

# Population genetics of *Pinus pinea*, a conifer with very low genetic variation

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# Talk summary

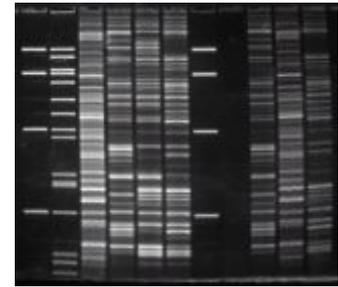
Introduction

Low levels of genetic variation in *P. pinea*

Comparison with other Mediterranean pines

Demographic inferences

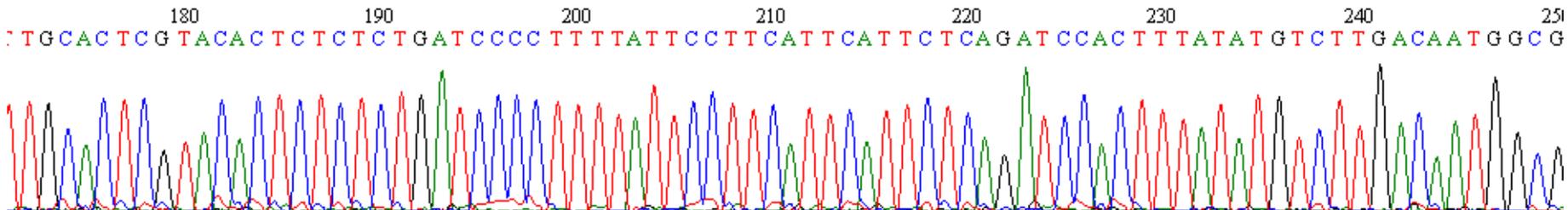
Many misteries to solve...



# Molecular marker types

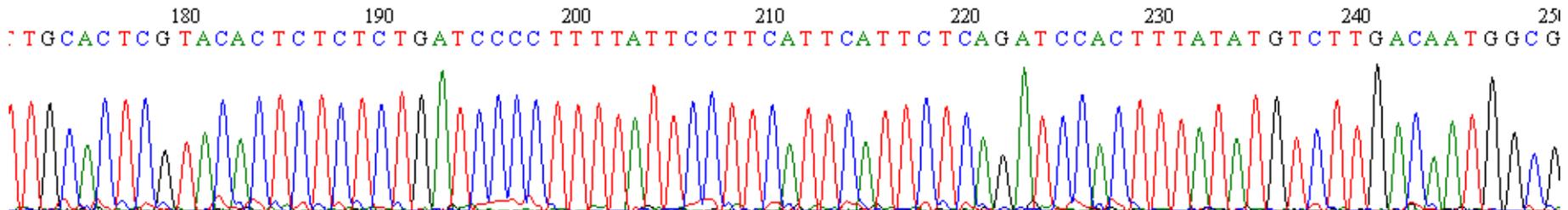
- **Neutral markers:** they are not affected by selection.
  - Provide information about evolutionary history.
  - Estimate changes of effective population number ( $N_e$ ).
- **Functional markers:** loci underlying adaptive traits.
  - Give information about selective processes and the process of adaptation to environment.

*Candidate genes* = genes with a known biological function



# The central dogma of **conservation genetics**

- **Genetic variation** is a prerequisite for adaptation to new environmental conditions. Thus, a great research effort has been invested in surveying genetic variation of natural populations and studying the processes that reduce levels of variability.



# One of the most famous cases is that of the African cheetah

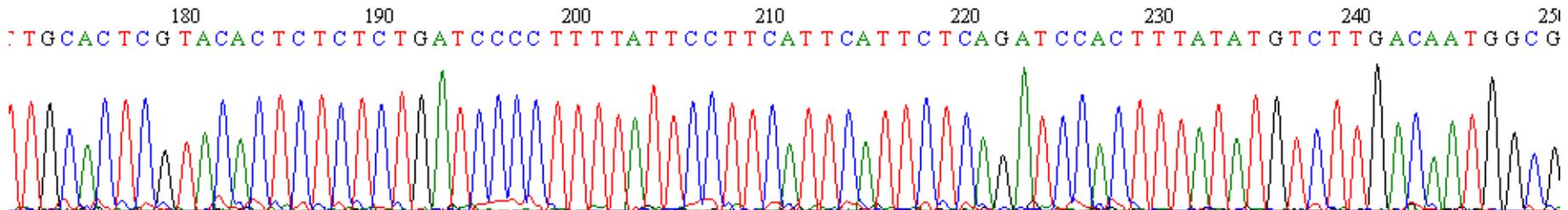
(O'Brien et al. 1985)



- This species would have experienced a prolonged bottleneck resulting in extreme inbreeding during the Pleistocene when large mammals such as the giant ground sloth or cave bears became extinct (O'Brien 1994).
- Although the cheetah has regained a large distribution range, it has poor sperm quality and poor reproductive success (Lehman 1998; Amos and Balmford 2001).

# The central dogma of **conservation genetics**

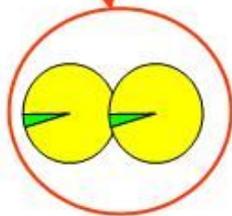
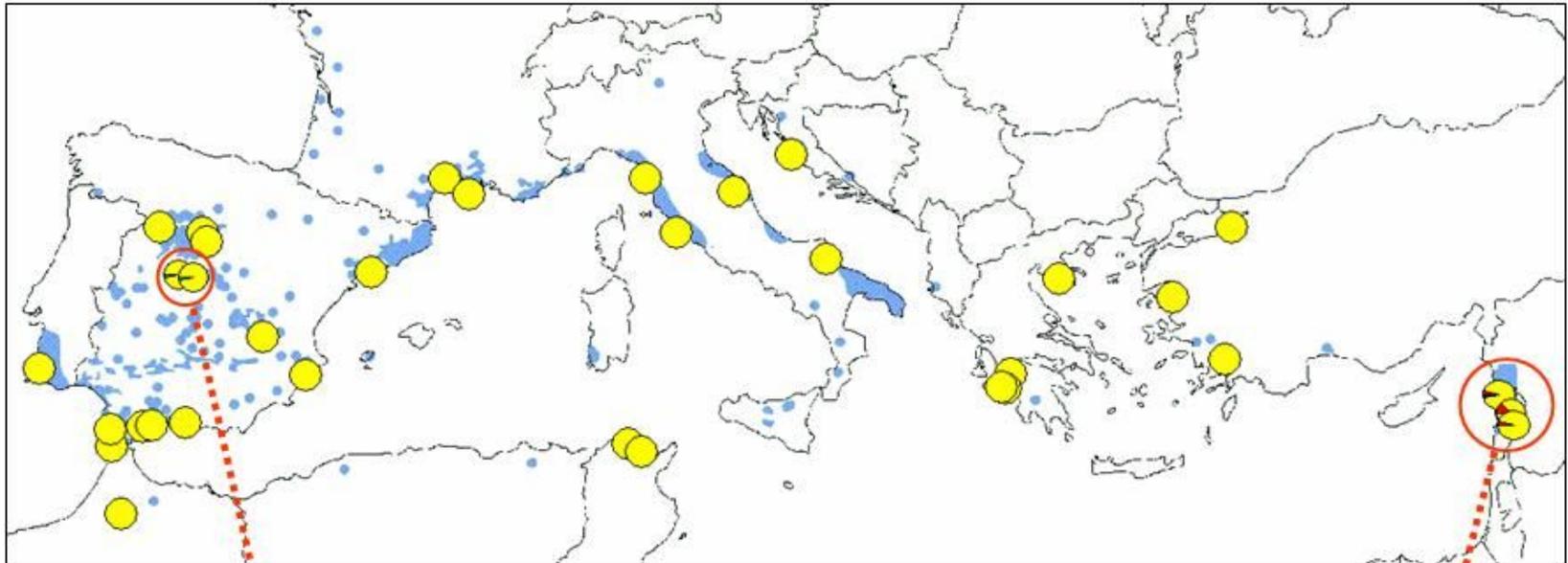
- Genetic variation is a prerequisite for adaptation to new environmental conditions. Thus, a great research effort has been invested in surveying genetic variation of natural populations and studying the processes that reduce levels of variability.
- However, the discovery of genetically depauperate but geographically widespread (and apparently healthy) species, such as *Pinus pinea*, challenges this idea.



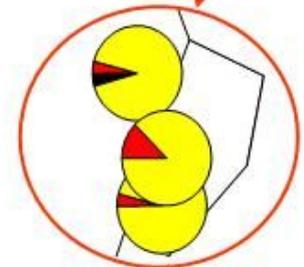
# Chloroplast variation in *Pinus pinea*

(Vendramin et al. 2008, *Evolution*)

Haplotype map based on 12 cpSSRs

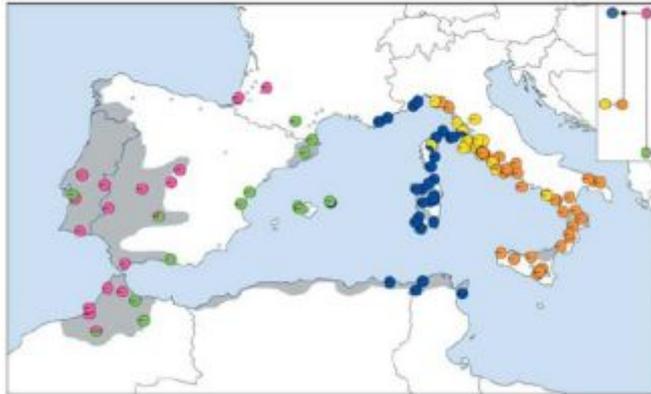


Only a few rare variants appear in **central Spain** and **Lebanon!**



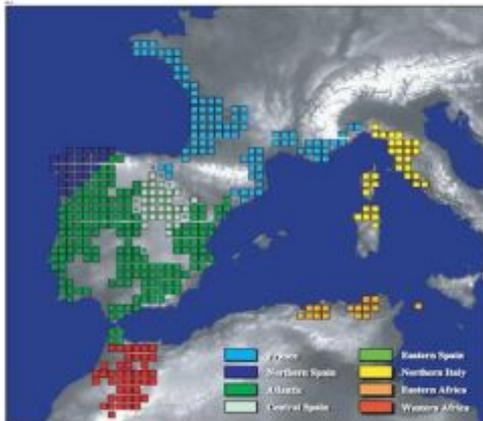
# But, how does it look in other Mediterranean trees?

*Quercus suber*



(Magri et al. 2007 Mol. Ecol.)

*Pinus pinaster*



(Bucci et al. 2007 Mol. Ecol.)

	<i>Pinus pinea</i>	Other conifers <sup>a</sup>
Number of loci per species (>6 repeats)	13	4.9
Proportion of polymorphic loci <sup>b</sup>	23%	71%
$H$ (haplotypic diversity)	0.019	0.37
$\hat{A}$ (mean number of alleles/locus)	1.23	4.62
$\hat{A}_{[100]}$ <sup>c</sup>	1.06	3.90
$\hat{A}_{[100;10]}$ <sup>d</sup>	1.08	2.58
Mean number of repeats per locus	10.0	13.0
Maximum number of repeats	14.0	15.1

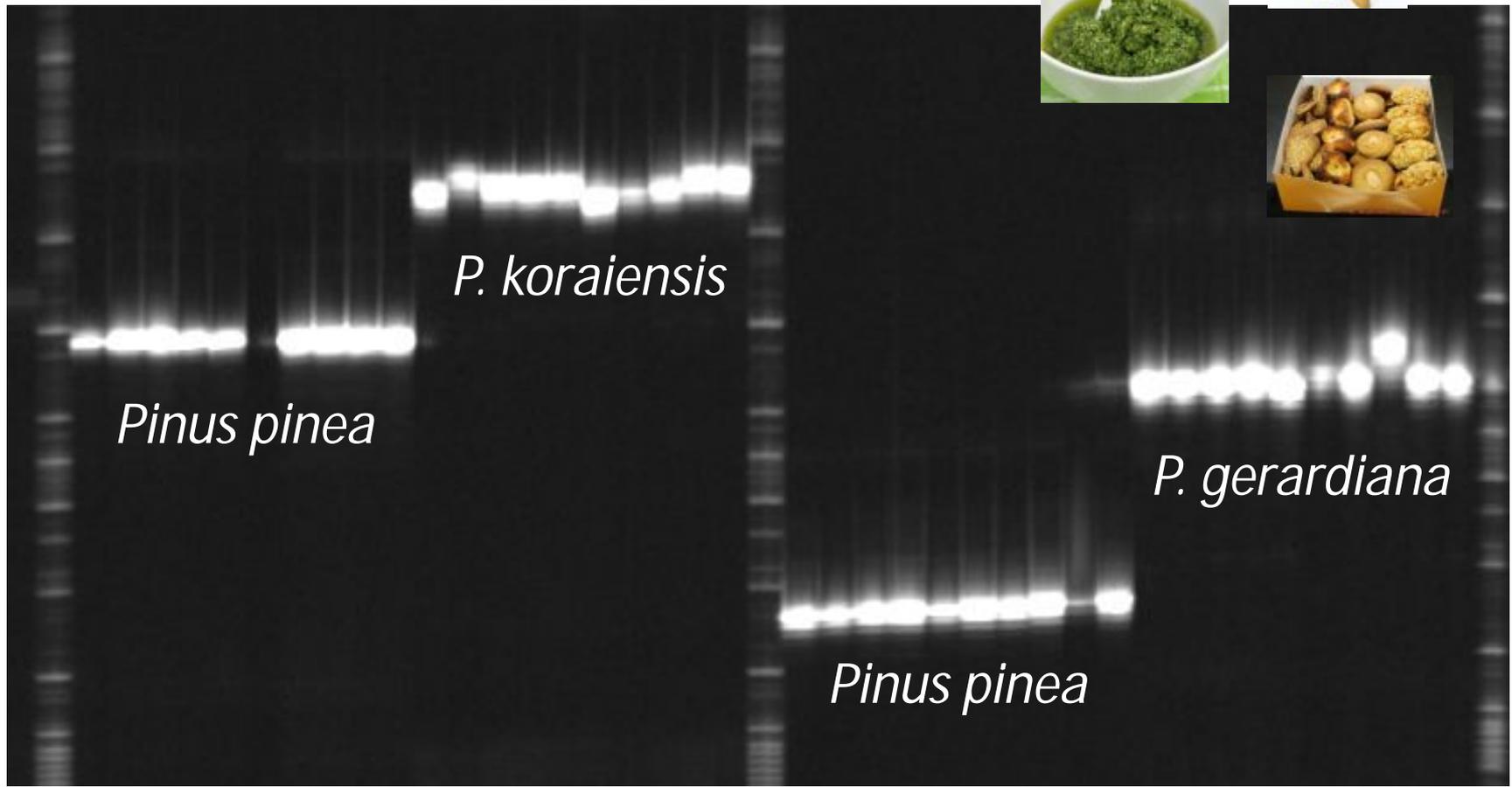
<sup>a</sup>Data for eight species combined: *Abies alba*, *Picea abies*, *Pinus brutia*, *P. cembra*, *P. halepensis*, *P. lambertiana*, *P. mugo*, and *P. pinaster*; see Petit et al. (2005).

<sup>b</sup>No threshold used.

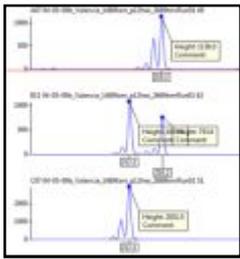
<sup>c</sup>Mean number of alleles per locus in equal-sized samples of 100 individuals.

<sup>d</sup>Mean number of alleles per locus in equal-sized samples of 100 individuals after standardization to a mean number of repeats per locus of 10.

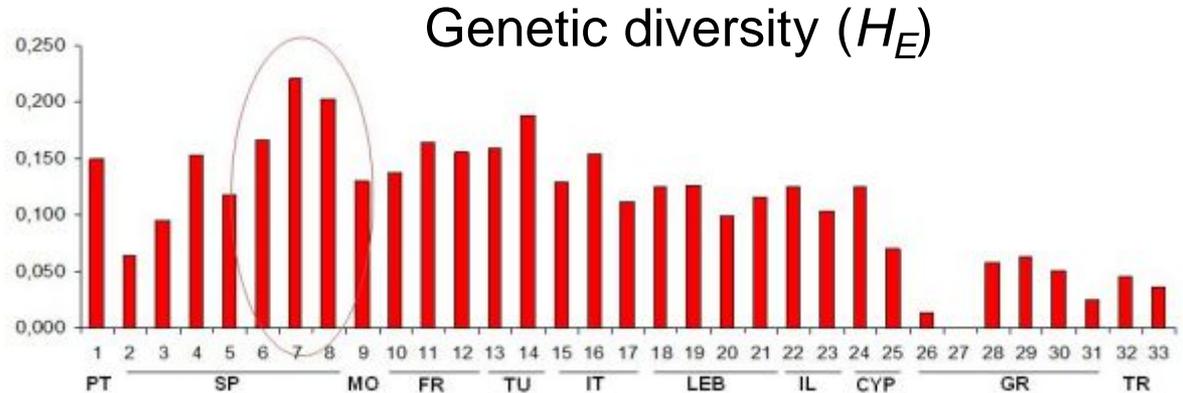
# Using the low diversity in *P. pinea* for identification of commercial pine nuts: a simple cpSSR-based test



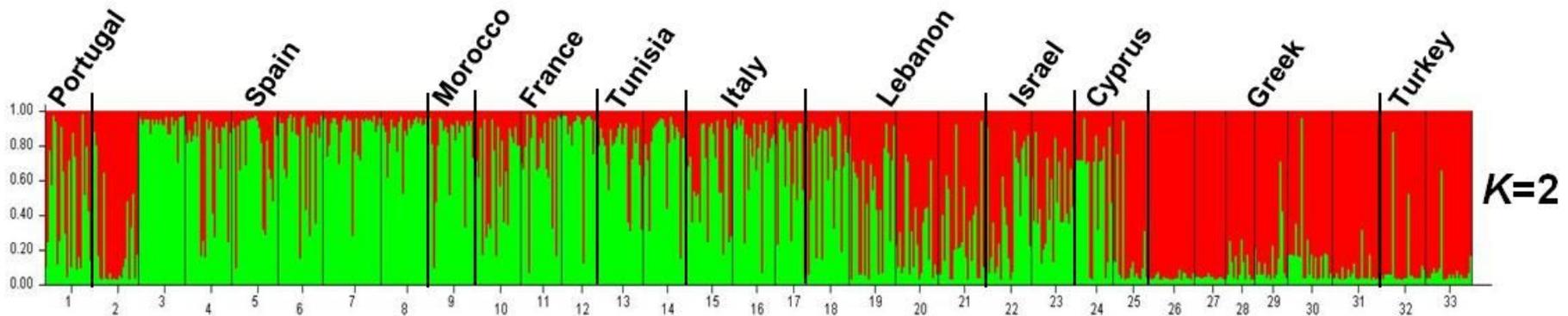
# Nuclear variation in *Pinus pinea*: nuSSRs



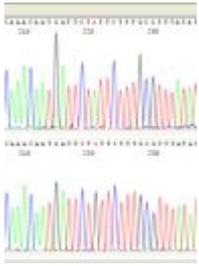
Species	$H_E$
<i>P. pinea</i>	0.11
<i>P. halepensis</i>	0.66
<i>P. pinaster</i>	0.82
<i>C. sempervirens</i>	0.68



Low diversity, slightly higher in some Iberian populations



Two gene pools at western and eastern Mediterranean



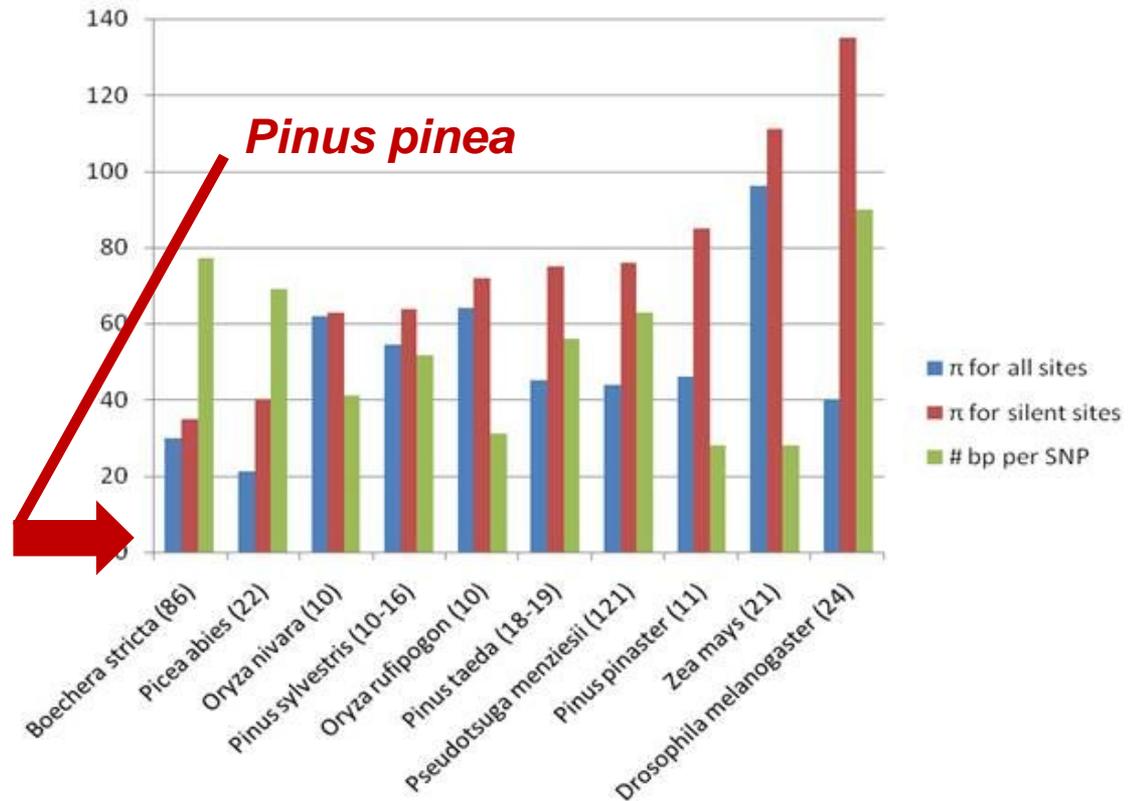
# Nuclear variation in *Pinus pinea*: SNPs and candidate genes

194 genes sequenced,  
only 19 polymorphic!

Species	$\pi$
<i>P. pinea</i>	0.00019
<i>P. halepensis</i>	0.00311
<i>P. pinaster</i>	0.00517
<i>P. taeda</i>	0.00531

(González-Martínez et al. 2011; Grivet et al. 2011)

Nucleotide diversity ( $\pi$ )



No population genetic structure for candidate genes

# List of genetically **depauperated** but **widespread** plant species, excluding clonal and self-fertilising plants, ranked by order of increasing diversity

Species <sup>a</sup>	Family	Stature <sup>b</sup>	Habit <sup>c</sup>	Pop <sup>d</sup>	Loci <sup>e</sup>	$H_{es}^f$	Reference
<u><i>Pinus resinosa</i></u>	Pinaceae	32	W	2	27	0.001	Allendorf et al. 1982
<u><i>Berchemia berchemiaefolia</i></u>	Rhamnaceae	17	W	4	14	0.001	Lee et al. 2003
<u><i>Schwalbea americana</i></u>	Scrophulariaceae	0.6	H	13	15	0.006	Godt and Hamrick 1998
<u><i>Pinus pinea</i></u>	Pinaceae	30	W	17	32	0.015	Fallour et al. 1997
<u><i>Lespedeza capitata</i></u>	Fabaceae	1.6	H	12	34	0.020	Cole and Biesboer 1992
<u><i>Juglans cinerea</i></u>	Juglandaceae	30	W	9	12	0.029	Morin et al. 2000
<u><i>Heuchera americana</i></u>	Saxifragaceae	0.6	H	12	14	0.039	Soltis 1985
<u><i>Desmodium nudiflorum</i></u>	Fabaceae	0.29	H	5	13	0.043	Schaal and Smith 1980
<u><i>Tsuga canadensis</i></u>	Pinaceae	35	W	17	10	0.043	Zabinski 1992

<sup>a</sup>Trees are underlined.

<sup>b</sup>Stature in meters.

<sup>c</sup>Habit (W: woody, H: herbaceous).

<sup>d</sup>Pop: number of populations sampled.

<sup>e</sup>Loci: number of loci scored.

<sup>f</sup> $H_{es}$ : expected heterozygosity at the species level.



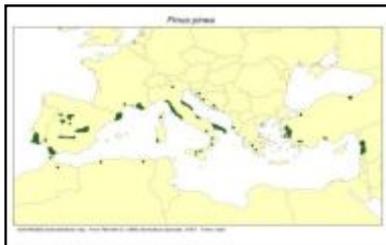
*Pinus pinea* displays a significant lower diversity than most plants ...

Why?

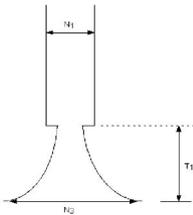


# Actually, we don't know, although we suspect that it may be a combination of the following factors:

- *Pinus pinea* has a **very fragmented range** and a relatively narrow ecological niche (compared to other conifers).
- **Low mutation rates** (but, actually, *P. pinea* has one of the largest known genomes).
- The relatively inbred mating system, along with other **reproductive traits** of the species (e.g., high energetic investment in seed production; abundant seed predation; the three-year cone maturation cycle; late age of first flowering) might have promoted low historical effective population sizes.
- Contrary to other pines, *P. pinea* ability to disperse seeds depends on the presence of **mutualistic birds**, not on wind.



- Evidence of an ancient and strong bottleneck.

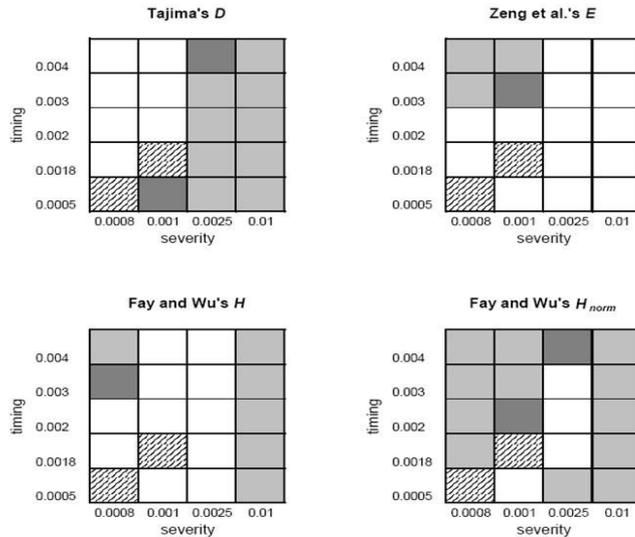


Approximate Bayesian Computation (ABC) modeling

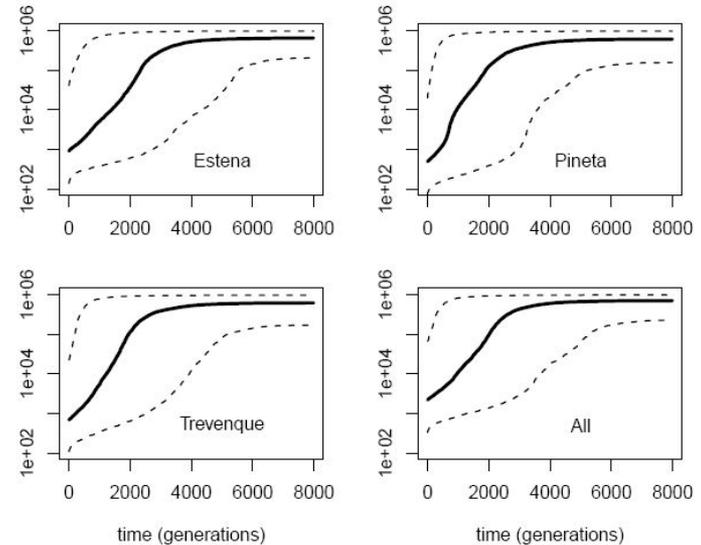
A reduction to 5% its effective population size some 50,000 generations ago

How does this bottleneck compare to other Mediterranean conifers that suffered historical reduction in effective population size?

*Pinus halepensis*



*Taxus bacatta*



Also strong bottlenecks but much more recent!!!

**But most amazing of all is that *P. pinea* was able to colonize such a **wide range** in absence of genetic variation!**

### **On going discussion of potential causes:**

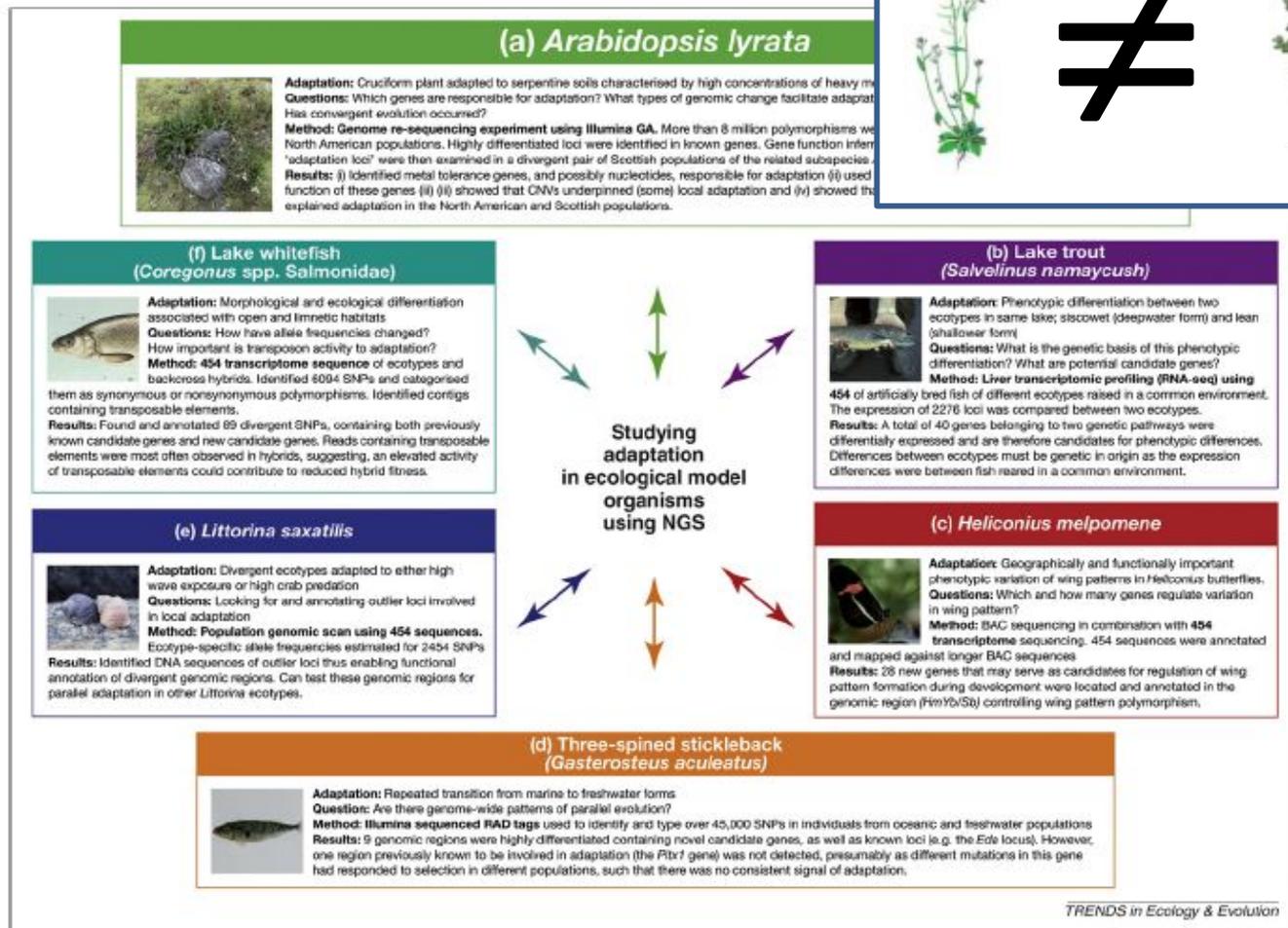
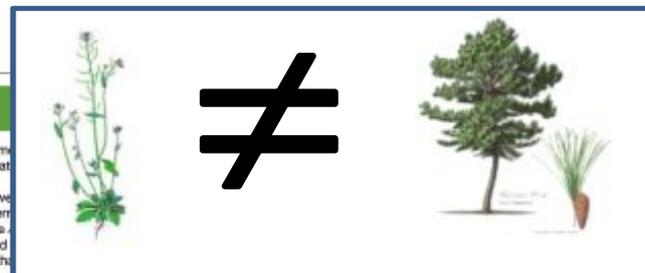
- Existence of a highly effective disperser as well as the **significant role of humans** (pine nuts consumed by Neanderthals; cultivation of the species since at least three millennia).
- **Loss of specific parasites and diseases** during the bottleneck phase (Amos and Balmford 2001). This 'release from enemies' hypothesis is similar to that proposed for the success of invasive species in their introduced range (Keane and Crawley 2002). As a matter of fact, *P. pinea* is known to have comparatively few parasites and diseases (Fady et al. 2004).

- Successful adaptation to new environmental conditions encountered during the expansion depends on the presence of variation at **phenotypic traits**, not on marker diversity (see talk by Sven Mutke).
- **Epigenetic variation** can also accumulate quickly after a bottleneck (Rapp and Wendel 2005); some preliminary evidence of epigenetic variation in *P. pinea* does exist, but still a mechanism to inherit this variation is not clear.
- *P. pinea* has a high level of **phenotypic plasticity** (Mutke et al. 2005), which could have helped it to colonize new environments despite reduced genetic variation. Phenotypic plasticity is seen that a force that allows a species to temporarily survive harsh environmental conditions favoring adaptation.

# Conclusions & Perspectives

- Stone pine has **low genetic variation** as estimated by a variety of molecular markers; however, after an ancient bottleneck, the species was able to colonize a **wide range**.
- However, we should keep in mind that even if some species can survive and thrive following a severe population bottleneck (such as stone pine, red pine or the northern elephant seal), these represent **the few lucky ones rather than the norm** (Godt and Hamrick 1997).
- Moreover, the time needed to re-establish variation is considerable, especially in long-lived species, and it is unclear how such species will react to **novel conditions**, not experienced during the bottleneck or recovery phase.
- The role of **plasticity** and **epigenetic variation** in *P. pinea* spread need to be further studied.

# Increasing availability of genomic resources for non-model species



(Stapley et al. 2010 TREE)

High throughput (HT) Sequencing (454, GAIIX) → The low variation of *P. pinea* as an opportunity.

# Acknowledgements

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....and you for your attention