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Molecular genetics segregate clones and provenances of stone pine, a tree rooted deep in the Alentejo

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PINEA SPOT CONGRESS
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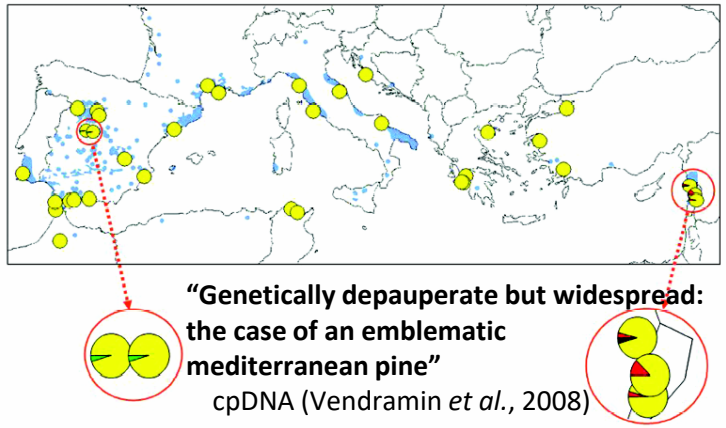
Introduction

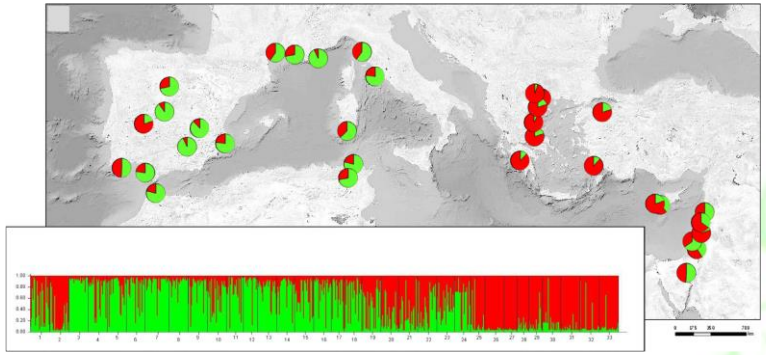
Pinus pinea

- A native Mediterranean forest tree, associated with mixed open woodlands ⊃ {pines, oaks, junipers, …}
- Since 18th c., one of the pines widely used in restoration, namely on coastal sands (doubling area from 0.3 to 0.7+ Mha)
- S. 20th c., breeding programs targeting edible pine nuts (cf. KN by F. Pérez, "Clones" by C. Guadaño afterwards)
- 20th-21st c., private planting of agroforestry and agronomic orchards (some 0.25+ Mha -> 1 Mha total area)
- Nevertheless, it is a relict species with **extremely low genetic variation**, both at quantitative and molecular traits

→ its breeding was limited by **low variation** to be captured and by a **lack of suitable genomic tools** for identification



"Genetically depauperate but widespread: the case of an emblematic mediterranean pine"
cpDNA (Vendramin *et al.*, 2008)



nuSSRs (Jaramillo-Correa et al. 2020)

Only 15 clones as basic materials in 2015 (ES)

7 SSRs (Pinzauti et al. 2012):

- Low allelic polymorphism
- 16% errors in genotyping
- Inconsistent results

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Introduction

Quantitative Genetics

- International common-garden provenance trial FAO *Silva Mediterranea* (since 1994)

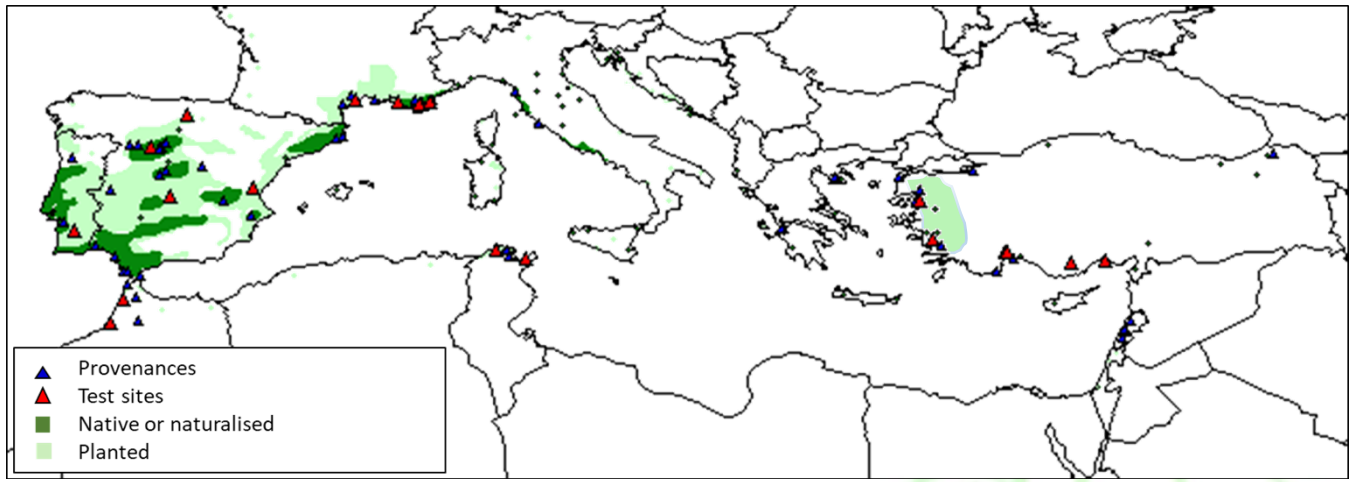
  …"extremely low genetic variation"

  Phenotypic plasticity >> Genetic local adaption

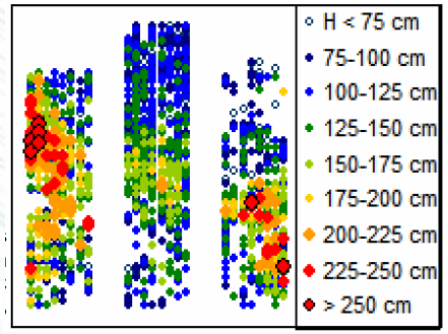
- Difference in cone production between clones

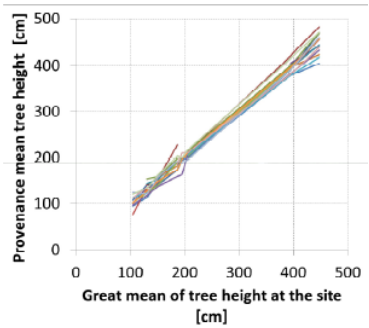
  (cf. presentation **Guadaño et al.**)

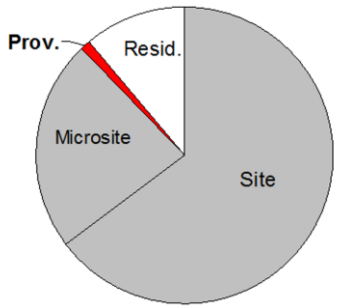
  …huge microsite effects mask genetic contribution

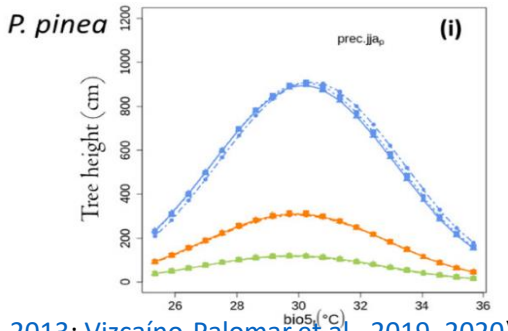


Tree height in a range of field trials varied within test sites, but did ***"nearly not"*** **depend** on the origin of the plants.









([Mutke *et al.*, 2010](), [2013](); [Vizcaíno-Palomar et al., 2019](), [2020]())

Material & methods

Olsson et al. (in rev.). **Managing genetic resources with genomic tools: the case of *Pinus pinea*, a multipurpose forest tree with low genome diversity**. For. Eco. Manage.

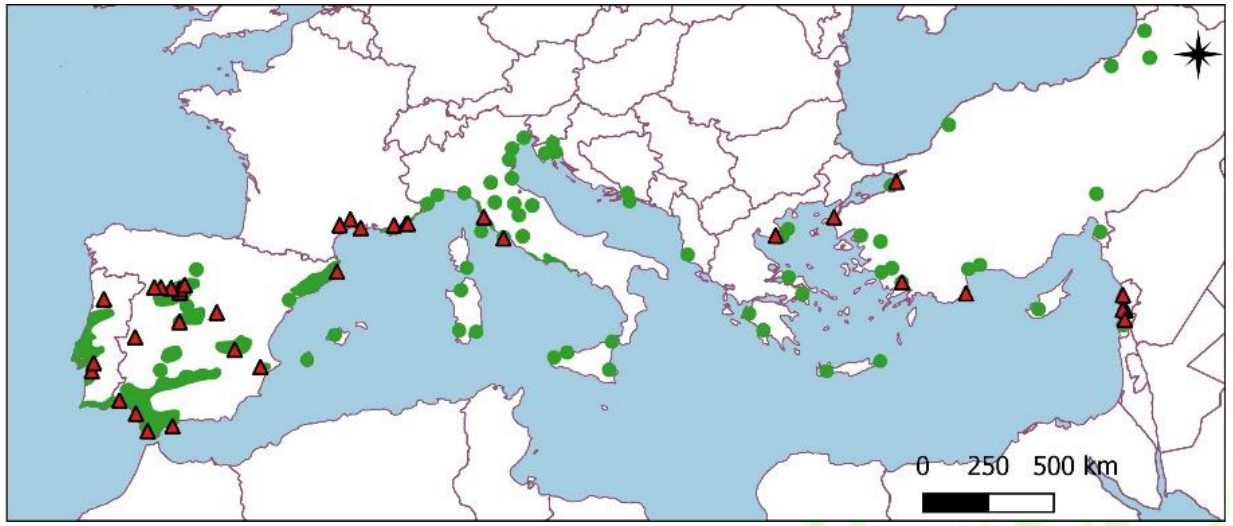
Aims: Clonal identification, Parentage analyses and genetic relationships, Population genetic structure

Materials: 1 925 individuals sampled

- 37 populations (full distribution range)
- 323 Spanish and Portuguese selected clones
- 16 progenies (half-sib families)

Methods: 2 245 markers retained in quality filtering

- from **Axiom´s 4TREE** array developed by B4EST project consortium



• species distribution, red triangles: sites sampled in this study

Axiom SNP scoring *Pinus pinea* (SNP – Single-Nucleotide Polymorphism)

SNPs submitted	128 279
SNPs retained	36 784
P.pa. SNPs in the TF **4TREEarray**:	
(PolyHRes+NoMHom) 5 671	

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Material & methods

Sampling scheme

1,925 samples

1,131 full distribution
(37 populations)

- 17 Greece (1 p.)
- 236 France (6 p.)
- 178 Italy (2 p.)
- 71 Lebanon (4 p.)
- 91 Portugal (3 p.)
- 467 Spain (17 p.)
- 71 Turkey (4 p.)

557 REINFFORCE arboreta
574 FAO *Silva Mediterranea* trial

268 clone samples ES
(252 individuals)

Provenance regions
- ES1, ES2, ES3, ES6

15 registered clones

10 - 11 ramets/ clone

91 unregistered clones

1 ramet/clone

64 clones PT

1 ramet/clone

462 Progenies
(half-sib families from 16 clones)

Best

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Results

Clonal identification

- More precise and reliable identification with 2,245 SNPs than with 7 SSRs
- Practical applications of results
  - Exclusion of 14 ramets from their corresponding genets (10%)
    (possible errors during grafting, nursery handling, planting or sampling)

    → Consensus genotypes as first reference entries in the stone pine breeding programme database

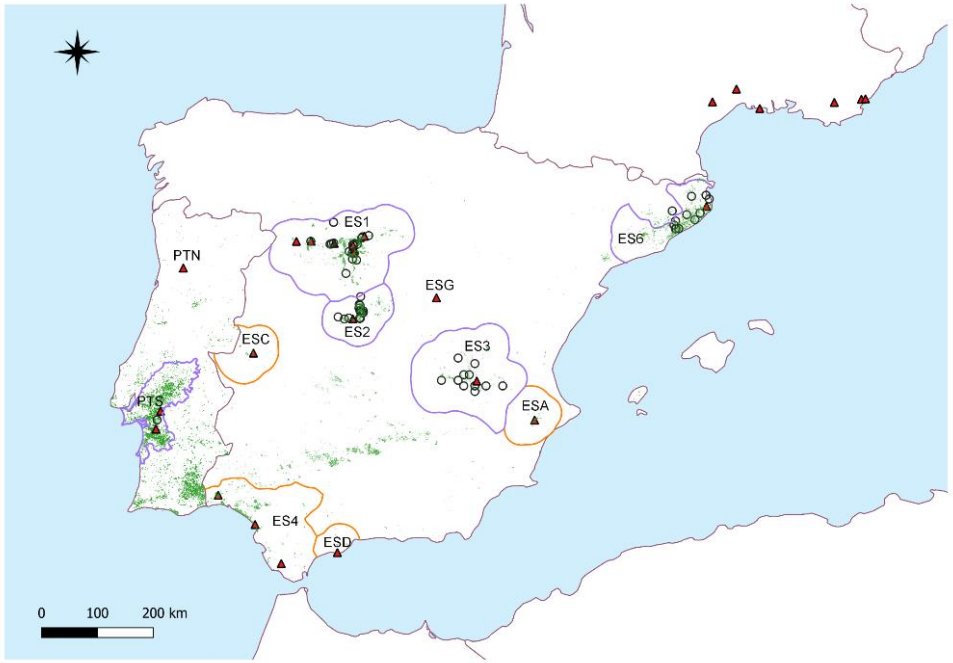
  - Registration of new elite clones (now distinguishable by SNP genotype)

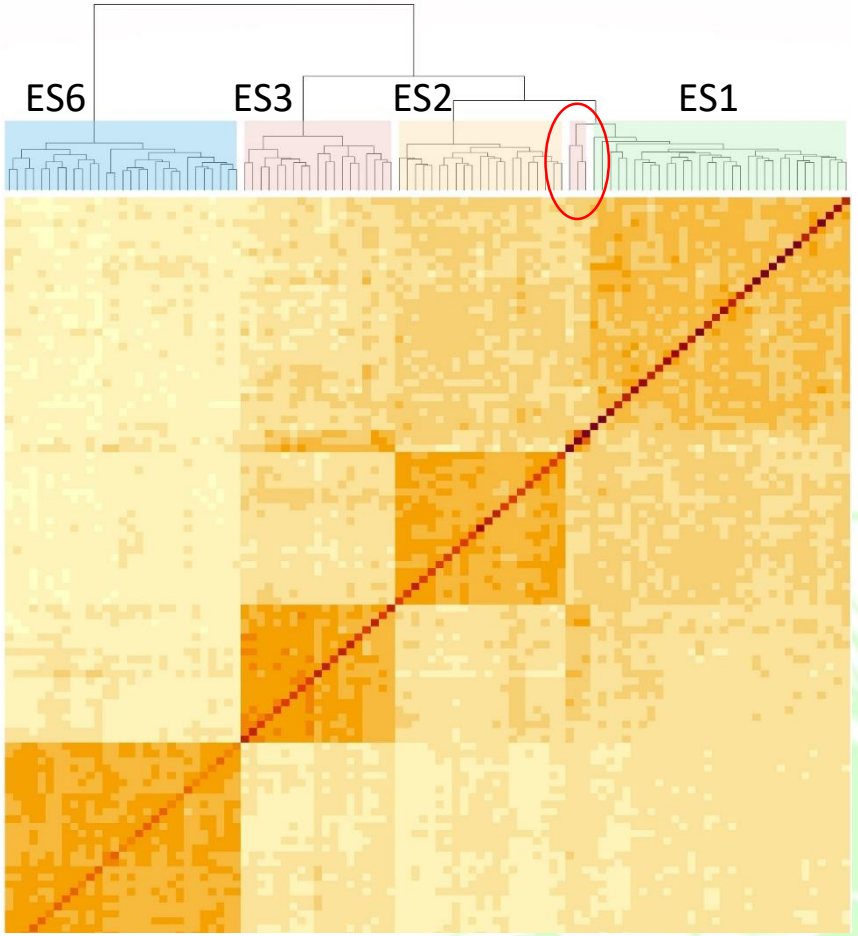
    upcoming ✔

Results

Parentage analysis and Genetic relationships

- Parentage analysis with *sequoia*: mother assigned for 442 out of 455 offspring (97%)
- GRM matrix of 104 clones mainly reflected expected population genetic structure (and reveals afforestations)





Additive GRM *sommer*

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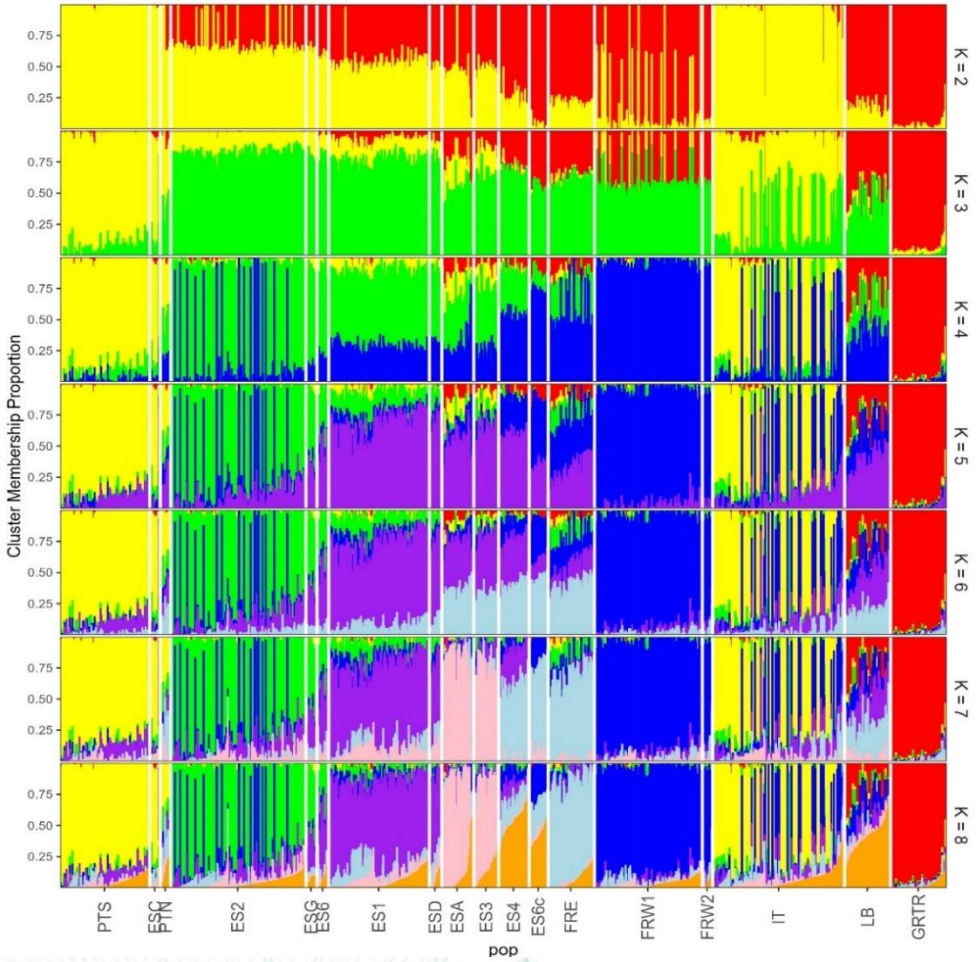
Results

Population genetic structure



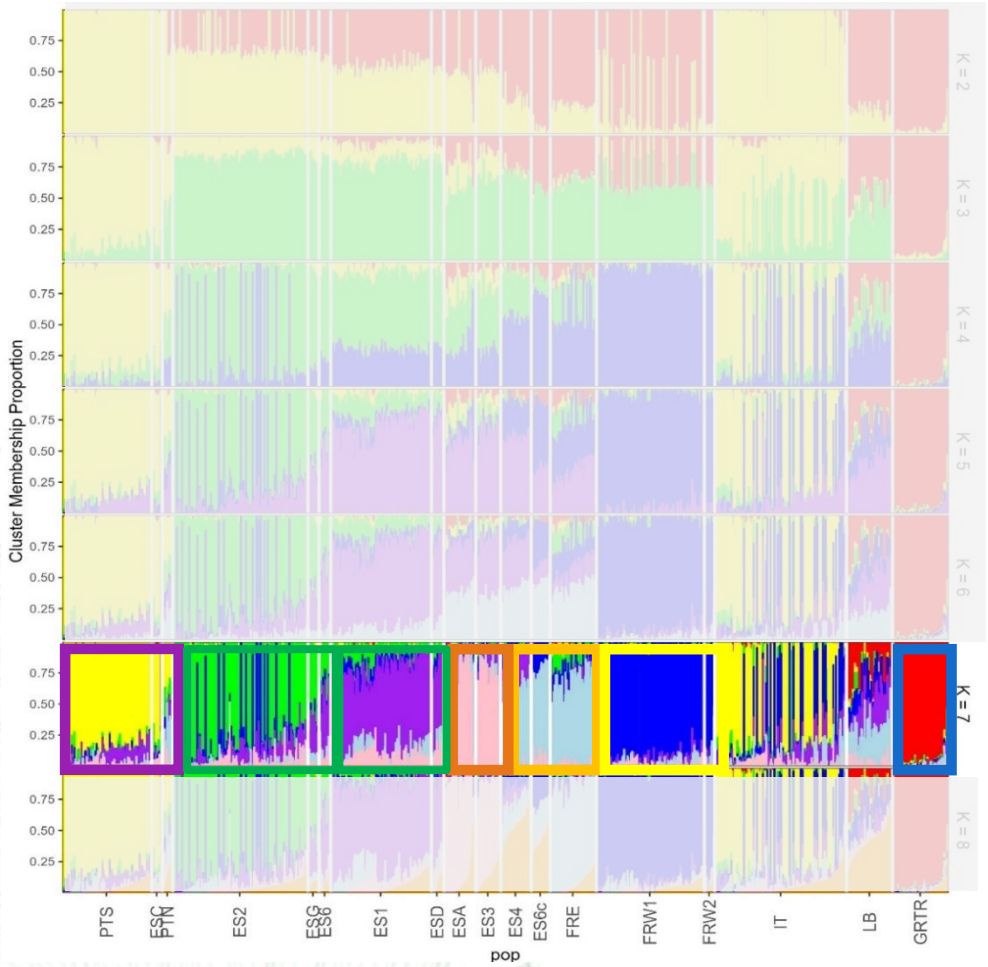
Individual ancestry coefficients *LEA: sNMF, CLUMPP, snpR*

K = 7

- No *a priori* knowledge
- Cross entropy
- Well defined regions

Results

Population genetic structure



Individual ancestry coefficients *LEA: sNMF, CLUMPP, snpR*

Portugal, Extremadura ES
¿ Tuscany ?

Inner Spain
(2 Reg of Prov.)

South eastern Spain

Andalucia, Catalonia, Provence
¿Lebanon?

K = 7

Languedoc-Roussillon (planted)

Aegean
¿minor in Lebanon?

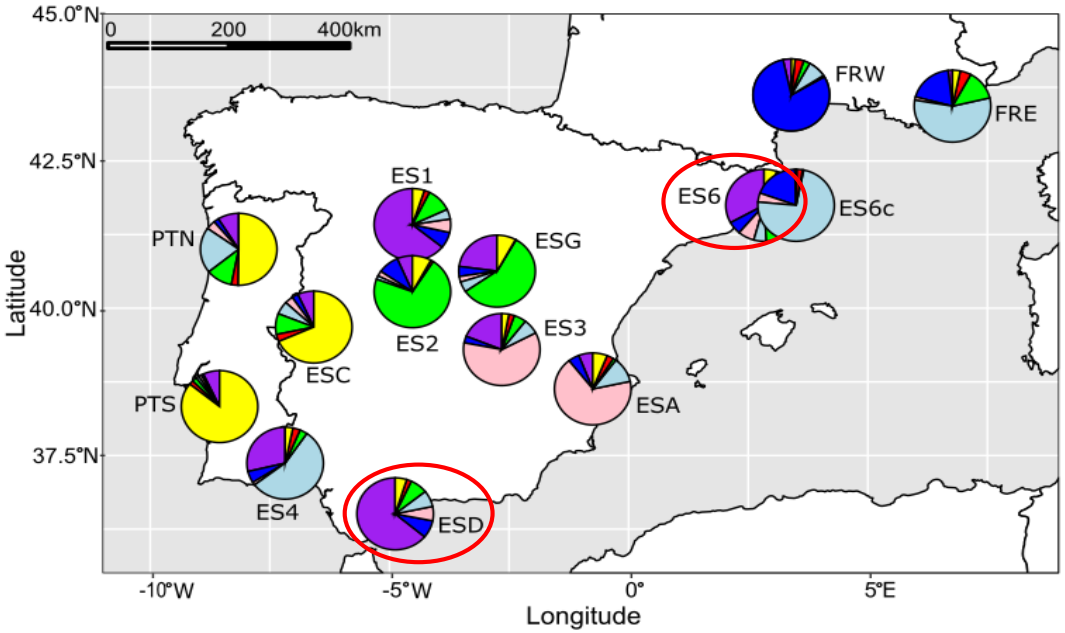
Results

Population genetic structure

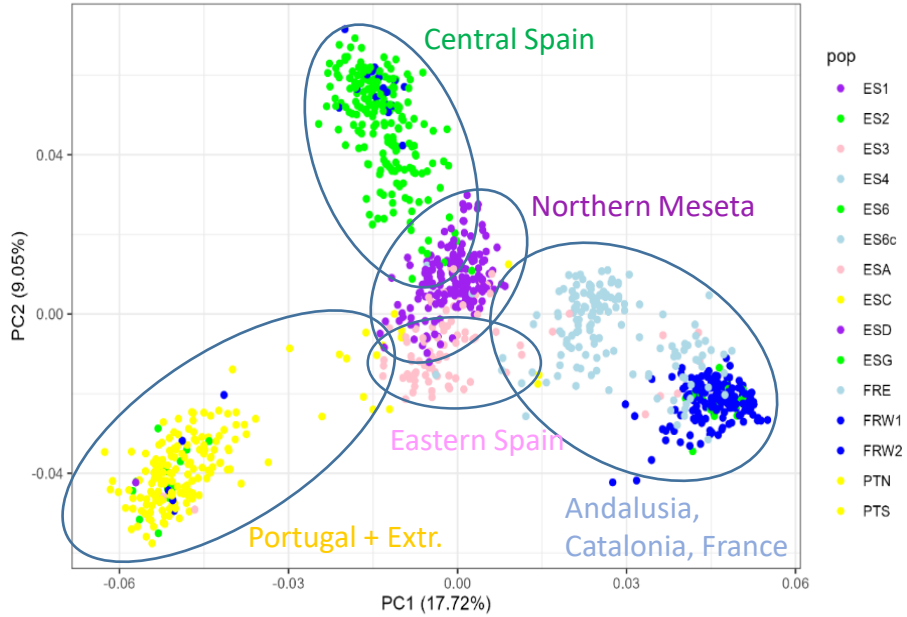
- Global analysis with K=7
- In the Western Mediterranean, 5 gene pools
- Unexpected patterns: 2 stands sampled, ESD (coastal Marbella) & ES6 (farmland edge)

putative historic introductions by forestation XIXth c. (*phylloxera* aftermaths)?

Individual ancestry coefficients *LEA: sNMF*



PCA (*snpR*)

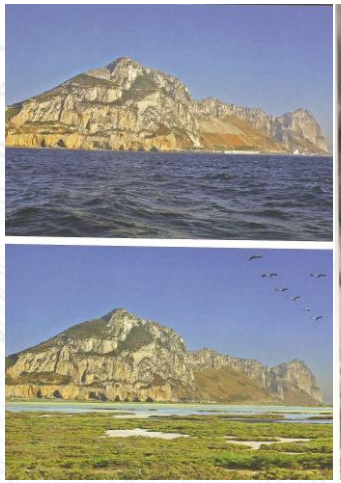


Discussion *Pinus pinea* – the "tame pine"?

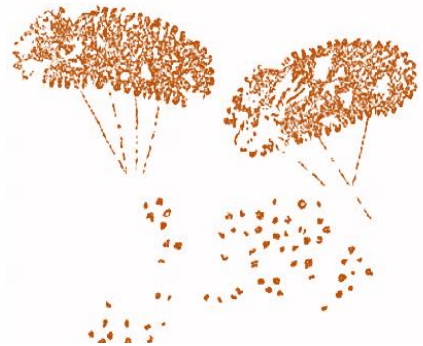
pino domestico, pino doncel, pinheiro manso,...

- Native Mediterranean tree, sparsely spread from the Portugal to the Black Sea and Lebanon
- Where is it native, archaeophyte or naturalised alien? Are the singled-out regional clusters glacial refugia, or mere bridgeheads?
- In Iberia, autochthony confirmed (cf. Prada et al., 1997; Blanco et al, 1997; Gil 1999; Martínez et al., 2003; Martínez & Montero 2004], especially for **open woodland landscapes** in the southwest, where its edible pine nuts have been foraged and consumed **since at least 100 000 BCE**, first by **Neanderthals**, later by **modern humans** (cf. Badal et al., Finlayson et al., Carrión et al., Cortés-Sánchez et al., Zilhão et al.,...)

The last ever Neanderthals lived "retired" in Gibraltar and at the *Costa del Sol* on seafood, snails, …and pine nuts (<30 ka)



Broodbank, 2013

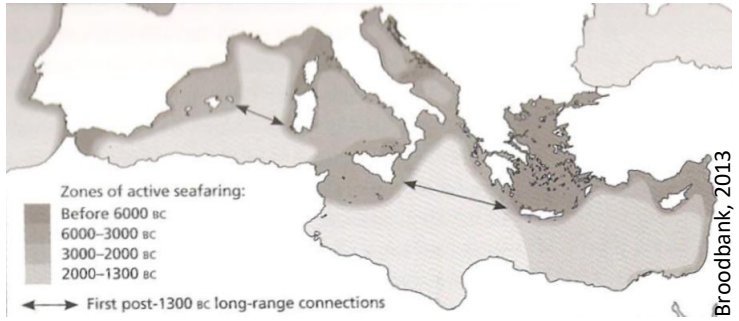


Neolithic rock art, Alcoi, Alicante, ES (Hernández et al., 2007)

Discussion *Pinus pinea* – the "tame pine"?

pino domestico, pino doncel, pinheiro manso,...

linked to the cultural landscapes of local **Megalithic, Tartessian** and **Phoenician sites**

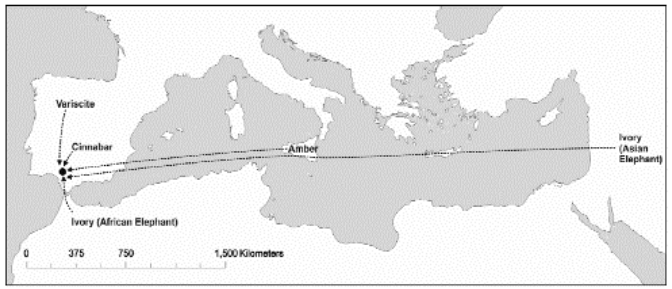


Broodbank, 2013

~Neolithic 6 000-5 000 BCE (~cardial ware)

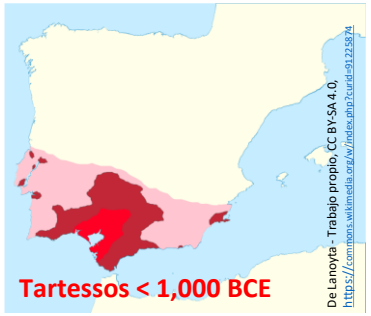
One sea but many routes to Sail. The early maritime dispersal of Neolithic crops from the Aegean to the western Mediterranean (de Vareilles et al., 2020; Zilhão, 2014),

…while in S-Portugal, local Mesolithic hunter-gatherers still lived on pine nuts (Soares & Tavares da Silva, 2018)



Ivory vessel (Asian elephant tusk)

Copper Age ~2 500 BCE (García Sanjuan et al., 2013)



De Lanoyta - Trabajo propio, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=91225874

rooted deep in the Alentejo

Soares & Tavares da Silva, 2018

Mesolithic hunter-gatherers living on pine nuts

Discussion *Pinus pinea* – the "tame pine"? (s. 1 000 BCE)

Linked to the **Megalithic, Tartessian** and **Phoenician** cultures

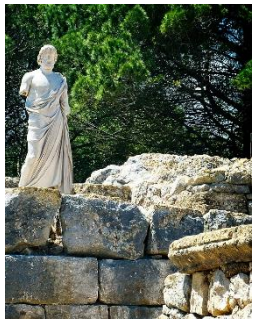
A. Tartessos (Andalusia, ES) Neanderthal 50 kyr

B. Alcácer do Sal (Tagus & Sado estuaries, PT)

Neanderthals 100 kyr (Zilhão, 2020), Phoenician *Bevipo/Keition*

C. Inner Spain Bronze 3.5 ka

By Caudullo et al, 2017
doi:10.1016/j.dib.2017.05.007
cf. commons.wikimedia.org



By Mary Harrsch - Own work, CC BY-SA 4.0,
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1. Emporion (Catalonia, ES)
Greek Emporion (trade post) between →Massalia and →Tartessos

2. Nora (Sardegna, IT) Phoenician colony
[or *Tartessian*, founded by "*king Nórax, grandson of Geryon*"]

3. Massalia (Bouches-du-Rhône, FR)
Greek colony founded from →Phocaea

4. Phocaea (Foça, Izmir, TR) [*"Arganthonios, king of Tartessos, funded their city wall"*]
Greek city 80 km south of →Pergamon (Bergama), capital famous for its Cybele temple

5. Kozak (Izmir, TR) in 1920 "the most relevant stone pine forest in Turkey", but in 1930 < 10.000 ha
20 km north of →Bergama

6. and, of course, the very Phoenicia itself (Lebanon)

Pergamon (by Klaus-Peter Simon - Own work, CC BY-SA 3.0,
https://commons.wikimedia.org/w/index.php?curid=1276709)

…and we know that, and even *when*, Phoenicians did introduce stone pine first in Sicily [Stika et al., 2007; Moricca et al. 2021]
In Classic empires it was widely planted, as sacred tree of →Cybele, cones were ceremonial offers and grave good...
(and its closed cones were used as stoppers for shipped wine amphoras!)

Conclusions

- We succesfully **identified clones**, genetic relationships and **gene pools**
- Deep insight into the **natural and cultural story** of this pine
- Better **traceability** of genetic material and **knowledge-based conservation**
- Genome-wide markers will allow to develop low-input breeding strategies

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