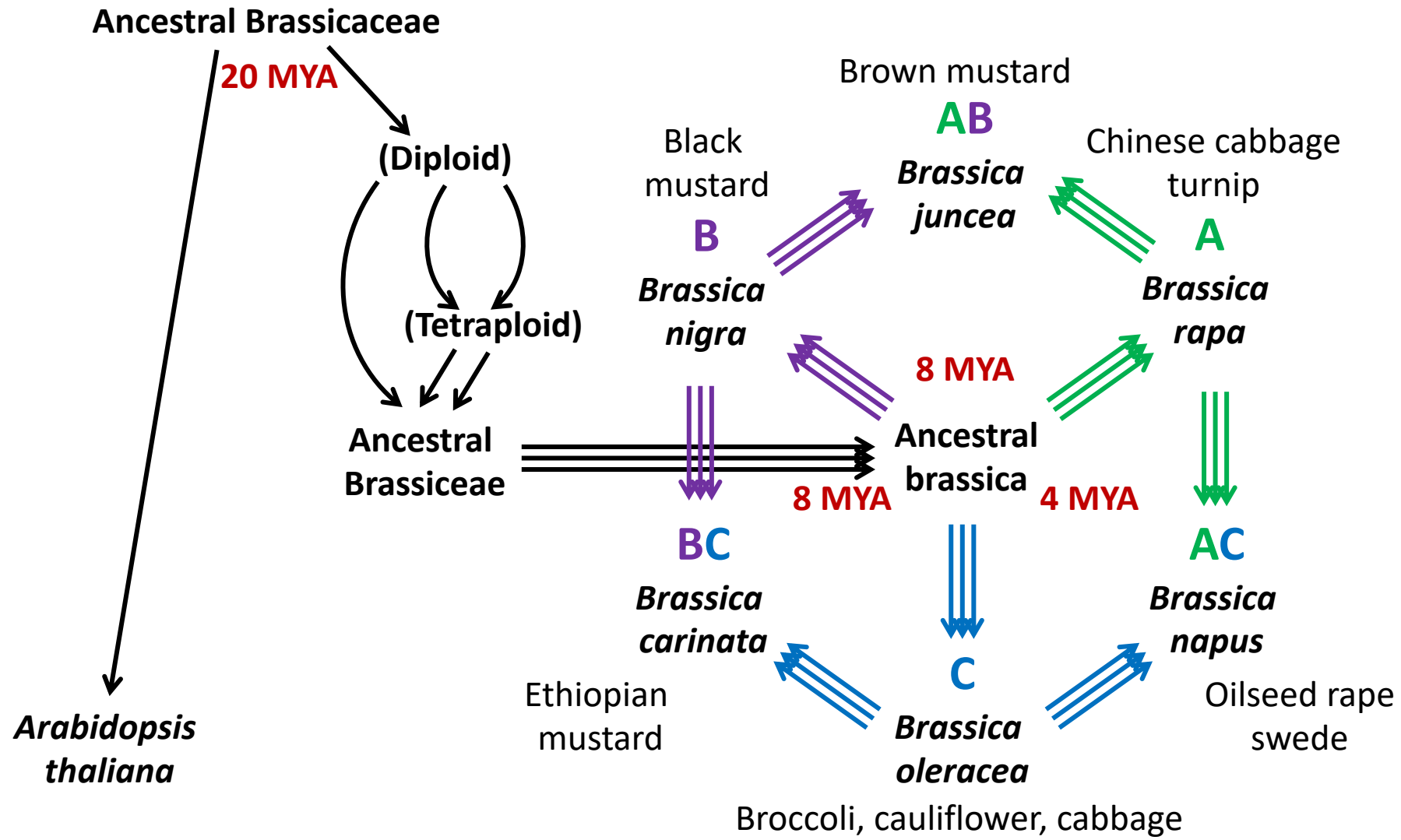
Natural and induced genome structural variation in oilseed rape

Ian Bancroft

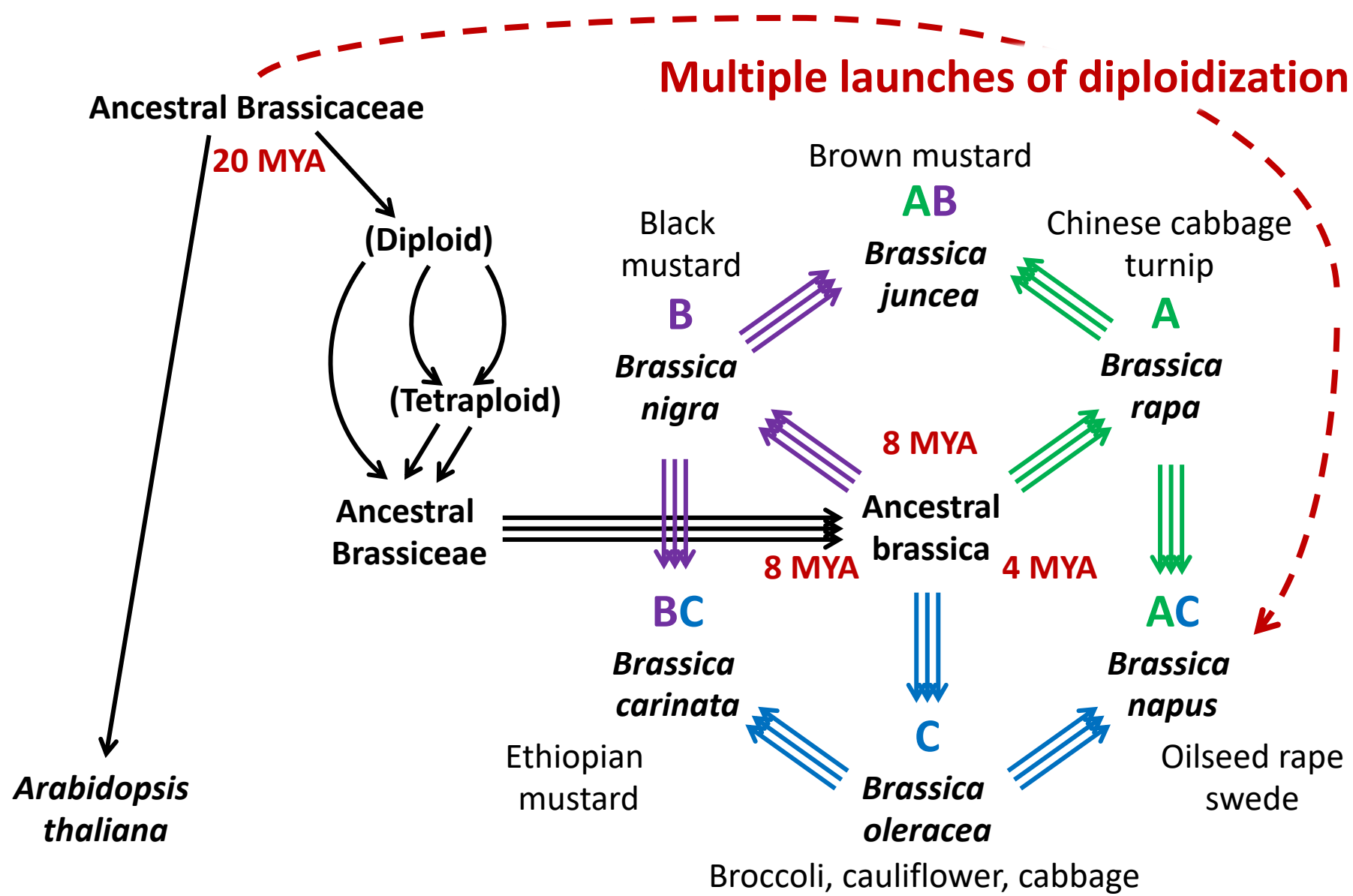
17th June, 2019

- **Understanding and dealing with genome complexity in *Brassica* species**
- **Genome structural variation resulting from homoeologous exchange**
- **Genome structural variation resulting from ionising radiation**

Genome complexity in the cultivated *Brassica* species



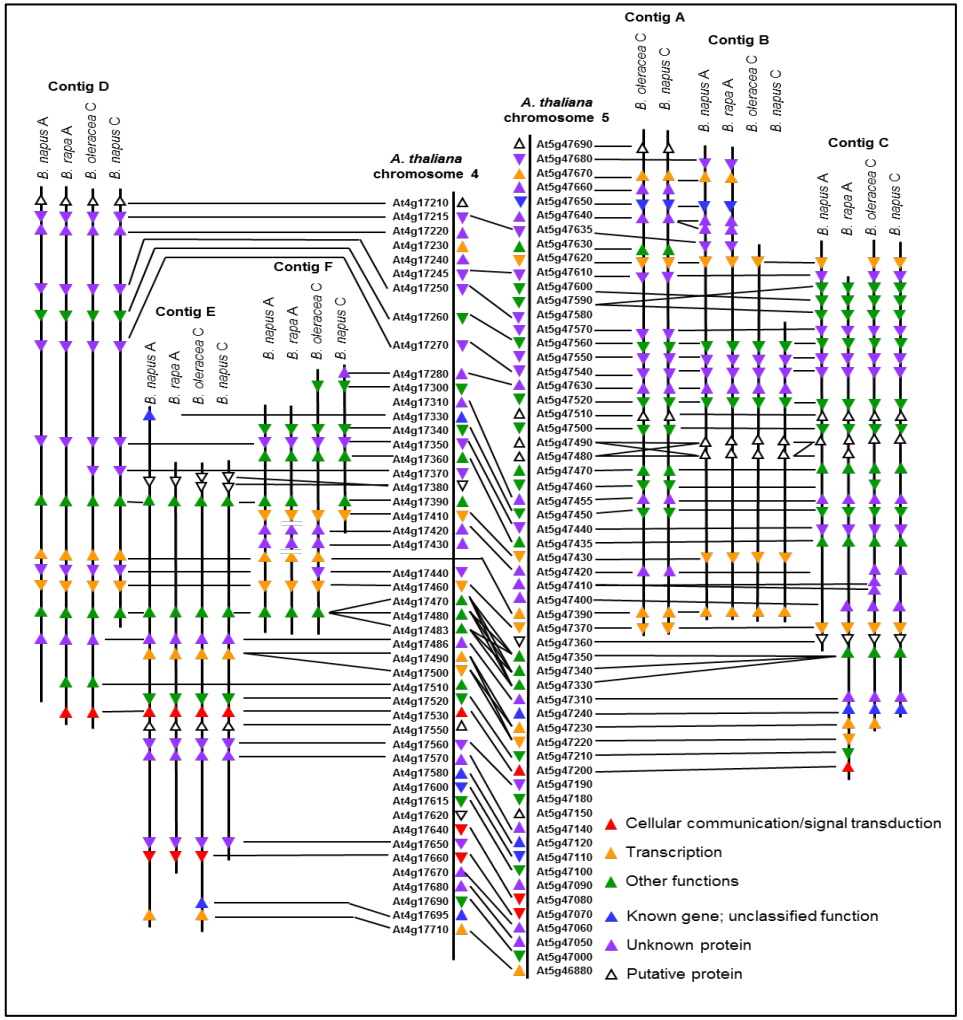
Genome complexity in the cultivated *Brassica* species



Genome complexity in the cultivated *Brassica* species

Targeted high quality genome sequencing showed:

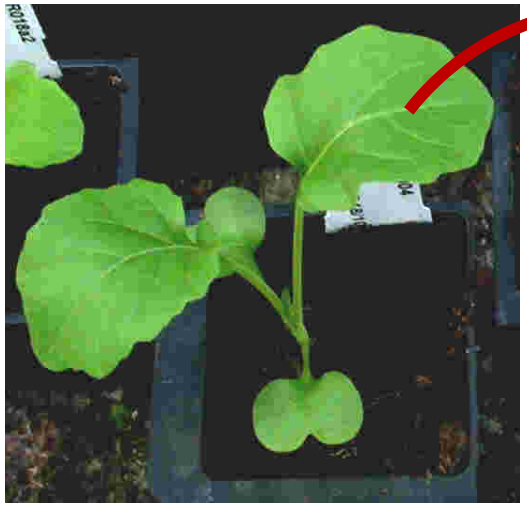
- **Conserved synteny between protein-coding genes**
- **Interspersed gene loss results in ~4 orthologues in *B. napus* for each gene in Arabidopsis**



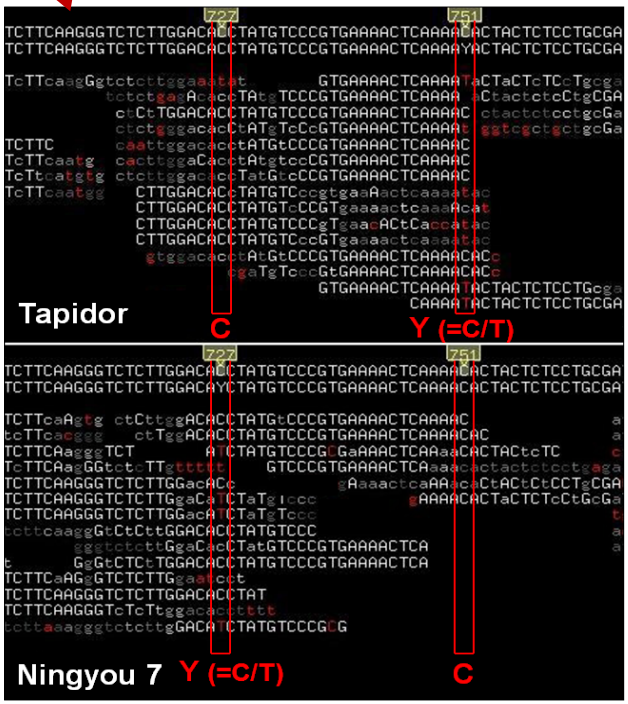
THE PLANT CELL
AMERICAN SOCIETY OF PLANT BIOLOGISTS

Cheung, et al. Comparative analysis between genome segments of *Brassica napus* and its progenitor species reveals extensive sequence-level divergence. Plant Cell 21:1912-1928, 2009

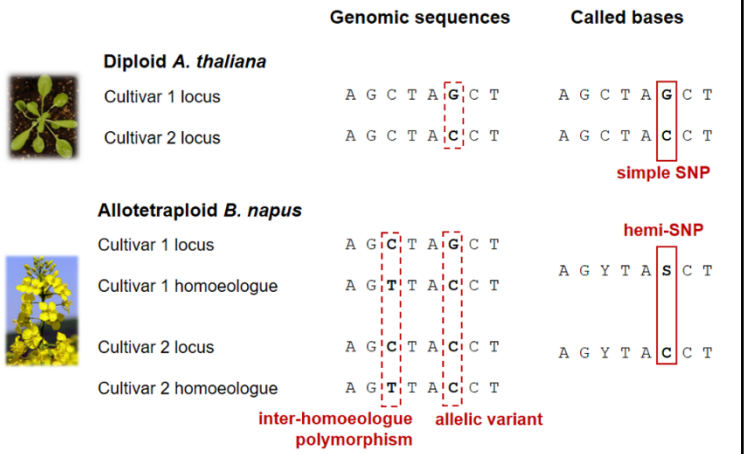
“Functional genotypes” from mRNAseq data



~3 Gb mRNAseq / plant



A need to overcome stochastic sampling effect

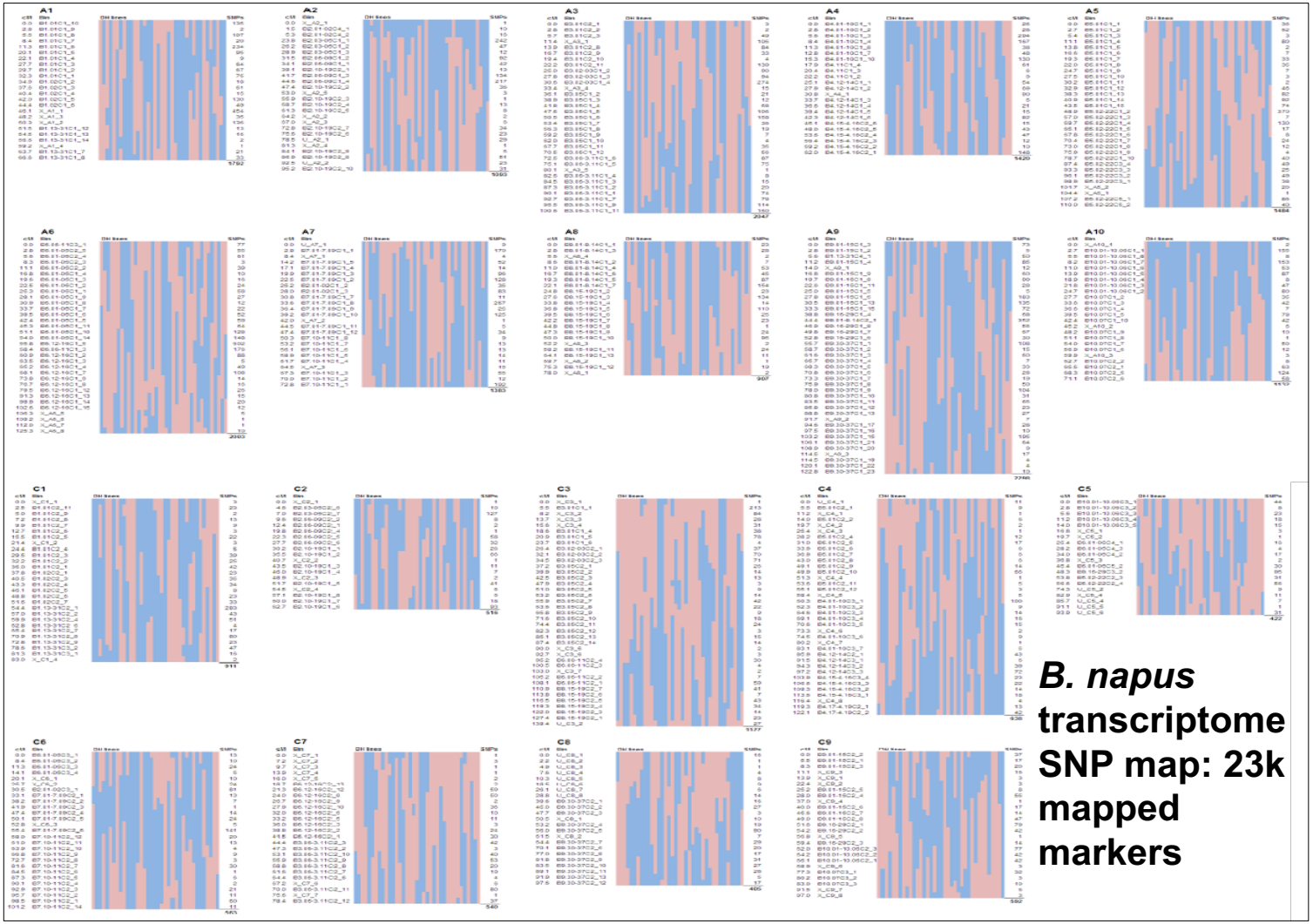


Map to unigene or CDS reference for SNP-calling & quantification

Plant Biotechnology Journal

Trick, et al. SNP discovery in the polyploid *Brassica napus* using Solexa transcriptome sequencing Plant Biotechnology J. 7:334-346, 2009

Transcriptome SNPs as molecular markers in *B. napus*

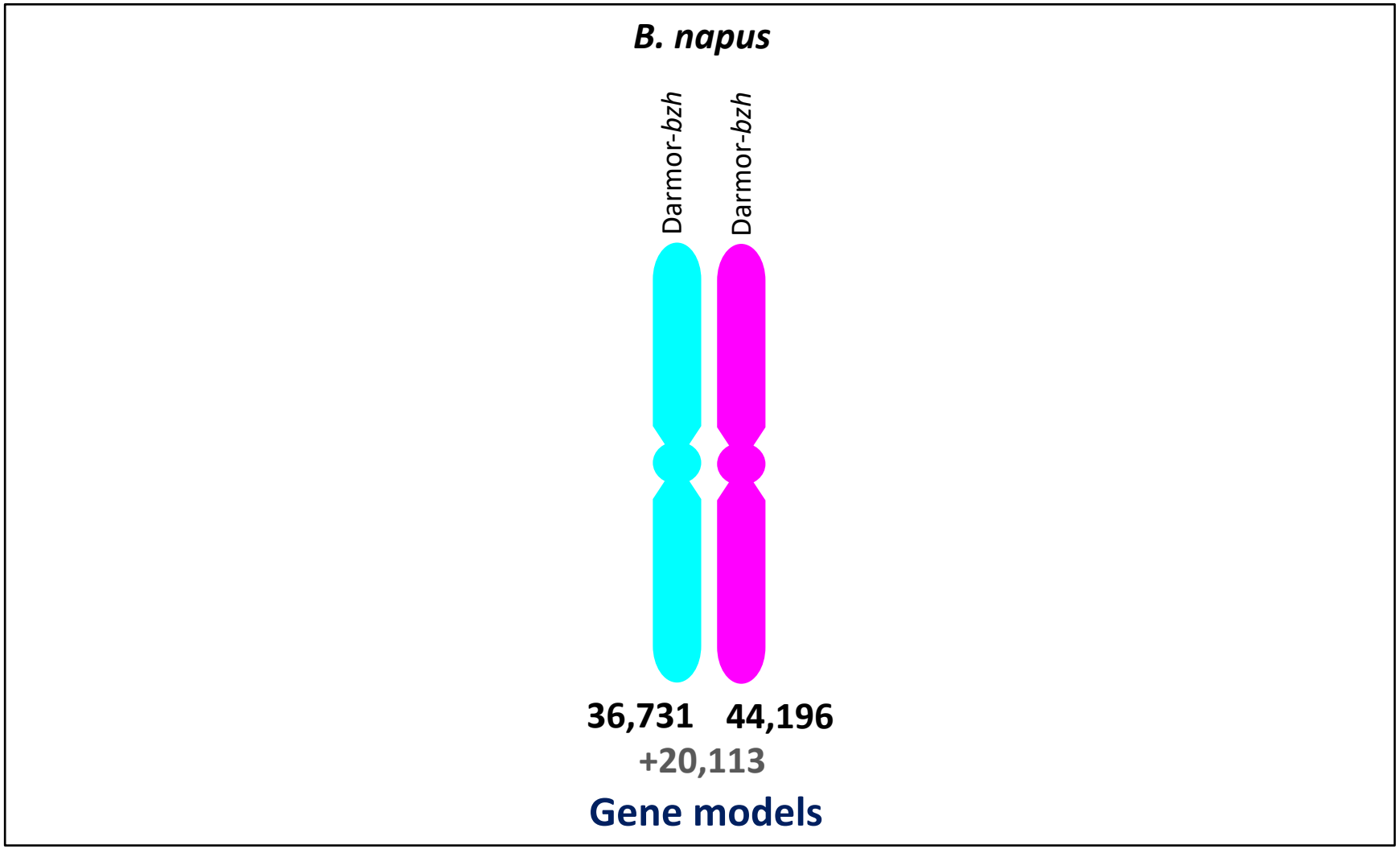


B. napus **transcriptome SNP map: 23k mapped markers**

nature biotechnology

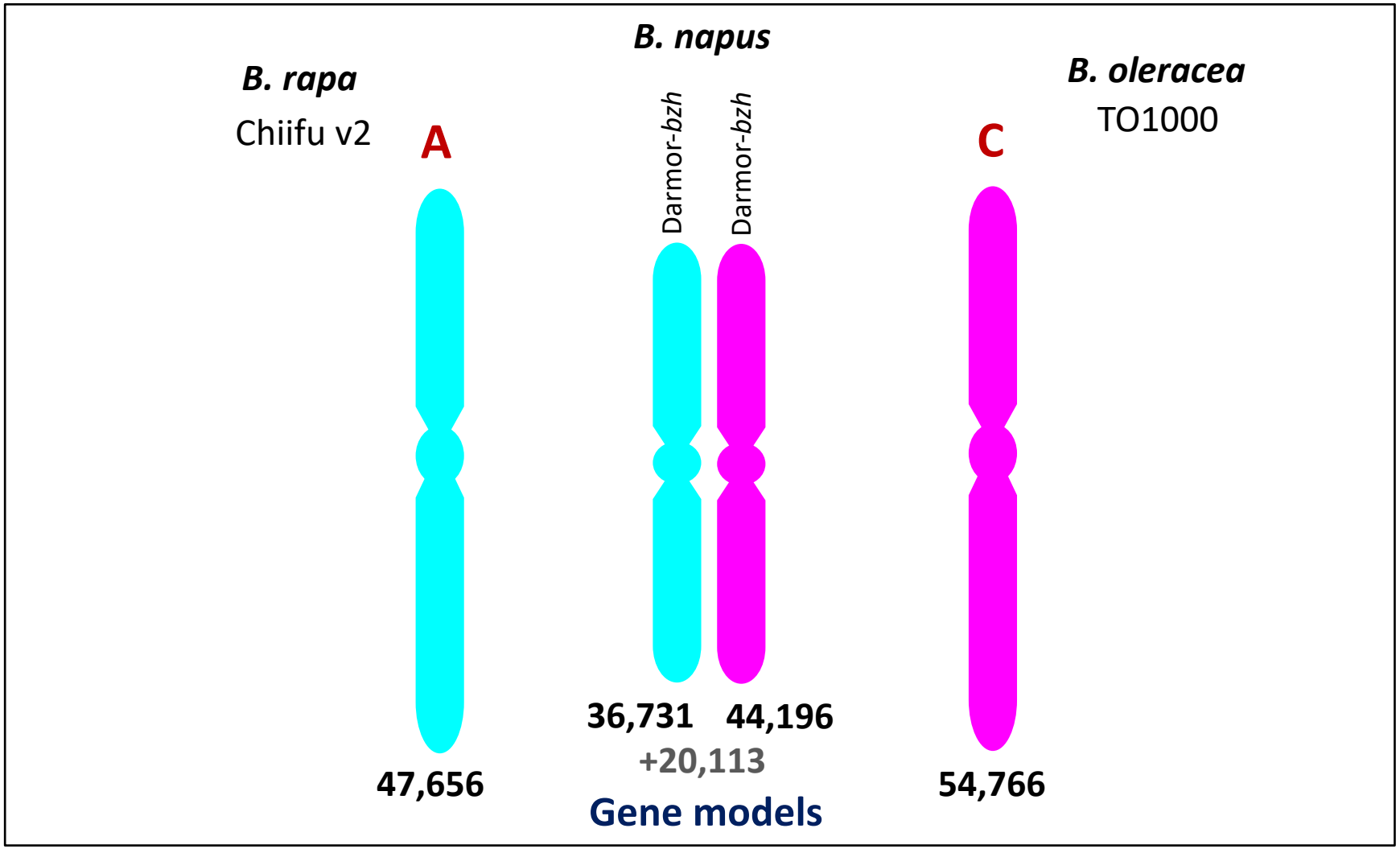
Bancroft, et al. Dissecting the genome of the polyploid crop oilseed rape by transcriptome sequencing Nature Biotechnology 29:762-6, 2011

B. napus genome sequence was difficult to assemble

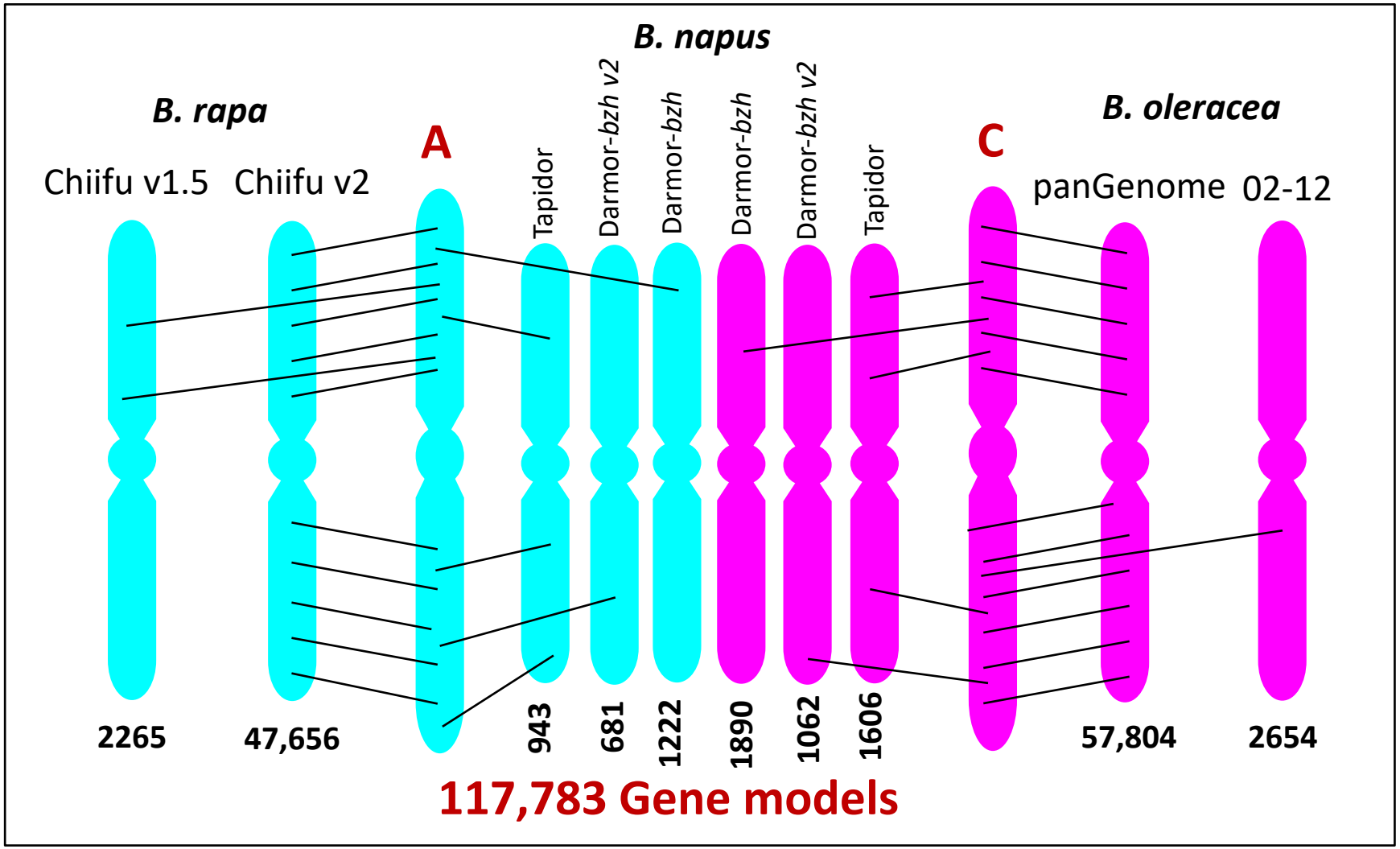


Chalhoub, et al. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* 345:950-953, 2015

Genome assignment completely secure in progenitor species



AC pan-transcriptome reference sequence for *B. napus*



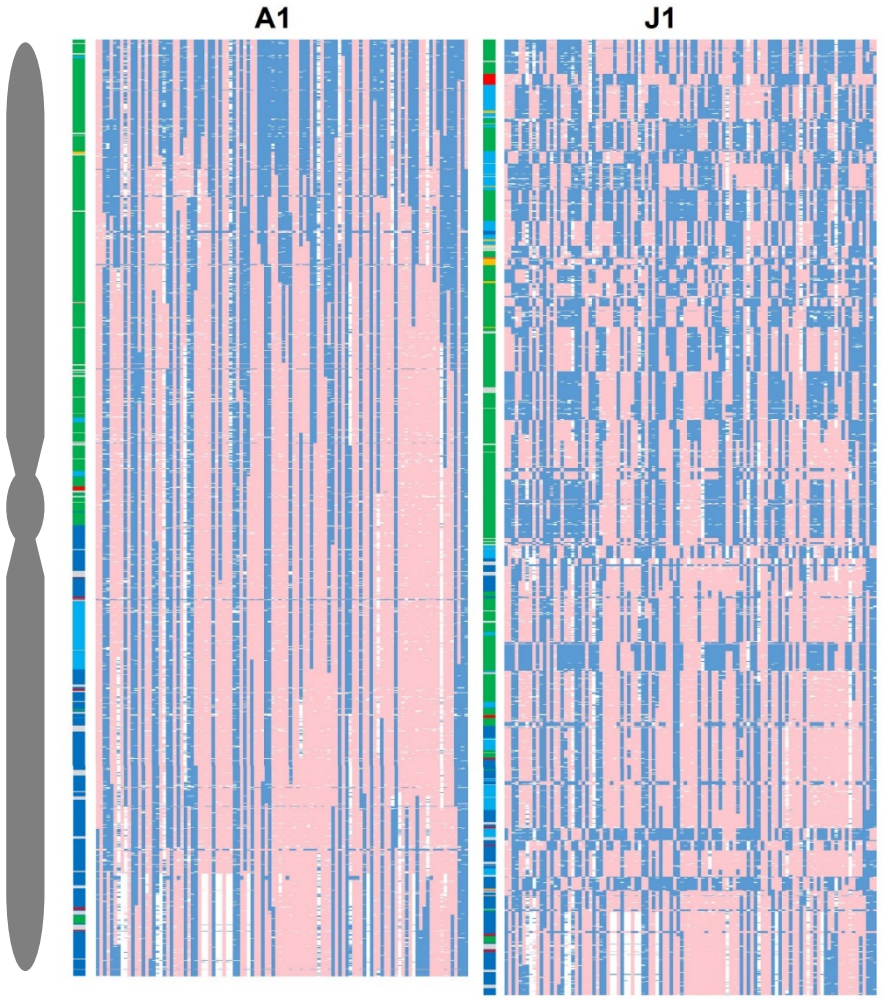
He, et al. Construction of *Brassica* A and C genome-based ordered pan-transcriptomes for use in rapeseed genomic research. *Data in brief* 4: 357-362, 2015.

Improving the organisation of *Brassica* genome sequences

Chimaeric and mis-mapped scaffolds are common in genome sequences

Allotetraploid *Brassica* genomes pose particular problems

Genome Ordered Graphical Genotypes (GOGGs) proposed as part of quality control process for genome sequence assembly



nature genetics

He and Bancroft. Organisation of the genome sequence of the polyploid crop species *Brassica juncea* Nature Genetics 50:1496-1498, 2018

GOGGs for *B. napus* using AC diploid CDS reference pan-genome

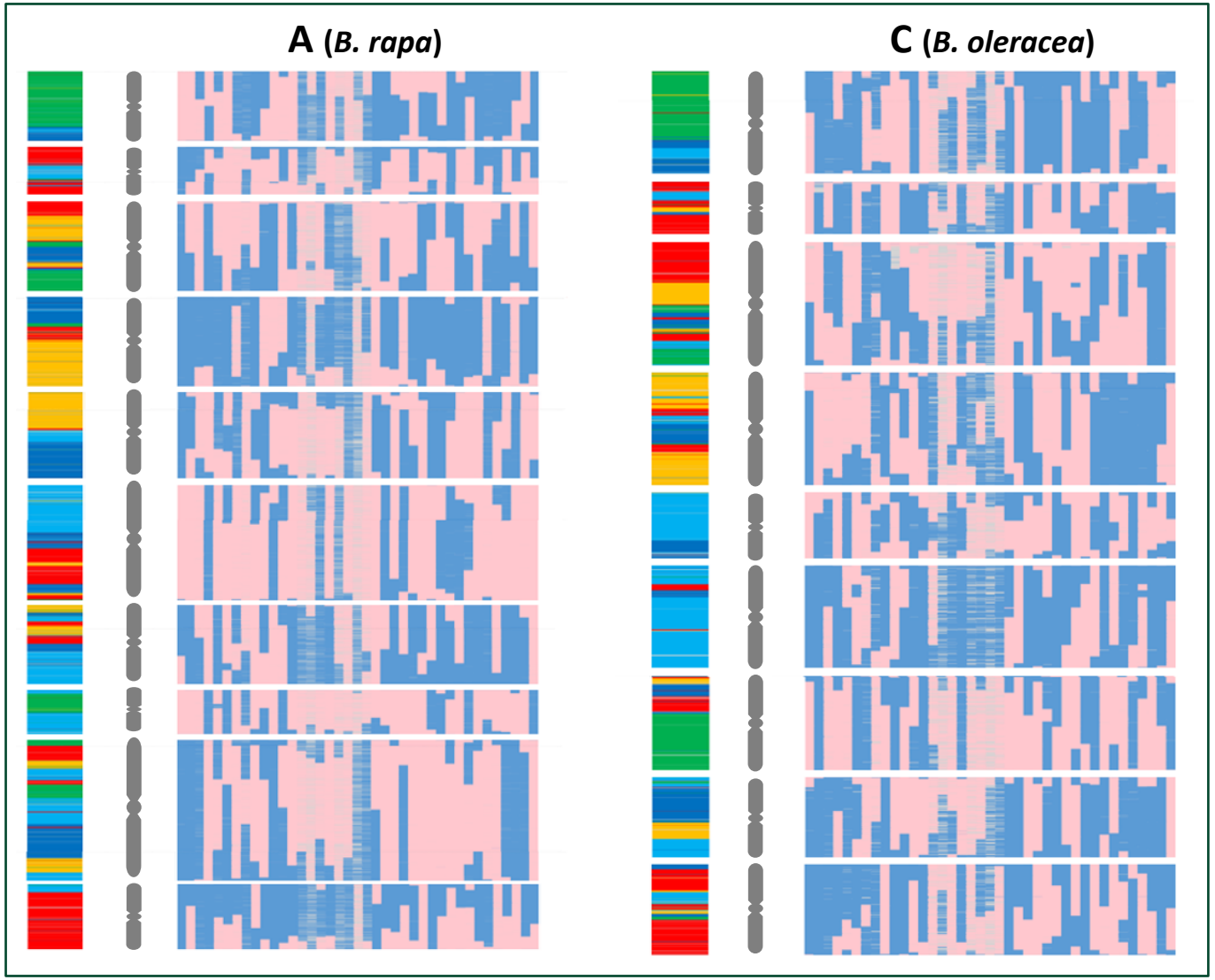
Graphical genotypes for TNDH population

39 lines used for transcriptome SNP mapping

Only simple SNPs used for mapping

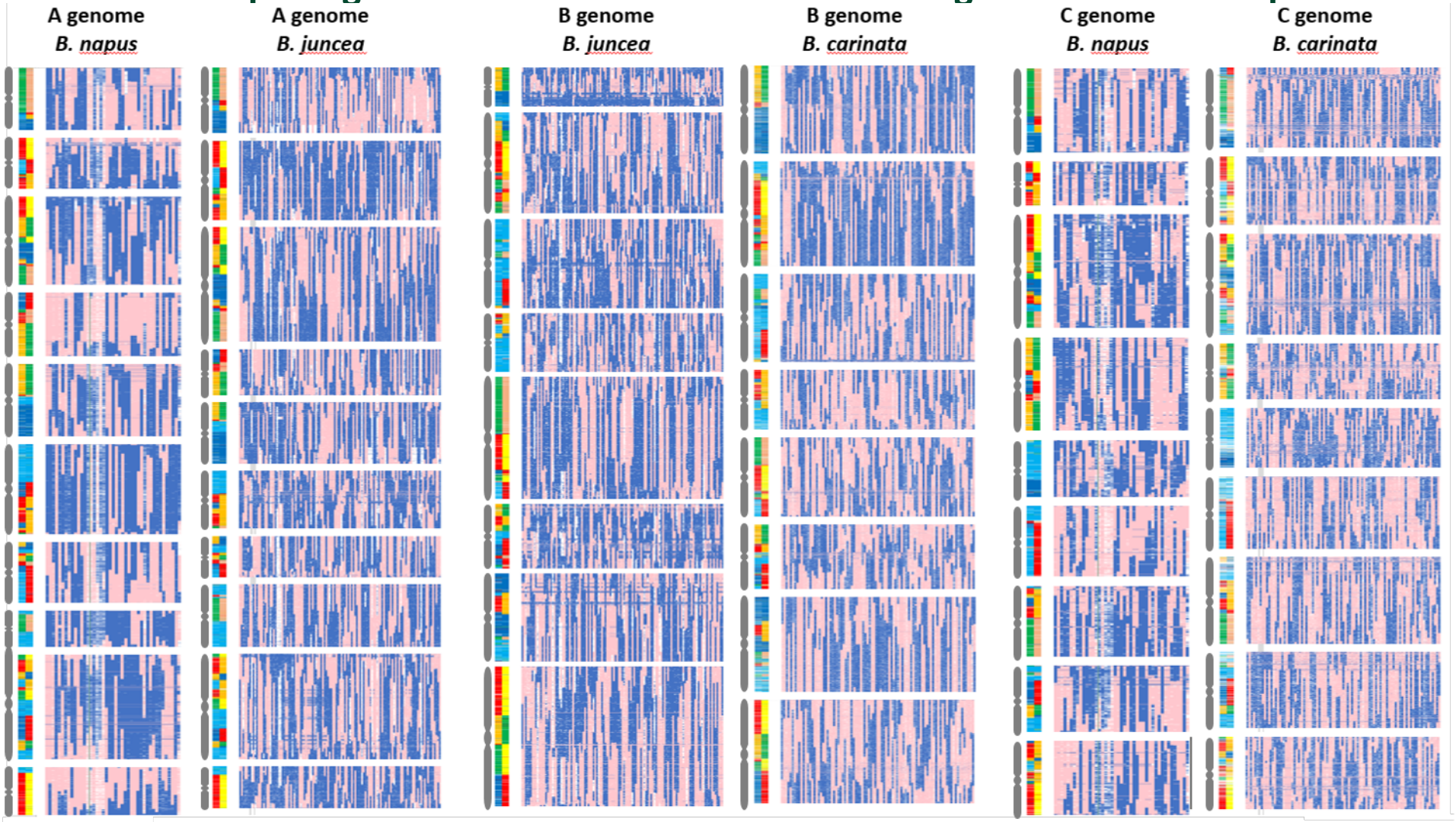
4509 markers in different genes mapped in A genome

2084 markers in different genes mapped in C genome



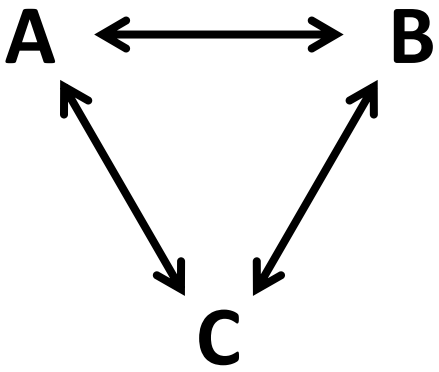
Diploid genomes collinear in both allotetraploid populations

GOGGs for diploid genomes indicate conservation of organisation across species



Identifying orthologous gene triplets across genomes

Identify triplets of orthologues between *Brassica* A, B and C genomes, as 3-way reciprocal top BLAST hits between CDS gene models



21,359 reciprocal top BLAST hit triplets

	A gene	B gene	C gene	A_position	B_position	C_position	Arabidopsis thaliana	Thellungiella parvula
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7	BraA01g000090.	maker-scaff_95	Bo1g001100.1	A01_000029350_000032812	B05_000042508_000042718	C01_000039996_000043628	AT4G38470.1	Tp7g37310
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9	BraA01g000130.3	fgenesh_maske	Bo1g002190.1	A01_000079510_000079806	B05_000030822_000031118	C01_000106126_000106422	AT4G38840.1	Tp7g36990
10	BraA01g000150.3	fgenesh_maske	Bo1g002200.1	A01_000088205_000088522	B05_000023813_000024130	C01_000117495_000117812	AT4G38860.1	Tp7g36970
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14	BraA01g000190.3	maker-scaff_95	Bo1g002250.1	A01_000101158_000104774	B05_000010670_000011250	C01_000130721_000134516	AT4G38950.2	Tp7g36890
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28	BraA01g000400.	fgenesh_maske	Bo1g002510.1	A01_000180669_000181013	B05_000168141_000168494	C01_000216702_000217046		
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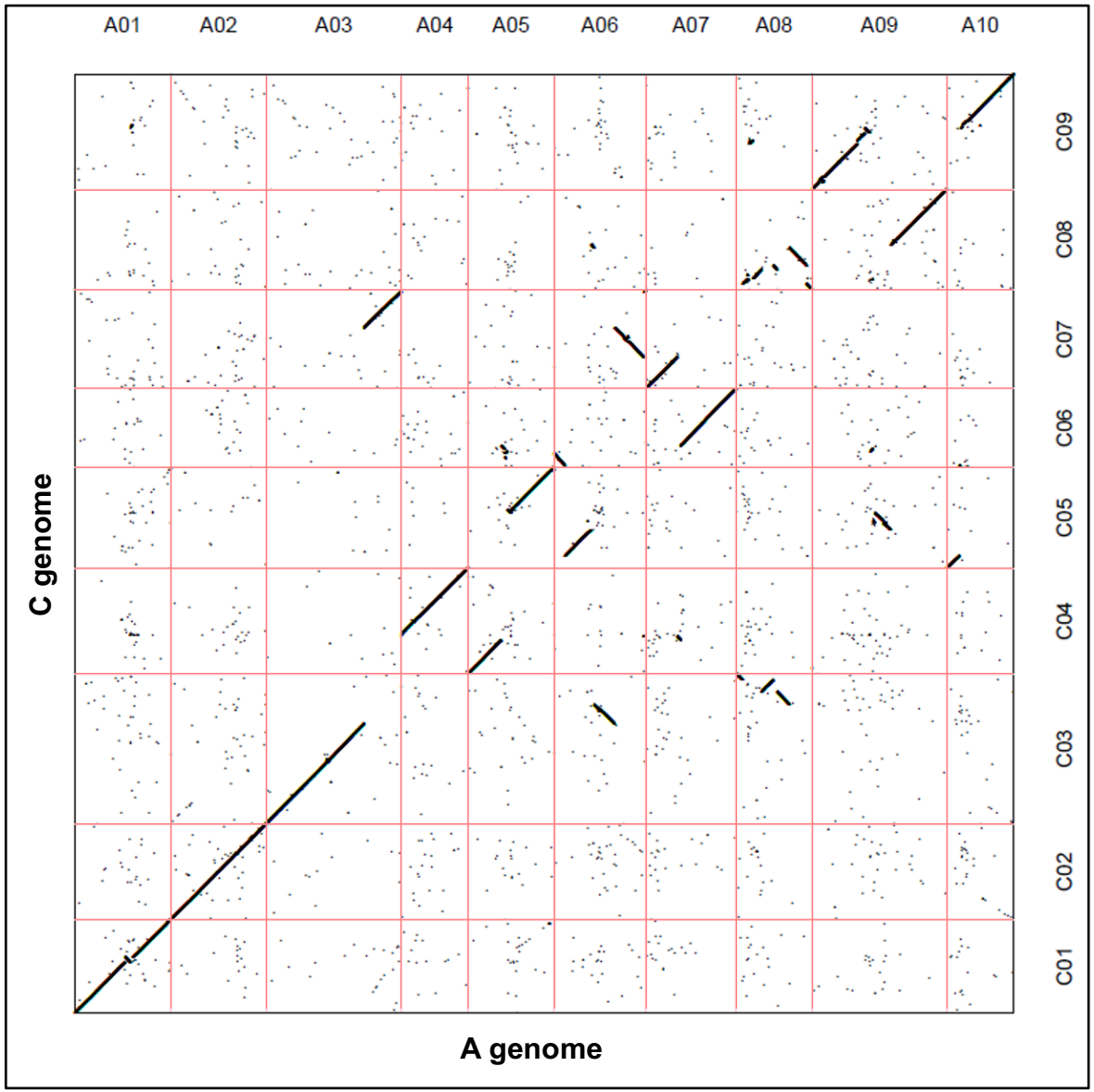
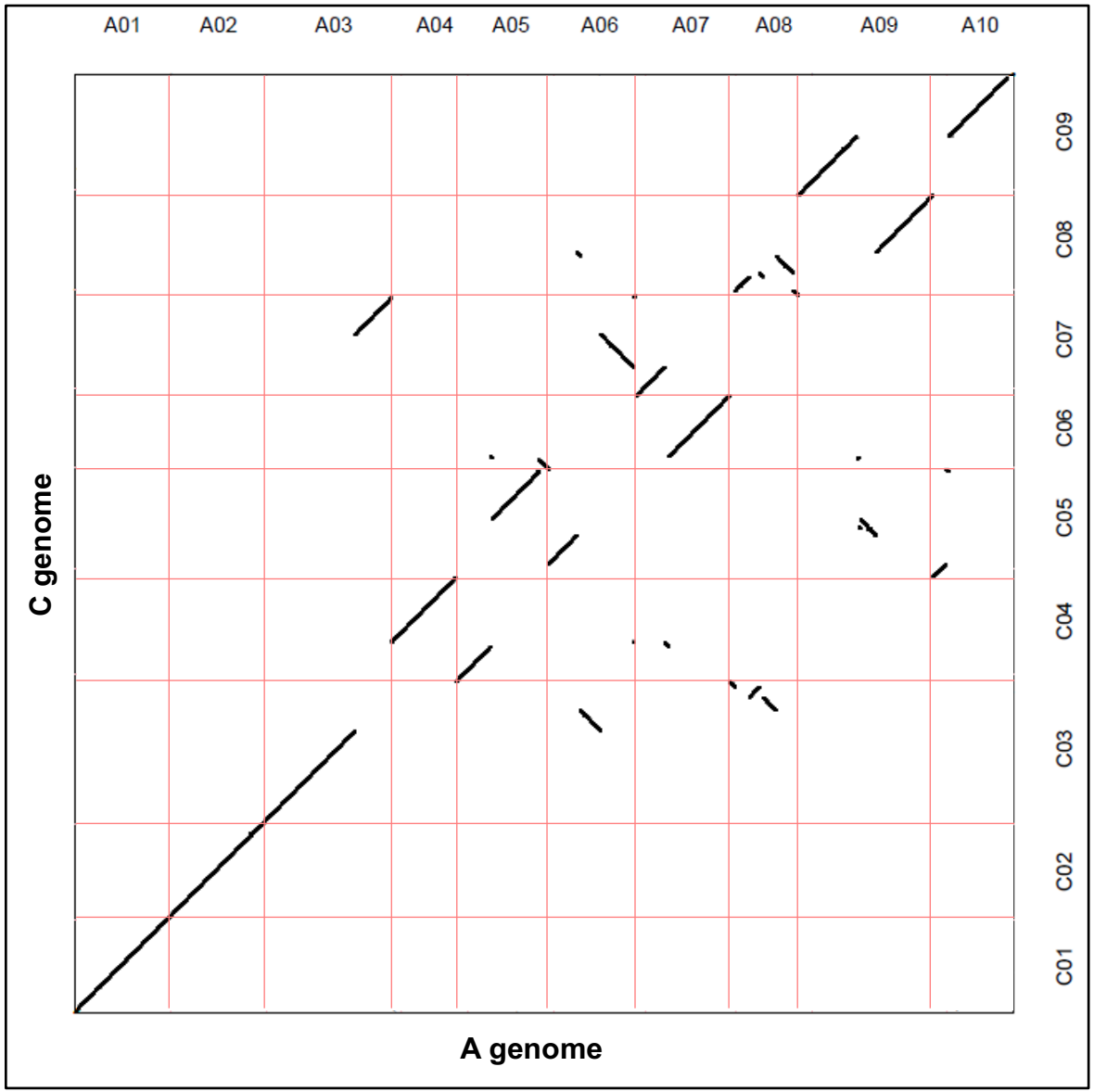
Homoeology relationships between A and C genomes



Based on 31,591 reciprocal top BLAST hit pairs between A and C genome

Homoeology relationships between A and C genomes



Based on 21,359 reciprocal top BLAST hit triplets between A, B and C genomes

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Ian Bancroft

17th June, 2019

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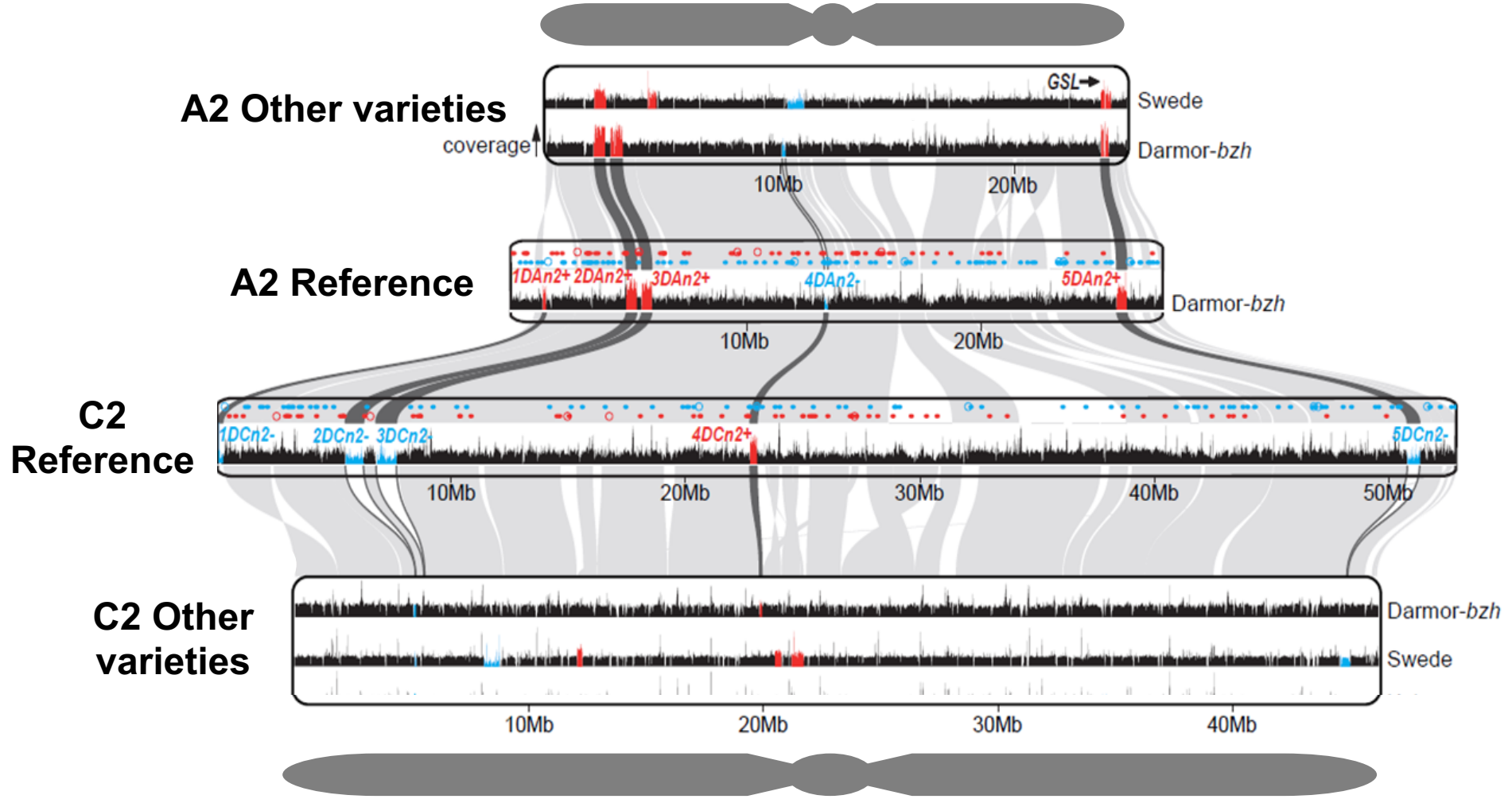
- **Genome structural variation resulting from homoeologous exchange**
- Genome structural variation resulting from ionising radiation

Conventional visualisation of genome dosage



Science AAAS

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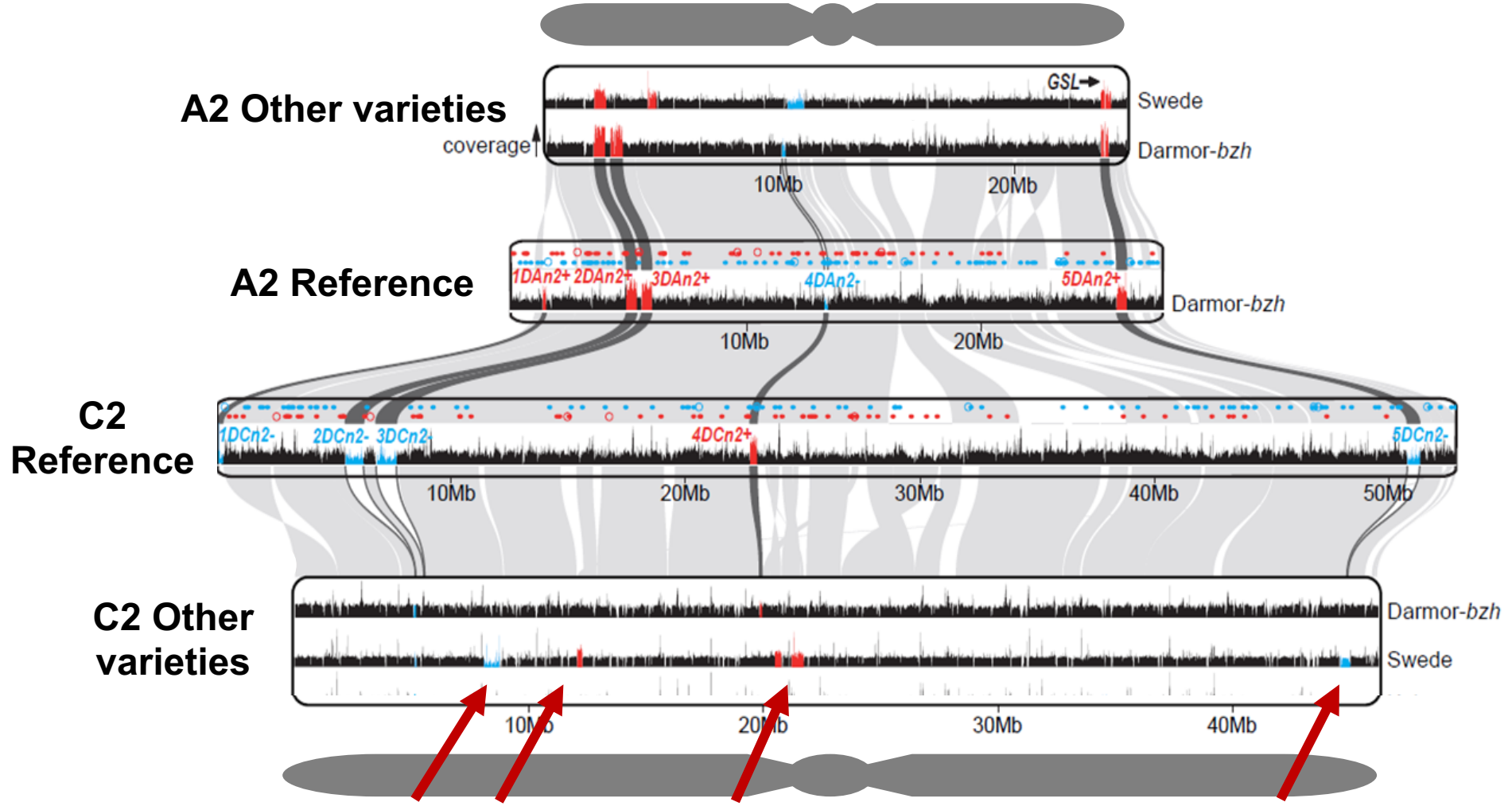


Genome dosage difficult to determine, especially when HEs in reference

Science AAAS

Chalhoub, et al. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* 345:950-953, 2015

Visualizing genome dosage variation using mRNAseq data

Quantify transcript abundance (RPKM) using
AC pan-transcriptome reference sequence

↓

Filter data for reciprocal top BLAST hit gene pairs

↓

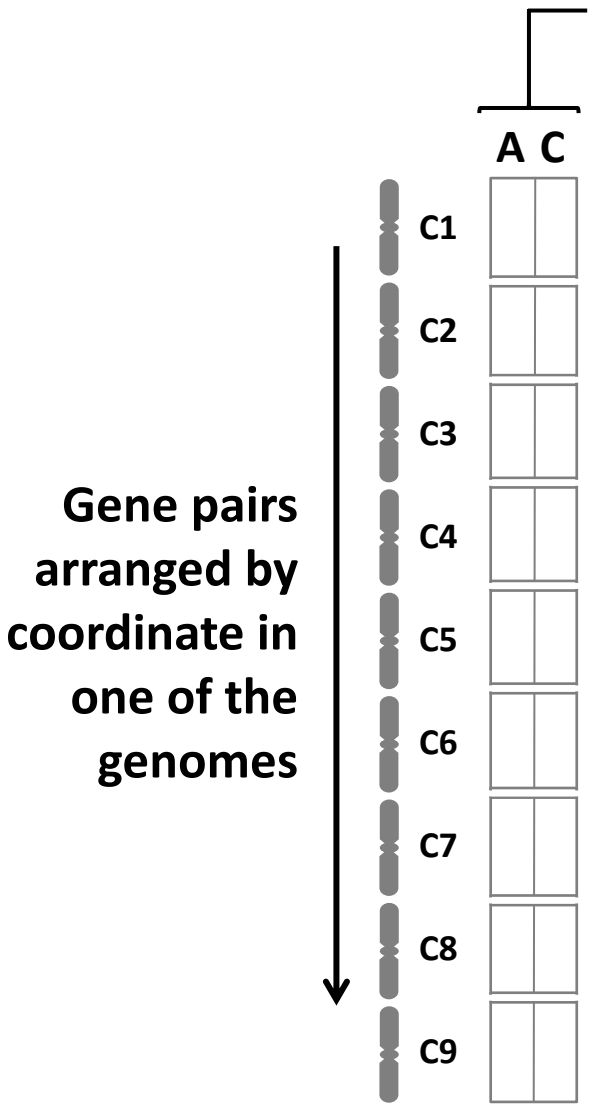
Code intensity of shading based on RPKM values

↓

Visualise using tile plots with gene pairs ordered by one genome

Visualizing genome dosage variation using mRNAseq data

Positions of 31,591 homoeologous gene pairs, arranged by the order in the C genome of the C genome homoeologoue



	A genome homoeologue	Position in A genome	Order in A	Order in C	Position in C genome	C genome homoeologue
2	Cab020597.1	A01_000007596_000004363	1	1	C01_000004096_000006192	Bo1g001030.1
3	Cab020596.1	A01_000009147_000008191	2	25377	C07_048354208_048355539	Bo7g120870.1
4	Cab020595.2	A01_000011403_000009778	3	2	C01_000007519_000009142	Bo1g001040.1
5	Cab020594.1	A01_000012765_000011917	4	3	C01_000009720_000010577	Bo1g001050.1
6	Cab020592.1	A01_000021944_000019956	5	5	C01_000037620_000038611	Bo1g001080.1
7	Cab020591.1	A01_000024271_000022281	6	6	C01_000039858_000040949	Bo1g001090.1
8	Cab020590.1	A01_000032446_000028836	7	7	C01_000046852_000050484	Bo1g001100.1
9	Cab020589.1	A01_000033293_000032595	8	30597	C09_021661539_021662053	Bo9g071600.1
10	Cab020588.1	A01_000035983_000033952	9	9	C01_000068965_000070059	Bo1g001140.1
11	Cab020581.1	A01_000063572_000063039	10	10	C01_000103201_000103782	Bo1g002150.1
12	Cab020579.1	A01_000079019_000078723	11	11	C01_000112982_000113278	Bo1g002190.1
13	Cab020577.1	A01_000088358_000088041	12	12	C01_000124351_000124668	Bo1g002200.1
14	Cab020576.1	A01_000094861_000093230	13	13	C01_000128563_000130200	Bo1g002210.1
15	Cab020575.1	A01_000097996_000095110	14	14	C01_000130440_000133386	Bo1g002220.1
16	Cab020574.1	A01_000100108_000098057	15	15	C01_000134318_000135251	Bo1g002230.1
17	Cab020573.1	A01_000105326_000101322	16	16	C01_000137577_000141372	Bo1g002250.1
18	Cab020571.1	A01_000115025_000112704	17	17	C01_000148961_000151258	Bo1g002280.1
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21	Cab020568.1	A01_000135472_000131405	20	20	C01_000172779_000174206	Bo1g002330.1
22	Cab020567.1	A01_000143020_000139039	21	21	C01_000175196_000180272	Bo1g002340.1
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24	BnaA01g05340D	A01_000146772_000145212.00	23	24	C01_000184847_000187483	Bo1g002370.1
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42	Cab020543.1	A01_000244118_000242423	41	43	C01_000305351_000307099	Bo1g002590.1
43	Cab020540.1	A01_000247210_000246059	42	44	C01_000312999_000314150	Bo1g002620.1
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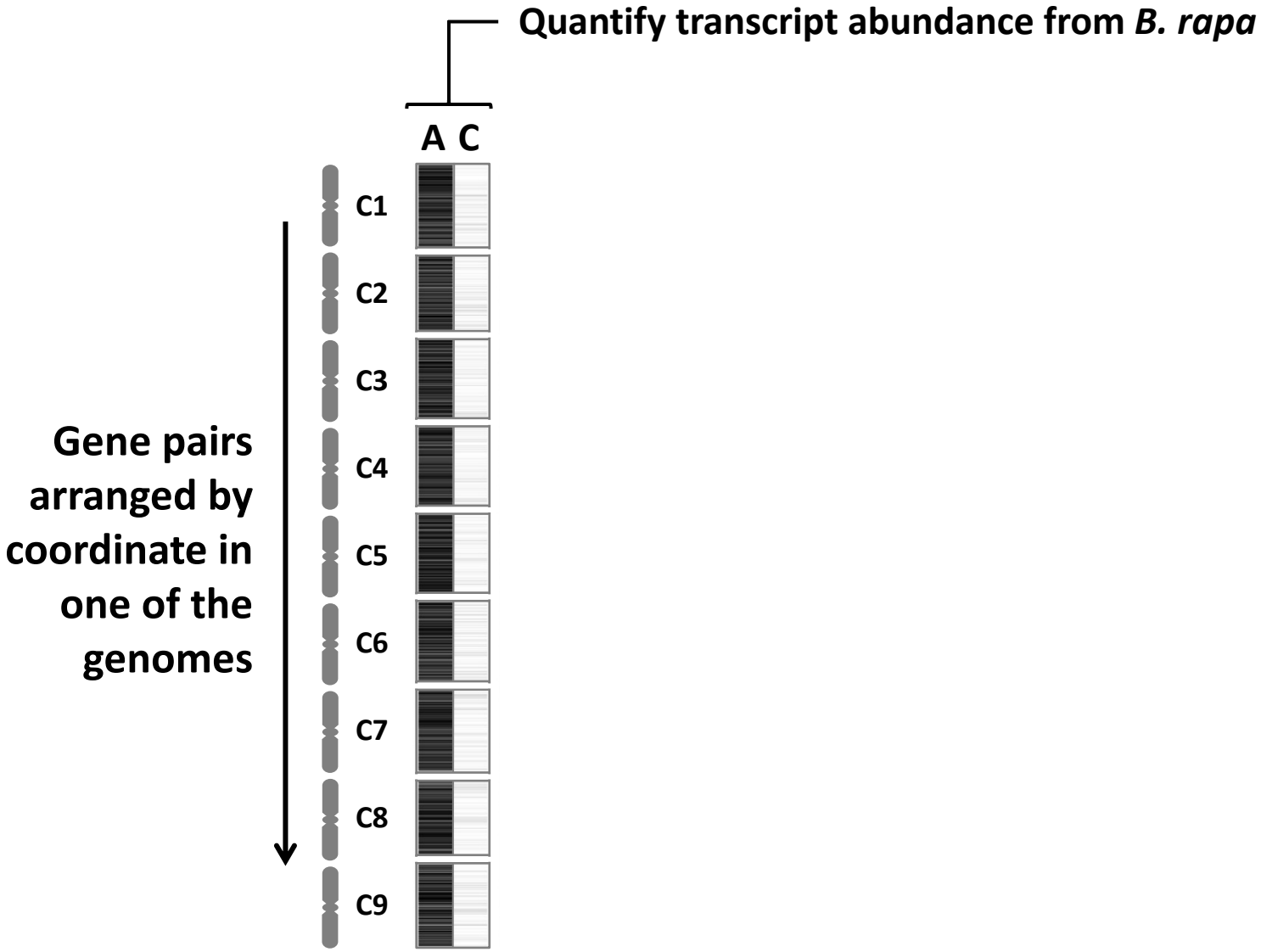
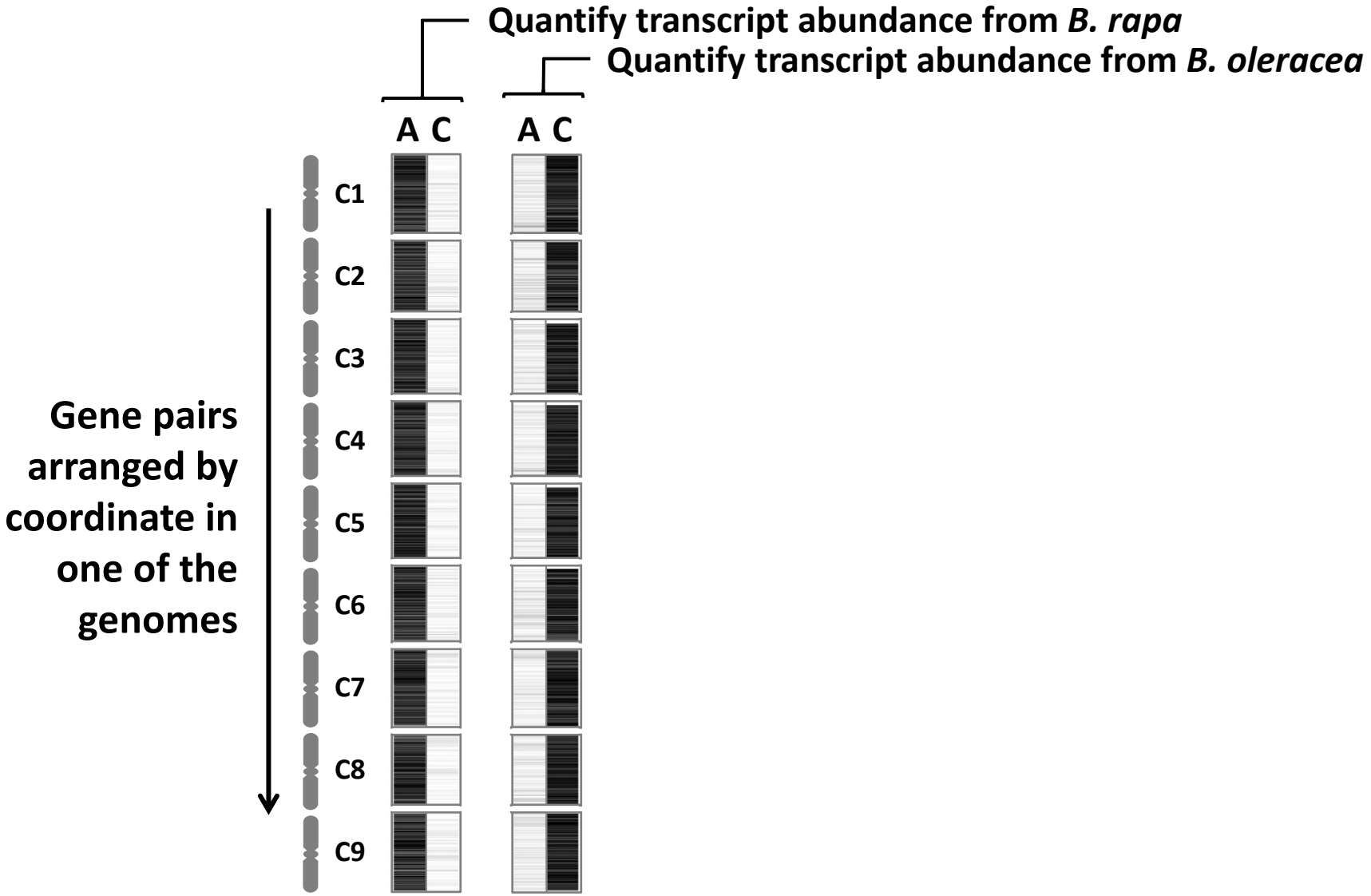
Visualizing genome dosage variation using mRNAseq data



Visualizing genome dosage variation using mRNAseq data



Visualizing genome dosage variation using mRNAseq data

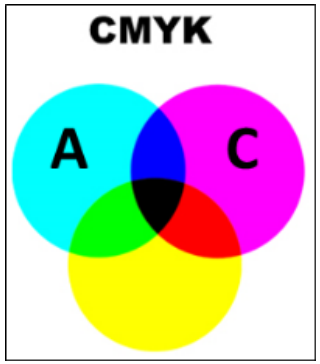
Quantify transcript abundance (RPKM) using AC pan-transcriptome reference sequence

↓

Filter data for reciprocal top BLAST hit gene pairs

↓

Use CMYK colour-coding based on RPKM values



↓

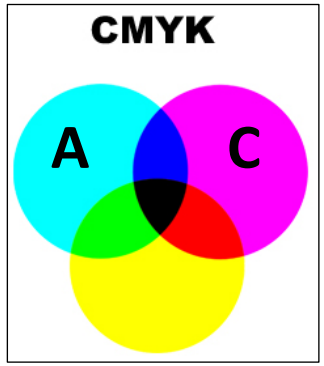
Visualise using tile plots with gene pairs ordered by one genome

Plant Biotechnology Journal

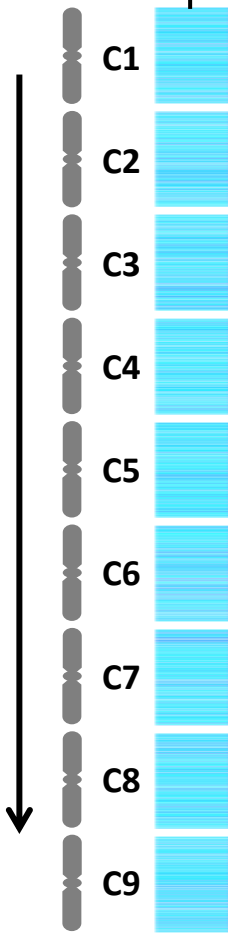
Harper, et al. Genome distribution of differential homoeologue contributions to leaf gene expression in bread wheat. Plant Biotechnology Journal 14:1207-1214, 2016

Visualizing genome dosage variation using mRNAseq data

A (*B. rapa*) = cyan



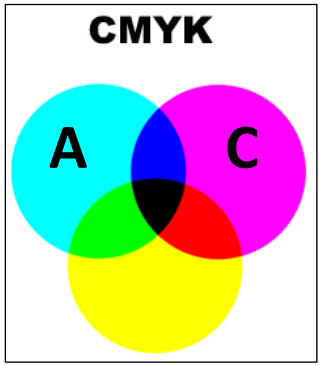
Gene pairs arranged by coordinate in one of the genomes



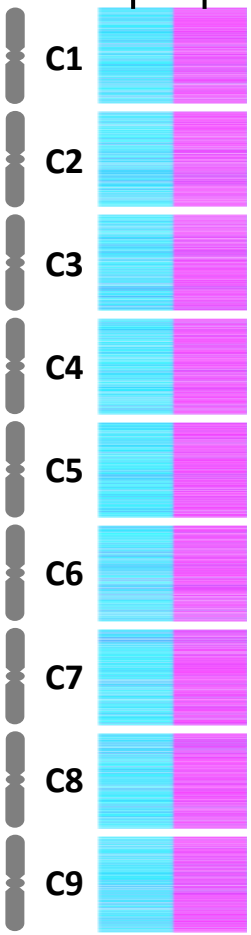
Visualizing genome dosage variation using mRNAseq data

A (*B. rapa*) = cyan

C (*B. oleracea*) = magenta



Gene pairs arranged by coordinate in one of the genomes

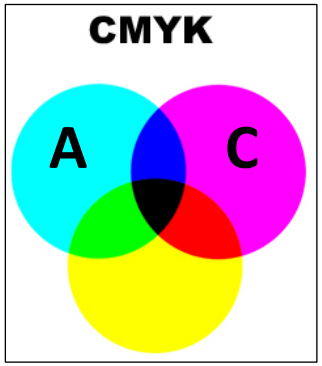


Visualizing genome dosage variation using mRNAseq data

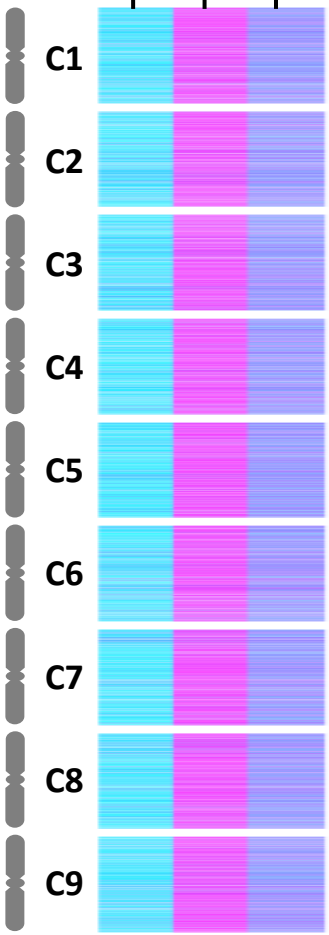
A (*B. rapa*) = cyan

C (*B. oleracea*) = magenta

in silico A + C (*B. napus*) = blue



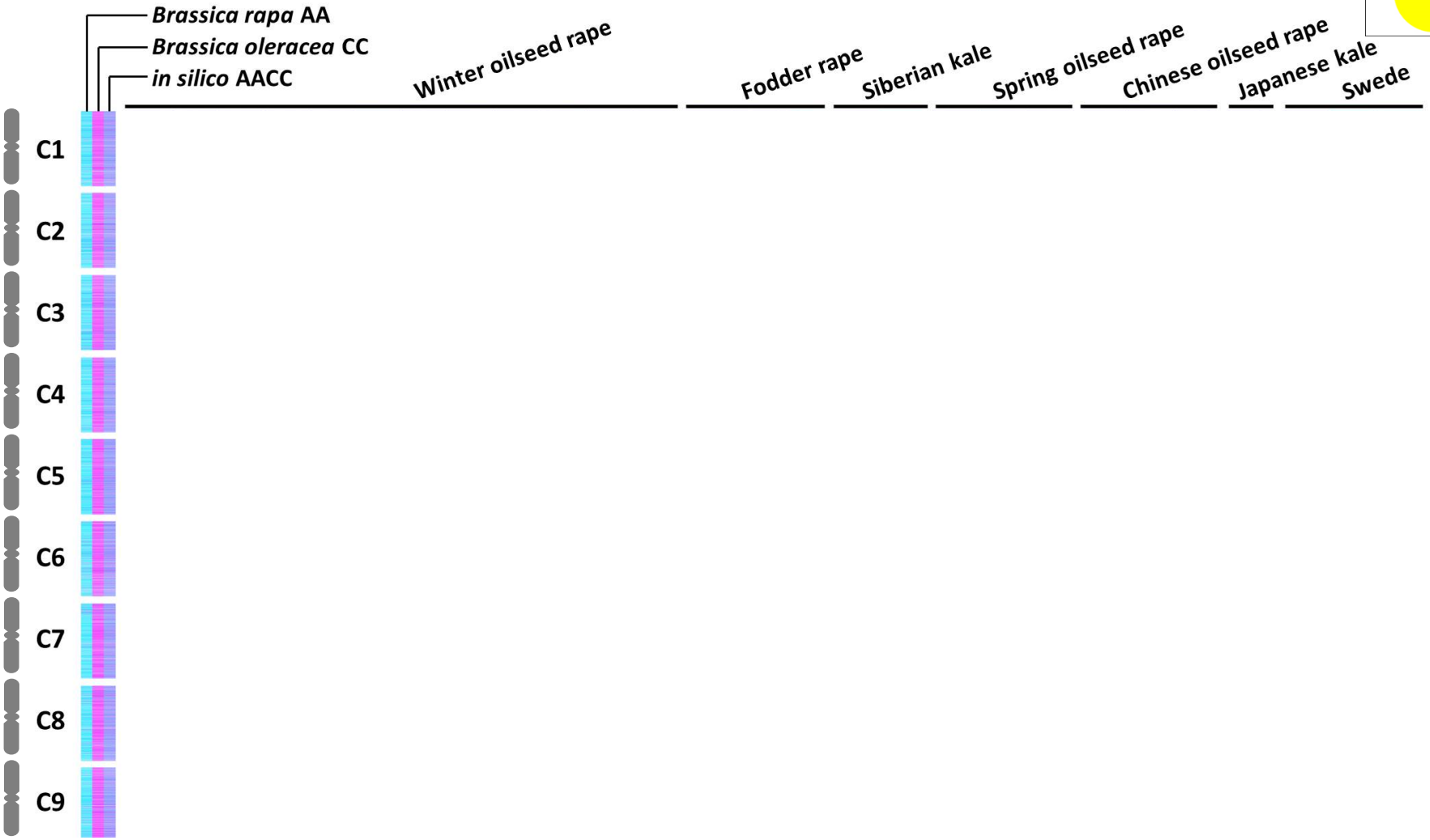
Gene pairs arranged by coordinate in one of the genomes

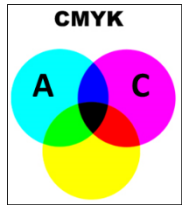


Assessment of a panel of *B. napus* varieties by mRNAseq

27 varieties selected across all crop types; x 4 biological replicates

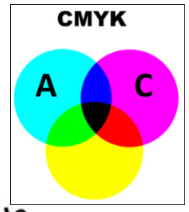
Expect to see blue signal throughout the genome

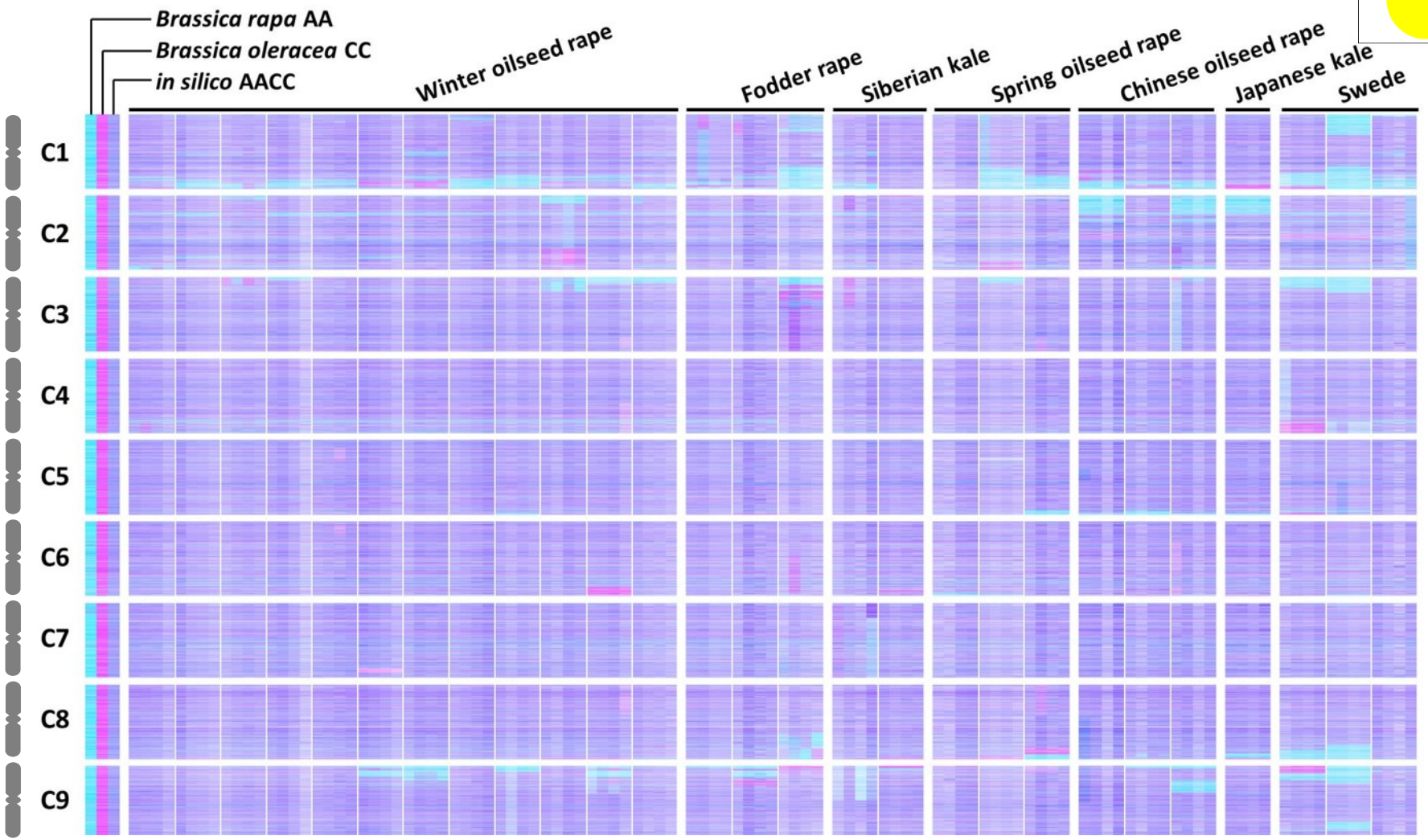




Assessment of a panel of *B. napus* varieties by mRNAseq

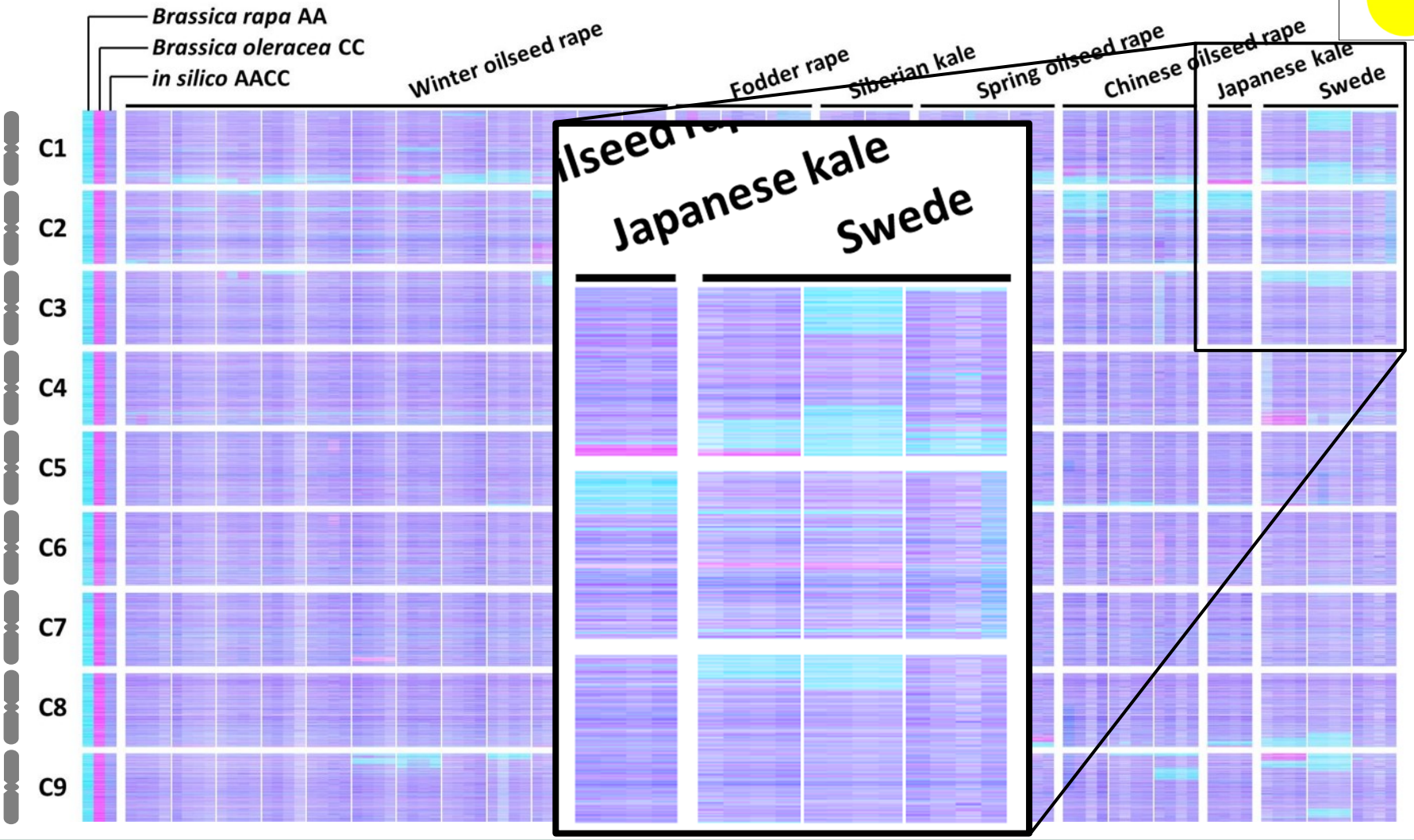
Homoeologous exchanges duplicating the A genome appear cyan, those duplicating the C genome appear magenta





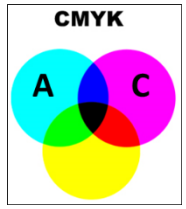
Assessment of a panel of *B. napus* varieties by mRNAseq

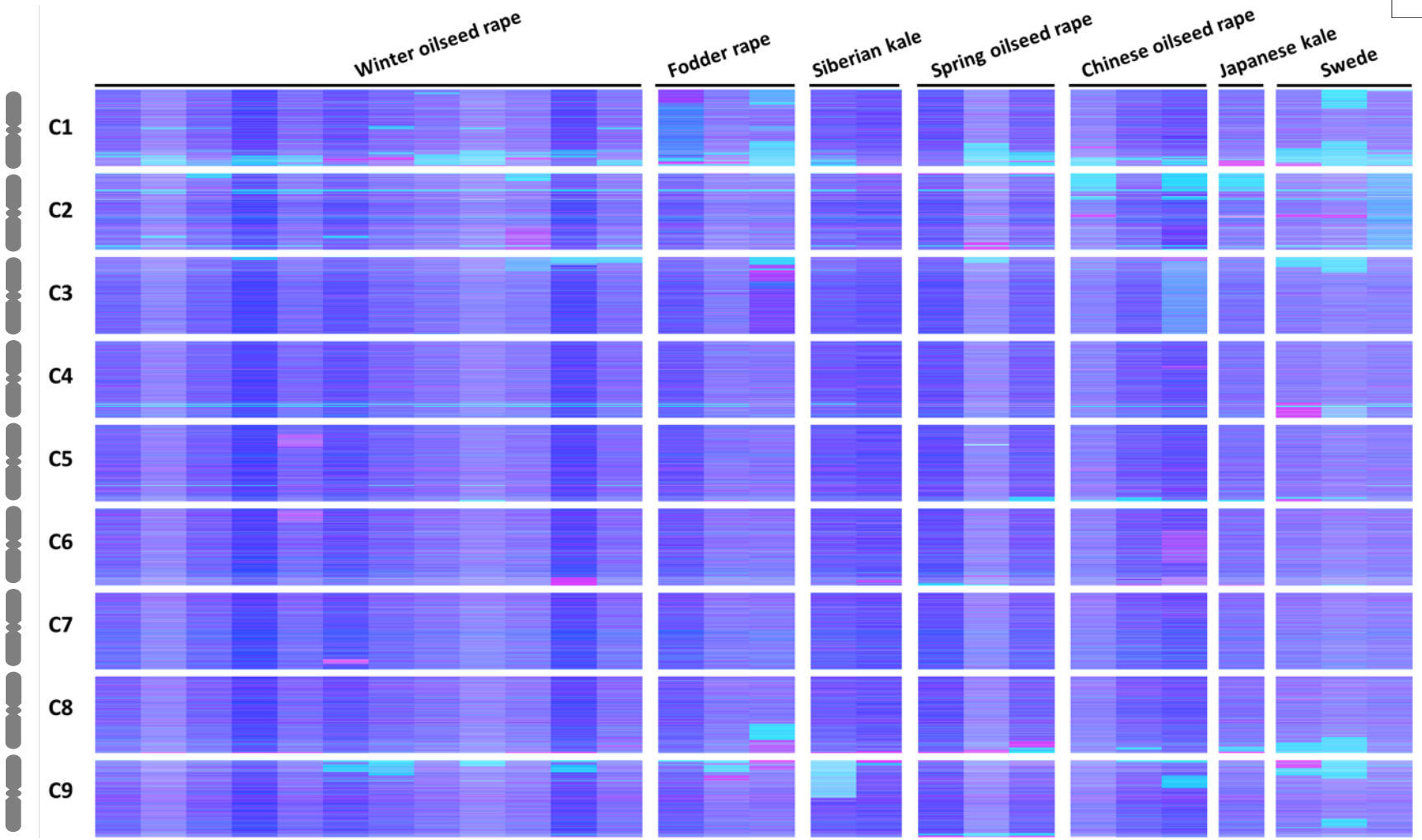
Homoeologous exchanges duplicating the A genome appear cyan, those duplicating the C genome appear magenta



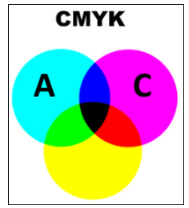
Confirmed by genome sequencing as structural variation

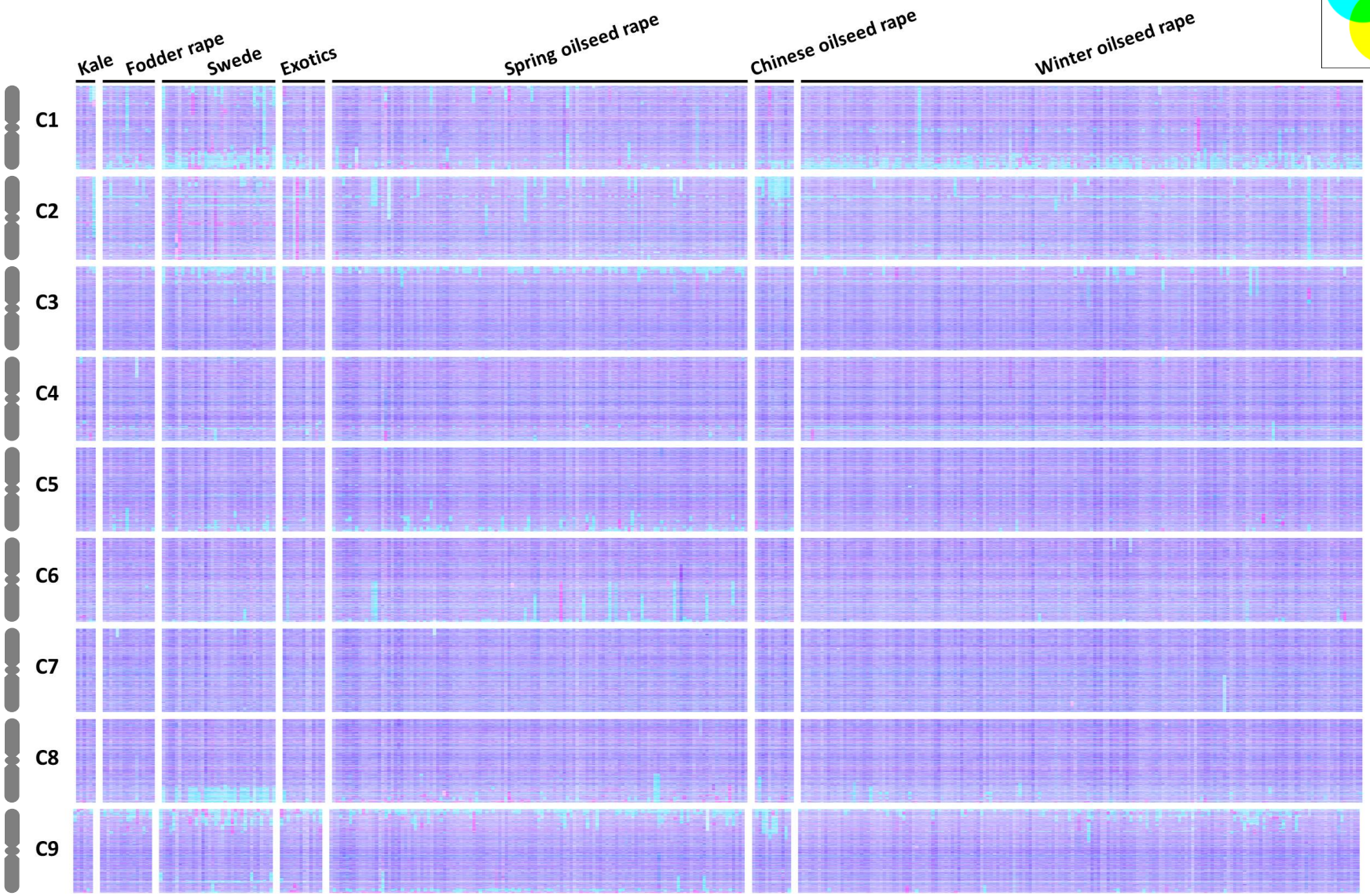
Homoeologous exchanges confirmed by genome skim sequencing





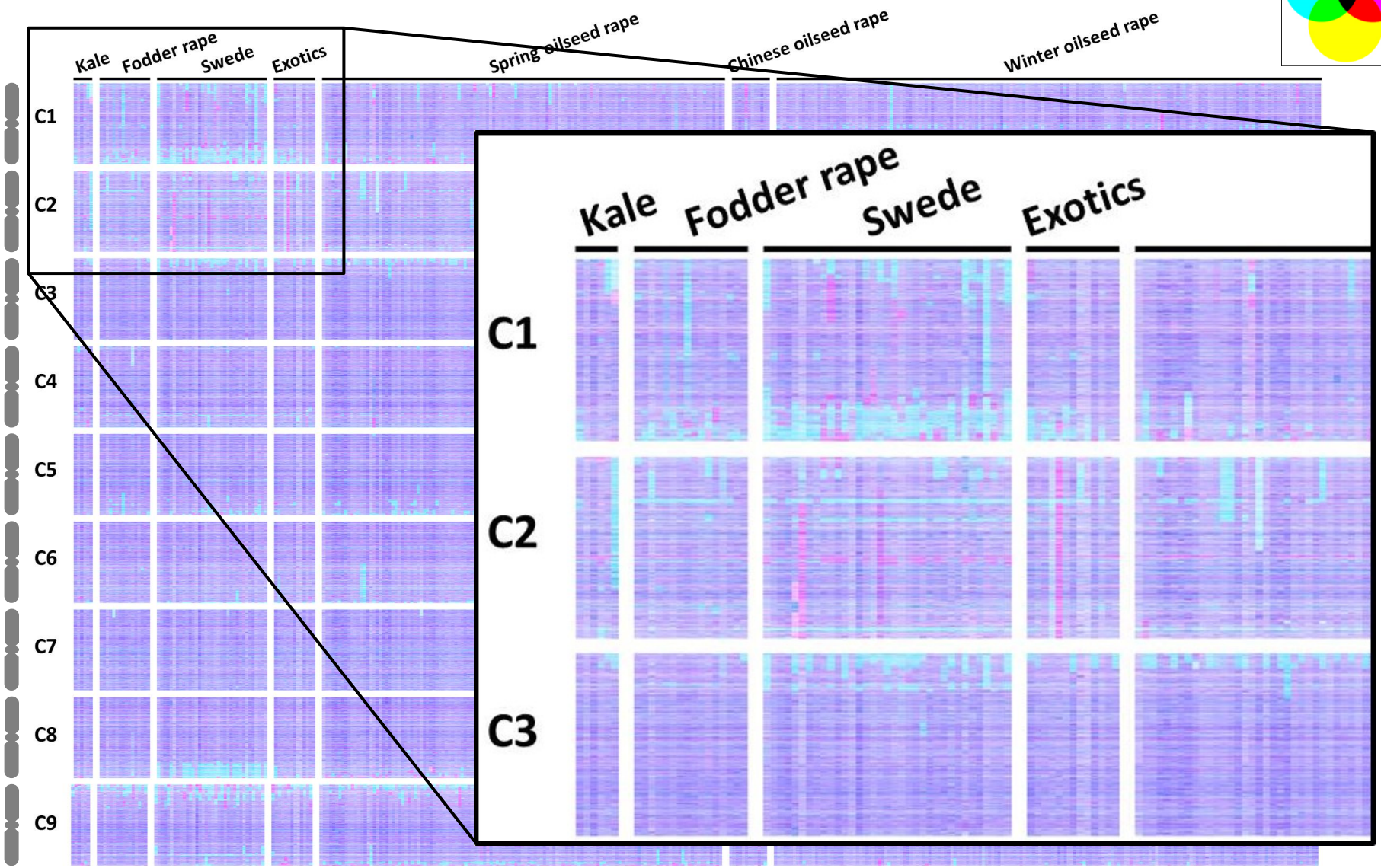
Homoeologous exchanges in *B. napus* RIPR diversity panel





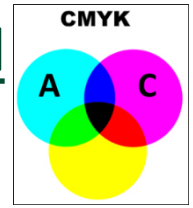
383 *B. napus* accessions

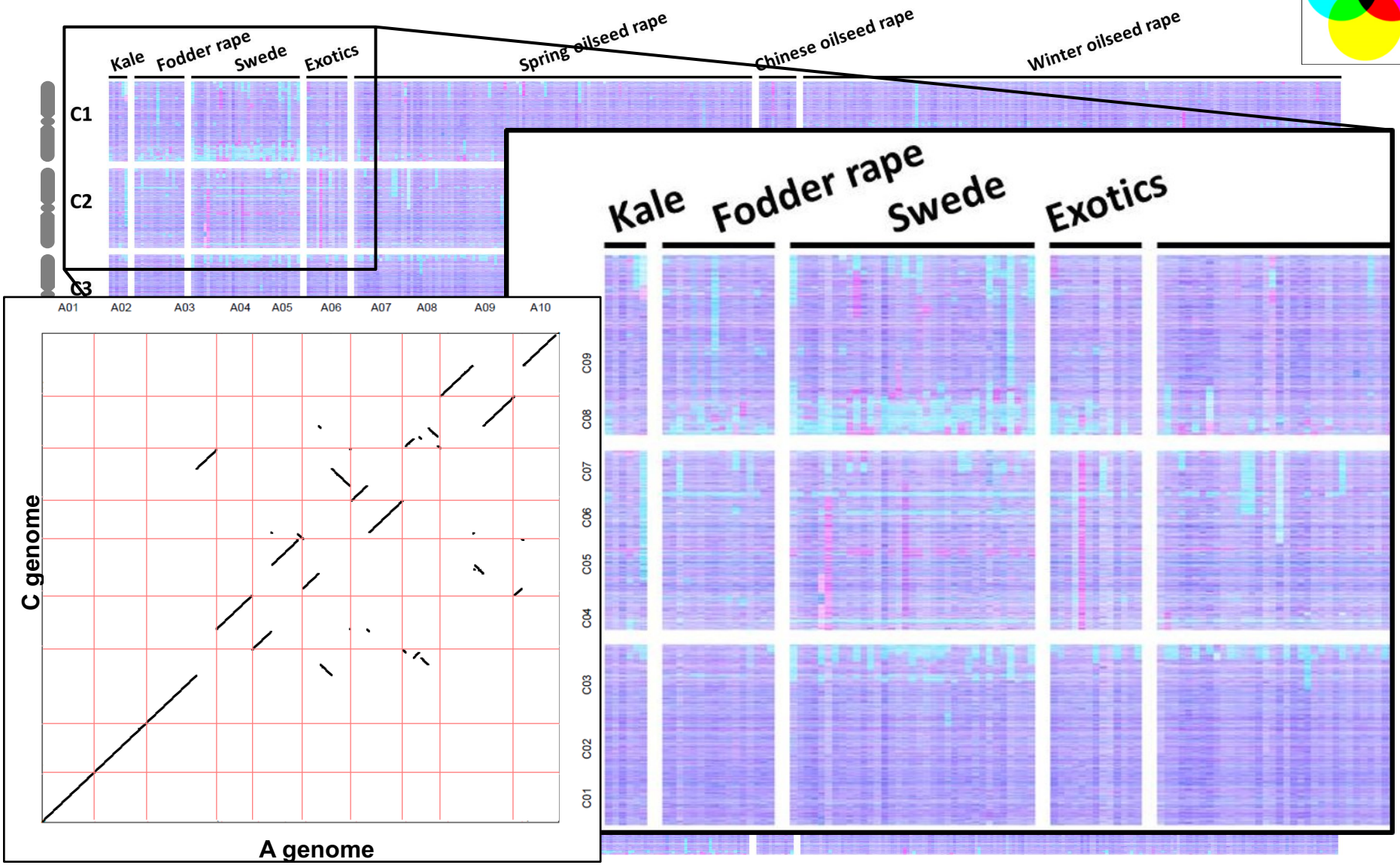
Homoeologous exchanges in *B. napus* RIPR diversity panel



383 *B. napus* accessions

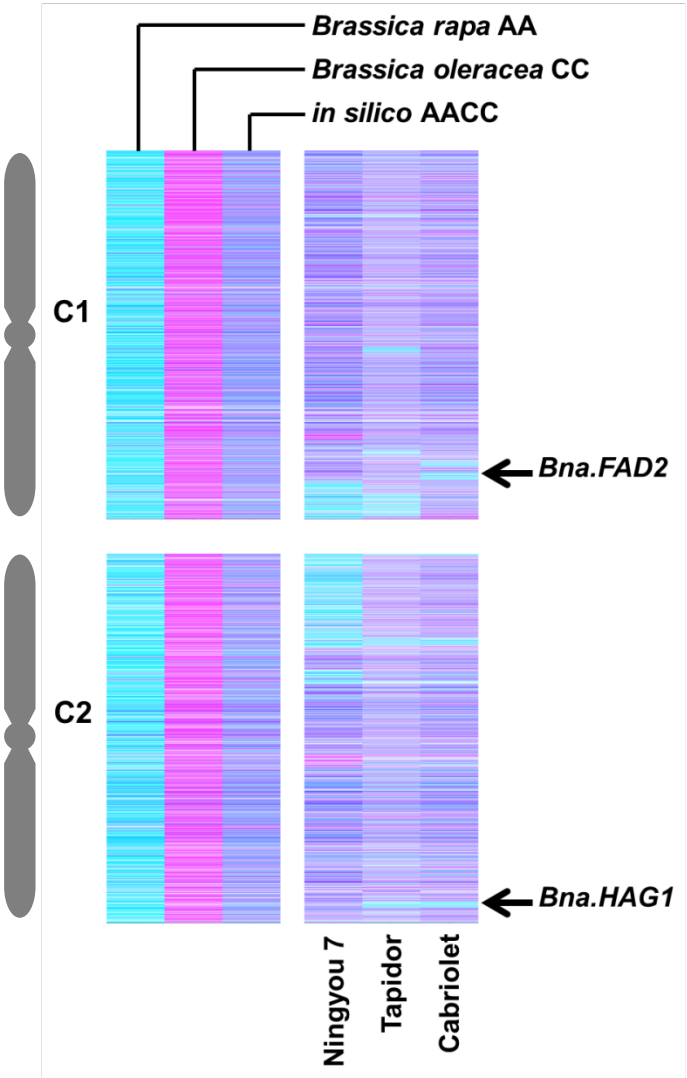
Homoeologous exchanges in *B. napus* RIPR diversity panel





383 *B. napus* accessions

Homoeologous exchanges affect crop traits



Substitution of active orthologue of *FAD2* by inactive orthologue reduces seed oil PUFA content

Substitution of active orthologue of *HAG1* by inactive orthologue reduces seed glucosinolate content

Plant Biotechnology Journal

Extensive homoeologous genome exchanges in allopolyploid crops revealed by mRNAseq-based visualization. Plant Biotechnology J. 15:594-604, 2017

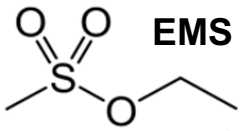
Natural and induced genome structural variation in oilseed rape

Ian Bancroft

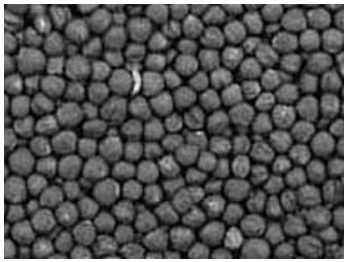
17th June, 2019

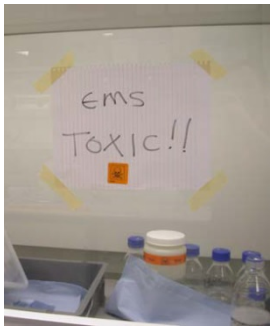
- Understanding and dealing with genome complexity in *Brassica* species
- Genome structural variation resulting from homoeologous exchange
- **Genome structural variation resulting from ionising radiation**

Genomics-led predictive mutation breeding



Treat seeds

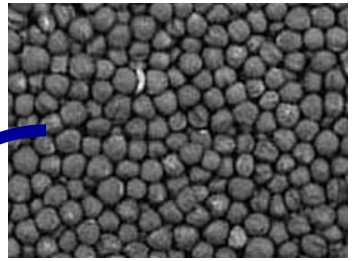




Sow at high density

Grow and self plants from treatments with ~50% kill

Collect seed from M_1 plants



Grow

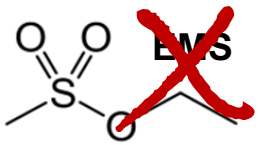
Make DNA from a leaf of each M_2 plant from which seeds will be collected

Collect selfed seed from one M_2 plant per M_1 individual

Panel of C/G to T/A mutations

Seed stocks

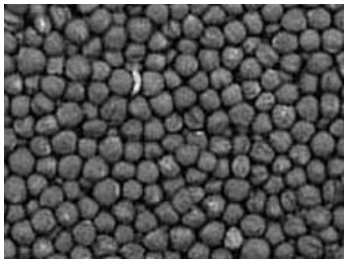
Genomics-led predictive mutation breeding

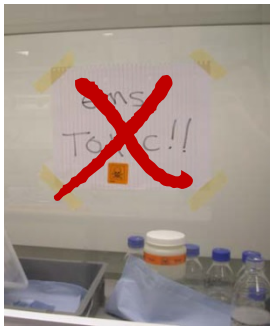


γ Radiation

IAEA
International Atomic Energy Agency

Treat seeds

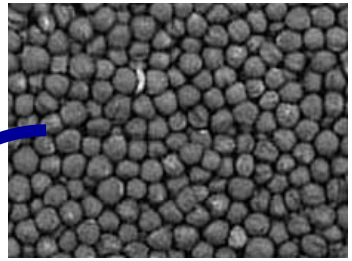




Sow at high density

Grow and self plants from treatments with ~50% kill

Collect seed from M_1 plants



Grow

Make DNA from a leaf of each M_2 plant from which seeds will be collected

Collect selfed seed from one M_2 plant per M_1 individual

Point, frame-shift and deletion mutations

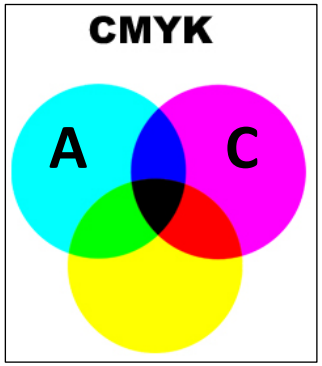
Panel of C/G to T/A mutations

Seed stocks

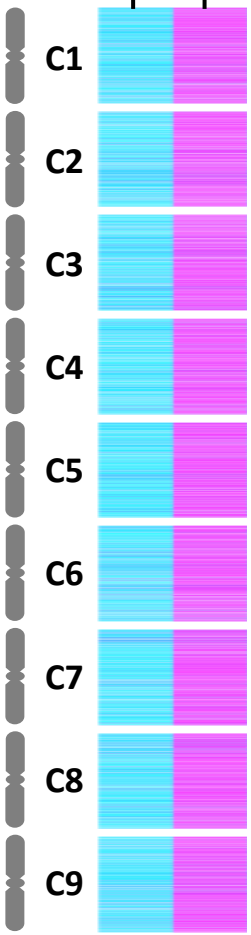
Visualizing genome dosage variation using mRNAseq data

A (*B. rapa*) = cyan

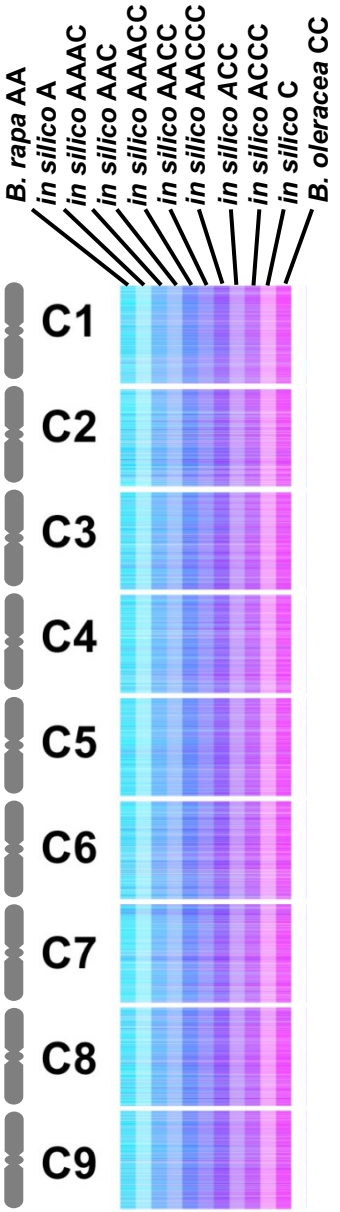
C (*B. oleracea*) = magenta

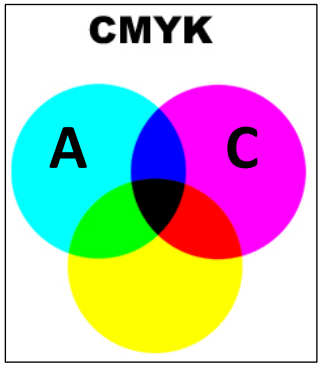


Gene pairs arranged by coordinate in one of the genomes

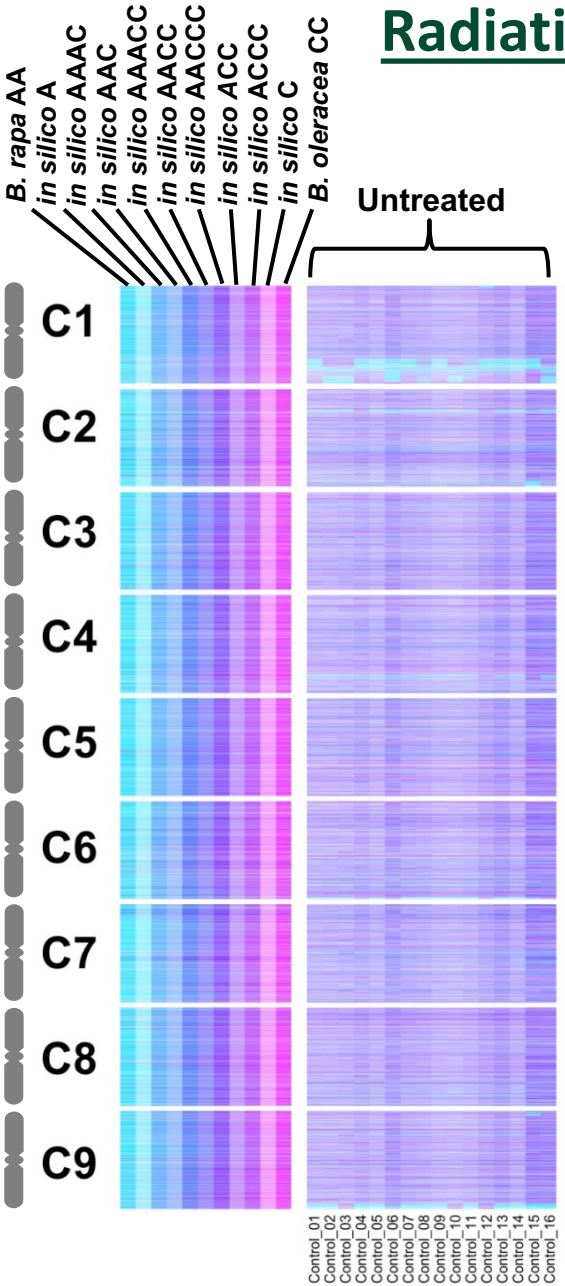


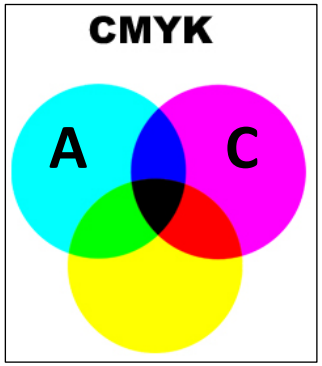
Radiation-induced structural variation





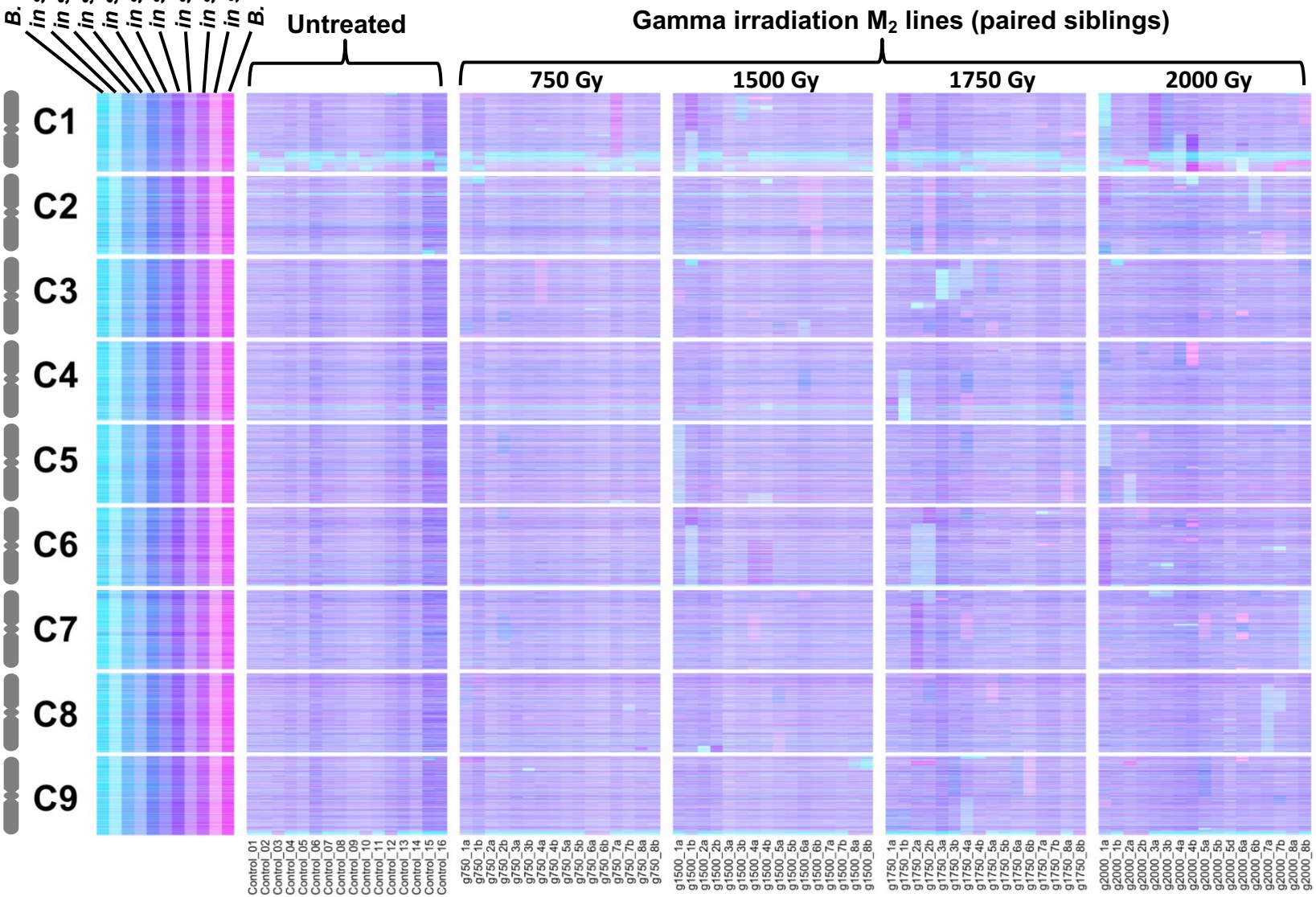
Radiation-induced structural variation





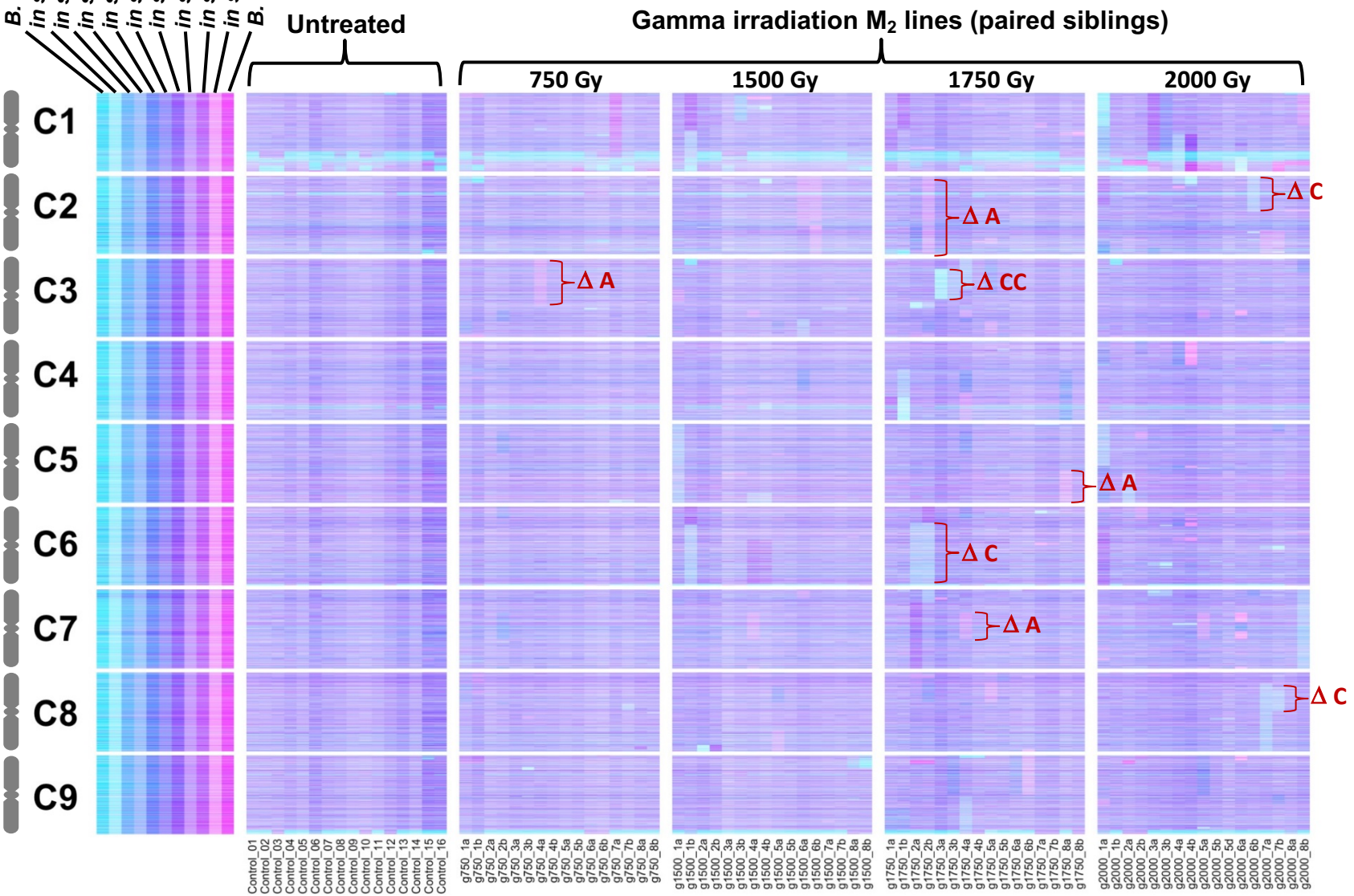
Radiation-induced structural variation

More lesions induced at higher doses



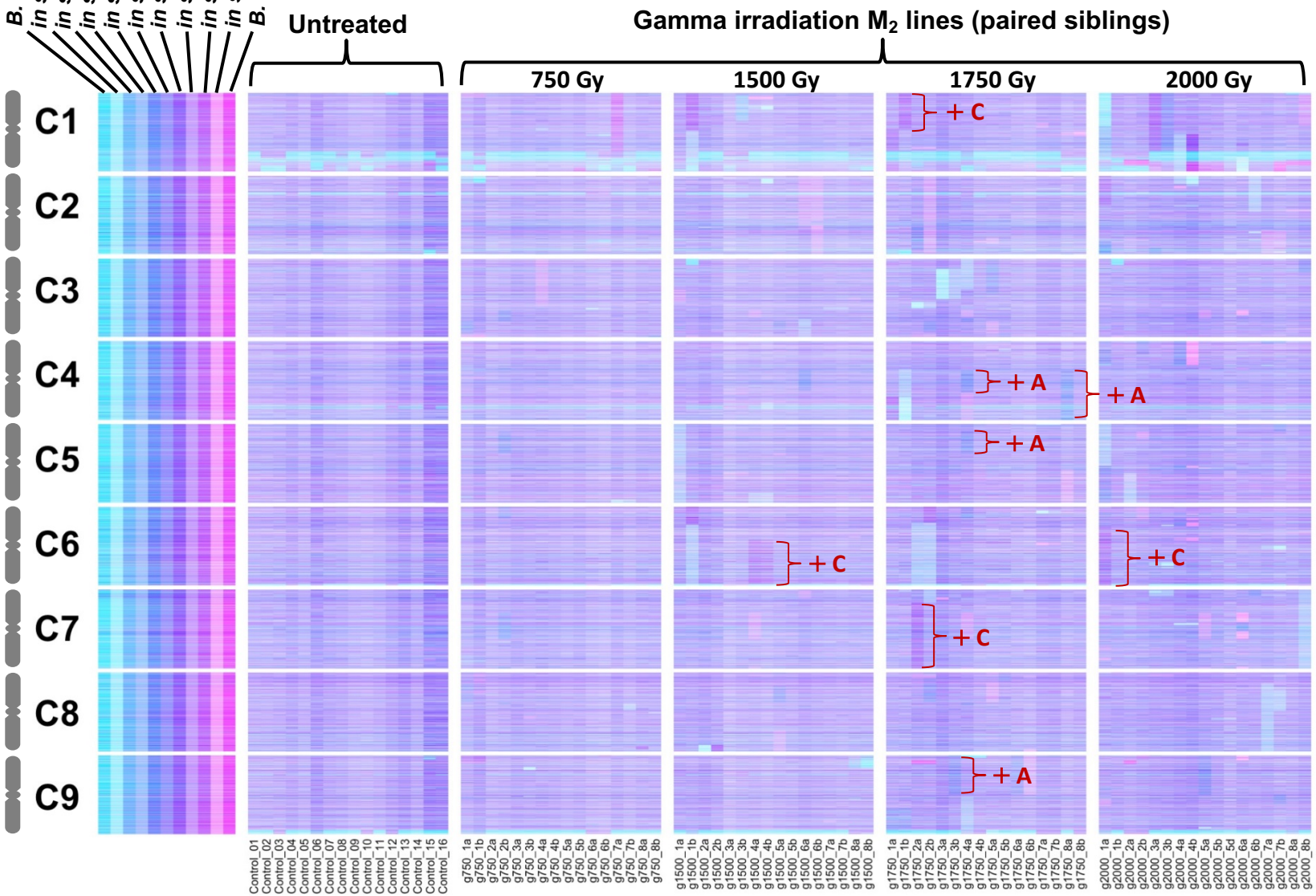
Radiation-induced structural variation

Large-scale radiation-induced deletions



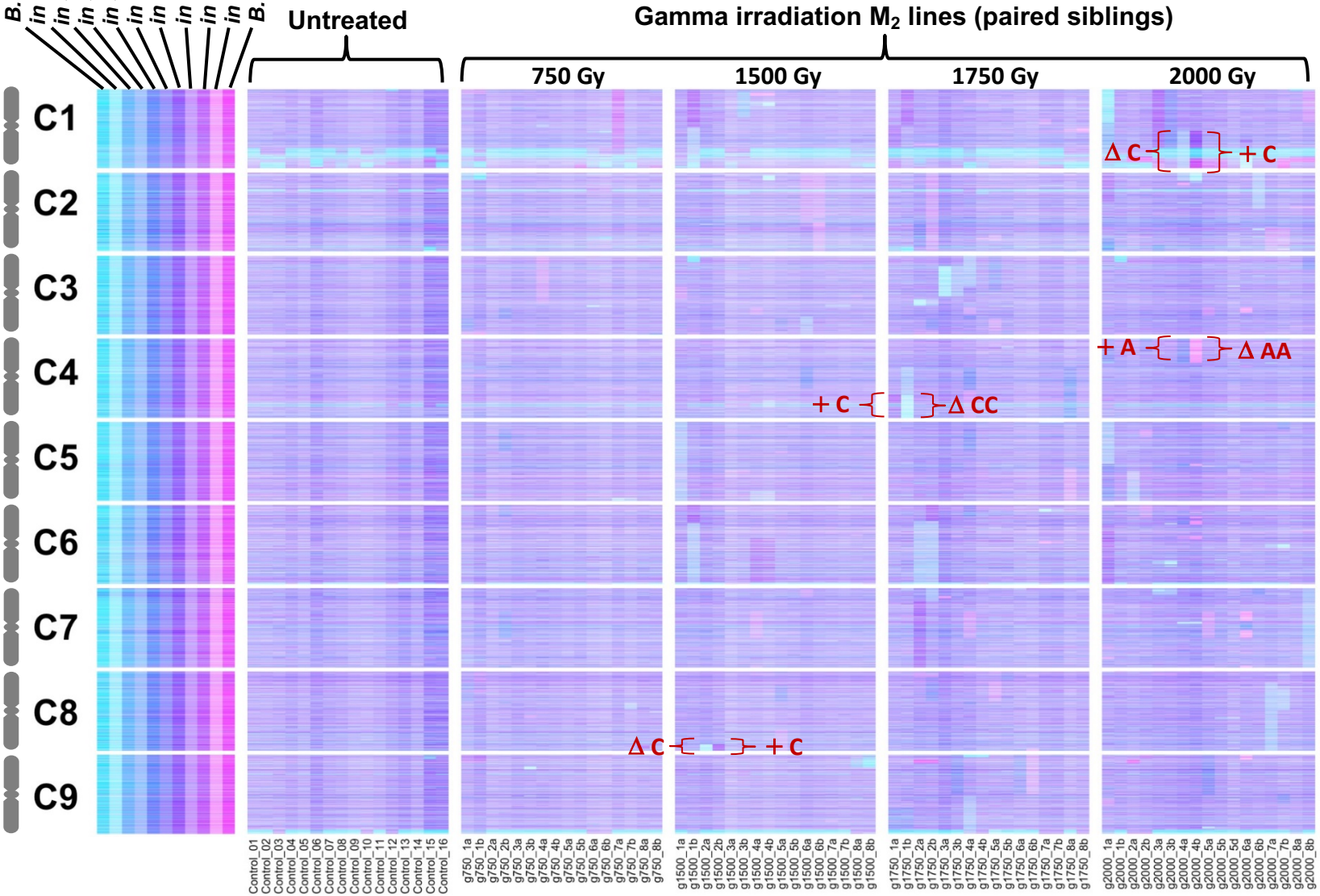
Radiation-induced structural variation

Large-scale radiation-induced duplications



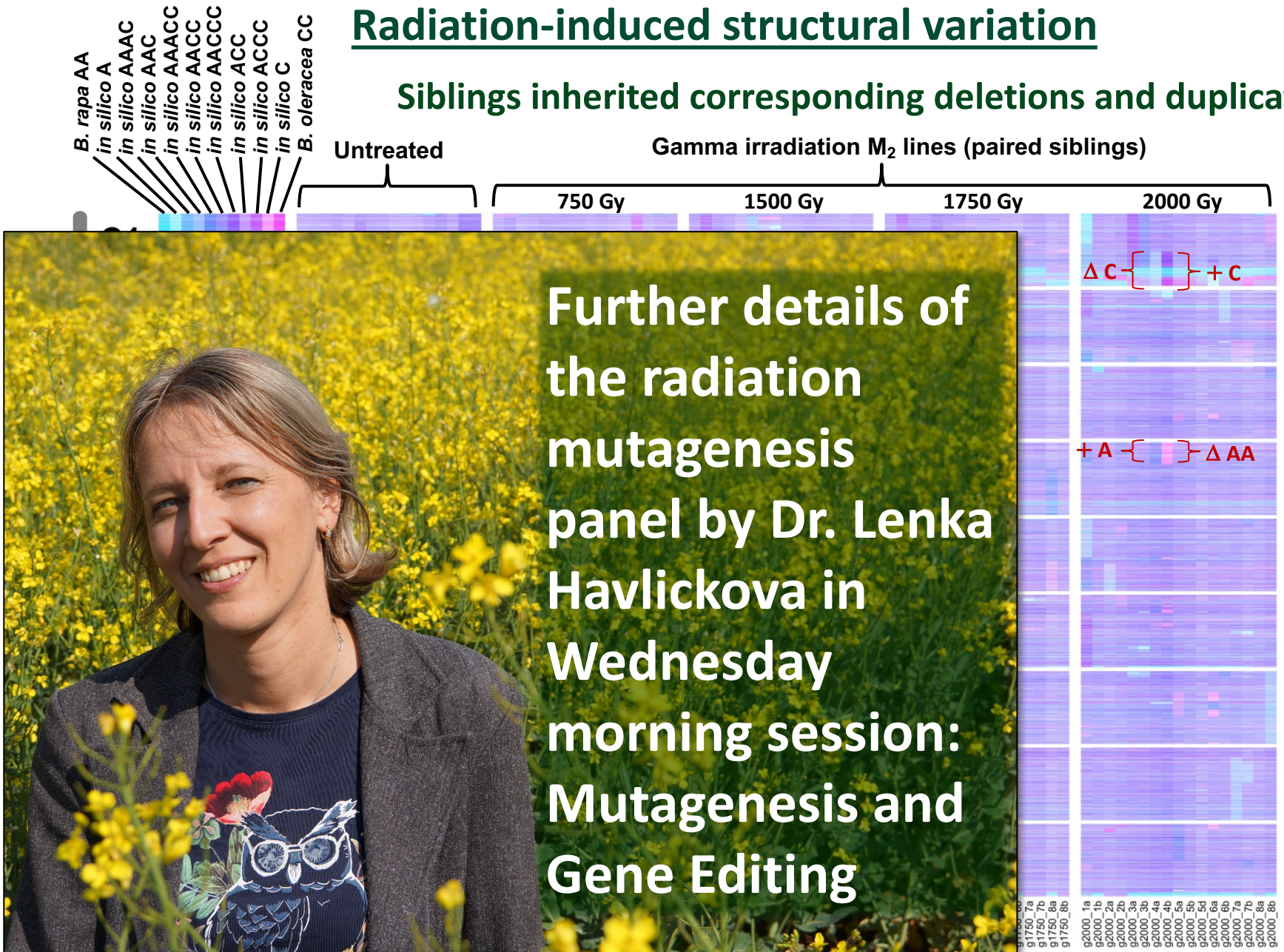
Radiation-induced structural variation

Siblings inherited corresponding deletions and duplications



Radiation-induced structural variation

Siblings inherited corresponding deletions and duplications



Acknowledgements

BBSRC BB/L027844/1 EvoGenapus; BBSRC BB/L002124/1 RIPR; BBSRC BB/L011751/1 CGAT

National Capability in Genomics (BB/J010375/1) Platforms and Pipelines Group at The Genome Analysis Centre

High-Throughput Genomics Group at the Wellcome Trust Centre for Human Genetics (Wellcome Trust 090532/Z/09/Z)