

The *Ph1*/*ZIP4* story:

Stabilization of wheat as a polyploid and preservation of grain number

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John Innes Centre

Unlocking Nature's Diversity



Why is *Ph1*/*ZIP4* so relevant?

“The *Ph1* locus presumably appeared during wheat polyploidization stabilizing the wheat genome during meiosis”



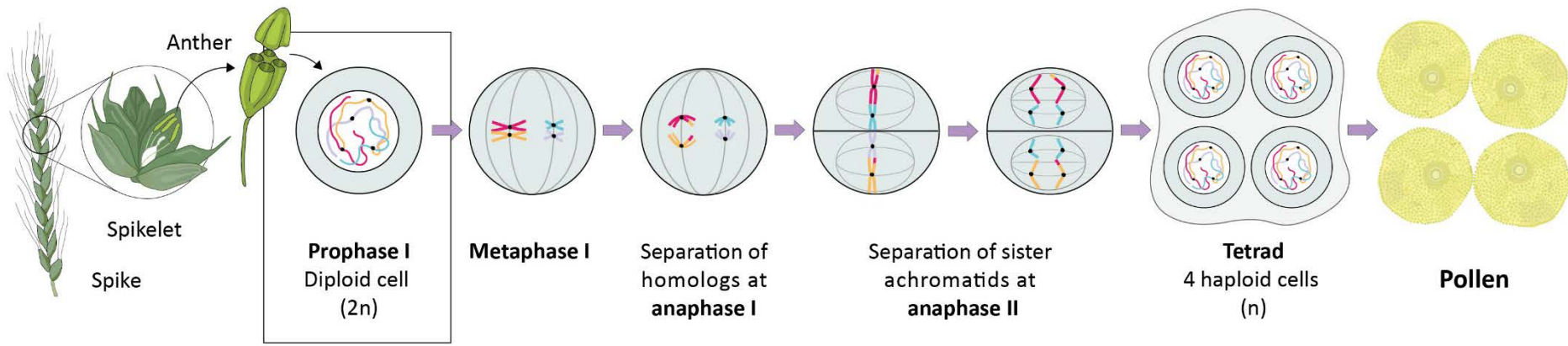
High fertility and stability
of polyploid wheat



Prevention of recombination between
polyploid wheat and its **wild relatives**

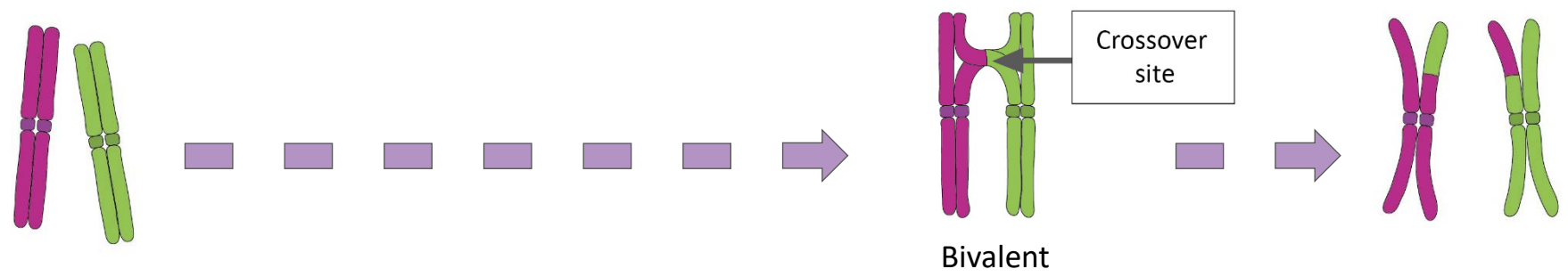


Meiosis

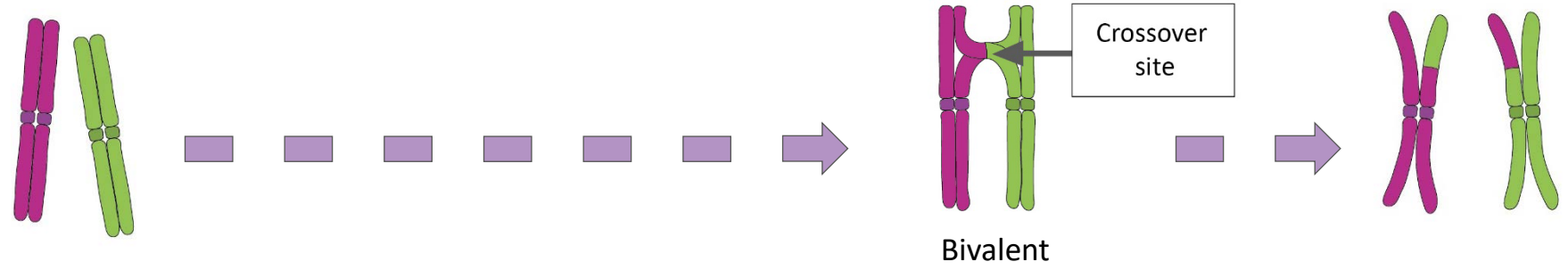
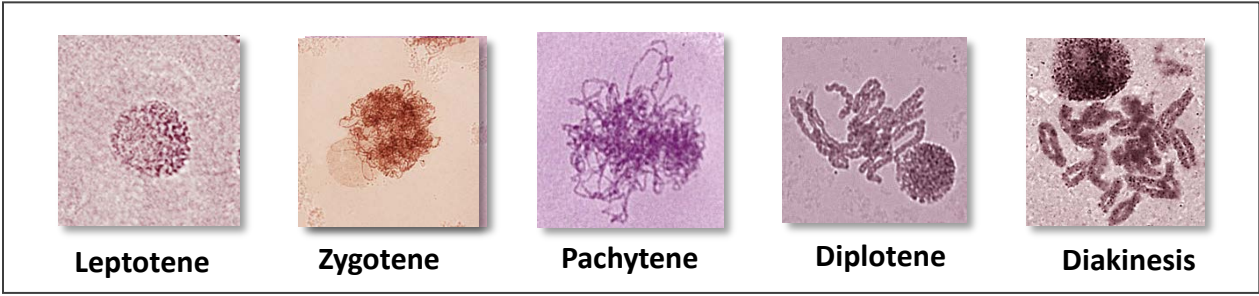
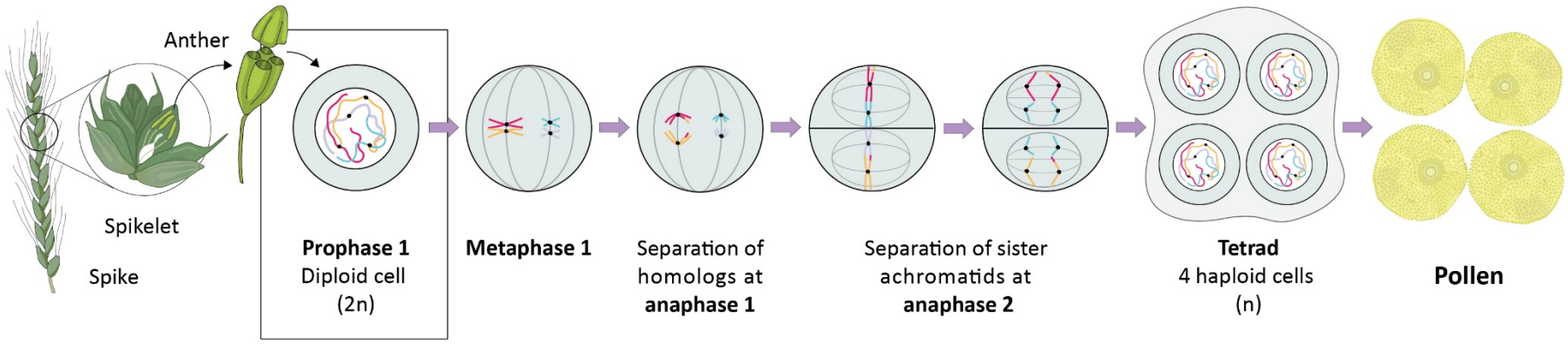


Homologous recombination

- Ensure correct segregation of homologs
- Creates new combination of genes

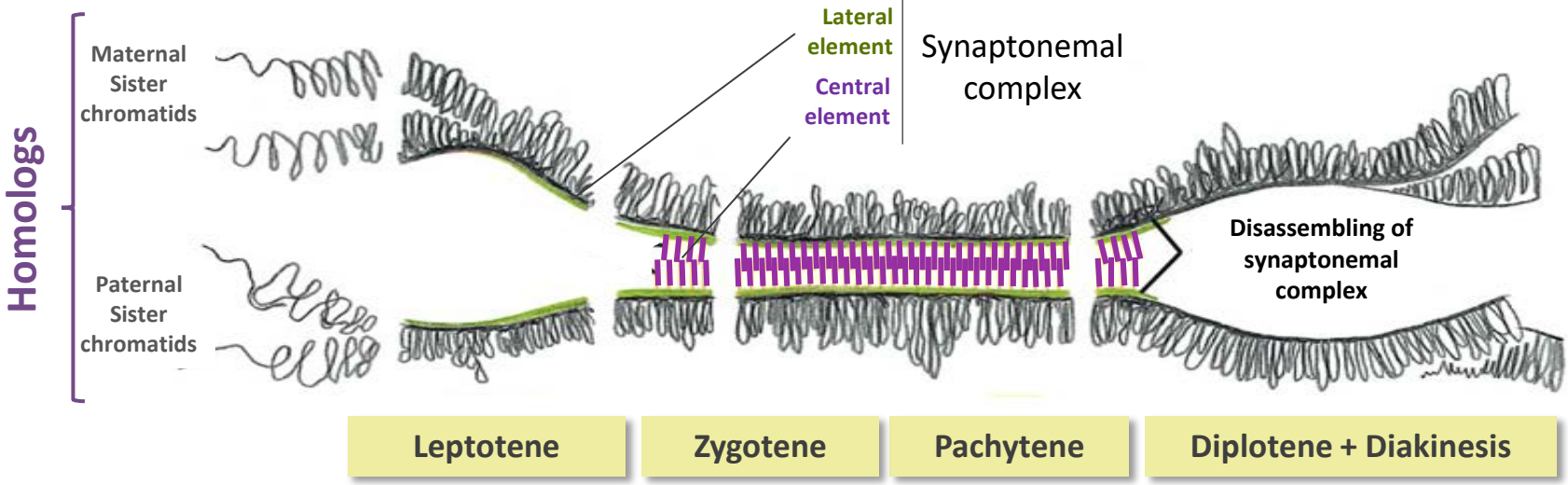
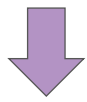
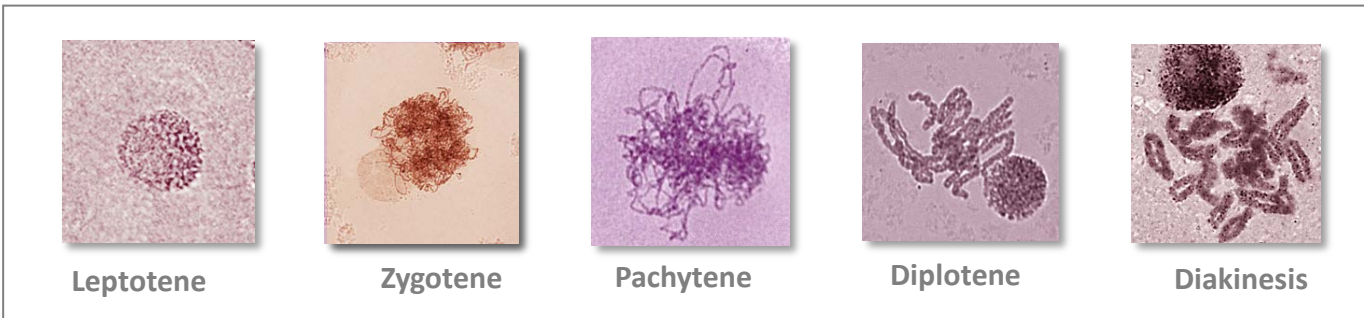


Meiosis



Process of Synapsis

PROPHASE I



THE BREAD WHEAT GENOME

1. Genome size

***T. aestivum*: 16.000 Mbp** ($6n=2x=42$)

S. cerevisiae: 12 Mbp ($2n=2x=32$)

C. elegans: 100 Mbp ($2n=2x=12$)

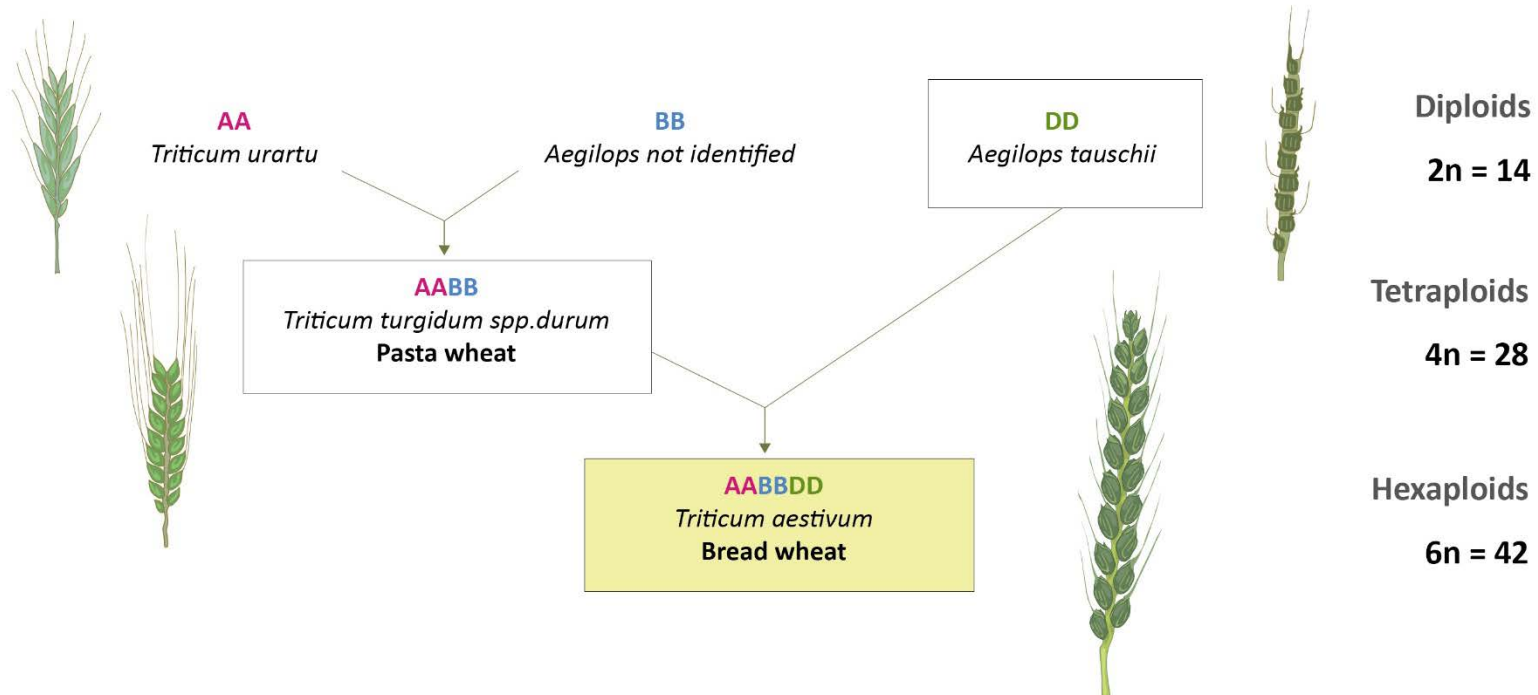
A. thaliana: 135 Mbp ($2n=2x=10$)

Human: 3.235 Mbp ($2n=2x=46$)

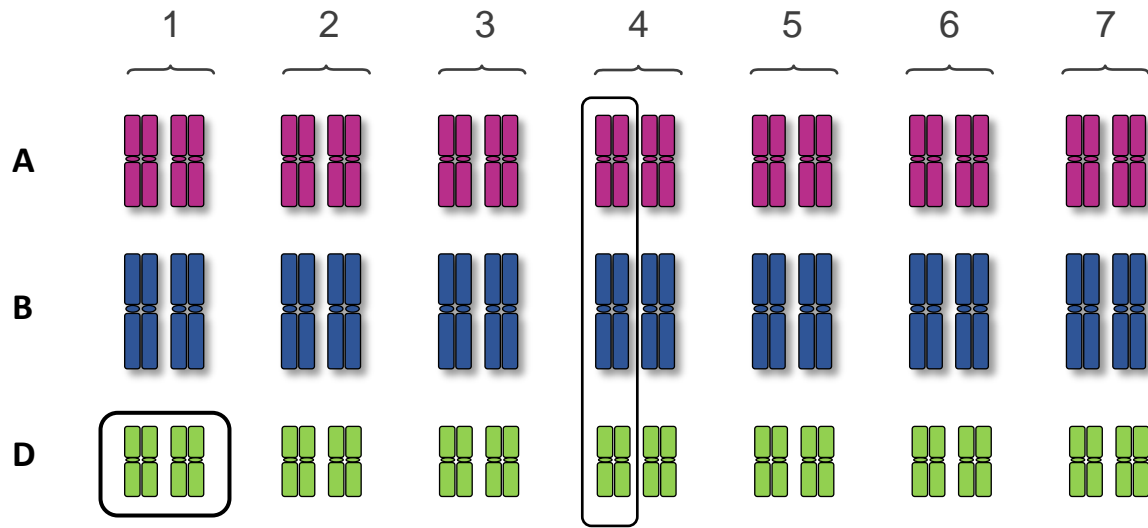
2. Highly repetitive

- > 85% is made of repetitive DNA
- Genes represent only 2% of the genome

3. Polyploid: Allohexaploid ($6x = 2n = 42$, AABBDD)



Wheat diploid like behaviour during meiosis



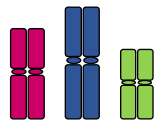
Homologs



1D 1D

Homeologs

or **“related chromosomes”**



4A 4B 4D

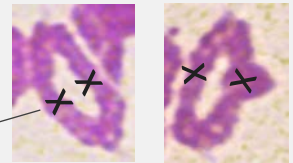
~95-98 %
sequence similarity

Rod bivalents

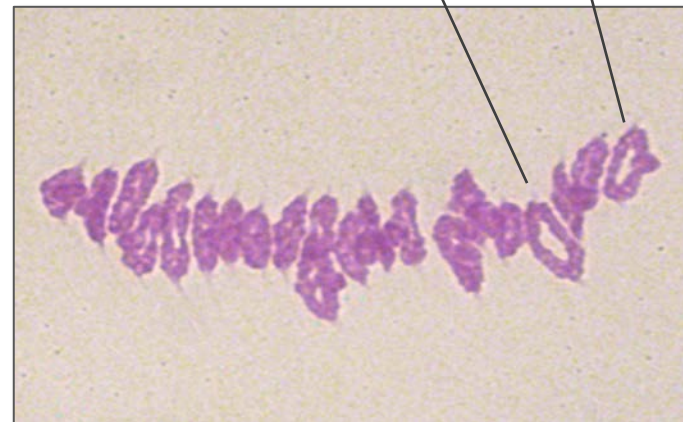


CO

Ring bivalents



Crossover
site (CO)

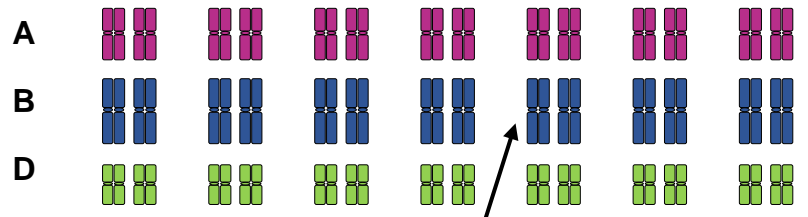


Meiotic metaphase I with 21 ring bivalents

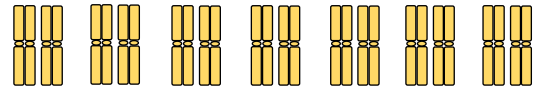
Discovery of the *Ph1* locus in wheat in 1958

Wheat 2n= 42

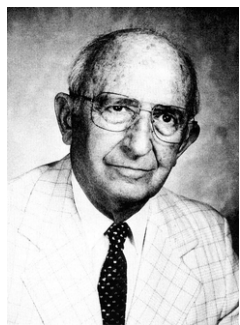
Rye 2n= 14



X



Ph1 locus
(Pairing homoeologous 1)
Controls the diploid-like
behaviour of hexaploid wheat



Ernie Sears



Ralph Riley

WT wheat-rye hybrid



Metaphase I

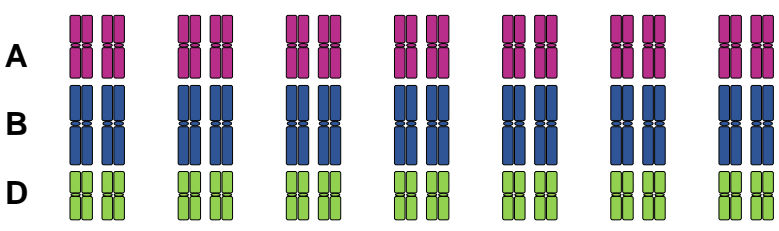
Wheat-rye hybrid in absence of *Ph1*



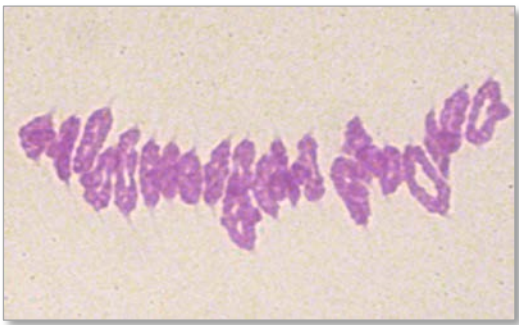
Metaphase I

Double effect of the *Ph1* locus on wheat breeding

Wheat 2n= 42

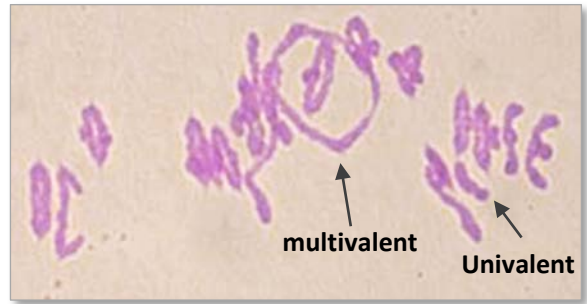


All bivalent



WT wheat

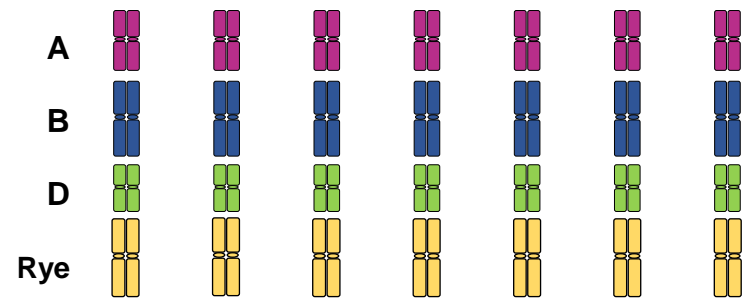
Mostly bivalents but also univalent + multivalents



Absence of *Ph1*

Ph1 is necessary for wheat genome stability

Wheat- rye hybrid n = 28

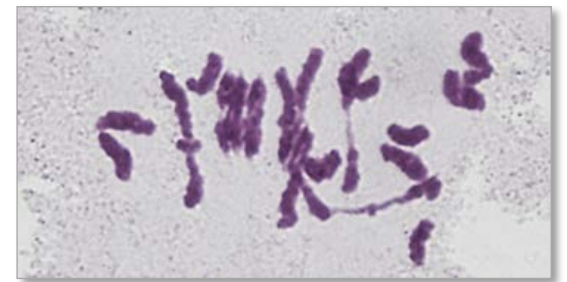


All univalents



WT hybrid

Univalents + bivalents + multivalents

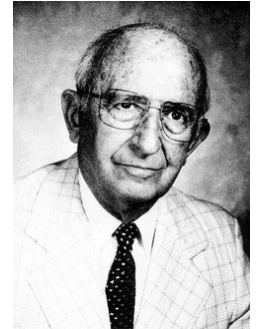
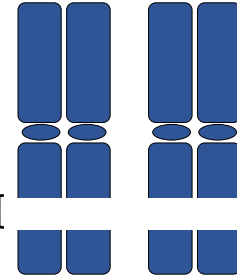


Absence of *Ph1*

Ph1 is a barrier for wild relatives introgressions

Use of *Ph1* locus deletion in breeding

- ***Ph1b* mutant** → Hexaploid wheat with a 70 Mb deletion on the long arm of chromosome 5B (Sears, 1977)



(Ernie Sears)

1187 genes

59.3 Mb {

- ***Ph1* mutant successfully used:**

- *Aegilops peregrina*
- *Aegilops variabilis*
- *Ae. Geniculata* (leaf and stripe rust resistance)
- *Ae. Triuncialis* (leaf rust resistance)
- *Amblyopirum muticum*
- *Thinopyrum bessarabicum* (salinity tolerant and disease resistance)
- *Aegilops speltoides*, *Triticum timopheevii*, *Triticum urartu*, *Thinopyrum intermedium* and *Thinopyrum elongatum*

Under way



Aegilops



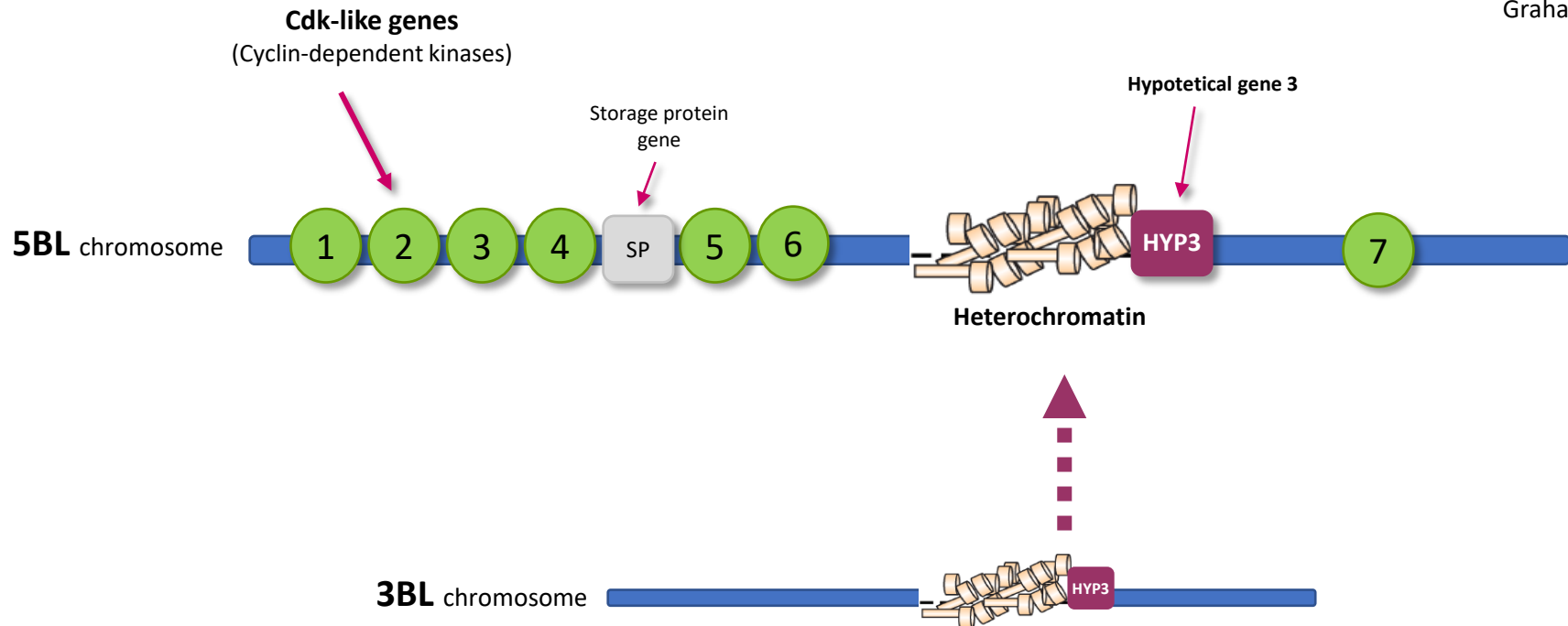
Thinopyrum

Screening overlapping deletions with the *Ph1* deletion phenotype

0.5 Mb region including a cluster of **Cdk-like genes**, with closest homology to CDK2 in mammals, with a block of **heterochromatin from 3B** inserted in the middle
(Griffith et al, 2006. *Nature* 439, 749-752; Al-kaff et al, 2008. *Ann. Bot.* 101(6):863-72)

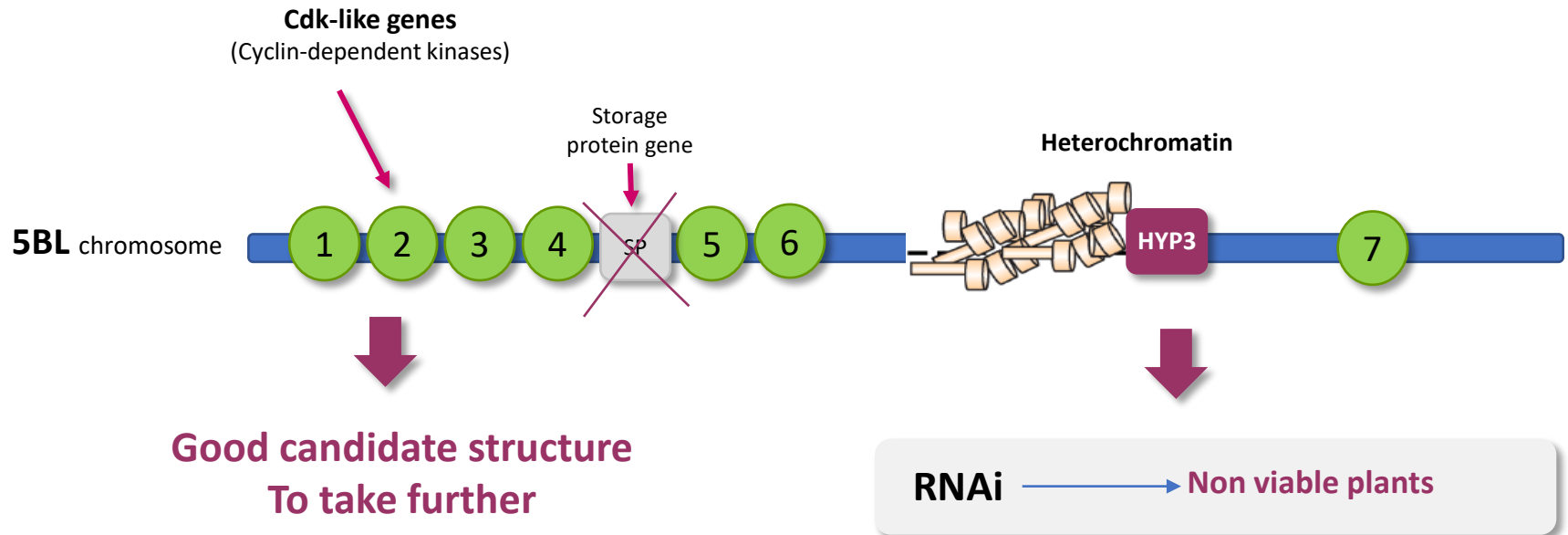


Graham Moore



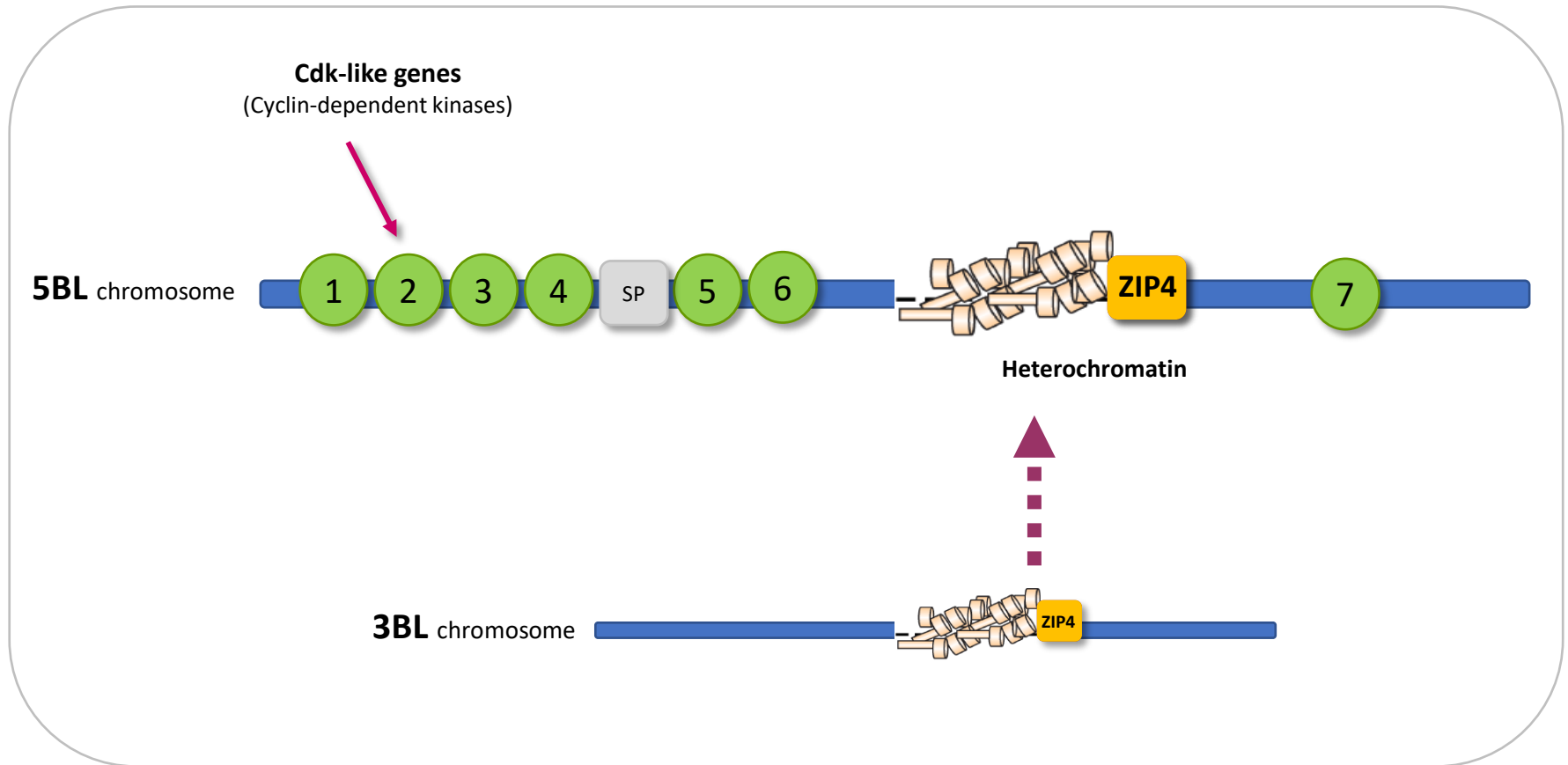
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(Griffith et al, 2006. *Nature* 439, 749-752; Al-kaff et al, 2008. *Ann. Bot.* 101(6):863-72)



HYP3 was reannotated as the meiotic gene *ZIP4*

Ph1 locus is defined to a cluster of Cdk-like genes with a duplicated segment of heterochromatin from 3B, which contains a copy of the meiotic gene *ZIP4* (Martín et al, 2017. *Chromosoma* 126(6):669-680)



Release of the IWGSC RefSeq v1.0

- 1958 → *Ph1* locus discovered on the long arm of 5B
- 1977 → *ph1b* mutant: wheat with a 70 Mb (59.3 Mb) deletion
- 2006 → *ph1* mutant is defined to a 2.5 Mb region
- 2008 → *ph1* mutant is defined to a 0.5 Mb region

~7000 genes

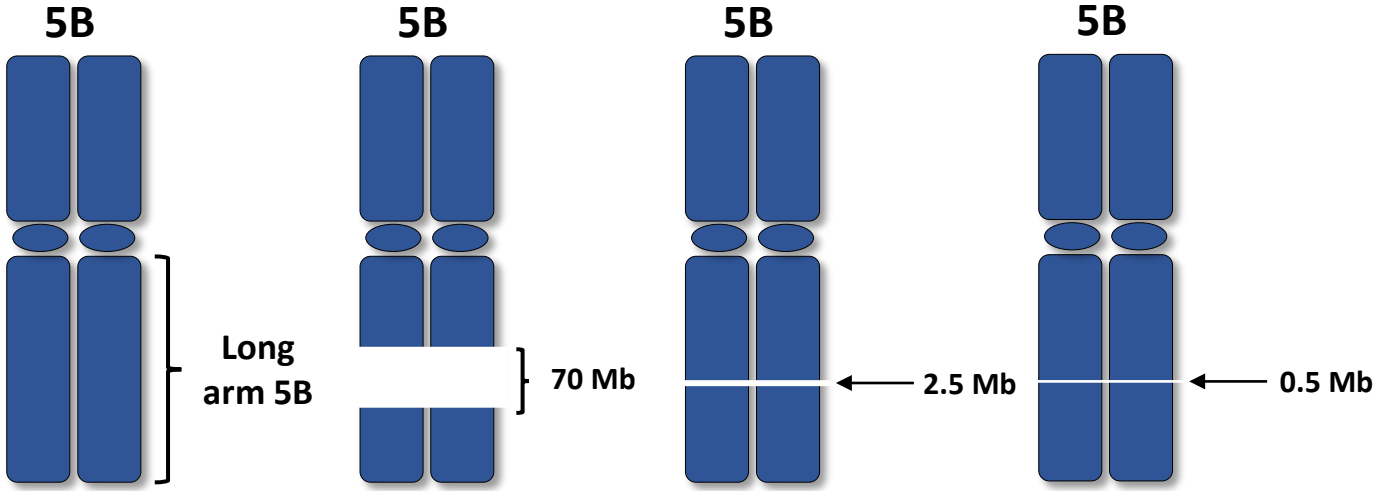
1187 genes

145 genes

25 genes

2 genes expressed in meiosis:

- **TaZIP4-B2** (TraesCS5B02G255100)
- Unknown function (TraesCS5B01G254900)



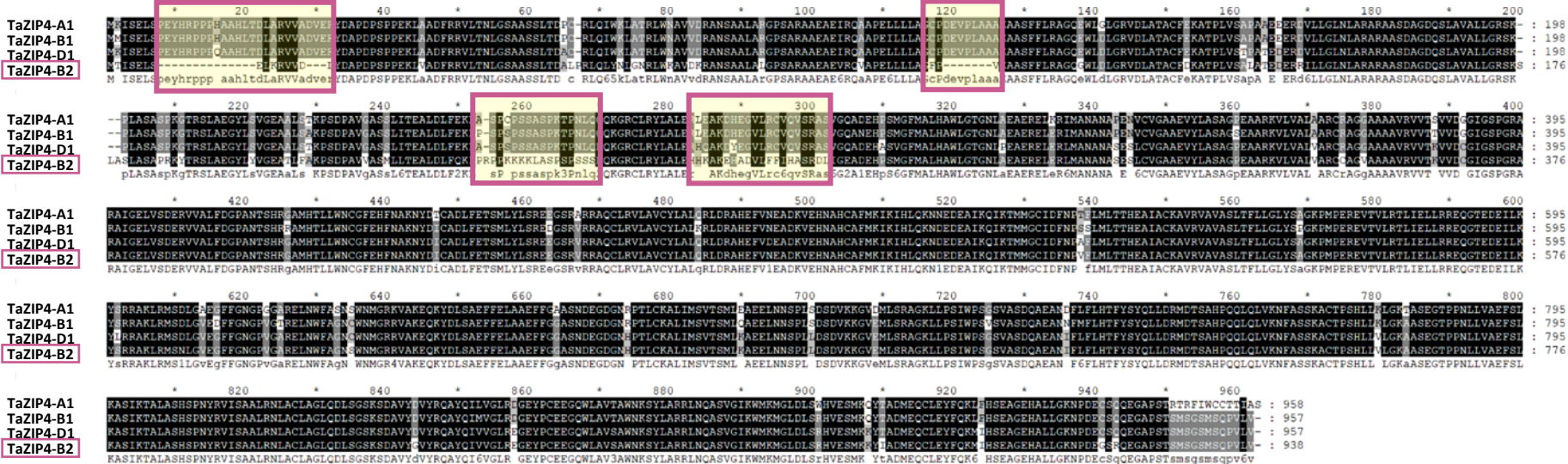
Release of the IWGSC RefSeq v1.0

4 ZIP4 copies in wheat

- **3A** → *TaZIP4-A1* (TraesCS3A02G401700)
- **3B** → *TaZIP4-B1* (TraesCS3B02G434600)
- **3D** → *TaZIP4-D1* (TraesCS3D02G396500)
- **5B** → *TaZIP4-B2* (TraesCS5B02G255100)

Sequence Identity Matrix:

	3A	3D	3B	5B
TraesCS3A02G401700	ID			
TraesCS3D02G396500	0.949	ID		
TraesCS3B02G434600	0.936	0.949	ID	
TraesCS5B02G255100	0.843	0.858	0.885	ID



Zip4 TILLING Mutants

TILLING lines in hexaploid wheat cv. 'Cadenza' available using the www.wheat-tilling.com database (Krasileva et al. 2017)

Line	Scaffold	WT base	Alt_base	Consequence	CDS position	Amino acids	Codons	Sift score	domain	Het/hom
Cadenza0198	TRIAE_CS42_5BL_TGACv1_404600_AA1305800	C	T	missense_variant	107	T36I	aCc/aTc	NO	spo22	hom
→ Cadenza1691	TRIAE_CS42_5BL_TGACv1_404600_AA1305801	C	T	missense_variant	500	A167V	gCc/gTc	NO	spo22	hom
Cadenza1363	TRIAE_CS42_5BL_TGACv1_404600_AA1305802	C	T	missense_variant	1243	H415Y	Cat/Tat	NO	spo22 TPR	het
Cadenza1866	TRIAE_CS42_5BL_TGACv1_404600_AA1305803	C	T	missense_variant	1445	A482V	gCc/gTc	NO	TPR	het
Cadenza1127	TRIAE_CS42_5BL_TGACv1_404600_AA1305804	G	A	missense_variant	1766	G589D	gGc/gAc	NO		hom
Cadenza1007	TRIAE_CS42_5BL_TGACv1_404600_AA1305805	G	A	missense_variant	1805	R602H	cGt/cAt	NO		hom
→ Cadenza0348	TRIAE_CS42_5BL_TGACv1_404600_AA1305806	G	A	stop_gained	1836	W612*	tgG/tgA			het

Wheat cv. Cadenza
2n = 42

X

Aegilops variabilis
2n = 28



Wheat- *Ae. variabilis* hybrid
n = 35
No homologs present



Zip4 TILLING Mutants

Line	No. of cell examined	Univalents	Rod bivalents	Ring bivalents	Multivalents	Chiasma frequency
Cad0000 x <i>Ae. variabilis</i> hybrids	128	32	1.5	0	0	1.5
Cad1691 x <i>Ae. variabilis</i> hybrids	117	14.7	6.7	1.2	1.3	12.2
Cad0348 x <i>Ae. variabilis</i> hybrids	102	14.6	6.6	1.1	1.5	12.2
Ph1b deletion CS x <i>Ae. variabilis</i> hybrids	136	12.4	6.5	1.8	1.8	14.15

➔ Wild type

➔ *Zip4* missense

➔ *Zip4* stop codon

➔ *Ph1b* deletion



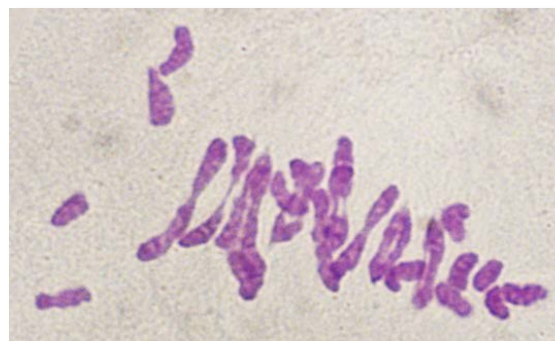
Lola Rey

Cad0000 x *Ae. Variabilis*
Wild type hybrids



Wild type

Cad1691 x *Ae. Variabilis*
***Zip4* Missense mutation hybrids**



Cad0348 x *Ae. Variabilis*
***Zip4* Stop codon hybrids**



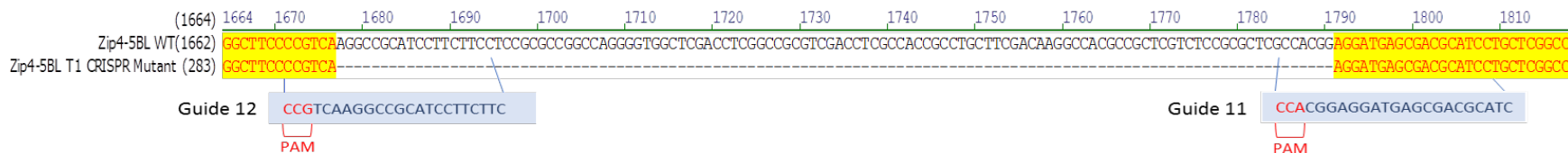
***zip4* mutants**

ZIP4 edition by CRISPR/Cas9 system

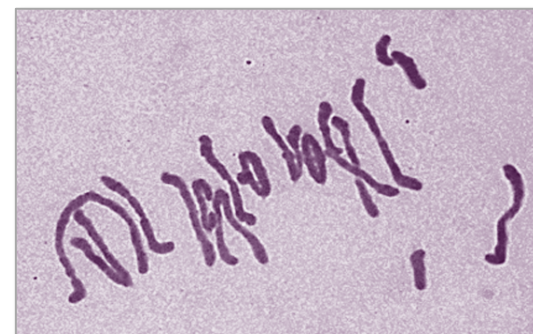
Alignment of all copies of the ZIP4 gene in wheat showing positions of the three sgRNAs



Localization of the large deletion (114 bp) in *TaZIP4-B2*.



Line	No. of cell examined	Univalents	Rod bivalents	Ring bivalents	Multivalents	Chiasma frequency
Fielder x <i>Ae. variabilis</i> hybrids	172	29	2.6	0	0.2	3.1
CRISPR Fielder x <i>Ae. variabilis</i> hybrids	124	9.6	5.6	1.9	3.1	16.7



A single gene, *ZIP4* on 5B, is responsible for the *Ph1* deletion phenotype on recombination

- 1958 → *Ph1* locus discovered on the long arm of 5B
- 1977 → *ph1b* mutant: wheat with a 70 Mb (59.3 Mb) deletion
- 2006 → *ph1* mutant is defined to a 2.5 Mb region
- 2008 → *ph1* mutant is defined to a 0.5 Mb region
- 2018 → A single meiotic gene: *TaZIP4-B2*

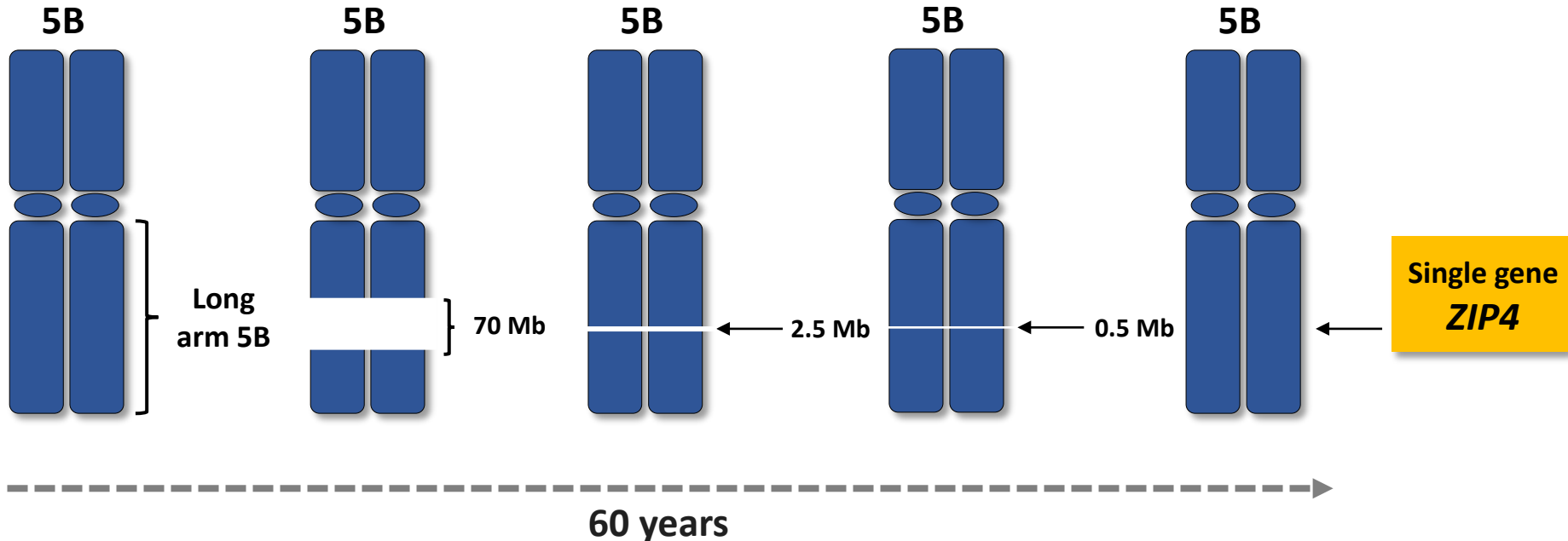
~7000 genes

1187 genes

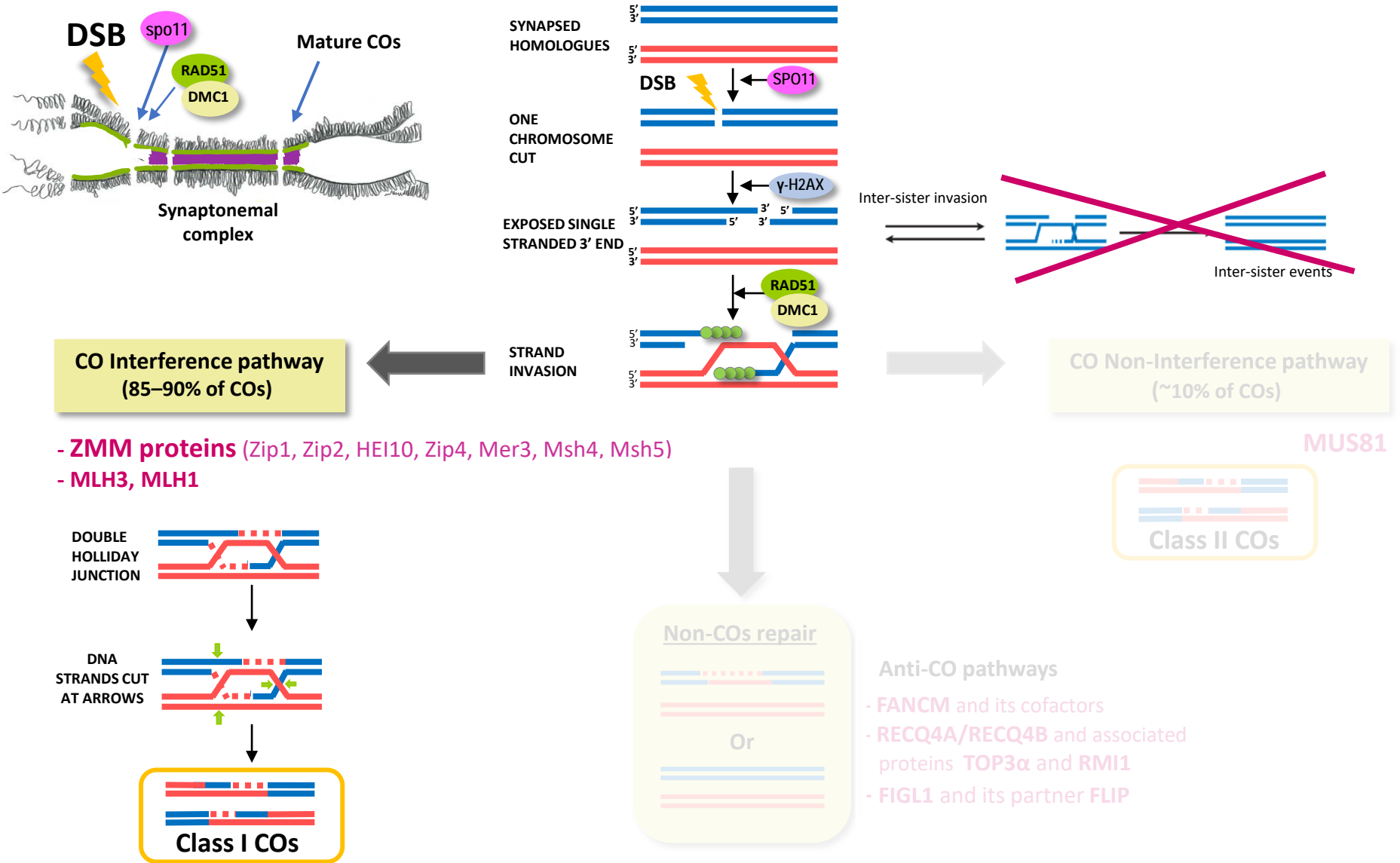
145 genes

25 genes

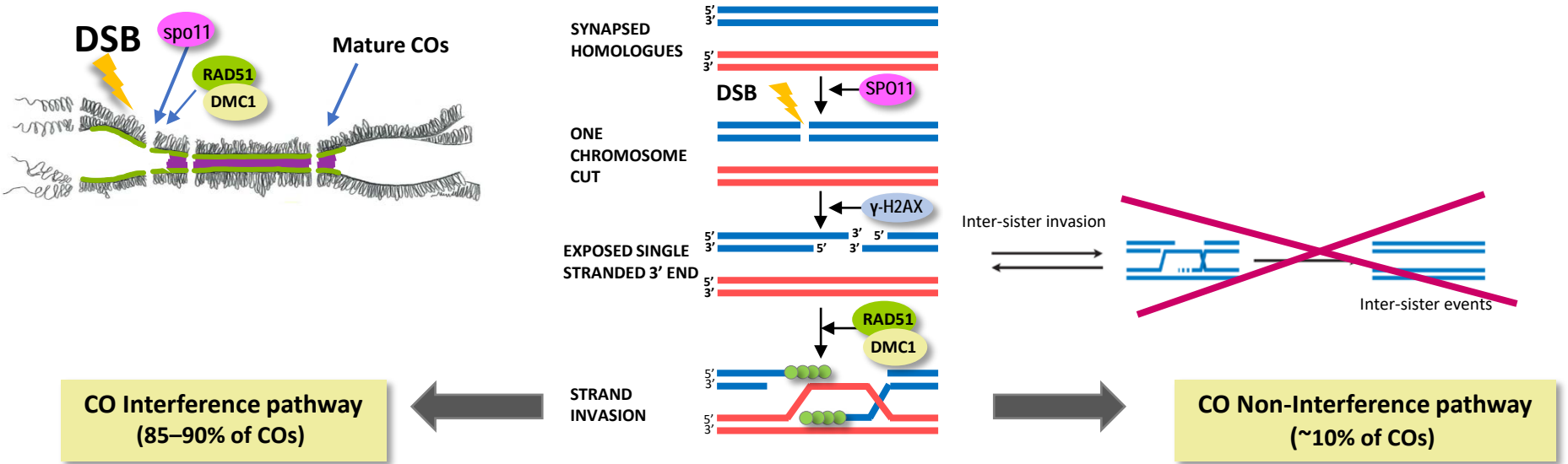
1 gene



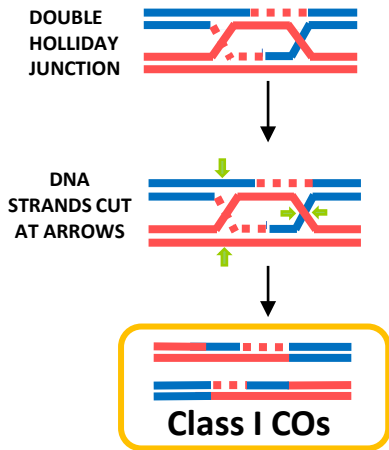
Crossover formation: a fragile balance between controlled DNA damage and repair



Crossover formation: a fragile balance between controlled DNA damage and repair



- **ZMM proteins** (Zip1, Zip2, HEI10, Zip4, Mer3, Msh4, and Msh5)
- **MLH3, MLH1**



ZIP4

- Is a tetra-tricopeptide repeat (TPR)
- In yeast it has a **scaffolding activity**: it interacts with other ZMM proteins, as well as with components of the meiotic chromosome axis
- Arabidopsis and rice **ZIP4** mutant **➔ Reduced CO** number between homologs and different degrees of sterility



MUS81

Does *Ph1* affect synapsis between related chromosomes?

■ Plant material: Wheat-Rye hybrids $n = 28$



No homologs present

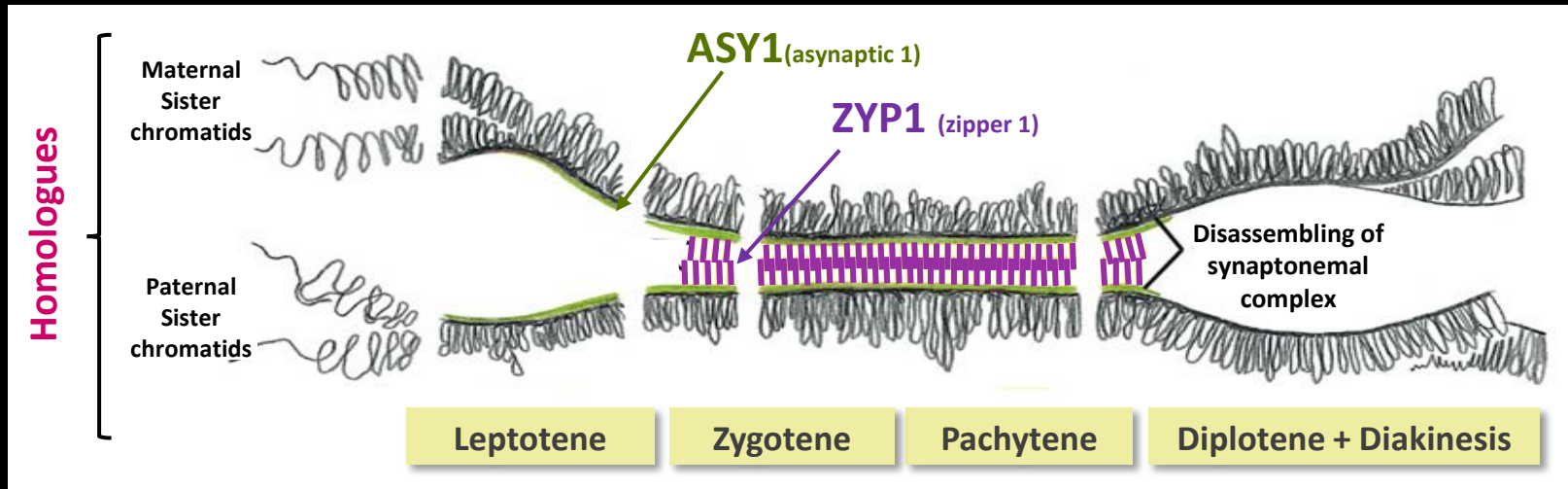
ph1b mutant



WT



■ Immunolabeling

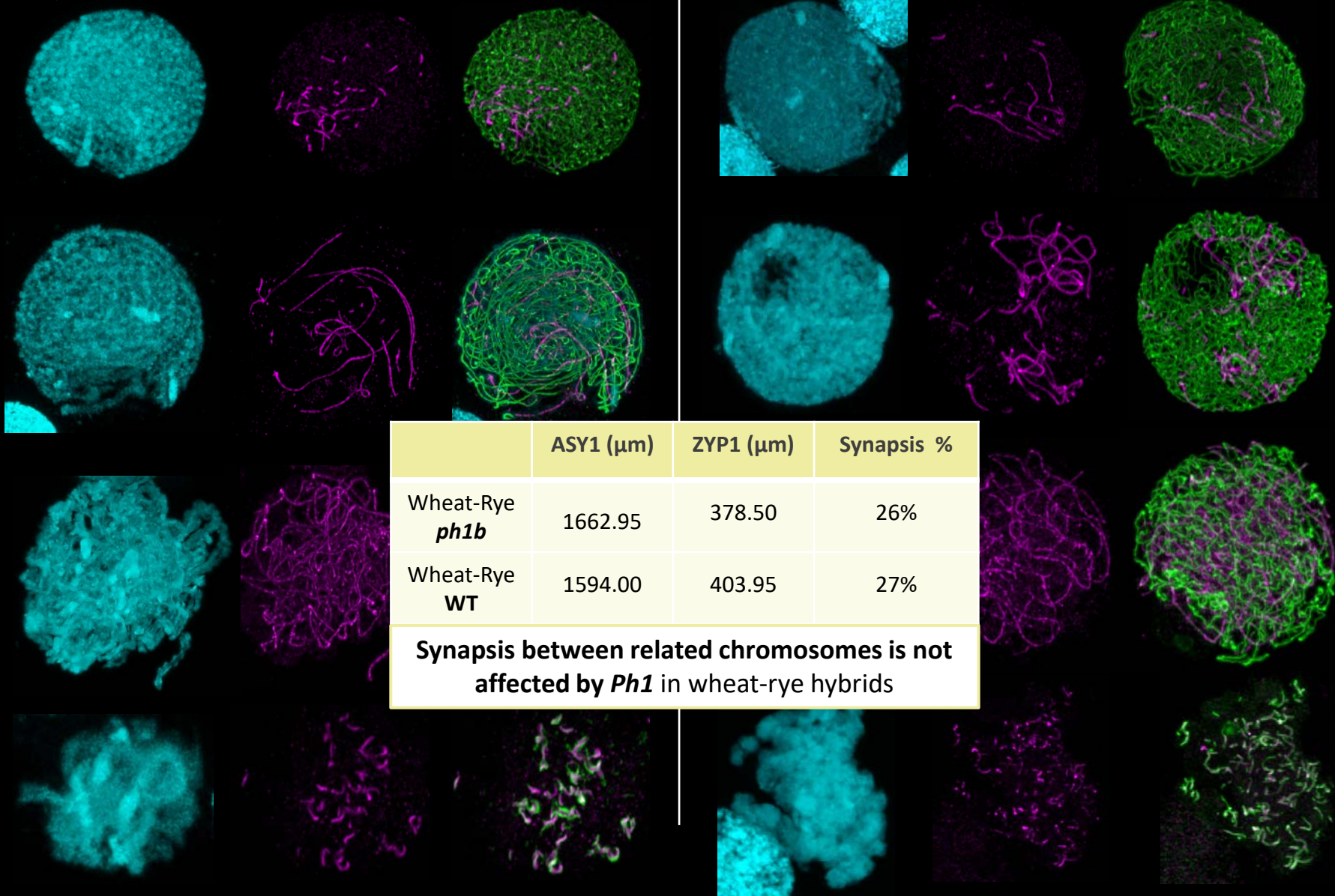


Wheat-rye hybrid *ph1b* mutant

Wheat-rye hybrid *WT*

DAPI - DNA
 ASY1- Lateral element
 ZYP1- Synapsis

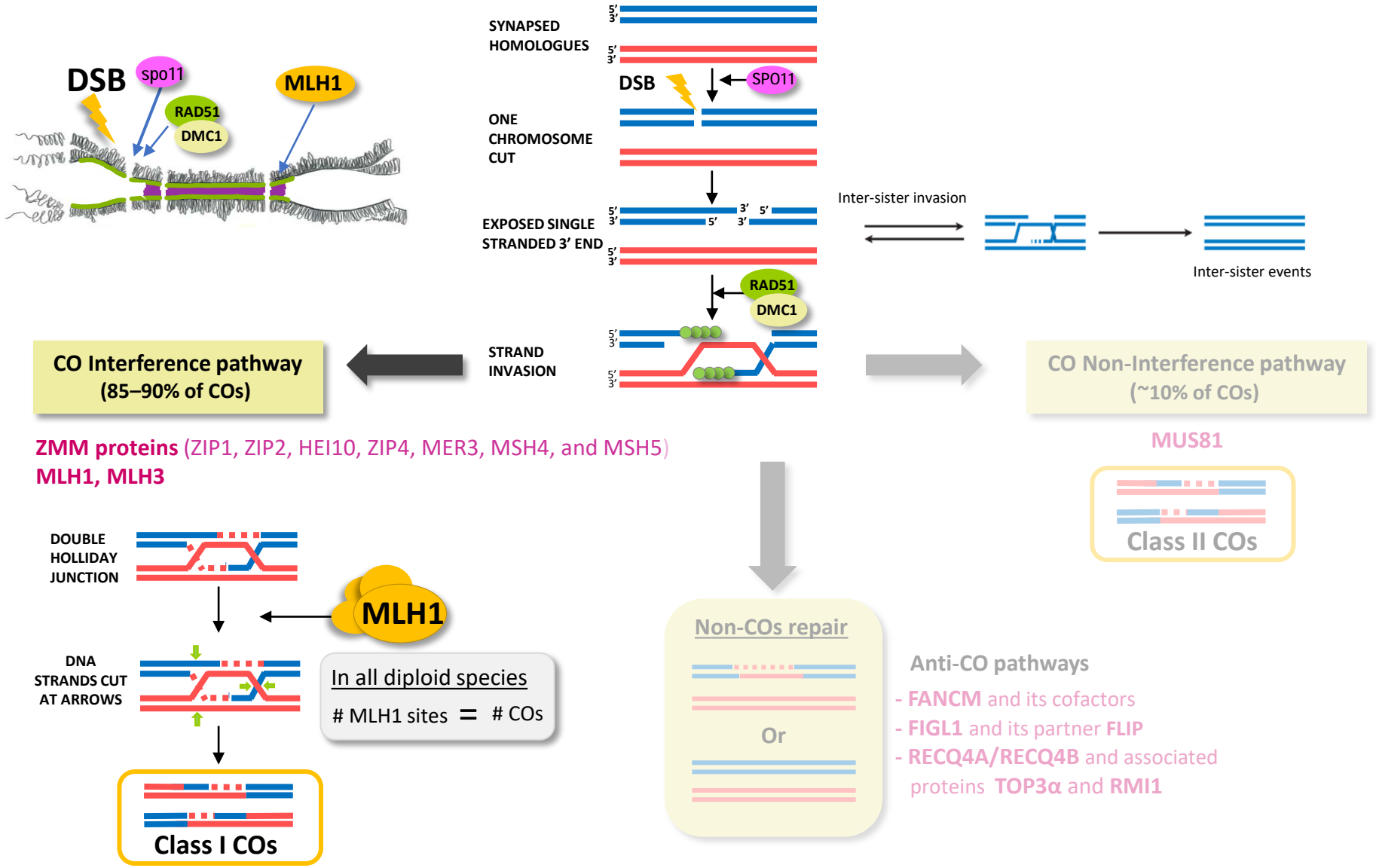
Beginning of zygotene
 Zygotene
 "Pachytene"
 Diplotene



	ASY1 (μm)	ZYP1 (μm)	Synapsis %
Wheat-Rye <i>ph1b</i>	1662.95	378.50	26%
Wheat-Rye WT	1594.00	403.95	27%

Synapsis between related chromosomes is not affected by *Ph1* in wheat-rye hybrids

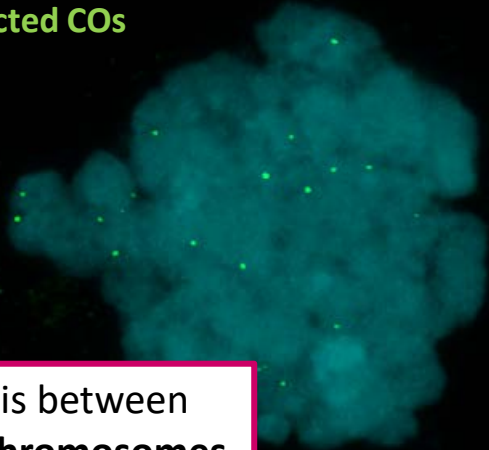
Crossover formation: a fragile balance between controlled DNA damage and repair



Wheat-rye *ph1b* mutant nc= 28

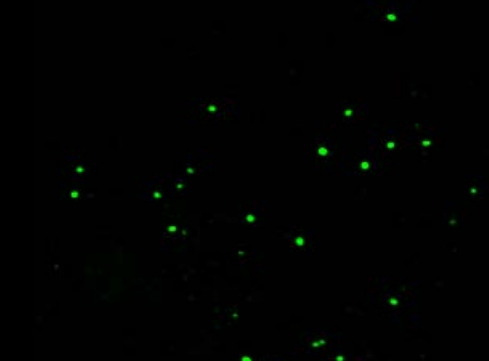
DAPI - DNA

MLH1 - Expected COs



Synapsis between
related chromosomes

- Number of COs \approx 7-8
- MLH1 number observed = 20-22

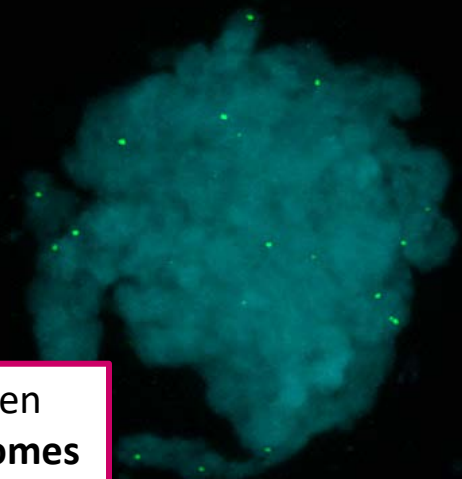


■ Absence of the *Ph1* locus can alleviate to some extent the MLH1 stalling

Wheat-rye WT nc = 28

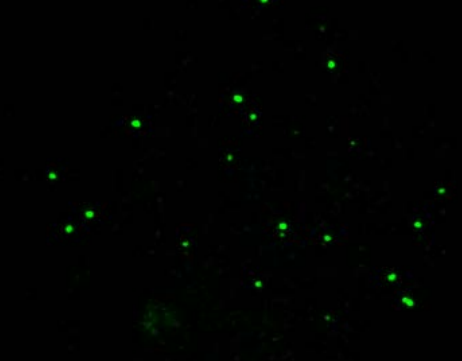
DAPI - DNA

MLH1 - Expected COs



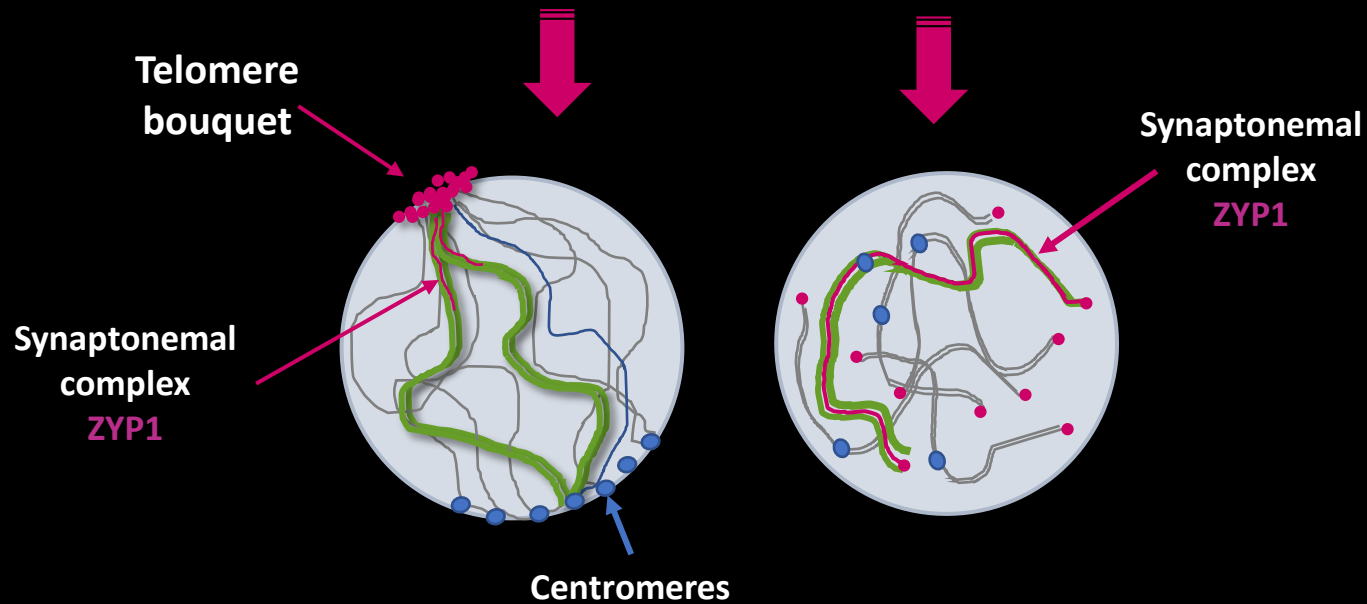
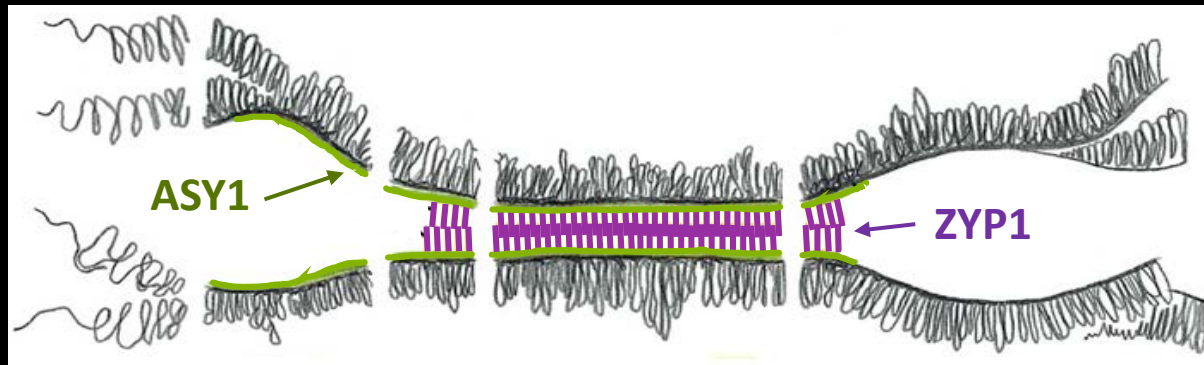
Synapsis between
related chromosomes

- Number of COs \approx 0-1
- MLH1 number observed = 20-22



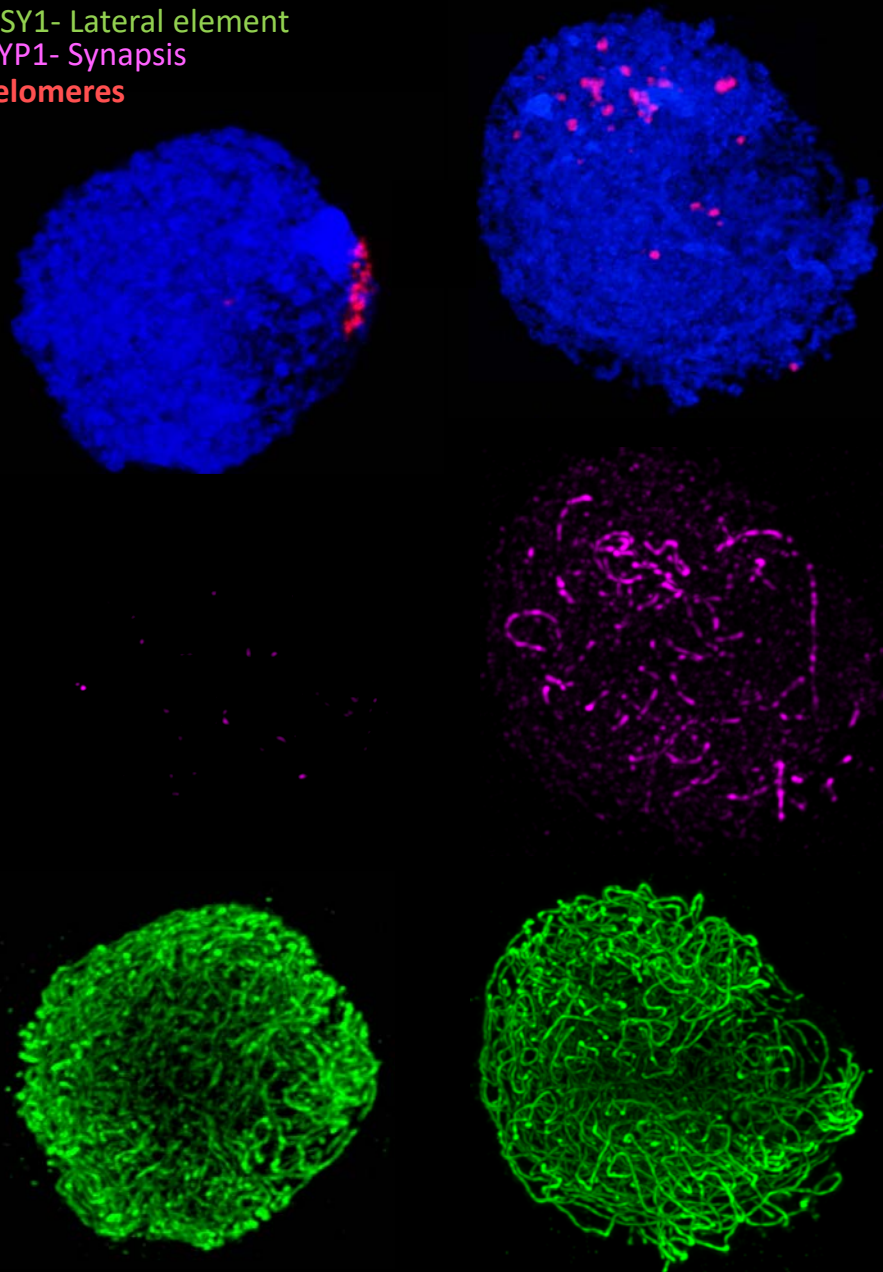
■ MLH1 don't correlate with the number of COs when synapsis occurs between related chromosomes

Timing of synapsis in relation to the telomere bouquet

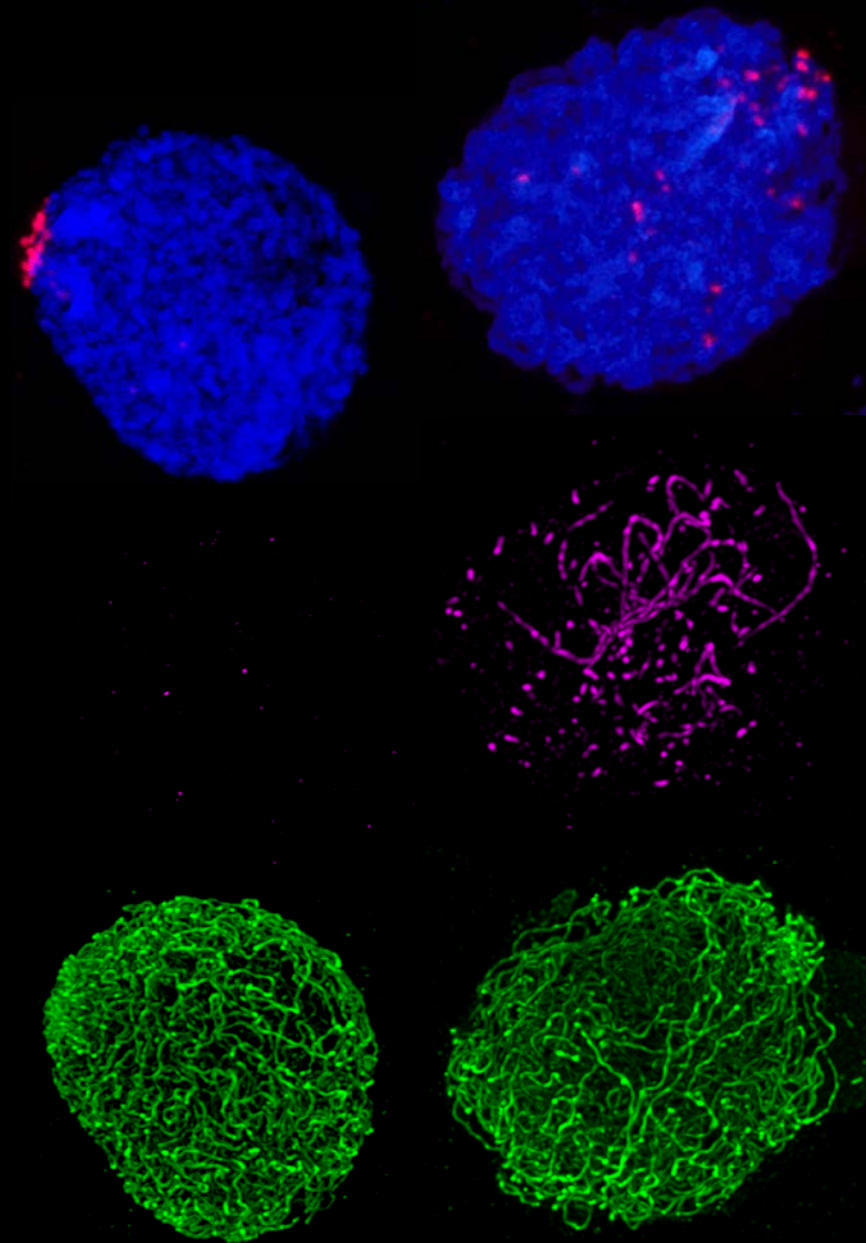


Wheat-rye hybrid *ph1b* mutant

DAPI - DNA
ASY1 - Lateral element
ZYP1 - Synapsis
Telomeres

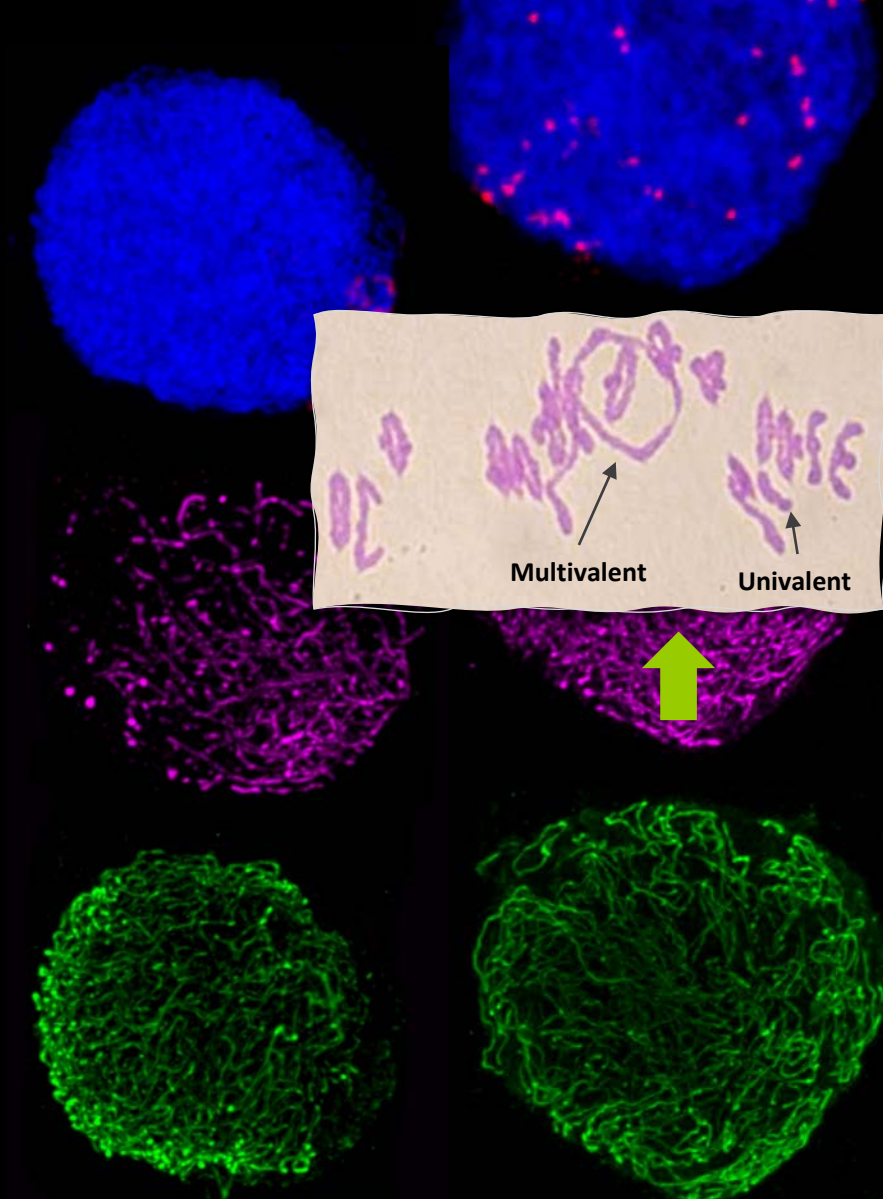


Wheat-rye hybrid WT

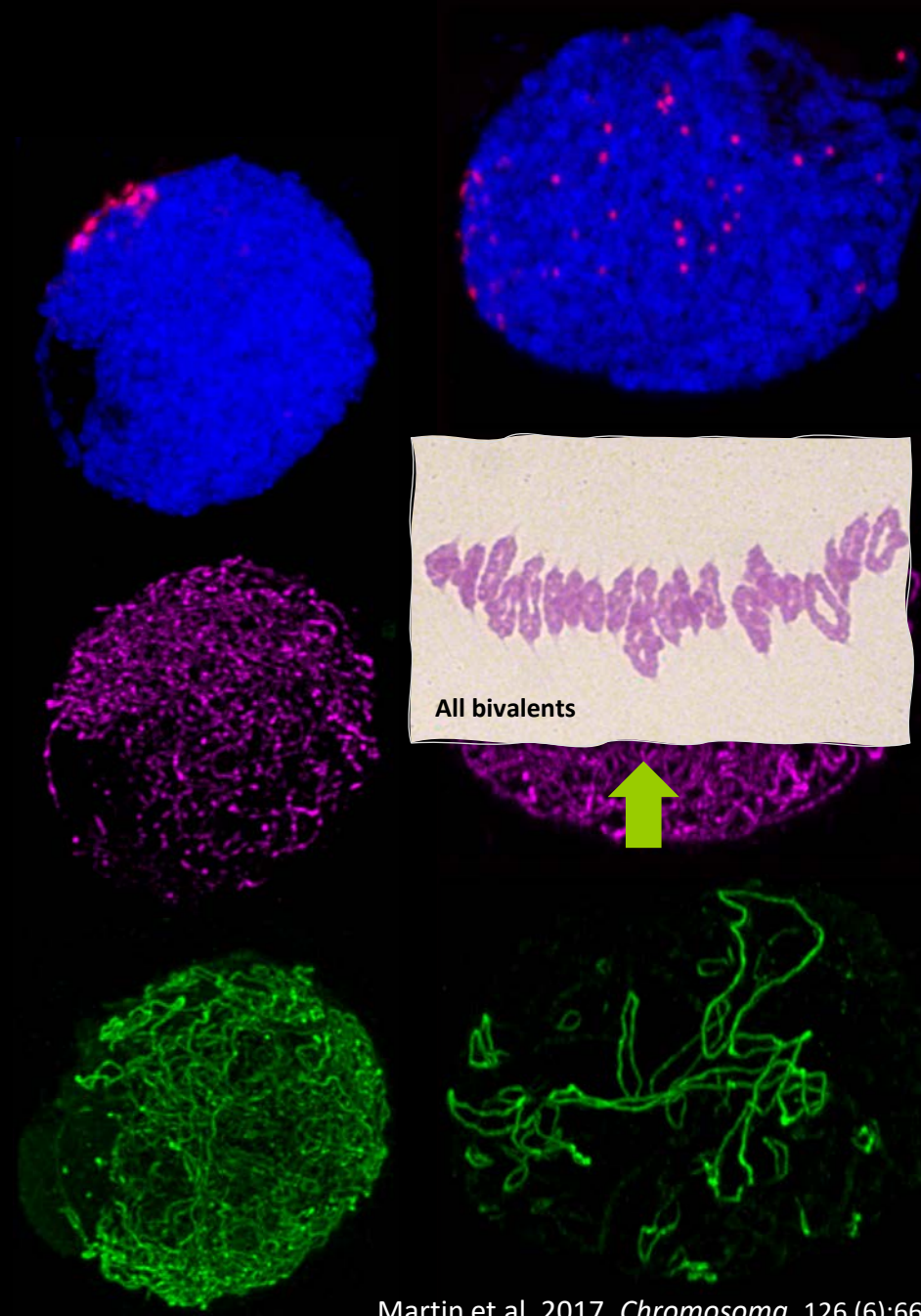


Wheat *ph1b* mutant

DAPI - DNA
ASY1- Lateral element
ZYP1- Synapsis
Telomeres



Wheat WT

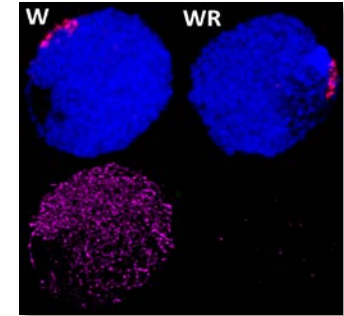


Cytological diploidization of polyploid wheat

- Independently of *Ph1* (*ZIP4-B2*), synapsis between related chromosomes does not take place during the telomere bouquet: only homologous synapsis can happen



Upon polyploidization, wheat already had a mechanism to sort homologs from related chromosomes



- Ph1* (*ZIP4-B2*) provided the “fine-tuning” needed for the meiotic adaptation observed in hexaploid wheat

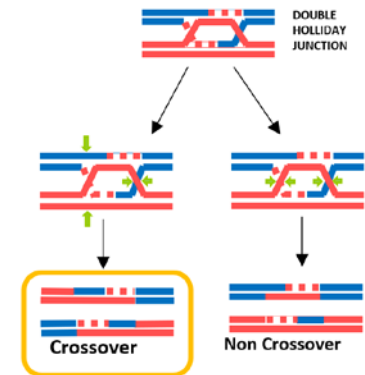


Dual effect of *Ph1* (*ZIP4-B2*) on synapsis and crossover

Ph1 promotes early homologous **synapsis**

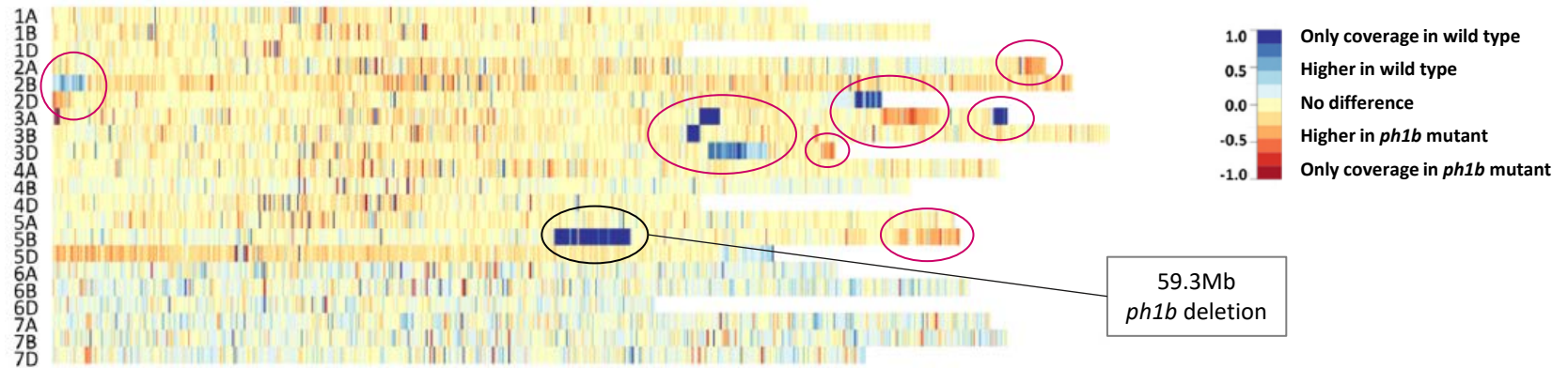
+

Prevents (MLH1) sites on related chromosomes from becoming **COs**

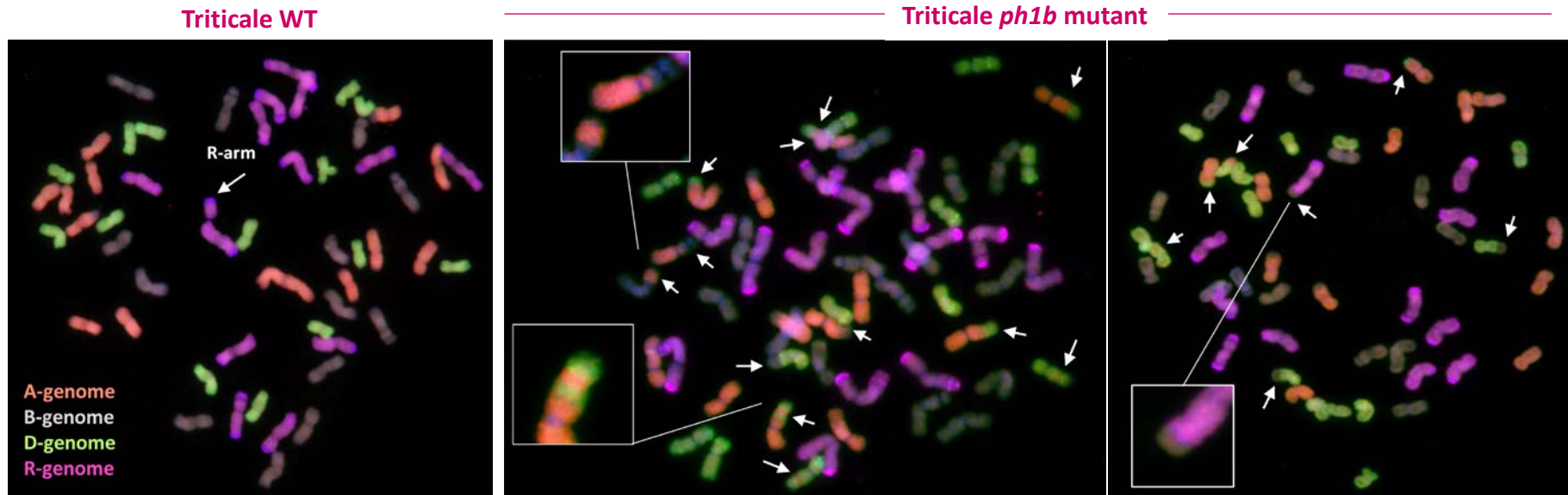


Multiple chromosome rearrangements in the absence of *Ph1*/ZIP4-B2

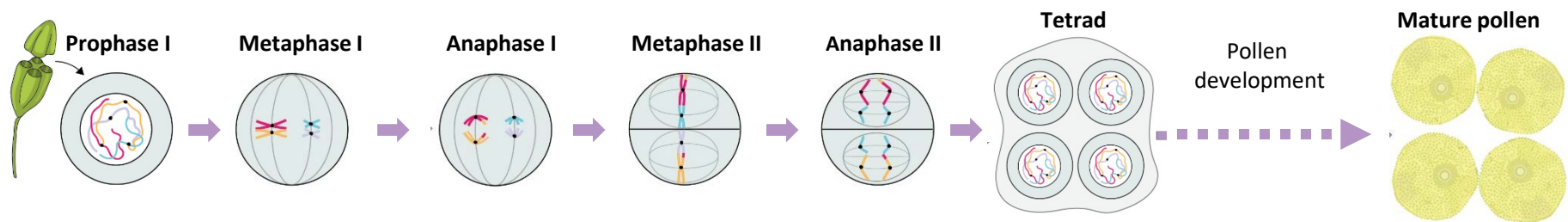
Heatmap comparing transcription in wheat and the *ph1b* mutant.



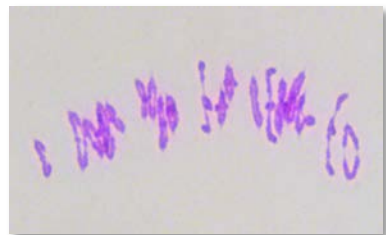
Root-tip metaphases of octoploid triticales ($2n = 8x = 56$) in the presence and absence of *Ph1*



Effect of the *TaZIP4-B2* deletion on meiotic and tetrad stages



WT Wheat
(Fielder)



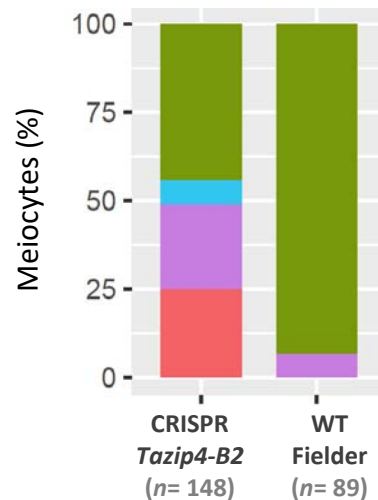
CRISPR *Tazip4-B2*
(Fielder)



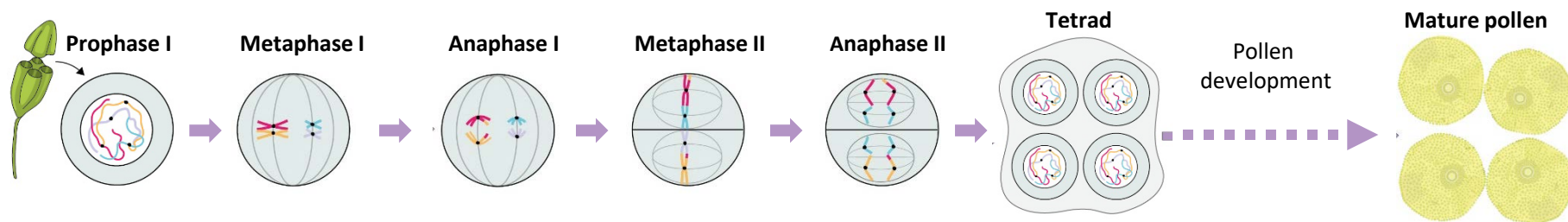
56% exhibit meiotic abnormalities

Metaphase I

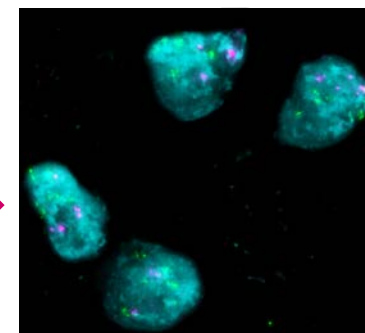
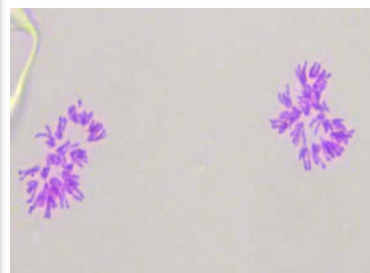
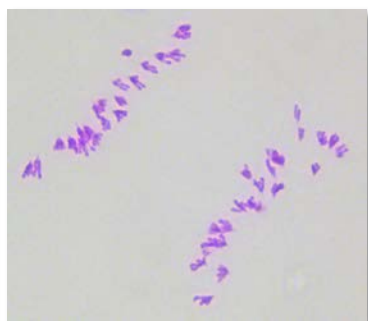
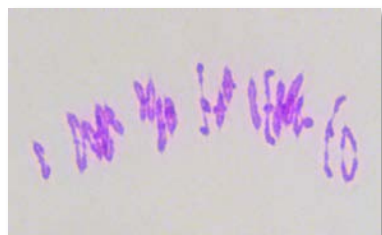
■ Univalents+Multivalents ■ Only multivalents
■ Bivalents ■ Only univalents



Effect of the *TaZIP4-B2* deletion on meiotic and tetrad stages



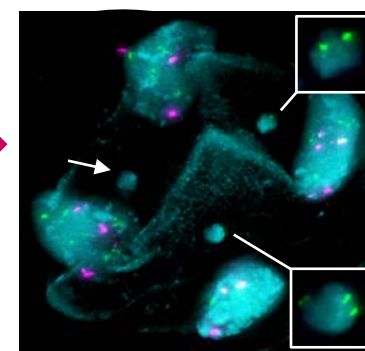
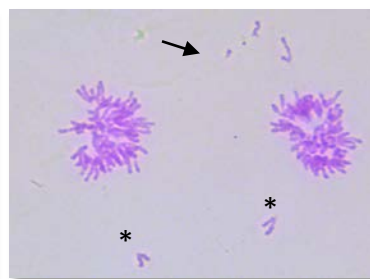
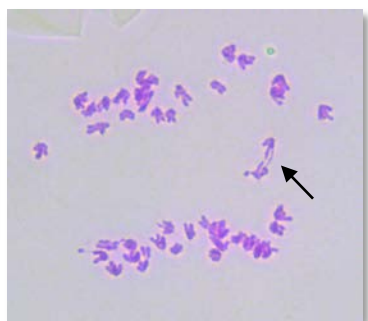
WT Wheat
(Fielder)



CRISPR
Tazip4-B2
(Fielder)



56% exhibit meiotic abnormalities



Metaphase I

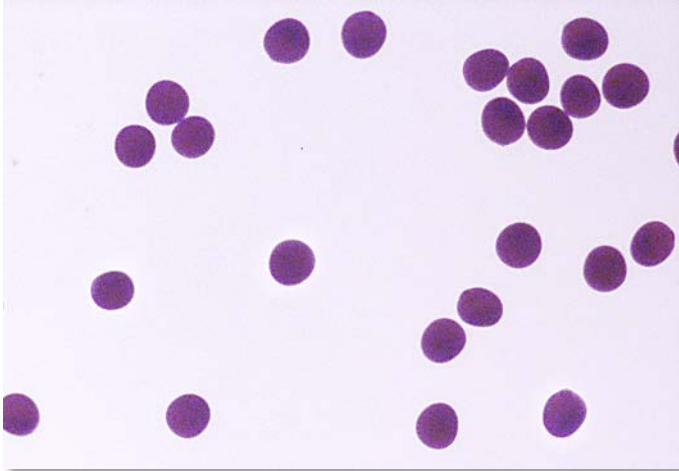
Anaphase I

Tetrads

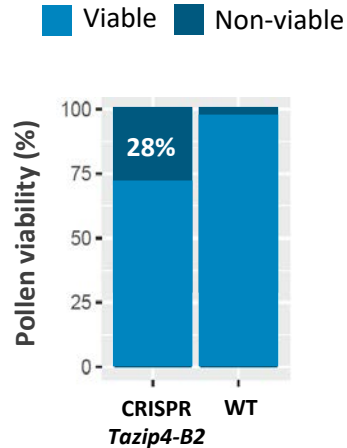
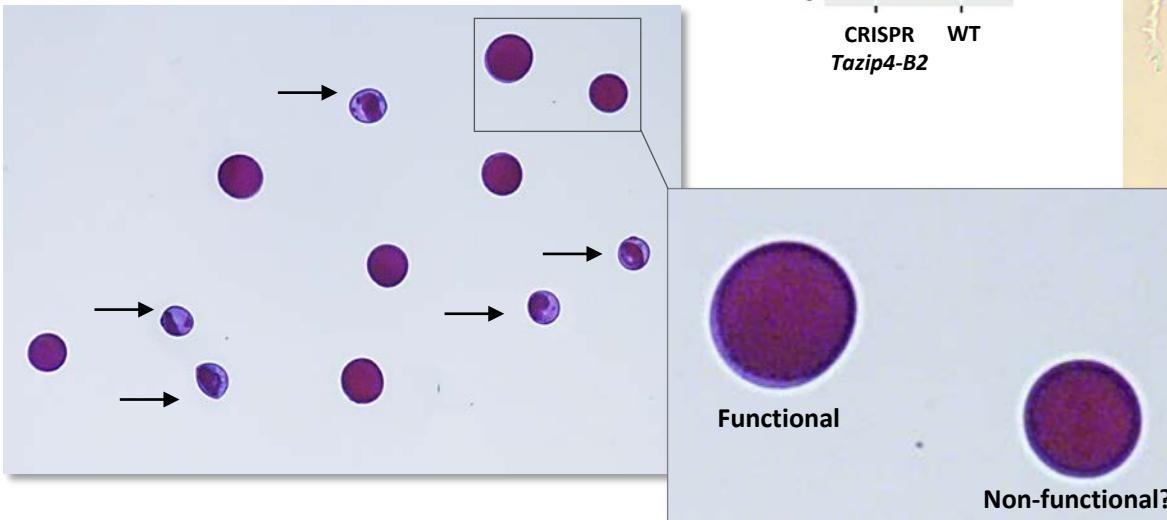
Pollen viability in the CRISPR-*Tazip4* mutant

Alexander stain

WT Wheat

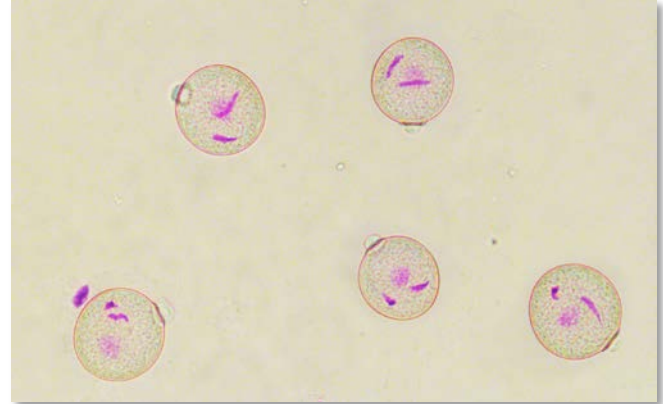


CRISPR *Tazip4-B2*

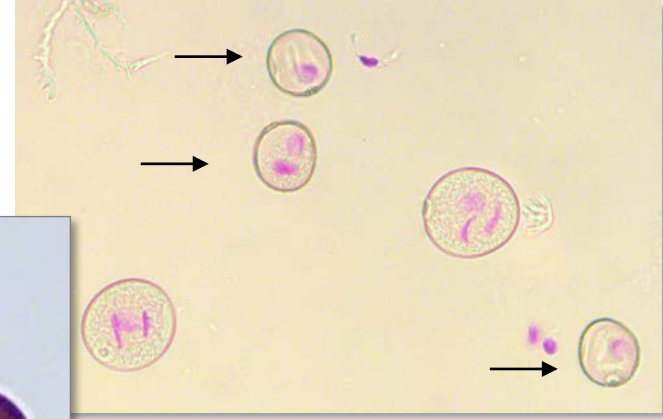


Feulgen stain

WT Wheat



CRISPR *Tazip4-B2*

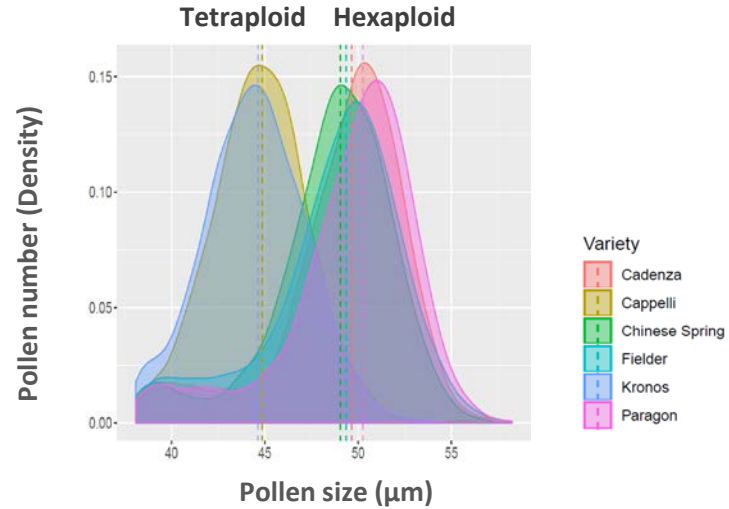


Pollen profiling method: pollen size and number

Particle sizer and counter



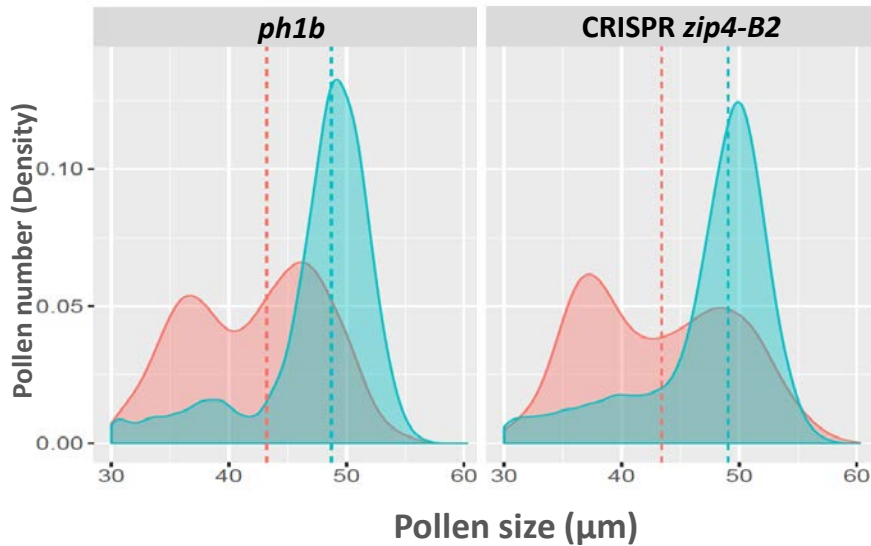
Multisizer 4e (Beckman Coulter Inc.)



Abdul Kader Alabdullah

Pollen profile

Wild type *Tazip4-B2* mutant



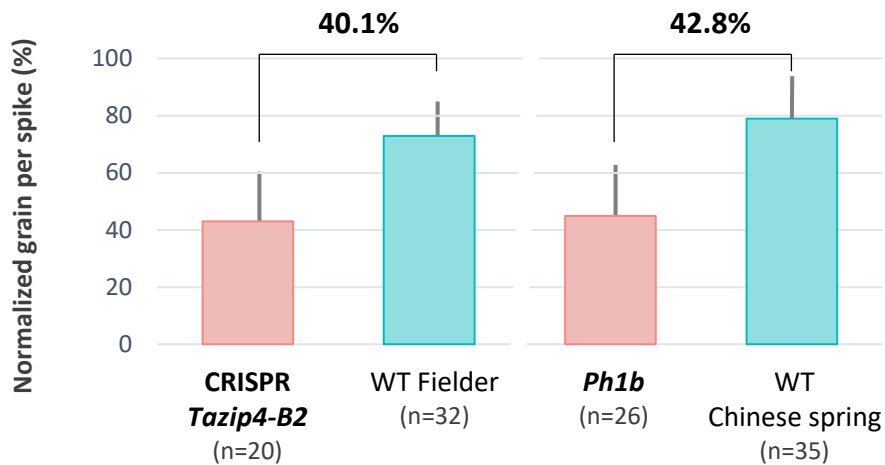
Open Access Editor's Choice Article

A Duplicated Copy of the Meiotic Gene *ZIP4* Preserves up to 50% Pollen Viability and Grain Number in Polyploid Wheat

Abdul Kader Alabdullah ^{1b}, Graham Moore * and Azahara C. Martín ^{1b}

Effect of *TaZIP4-B2* on fertility and grain number

● **Grain setting** → Over 40% reduction in grain set.



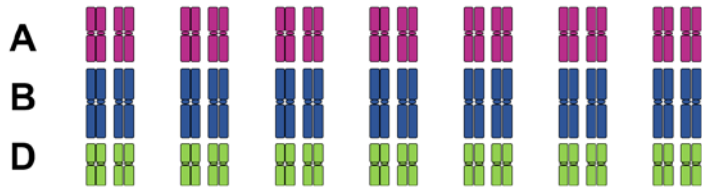
The elimination of the duplicated *TaZIP4* on 5B resulted in:

- 56% of meiocytes exhibiting meiotic abnormalities
- 50% of tetrads exhibiting micronuclei
- 48% of smaller pollen grains
- Over 40% reduction in grain set.

Extraordinary value of the wheat *ZIP4* duplication on fertility and grain number

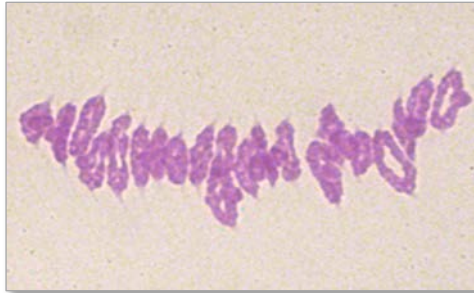
Can we decouple these 2 effects of *TaZIP4-B2*?

Wheat 2n= 42



All bivalents

WT wheat



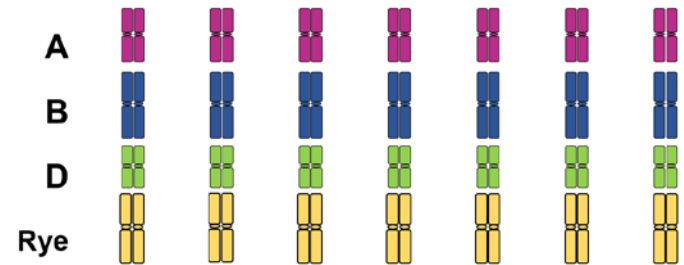
Mostly bivalents but also univalent + multivalents

TaZIP4-B2 mutant



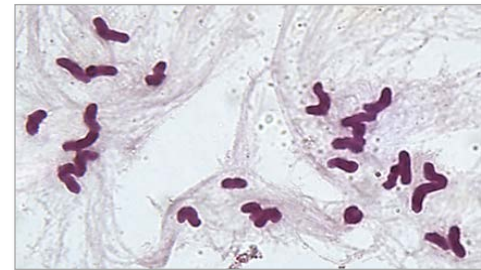
Ph1 is necessary for wheat genome stability

Wheat-rye hybrid n = 28



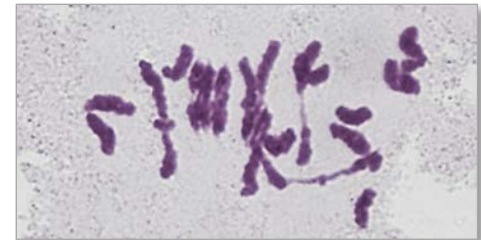
All univalents

WT hybrid



Univalents + bivalents + multivalents

TaZIP4-B2 mutant



Ph1 is a barrier for wild relatives introgressions

Cad1691 – *Tazip4-B2* TILLING mutant

TaZIP4-B2 performs 2 key meiotic functions:

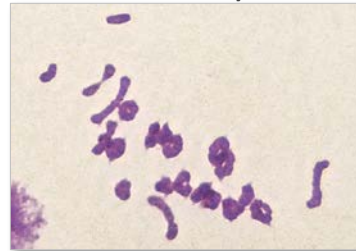
- A** Promotion of chromosome synapsis between homologs – early prophase I
- B** Suppression of COs between related chromosomes – Late prophase I

CRISPR *Tazip4-B2*



56% meicytes with univalent + multivalents

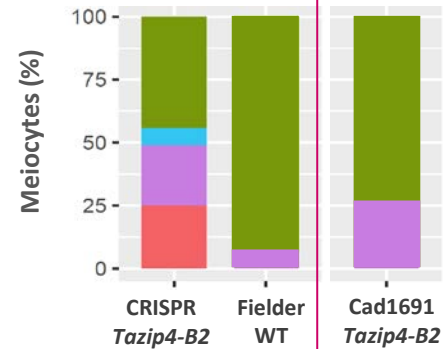
Cad1691 *Tazip4-B2*



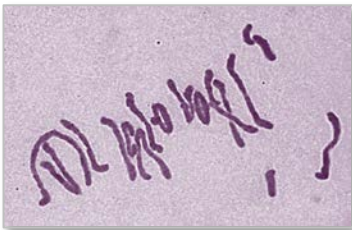
26% meicytes with univalents

No multivalents present

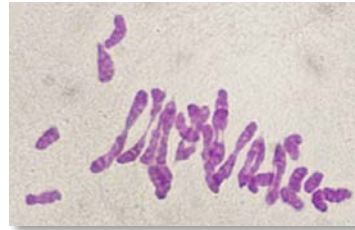
Univalents+Multivalents (red)
Bivalents (green)
Only multivalents (cyan)
Only univalents (purple)



CRISPR *Tazip4-B2* x *Ae. variabilis*



Cad1691 *Tazip4-B2* x *Ae. variabilis*



B ~~Suppression of COs between related chromosomes~~

Cad1691 – *Tazip4-B2* TILLING mutant

TaZIP4-B2 performs 2 key meiotic functions:

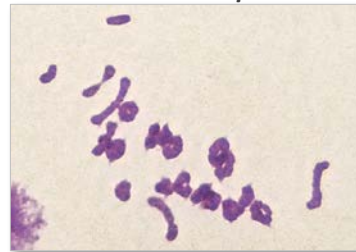
- A** Promotion of chromosome synapsis between homologs – early prophase I
- B** Suppression of COs between related chromosomes – Late prophase I

CRISPR *Tazip4-B2*



56% meiocytes with univalent + multivalents

Cad1691 *Tazip4-B2*



26% meiocytes with univalents

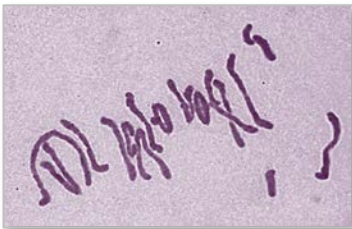
No multivalents present



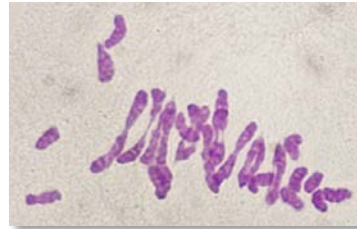
A Promotion of chromosome synapsis between homologs

Cad1691 *Tazip4-B2* mutant represents a 'separation-of-function' *Tazip4-B2* mutant *zip4-ph1d* mutant

CRISPR *Tazip4-B2* x *Ae. variabilis*



Cad1691 *Tazip4-B2* x *Ae. variabilis*



B ~~Suppression of COs between related chromosomes~~

Crossovers take place only between homologs in the *zip4-ph1d* mutant

GISH (Genomic *in situ* hybridization)



zip4-ph1d mutant

A genome

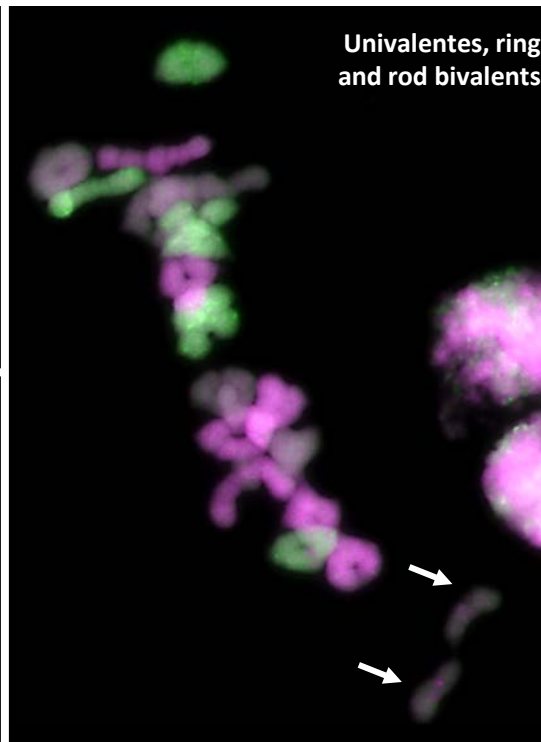
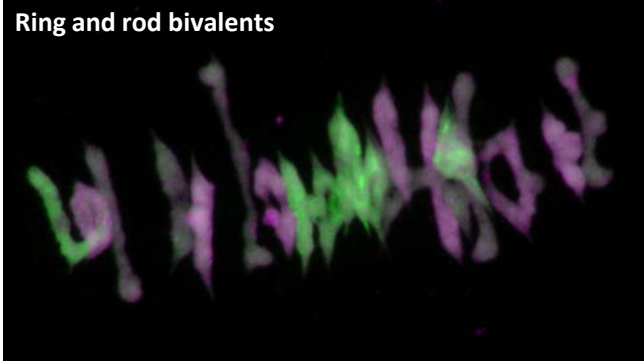
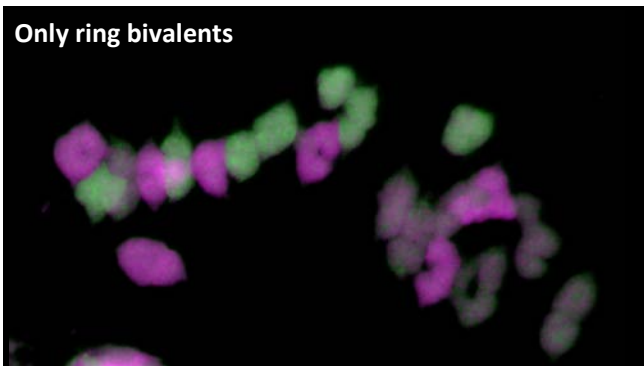
B genome

D genome

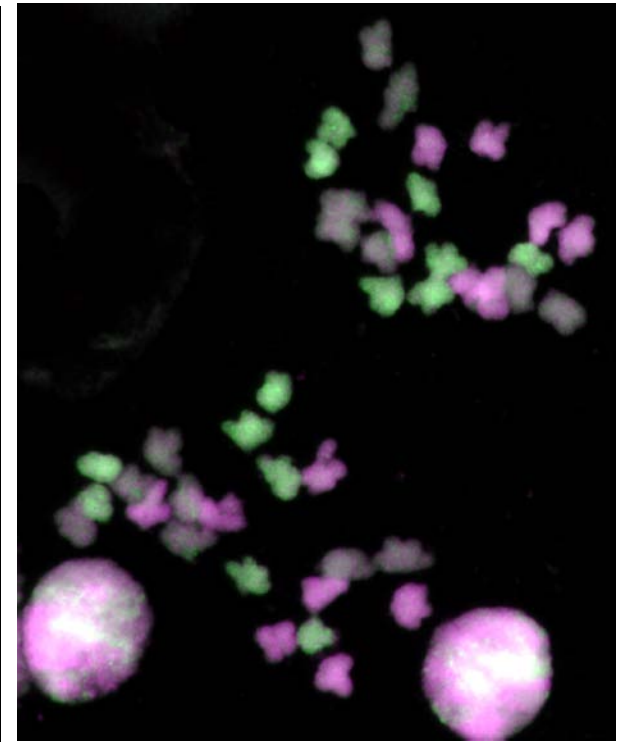
'separation-of-function'

Tazip4-B2 mutant

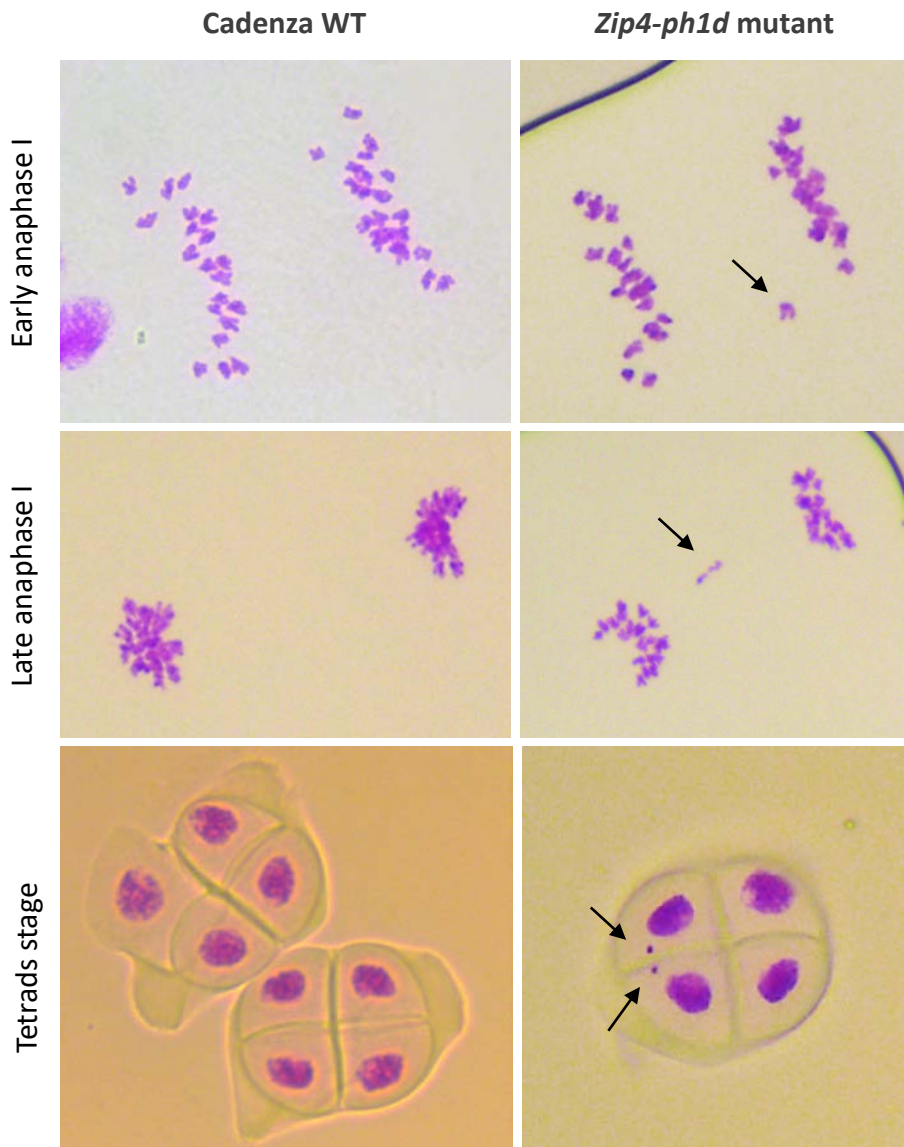
Meiotic metaphase I



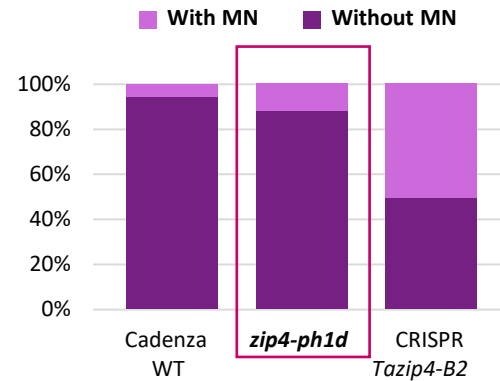
Meiotic anaphase I



There is mostly balanced chromosome segregation in the *zip4-ph1d* mutant



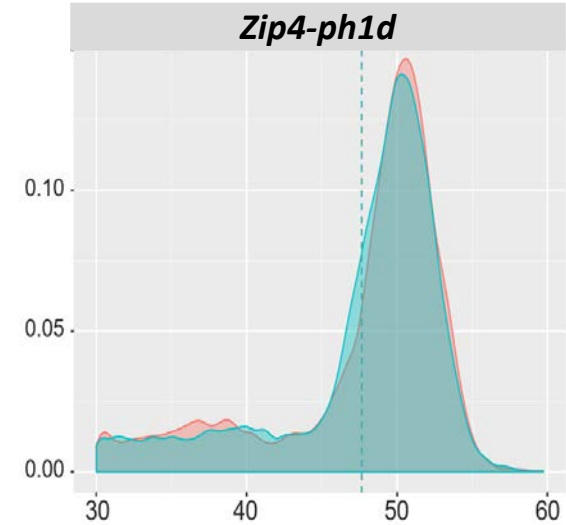
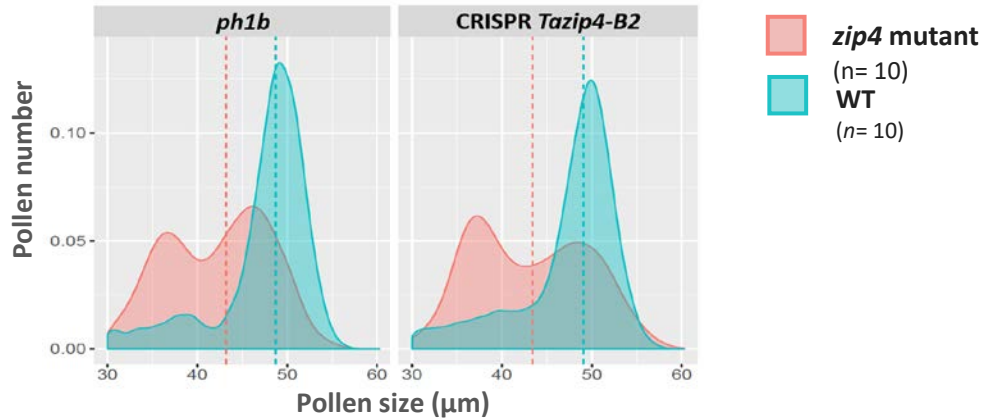
- ➔
- 8.6% laggards
 - No splitting of sister chromatids
 - No chromosome fragmentation



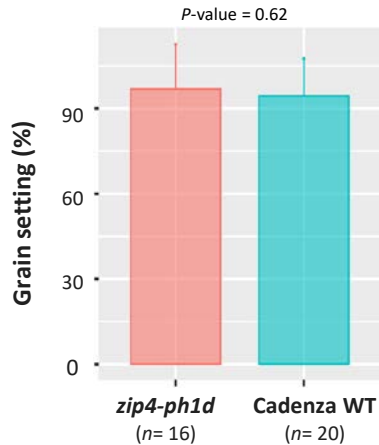
	Cadenza WT	<i>zip4-ph1d</i> mutant
% Tetrads with MN	5.4	12.2
Total No. Tetrads	710	742
No. Tetrads with MN%	39	91

Fertility is the same in the *zip4-ph1d* mutant and WT wheat

Pollen size and number - Multisizer 4e



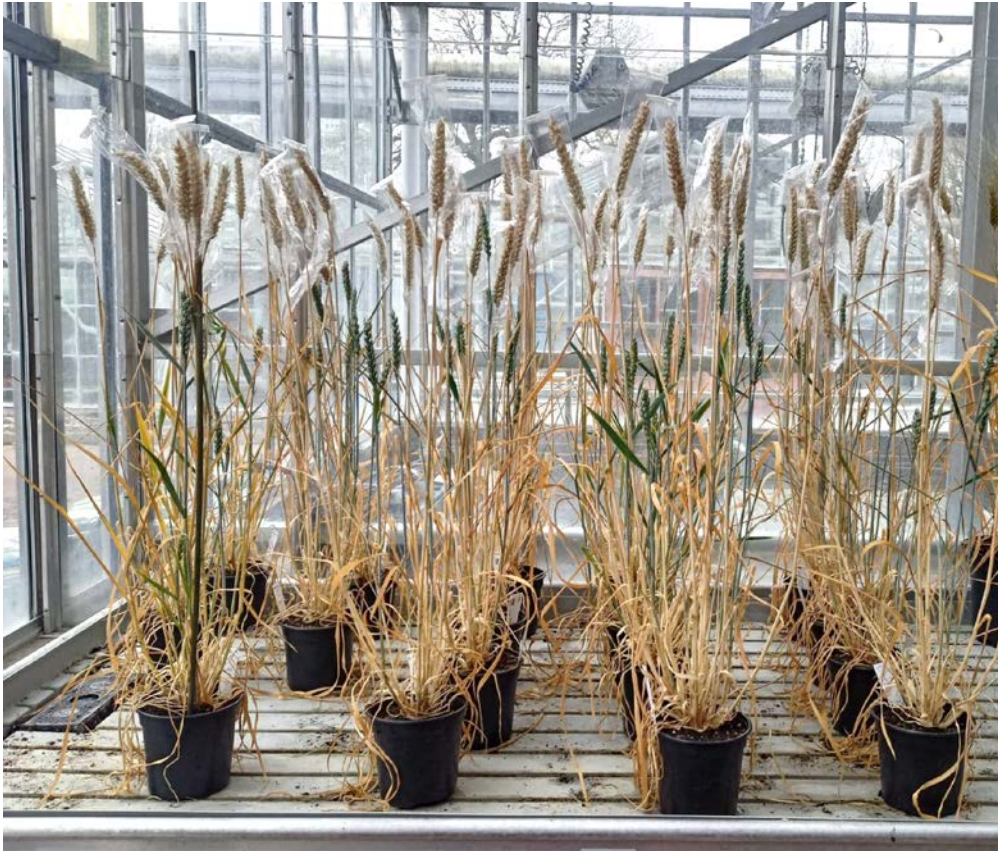
Grain number



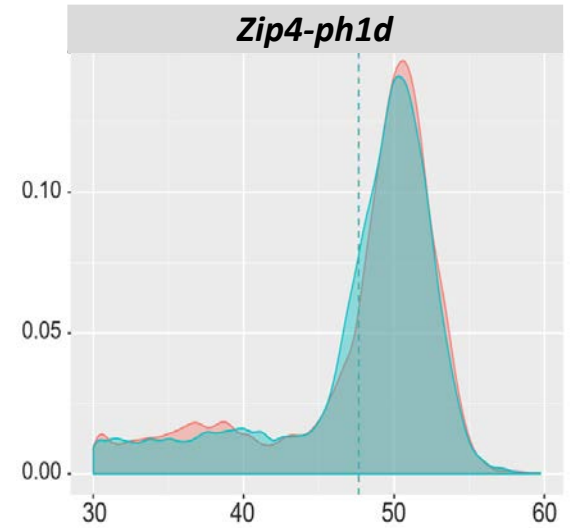
zip4-ph1d mutant

Cadenza WT

We **recommend the use** of the *zip4-ph1d* mutant in wheat breeding rather than previous described *ph1* mutants



zip4-ph1d mutants

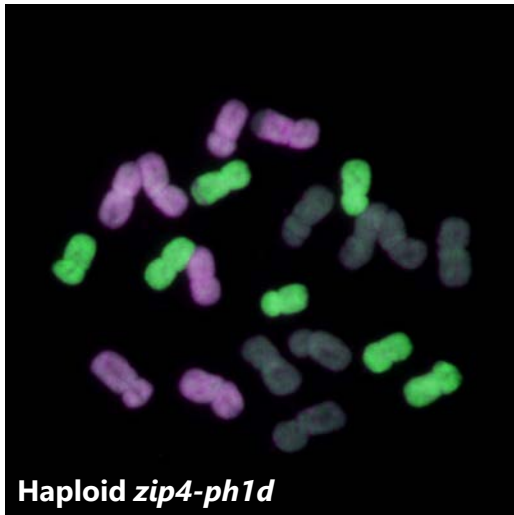


zip4-ph1d mutant

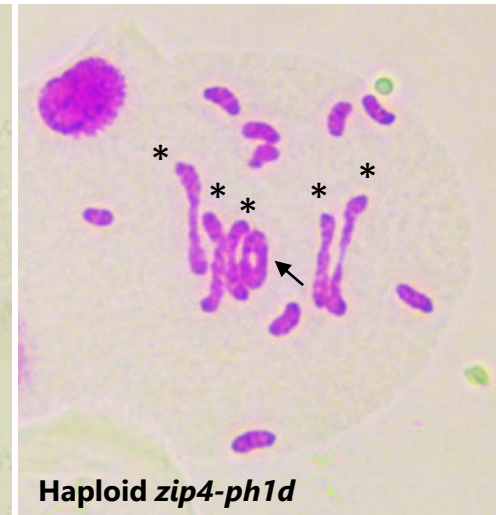
Cadenza WT

The *zip4-ph1d* mutation allows crossover between related chromosomes in wheat haploids

Root mitotic metaphase



Meiotic metaphase I



Line	No. of cells examined	Rod bivalents		Ring bivalents		Multivalents		CO frequency per cell
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Haploid Cad-WT	566	0.60 ± 0.04	0-3	0.01 ± 0.003	0-1	0.005 ± 0.005	0-1	0.63 ± 0.03
Haploid <i>zip4-ph1d</i>	433	3.36 ± 0.04	0-6	0.66 ± 0.07	0-4	0.68 ± 0.08	0-3	6.26 ± 0.12


CONCLUSIONS



1 A single meiotic gene, **ZIP4** on 5B (*TaZIP4-B2*), is responsible for the *Ph1* deletion phenotype on recombination

2 **Provides** (at least) **2 key function:**

- Promotes pairing-synapsis between homologs
- Suppresses crossover between related chromosomes



Preservation of wheat stability and fertility (grain number)

3 **Several *Tazip4-B2* mutants lines available** www.jic.ac.uk/research/germplasm-resources-unit

*CRISPR Tazip4-B2*mutant

Cad0348 Tazip4-B2 mutant

***Zip4-ph1d* mutant**
(accession no. W10349)



**Recommended
for breeding**

WHAT'S NEXT?

- What is the role of the *TaZIP4* copies on group 3 chromosomes?
 - Which are the ZIP4-B2 protein interacting partners?
 - What is the effect of the A¹⁶⁷V substitution in the *zip4-ph1d* mutant?
- Is there any genetic variability in *TaZIP4-B2* that we can explore?
 - Can we obtain new *TaZIP4-B2* mutants with useful phenotypic variation?

Thanks to:

Graham Moore

Peter Shaw

Lola Rey

Tracie Draeger

Ali Pendle

Abdul Kader Alabdullah

Dylan Phillips (Aberystwyth University)

RNAseq

Philippa Borrill

Janet Higgins (Earlham Institute)

CRISPR

Sadiye Hayta

Mark Smedley

Tilling mutants

Cristóbal Uauy



Unlocking Nature's Diversity



Biotechnology and
Biological Sciences
Research Council

