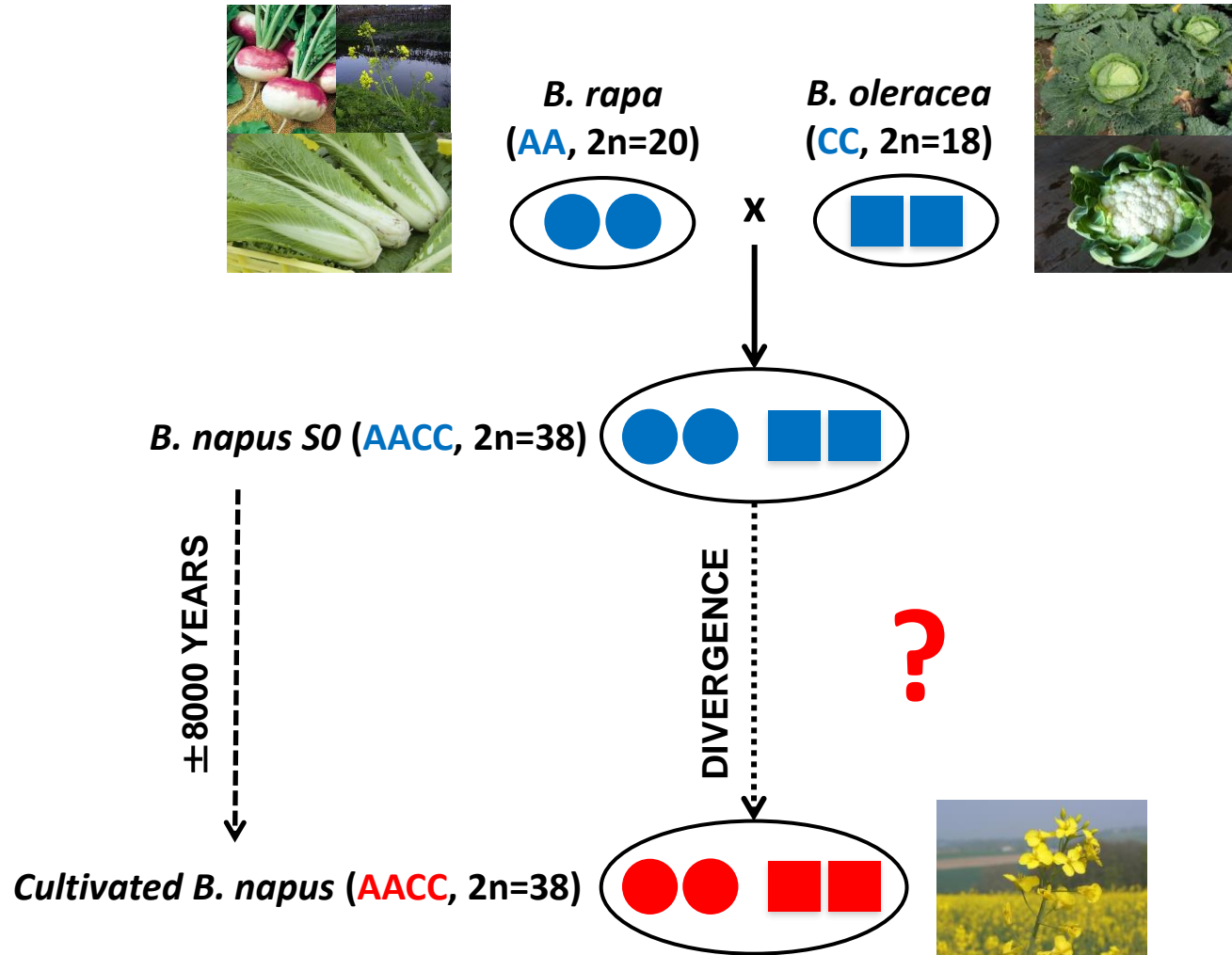


# Can a poor lonesome A genome of *Brassica napus* survive at diploid stage?

**Alexandre Pelé**<sup>1</sup>, Gwenn Trotoux<sup>1</sup>, Frédérique Eber<sup>1</sup>, Sylvie Negre<sup>1</sup>, Jérôme Morice<sup>1</sup>, Maryse Lodé-Taburel<sup>1</sup>, Marie-Madeleine Gilet<sup>1</sup>, Cyril Falentin<sup>1</sup>, Mathieu Rousseau-Gueutin<sup>1</sup>, Anne-Marie Chèvre<sup>1</sup>

<sup>1</sup>IGEPP, INRA, Agrocampus-Ouest, Université de Rennes 1, Le Rheu, France

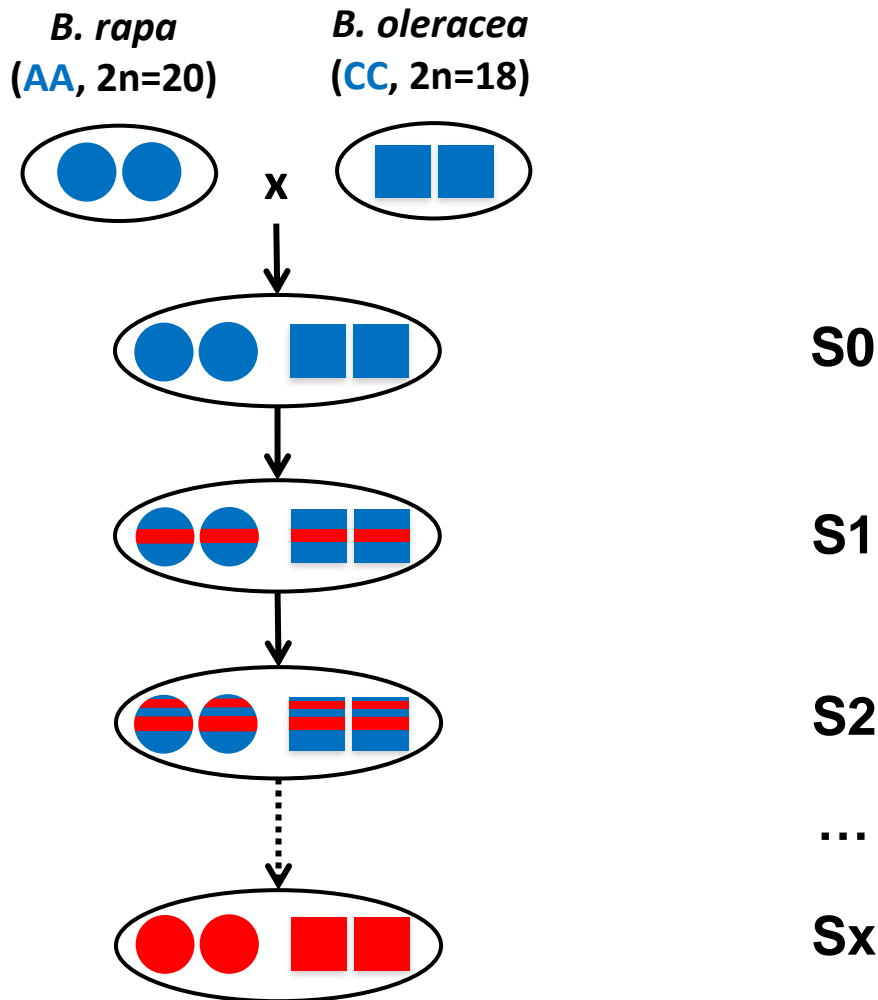
# Divergence occurred between *B. napus* formation and cultivated *B. napus*



# How can we explain the stabilization of *B. napus*?

## 2 Ways

### Newly Formed Allopolyploids



Cultivated *B. napus* (AACC, 2n=38)

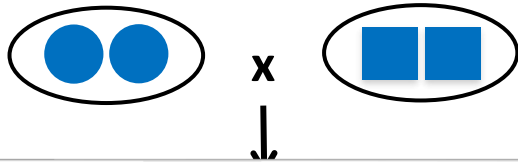
# How can we explain the stabilization of *B. napus*?

## 2 Ways

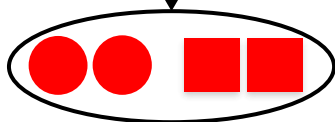
### Newly Formed Allopolyploids

*B. rapa*  
(AA, 2n=20)

*B. oleracea*  
(CC, 2n=18)



Formation and stabilization from  
diploid species



S0

S1

S2

...

Sx

Cultivated *B. napus* (AACC, 2n=38)

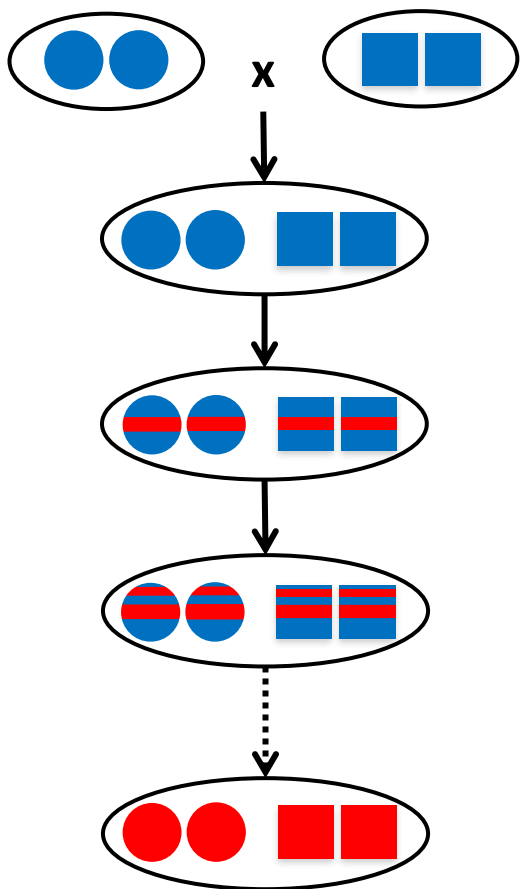
# How can we explain the stabilization of *B. napus*?

## 2 Ways

### Newly Formed Allopolyploids

*B. rapa*  
(AA, 2n=20)

*B. oleracea*  
(CC, 2n=18)



### S0 Functional & Epigenetics Changes

(Lukens *et al.*, 2006; Albertin *et al.* 2006; Gaeta *et al.*, 2007 ; Xu *et al.*, 2009)

### S1 Burst of Genetic Changes

(Song *et al.*, 1995; Gaeta *et al.*, 2007 ; Szadkowski *et al.*, 2010;2011; Xiong *et al.*, 2011)

S2

...

Sx

Cultivated *B. napus* (AACC, 2n=38)

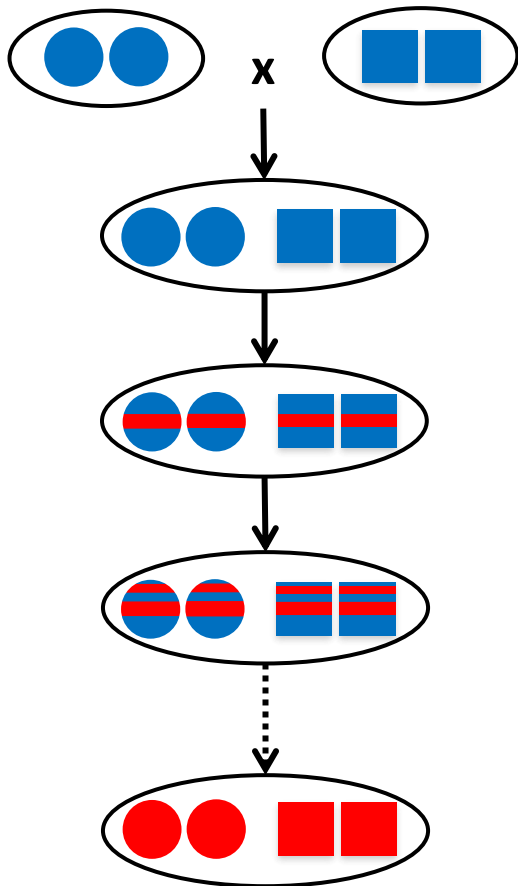
# How can we explain the stabilization of *B. napus*?

## 2 Ways

### Newly Formed Allopolyploids

*B. rapa*  
(AA, 2n=20)

*B. oleracea*  
(CC, 2n=18)

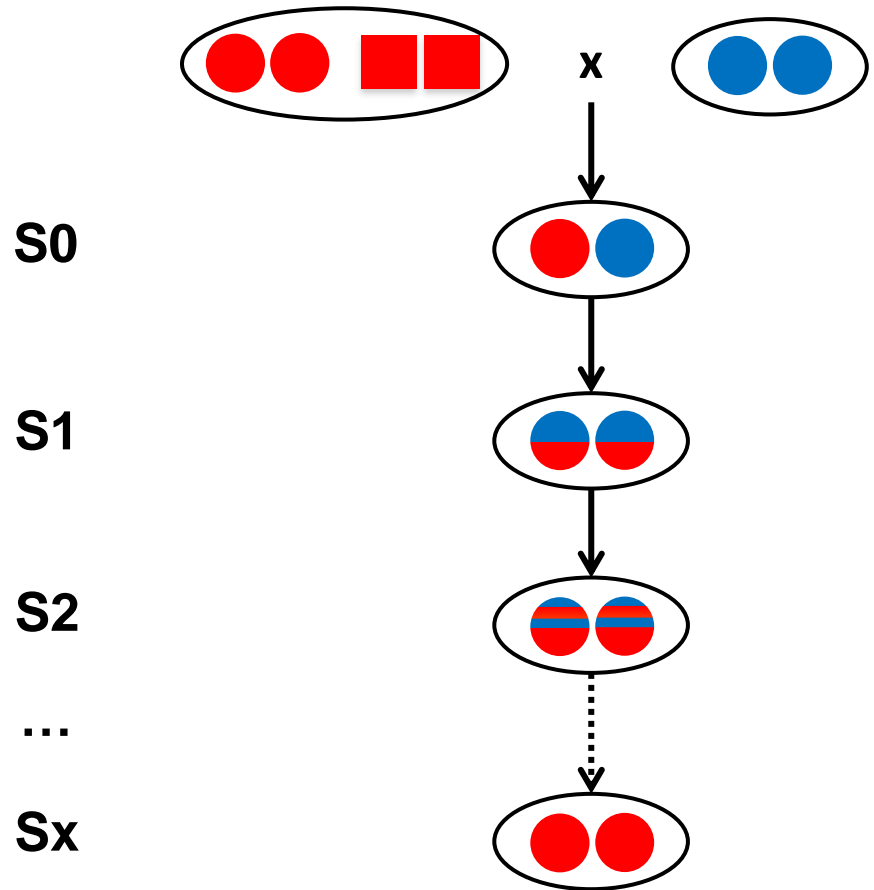


*Cultivated B. napus* (AACC, 2n=38)

### Subgenome Extraction

*Cultivated B. napus*  
(AACC, 2n=38)

*B. rapa*  
(AA, 2n=20)



S0

S1

S2

...

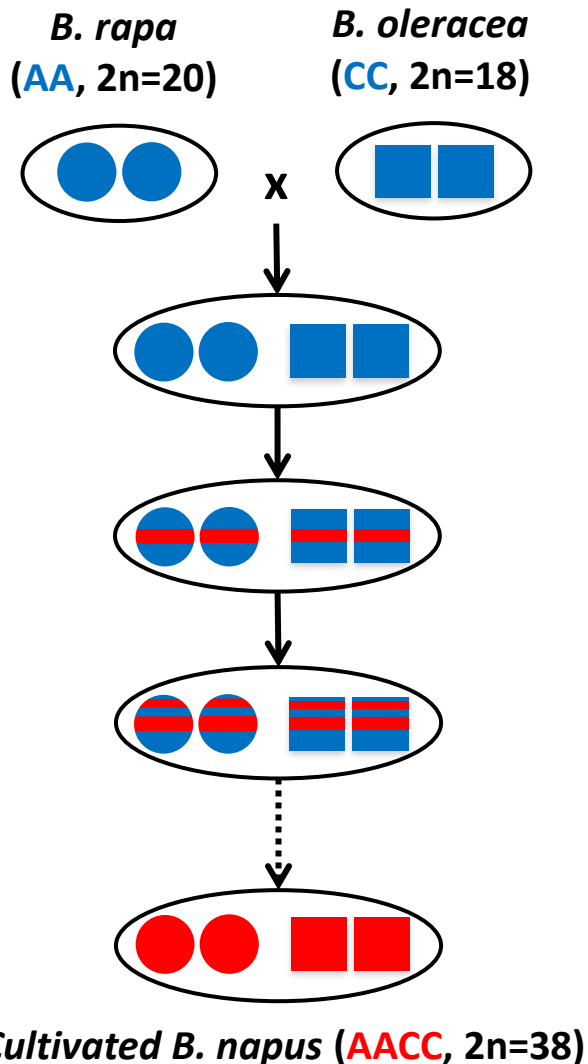
Sx

*Extracted A genome* (AA, 2n=20)

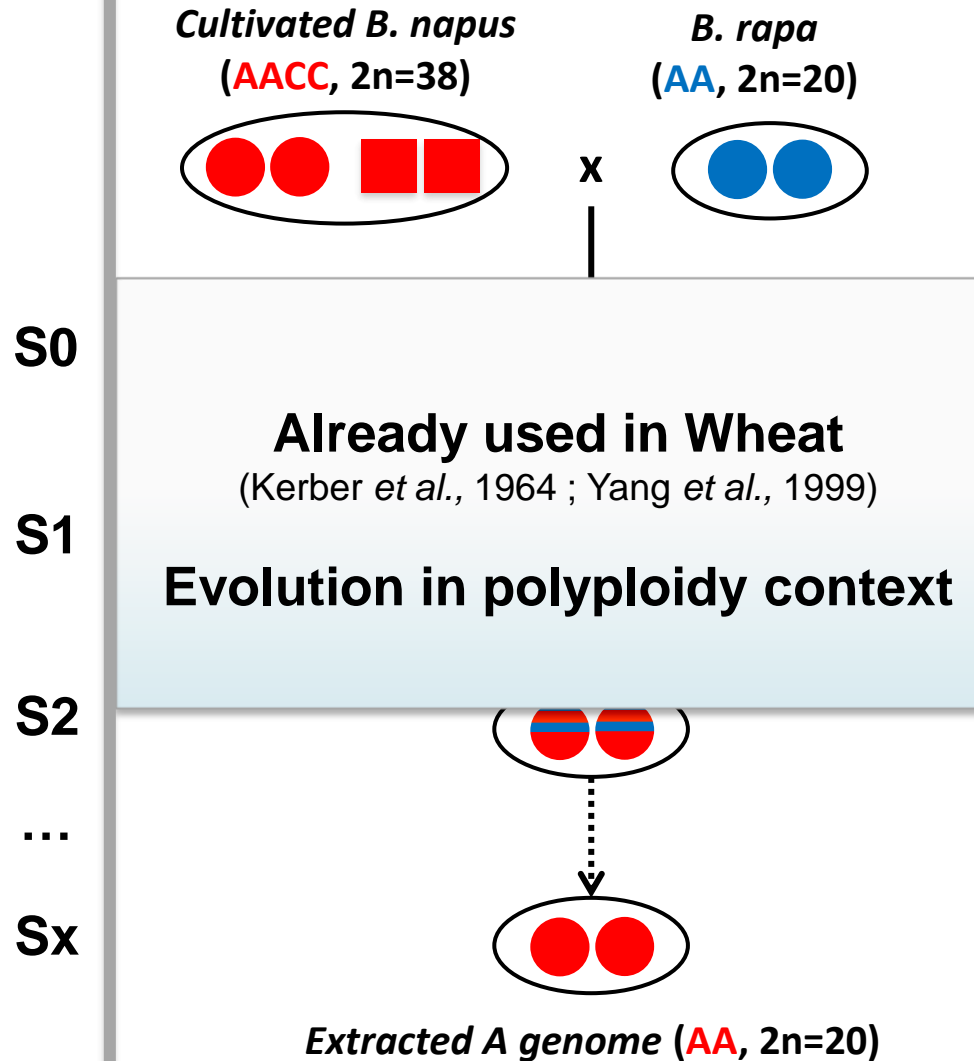
# How can we explain the stabilization of *B. napus*?

## 2 Ways

### Newly Formed Allopolyploids



### Subgenome Extraction



# Extraction of *B. napus* A Subgenome

## Strategy 1

*B. rapa* cv. C1.3  
(AA, 2n=20)



*B. napus* cv. Darmor  
(AACC, 2n=38)



X

AAC  
(2n=29)



50%

Theoretical %  
of A Darmor  
in AAC hybrids



# Extraction of *B. napus* A Subgenome

## Strategy 1

*B. rapa* cv. C1.3  
(AA, 2n=20)



X

AAC  
(2n=29)

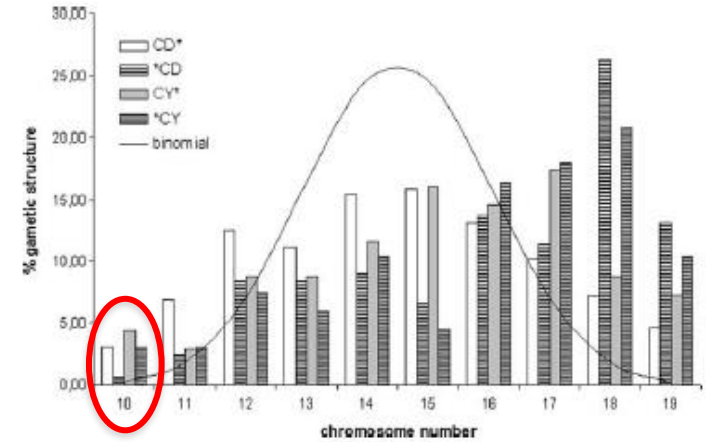


X

75%



*B. napus* cv. Darmor  
(AACC, 2n=38)



Leflon *et al.*, 2006

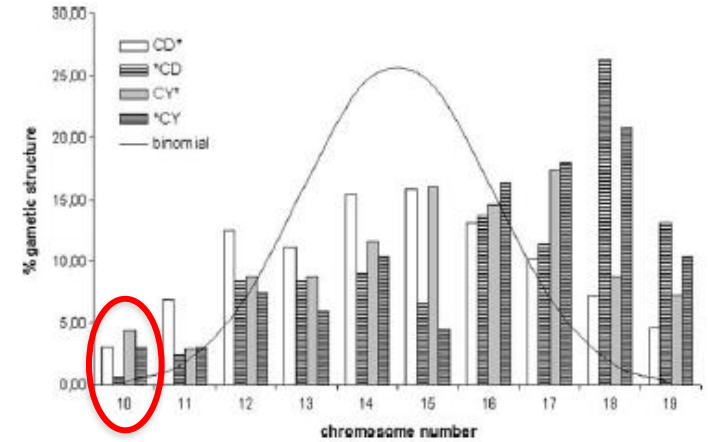
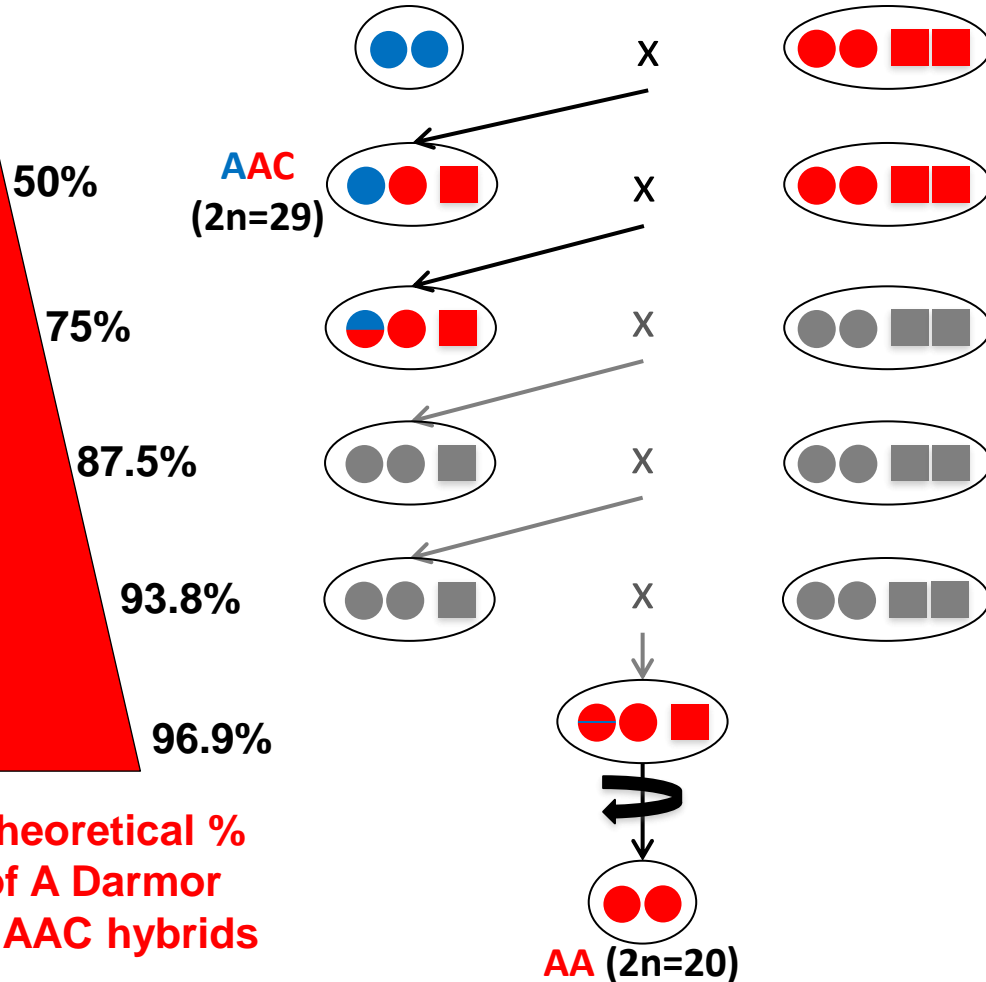
Theoretical %  
of A Darmor  
in AAC hybrids

# Extraction of *B. napus* A Subgenome

## Strategy 1

*B. rapa* cv. C1.3  
(AA, 2n=20)

*B. napus* cv. Darmor  
(AACC, 2n=38)



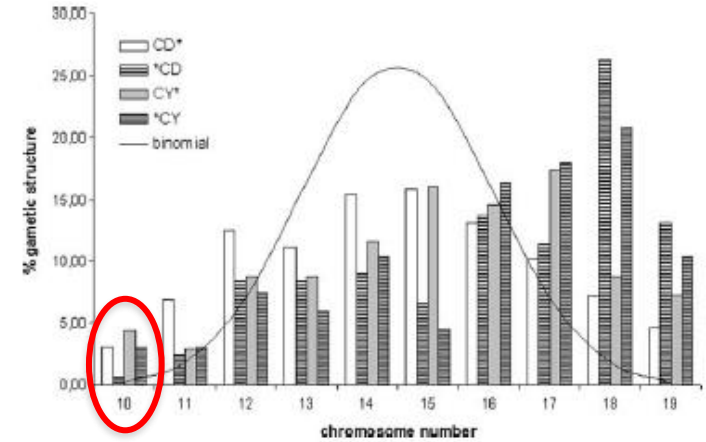
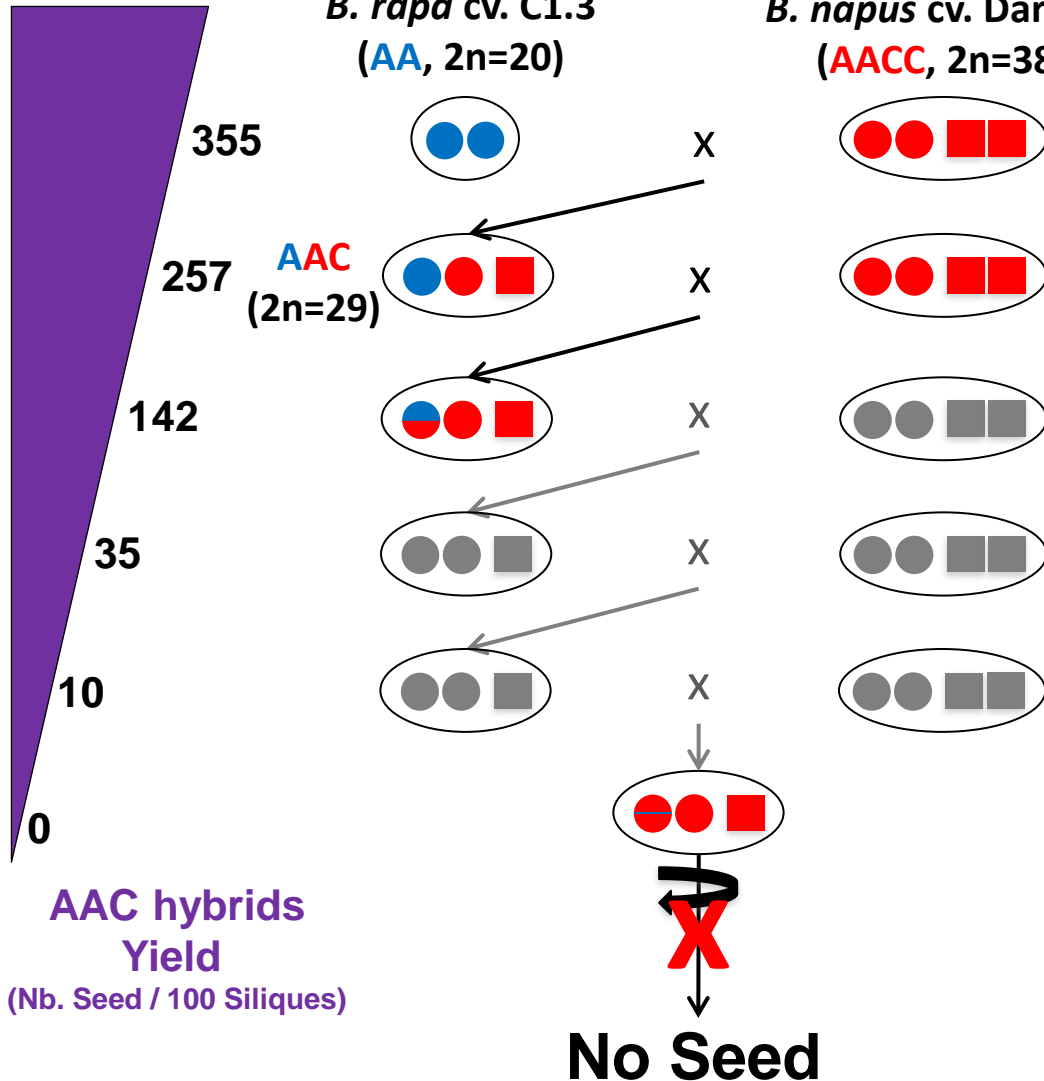
Leflon *et al.*, 2006

# Extraction of *B. napus* A Subgenome

## Strategy 1

*B. rapa* cv. C1.3  
(AA, 2n=20)

*B. napus* cv. Darmor  
(AACC, 2n=38)



Leflon *et al.*, 2006

# Extraction of *B. napus* A Subgenome

## Strategy 1

*B. rapa* cv. C1.3  
(AA, 2n=20)



*B. napus* cv. Darmor  
(AACC, 2n=38)



355

X



Genome part of *B. rapa* could be necessary for plant survive at diploid stage

0

AAC hybrids

Yield

(Nb. Seed / 100 Siliques)

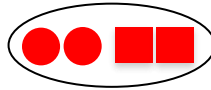


No Seed

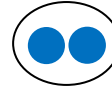
# Extraction of *B. napus* A Subgenome

## Strategy 2

*B. napus* cv. Darmor  
(AAC, 2n=38)



*B. rapa* cv. C1.3  
(AA, 2n=20)



x

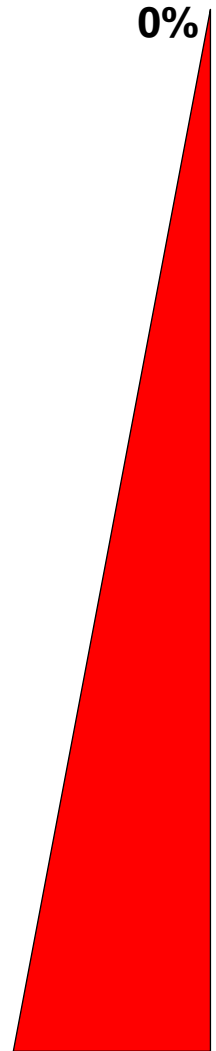
Cycle 1

AAC (2n=29)



Theoretical %  
of A Darmor  
in AA hybrids

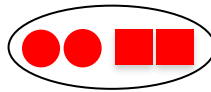
0%



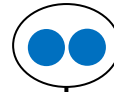
# Extraction of *B. napus* A Subgenome

## Strategy 2

*B. napus* cv. Darmor  
(AACC, 2n=38)



*B. rapa* cv. C1.3  
(AA, 2n=20)



Cycle 1

AAC (2n=29)



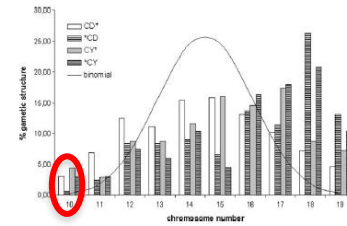
X



X



1<sup>st</sup> Generation  
AA (2n=20)

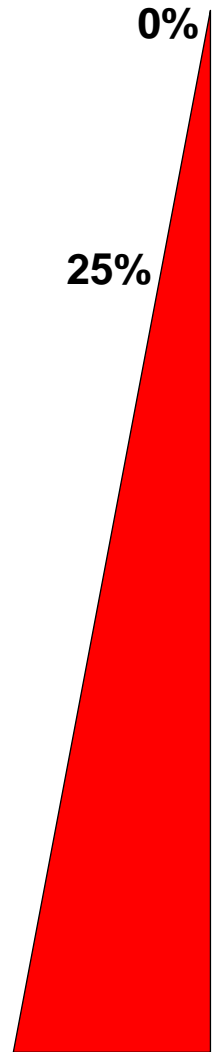


Leflon et al., 2006

Theoretical %  
of A Darmor  
in AA hybrids

0%

25%



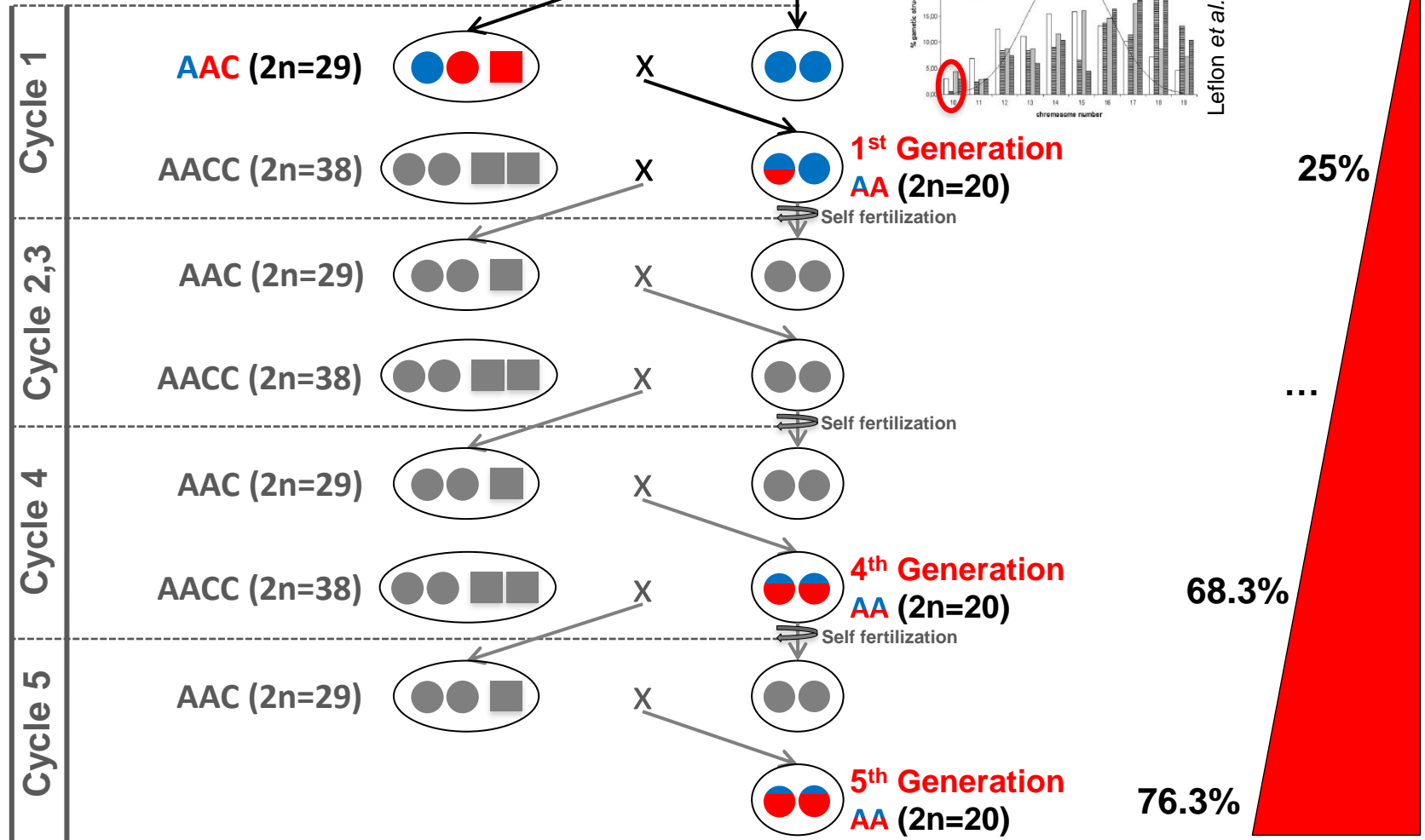
# Extraction of *B. napus* A Subgenome

## Strategy 2

*B. napus* cv. Darmor  
(AACC, 2n=38)

*B. rapa* cv. C1.3  
(AA, 2n=20)

Theoretical %  
of A Darmor  
in AA hybrids



# Extraction of *B. napus* A Subgenome

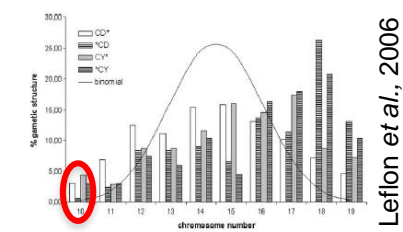
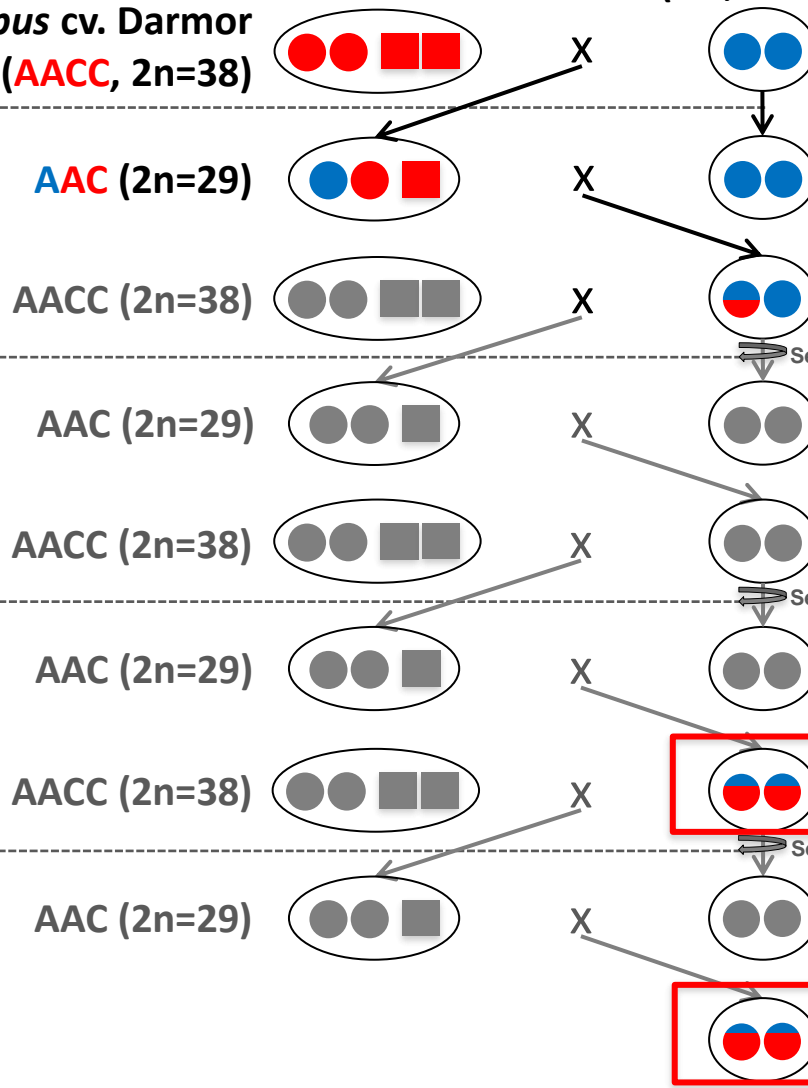
## Strategy 2

*B. napus* cv. Darmor  
(AACC, 2n=38)

*B. rapa* cv. C1.3  
(AA, 2n=20)

Theoretical %  
of A Darmor  
in AA hybrids

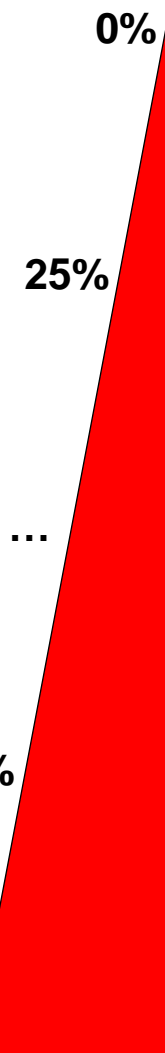
Cycle 1  
Cycle 2,3  
Cycle 4  
Cycle 5



1<sup>st</sup> Generation  
AA (2n=20)

4<sup>th</sup> Generation  
AA (2n=20)

5<sup>th</sup> Generation  
AA (2n=20)



1 plant / >100

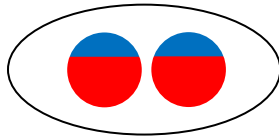
2 plant / >100



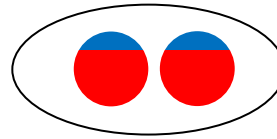
# Cytogenetic characterization of the last three plants $2n=20$

## Strategy 2

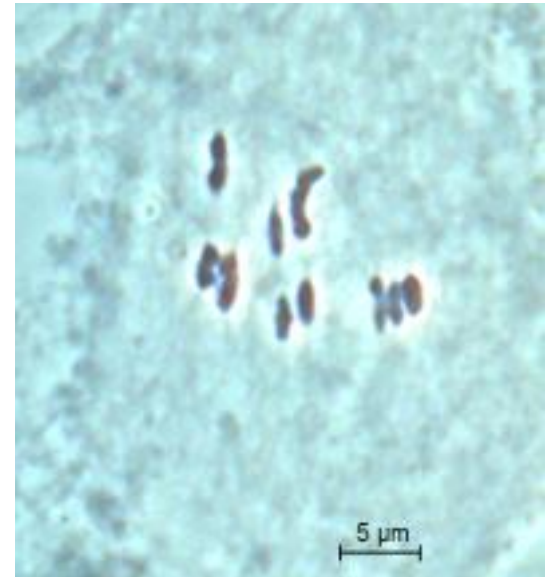
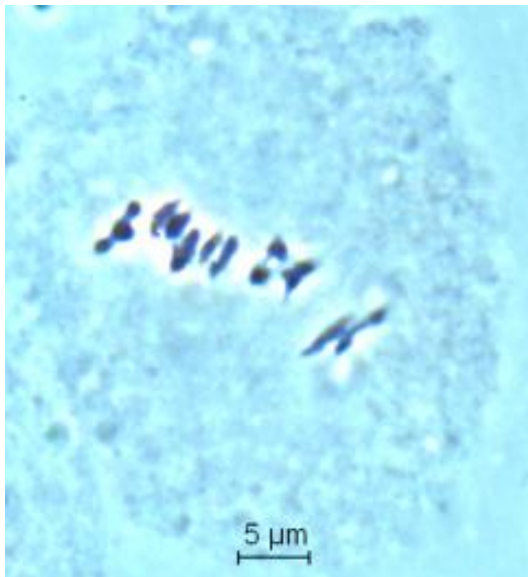
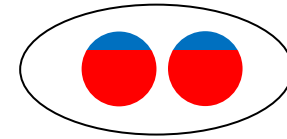
AA ( $2n=20$ )  
Cycle 4



AA ( $2n=20$ )  
Cycle 5.1



AA ( $2n=20$ )  
Cycle 5.2



Stable meiosis for all  $2n=20$

Pollen Fertility decrease compare to Darmor (between 59 & 93%)

# Molecular characterization of the last three plants $2n=20$

## Strategy 2

60 K Illumina Array<sup>®</sup>  
(52127 SNP)

Polymorphism between  
*B. rapa* (C1.3) and *B. napus*  
(Darmor)

7846 SNP

Best Physical location on A  
and/or C subgenome of  
*B. napus* (Darmor)

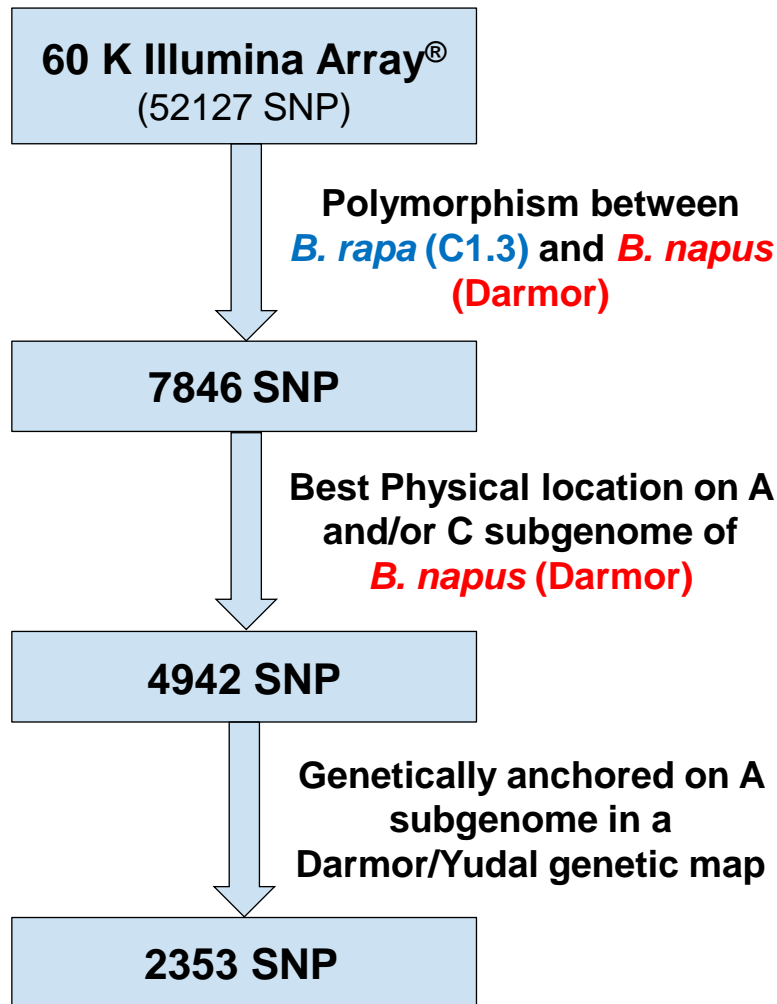
4942 SNP

Genetically anchored on A  
subgenome in a  
Darmor/Yudal genetic map

2353 SNP

# Molecular characterization of the last three plants $2n=20$

## Strategy 2



	% Darmor theoretical	% Darmor observed
AA Cycle 4	68%	57%
AA Cycle 5.1	76%	65%
AA Cycle 5.2	76%	68%

**Extraction of Darmor is significantly lower than expected in the three last plants ( $\chi^2$  test,  $\alpha < 0.001$ )**

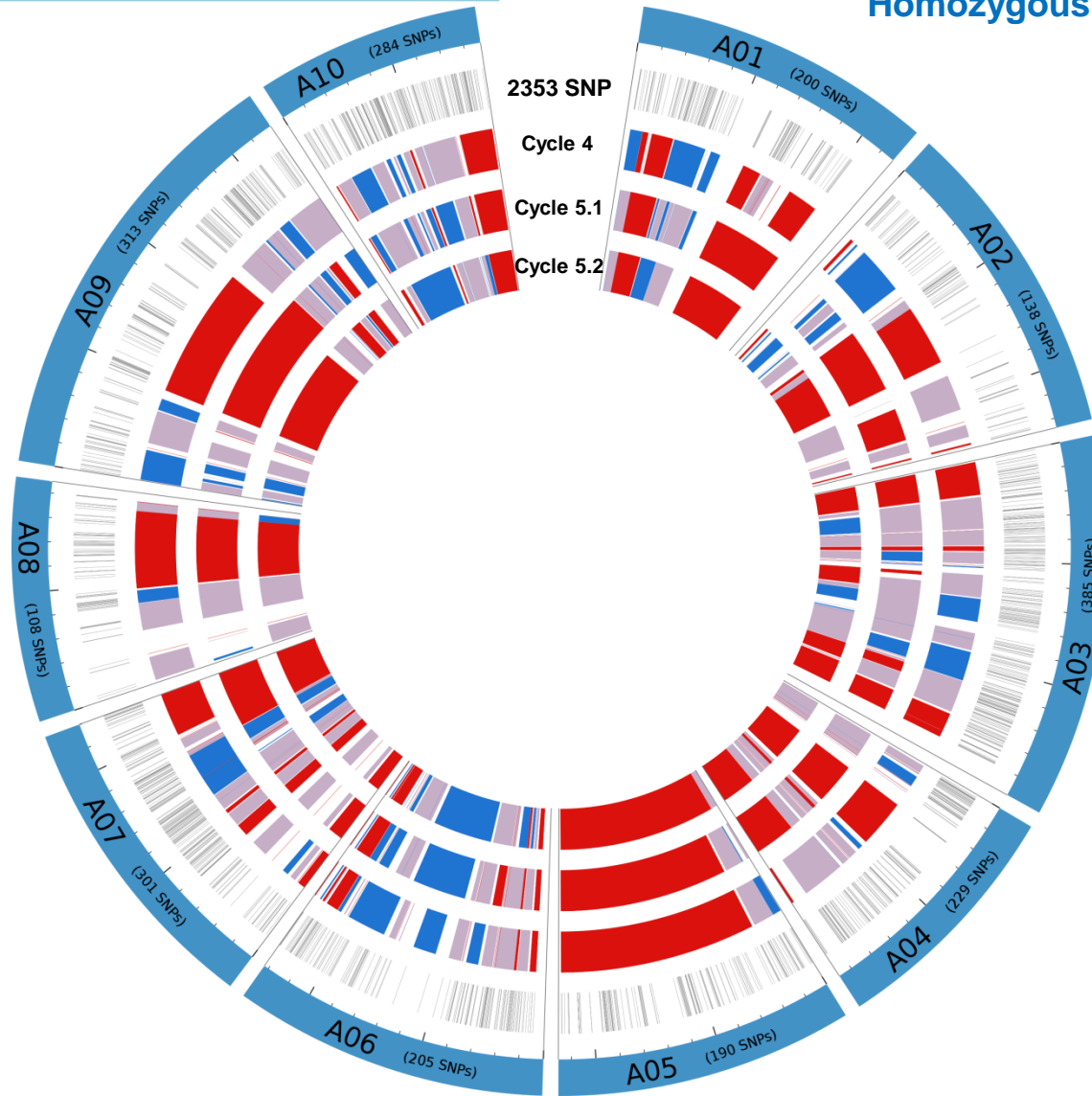
# Molecular characterization of the last three plants $2n=20$

## Strategy 2

Homozygous SNP of *B. napus* (Darmor)

Homozygous SNP of *B. rapa* (C1.3)

Heterozygous SNP

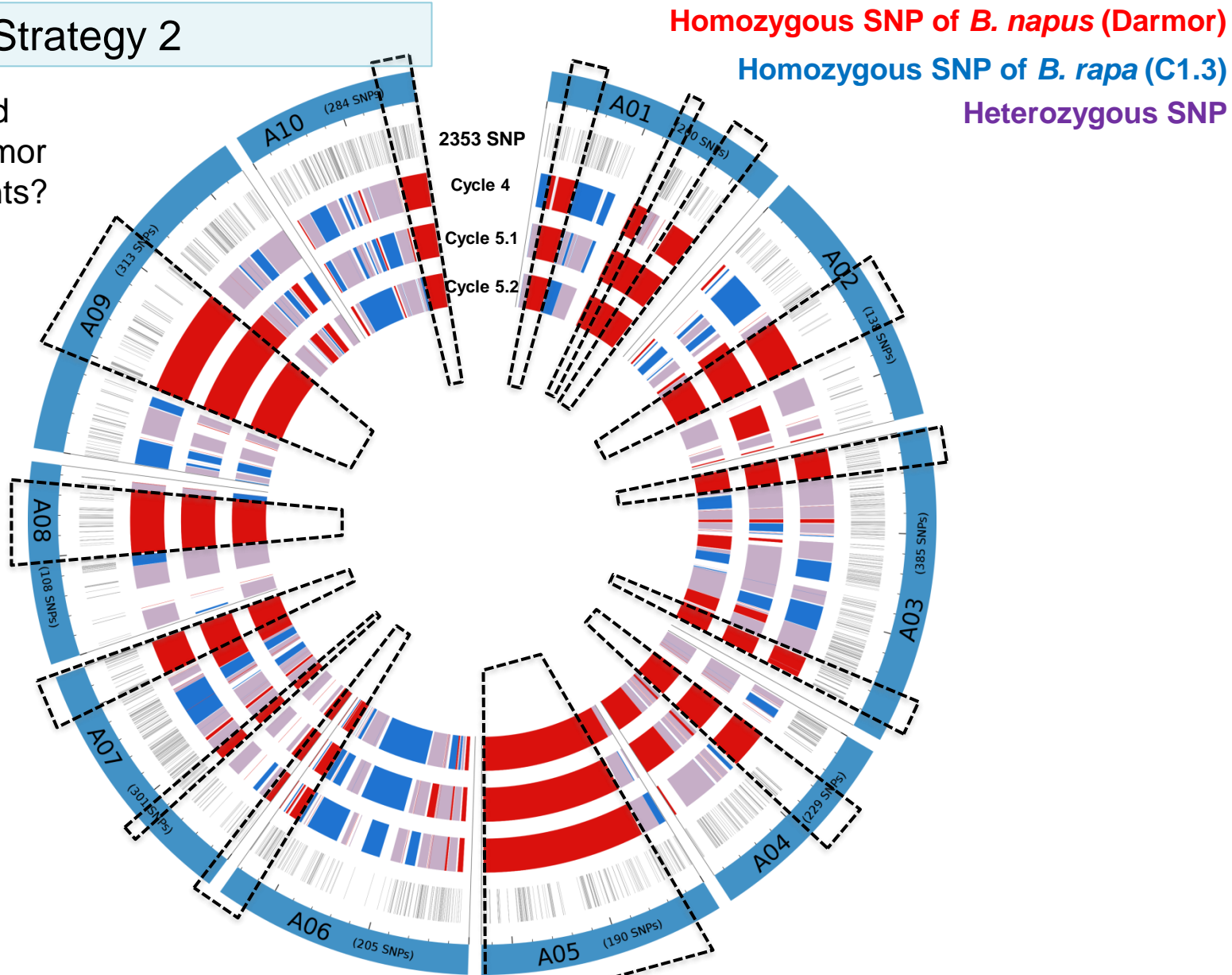


# Molecular characterization of the last three plants $2n=20$

## Strategy 2

Where are located homozygous Darmor regions in AA plants?

**In all 10 A chromosomes**



# Molecular characterization of the last three plants $2n=20$

## Strategy 2

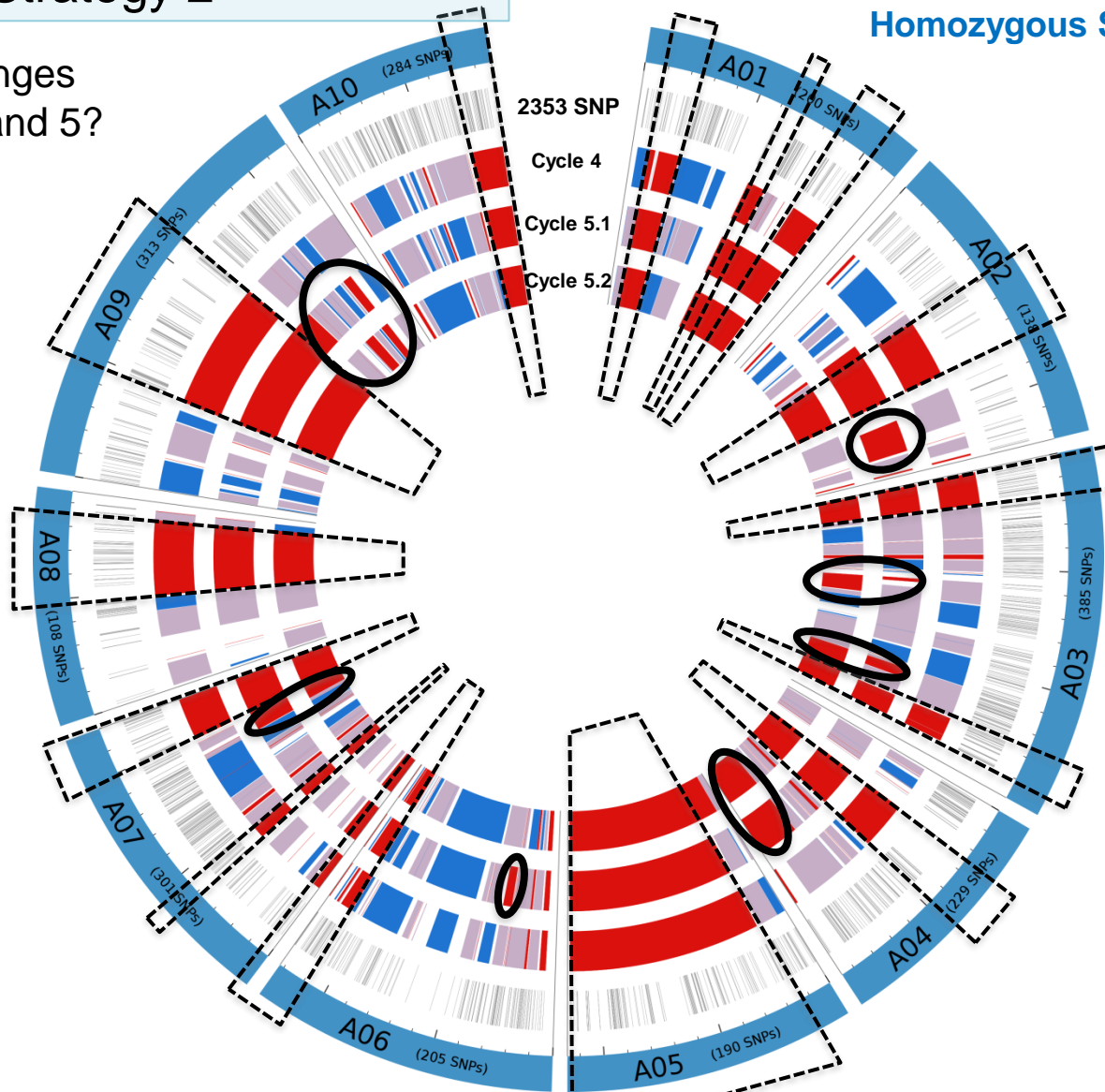
What are the changes between cycle 4 and 5?

**Increase of homozygous Darmor rate depends on chromosome**  
**36% to 48%**

**Homozygous SNP of *B. napus* (Darmor)**

**Homozygous SNP of *B. rapa* (C1.3)**

**Heterozygous SNP**



# Molecular characterization of the last three plants $2n=20$

## Strategy 2

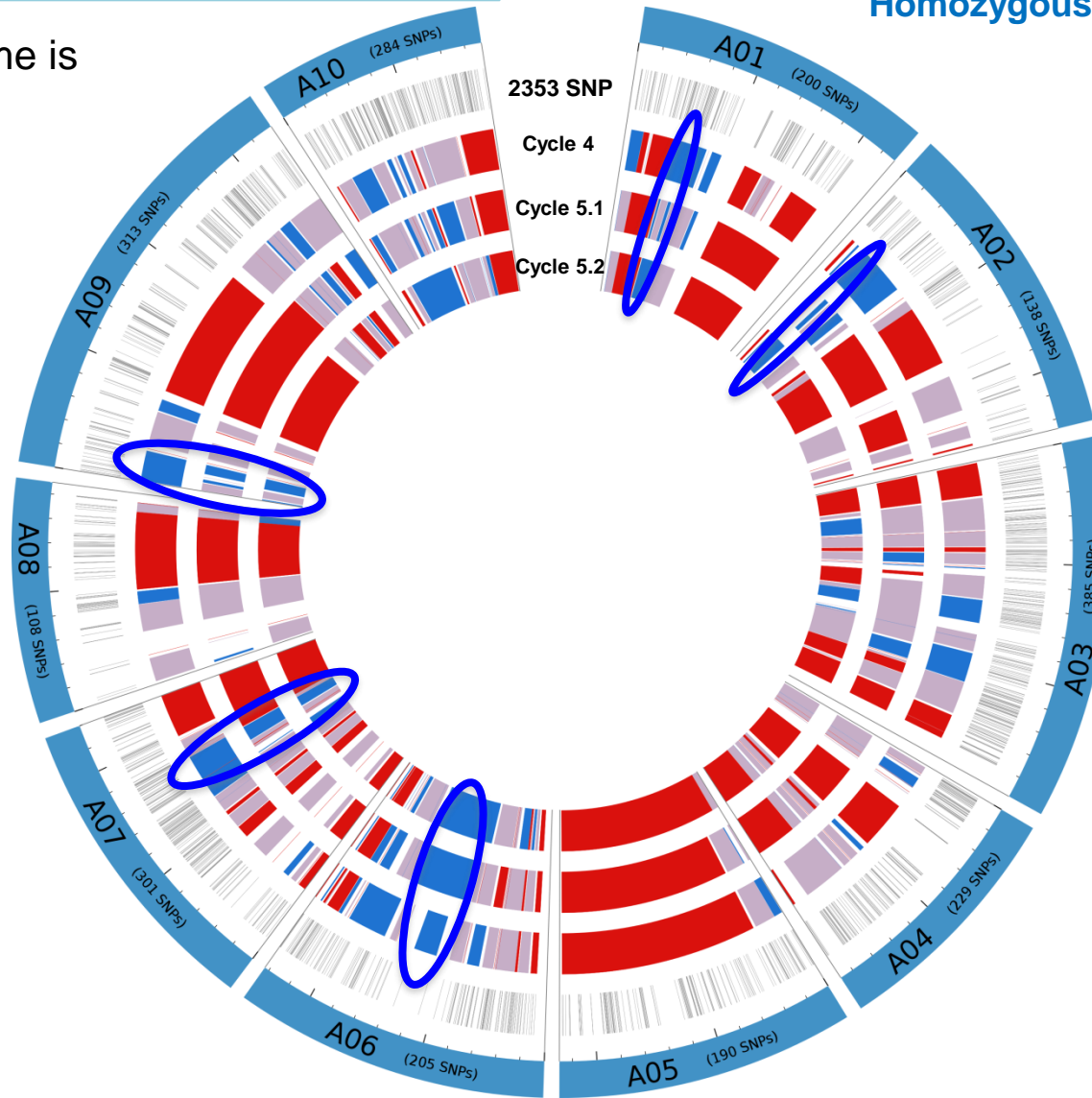
Majority of A genome is heterozygous or homozygous C1.3

12 to 23% are fixed at *B. rapa* homozygous stage

Homozygous SNP of *B. napus* (Darmor)

Homozygous SNP of *B. rapa* (C1.3)

Heterozygous SNP



# Molecular characterization of the last three plants $2n=20$

## Strategy 2

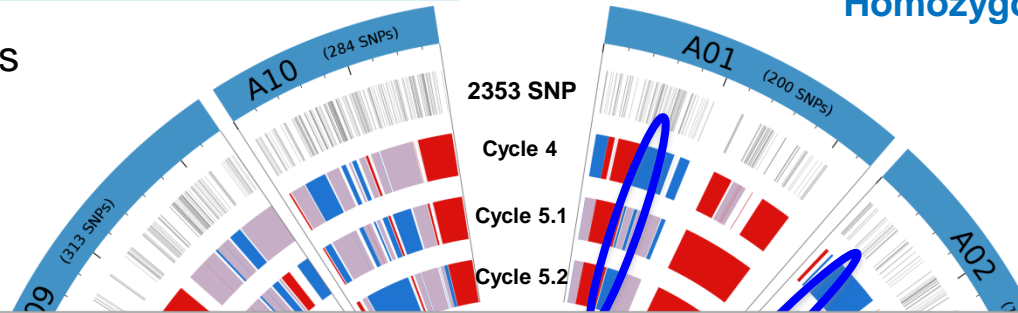
Majority of A genome is  
heterozygous or  
homozygous C1.3

12 to 23% are  
fixed at *B. rapa*  
homozygous  
stage

Homozygous SNP of *B. napus* (Darmor)

Homozygous SNP of *B. rapa* (C1.3)

Heterozygous SNP



Some genomic regions seem to be fixed at *B. rapa* homozygous stage:

Are they essential for plant viability?



# Molecular characterization of the last three plants $2n=20$

## Strategy 2

### Structural changes?

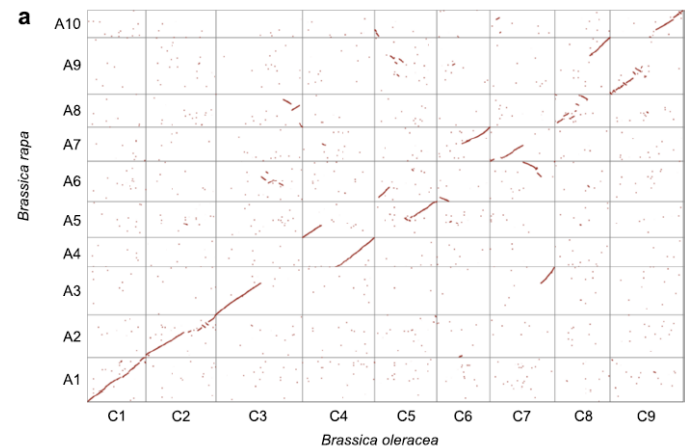


With 4807 SNP only of C subgenome and mapped on *B. napus*

	C Subgenome SNP detected	% of C subgenome introgressed
AA Cycle 4	41	<1%
AA Cycle 5.1	41	<1%
AA Cycle 5.2	30	<1%



- ❖ Small telomeric genomic regions of C1 and C2
- ❖ 20 potential gene conversions



Parkin *et al.*, (2014)

# CONCLUSION

- ❖ Difficulty to extract A genome at diploid stage: Strategy 1 failed, Strategy 2 efficient but slower
- ❖ % of *B. napus* A genome extracted < % expected with retention of *B. rapa* SNP at homozygous stage (12 to ~25%)
- ❖ Introgressions of some C genomic regions from C1 and C2 (homoeologous of A01 and A02) and gene conversions

➡ **It will be difficult to get a pure A genome of *B. napus* at diploid stage**

↪ we will continue new cycles and molecular selection to try to increase level of Darmor A genome extracted

➡ **Some genomic regions probably evolved in a polyploid context**

↪ Structural and functional characterization of *B. rapa* homozygous regions that could have the most diverged in a polyploid context

# Biodiversity & Polyploidy Team :

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M. Lodé

G. Trotoux

A. Pelé (PhD)

A.M. Chèvre

J. Morice

M. Gilet

G. Deniot

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