

New insights on homologous recombination in polyploids: the striking case of Brassica allotriploids

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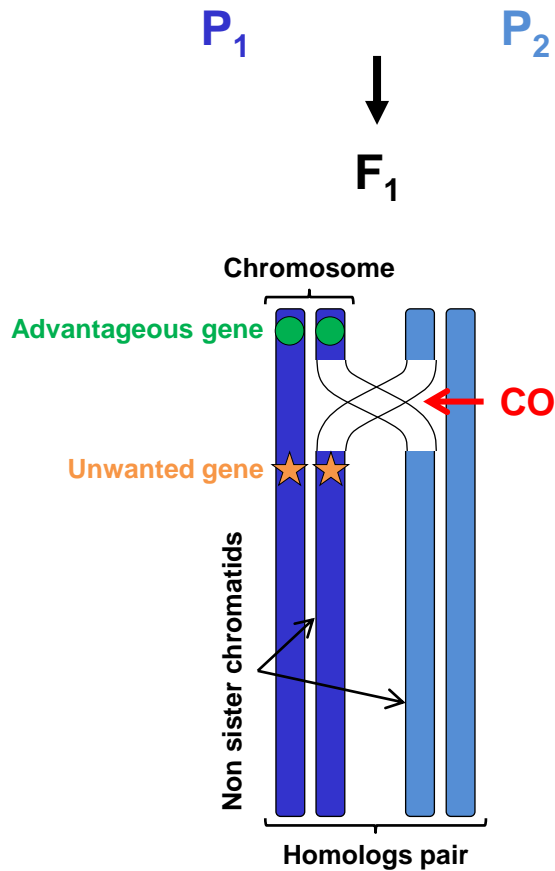
²GQE-LeMoulon, INRA, Université Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Gif sur Yvette, France

³GDEC, INRA, Université de Clermont-Ferrand, France

What is the interest of Meiotic Crossovers (COs)?

ESSENTIAL FOR PLANT BREEDING

Allow the generation of diversity



Selection in progeny

SCIENTIFIC CONTEXT

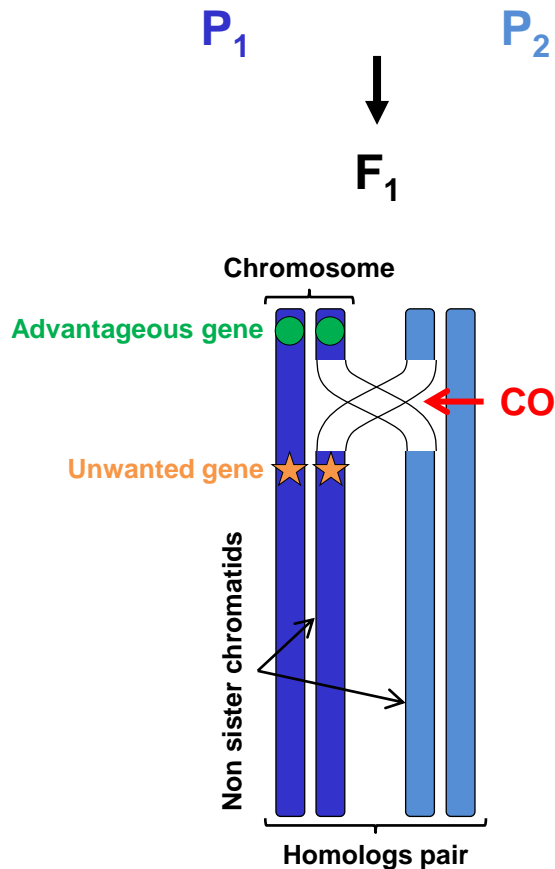
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Allow the generation of diversity

HOWEVER:

- Per pair of homologs, rarely more than 3 COs occur

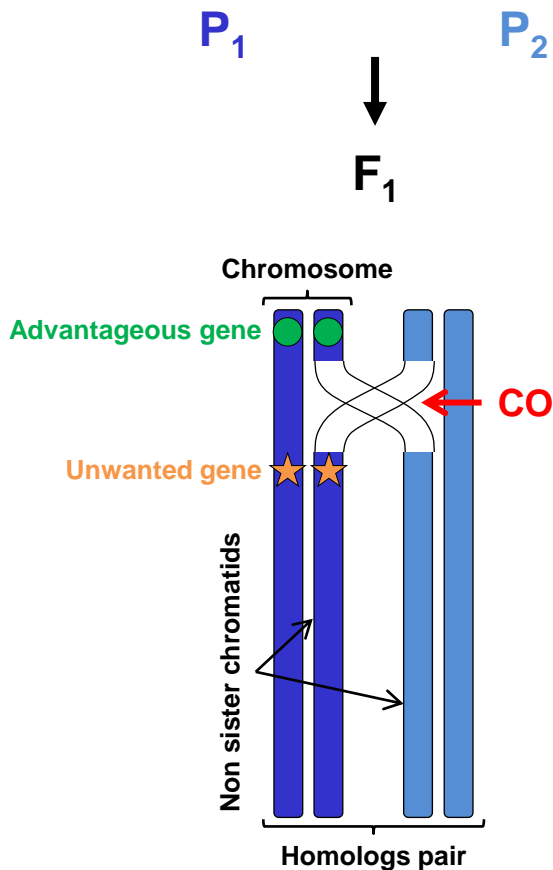


Selection in progeny

What is the interest of Meiotic Crossovers (COs)?

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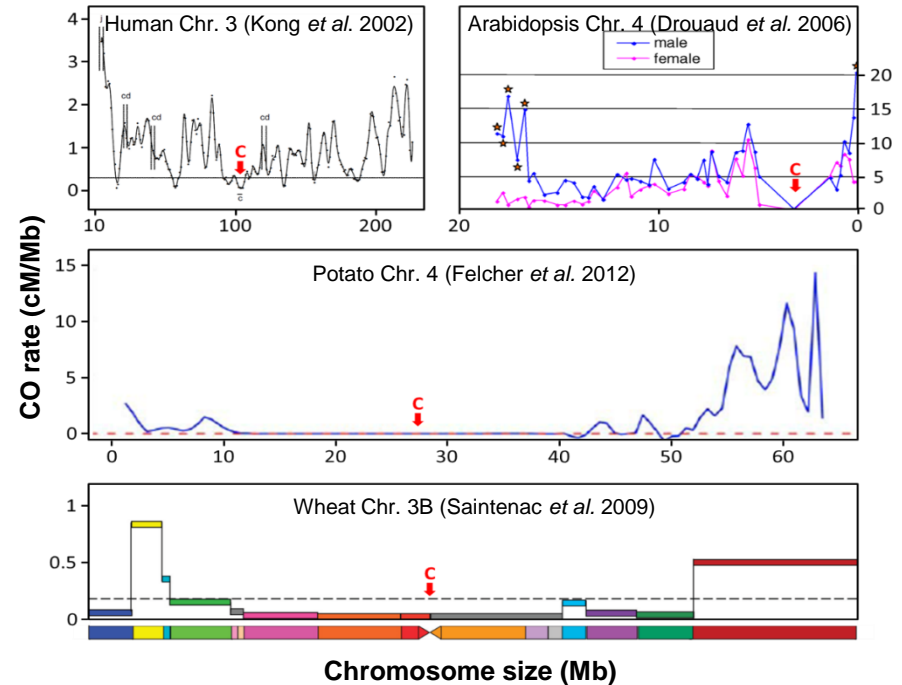
Allow the generation of diversity



Selection in progeny

HOWEVER:

- Per pair of homologs, rarely more than 3 COs occur
- COs distribution is NOT homogenous along the chromosomes



- The higher rates of recombination are often located on distal parts of the chromosomes
- No CO are formed in the vicinity of centromeres

SCIENTIFIC CONTEXT

What is the interest of Meiotic Crossovers (COs)?

ESSENTIAL FOR PLANT BREEDING

Allow the generation of diversity

BREEDING CONSTRAINTS:

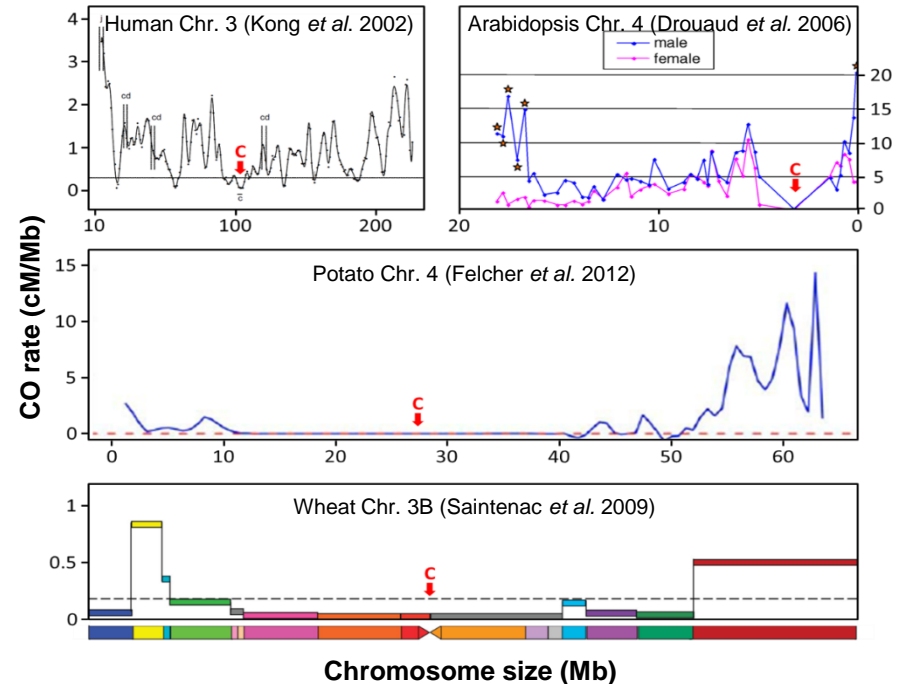
- Generation of large progenies
- Impossibility to separate some genes

INTEREST:

To control the number and distribution of COs events

HOWEVER:

- Per pair of homologs, rarely more than 3 COs occur
- COs distribution is NOT homogenous along the chromosomes



- The higher rates of recombination are often located on distal parts of the chromosomes
- No CO are formed in the vicinity of centromeres

SCIENTIFIC CONTEXT

Why the rapeseed is a good model for studying meiotic recombination?



- Formed 7,500 years ago
- 1st oleaginous species in Europe and 3rd in the world (70 Mt)
- Use for: Oil production & Seed cake



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

(Wang *et al.*, 2011)

X



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

(Liu *et al.*, 2014)



***B. napus*
AACC, 2n=38**

(Chalhoub *et al.*, 2014)

SCIENTIFIC CONTEXT

Why the rapeseed is a good model for studying meiotic recombination?



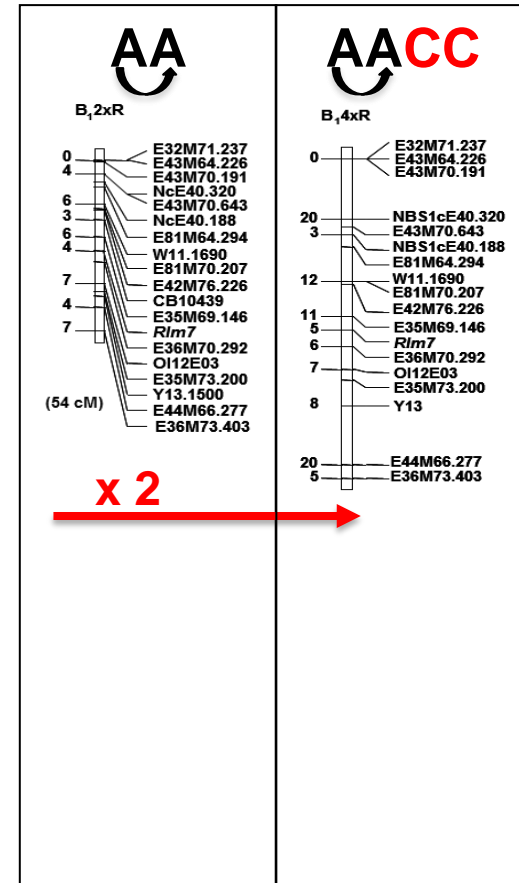
- Formed 7,500 years ago
- 1st oleaginous species in Europe and 3rd in the world (70 Mt)
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CO rate between homologous A07 chromosomes is 2 fold-higher in AA**CC** ($2n=38$) than AA ($2n=20$) hybrids

B. rapa AA,
 $2n=20$
(Wang *et al.*, 2011)

B. oleracea
CC, $2n=18$
(Liu *et al.*, 2014)

B. napus
AAC**C**, $2n=38$
(Chalhoub *et al.*, 2014)



SCIENTIFIC CONTEXT

Why the rapeseed is a good model for studying meiotic recombination?



- Formed 7,500 years ago
- 1st oleaginous species in Europe and 3rd in the world (70 Mt)
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Strikingly, CO rate between homologous A07 chromosomes is much higher in AAC hybrids

B. rapa AA
2n=20

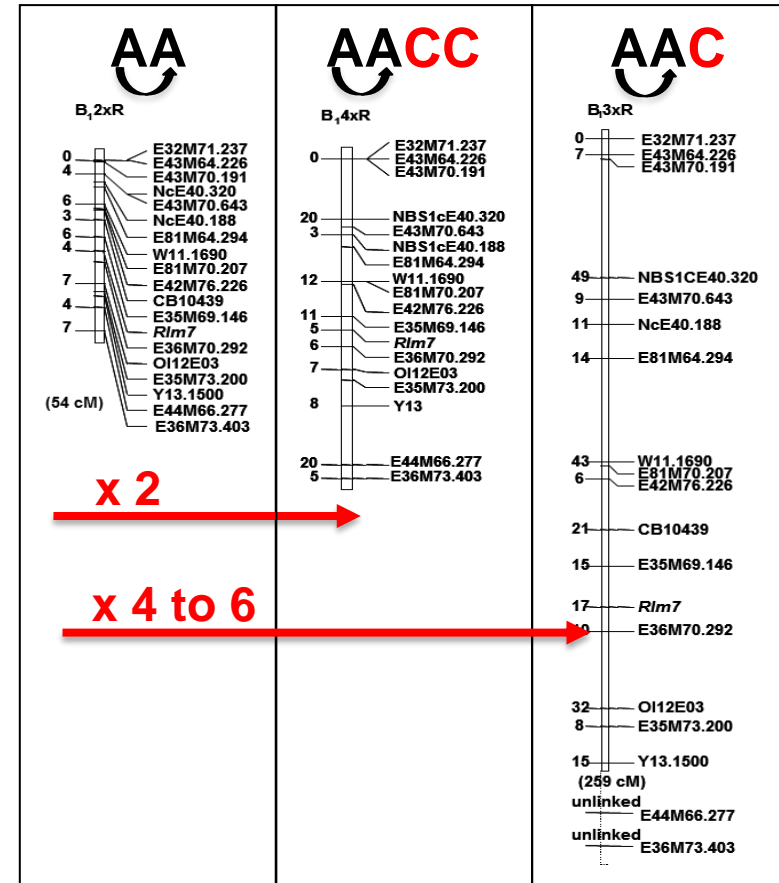
B. oleracea CC
2n=18

(Leflon *et al.*, 2010)

QUESTIONS

What about the rate and distribution of COs between the 10 homologous A chromosomes in AAC hybrids?

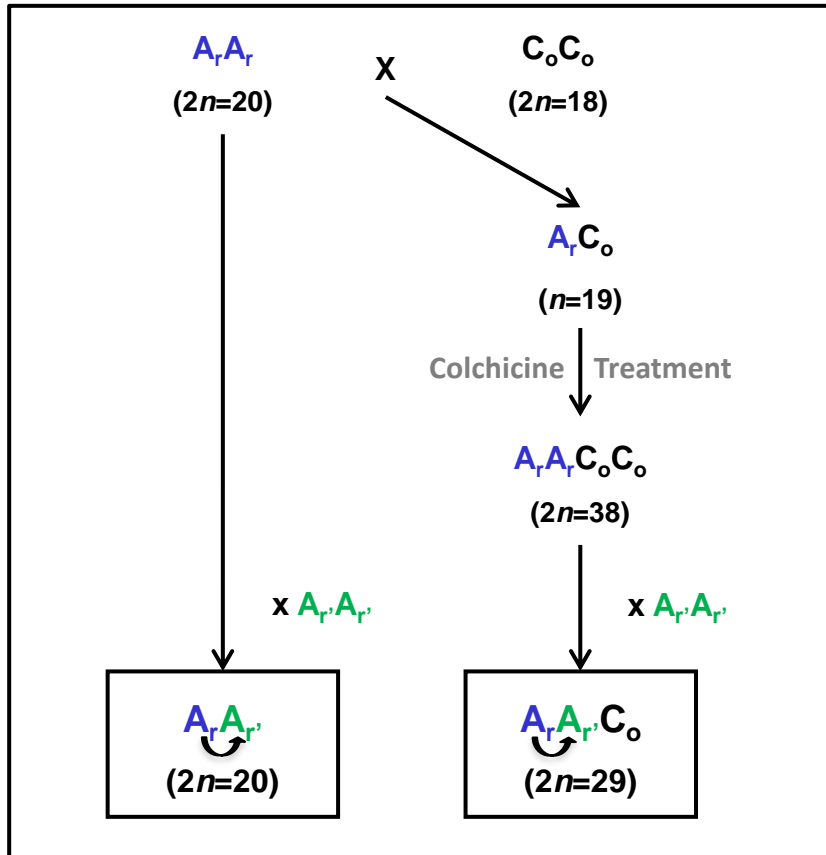
AACC 2n=38



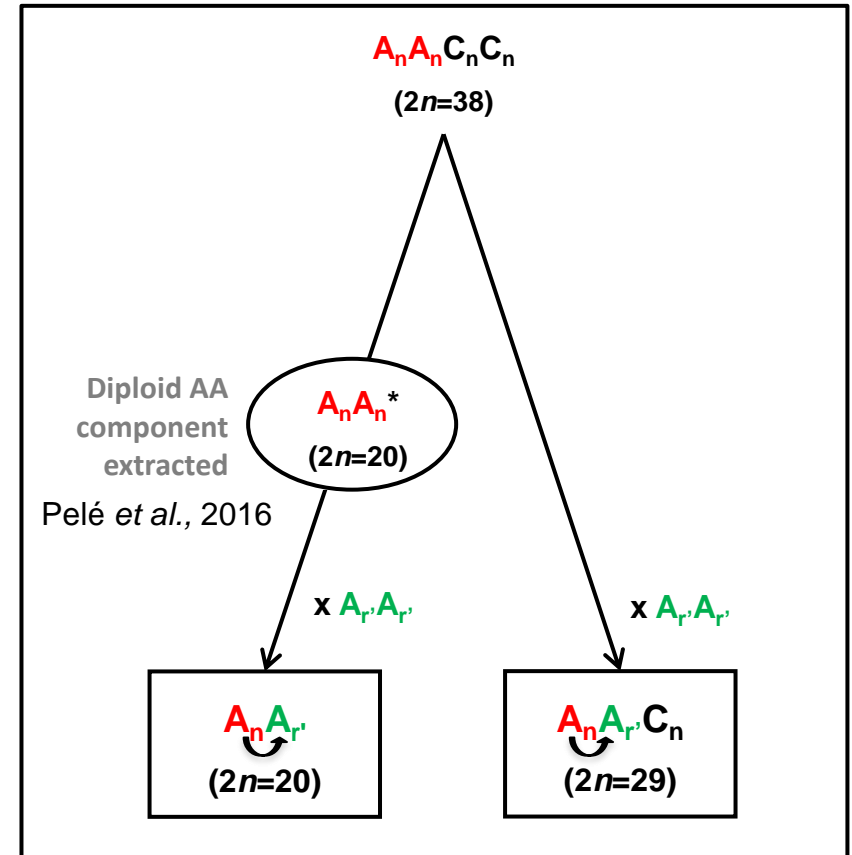
(Leflon *et al.*, 2010)

RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

Production of AA and AAC hybrids



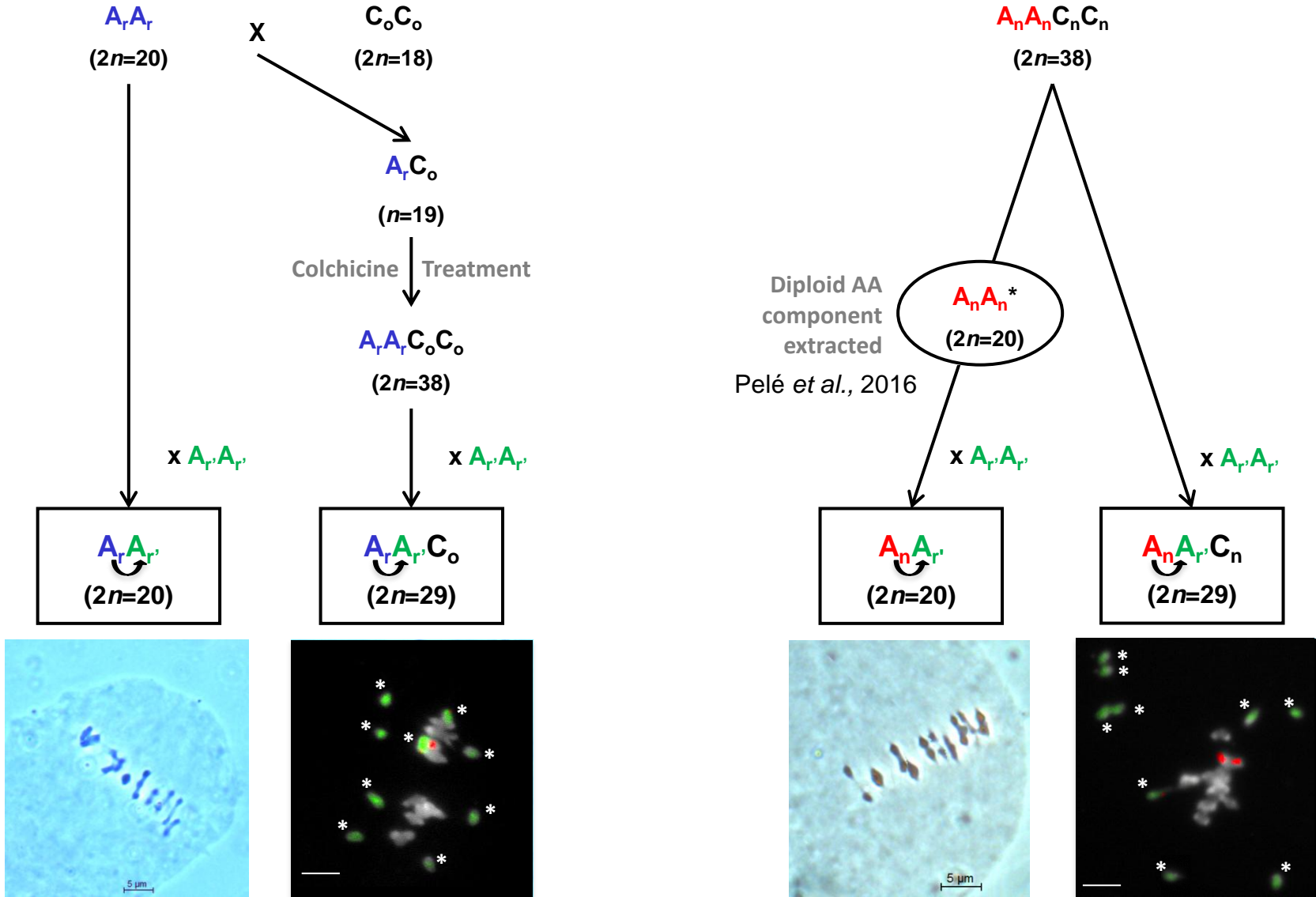
- $A_r A_r$ = *B. rapa* cv. C1.3
- $A_r' A_r'$ = *B. rapa* cv. Chiifu (Sequenced)
- $C_o C_o$ = *B. oleracea* cv. RC34.



- $A_n A_n$ = A genome of *B. napus* cv. Darmor extracted
- $A_r' A_r'$ = *B. rapa* cv. Chiifu (Sequenced)
- $A_n A_n C_n C_n$ = *B. napus* cv. Darmor.

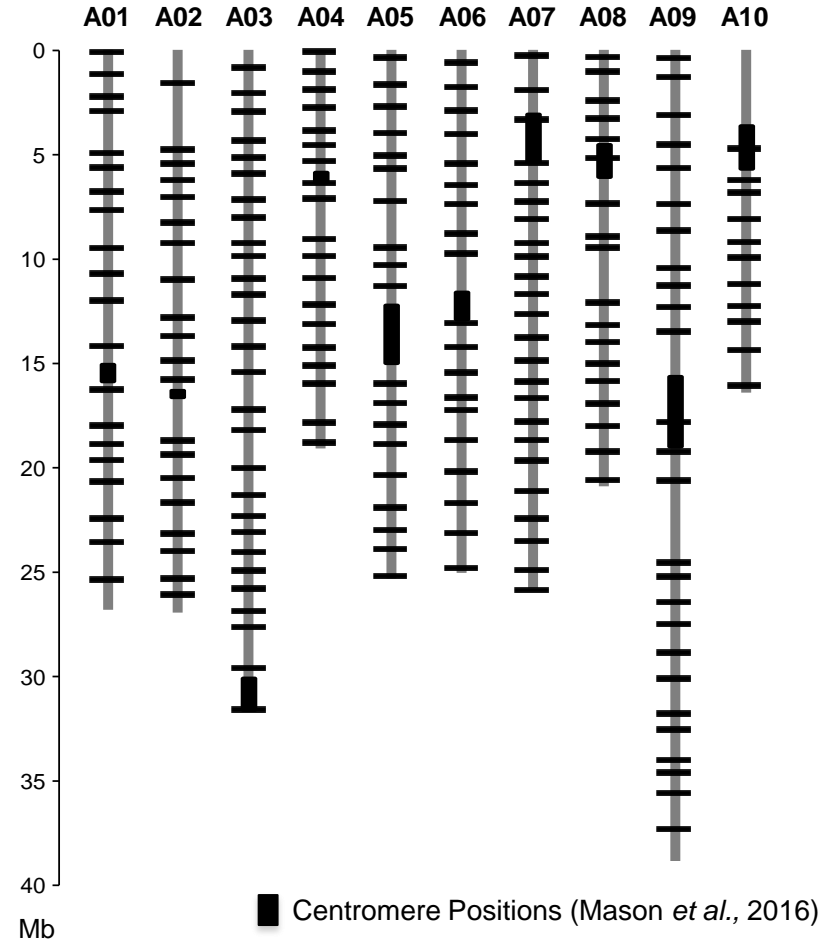
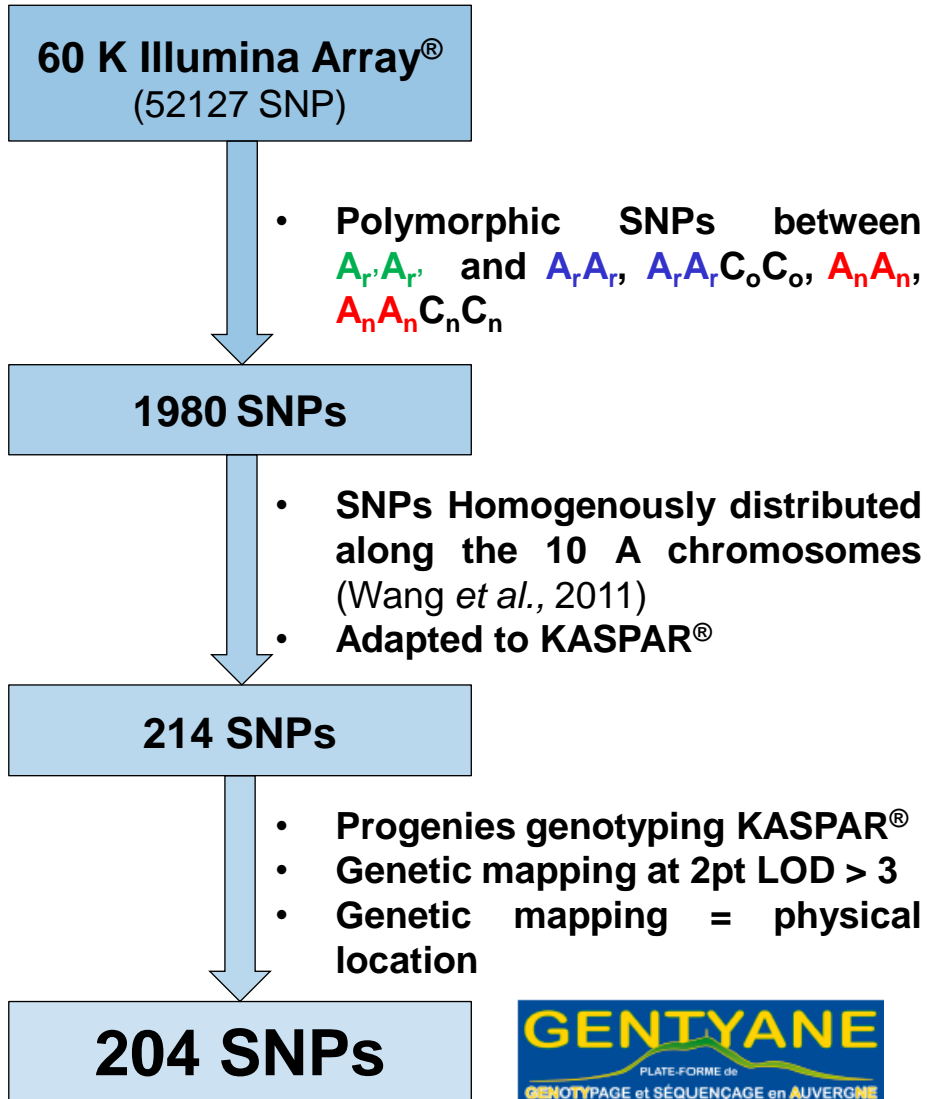
RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

Cytogenetic characterization of AA and AAC hybrids



RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

Selection of polymorphic SNP markers covering the whole A genome

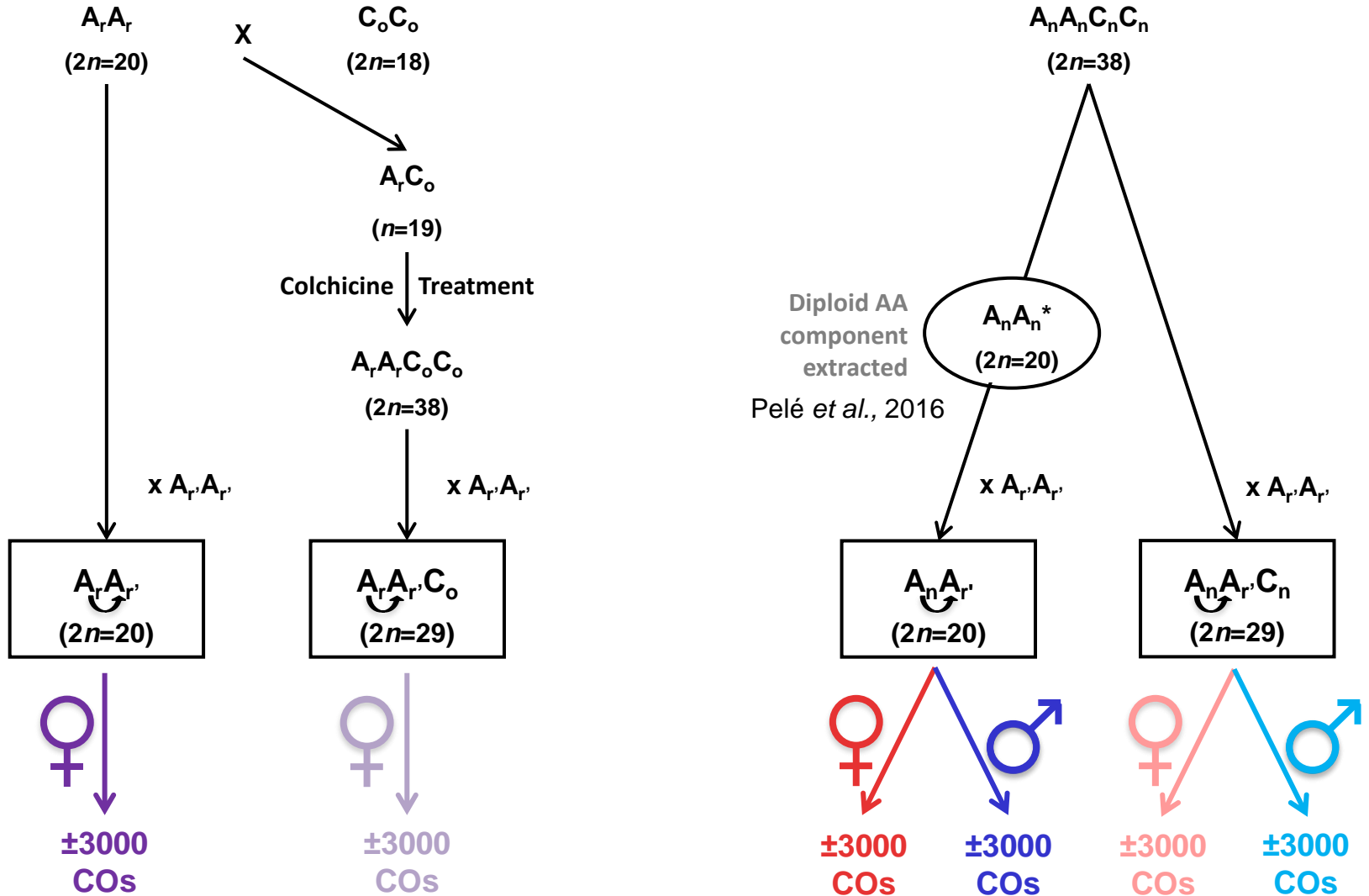


1 SNP / 1.25 Mb (SE = 0.04, n = 194)

Covering 94% of the A genome

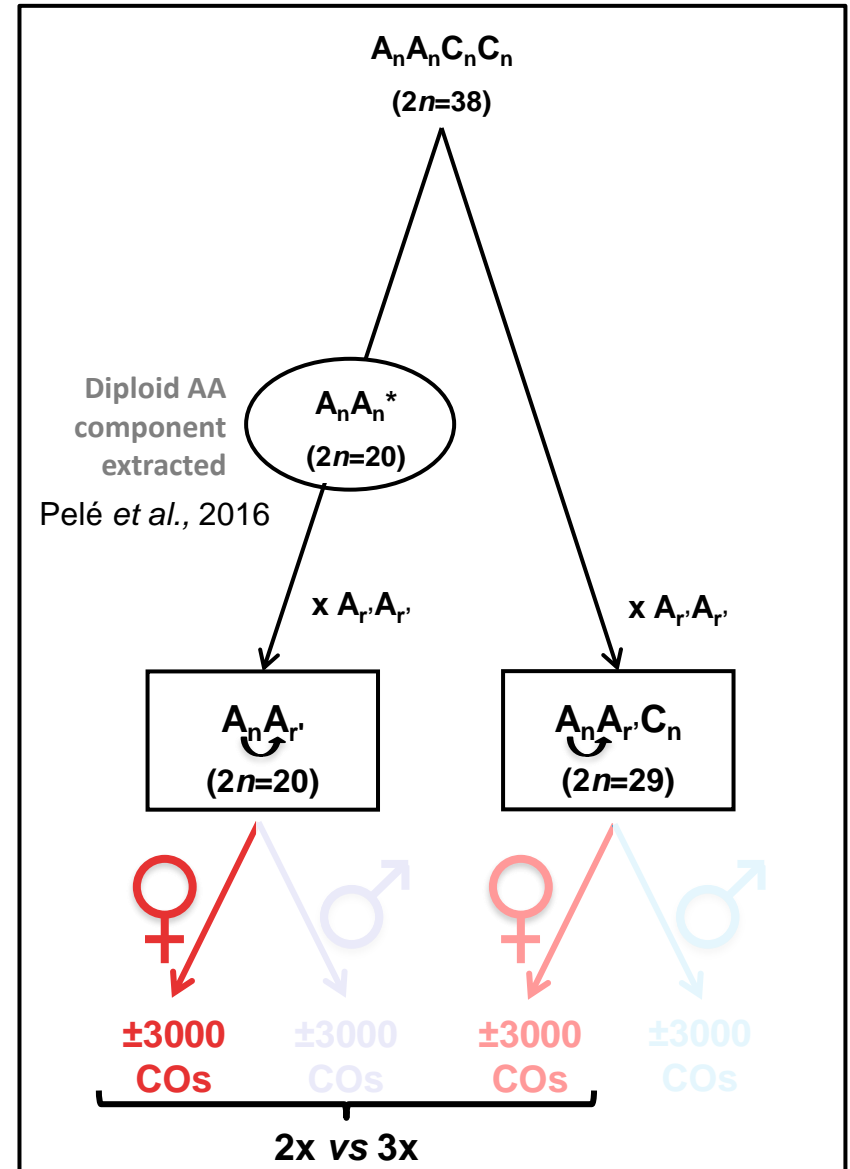
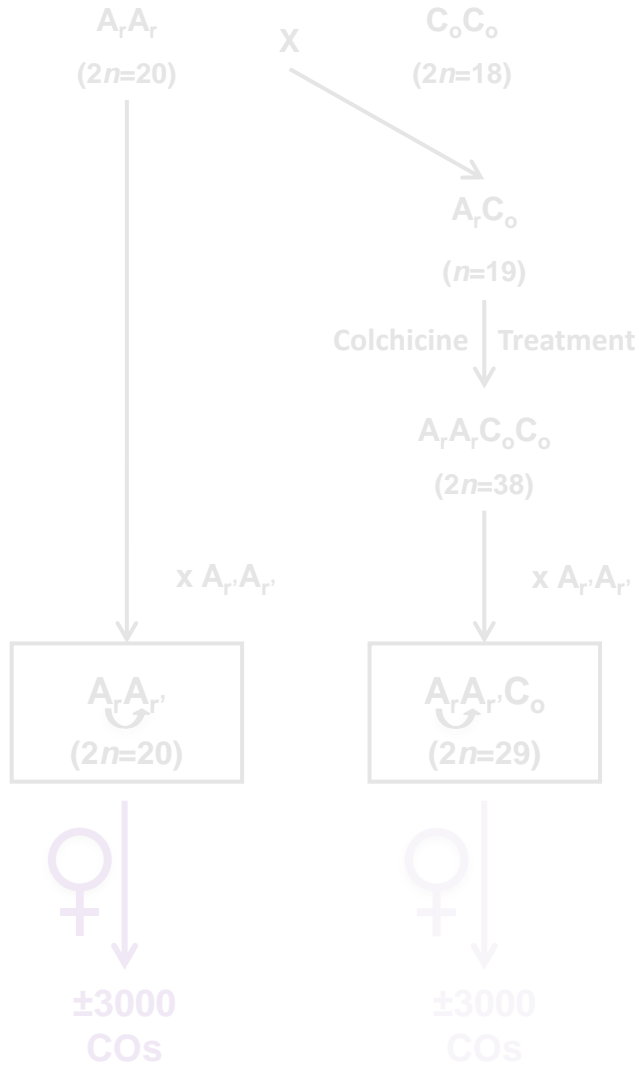
RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

Detection of COs on progenies of AA and AAC



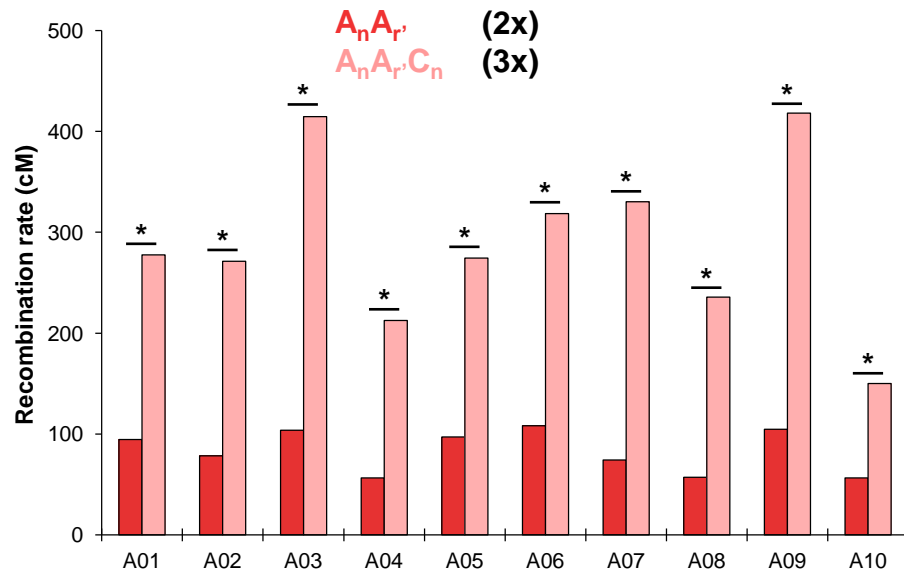
RATE AND DISTRIBUTION OF COs IN AA vs AAC HYBRIDS

Detection of COs on progenies of AA and AAC



RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

CO rates between the homologous A chromosomes in AA and AAC hybrids

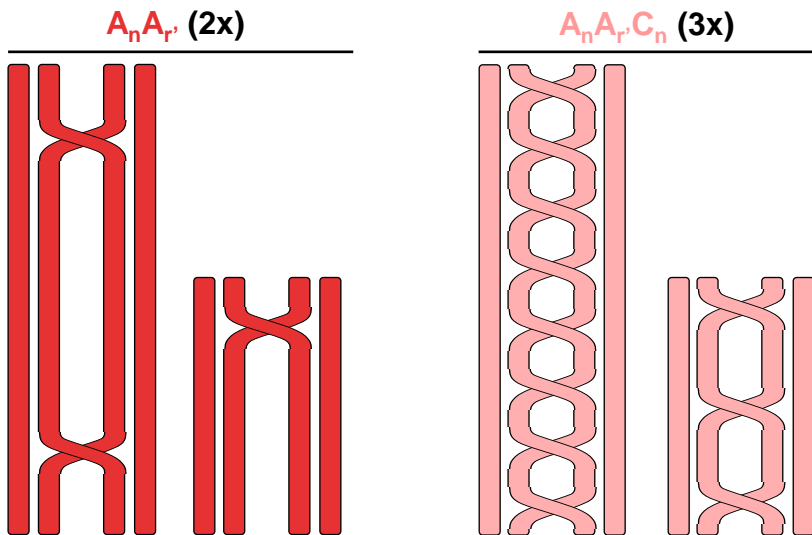
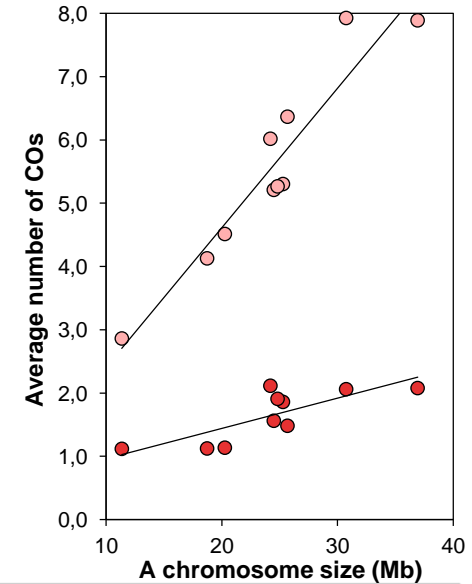
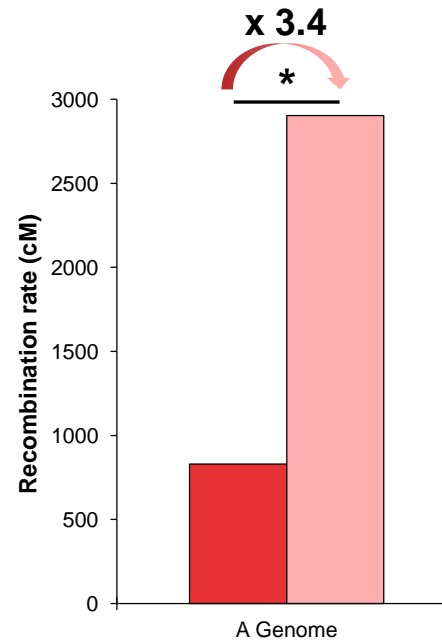
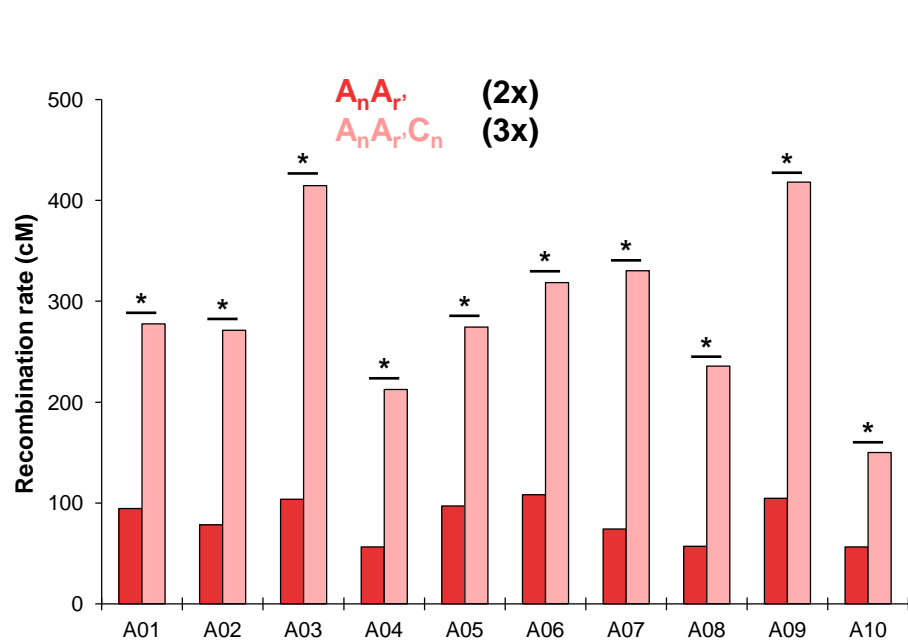


CO rate: AA <<< AAC

Between all homologous A chromosomes,
extending at the whole A genome the
results of Leflon *et al.* (2010)

RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

CO rates between the homologous A chromosomes in AA and AAC hybrids



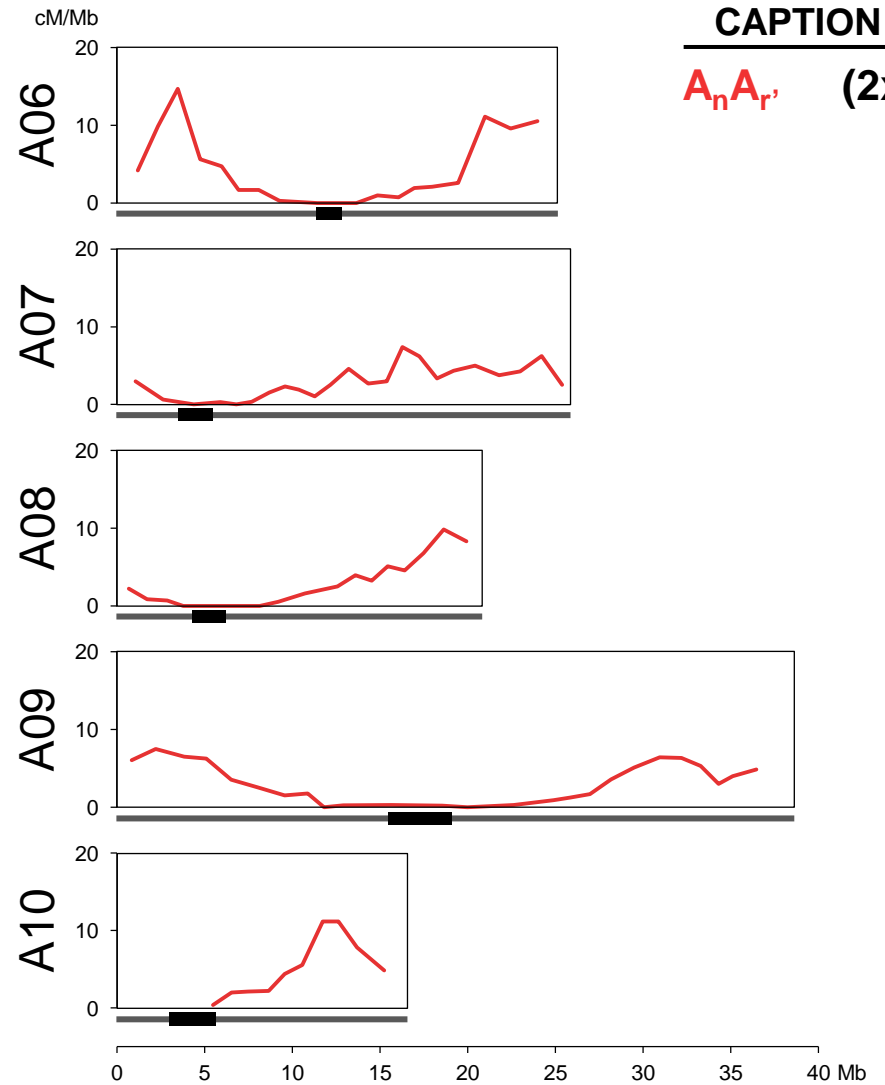
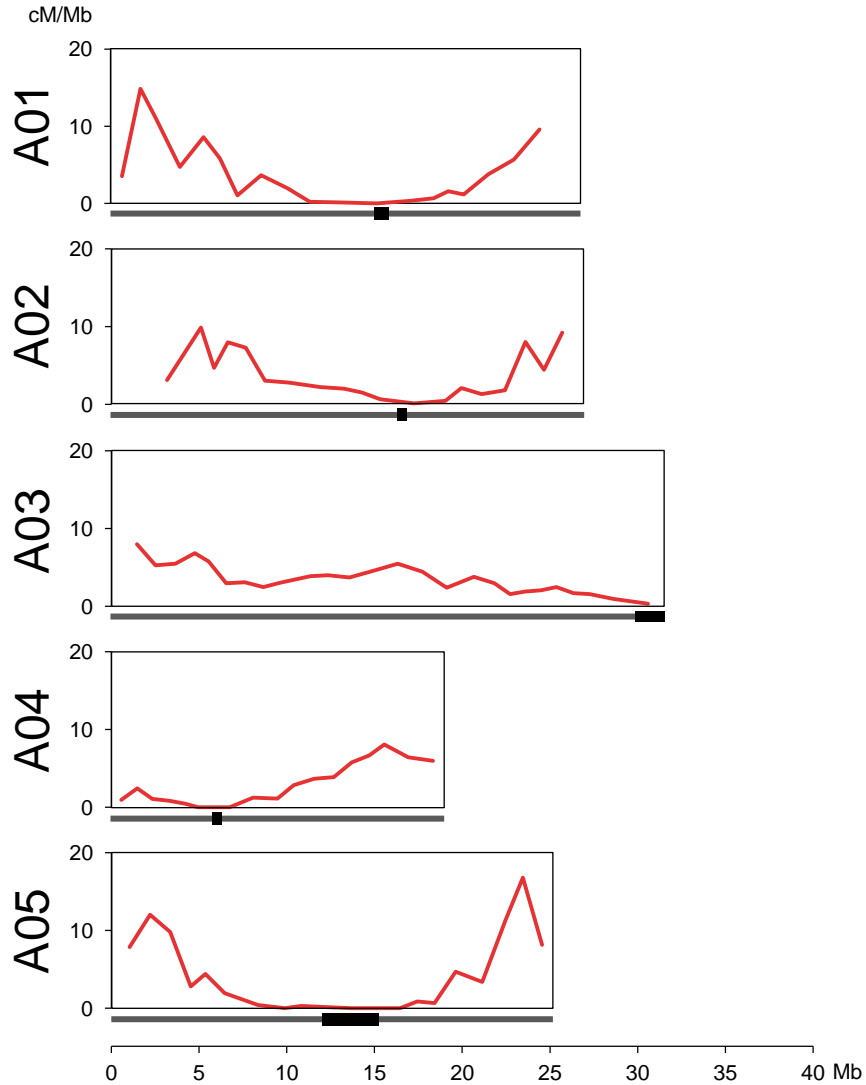
CO rate: AA <<< AAC

At the whole A genome the number of COs is 3.4-fold higher in AAC hybrid

The number of COs is positively correlated with the A chromosome size

RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

COs distribution along the A chromosomes in AA and AAC hybrids

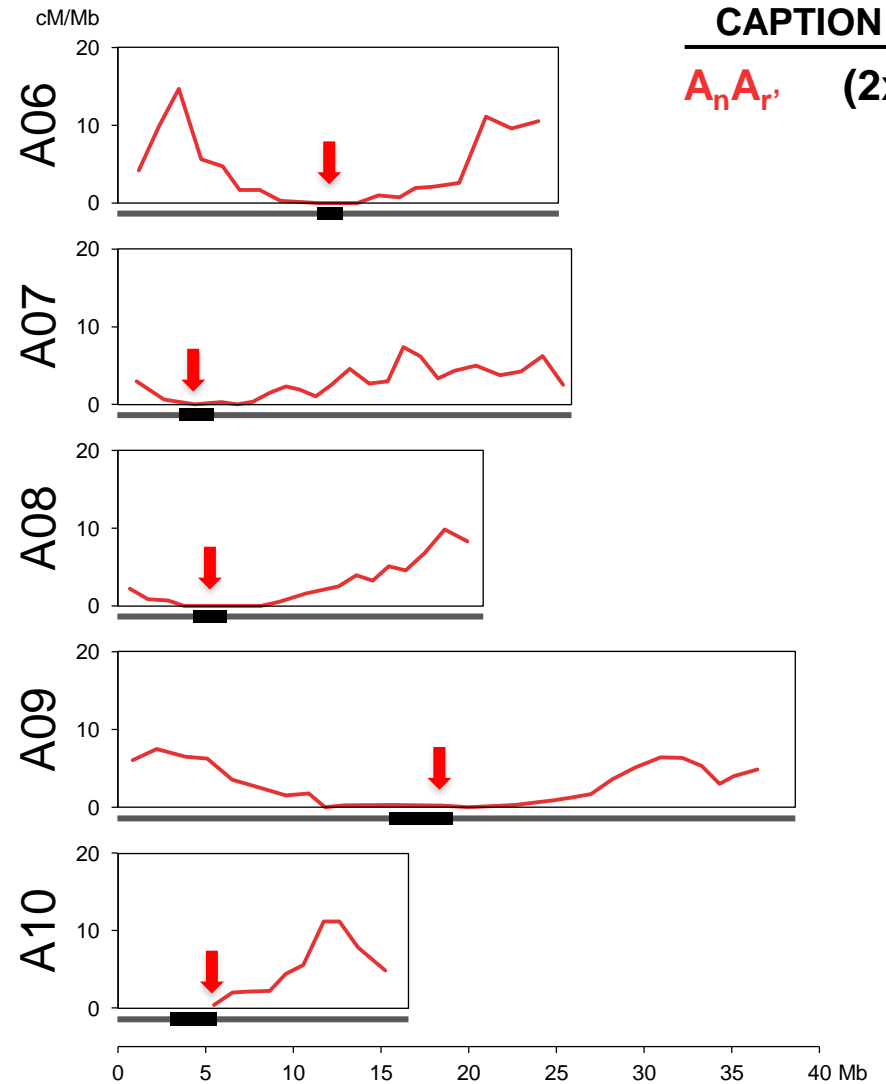
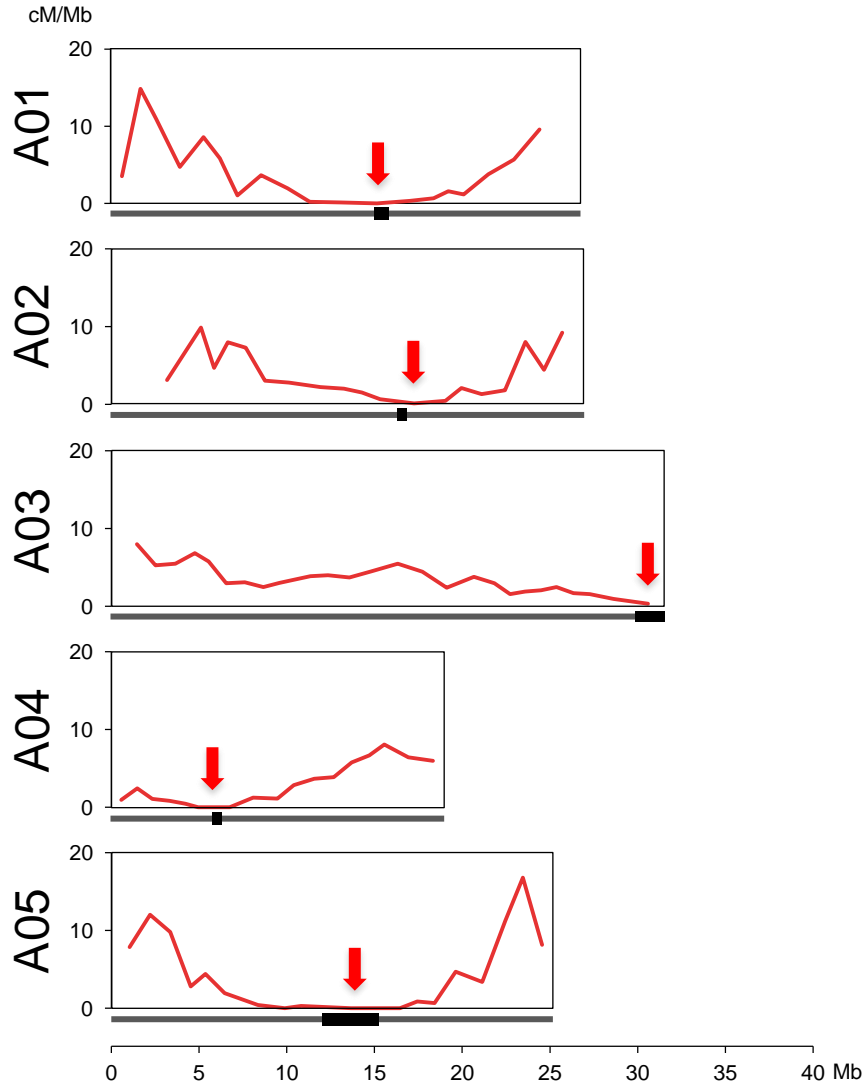


CAPTION
 $A_n A_{r'}$ (2x)

RATE AND DISTRIBUTION OF COs IN AA vs AAC HYBRIDS

COs distribution along the A chromosomes in AA and AAC hybrids

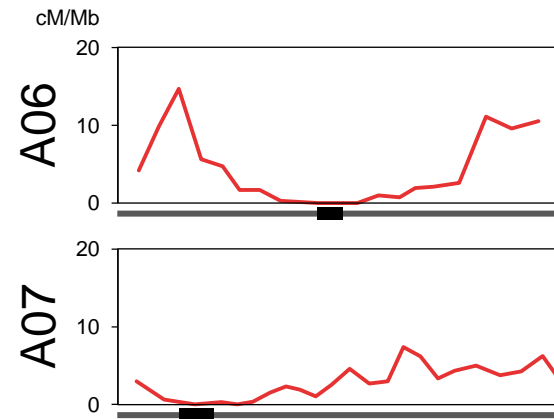
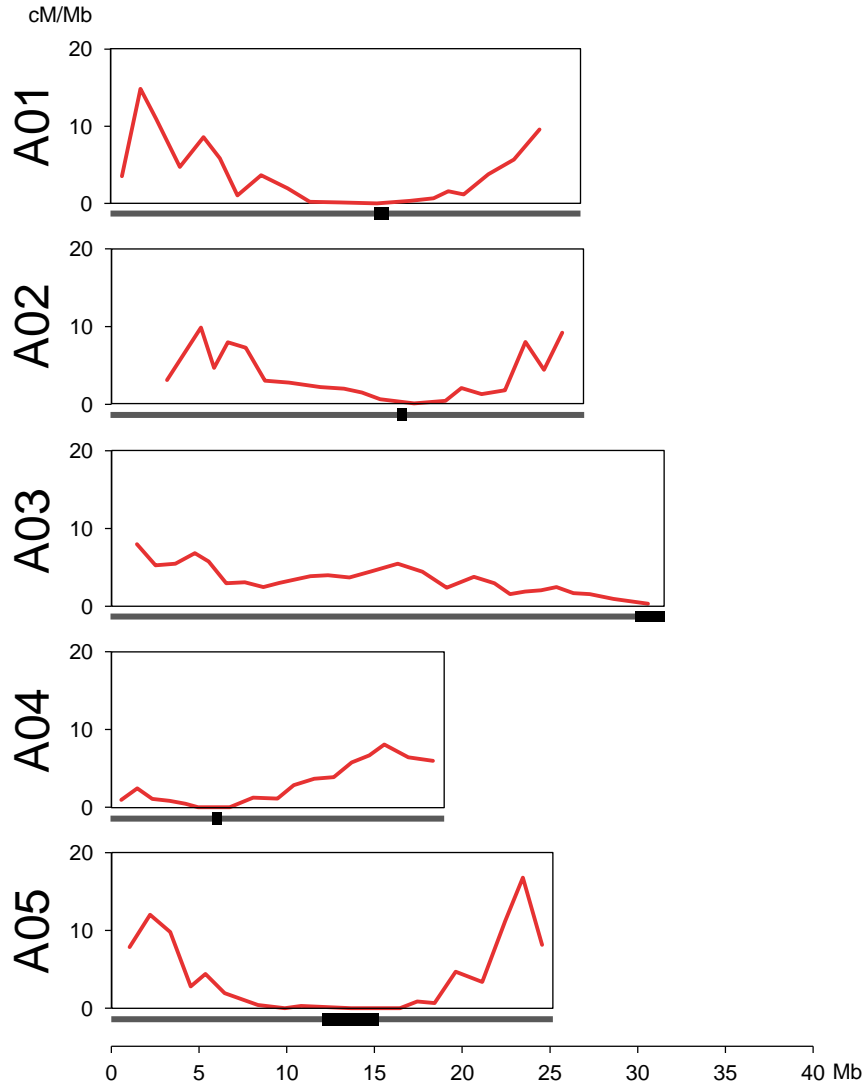
- ❖ In AA hybrid, ~10% of the A genome is totally deprived of COs, especially close to the centromeres



CAPTION
 $A_n A_{r'}$ (2x)

RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

COs distribution along the A chromosomes in AA and AAC hybrids



CAPTION
 $A_n A_{r'}$ (2x)

In AA hybrid

Expected Recombination landscapes are observed along each A chromosome

With:

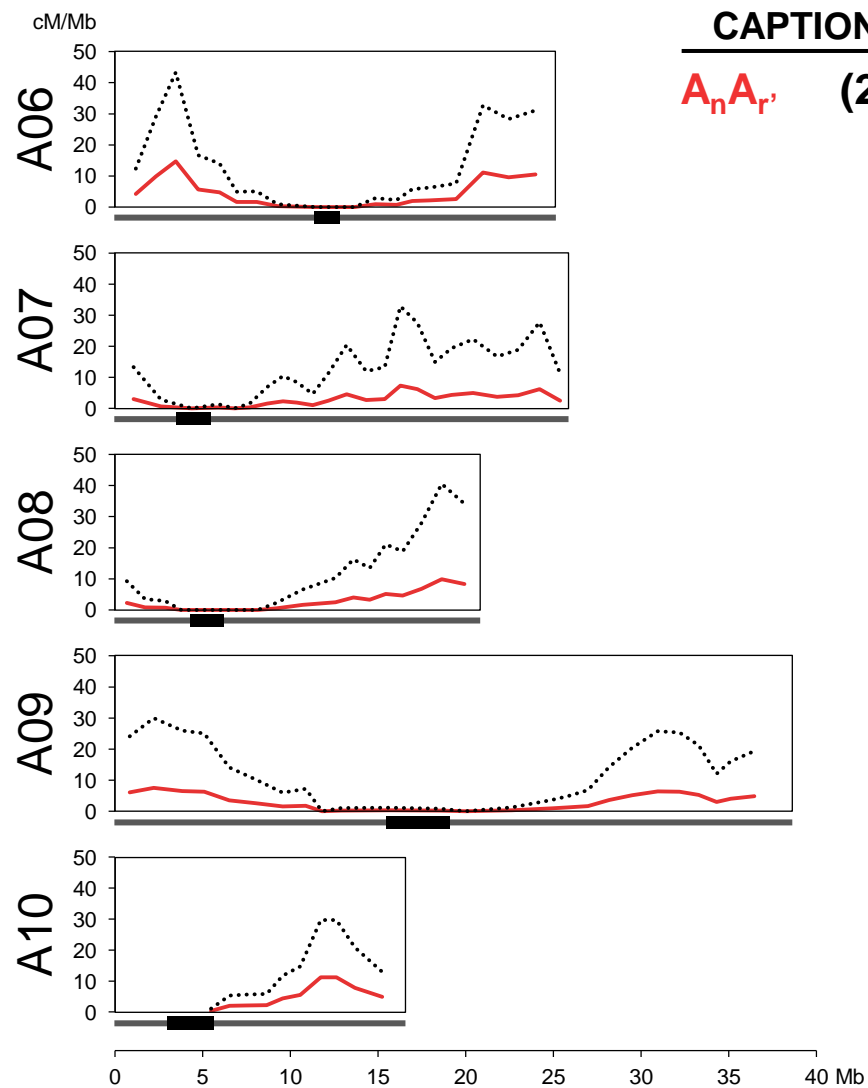
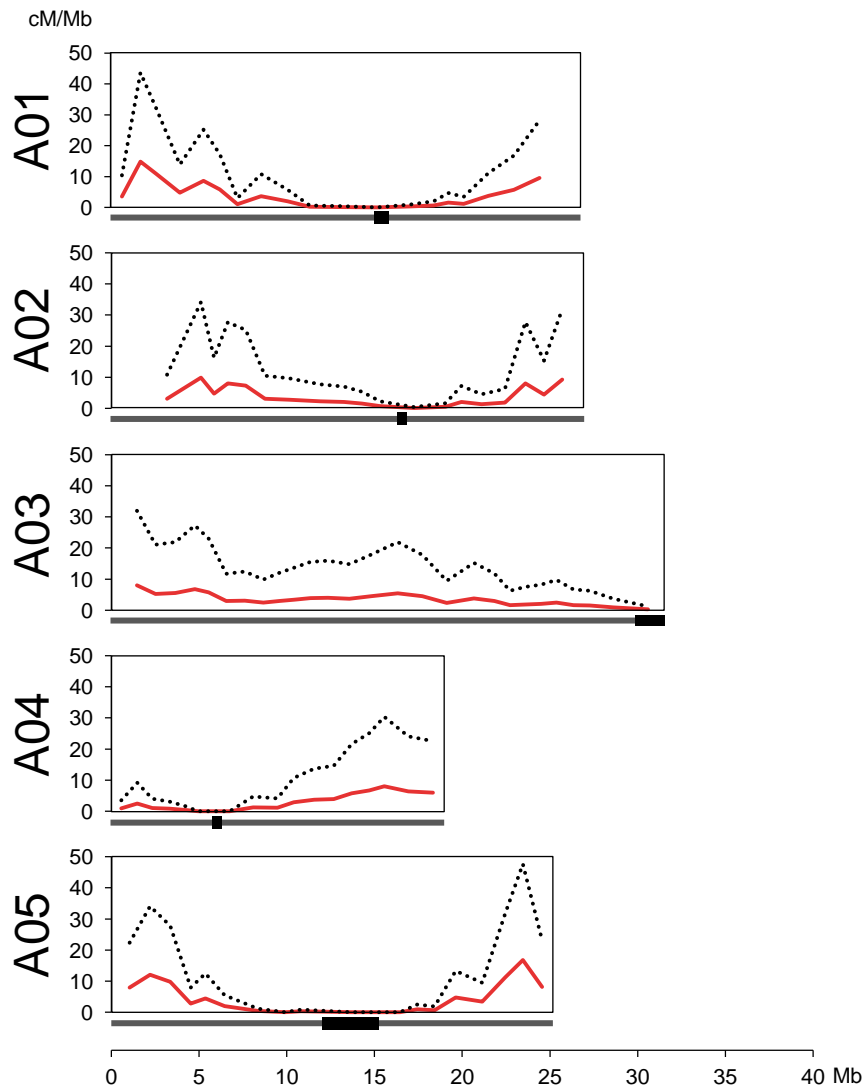
- ✓ No CO in the vicinity of centromeres
- ✓ Gradient for CO rates along chromosome arms

What is going on in AAC hybrid?

RATE AND DISTRIBUTION OF COs IN AA vs AAC HYBRIDS

COs distribution along the A chromosomes in AA and AAC hybrids

❖ Does CO rates increase proportionally along the A chromosomes compared to AA hybrid?

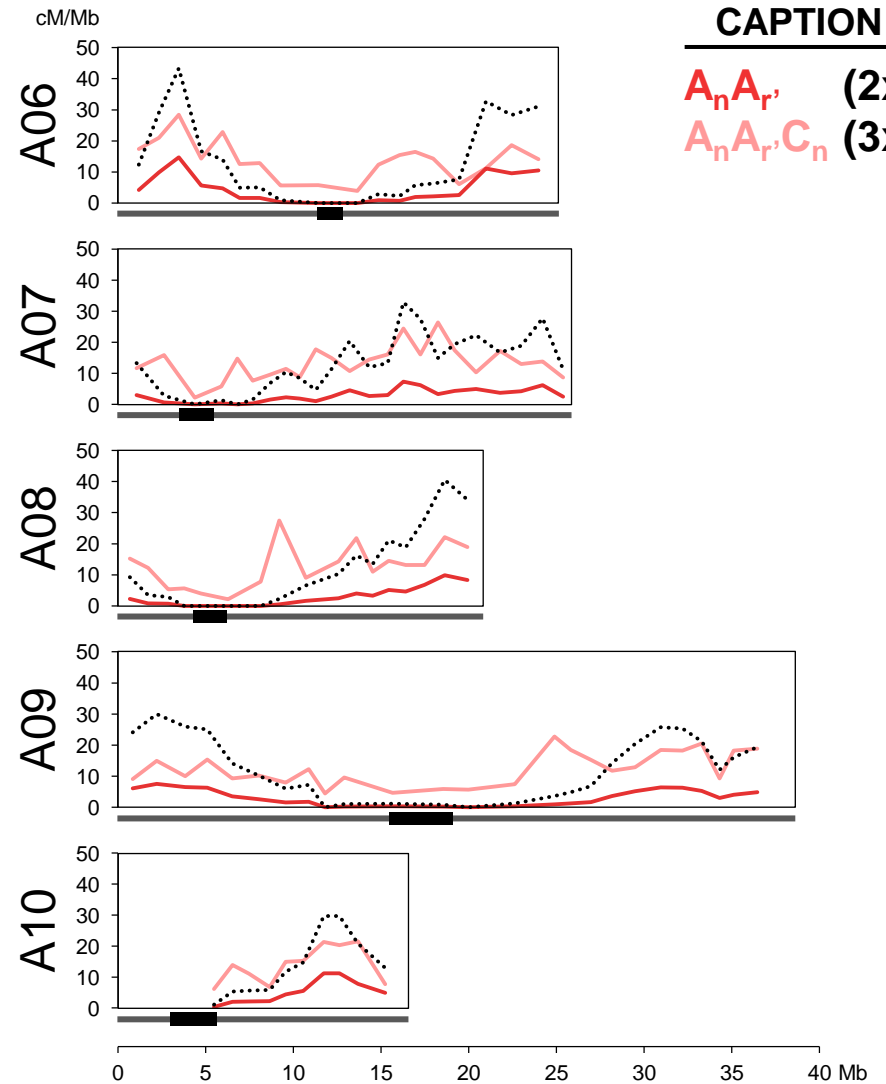
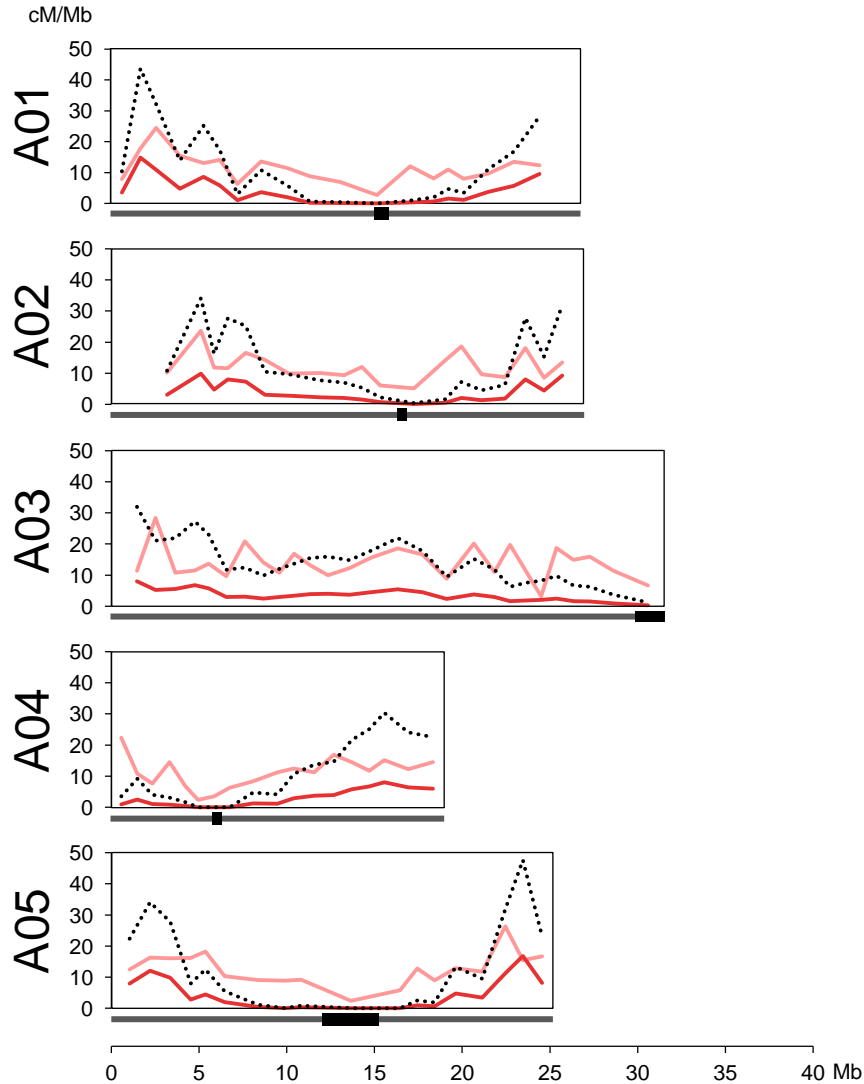


CAPTION
 $A_n A_{r'}$ (2x)

RATE AND DISTRIBUTION OF COs IN AA vs AAC HYBRIDS

COs distribution along the A chromosomes in AA and AAC hybrids

❖ Does CO rates increase proportionally along the A chromosomes compared to AA hybrid? **NO**



CAPTION

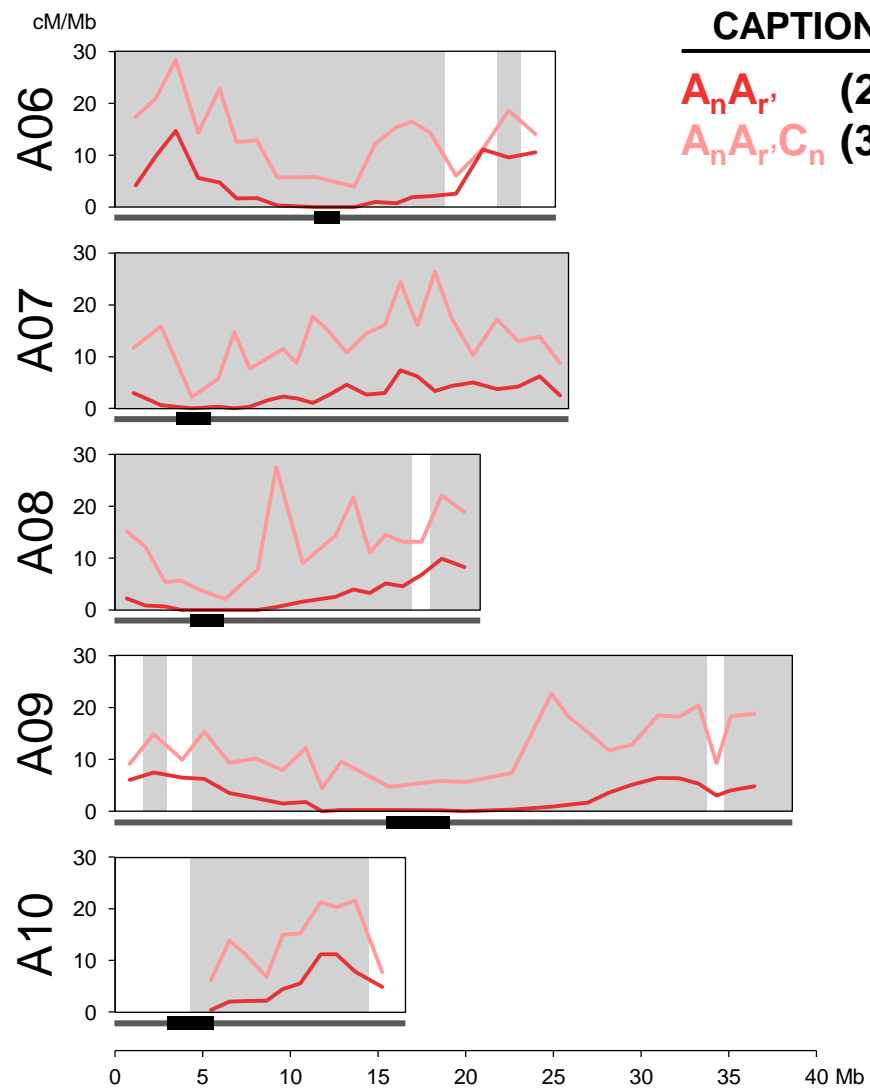
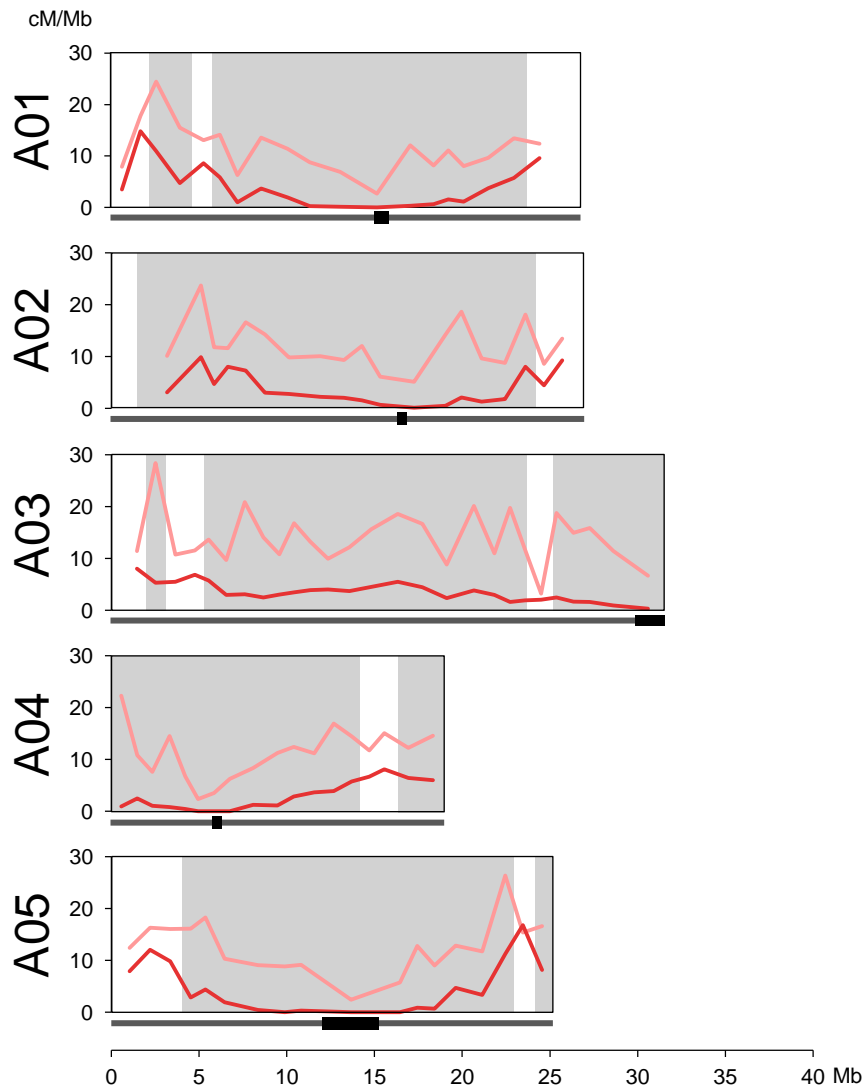
$A_n A_{r'}$ (2x)

$A_n A_{r'} C_n$ (3x)

RATE AND DISTRIBUTION OF COs IN AA vs AAC HYBRIDS

COs distribution along the A chromosomes in AA and AAC hybrids

❖ In AAC hybrid, CO rates increase significantly in most intervals compared to AA hybrids



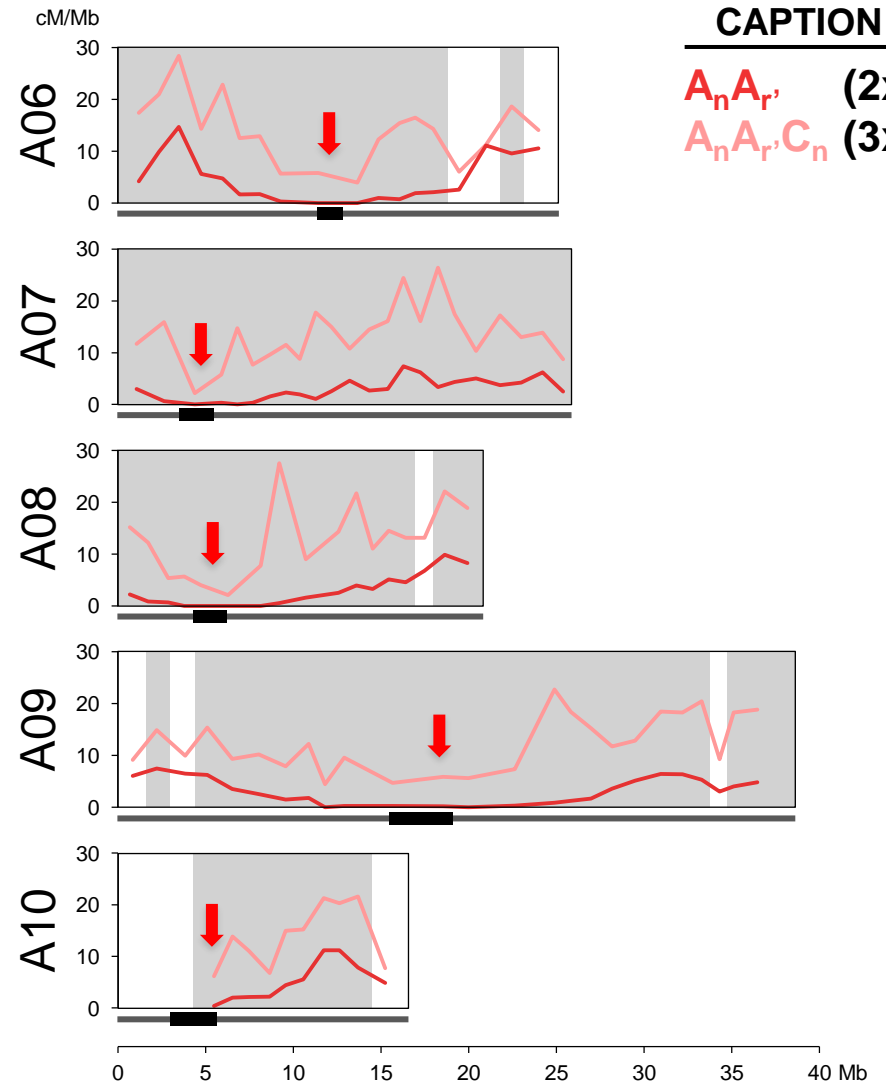
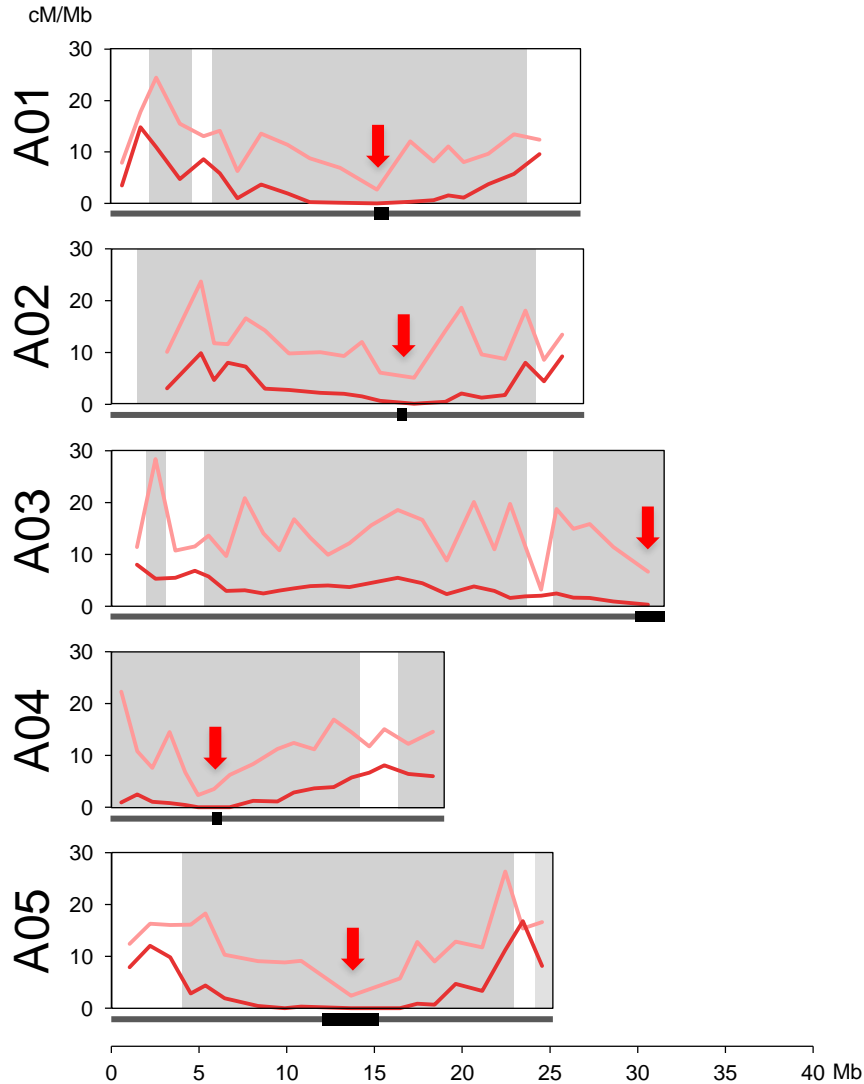
CAPTION

$A_n A_{r'}$ (2x)
 $A_n A_{r'} C_n$ (3x)

RATE AND DISTRIBUTION OF COs IN AA vs AAC HYBRIDS

COs distribution along the A chromosomes in AA and AAC hybrids

❖ Even in intervals close or including the **centromeres**, where **new recombining regions** are formed



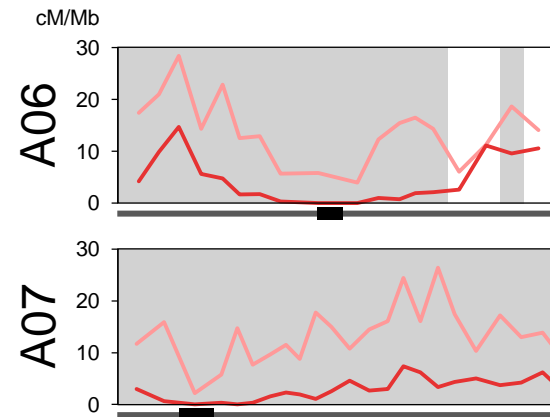
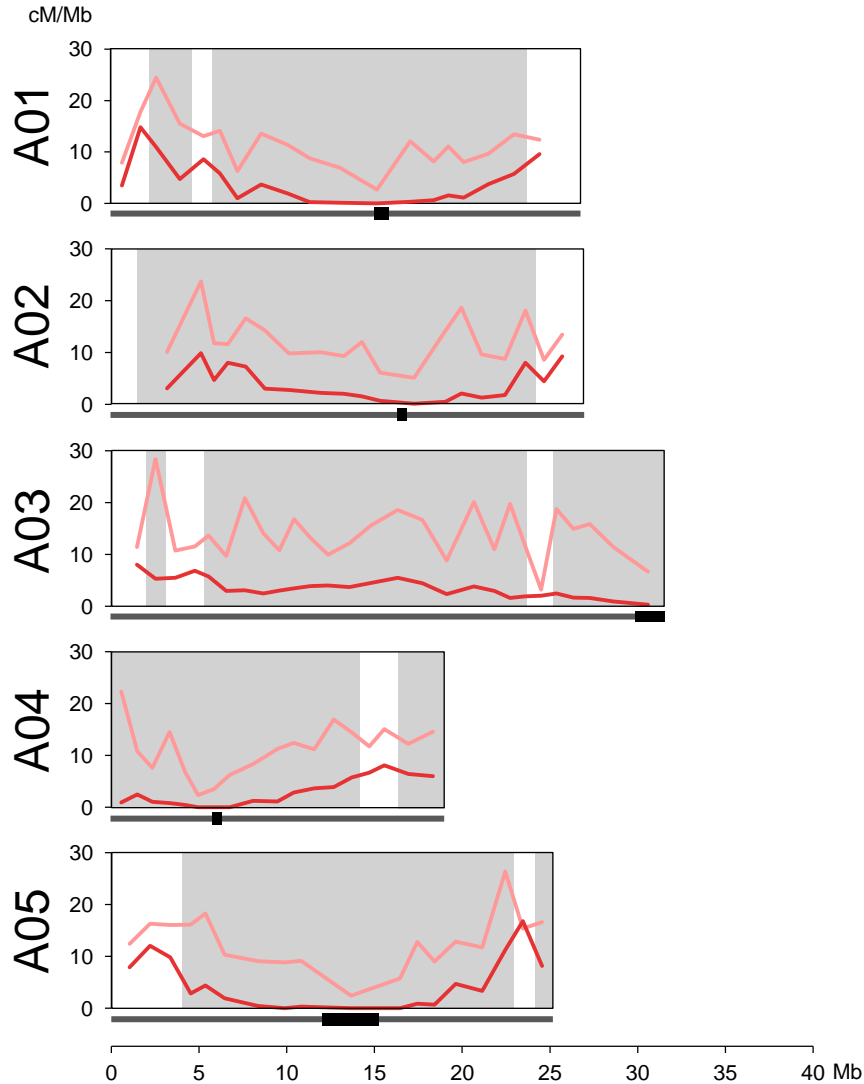
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$A_n A_{r'}$ (2x)

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RATE AND DISTRIBUTION OF COs IN AA vs AAC HYBRIDS

COs distribution along the A chromosomes in AA and AAC hybrids



CAPTION

$A_n A_{r'}$ (2x)
 $A_n A_{r'} C_n$ (3x)

In AAC hybrid

Recombination landscapes along each of the A chromosomes are \neq AA hybrid

- ✓ COs are formed all along the A genome, even in the vicinity of the centromeres
- ✓ No Gradient for CO rates along chromosome arms

RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

In conclusion, the additional C genome allows to change:

	♀		♂
	C genome from <i>B. oleracea</i>	C genome from <i>B. napus</i>	C genome from <i>B. napus</i>
❖ COs number :	$A_r A_{r'} \lll A_r A_{r'} C_o$	$A_n A_{r'} \lll A_n A_{r'} C_n$	$A_n A_{r'} \lll A_n A_{r'} C_n$
❖ COs distribution :	$A_r A_{r'} \neq A_r A_{r'} C_o$	$A_n A_{r'} \neq A_n A_{r'} C_n$	$A_n A_{r'} \neq A_n A_{r'} C_n$

All these results were confirmed whatever:

- ✓ The C genome origin
- ✓ The sex of meiosis

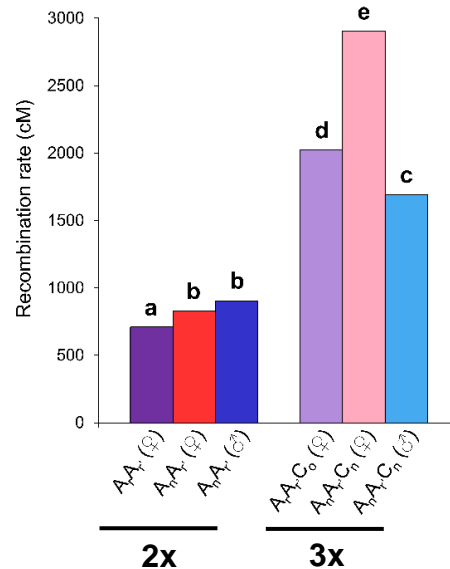
RATE AND DISTRIBUTION OF COs IN AA VS AAC HYBRIDS

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BUT:

Variations were observed only for the CO rates between AAC hybrids



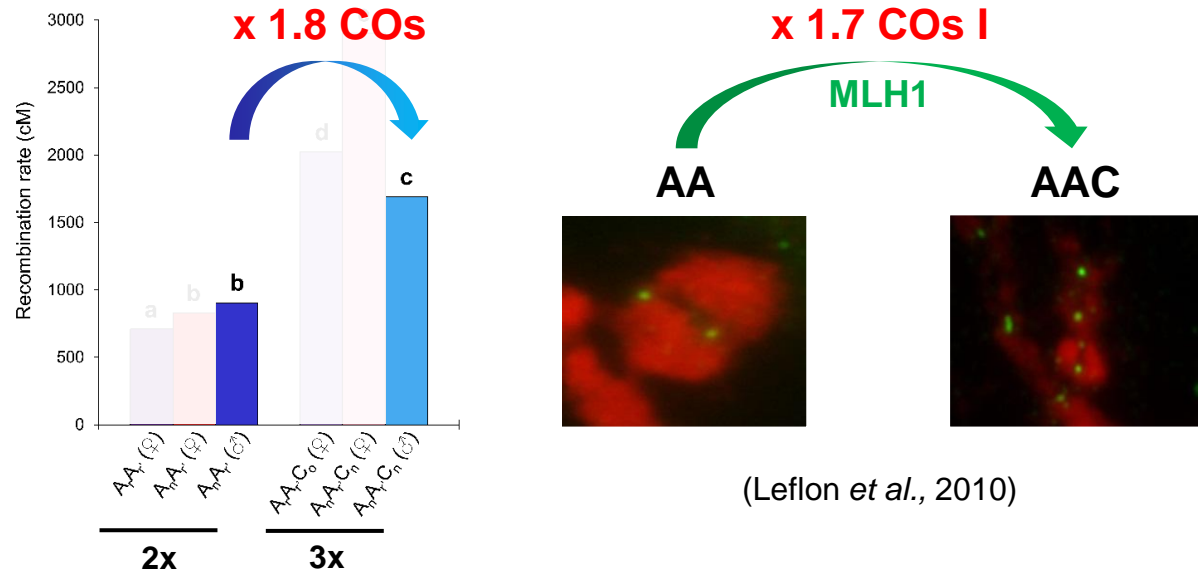
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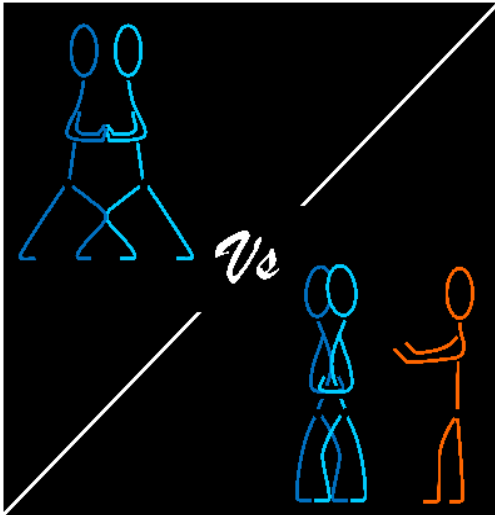
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❖ COs distribution :	$A_r A_r' \neq A_r A_r' C_o$	$A_n A_r' \neq A_n A_r' C_n$	$A_n A_r' \neq A_n A_r' C_n$

ASTONISHINGLY:

Comparing genetic and cytogenetic data, we found that in male AAC hybrids, extra-COs formed compared to AA hybrids belong mainly or only to the Class I





RESEARCH ARTICLE

Amplifying recombination genome-wide and reshaping crossover landscapes in *Brassicas*

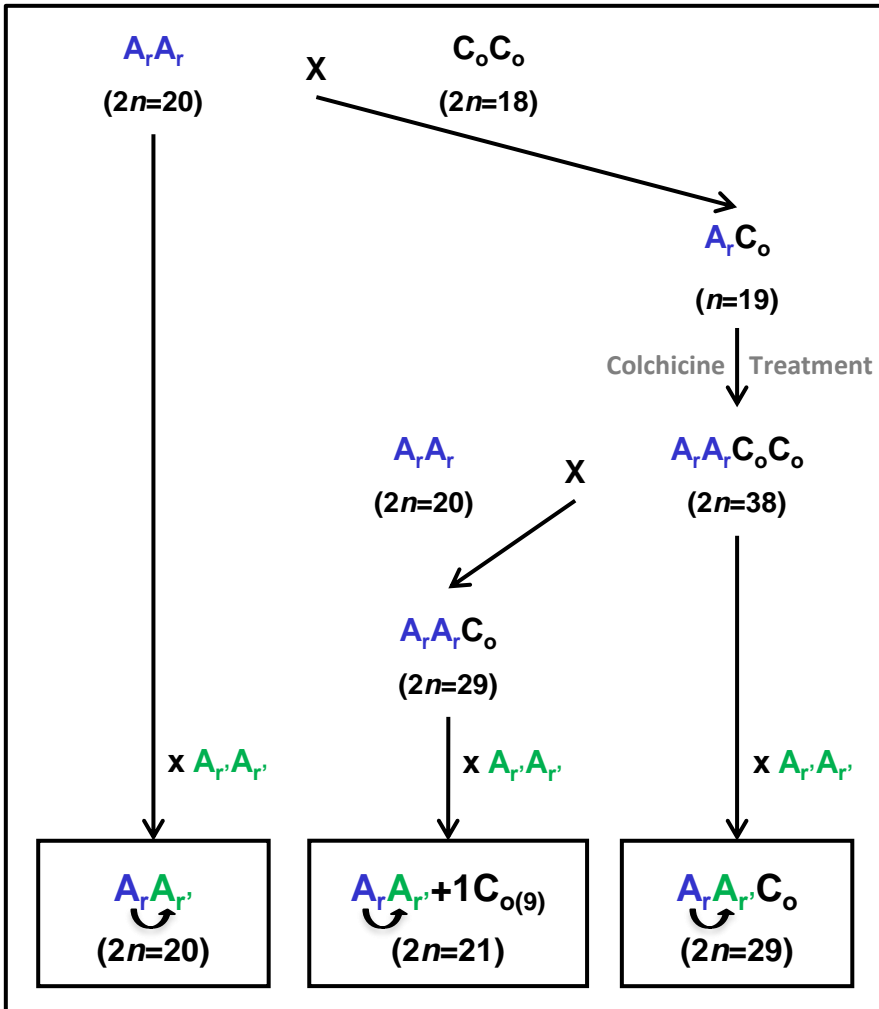
Alexandre Pelé¹, Matthieu Falque², Gwenn Trotoux¹, Frédérique Eber¹, Sylvie Nègre¹, Marie Gilet¹, Virginie Huteau¹, Maryse Lodé¹, Thibaut Jousseau¹, Sylvain Dechaumet¹, Jérôme Morice¹, Charles Poncet³, Olivier Coriton¹, Olivier C. Martin², Mathieu Rousseau-Gueutin¹, Anne-Marie Chèvre^{1*}

1 IGEPP, INRA, Agrocampus Ouest, Université de Rennes 1, Le Rheu, France, **2** GQE-Le Moulon, INRA, Université Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, Gif sur Yvette, France, **3** GDEC, INRA, Université de Clermont-Ferrand, France

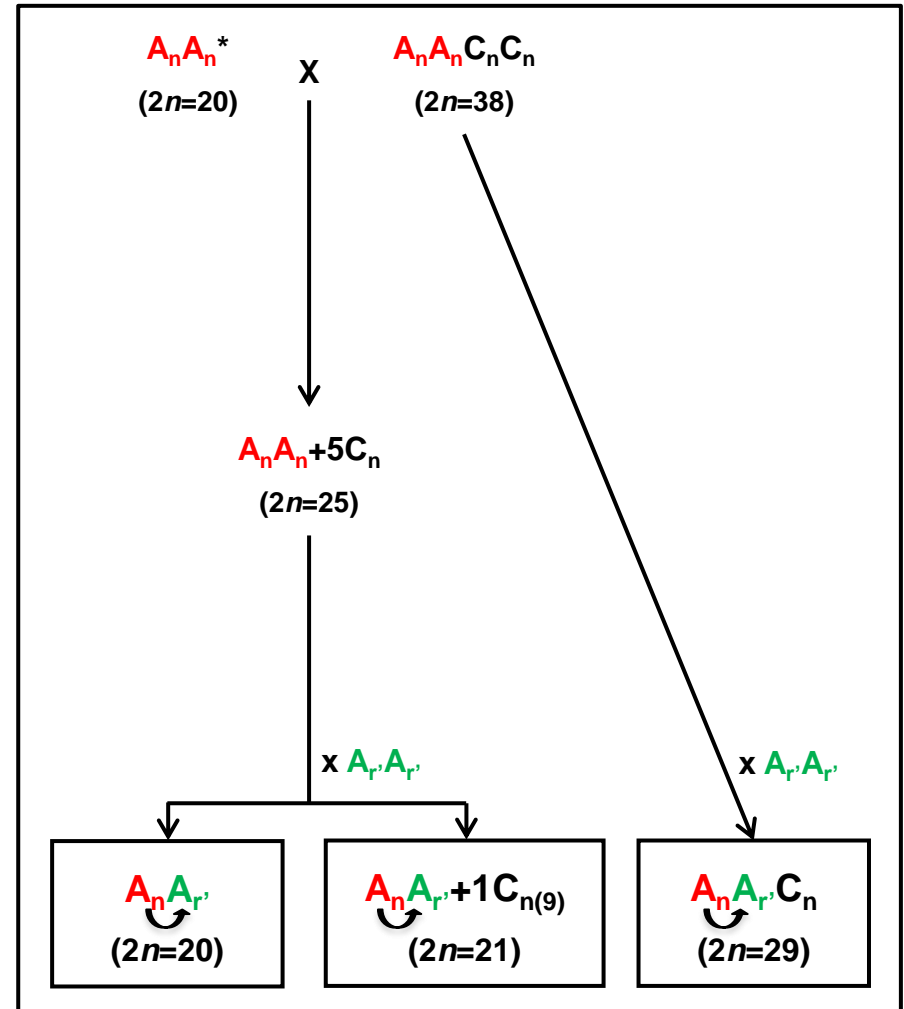
What about the regulation of homologous recombination in AAC hybrids?

REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Production of AA+C09 hybrids



- $A_r A_r$ = *B. rapa* cv. C1.3
- $A_r A_r$ = *B. rapa* cv. Chiifu (Sequenced)
- $C_o C_o$ = *B. oleracea* cv. RC34.

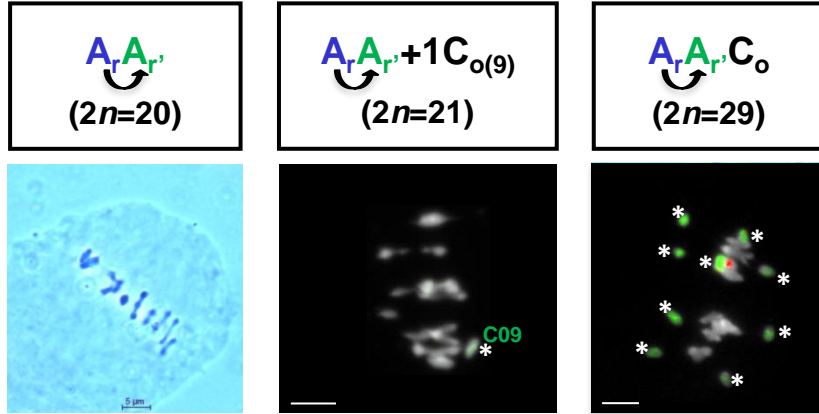


- $A_n A_n$ = A genome of *B. napus* cv. Darmor
- $A_r A_r$ = *B. rapa* cv. Chiifu (Sequenced)
- $A_n A_n C_n C_n$ = *B. napus* cv. Darmor.

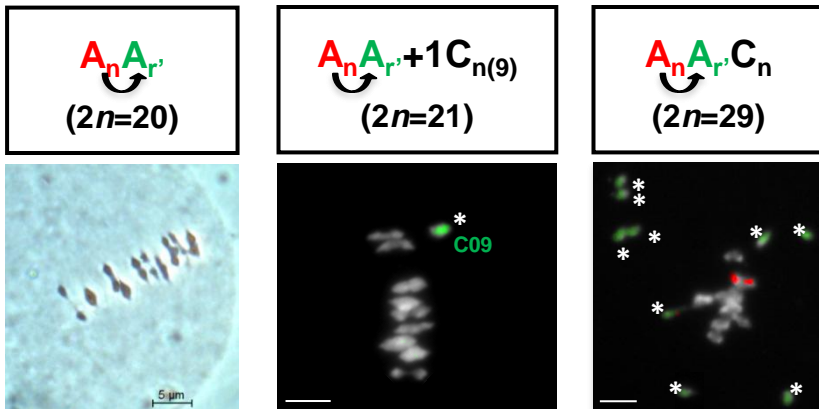
REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Cytogenetic characterization of AA+C09 hybrids

C09 chromosome or C genome from *B. oleracea*



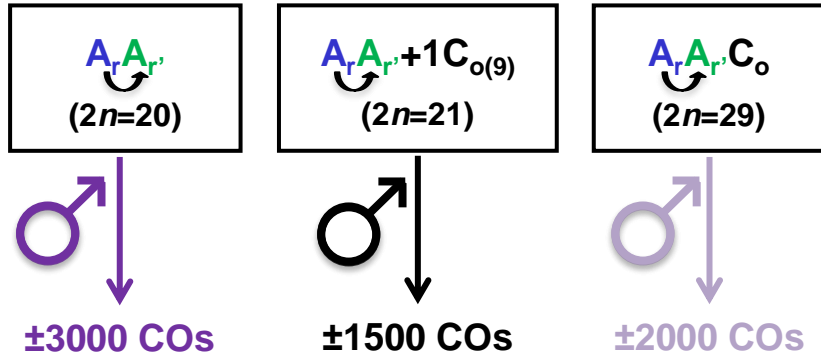
C09 chromosome or C genome from *B. napus*



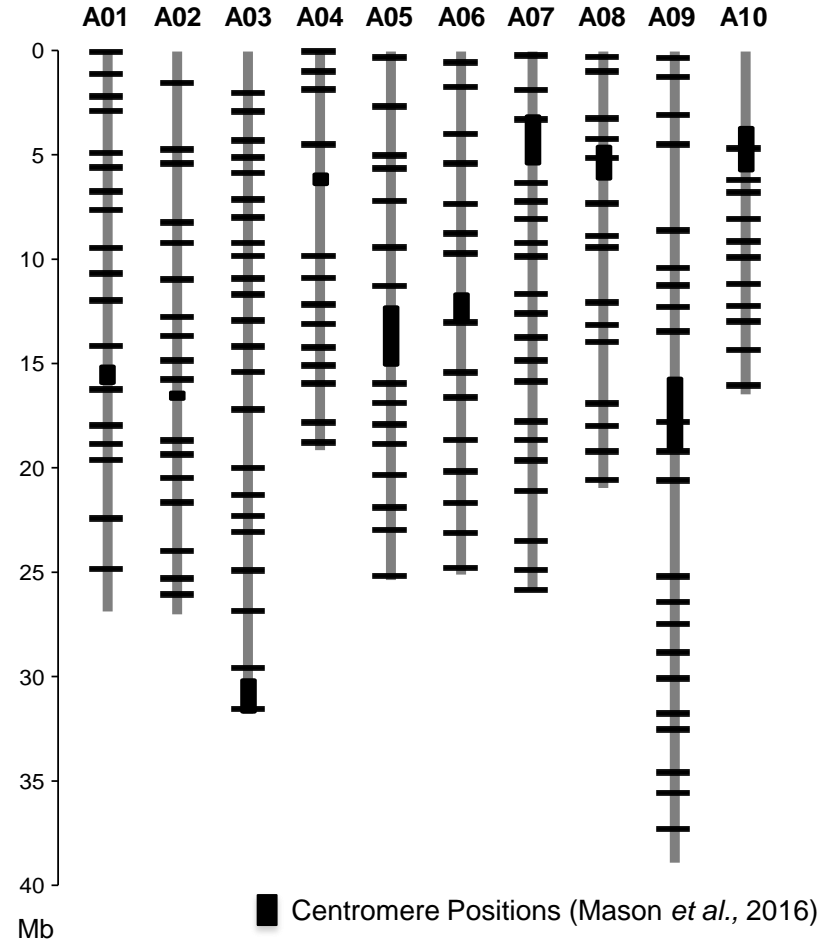
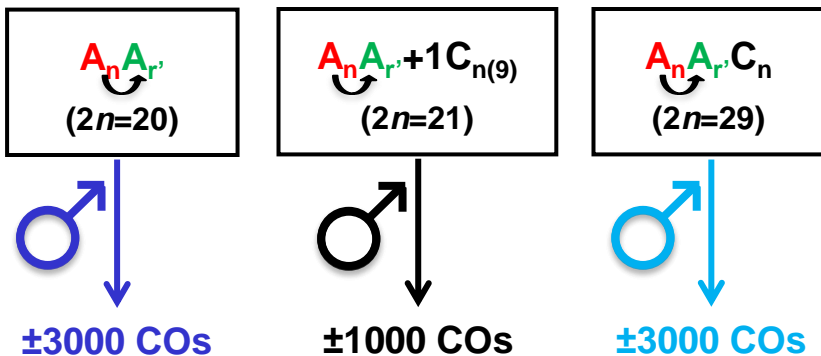
REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Detection of COs on progenies of AA+C09 hybrids

C09 chromosome or C genome from *B. oleracea*



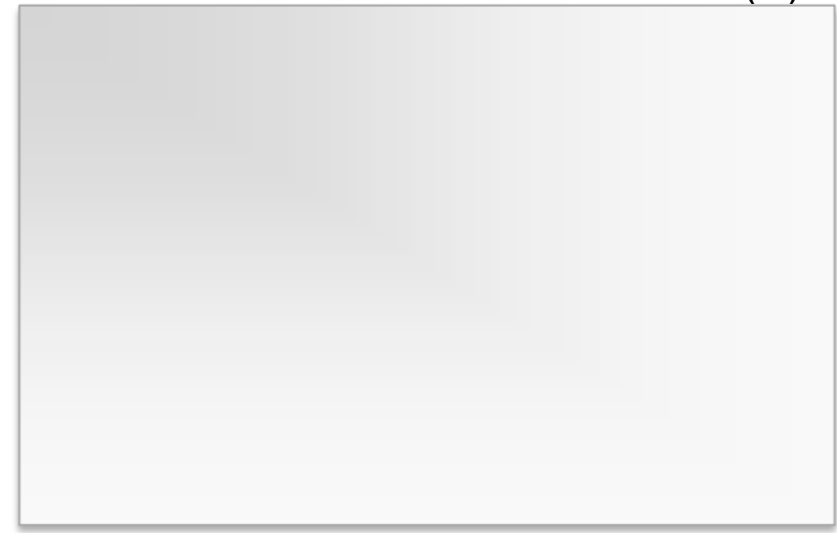
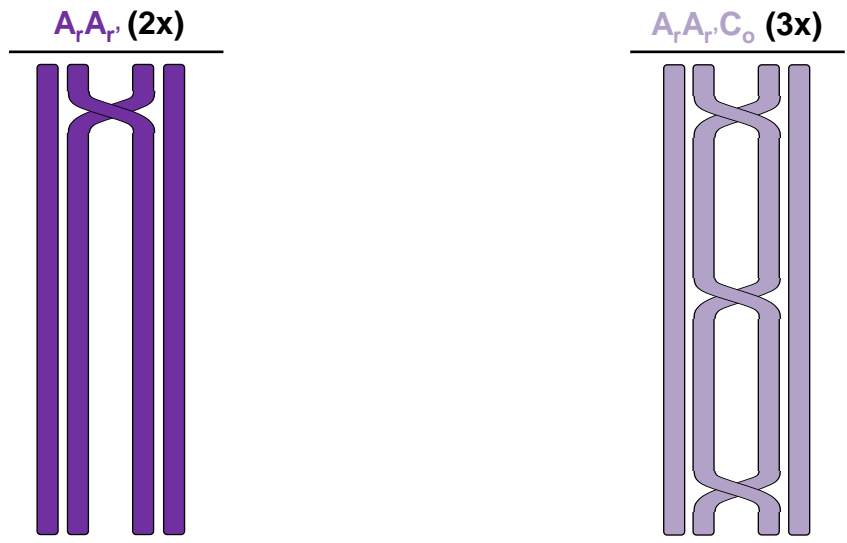
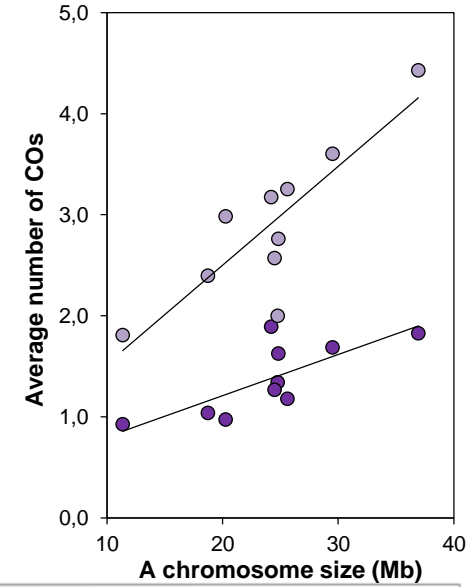
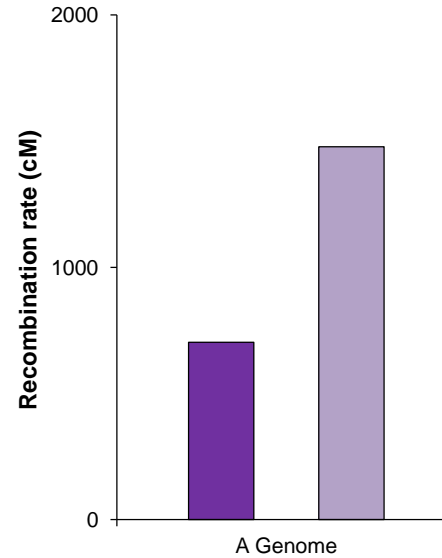
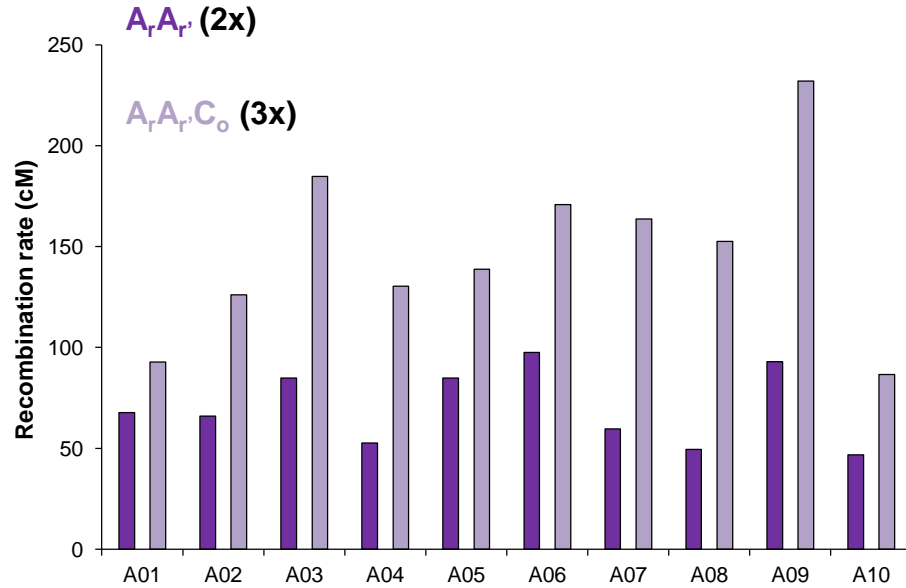
C09 chromosome or C genome from *B. napus*



169 SNPs
 1 SNP / 1.5 Mb (SE = 0.07, n = 159)
 Covering 94% of the A genome

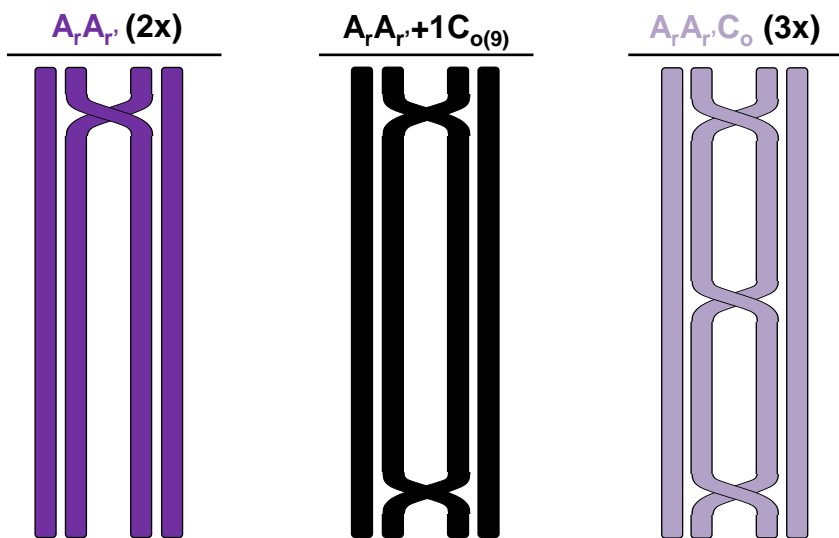
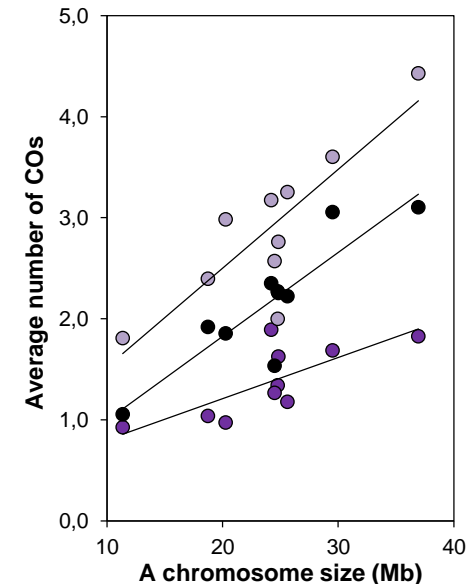
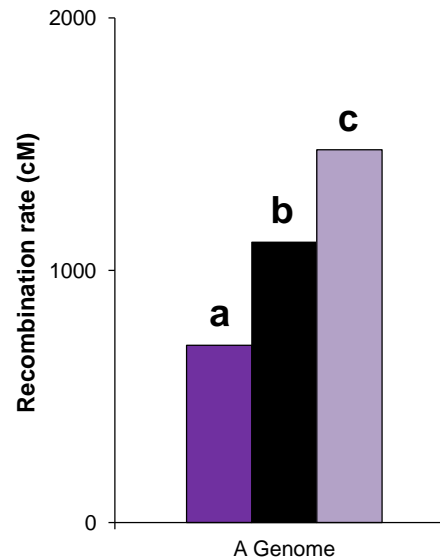
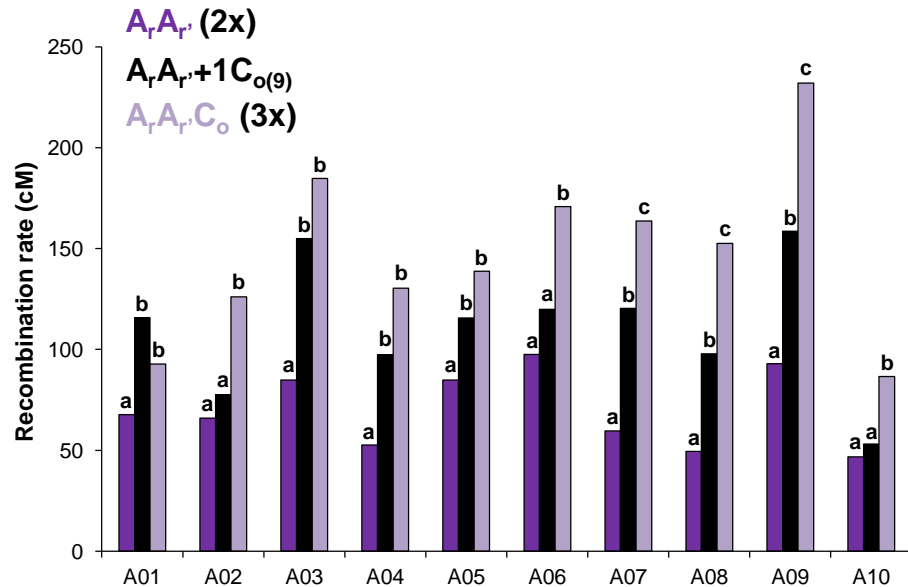
REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. oleracea* C09 chromosome on CO rates between the homologous A chromosomes



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Effect of the *B. oleracea* C09 chromosome on CO rates between the homologous A chromosomes

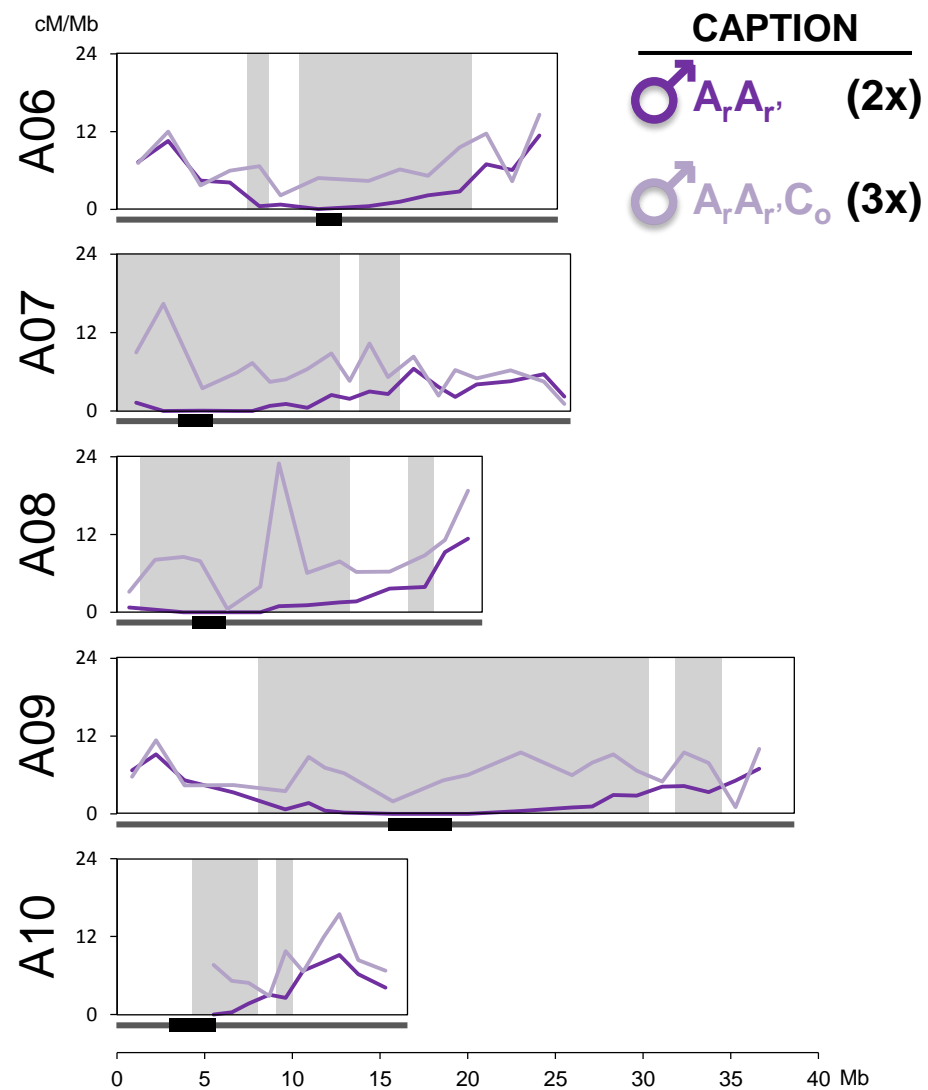
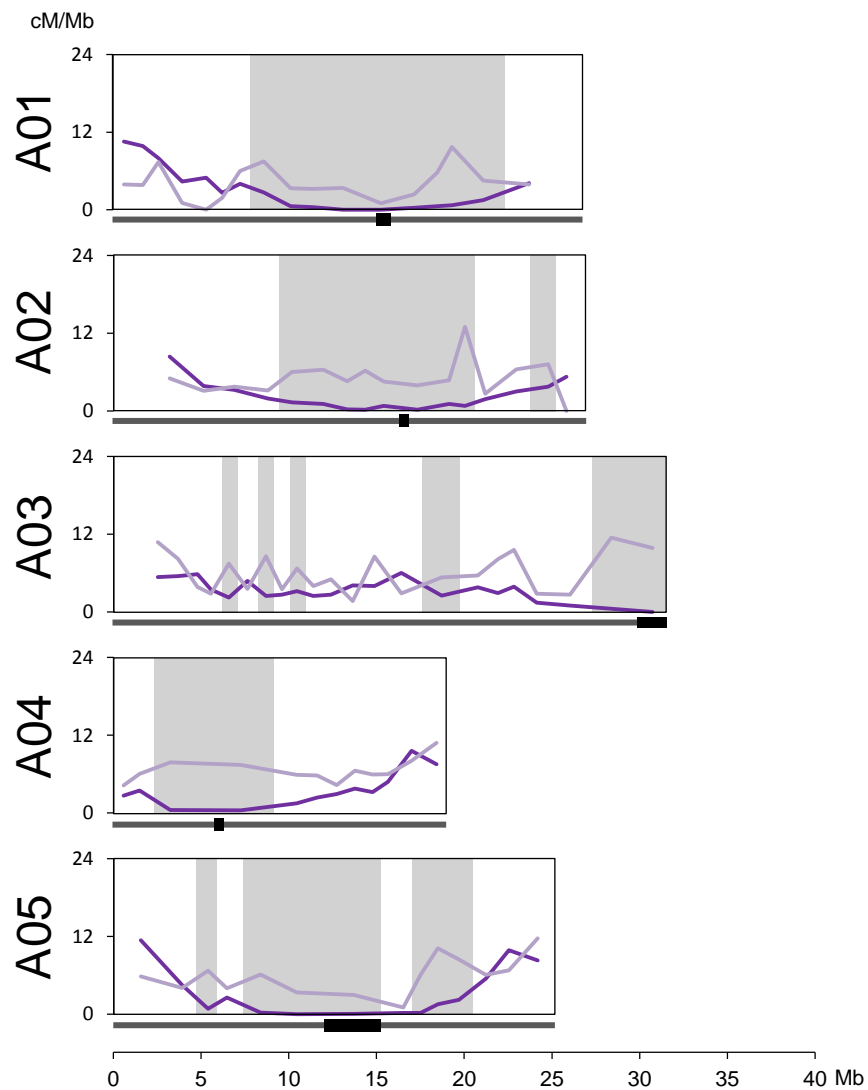


The *B. oleracea* C09 chromosome explains ~50% of the CO rate variation between AA and AAC hybrids

Between almost all homologous A chromosomes, extending at the whole A genome the results of Suay *et al.* (2014)

REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. oleracea* C09 chromosome on CO distribution along the A chromosomes



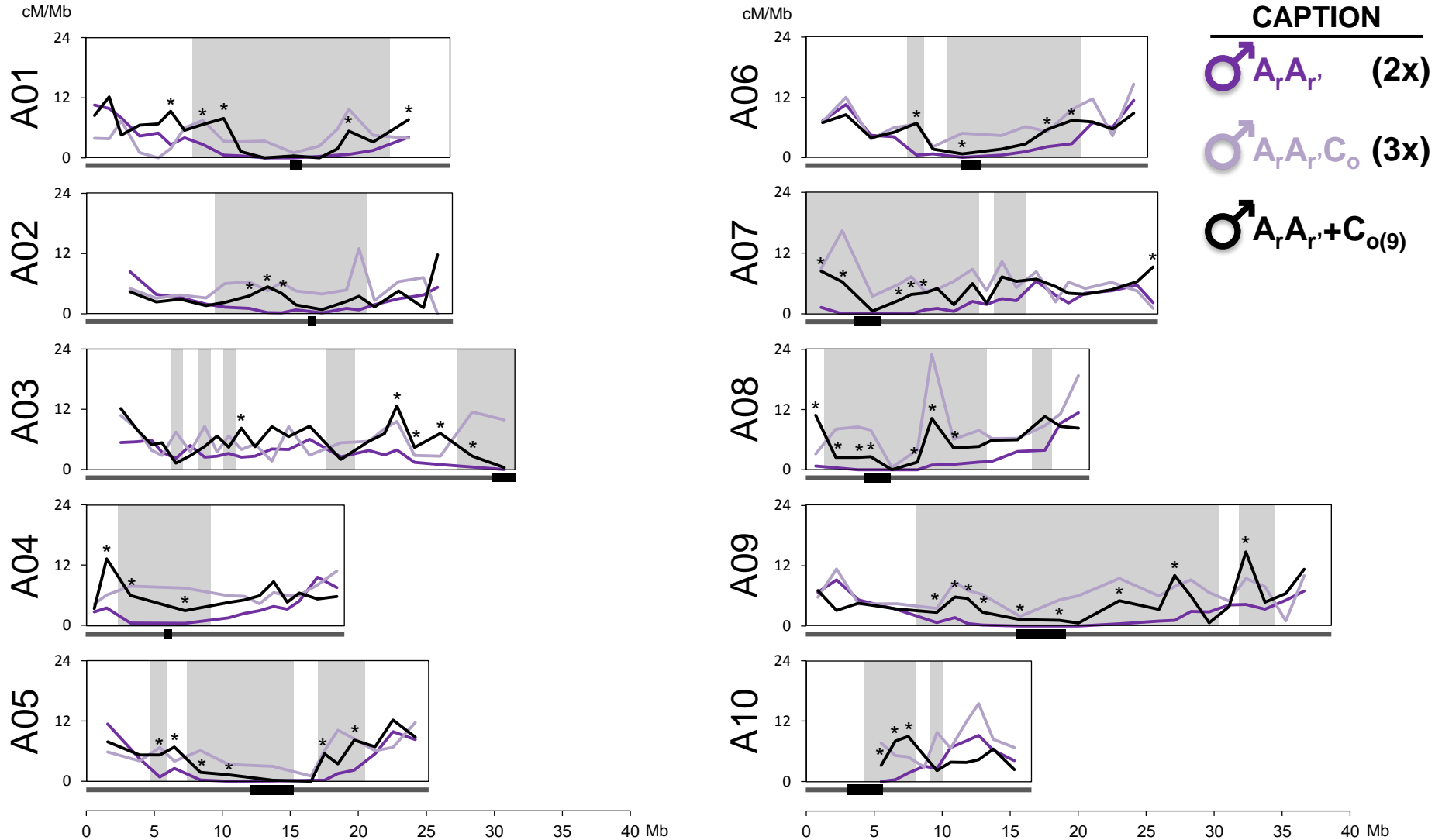
CAPTION

$\text{♂ } A_rA_{r'}$ (2x)
 $\text{♂ } A_rA_{r'}C_o$ (3x)

REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. oleracea* C09 chromosome on CO distribution along the A chromosomes

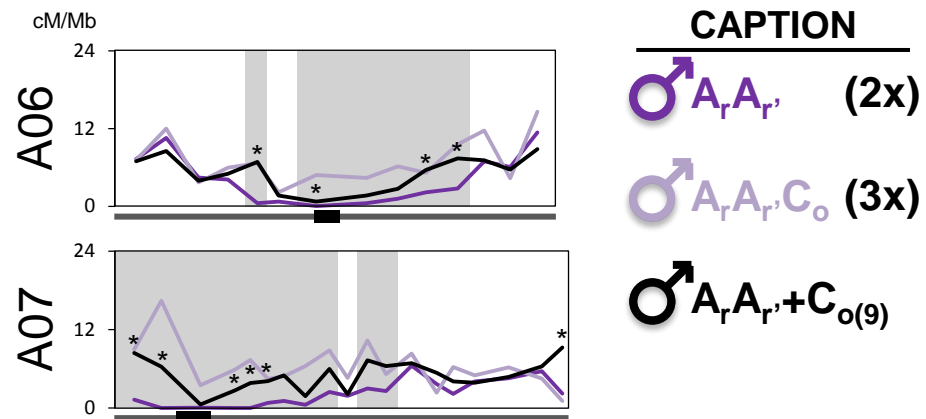
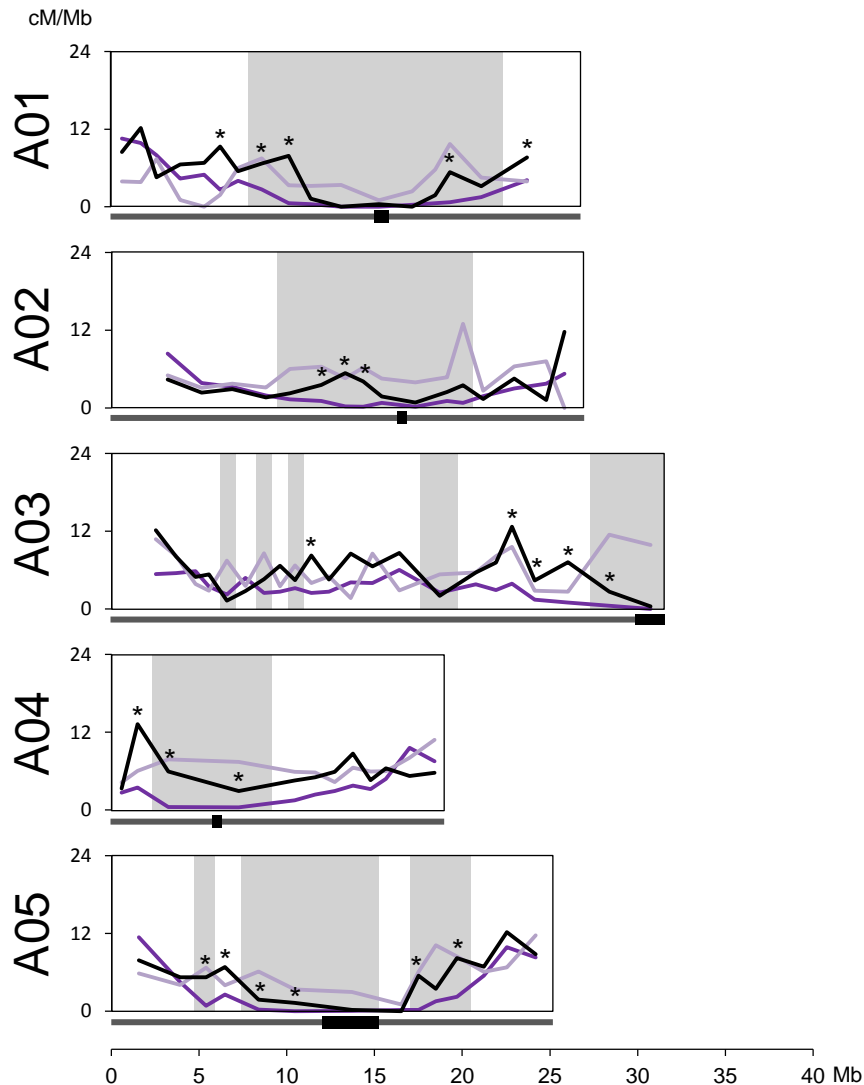
❖ The additional C09 chromosome from *B. oleracea* modify the COs distribution similarly to the C genome



REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. oleracea* C09 chromosome on CO distribution along the A chromosomes

❖ The additional C09 chromosome from *B. oleracea* modify the COs distribution similarly to the C genome



CAPTION

♂ $A_r A_r'$ (2x)

♂ $A_r A_r' C_o$ (3x)

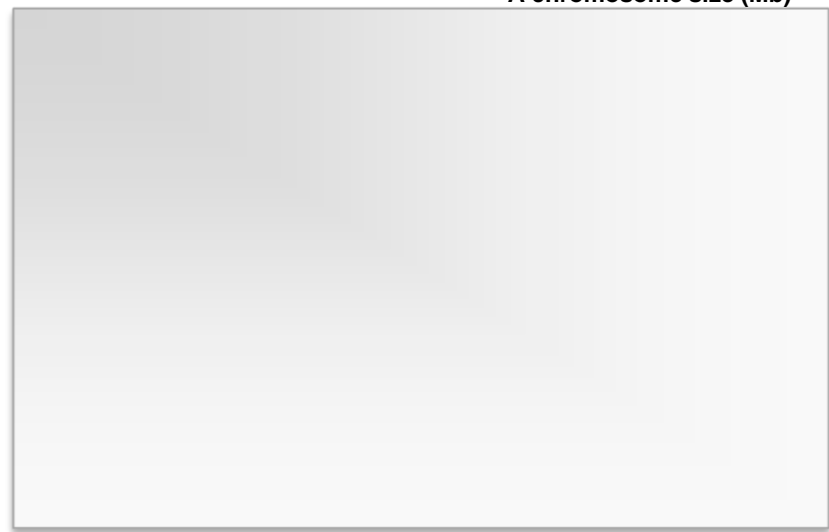
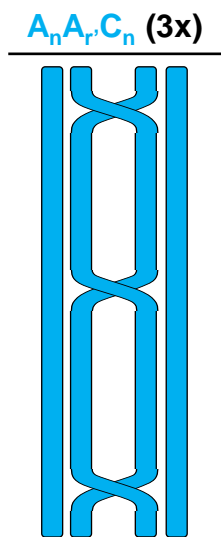
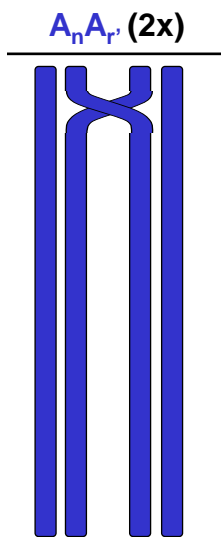
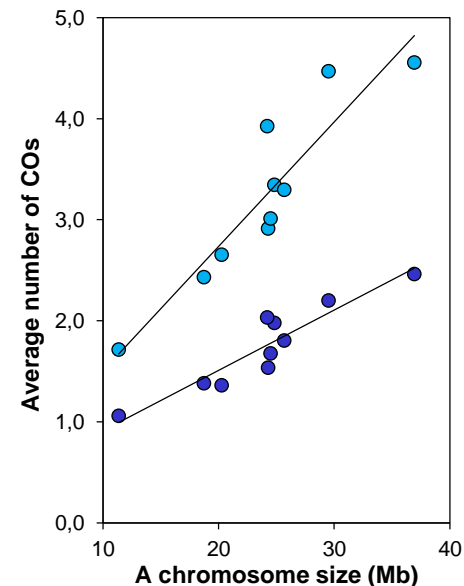
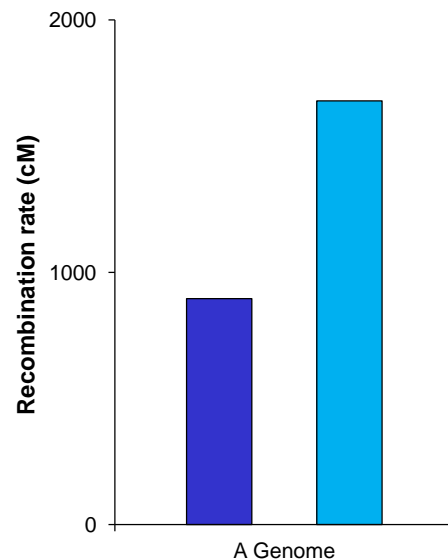
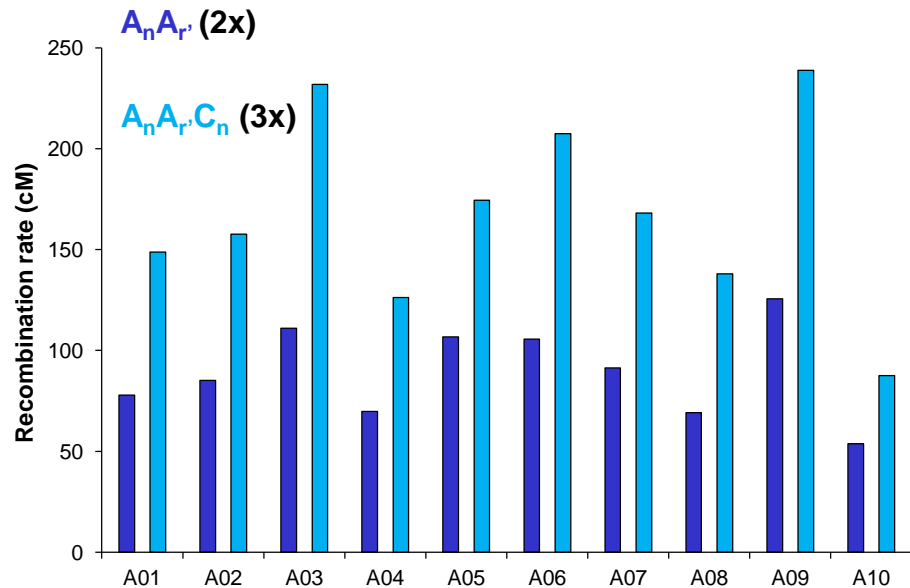
♂ $A_r A_r' + C_o(9)$

The C09 chromosome from *B. oleracea* is responsible for the modification of COs distribution observed in AAC hybrids

What happens when it comes from *B. napus*?

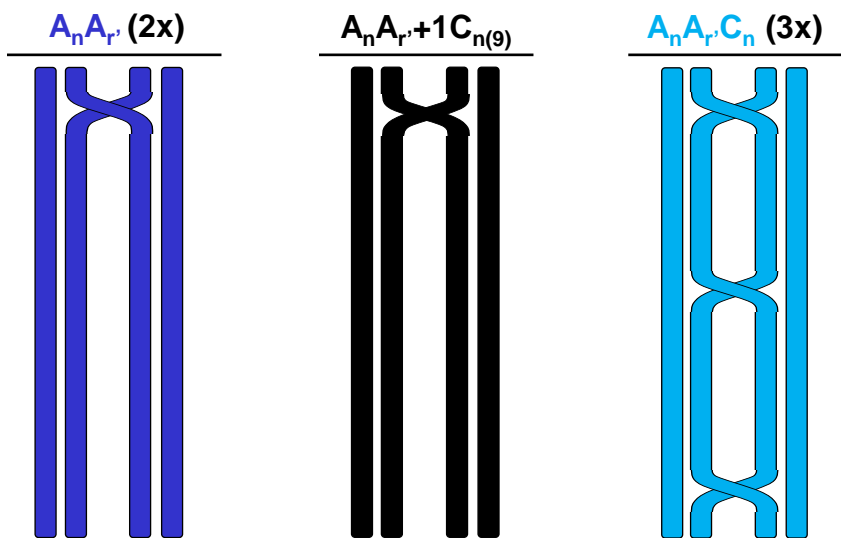
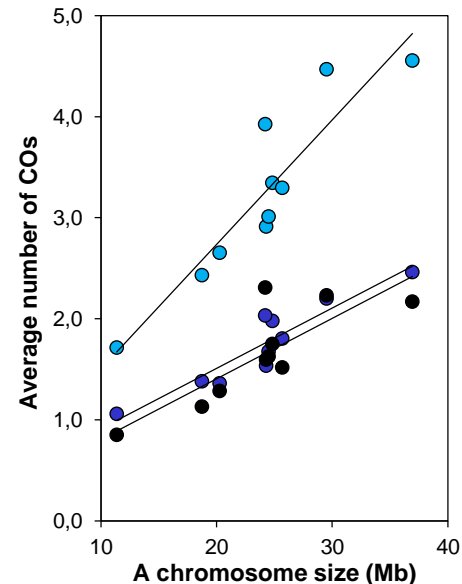
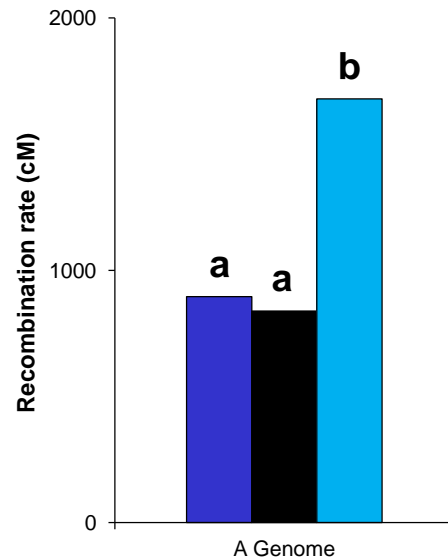
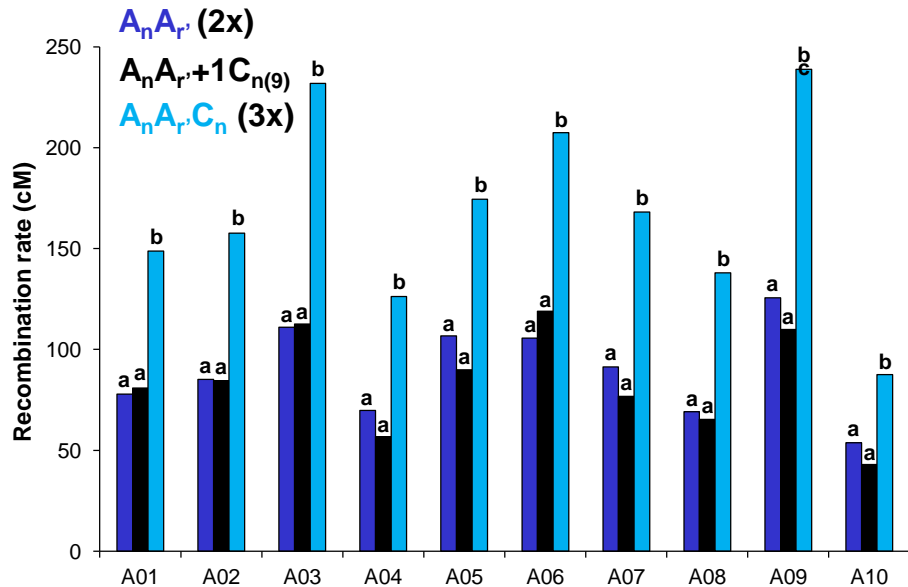
REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. napus* C09 chromosome on CO rates between the homologous A chromosomes



REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. napus* C09 chromosome on CO rates between the homologous A chromosomes



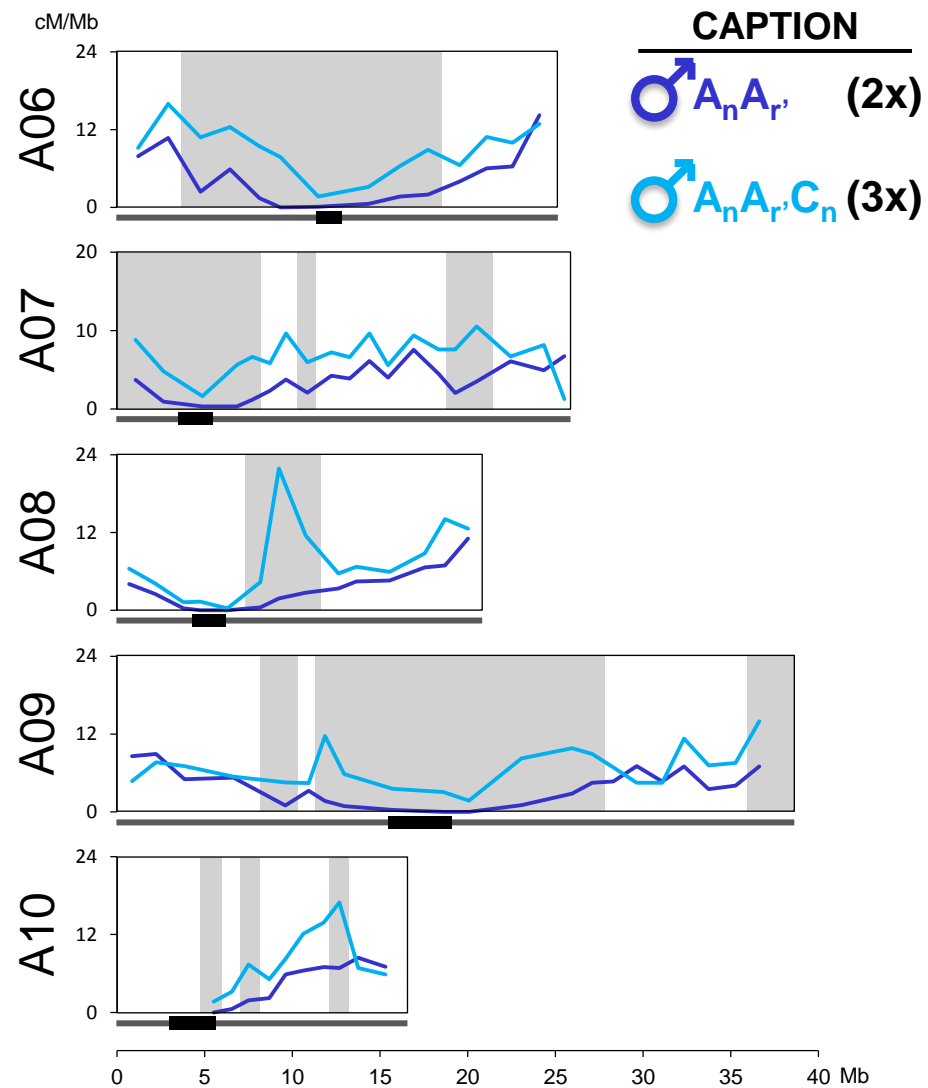
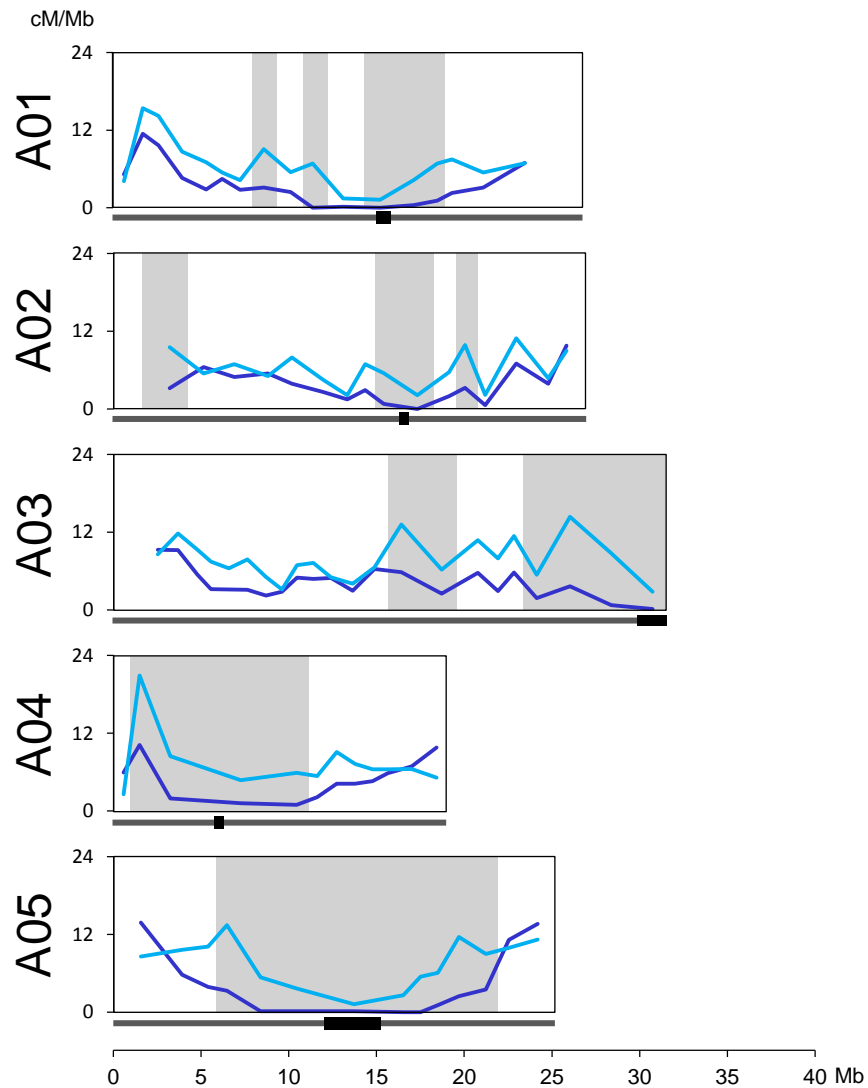
The *B. napus* C09 chromosome has no effect on CO rates

Between all homologous A chromosomes

And on COs distribution?

REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. napus* C09 chromosome on CO distribution along the A chromosomes



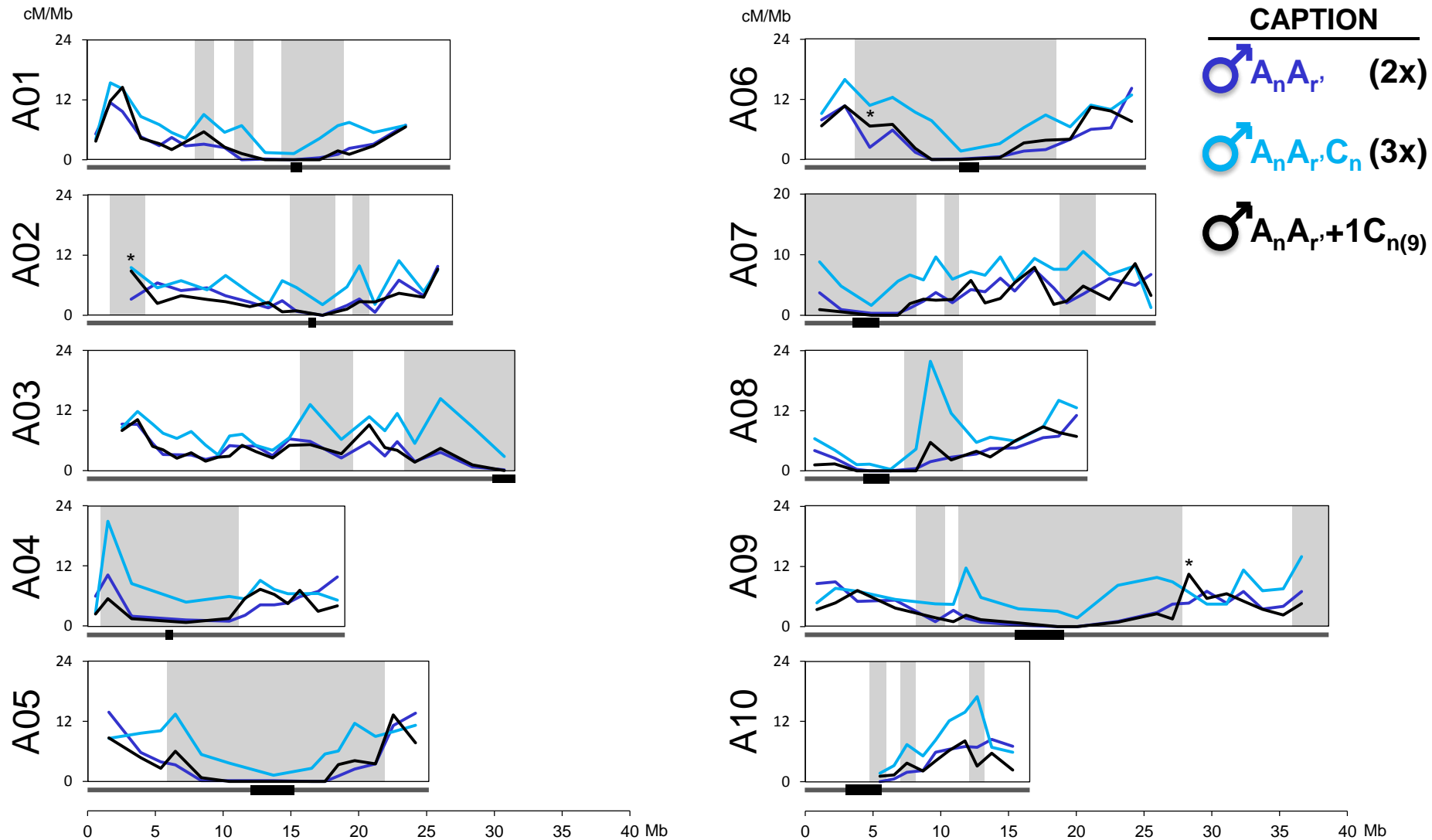
CAPTION

$\text{♂ } A_n A_r$ (2x)
 $\text{♂ } A_n A_r C_n$ (3x)

REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. napus* C09 chromosome on CO distribution along the A chromosomes

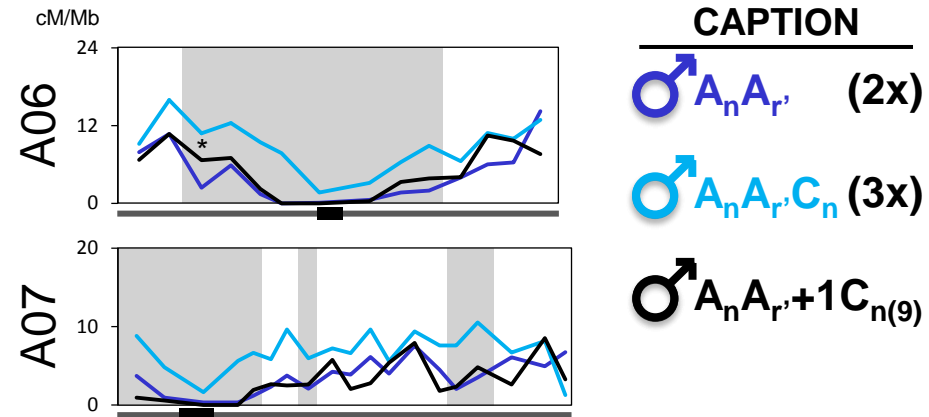
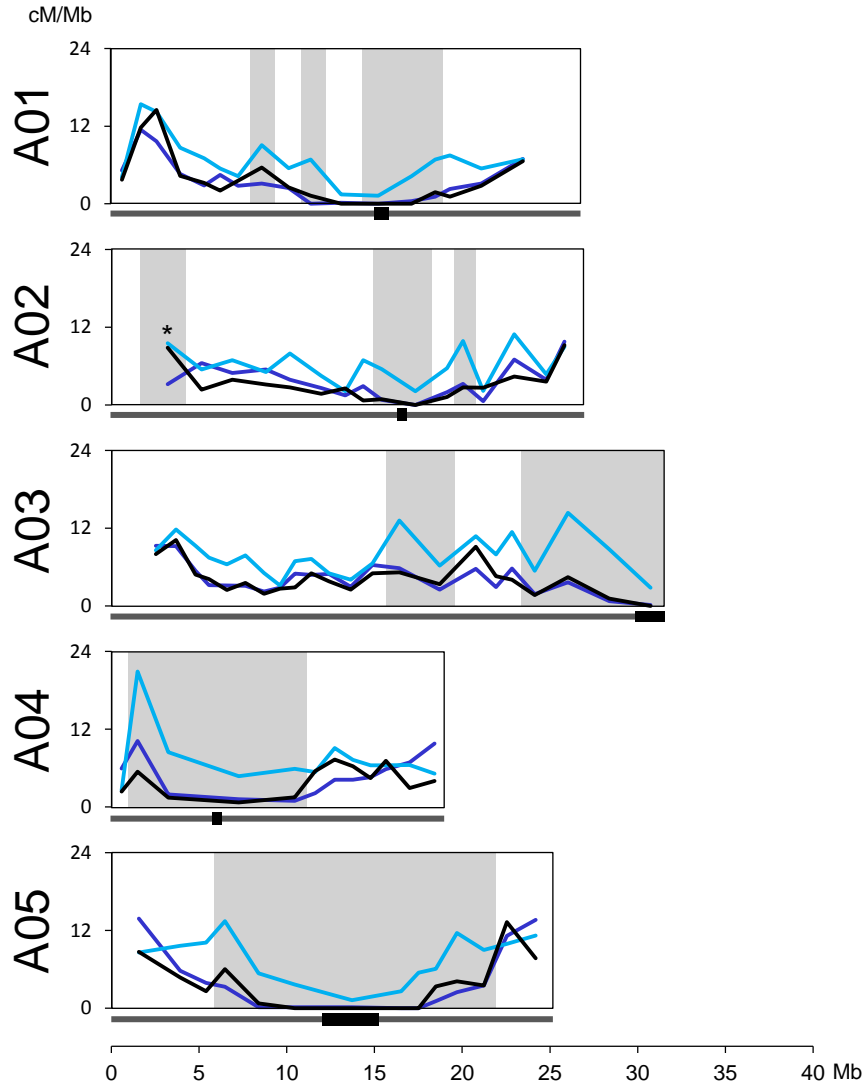
❖ The additional C09 chromosome from *B. napus* **DO NOT** modify the COs distribution



REGULATION OF HOMOLOGOUS RECOMBINATION IN AAC HYBRIDS

Effect of the *B. napus* C09 chromosome on CO distribution along the A chromosomes

❖ The additional C09 chromosome from *B. napus* **DO NOT** modify the COs distribution



CAPTION

♂ $A_n A_r$ (2x)

♂ $A_n A_r C_n$ (3x)

♂ $A_n A_r + 1 C_n(9)$

The C09 chromosome from *B. oleracea* and *B. napus* have a contrasted effect on homologous recombination

Probably due to the genome evolution in a polyploid context

CONCLUSIONS

❖ AAC vs AA hybrids, whatever genetic background and male/female meiosis:

- Increase of CO rates
- Change of COs distribution
- **Due to Class I COs in male meiosis**



Allowing to break the linkage disequilibrium in rapeseed breeding programs by using AAC hybrids

❖ The additional C09 chromosome of *B. oleracea* explains:

- 1/2 of CO rates variation
- Change of COs distribution



Suggesting a genetic control

❖ The additional C09 chromosome of *B. napus* DOESN'T explain:

- CO rates variation
- Change of COs distribution



Possibly due to genome evolution in a polyploid context

Prospect:

- ❑ Use in breeding programs
- ❑ What are the mechanisms responsible for modifications of CO rate and distribution? Epigenetics? (Melamed-Bessudo and Levy, 2012 ; Mirouze *et al.*, 2012; Yelina *et al.*, 2012)
- ❑ What is/are the gene(s) on the C09 chromosome of *B. oleracea* involved in the reshaping of homologous recombination in AAC hybrids

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...And thank you for your attention

