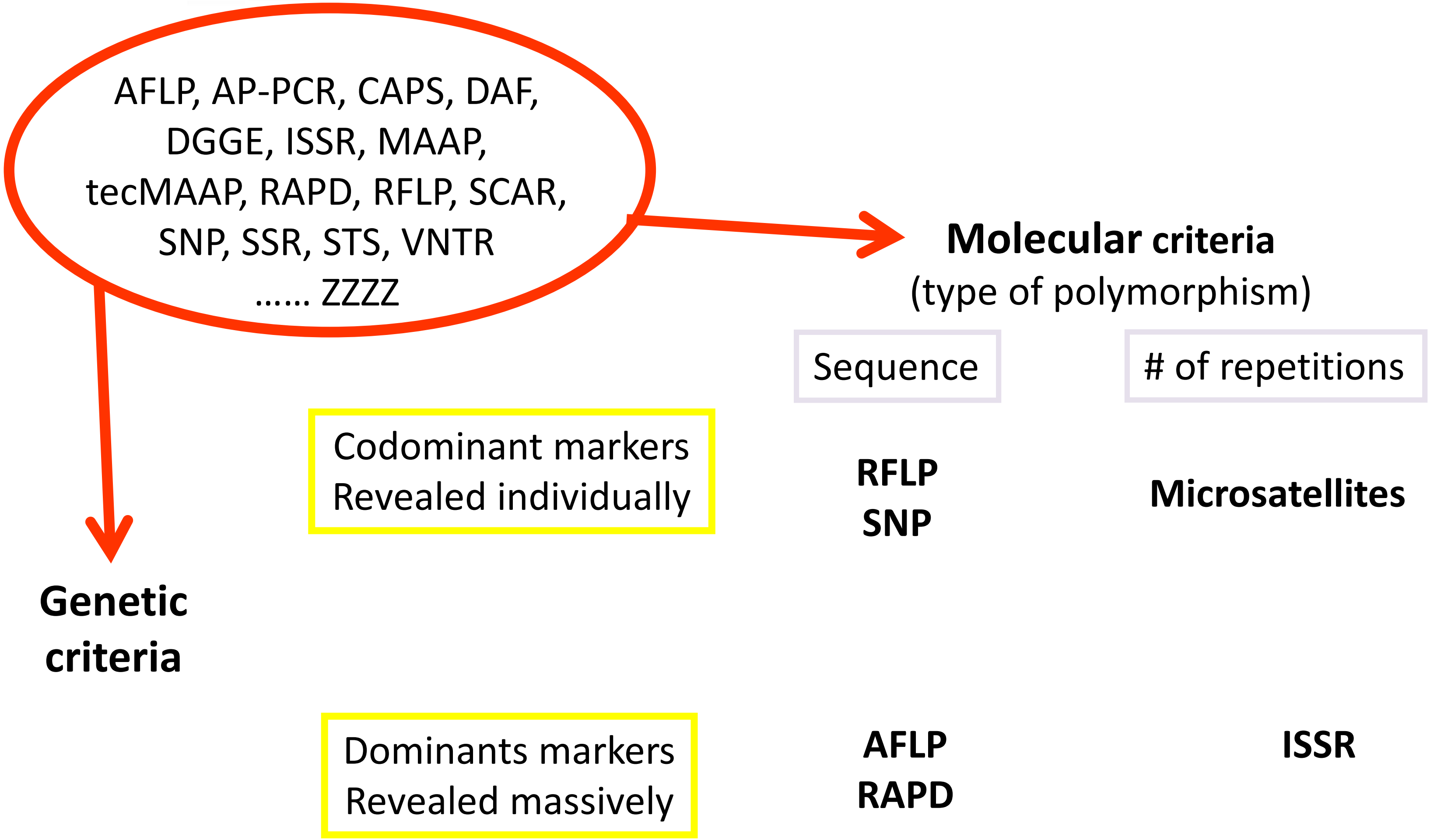
12 Réserves naturelles

EUFORGEN

_02

Marker development

Classification of molecular markers



AFLP, AP-PCR, CAPS, DAF,
DGGE, ISSR, MAAP,
tecMAAP, RAPD, RFLP, SCAR,
SNP, SSR, STS, VNTR
…… ZZZZ

Molecular criteria
(type of polymorphism)

Genetic criteria

	Sequence	# of repetitions
Codominant markers Revealed individually	**RFLP** **SNP**	**Microsatellites**
Dominants markers Revealed massively	**AFLP** **RAPD**	**ISSR**

Co-dominant markers, different type of polymorphism

SSR

Multiallelic, ~4000 available on the reference genome *Populus trichocarpa*

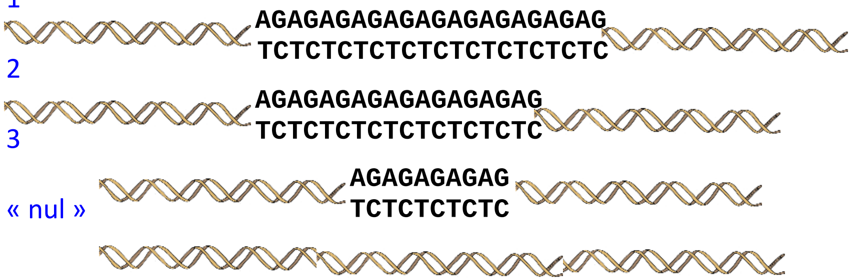
Allèle 1

Allèle 2

Allèle 3

Allèle « nul »

…



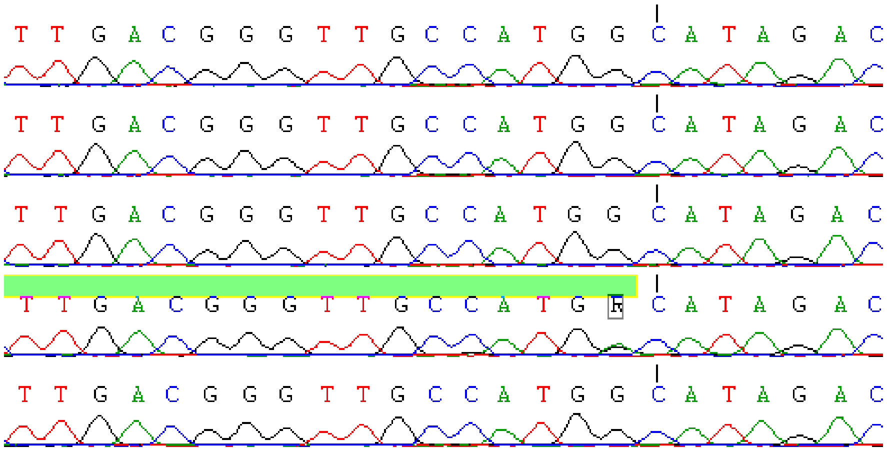
AGAGAGAGAGAGAGAGAGAGAG
TCTCTCTCTCTCTCTCTCTCTC

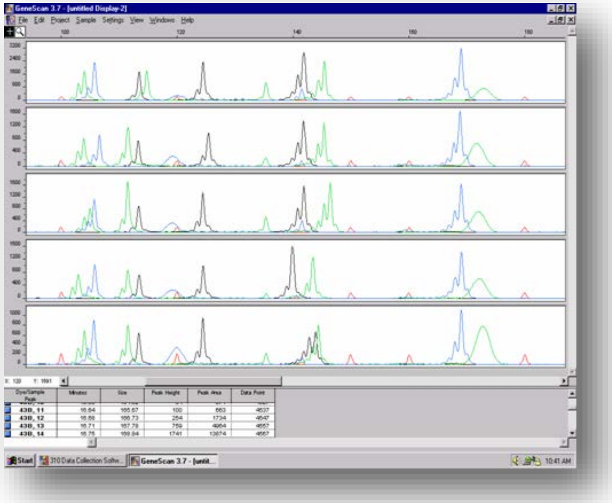
AGAGAGAGAGAGAGAGAG
TCTCTCTCTCTCTCTCTC

AGAGAGAGAG
TCTCTCTCTC

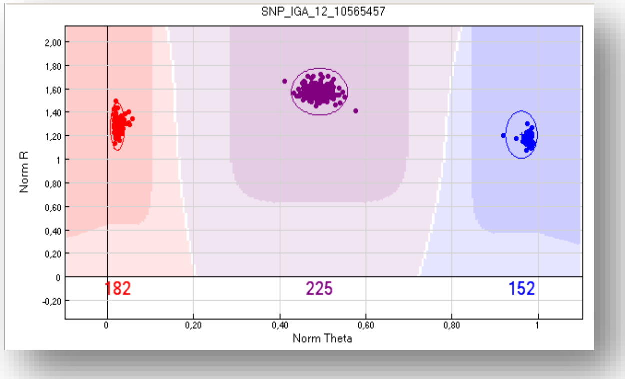
SNP

Biallelic, seveal millions (in poplar 1 SNP/ 90 pb)



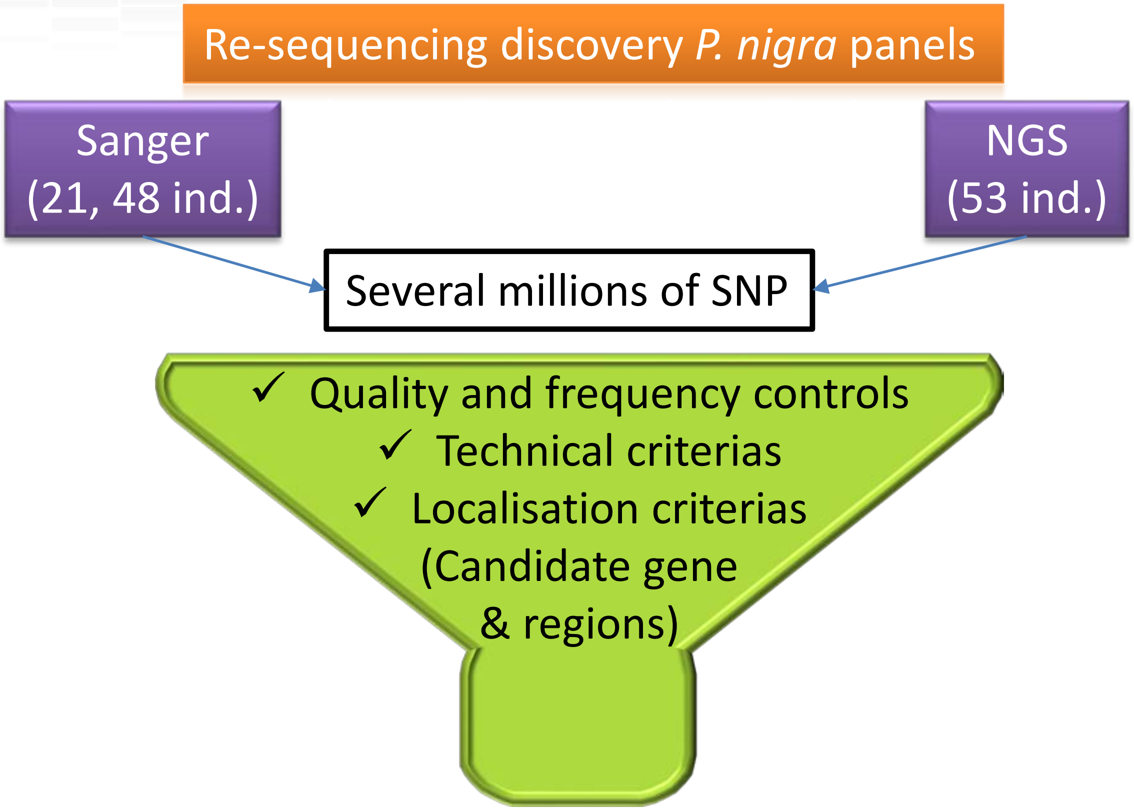


Genotyping
Many techniques



Development of SNP markers:

2 steps : discovery, genotyping



Re-sequencing discovery *P. nigra* panels

Sanger
(21, 48 ind.)

NGS
(53 ind.)

Several millions of SNP

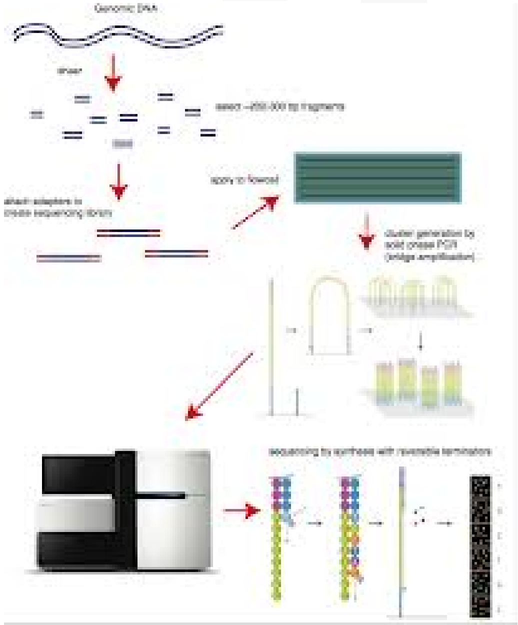
- ✓ Quality and frequency controls
- ✓ Technical criterias
- ✓ Localisation criterias (Candidate gene & regions)

10 332 SNP bead-chip used for genotyping a *P. nigra* association population
9127 SNPs technically available
8259 polymorphic SNP

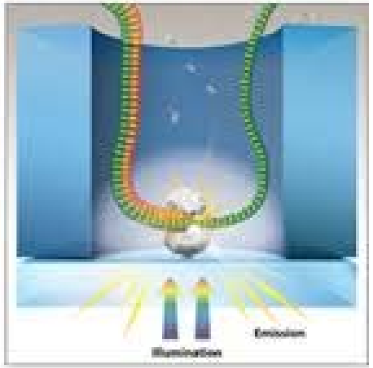
(Faivre-Rampant et al, Mol Ecol Res 2016)

Next Generation Sequencing (NGS)

1 step : discovery and genotyping



Hiseq Illumina



SMRT – Pacific bioscience

ion torrent
by Life technologies

New opportunity for characterization of forest genetic resources?

_03

Characterization of Genetic Resources

_03a

Characterization of Genetic Resources

Structuration of diversity

Management of Forest Genetic Resources

Specific aspects

Ex situ

In situ

Question

❖ How to sample (define a network, populations, individuals).

To answer

Knowledge of existing variability, structuration and evolution potential

How to measure genetic diversity?

Measures *in situ*

Sex, phenology

Measures *ex situ* :

Growth and biomass
Foliar traits
Phenology
Wood properties

Pest resistance

Neutral

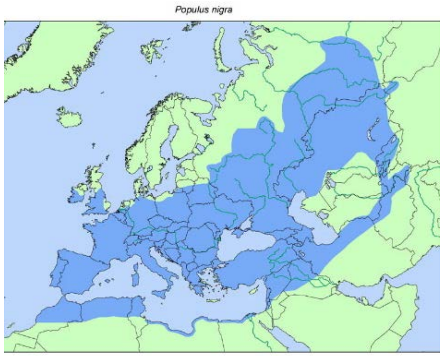
Molecular markers

SSR
SNP

How to measure genetic diversity?

Measures *ex situ*

Sampling in natural area



(Riparian forest)

Comparison in 1 or several environments

Clonal trial

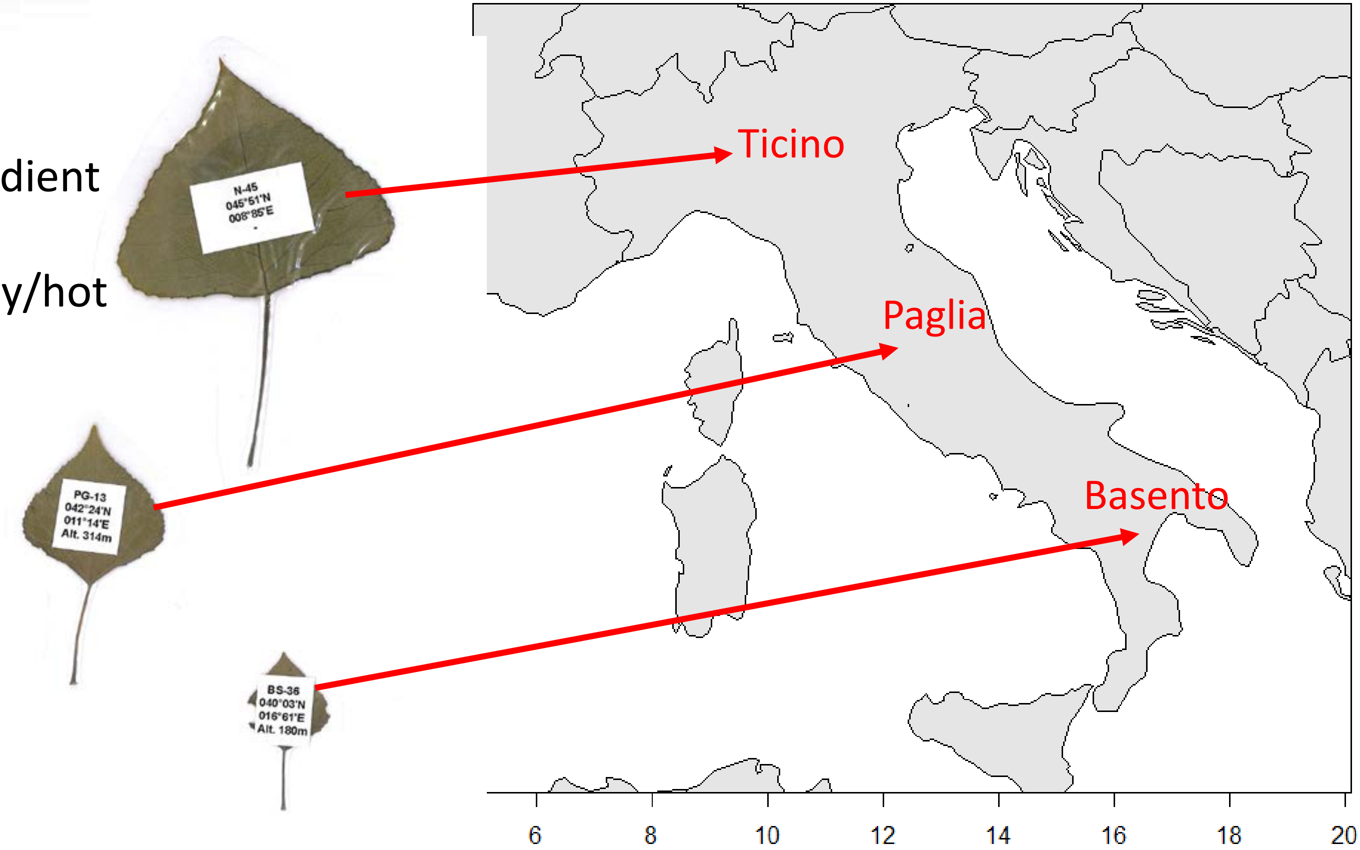
Observations/Measures

For ex. : Leaf size

How to measure genetic diversity?

Leaf size variation reflect local adaptation

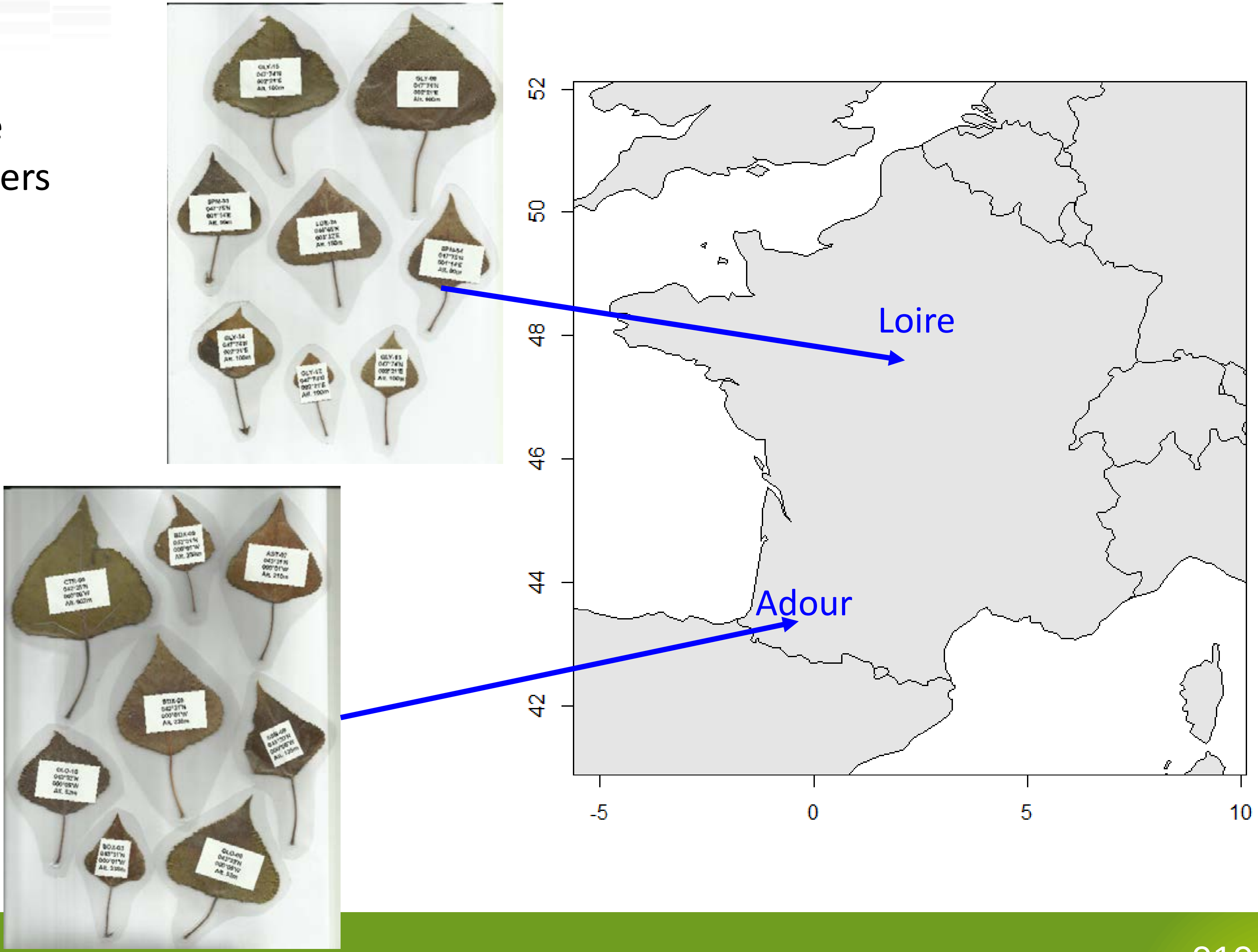
- Origin : Italy
- North-South gradient
- Small leaves = adaptation to dry/hot climate



How to measure genetic diversity?

Leaf size variation reflect population dynamics

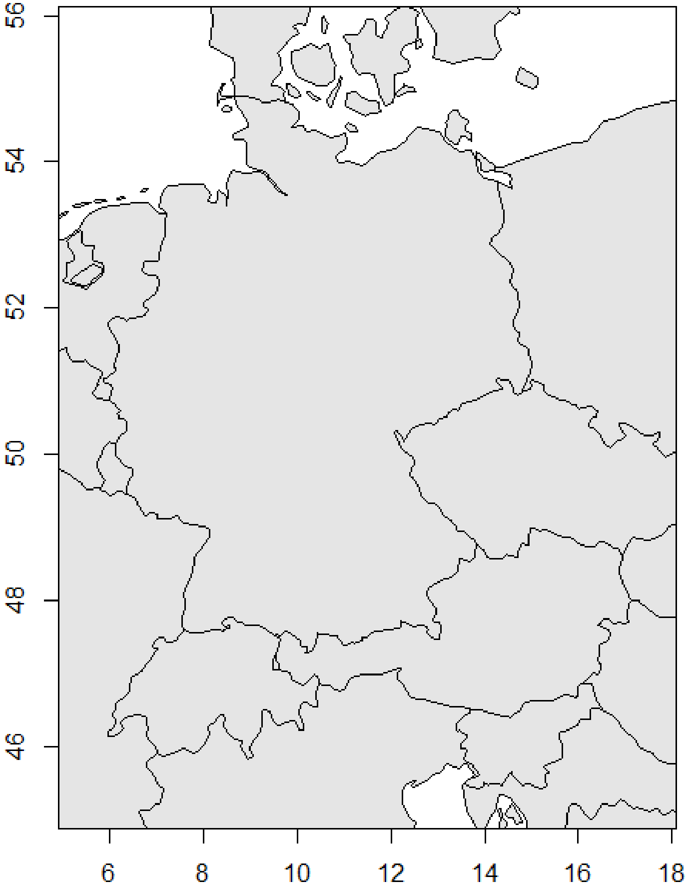
- Origin : France
- 2 dynamics rivers systems



How to measure genetic diversity?

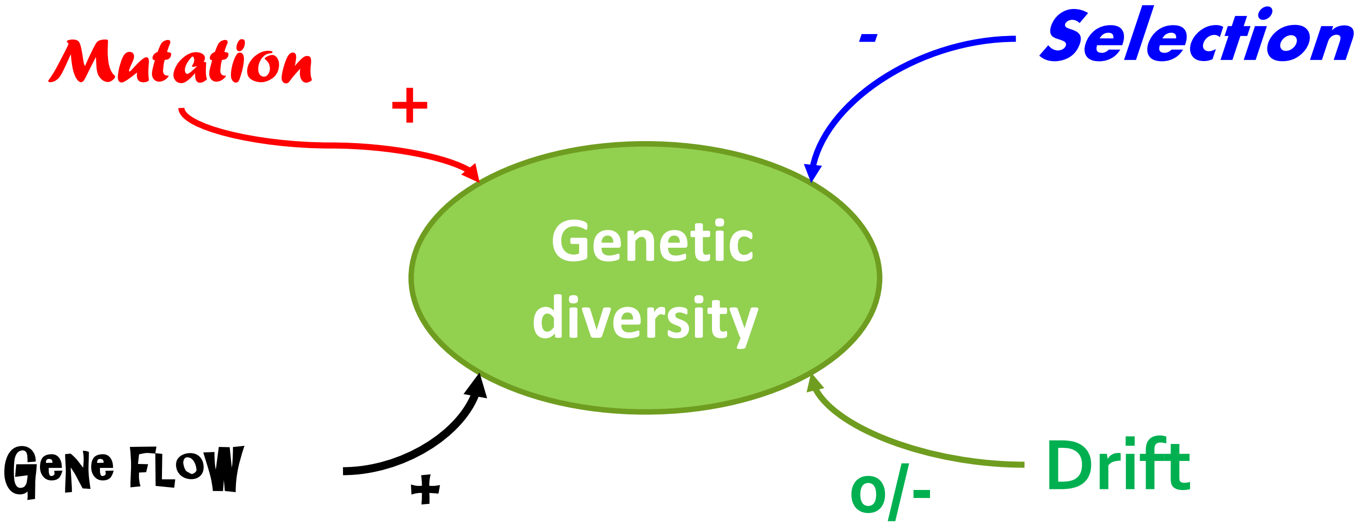
Leaf size variation reflect population dynamics

- Origin : Germany
- Rhine: no river dynamics



How to measure genetic diversity?

What evolutionary forces have an impact on diversity ?



Mutation

+

Selection

-

Genetic diversity

Gene Flow

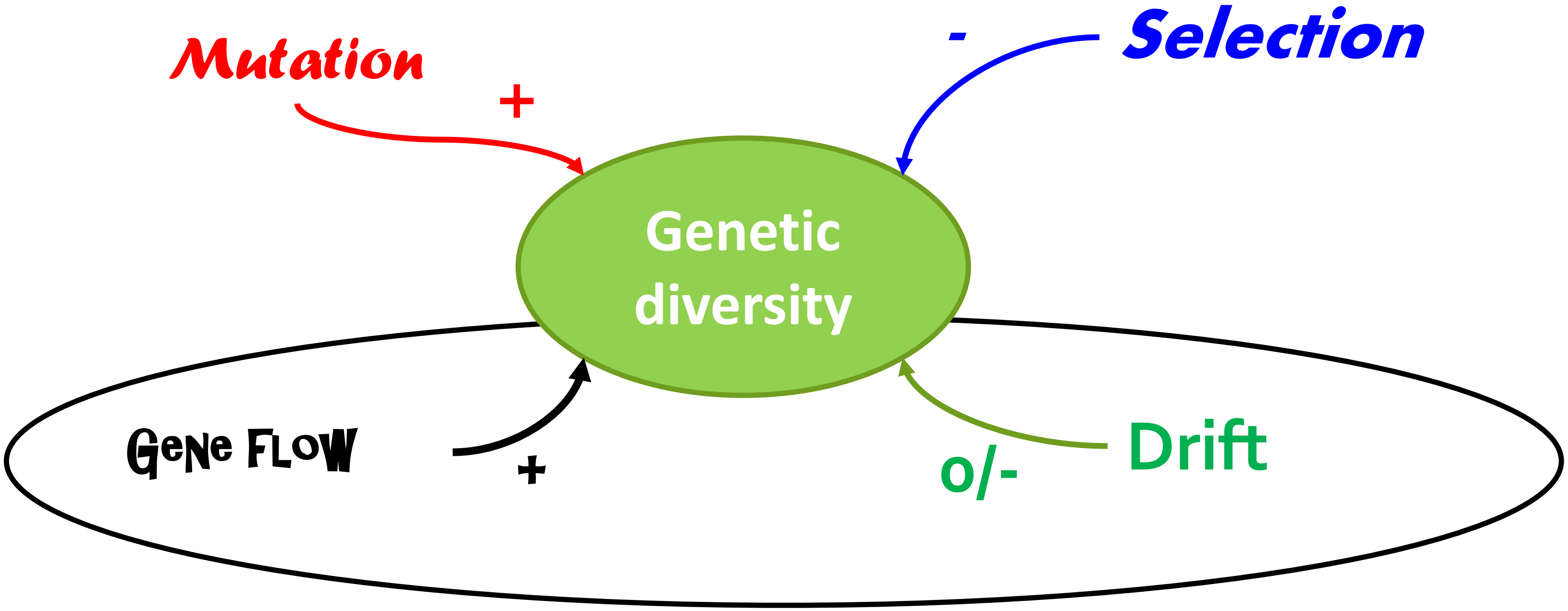
+

Drift

o/-

How to measure genetic diversity?

Measure genetic diversity with molecular markers



In general….

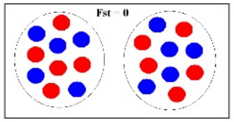
Diversity descriptors

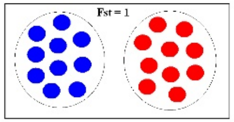
Examples

- **Expected heterozygosity (*He*)**
  - ☐ Probability for 2 alleles randomly drawn to be different
  - ☐ An estimator : $\widehat{H} = 1 - \sum x_i^2$ ← Frequence of i allele
  - ☐ Vary from 0 to 1

- **Genetic distances**
  - ☐ Degree of genetic divergence between populations.
  - ☐ Nei (1978), ...

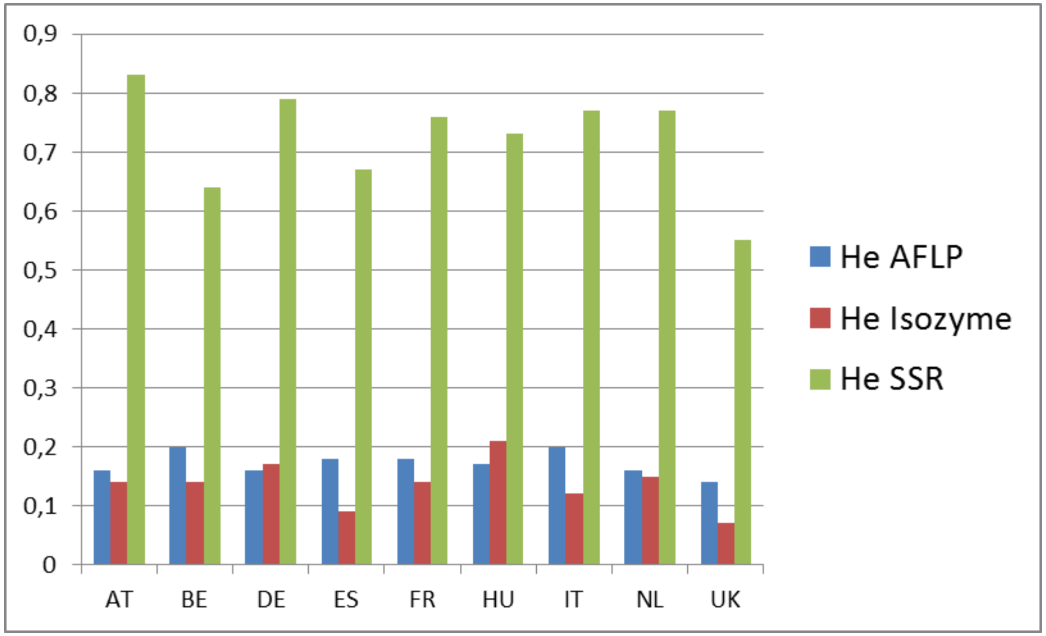
- **Fst**
  - ☐ Loss of heterozygotes due to sous-population subdivision
  - ☐ An estimator : $F_{st} = \frac{Ht - Hs}{Ht}$
  - ☐ Vary from 0 (no differences bewteen sub-populations) to 1 (populations completely different)

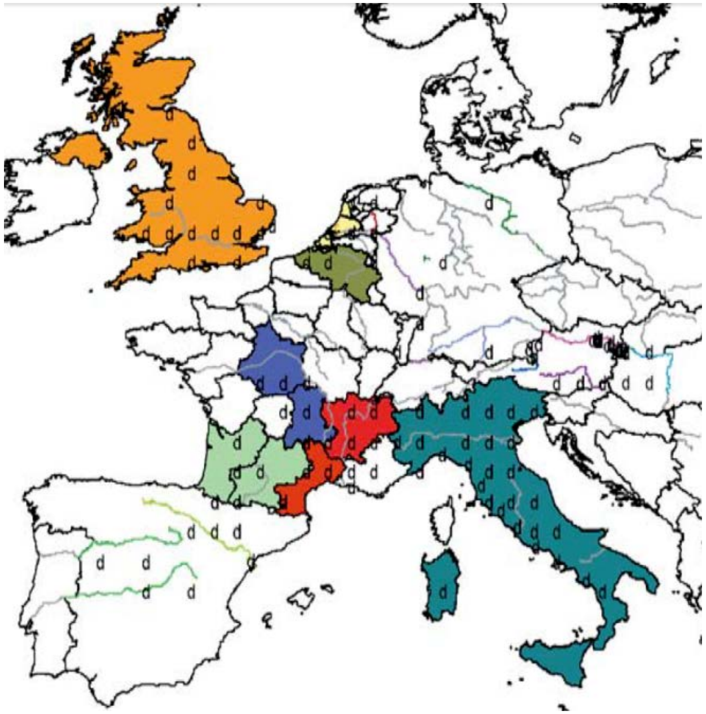




Diversity descriptors and type of markers

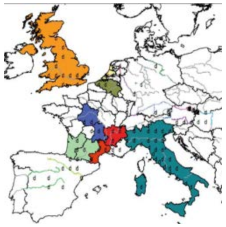
Populus nigra





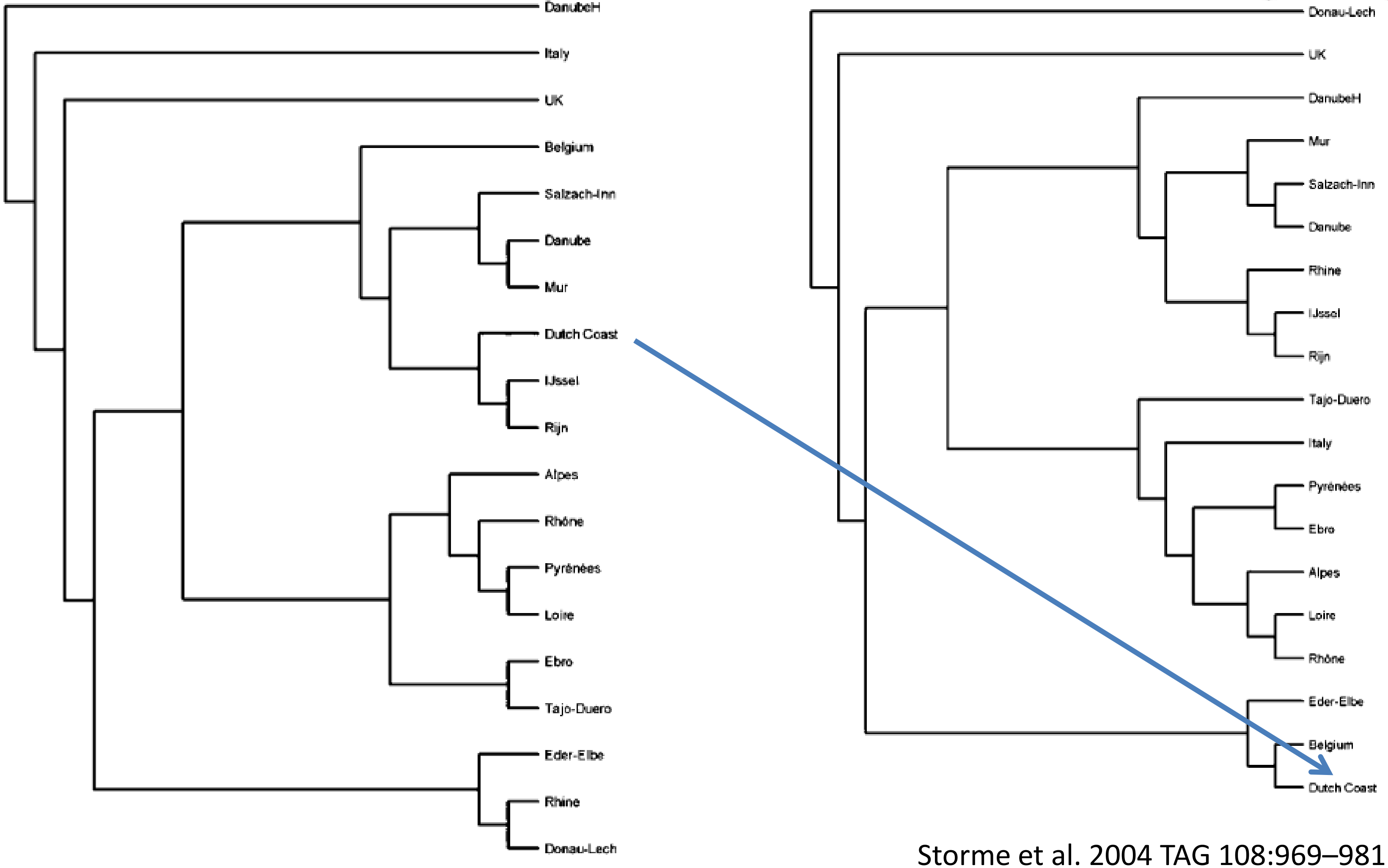
Storme et al. 2004 TAG 108:969–981

Structuration variable depending on type of marker



AFLP

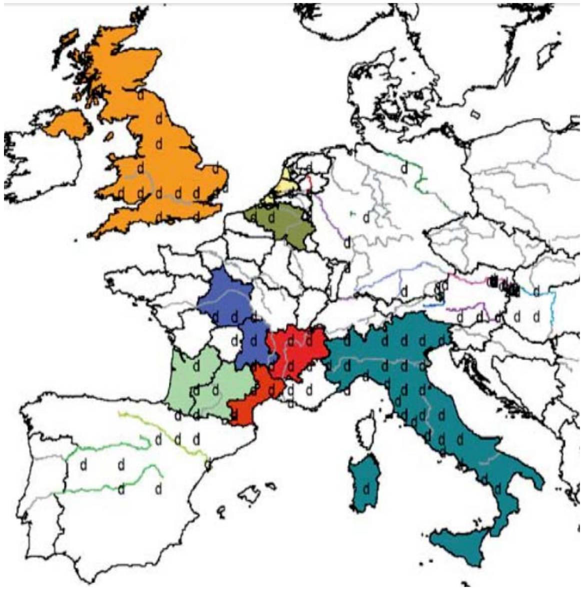
SSR + Isozymes



Storme et al. 2004 TAG 108:969–981

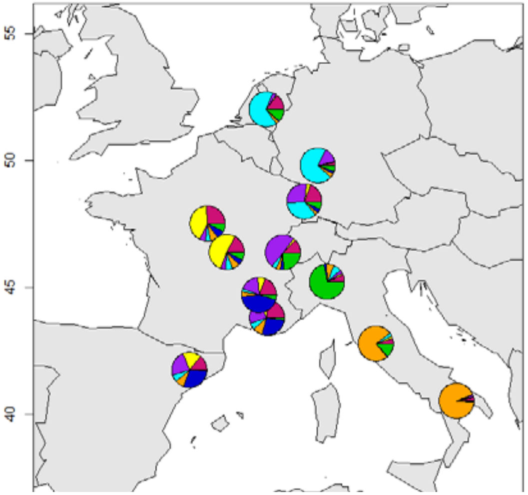
Structuration variable depending on type of marker

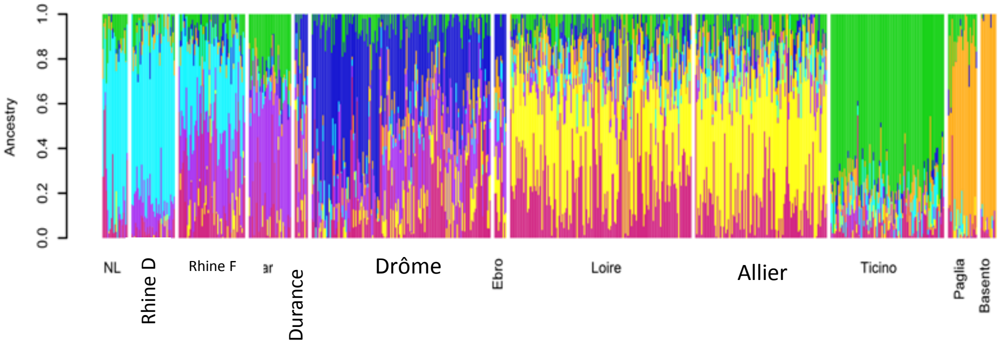
19 populations
5 SSR
Fst = 0.11



(Storme et al. 2004 TAG 108:969–981)

12 populations
600 SNP
Fst = 0.058 (Jost D)



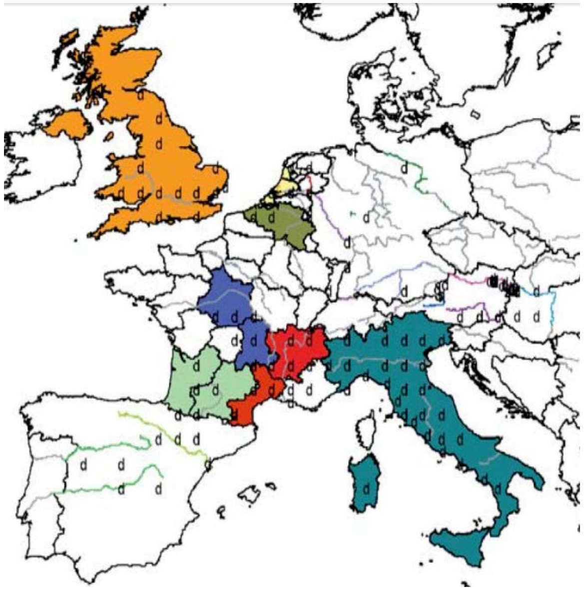


(Faivre et al 2016, Mol Ecol Res)

Structuration variable depending on geographical scale

Fst

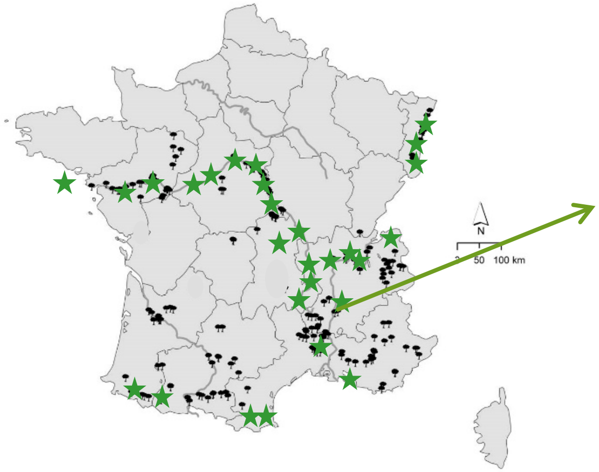
0.11



19 populations
5 SSR

Storme et al. 2004 TAG 108:969–981

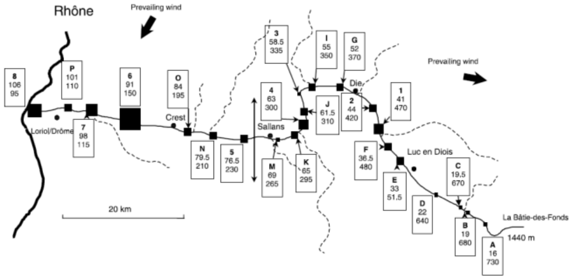
0.0391



8 populations
10 SSR

Jorge et al, unpublished

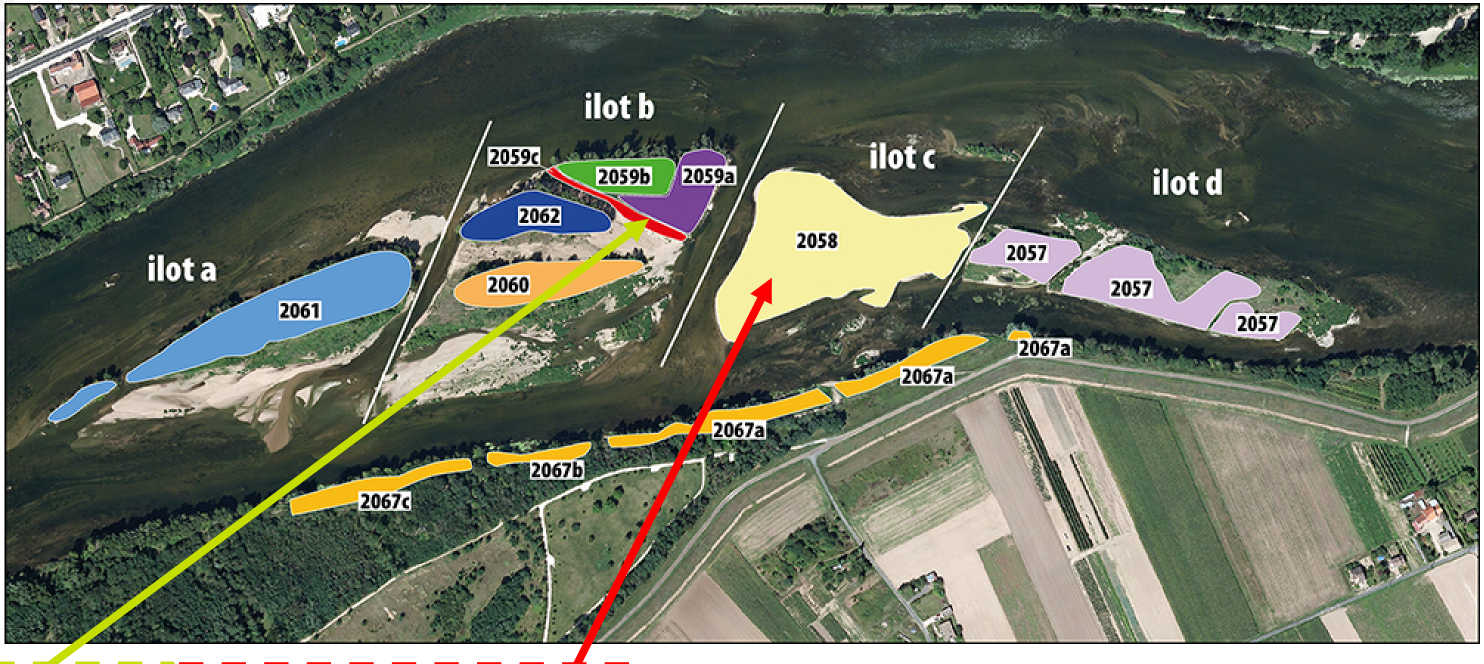
0.047



22 populations
6 SSR

Imbert et Lefevre, 2003
Journal of Ecology 91: 447–456

Structuration variable depending on geographical scale



Photos M. Villar

32 seedlings x 51 spots x 5 ecotopes
10 SSR

Fst (between ecotopes) = 0,017**

Project Biomareau
(Marin et al., 2014; Poster IPSVI)

_03b

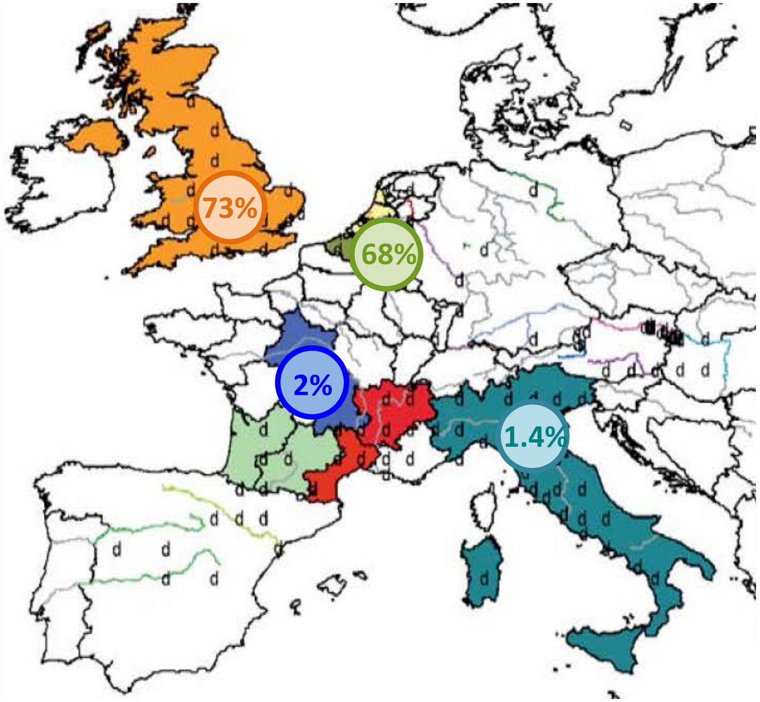
Characterization of Genetic Resources

Clonality

Estimation of rate of clonality in *Populus nigra* with molecular markers

- Poplars are easy vegetative propagation tree species.
- In wild stands, clonalty rate vary between 0% to 97% (proportion of identical genotype individuals [1]).
- Example : in national *ex situ* collection (Storme et al 2004):

Markers SSR, AFLP

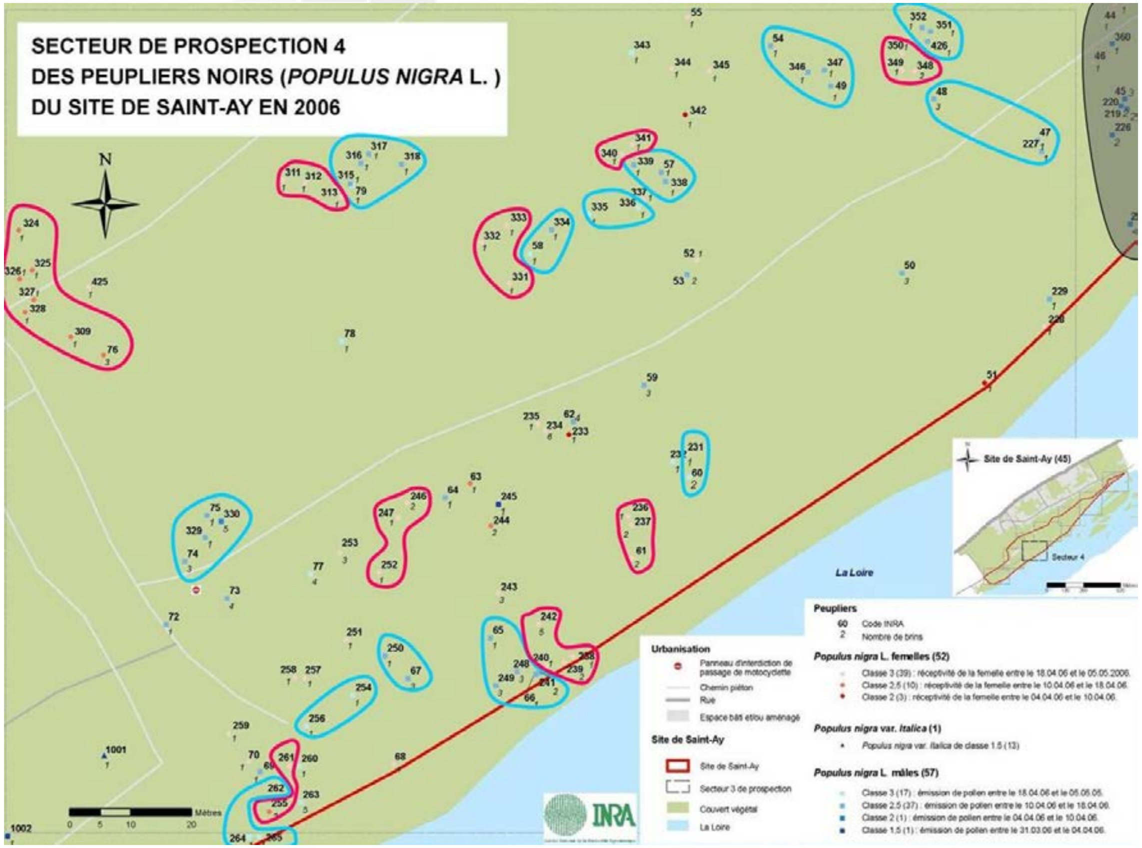


Resprout form tree stump, Populus spp.,Canada

[1] Arens et al. 1998; Barsoum 2002; Barsoum et al. 2004; Cottrell et al. 1997; Koskela et al. 2004; Legionnet 1997; Pospiskova and Bartakova 2004; Pospiskova and Salkova 2006; Rathmacher et al. 2010; Smulders et al. 2008b; Storme et al. 2004; Winfield et al. 1998

Estimation of rate of clonality in *Populus nigra* with molecular markers

11 SSR markers, in situ




© N. Chenault

- Identification of **clonal spots**
- Human action, floods?

Estimation of rate of clonality in *Populus nigra* with molecular markers

Power of markers

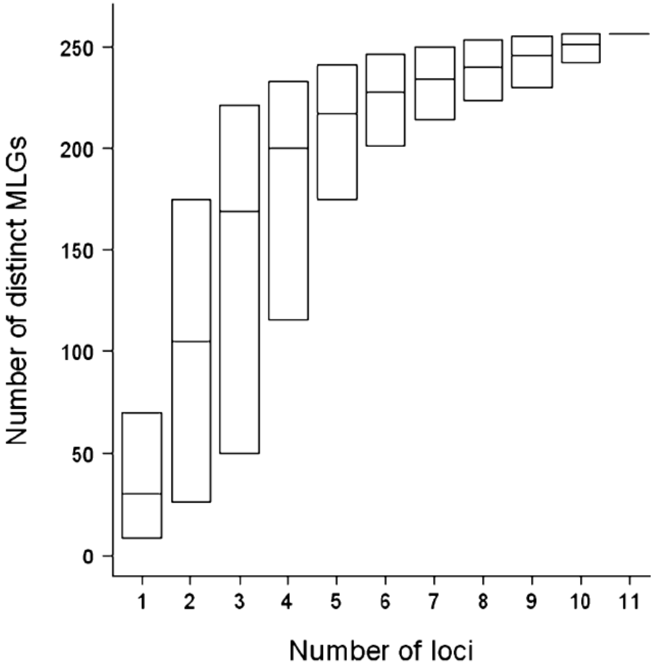
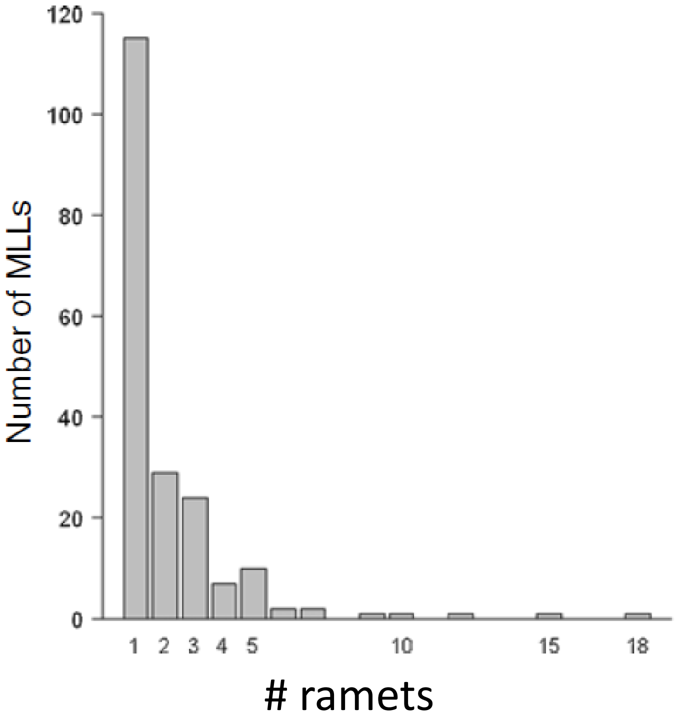


Fig. 2 Genotypic resolution associated with each possible SSR combination. The *boxes* are bounded by the most and least informative combinations of loci. The *inner line* represents the mean value



MLG : Multi Locus Genotype ; MLL : Multi Locus Lineage

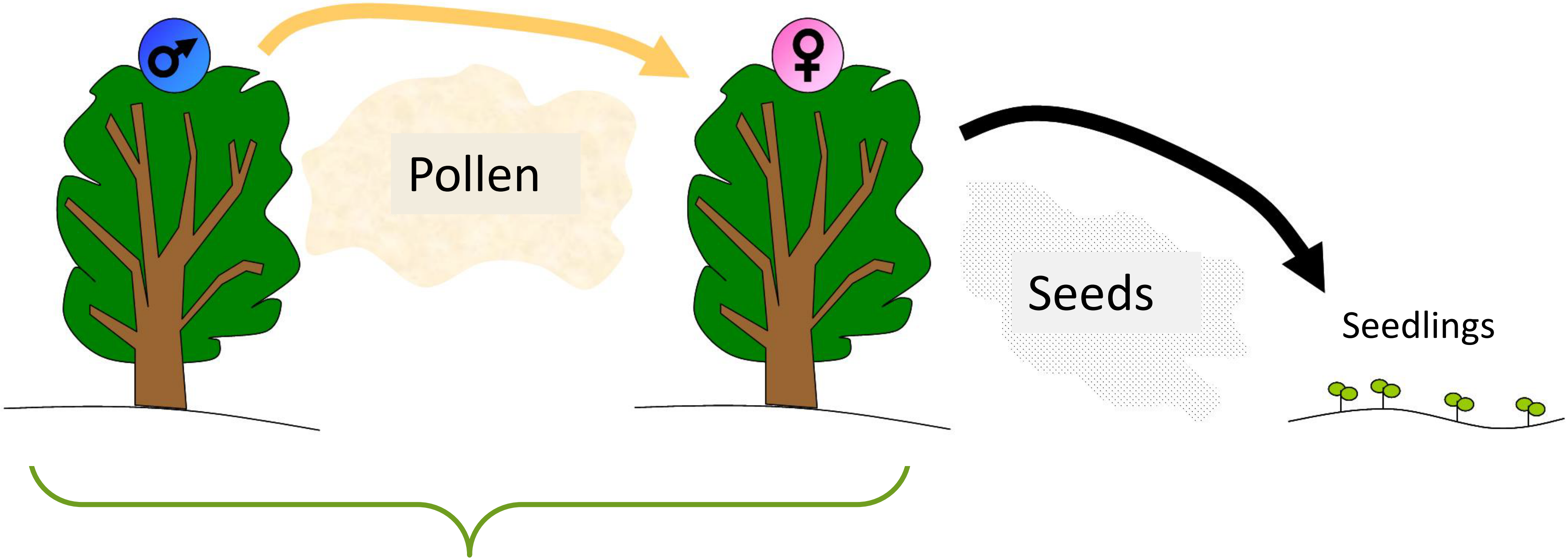
(Chenault et al 2011, TGG)

_03c

Characterization of Genetic Resources

Gene flow

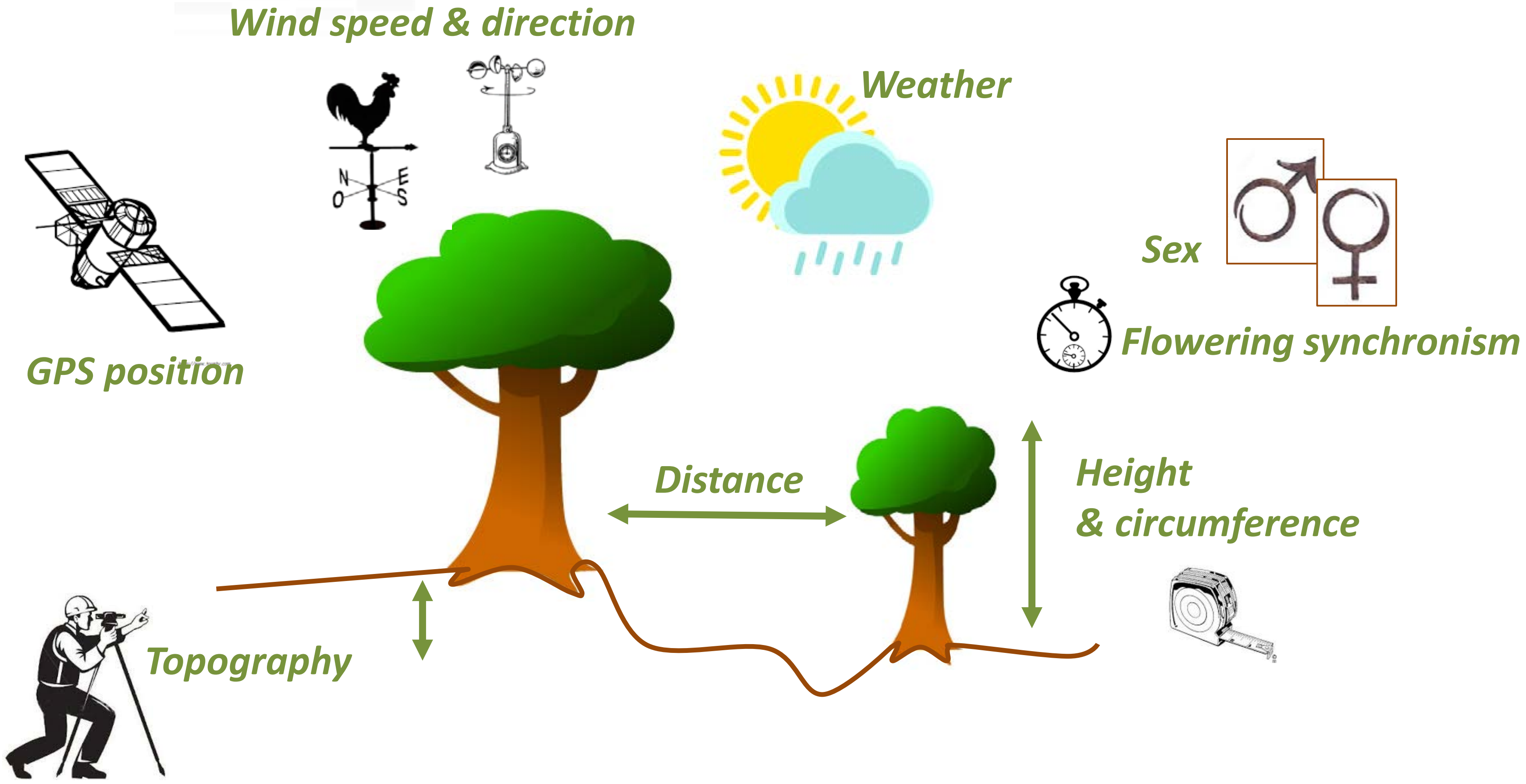
Gene flow



Pollen flow

Models for gene flow

Parameters



Wind speed & direction

Weather

Sex

Flowering synchronism

GPS position

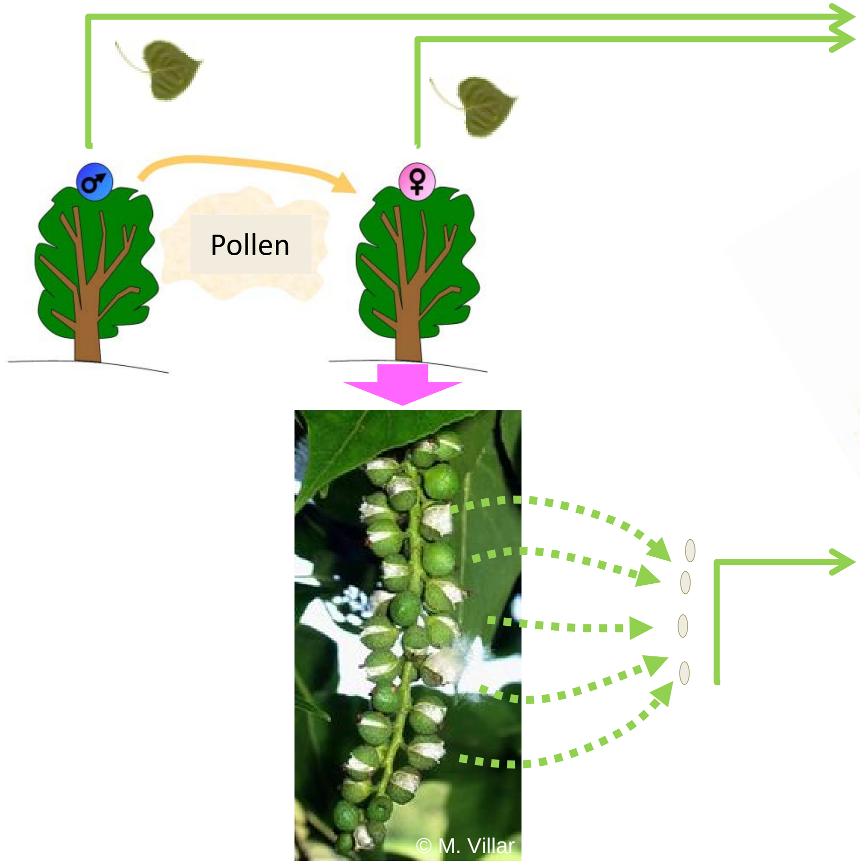
Distance

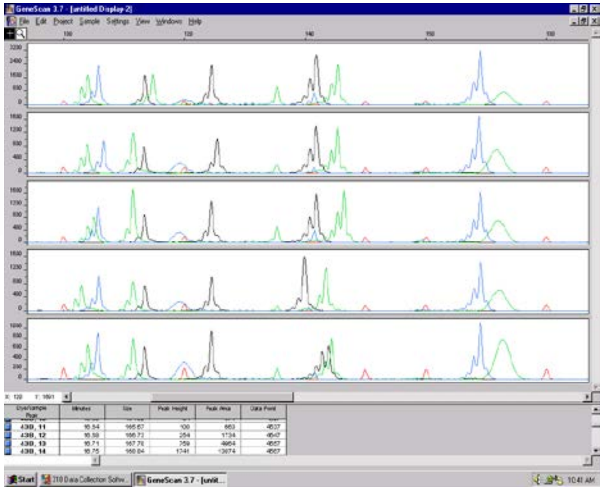
Height
& circumference

Topography

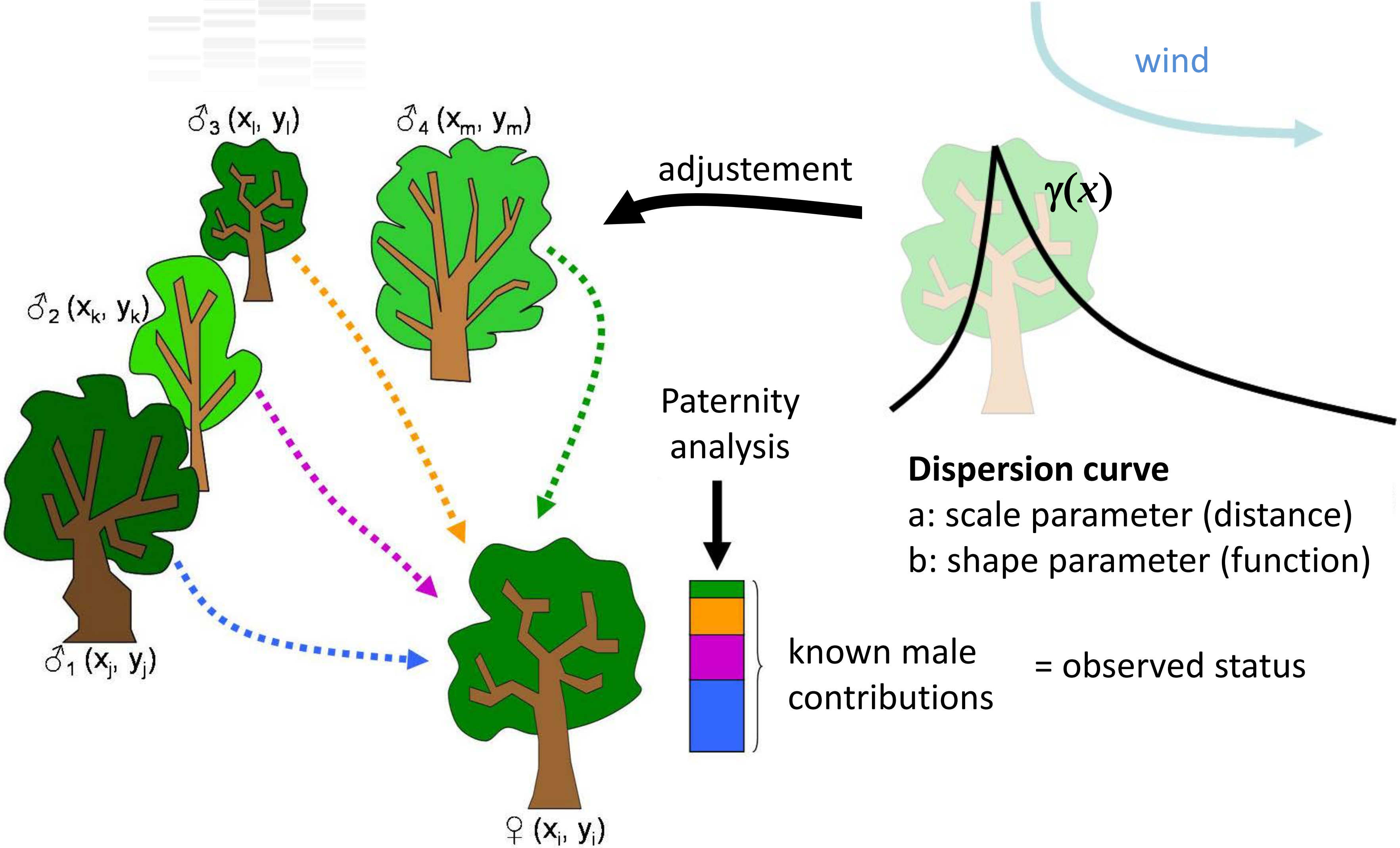
Models for gene flow

Paternity analysis with molecular markers





Models for gene flow



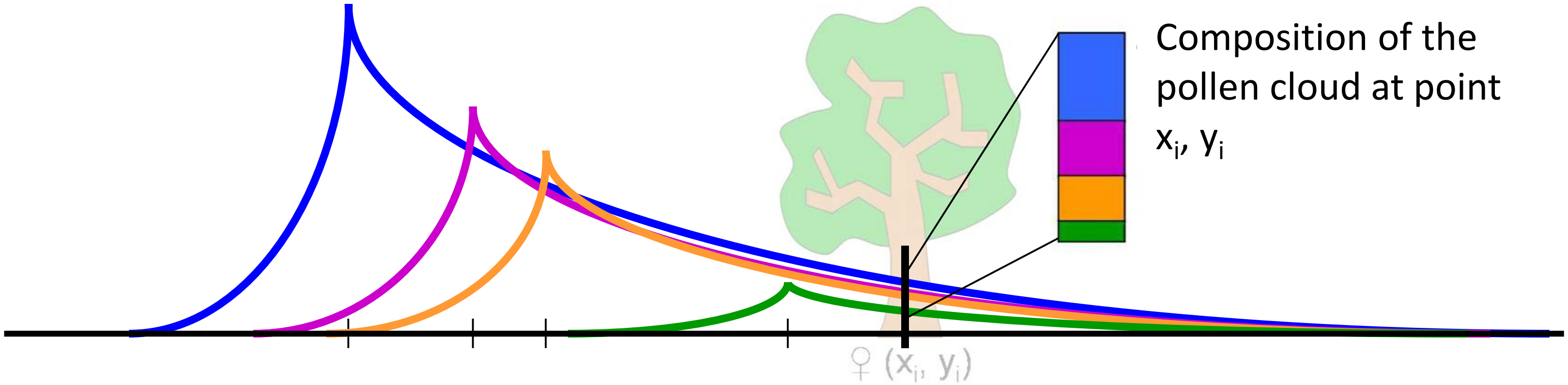
adjustement

Paternity analysis

Dispersion curve
a: scale parameter (distance)
b: shape parameter (function)

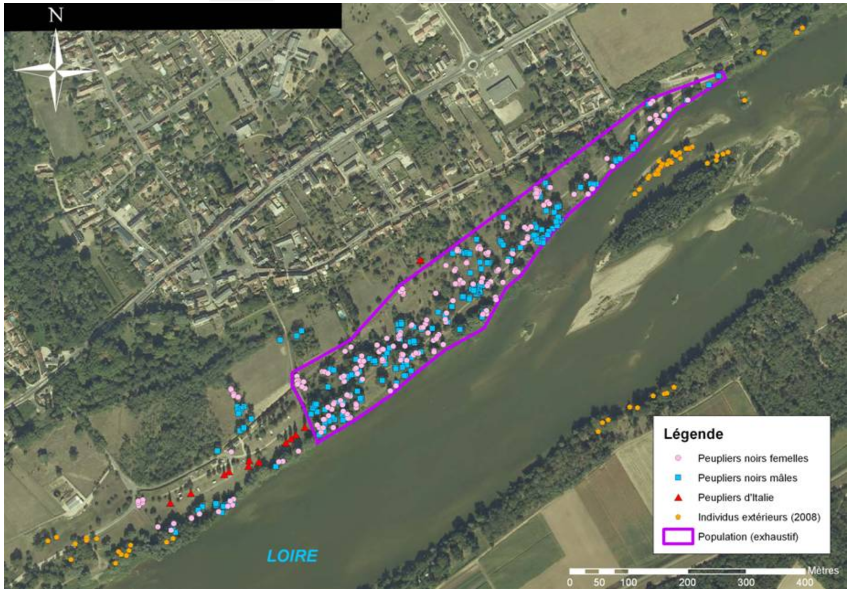
known male contributions = observed status

Models for gene flow



Composition of the pollen cloud at point x_i, y_i

Models for gene flow

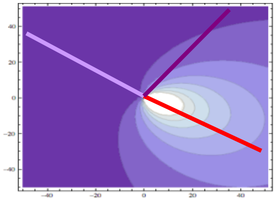


255 ***Black poplars*** ♂

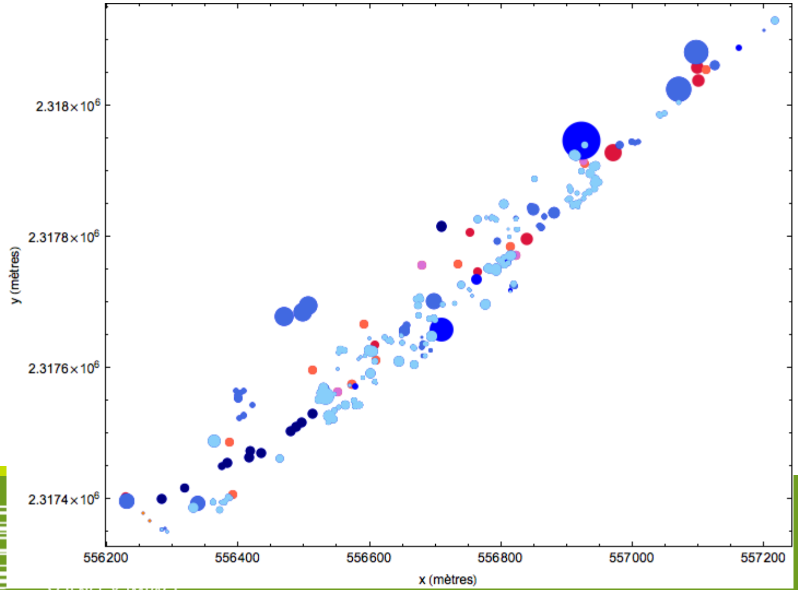
229 ***Black poplars*** ♀

1350 seeds (2006)

- Large part of pollen flow outside the site (**51,3%**)
- Pollen dispersal at long distances confirmed (**> 1000 m**)
- Evidences of anisotropic dispersion of pollen



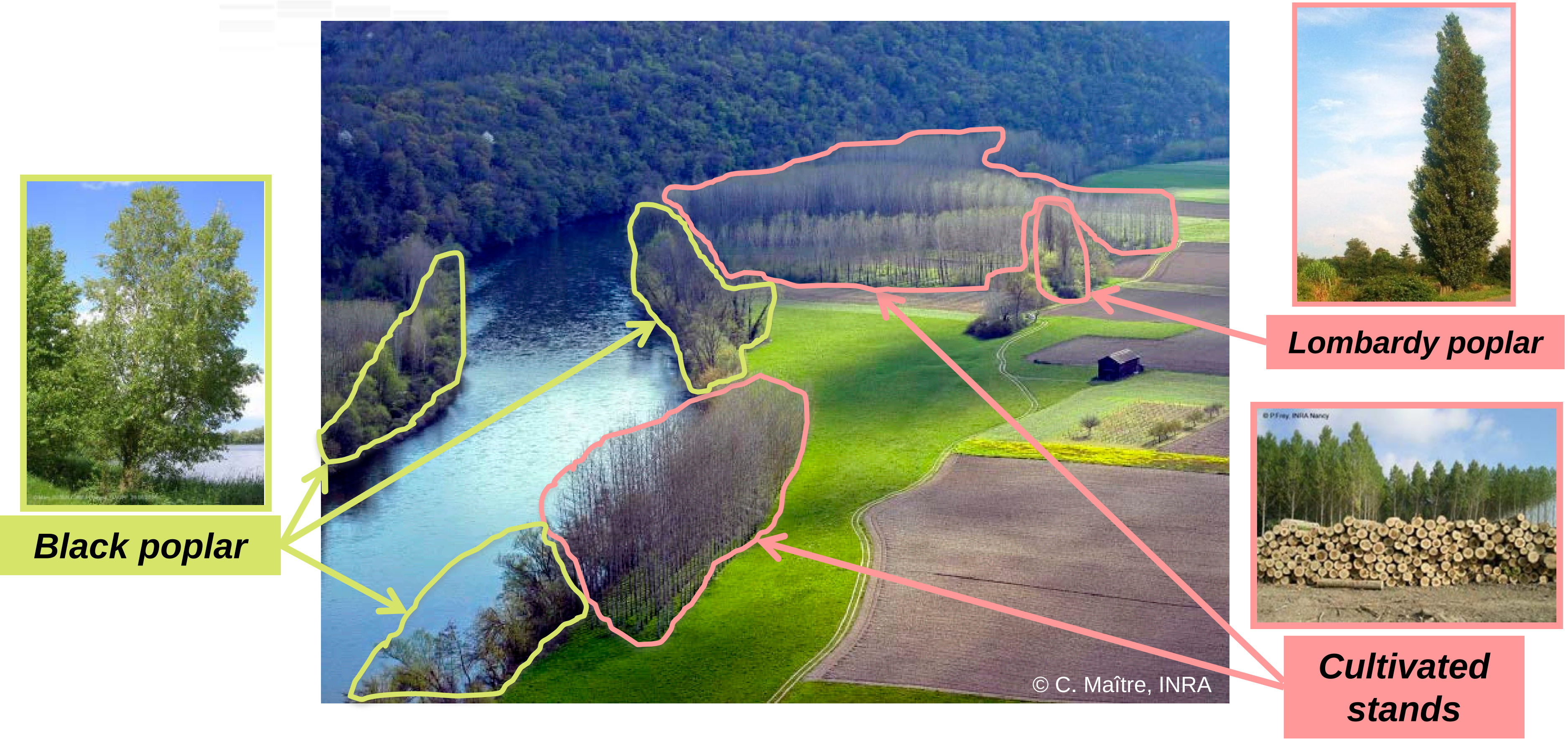
Male contributions



Dowkiw et al, unpublished

Poplars in European landscapes

Cultivated poplar stands and wild stands strongly intermingled ….



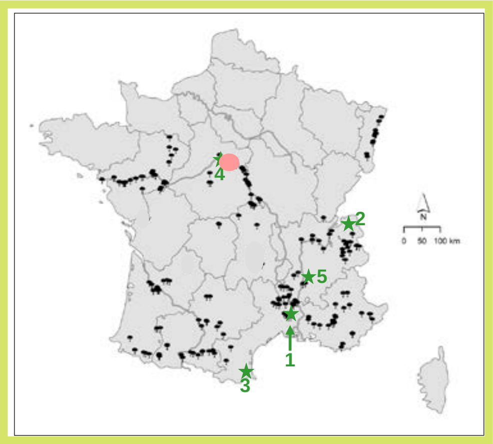
(After C. Bastien, 2009)

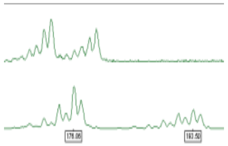
Gene flow ‘Cultivated’ => ‘wild’

Quantification of F1 hybrids with lombardy poplar at different scales

Pollen flow

Lombardy poplar(♂)





10 SSR markers

♣ In ex situ national collection
(310 individuals)

6 %

★ In 5 natural area
(230 individuals)

4,3 %

● In a intensive study site11 ha
(1350 seeds 2006)

1,9 %

(after C. Bastien, 2009)

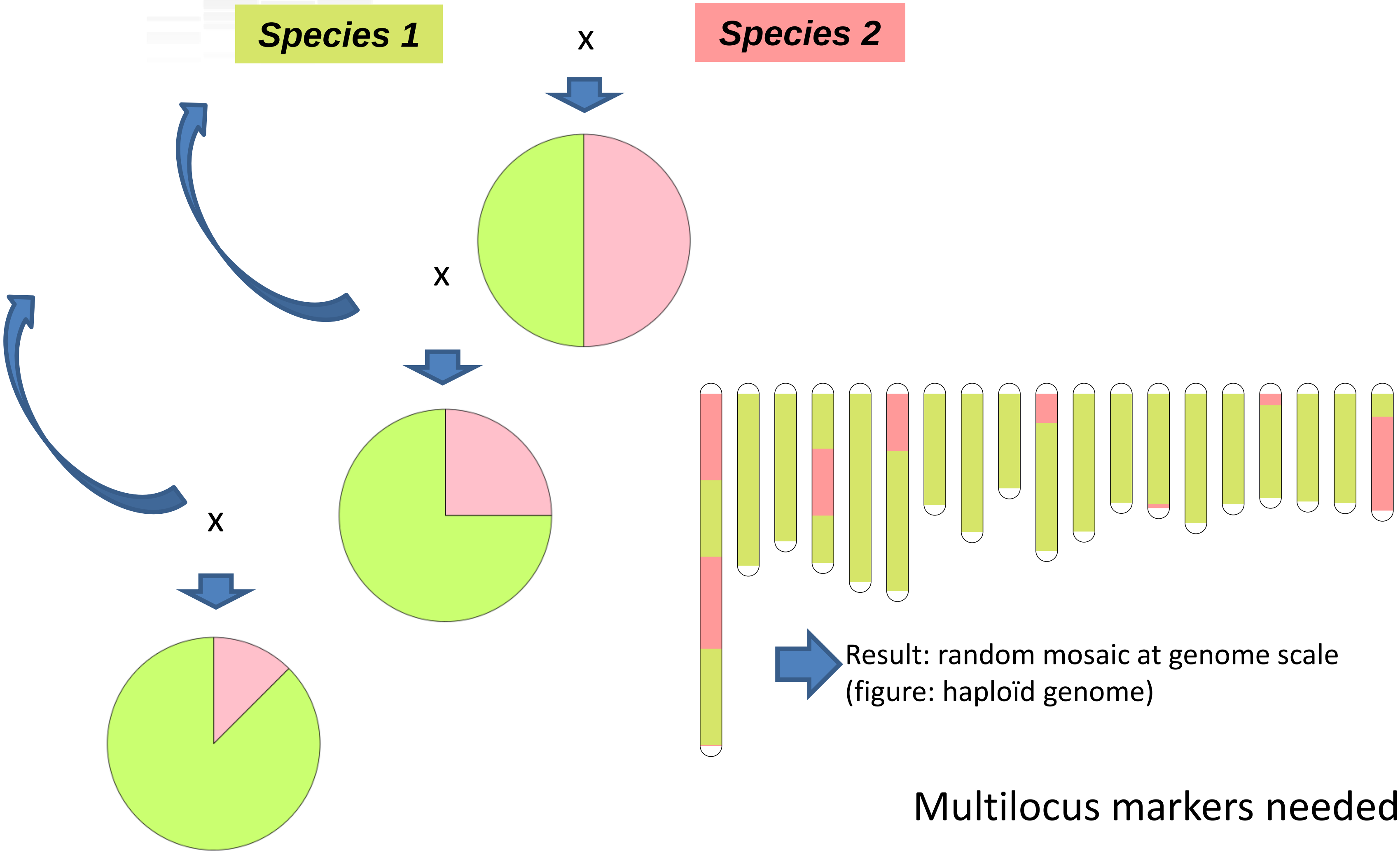
Gene flow ‘Cultivated’ => ‘wild’

Populus nigra

Populus deltoides
P. deltoides x P. trichocarpa
P. deltoides x P. nigra

- **Species specific markers**
- **Hybridation / Introgression**

Markers to detect introgression

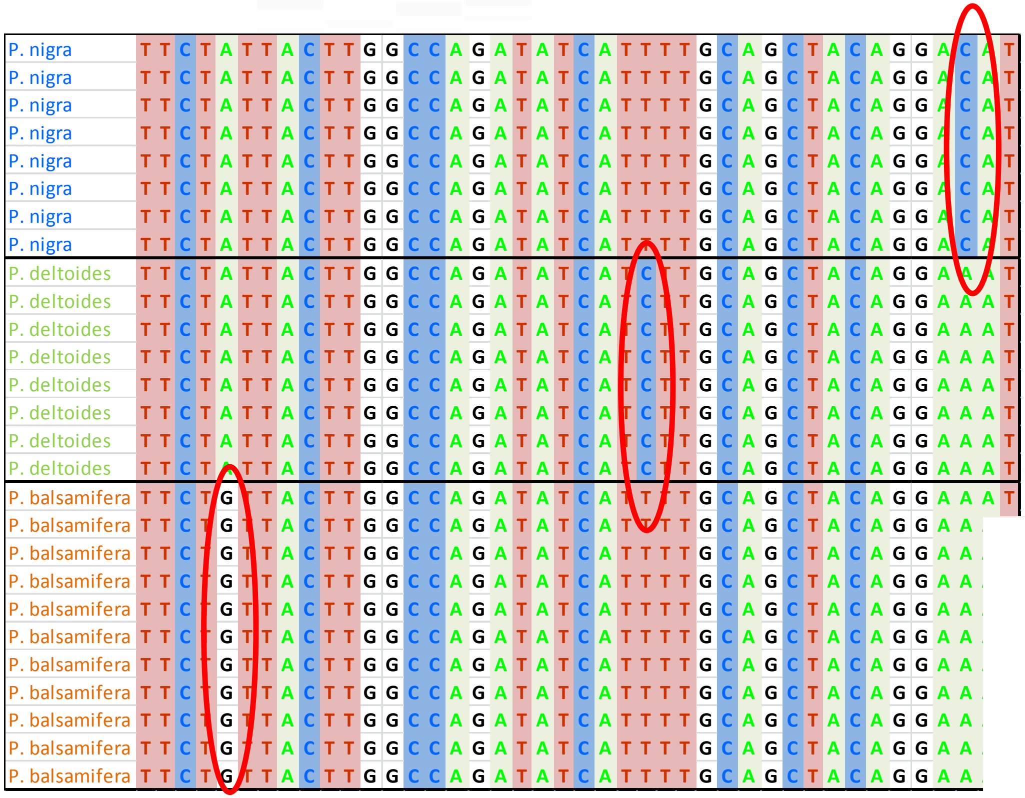


Result: random mosaic at genome scale (figure: haploïd genome)

Multilocus markers needed

Markers to detect introgression

SNP (Single Nucleotide Polymorphism)



P. nigra	T T C T A T T A C T T G G C C A G A T A T C A T T T T G C A G C T A C A G G A C A T
P. nigra	T T C T A T T A C T T G G C C A G A T A T C A T T T T G C A G C T A C A G G A C A T
P. nigra	T T C T A T T A C T T G G C C A G A T A T C A T T T T G C A G C T A C A G G A C A T
P. nigra	T T C T A T T A C T T G G C C A G A T A T C A T T T T G C A G C T A C A G G A C A T
P. nigra	T T C T A T T A C T T G G C C A G A T A T C A T T T T G C A G C T A C A G G A C A T
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P. nigra	T T C T A T T A C T T G G C C A G A T A T C A T T T T G C A G C T A C A G G A C A T
P. nigra	T T C T A T T A C T T G G C C A G A T A T C A T T T T G C A G C T A C A G G A C A T
P. deltoides	T T C T A T T A C T T G G C C A G A T A T C A T C T T G C A G C T A C A G G A A A T
P. deltoides	T T C T A T T A C T T G G C C A G A T A T C A T C T T G C A G C T A C A G G A A A T
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P. deltoides	T T C T A T T A C T T G G C C A G A T A T C A T C T T G C A G C T A C A G G A A A T
P. deltoides	T T C T A T T A C T T G G C C A G A T A T C A T C T T G C A G C T A C A G G A A A T
P. balsamifera	T T C T G T T A C T T G G C C A G A T A T C A T T T T G C A G C T A C A G G A A A T
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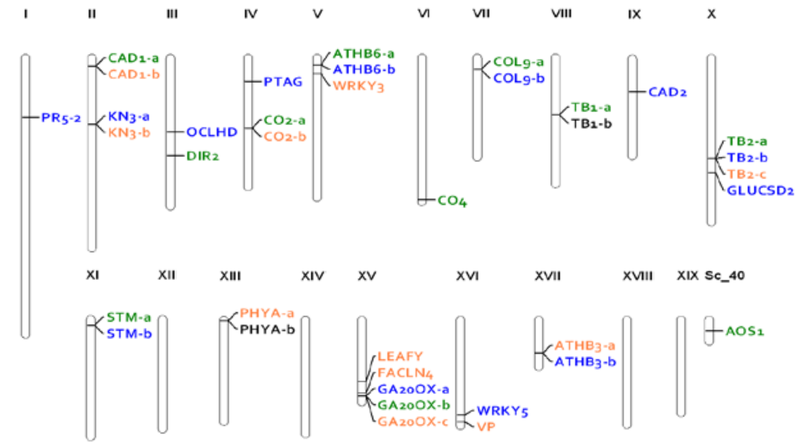
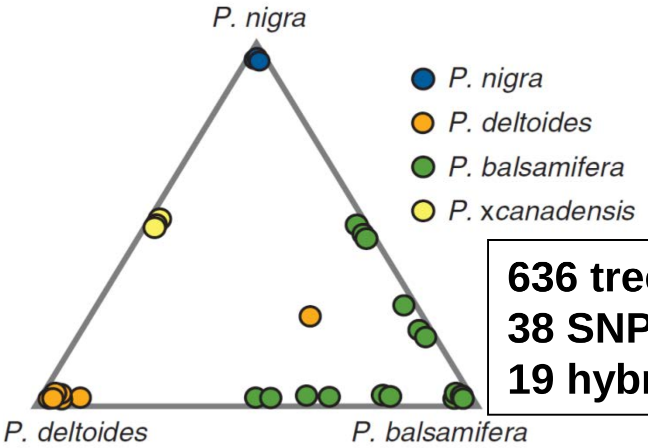


14 *P. nigra* **11 *P. deltoides*** **11 *P. balsamifera***



636 trees
38 SNPs
19 hybrids: 3%

Thompson et al 2010, Mol Ecol 19, 132–145

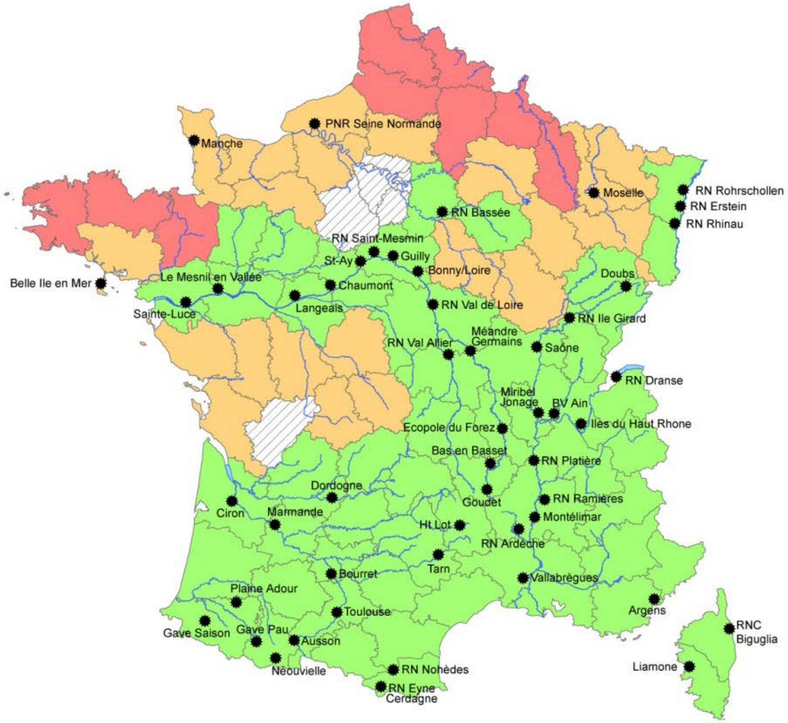
Management of genetic resources for *Populus nigra* in France

In situ

- Inventory and characterisation of natural stands
- Involvement of Natural reserves in the in conservation program
- Sampling
- Molecular and phenotypis charcterisation
- Ex situ collection

Ex situ


AGPF



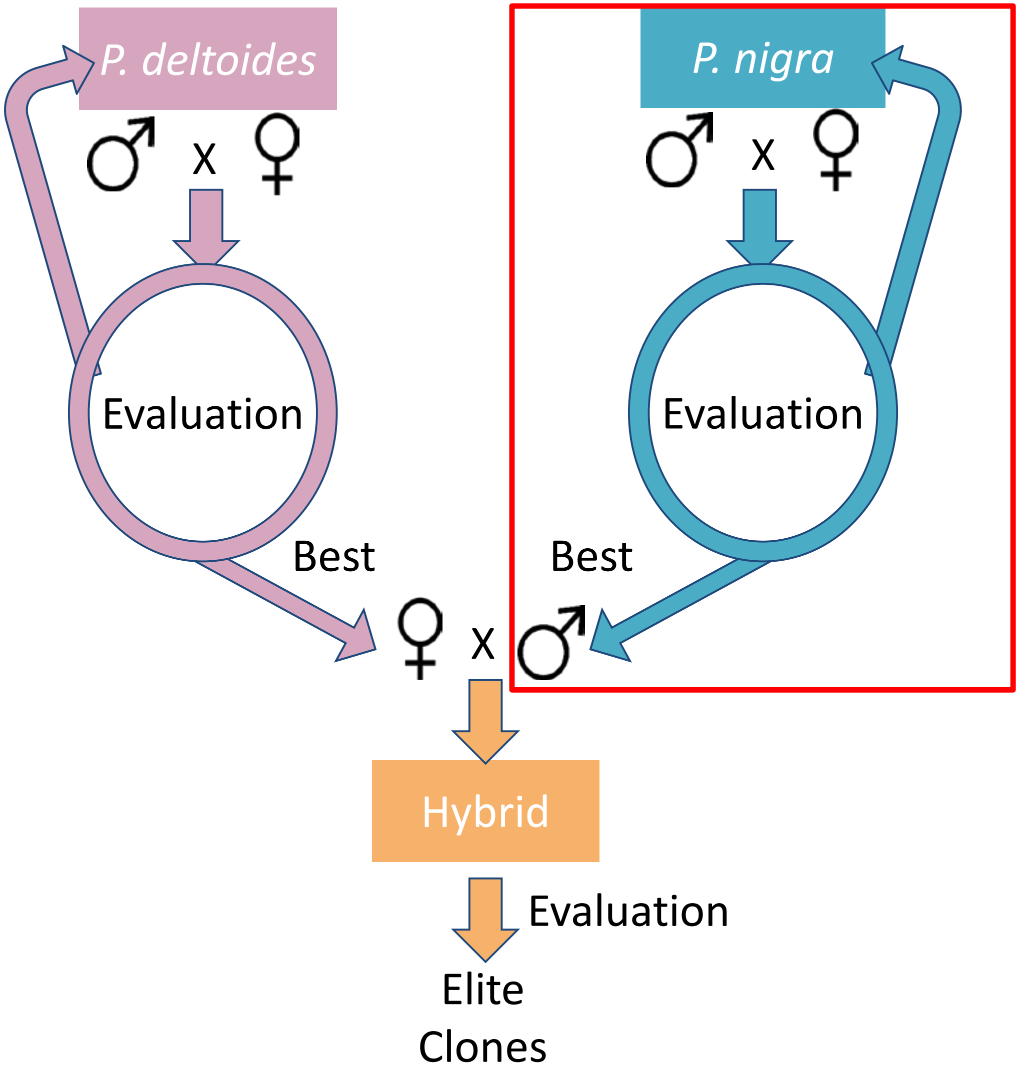
Occurence of the species:
- 🟩 Present in riparian forests
- 🟧 Present as isolated trees
- 🟥 absent

_04

Breeding

Poplar breeding program

Crossing & intraspecific/hybrid evaluations



P. deltoides

♂ X ♀

Evaluation

Best

P. nigra

♂ X ♀

Evaluation

Best

♀ X ♂

Hybrid

Evaluation

Elite
Clones

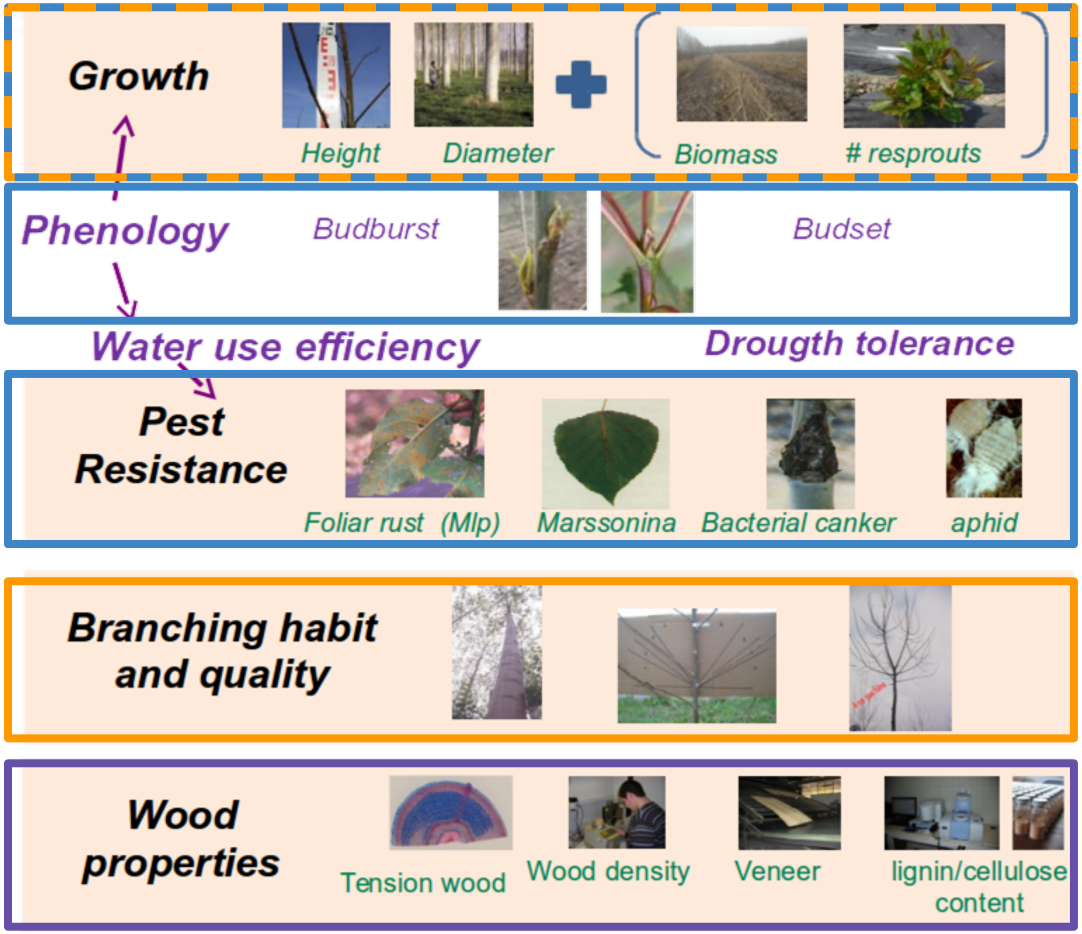
Poplar breeding program

Selection criteria

< 5 years

5-10 years

> 10 years



Growth

Height

Diameter

Biomass

resprouts

Phenology

Budburst

Budset

Water use efficiency

Drougth tolerance

Pest Resistance

Foliar rust (Mlp)

Marssonina

Bacterial canker

aphid

Branching habit and quality

Wood properties

Tension wood

Wood density

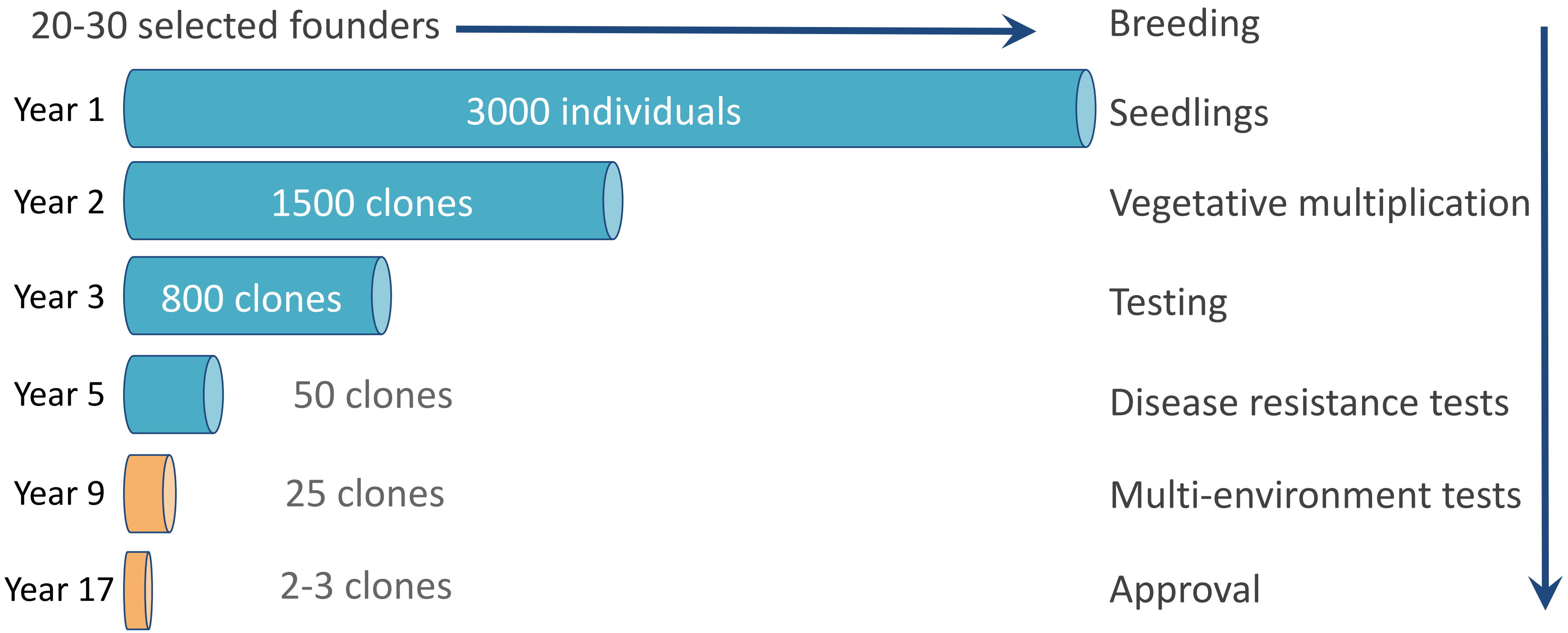
Veneer

lignin/cellulose content

Coll. LBLGC, Univ Orléans

Poplar breeding program

Time and selection rate



20-30 selected founders → Breeding

Year 1 — 3000 individuals — Seedlings

Year 2 — 1500 clones — Vegetative multiplication

Year 3 — 800 clones — Testing

Year 5 — 50 clones — Disease resistance tests

Year 9 — 25 clones — Multi-environment tests

Year 17 — 2-3 clones — Approval

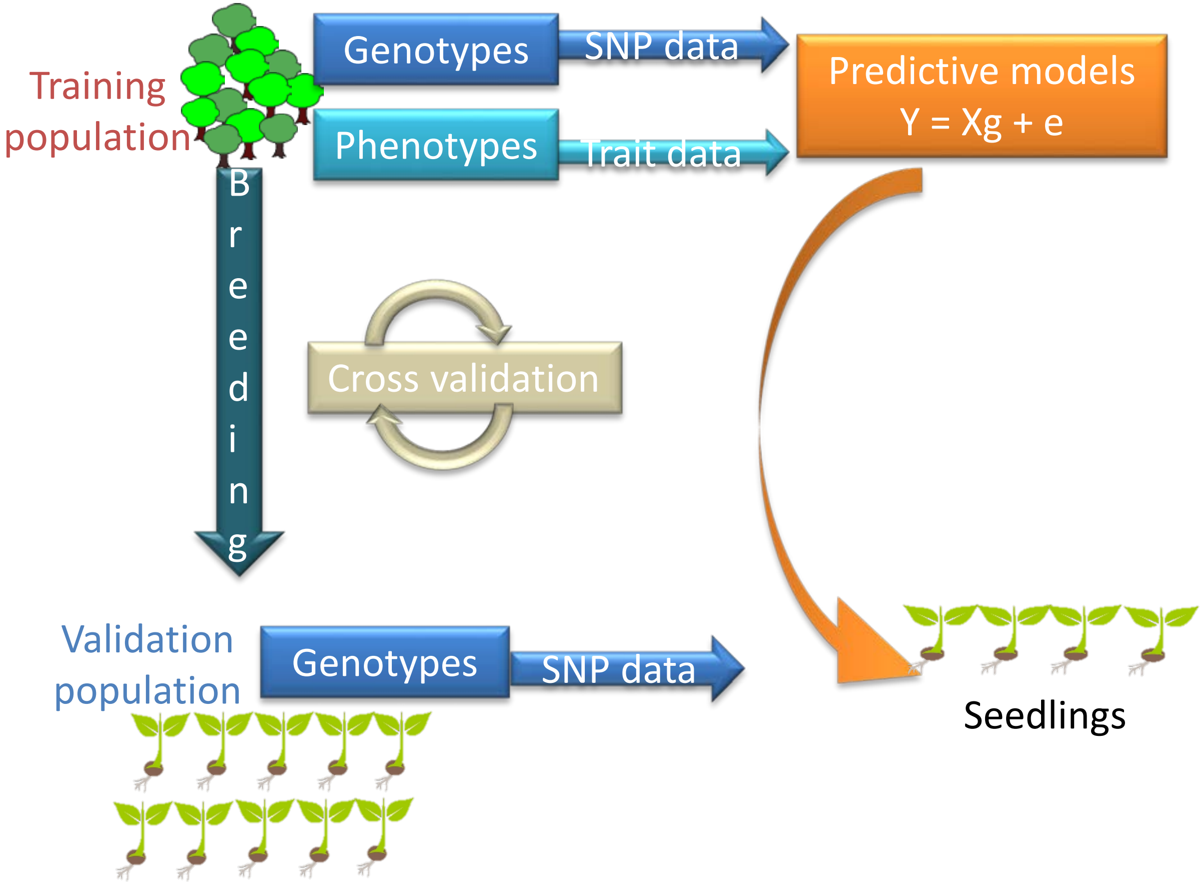
Poplar breeding program

	Strengths	**Weaknesses**
Biological	Controlled crossings in greenhouses	Long selection cycle> 15 years First flowering between 4 and 7 years ↘
	A large number of available progenies ↗	But not used
	High vegetative multiplication	
	Strong correlation between the parent's breeding value and that of his offspring in hybridization	traits evaluated late and expensive ☺
Operational	Strong selection intensity	Multi-steps selection ☺
	Precise phenotypic evaluation	Poor genetic diversity within clones in production ☺

What solution can bring genomic selection ?

Poplar breeding program

Genomic selection



Training population

Genotypes

SNP data

Phenotypes

Trait data

Predictive models
Y = Xg + e

Breeding

Cross validation

Validation population

Genotypes

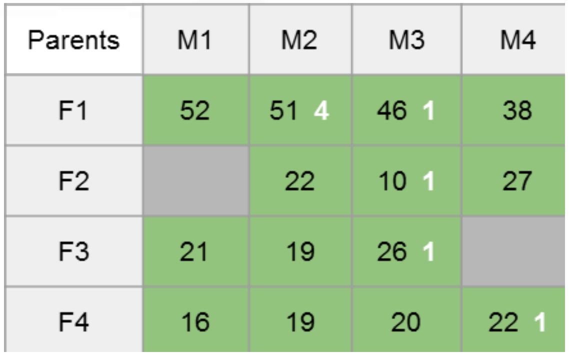
SNP data

Seedlings

Poplar breeding program

Genomic selection

Training population = factorial mating design

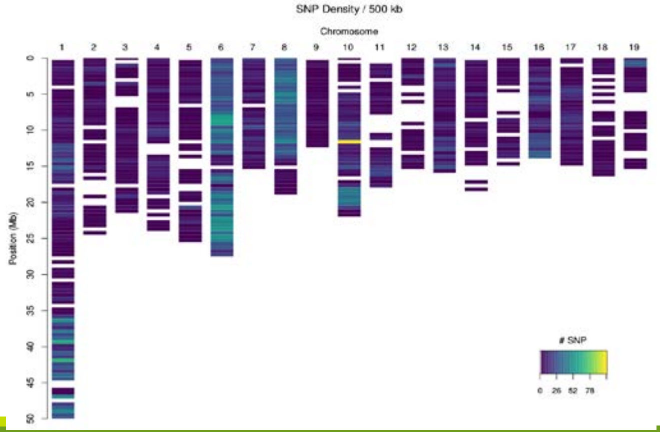


Parents	M1	M2	M3	M4
F1	52	51 4	46 1	38
F2		22	10 1	27
F3	21	19	26 1	
F4	16	19	20	22 1

Genotypes

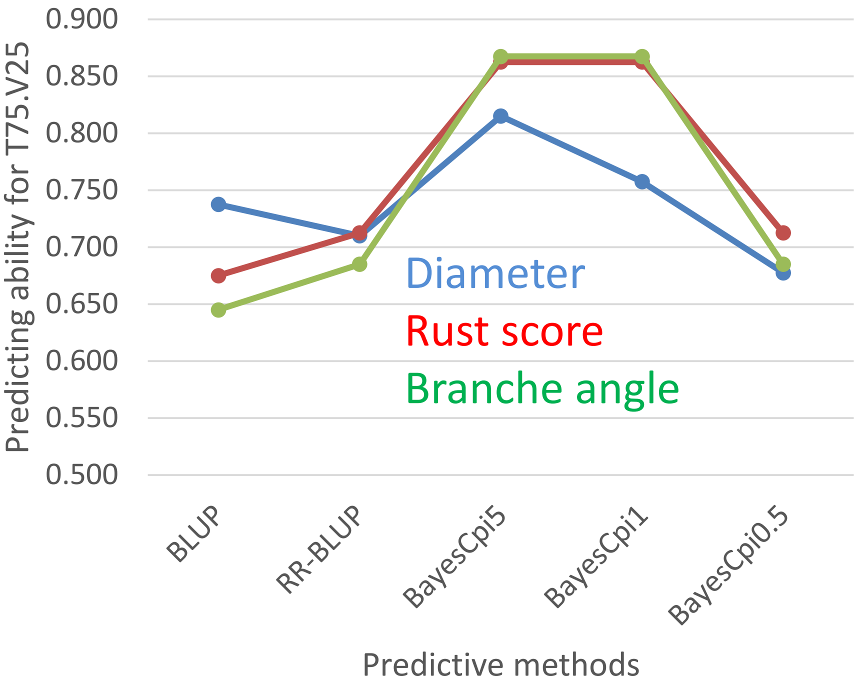
➔12K beadchip illumina (Faivre-Rampant et al., 2016)

➔After trimming 7300 SNPs



Testing

Predictive models
$Y = Xg + e$

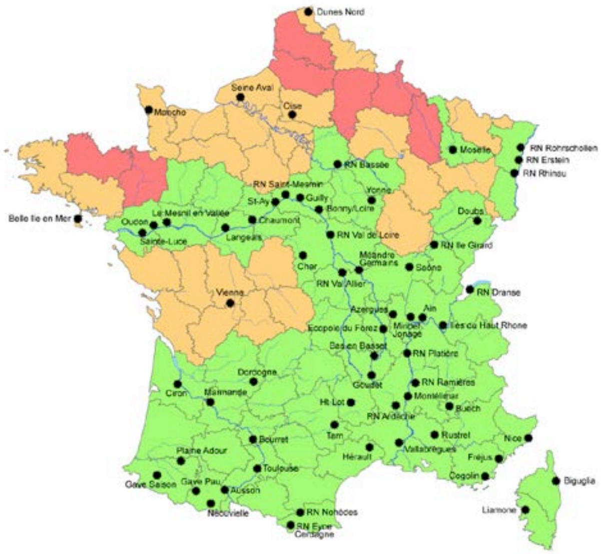


M. Pégard, PhD thesis

Development of cost- and time-effective breeding methods

Multi-**C**lonal **V**arieties of *Populus nigra*

Target in deployment = ecological restoration of river banks



Occurence of the species:

🟩 Present in riparian forests

🟧 Present as isolated trees

🟥 absent

General rules of a MCV of black poplar

- Individuals of the species *Populus nigra !*
- Variety by river basin (six in France)
- Largest diversity as possible (geographic, ecologic and genetic), representative of the river basin
- Choice of 25 genotypes per variety

After M. Villar, coord. National Conservation Programme of *Populus nigra*

Development of cost- and time-effective breeding methods

Multi-**C**lonal **V**arieties of *Populus nigra*

Target in deployment = ecological restoration of river banks

Species descriptors and introgression

- In situ phenotype : tree shape, bark characteristics, stem characteristics, leaf shape, bud shape, male and female catkins
- SSR and SNP markers

Populus nigra var. Italica

One variety per river basin & large ecological regions

GRECO : 'large ecological regions' of the French National Forest Inventory

Largest diversity as possible

- Sex ratio
- No clone (SSR markers)

Traits evaluated in trials :

- Phenology: max. variability
- Height and diameter: eliminate extremes

to avoid competiton

- Branching patterns: avoid fastigiate

After M. Villar, coord. National Conservation Programme of *Populus nigra*

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Etienne KLEIN

Thanks