

# ***Brassica* C Genome Centromere Locations Revealed Through Half-Tetrad Analysis**

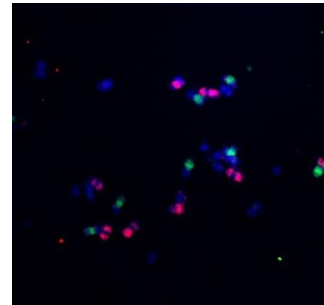
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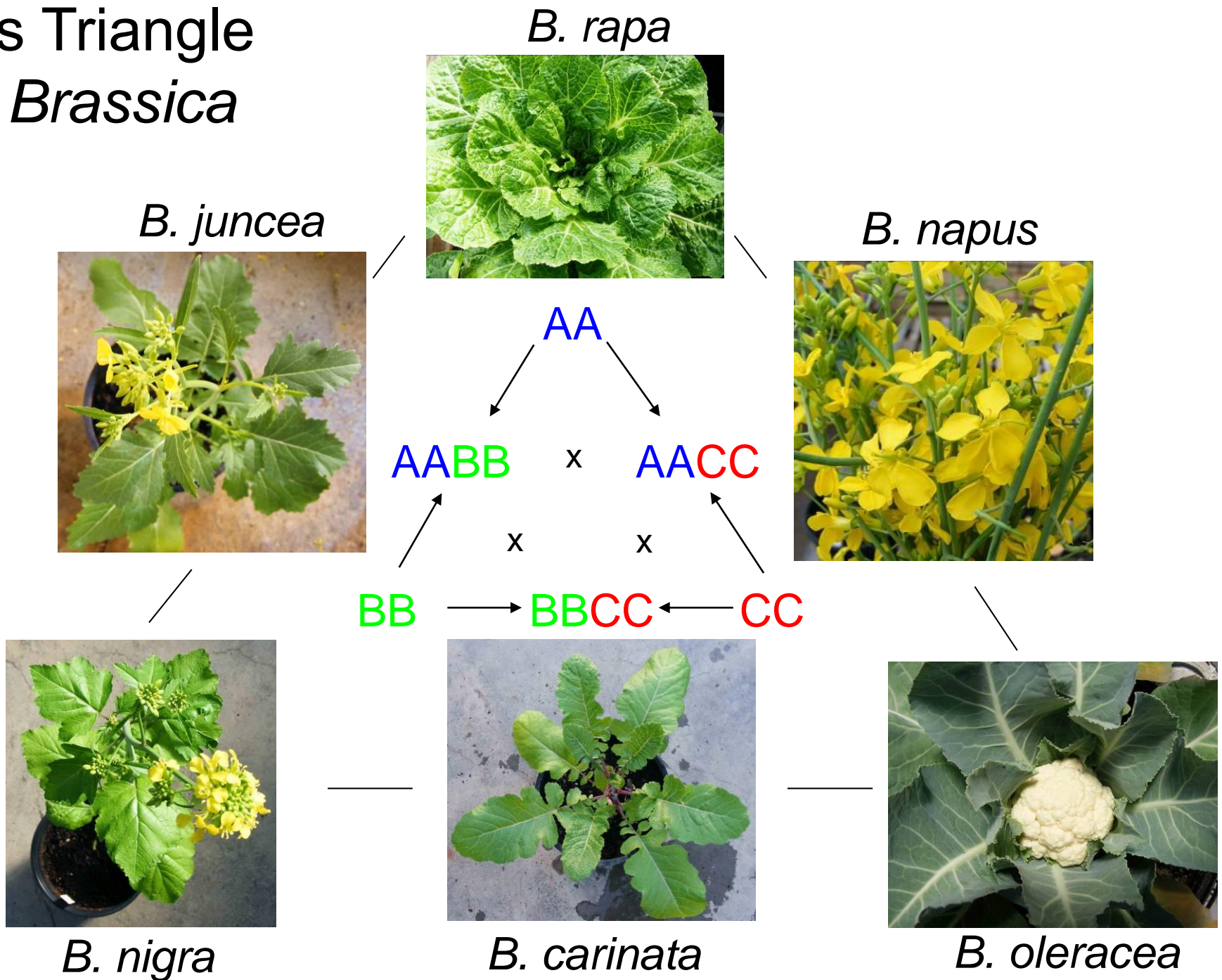


# Outline



- Some unexpected results
- What is half-tetrad analysis?
- Genotyping using the Illumina 60K SNP chip
- Centromere positions
- Utility of this approach for other species

# U's Triangle of *Brassica*





X



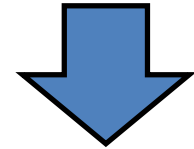
*B. napus*

*B. carinata*



CCAB  
hybrids

Molecular  
marker analysis



Microspores  
(male gametes)



Embryos



Plants



Derivation of experimental progeny through microspore culture

# Results: unreduced gametes!

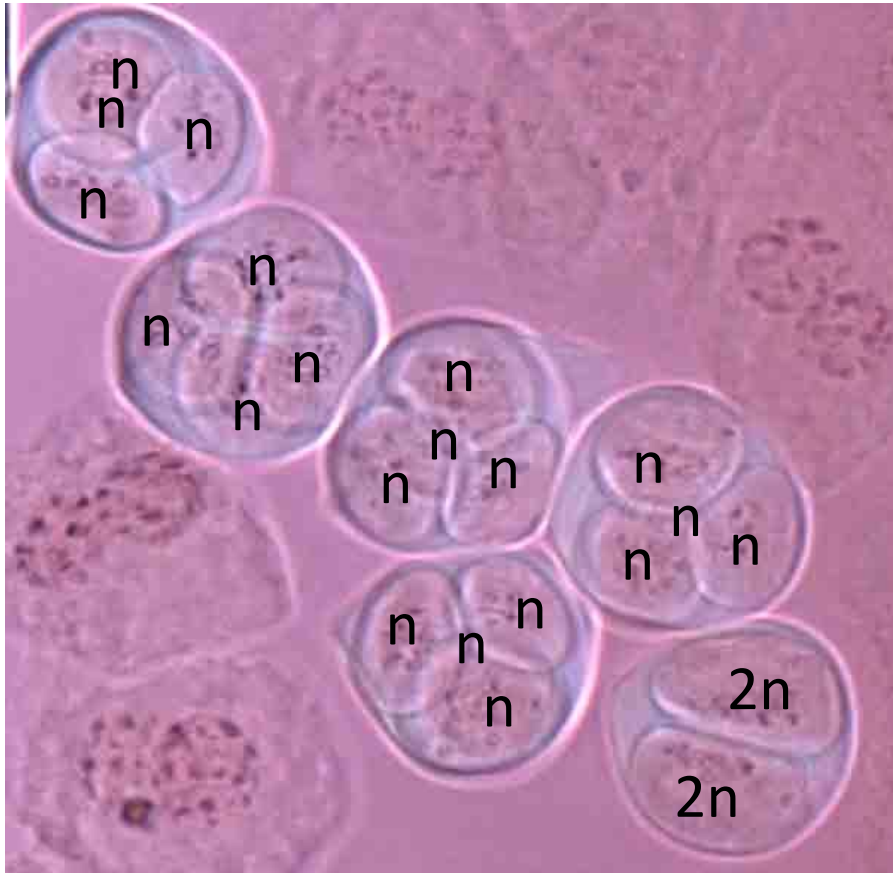
- Of 84 progeny derived from microspore culture of *B. napus* x *B. carinata* hybrids...
  - 13 were from reduced gametes
  - 71 were from unreduced gametes!
    - Additionally, 22 of these were clones...



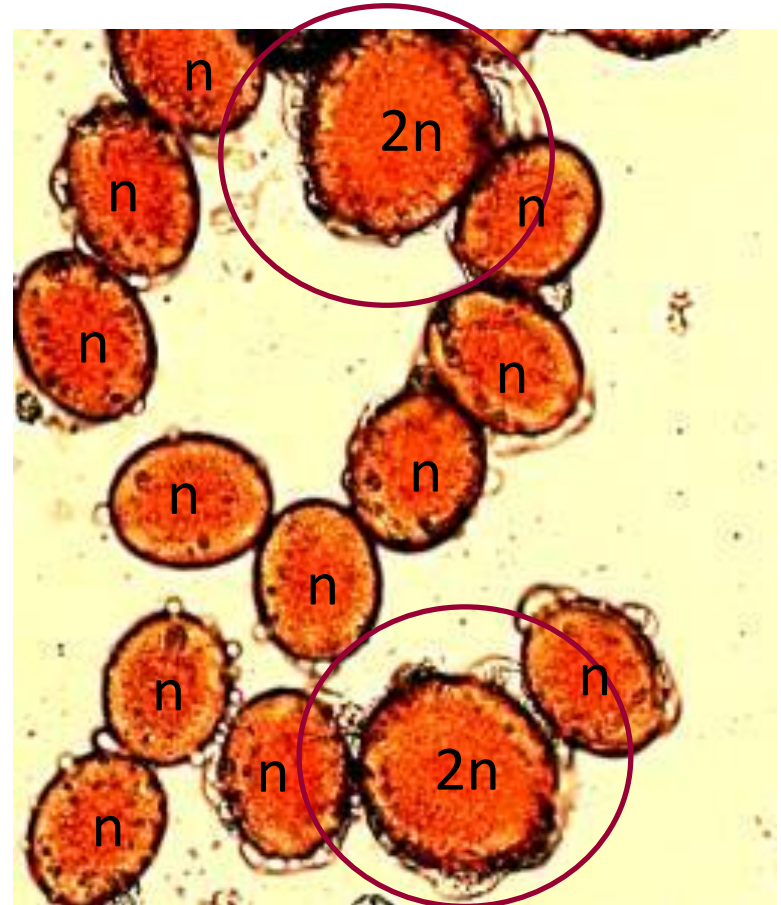
Cousin and Nelson (2009) *Plant Cell Reports* 28:831-835

Mason et al. (2011) *Theor Appl Genet* 122:543-553

# Unreduced gametes

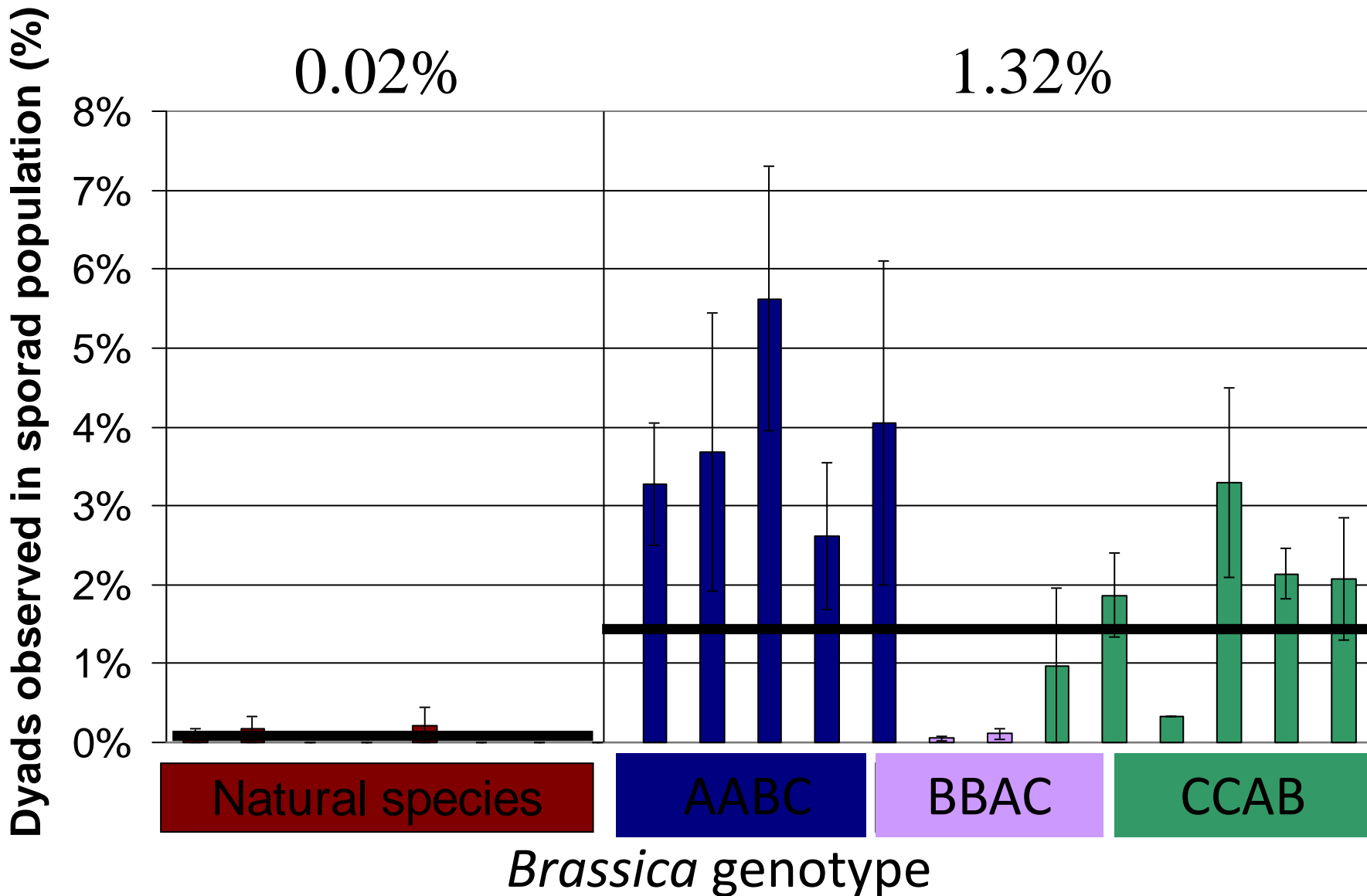


Sporads in *B. napus*: one dyad (2 x unreduced nuclei) and five tetrads (4 x reduced nuclei)



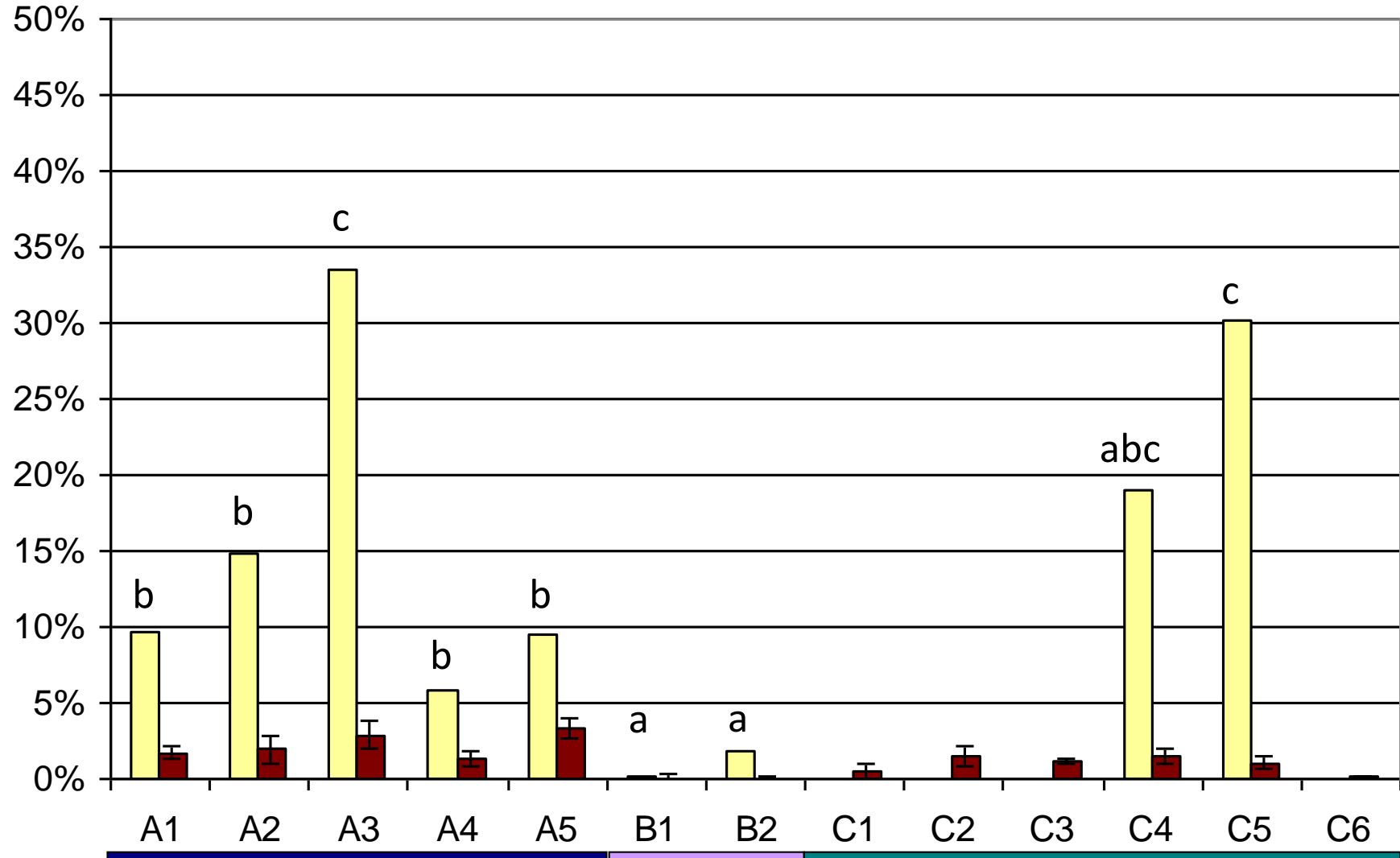
Pollen grains in *B. napus*: two giant pollen (unreduced) are circled

# Interspecific hybrids produce more unreduced gametes than their parent species



# Genotypic differences

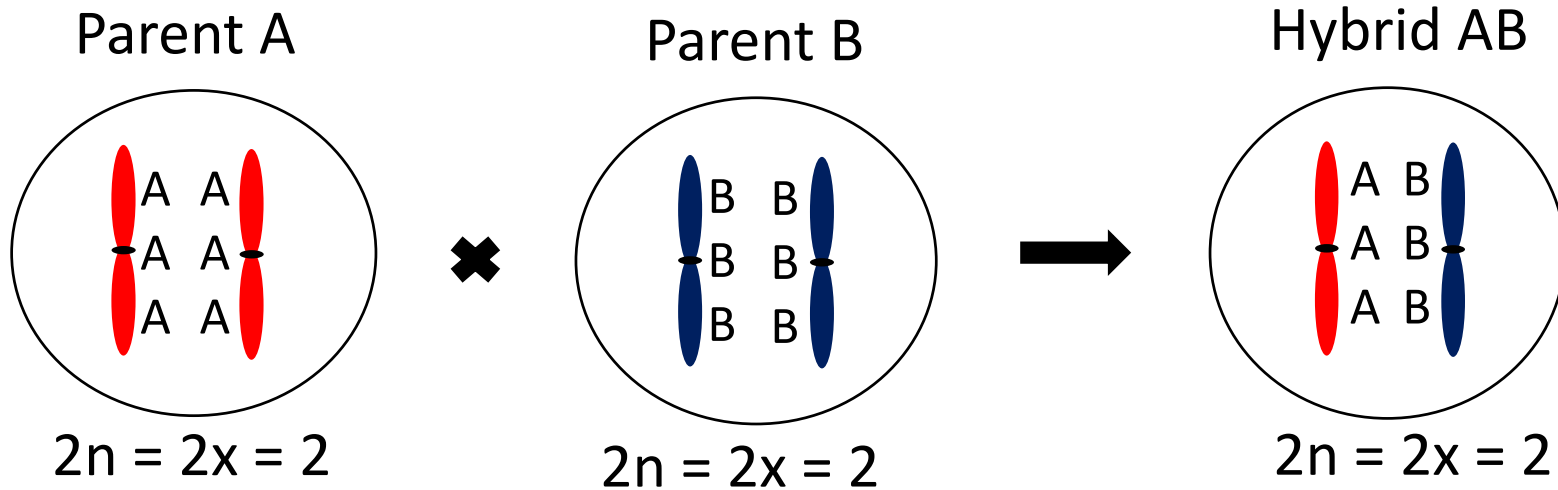
- 2n gametes in viable pollen
- 2n gametes in sporads



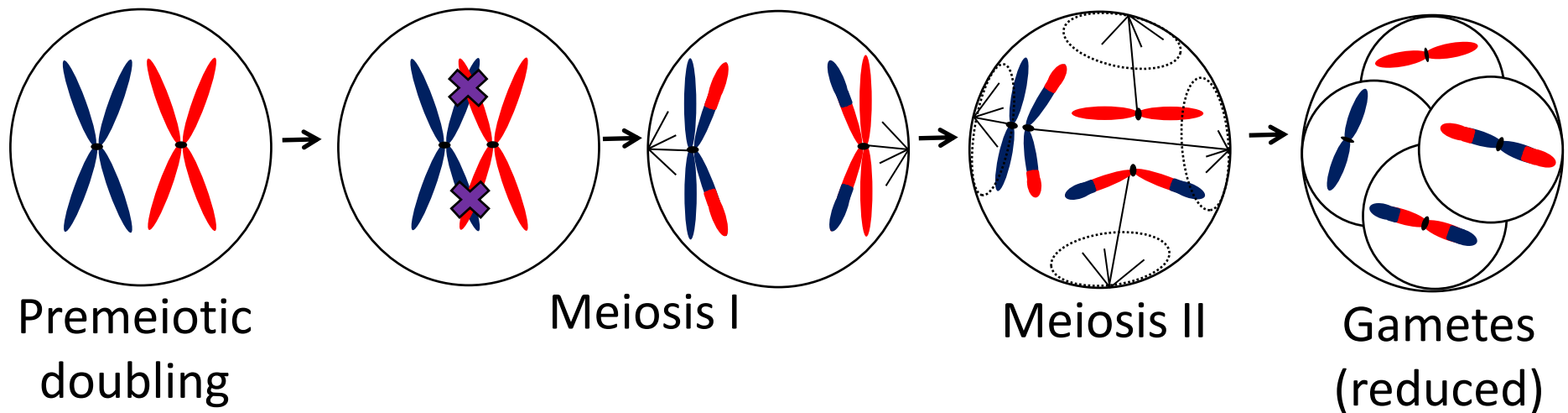
Hybrid genotype



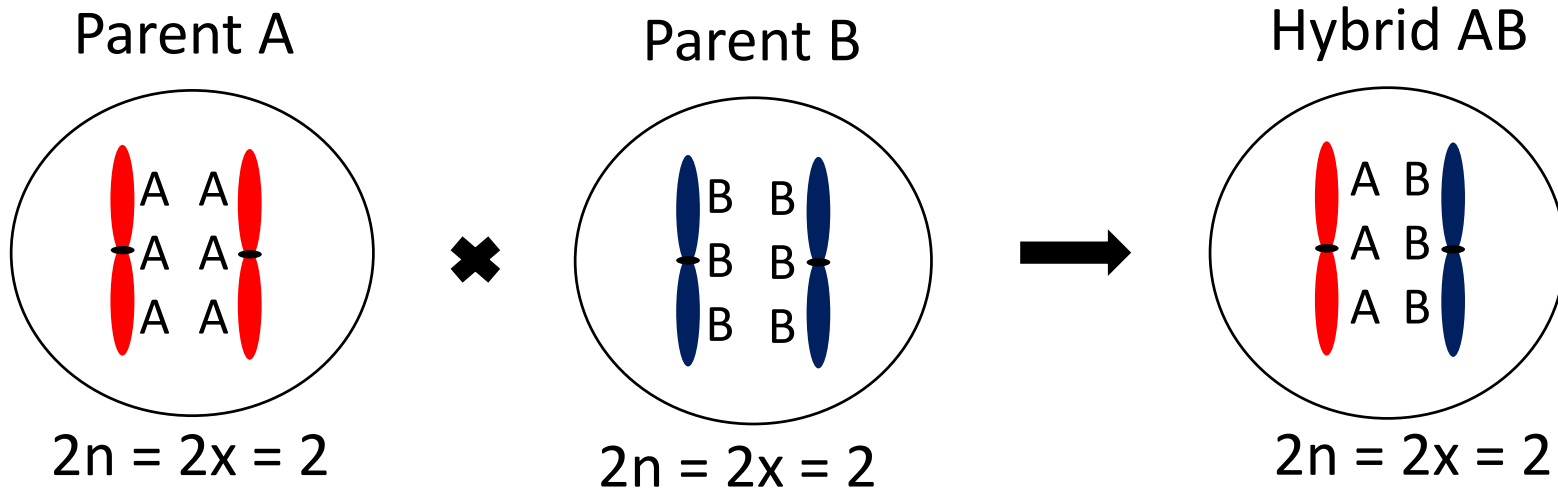
# Production of a hybrid heterozygous for parent alleles



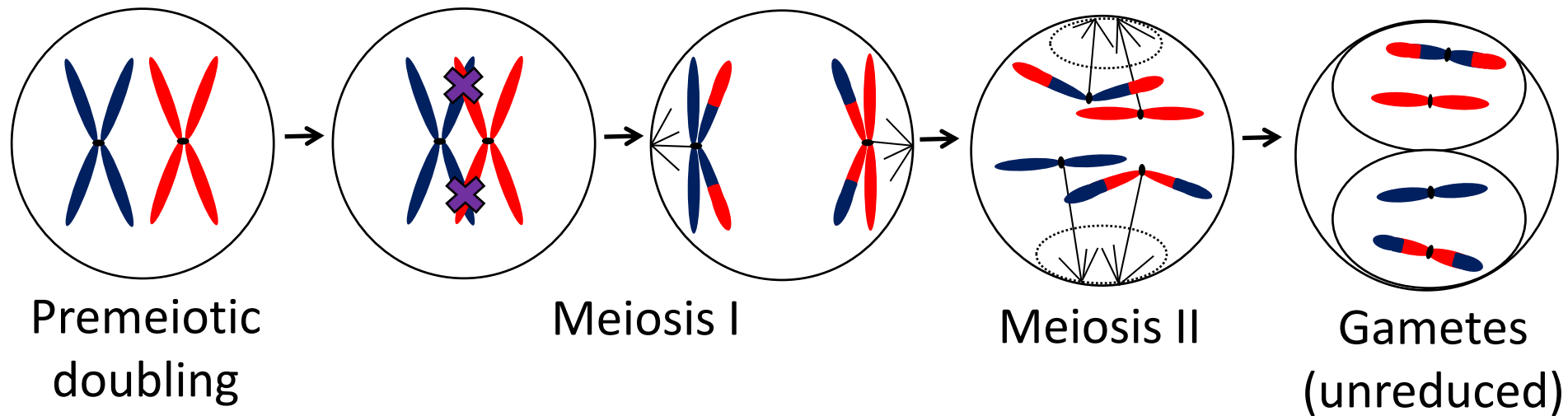
## Normal meiosis in Hybrid AB



# Production of a hybrid heterozygous for parent alleles



## Unreduced gamete formation (parallel spindles with crossovers)



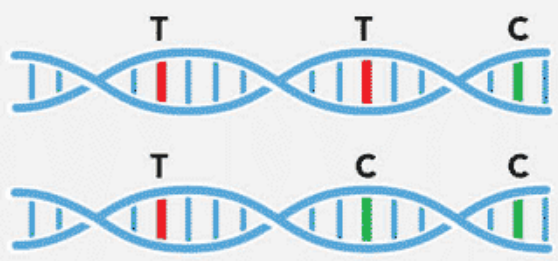


# Half-tetrad analysis

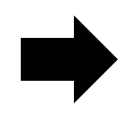
- Analysis of half of the meiotic tetrad – derived from the text book example of tetrad analysis in fungi
- Presence of half the meiotic products in one cell allows significantly more inference about meiotic behaviour in the parent
  - Many examples in *Arabidopsis*
- Genetic analysis using unreduced gametes!

# Brassica 60K SNP array now available

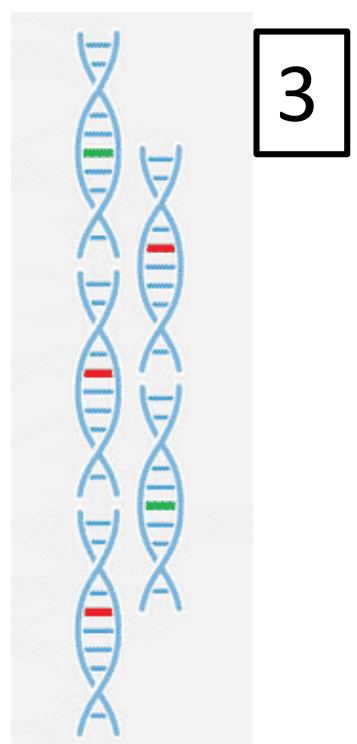
Infinium HD Assay 1



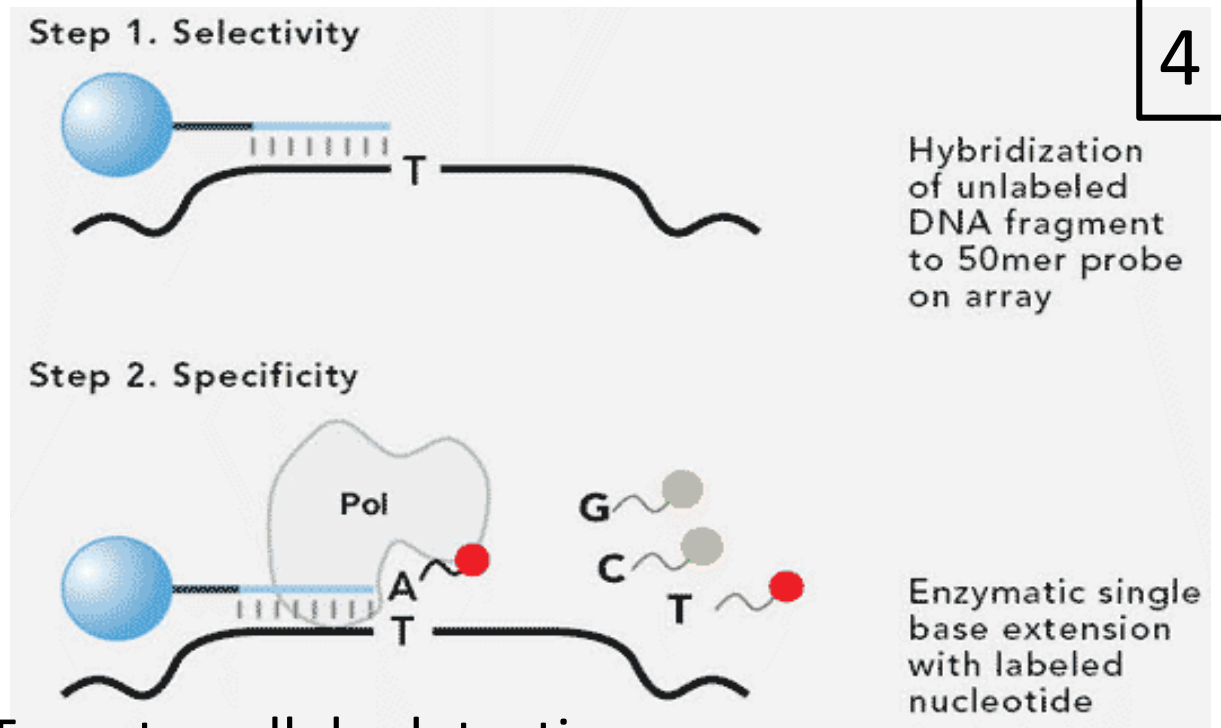
Genomic DNA (200-400 ng)



PCR-free whole-genome amplification



Fragmentation



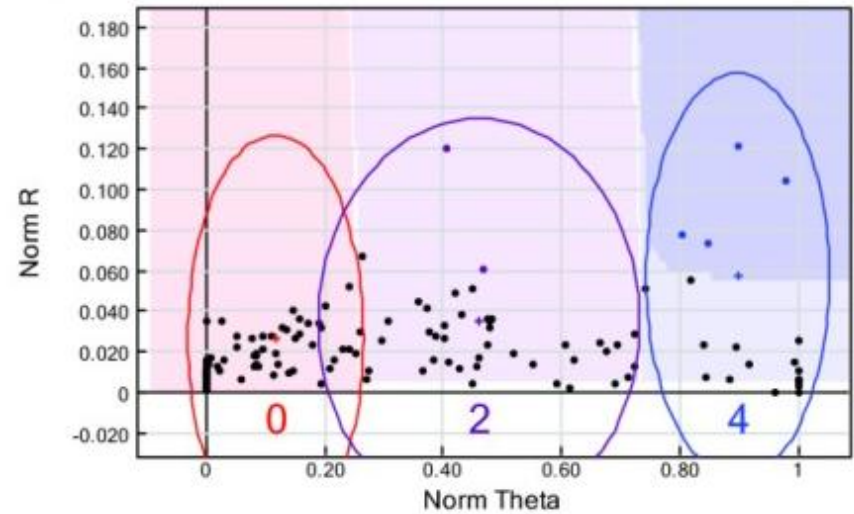
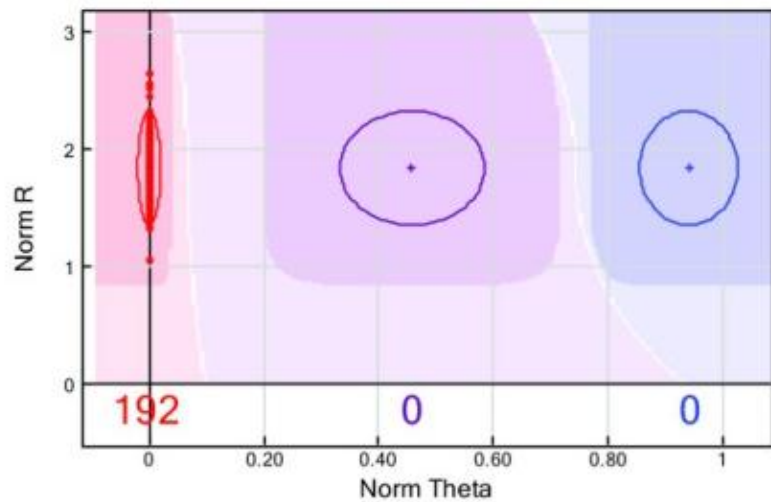
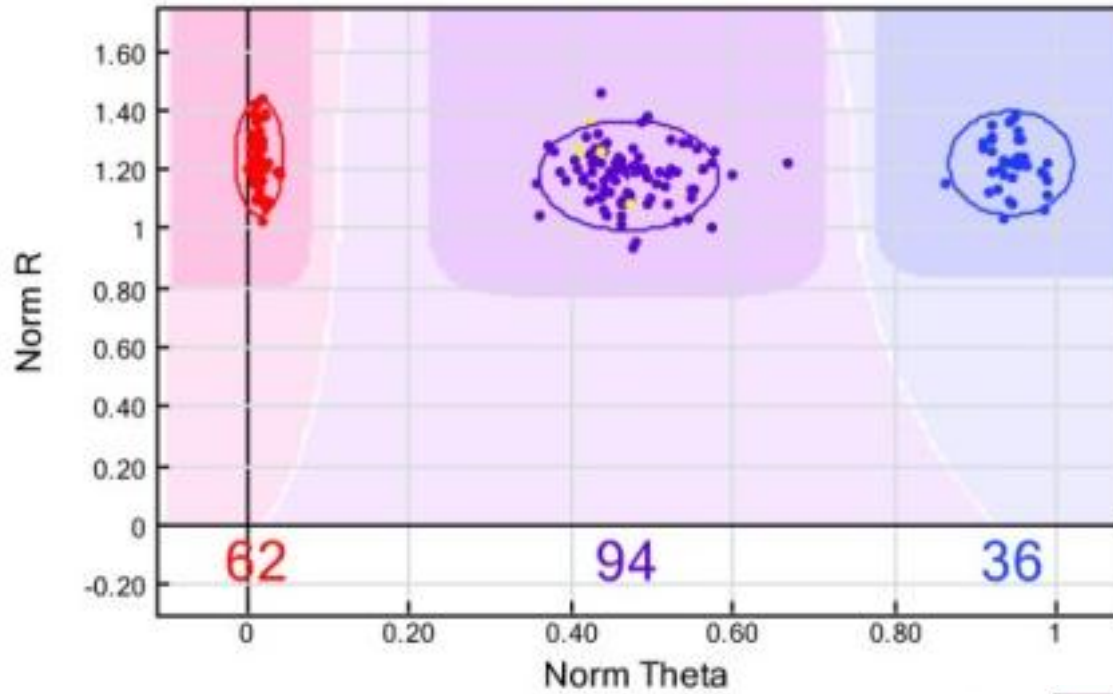
Two-step allele detection



AA  
(e.g. A/A)

AB  
(e.g. A/C)

BB  
(e.g. C/C)



# SNP data analysis workflow

52 157 SNPs



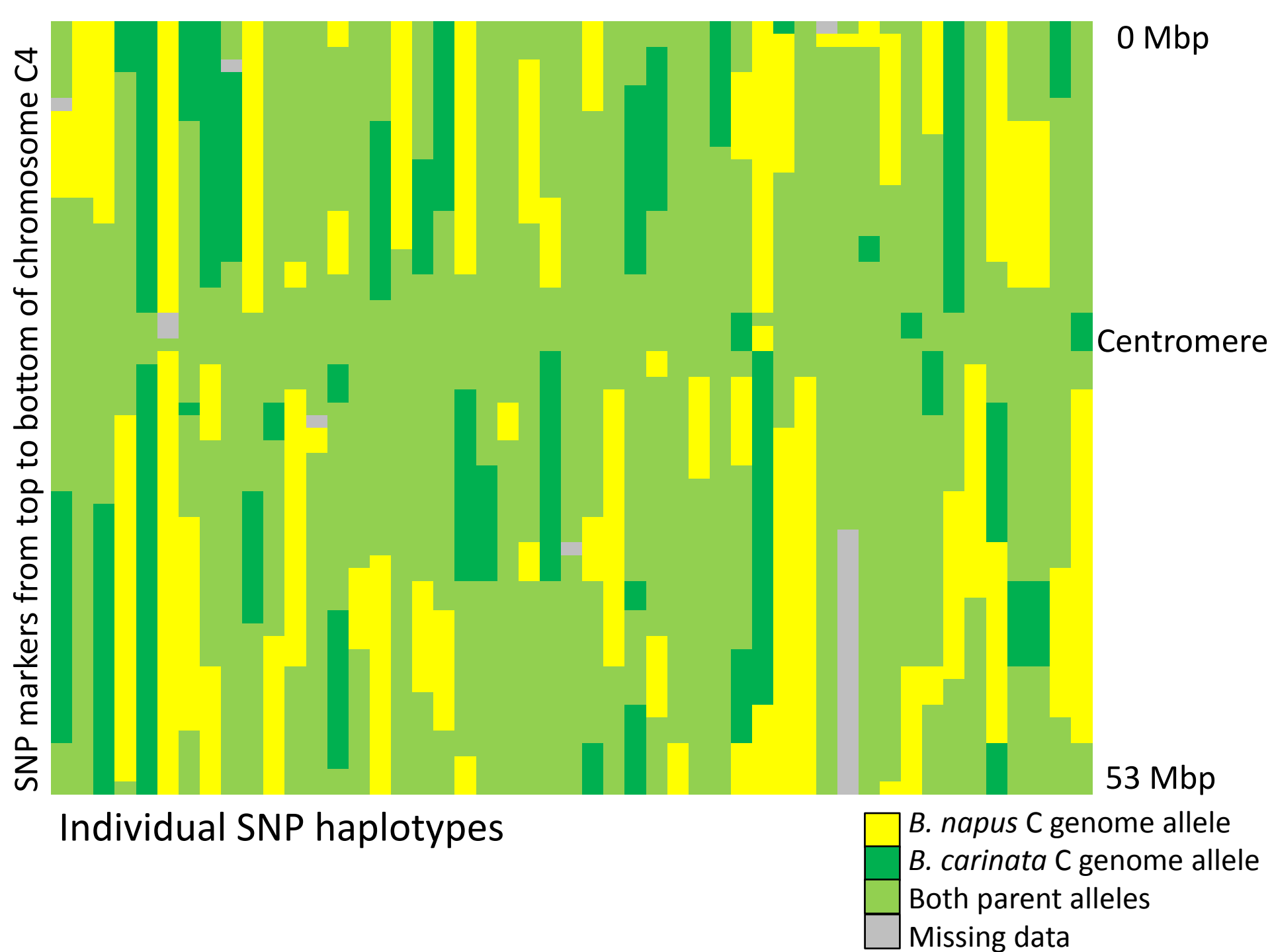
Sort for SNPs heterozygous between the two parent genotypes in the C genome (generally 5 – 10 000 SNPs)



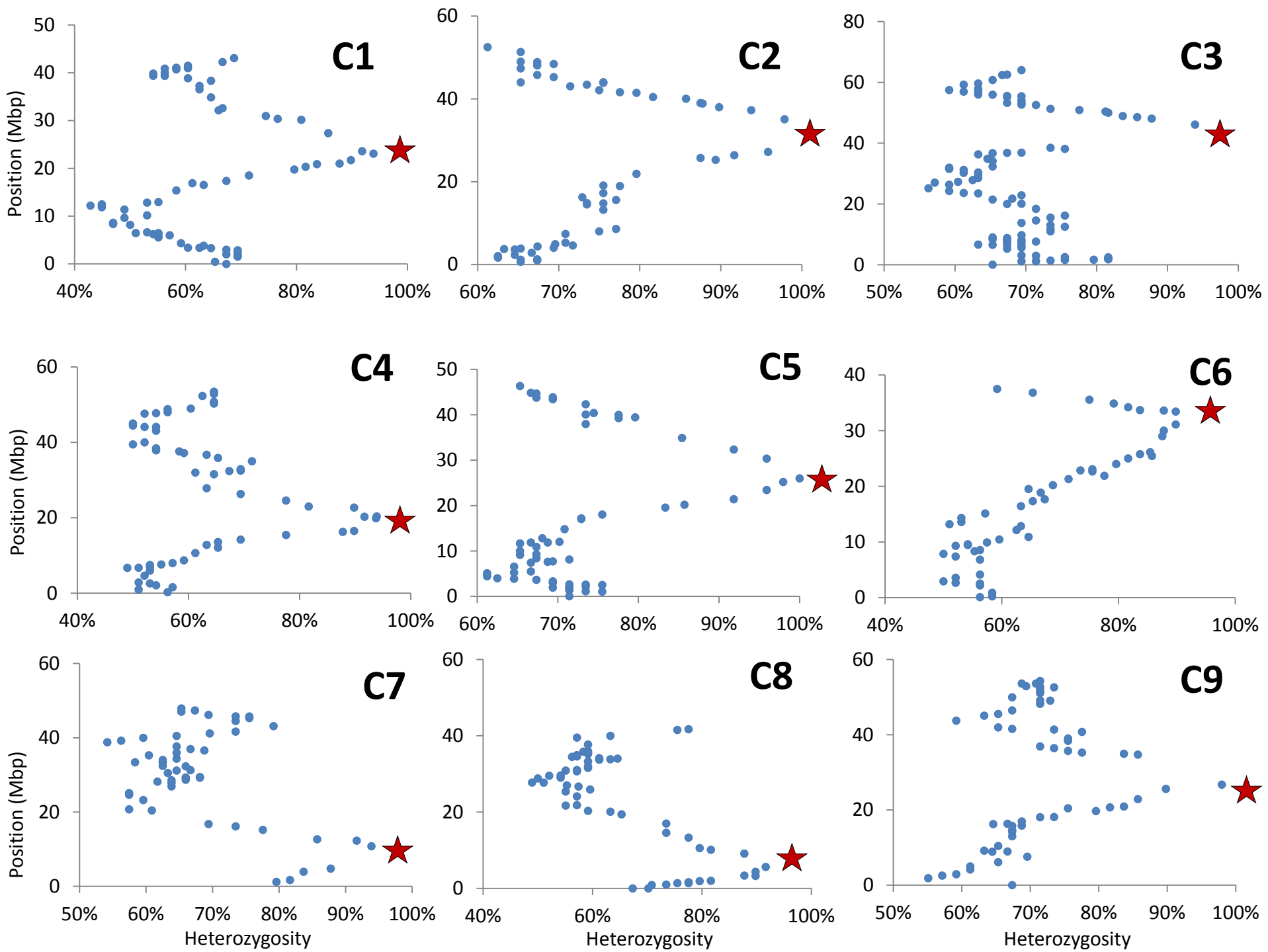
Data cleaning: remove dodgy SNPs (amplifying two loci, high proportion missing values etc.)



Run custom script in R to condense SNP marker data into one representative per haplotype block







## Centromere locations in the *Brassica* C genome chromosomes

Chromosome	Start (Mbp)*	End (Mbp)*	Total length (Mbp)**	Centromere location as % distance along assembled chromosome	Size of centromere-containing region in sequence assembly (Mbp)
C1	18.6	19.3	43.1	44%	0.7
C2	30.3	35.1	52.8	62%	4.8
C3	38.4	48.1	64.8	67%	9.6
C4	18.4	19.9	53.5	36%	1.5
C5	25.6	27.5	46.3	57%	1.8
C6	26.1	31.1	39.7	72%	4.9
C7	7.3	9.8	47.9	18%	2.5
C8	5.0	7.8	41.7	15%	2.8
C9	22.9	34.7	54.6	53%	11.7

\* Represents the conservative outer boundaries within which the active centromeric region must fall. Data shared with Parkin et al. (submitted).

\*\* As covered by polymorphic SNPs in this population in the assembled C genome



# Centromere mapping through half-tetrad analysis in other species

- Unreduced gametes
  - Are produced at low levels in almost all species
  - Can be selected for by crosses between species and ploidy levels, particularly in *Brassica*
- Availability of physically mapped SNP markers
  - Illumina Infinium array, skim genotyping-by-sequencing, assembled genomes
  - Not actually necessary to have mapped markers but makes this analysis very straightforward!

# Acknowledgements

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**Australian Research Council**



Matt Nelson



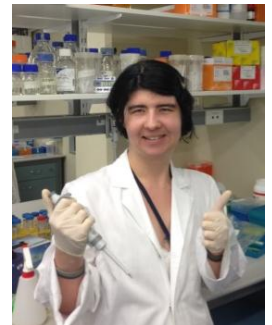
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OF QUEENSLAND  
AUSTRALIA



Jess Dalton-Morgan

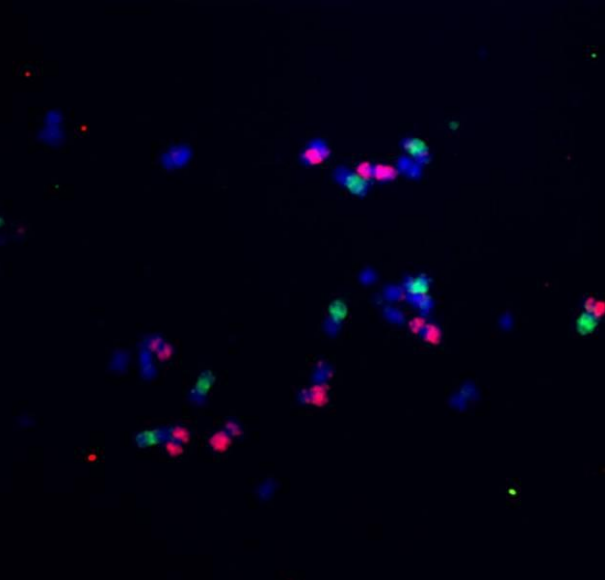
Isobel Parkin



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Thank you!

