National programme on conservation of genetic resources of *Populus nigra* in France: results of 15 years of *in situ* and *ex situ* programmes

Marc VILLAR, INRA Orléans, France
marc.villar@inra.fr
http://peupliernoir.orleans.inra.fr

Olivier FORESTIER, ONF Guémené Penfao, France
olivier.forestier@onf.fr

National programme on conservation of genetic resources of *Populus nigra* in France

Status (inventory, introgression with other *Populus spp.*)

Evaluation of genetic diversity

***Ex situ* core collection / in stool beds and populetum**

***In situ* Genetic Conservation Units**

Ecology of the riparian Salicaceae forest (BioMareau project 2012-2019 along the Loire river)

Status (inventory, introgression with other *Populus spp.*)

Evaluation of genetic diversity

***Ex situ* core collection**

***In situ* Genetic Conservation Units**

Ecology of the riparian Salicaceae forest (BioMareau project 2012-2019 on the Loire river)

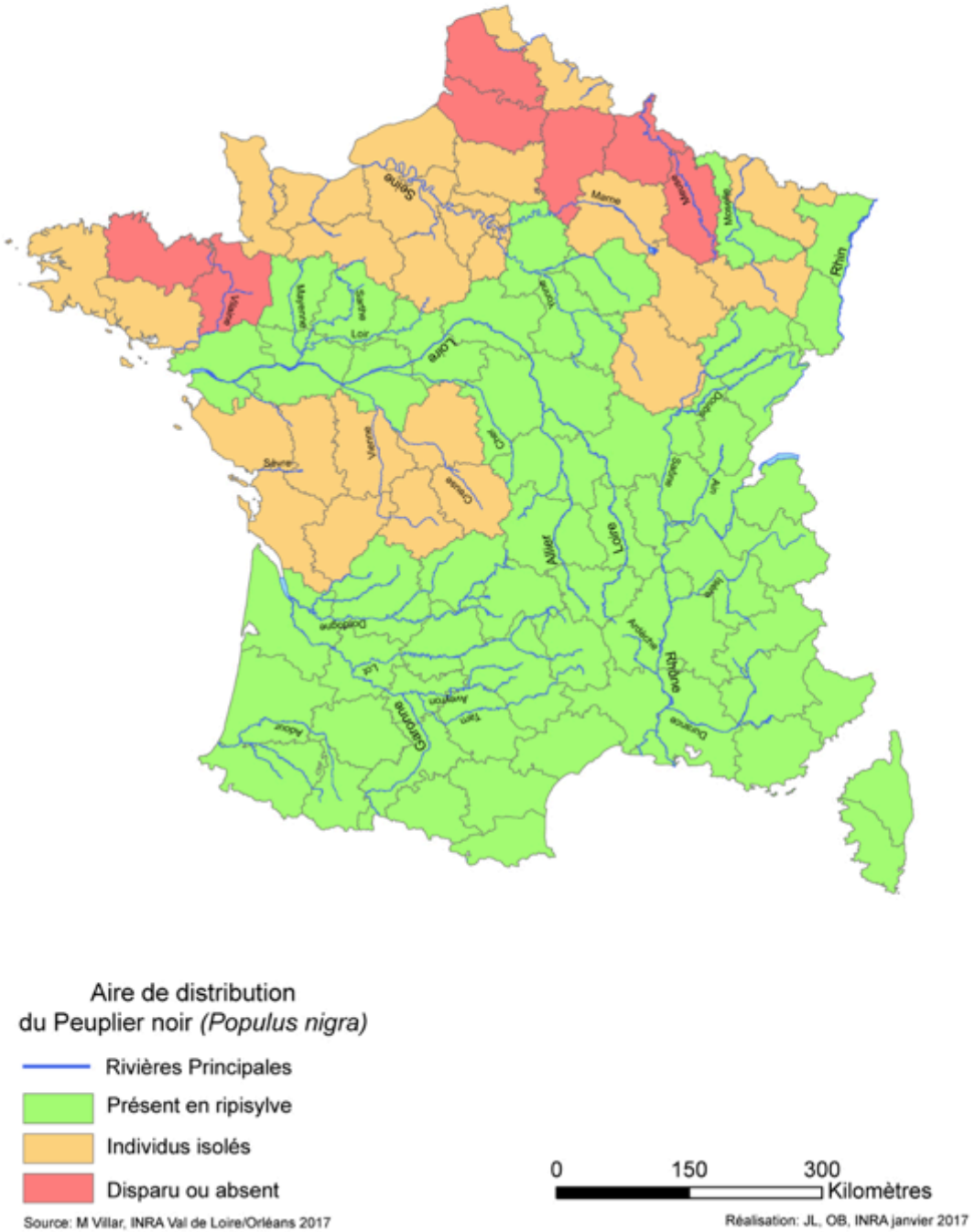
Status in France of *Populus nigra*

in continuous riparian forest
(example Loire river : 1580 trees in 9 ha in Mareau-aux-Prés islands)

as scattered trees
(example Seine river : only 23 trees on a portion of 28 kms of the river)

present in the past and destroyed ?
or absent ?

Inventory



France

(Villar and Forestier 2017)

Status / introgression with other *Populus spp.*

Gene flow between native *Populus nigra* and ornemental Populus nigra cv. Italica

Male clone (pollen)

Loire, St Luce, 44

Tree Genetics & Genomes (2011) 7:1249–1262
DOI 10.1007/s11295-011-0410-6

ORIGINAL PAPER

SSR-based analysis of clonality, spatial genetic structure and introgression from the Lombardy poplar into a natural population of *Populus nigra* L. along the Loire River

Nicolas Chenault · Sophie Arnaud-Haond · Mary Juteau · Romain Valade · José-Luis Almeida · Marc Villar · Catherine Bastien · Arnaud Dowkiw

Molecular detection of F_1 hybrids at seedling and adult stages

1.6% (1/63) in a natural site *Vanden Broeck et al. 2004*

2.6% (5/194) in a natural site *Chenault et al. 2011*

6% (18/310) in national gene bank *Jorge et al., unpubl.*

4.3% (10/230) in 5 natural pop. *Jorge et al.,unpubl.*

For more info …

Ann. For. Sci. 62 (2005) 601–613

DOI: 10.1051/forest:2005072

Review

Natural hybridization between cultivated poplars and their wild relatives: evidence and consequences for native poplar populations

An VANDEN BROECK[a*], Marc VILLAR[b], Erik VAN BOCKSTAELE[c], Jos VAN SLYCKEN[a]

Status (inventory, introgression with other *Populus spp.*)

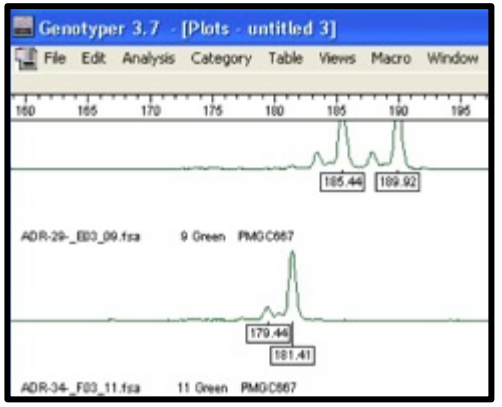
Evaluation of genetic diversity

Ex situ core collection and populetum establishment

In situ Genetic Conservation Units

Ecology of the riparian Salicaceae forest (BioMareau project 2012-2019 on the Loire river)

Evaluation of adaptive traits *in situ*



DNA markers SSR, SNP

Evaluation of genetic diversity

in experimental nursery trails

Evaluation of adaptive traits *ex situ*

Evaluation of adaptive traits *in situ*

FLOWERING

Two black poplars
along the Loire river

8th april 2008

Populus spp. : dioecious

Adaptive trait : leaf phenology / <u>spring bud flush</u>

River Drôme 13th March 2017

Two black poplars
along the Loire river

8th april 2008

flowering

another adaptive trait :

<u>spring bud flush</u>

29th april 2008

Adaptive trait : leaf phenology / autumn leaf senescence

11 october 2011, RNN St-Mesmin, Loire

Evaluation
of adaptive traits
in situ

Evaluation of genetic diversity

DNA markers
SSR, SNP

in experimental nursery trails

Evaluation of adaptive traits *ex situ*

Collect of cuttings in winter

Vegetative propagation

Vegetative propagation

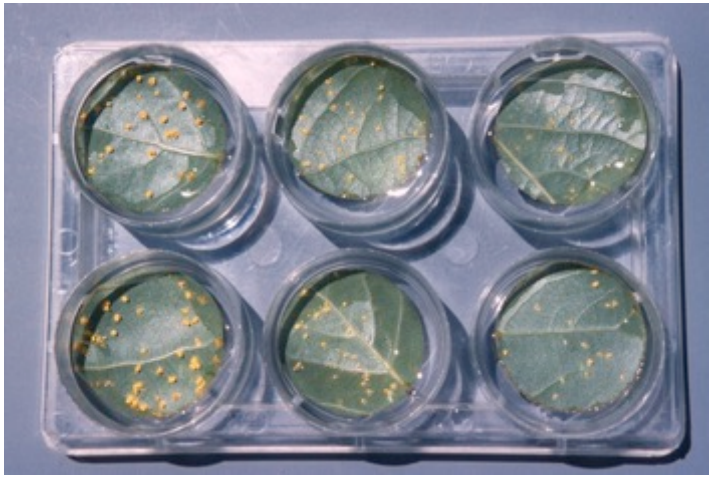
Plantation in stoolbed / trial

Evaluation of adaptive traits
(in experimental trial, from cuttings)

Nursery trials / 2 years old

Pots *(greenhouse, outside, transport)*
4 months old

Lab trial (leaf disk : test for rust susceptibility)



Evaluation of adaptive traits

Growth (height, diameter)

Susceptibility to diseases

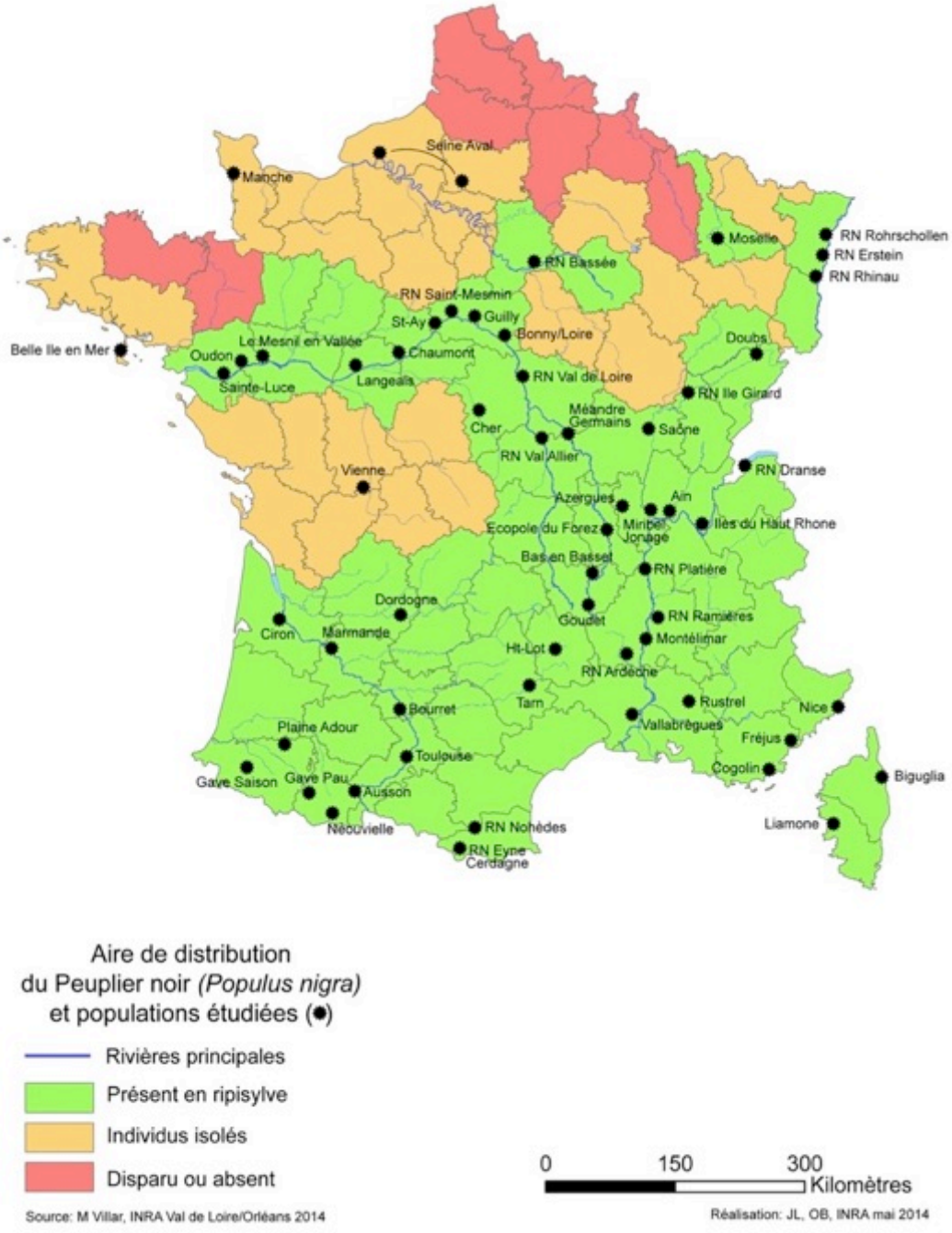
Phenology (budburst and budset)

Foliar traits (surface, WUE …)

Architecture

Etc…

Evaluation of adaptive traits



Populus nigra **programme in 2019 : evaluation of 60 populations (7 nursery trials)**

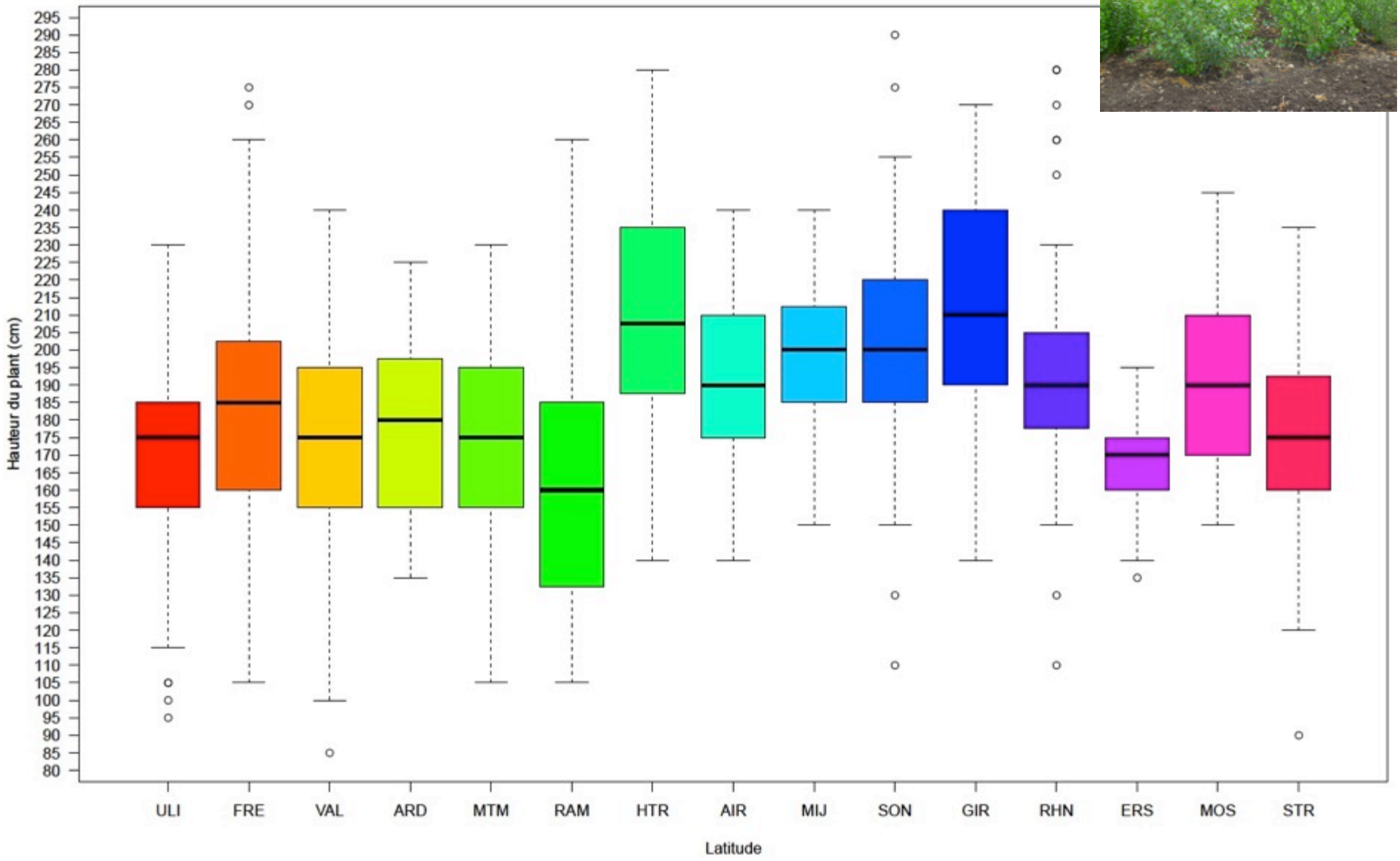
<u>Total</u> : 2000 clones

RESULTS : nursery trial / *15 Pops*

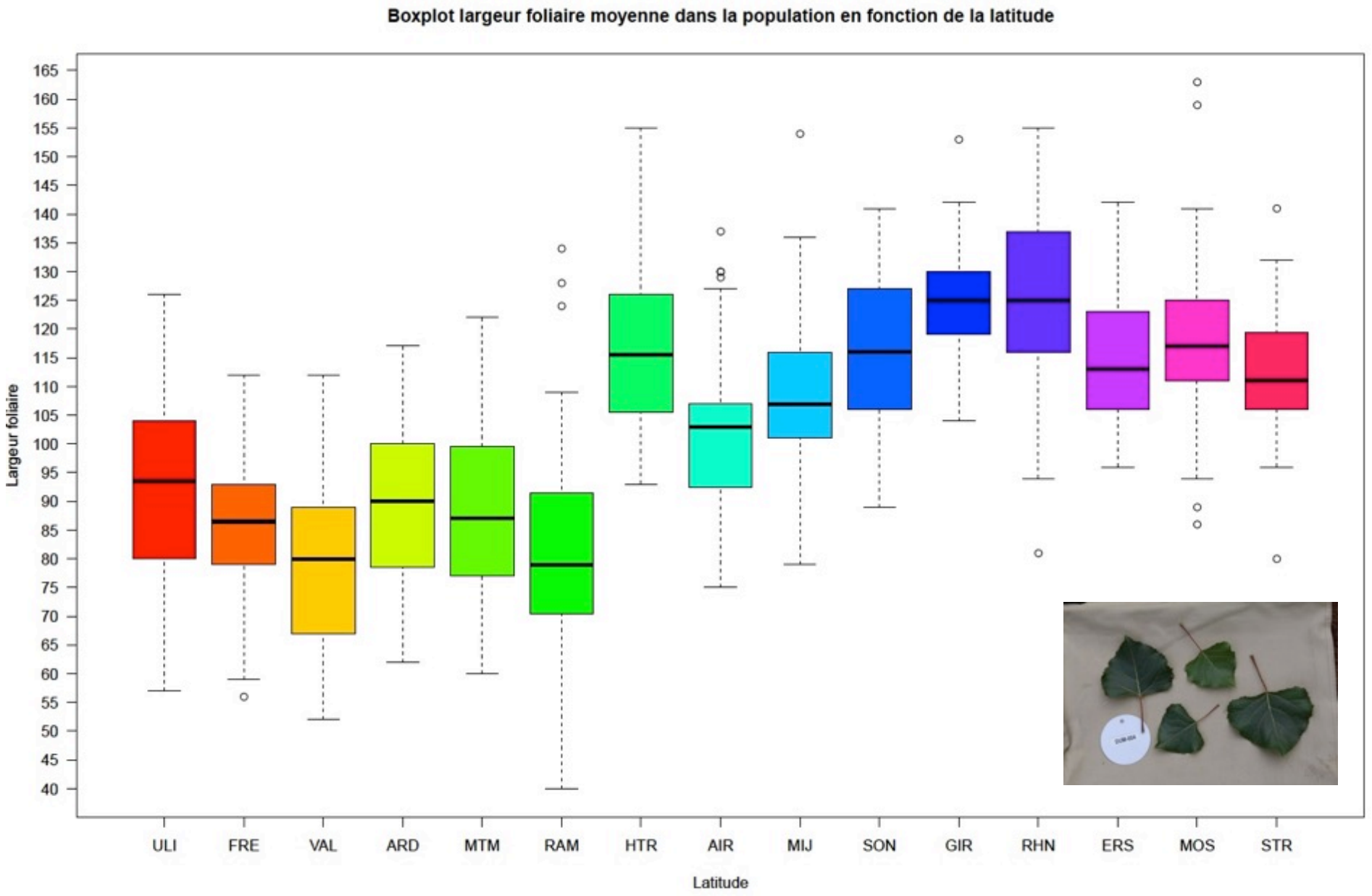
EXAMPLE :
Population study :

along a latitudinal gradient
Rhône / Saône / Rhin

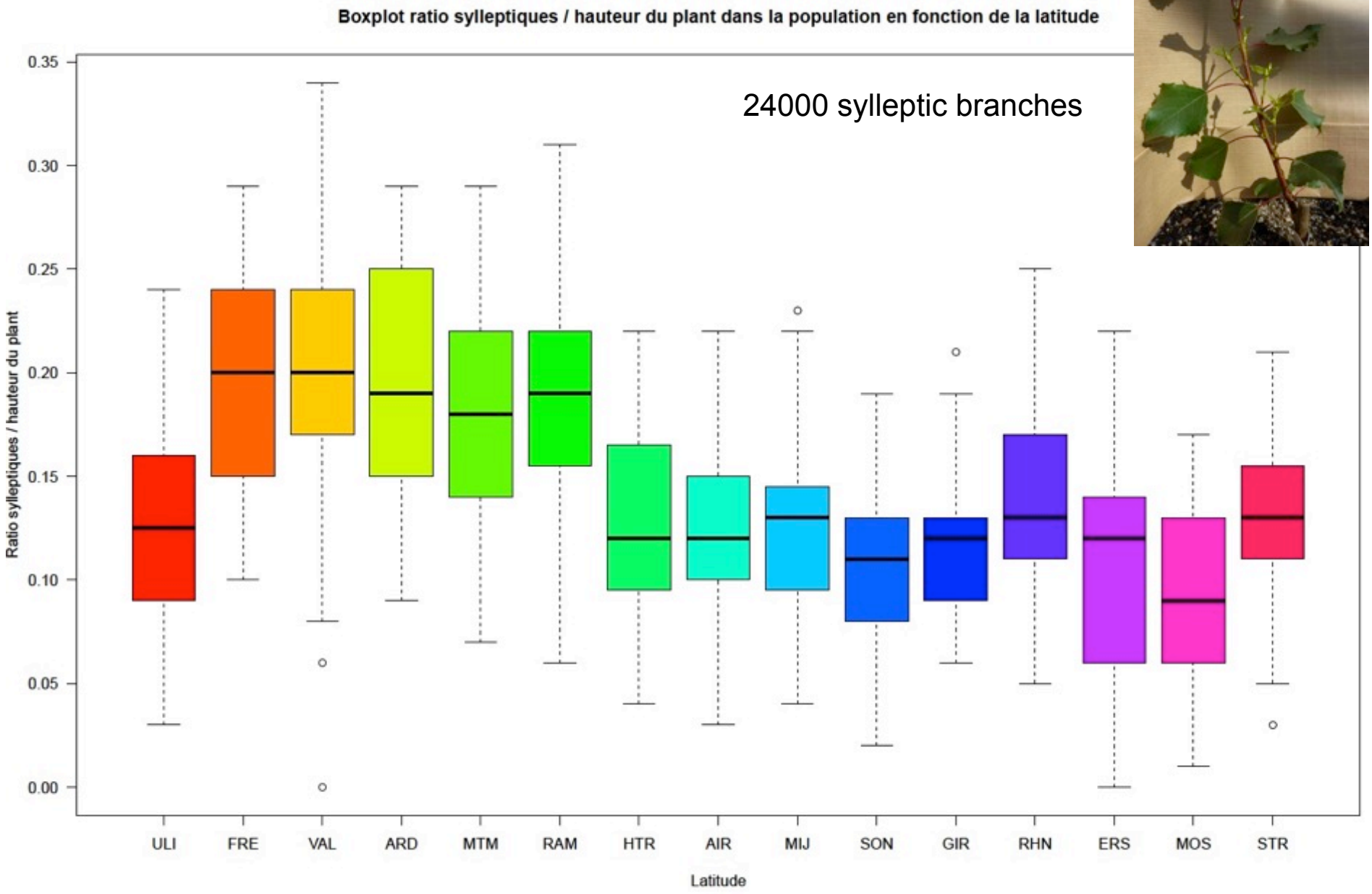
(30 clones per pop)



Origin : South → Origin : North

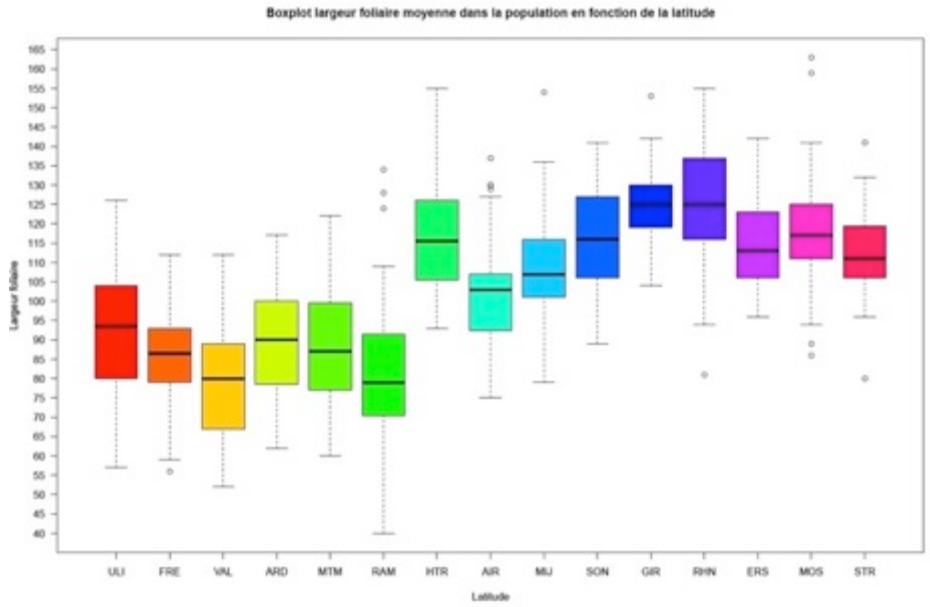


Origin : South ⟶ Origin : North

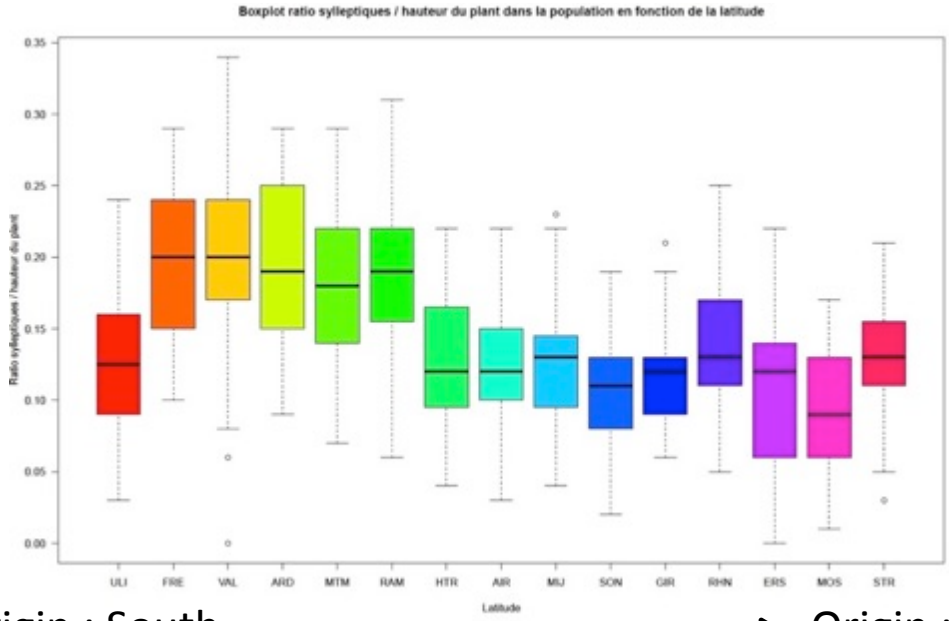


Origin : South → Origin : North

Largeur foliaire (max)



Nombre rameaux sylleptiques



Origin : South ⟶ Origin : North

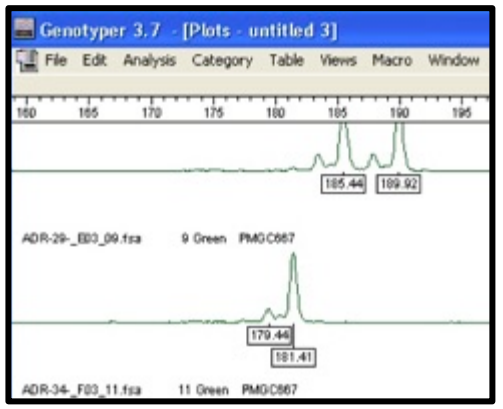
POPs from North France :
larger leaves
but with less branches

POPs from Sud :
smaller leaves
but with more branches

Future : same total leaf area ?

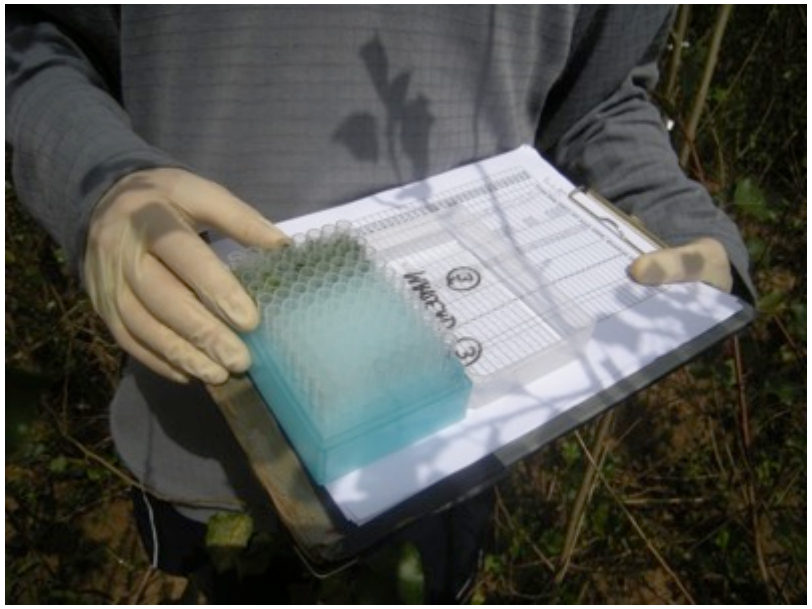
Evaluation
of adaptive traits
in situ

Evaluation of genetic diversity

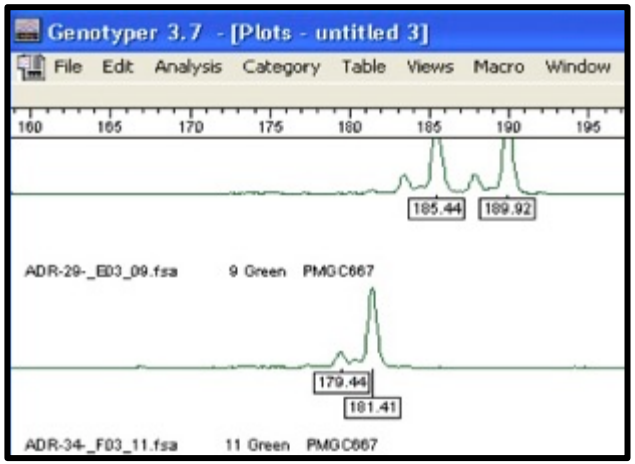


DNA markers SSR, SNP

Evaluation
of adaptive traits
ex situ



Genotyping with foliar DNA (microsatellite and SNP markers)



DNA genetic study at different scales :

Europe : European collection (EU projects Evoltree / NovelTree)

Faivre Rampant et al. Mol Ecol. Res 2016

France : 6 river bassins. Ministry of Agriculture projects

Loire river : 15 populations upstream / downstream (InterRegional projects) / 900 kms long

Loire river local : 53 kms long, regeneration 2015 (Regional project BioMareau)

Loire river microlocal : Mareau-aux-prés islands (3 ha), comparison among regenerations 2013, 2015, 2017 (Regional projects BioMareau).

Roger et al. in prep

Intraspecific genetic diversity / DNA

Nloci	Npop	N	A	Ae	**Ho**	He	Fis	Fst	Popuations	Références
6	22	574	12.1		**0.78**	0.73	0.077	0.047	6 populations de la Drôme	Imbert and Lefèvre 2003
5	19	465	15.6	6.02		0.72			Collections européennes	Storme *et al.* 2004
12	10	308	19.33	5.7	**0.66**	0.74	0.103	0.028	Collection française divisée en 10 sous populations	Brachet *et al.* 2006
12	5	218	9	4.4		0.71	0.03	0.05	5 populations françaises	Jorge *et al.* unpublished
7	17	915	15.7		**0.74**	0.76	0.027	0.081	17 populations européennes	Smulders *et al.* 2008

Etude de diversité via les marqueurs microsatellite chez *Populus nigra*

Nloci : nombre de loci étudié

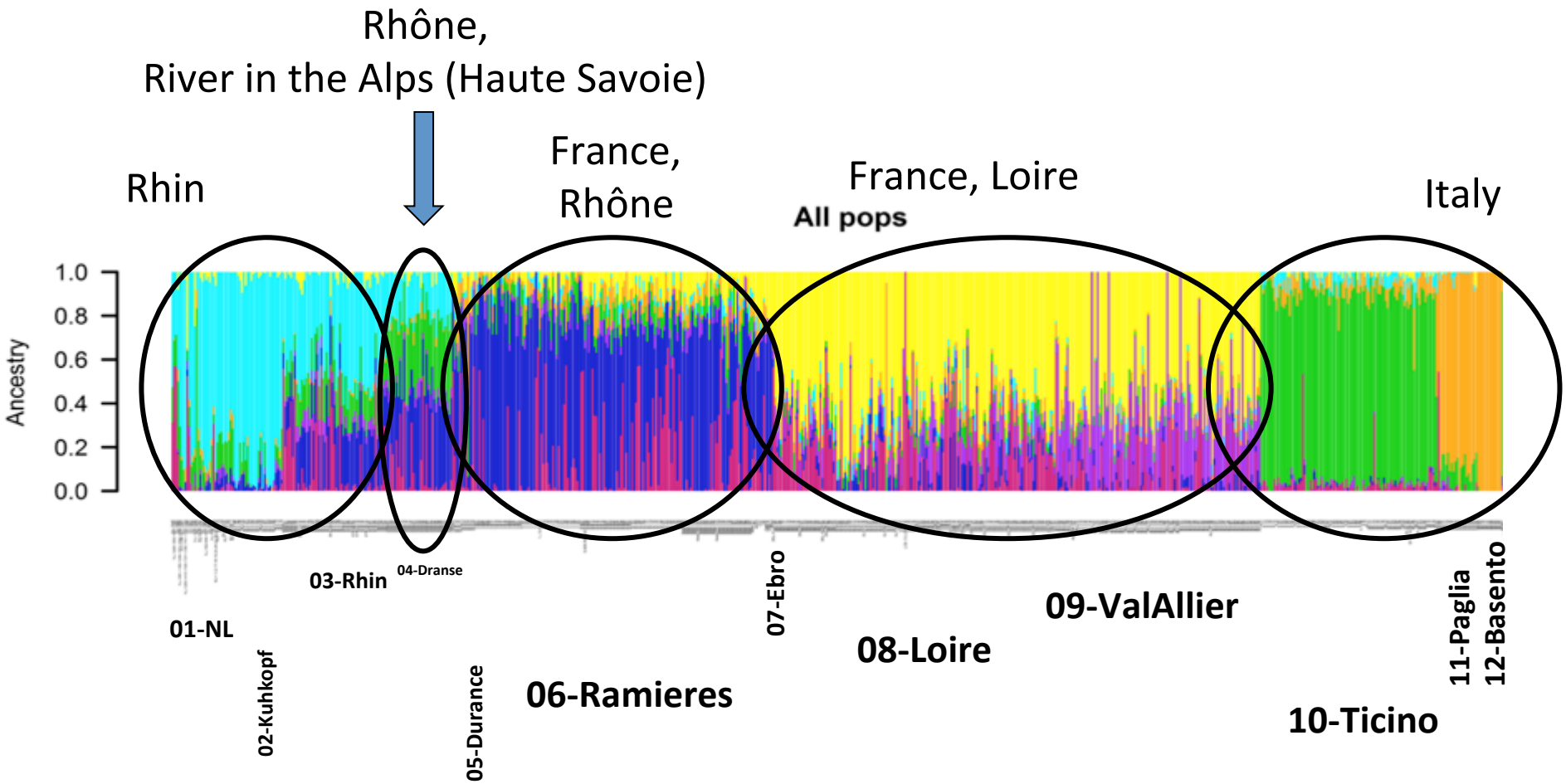
Npop : nombre de populations étudiées
N : nombre de génotypes étudiés

A : nombre moyen d'allèles par locus
Ae : nombre moyen d'allèles efficaces

Ho : heterozygotie observée
He : hétérozygotie attendue

Fis : index de fixation
Fst : index de différentiation interpopulation

Population structure (K=7) of 721 *P. nigra* genotypes (admixture)



Faivre Rampant *et al*,
Mol Ecol. Res 2016

Status (inventory, introgression with other *Populus spp.*)

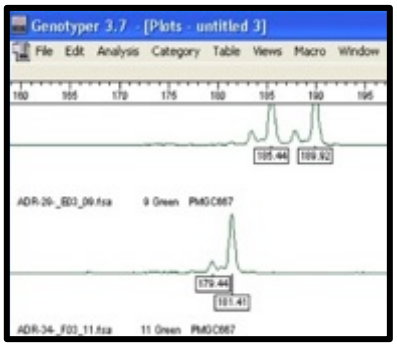
Evaluation of genetic diversity

***Ex situ* core collection / in stool beds and populetum**

In situ Genetic Conservation Units

Ecology of the riparian Salicaceae forest (BioMareau project 2012-2019 on the Loire river)

Evaluation of adaptive traits *in situ*



DNA markers SSR, SNP

Evaluation of genetic diversity

Evaluation of adaptive traits *ex situ*

Constitution of Core Collection (+ ecological data)

Core Collection :
max. 500 clones

- **In stool beds (in nursery / one year old stem)**
- **in populetum (adult trees)**

Status (inventory, introgression with other *Populus spp.*)

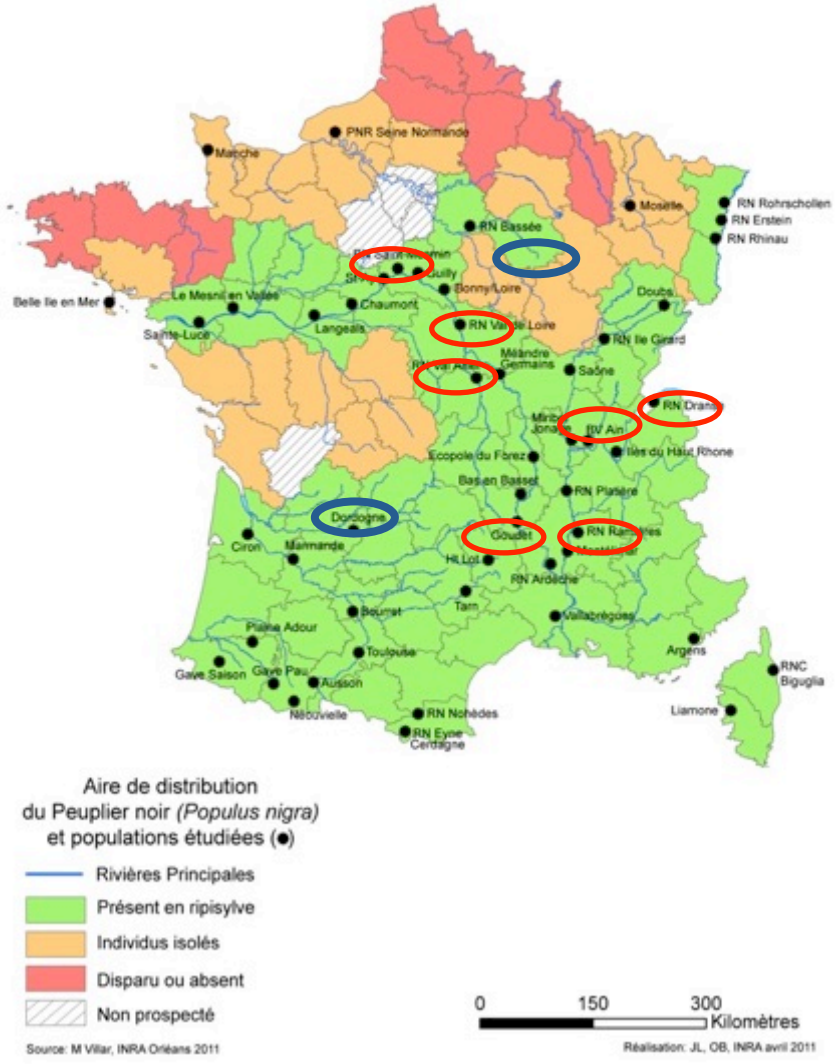
Evaluation of genetic diversity

Ex situ core collection

***In situ* Genetic Conservation Units**

Ecology of the riparian Salicaceae forest (BioMareau project 2012-2019 on the Loire river)

In situ conservation : network of Genetic Conservation Units (GCUs)



Data required :

- Genetic diversity
- 500 ind. of different age groups
- River dynamics for sexual regeneration
- Large surface as *Populus nigra* forms metapopulations
- Protected sites : from Natura 2000 to National Natural Reserve
- Network of different env : atlantic, mediterranean, mountain

7 GCUs selected
validated by CRGF,
4 management charts signed
2 GCUs in project

Units on Google Maps

Clusters of units are indicated as red circles. Numbers inside the circles show how many units each cluster includes. Yo

☑ Clustered markers ☐ Show all

Units that match the search criteria Units that are excluded by the search criteria

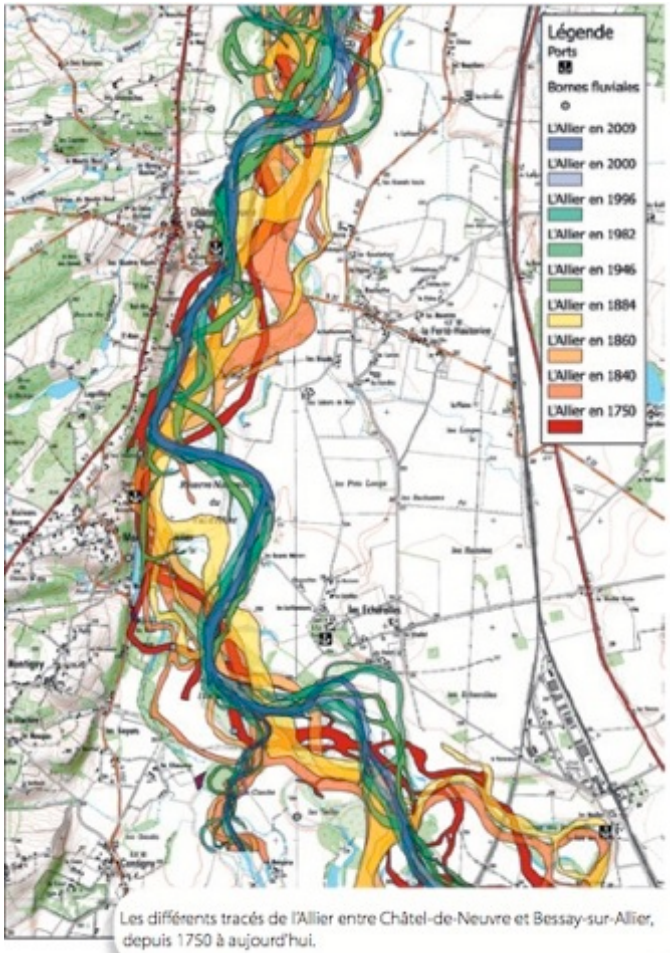
1st GCU : Mareau/St-Mesmin, NNR de St-Mesmin,
Loire river bassin

Drôme

Photo JM Faton

2nd GCU : NNR Ramières Val de Drôme (Rhône river bassin) mediterranean context

3rd GCU : NNR Val d'Allier (Loire river basin)
High morphodynamics of the river



Les différents tracés de l'Allier entre Châtel-de-Neuvre et Bessay-sur-Allier, depuis 1750 à aujourd'hui.

4th GCU : NNR Delta de la Dranse
(Rhône river bassin)
mountain context

Status (inventory, introgression with other *Populus spp.*)

Evaluation of genetic diversity

Ex situ core collection

In situ Genetic Conservation Units

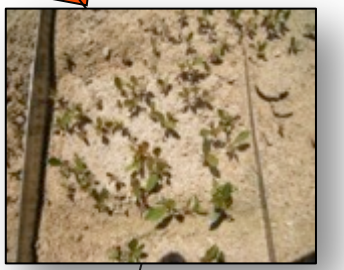
Ecology of the riparian Salicaceae forest (BioMareau project 2012-2019 on the Loire river)

Populus nigra and its environment !

SYNCHRONISATION OF SEED DEHISCENCE
AND AVAILABILITY OF GERMINATION SITES

Dissémination de graines (fin avril à mi juillet) germination immédiate

COORDINATION BETWEEN ROOT GROWTH AND LOWERING OF THE WATER LEVEL



Quelques semaines après germination

Forte croissance racinaire

HIGH TEMPERATURE

6 mois après germination

FLOODING

Quelques années plus tard : arbres adultes

DIOECIOUS,
SYNCHRONISATION OF FLOWERING TIME

Populus nigra and its environment !

Fluvial management operations within the GCU Mareau-St-Mesmin

Foraging activities of the beavers *(Castor fiber)* within the GCU Mareau-St-Mesmin

Consequences on the genetic resources of *Populus nigra* (I)

Populus nigra and its environment !

Sediment (morpho)dynamics from the river

Consequences on the genetic resources of *Populus nigra* (II)

= Project **BioMareau** (2012-2019) : Consequences of fluvial maintenance operations on the sediment morphodynamic and the associated **BIO**diversity in the mosaic of **Mareau**-aux-prés islands

To be complete …

Commercial black poplar varieties : **Variety Clonal Mix (25 clones / variety)**

VMC Loire registration 2009
VMC Garonne registration 2009
VMC Rhin registration 2009
VMC Seine registration 2014
2 VMC Rhône-Méditerranée and VMC Rhône-Saône 2017

http://peupliernoir.orleans.inra.fr/

Cette opération est cofinancée par l'Union européenne. L'Europe s'engage sur le bassin de la Loire avec le Fonds européen de Développement Régional.

ETABLISSEMENT PUBLIC LOIRE

Zone Atelier LTER FRANCE LOIRE
http://www.za-inee.org/

UNION EUROPÉENNE
Fonds Européen de Développement Régional

L'EUROPE s'engage en région Centre-Val de Loire avec le FEDER

PLAN LOIRE Grandeur Nature

Région Centre-Val de Loire