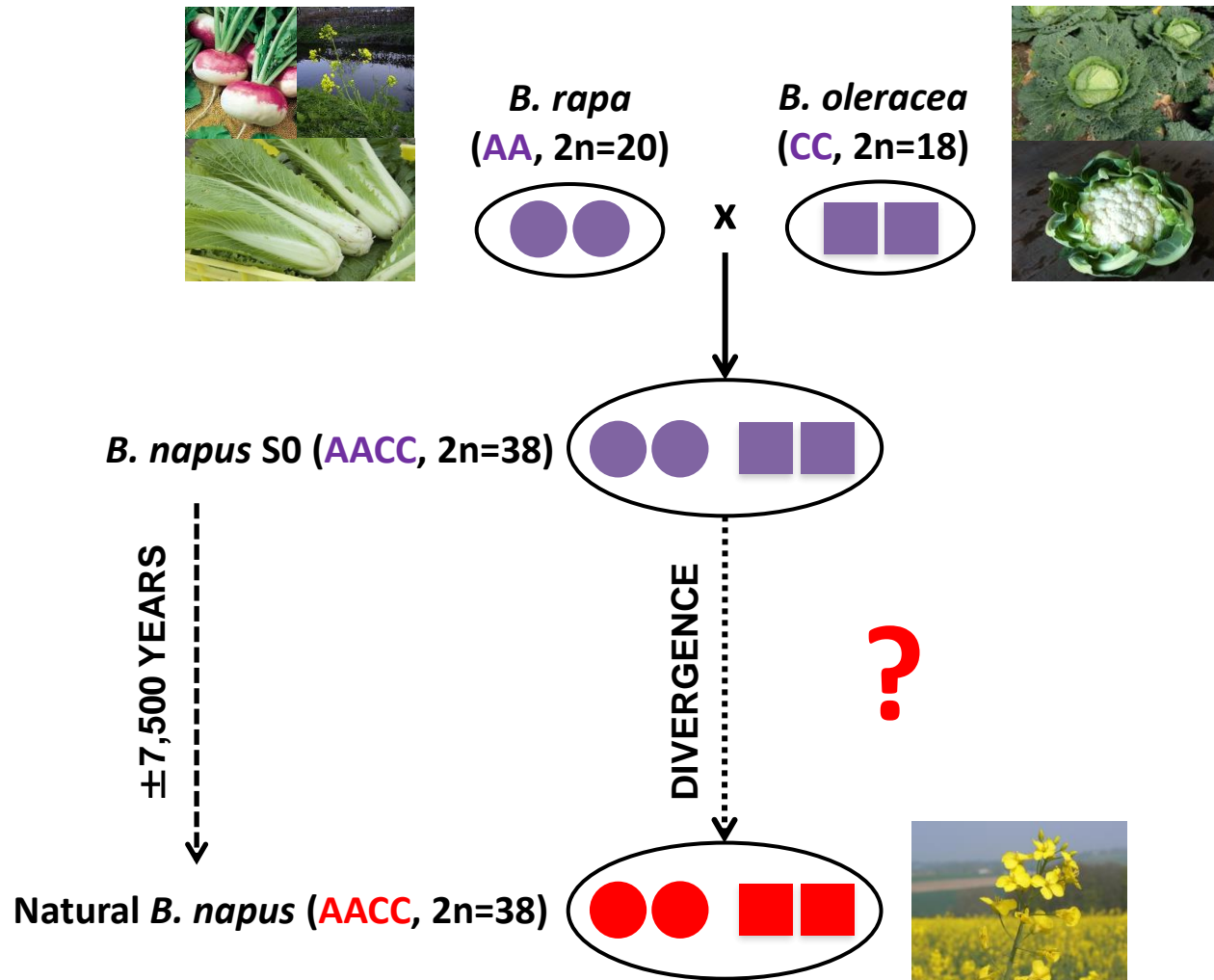


# The poor lonesome A subgenome of *Brassica napus* L. may not survive without its mate

**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>

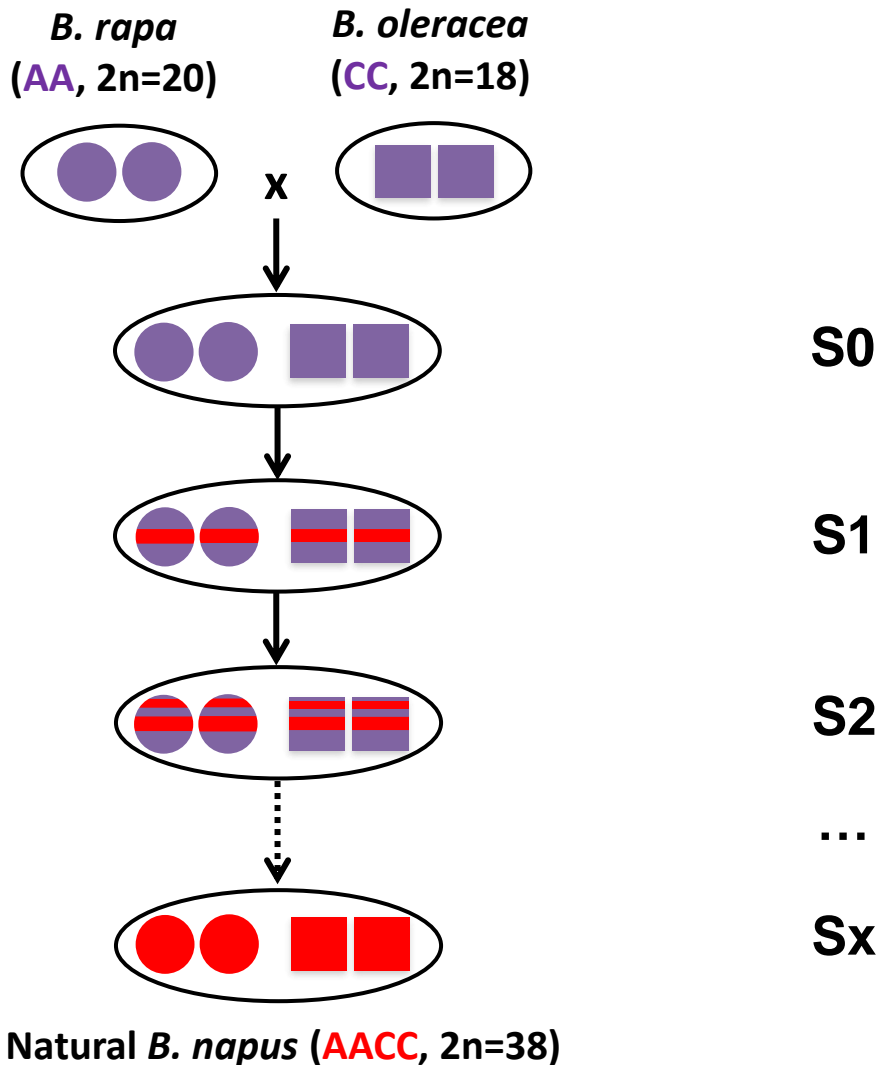
<sup>1</sup>IGEPP, INRA, Agrocampus-Ouest, Université de Rennes 1 , Le Rheu, France

# Divergence occurred since *B. napus* formation



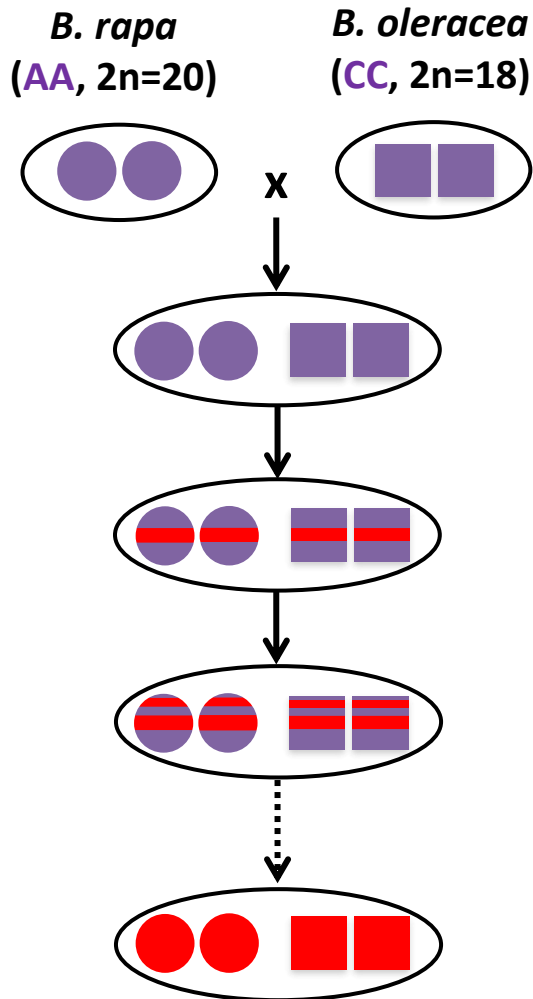
# How can we study this evolution?

Short-term: Resynthesized *B. napus*



# How can we study this evolution?

Short-term: Resynthesized *B. napus*



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

## S0 Functional & Epigenetic 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

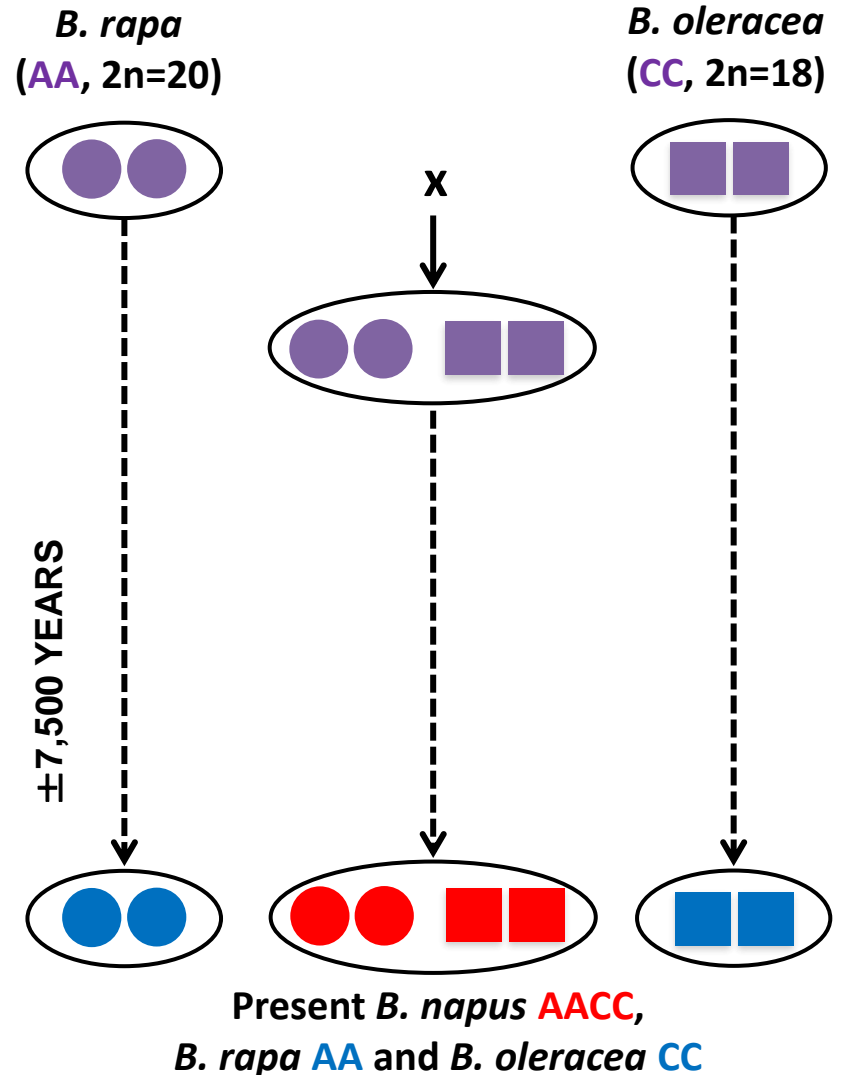
...

Sx

Limited to the first few generations

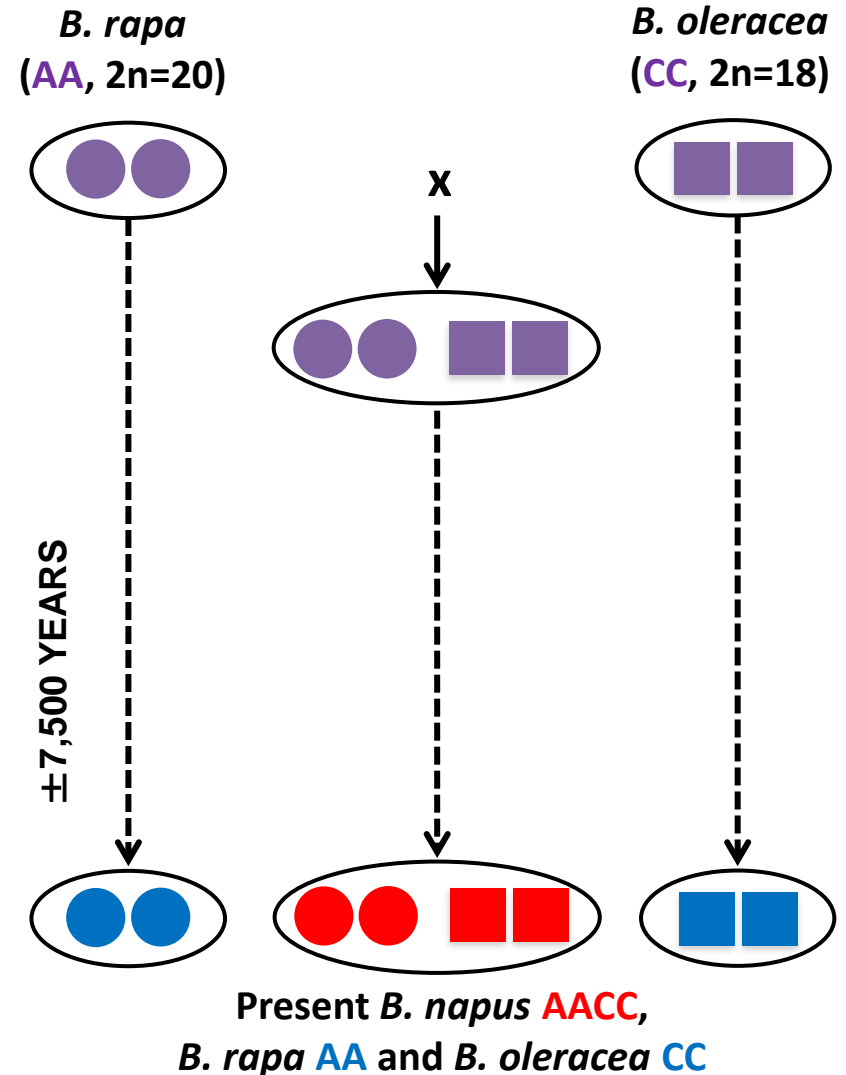
# How can we study this evolution?

**Long-term:** Comparing *B. napus* to the present *B. rapa* / *B. oleracea*



# How can we study this evolution?

**Long-term:** Comparing *B. napus* to the present *B. rapa* / *B. oleracea*



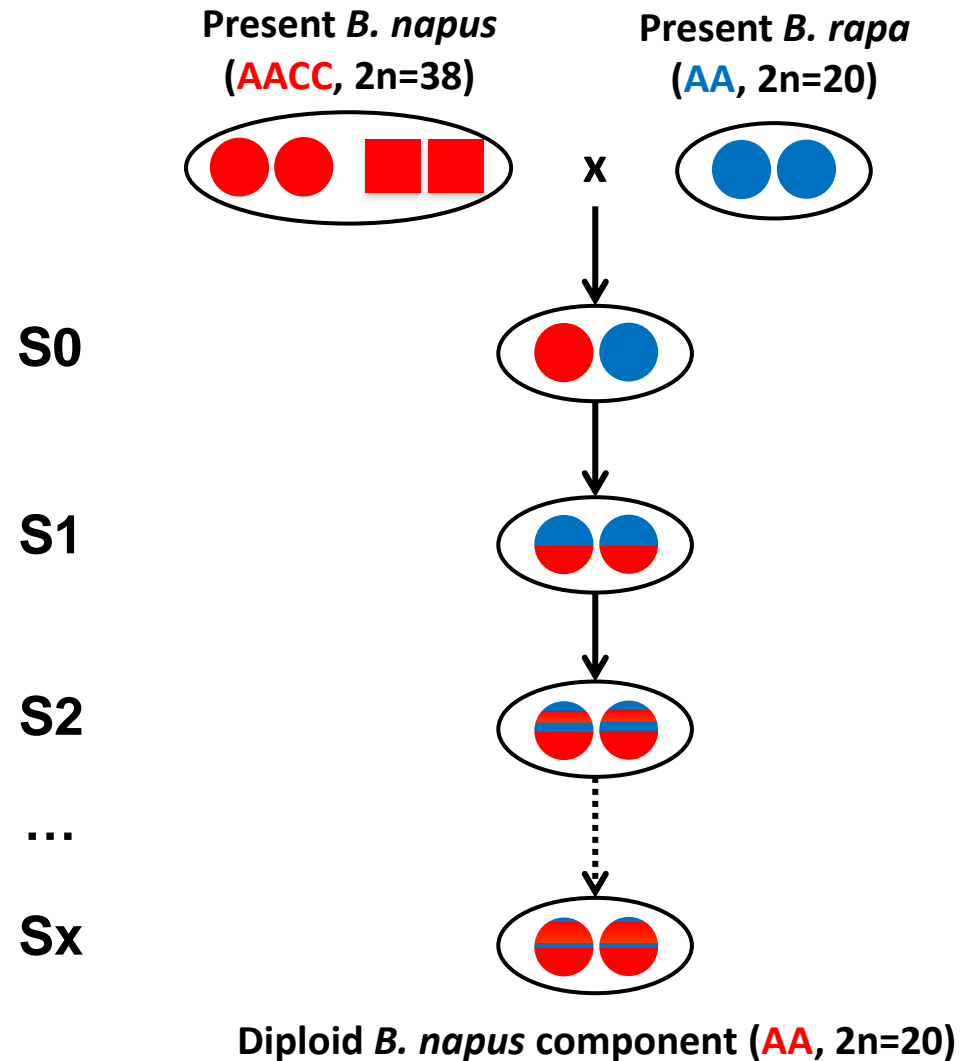
**But progenitors have evolved independently since *B. napus* formation**

**Genic conversion  
Gene Loss / Gain  
Rearrangements**

(Wang *et al.*, 2011 ; Chalhoub *et al.*, 2014, Liu *et al.*, 2014)

# How can we study this evolution?

## Long-term: Subgenome Extraction



# How can we study this evolution?

## Long-term: Subgenome Extraction

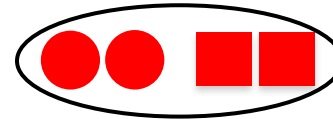
Only used in AABBDD Wheat  
for extracting the AABB  
component  
(Kerber *et al.*, 1964)

Evolution in polyploid context



Interdependency between each  
original genome?

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

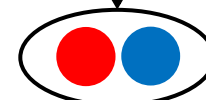


Present *B. rapa*  
(AA, 2n=20)



x

S0



S1



S2



...

Sx



Diploid *B. napus* component (AA, 2n=20)



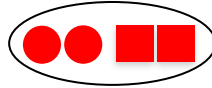
# Extraction of the *B. napus* AA component

## Strategy 1

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

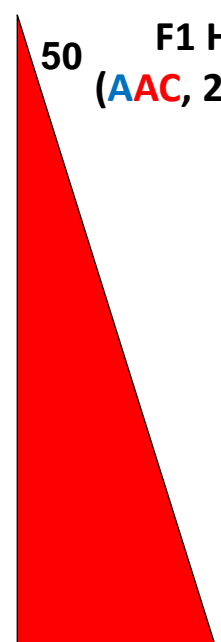


*B. napus* cv. Darmor  
(AACC, 2n=38)



x

50  
F1 Hybrid  
(AAC, 2n=29)

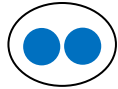


Theoretical %  
of *B. napus* A  
subgenome in  
AAC hybrids

# Extraction of the *B. napus* AA component

## Strategy 1

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



X

F1

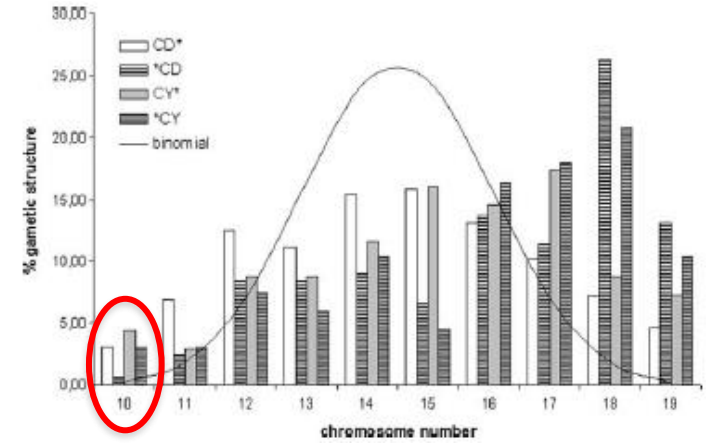
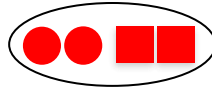


X

G1



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



Leflon *et al.*, 2006

50

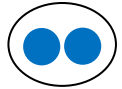
75

Theoretical %  
of *B. napus* A  
subgenome in  
AAC hybrids

# Extraction of the *B. napus* AA component

## Strategy 1

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



X

F1



X

G1



X

G2



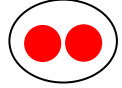
X

G3



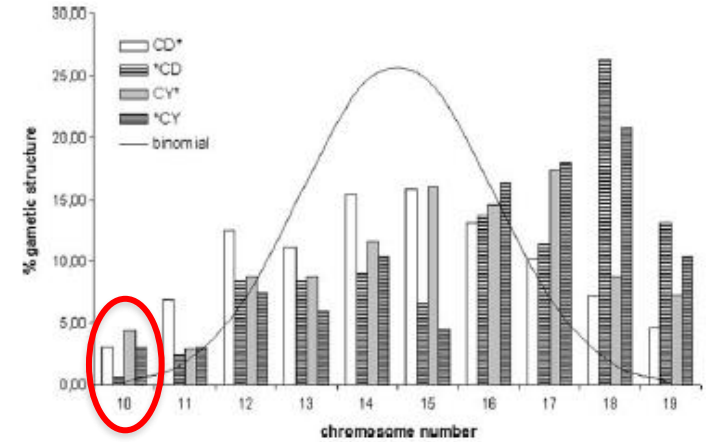
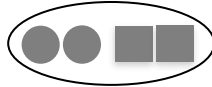
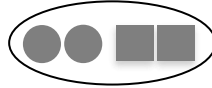
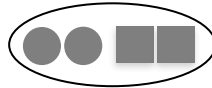
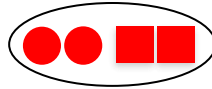
X

G4



AA component (2n=20)

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



Leflon *et al.*, 2006

50

75

87.5

93.8

96.9

Theoretical %  
of *B. napus* A  
subgenome in  
AAC hybrids

# Extraction of the *B. napus* AA component

## Strategy 1

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



X

F1



X

G1



X

G2



X

G3



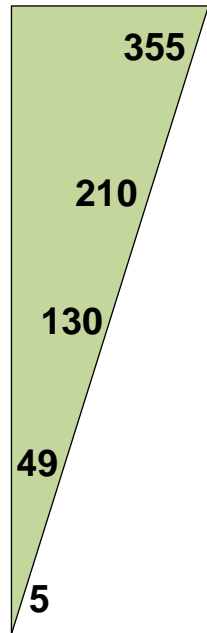
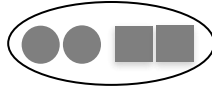
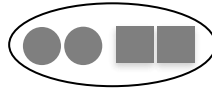
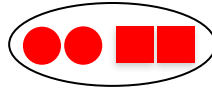
X

G4

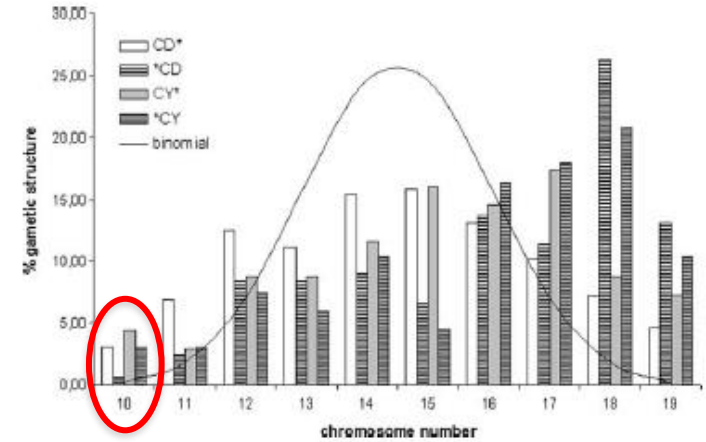


No Seed

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



AAC hybrids Yield  
(Nb. Seed / 100  
Pollinated Flowers)

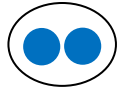


Leflon *et al.*, 2006

# Extraction of the *B. napus* AA component

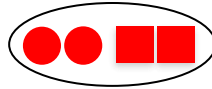
## Strategy 1

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



X

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

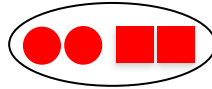


X

F1



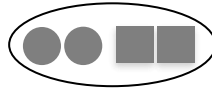
X



G1



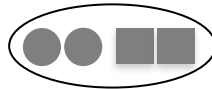
X



G2



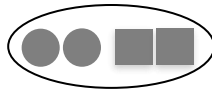
X



G3



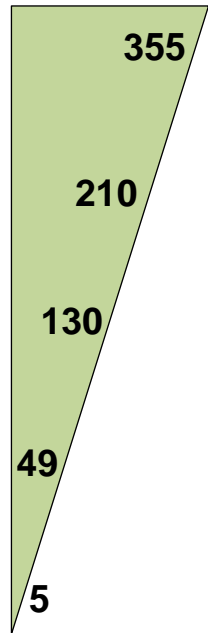
X



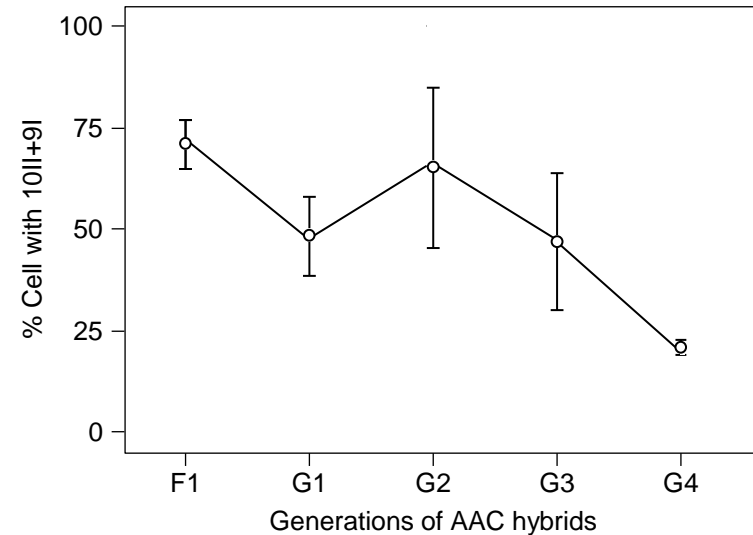
G4



No Seed



AAC hybrids Yield  
(Nb. Seed / 100  
Pollinated Flowers)



**Impossibility to obtain the pure AA component of *B. napus*:**

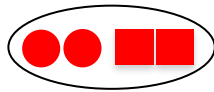
- Rearrangements ?
- Evolution in a polyploid context of the *B. napus* A subgenome ?

# Extraction of the *B. napus* AA component

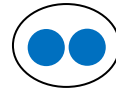
## Strategy 2

Theoretical % of *B. napus* A subgenome in AAC hybrids

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



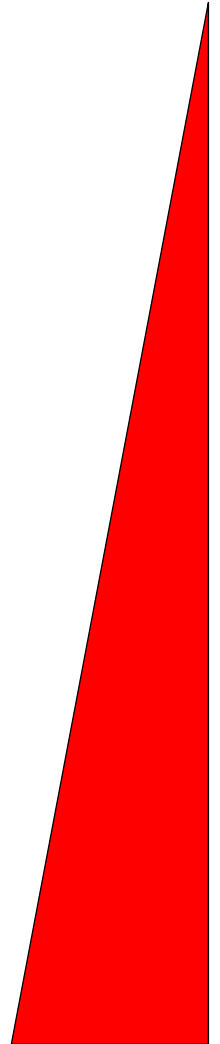
X



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

Cycle 1

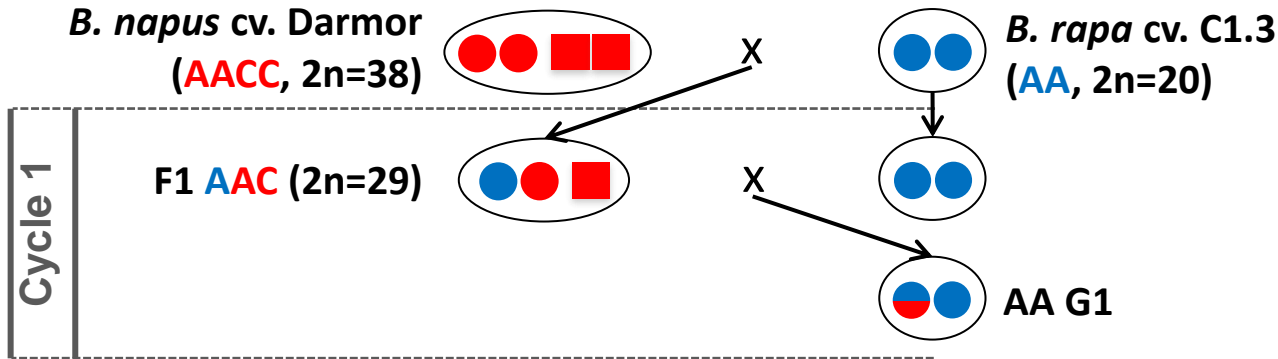
F1 AAC (2n=29)



# Extraction of the *B. napus* AA component

## Strategy 2

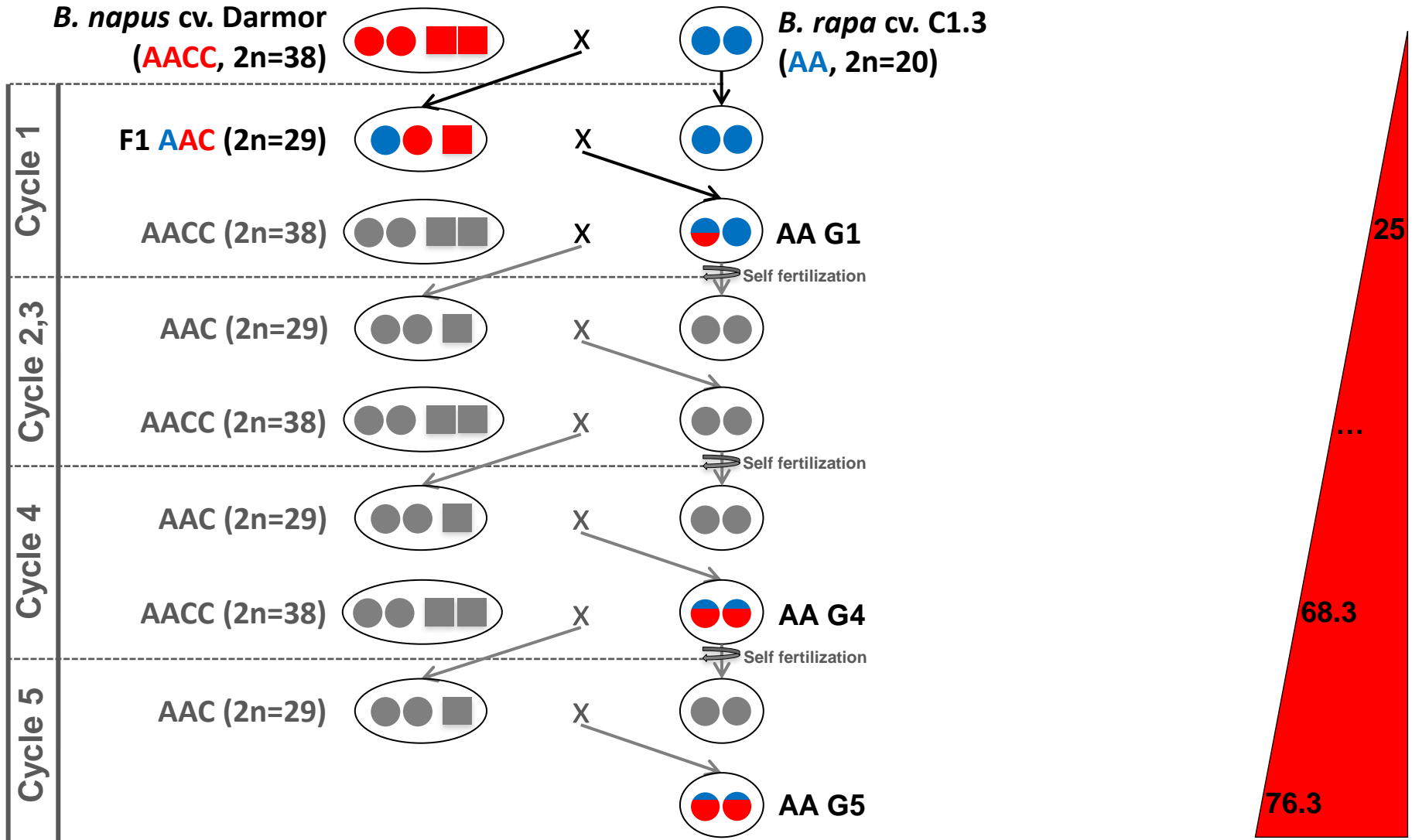
Theoretical % of *B. napus* A subgenome in AAC hybrids



# Extraction of the *B. napus* AA component

## Strategy 2

Theoretical % of *B. napus* A subgenome in AAC hybrids

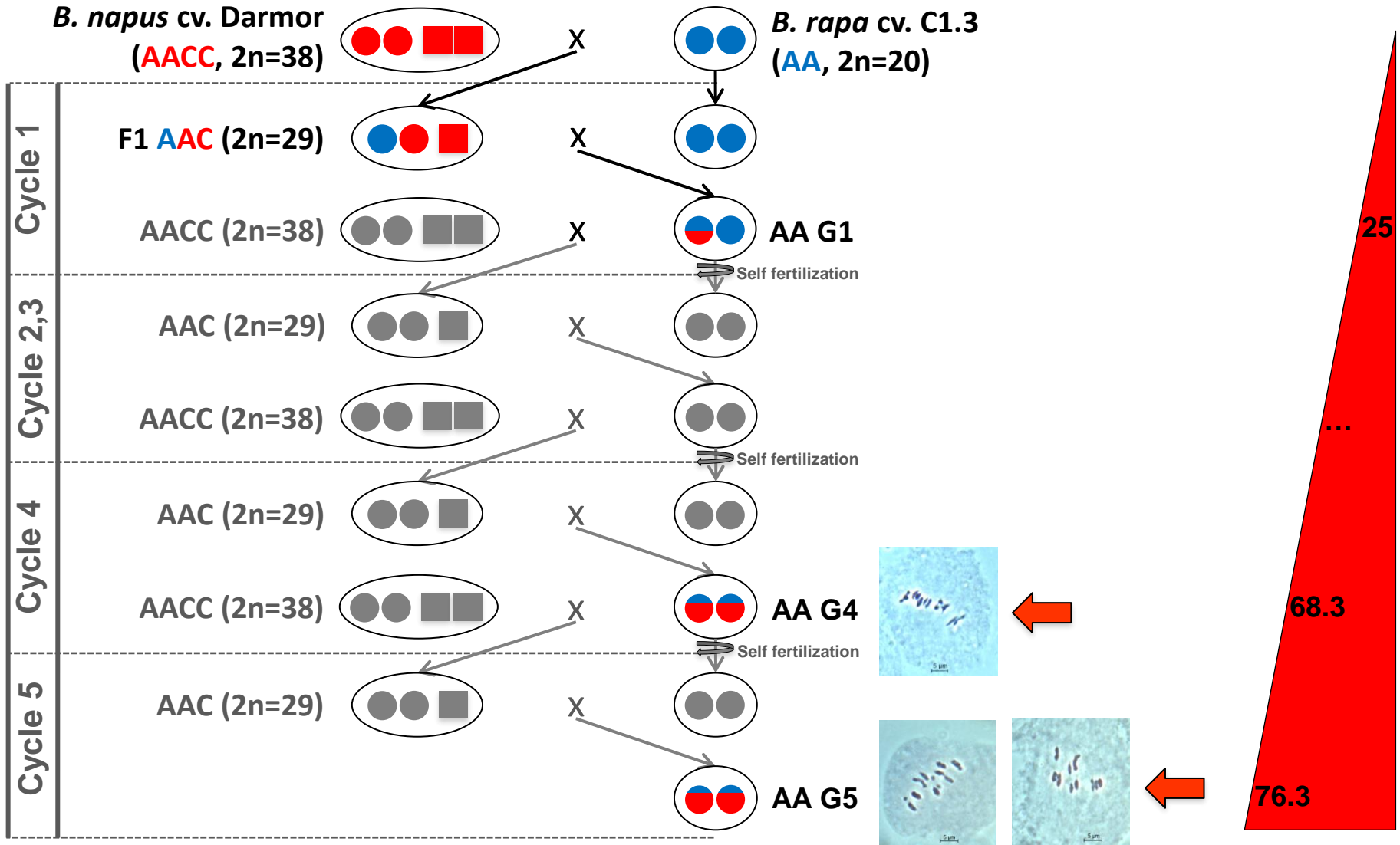




# Extraction of the *B. napus* AA component

## Strategy 2

Theoretical % of *B. napus* A subgenome in AAC hybrids



# Molecular characterization of the G4 and G5 AA plants

% *B. napus* A subgenome extracted

60 K Illumina Array®  
(52,127 SNP)

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

7,846 SNP

Genetically & physically  
anchored on A subgenome  
(Darmor/Yudal genetic map)

2,290 SNP

*B. napus* C subgenome introgressions

60 K Illumina Array®  
(52127 SNP)

Polymorphism :  
*B. napus* (Darmor) ++  
*B. rapa* (C1.3) --

18,674 SNP

Genetically & physically  
anchored on C subgenome  
(Darmor/Yudal genetic map)

4,807 SNP

# Molecular characterization of the G4 and G5 AA plants

## % *B. napus* A subgenome extracted

	% <i>B. napus</i> theoretical	% <i>B. napus</i> observed
<b>AA G4</b>	<b>68%</b>	<b>57%</b>
<b>AA G5 pI1</b>	<b>76%</b>	<b>65%</b>
<b>AA G5 pI2</b>	<b>76%</b>	<b>68%</b>

**Extraction of *B. napus* A subgenome is significantly lower than expected in all AA plants ( $\chi^2$  test,  $\alpha < 0.001$ )**

## *B. napus* C subgenome introgressions

	C01 start (0.53 Mbp)	C02 start (4.64 Mbp)	C02 end (2.27 Mbp)
<b>AA G4</b>	<b>X</b>	<b>X</b>	
<b>AA G5 pI1</b>		<b>X</b>	
<b>AA G5 pI2</b>		<b>X</b>	<b>X</b>

**3 introgressions detected and validated (sequencing) with variations between G4 & G5**

# Molecular characterization of the G4 and G5 AA plants

## % *B. napus* A subgenome extracted

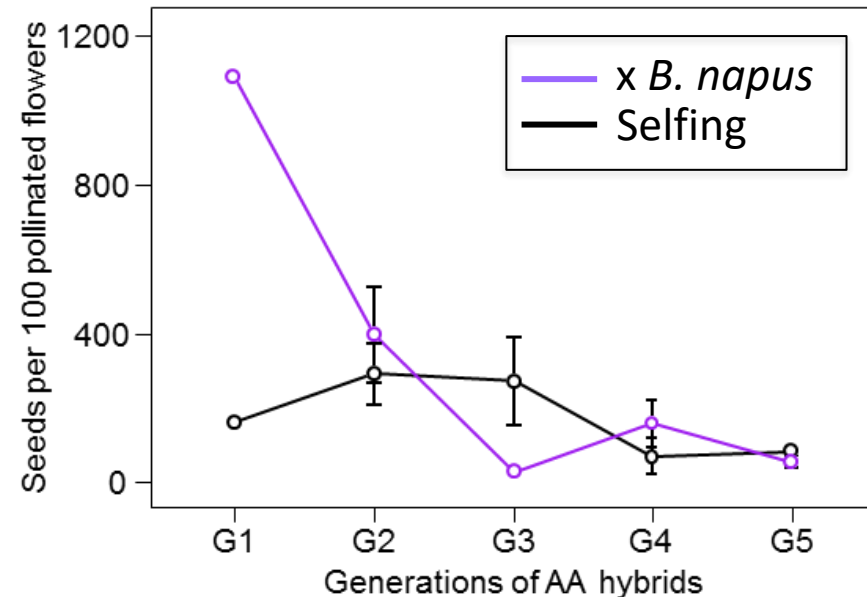
	% <i>B. napus</i> theoretical	% <i>B. napus</i> observed
AA G4	68%	57%
AA G5 pl1	76%	65%
AA G5 pl2	76%	68%

Extraction of *B. napus* A subgenome is significantly lower than expected in all AA plants ( $\chi^2$  test,  $\alpha < 0.001$ )

Pure *B. napus* AA plant may not survive deprived of C subgenome?

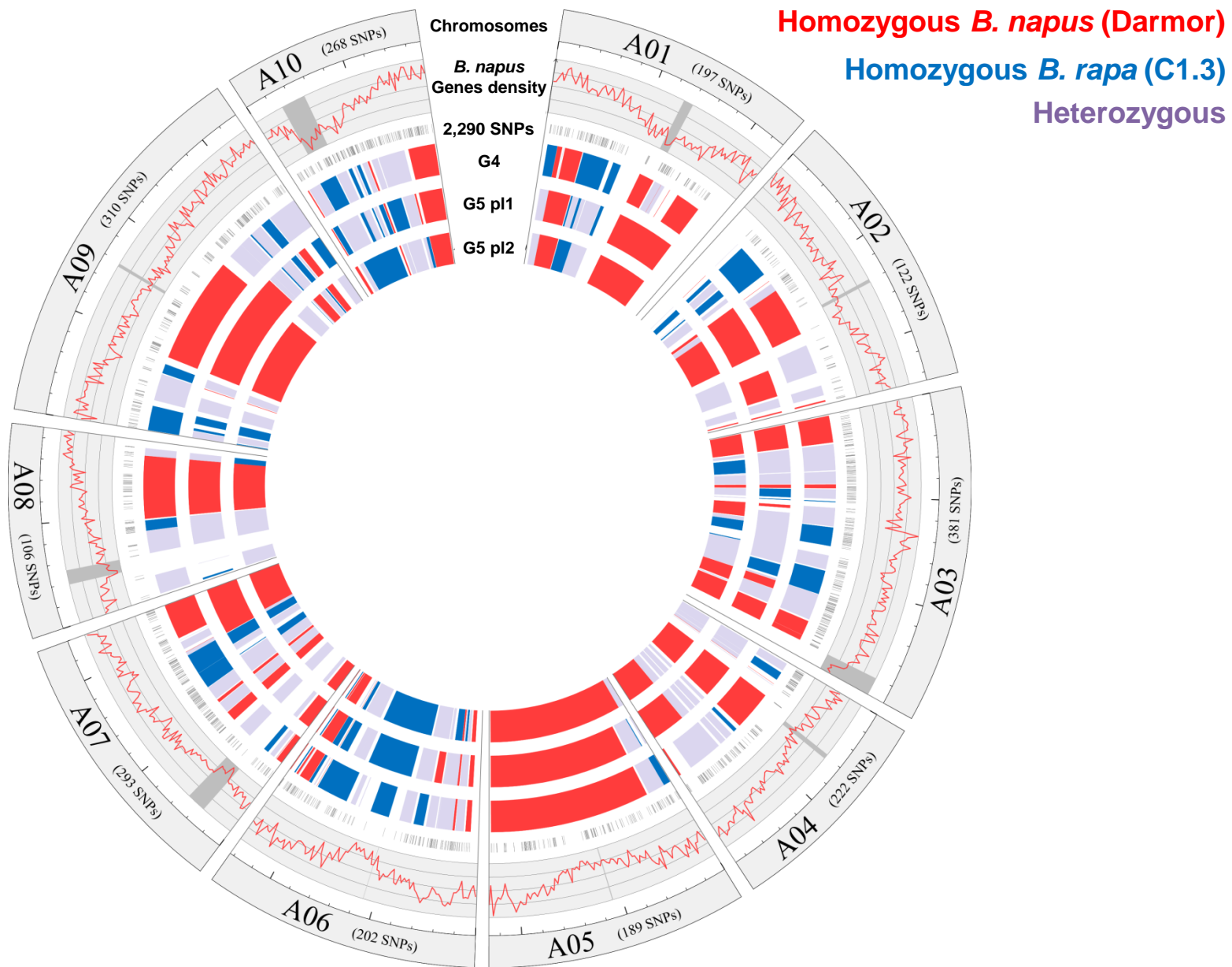
## *B. napus* C subgenome introgressions

	C01 start (0.53 Mbp)	C02 start (4.64 Mbp)	C02 end (2.27 Mbp)
AA G4	X	X	
AA G5 pl1		X	
AA G5 pl2		X	X



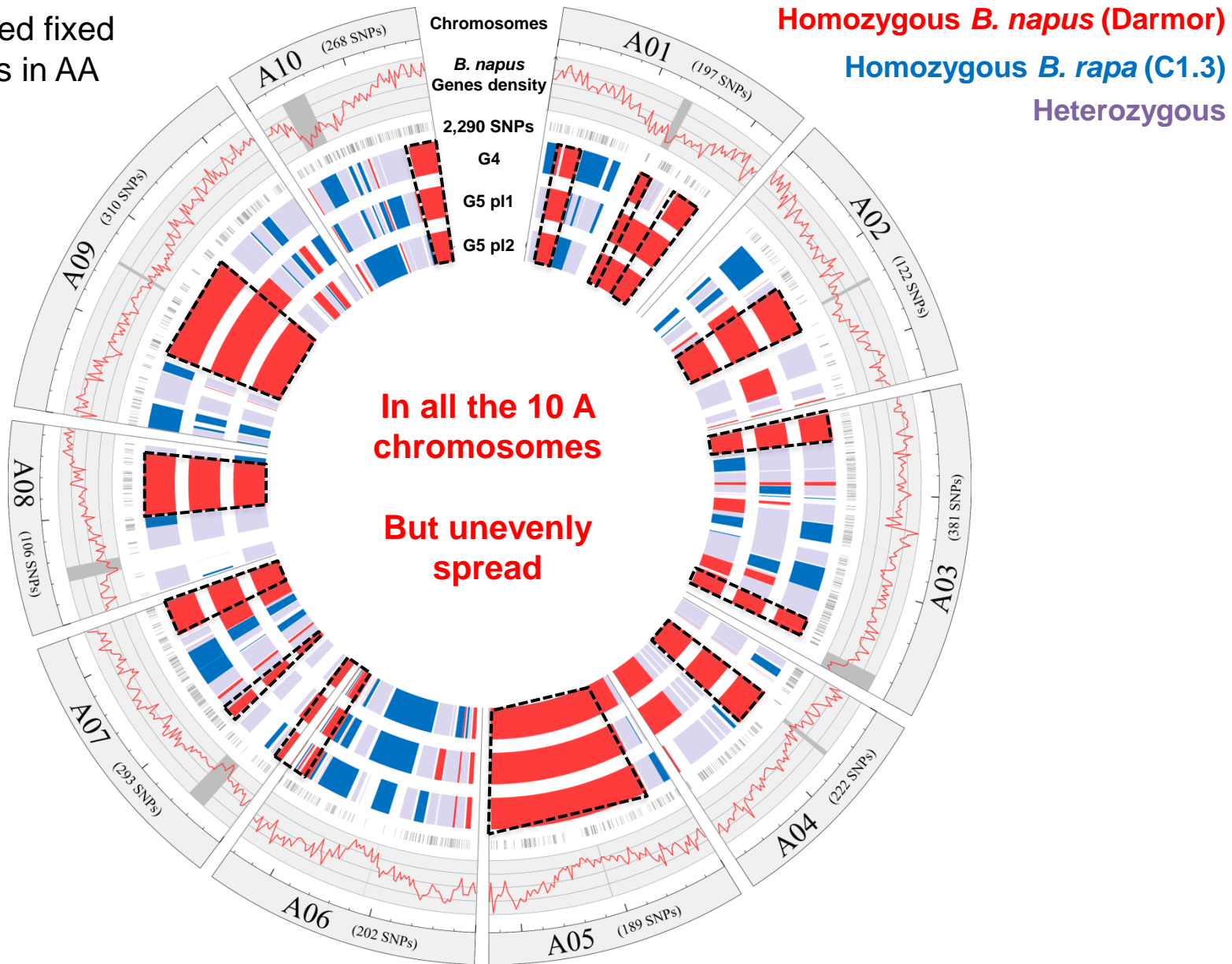
Introgressions of *B. napus* C subgenome don't impact the yield

# Molecular characterization of the G4 and G5 AA plants



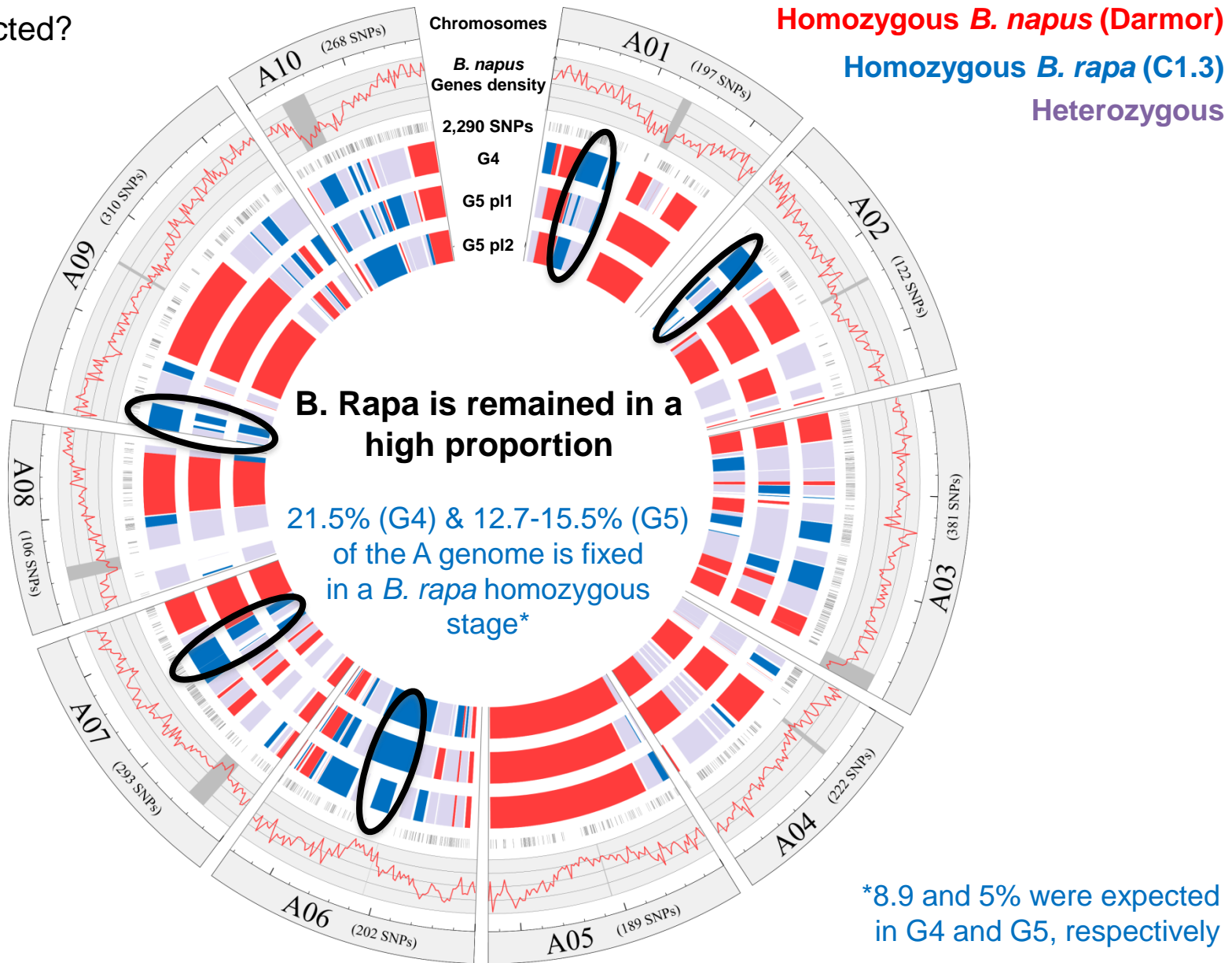
# Molecular characterization of the G4 and G5 AA plants

Where are located fixed *B. napus* regions in AA plants?



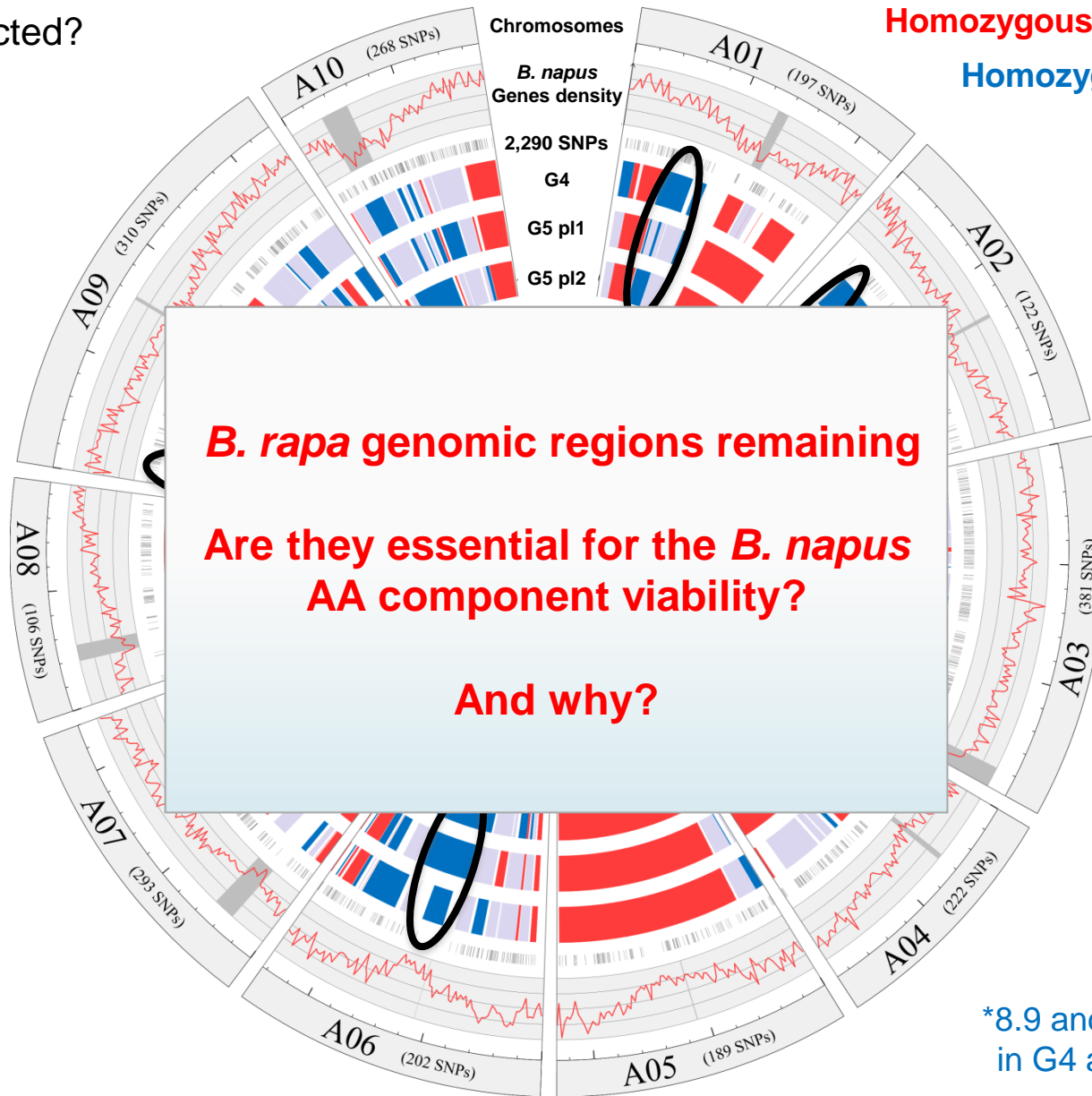
# Molecular characterization of the G4 and G5 AA plants

What is unexpected?



# Molecular characterization of the G4 and G5 AA plants

What is unexpected?



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

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

**Heterozygous**

***B. rapa* genomic regions remaining**

**Are they essential for the *B. napus*  
AA component viability?**

**And why?**

\*8.9 and 5% were expected  
in G4 and G5, respectively



# Molecular characterization of the G4 and G5 AA plants

*B. napus* S0

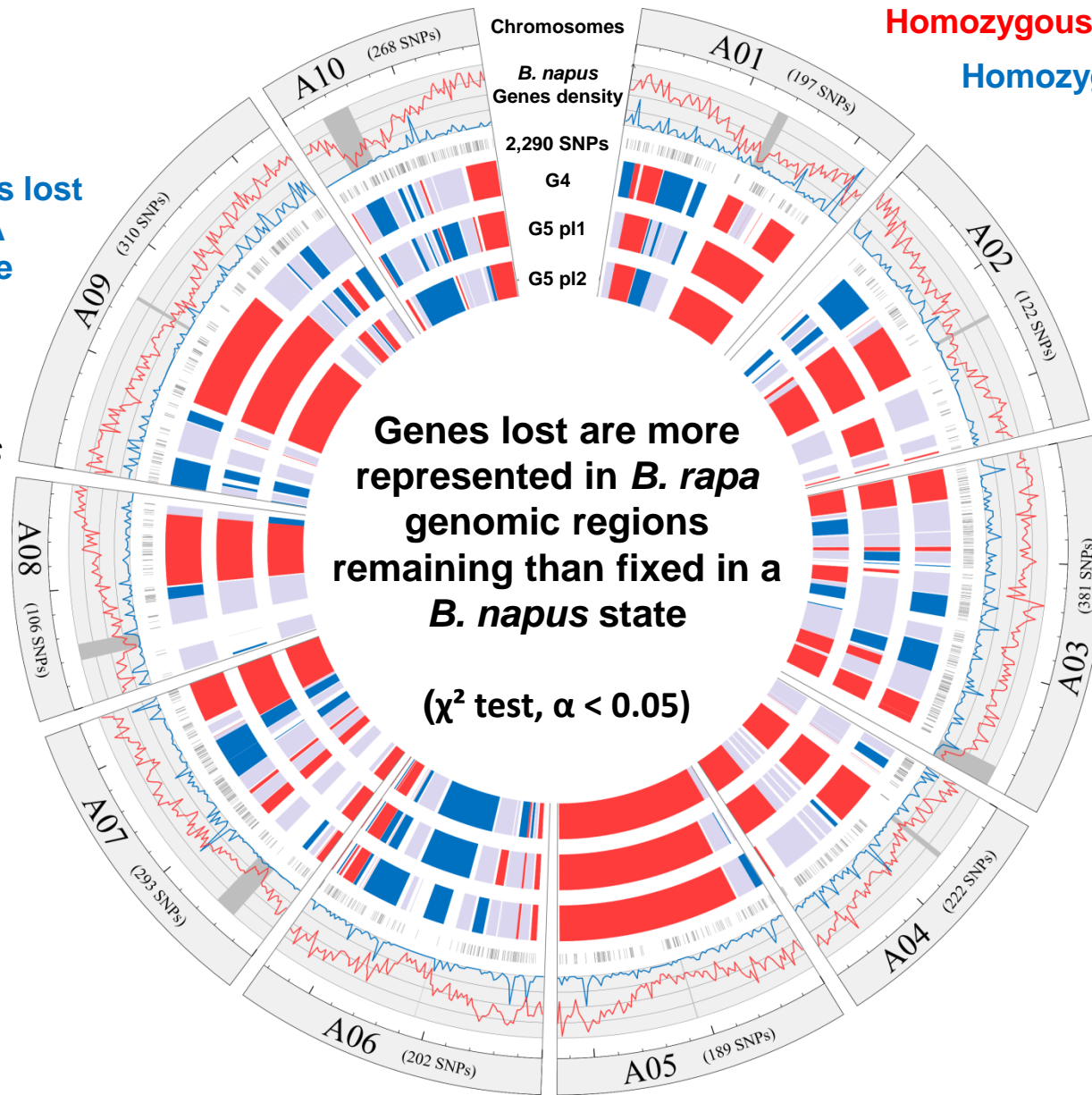
±7,500 YEARS



1,925 genes lost  
along the A  
subgenome

Natural *B. napus*

(Chalhoub *et al.*, 2014)

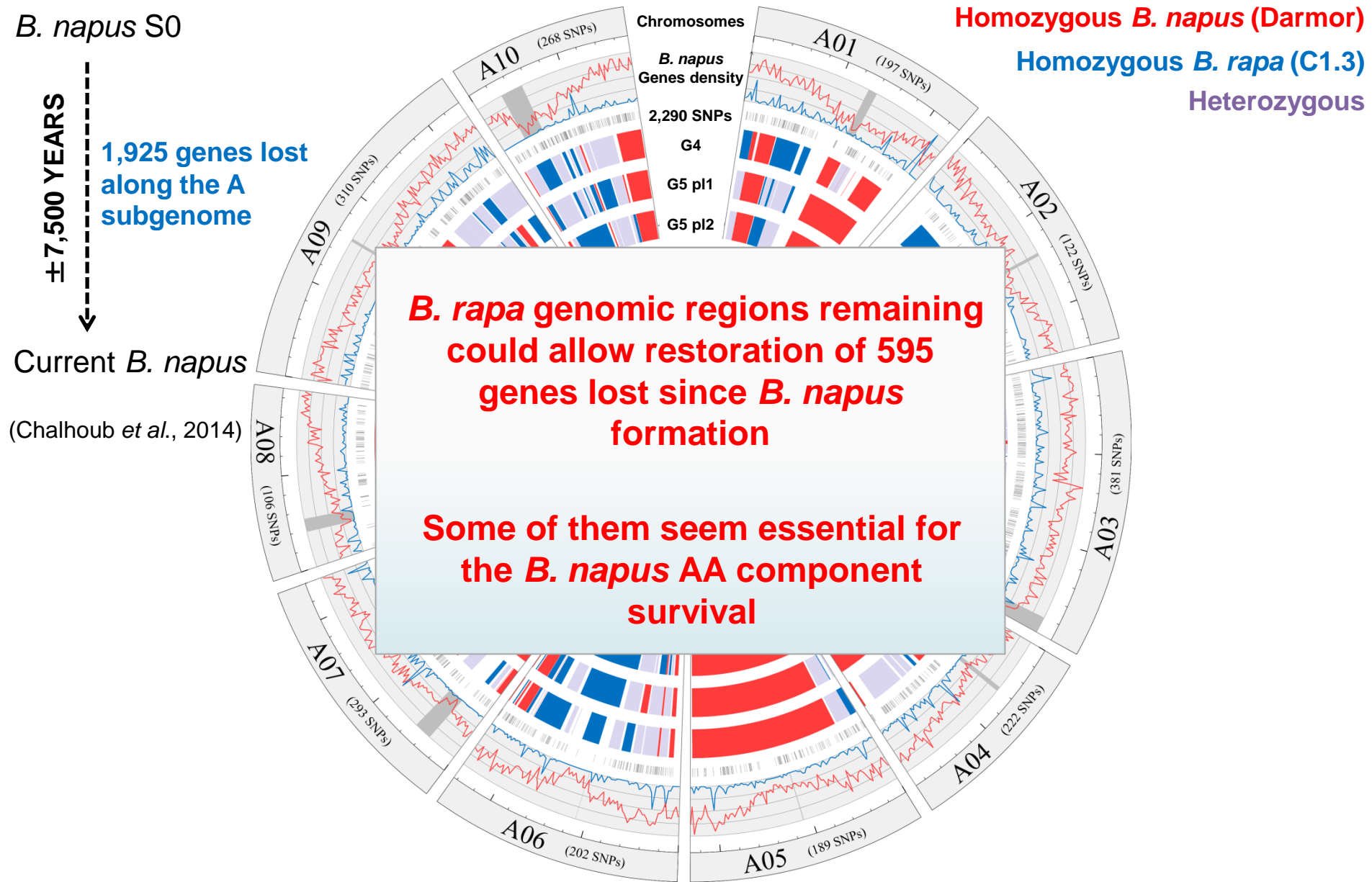


Homozygous *B. napus* (Darmor)

Homozygous *B. rapa* (C1.3)

Heterozygous

# Molecular characterization of the G4 and G5 AA plants



# CONCLUSION

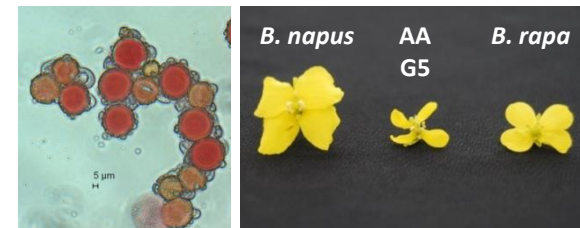
- ❖ We were unable to produce pure *B. napus* AA plants and AA plants generated (G4 & G5) presented a lower proportion of *B. napus* extracted than expected
- ❖ These results were related to the *B. napus* structural evolution in a polyploid context (genes lost)
- ❖ And suggest that only 7,500 years of coevolution leads to interdependency between the original subgenome

→ **68% of the *B. napus* A subgenome extracted**

↪ How far can we go? New cycle ongoing but:

→ **595 candidates genes**

↪ Functional characterization of the genes restored by the *B. rapa* genomic regions remaining in AA plants



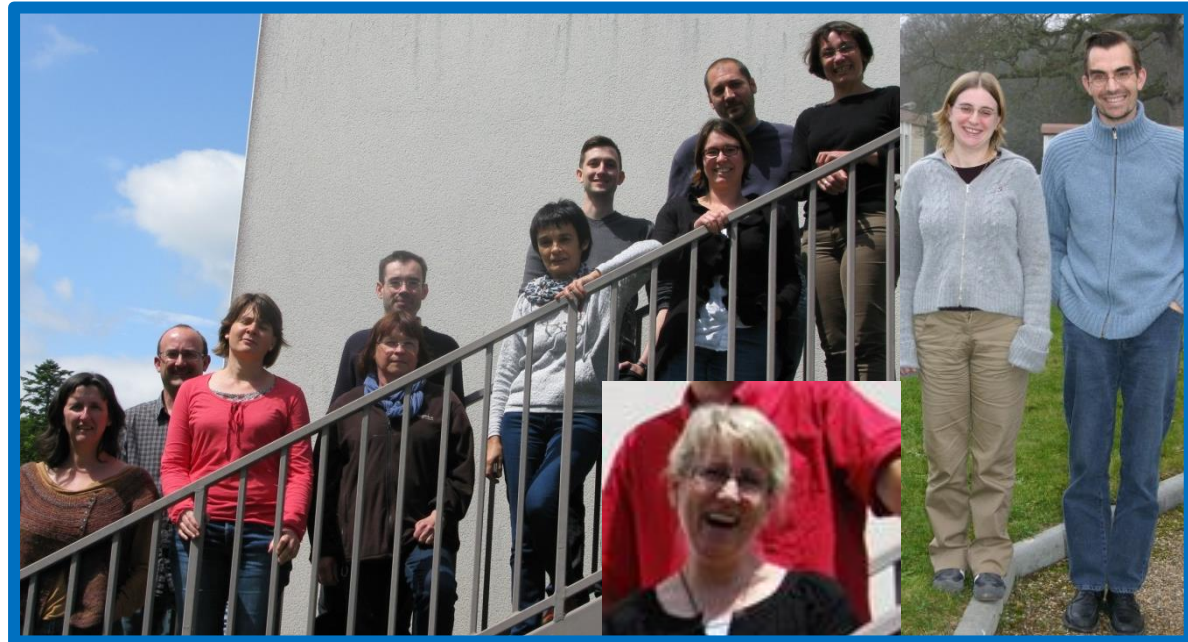
Low pollen  
fertility

Poor  
Vigor

# ACKNOWLEDGMENT

## Biodiversity & Polyploidy Team:

Anne-Marie CHEVRE  
Mathieu ROUSSEAU-GUEUTIN  
Gwenn TROTOUX  
Jérôme MORICE  
Sylvie NEGRE  
Maryse LODÉ  
Marie GILET  
Olivier CORITON  
Virginie HUTEAU  
Frédérique EBER



**...And thank you for your attention**

... I'M A POOR LONESOME  
GENOME, AND A LONG  
WAY FROM HOME...

**END**

