Genomic resources and gene/QTL discovery in cereals

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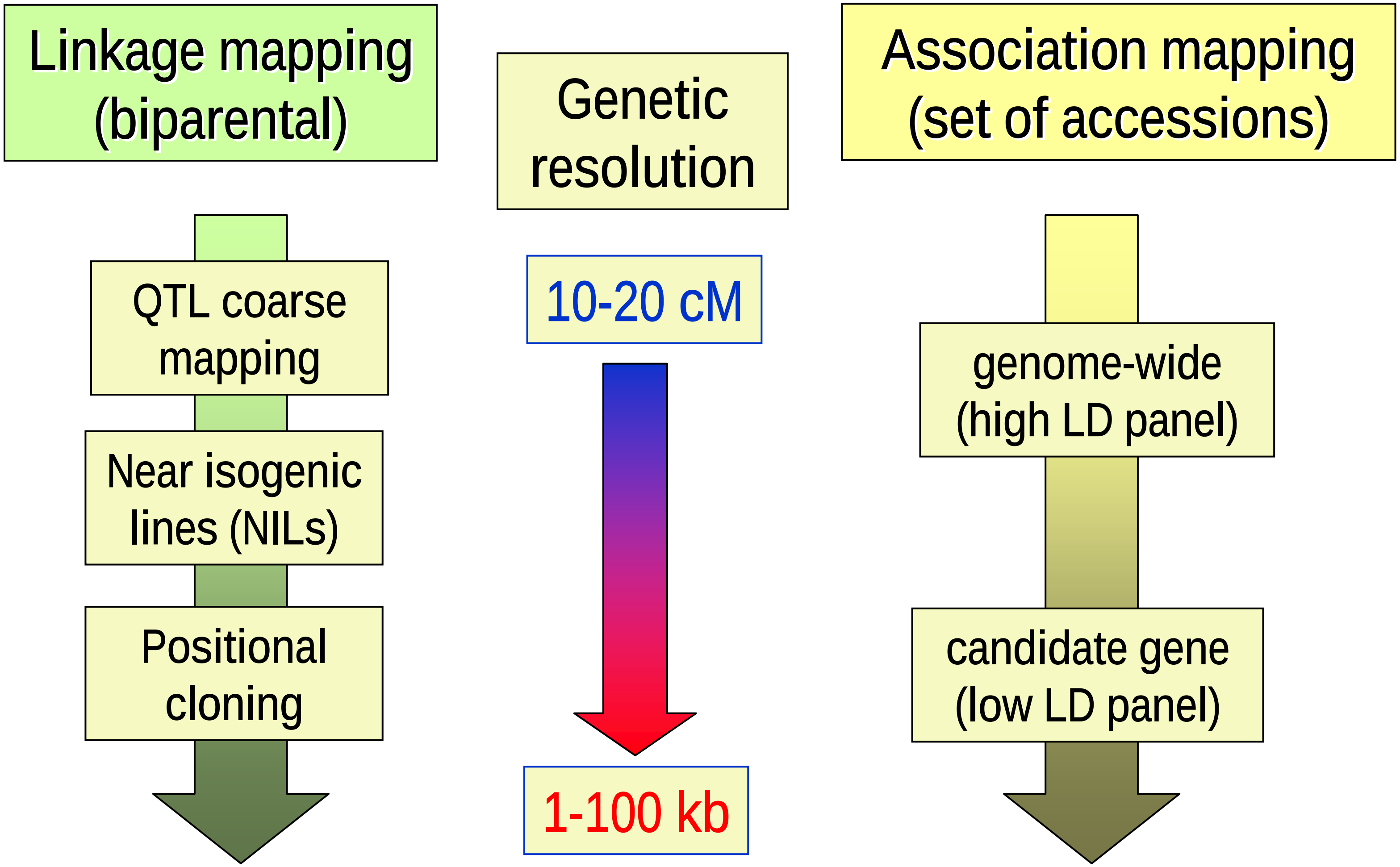
Outline

- Genomics approaches
- Available genetic resources
- Examples of target traits
- Perspectives

QTL mapping and cloning strategies



Linkage mapping (biparental)

Genetic resolution

Association mapping (set of accessions)

QTL coarse mapping

Near isogenic lines (NILs)

Positional cloning

10-20 cM

1-100 kb

genome-wide (high LD panel)

candidate gene (low LD panel)

Candidate gene validation

Genetic resources for gene/QTL discovery in cereals

	Biparental RILs	Introgression libraries	AB-QTL populations
Barley	+ + + +	+	+ +
Maize	+ + + +	+ +	no
Millets	+	no	no
Rice	+ + + +	+ +	+ +
Sorghum	+ +	no	no
Rye	+	no	no
Triticale	+	no	no
Wheat	+ + + +	+	no

Genetic resources for gene/QTL discovery in cereals

	Biparental RILs	Introgression libraries	AB-QTL populations	Mini-core collections
Barley	+ + + +	+	+ +	+ + +
Maize	+ + + +	+ +	no	+ + +
Millets	+	no	no	+ +
Rice	+ + + +	+ +	+ +	+ + +
Sorghum	+ +	no	no	+ +
Rye	+	no	no	+ +
Triticale	+	no	no	+
Wheat	+ + + +	+	no	+ + +

Genetic resources for gene/QTL discovery in cereals

	Biparental RILs	Introgression libraries	AB-QTL populations	Mini-core collections	Mutagenized collections
Barley	+ + + +	+	+ +	+ + +	+ + + +
Maize	+ + + +	+ +	no	+ + +	+ + + +
Millets	+	no	no	+ +	+
Rice	+ + + +	+ +	+ +	+ + +	+ + + +
Sorghum	+ +	no	no	+ +	+ +
Rye	+	no	no	+ +	+
Triticale	+	no	no	+	+
Wheat	+ + + +	+	no	+ + +	+ + + +

TILLING

Targeting Induced Local Lesions IN Genomes

CHEMICAL MUTAGENESIS

\+

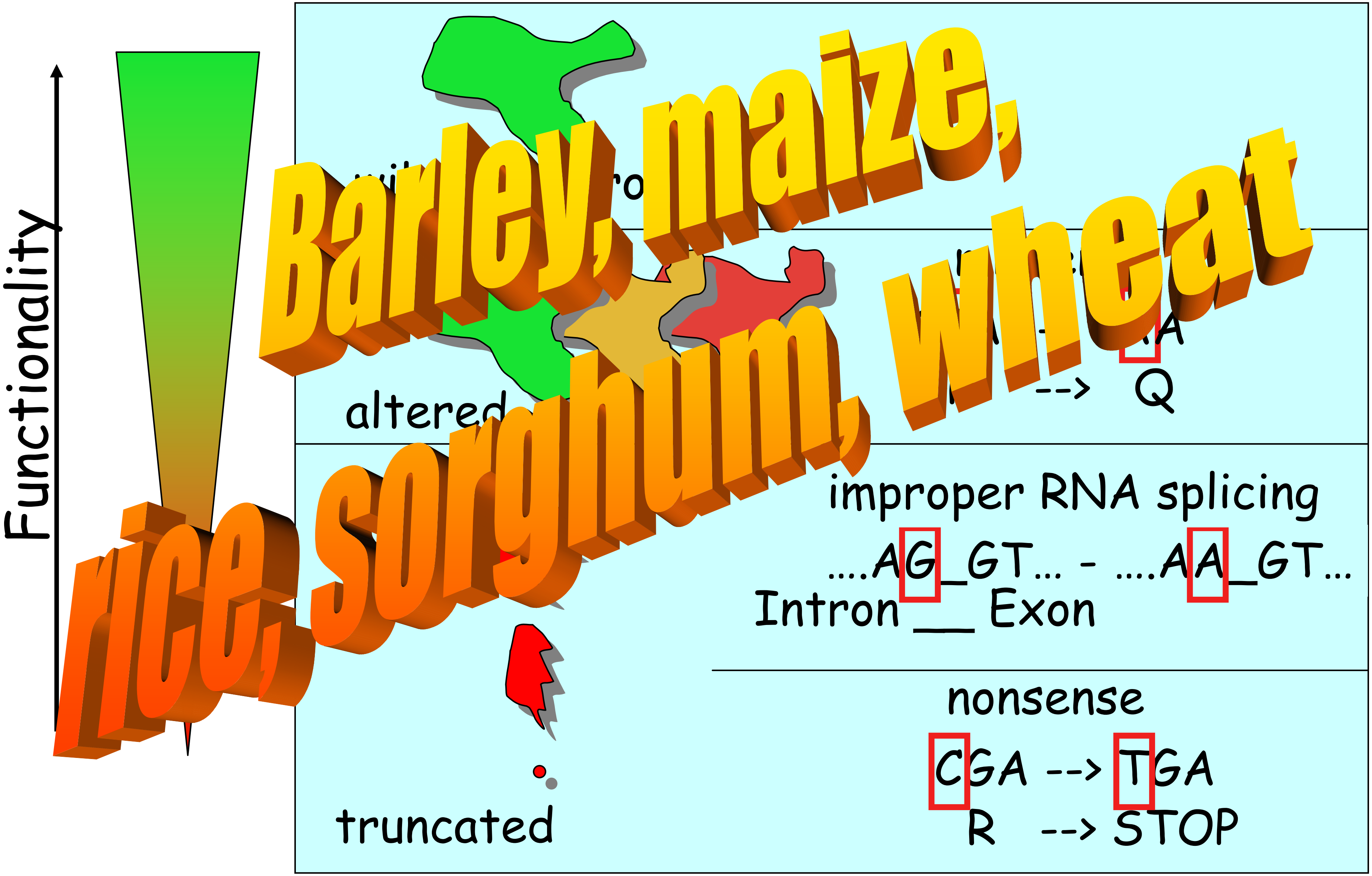
PCR-BASED SCREENING

IDENTIFICATION OF POINT MUTATIONS IN REGIONS OF INTEREST

With TILLING, a library of DNA samples from thousands of individuals is screened for artificially-induced or naturally-occurring (EcoTILLING) single-nucleotide polymorphisms (SNPs).

McCallum CM et al. (2000). Nature Biotechnology 1: 455–457.

TILLING recovers a range of mutation types



Barley, maize,

rice, sorghum, wheat

Functionality

altered

--> Q

truncated

improper RNA splicing

....AG_GT... - ....AA_GT...

Intron __ Exon

nonsense

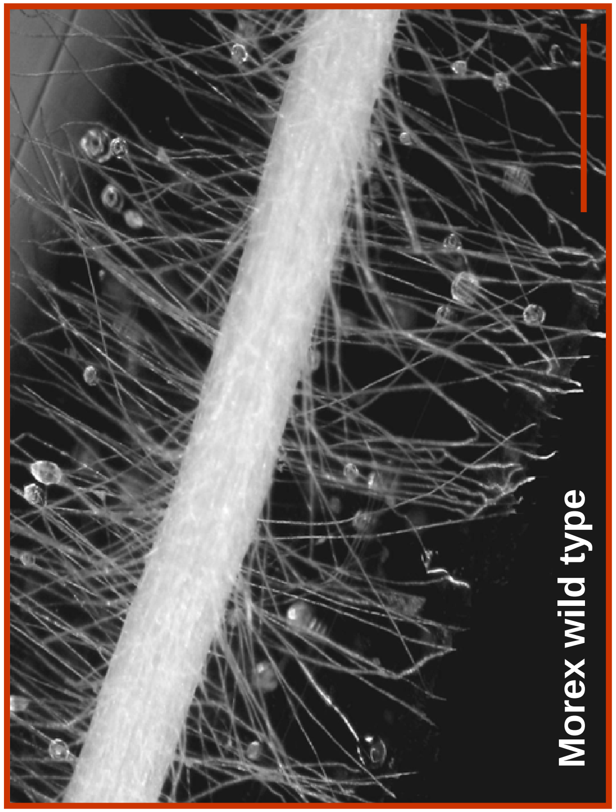
CGA --> TGA

R --> STOP

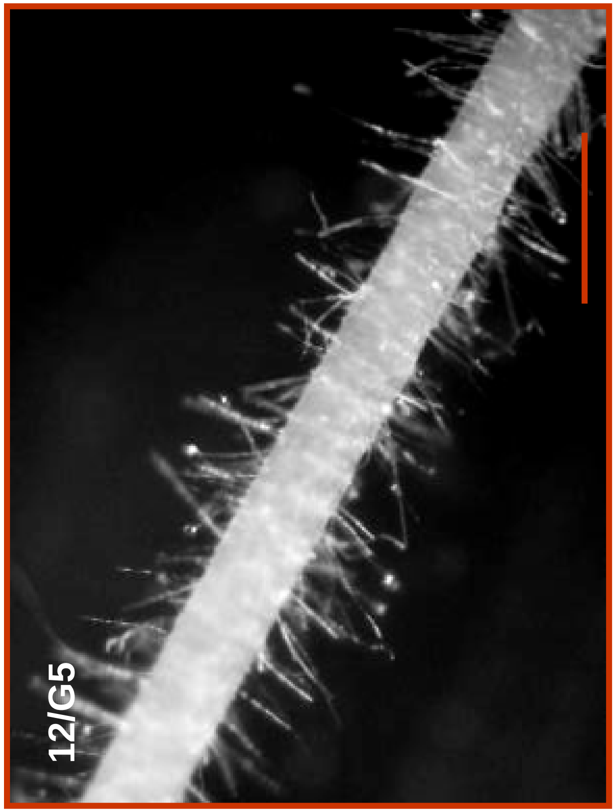
Courtesy of L. Comai, modified

ROOT MUTANTS – Reverse analysis

TILLING with TILLMore in barley



Wild-type



12/G5

37/C7

Photos: courtesy of Iwona **Szarejko**

Cloning genes and QTLs

… looking for a needle in a haystack …

To clone or not to clone QTLs?

QTL cloning as an essential step towards:

- More effective marker-assisted selection
- Understanding the functional basis of traits
- Genetic engineering of quantitative traits
- Unlocking the allelic richness at target loci via direct sequencing of germplasm

Salvi & Tuberosa (2005) Trends in Plant Science, 10: 297-302.

Summary of QTL cloning for abiotic stress tolerance in cereals

Stress	Barley	Maize	Rice	Sorghum	Wheat
Drought	in progress	in progress	in progress	in progress	in progress
Flooding	-	in progress	yes	-	-
Salinity	in progress	-	yes	-	yes
Low N	in progress	yes	in progress	-	in progress
Low P	-	in progress	in progress	-	-
Aluminum	-	yes	in progress	yes	in progress
Boron	yes	-	-	-	yes
Low temps	yes	-	-	-	yes
High temps	-	-	-	-	in progress

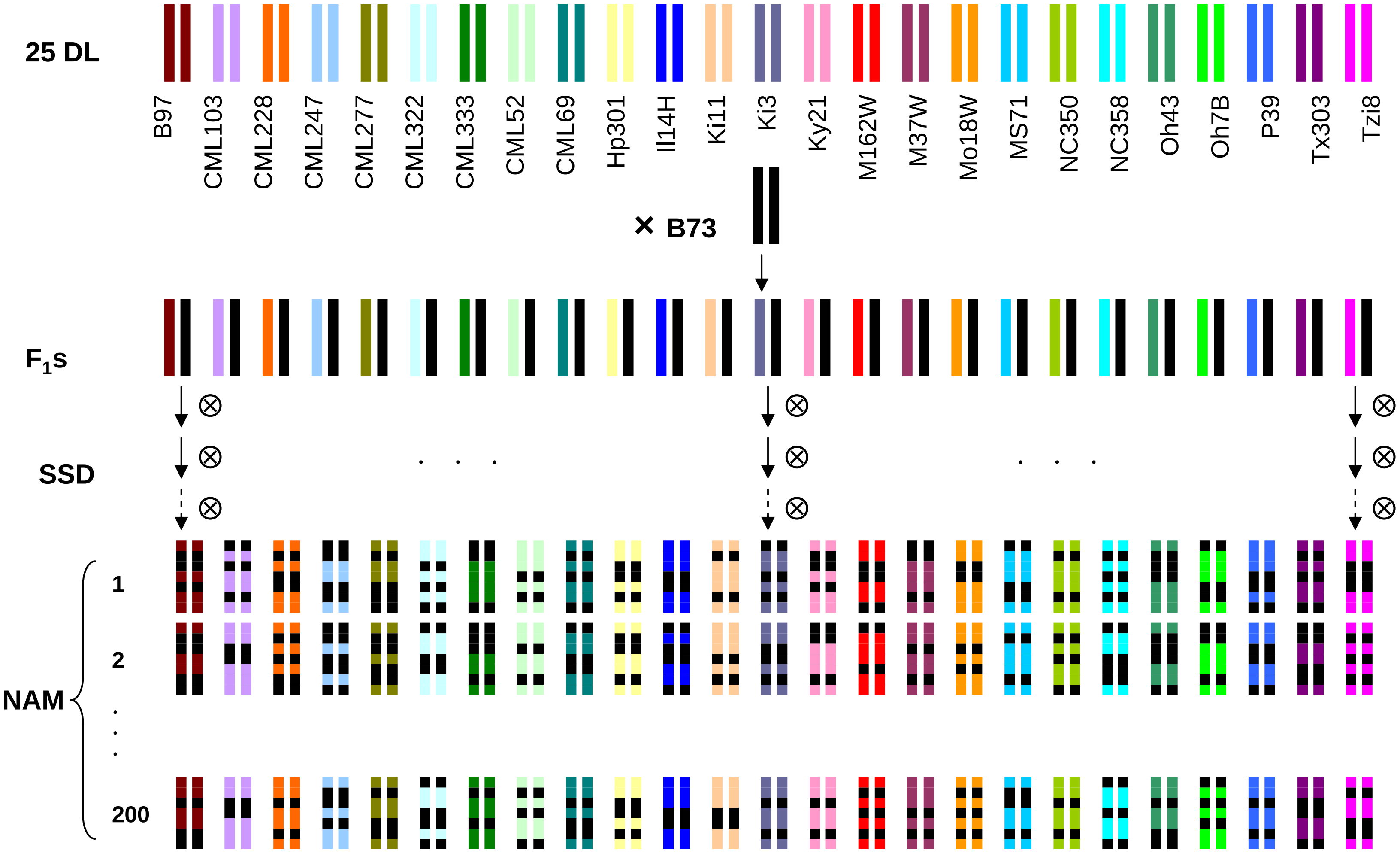
Perspectives in gene/QTL mapping and cloning

Maize Diversity Project Team

The Nested Association Mapping (NAM) project

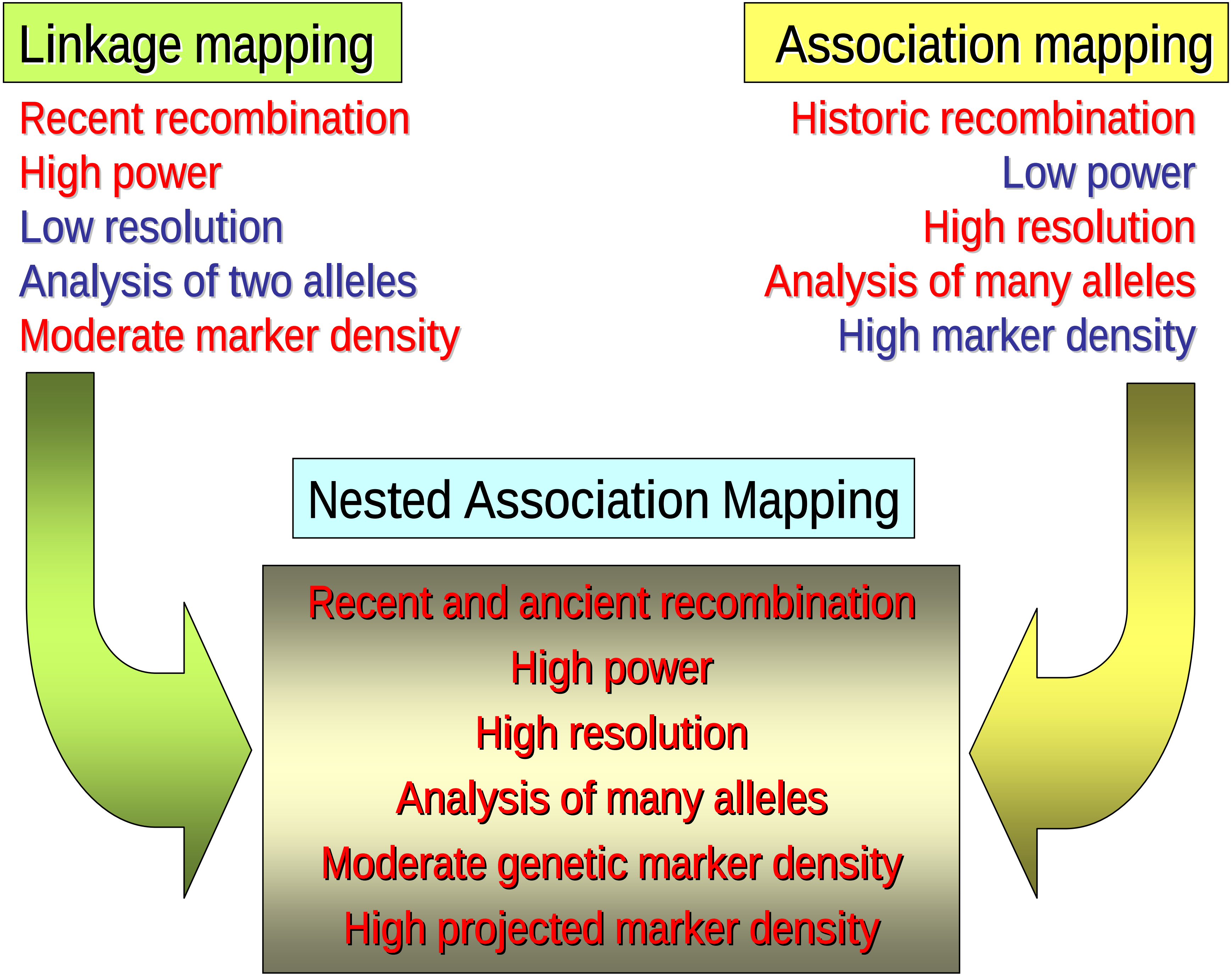
Courtesy of Mike McMullen

Nested Association Analysis



Courtesy of Mike McMullen

Yu et al. (2008) Genetics 178: 539



Linkage mapping

Recent recombination
High power
Low resolution
Analysis of two alleles
Moderate marker density

Association mapping

Historic recombination
Low power
High resolution
Analysis of many alleles
High marker density

Nested Association Mapping

Recent and ancient recombination
High power
High resolution
Analysis of many alleles
Moderate genetic marker density
High projected marker density

Conserved Orthologous Set (COS) markers
(J. Salse & coworkers, INRA, France)

- Set of ca. 170,000 gene sequences available for cereals
- Rice, maize, sorghum, *Brachypodium* genomes have been sequenced

COS markers have been derived from orthologous Expressed Sequence Tags (EST) that have been conserved across evolutionary related species.

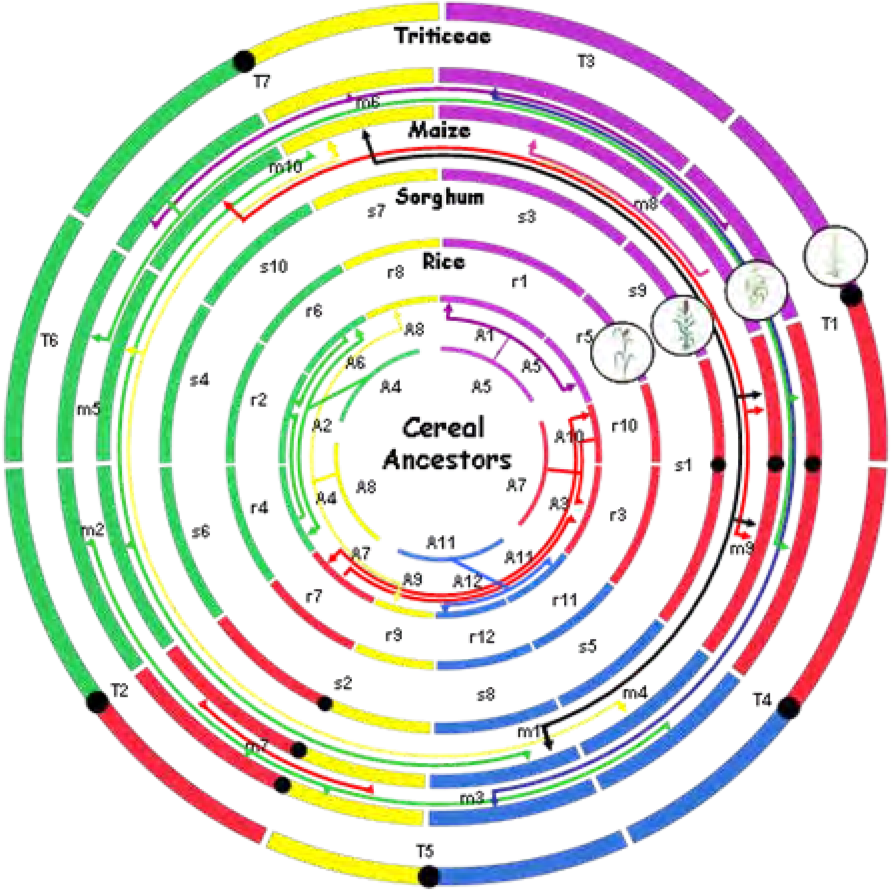
Advantages

- Transferable among species
- Mostly codominant
- Gene-based markers

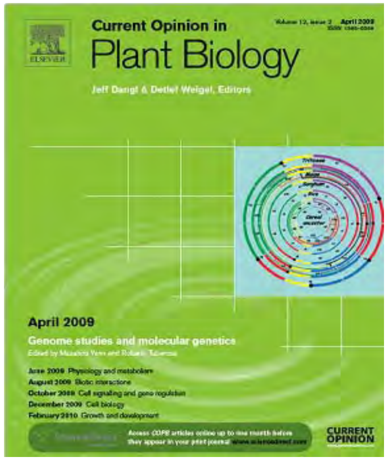
Disadvantages

- Need expertised inference of synteny
- Low level of polymorphism

Salse & co-workers: Paleogenomics in cereals for trait improvement



Salse & co-workers (INRA, France)
9,138 COS markers for the monocots



Current Opinion in
Plant Biology

Jeff Dangl & Detlef Weigel, Editors

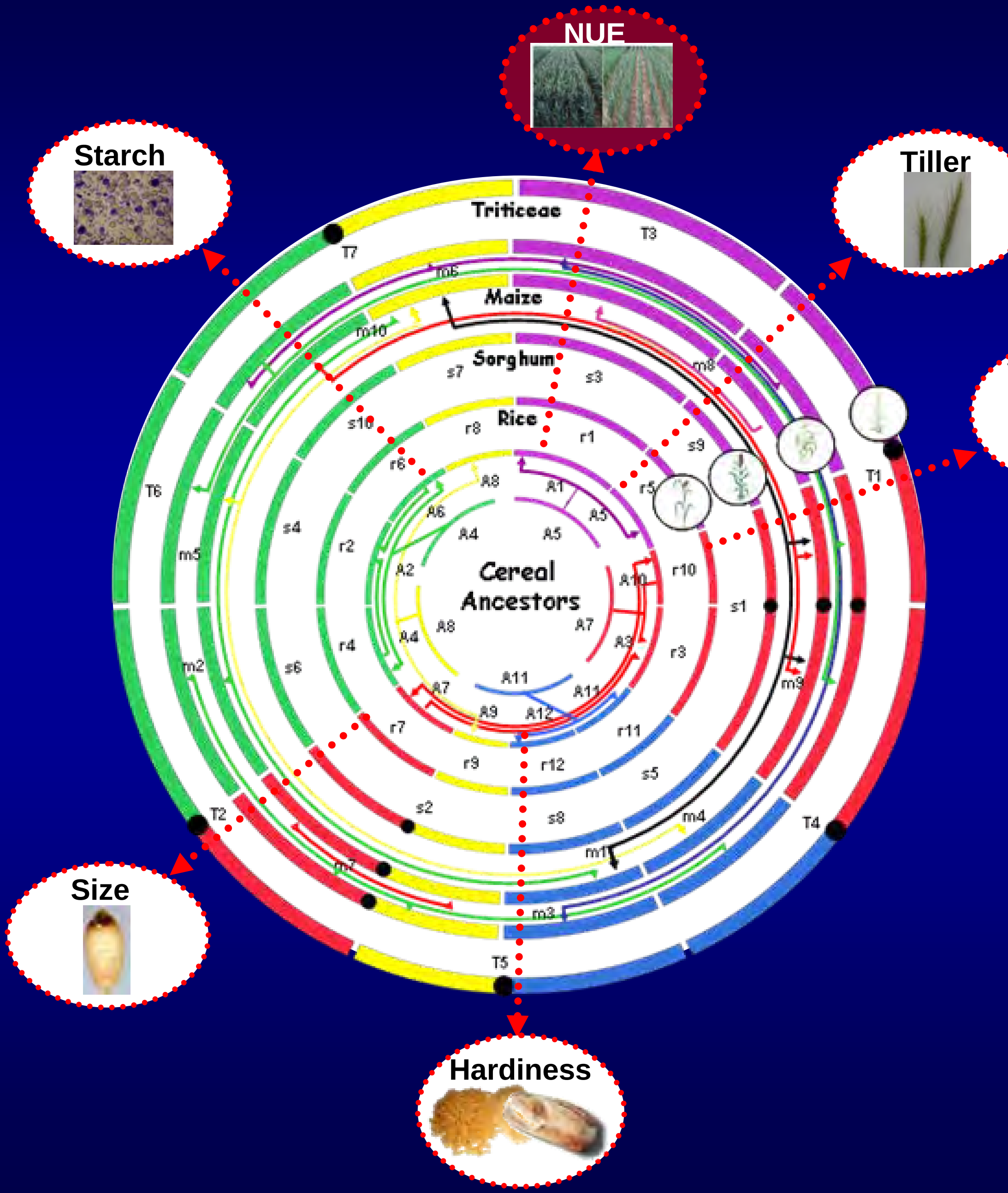
April 2009

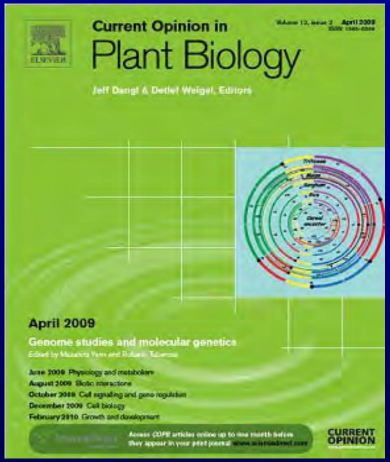
Genome studies and molecular genetics

June 2009 Physiology and metabolism
August 2009 Biotic interactions
October 2009 Cell signaling and gene regulation
December 2009 Cell biology
February 2010 Growth and development

CURRENT OPINION

Bolot et al. (2009)
COPB, 12: 11-15





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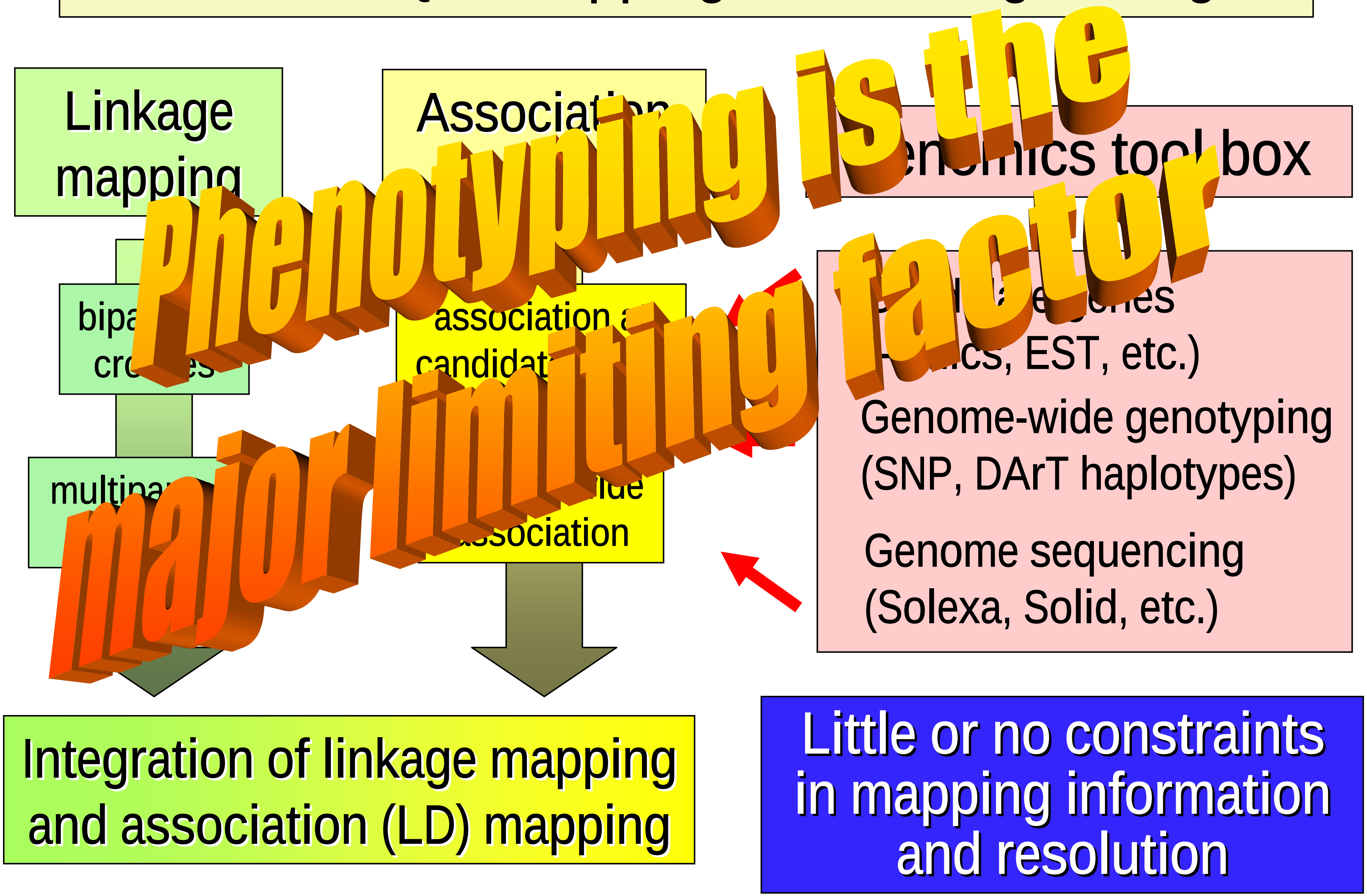
Future challenges/opportunities for unlocking valuable natural variation for target traits

- High-throughput genotyping (SNPs) & phenotyping
- Resequencing and managing the data
- *In silico* analysis, annotation and comparative genomics
- Tissue-specific expression profiles
- Functional maps (ESTs, FLcDNAs, eQTL)
- QTL cloning

Evolution of QTL mapping and cloning strategies



Phenotyping is the major limiting factor

Linkage mapping

Associat n

enomics tool box

bipa

cr es

association a

candidat

multipa

ide

ssociation

ics, EST, etc.)

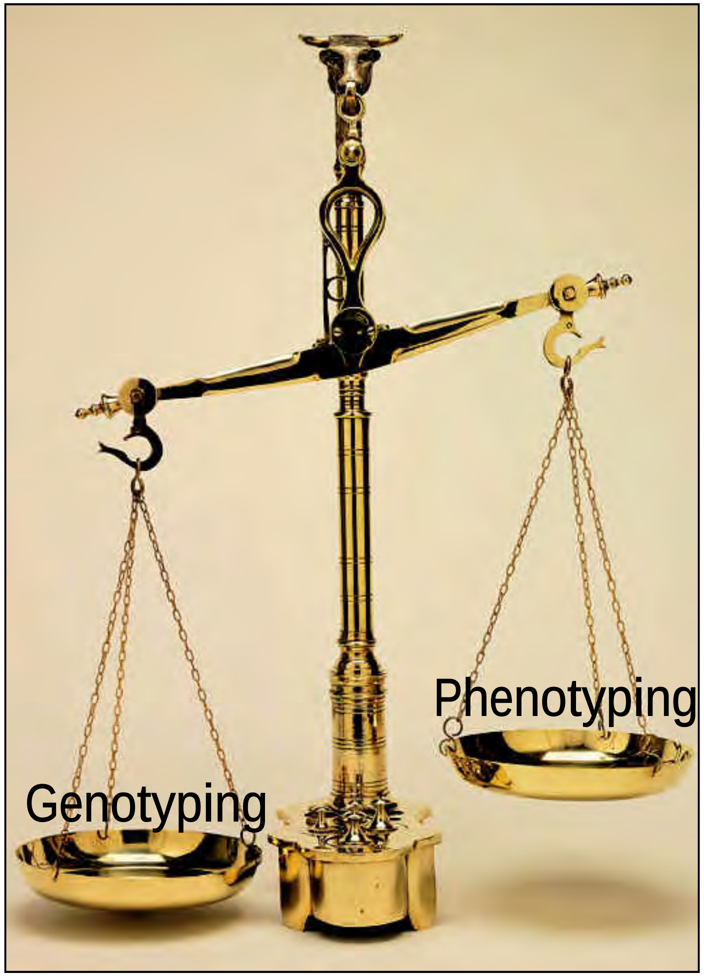
Genome-wide genotyping (SNP, DArT haplotypes)

Genome sequencing (Solexa, Solid, etc.)

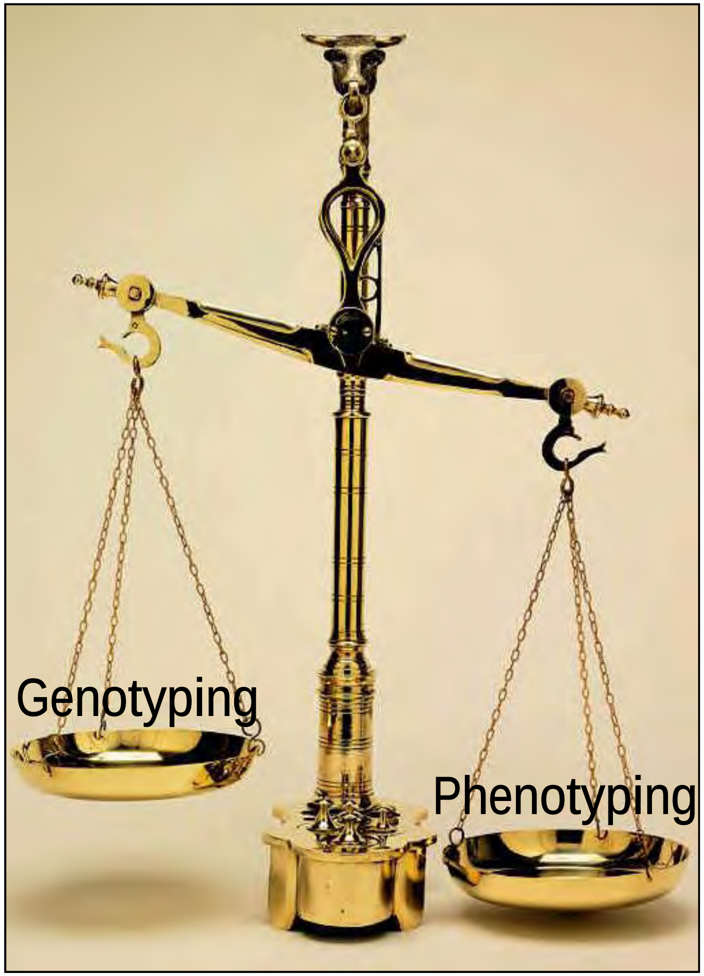
Integration of linkage mapping and association (LD) mapping

Little or no constraints in mapping information and resolution

Past



Future



Conclusions

- Plenty of genetic resources are available in cereals for gene/QTL discovery.
- Devising the right phenotyping screening and choosing the right materials is key to the discovery of loci relevant for the desired trait in the target environment.
- The cost of molecular profiling and sequencing will continue to decrease, making genomics approaches more affordable.
- Association mapping will play an increasingly important role for relatively simple traits; it is more questionable to what extent it will work with physiologically complex traits.
- The contribution of wild germplasm to gene discovery will increase.

Thanks for your attention!