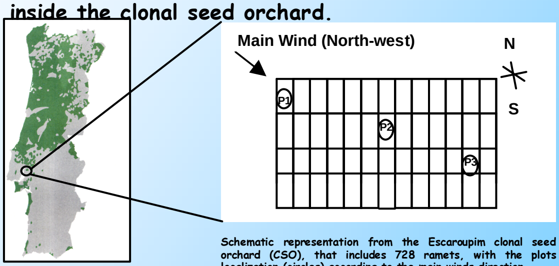
Pollen contamination in a maritime pine clonal seed orchard using microsatellites

Fernandes L.(1), Rocheta M.(1), Cordeiro J.(1), Pereira S.(1), Ribeiro M. M.(2), (1) IBET/ITQB, Quinta do Marquês, Ap. 12, 2780 – 901 Oeiras, Portugal (2) Departamento de Silvicultura e Recursos Naturais, Escola Superior Agrária de Castelo Branco, Quinta da Senhora de Mércules, 6001 – 909 Castelo Branco

Introduction: Maritime pine (*Pinus pinaster* Ait.) is a very important species in Portugal, for it occupies about 1/3 of the forest area. Seed orchard's pollen contamination estimate is fundamental to compute the decrease in the expected genetic gain due the contamination with foreign pollen from the outside sources. The aims of this study were: to identify the parental clones with a pre-determined confidence level, to estimate pollen contamination and to study the mating system inside the clonal seed orchard.



Schematic representation from the Escaroupim clonal seed orchard (CSO), that includes 728 ramets, with the plots localization (circles) according to the main winds direction.

To fingerprint the 60 different genotypes established in Escaroupim clonal seed orchard (CSO), and the 206 seeds collected from 7 trees from each one of the three *plots* distributed according to the main winds direction.

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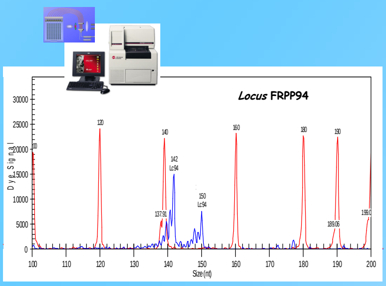
We used 3 nuclear microsatellites (nuSSR) to perform the paternity and pollen contamination analysis.

The seeds

The needles

→ DNA Extraction → Fragment analysis →



→ **Fragment Analysis Results**: the 3 *loci* revealed to be enough to distinguish all the 60 clones but 2 pairs, which shared the same genotype.

Paternity Analysis

Software FaMoz (Gerber *et al.*, 2003)

Parameters	*Plot* 1	*Plot* 2	*Plot* 3	Total
No likely father inside the stand	**25**	**37**	**33**	**95**
Father among the genotyped one	30	25	14	69
Single matching father	11	8	23	42
Total offspring	66	70	70	206

The number of seeds with father outside the seed orchard was 95 in 206 seeds

↓

The minimum estimate of pollen contamination was 46%

Genetic Flow Estimation

N=200
G=60

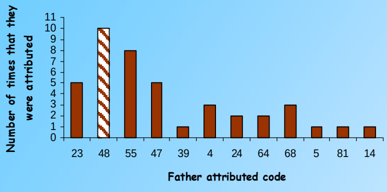
→ The genetic flow was estimated by simulations from 400 gametes

Genetic flow (%)	*plot* 1	*plot* 2	*plot* 3	Total
True	58	51	61	56
Apparent	58	34	61	47
Criptic	13	18	13	13

True genetic flow → The proportion of gametes generated according to allele frequencies, from outside the study stand, produced one of the offspring

Pollen Contamination → [46% ; 56%]

Paternity Analysis Results: Pollen contamination estimates ranged from 46% to 56%. Those values could be explained by the presence of a *P. pinaster* stand at a distance lower than 2 Km and the existence of natural regeneration of this species on the orchard's isolation zone. Pollen contamination was higher in *plots* 1 and 3 than in interior *plot* (n° 2).



Number of times the fathers were attributed. The broken line bar enhance the father/clone that more contributed for the pollinization

In this study, only 20% of the clones contributed for the next generation, which may lead to a reduction of seeds' genetic variability. However, an asymmetrical sampling from the orchard (3 *plots*) was made, which might have biased the results and, the effective number of clones participating in the next generation, might be higher.

Mating System Results: The outcross pollination rate was found to be very high - 90.2% -. The minimum estimate of biparental inbreeding was 21.2%, a not so high value if we consider the number of seeds sampled and the orchard size.

Future Research: to obtain the allelic frequencies from outside the study stand for better estimate the cryptic genetic flow and to have a more accurate estimate of the true genetic flow.

Acknowledgements: Thanks to Engª Manuela Veloso from Estação Agronómica Nacional, Oeiras, Portugal. This work could not have been done without the plant material kindly provided by INIA's Estação Florestal Nacional research team, Oeiras, Portugal. This study was supported by European Social Funds and Education Minister integrated on PRODEP III.

Reference: Gerber S. *et al.* 2003. FaMoz: a software for parentage analysis using dominant, codominant and uniparentally inherited markers. *Molecular Ecology Notes* 3: 479-481.

Poster presented in the Conference: XXXI Jornadas Portuguesas de Genética, Lisbon, 5-6 February 2004