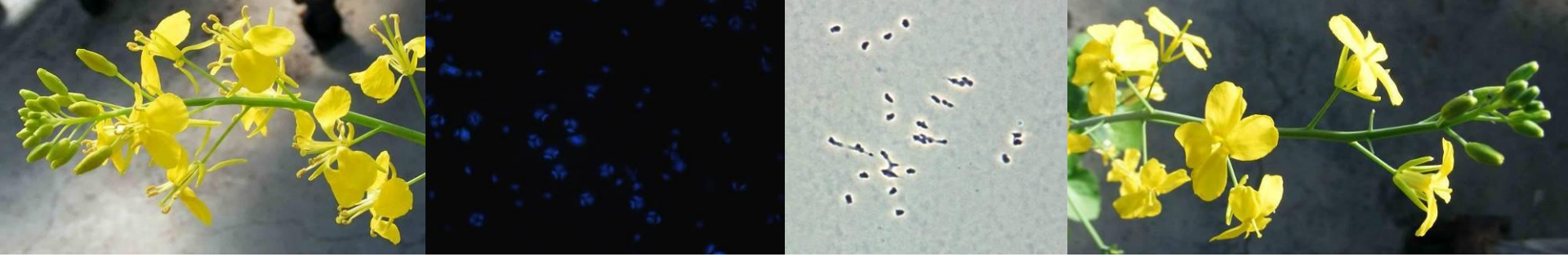

Unreduced Gametes in *Brassica*: Effects of Genotype, Temperature and Interspecific Hybridization

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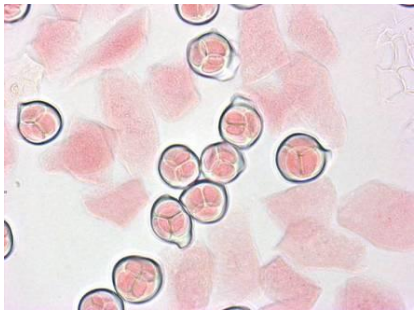


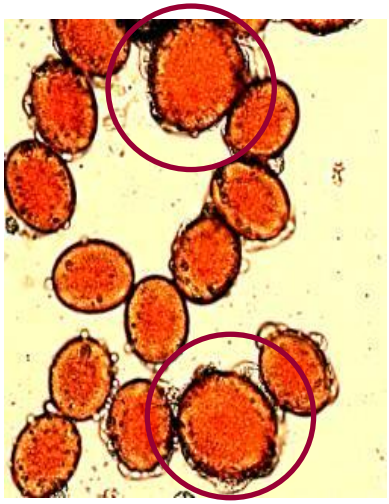
Guijun Yan, The University of Western Australia

Wallace A. Cowling, Canola Breeders Western Australia

Matthew N. Nelson, The University of Western Australia

Summary

- What is an unreduced gamete?
- Why are unreduced gametes interesting?
- Experimental material
- Hypotheses: hybridisation, genotype and temperature effects
- Results from pollen measurements
- Results from sporad observations
- Results from hybridisation experiment



What is a reduced gamete?

Maternal chromosome

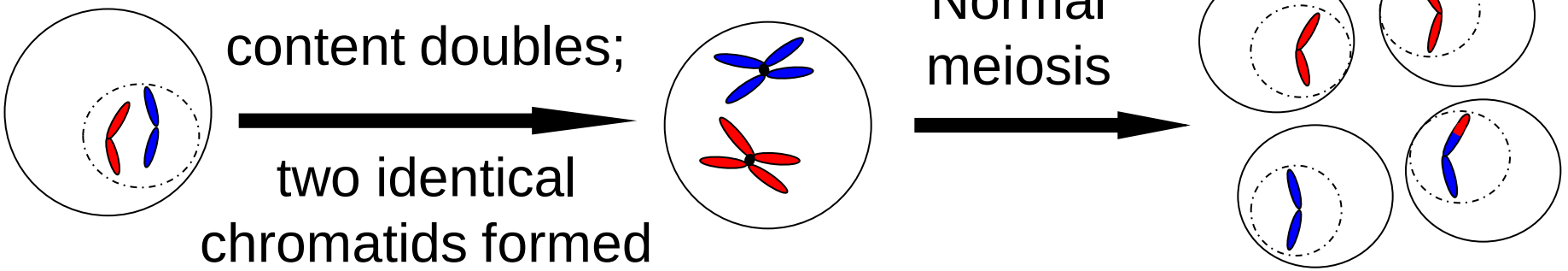
Paternal chromosome

Reduced gametes

Pre-meiosis DNA content doubles;

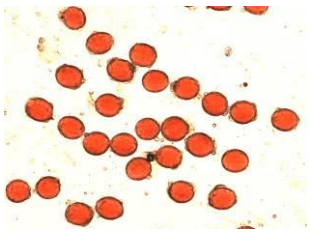
two identical chromatids formed

Normal meiosis



“2n” DNA content

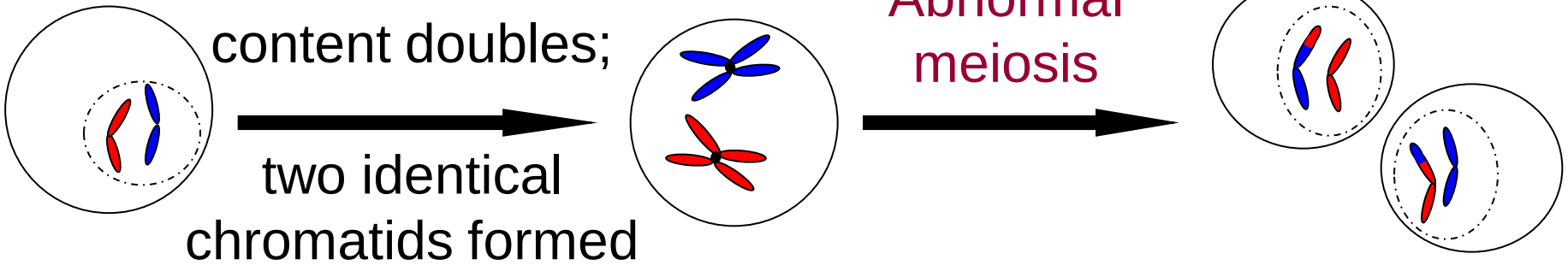
“n” DNA content



What is an unreduced gamete?

Maternal chromosome

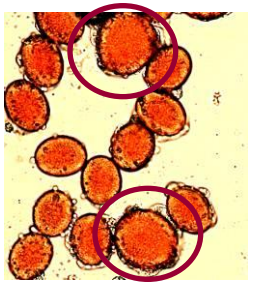
Paternal chromosome



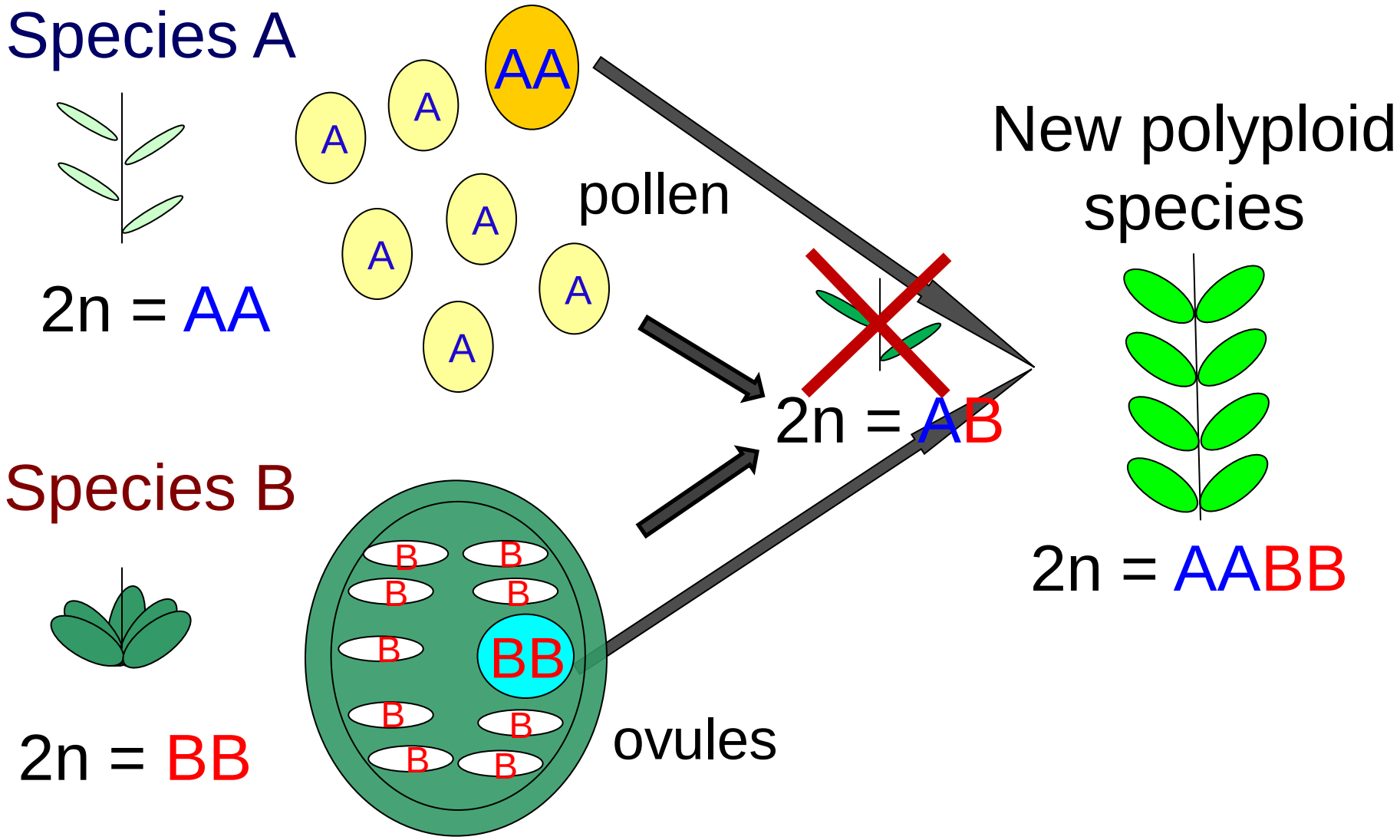
Unreduced gametes

"2n" DNA content

"2n" DNA content



Evolution of polyploid plants through union of unreduced gametes



U's Triangle of *Brassica*

B. rapa

B. juncea

B. napus

AA

AABB AACC

BB BBCC CC

B. nigra

B. carinata

B. oleracea

U's Triangle of *Brassica*

B. rapa

B. juncea

B. napus

AA

AABB x AACC

x x

BB → BBCC ← CC

B. nigra

B. carinata

B. oleracea

B. juncea

x

B. napus

x

B. carinata

x

Interspecific hybrids

~2400 bud pollinations

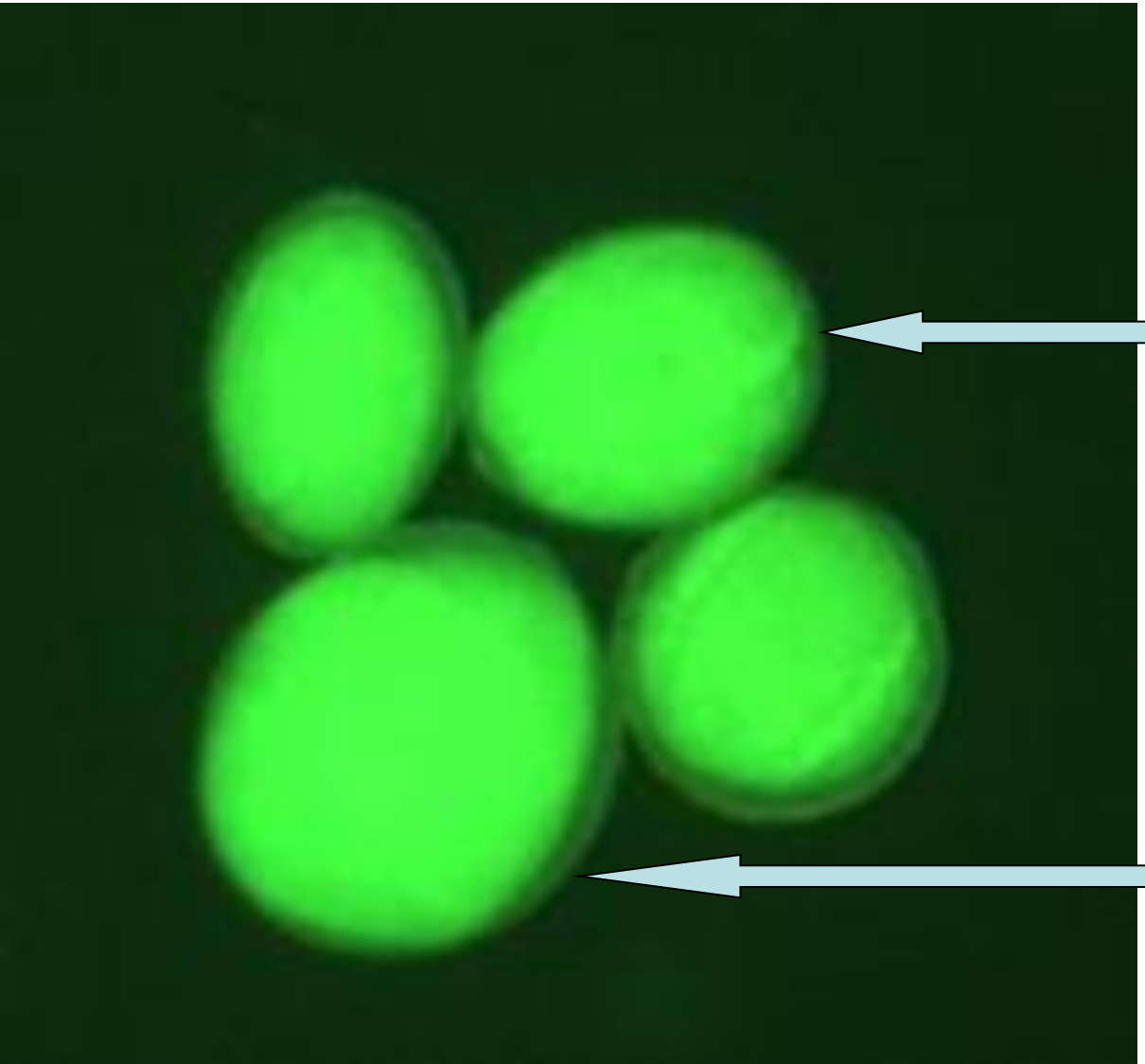
13 genotypes total

Unreduced gametes: hypotheses

- Do interspecific hybrid *Brassica* produce more unreduced gametes than their natural parents?
- Are there differences between *Brassica* genotypes in unreduced gamete production?
- Will unreduced gametes contribute to form higher ploidy progeny in interspecific crosses?
- Does temperature influence unreduced gamete production?

Estimating unreduced gamete production

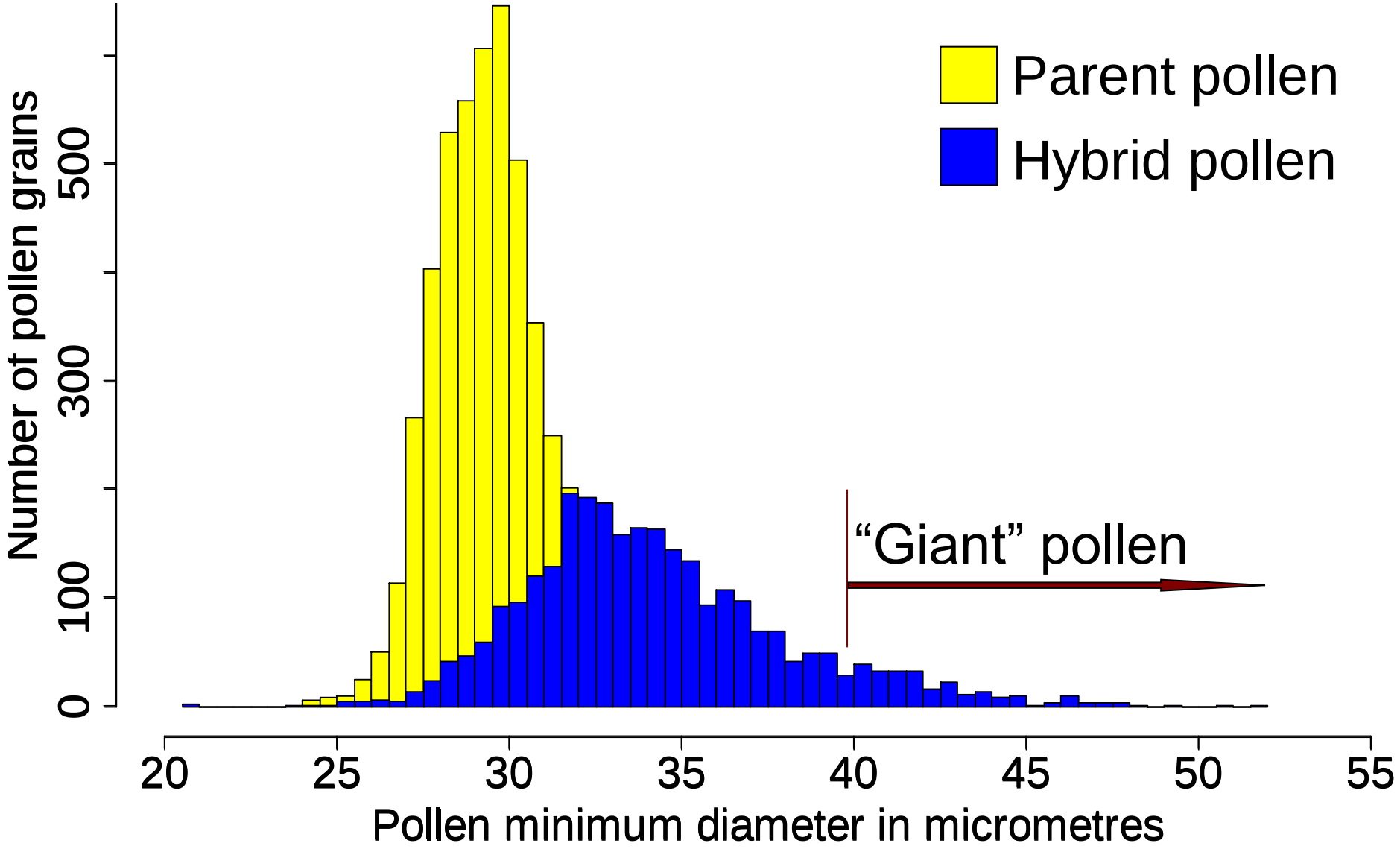
Method 1: Pollen measurements



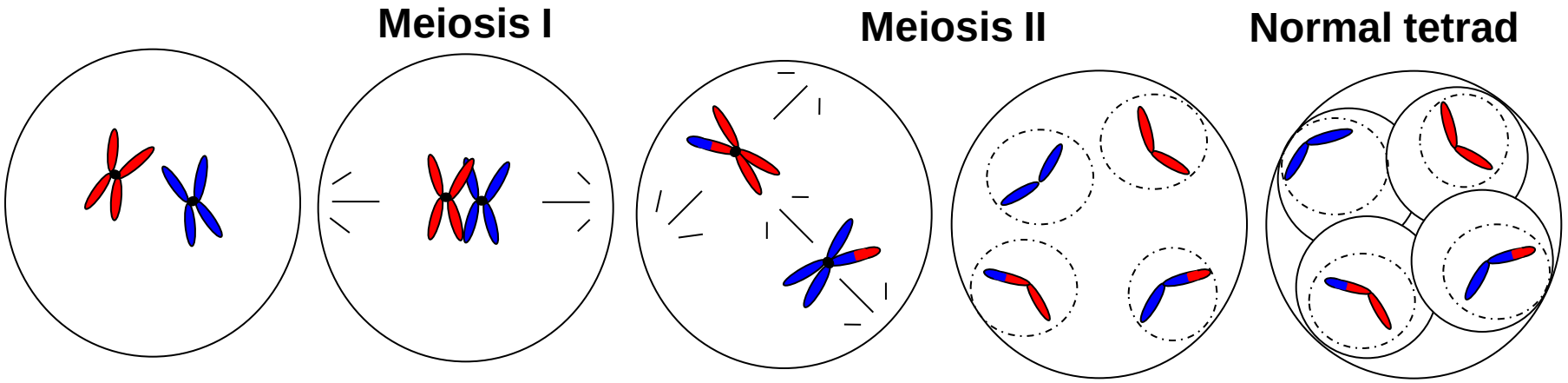
“Normal” viable pollen (reduced)

“Giant” viable pollen (unreduced)

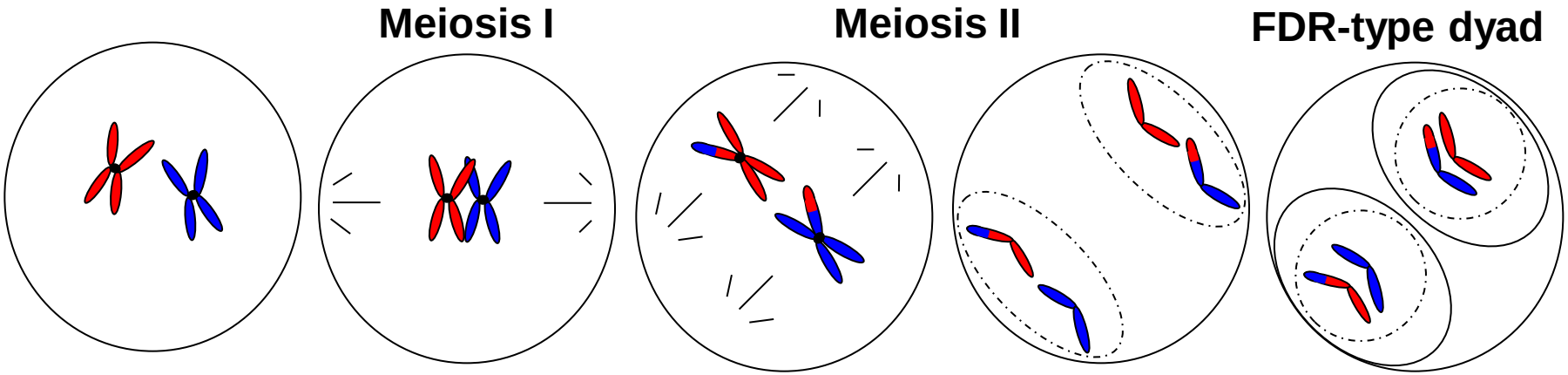
Hybrids produce high frequencies of viable unreduced pollen grains



Normal Meiosis



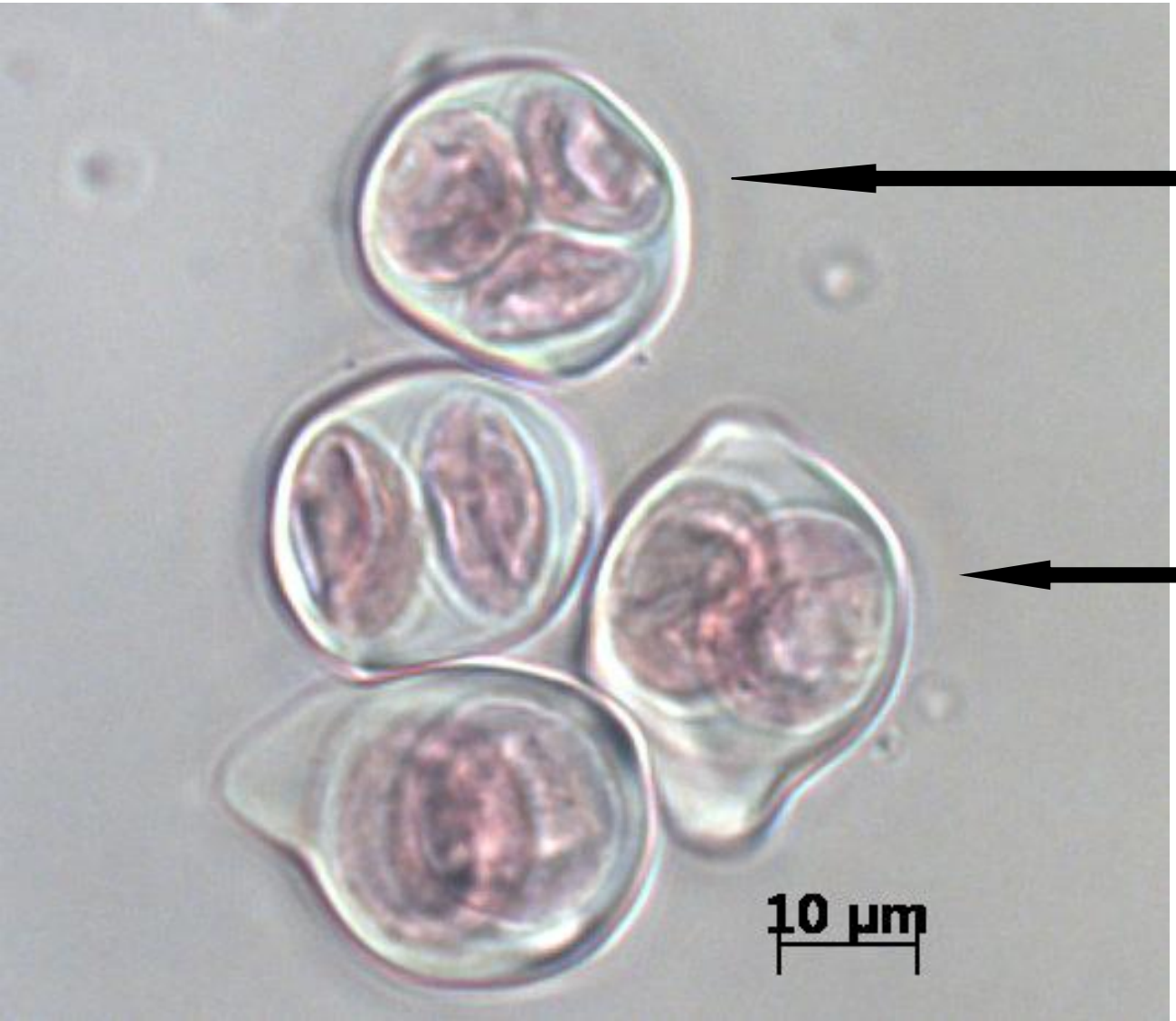
Parallel Spindles (a common means of 2n gamete formation)



Dicotyledonous plants

Estimating unreduced gamete production

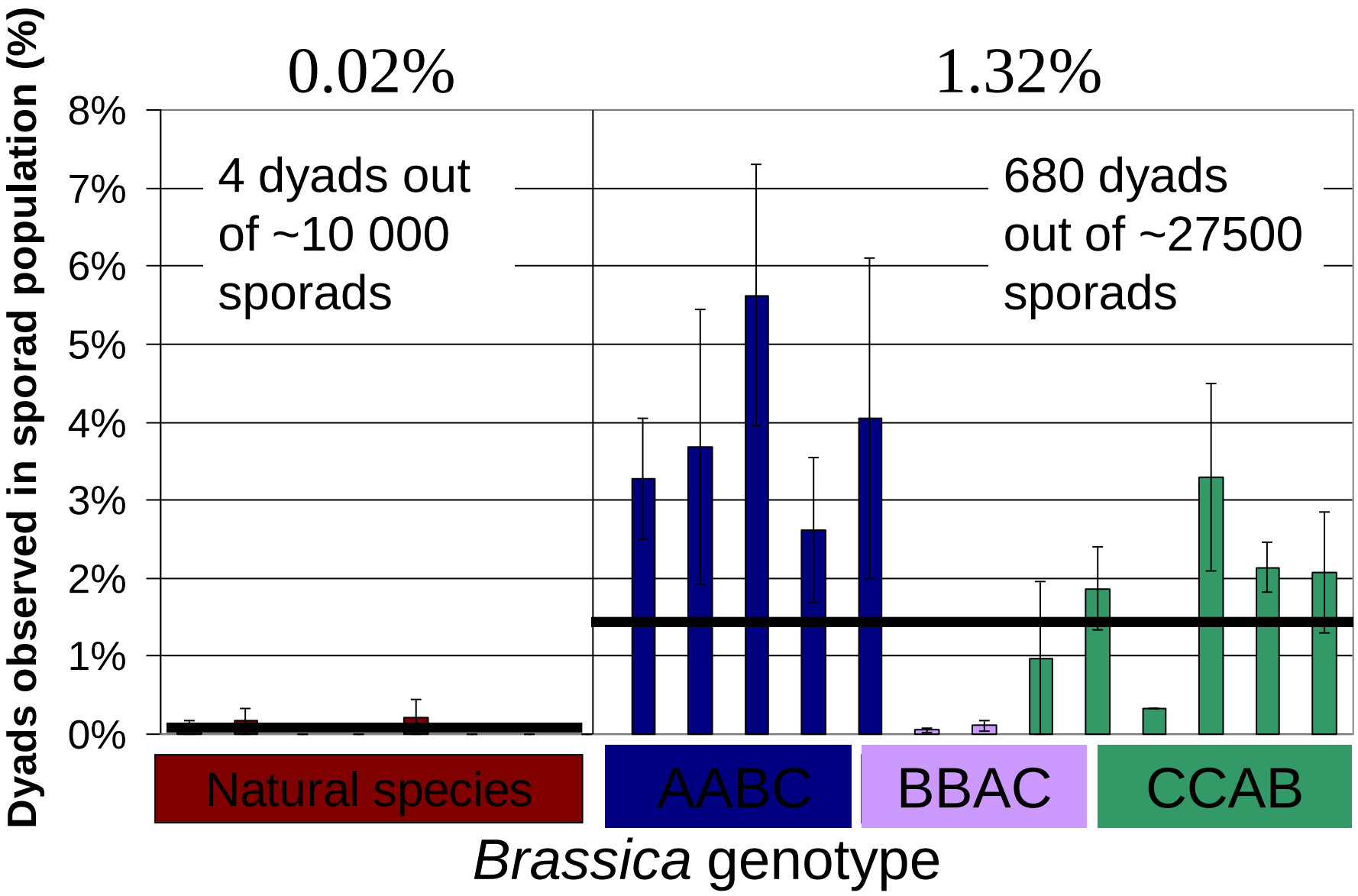
Method 2: Sporad observation



Tetrad
(4 nuclei)
reduced

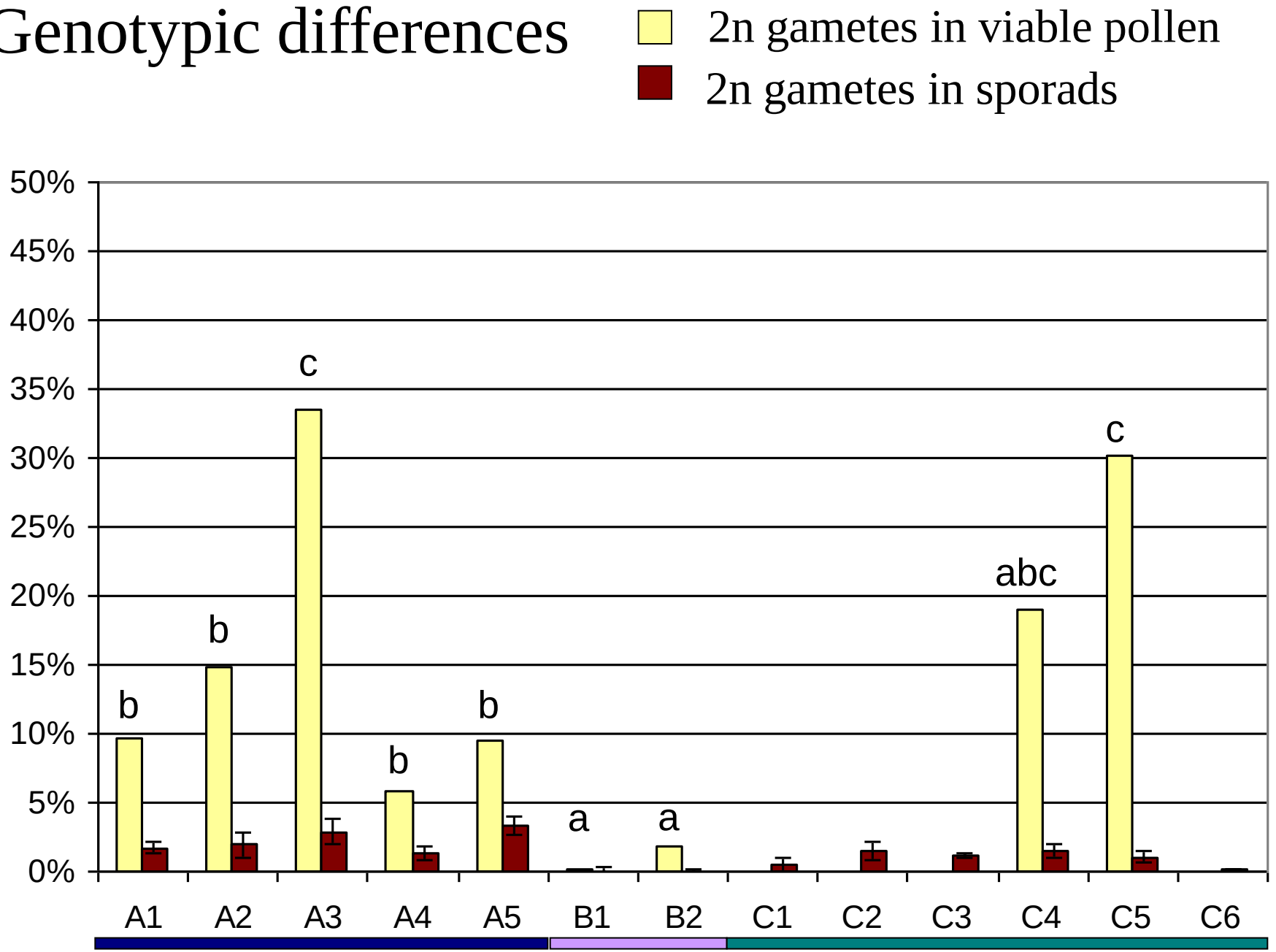
Dyads
(2 nuclei)
unreduced

Sporad results: interspecific hybrids produce more unreduced gametes than their parent species



Mason et al (2011) BMC Plant Biology 11:103

Genotypic differences



Mason et al (2011) BMC Plant Biology 11:103

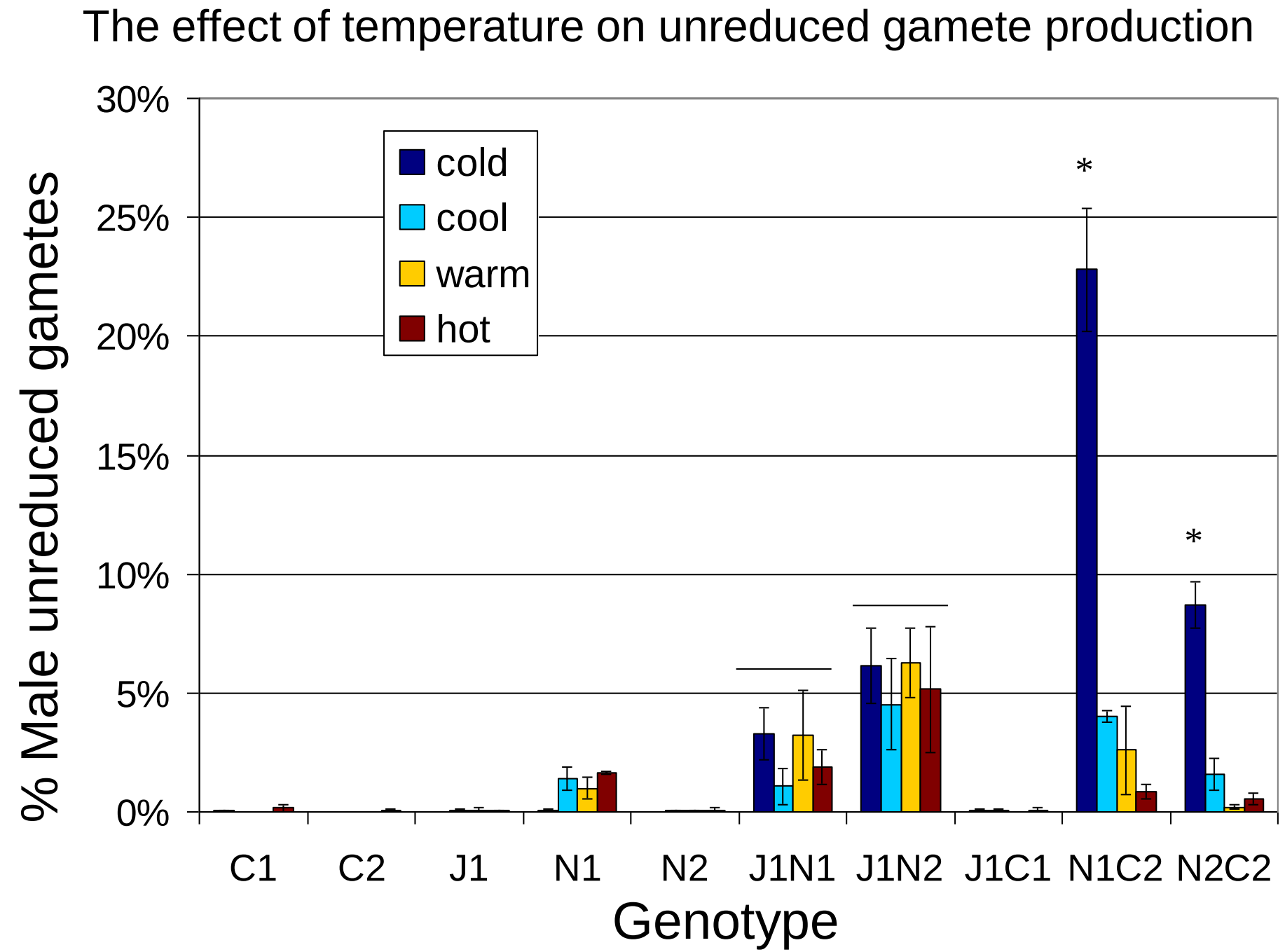
Does temperature affect unreduced gamete production?

Temperature treatments – four controlled environment rooms, night/day temperature

- "cold" – 5°C / 10°C
- "cool" – 13°C / 18°C
- "warm" – 15°C / 25°C
- "hot" – 25°C / 30°C

- Five genotypes (*B. napus*, *B. juncea*, *B. carinata*) and five of their interspecific hybrids
- Two plants x 10 genotypes in each room
- 2 x 300 sporad counts per plant

Mason et al (2011) BMC Plant Biology 11:103



Mason et al (2011) BMC Plant Biology 11:103

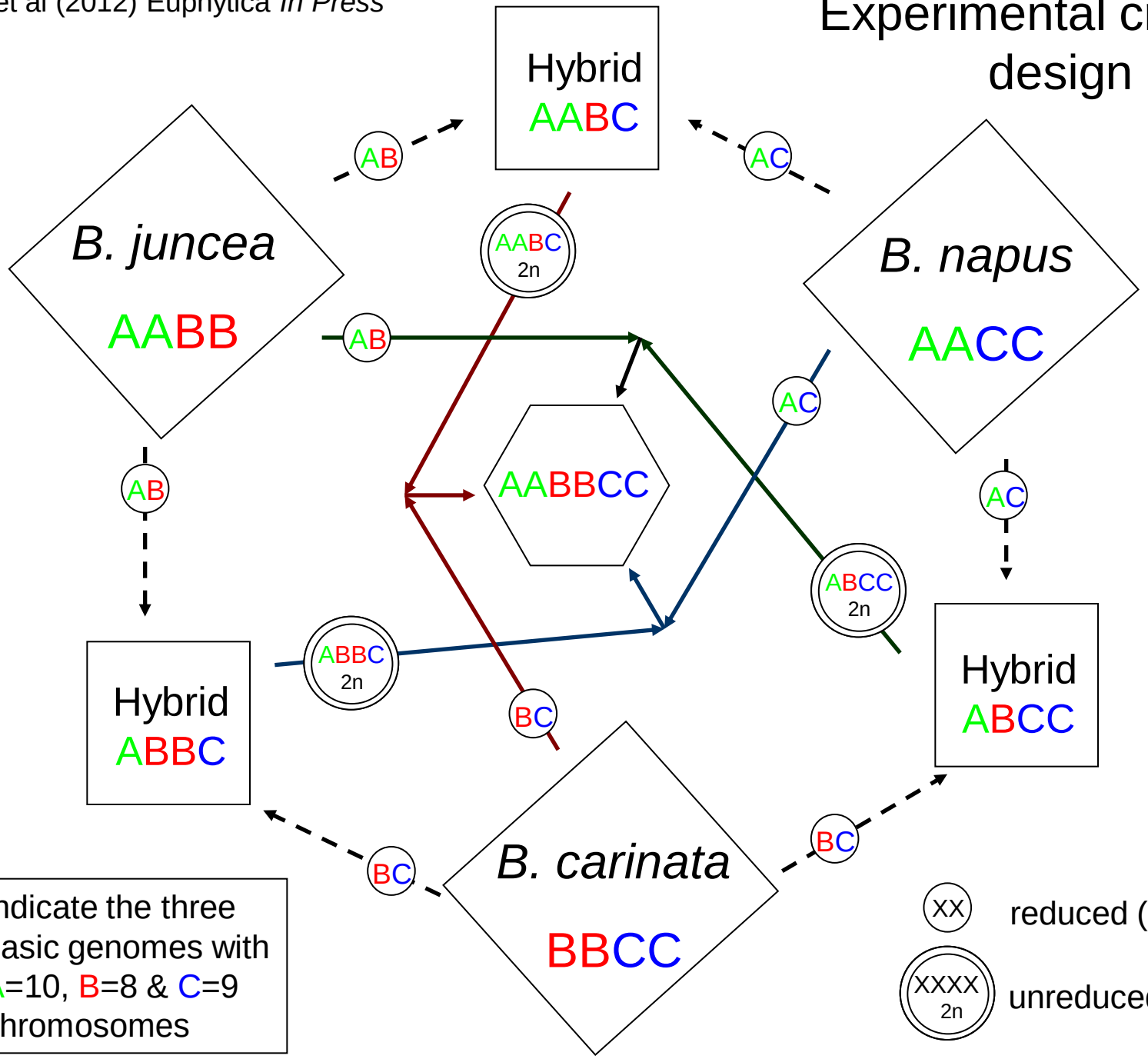
Allohexaploid *Brassica*

B. rapa AA	*B. nigra* BB	*B. oleracea* CC	Diploid $2n = 2x$
B. juncea AABB	*B. napus* AACC	*B. carinata* BBCC	Tetraploid $2n = 4x$
	AABBCC?		Hexaploid $2n = 6x$

Can we use unreduced gametes to produce this hexaploid from the tetraploid species?

Mason et al (2012) Euphytica *In Press*

Experimental crossing design



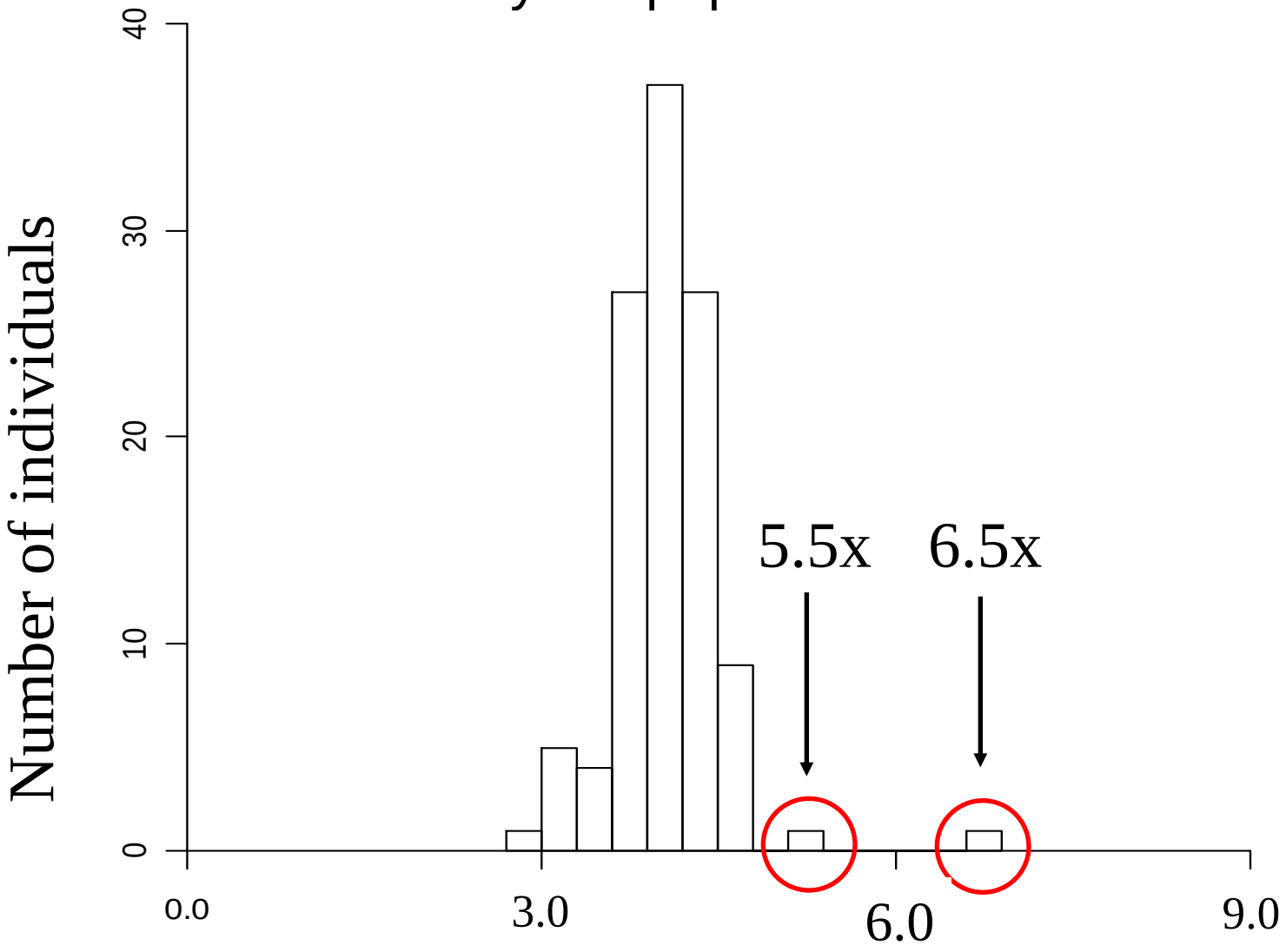
AA
BB
CC

Indicate the three basic genomes with A=10, B=8 & C=9 chromosomes

XX reduced (n) gamete

XXXX 2n unreduced (2n) gamete

DNA content (estimated by flow cytometry) for second cross hybrid population



Relative DNA content: ~AABBCC = 6.0x

Approximate ploidy level of progeny

Cross	Plants	
AABC x BBCC	46	All ~4x
BBAC x AACC	64	All ~4x
CCAB x AABB	2	**1 plant ~5.5x** **1 plant ~6.5x**

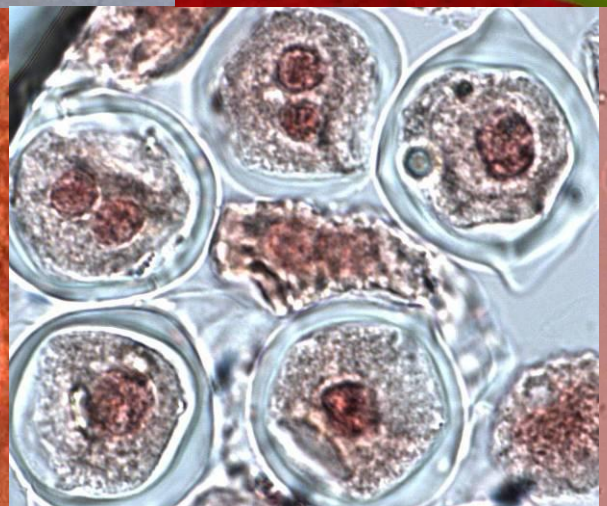
Mason et al (2012) Euphytica *In Press*

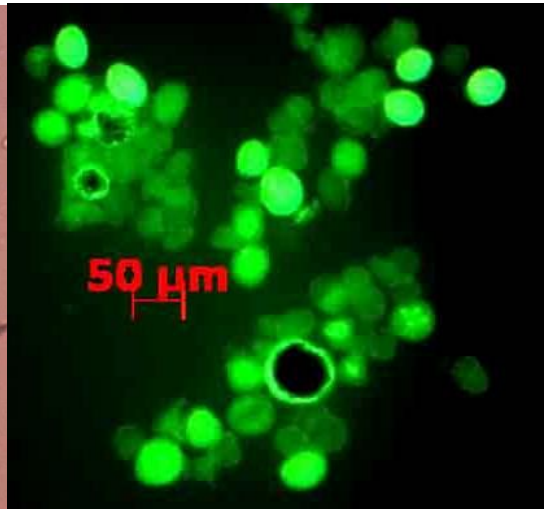
Phenotype of “~6.5x” plant

Pollen viability: **0-22%**

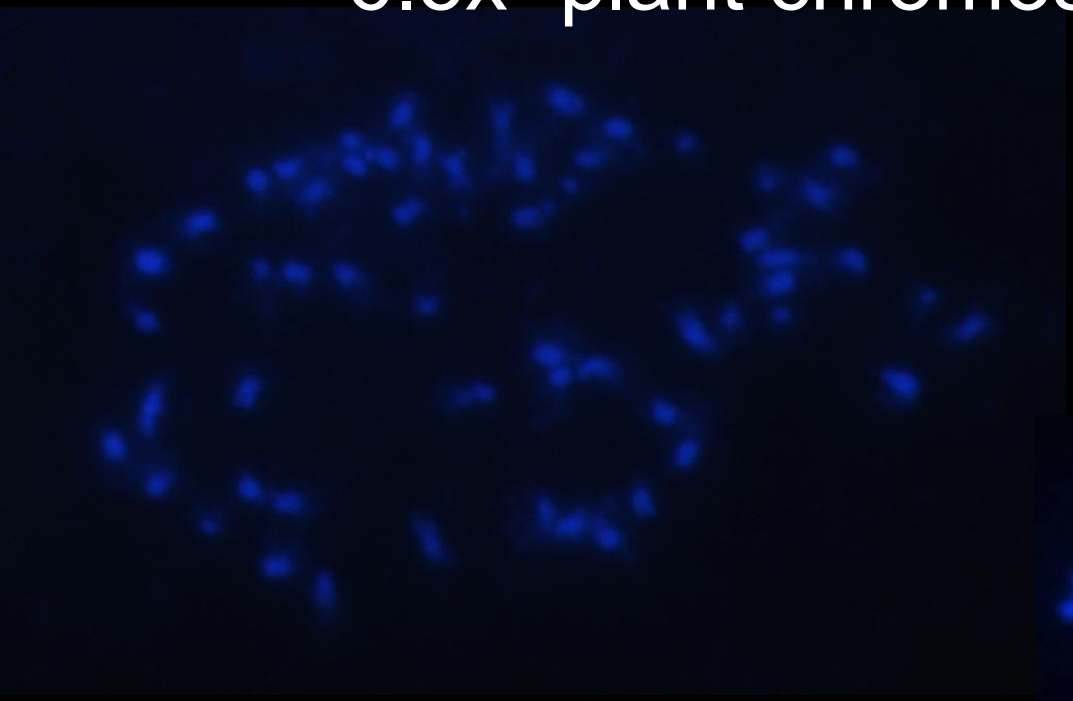
Self seed set: **0**

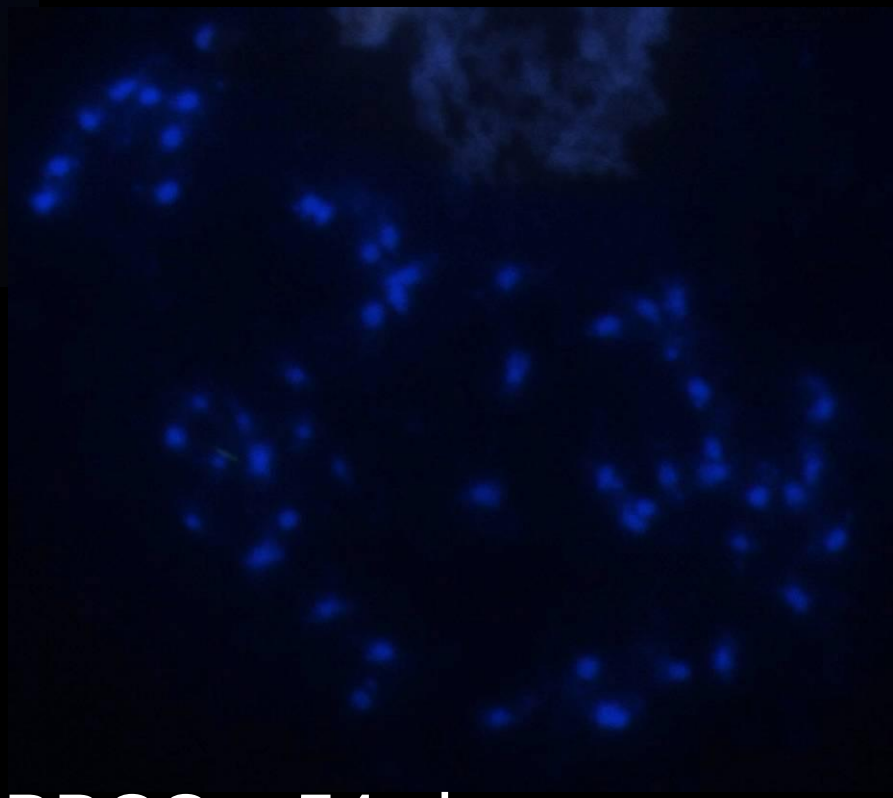
OP seed set: **13**





“~6.5x” plant chromosome spreads





2n ~ 58

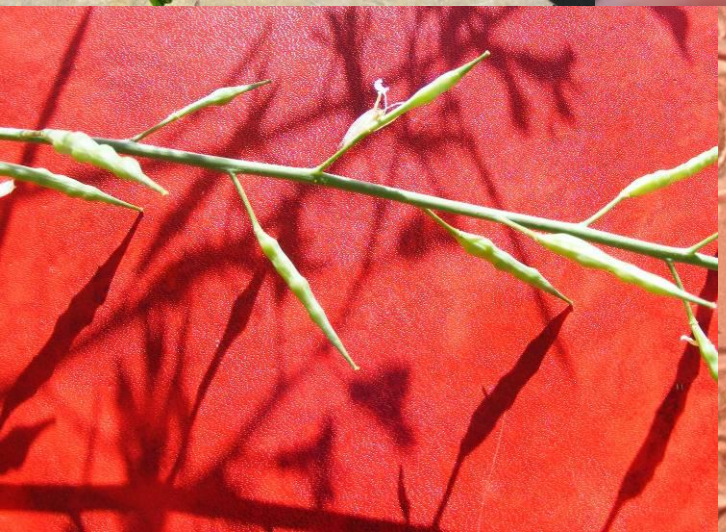
Expected AABBCC = 54 chromosomes

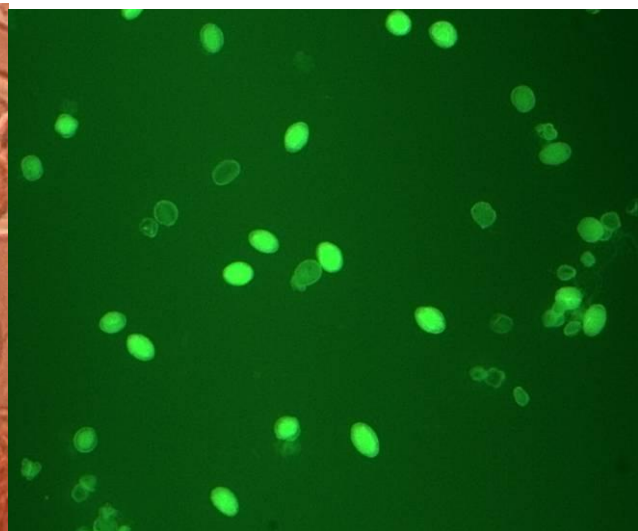
Phenotype of “5.5x” plant

Pollen viability: **25%**

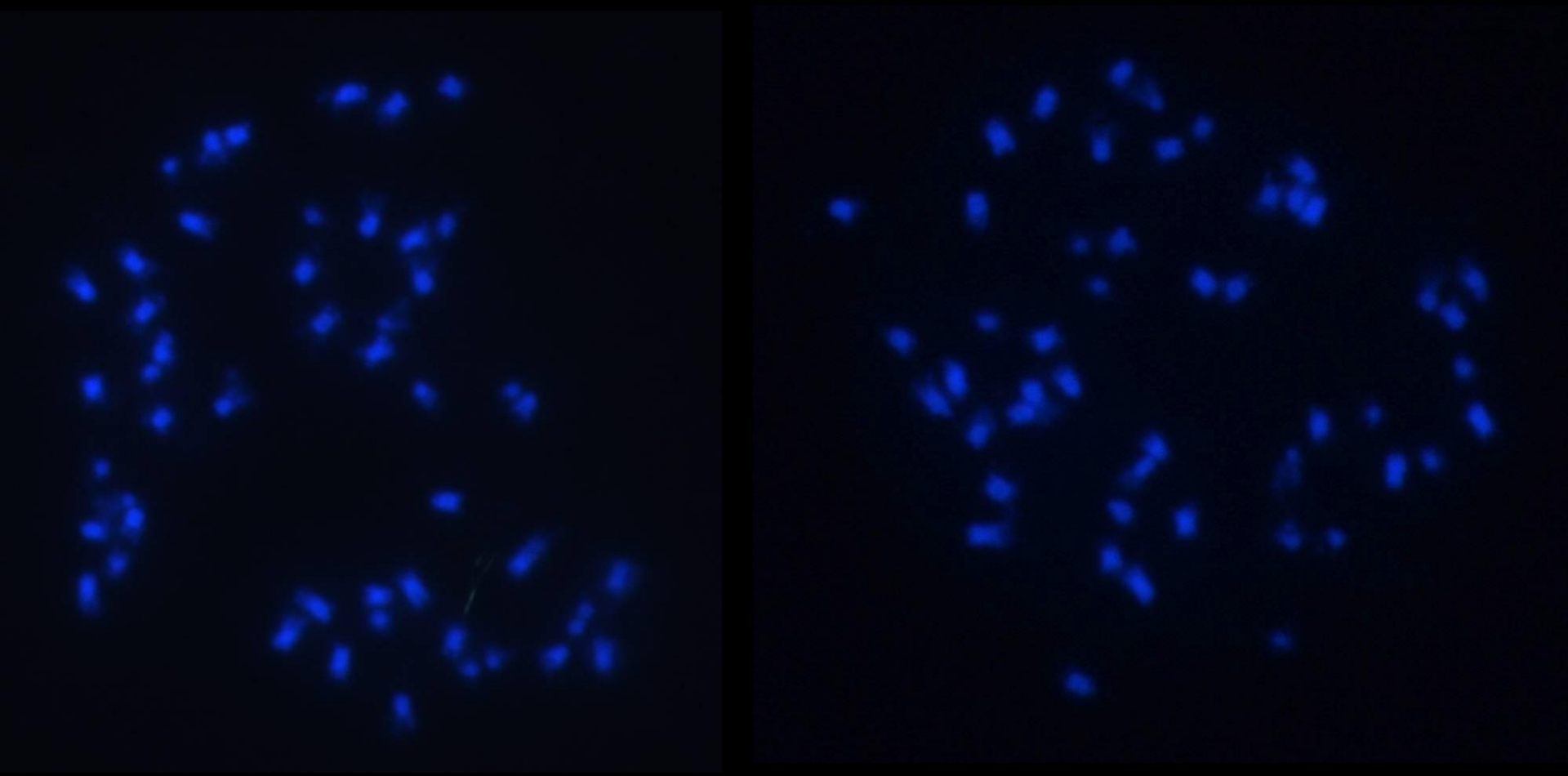
Self seed set: **166**

OP seed set: **458**





“5x” plant chromosome spreads



2n ~ 50

Expected AABBCC = 54 chromosomes

Molecular marker results

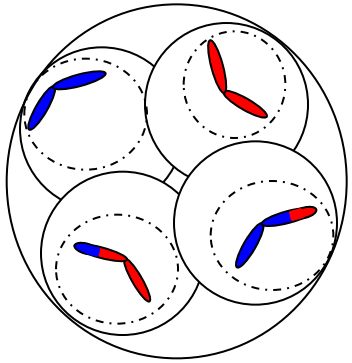
6.5x plant

A^jB^j from *B. juncea* + 2(0.5A + 0.5B + $C^{n\ or\ c}$)

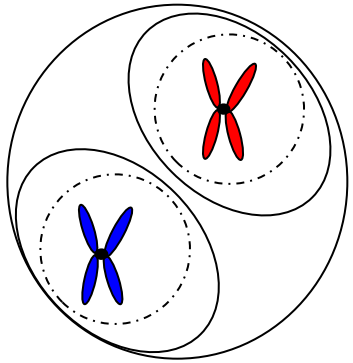
from $C^nC^cA^nB^c$ hybrid

Explanation: Meiosis II failed to separate sister chromatids

Normal tetrad



SDR-type dyad



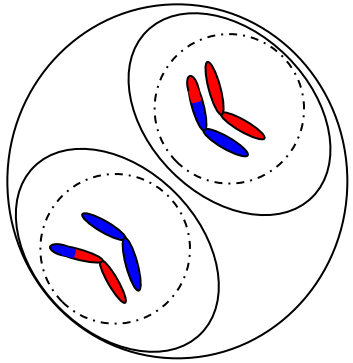
5.5x plant

A^jB^j from *B. juncea* + $C^{n/c}C^{n/c}A^nB^c$ from $C^nC^cA^nB^c$ hybrid (minus a few chromosomes)

= $A^jA^nB^jB^cC^nC^c$

Explanation: Meiosis I failed to separate homologous chromosomes

FDR-type dyad



Unreduced gametes: conclusions

- Interspecific hybrid *Brassica* produce more unreduced gametes than their natural parents
- Genotype influences unreduced gamete production
- Unreduced gametes can be used to produce higher ploidy progeny in interspecific crosses, depending on genotype
- Cold ambient temperature increases unreduced gamete production in some genotypes

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