

# Application of Molecular Markers to an Important Crop Species : Maize

Pierre RUAUD, Limagrain Verneuil Holding



# Limagrain = 4<sup>th</sup> largest world seed company

## Field seeds



■ Limagrain is the **European leader for corn and wheat seeds**, with the brands **Anjou, LG** and **Nickerson**. Through its company **AgReliant**, the Group is also an important player on the North American market.

## Bakery products and cereal ingredients



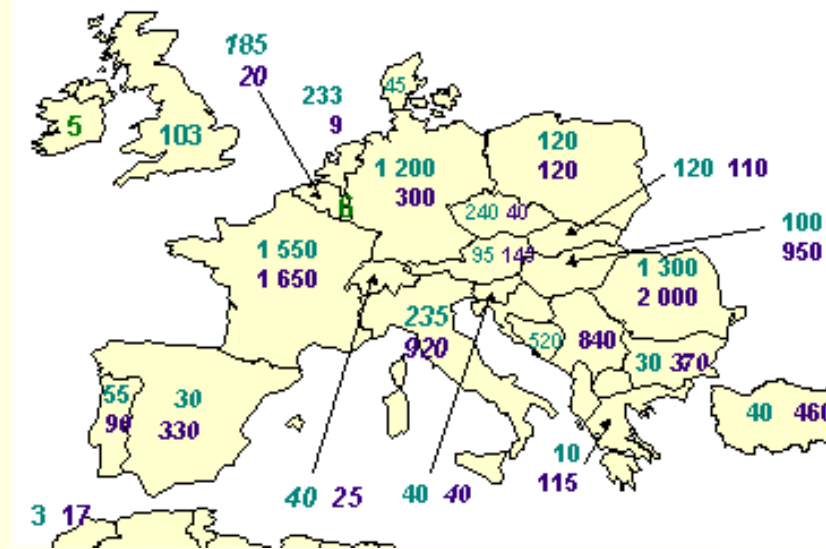
■ With **Jacquet**, Limagrain is the **second largest industrial baker in France**. With the foundation of **Limagrain Céréales Ingrédients**, the Group has now taken up strong positions on the market for cereal ingredients with the brands **FCI, Dafa, MCT** and **Sofalia**.

## Vegetable seeds and garden products



■ With **Vilmorin Clause & Cie**, the Group is the **world leader for Home Garden seeds** and is the **second largest company in the world for seeds intended for Professional growers**.

# Uses of Maize



55 % Grain Maize

45 % Silage Maize

42 % Feed

5 % Milling

8 % Starch



# Specificity of Maize for a Molecular Biologist

- Breeding material is highly structured into « combination groups » preserving combining ability
  - High Linkage Disequilibrium inside groups, low LD between
- Maize genome is diploid (but duplicated) and very polymorph
  - Easy development for all kind of marker (RFLP, AFLP, SSR, SNP...)
- Major traits are complex and polygenic
  - Yield, grain moisture, lodging
  - What is heterosis ?
- Thanks to its huge market, Maize benefits from a great research effort

# Molecular Markers in Maize Breeding

Molecular Markers are a source of information for Breeders

- Information from a panel of neutral markers  
→ this panel must be designed in order to cover the whole genome
- Information from specific markers linked to a trait of interest  
→ Former development study is required to establish the link
- Applications of Molecular Markers depend on their neutral / informative status.

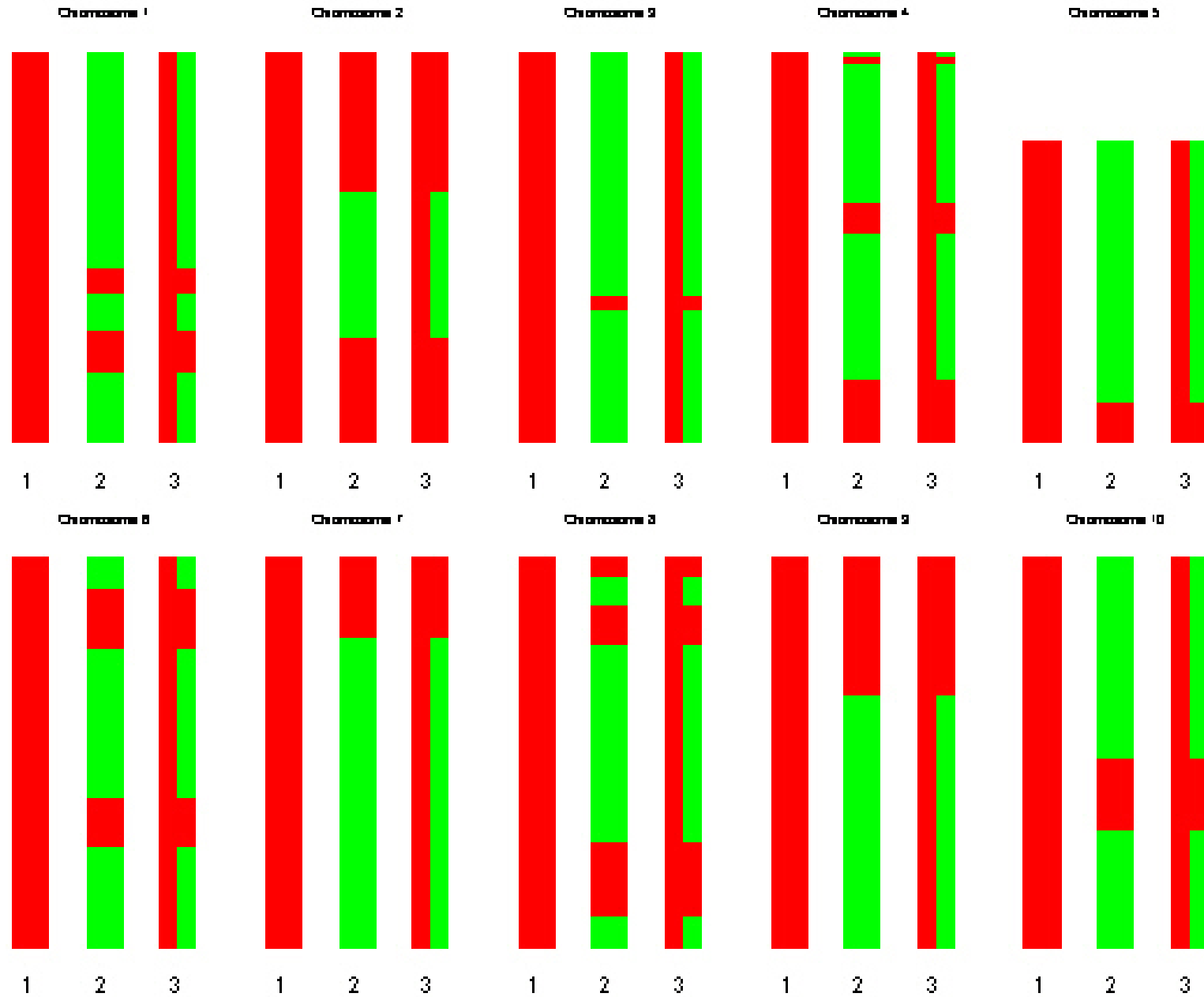
# Neutral Markers in Maize Breeding

## 1 - Fingerprinting

Our first routine application (RFLP → SSR)

Type of Information Breeders obtain from fingerprinting :

- seed purity and homogeneity control
- pedigree verification



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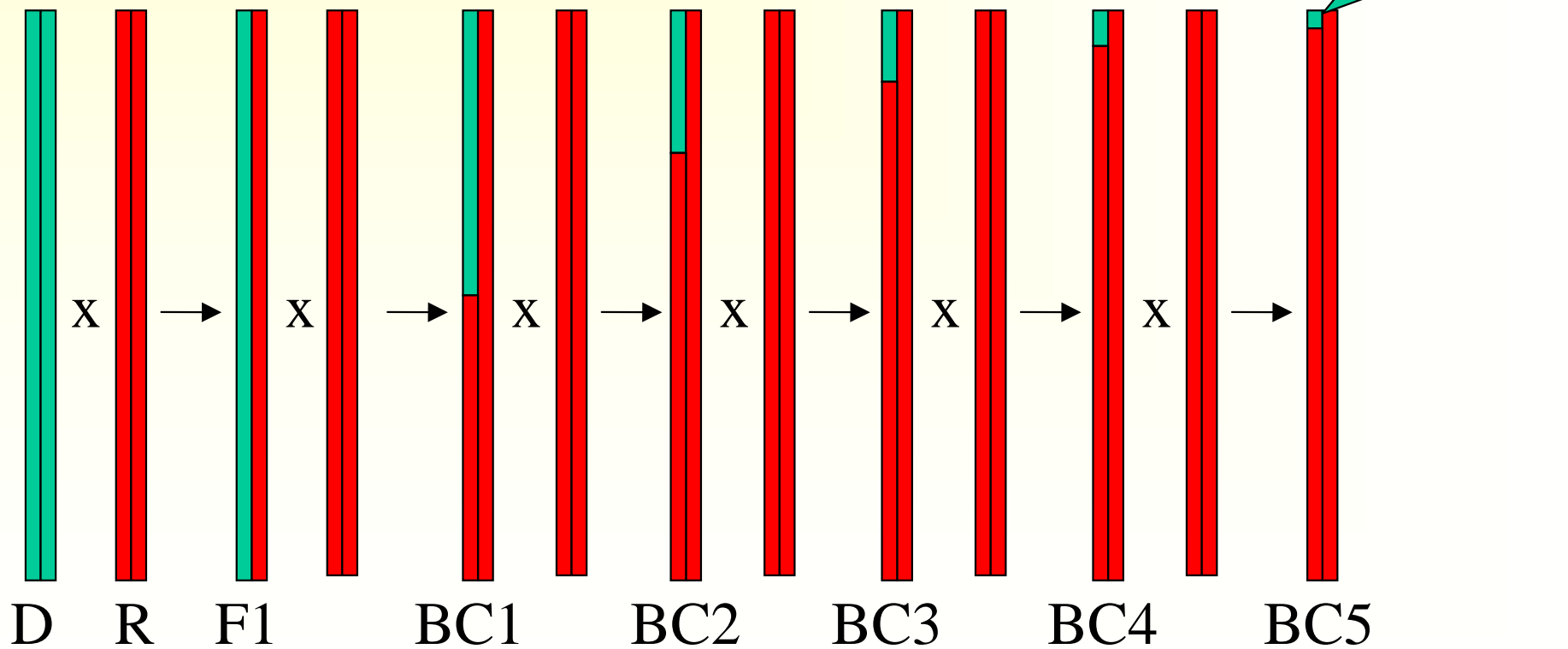
Type of Information Breeders obtain from fingerprinting :

- seed purity and homogeneity control
- pedigree verification
- IP and market survey

# Neutral Markers in Maize Breeding

## 2 - Genome recovery

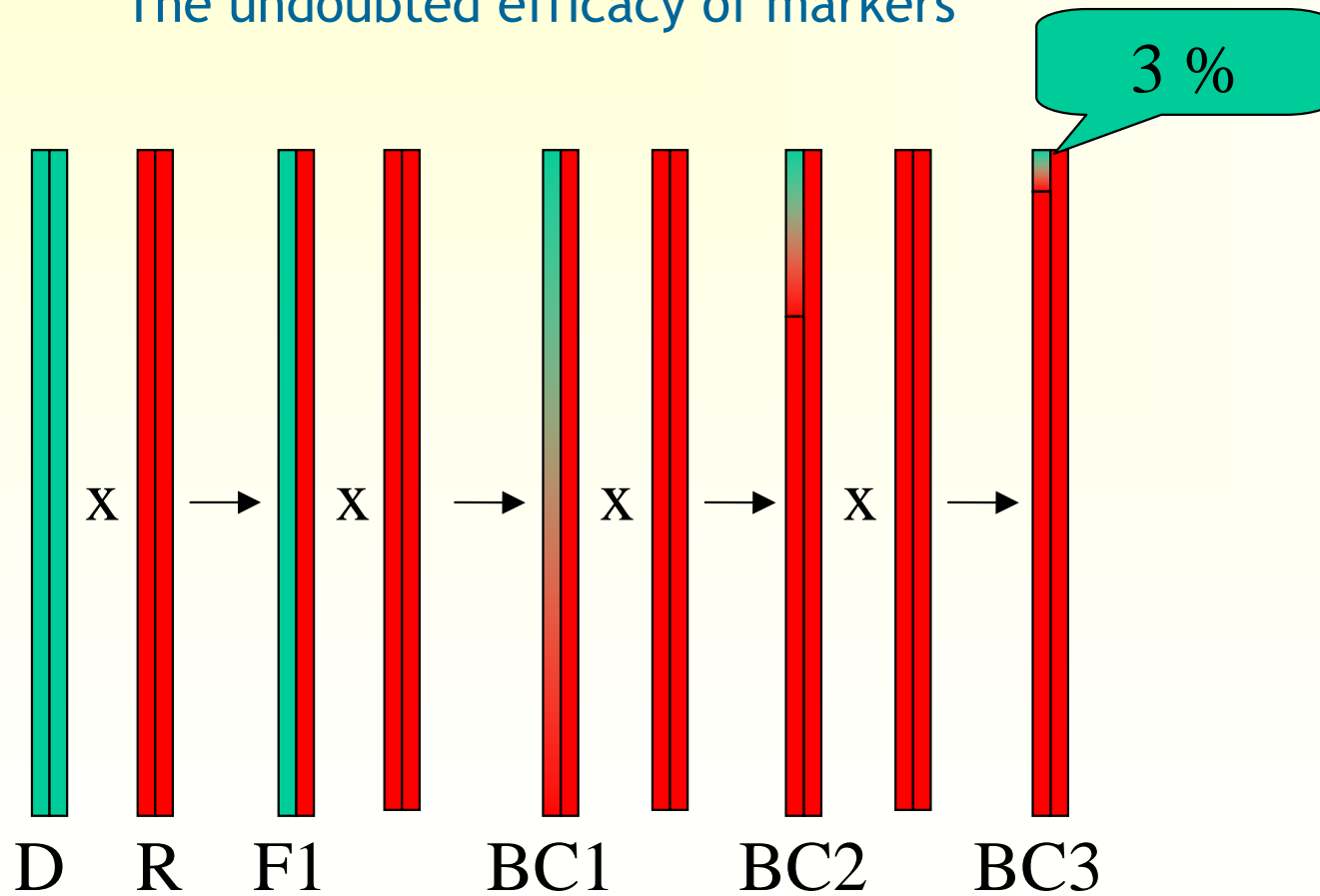
The undoubted efficacy of markers



# Neutral Markers in Maize Breeding

## 2 - Genome recovery

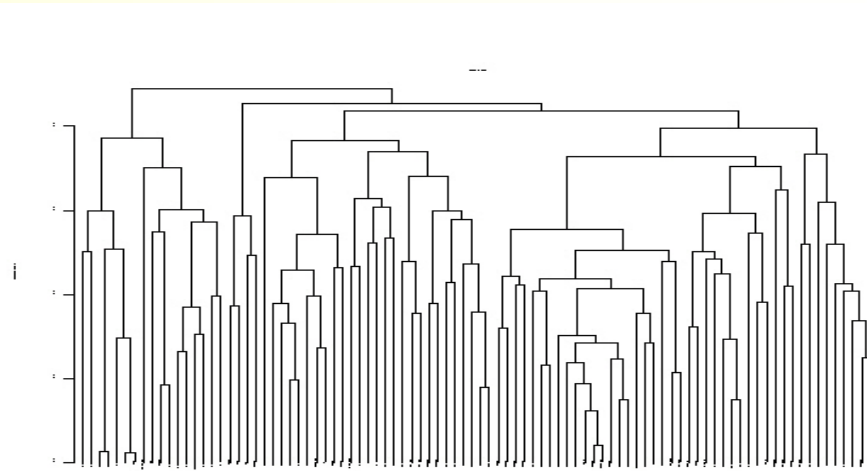
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# Neutral Markers in Maize Breeding

## 3 - Panelling

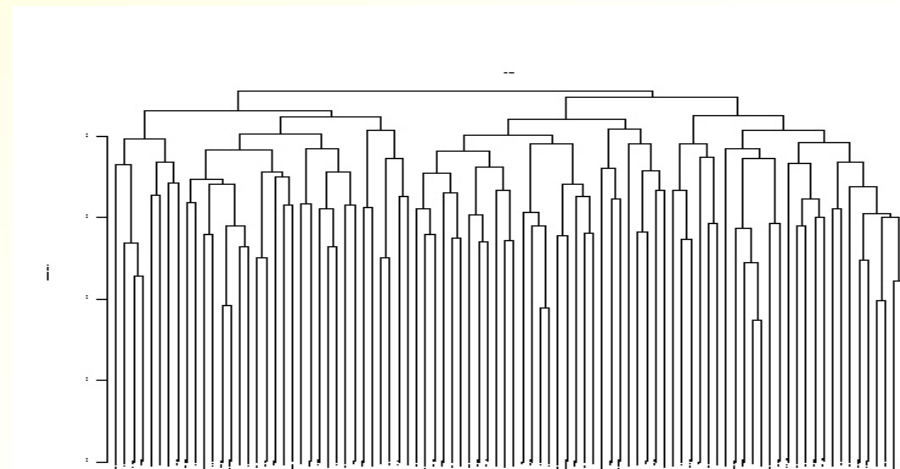
- understand the structure of an existing panel. This is necessary to exploit existing data for association genetics.
- design large collection minimizing Linkage Disequilibrium. This is a way to better explore association genetics on phenotyping platforms.
- create core-collections for genetic resources preservation and allele discovery.



Collection of 96 top elite inbred lines

Panelling with markers allows :

- to eliminate redundancy
- to equilibrate allelic distribution



Core-collection of 96 adapted inbred lines

# Informative Markers in Maize Breeding

1 - Monogenic traits in Maize = a rare situation

→ specialty maize (sweet, waxy or high-amylose, high protein...)

→ grain maize in Europe ...

E.turcicum resistance genes

CMS restoration genes

non-GMO herbicide resistance

GMO's



## Why CMS ?

Hybrid seed production:  
male rows give pollen  
female rows give seed



Female rows have to be completely sterile :  
Castration is manual, mechanical or genetic.



Genetic Sterility is due to cytoplasmic genes  
Nuclear gene (Rf) is able to restore anther development

# Converting fertile restorer female lines to CMS

How to know a maize plant is Rf (restorer) or rf (maintainer) ?

- 1- Cross with a C-rf line.
- 2- Observe F1 progeny during flowering year 2.



All Rf progenies are eliminated

- 1- sample leaf tissue on plantlet



- 2- check for rf specific peak



- 3- information is provided before flowering year 1

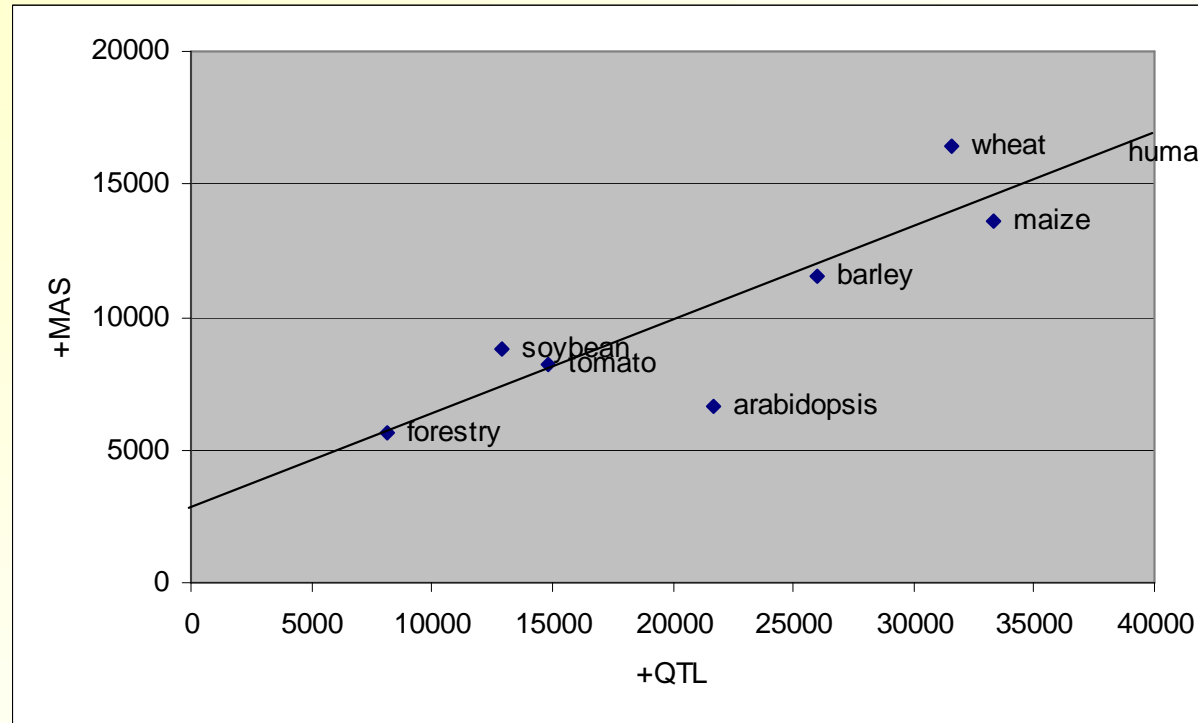
# Informative Markers in Maize Breeding

## 2 - Multigenic traits in Maize = a developing world

- past, present and future of QTLs
- Association genetics and High Throughput Genotyping



# Past, Present and Future of QTLs



Comparative search on Google with keywords :

<species> + QTL vs <species> + Marker Assisted Selection

# Past, Present and Future of QTLs

It is easy to find high effect QTLs :

- . Build a recombinant population from 2 unrelated inbred lines
- . Self to a few hundreds of F6 avoiding selection pressure
- . Then you obtain and map high effect QTLs

What are the limits of such a work ?

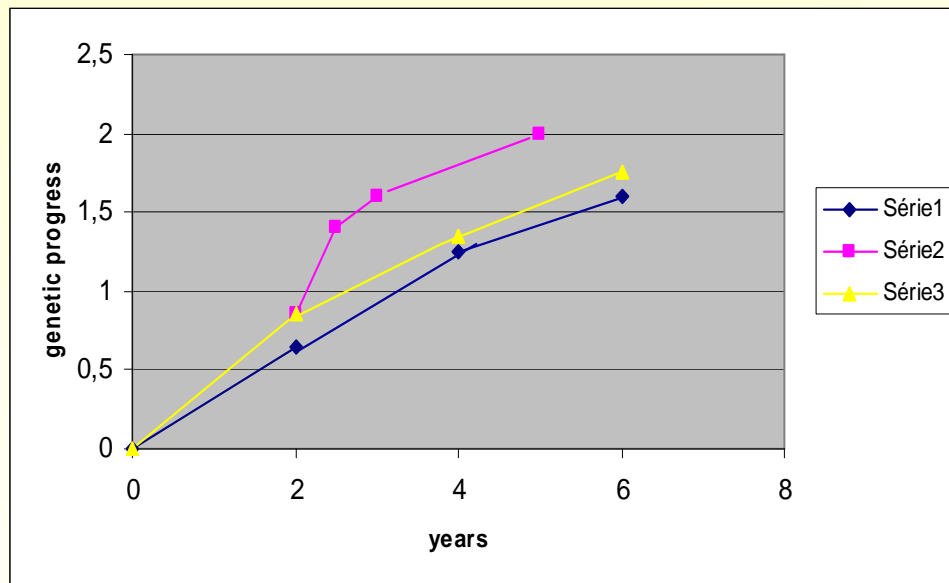
# Past, Present and Future of QTLs

- 1- Maize breeders create small populations from related inbred lines
- 2- They apply selection pressure on early generations
- 3- QTLs are mapped with a bad resolution (expansive fine-mapping is required)
- 4- QTLs are not repeatable (genotype and environment-dependant)

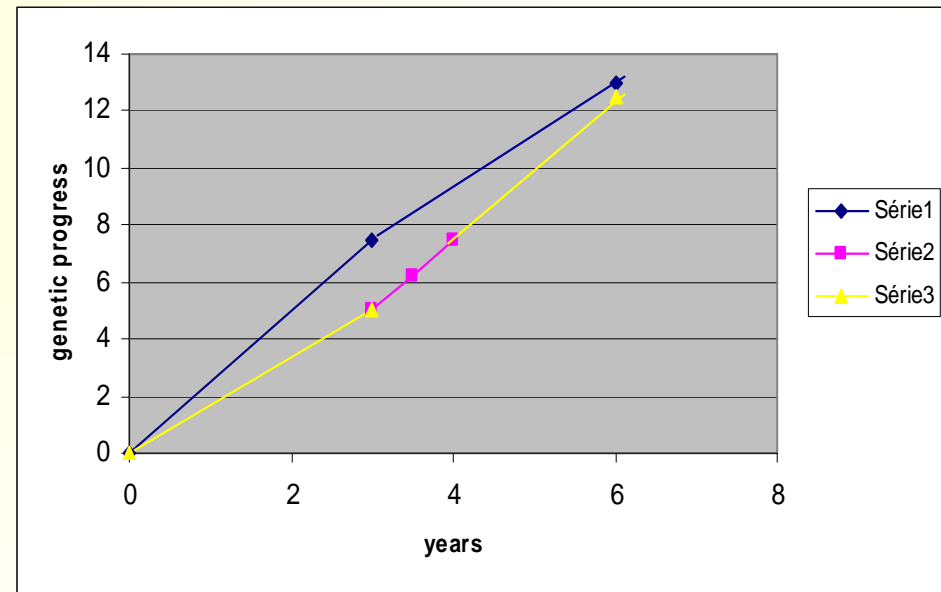
## A 10 years program with ProMaïs / INRA

### Comparison of Phenotypic Recurrent selection

- with **Marker-Assisted Recurrent Selection**
- and **Marker-only breeding cycles**



Theoretical (Hospital *et al*, 1997)



Observed Yield index (Moreau *et al*, 2001)

# Past, Present and Future of QTLs

But there is still a window for QTL-MAS :

- 1- Adapt biometrics from one big population to several small
- 2- capitalize information over all genotypes
- 3- focus on oligogenics traits such as quality or disease resistance

# Association Genetics

A new science for crop breeding (coming from human genetics).

- Replace a biparental population with a panel of hundreds of inbred lines.



	QTL	Association Test
Allele #	2	N
Linkage disequilibrium	High	Low
Material	Recombinant population	Panel of varieties

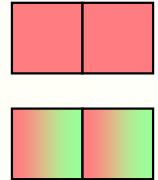
Allele	Value
A	Bad
B	Bad
C	Average
D	Average
E	Good

Resulting from Association study



Correspond to > 10 QTL studies :

2 with good resolution 

>2 with no effect 

6 with low resolution 

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- Saturate the genome with functional markers instead of a 20 cM interval coverage.

MaizeGDB: SSR Data in MaizeGDB - Microsoft Internet Explorer

Adresse <http://www.maizegdb.org/ssr.php>

**MaizeGDB**  
Maize Genetics and Genomics Database

Useful pages | docs | reports | browse data | search | tools | annotation | links

home | Search all records for Go!

### SSR Data In MaizeGDB

SSR Research Tools | SSR Browser | Discussion of SSR Data for the General Public

#### SSR Research Tools

**Simple SSR Search:** This search form allows you to just enter basic name or repeat info to quickly retrieve the desired SSR. Use % as a wild card. If you are having difficulties, click on the green question-mark box. A sample search is provided to help you.

submit (see a sample SSR query)

This search can be executed on every page on the site by utilizing the form in the upper right corner. Choose "SSR" from the drop-down menu, enter your search term in the field, and hit return to find the SSR you're looking for.

**Mapped SSRs:** This utility displays the map locations of the SSRs in MaizeGDB. You can obtain this information by chromosome (1-10, below).  
[1](#) | [2](#) | [3](#) | [4](#) | [5](#) | [6](#) | [7](#) | [8](#) | [9](#) | [10](#)

**Mapped & Anchored SSRs:** This utility lists SSRs that are both genetically mapped and also detect a BAC. You can obtain this information by chromosome (1-10, below).  
[1](#) | [2](#) | [3](#) | [4](#) | [5](#) | [6](#) | [7](#) | [8](#) | [9](#) | [10](#)

**Complete List of SSR Repeats:** Provides a complete list of SSR repeats available in MaizeGDB, linked to their respective full SSR records.

**SSRs Derived From Genes:** An up-to-date listing of SSRs and the genes from which they are derived, formatted in an easily-downloadable table.

You might also be interested in [visually browsing our gel patterns](#).

#### SSR Browser

This tool allows you to specify detailed search criteria, much like the other advanced searches, but the output is intended to allow you to dig more directly into the data rather than just visiting individual records.

Terminé

Démarrer | Microsoft PowerPoint - [e...] | MaizeGDB: SSR Data in...

Génoplante - Microsoft Internet Explorer

Adresse [http://www.genoplante.com/script/public/home\\_fr.php?nopic=0&js=1&langue=fr&sid=](http://www.genoplante.com/script/public/home_fr.php?nopic=0&js=1&langue=fr&sid=)

Programme fédérateur en génomique végétale

Accès membres Dernière mise à jour: le 08/02/2005

Flash info  
 Communiqué de presse  
 22 Septembre 2004  
 FLAGdb<sup>++</sup>: a database for the functional analysis of the *Arabidopsis* genome  
 Voir le résumé

Recherche  
  
 ok  
 Glossaire  
 Contacts

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Mentions légales  
 Liens utiles  
 Plan du site  
 English version  
 Plan d'accès  
 Crédits  
 Site sans images

La génomique végétale pour l'amélioration des plantes  
 Entrez dans Génoplante  
 Les résultats de Génoplante  
 Produits bioinformatiques  
 Travailler pour Génoplante

**GENOPLANTE**  
 Programme fédérateur en génomique végétale

Démarrer | Microsoft PowerPoint - [e...] | Génoplante - Microsof... | 12:59

from earth to life



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- Then you have to face new questions ...

# New Questions for a New Science

Choice 1 :

The more you search for correlations, the more you find. 100 statistical tests will deliver 5 false positives at a 5% risk threshold

- Bonferroni correction (from 5% risk to 0,1%) minimize spurious associations but miss small effect genes
- multigenic traits are managed by many small effect genes

Shall we solve the question of complex traits ?

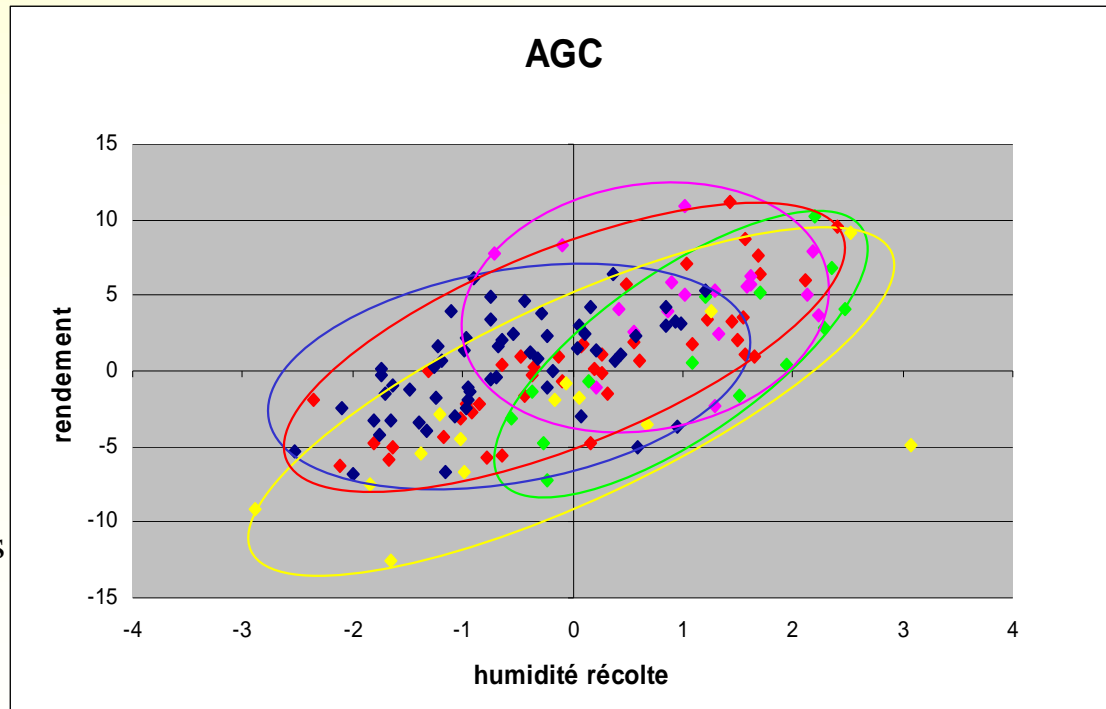
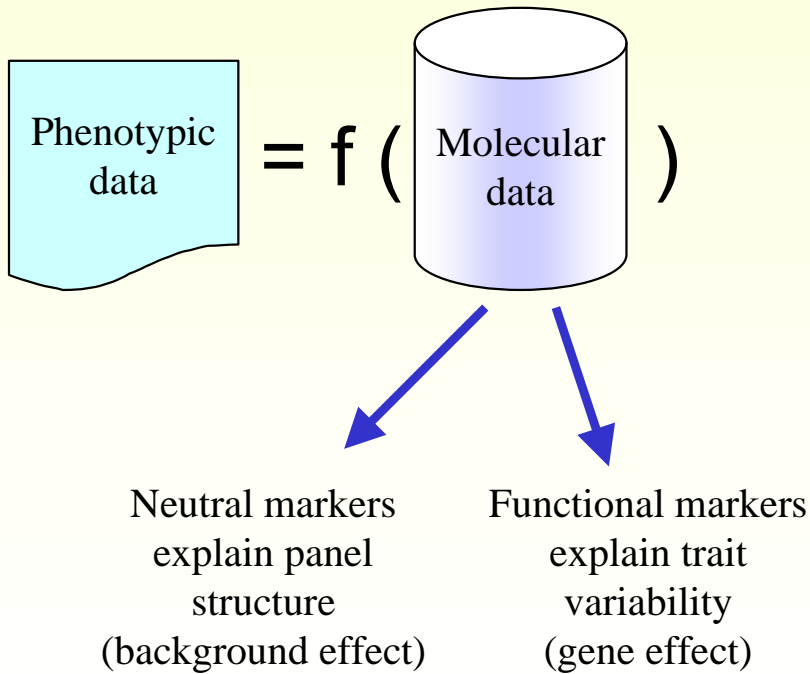
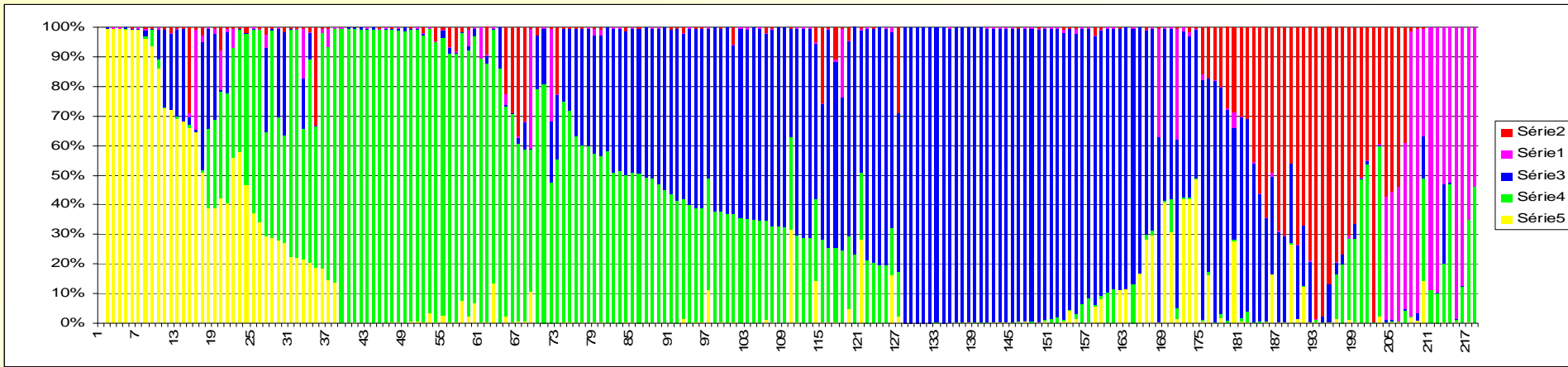
# New Questions for a New Science

Choice 2 :

Epistasy (effect of the whole genome on the targeted gene expression).  
→ unroll the panel structure to evaluate genetic background average interaction  
→ risk of missing favourable alleles which are group-specific.

Do we have to work only on low LD panels (eg. Genetic resources core-collections, excluding breeding material) ?

# How to clarify group x GCA interaction with Structure.



# Conclusions

Neutral Molecular Markers are routinely used for several purposes.

Informative markers are few but useful for a couple of targets.

QTL-Marker Assisted Selection had a very low impact on maize breeding. Thanks to genomics, functional markers are designed. Screened by association genetics, they must help breeders to decipher complex traits.

