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## Incompatibilities in crop breeding

Pierre Sourdille

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# Introduction

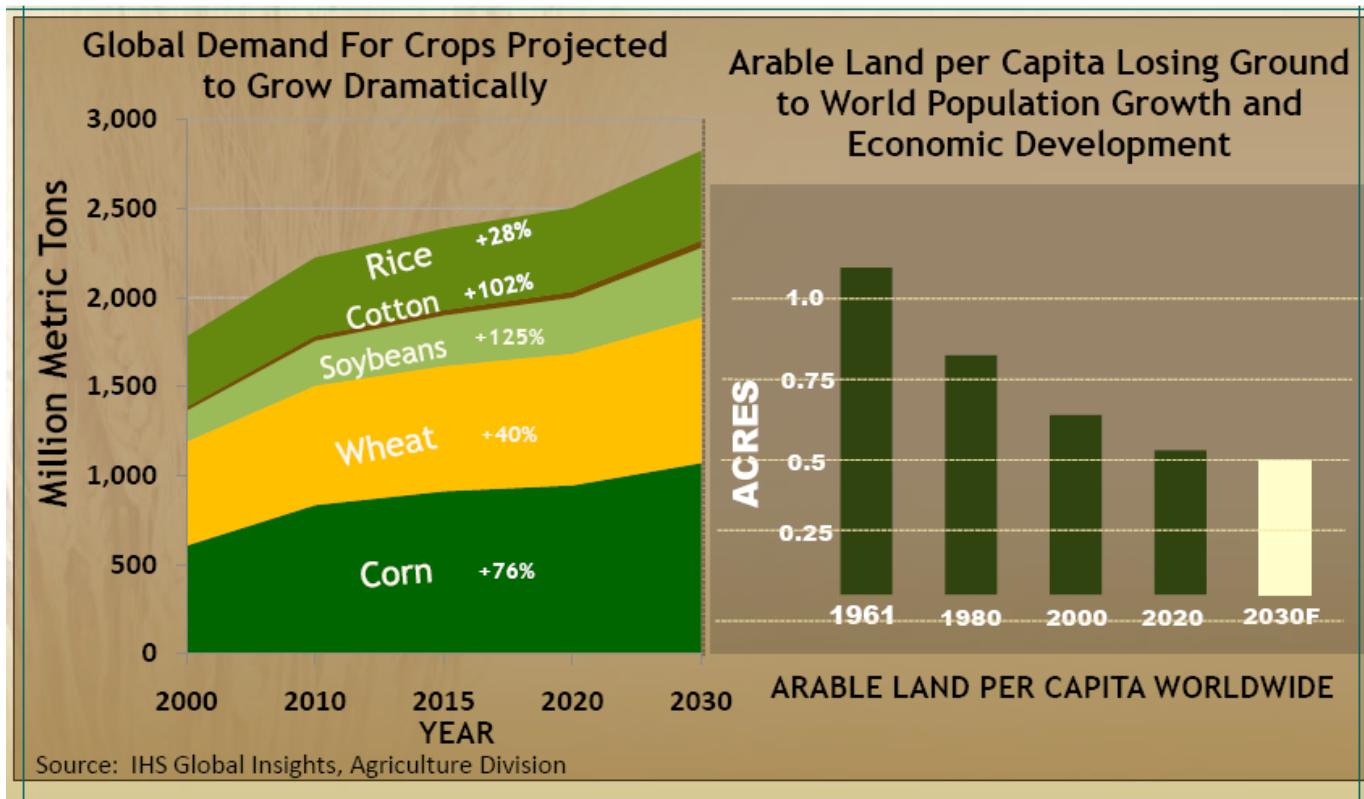
## Incompatibilities in crop breeding

Sourdille Pierre

UMR INRA-UBP Genetics, Diversity &  
Ecophysiology of Cereales (GDEC)

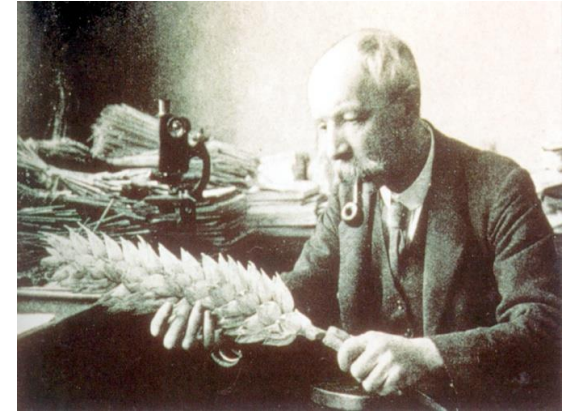
Clermont-Ferrand

# An increasing demand with less arable lands to produce: improving yield is a priority!



# How to improve yields?

- Identify genes of interest to improve
  - Genetic mapping & QTL detection
  - Association genetics (GWAS)
  - Positional cloning
- Search for original and powerful alleles
  - Exploitation of genetic resources
  - Explore wild relatives
- Realize crosses
  - Problem of crosses
  - Problem of chromosome pairing
  - Problem of recombination between chromosomes

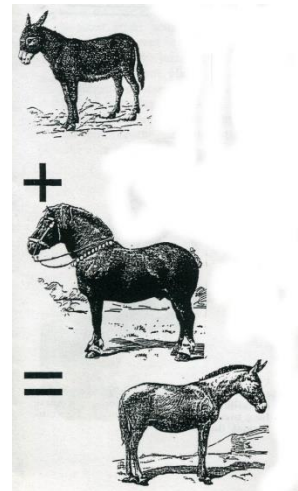


# Some troubles linked to the realization of crosses

- Species barrier
  - Definition of a species
    - Set of individuals (population) that can, under natural conditions:
      - Reproduce with each other i.e.
        - » Lead to a viable descent
        - » Lead to a fertile descent

# Some troubles linked to the realization of crosses

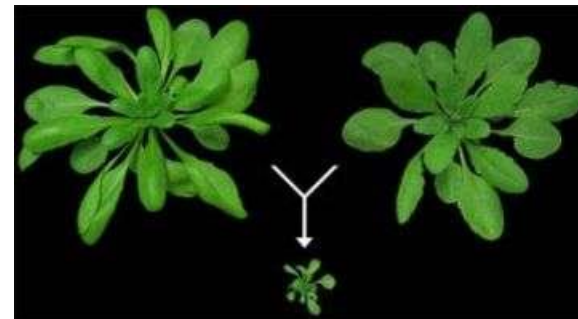
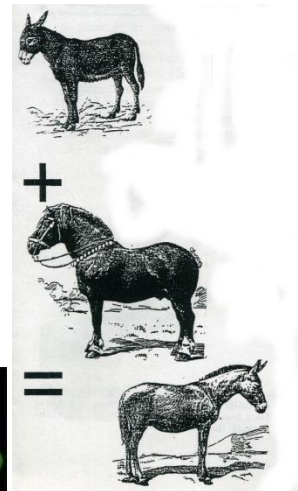
- Species barrier
  - Interspecific or intergeneric incompatibility
    - Impossible crosses
    - Sterile descent



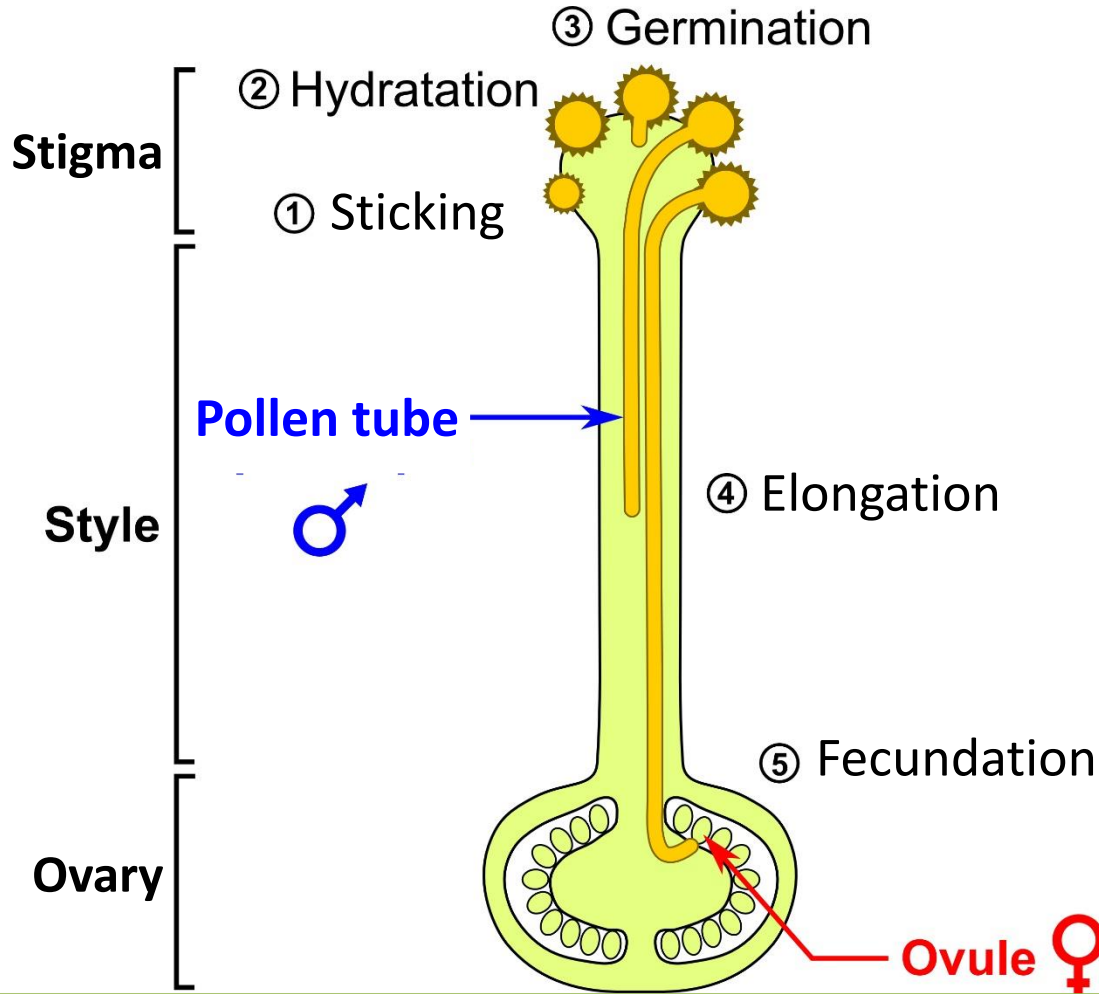


# Some troubles linked to the realization of crosses

- Species barrier
  - Interspecific or intergeneric incompatibility
    - Impossible crosses
    - Sterile descent
- Intra-varietal barrier
  - Self-Incompatibility
  - Inter-Incompatibility
    - Hybride Necrosis



# Fecundation in plants





# Gametes in plants

- Pollen grain

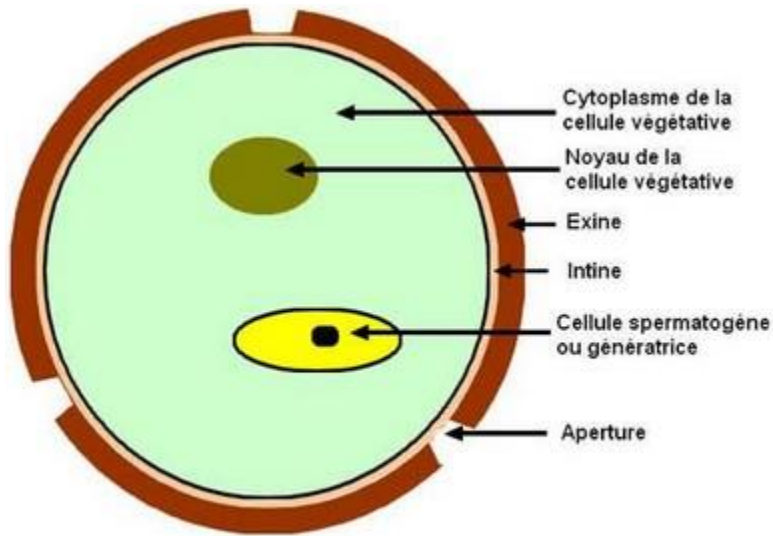


Schéma d'un grain de pollen

- Ovule

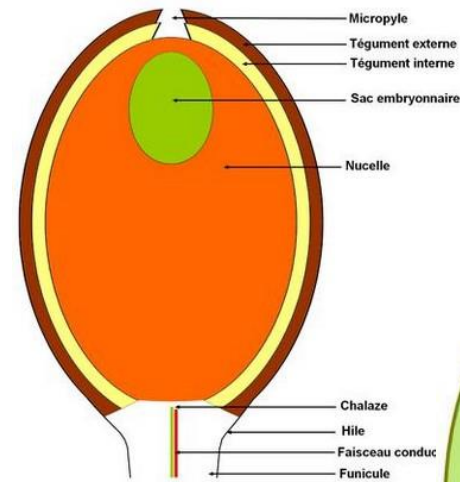


Schéma d'un ovule d'Angiosperme

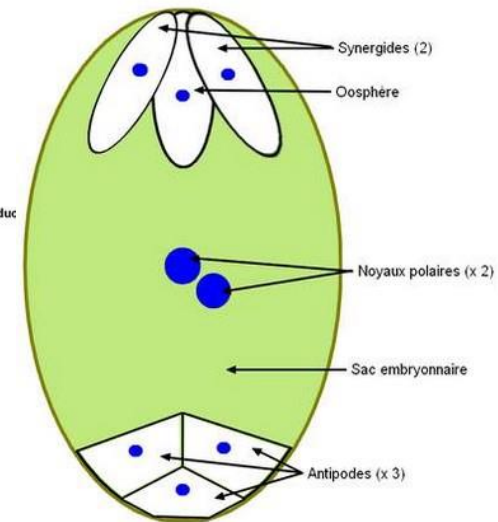
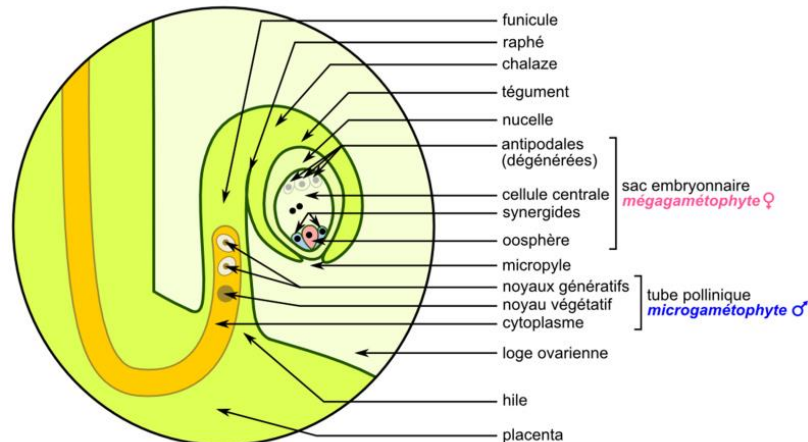


Schéma d'un sac embryonnaire



# Sexual reproduction in plants

- Autogamous
  - Fecundation of female gametes by male gametes of the same flower



# Sexual reproduction in plants

- Autogamous
  - Strictly autogamous = rare = residual allogamy (5% in wheat)





# Sexual reproduction in plants

- Cleistogamous
  - Reproduction before flowers open



# Sexual reproduction in plants

- Cleistogamous
  - Reproduction before flowers open
  - Reduction of gene flux but...
  - Some flowers may still open (instability of the trait) = not enough reliable to ensure biological lockdown (GMOs)

# Sexual reproduction in plants

- Allogamous
  - Definition: cross-fecundation between two distinct individuals
  - Avantages: favours genetic diversity

# Sexual reproduction in plants

- Allogamous
- Different cases:
  - Plants from the same species are either male or female = dioecious species





# Sexual reproduction in plants

- Allogamous
- Different cases:
  - Male and female flowers are distinct but on the same plant = monoecious species



# Sexual reproduction in plants

- Allogamous
  - Strictly allogamous: several cases
    - Pollen does not germinate if it falls on a female organ of the same flower





# Sexual reproduction in plants

- Allogamous
  - Strictly allogamous: several cases
    - Pollen dispersed before maturity of female flowers = protandry



# Sexual reproduction in plants

- Allogamous
  - Strictly allogamous: several cases
    - Ovaries mature before pollen dispersion = protogyny



# Sexual reproduction in plants

- Allogamous
  - Partially: production of a few grains after self-pollination





# Apomixis, a special case!

- Definition: asexual multiplication, without fecundation and with modification of the meiosis (related to parthenogenesis)
- Pollination = stimulation of the diploid cell of the ovule → reproduction of the maternal genotype
- Loss of diversity (transmission of somatic mutations only)
- Cultivated species not concerned





# Underlying questions behind these reproductive modes

- What factors control incompatibility?
  - Self-incompatibility
  - Interspecific incompatibility
- Results of unlocking incompatibility?
- Interest of introgressions in breeding?
- Impact of introgressions in breeding?



# Self-incompatibility in plants

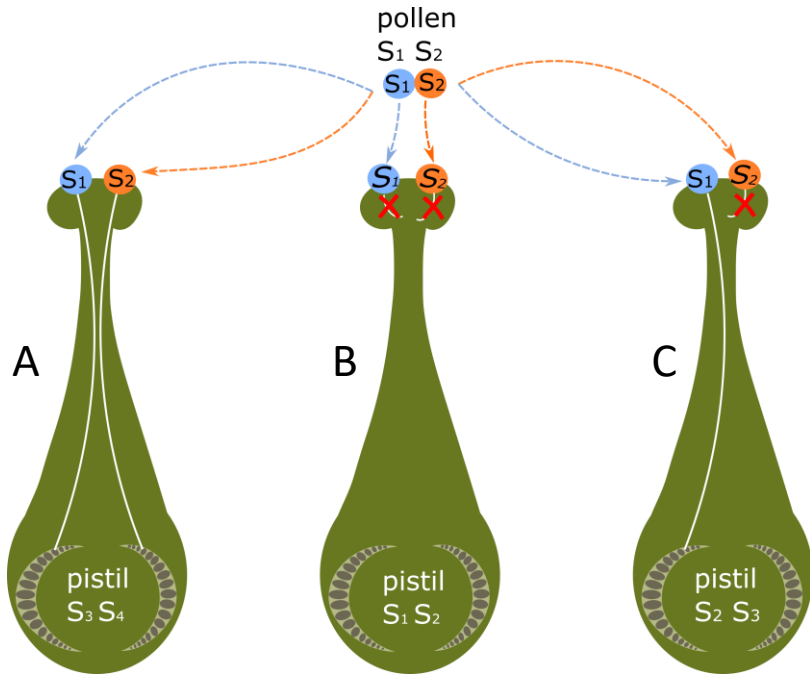
- Definition :
  - Physiological impossibility for a male gamete to fertilize a female gamete of the same hermaphroditic flower
  - Impossibility to produce seeds through selfing
- Examples
- $\frac{1}{2}$  angiosperms



# Self-incompatibility in plants

- Gametophytic self-incompatibility:
  - The allele of the male gametophyte = one of the two alleles from the ovary (diploid) from the female organ → development of the pollen tube blocked = fecundation cannot occur
  - Selfing impossible

# Self-incompatibility in plants



Laugerotte et al. 2022 Plant Biotech. J. doi: 10.1111/pbi.13784

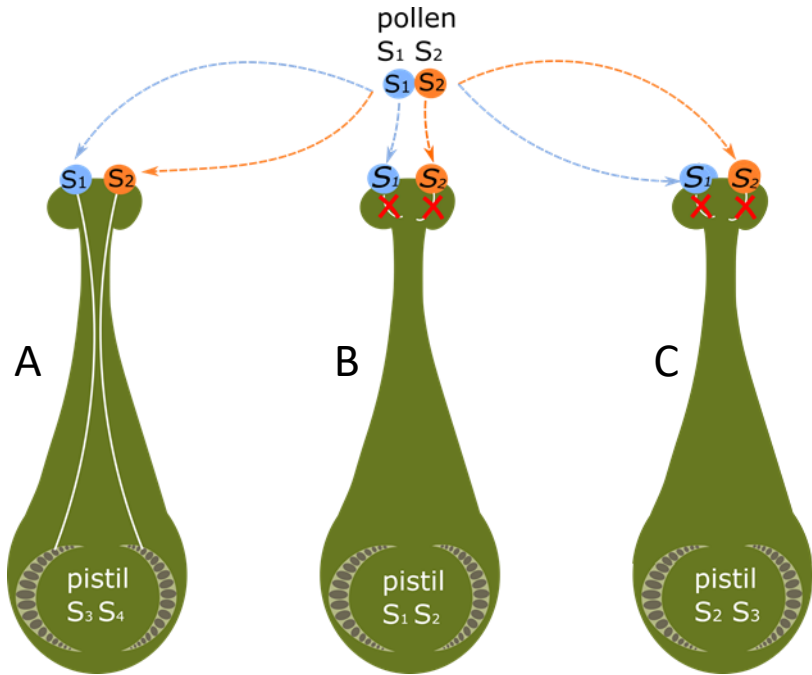
- Pollen S1/S2
  - Envelop (diploid) S1S2
  - Gametophyte (haploid) S1 or S2
- Plant A (S3/S4)
  - Pistil/ovary (diploid) S3S4
- Plant B (S1/S2)
  - Pistil/ovary (diploid) S1S2
- Plant C
  - Pistil/ovary (diploid) S2S3

- ➔ S1 pollen germinates and fertilize plant A (S3/S4) and C (S2/S3) but not B (S1/S2)
- ➔ S2 pollen fertilizes plant A only (S3/S4) but not B (S1/S2) or C (S2/S3)

# Self-incompatibility in plants

- Sporophytic self-incompatibility:
  - Cell wall of the sporophyte (pollen grain) = diploid
  - At least one of the two alleles of the male sporophyte = one of the two alleles of the ovary (diploid) of the female organ → development of the pollen tube blocked = no fecundation
  - Selfing impossible

# Self-incompatibility in plants



Laugerotte et al. 2022 Plant Biotech. J. doi: 10.1111/pbi.13784

- Pollen S1/S2
  - Envelop (diploid) S1S2
- Plant A (S3/S4)
  - Pistil/ovary (diploid) S3S4
- Plant B (S1/S2)
  - Pistil/ovary (diploid) S1S2
- Plant C
  - Pistil/ovary (diploid) S2S3

➔ S1 or S2 pollen germinate and fertilize plant A only (S3/S4) but not B (S1/S2) or C (S2/S3)

# Self-incompatibility in plants

- Example of olive tree
  - Six known alleles: R1 to R6
  - No homozygotes (known)
  - 15 combinations

	R1	R2	R3	R4	R5
R2	R1R2				
R3	R1R3	R2R3			
R4	R1R4	R2R4	R3R4		
R5	R1R5	R2R5	R3R5	R4R5	
R6	R1R6	R2R6	R3R6	R4R6	R5R6



<https://s2nhh.org/articles/2016/11/lautofecondation-et-lincompatibilite-des-croisements-chez-les-plantes-les-manifestations-chez-lolivier>

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# Self-incompatibility in plants

- Example of olive tree (sporophytic SI)
  - Dominance of alleles
    - $R6 > R2 > R1 = R3 = R5 > R4$  (R4 not found = R4R4)
    - Expression in mother cells = 1 pollen type delivered

	R1	R2	R3	R4	R5
R2	R1 <b>R2</b>				
R3	<b>R1</b> R3	<b>R2</b> R3			
R4	<b>R1</b> R4	<b>R2</b> R4	<b>R3</b> R4		
R5	<b>R1</b> R5	<b>R2</b> R5	<b>R3</b> R5	R4 <b>R5</b>	
R6	<b>R1</b> R6	<b>R2</b> R6	<b>R3</b> R6	<b>R4</b> R6	<b>R5</b> R6



<https://s2nhh.org/articles/2016/11/lautofecondation-et-lincompatibilite-des-croisements-chez-les-plantes-les-manifestations-chez-lolivier>

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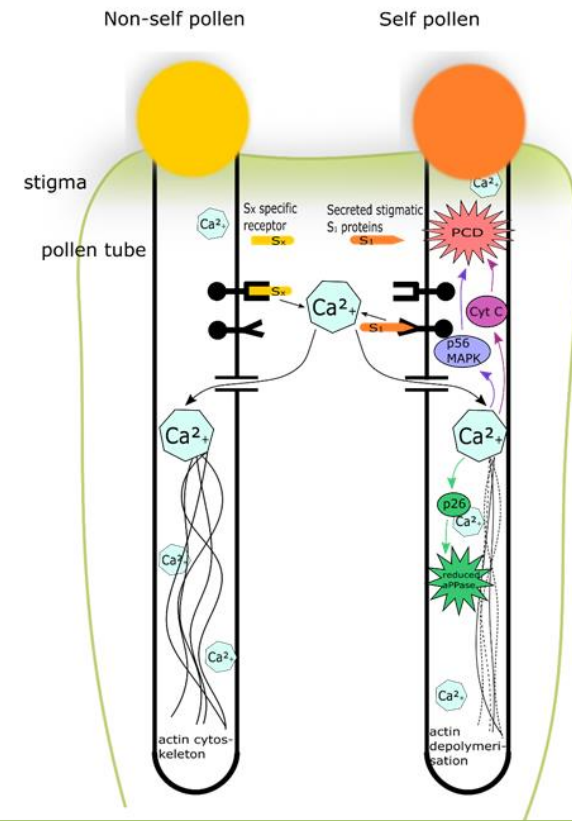
# Self-incompatibility in plants

- What genes for self-incompatibility?
  - Gametophytic
    - Mechanism with RNase
      - Identified in tobacco in 1989
      - Emission of ribonuclease (S-RNase) by the ♀ = degradation ♂ ribosomal RNA
      - Block pollen tube growth at 1/3 of style



# Self-incompatibility in plants

- What genes for self-incompatibility?
  - Gametophytic
    - Mechanism with S-glycoproteins
      - Identified in Papaveraceae in 1997
      - Interaction ♂ / ♀ = calcium influx
      - Immediate (1-2 min) stop of elongation



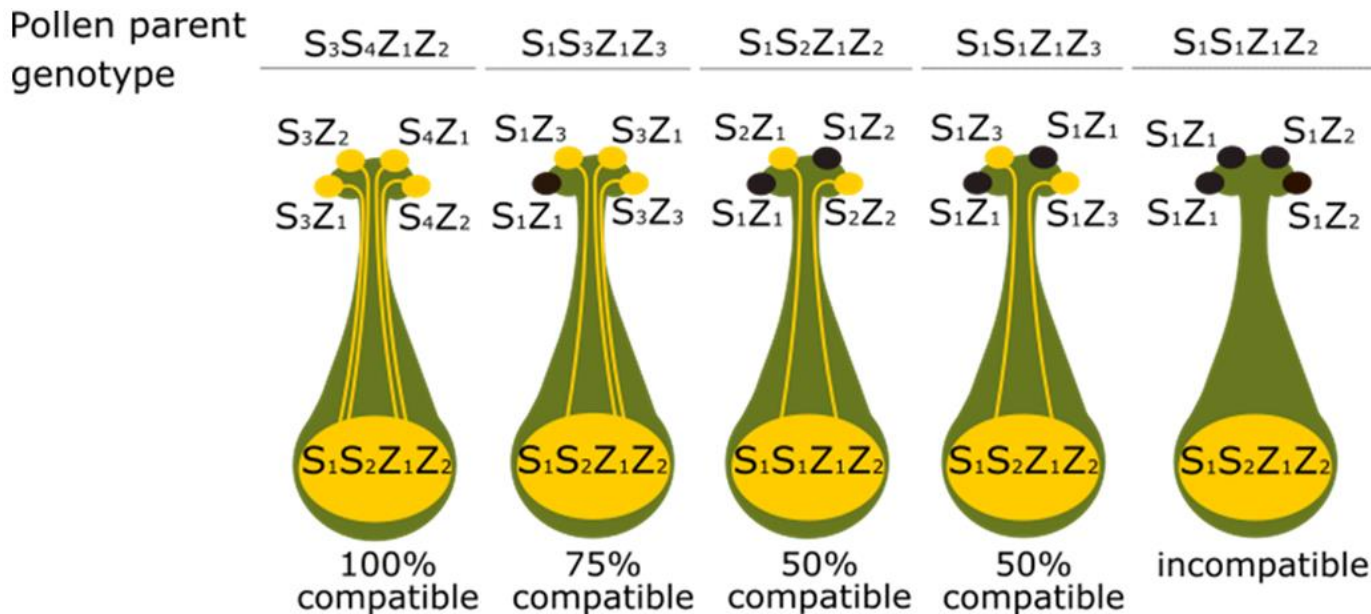
# Self-incompatibility in plants

- What genes for self-incompatibility?
  - Gametophytic
    - Mechanism with two loci
      - Identified in grasses in
      - Two loci: S & Z
      - If both ♂ expressed alleles = ♀ alleles, then incompatibility
      - Genes not cloned



# Self-incompatibility in plants

- What genes for self-incompatibility?
  - Gametophytic
    - Mechanism with two loci

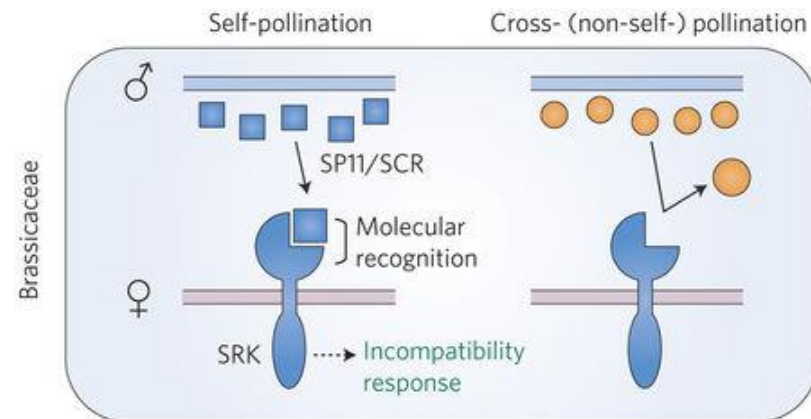


# Self-incompatibility in plants

- What genes for self-incompatibility?
  - Sporophytic

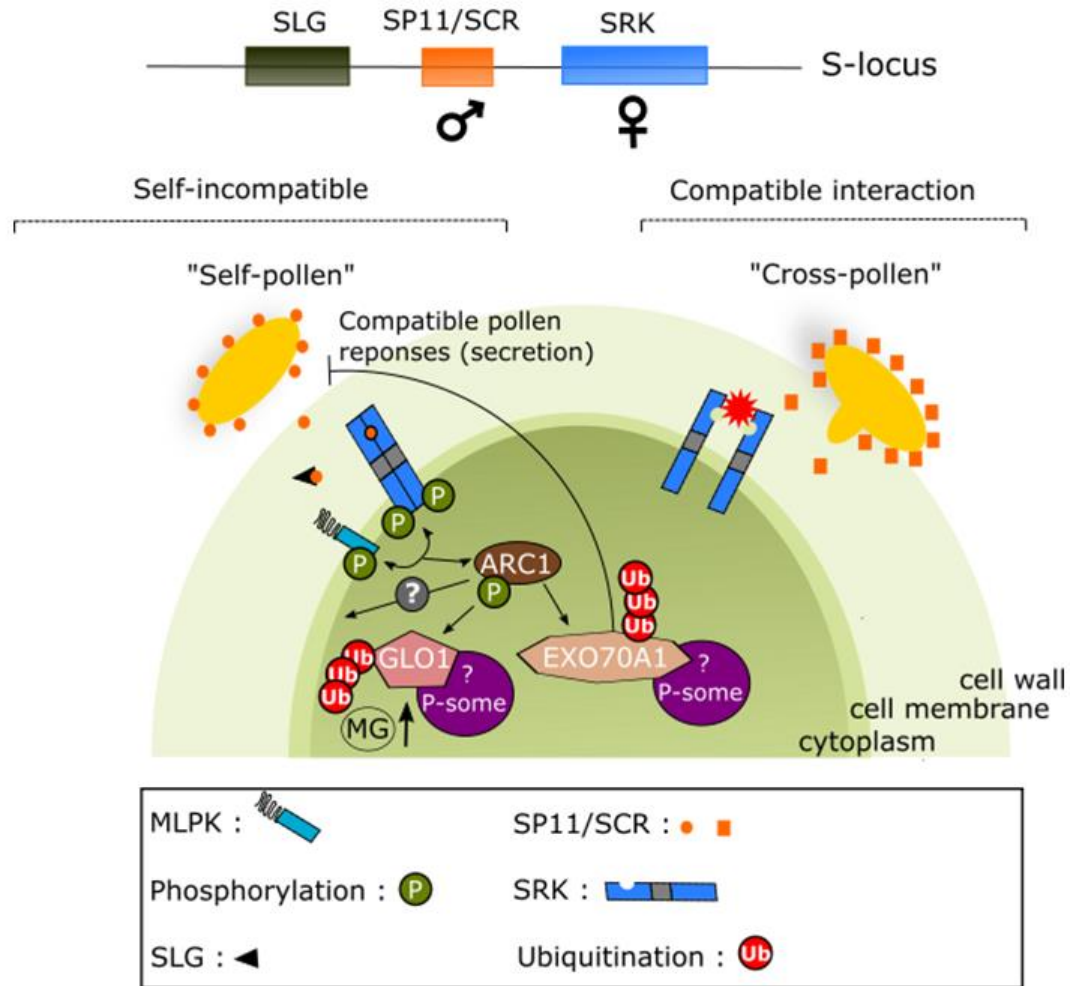


- Mechanism SI (self-incompatibility) in Brassica (1999)
  - Phenotype of pollen determined by the anther's genotype
  - Small Cystein-Rich (SCR) proteins from pollen envelop (or SP11)
  - Recognized by SRK
    - Variable external domain
    - Internal domain = kinase
  - Inhibition of pollen



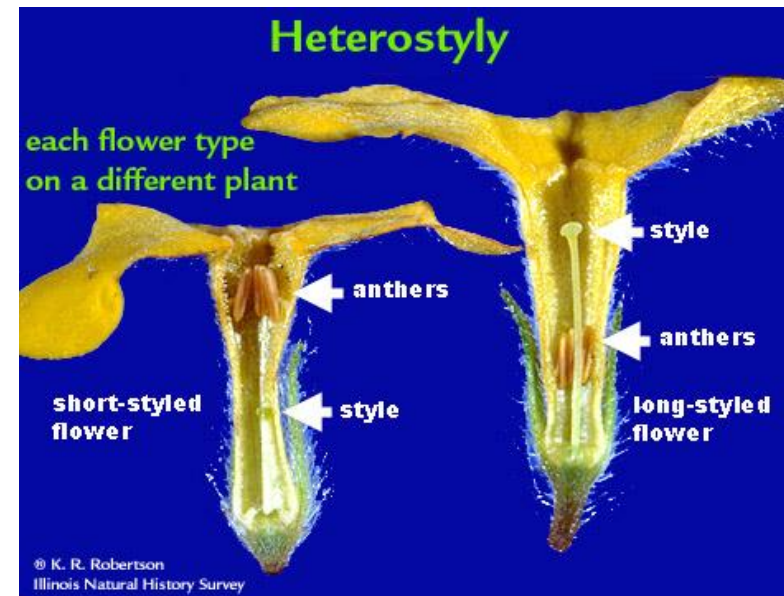


# Self-incompatibility in Brassica



# Self-incompatibility in plants

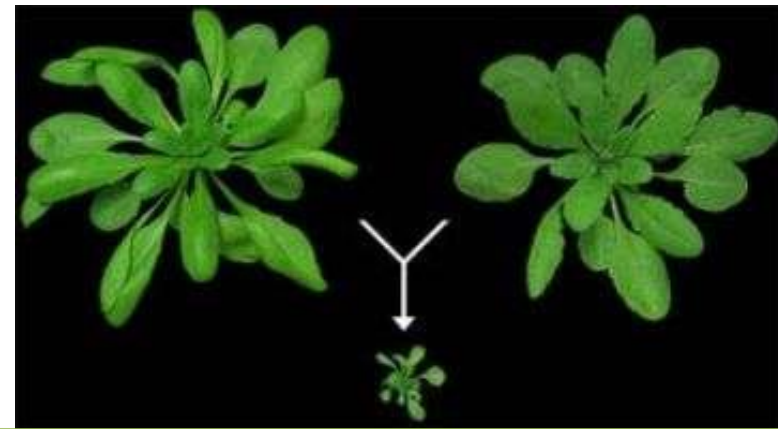
- What genes for self-incompatibility?
  - Sporophytic
    - Heteromorphic incompatibility
      - Ex.: egg-plant
      - Hetero-style flowers
      - 1 gene, 2 alleles (s/S ; 1971)
      - Gene not cloned





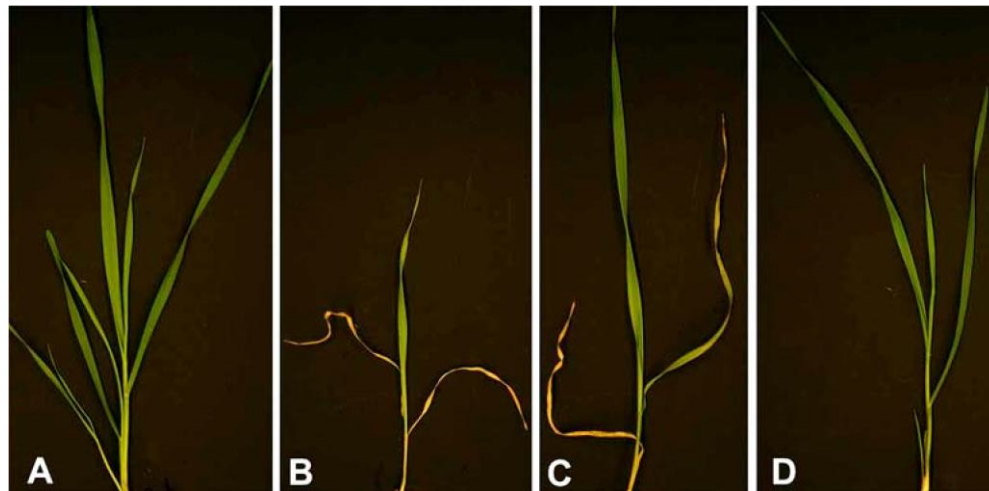
# Hybrid necrosis

- Crosses can be made
- Seeds are produced
- Seeds germinate
- But plantlet does not develop...





# Hybrid necrosis in wheat

- Frequently found in 6x x 4x crosses
- Lethal ou semi-lethal = yield ↓



# Hybrid necrosis in wheat

- Two genes involved:
  - Ne1 on 5BL; Ne1>ne1
  - Ne2 on 2BS; Ne2>ne2
- Four possible combinations

	Ne1Ne2	Ne1ne2	ne1Ne2	ne1ne2
Ne1Ne2				
Ne1ne2				
ne1Ne2				
ne1ne2				

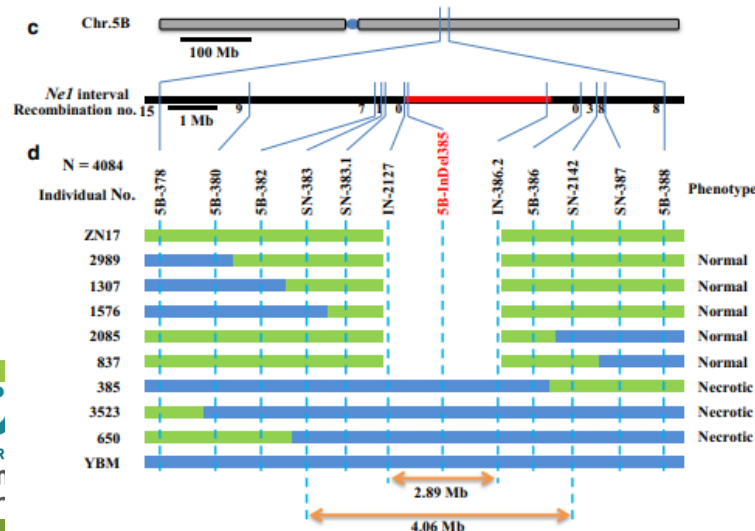
# Hybrid necrosis in wheat

- Genotypes ne1ne2 most frequent: 😊
- But not everywhere: 😞

	ne1ne2	Ne1ne2	ne1Ne2
Amérique du Nord	37,4%	7,5%	55,1%
Afrique	43,4%	47,9%	6,7%
Blé synthétiques	?	?	70%

# Hybrid necrosis in wheat

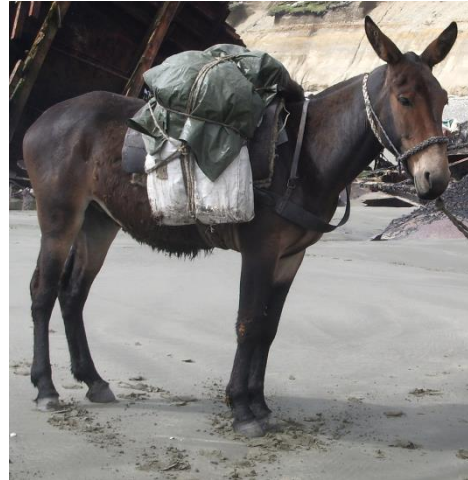
- Problem: how to know if Ne1 or Ne2?
- → Development of molecular markers
- Positional cloning of the two genes
  - Ne2 = CC-NBS-LRR (coiled coil-nucleotide-binding site-leucine-rich repeat) protein
  - Ne1?





# Interspecific incompatibility

- Interspecific hybridization quite frequent in animals
  - Polar bear x Grizzly
  - Wolf x Dog
  - Wild-board x Pig
  - Donkey x Horse
- Interest
  - Species improvement (hardiness)
  - Opportunities (diversity)
- Problem: sterility



# Interspecific incompatibility

- Interspecific hybridization less frequent in plants (except orchids)
- In general, controlled crosses
  - Wheat x Rye = Triticale
  - Tangerine x Orange = Clementine
  - Fescue x Ryegrass = Festulolium
- Interest
  - Species improvement (hardiness)
  - New species
  - Introduce diversity
- Problem: sterility but not always + vegetative multiplication in some cases



# Unlock interspecific incompatibility: example of Triticale

- Triticale ( $\times$ *Triticosecale* Wittm.)
- Derived from a wheat x rye cross
  - Wheat = ♀
- Interest
  - Hardiness (organic farming, low inputs)
  - Productivity (8t/ha)
- Outcomes
  - Animal feeding (pigs, poultry)
  - Biogaz, biofuel
  - No panification
- Variability: ~120 varieties in France, ~320 in Europe
- Production: ~350 kha in France (5 Mha for wheat!)



# Unlock interspecific incompatibility: example of Triticale

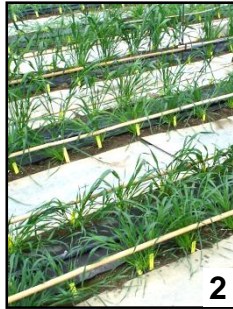
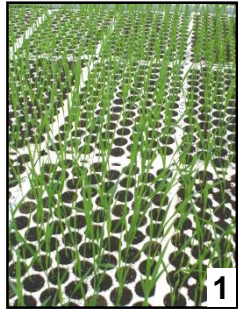
- First wheat/rye hybrids in the 19th century
- First fertile hybrid in 1888
- Breeding since 1930 in Poland (discovery of colchicin) but development in the 1980s only
  - 1982 Lasko
  - 1983 Clercal (INRA CFD)

# Unlock interspecific incompatibility: example of Triticale

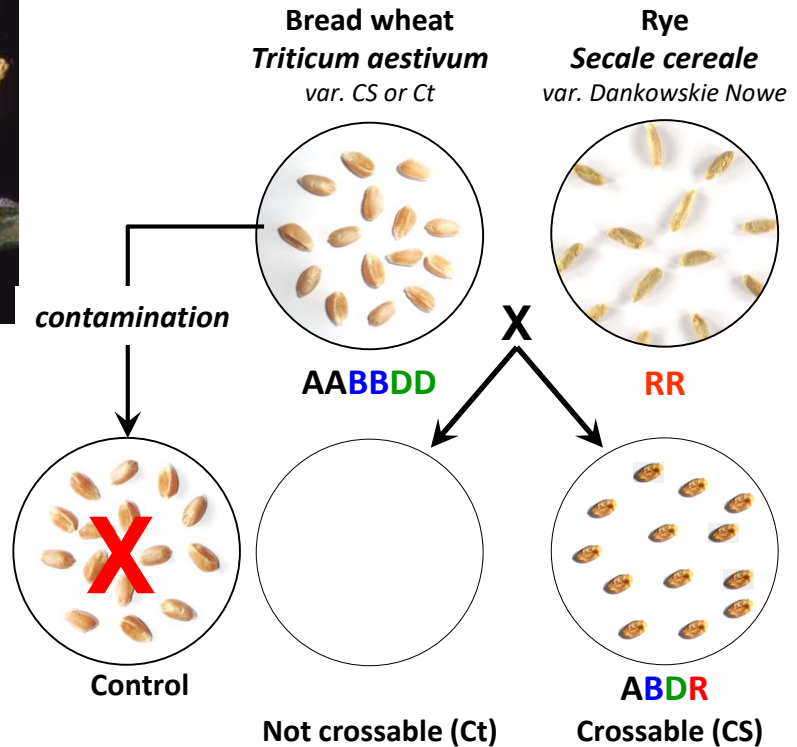
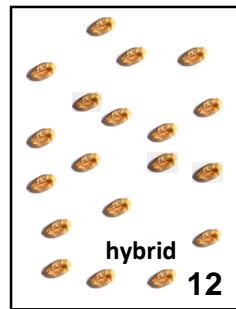
- Problems:
  - A few wheat varieties are compatible (European elite lines are incompatible)
  - Complex trait = polygenic
  - Incompatibility is dominant
  - Crosses
    - Wheat ♀ x Rye ♂ : 😊
    - Rye ♀ x Wheat ♂ : 😞
  - Tedious phenotyping 😞



# Phenotyping crossability



Rye pollen

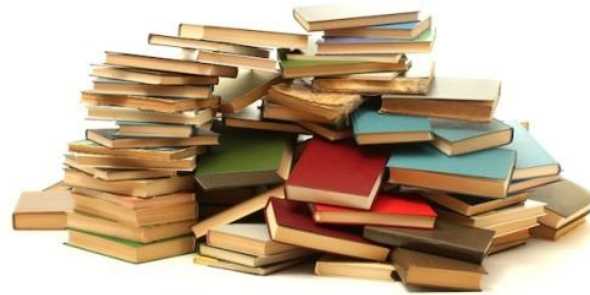


# Unlock interspecific incompatibility: example of Triticale

- Problems:
  - A few wheat varieties are compatible (European elite lines are incompatible)
  - Complex trait = polygenic
  - Incompatibility is dominant
  - Crosses
    - Wheat ♀ x Rye ♂ : 😊
    - Rye ♀ x Wheat ♂ : 😞
  - Tedious phenotyping: 😞
- ➔ It is therefore necessary to develop molecular markers or to clone the gene(s) involved!

# Genetic bases of wheat x rye crossability

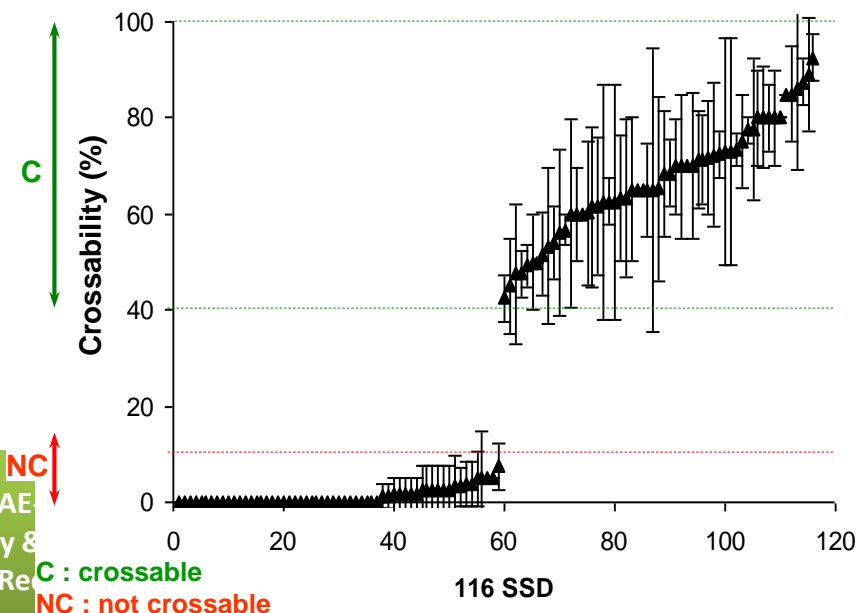
- First analyses beginning of 20<sup>th</sup> century (Backhouse, 1916)
- Wheat x rye crossability controlled by two major genes
  - *Kr1* & *Kr2*
  - Genes on 5AL & 5BL = homoeologues
- *Kr1* & *Kr2* also control *H vulgare* x *H bulbosum* crossability
- Minor genes
  - *Kr3* on 5DL
  - *Kr4* on 1AL



Lein (1943)  
Riley and Champan (1967)  
Krolow (1970)  
Lange and Riley (1973)  
Snape *et al.* (1979)  
Jalani and Moss (1980, 1981)  
Fedak and Jui (1982)  
Falk and Kasha (1983)  
Miller *et al.* (1983)  
Sitoh *et al.* (1985)  
Romero and Cuadrado (1992)  
Zheng *et al.* (1992)

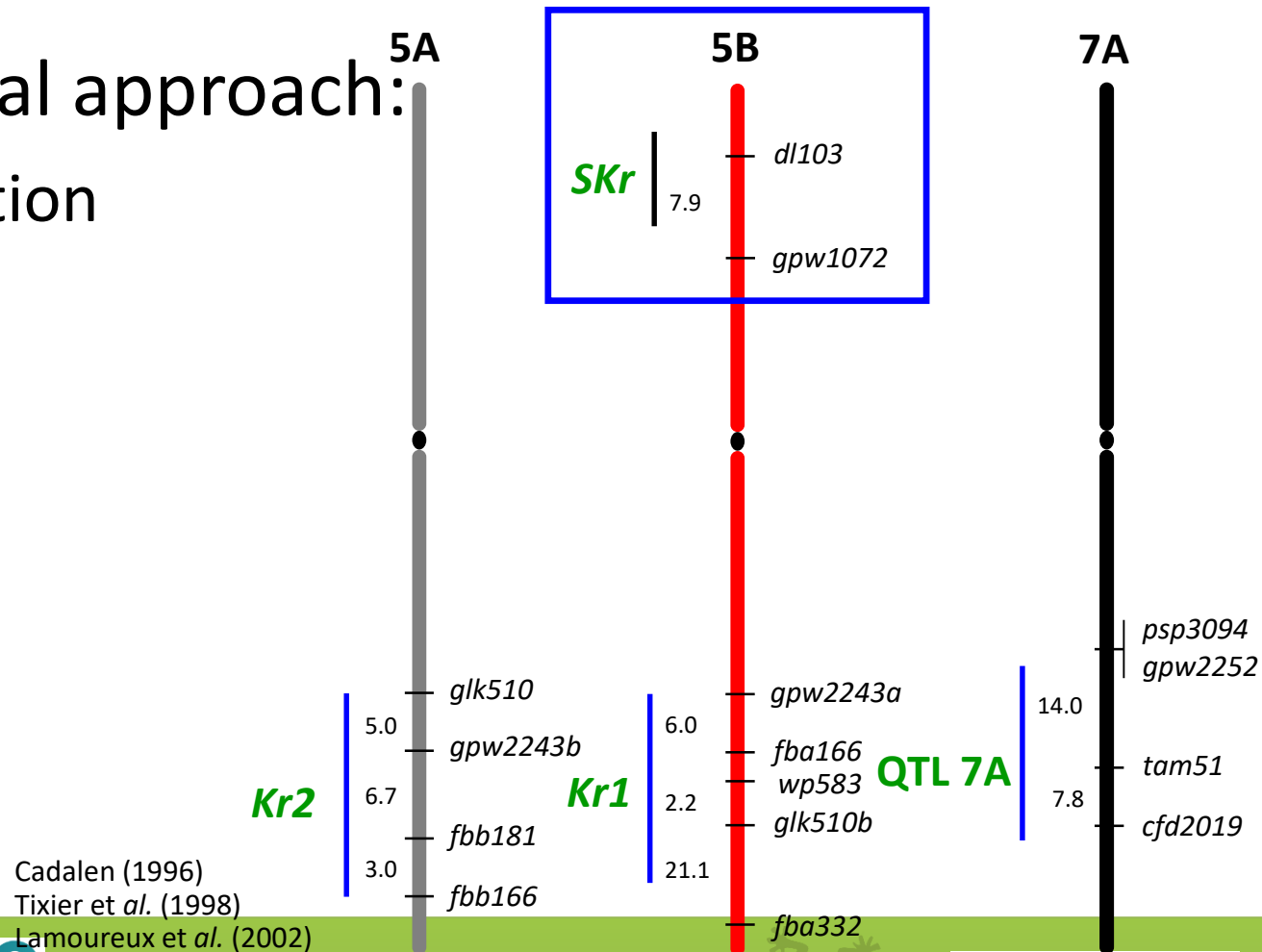
# Positional cloning of a gene involved in interspecific incompatibility: SKr

- Experimental approach:
  - Identification of contrasted parents
    - Courtot: French variety, not crossable (<5%)
    - Chinese Spring: Chinese variety, international reference, highly crossable (>90%)
  - Development of segregating populations
    - DH lines (~200)
    - SSD lines (~600)
    - HIF lines (~250)
  - Genetic mapping
    - RFLPs, SSRs, SNPs
  - Phenotyping



# Positional cloning of a gene involved in interspecific incompatibility: SKr

- Experimental approach:
  - QTL detection



Cadalen (1996)  
 Tixier et al. (1998)  
 Lamoureux et al. (2002)

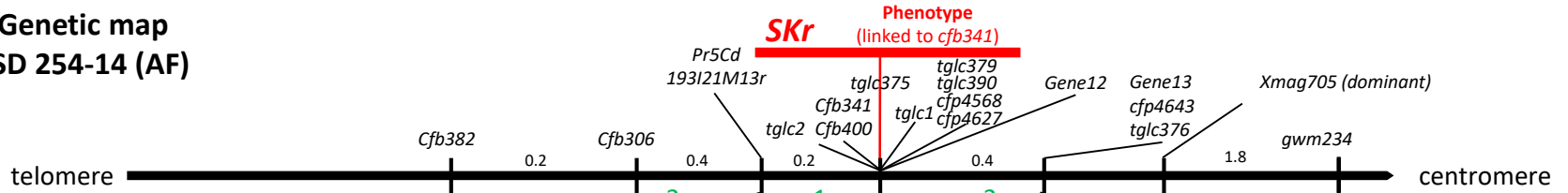


# Positional cloning of a gene involved in interpsecific incompatibility: *SKr*

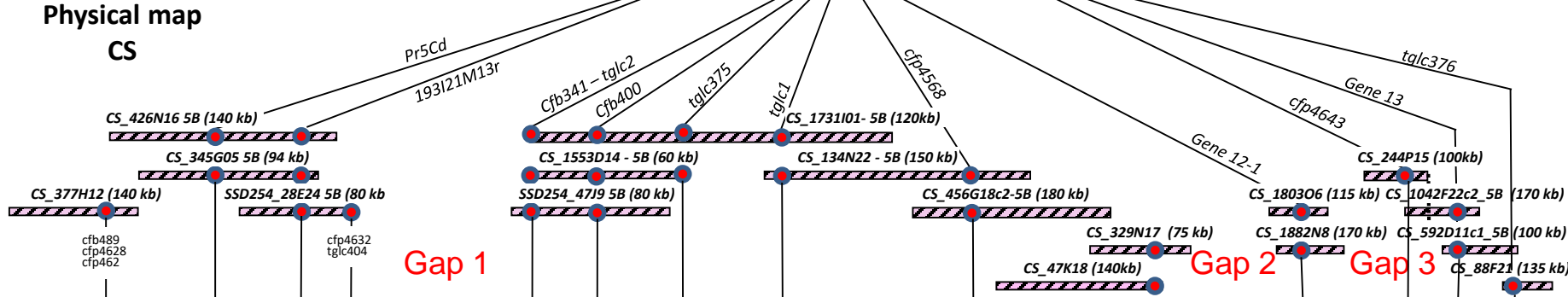
- Experimental approach:
  - Physical maps
    - In Courtot & Chinese Spring
  - Sequence

# Physical maps (V1)

## Genetic map SSD 254-14 (AF)



## Physical map CS



## Sequence (CS)

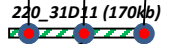
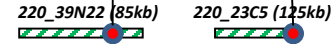
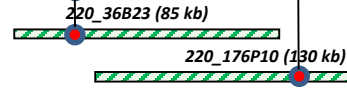
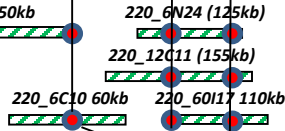
Contig 1

Contig 2

Contig 3

Contig 4

## Sequence (Ct)



## Annotation



## Wheat 5BS

## Barley 5H (1 Mb)

## Comparison



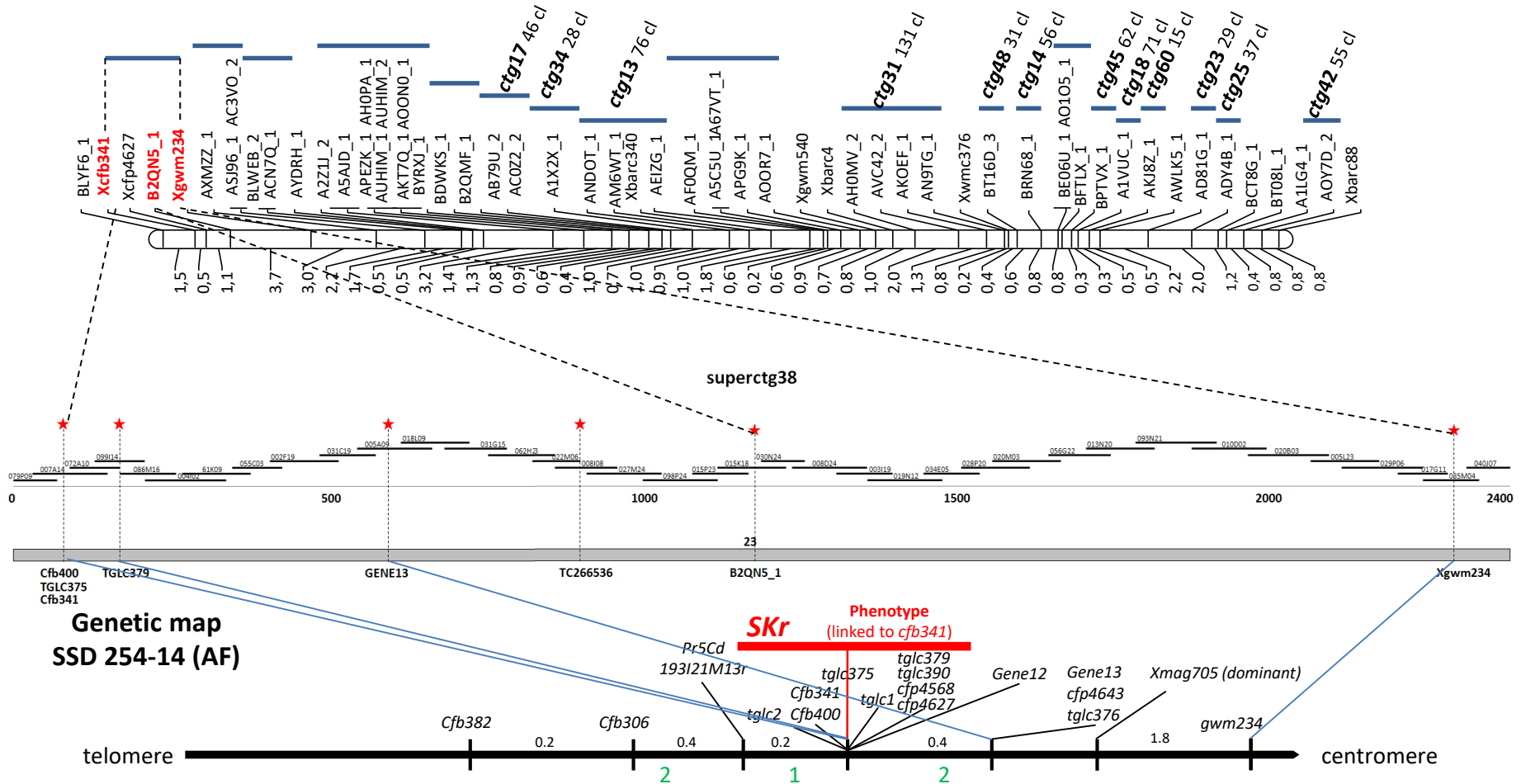
## Rice 12

## Comparison



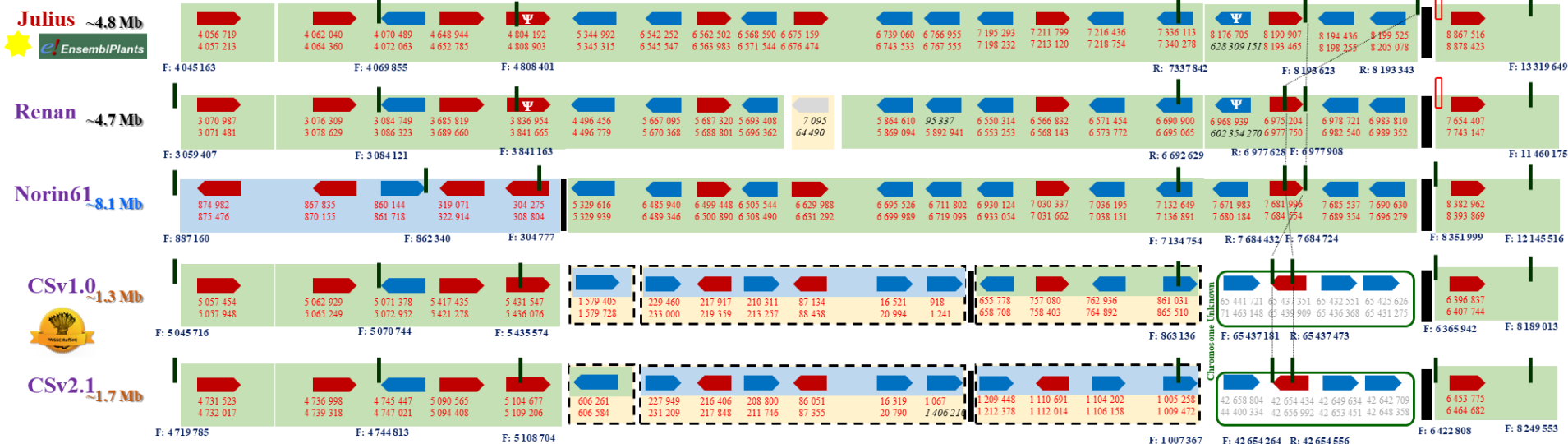
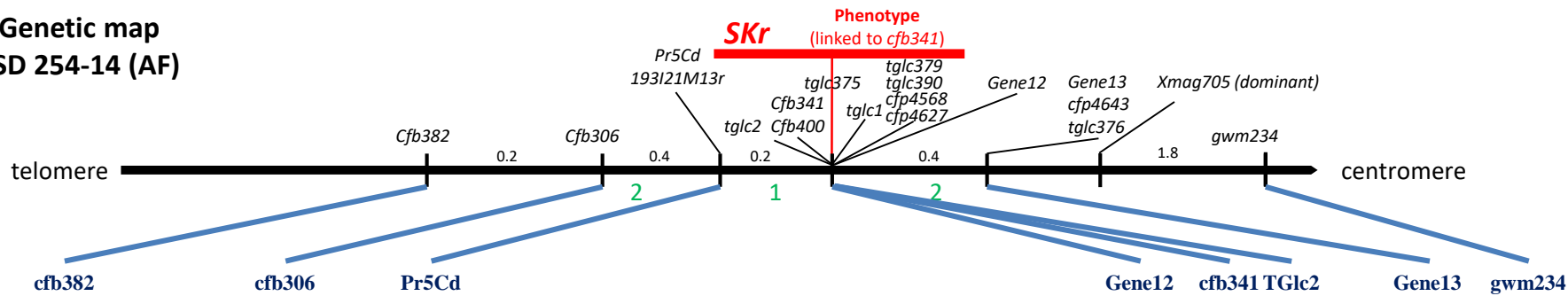
## Rice 12

# Chinese Spring physical map (V2)

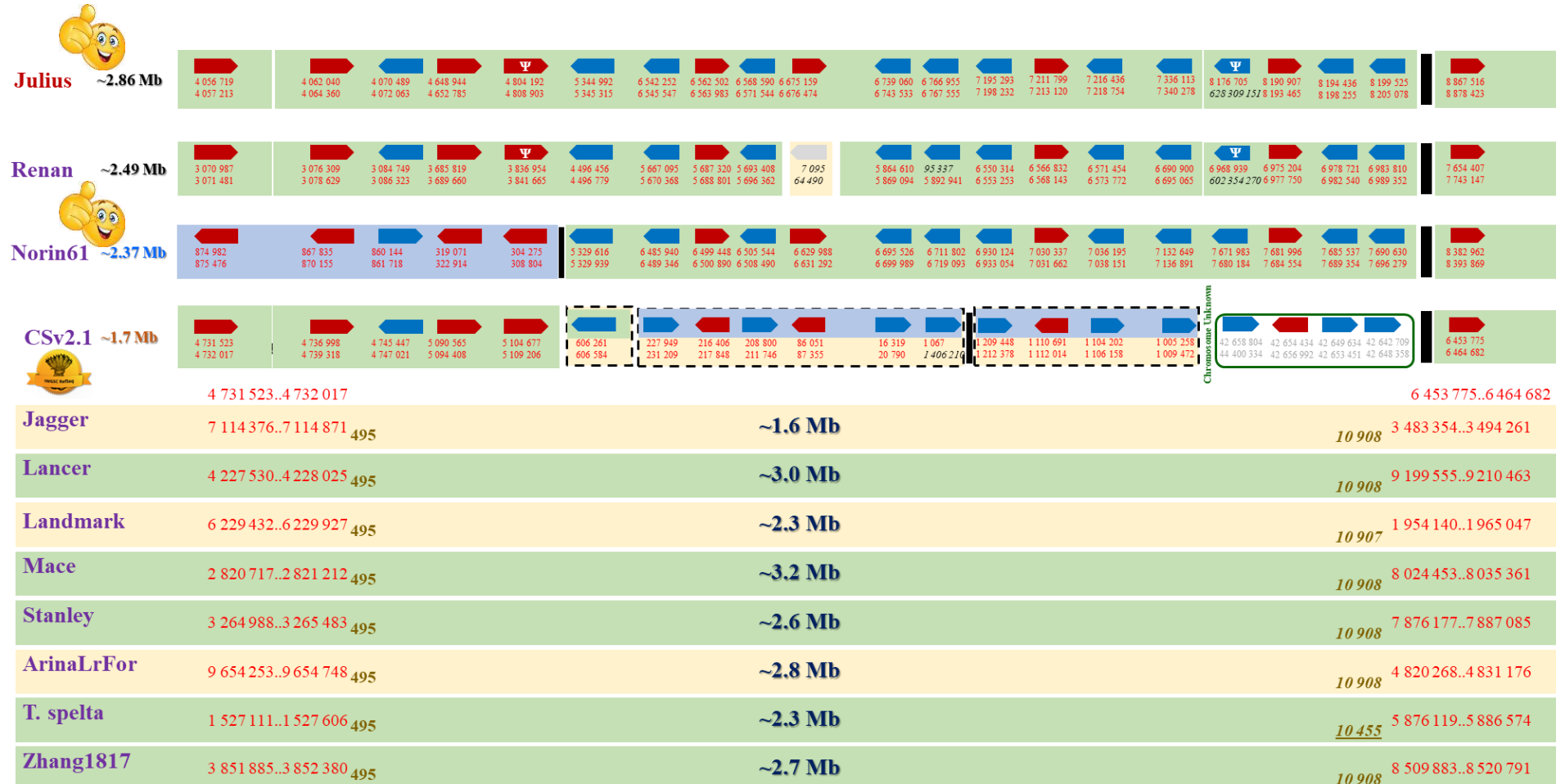


# Full sequence of the locus in four varieties

Genetic map  
SSD 254-14 (AF)



# Full sequence of the locus in 12 varieties





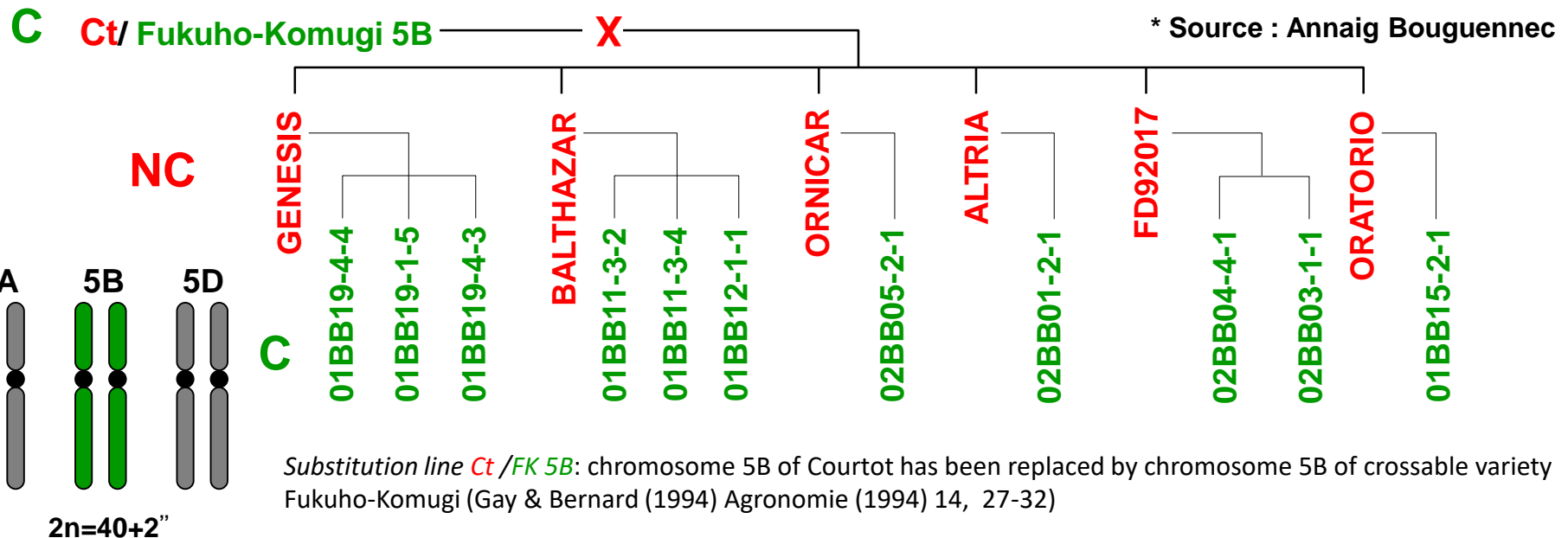


# Comparative analysis of the sequences

- Same gene content (16) between crossable and non crossable
  - Not presence/absence polymorphism
- Check for significant differences
  - Stop codon, splicing variants, mutants
  - Difference in expression
- Two high-quality & highly similar sequences
  - Julius (NC), Norin-61 (C) → new population?
- Functional validation
  - Knock-out
    - CRISPR/Cas9
    - VIGS
    - EMS
- Exploitation of results in breeding schemes

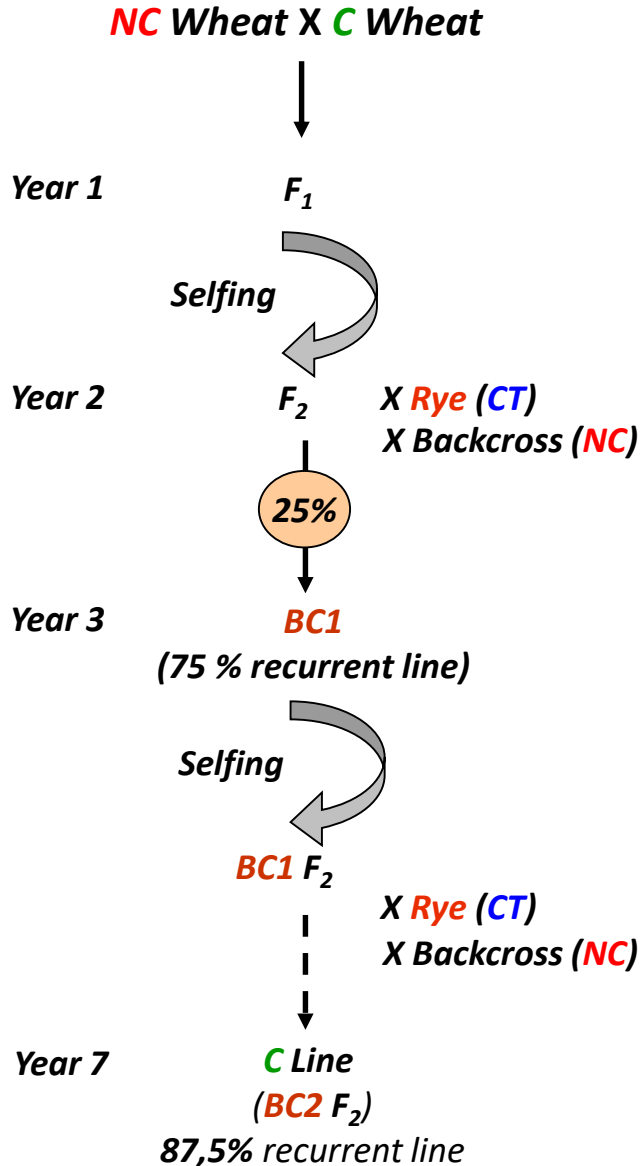
# Exploitation in breeding schemes

- Six **NC** French varieties x **C** substitution line  
= 11 progenies **C**



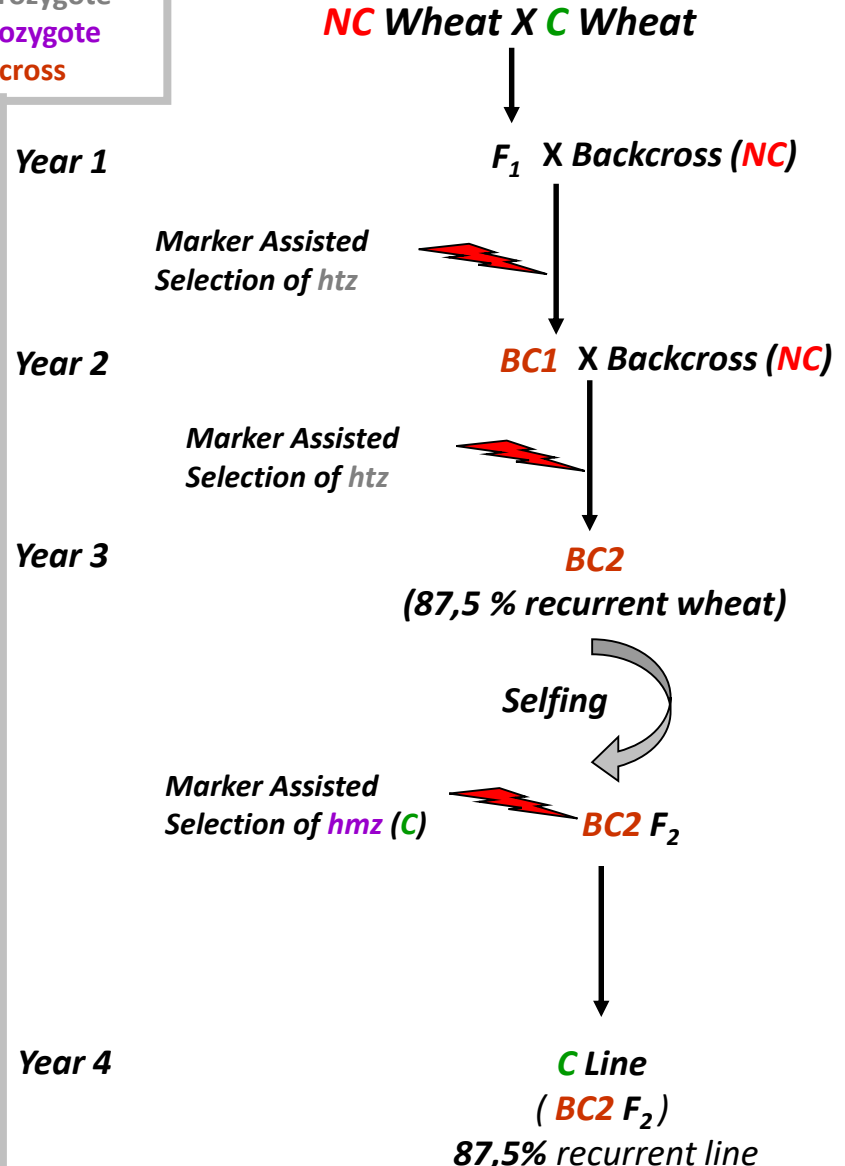
# Production of crossable lines

Without markers

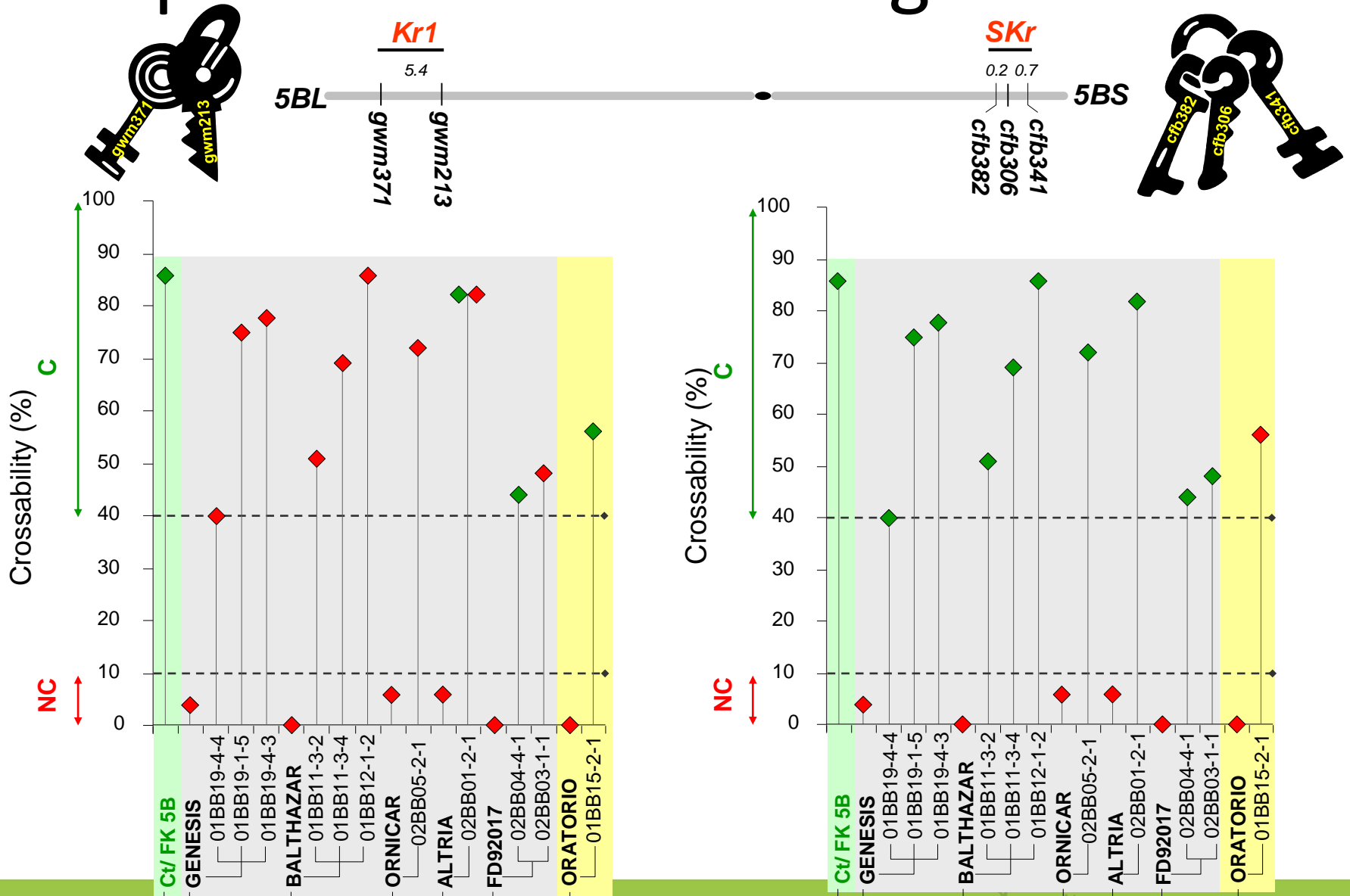


CT: crossability test  
 NC: non crossable  
 C: crossable  
 Htz: heterozygote  
 Hmz: homozygote  
 BC: backcross

With markers



# Exploitation in breeding schemes



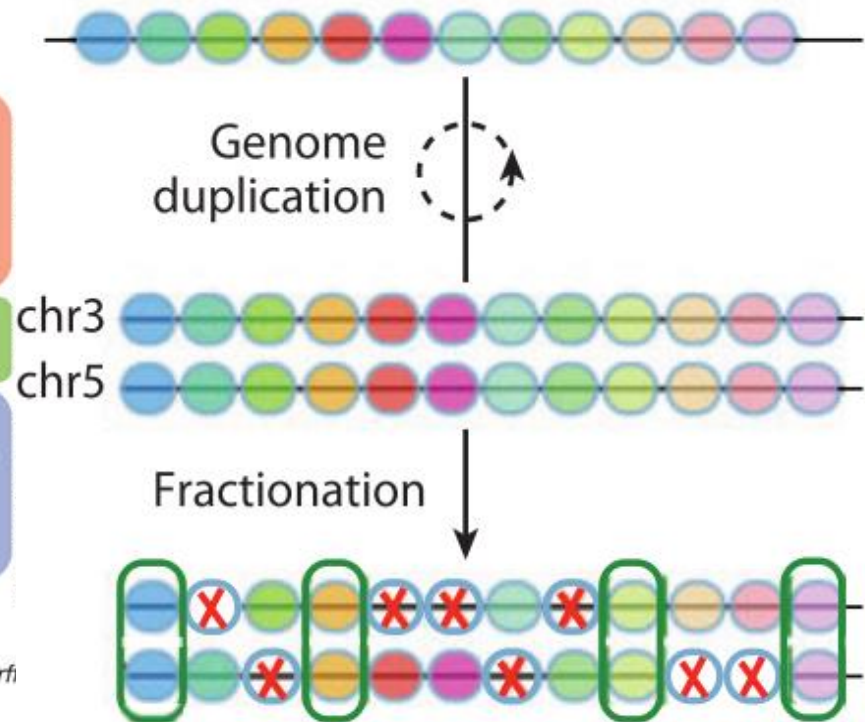
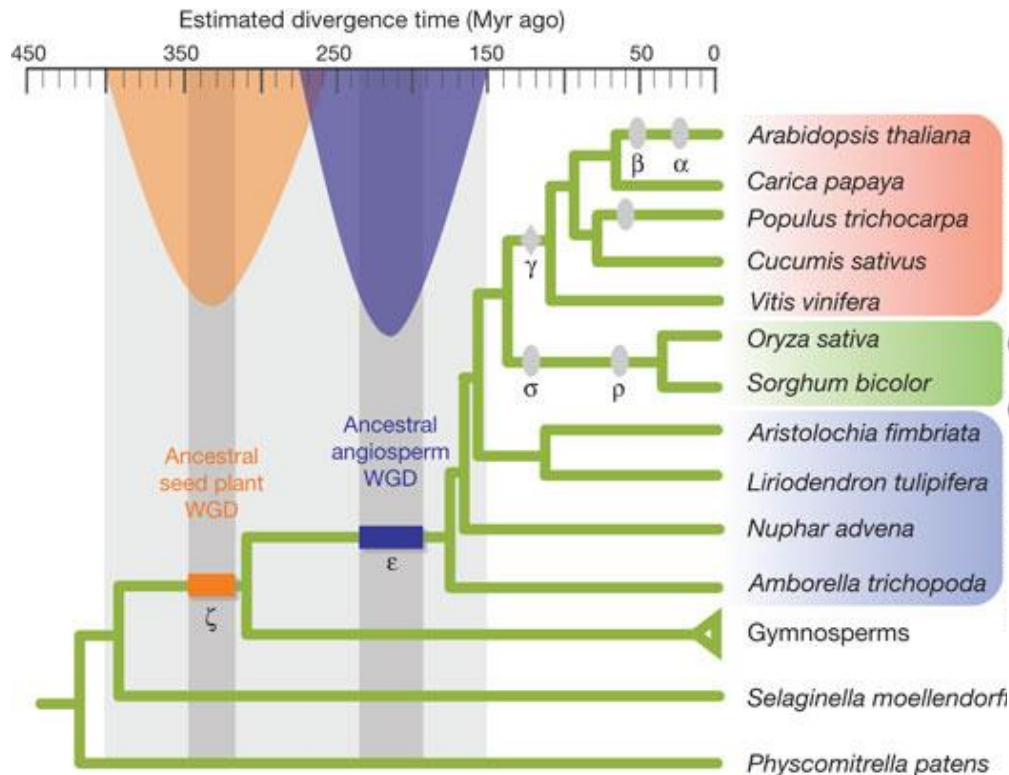


# Successful interspecific natural crosses: allopolyploids

- All angiosperme species have undergone (at least) two whole genome duplication (WGD) events
- They returned to diploid state during evolution (fractionation)

# Successful interspecific natural crosses: allopolyploids

Jiao *et al.* 2011; Freeling (2009)



# Successful interspecific natural crosses: allopolyploids

- ~30% of actual species are polyploids



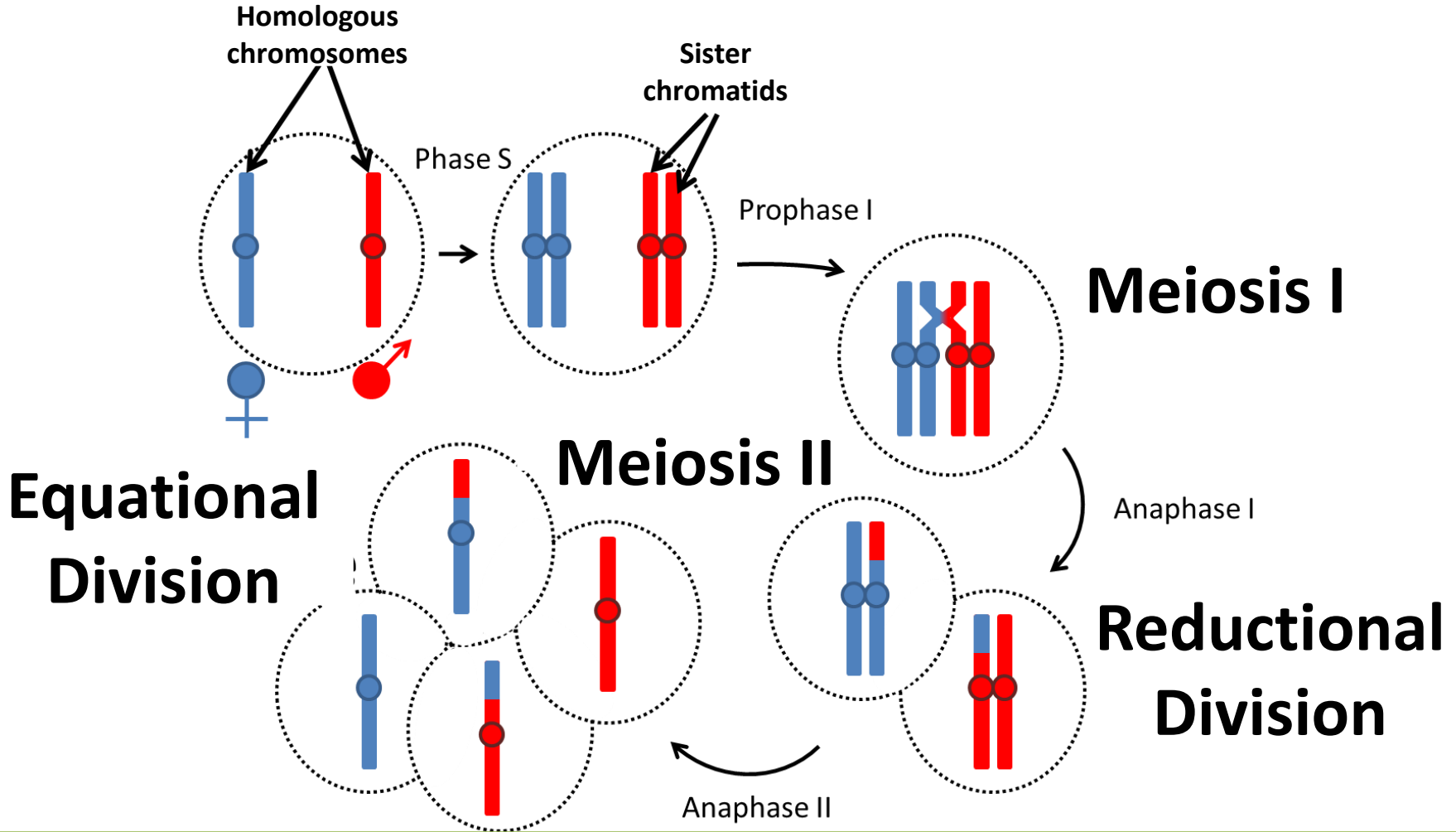
- Autopolyploids
  - Duplication of its own genome
- Allopolyploids
  - Combination of different genomes

# How the genome can duplicate?

- A diploid individual ← fusion of two (normal) haploid gametes
- A triploid individual ← fusion of one haploid gamete and one diploid gamete
- A tetraploid individual (doubling of the genome) ← fusion of two diploid gametes

**Gametes ← Meiosis**

# Progression of meiosis



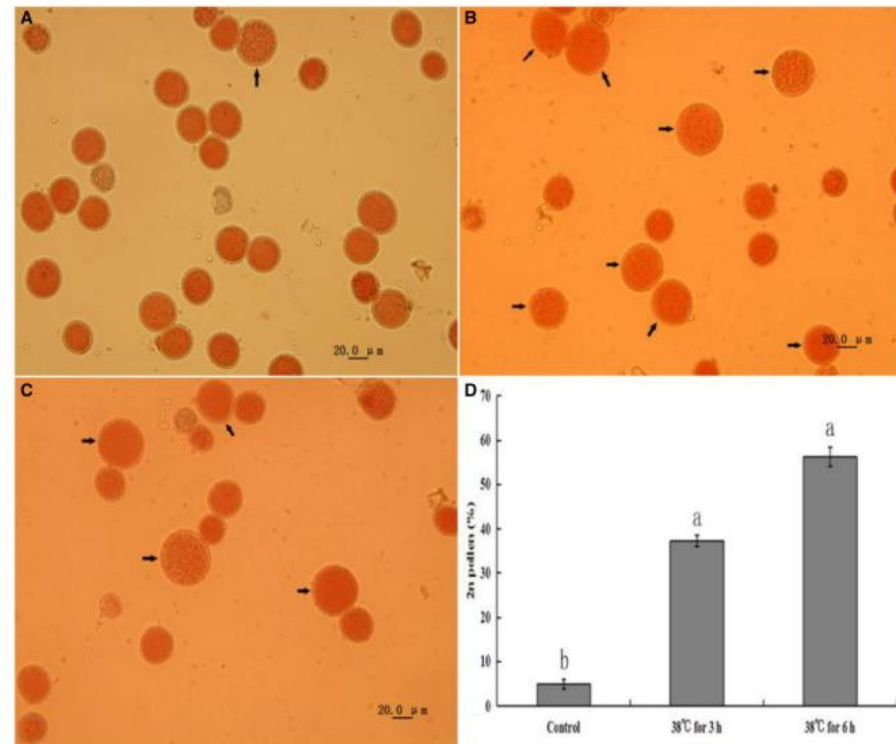


# Production of unreduced gametes

- Omission of the first or second division
- Mutation of meiotic genes → disruption of divisions = unreduced gametes
  - ♂ or ♀
  - Usually not 100% of gametes
  - Autogamous species = di-, tri- or tetraploids (autopolyploids)
  - Allogamous species = di-, tri- or tetraploids (allopolyploids)
  - Low fertility of mutants (1-10 seeds in At)
- Examples: DYAD, TAM, OSD1

# Production of unreduced gametes

- Omission of the first or second division
- External factors
  - High temperature
    - Time + intensity
  - Chemical treatments
    - Colchicine (not natural)



# Problems linked to the natural creation of polyploids

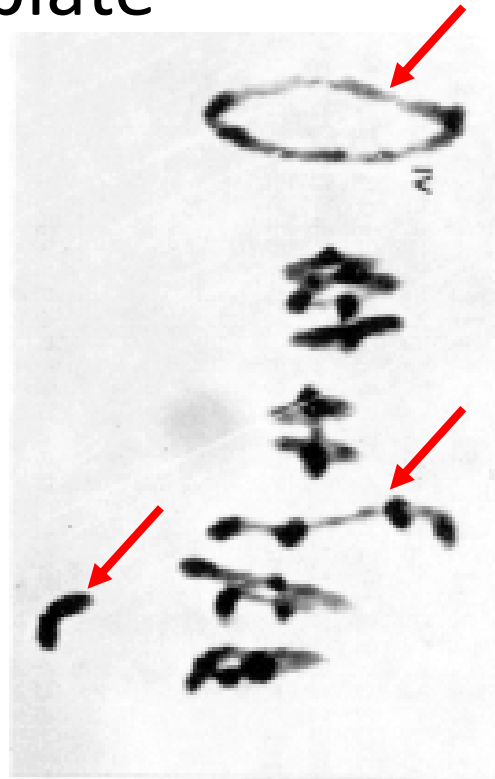
- First generation of polyploids = low fertility → loss of selective advantage = elimination through natural selection!
- Why fertility is lost?

# Disrupted meiosis in neopolyploids

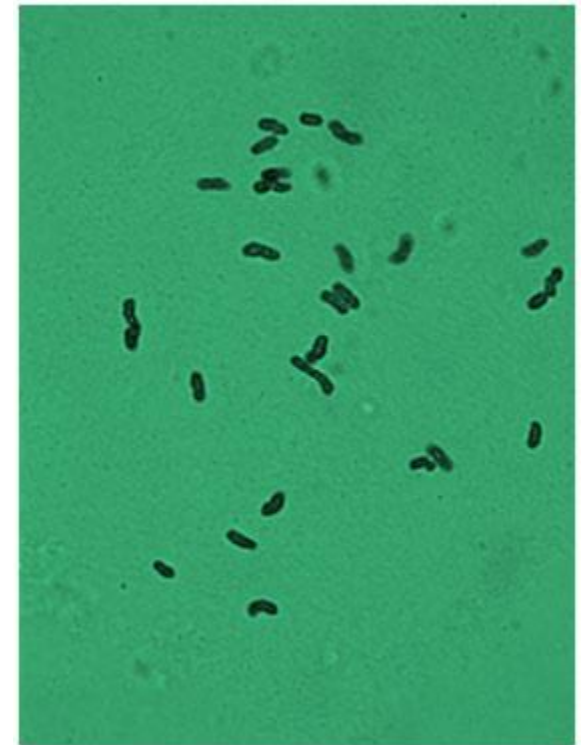
- Metaphase I plate



2X barley



4X barley



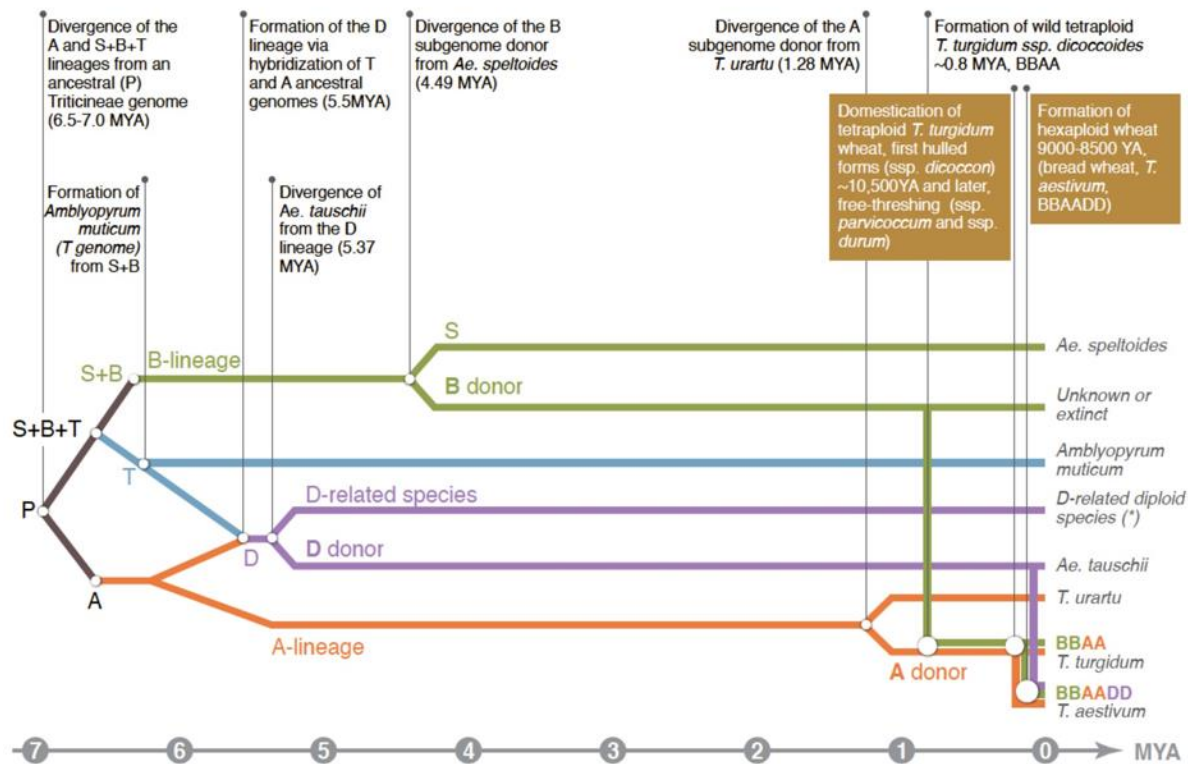
Primary Triticale ABDR

# Disrupted meiosis in neopolyploids

- To remain fertile:
  - Diploid gametes
  - Rapid return to a more stable state
    - “Diploidisation” → balanced number of chromosomes
    - Control of chromosome pairing:
      - Between homologues (autopolyploids)
      - Between related chromosomes (homoeologues; allopolyploids)

# Control of chromosome pairing in hexaploid wheat

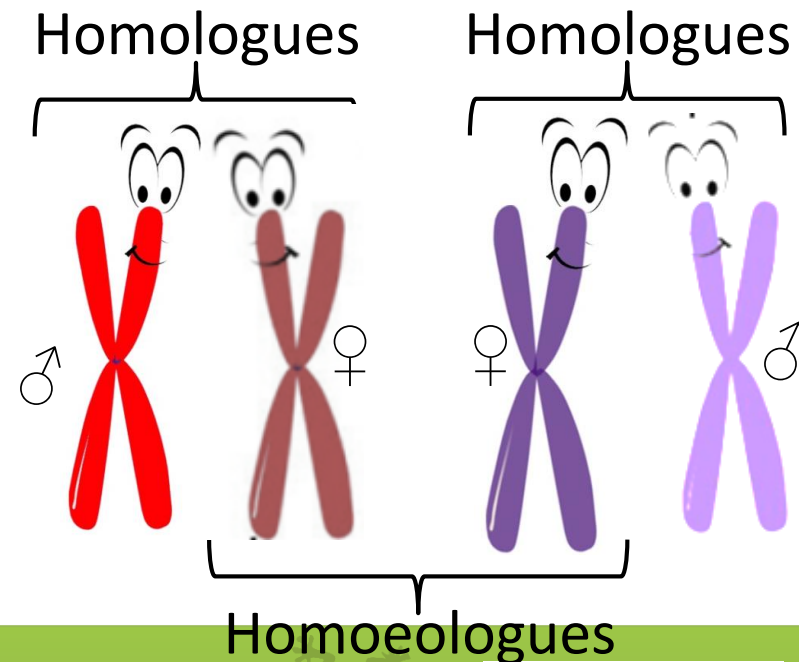
- Bread wheat, a recent allopolyploid





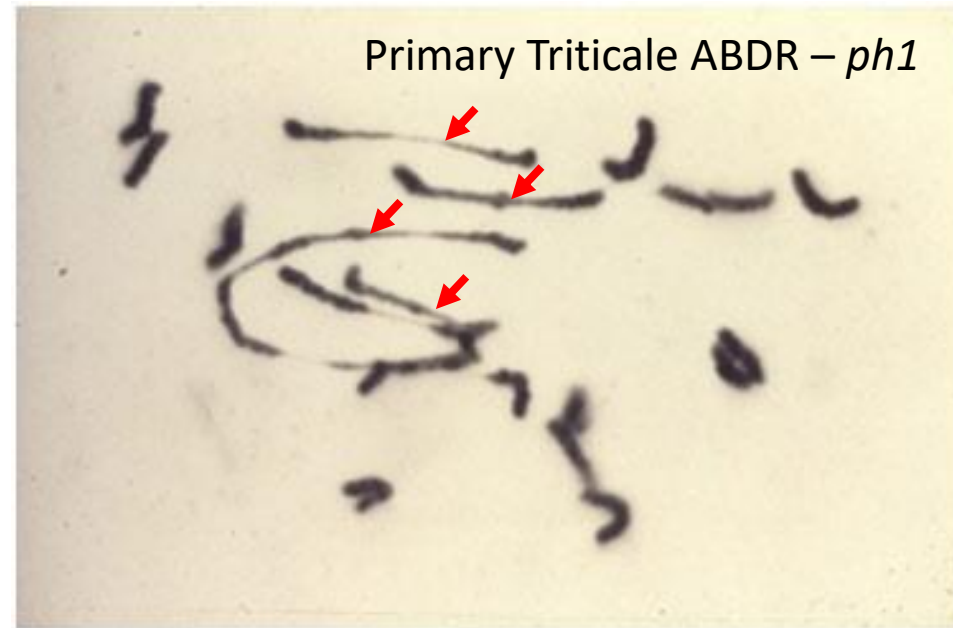
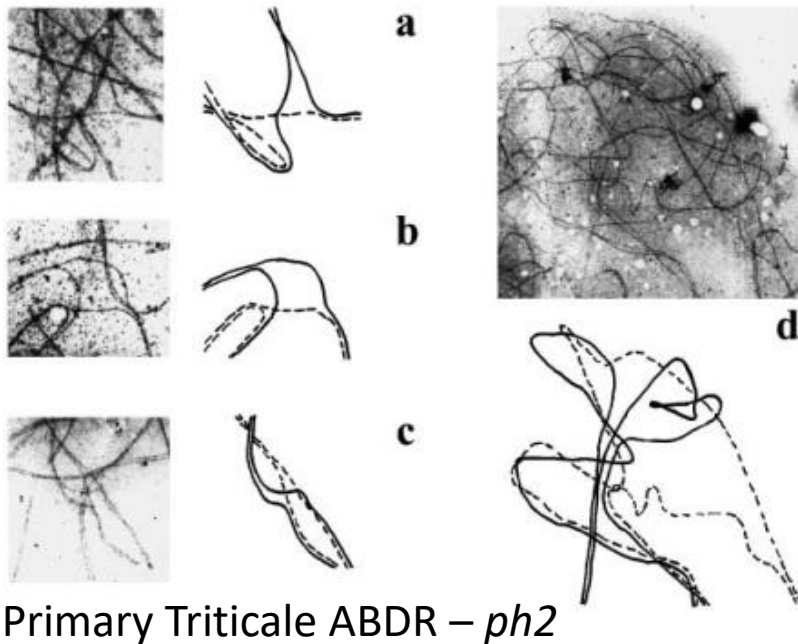
# Control of chromosome pairing in hexaploid wheat

- Hexaploid :
  - Three highly similar genomes = homoeologues
  - Same gene content & order
  - Divergence = TE
- Pairing controle
  - Two levels
    - Homologues
    - Homoeologues



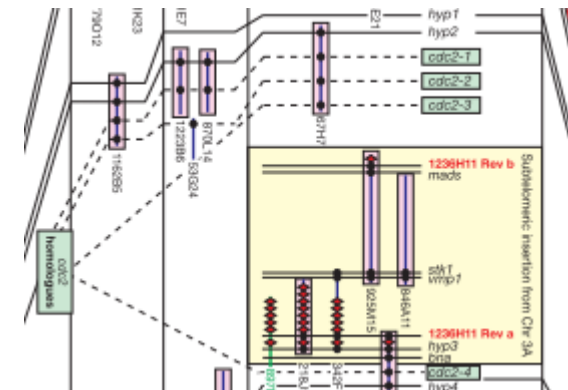
# Control of chromosome pairing in hexaploid wheat

- One major locus: *Ph1*
- Minor genes (*Ph2*)



# Control of chromosome pairing in hexaploid wheat

- One major locus: *Ph1*
  - On chromosome 5BL (Riley & Chapman 1958)
  - Cloning (Griffiths et al. 2006)
    - Complex of 4 *CDK2-L*
    - Heterochromatin bloc (3AL)
      - TaZIP4 (Rey et al. 2017)
  - Role of *Ph1*:
    - More visible in hybrids (wheat/relatives; wheat/rye)
    - Favours homologous pairing
    - Block *MLH1* between homoeologues



# Control of chromosome pairing in hexaploid wheat

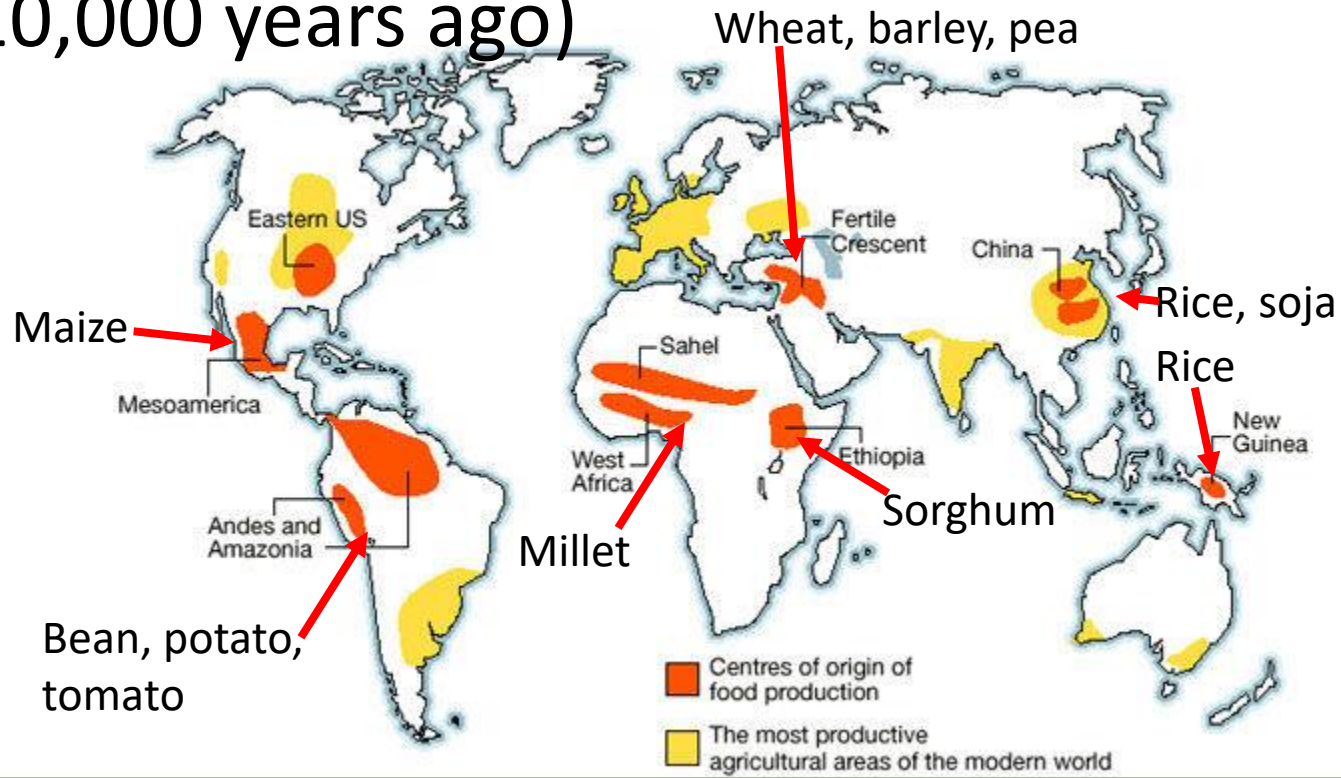
- In case of mutation of the locus (*ph1*)
  - In hexaploid
    - Homoeologues can pair
    - Recombination events (CO) may occur
    - But rarely
  - In wheat x relatives haploid hybrids
    - Homoeologues can pair
    - Recombination events (CO) may occur
    - More frequent phenomenon = translocations or introgressions

# Interest of introgressions

- Stress resistance (tolerance)
  - Biotic stresses (diseases, pests)
  - Abiotic stresses (drought, temperature, salinity)
  - Other traits

# Interest of introgressions

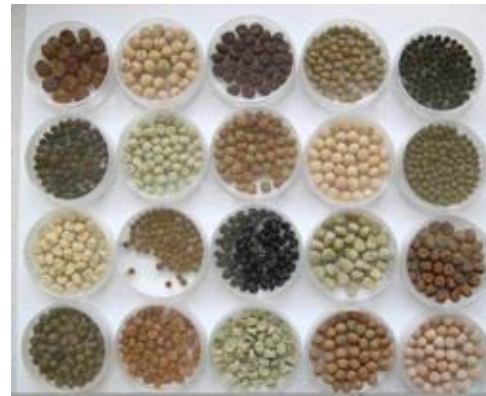
- Crop improvement starts with sedentarisation (10,000 years ago)





# Interest of introgressions

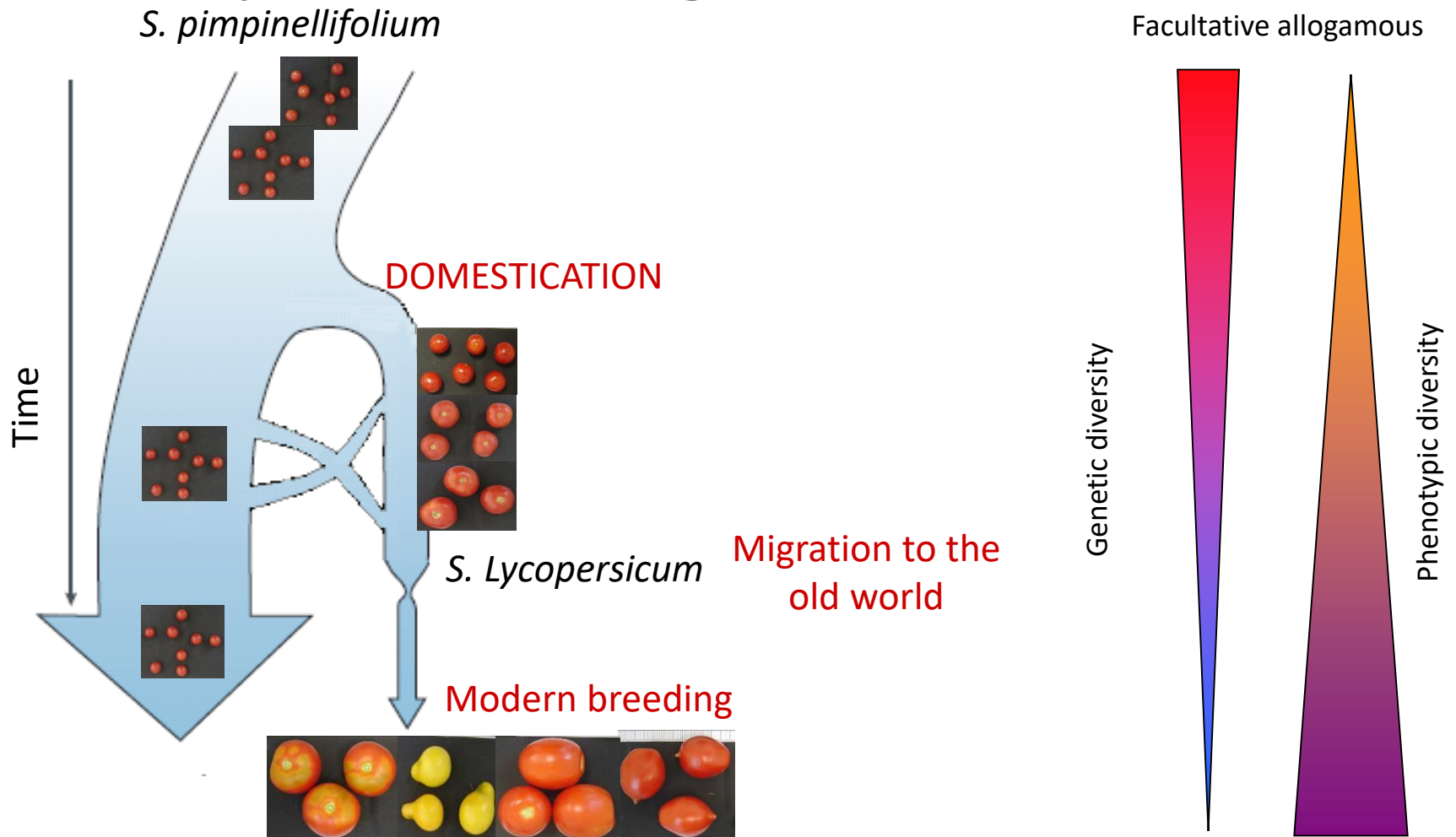
- Search for new diversity



# Interest of introgressions

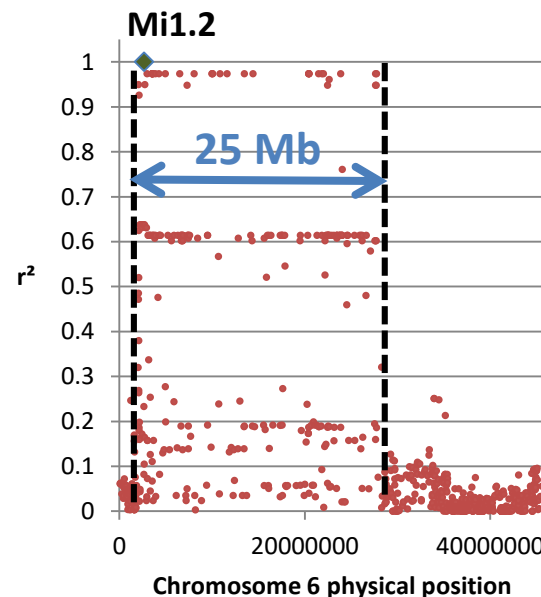
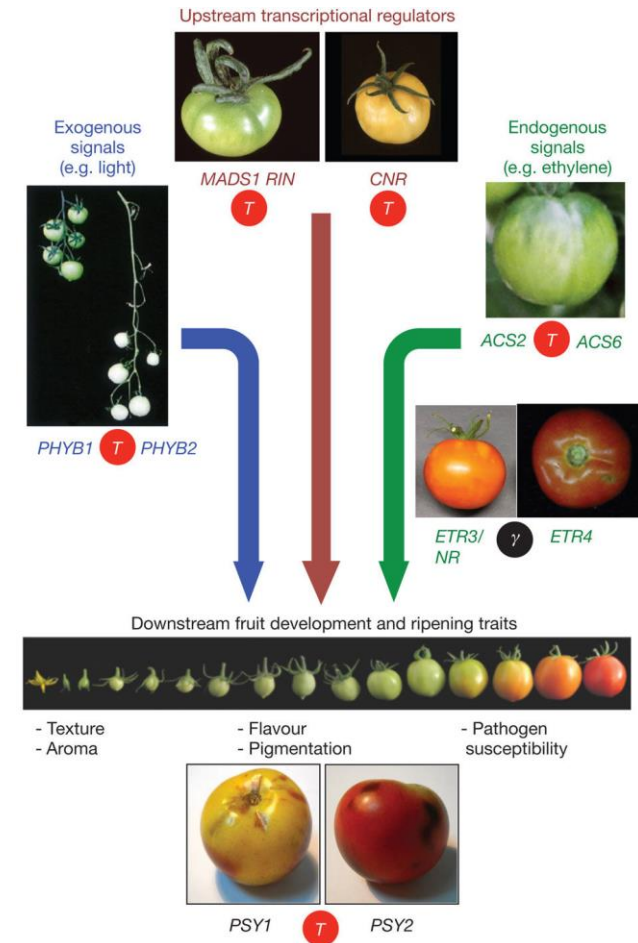
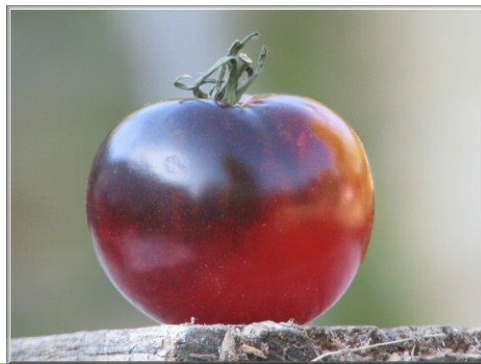
- Search for diversity
- Crosses between elites and wild relatives
- Identification of relevant individuals (resistance, color, taste...)
- Backcross to elite

# Examples of introgressions in tomato



# Examples of introgressions in tomato

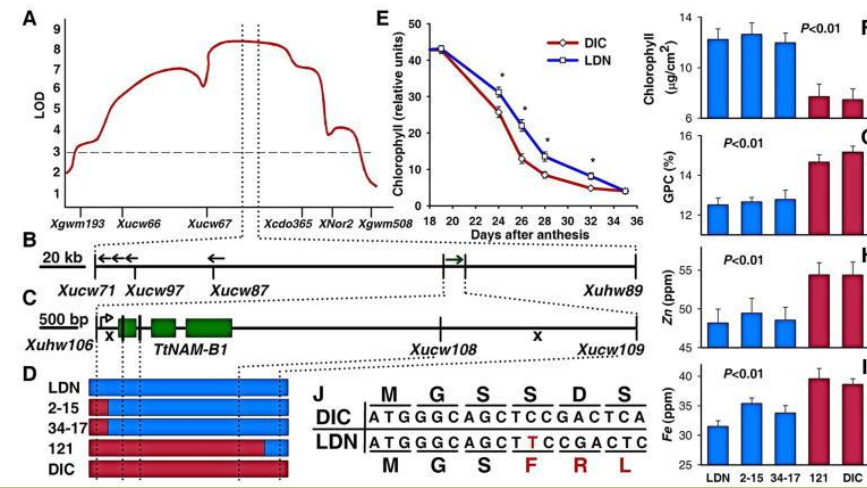
- Fruit colour
  - X blue wild x T cocktail
- Fruit maturation
  - Without ethylen
- Resistance





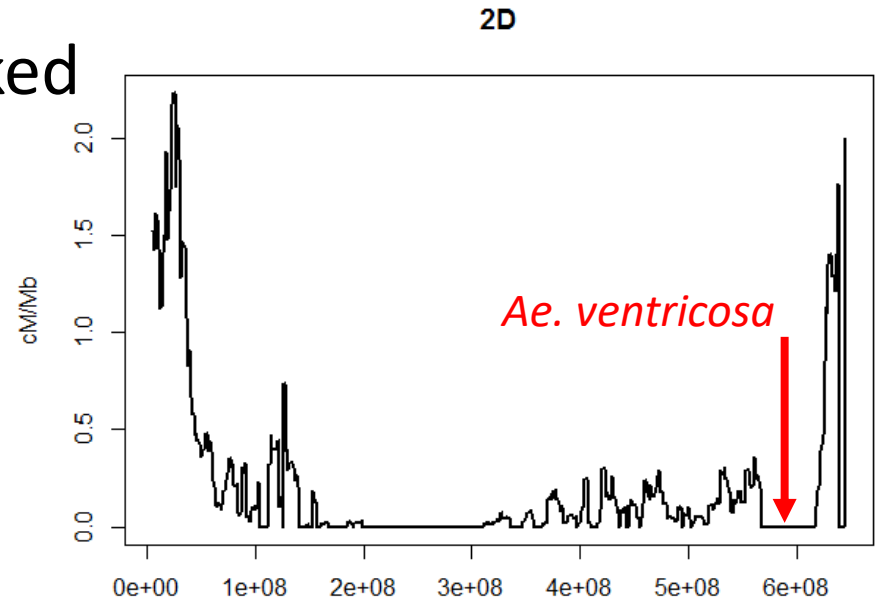
# Examples of introgressions in wheat

- Disease resistances
  - Eye-spot, downy mildew, powdery mildew, septoria, fusarium
  - *Aegilops* (*ventricosa* & others), *T. tauschii* (synthetics)
- Hardiness & resistance
  - Translocation 1RS/1BL
- Protein content, Zn, Fe



# Limites of introgressions

- Linkage drag
  - Deleterious traits together with traits of interest (e.g. yield loss and eye-spot resistance)
  - Recombination is blocked





# Takehome messages

- There is clearly an interest in crossing diverse species to improve variability in the species of interest
- Several barriers limit interspecific crosses
- Some of these barriers maybe overcome because of mutation of key genes
- Unlocking these barriers leads to breeding effects
  - Loss of fertility
  - Loss of recombination
    - Genes controlling chromosome pairing and homoeologous recombination may be engineered as well
- Introgressions could be favourable but ...
- They could lead to linkage drag