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## A brief evolutionary history of *Pinus pinea* L

Bruno Fady, Santiago C. Gonzalez Martinez, Giovanni G. Vendramin

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# *A Brief Evolutionary History of Pinus pinea L.*

Bruno Fady\*,  
Santiago C. Gonzalez-Martinez\*\*, Giovanni G. Vendramin\*\*\*



INRA – URFM, Avignon, France  
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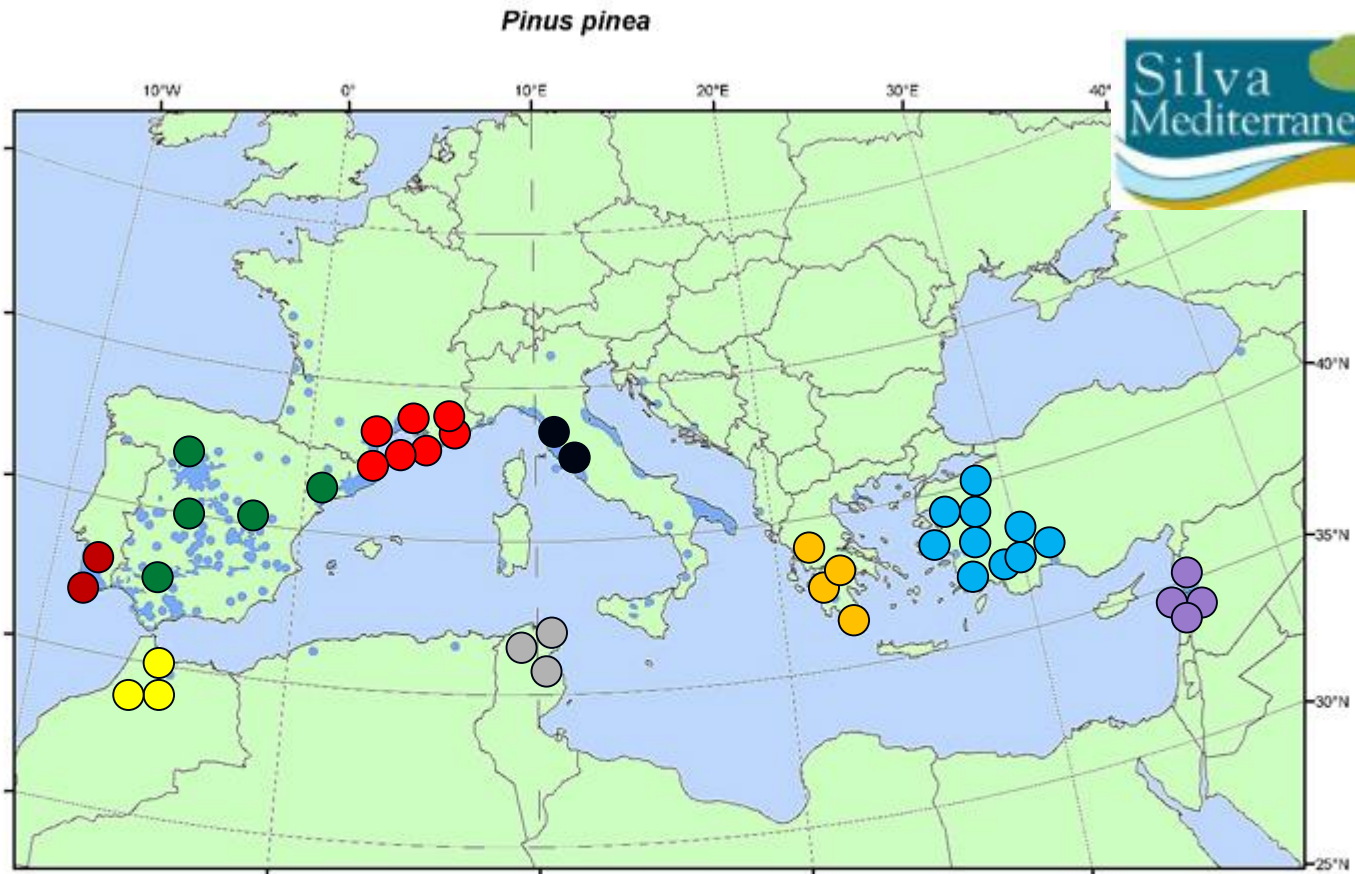


For me, it all started in 1992.....

# Collecting provenances from the entire geographic distribution range of *Pinus pinea* L.



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c/o Biodiversity International  
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More information  
and other maps at  
[www.euforgen.org](http://www.euforgen.org)



This distribution map, showing the natural distribution area of *Pinus pinea* was compiled by members of the EUFORGEN Networks

Citation: Distribution map of Italian stone pine (*Pinus pinea*) EUFORGEN 2009, [www.euforgen.org](http://www.euforgen.org).

First published online on September 2004 - Updated on 24 July 2008



# The genetic diversity of *Pinus pinea* L. at isozyme loci

Study on Isozyme Variation in *Pinus pinea* L.:  
Evidence for Low Polymorphism

Fallour et al. (*Silvae Genet.*) 1997

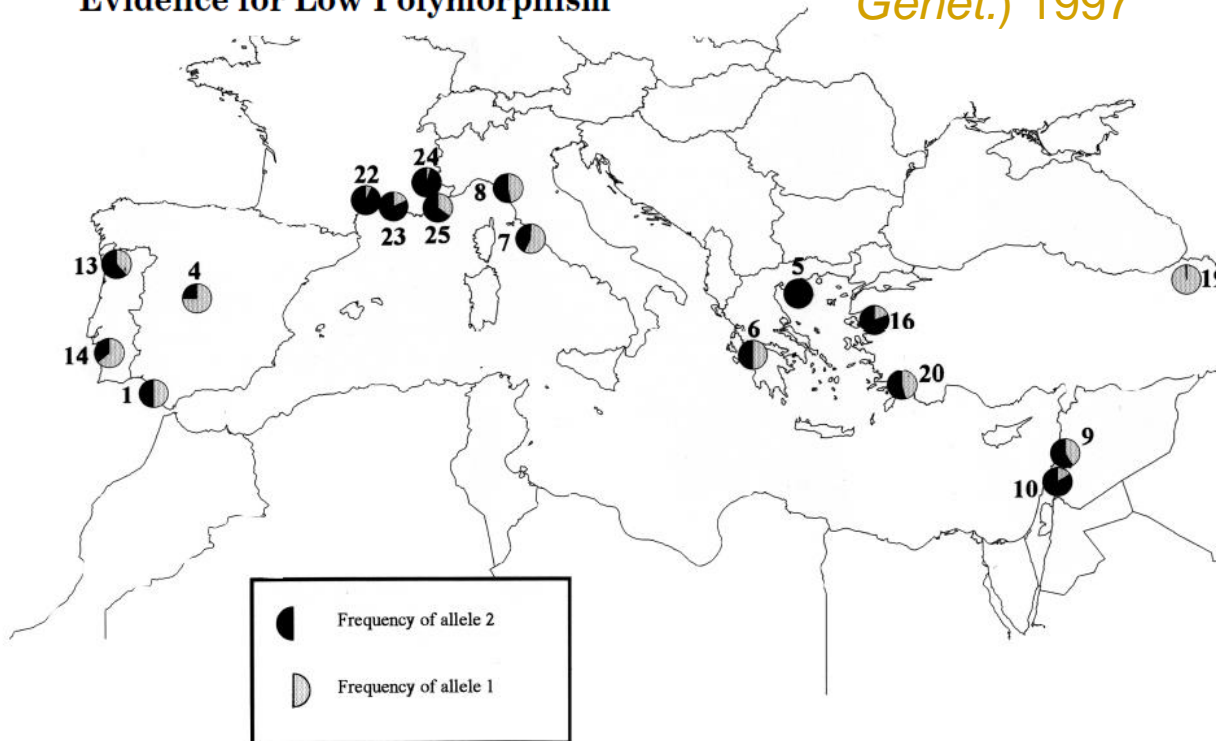


Figure 3. – Localization of the studied populations with their allele frequencies at the ME locus.

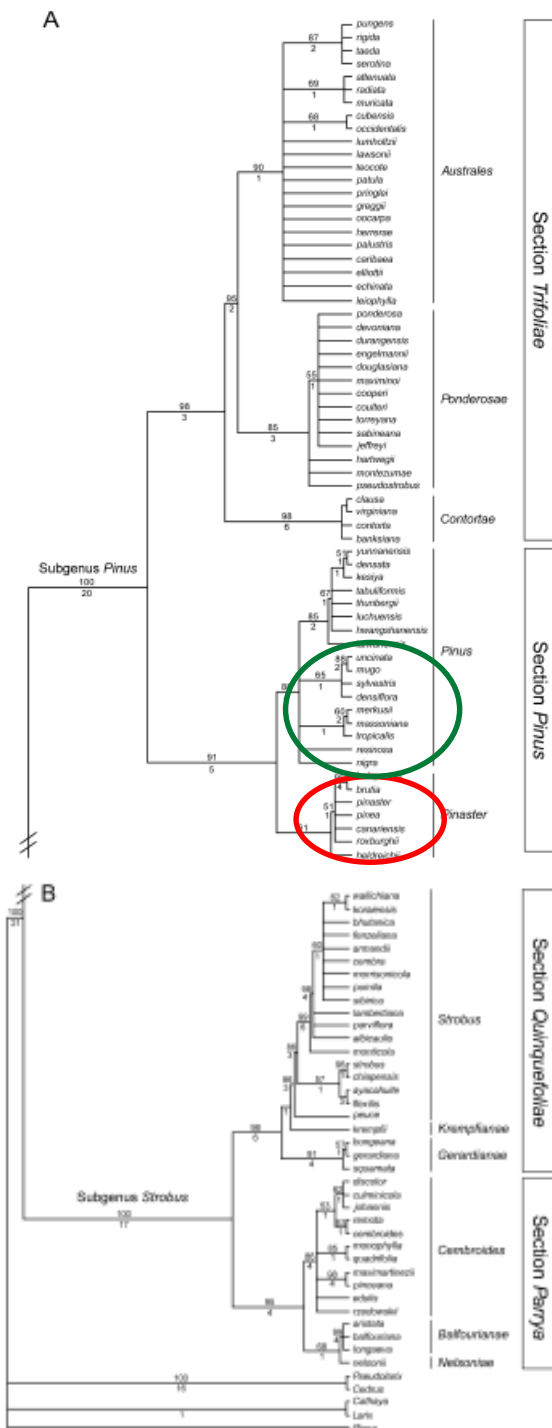
*P. nigra*  
 $He = 0.250$

*P. Sylvestris*  
 $He = 0.350$

*P. pinaster*  
 $He = 0.145$

*P. Halepensis*  
 $He = 0.100$

**$He = 0.011$  (31 loci, 25 populations)!!!**  
**No spatial pattern!**

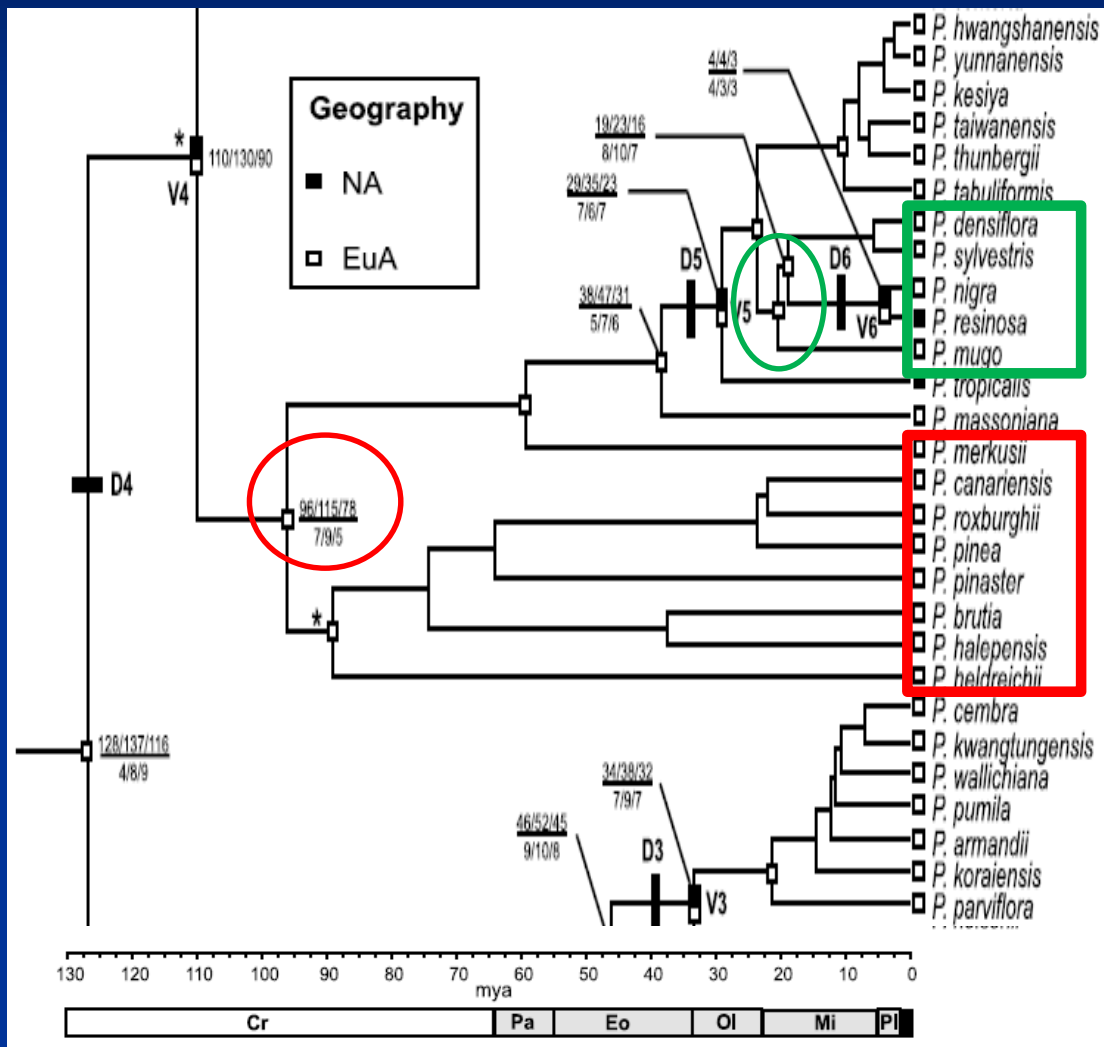


**Mediterranean pines have a common (Old World) phylogenetic origin**

**“True” Mediterranean pines (+ *P. heldreichii*): section *Pinus*, sub-section *Pinaster***

**“Mountain” Mediterranean pines: section *Pinus*, sub-section *Pinus***

# True Mediterranean pines are older than Mountain Mediterranean pines

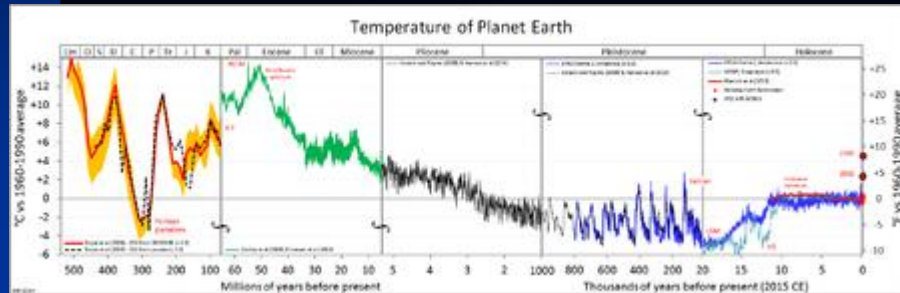


**Pines : a 128 MA-old Eurasian group**

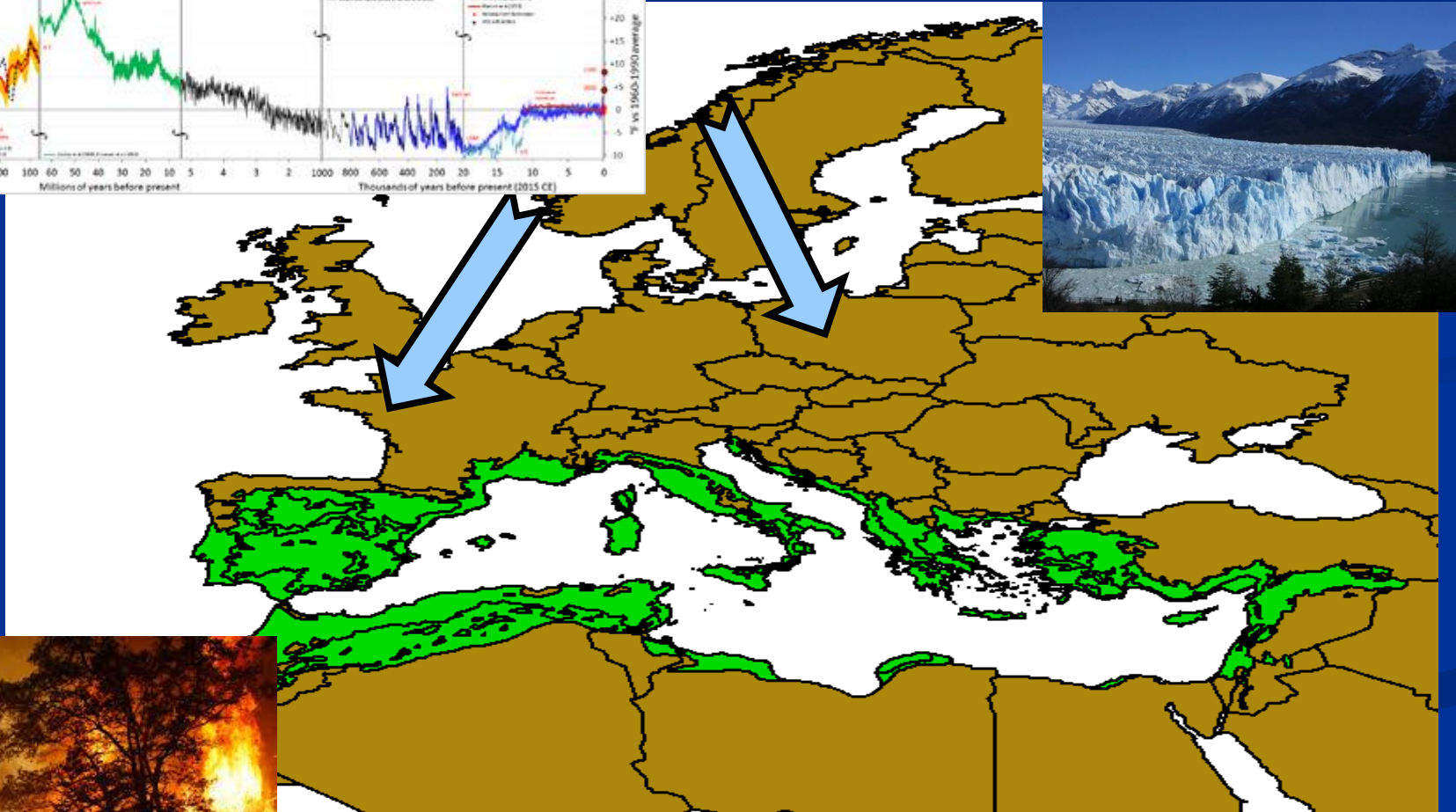
**“Mountain” Mediterranean pines: a 20 MA-old Oligocene group**

**“True” Mediterranean pines: a 96 MA-old Cretaceous group**

# Global climate cooling and fire during the Tertiary can explain a reduced genetic diversity in “true” Mediterranean pines



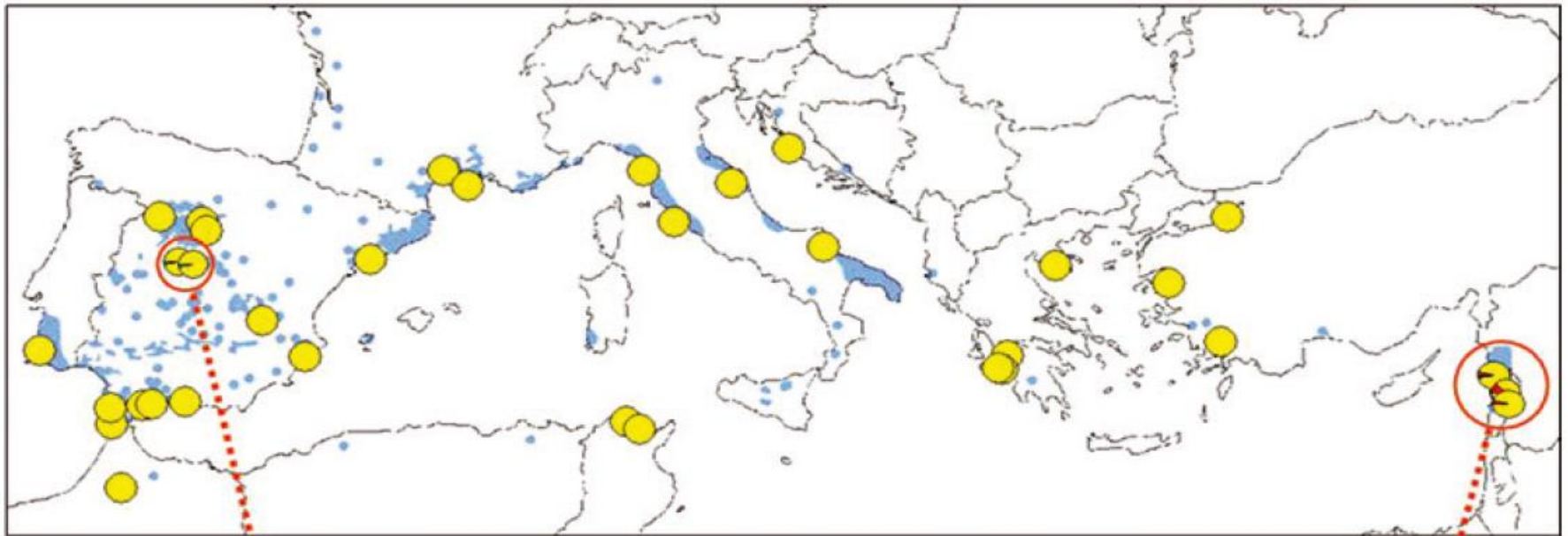
Fady (AFS) 2012



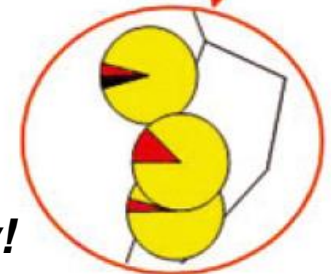
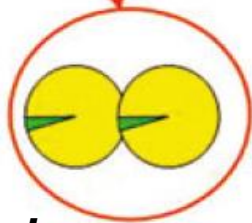
See papers by Pausas et al., by Keeley et al.



# ***Looking for diversity in other parts of the genome of *Pinus pinea*: the chloroplast genome***

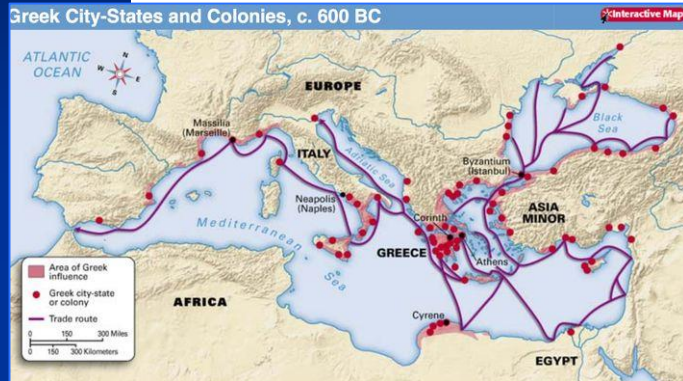


**Only 4 haplotypes in 13 cpSSRs  
across the entire range**

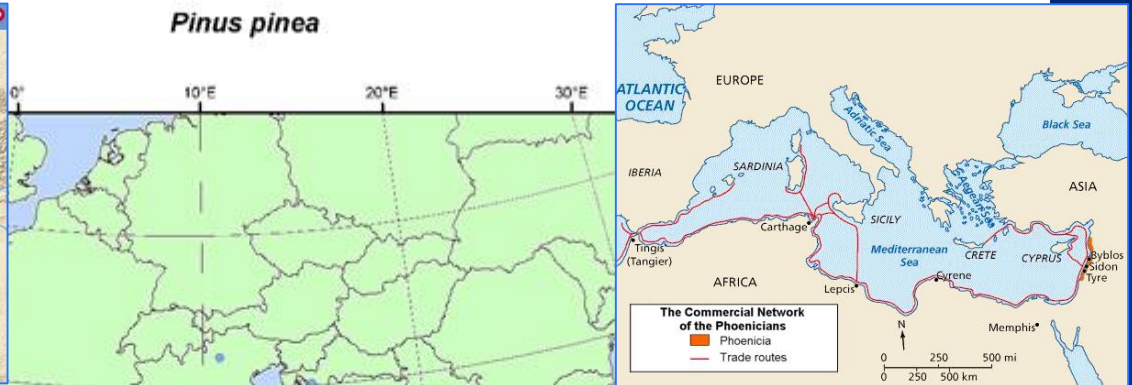


***Pinus pinea*: a xerothermic pine with extremely low diversity!**

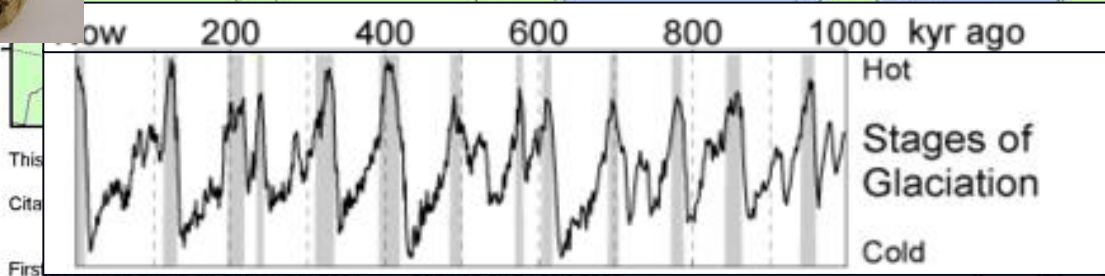
# Extreme low diversity: glacial cycles and human historical trading routes. Just an explanation?



*Pinus pinea*



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 and other maps at  
 www.eufegen.org



Vendramin et al. (*Evolution*) 2007

# ***Developing new genomic resources (still): the nuclear genome again!***

AMERICAN JOURNAL OF  
**Botany**

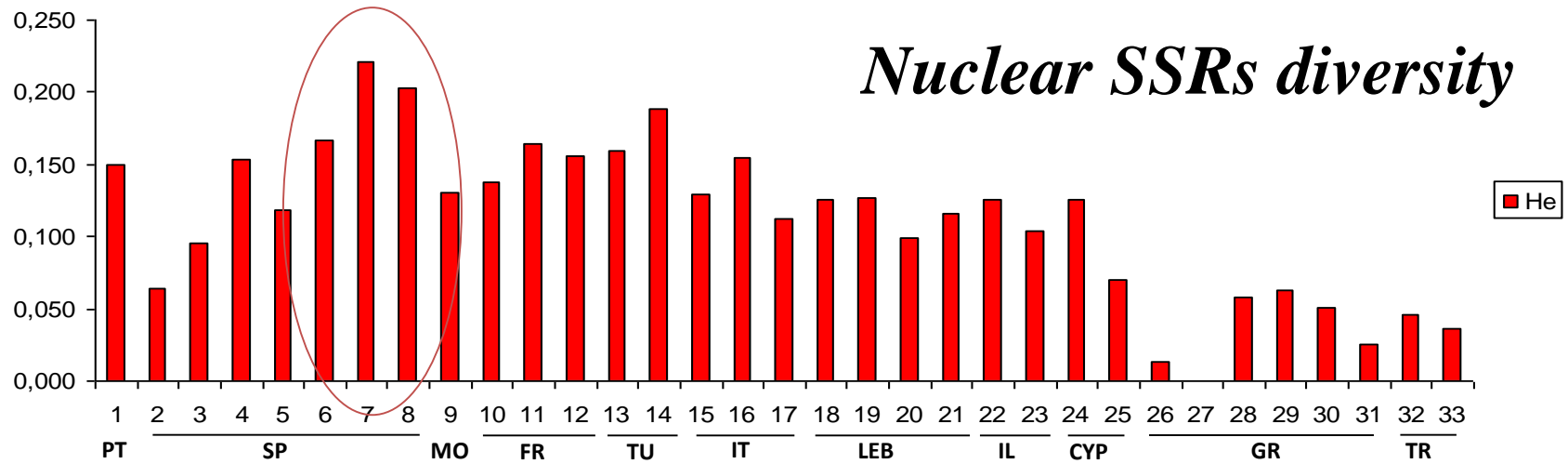
American Journal of Botany: e362–e365. 2012.

AJB PRIMER NOTES & PROTOCOLS IN THE PLANT SCIENCES

## **NUCLEAR MICROSATELLITES FOR *PINUS PINEA* (PINACEAE), A GENETICALLY DEPAUPERATE TREE, AND THEIR TRANSFERABILITY TO *P. HALEPENSIS*<sup>1</sup>**

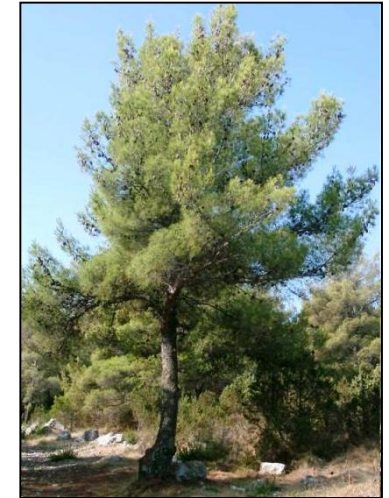
F. PINZAUTI<sup>2</sup>, F. SEBASTIANI<sup>2</sup>, K. B. BUDDE<sup>3</sup>, B. FADY<sup>4</sup>, S. C. GONZÁLEZ-MARTÍNEZ<sup>3</sup>,  
G. G. VENDRAMIN<sup>2,5</sup>

# Nuclear SSRs diversity

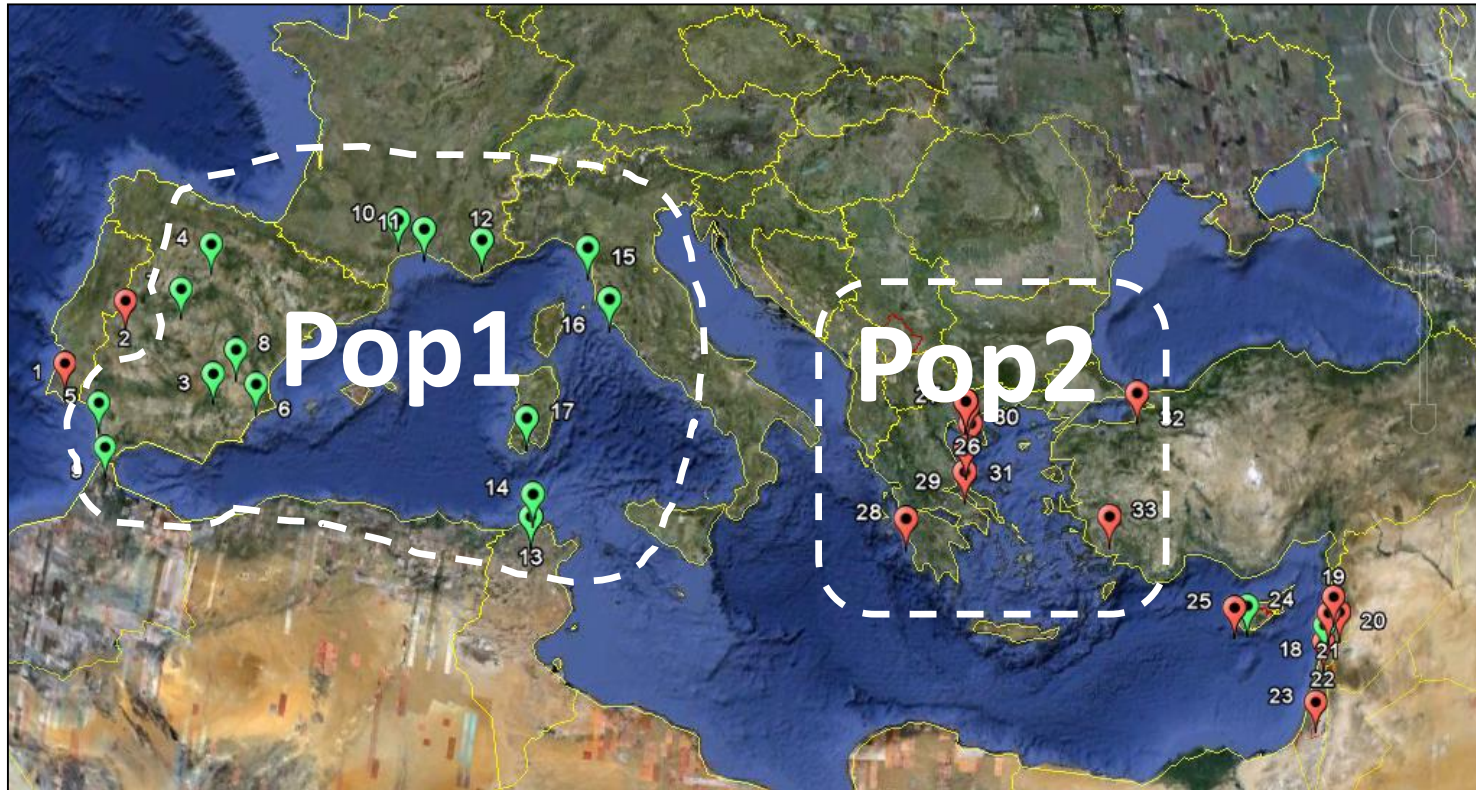
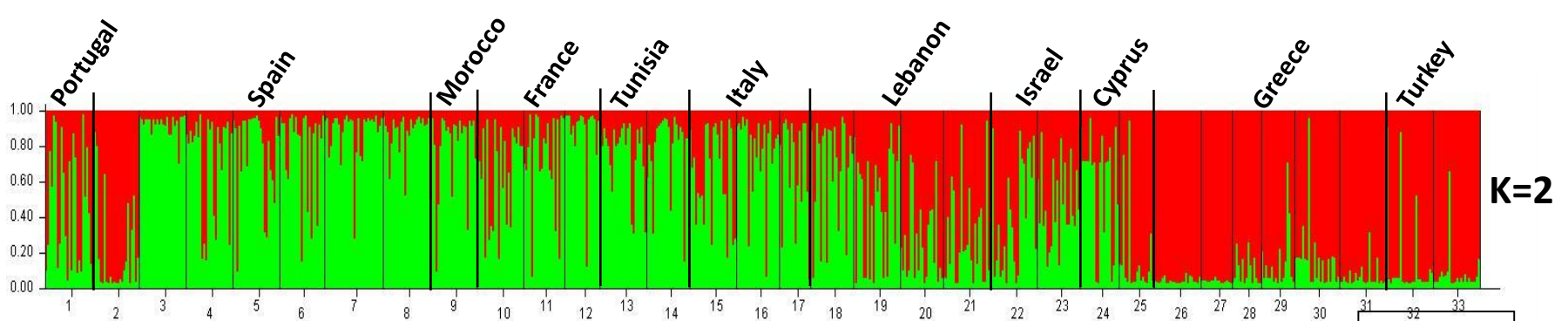


## Genetic diversity in Pinus species

Species	Heterozygosity
<i>Pinus pinea</i>	0.11
<i>Pinus halepensis</i>	0.66
<i>Pinus pinaster</i>	0.82
<i>Pinus sylvestris</i>	0.88



(GG Vendramin, SC Gonzalez-Martinez, et al. unpublished)



**33 pop.  
735 ind.**

1	Quinta do Lago	Portugal
2	Garovillas	Spain
3	Bogarra	Spain
4	Palafruguell	Spain
5	Donana	Spain
6	Biar	Spain
7	San Martín de V.	Spain
8	Tarazona	Spain
9	Cap Spartel	Morocco
10	Villeneuve/Herault	France
11	Saint Maries	France
12	Saint Aygulf	France
13	Cap Bon	Tunisian
14	Rimel	Tunisian
15	Migliarino	Italy
16	Feniglia	Italy
17	Sardegna/Pula	Italy
18	Bkassine	Lebanon
19	Beit Mounzer	Lebanon
20	Kornael	Lebanon
21	Qsaibe	Lebanon
22	Masade/Golden Heights	Israel
23	Quirit/Anavin	Israel
24	Lemessos	Cyprus
25	Pafos	Cyprus
26	Chalkidiki Agios	Greek
27	Parthenonas	Greek
28	Kaiafas	Greek
29	Strofilia	Greek
30	Skopelos	Greek
31	Skiathos	Greek
32	Bursa Yalova	Turkey
33	Yatagan Katranci	Turkey

**Run parameters:**

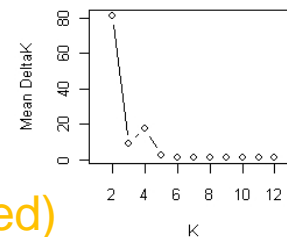
**735 individuals**

**13 nSSR loci**

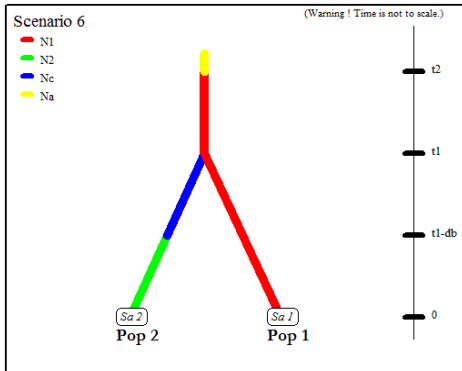
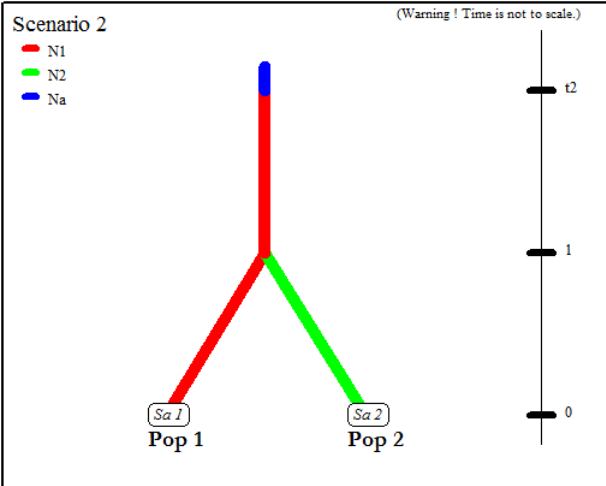
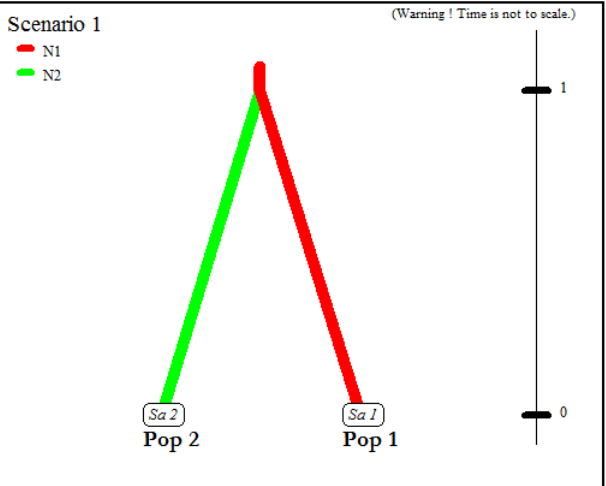
**K=13 populations assumed**

**500000 Burn-in period**

**100000 Reps**

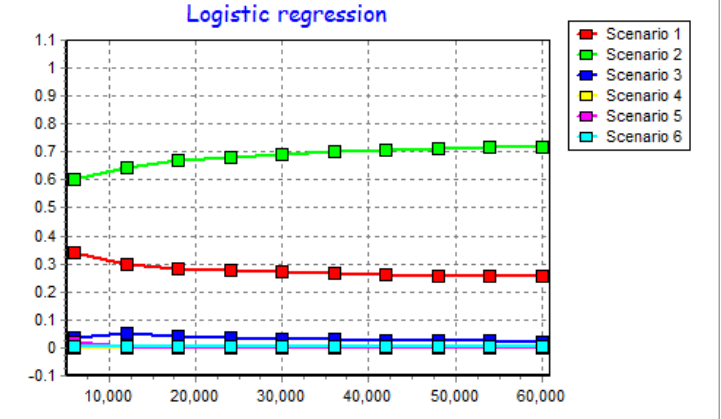
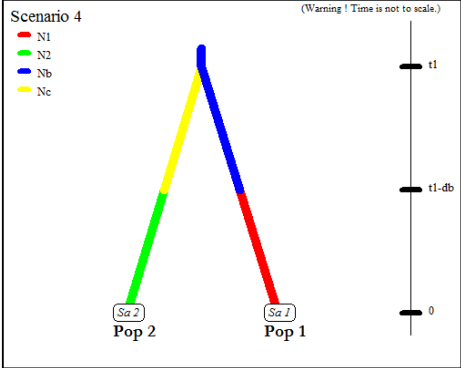
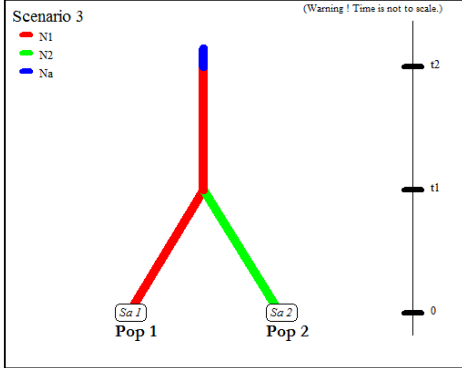
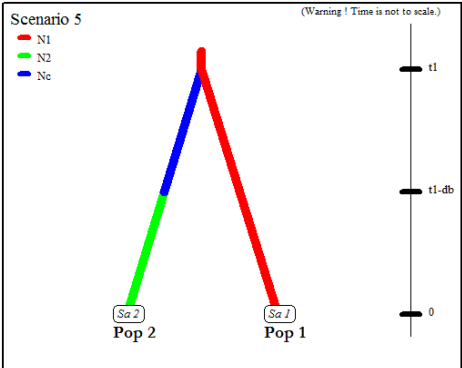


(GG Vendramin, SC Gonzalez-Martinez, et al. unpublished)

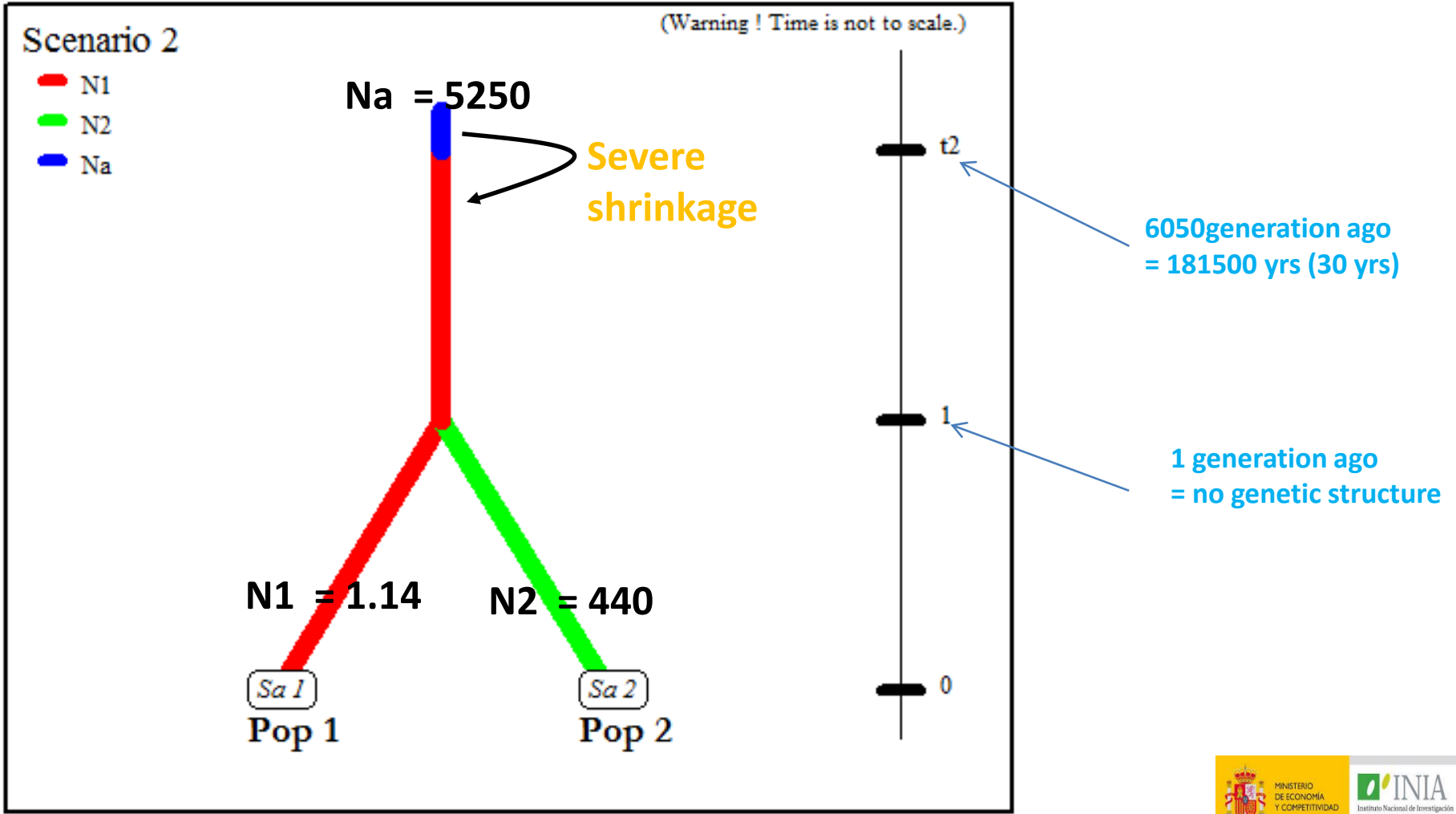


Null hypothesis I:  
no genetic structure  
Divergence occurred just 1 generation ago

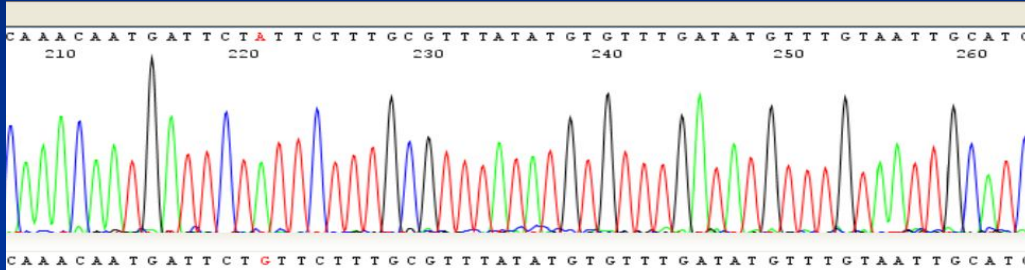
Null hypothesis II:  
no genetic structure with  
ancient bottleneck  
Divergence occurred just 1 generation ago



# Most likely scenario; No genetic structure with ancient population shrinkage



# Developing yet new genomic resources: yet again (almost) no diversity!!



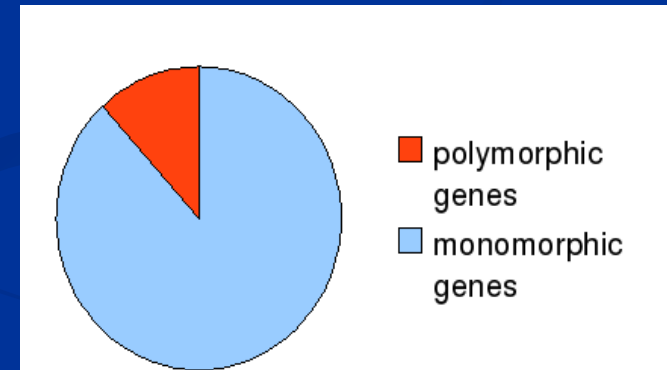
Resequencing 264 candidate genes identified in *P. taeda* => 194 gene sequences in *P. pinea*

*P. pinaster*  $\pi = 0.0024$

*P. radiata*  $\pi = 0.0019$

*P. taeda*  $\pi = 0.0051$

(Gonzalez-Martinez S. et al. 2006)

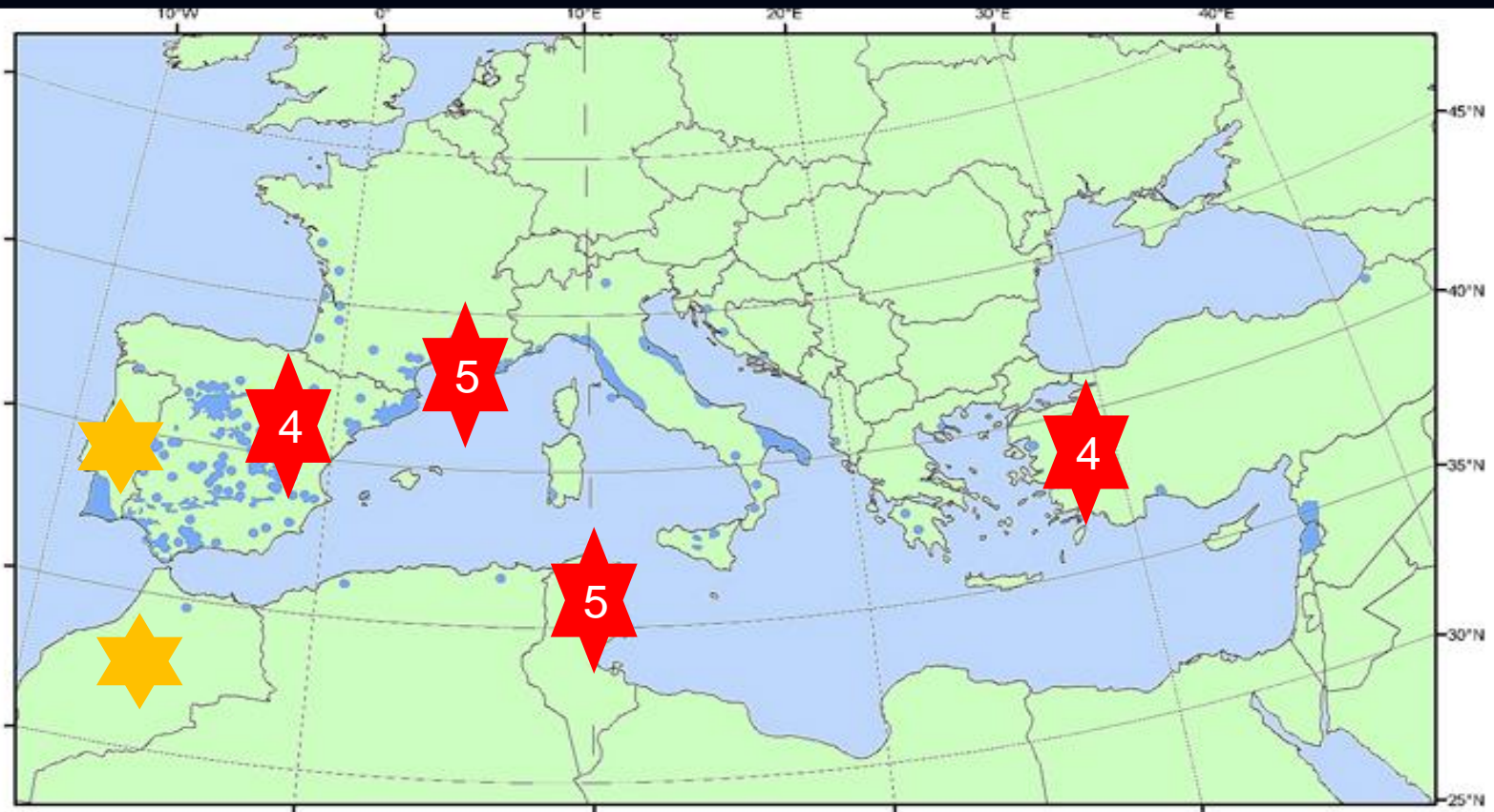


$\pi = 0.000197$   
Total SNPs = 31  
1 SNP every 1439 bases

(GG Vendramin, SC Gonzalez-Martinez, et al. unpublished)



# Does (almost) no DNA diversity affects phenotypic traits? Using provenance tests with similar genetic material in the Mediterranean



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This distribution map, showing the natural distribution area of *Pinus pinea* was compiled by members of the EUFORGEN Networks

Citation: Distribution map of Italian stone pine (*Pinus pinea*) EUFORGEN 2009, [www.euforgen.org](http://www.euforgen.org).

First published online on September 2004 - Updated on 24 July 2008



# ***Low genetic diversity for growth, high plasticity***

## **Low genetic and high environmental diversity at adaptive traits in *Pinus pinea* from provenance tests in France and Spain**

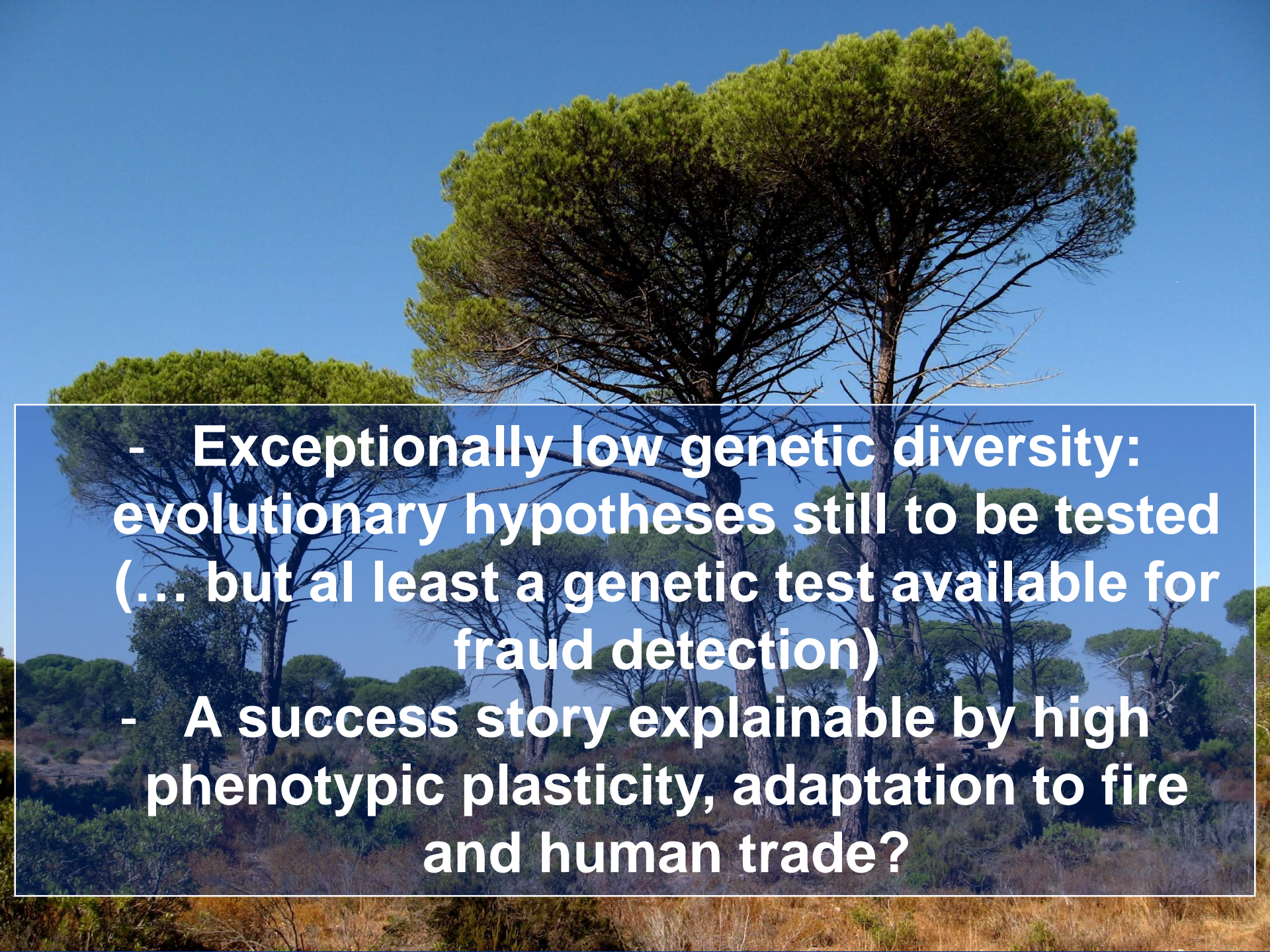
*Options Méditerranéennes*, A, no. 105, 2013  
*Mediterranean Stone Pine for Agroforestry*

S. Mutke<sup>\*1</sup>, J. Gordo <sup>\*\*</sup>, M.L. Khouja and B. Fady<sup>\*\*\*\*</sup>



Within site among provenance  
variance: 3 – 12% total variance

Plasticity within-provenance among  
sites: Up to 3 fold differences

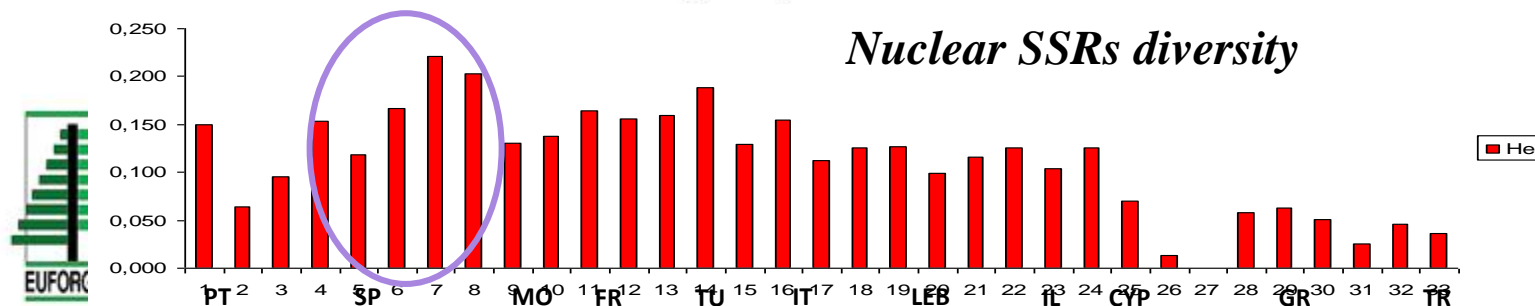
- 
- Exceptionally low genetic diversity: evolutionary hypotheses still to be tested (... but at least a genetic test available for fraud detection)
  - A success story explainable by high phenotypic plasticity, adaptation to fire and human trade?



**Issues to resolve linked to exceptionally low genetic diversity :**

- **Conservation (of what FGR)?**
- **Breeding and vulnerability**

# Where to conserve FGR and look for useful genotypes for breeding?



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A photograph of a pine forest, likely Pinus pinea, with several large, mature trees in the foreground and a dense stand in the background. The sky is clear and blue. The text is overlaid on the image in two white boxes with black borders.

**Thank you for your attention**

**Issues to resolve linked to *Pinus pinea*'s exceptionally low genetic diversity :**

- **Conservation (of what FGR)?**
- **Breeding : possible for what traits?**