

Conservation of genetic diversity in artificially regenerated holm oak populations



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International (i.e. Strasbourg Resolution S2, 1990; Directive 1999/105/CE) and national legislation give precise directives on the conservation of forest genetic resources.

- Maintaining sufficient variation for natural evolution of the ecosystems;
- Protection against contamination and loss of genetic resources.

It implies

- selection of proper species;
- maintaining a suitable level of diversity after restoration actions;
- control of the origin of reproductive material.

Few data on the impact of silvicultural practices on natural genetic diversity, and on the minimal amount of genetic diversity represented in an artificially regenerated stand are available.



Quercus ilex



- play an important role in composition and structure of mediterranean ecosystems;
- one of the autoctonous species most used for forest restoration;
- reliable information on the origin of seedlings for afforestation.

Objectives



- Quantify the change of genetic diversity from the natural seed-origin population to artificial stands;
- Explore the effects of seed sampling strategies on the genetic diversity of artificial stands;
- Investigate the possibility of genetic contamination because of introduction of foreign material.

Materials and methods
(1)



TYPE OF POPULATION

CRITERIA

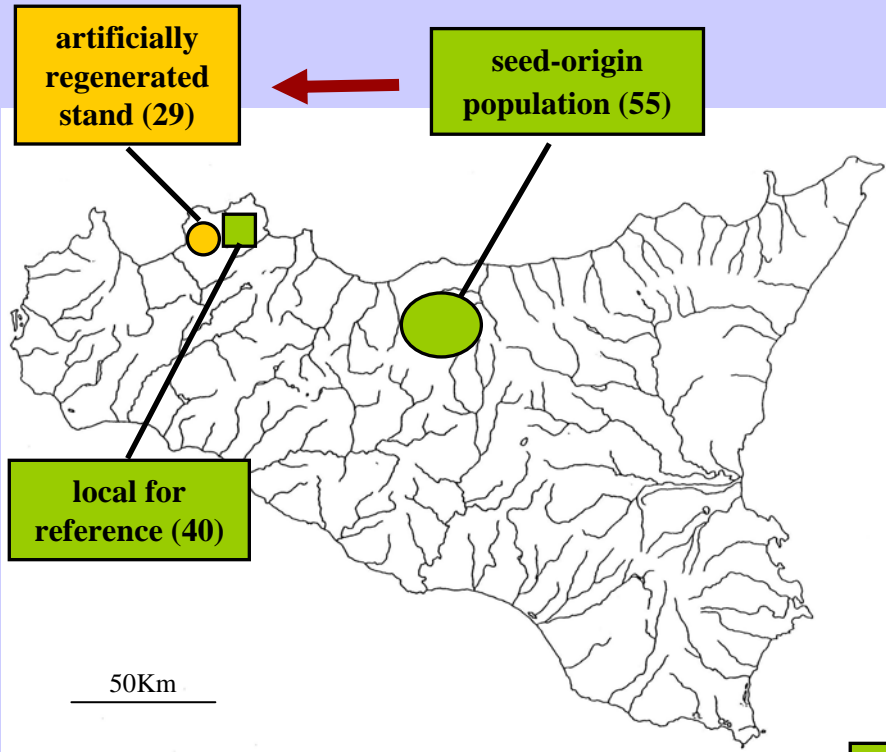
natural seed-origin	official source of seeds
artificially regenerated	known seed-origin
natural local	close to the artificially regenerated

NUMBER OF SAMPLED INDIVIDUALS PER POPULATION

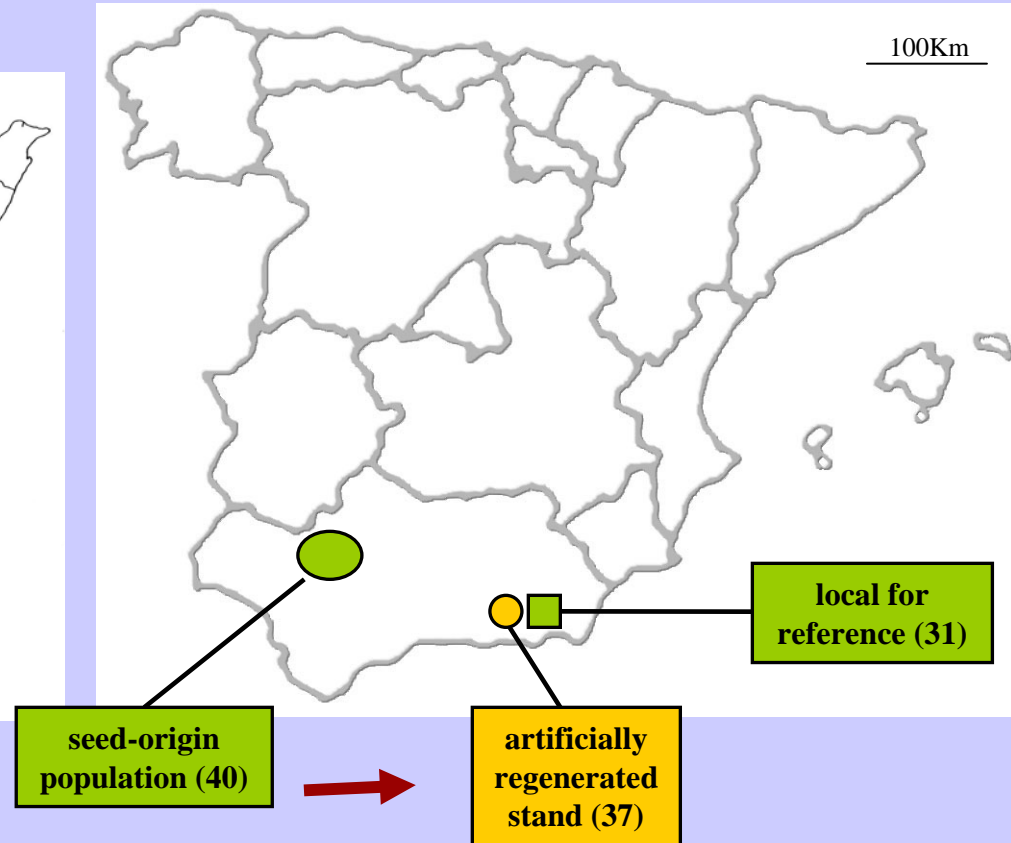
40 (30): average distance between individuals 50 m for natural populations




Materials and methods (2)

SICILY



ANDALUSIA



-  natural population
-  artificial stand
-  seed/seedling movement



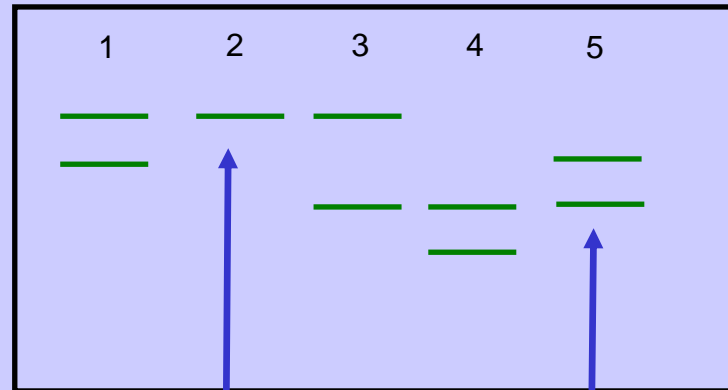
GENETIC CHARACTERIZATION

6 nuclear microsatellites *loci*

- very repetitive DNA sequences;
- numerous in the genome;
- high length polymorphism;

ANALYSIS STEPS

- ⇒ DNA extraction
- ⇒ amplification (PCR)
- ⇒ electrophoresis



homozygote

heterozygote



How much genetic diversity from seed-origin population is conserved in the regenerated stands?

	SICILY		ANDALUSIA	
	A	He	A	He
seed-origin population	8.37	0.65	12.1	0.76
artificially regenerated stand	6.0 (71%)	0.52 (80%)	10.2 (84%)	0.72 (94%)

A, allelic richness: weighted mean allele number per *locus*.

He, expected heterozygosity: expected proportion of heterozygous individuals.

Results

(2)



Does genetic diversity of artificial regenerated stands depend on the number of mother trees?

effective population size, **Ne**
of artificially regenerated stand

35.4 in Sicily

102.9 in Andalusia

Effective population size, N_e , corresponds to the number of generations after which a 50% reduction in the heterozygosity, due to genetic drift, would be produced (Wright, 1978).

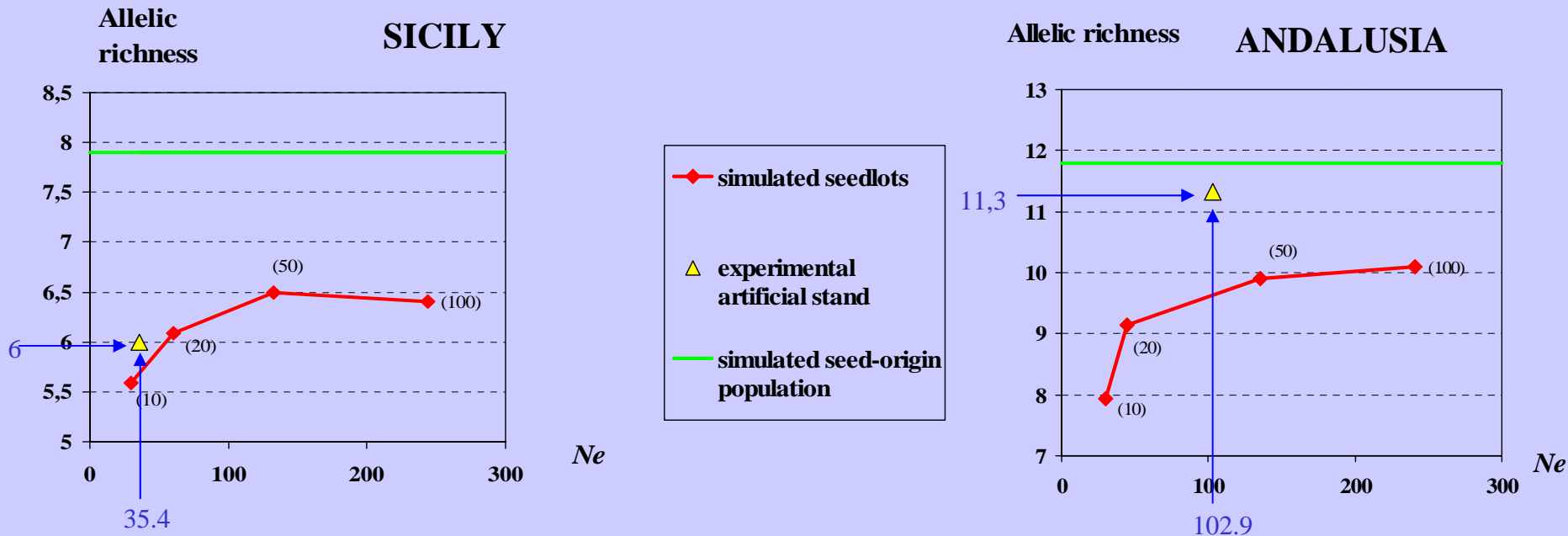
SIMULATION (based on the allelic frequencies of the natural seed-origin population)

- seed-origin population of 500 individuals, within an unlimited population;
- fictitious seed collections (1000 seeds);
- different numbers of randomly selected mother trees (10, 20, 50, 100);
- for each simulated seedlot, N_e and A have been calculated.

Results (2)



Allelic richness of fictitious seedlots goes up when the number of seed parents increases, because of progressively higher effective population size.



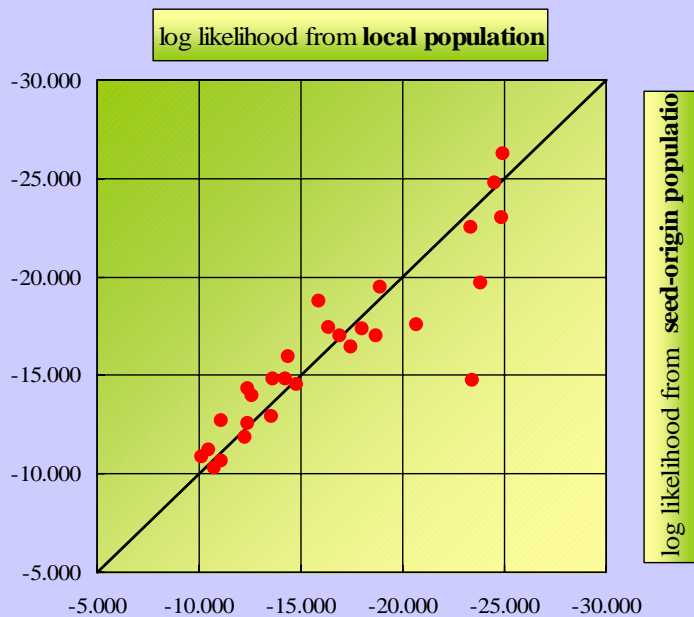
Possibly genetic drift occurs in the simulated following generation, particularly if seed-collection involves a limited number of trees.



Are there risks of genetic contamination?

The **assignment test** matches each genotype to the population where its expected frequency is highest, that is, where it has the greatest possibility of occurrence (Waser and Strobeck, 1998).

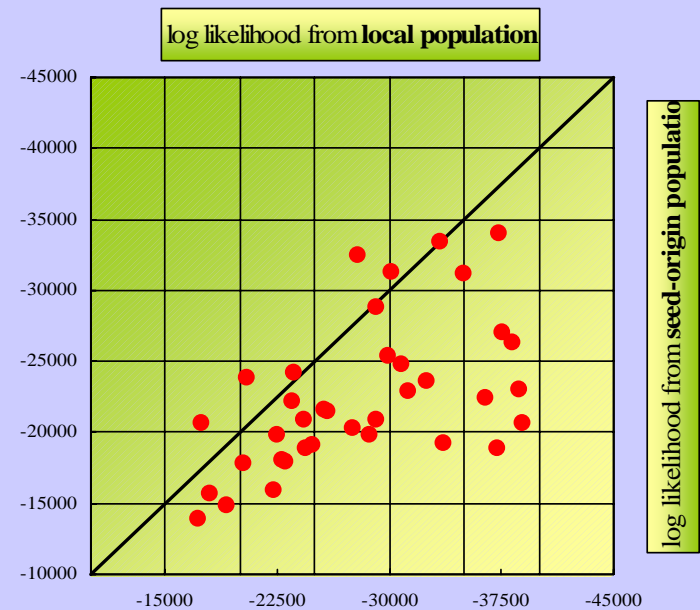
SICILY



• artificially regenerated stand

very low or no contamination at all

ANDALUSIA



possible genetic contamination

Conclusions



- ❖ Artificially regenerated stands studied seem to have been subject to a genetic process which natural equivalent is a **bottleneck or a founder effect**.
This is probably caused by an inappropriate forestry seed collection strategy limited to a few trees, that might have been accentuated by specific mating system properties.
- ❖ **Optimal sampling size** could be different for the two regions, being higher in Andalusia, which seems genetically more diverse.
- ❖ **Genetic contamination** is a real risk. It depends on the genetic characteristics of the population in each area.
- ❖ **Nuclear microsatellites markers** are useful tools to investigate genetic diversity of artificially regenerated stands of holm oak, including the cases when genotype information of parent identity is lacking.
- ❖ Forestry management cannot be easily generalized and for the single species in each area **specific strategies** are required.

Thank you for your attention!

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