



Demographic and evolutionary history of the European black pine (*Pinus nigra* Arn)

Guia Giovannelli, Caroline Scotti-Saintagne, Ivan Scotti, Anne Roig, Ilaria Spanu, Giovanni Giuseppe Vendramin, Frédéric Guibal, Bruno Fady

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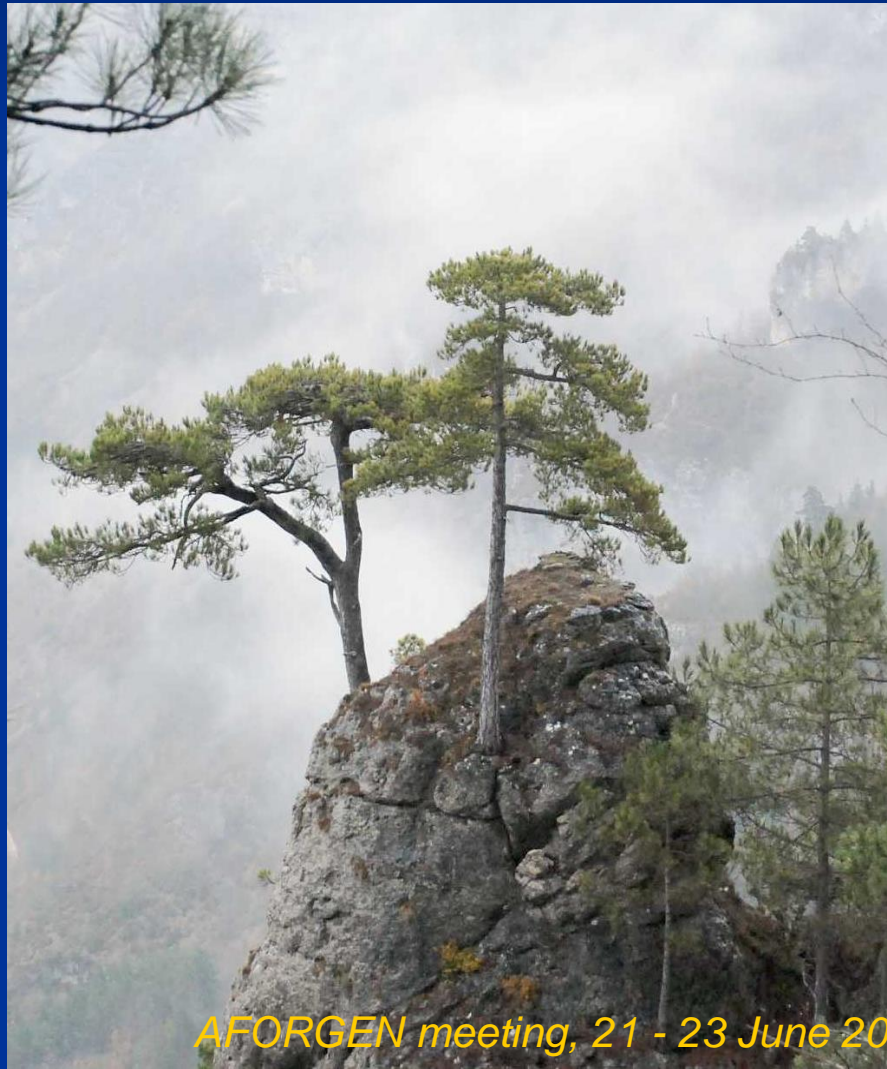
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Guia **Giovannelli**,
Caroline **Scotti-
Saintagne**, Ivan
Scotti, Anne **Roig**,
Ilaria **Spanu**, Giovanni
Giuseppe **Vendramin**,
Frédéric **Guibal**, Bruno
Fady

INRA – URFM,
Ecologie des Forêts
Méditerranéennes
Avignon, France

(bruno.fady@inra.fr)



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Do you know the European black pine?

***The conifer tree that everyone loves to hate,
and which foresters plant everywhere...***

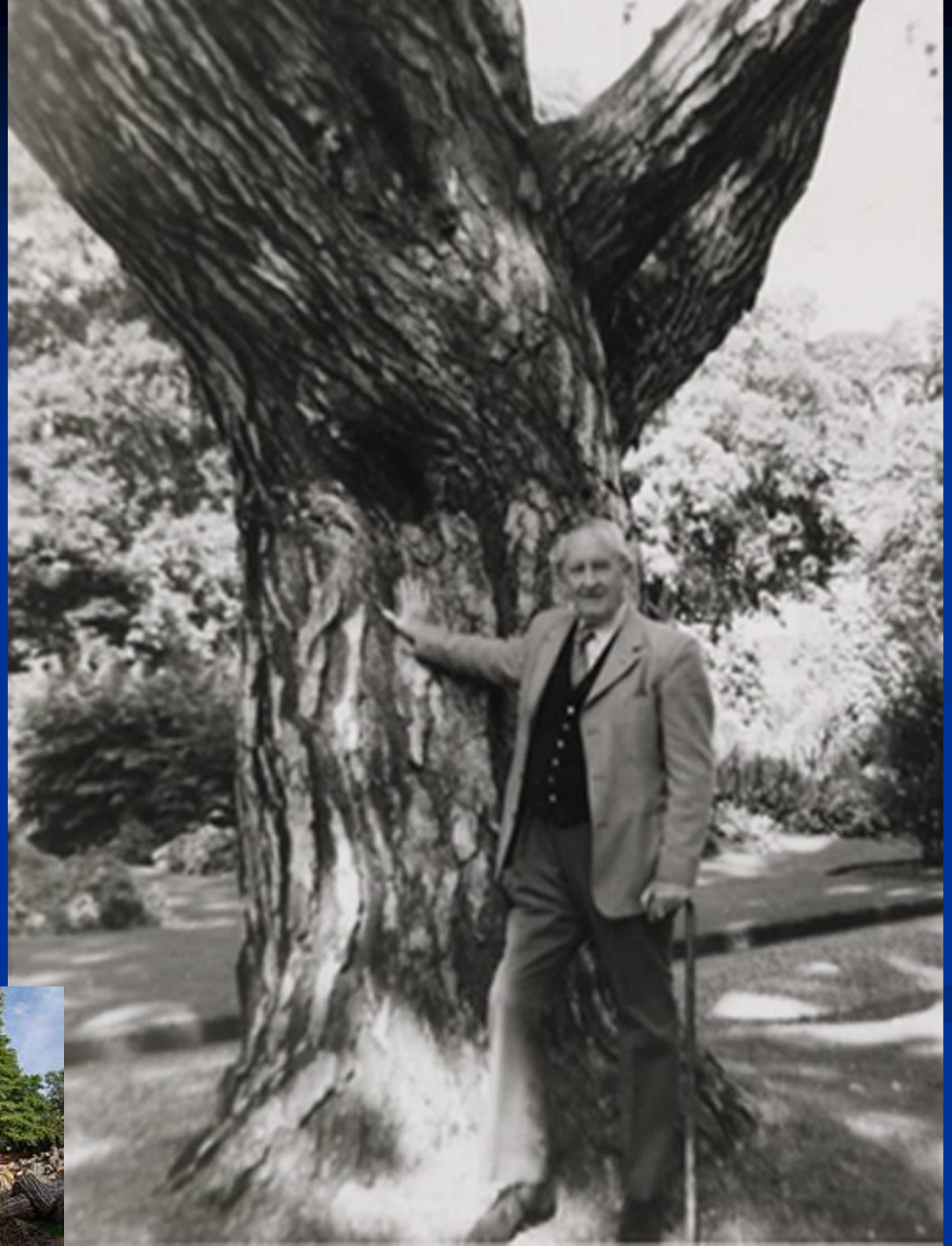


*This is a beauty
of dissonance,
this resonance
of stony strand,
this smoky cry
curled over a **black pine**
like a broken
and wind-battered branch
when the wind
bends the tops of the pines
and curdles the sky
from the north.*

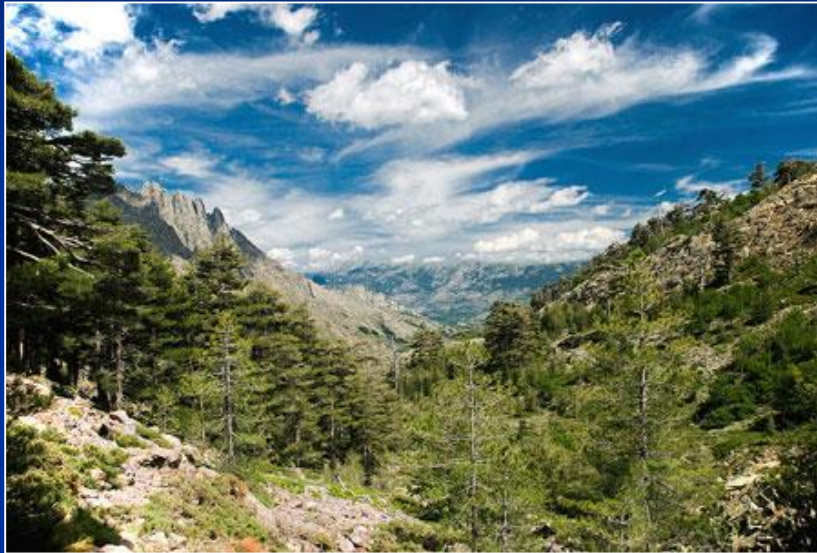
A.J.M. Smith
Canadian poet
(1902-1980)



One tree to rule them
all: JRR Tolkien with
the *black pine* in
Oxford Botanic
Garden



A pioneer species with low ecological requirements



Resistance to drought



Low soil pH and substrate specificity

One of the most productive, multiple purpose, native European conifers

- Over 3.5 million hectares of native forests
- Mean productivity from 6-10 m³/ha/year (natural conditions) to 8-20 m³/ha/year (monoculture)
- Durable wood, high resin content, easy to work



- Ecological restoration
- Erosion control
- Reforestation
- Parks and recreation



... But whose evolutionary history we actually know little about!

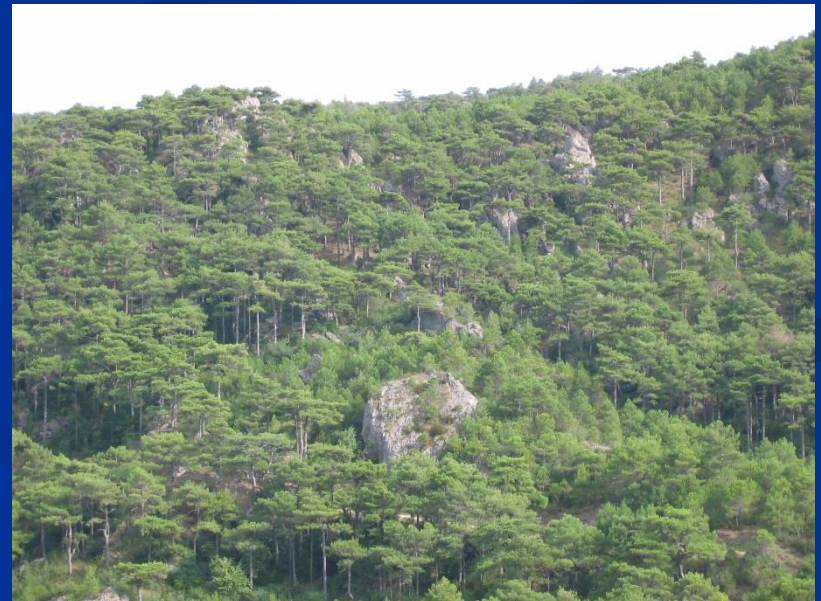
A habitat at stakes: uniqueness, risks and protection needs

- Priority habitat under “Habitats directive D. 92/43 CEE” (21 May 1992) : « (Sub-) Mediterranean pine forests with endemic black pines »
- European, national and regional issue: conservation and sustainable use within the national and regional biodiversity strategies.

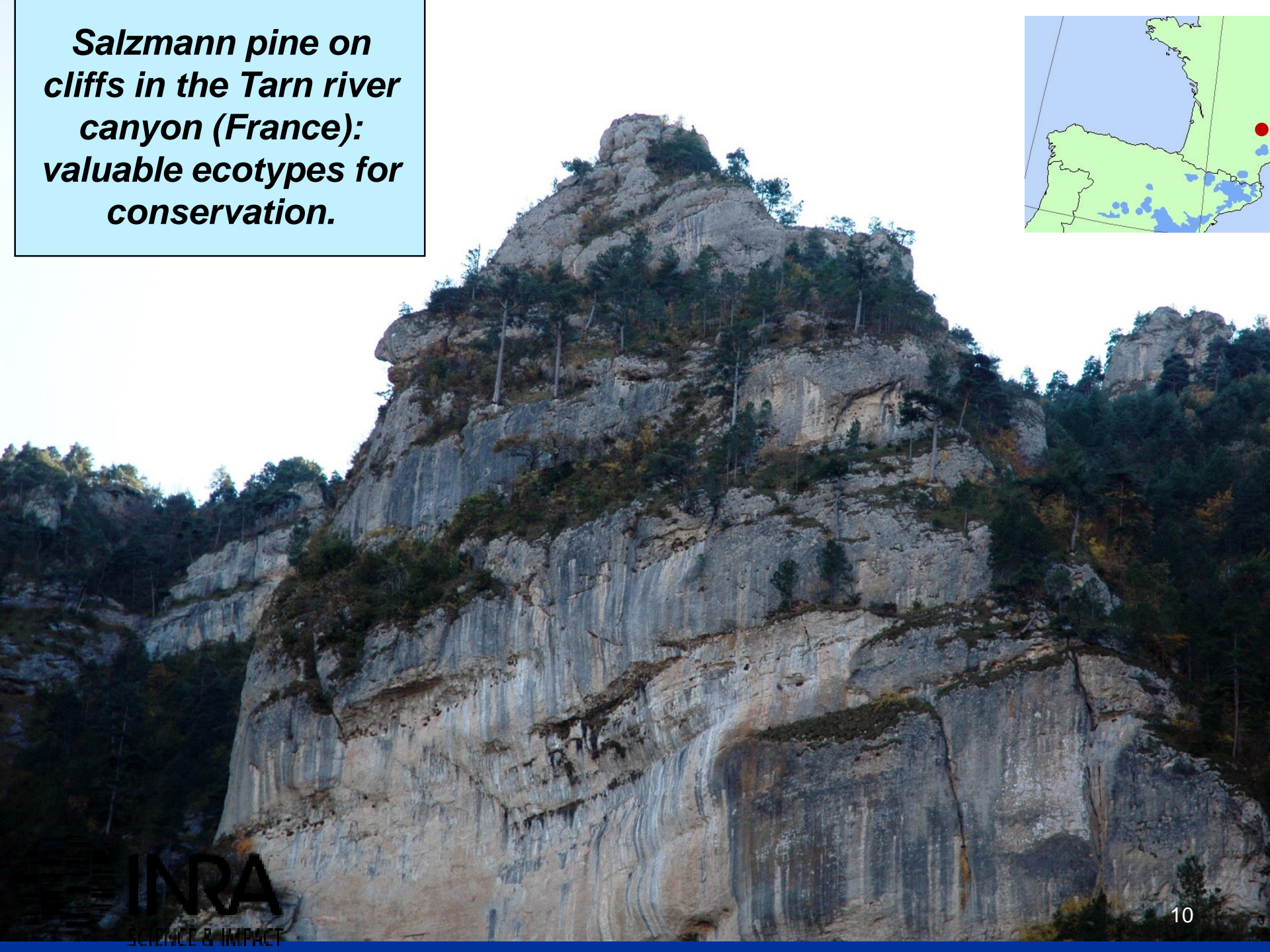
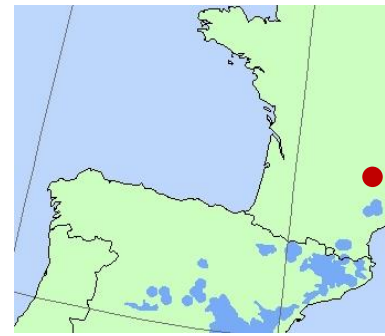


Also an ecological asset: uniqueness, risks and protection needs

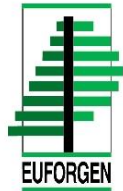
- Wild fires and climate change: risks on habitat (loss)
- Hybridization: potential risk (benefit?) for genetic resources. In France, 5 000 ha **native** forests versus 300 000 ha **planted** Austrian and Corsican black pine forests



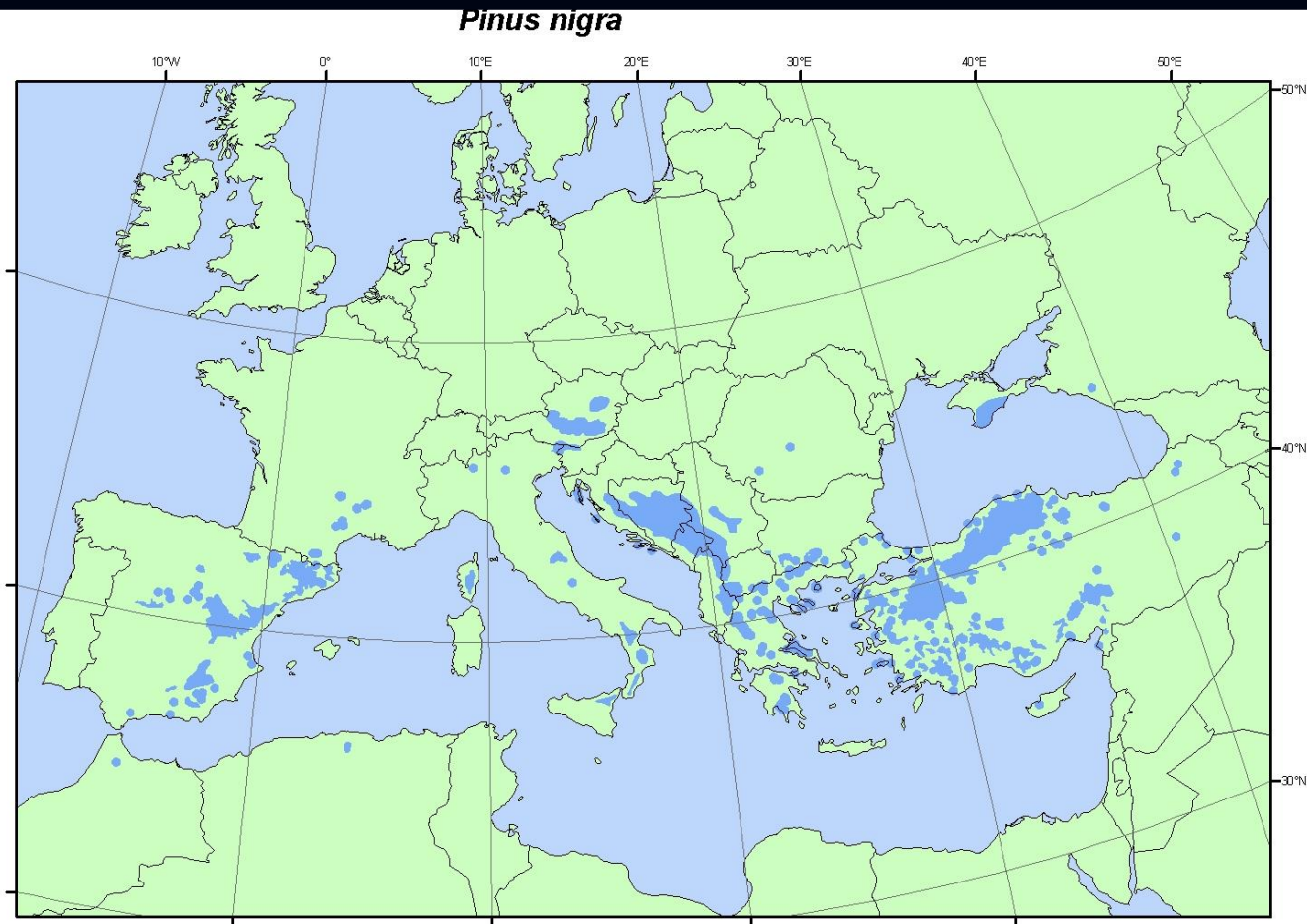
***Salzmann pine on
cliffs in the Tarn river
canyon (France):
valuable ecotypes for
conservation.***



Chorology of black pine and its 3.5 million ha of forests: fragmentation rules!



EUFORGEN Secretariat
c/o Biodiversity International
Via dei Tre Denari, 472/a
00057 Maccarese (Fiumicino)
Rome, Italy
Tel. (+39)066118251
Fax (+39)0661979661
euf_secretariat@cgiar.org
More information
and other maps at:
www.euforgen.org



This distribution map, showing the natural distribution area of *Pinus nigra* was compiled by members of the EUFORGEN Networks and was published in: Isajev, V., B. Fady, H. Semerci and V. Andonovski. 2004. EUFORGEN Technical Guidelines for genetic conservation and use of European black pine (*Pinus nigra*). International Plant Genetic Resources Institute, Rome, Italy. 6 pages

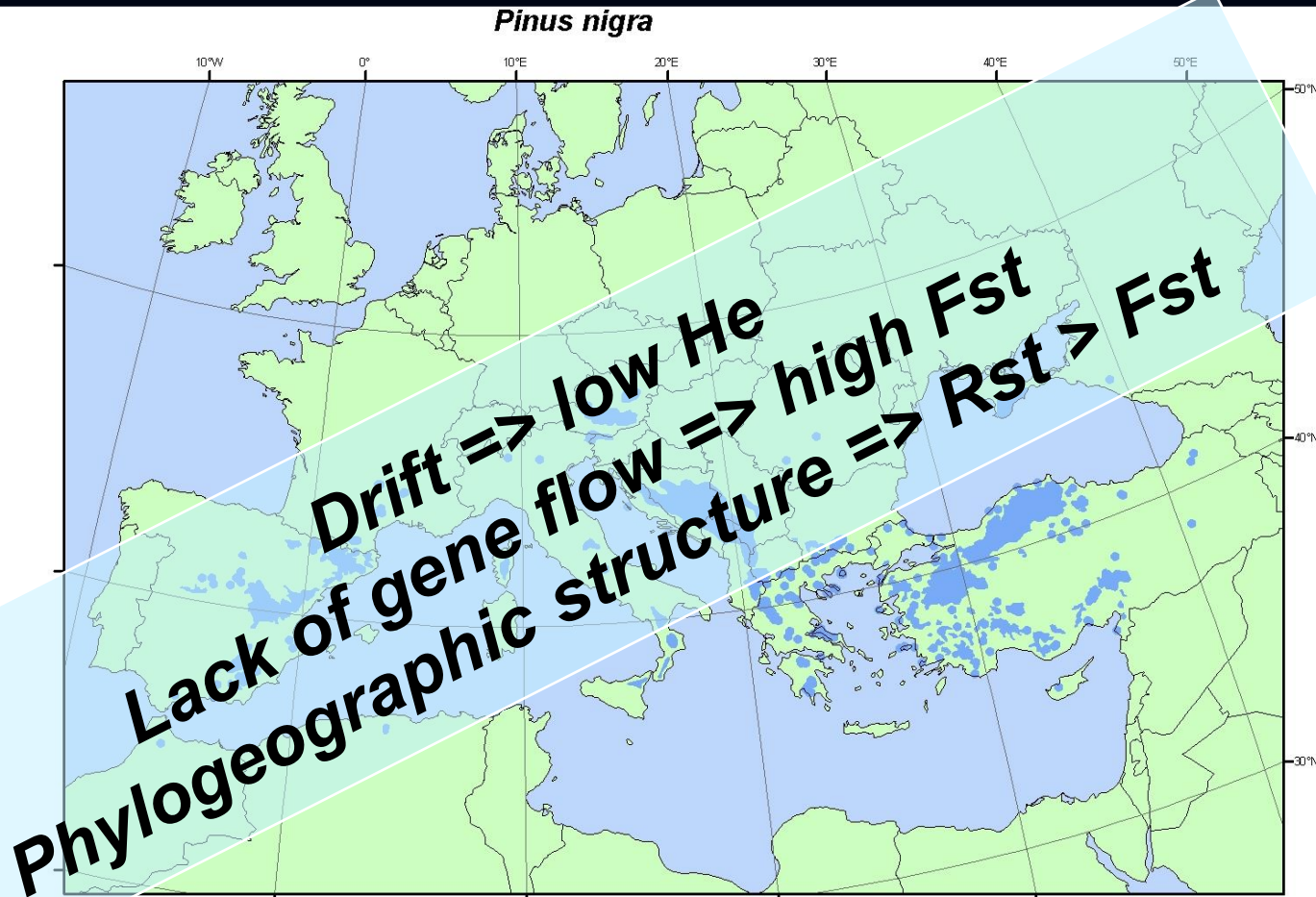
First published online on 26 March 2005 - Updated on 30 July 2008

Km
0 250 500 1,000

Expectations under long-term, strong fragmentation and a common ancestor

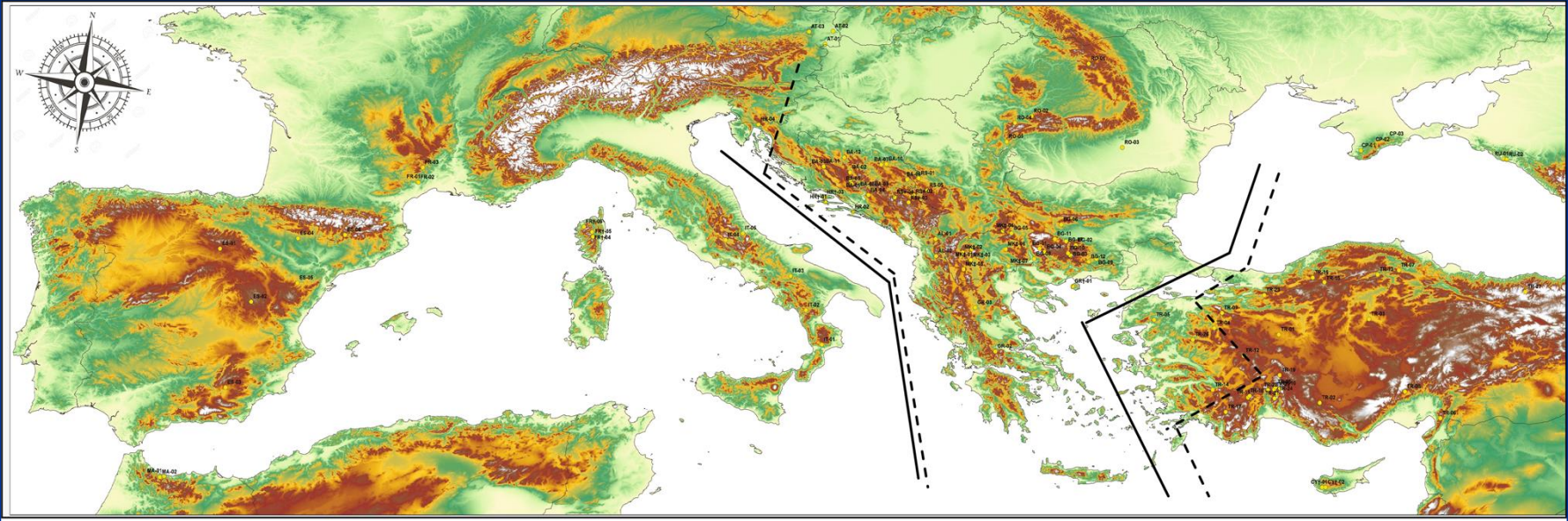


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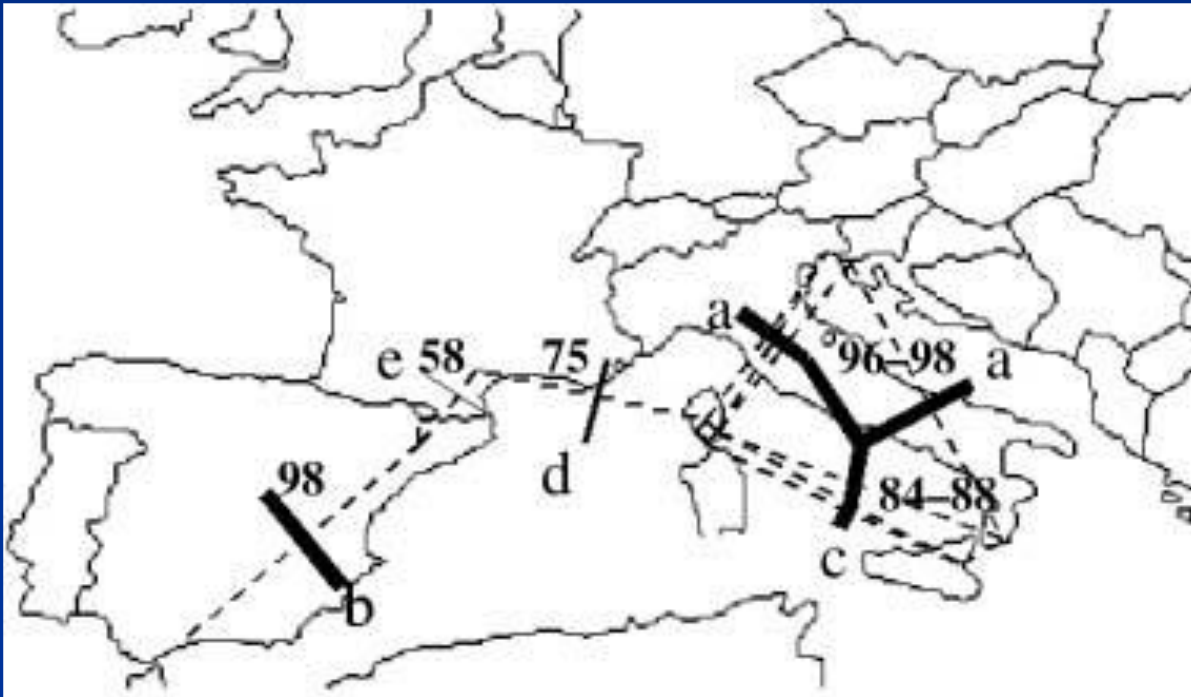
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Contradictory demographic scenarios (1)



Naydenov et al., 2016, 2017 (cpSSR sequences):
Ancient split into 3 lineages, between 4,37 and 0,93 My ago
272 haplotypes, $G_{st} \sim 0.177$, $H \sim 0.29$

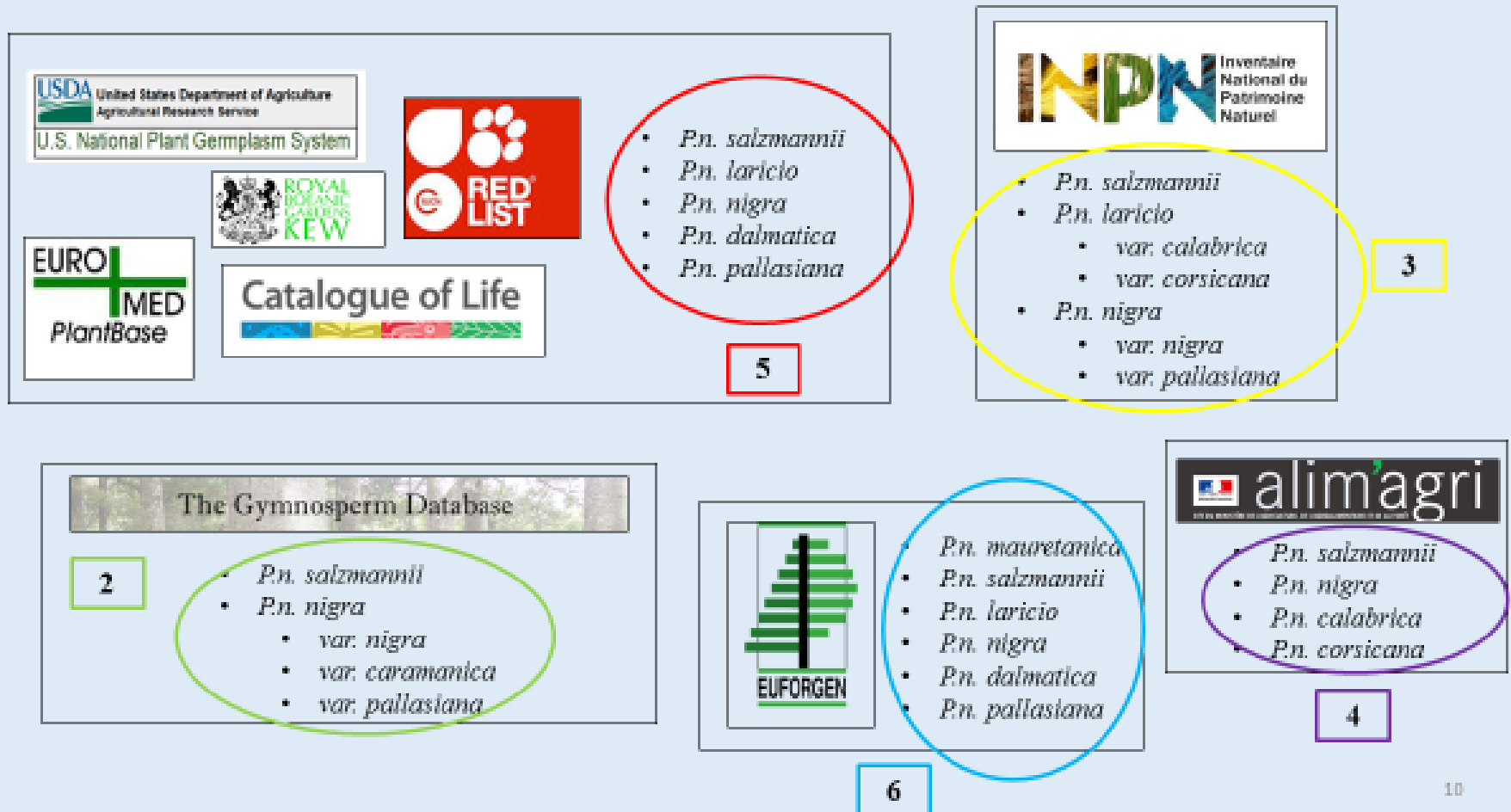
Contradictory demographic scenarios (2)



Fady et al., 2010
(cpSSR length polymorphism):
229 haplotypes,
 $F_{st} \sim 0.11$,
 $H_e \sim 0.95$

Afzal-Rafii and Dodd, 2007 (cpSSR length polymorphism):
Recent split into 6 lineages, between 150 and 30 kY
235 haplotypes, $PH_{lct} \sim 0.35$, $H_e \sim 0.95$

Taxonomic uncertainties



Differentiation and the evolutionary history of black pine

Sampling:

19 populations
(12 trees per population)



DNA markers:

4 barcoding (organelle) genes
4 cpSSR (Vendramin & al. 1996),
14 nSSR (Giovannelli & al. 2017),
14 « candidate » genes (SNP) (Mosca & al. 2012),

Differentiation and the evolutionary history of black pine

Sampling:

19 populations
(12 trees per population)



Results:

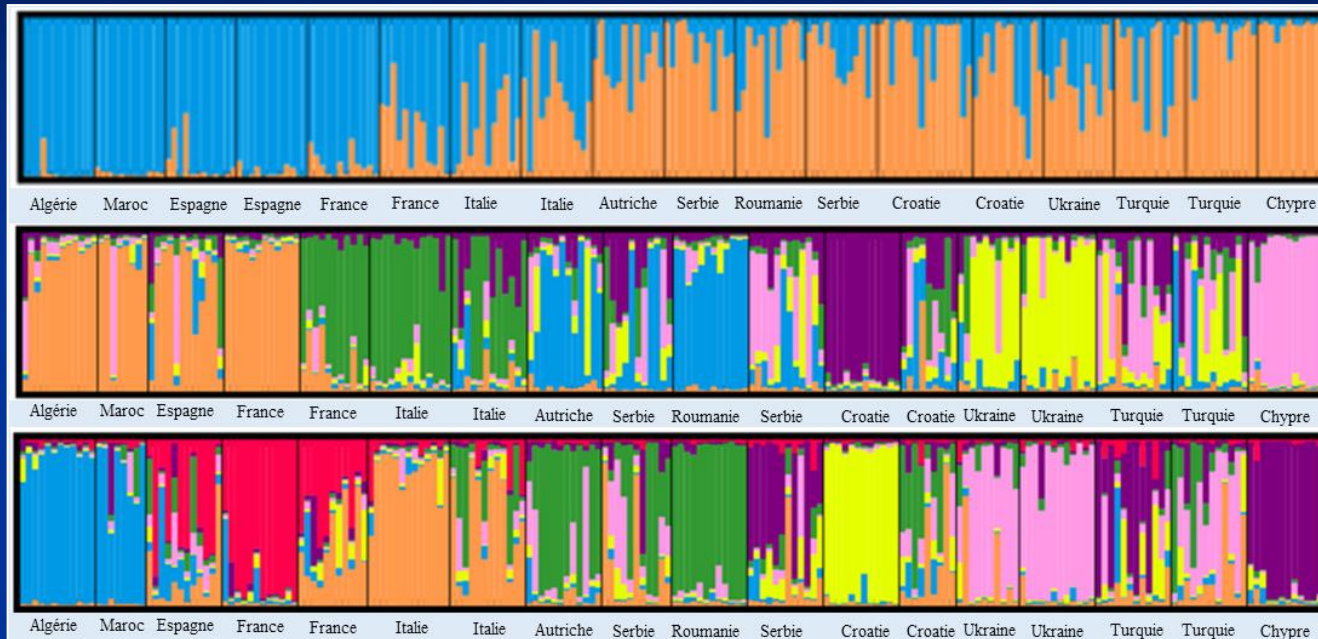
Barcoding genes: no diversity

cpSSRs: 143 haplotypes, $H_e \sim 0,86$, $F_{st} \sim 0,05$

nSSRs: $0,30 < H_e < 0,85$, $F_{st} \sim 0,138$

“Candidate genes”: $0,53 < H_e < 0,75$, $F_{st} \sim 0,12$

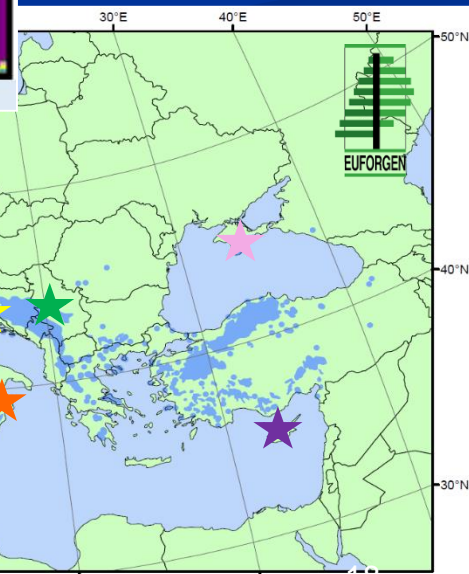
The most likely genetic structure of black pine range-wide



SNP, $K = 2$

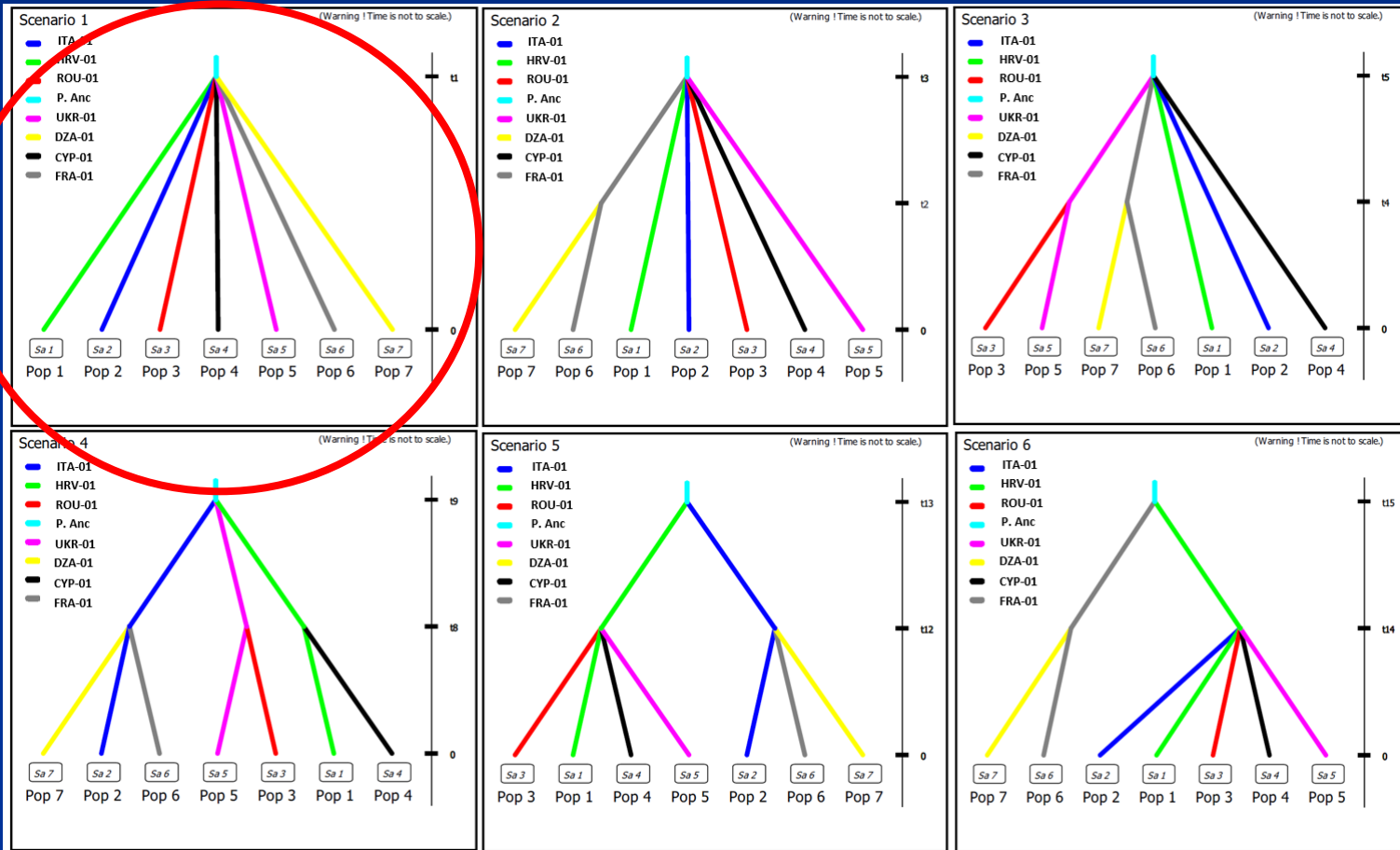
nSSR, $K = 6$

All, $K = 7$

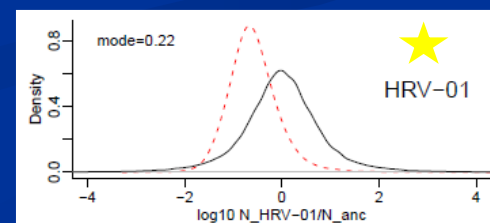
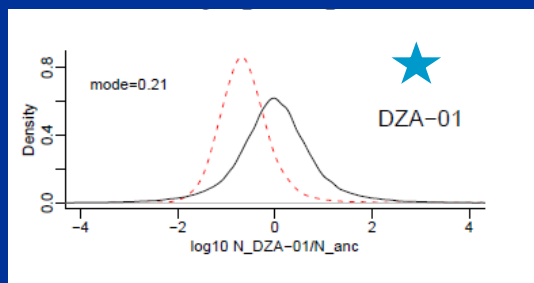
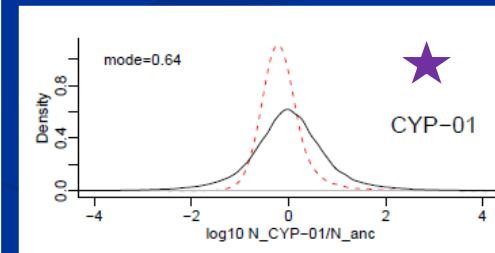
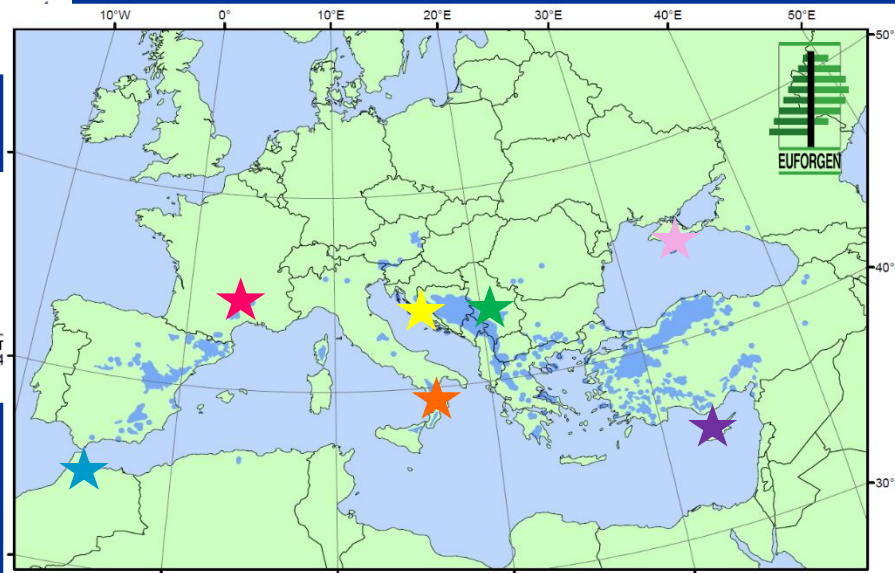
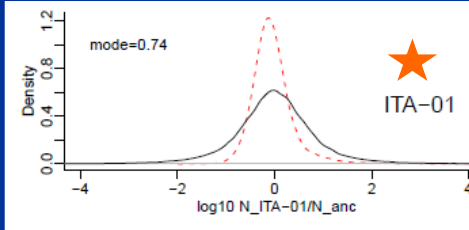
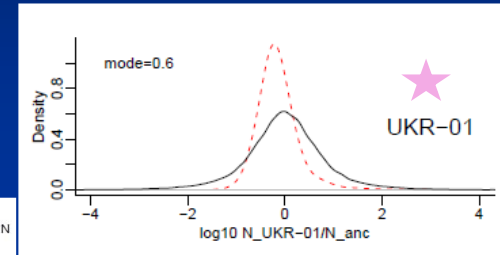
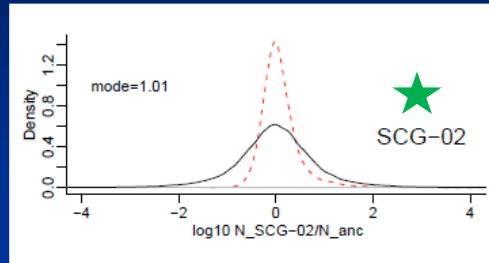
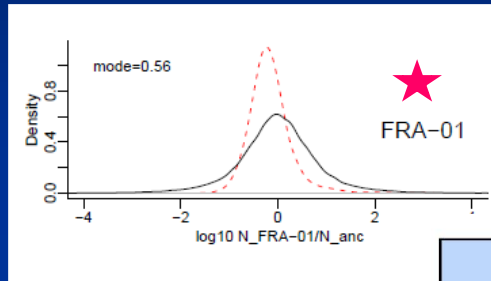


- An east – west cline
- (2 to) 7 different lineages
- High gene flow among populations

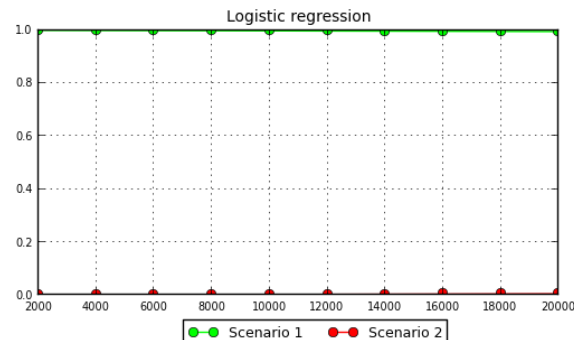
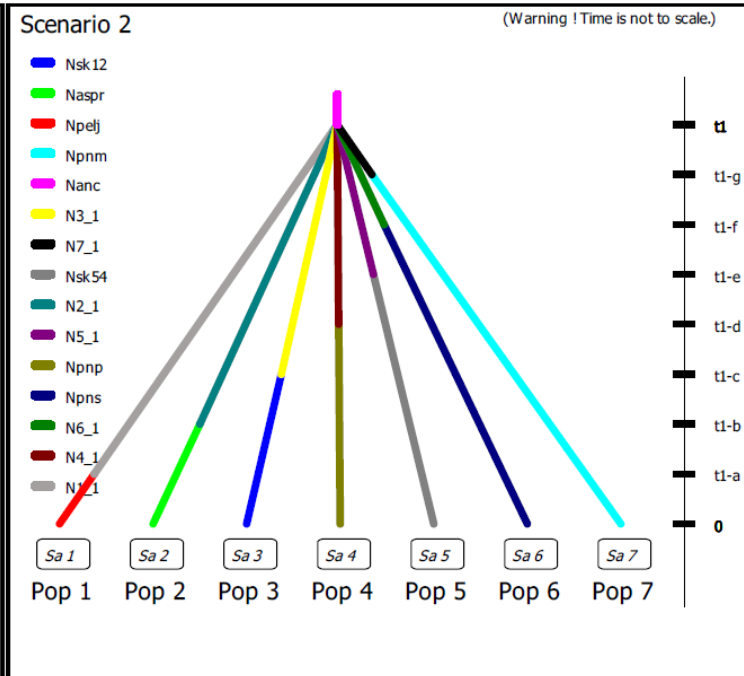
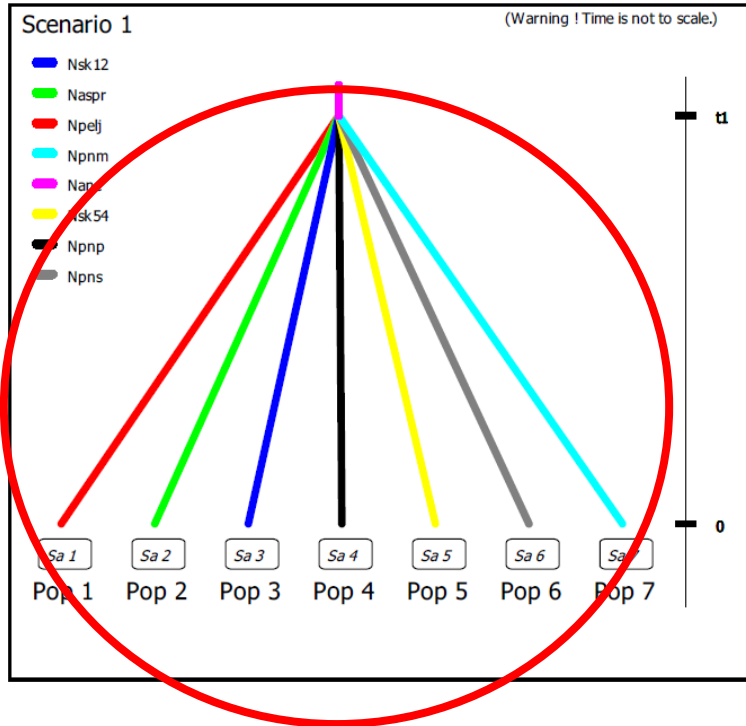
The genetic structure of black pine is best explained by a last-glaciation-time demographic split into 7 lineages



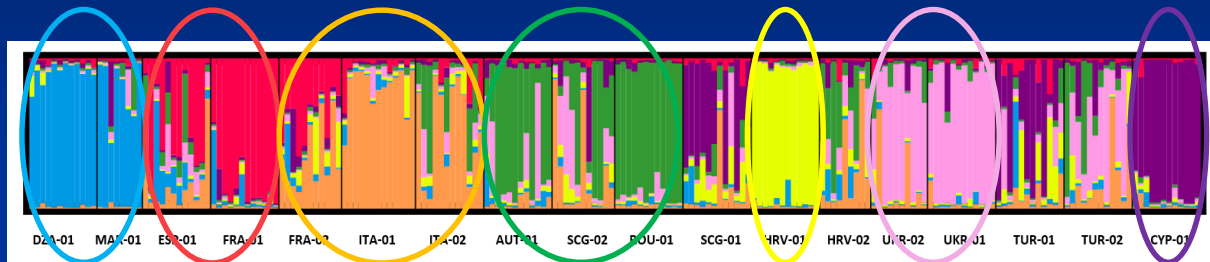
Expansion or contraction in the 7 lineages



... And no bottlenecks



Identification of 7 different lineages



Black pine and its sub-species (?):

- *P.n. mauretanica*
- *P.n. salzmannii*
- *P.n. laricio*
- *P.n. nigra*
- *P. n. pallasiana*
- *P. n. dalmatica*
- *P.n. caramanica*

Algeria, Morocco
(*P.n. mauretanica*)

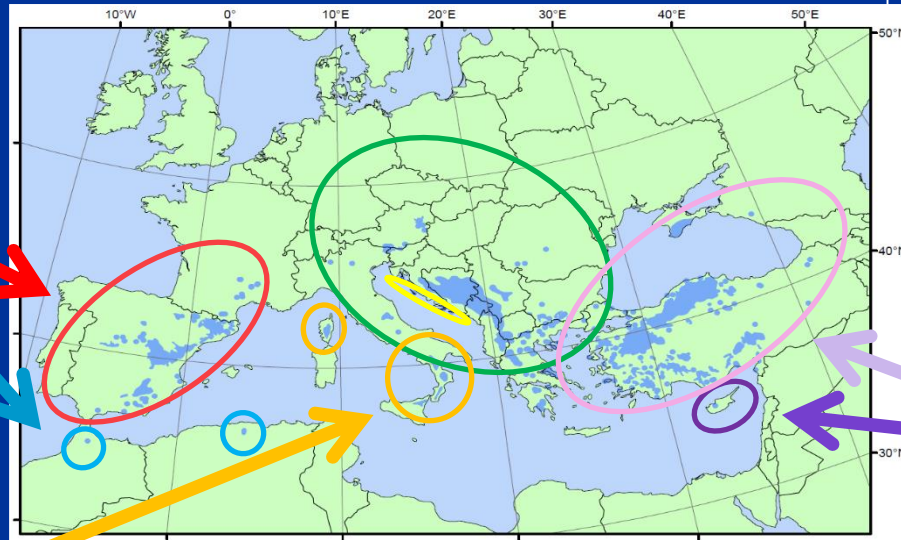
≠ or =

France, Spain
(*P.n. salzmannii*)

Corsica (*P.n. laricio*)

=

Calabria (*P.n. laricio*)



Turkey/Ukraine
(*P.n. pallasiana*)

≠

Cyprus (*P.n. caramanica*)

The evolutionary history of the European black pine

- High levels of gene flow despite fragmentation and moderate gene diversity ($F_{st} \sim 0.06-0.10$; $H_e \sim 0.8$);
- Weak phylogeographic and past climate signals;
- A relatively recently (Holocene) structured genetic diversity of 7 lineages;
- A few genetically depauperate lineages

The evolutionary history of the European black pine

- Some opportunities for taxonomic fingerprinting:
 - => detecting man-made translocation events;
 - => selecting gene conservation units outside of man-made hybrid zones;
- A need to revise taxonomy and possibly legal (forest reproductive material) documents;
- Outlook: SNP from transcriptome sequences.

The European black pine: there is more than meets the eye...

A structured diversity across its distribution range that can guide gene conservation strategies and forest management

