The use of markers and perspectives for genomic selection in maritime pine

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The use of markers and perspectives for genomic selection in maritime pine (*Pinus pinaster*)

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Journée thématique "Sélection génétique de Hévéa"
4 septembre 2020

Outlines

1/ French maritime pine breeding programme

2/ Molecular markers available

3/ Identity and pedigree checking
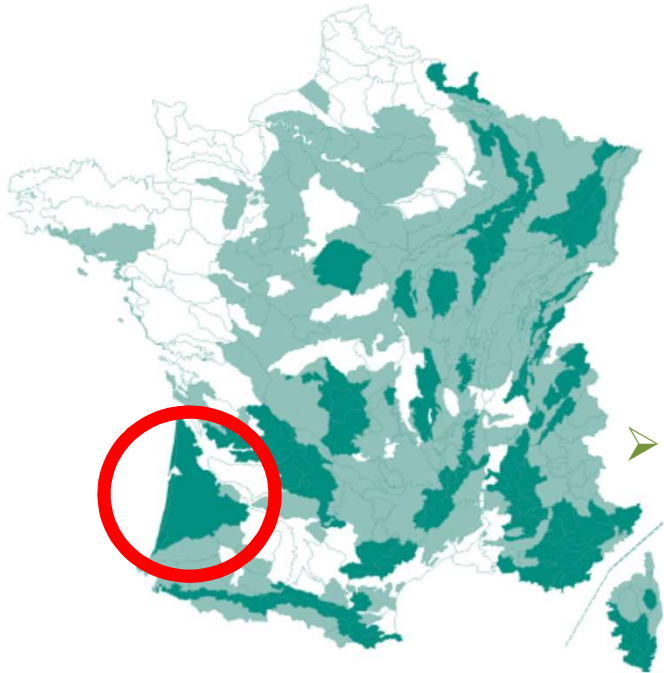
4/ Mating designs based on pedigree recovery

5/ GS proof-of-concept in maritime pine

6/ Perspectives for GS in maritime pine

1/ French maritime pine breeding programme

Maritime pine forests in southwestern France

- ➢ **Maritime pine forest established at the end of the 19th century**
  - Native species
  - Poor soils (sandy podzol)
  - Dry summers / wet winters (hydromorphic soils)

- ➢ **0.8 million hectares forest (7% of the French forest)**
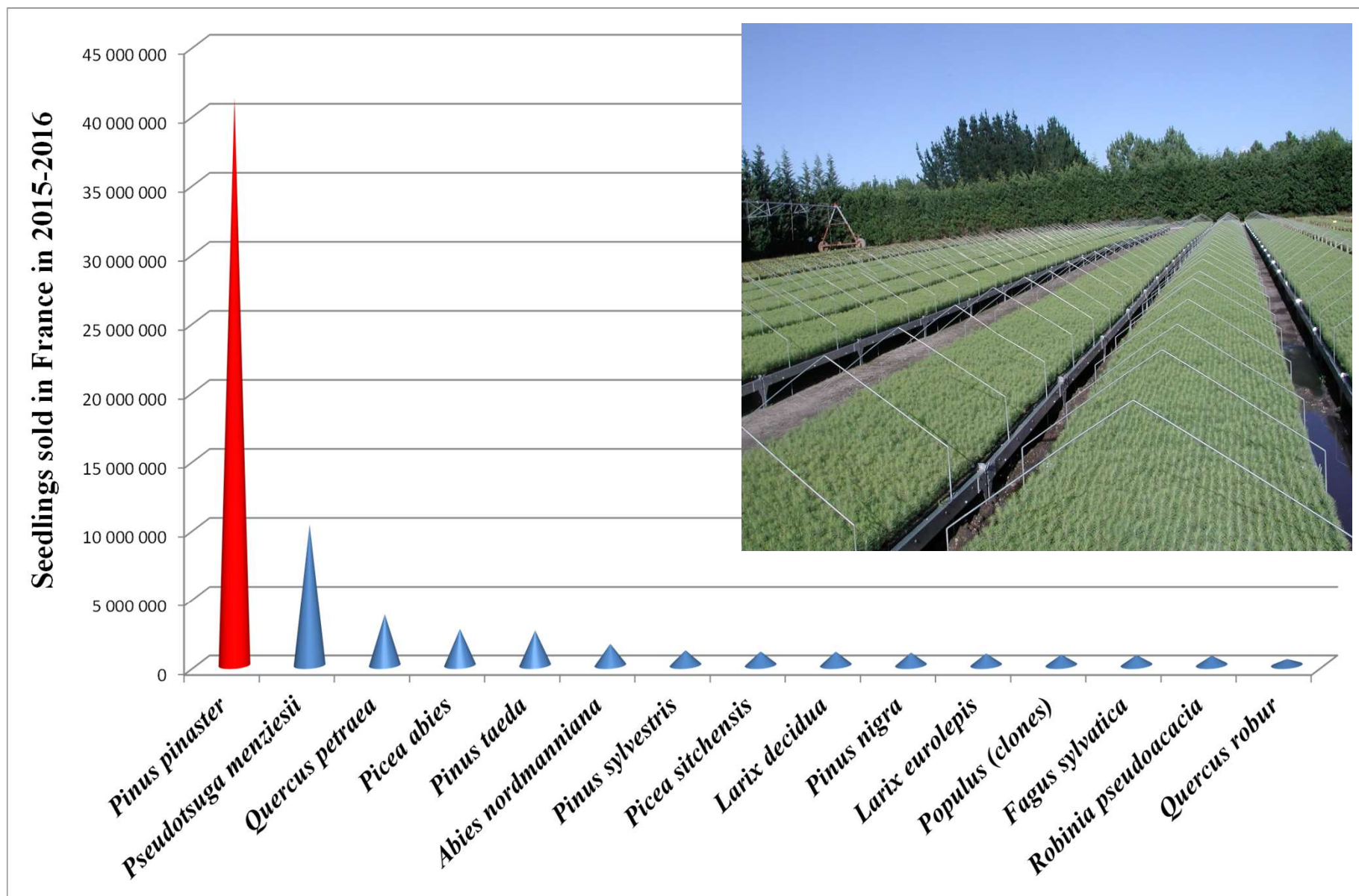 - Maritime pine plantations (>90% improved seedlings) except in sand dunes (natural regeneration)

- ➢ **24% of French wood harvest**
  - 11 m3/ha/year in average
  - 8.5 million m³ harvested /year
  - 60% saw timber / 40% industrial wood

Maritime pine = 1ˢᵗ plantation species in France

Chart: Seedlings sold in France in 2015-2016

Y-axis: Seedlings sold in France in 2015-2016
Values: 0, 5 000 000, 10 000 000, 15 000 000, 20 000 000, 25 000 000, 30 000 000, 35 000 000, 40 000 000, 45 000 000

X-axis categories: *Pinus pinaster*, *Pseudotsuga menziesii*, *Quercus petraea*, *Picea abies*, *Pinus taeda*, *Abies nordmanniana*, *Pinus sylvestris*, *Picea sitchensis*, *Larix decidua*, *Pinus nigra*, *Larix eurolepis*, *Populus (clones)*, *Fagus sylvatica*, *Robinia pseudoacacia*, *Quercus robur*

Breeding program managed by a consortium

- ➢ **Base population established in 1960's by INRA**

- ➢ **Two breeding programs (INRA, FCBA)**

- ➢ **Consortium since 1995 = " Groupe Pin Maritime du Futur "**

« Recurrent » fundings = Ministry of Agriculture + Région « Nouvelle Aquitaine »
Specific studies = European Union + Ministry of Education and Research (ANR projects)

Stakeholders for maritime pine breeding

Maritime pine breeding program
Managed by GPMF (« Groupe Pin Maritime du Futur »)
IP = INRA + FCBA

Seed Orchards

Composition + Design
GPMF but SO must be validated by a national public authority (CTPS)

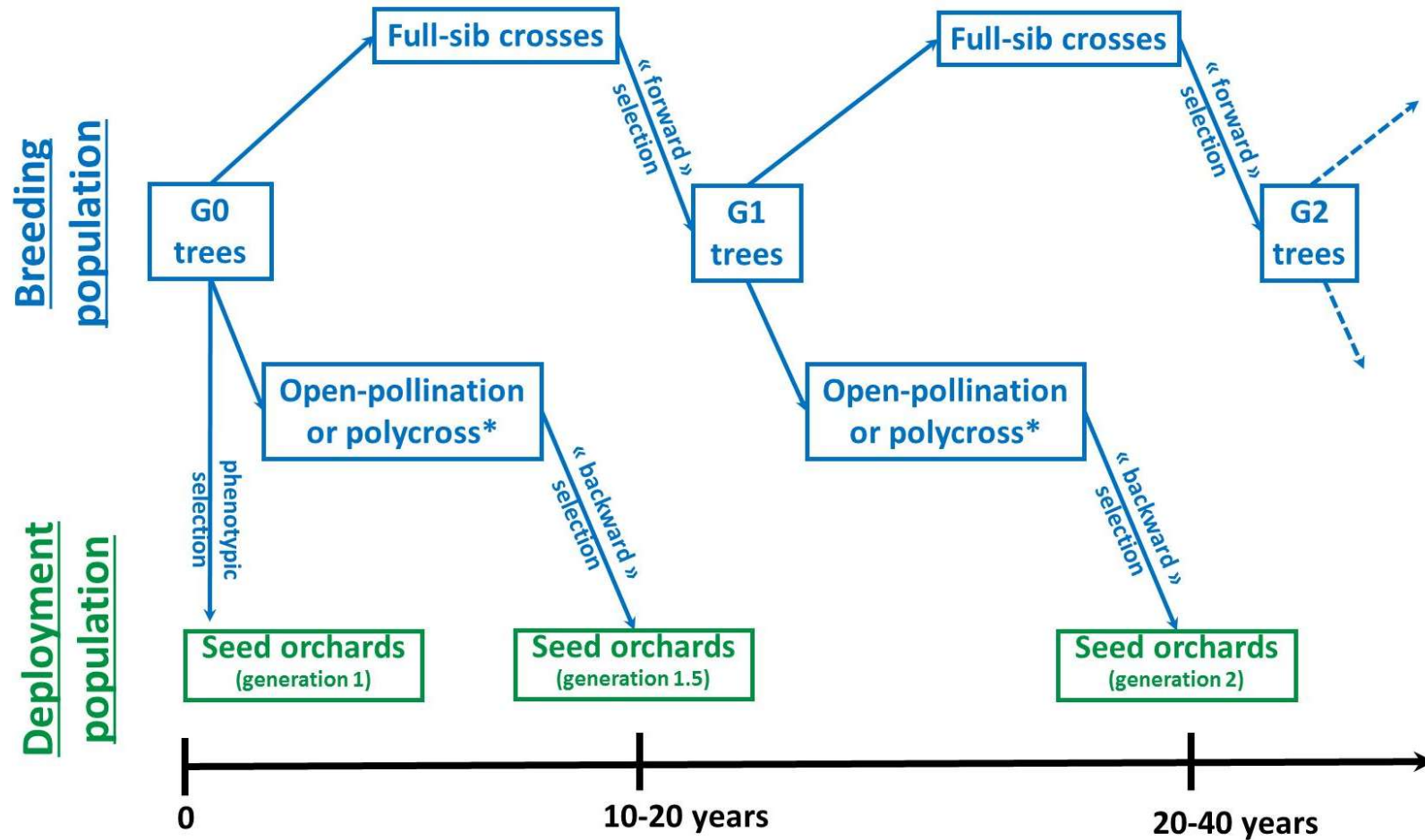**Ownership + Management**
Public (ONF) and private companies

royalties

Nurseries
Private companies
(Forelite, Planfor, Naudet, Robin)

Recurrent selection scheme

> **Selection criteria:**
> - Volume (girth, height)
> - Stem straightness
> - Rust resistance
> - Wood quality traits (branching quality, wood density, spiral grain)

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Maritime pine breeding: the latest advances

➤ **BLUP evaluation** (individual mixed model based on pedigrees and phenotypes)
  - database = 600,000 trees including historical trials (first one in 1962)
  - powerful genetic evaluation

➤ **Seed orchards renewed more often**
  - previously: one new SO composition every 15 years based on backward selections
  - currently: faster turnover (~3 years) based on backward and forward selections

➤ **New selection criteria under study**
  - pest resistance (pine wood nematode)
  - climate change (drought resistance)

➤ **New provenances**
  - Genetic diversity infusion with southern provenances for drought resistance

➤ **Rolling front strategy**
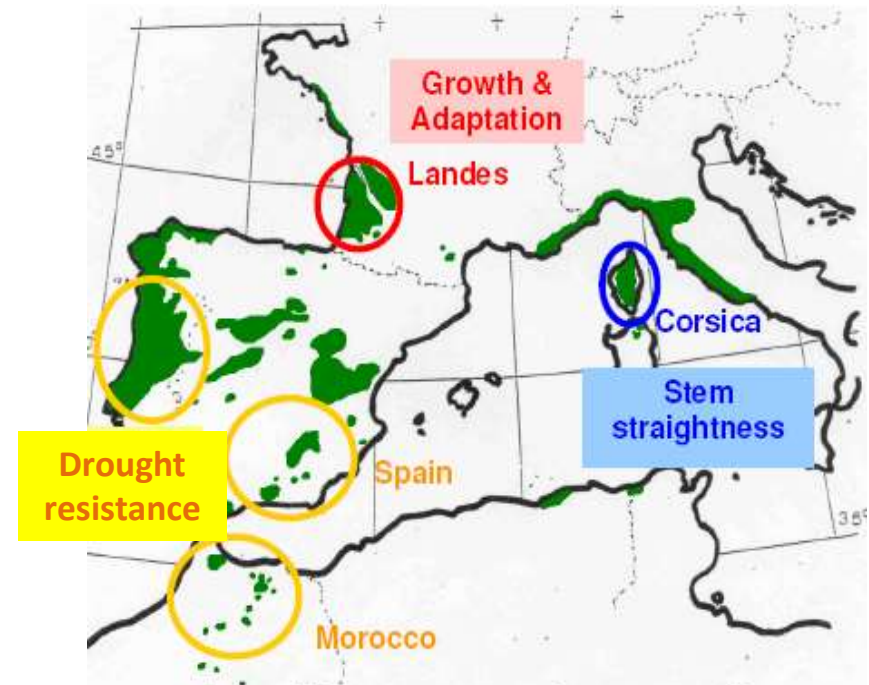
2/ Molecular markers available

High density genotyping

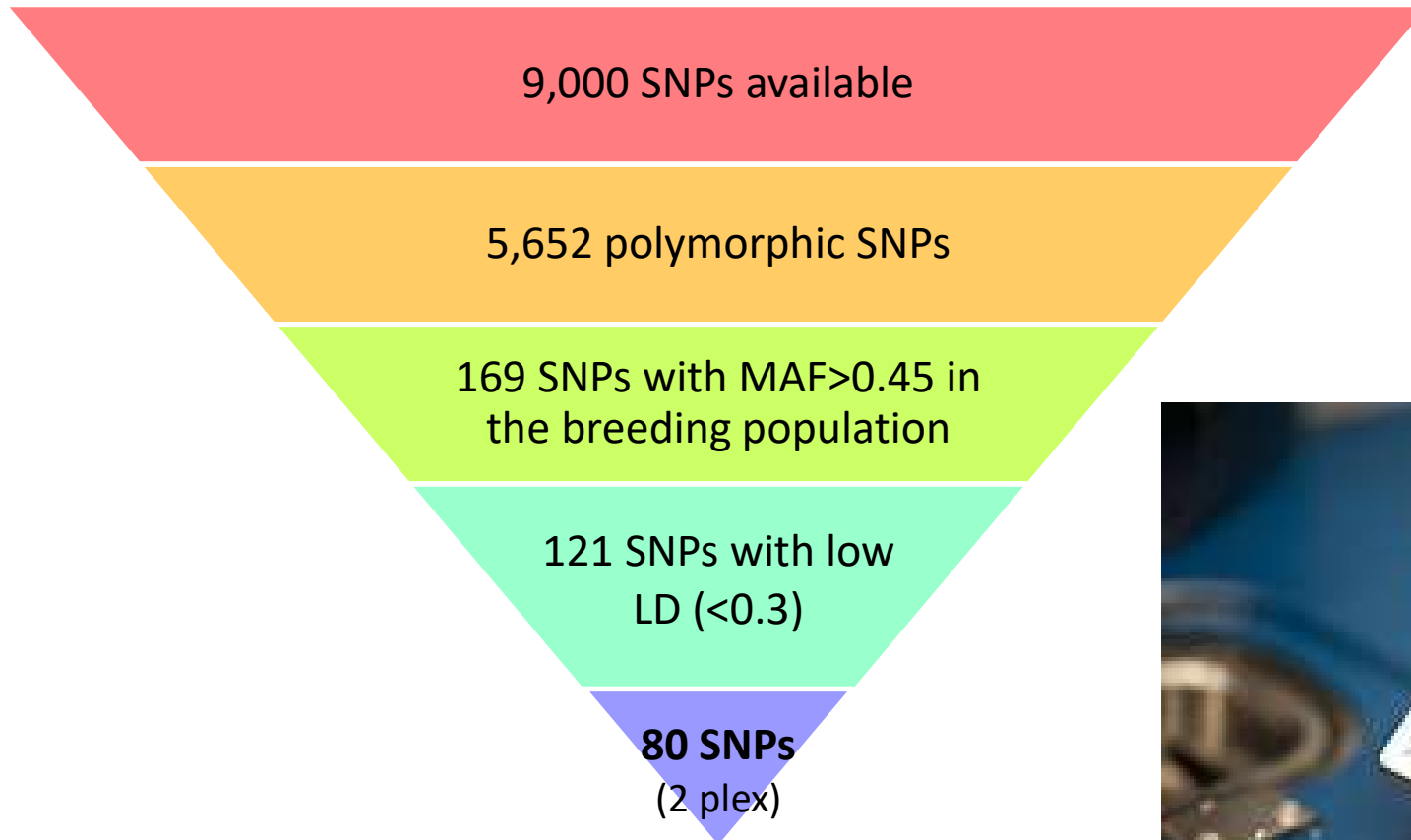## High-density SNP assay development for genetic analysis in maritime pine (*Pinus pinaster*)

C. PLOMION,*† J. BARTHOLOMÉ,*† I. LESUR,*‡ C. BOURY,*† I. RODRÍGUEZ-QUILÓN,§ H. LAGRAULET,*† F. EHRENMANN,*† L. BOUFFIER,*† J. M. GION,*¶ D. GRIVET,§ M. DE MIGUEL,*† N. DE MARÍA,§ M. T. CERVERA,§ F. BAGNOLI,** F. ISIK,†† G. G. VENDRAMIN** and S. C. GONZÁLEZ-MARTÍNEZ§

➢ Currently: **9,000** Illumina Infinium SNP array (transcriptome data + candidate genes)

➢ In 2020: **16,000** SNPs available (B4EST project) with a multi-species Axiom array (ThermoFischer) ~50€/sample

Low density genotyping (80 SNPs)

9,000 SNPs available

5,652 polymorphic SNPs

169 SNPs with MAF>0.45 in the breeding population

121 SNPs with low LD (<0.3)

80 SNPs
(2 plex)

Genotyping using Sequenom's Mass Array technology (mass spectroscopy)

(cost ~4€ per sample for 1 plex)

Applications High / Low density genotyping

➢ **High density genotyping for:**
- linkage mapping
- association studies
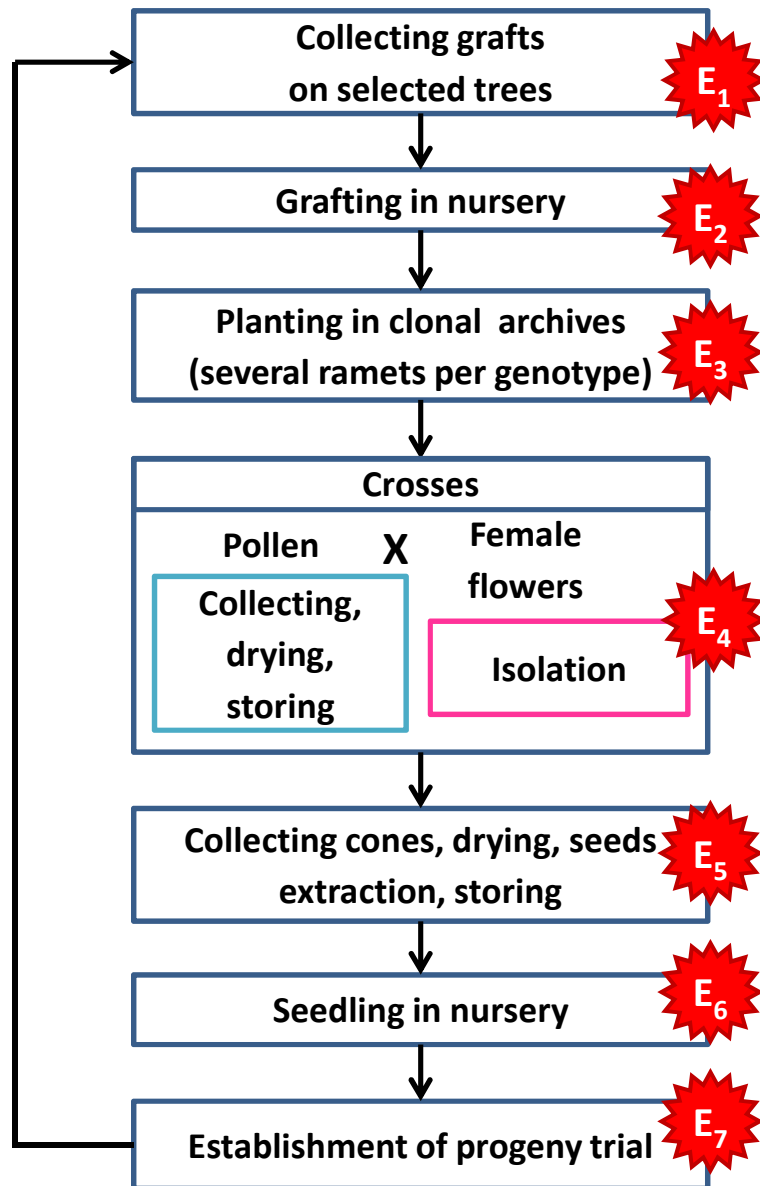- genomic selection

➢ **Low density genotyping for:**
- id checking
- pedigree recovery
- pollen contamination / parental contribution in SO
- seedlot certification (provenance)
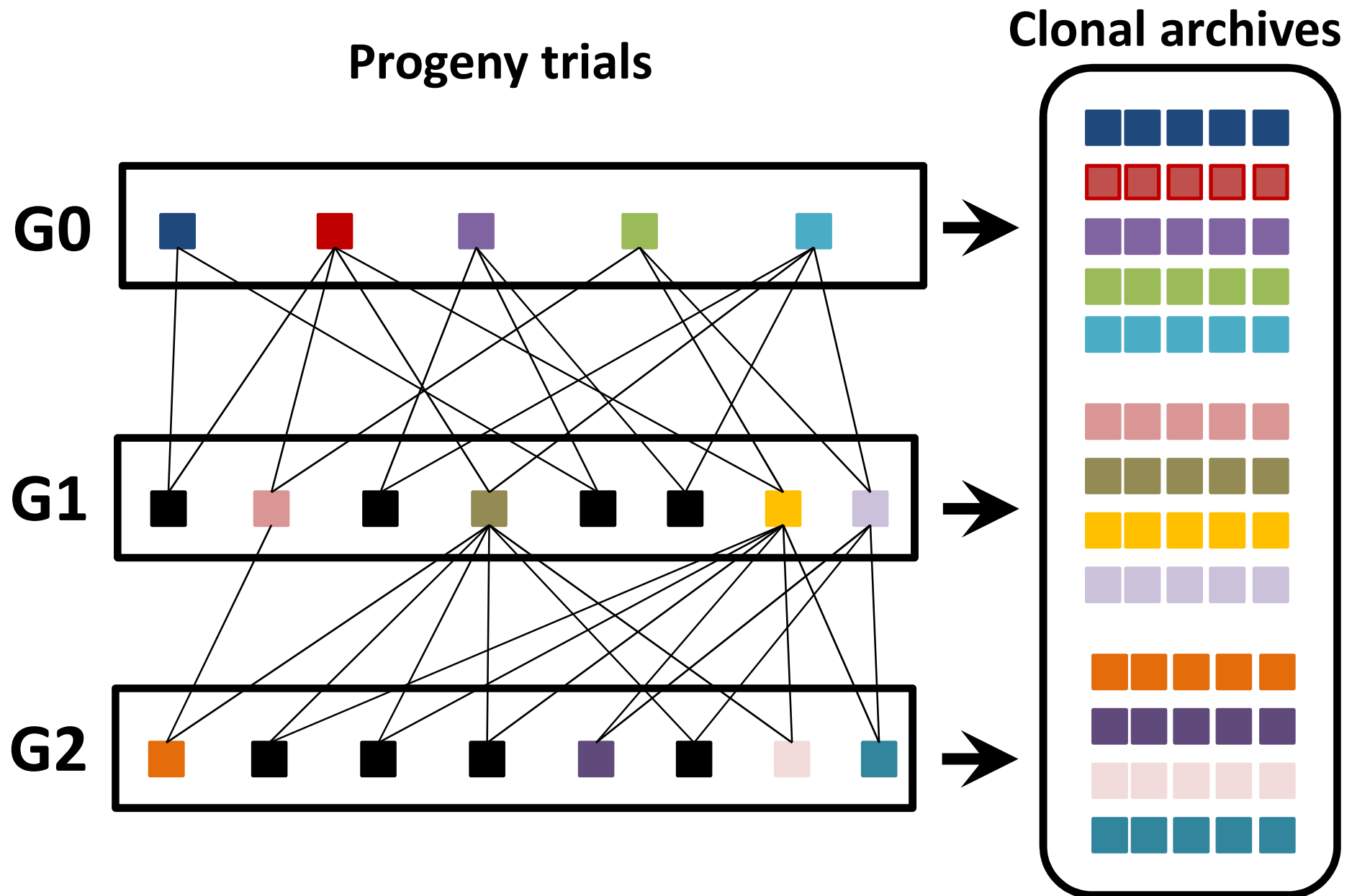
3/ Identity and pedigree checking

Various sources of id errors

(from Marjorie Vidal PhD, 2016)

➢ Cumulative process over generations

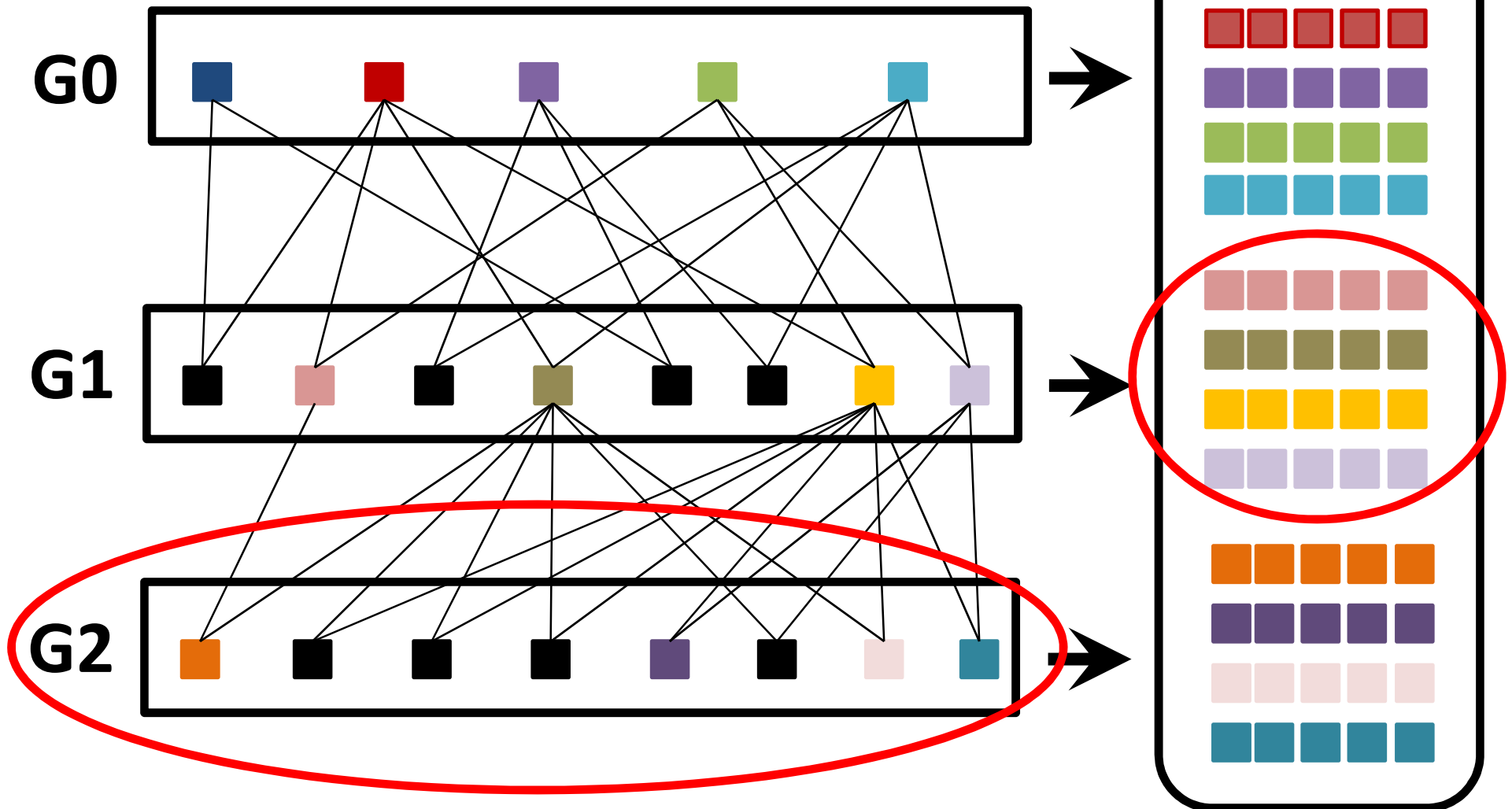➢ Markers = the only way to check id
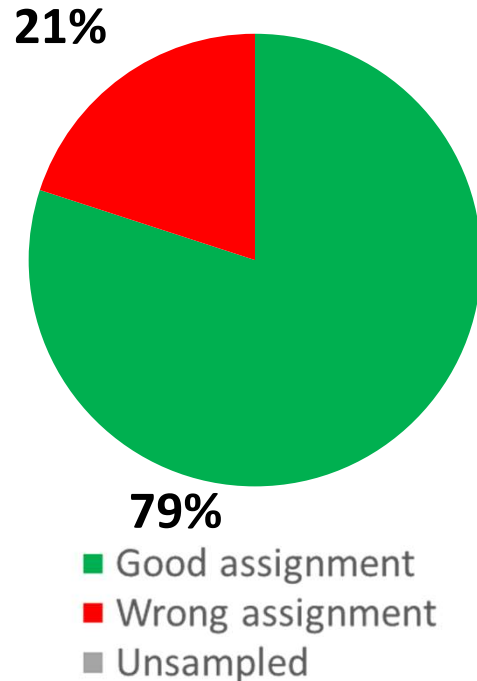
15

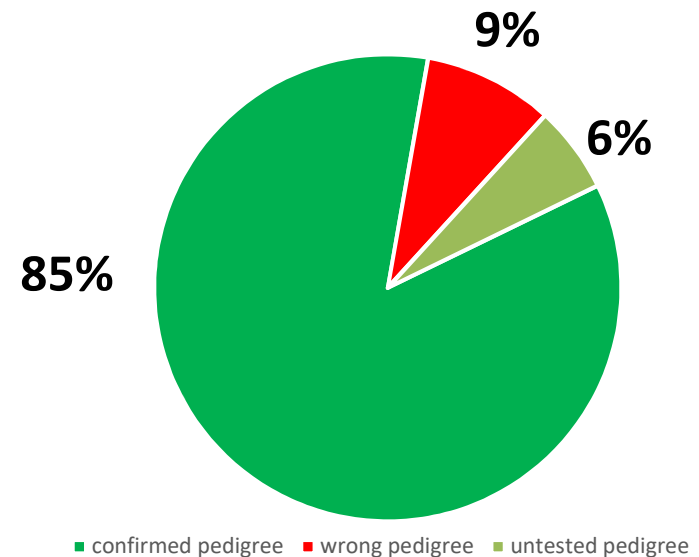Id errors in progeny trials and clonal archives

Id errors in progeny trials and clonal archives

Estimation of pedigree errors in the maritime pine breeding population (80 SNPs)

G2 progeny trials
(3,009 trees)

G1 clonal archives
(1061 G1 trees x 3-5 ramets / tree)
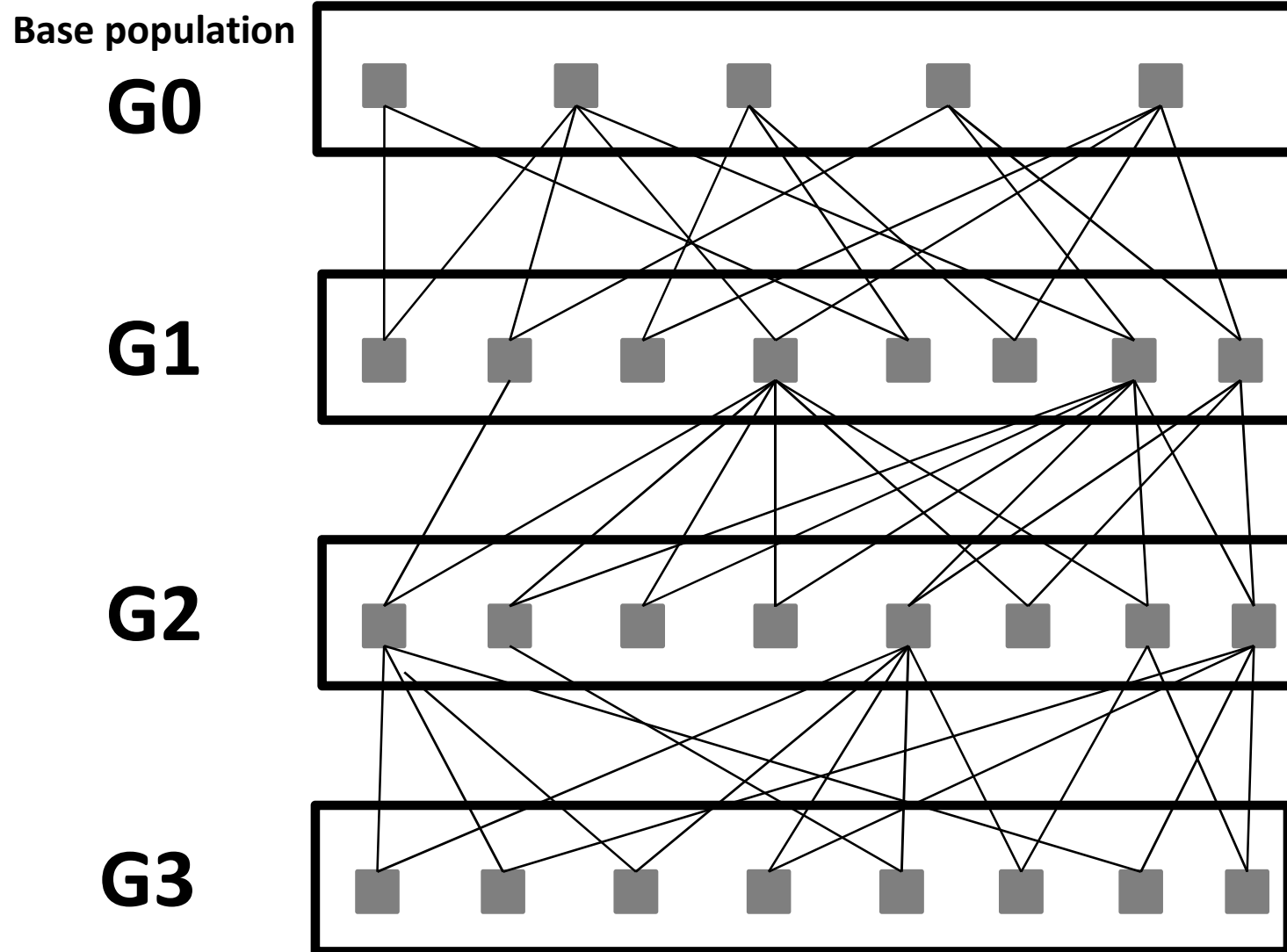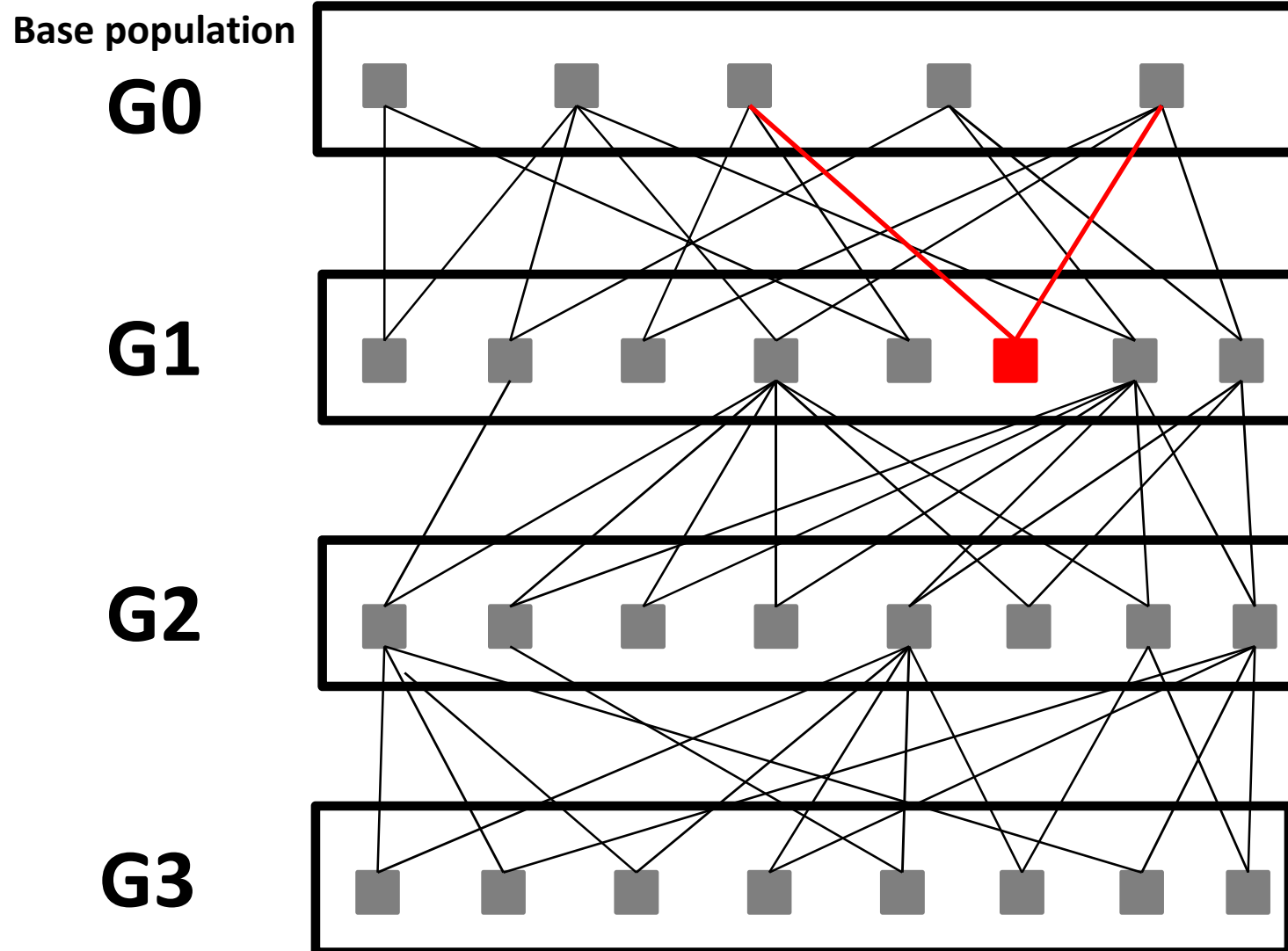
21%

79%

- Good assignment
- Wrong assignment
- Unsampled

9%

6%

85%

- confirmed pedigree
- wrong pedigree
- untested pedigree

Consequences of id errors

Base population
G0

G1

G2

G3

Consequences of id errors

Base population

G0

G1

G2

G3

Consequences of id errors

Base population
G0

G1

G2

G3

Consequences of id errors

Base population

G0

G1

G2

G3
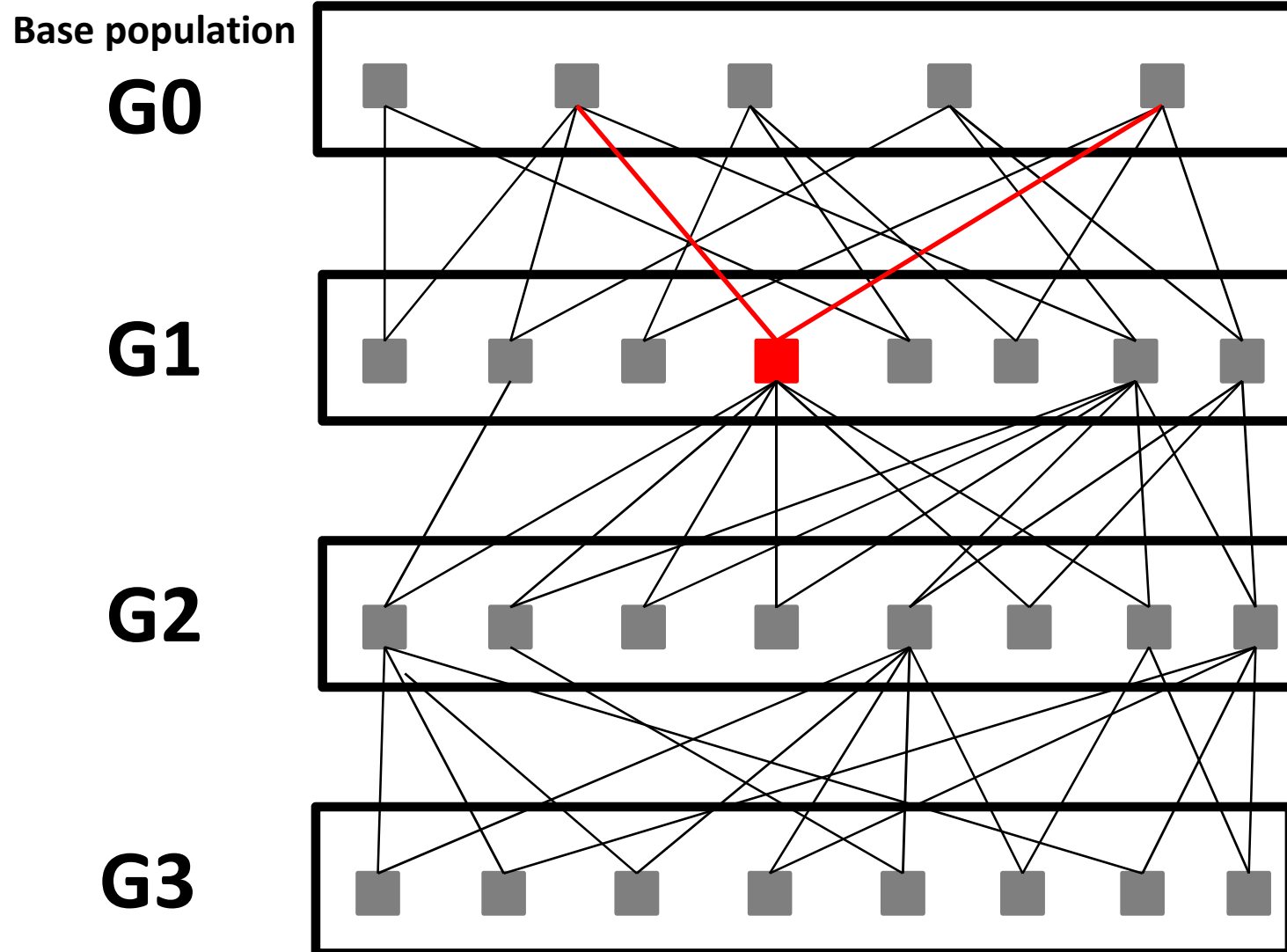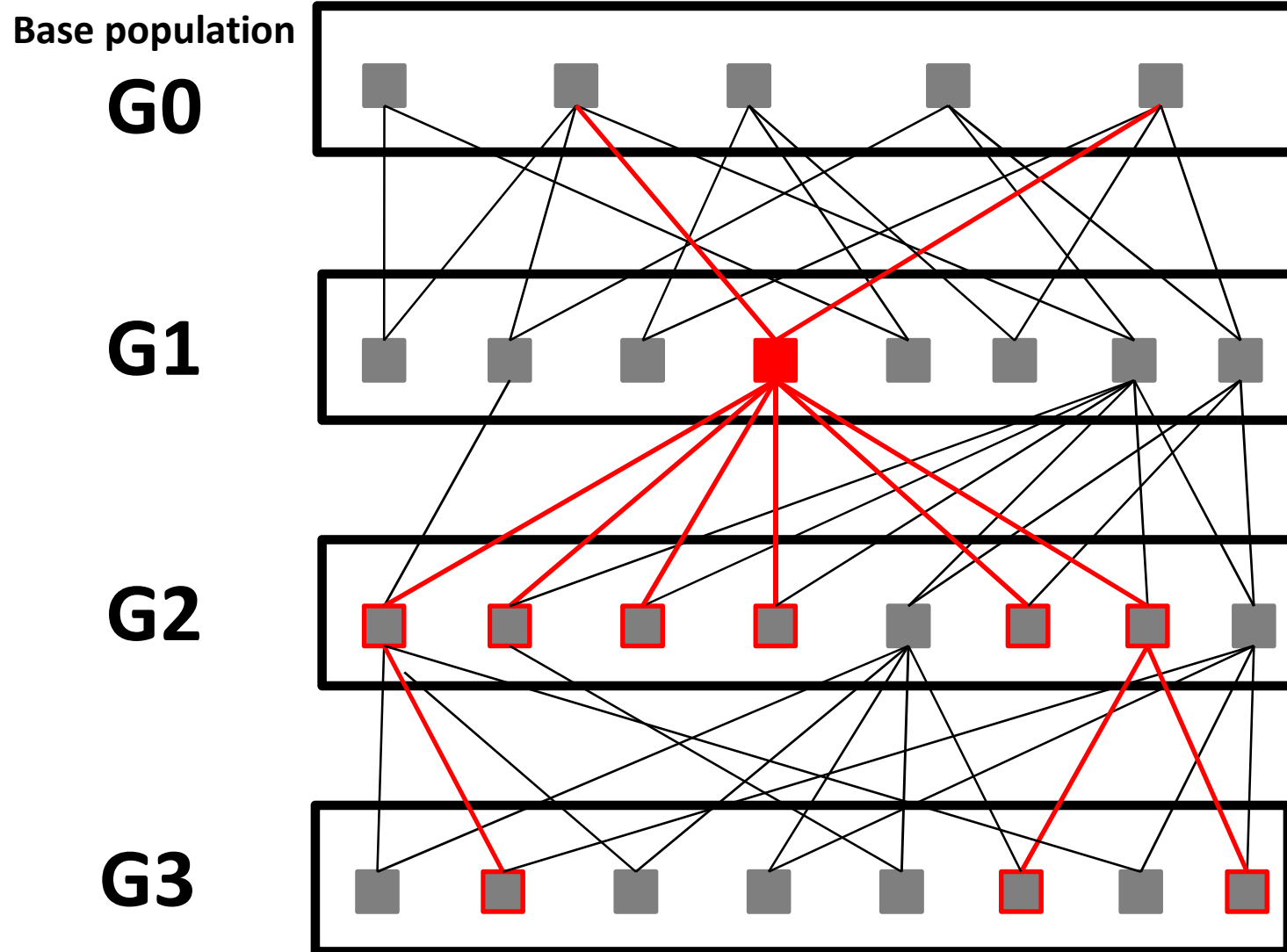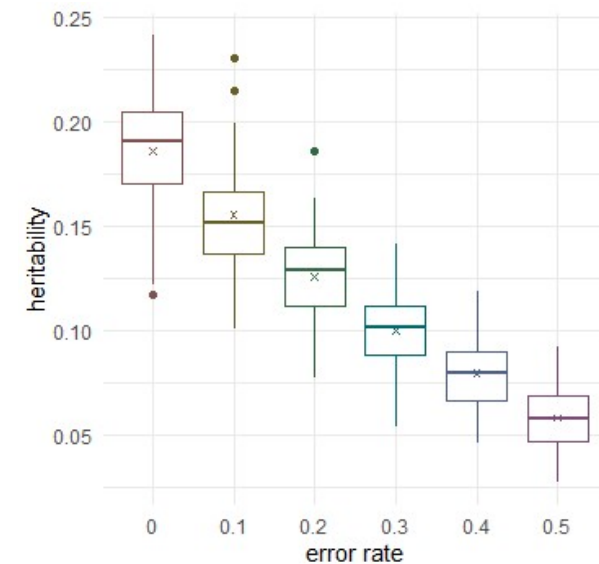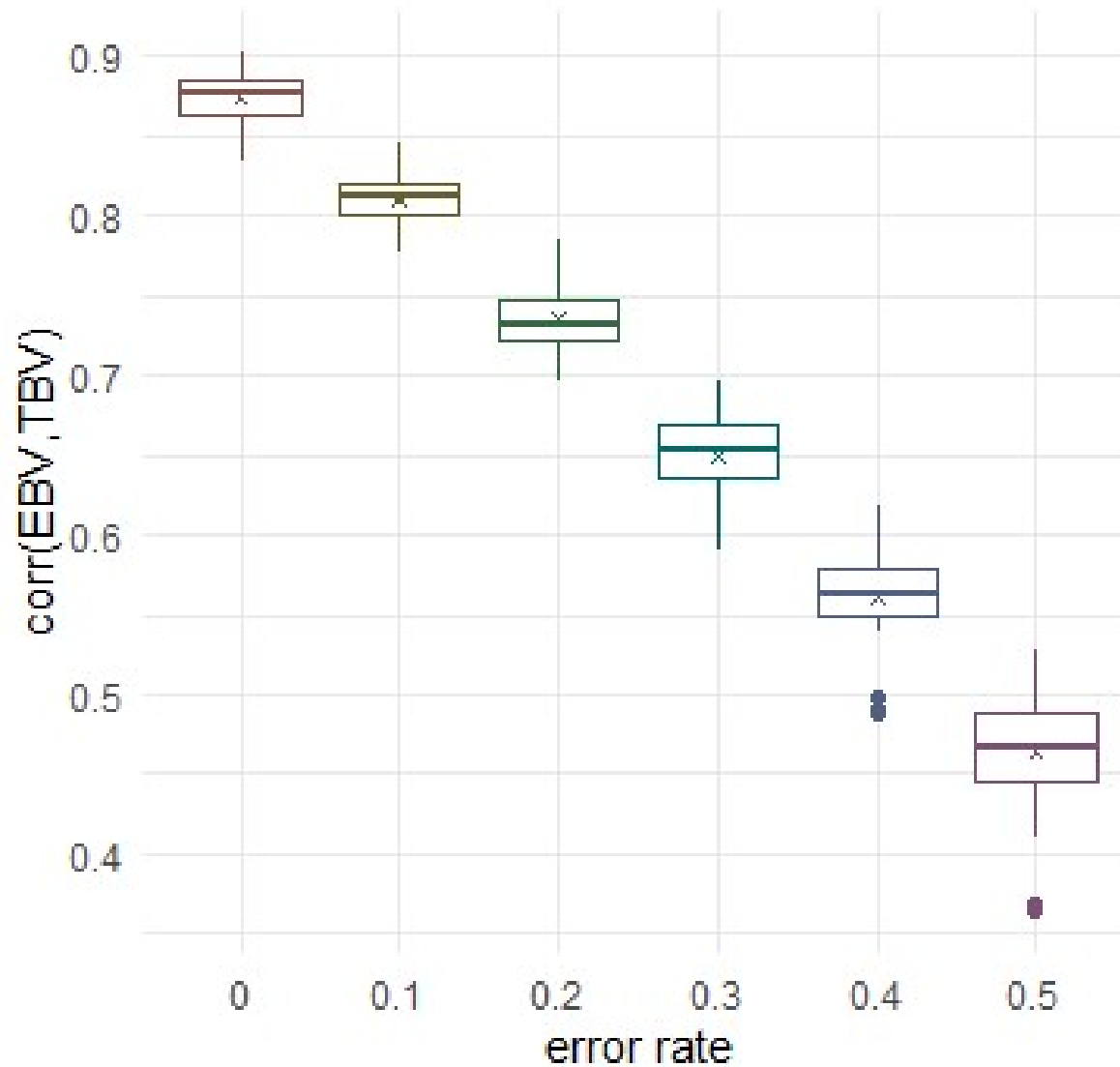
> **Higher genetic gains when the pedigree is cleansed**

« Id card » in clonal archives

➢ **3-year-project (2018-2020) to genotype 8,000 trees with 80 SNPs :**
  - « id card » for each genotype in clonal archives
  - correct (when possible) for pedigree mistakes in the database and in the field
  - field labelling with bar codes

➢ **Easier to implement during the first generations**

➢ **First mandatory step to go further with markers implementation in the breeding programme**

4/ Alternative mating design based on pedigree recovery
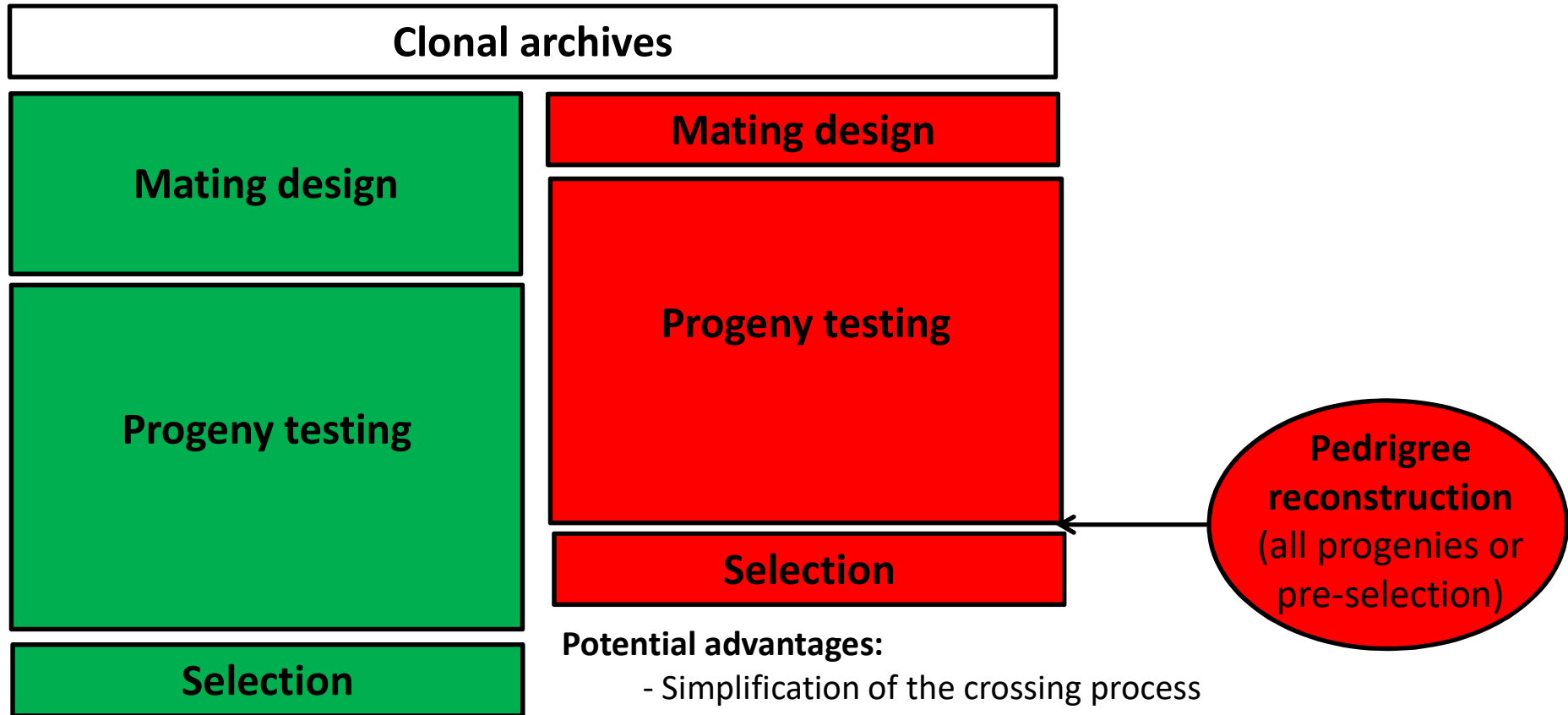
Mating design based on pedigree recovery

Bi-parental mating   **Polymix or open-pollination**

Clonal archives

Mating design

Progeny testing

Selection

Mating design

Progeny testing

Selection

Pedigree reconstruction
(all progenies or pre-selection)

Potential advantages:
- Simplification of the crossing process
- Generation of a large number of families
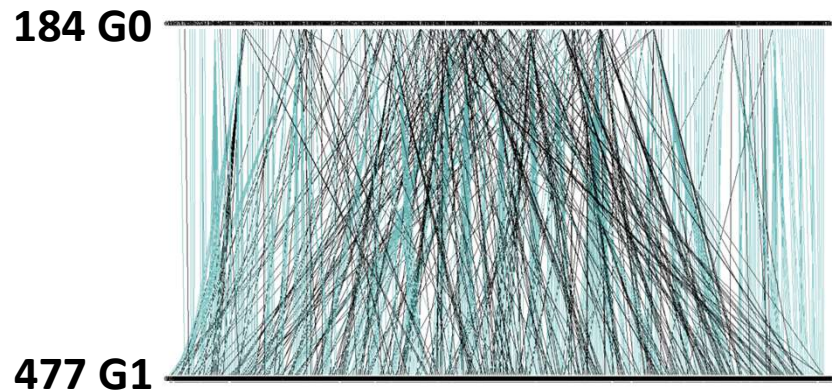- Verification of id for selected genotypes

➢ **Comparisons through simulations: no advantages to generate a lot of families**
➢ **Simulations in progress to carry out comparison including the costs of crosses and genotyping**

5/ GS proof-of-concept in maritime pine
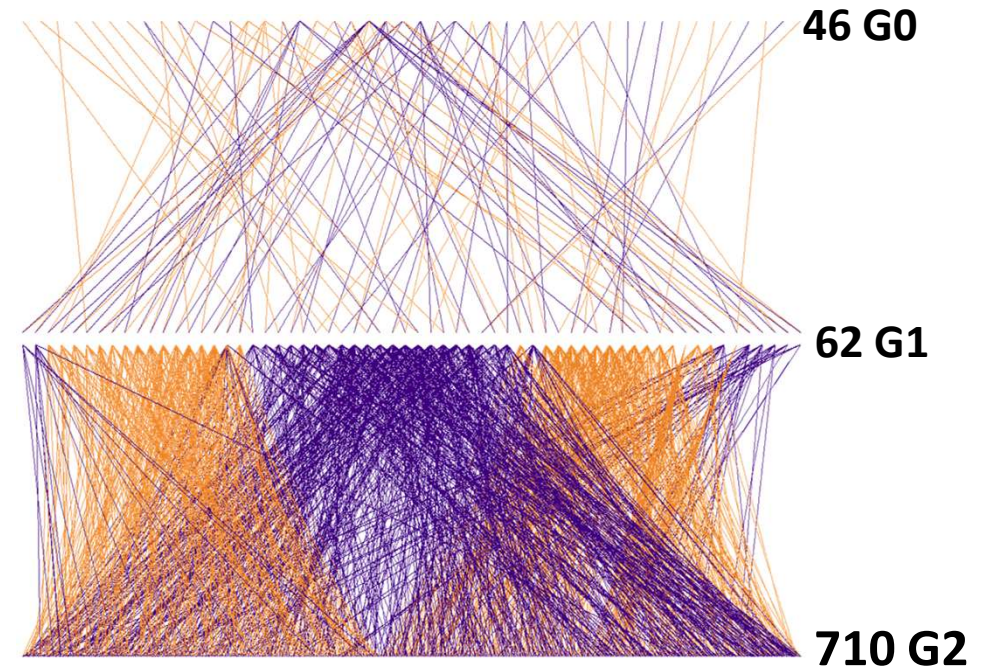
2 GS populations (sampled from the breeding pop)

Population 1

184 G0

477 G1

Population 2

46 G0

62 G1

710 G2

- 661 individuals (Ne≈100)
- G0 + G1
- G1: 191 HS families (≈ 2.5 individuals)
- 2,500 SNPs
- Pseudo-phenotypes (BLUP)
- Growth, sweep

- 818 individuals (Ne=24)
- G2 + all their ancestors (G0 + G1)
- G2: 32 HS families (≈ 22 individuals)
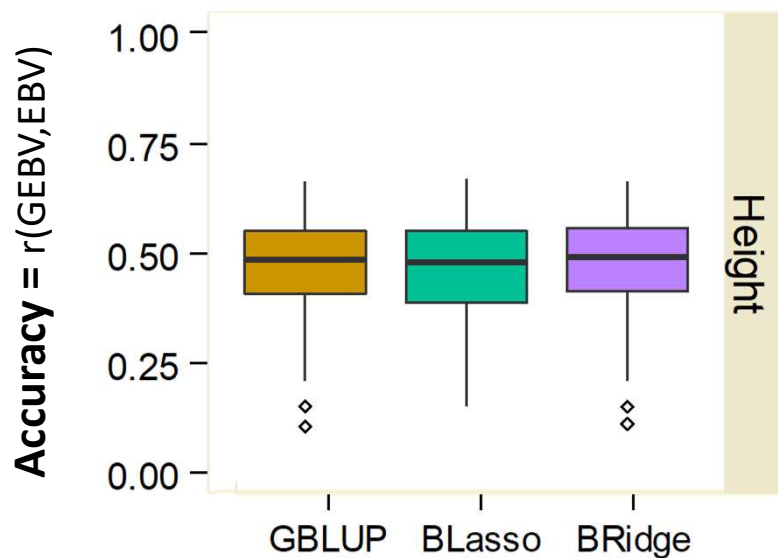- 4,300 SNPs
- Pseudo-phenotypes (BLUP)
- Growth, sweep

Isik *et al.* 2015

Bartholome *et al.*, 2016

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Prediction accuracy

Population 1

Training: G0 + 90% G1
Validation: 10% G1

Population 2

Training: 80% G2
          G0 + G1 + 80% G2

Validation: 20% G2
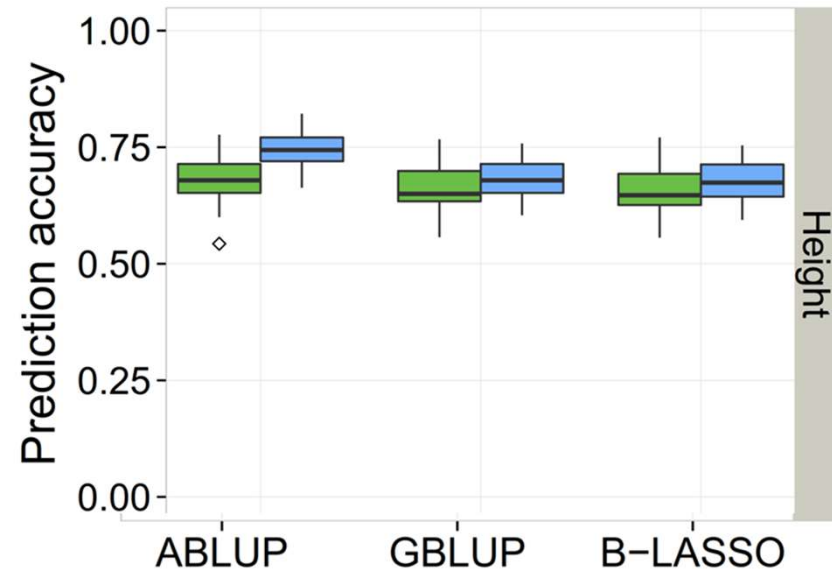
- ➤ Accuracy from 0.5 to 0.75 despite low linkage disequilibrium and low marker coverage of the genome (2.5 SNPs/cM in Pop2)
- ➤ Higher accuracy in Pop2 (lower effective size, all ancestors genotyped)
- ➤ Similar results whatever the method (GBLUP vs Bayesian methods)
- ➤ Similar accuracy for the pedigree-based method (A-BLUP) and marker-based methods

Mendelian sampling prediction: A-BLUP vs G-BLUP
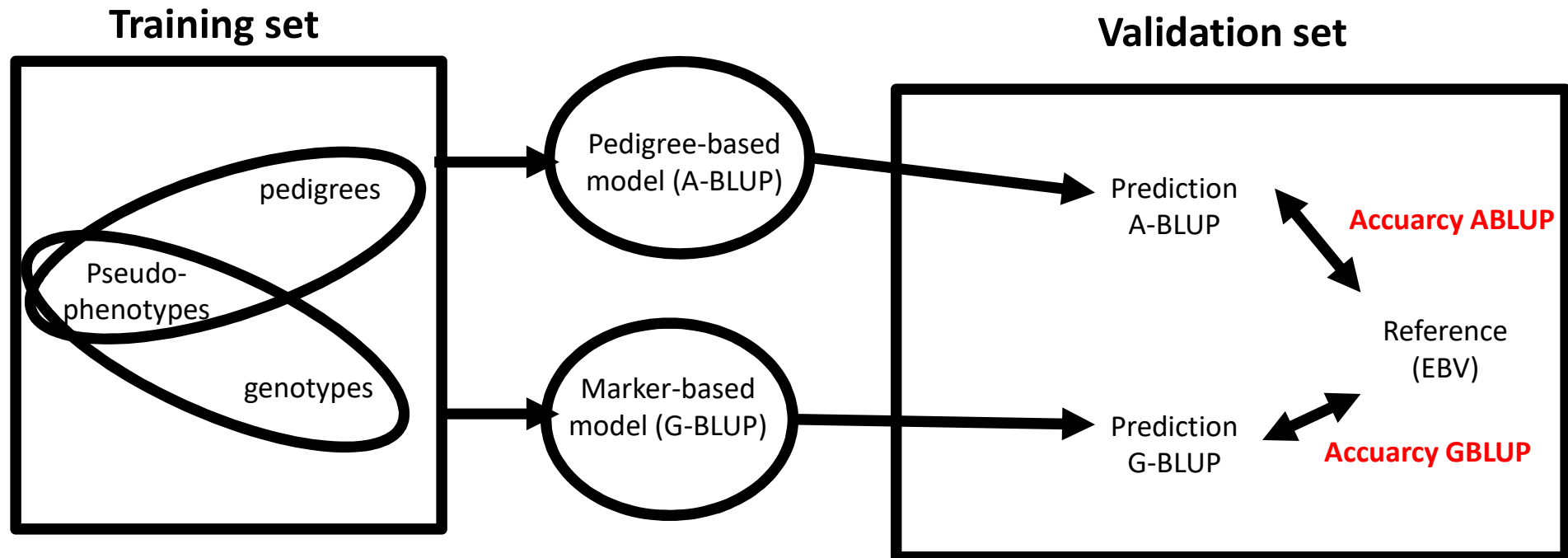
Training set

Validation set

pedigrees

Pseudo-phenotypes

genotypes

Pedigree-based model (A-BLUP)

Marker-based model (G-BLUP)
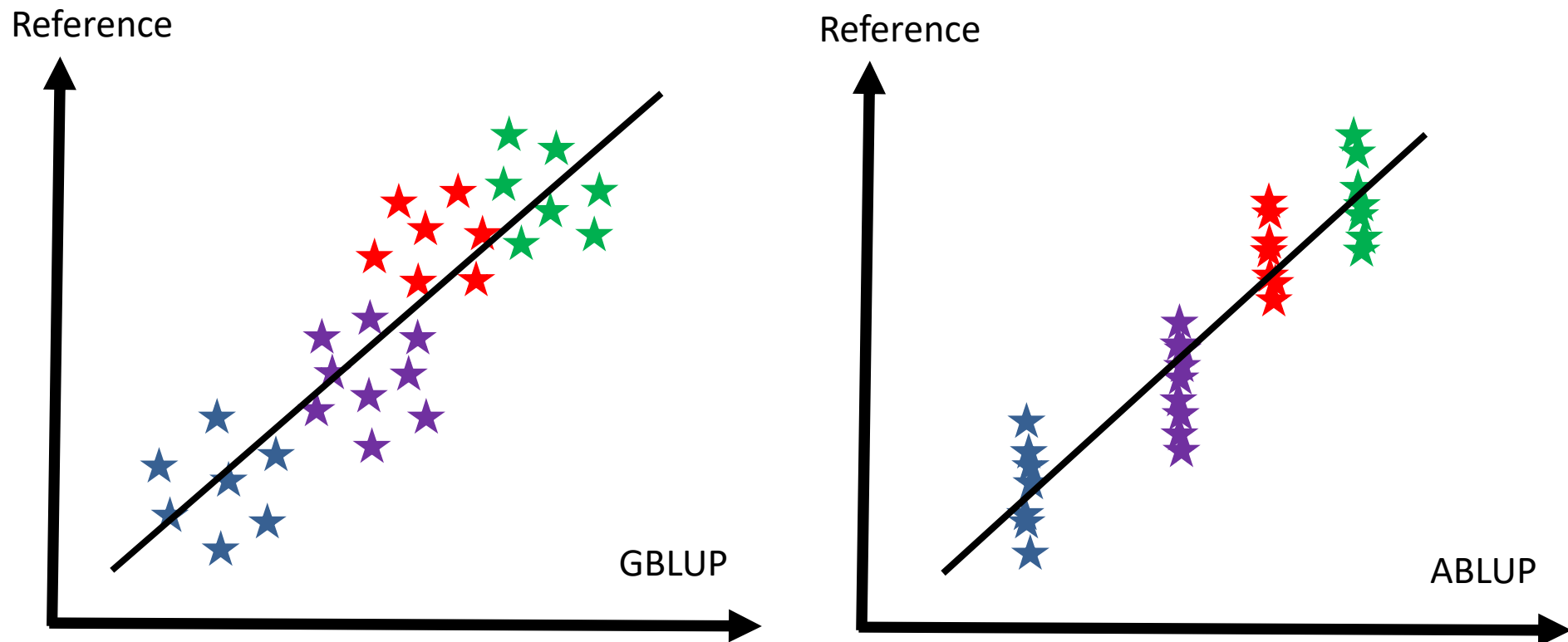
Prediction A-BLUP

Accuarcy ABLUP

Reference (EBV)

Prediction G-BLUP

Accuarcy GBLUP

- A-BLUP evaluation: all trees from one family have the same BV if no individual phenotype / progeny (= mid-parent value)
- GS models: can predict a different BV for each tree of a family even with no phenotype (variation around mid-parent value = Mendelian sampling)

Mendelian sampling prediction: A-BLUP vs G-BLUP

GS predictive accuracy is not directly linked with the ability to predict the Mendelian sampling (GS accuracy comes from relatedness and LD markers-QTLs)

Depending on the population studied, GS models can have high accuracy even without any ability to predict Mendelian sampling

Mendelian sampling prediction: A-BLUP vs G-BLUP

➢ **In the forest trees litterature:**

- Ability to predict Mendelian sampling is generally poorly discussed

- GS accuracy mainly based on relatedness (and not LD markers-QTLs)

- G-BLUP > A-BLUP when G-matrix allows correcting (pedigree errors) or completing kinship (full pedigree unknown, structuration in the base population not taken into account with A-BLUP)

➢ **For maritime pine, the similar accuracy from A-BLUP and G-BLUP can be explained by:**
  - a limited number of markers in comparison to the large size of conifer genome (more than 20 Gb)
  - the reliability of the pseudo-phenotypes considered as the reference to estimate the genomic selection accuracy
  - **the design of the training population, generally with a low number of trees per family**

6/ Perspectives for GS in maritime pine
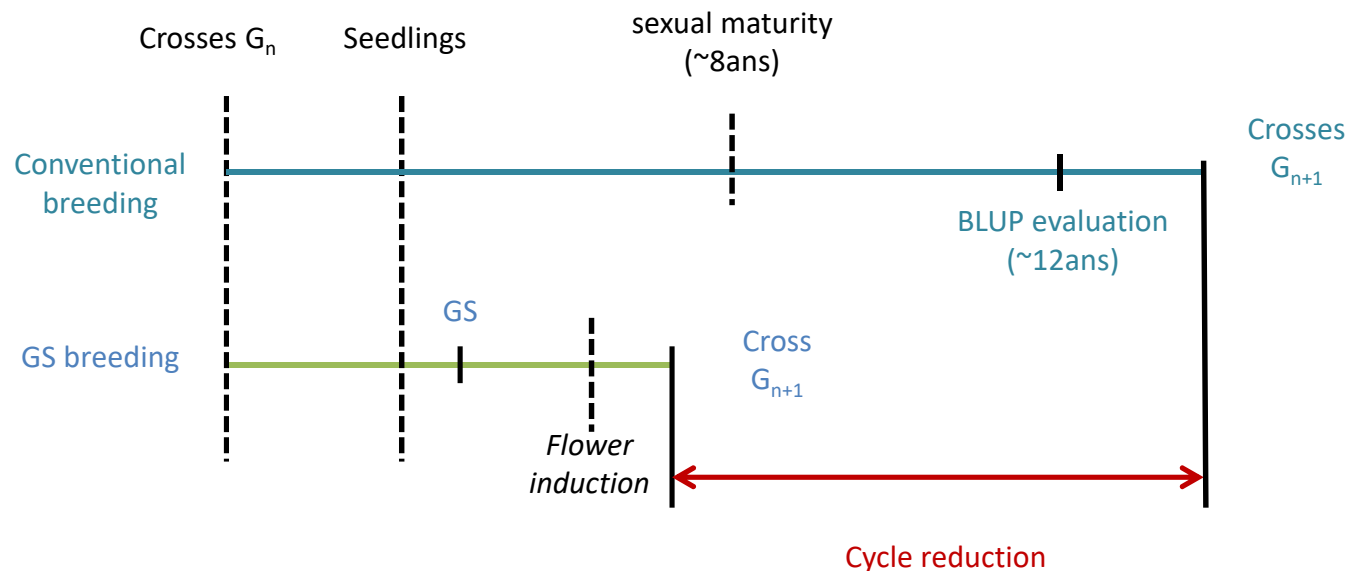
GS perspectives

A/ From pedigree based matrix (A-matrix) to markers based matrix (G-matrix)

– Keep the same BLUP methodology but substitute G-matrix (or H-matrix) for A-matrix

– Increase of accuracy as:
  • realized relationship is substituted to expected relationship (or selection earlier for the same accuracy)
  • Particularly efficient for hidden relationship (or incomplete pedigree, or structure base population)

– This arises 2 issues:
  • Merge genotyping data with various nber SNP (➜ implementation)
  • Strategies to genotype the « key » trees

GS perspectives

## B/ Selection without phenotyping (decrease breeding cycle length)

- The more attractive perspective but depends on the sexual maturity age (or associated with flowering induction)

- Can be used for pre-screening (ie selection intensity increase)

C/ Selection for costly traits

– For instance: pest resistance, drought resistance…

– Phenotyping focused in the training population

– Multitrait evaluation

Multi-trait model + phenotypes of correlated traits

Multi-trait model

Single-trait model
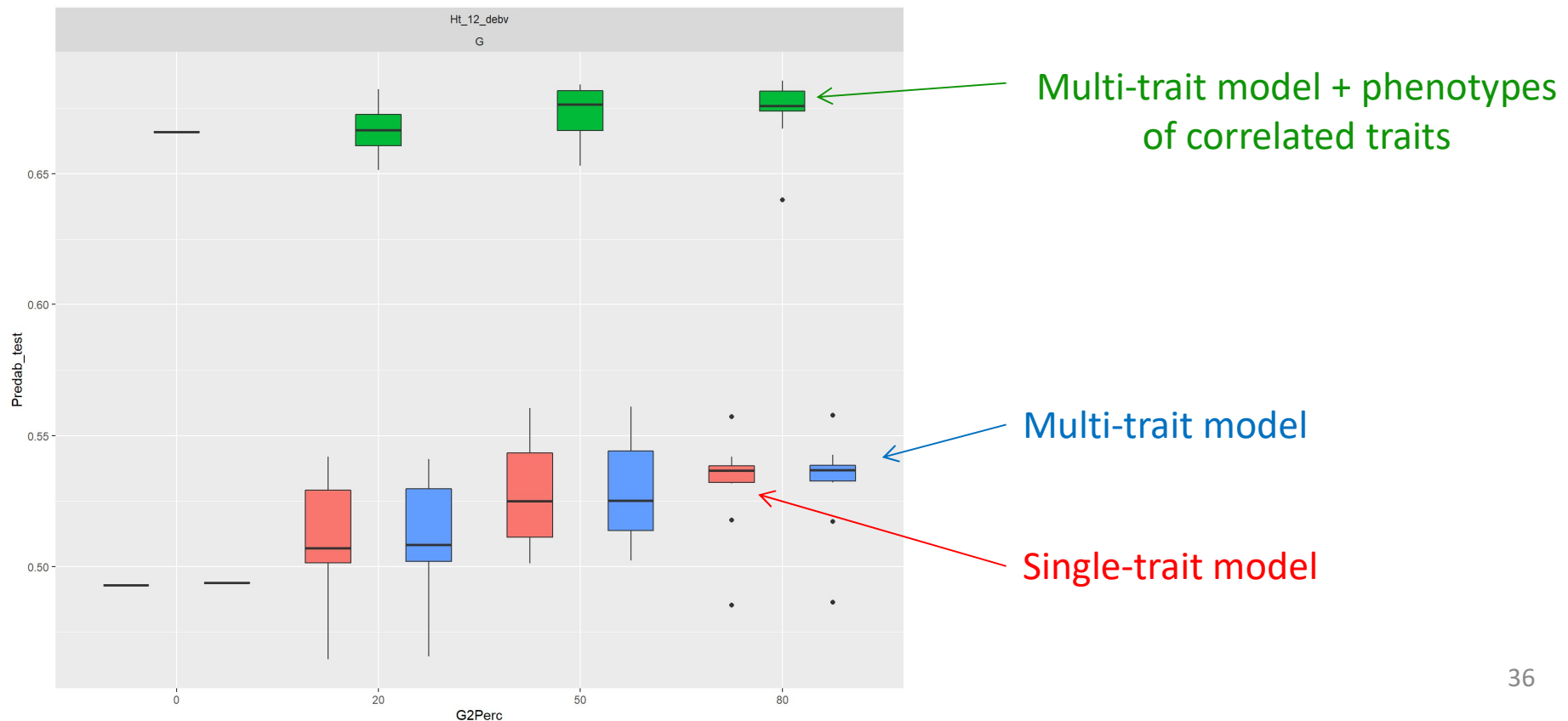
Conclusion

➢ **Even without GS, use of markers increases genetic gains and open opportunities for new breeding strategies**

➢ **Main GS objectives in maritime pine:**
   - shortening of breeding cycle duration
   - selection for costly traits (disease resistance, climate change)

➢ **Main research area in GS:**
   ability to predict the variability within families i.e. Mendelian sampling (design of the training population, phenotypes reliability, number of markers)

➢ **Optimal breeding strategy vs. the one we can effectively apply!**
   • GS implies: new field work, new databases, new skills…
   • implementation of markers as a first step (id checking, pedigree recovery)

Thanks for your attention!

Acknowledgment