# Optimizing the choice of microsatellites for fingerprinting *Eucalyptus*

L. SANCHÉZ[1], M.M. RIBEIRO[2], C. RIBEIRO[3], J.A. ARAÚJO[3], N. BORRALHO[3], C.M. MARQUES[3]

[1] *Unité Amélioration, Génétique et Physiologie Forestières, INRA Centre d'Orléans, 45166 Olivet, France.*
[2] *Unidade Departamental de Silvicultura e Recursos Naturais, Escola Superior Agrária, 6001-909 Castelo Branco, Portugal.*
[3] *RAIZ-Centro de Investigação Florestal, ITQB II, 2781-901 Oeiras, Portugal.*
*E-mail: leopoldo.sanchez@orleans.inra.fr*

ESCOLA SUPERIOR AGRÁRIA
INSTITUTO POLITÉCNICO DE CASTELO BRANCO

INRA
www.orleans.inra.fr

www.raiz-iifp.pt

grupo Portucel Soporcel
RAIZ
Instituto de Investigação da Floresta e Papel

## PURPOSE OF THE STUDY

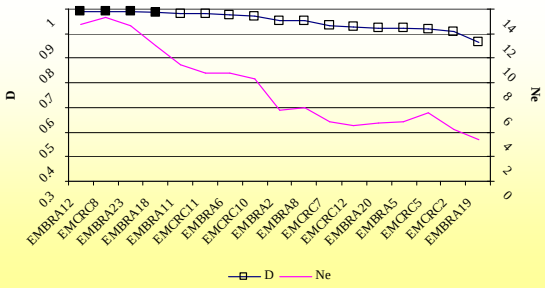
**[A] What is the Minimum set of Discriminant Markers (MSDM) ...**
- **... for a given set of known parental genotypes ?**
- **... for hypothetical offspring comprising Selfs, Full-sibs, Half-sibs and unrelated individuals ?**

**[B] What are the attributes of a discriminant marker ?**

**[C] Does the presence of null alleles affect the result ?**

## RESULTS

**Figure 1**: Discriminant power (D) and effective number of alleles (Ne) for the 17 SSR and 140 élites used in this study, with a 4 SSR MSDM (filled symbols).

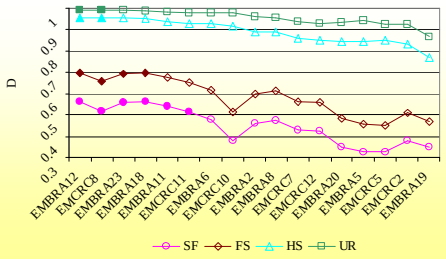



**[A] MSDM**
**4 SSR: C=2x10^(-4)**
**6 SSR: C=10^(-4)**
**12 SSR: C<<10^(-6)**

**[B] MSDM comprised systematicaly highly polymorphic SSR with Ne >=11, i.e. high polymorphism + low variance of allelic frequencies.**

**Figure 2**: Discriminant power (D), with MSDM (filled symbols) for simulated offspring, with pairs of unrelated individuals (UR; upper line), half-sibs (HS), full-sibs (FS) and pairs of sefs (SF; lower line).

**[A] MSDM**
**6 SSR for SF: C=10^(-2)**
**4 SSR for FS: C=10^(-2)**
**2 SSR for HS: C=10^(-4)**
**2 SSR for UR: C<<10^(-6)**



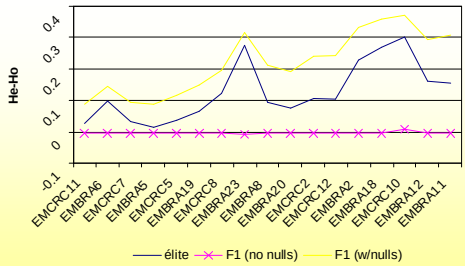
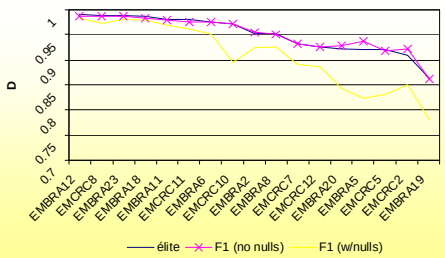




**Figure 3**: Difference between expected (He) and observed heterozygosity (Ho), for élites F0, simulated F1 from random mating of élites with no presence of null alleles, and simulated F1 from random mating of élites with null alleles (100% of all the homozygotes).

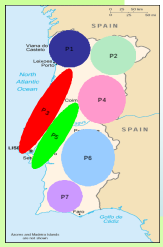
**[C] The élite's deficit in H could be partly explained by the presence of null alleles. Note that this deficit followed a similar pattern to that shown by the F1 with null alleles.**

**Figure 4**: D for same cases as in Fig. 3.

**[C] For a limited number of scenarios, the presence of null alleles in the élites increased D in their simulated offspring (ex., case of parental carriers of 1 null allele). In general, however, null alleles decreased D in the offspring, especially for those SSR of low polymorphism.**

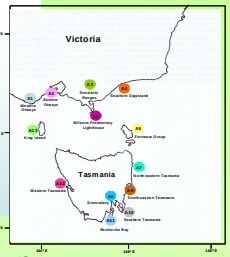



## MATERIAL AND METHODS




**RAIZ breeding Population (500 élites)**

**Sample of 140 élites and use of publicy available molecular markers (see ref.): 17 nuSSRs**

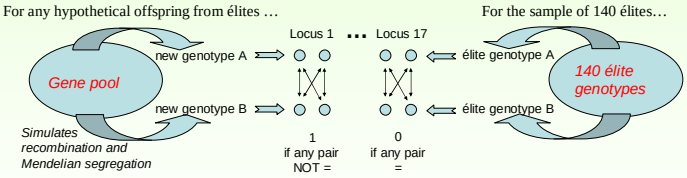



**RAIZ: 7 regions from P1 to P7 (seed introduction after 18th century)**

**13 provenances**

### Methodological approach

Development of ZETA(*) Monte-Carlo simulation software to obtain…
- …basic genetic descriptors for each marker (heterozygosity, effective number of alleles, measures of relatedness, etc);
- …MSDM with error = 0 (if enough markers) for the sample of 140 élites;
- …MSDM with error > 0 for any hypothetical set of offspring from élites parents (or parental *gene pool*);
- …and MSDM with the presence of null alleles.




Monte-Carlo D was obtained by **bootstrap of 10^(+6) pairs of genotypes:** (i) of non-related, half-sibs, full-sibs and selfs; (ii) existing élite genotypes.

**Discriminant power (D)** = proportion of 1's across bootstrapped cases.
Thus, the frequency of pairwise comparisons for which a marker presents distinguishable genotypic patterns over the assemble of comparisons across the sample under study.
The **error of confusion** (**C**) is equal to 1-D.

## CONCLUSIONS

**We presented a useful tool to analyse the discrimination power (D) and to minimize the subsequent risk of confusion (C) in the fingerprinting of genotyped breeding populations.**

**For the present case, *safe* fingerprinting was possible with a limited number of highly polymorphic SSR. The fingerprinting capacity of these SSRs was stable across sub-samples of distinct origin within the élite, and also between *simulated* offspring.**

**Fingerprinting capacity may be reduced by the presence of null alleles. The choice of highly polymorphic SSR could eventually minimize this effect.**

**(*)** publicy available user-friendly version of ZETA in preparation…

**References** : Brondani, R.P.V., Brondani, C., Tarchini, R. and Grattapaglia, D. (1998). *Theor Appl Genet* **97**: 816 – 827. Brondani, R.P.V., Brondani, C., and Grattapaglia, D. (2002). *Mol Genet Genomics* **267**: 338-347. Jones, R.C., Steane, D.A., Potts, B.M. and Vaillancourt, R. (2002). *Can J For Res* **32**: 59-66. Steane, D.A., Vaillancourt, R.E., Russell, J., Powell, W., Marshall, D. and Potts, B.M. (2001). *Silvae Genetica* **50**: 89-91.

**Poster presented in the International Conference: 'Eucalyptus in a changing world', Aveiro, 11-15 October 2004**