Article:
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Organisation of the genome sequence of the polyploid crop species *Brassica juncea*

To the Editor:

A draft genome sequence of *Brassica juncea*, a member of the Brassicaceae and therefore a species benefiting from the functional genomics advances in the "model" species *Arabidopsis thaliana*, was reported recently by Yang et al[1]. *B. juncea* is a recently-formed allotetraploid, the diploid progenitors of which were mesohexaploids: *B. rapa* (which contributed the A genome) and *B. nigra* (which contributed the B genome). In addition to underpinning future trait-oriented work in this important crop species, which includes both vegetable and oil types, the sequences were analysed for characteristics of genome evolution under crop selection. For both purposes, the genome sequences must represent with high fidelity (though not perfectly in "draft" form), both the gene complement and gene order of the species. As a model for addressing the challenges of achieving an adequate representation of the latter for allopolyploid crops, the construction methodology employed short shotgun sequence reads, single-molecule long reads, BioNano sequencing and high-resolution genetic mapping.

A particular problem in genetic mapping in polyploids is the confounding effects of single nucleotide polymorphisms (SNPs) resulting from inter-homoeologue polymorphisms (IHPs), which are much more abundant than the allelic SNPs that are needed for genetic (linkage) mapping. In species such as *B. juncea* and *B. napus* (which also contains the A genome contributed by a *B. rapa* progenitor, but in this species in combination with the C genome contributed by a *B. oleracea* progenitor), further complications arise from the mesohexaploid nature of the genomes of the diploid progenitors, resulting in inter-paralogue polymorphisms (IPPs). However, so long as sufficient sequencing redundancy has been obtained to overcome stochastic sampling effects and differentiate allelic SNPs (which will segregate across a linkage mapping population) from IHPs and IPPs (which should be invariant), the confounding effects can be overcome. Even using transcriptome sequence data, robust methodologies have been developed in *B. napus* to score allelic SNPs for high resolution linkage map construction and to underpin association genetics[2-4].

We aimed to test the fidelity with which the genome sequence reported by Yang et al[1] represents the gene order of *B. juncea* by comparing that with our own estimates using an AB *Brassica* genomics platform constructed as for our AC *Brassica* genomics platform[5], based on the sequences of the progenitor species *B. rapa* (A genome) and *B. nigra* (B genome) (Supplementary Note). For the test, we used the CDS gene models from (1) the AB *Brassica* genomics platform and (2) the *B. juncea* genome sequence of Yang et al[1]

(denoted J genome) as the reference sequences for mapping Illumina mRNAseq reads from 106 lines of the *B. juncea* VHDH mapping population[6, 7] with variant-calling essentially as described previously for *B. napus*[2, 3, 4] (Supplementary Note, Life Sciences Reporting Summary). The SNP scoring strings were filtered to retain only simple SNPs (i.e. polymorphisms between resolved bases) and displayed in genome sequence order as genome-ordered graphical genotypes (GOGGs). If the order in the genome sequence of the genes in which the polymorphisms are scored is correct, the result should resemble a genetic linkage map, i.e. with few instances of nearby alternating parental alleles in individual recombinant lines. The GOGGs generated comprised 33,059 scored SNP markers for the AB *Brassica* genomics platform and 29,834 scored SNP markers for the *B. juncea* genome sequence reported by Yang et al[1] (Supplementary Figure 1). An example, for chromosome J1 of Yang et al[1] compared with A1 from the AB *Brassica* genomics platform, is shown in Figure 1. The results of this simple quality control assessment show that the authentic arrangement of genes in *B. juncea* matches very well that of their orthologues in the AB reference, and hence in the progenitor species, but they also show that the *B. juncea* genome sequence reported by Yang et al[1] is extensively mis-assembled. We note also that the internationally-agreed nomenclature for B genome chromosomes[8], which we followed for the AB resource, was not followed for the *B. juncea* genome sequence.

The assembly and validation methodology described by Yang et al[1] sounds plausible and may well be taken as a model to follow for other polyploid crops, so why was it ineffective? Detailed inspection of the GOGGs suggests two problems: chimeric assemblies (where collinearity with the genome of *A. thaliana* breaks down) and mistaking IHPs or IPPs for allelic SNPs when undertaking the linkage mapping with the 5,333 “bin markers” or in the pre-existing linkage map (where collinearity with the genome of *A. thaliana* is maintained). The bin markers appear to have been scored on the basis of only ~0.7-fold redundant genome re-sequencing, which wouldn’t be sufficient (in SNP scoring) to differentiate the differing types of polymorphisms (IHPs, IPPs and allelic SNPs) in polyploid genomes. It is less clear why use of the single-molecule long reads and BioNano sequencing failed to detect the chimerism.

Although the draft of the *B. juncea* genome sequence reported by Yang et al[1] does not appear to faithfully represent the organization of that genome, undermining analyses requiring positional information (such as illustrated in Figures 1, 2a, 3 and 4a in the report of Yang et al[1]), it could easily be improved by exploiting the linkage mapping information depicted by the GOGGs. Indeed, the B genome component of our AB *Brassica* genomics

platform was based on the *B. nigra* genome sequence reported by Yang et al[1] alongside that of *B. juncea* and was developed by splitting it (into 175 segments) and re-organising based on the transcriptome SNPs scored across the *B. juncea* VHDH mapping population. The assessment of genome assemblies based on GOGGs therefore not only represents an important quality control measure, it also provides a solution where problems are found. Linkage mapping populations have been a fundamental resource for the genetic analyses of traits in crop so will usually be available already in crop species for which genome sequencing is being undertaken. To help assure the quality of genome sequences, we would like to propose an expectation that validation by means of GOGGs should be incorporated into the assembly workflow for polyploid crop genomes.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

Z.H. and I.B. designed the study and analysed the data. I.B. wrote the manuscript and Z.H. read and approved the manuscript for publication.

COMPETING FINANIAL INTERESTS

The authors declare no competing financial interests.

DATA AVAILABILITY

The *B. juncea* mRNAseq data used for production of the graphical genotypes have been deposited in the SRA data library under project ID PRJNA471033.

Zhesi He & Ian Bancroft

Department of Biology, University of York, Heslington, York, YO10 5DD, UK

Email: ian.bancroft@york.ac.uk

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2. Trick, M. *et al, Plant Biotechnol. J.* **7**, 334-346 (2009).
3. Bancroft, I *et al, Nat. Biotechnol.* **29**, 762-766 (2011).
4. Harper, A.L. *et al, Nat. Biotechnol.* **30**, 798-802 (2012).

5. He, Z. *et al. Data in Brief* **4**, 357-362 (2015).
6. Paritosh *et al. BMC Genomics* **15**, 396 (2014).
7. He, Z. *et al. Plant Biotechnol. J.* **15**, 594-604 (2017).
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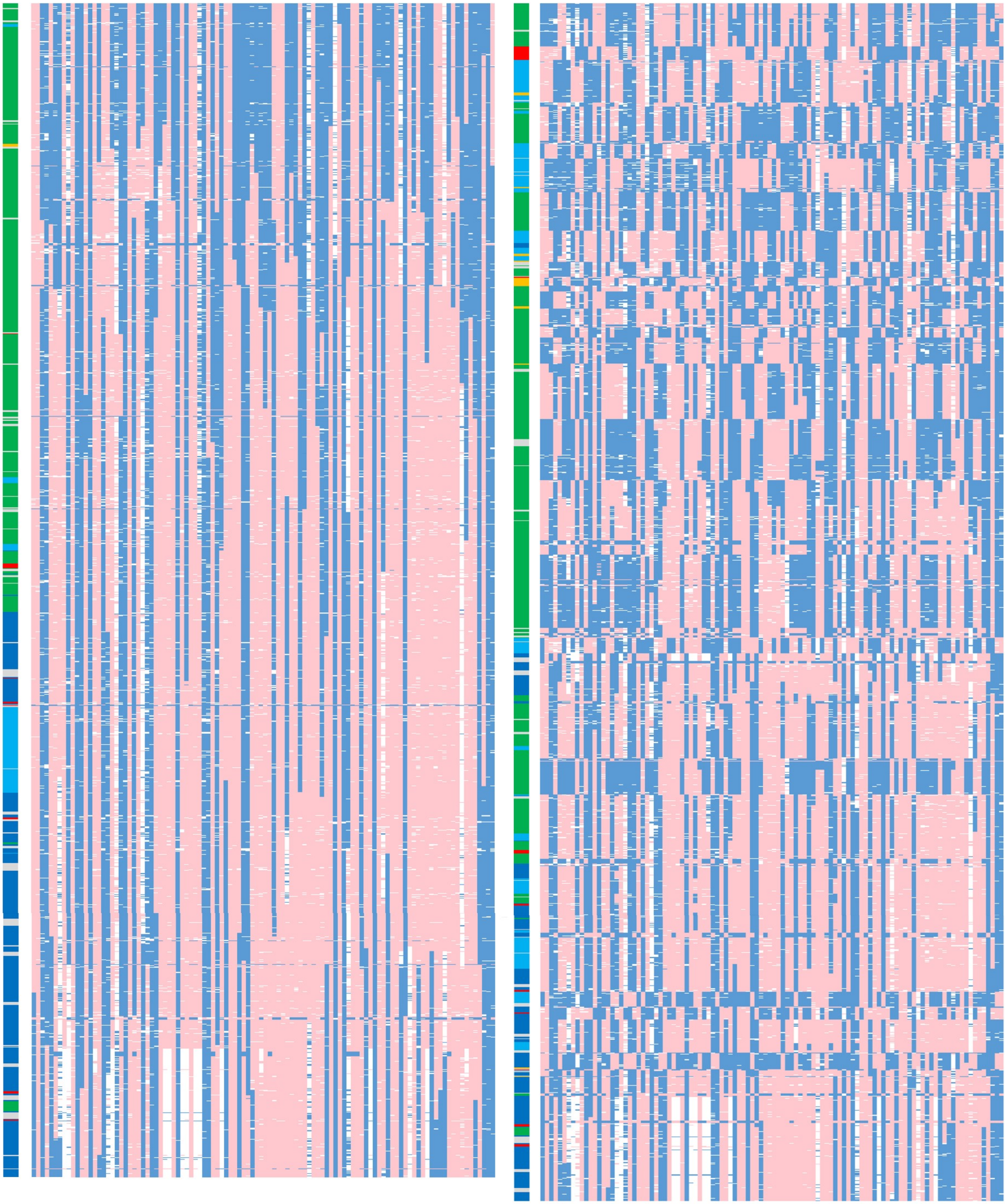
FIGURE LEGENDS

Figure 1. Quality control assessment of genome sequence organisation of *B. juncea* using genome-ordered graphical genotypes, chromosomes A1 and J1 as an example. Graphical genotypes are shown for transcriptome SNP markers scored across 106 lines of the VHDH mapping population with Heera alleles in coral, Veruna alleles in blue and missing scores in white. The genotypes for 2004 and 2040 markers are shown for chromosomes A1 and J1, respectively. The multi-coloured bars are colour-coded to the chromosome of the top BLAST sequence similarity match in *Arabidopsis thaliana* of the *Brassica* gene model in which the SNP is scored (light blue = chromosome 1, orange = chromosome 2, dark blue = chromosome 3, green = chromosome 4, red = chromosome 5, light grey = no BLAST hit with E-value < 1e30).


A1
J1

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Zhesi He & Ian Bancroft
Department of Biology, University of York, Heslington, York, YO10 5DD, UK
Email: ian.bancroft@york.ac.uk

Supplementary note

Production of genome-ordered graphical genotypes for *B. juncea*

For our genomics platform, we used the A genome component of the *B. napus* AC Pan-transcriptome resource[1], which was based on the version 2.0 *B. rapa* genome sequence[2], with minor updates, and a newly-developed B genome component. A total of 88,713 CDS models were extracted from the genome resources to form the AB transcriptome reference sequence. Illumina mRNAseq reads from 106 lines of the *B. juncea* VHDH mapping population[3] were mapped with this AB transcriptome reference and SNPs scored using methodology developed and described previously for *B. napus*[4-7]. The SNP scoring strings were filtered to remove hemi-SNPs (i.e. instances where the most frequent or second most frequent allele scored is an ambiguity code representing more than one base). The remaining SNPs were output to MS Excel files with each row representing, in order: (1) the SNP identifier; (2) genome coordinate (chromosome_start nucleotide_end nucleotide) of the CDS gene model in which the SNP was scored; (3) best BLAST nucleotide sequence similarity match of the gene model with *Arabidopsis thaliana* gene models (with conditional formatting coded to the chromosome of the *A. thaliana* gene model); (4) the name of the gene model in which the SNP was scored; (5) simple SNP flag; (6) nucleotide allele in Heera parent; (7) nucleotide allele in Veruna parent; (8-113) the graphical genotypes as the parental allele calls for each of the 106 lines of the VHDH mapping population (with A corresponding to the Heera allele, B corresponding to the Veruna allele and conditional formatting coded to A or B allele). The spreadsheet was sorted by genome coordinate of the gene models in which the SNPs were scored, row height was set to 1 pixel and screen shot images compiled in MS PowerPoint to display the genome-ordered graphical genotypes (GOGGs).

Re-assembly of the *B. nigra* genome

The *B. nigra* genome sequence as reported by Yang et al[8] was first imaged as a GOGG, in combination with the *B. rapa*-derived A genome and using Illumina mRNAseq reads from 106 lines of the *B. juncea* VHDH mapping population[3], as described above. This revealed extensive mis-assembly as disjoint blocks of markers with consistent graphical genotypes.

The mis-assembled blocks of scored markers were rearranged manually in the MS Excel spreadsheet underlying the GOGG. Based on the end-most genes in the blocks with consistent genotypes, positions in the chromosome assemblies were identified visually for splitting, using an MS Excel spreadsheet list of gene models arranged by genome coordinate. The split sites were chosen either as the mid-point between genes representing the positions of discontinuities in collinearity with the *A. thaliana* genome (indicative of chimeric scaffolds) or, where collinearity with *A. thaliana* was maintained, as the mid-point between genes representing the positions of discontinuities in gene model nomenclature (indicative of mis-mapping of scaffolds to homoeologous/paralogous positions). We developed the new B genome resource by splitting the published genome into 175 segments and re-concatenating them to be consistent with the linkage mapping shown by the SNP scoring strings in the graphical genotypes (Supplementary Table 1). The chromosomes were then re-numbered to match the international convention[9]. New coordinates for the gene models were generated based on best BLAST similarity match in the B genome re-assembly (E-value < 1e30). Finally, the assembly was validated by producing a GOGG based on the new genome coordinates of the gene models, as shown in the B genome section of Supplementary Figure 1.

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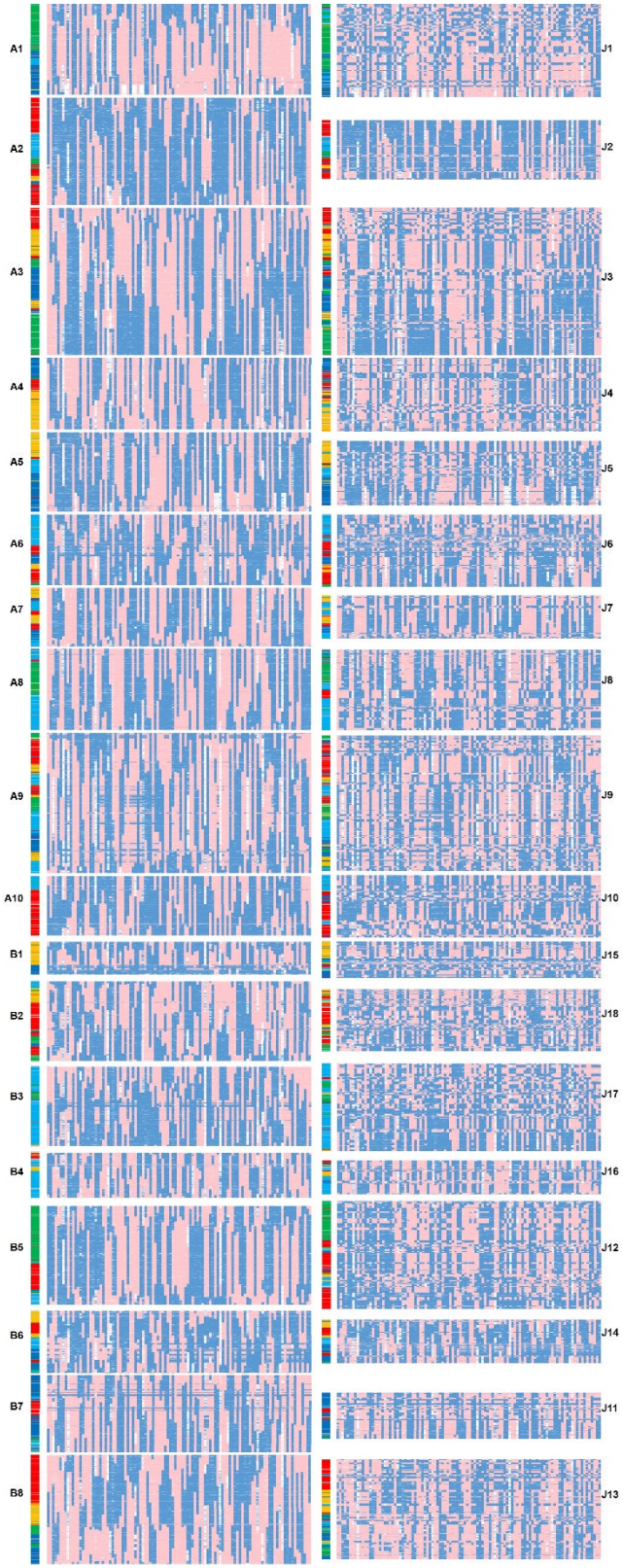
9. King, G. https://dx.doi.org/10.4226/47/5afb8519d194c (2010).

Supplementary Figures



Supplementary Figure 1. Quality control assessment of genome sequence organisation of *B. juncea* using genome-ordered graphical genotypes. Graphical genotypes are shown for transcriptome SNP markers scored across 106 lines of the VHDH mapping population with

Heera allele in coral, Veruna alleles in blue and missing scores in white. The graphical genotypes are organised by linkage group and labelled using the international convention for *Brassica* chromosome nomenclature (A1 to A10 and B1 to B8; genotypes for 33,059 markers shown) or the nomenclature used by Yang et al[8] (J1 to J18; genotypes for 29,834 markers shown). The multi-coloured bars are colour-coded to the chromosome of the top BLAST sequence similarity match in *Arabidopsis thaliana* of the *Brassica* gene model in which the SNP is scored (light blue = chromosome 1, orange = chromosome 2, dark blue = chromosome 3, green = chromosome 4, red = chromosome 5, light grey = no BLAST hit with E-value < 1e30).

Supplementary tables

Supplementary Table 1. Re-build specification for the *B. nigra* genome. Nucleotide coordinates for blocks of genome sequence refer to the original chromosome assemblies of Yang et al[8]. The chromosome (Chr) nomenclature in the re-assembly corresponds to that of Yang et al[8]. The international nomenclature[9] B1, B2, B3, B4, B5, B6, B7 and B8 correspond to Yang et al[8] chromosomes B5, B8, B7, B6, B2, B4, B1 and B3, respectively.

Chr	Block	Start nucleotide	Stop nucleotide	Orientation
B01	1	B03_007582801	B03_007885645	fwd
B01	2	B08_032495400	B08_032871719	fwd
B01	3	B01_009064427	B01_009778984	rev
B01	4	B01_007582779	B01_008477874	rev
B01	5	B01_007217776	B01_007582778	fwd
B01	6	B01_006942609	B01_007217775	rev
B01	7	B01_008477875	B01_009064426	rev
B01	8	B01_002065798	B01_006485396	rev
B01	9	B01_000000001	B01_000498900	fwd
B01	9.5	B02_013890010	B02_014921067	fwd
B01	10	B08_018602377	B08_018802048	fwd
B01	11	B06_010596212	B06_010889573	fwd
B01	12	B06_027053519	B06_027293964	rev
B01	13	B07_004574023	B07_004606846	fwd
B01	14	B06_002493704	B06_002720701	fwd
B01	15	B04_015647478	B04_015901751	fwd
B01	16	B01_009778985	B01_010343995	rev
B01	17	B01_000498901	B01_002065797	fwd
B01	18	B01_012483451	B01_013591901	fwd
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B01	23	B04_031974885	B04_032085266	rev
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B01	25	B01_029798228	B01_030315192	fwd
B01	26	B01_030411022	B01_030653157	rev
B01	27	B01_030653158	B01_999999999	rev
B02	28	B07_021173313	B07_022247982	fwd
B02	29	B02_003989485	B02_005436514	rev
B02	30	B02_005436515	B02_006692969	rev
B02	31	B02_002437988	B02_003989484	rev
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B02	33	B06_017792993	B06_017875548	fwd
B02	34	B02_000450561	B02_002437987	fwd
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B03	69.2	B03_016414470	B03_017979820	fwd
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B03	69.6	B03_012624233	B03_012929964	rev
B03	69.7	B06_026686062	B06_026697744	rev
B03	69.8	B03_009392611	B03_011730707	rev
B03	69.9	B03_007885646	B03_008708174	rev
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B08	160	B05_031539984	B05_032225571	fwd
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B08	162	B08_025092391	B08_030117930	fwd
B08	163	B08_032871720	B08_032887280	fwd
B08	164	B08_030117931	B08_030818427	fwd
B08	165	B08_032887281	B08_037718028	fwd
B08	166	B08_037955294	B08_999999999	fwd