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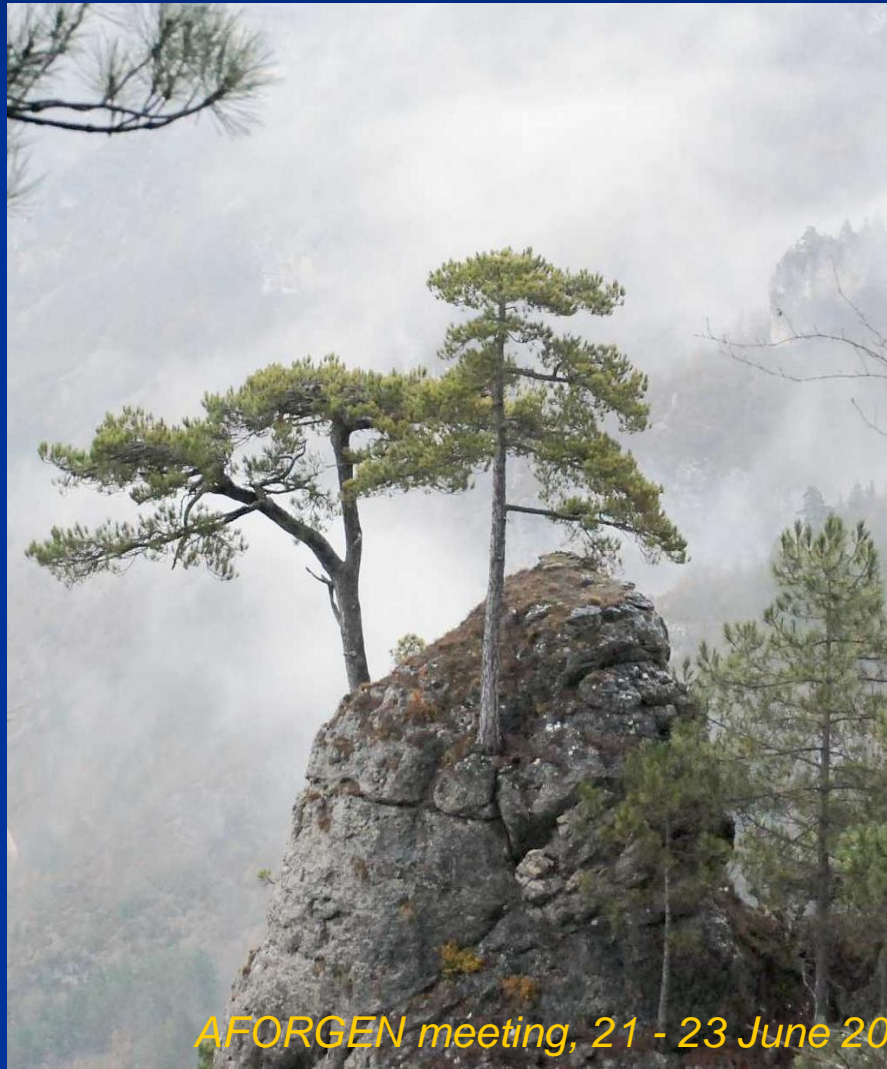
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# Demographic and evolutionary history of the European black pine (*Pinus nigra* Arn)



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***Do you know the European black pine?***

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



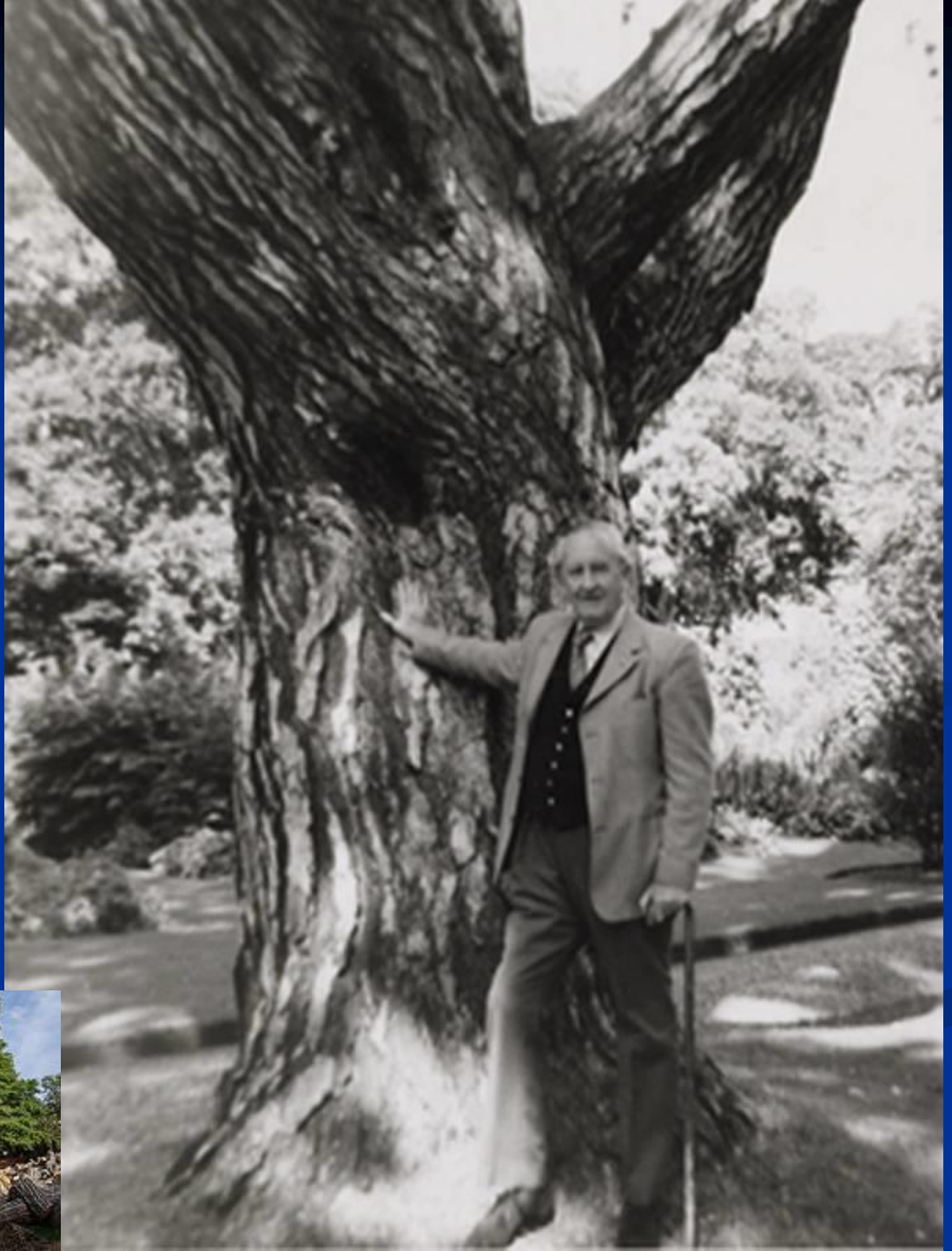


*This is a beauty  
of dissonance,  
this resonance  
of stony strand,  
this smoky cry  
curled over a **black pine**  
like a broken  
and wind-battered branch  
when the wind  
bends the tops of the pines  
and curdles the sky  
from the north.*

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

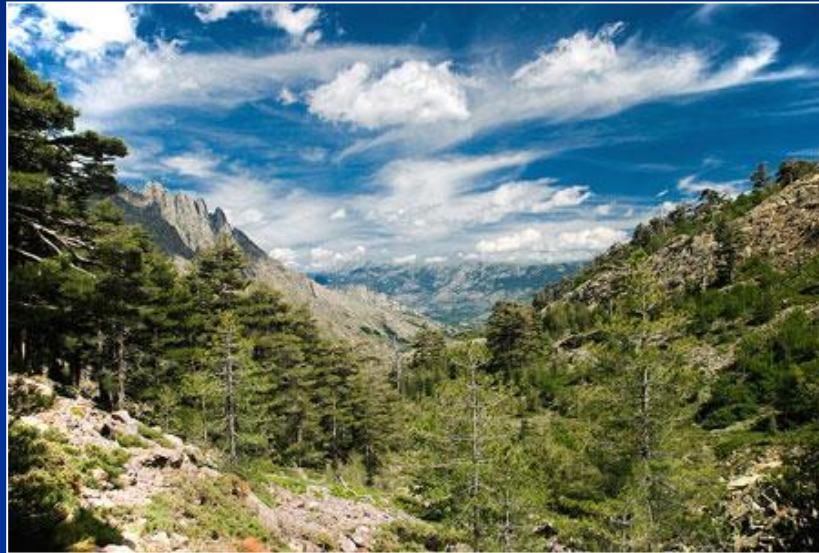


One tree to rule them all: JRR Tolkien with the *black pine* in Oxford Botanic Garden





# *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 m<sup>3</sup>/ha/year (natural conditions) to 8-20 m<sup>3</sup>/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!***



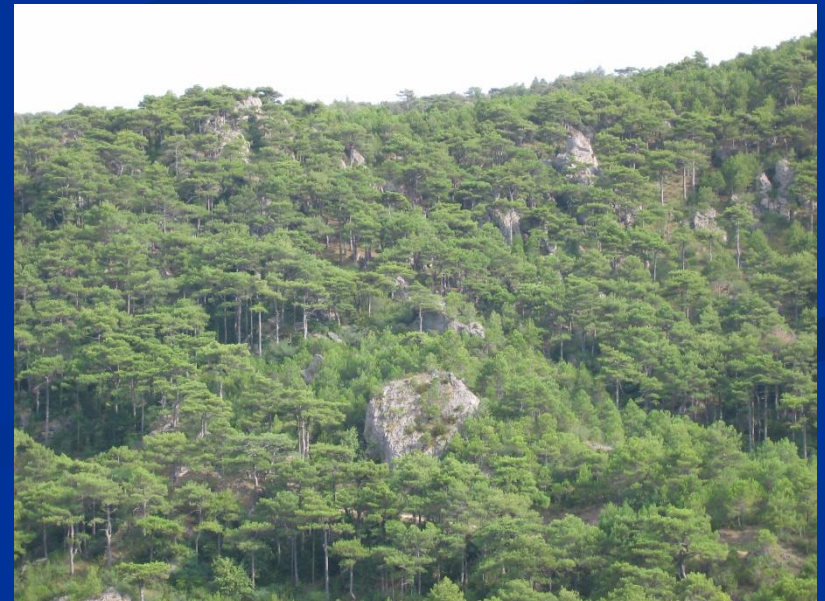
# *A habitat at stakes: uniqueness, risks and protection needs*

- 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.***

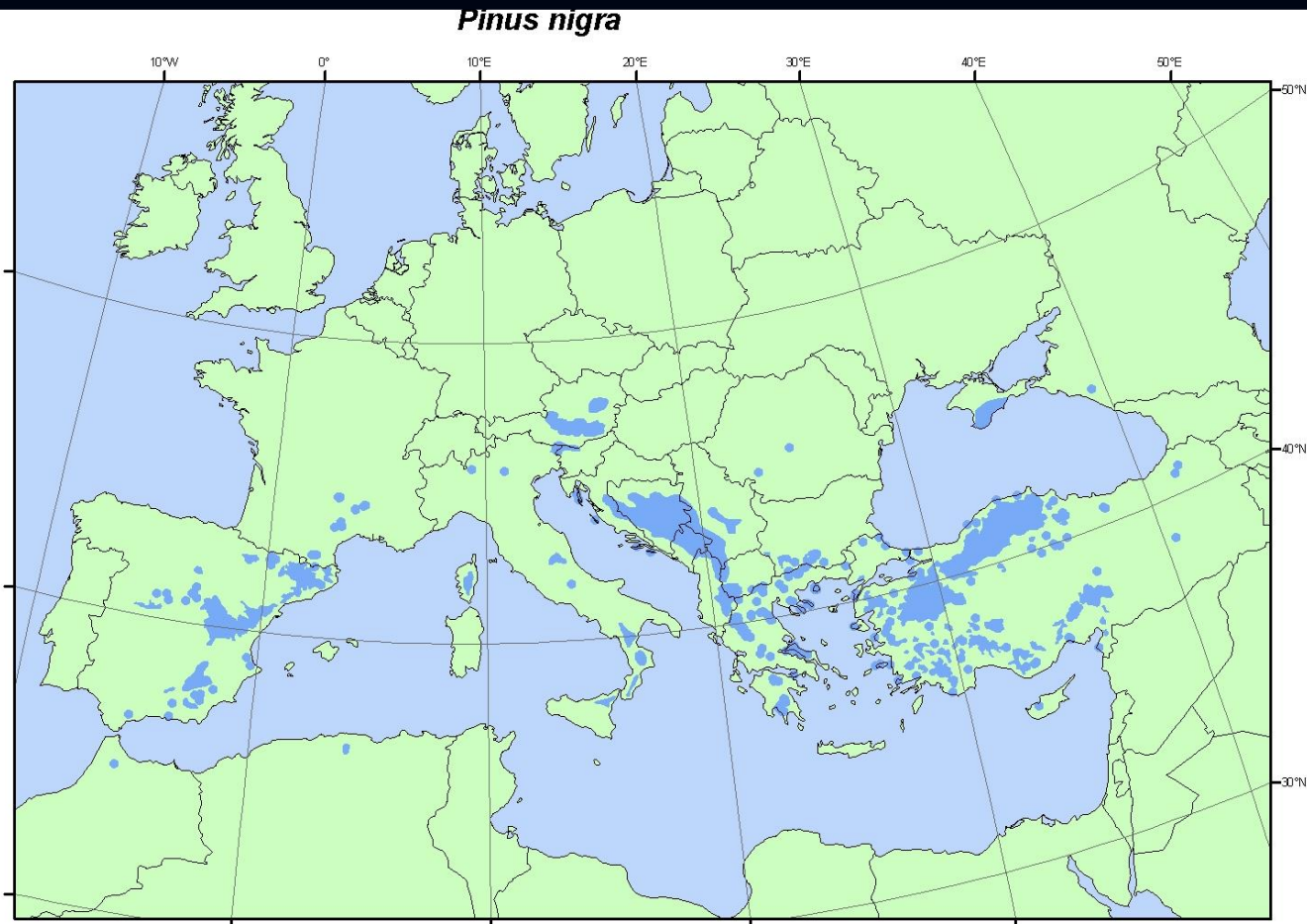




# Chorology of black pine and its 3.5 million ha of forests: fragmentation rules!



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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

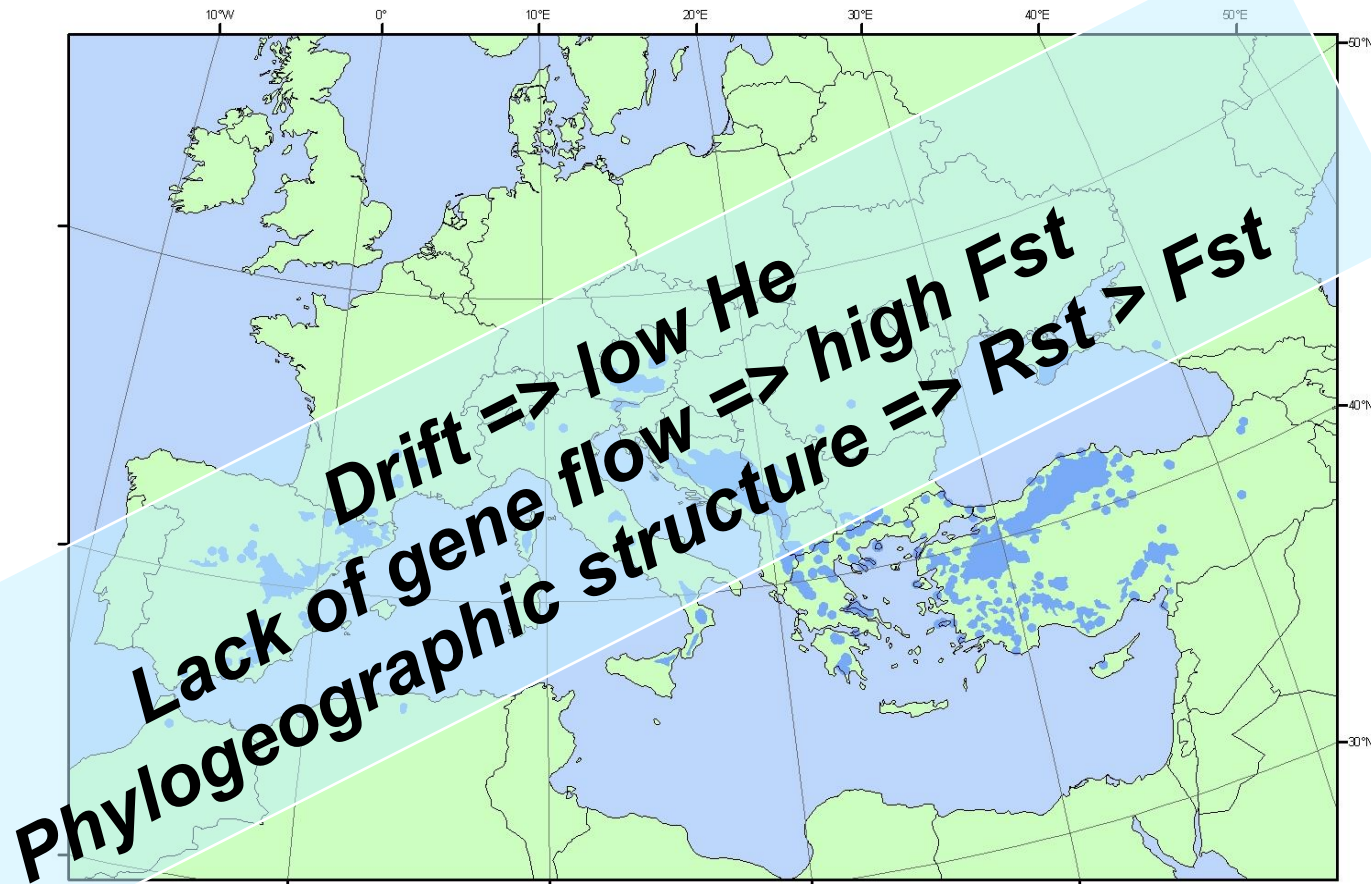
First published online on 26 March 2005 - Updated on 30 July 2008

0 250 500 1,000 Km

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



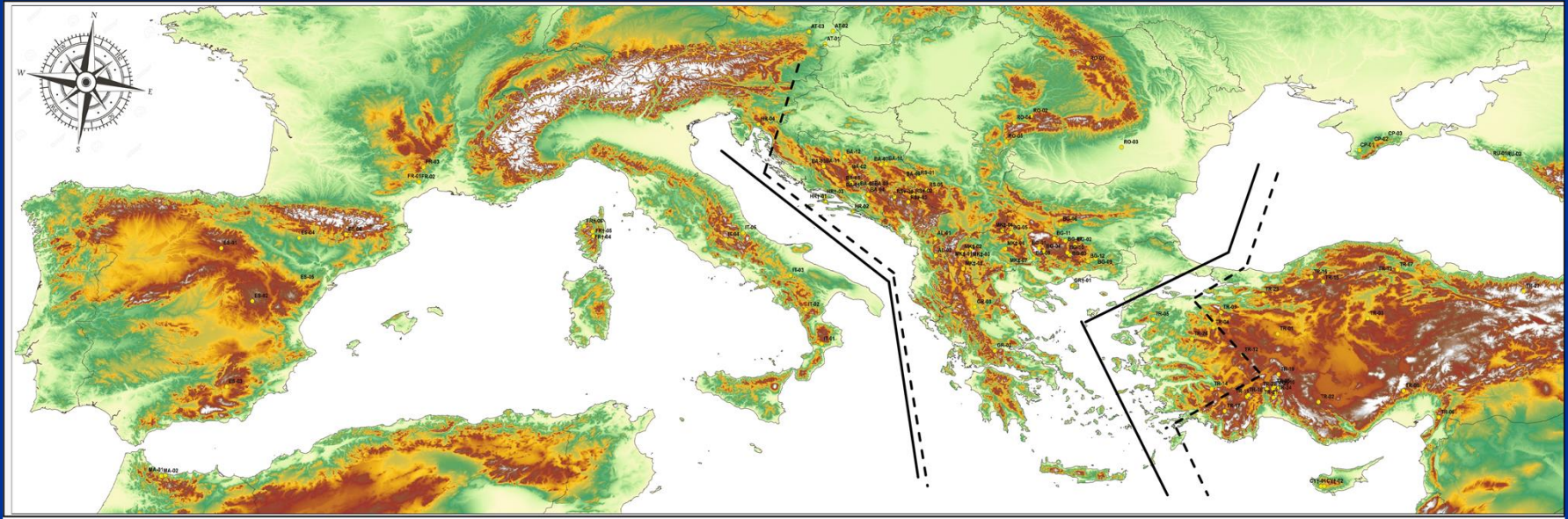
## *Pinus nigra*



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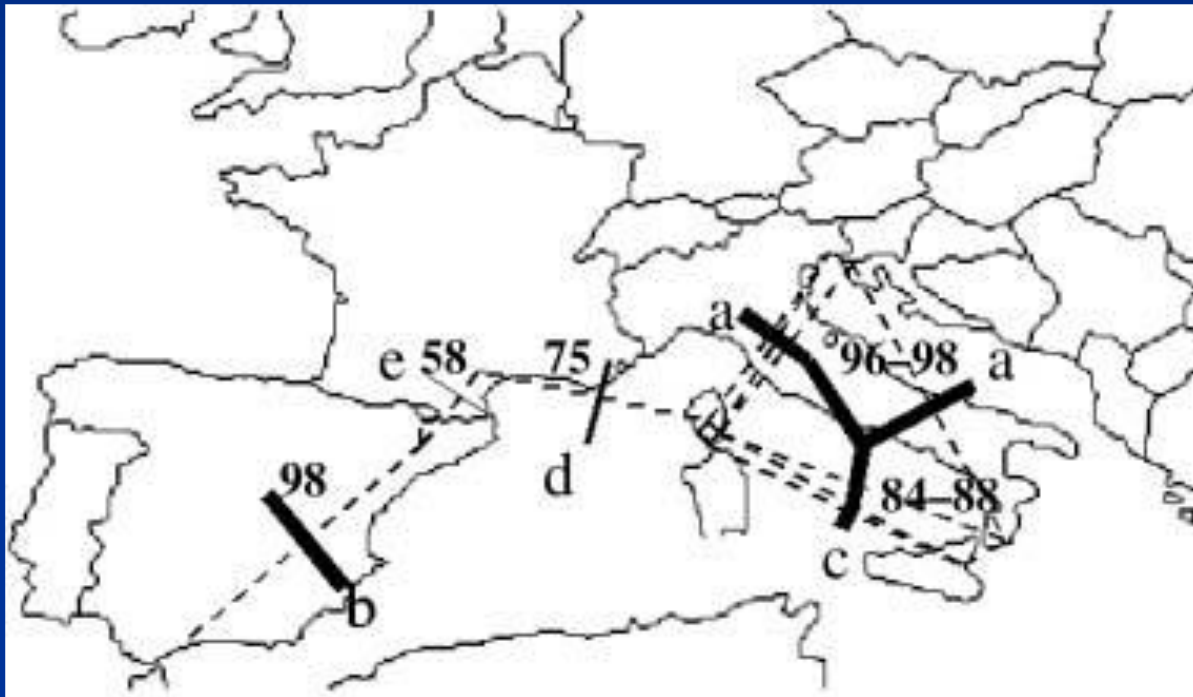
# Contradictory demographic scenarios (1)



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



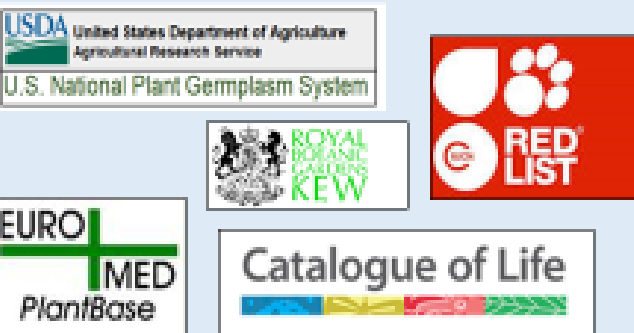
## Contradictory demographic scenarios (2)



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



USDA United States Department of Agriculture  
Agricultural Research Service  
U.S. National Plant Germplasm System

EURO+MED  
PlantBase


ROYAL BOTANIC GARDENS  
KEW

RED LIST

Catalogue of Life

- *Pn. salzmannii*
- *Pn. laricio*
- *Pn. nigra*
- *Pn. dalmatica*
- *Pn. pallasiana*

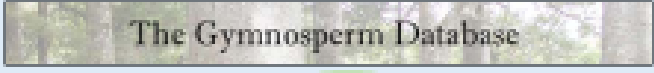
5



INPN Inventaire National du Patrimoine Naturel

- *Pn. salzmannii*
- *Pn. laricio*
  - var. *calabrica*
  - var. *corsicana*
- *Pn. nigra*
  - var. *nigra*
  - var. *pallasiana*


3



The Gymnosperm Database

2


- *Pn. salzmannii*
- *Pn. nigra*
  - var. *nigra*
  - var. *caramanica*
  - var. *pallasiana*



EUFORGEN

- *Pn. mauretanic*
- *Pn. salzmannii*
- *Pn. laricio*
- *Pn. nigra*
- *Pn. dalmatica*
- *Pn. pallasiana*

6



alimagri

- *Pn. salzmannii*
- *Pn. nigra*
- *Pn. calabrica*
- *Pn. corsicana*

4

# 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:

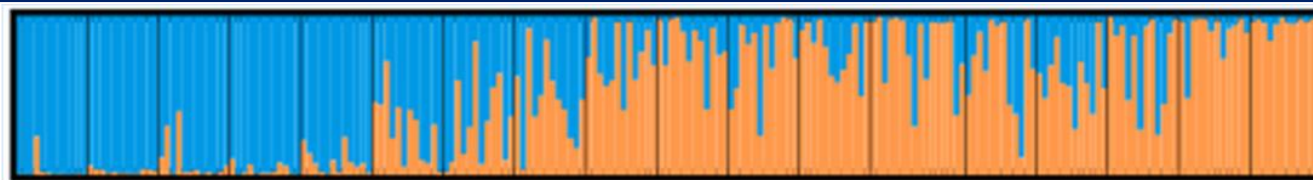
Barcoding genes: no diversity

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

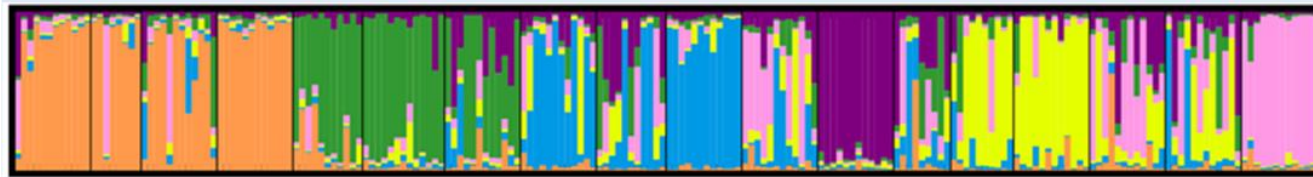
nSSRs:  $0,30 < H_e < 0,85$ ,  $F_{st} \sim 0,138$

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

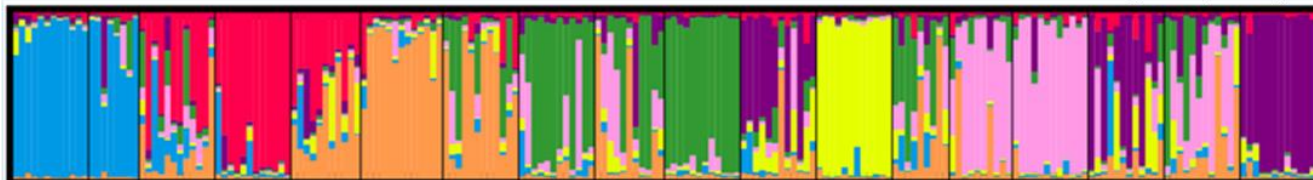
# The most likely genetic structure of black pine range-wide



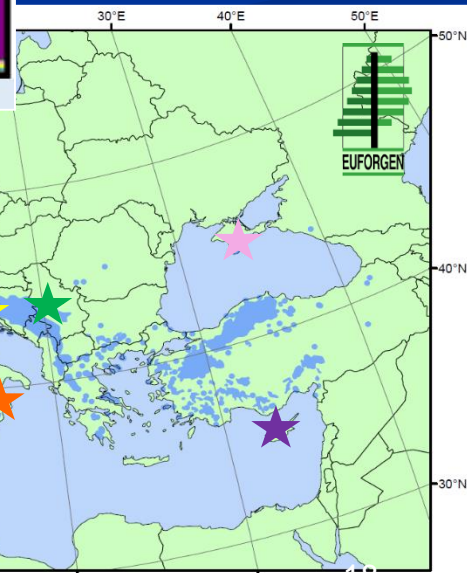
Algérie Maroc Espagne Espagne France France Italie Italie Autriche Serbie Roumanie Serbie Croatie Croatie Ukraine Turquie Turquie Chypre



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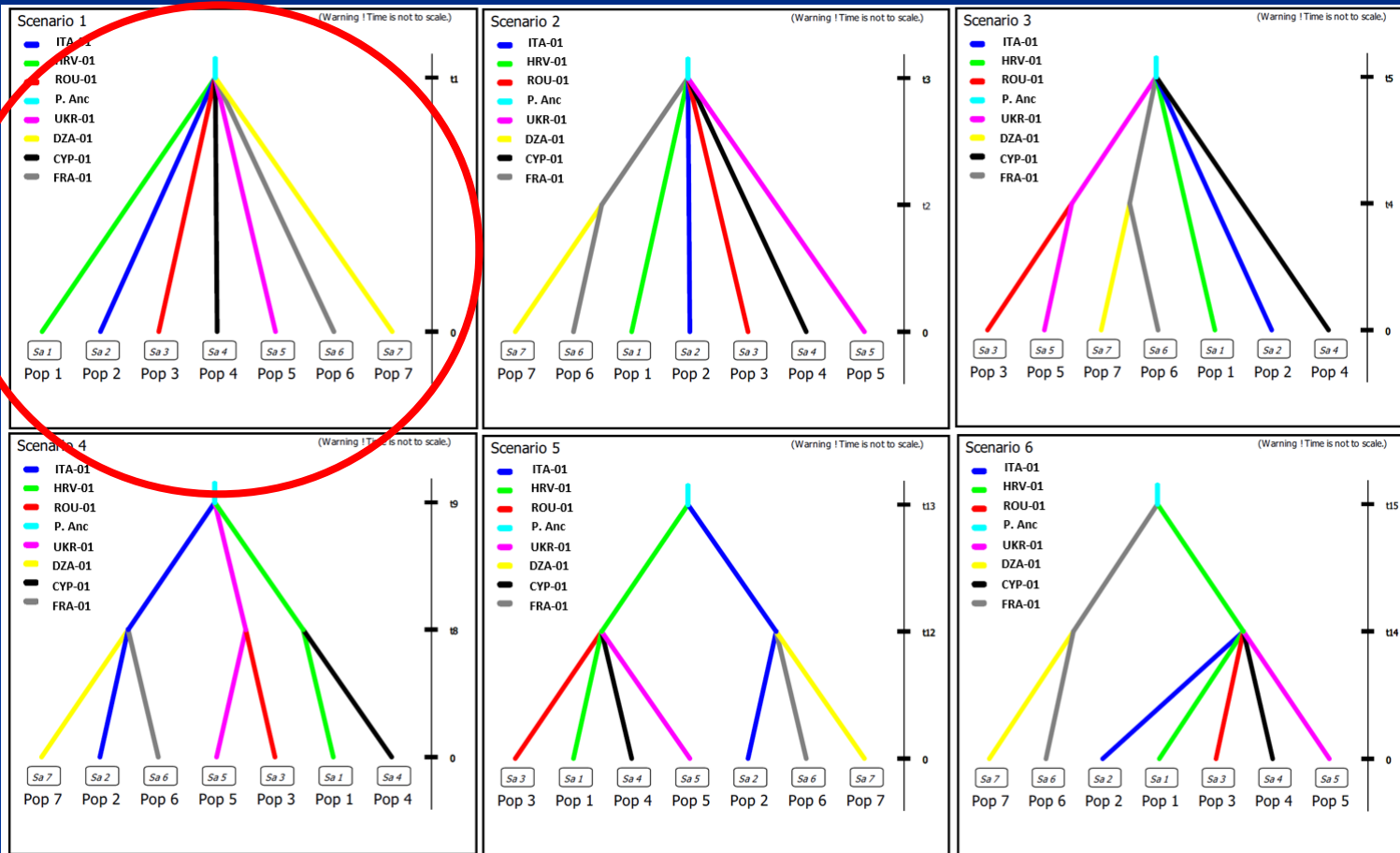


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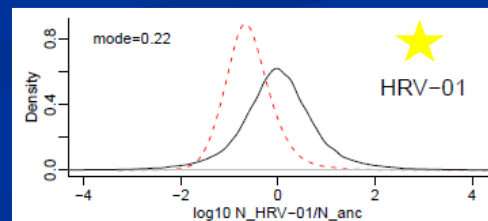
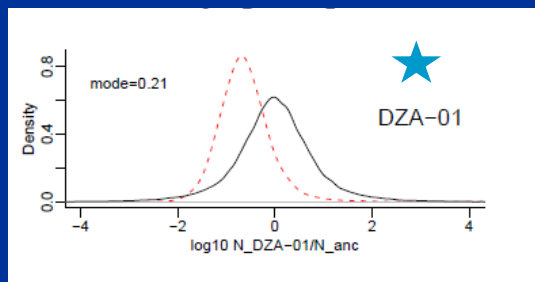
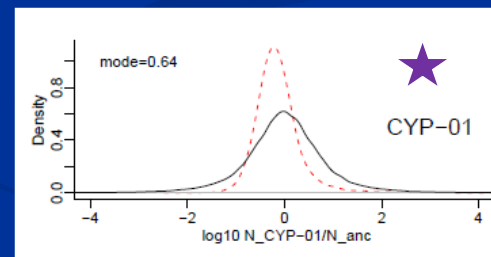
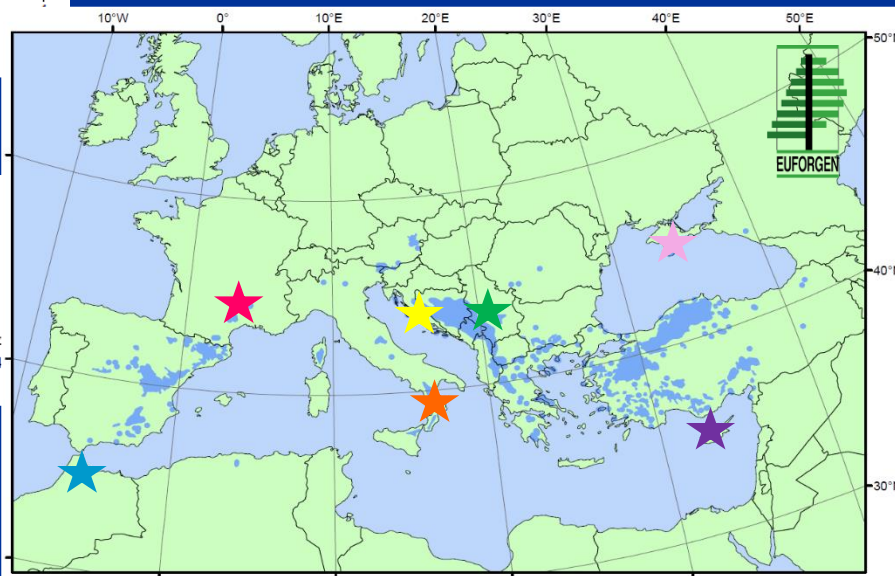
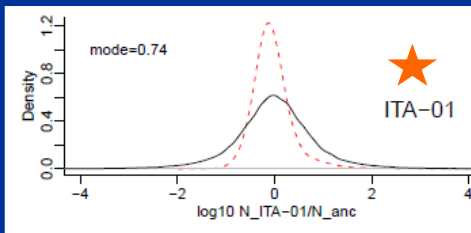
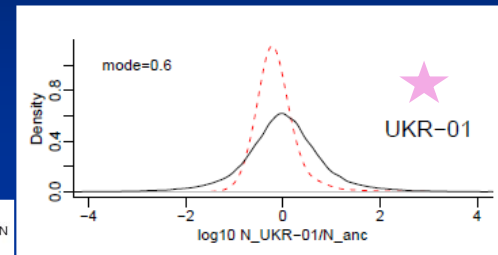
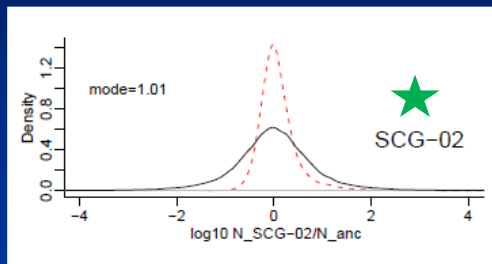
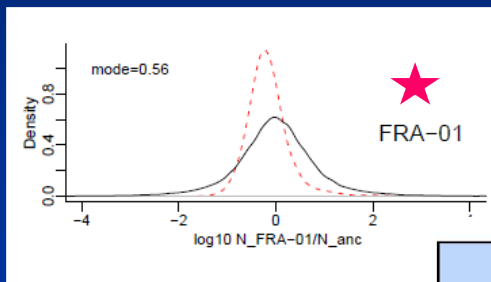
- An east – west cline
- (2 to) 7 different lineages
- High gene flow among populations

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

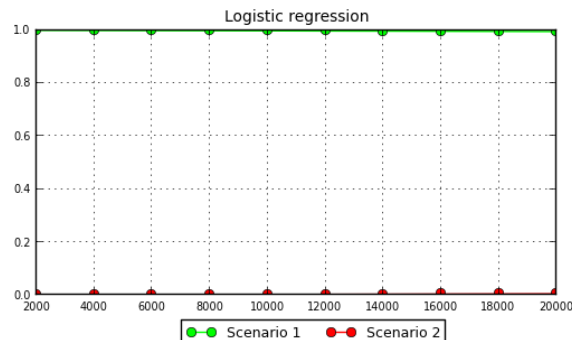
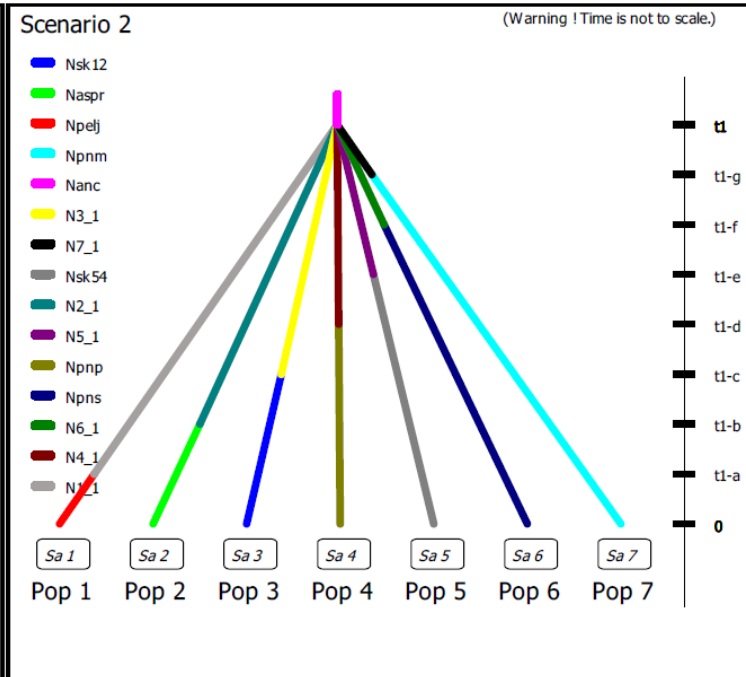
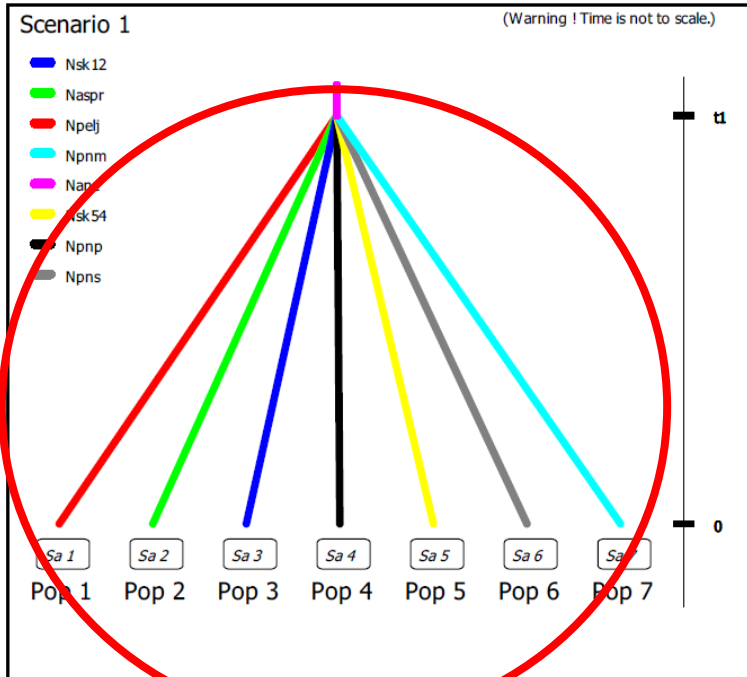




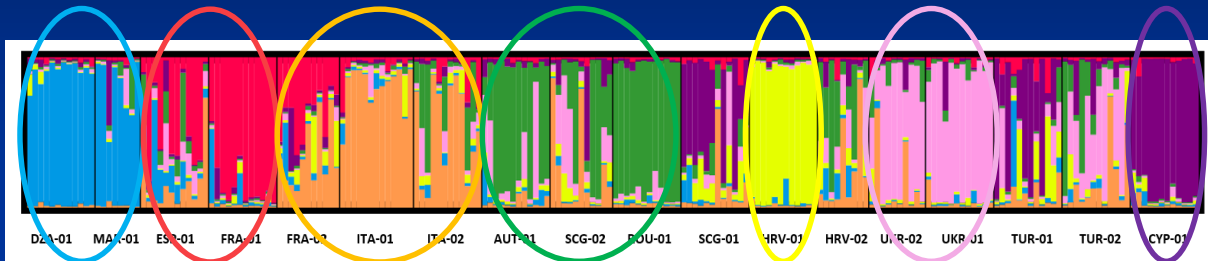
# Expansion or contraction in the 7 lineages



# ... 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*)

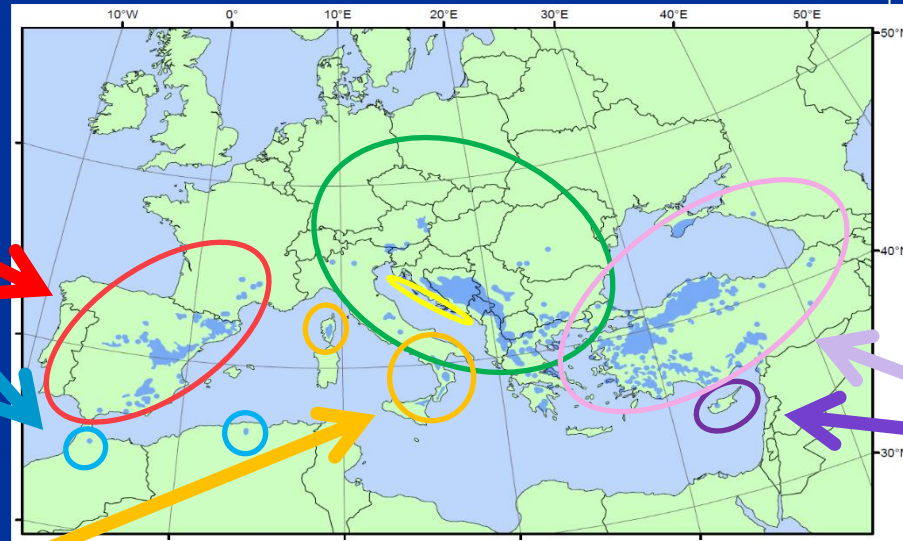
≠ or =

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***

