

Genes derived from ancient polyploidy have higher genetic diversity and are associated with domestication in *Brassica rapa*

Xinshuai Qi^{1*} , Hong An^{2*} , Tara E. Hall¹, Chenlu Di¹, Paul D. Blischak¹ , Michael T. W. McKibben¹ , Yue Hao³ , Gavin C. Conant^{3,4} , J. Chris Pires²  and Michael S. Barker¹ 

¹Department of Ecology & Evolutionary Biology, University of Arizona, Tucson, AZ 85721, USA; ²Division of Biological Sciences, University of Missouri, Columbia, MO 65211, USA;

³Bioinformatics Research Center, North Carolina State University, Raleigh, NC 27695, USA; ⁴Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695, USA

Summary

Author for correspondence:
Michael S. Barker
Email: msbarker@arizona.edu

Received: 4 June 2020
Accepted: 30 November 2020

New Phytologist (2021) 230: 372–386
doi: 10.1111/nph.17194

Key words: *Brassica*, domestication, genetic diversity, genomics, polyploidy, whole genome duplication.

- Many crops are polyploid or have a polyploid ancestry. Recent phylogenetic analyses have found that polyploidy often preceded the domestication of crop plants. One explanation for this observation is that increased genetic diversity following polyploidy may have been important during the strong artificial selection that occurs during domestication.
- In order to test the connection between domestication and polyploidy, we identified and examined candidate genes associated with the domestication of the diverse crop varieties of *Brassica rapa*. Like all 'diploid' flowering plants, *B. rapa* has a diploidized paleopolyploid genome and experienced many rounds of whole genome duplication (WGD). We analyzed transcriptome data of more than 100 cultivated *B. rapa* accessions.
- Using a combination of approaches, we identified > 3000 candidate genes associated with the domestication of four major *B. rapa* crop varieties. Consistent with our expectation, we found that the candidate genes were significantly enriched with genes derived from the Brassicaceae mesohexaploidy. We also observed that paleologs were significantly more diverse than non-paleologs.
- Our analyses find evidence for that genetic diversity derived from ancient polyploidy played a key role in the domestication of *B. rapa* and provide support for its importance in the success of modern agriculture.

Introduction

Polyploidy, or whole genome duplication (WGD), has long been associated with crop domestication and diversity (Anderson, 1969; Lewis, 1980; Heiser, 1990; Hilu, 1993; Paterson, 2005; Udall & Wendel, 2006; Meyer *et al.*, 2012; Renny-Byfield & Wendel, 2014). Many desirable crop traits such as larger seed size, greater stress tolerance and increased disease resistance often are attributed to polyploidy (Lewis, 1980; Levin, 1983). A recent phylogenetic analysis found that domesticated plants have experienced significantly more polyploidy than their wild relatives (Salman-Minkov *et al.*, 2016). Polyploidy often precedes domestication and crops are nearly twice as likely to be domesticated in lineages with a relatively recent WGD compared to those without (Salman-Minkov *et al.*, 2016). Among the potential explanations for the relationship between polyploidy and domestication, the expanded genetic diversity and plasticity of polyploid plants may be especially advantageous during domestication and crop improvement (Otto & Whitton, 2000; Shimizu-Inatsugi *et al.*, 2017; Baduel *et al.*, 2018; Paape *et al.*, 2018; Monnahan *et al.*, 2019). Analyses in yeast have shown that

polyploid lineages not only have higher genetic diversity, but also adapt to new environments faster than their lower ploidal level relatives (Selmecki *et al.*, 2015). Likewise, the niches of polyploid plants evolve faster than their diploid relatives (Baniaga *et al.*, 2020). These features may collectively give polyploids unique advantages over diploids during domestication and the global spread of crops that occurred with human population expansion.

Although nearly 30% of plant species are recent polyploids, all flowering plants are paleopolyploids with varying histories of WGD (One Thousand Plant Transcriptomes Initiative, 2019). Given that the genetic consequences of polyploidy play out over time as genomes diploidize and paralogs fractionate (Otto, 2007; Arrigo & Barker, 2012; Baduel *et al.*, 2018), we may expect that the effects of polyploidy extend to diploidized species. Here, we sought to test whether past polyploidy is associated with increased diversity and domestication in the crop varieties of *Brassica rapa*. Like all flowering plants, the genome of *B. rapa* has been multiplied and fractionated many times over. The most recent polyploidization in the ancestry of *B. rapa* was a mesohexaploidy that occurred *c.* 9–28 Ma (Lukens *et al.*, 2004; Lysak *et al.*, 2005; Beilstein *et al.*, 2010; Wang *et al.*, 2011; Arias *et al.*, 2014; Cheng *et al.*, 2014; Franzke *et al.*, 2016). Further, *B. rapa* has been domesticated into many different crop varieties across

*These authors contributed equally to this work.

Europe and Asia. These include turnips, oil seeds, pak choi, Chinese cabbage, and muSTARD seeds. Many researchers have suggested that there is a connection between the mesohexaploidy and the diversity of *B. rapa* crop varieties (Cheng *et al.*, 2014, 2016), but the relationship has never been tested explicitly. Using recently sequenced transcriptomes from a diverse array of *B. rapa* accessions (Qi *et al.*, 2017), we tested if polyploid-derived regions of the genome are enriched with candidate genes associated with domestication. We also compared genetic variation in the polyploid vs non-polyploid derived regions of the *B. rapa* genome. Given the frequency of ancient polyploidy and its contribution to the evolution of plants, our analyses demonstrate the key role of polyploidy in the domestication of *B. rapa* and provide support for its importance in the success of modern agriculture.

Materials and Methods

Data sources

RNA-seq data for this study were generated previously by our group (Qi *et al.*, 2017). Based on previous population genomics analyses (Qi *et al.*, 2017), a total of 102 *Brassica rapa* accessions (Supporting Information Table S1) were selected from the USDA GRIN database (<http://www.ars-grin.gov/>) or from the author's collection to represent the five major *B. rapa* genetic groups. The five genetic groups include an earlier derived Europe and Central Asia group, represented by turnip (TN, *B. rapa* and *B. rapa* subsp. *rapa*, 22 accessions), and four derived *B. rapa* groups represented by: Pak choi (PC, *B. rapa* subsp. *chinensis*, 25 accessions); Chinese cabbage (CC, *B. rapa* subsp. *pekinensis*, 28 accessions); Indian sarson (IS, *B. rapa* subsp. *trilocularis* and *B. rapa* subsp. *dichotoma*, 20 accessions); and toria (TO, seven accessions). The last four genetic groups diverged from the TN group *c.* 2400–4100 yr ago after the initial *B. rapa* domestication in European-Central Asia (Qi *et al.*, 2017). Two *B. oleracea* accessions (SRR630924 and SRR1032050) from the NCBI Sequence Read Archive (SRA) were used as outgroups for analyses.

RNA-seq variant calling

Raw reads were cleaned using TRIMMOMATIC v.0.32 (Bolger *et al.*, 2014), and mapped to the reference *B. rapa* genome (v.1.5, <http://brassicadb.org/brad/>) with TOPHAT v.2.0.14 (Trapnell *et al.*, 2009). SNP calling was performed using SAMTOOLS v.0.1.18 and BCFTOOLS v.0.1.17 (Li *et al.*, 2009; Li, 2011). The resulting VCF files were filtered with the vcfutils.pl script and vcfilter. Only single nucleotide polymorphisms (SNPs) with depth > 10 and variant quality (QUAL) > 30 were retained, which included 1.32 million SNPs. Details about sequencing and variant calling were described previously (Qi *et al.*, 2017).

Identifying genes derived from the mesohexaploidy event (Paleologs)

We used two different approaches to identify paleologs – genes derived from an ancient polyploid event – retained from the

Brassicaceae mesohexaploidy, the most recent polyploidy in the ancestry of *B. rapa*. Both approaches aim to distinguish genes that were born from the mesohexaploidy from genes derived from other types of duplication events, such as tandem, segmental and other small-scale duplications. Genes that we identify as derived from the Brassicaceae mesohexaploidy were labelled as paleologs, and other genes were labelled as non-paleologs. The first approach used synteny and gene age distribution data from only *B. rapa* to infer paleologs. To estimate the age distribution of gene duplications (also called a K_s plot) for *B. rapa* we obtained CoDing Sequence (CDS) from the *Brassica* database (<http://brassicadb.org/brad/>). The synonymous substitution rate (K_s) of each gene duplication was calculated using DUPPIPE (Barker *et al.*, 2008, 2010). The gene age distribution was visualized in R using histograms. The boundaries of the hexaploid peak were determined using EMMIX (McLachlan *et al.*, 1999) by fitting a mixture model of normal distributions to the K_s data (Tiley *et al.*, 2018). One hundred random STARTING POINTs and 10 k-means STARTING POINTs were used to identify the number of normal distributions (from 1 to 10). The best-fit model was selected based on the Bayesian information criterion (BIC) value. K_s nodes with > 50% likelihood assignment to the mesohexaploidy peak were considered as gene pairs derived from the *Brassica* hexaploidy event. We combined these age-based inferences of paleologs with syntenic data for *B. rapa*. Syntenic gene sets were generated by CoGe SYNMAP (genomevolution.org/CoGe/SynMap.pl) with a 2 : 2 quota-align ratio and default parameters (Tang *et al.*, 2011) using the unmasked *B. rapa* genome (v.1.5). These two paleolog lists were integrated with an independently estimated *B. rapa* paleolog list (Cheng *et al.*, 2012). Genes present in at least two of these three lists were extracted using the VENN online tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) and were considered to be high confidence paleologs. Genes present in only one of these three lists were considered to be low confidence paleologs and were not used in our study. Genes not present in any of the above three paleologs lists were considered non-paleologs.

We also developed a second set of paleologs and nonpaleologs from a recent multigenome comparative analysis with POINT, the Polyploidy Orthology Inference Tool (Hao *et al.*, 2020). The POINT analysis modeled the history of genome evolution following the Brassicaceae mesohexaploidy by comparing the genomes of *Arabidopsis thaliana*, *Brassica oleracea*, *B. rapa*, *Crambe hispanica* and *Sinapis alba*. Briefly, the POINT analysis compared each of the Brassicaceae species genomes to *A. thaliana* to identify blocks of triple conserved synteny across each of the four genomes. Blocks from each genome were then merged into 'pillars' that contain one to three surviving paralogs in each species from the mesohexaploidy. POINT uses a probabilistic approach to assess if a gene belongs to a paleolog pillar. We filtered for genes in the *B. rapa* genome that had a > 95% probability of assignment to one of these mesohexaploid pillars from the Hao *et al.* (2020) analysis. These data were used to develop a list of *B. rapa* paleologs that were retained in single, double or triple copy from the ancient hexaploidy. All other genes in *B. rapa* then were classified as non-paleologs.

Selection analyses

We used two different methods to identify genes with signals of positive selection in the four derived *B. rapa* genetic groups. Genomic regions with evidence consistent with selective sweeps were detected using SWEED 3.0 (Pavlidis *et al.*, 2013) based on the composite likelihood ratio (CLR) test of SNP site frequency spectrum (SFS) patterns. A total of 1.32 million SNPs were used in the SWEED tests for the TN group and each of the four derived *B. rapa* genetic groups. These analyses were performed with the default settings except each chromosome was divided to 60 000 grids. The CLR was calculated for each equally generated relative position on each chromosome. Only the top 1% significant outlier regions were considered. Genes within the outlier regions were then annotated based on *B. rapa* genome v.1.5.

We also used the McDonald–Kreitman test (M–K test) (McDonald & Kreitman, 1991) to identify genes experiencing positive selection. This method is based on the proportion of synonymous and nonsynonymous substitutions within and between species that are due to directional selection. For the M–K test, we compared SNPs specific to each of the four *B. rapa* crop groups with the two *B. oleracea* outgroup accessions. For each separate SNP dataset, we annotated synonymous and nonsynonymous SNPs using SNPeff v.4.2 (Cingolani *et al.*, 2012). The reference genome database was created using *B. rapa* genome v.1.5. The significance of the M–K test was evaluated using Fisher's exact test.

Differential gene expression analysis

Differential gene expression analysis was performed following the Tuxedo protocol (Trapnell *et al.*, 2012). Briefly, cleaned reads were first mapped to the *B. rapa* genome with TOPHAT (Trapnell *et al.*, 2009), then mapped BAM files were assembled in CUFFLINKS (Trapnell *et al.*, 2010), merged with CUFFMERGE, quantified in CUFFDIFF (Trapnell *et al.*, 2013) (CUFFLINKS v.2.2.1) with multiple-testing corrected *q*-values of 0.05, and finally indexed and visualized in CUMMERBUND v.2.14 (Goff *et al.*, 2012). Genes significantly differentially expressed between the TN group and the four derived *B. rapa* groups were identified using the getSig function in CUMMERBUND (Goff *et al.*, 2012).

Candidate genes from the literature

In order to confirm our findings, we compared our observations of paleolog enrichment with a list of candidate genes from published studies of *B. rapa*. We surveyed the literature for fine mapping and bulk segregant analyses of *B. rapa* that mapped traits to one or a few candidate genes. These studies and the candidate genes are listed in Table S7. Overall, our survey identified 40 candidate genes in *B. rapa* that have been mapped to the location of classical loci associated with crop traits.

Statistical analyses

To test for evidence of paleolog enrichment in our candidate gene lists, we used 2×2 chi-square tests. We compared the numbers

of paleologs and non-paleologs that were not candidate genes to the numbers of paleolog and non-paleolog candidate genes. For each candidate gene list, we aggregated the candidate genes of the four derived *B. rapa* genetic groups. The ratio of paleologs and non-paleologs among non-candidate genes in the *B. rapa* genome was used as the expected value for the chi-square test to determine if the candidate gene lists contained more paleologs than expected.

In order to demonstrate the logical relations among these lists, Venn diagrams were generated using the online Venn diagram tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). The density distribution of the identified paleologs, non-paleologs, SWEED outlier gene, M–K test outlier gene, and differentially expressed gene (DEG) were visualized in CIRCOS with a bin size of 100 kbp. We used the dhyper function in R to test for significant overlap among the different candidate gene lists.

Nucleotide diversity of *B. rapa* genes

The nucleotide diversity (π) of each paleolog and non-paleolog gene was estimated using a custom script from the output of VCFTOOLS (Danecek *et al.*, 2011). The results were summarized and visualized in R v.3.6.1 (R Core Team, 2019).

As a secondary verification of our calculations for nucleotide diversity in paleologs vs non-paleologs, we remapped the filtered RNA-seq reads to the *B. rapa* reference genome using STAR v.2.7.3a (Dobin *et al.*, 2013). We then used the resulting BAM files to calculate nucleotide diversity using genotype likelihoods with ANGSD v.0.921 (Korneliussen *et al.*, 2014). This procedure was done twice, once filtering for uniquely mapping reads and once without, to see if the results differed based on potential read mismapping caused by gene duplicates. For these calculations, we first inferred the folded site frequency spectrum ($-\text{doSaf } 1, -\text{fold } 1$) and used this as a POINT estimate for estimating theta ($-\text{doThetas } 1$) at each site separately for all paleologs and non-paleologs (Nielsen *et al.*, 2012; Korneliussen *et al.*, 2013). We then used the thetaStat program within ANGSD to calculate nucleotide diversity for each gene. We also compared the distributions of nucleotide diversity between paleologs and non-paleologs using both Student's *t*-tests and nonparametric Mann–Whitney *U*-tests within R v.3.6.1 (R Core Team, 2019).

Results

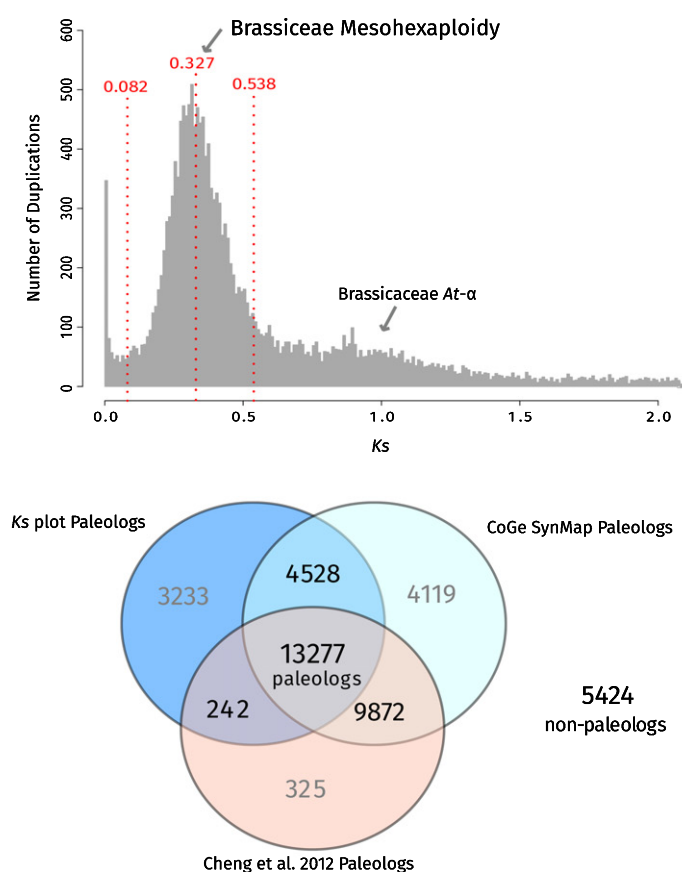
Partitioning the *B. rapa* genome into paleologs vs non-paleologs

In order to test the contribution of paleopolyploidy to the domestication of *B. rapa*, we used two different approaches to classify genes as paleologs – genes derived from the *Brassica* meso-hexaploidy – or non-paleologs. In the first approach, we used gene age distributions and synteny in *B. rapa*. An initial list of putative paleologs was generated from pairs of genes with synonymous divergence (K_s) values that correspond to the peak of duplication associated with the Brassiceae mesohexaploidy. This approach identified 21 280 genes that are likely derived from the

mesohexaploidy (Fig. 1a). This result is consistent with previous K_s estimates (Barker *et al.*, 2009; Cheng *et al.*, 2013). Ancient WGDs and their associated paralogs may also be classified by identifying syntenic blocks of duplication. Syntenic analyses with CoGe recovered 19 810 syntenic gene sets that contained 31 796 paleologs. Finally, we compared these two lists of putative paleologs with a previously curated list of 23 716 paleologs reported by an independent research group (Cheng *et al.*, 2012). Genes that appeared at least twice in these three lists were classified as paleologs and were used in further analyses (Fig. 1b). The final paleolog list included 27 919 genes (Table S2), which represents 68.06% of the 41 020 annotated genes in the *B. rapa* reference genome (v.1.5). We also considered the 5424 genes that were not present in any of the above three paleolog lists as non-paleologs (Fig. 1b), which represents 13.22% of the total *B. rapa* genes. Paleologs and non-paleologs are distributed throughout the *B. rapa* genome (Fig. 2).

We also used results from a recent multi-genome comparative analysis (Hao *et al.*, 2020) to infer paleologs in *B. rapa*. A recent POINT analysis of the genomes for four Brassicaceae species (*B. oleracea*, *B. rapa*, *C. hispanica* and *S. alba*) uncovered evidence for 14 050 regions ('pillars' in POINT terminology) of triple conserved synteny from the Brassicaceae mesohexaploidy across the four genomes. Genes in these regions of conserved synteny are present in one to three copies in each genome. After parsing these output for *B. rapa* genes that were assigned to these regions with >95% probability by POINT, we identified 4818 paleologs retained as single copy genes, 6128 paleologs retained in duplicate, and 2553 retained in triplicate (Fig. 1b; Table S2). The remaining 27 527 genes in the *B. rapa* genome were considered non-paleologs. Using this approach, only 32.9% of the *B. rapa* genome were identified as paleologs, a much lower fraction than the above synteny + K_s *B. rapa* only method. Despite the difference in the number of inferred paleologs, there was a high overlap

(a) Synteny + K_s *B. rapa* only Paleolog Inference



(b) POINT Multi-Genome Paleolog Inference

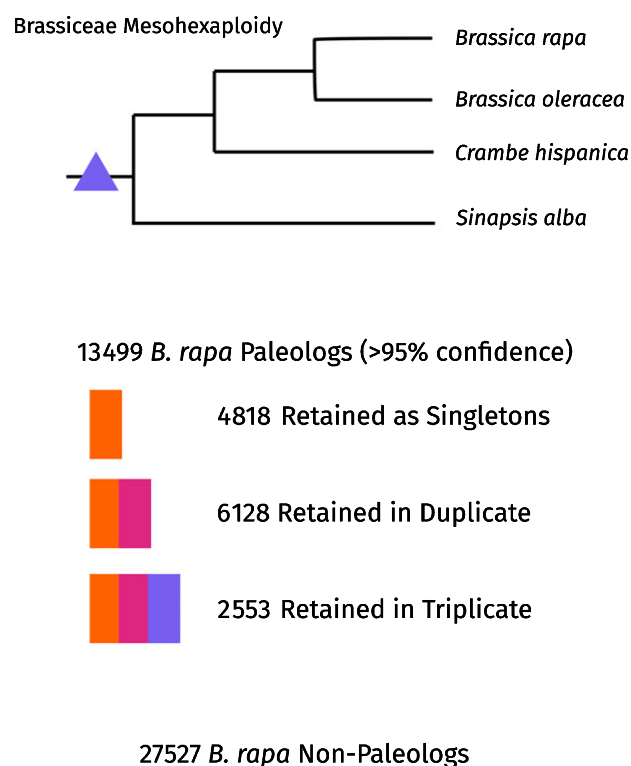


Fig. 1 Identifying paleologs derived from the *Brassica* hexaploidy event. (a) The age distribution of gene duplications from 41 020 CoDing Sequences (CDS) in the *B. rapa* genome (v.1.5). The x-axis represents the synonymous divergence of duplication events (K_s value), whereas the y-axis represents the number of duplications. Gene pairs with K_s divergence 0.082–0.538 were identified as putative paleologs. Venn diagram showing the overlap among our two *B. rapa* paleolog lists with a previously reported *B. rapa* paleolog list. Genes that appeared at least twice in these three lists were considered high confidence paleologs and were used in further analyses. Genes that were not present in any of the three paleologs lists were considered to be non-paleologs. (b) Phylogeny of species's genomes used for the POINT multi-genome analysis to identify paleologs in *B. rapa* from Hao *et al.* (2020). A total of 13 499 paleologs and 27 527 non-paleologs were identified in *B. rapa*. Colored blocks indicate the retention category of paleologs and the number of genes in each category.

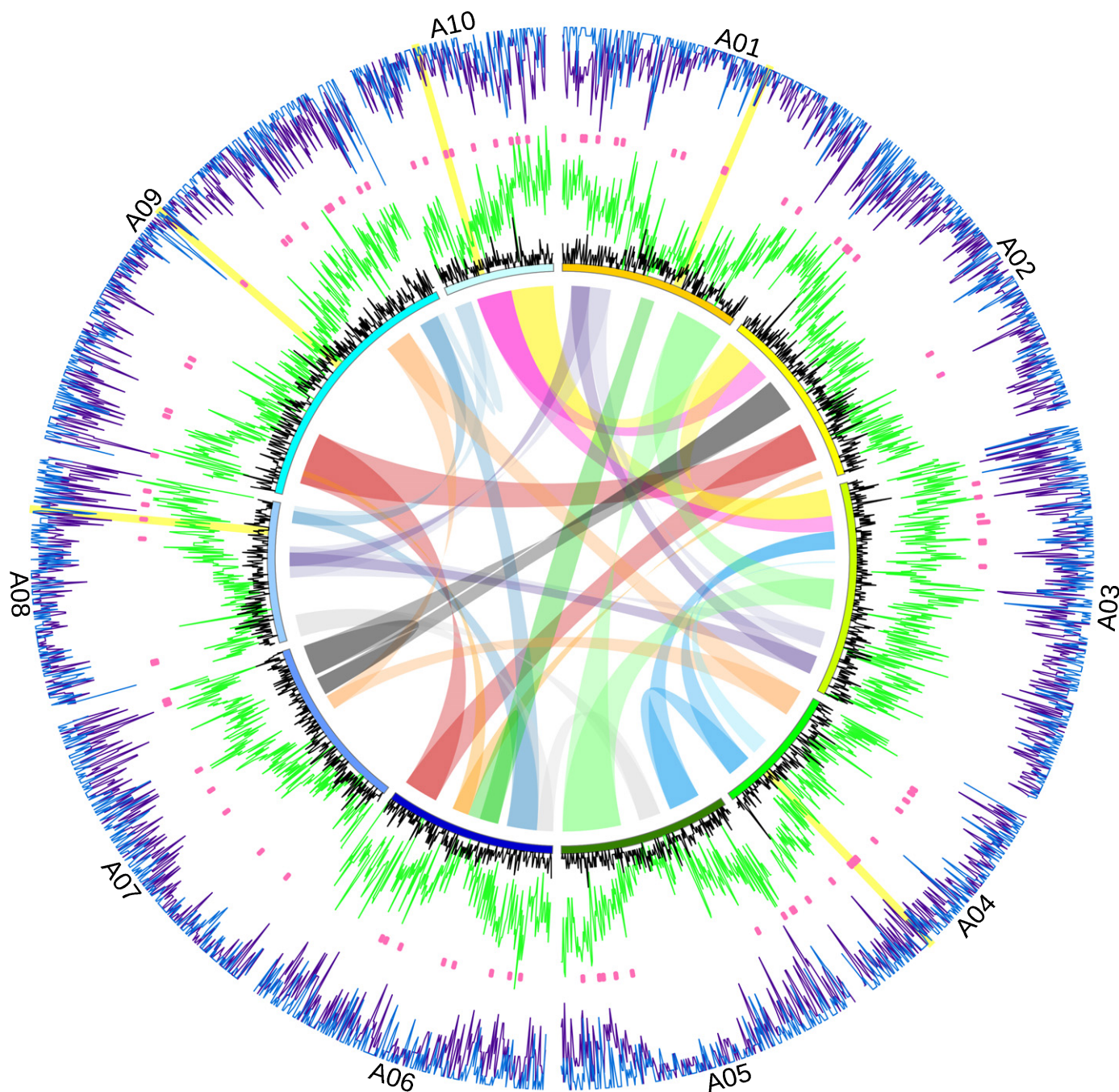


Fig. 2 CIRCOS plot of the distribution of surveyed genes in this study. Highlighted on the outside of the plot are the *Brassica* hexaploidy paleologs identified by the *B. rapa* only synteny + K_s analysis (light green lines), non-paleologs (black lines) and candidate genes identified by SWEED (blue lines), the McDonald–Kreitman test (pink dots), and the significantly differentially expressed genes between the Eurasian–Central Asia (EU–CA) group and the four derived *B. rapa* groups (purple lines). Heights of the lines indicate the relative density of genes in each category in the sliding window at each location. A01–A10 represent the 10 chromosomes of *B. rapa*. The rainbow ribbons in the center represent the syntenic regions among chromosomes. The yellow bars represent the location of the five *B. rapa* domestication candidate genes listed in Supporting Information Table S6.

in the lists with 89.2% of the paleologs inferred by POINT also found by the *B. rapa* only approach. Most of the difference in the overall number is likely due to the POINT approach's initial filtering for regions of the genome that have been retained in triplicate and requiring that each of these regions is found across all species, greatly reducing the number of paleologs identified. In contrast, the *B. rapa* only approach recovers more paleologs

because it only requires regions to be retained in duplicate in the genome of only one species (*B. rapa*). Notably, a less stringent version of the POINT results (not presented) that included all possible identified paleologs regardless of assignment probability identified 22 774 paleologs with 89.5% overlap with the *B. rapa* only list. Thus, these methods appear to be largely identifying the same genes as paleologs in the *B. rapa* genome. These methods

provide two different assessments of paleolog identity that we used to further explore the relationship between polyploidy and domestication of *B. rapa*.

In order to assess the similarity of the identified paleologs and non-paleologs with more global analyses of post-polyploid gene retention, we compared our gene lists with those of Li *et al.* (2016) and Tasdighian *et al.* (2017). Li *et al.* (2016) previously identified a set of 'core single copy' gene families across multiple plant species that consistently returned to single copy status within tens of millions of years following WGDs. Of the 6807 'core' single gene families identified in *B. rapa* (Li *et al.*, 2016), most were identified as paleologs in our analyses and many were still present in duplicate or triplicate (Table S3). This is consistent with Li *et al.* (2016) as they noted that many of these genes have not yet returned to single copy in *B. rapa*. We also found that the patterns of paleolog retention in *B. rapa* are consistent with retention patterns from across multiple angiosperms (Tasdighian *et al.*, 2017). We compared our POINT gene lists of single, double and triple retained paleologs to the reciprocal retention ranks of Tasdighian *et al.* (2017). As expected, the POINT lists followed the expected retention patterns with genes retained in triplicate composed of gene families with the highest reciprocal retention ranks, double copy next and single copy with the lowest retention rank scores (Fig. S1). Thus, our paleolog and non-paleolog inferences are consistent with previous analyses of gene family evolution following polyploidy.

Analyses of selection identify candidate genes associated with domestication of *B. rapa* crop varieties

We used a diverse collection of sequenced transcriptome data from across five groups to identify candidate genes associated with the domestication and improvement of *B. rapa* crop varieties. In total, 1.32 million SNPs from transcriptome data with $\geq 25\times$ coverage from 102 *B. rapa* accessions were analyzed (Table S1). Based on our previous population genomic analyses of these data (Qi *et al.*, 2017), the accessions comprised a European-Central Asian *B. rapa* population TN and four derived groups (PC, CC, IS and TO). We analyzed these data with an ensemble of molecular evolution and population genomic approaches to identify candidate genes associated with each group.

During domestication and crop improvement, we expect genetic variation important for agricultural traits to be selected in crop populations. To identify genes or genomic regions that have experienced recent positive selection, we used two different approaches. First, we used a selective sweep test, SWEED (Pavlidis *et al.*, 2013), to identify regions associated with significantly reduced variation consistent with a recent selective sweep in each of the crop varieties. We identified 3387 unique genes within the identified selective sweep regions (Figs 3a, S2, S3; Tables S4, S5). This included 1072 genes in *chinensis*, 687 genes in *pekinensis*, 1048 genes in *toria*, 1228 genes in the *sarsons*, and 1713 genes in European-Central Asian group. On average 70% of these genes were found in only one crop variety (Fig. 3a), indicating that many of these genes may have swept during the putative

independent domestication of each crop variety. We also used the M-K test (McDonald & Kreitman, 1991) to identify coding regions with a significant excess of fixed amino acid substitutions, a different signature of positive selection. We used *B. oleracea* as an outgroup. Analyzing each crop variety with the M-K test, we found 92 genes in total with a molecular evolutionary signature of positive selection (Fig. 3b; Tables S4, S5). All but one of these genes was uniquely identified in a different crop variety, similar to the selective sweep analysis and consistent with independent domestication and differentiation of these *B. rapa* crops. Overall, we identified 3479 candidate genes that may be associated with the domestication and improvement of the five *B. rapa* crop groups.

We also may expect significant changes in gene expression to occur during domestication and crop improvement that may not be apparent in analyses of positive selection. To identify candidate genes with changes in gene expression during domestication, we performed differential gene expression (DGE) analyses with the four derived *B. rapa* genetic groups. RNA-seq data for each individual was collected 20 days after germination and the expression levels of genes in each group were compared to gene expression levels in the TN group. We identified a total of 7813 genes with significant differential expression when comparing each group with the TN population (Figs 3c, S3, S4; Tables S4, S5). Unlike the analyses of selection above, the number of unique DEGs varied widely among the different crop varieties. The IS were the most differentiated from TN and the other crops with $>61\%$ of their DEGs unique. The *sarsons* also had the largest number of DEGs at 5804. In contrast, *pak choi* (PC) had the smallest number of DEGs, 1301, and the lowest percentage that were uniquely different at 14.2%. Other crop varieties fell between these values. The genes identified by all three methods were largely different among all the crops (Fig. 3d) with only a small number of genes identified by one or more of these approaches (i.e. sweep test, M-K test or DGE analyses). However, the number of overlapping genes found by each of these approaches was significant in all cases based on the expected numbers from a hypergeometric distribution (MK:SweeD overlap $P=0.0013$; MK:DGE overlap $P<0.0001$; SweeD:DGE overlap $P<0.0001$). This suggests that we were detecting shared signatures of domestication across these different candidate gene approaches. Likewise, most of the genes found to be associated with recent positive selection or differential gene expression were unique to a particular crop lineage as expected with independent domestication and differentiation of these distinct crop varieties.

Across all of the analyses, only five genes were repeatedly present in tests among one or more of the crop varieties (Fig. 3d; Table S6). These genes – *Bra010933*, *Bra023190*, *Bra031452*, *Bra003055* and *Bra035693* – were identified in some combination of the three inference methods with evidence for both signatures of positive selection and changes in gene expression. Considering that we recovered these five genes using different approaches, they may play an important role during the differentiation and improvement of *B. rapa* crop varieties. Searches of these genes and their *Arabidopsis* homologs in the STRING (Szczlarczyk *et al.*, 2019) and UniProt (UniProt Consortium, 2018)

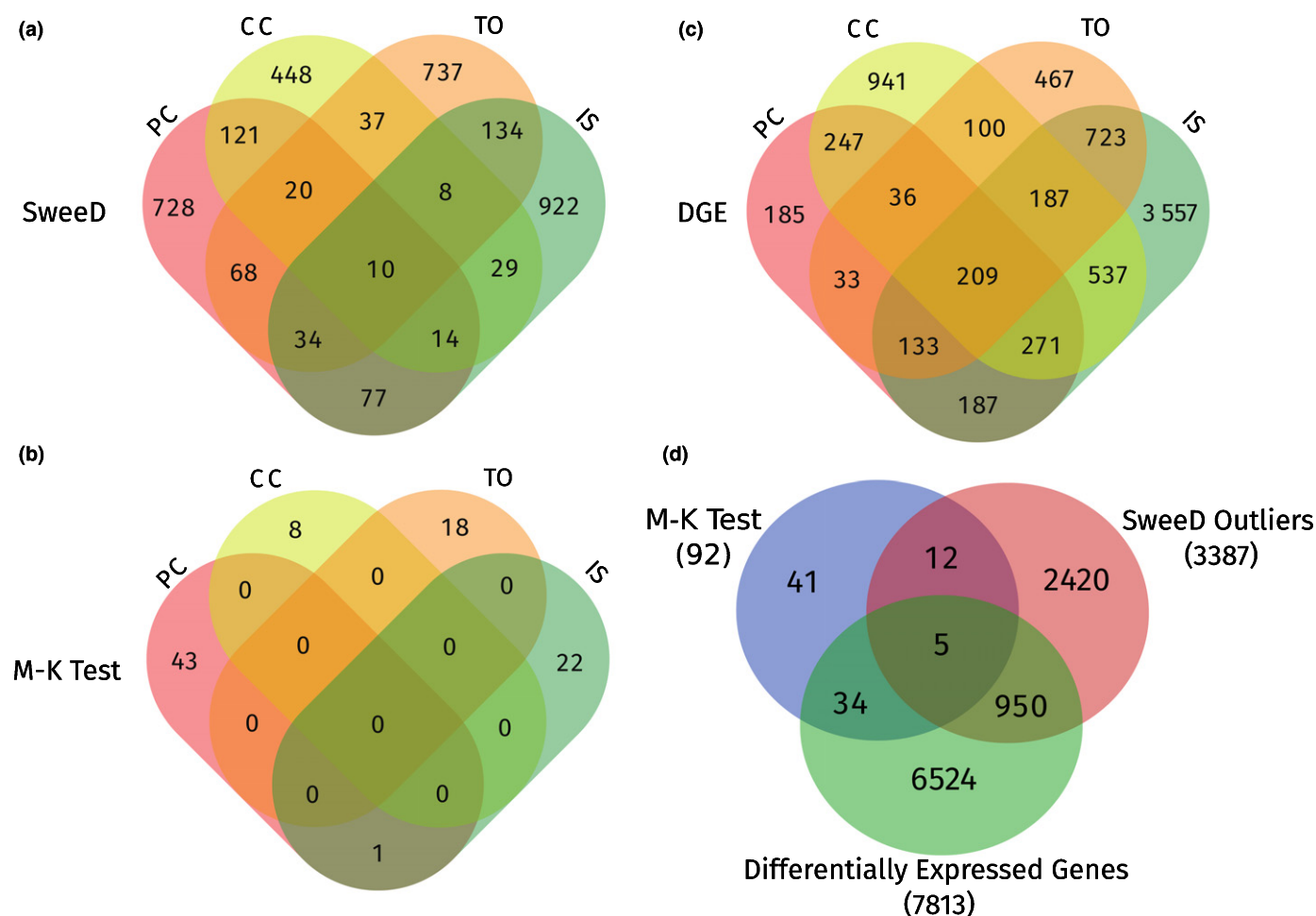


Fig. 3 Overlap among domestication candidate genes: PC, pak choi; CC, Chinese cabbage; TO, toria; and IS, Indian sarson. Number and overlap of candidate genes for each crop variety inferred by (a) SWEED, (b) the McDonald–Kreitman test and (c) differential gene expression (DGE) analyses. (d) Total number and overlap of candidate genes inferred by different methods across all crop varieties. Gene lists for each category are available in Supporting Information Table S4.

databases recovered a diverse range of potential functions. *Bra023190* is uncharacterized in *B. rapa*, but is homologous with *SGR2* in *Arabidopsis thaliana*, a gene associated with negative gravitropism and leaf movement in darkness (Kato *et al.*, 2002; Mano *et al.*, 2006). Notably, this gene was identified as a candidate gene in both of the cabbage crops analyzed here, *B. rapa chinensis* and *pekinensis*. Other genes are homologs with *A. thaliana* genes implicated in responses to oxidative stress from photooxidation (*Bra010933*), salt stress (*Bra031452*; Tuteja *et al.*, 2012), and a vacuolar V-type proton ATPase (*Bra035693*). Finally, *Bra003055*, identified by all three candidate gene approaches in the sarsons, was previously found to be overexpressed in *B. rapa* in soils that are deficient in iron and with an excess of zinc (Li *et al.*, 2014).

Paleologs from the *Brassica* mesohexaploidy and domestication

In order to test if the candidate genes associated with domestication of the *B. rapa* crop varieties are enriched with paleologs, we

compared the number of paleologs in the candidate gene lists to the expected number from our two genome-wide surveys (Fig. 1). We found that the candidate genes were significantly enriched with paleologs from the Brassiceae mesohexaploidy using either paleolog inference method (Fig. 4). Using paleologs inferred with the *B. rapa* only synteny + K_s approach (Fig. 1a), genes from all three candidate gene approaches were enriched across all crops with paleologs comprising 78.54% of SWEED ($\chi^2=160.06$, $P<0.001$), 83.7% of M-K ($\chi^2=10.33$, $P=0.0013$), and 78.6% of DEG candidates ($\chi^2=345.40$, $P<0.001$). Likewise, we found that candidate genes were significantly enriched with paleologs inferred with the POINT multi-genome approach (Fig. 1b). Using the POINT defined paleologs, we found that 42.6% of SWEED ($\chi^2=157.84$, $P<0.001$), 53.2% of M-K ($\chi^2=16.40$, $P<0.001$), and 40.3% of the DEG candidates ($\chi^2=239.04$, $P<0.001$) were paleologs. The POINT method allowed us to identify genes retained in single, double, and triple copy from the Brassiceae mesohexaploidy and we found that these varied in their enrichment among the candidate gene lists (Fig. 4; Table S7). Paleologs that have returned to single copy in *B. rapa*

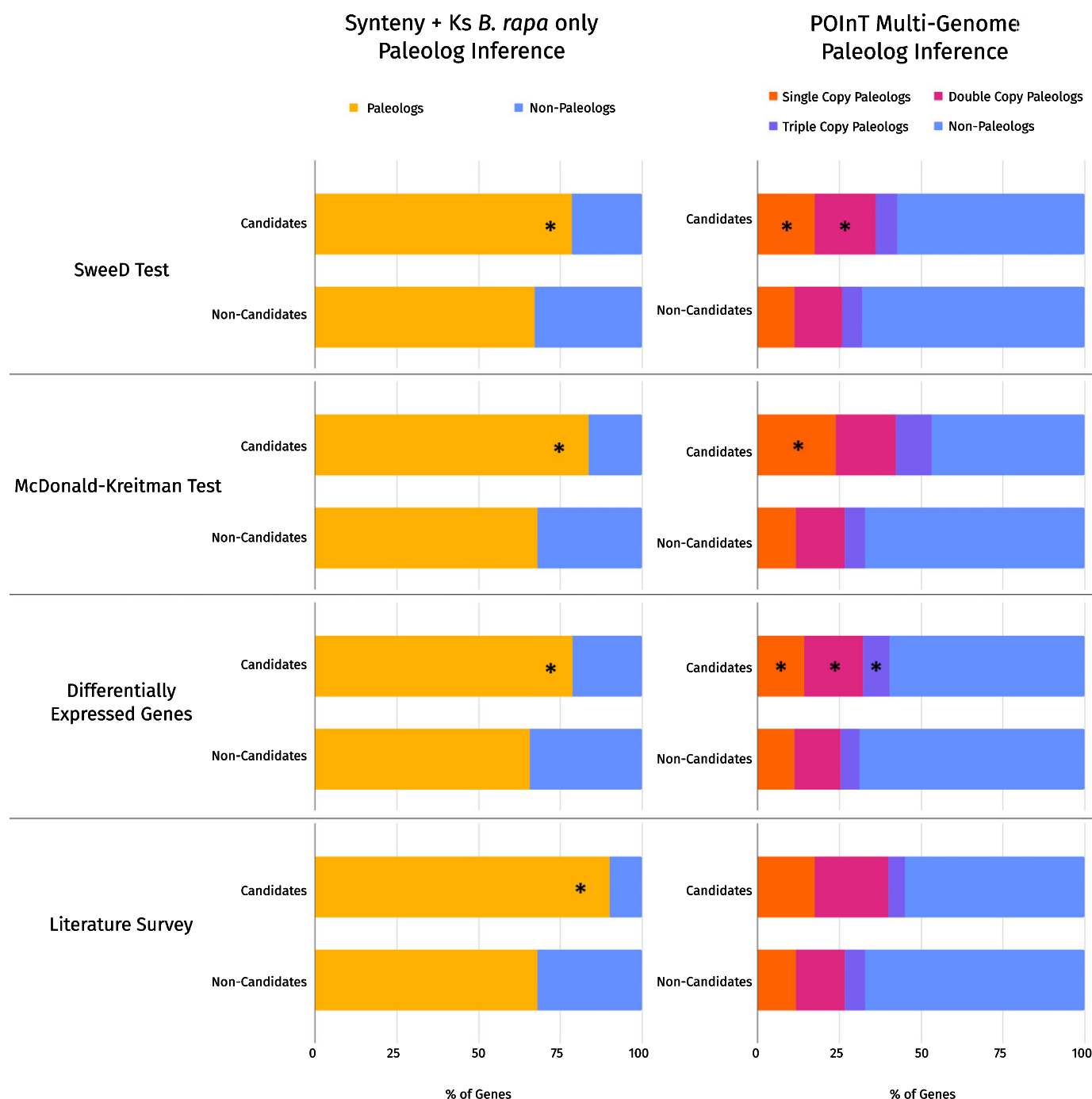


Fig. 4 Comparison of paleolog percentages across candidate gene lists. Percentage of paleologs in each candidate gene list compared to the rest of the *Brassica rapa* genome. Bar charts on the left are the paleologs (yellow) and non-paleologs (blue) identified by the *B. rapa* only synteny + K_s paleolog inference approach. Bar charts on the right are paleologs identified by the POInT multi-genome approach and include paleologs retained in single (orange), double (magenta) and triple (purple) copy as well as the percentage of the *B. rapa* genome that are non-paleologs (blue). The asterisks indicate significant deviation from the null based on chi-square tests ($P < 0.05$).

were significantly enriched in all of the candidate gene lists, whereas paleologs that are retained in duplicate were enriched in the SWEED and DEG lists. By contrast, paleologs that have retained all of their copies since the mesohexaploidy – those that are retained in triplicate – were found to be significantly enriched only in the DEG candidate list, but not in either candidate gene

list where there was evidence of significant protein coding changes among the genes. Likewise, we observed that the candidate gene lists were enriched with the ‘core single copy’ genes identified by Li *et al.* (2016) in *B. rapa* (Table S3). As noted above, many of these are still present in multiple copies in *B. rapa* and most were classified as paleologs in our analyses. Overall,

these results suggest that genes derived from the Brassiceae meso-hexaploidy were preferentially selected during the domestication of the *B. rapa* crops.

In order to further test if domestication genes in *B. rapa* are significantly enriched with paleologs, we also developed a list of candidate genes from the literature. We focused on studies published over the last 10 years that identified genes through other approaches, such as fine mapping or bulk segregant analysis, to better establish a causal relationship between loci and crop traits. In total, we identified 40 candidate genes that fit these criteria from the literature (Table S8). Many of these genes are associated with leaf and seed color variation, clubroot resistance and cuticular wax biosynthesis. Notably, 15 of these genes were recovered in our candidate gene scans (Table S8). Four of these genes were identified in our selective sweep and differential gene expression analyses. Mapping studies previously identified these genes as being associated with leaf color variation (*Bra006208*; Fu *et al.*, 2019), cuticular wax biosynthesis (*Bra011470* and *Bra032670*; Wang *et al.*, 2017, 2019), and clubroot resistance (*Bra019410*; Yu *et al.*, 2016). For other loci, mapping studies have identified a small collection of candidate genes in target regions. For example, *Rcr5* is a gene of major effect for clubroot resistance in *B. rapa*. A recent bulk segregant analysis and fine mapping study found eight genes in the *Rcr5* target region of *B. rapa* (Huang *et al.*, 2019). In our analyses, three genes in the center of their region were found to be significantly differentially expressed. These results suggest that future mapping studies in *B. rapa* may be able to leverage our candidate gene lists to improve gene identification.

How many of the candidate genes from our literature survey were paleologs? Of the 40 genes identified in the literature, 36 were paleologs with the *B. rapa* synteny + K_s method and 18 were paleologs with the POINT approach (Fig. 4; Table S8). The literature candidate genes were significantly enriched with paleologs defined by the *B. rapa* only approach ($\chi^2 = 8.85$, $P = 0.0029$) similar to our three different candidate gene lists. Although the percentage of POINT paleologs was higher in the literature candidate gene list than the rest of the genome (Fig. 4), it was not a significant difference ($\chi^2 = 2.13$, $P = 0.1441$). Given that these literature-based candidate genes were identified largely with mapping based approaches rather than genomic scans, these results suggest that the paleolog enrichment observed in our analyses is not likely to be an artifact of the inference methods. Overall, our results indicate that paleologs were an important source of variation for domestication in *B. rapa*.

Why did artificial selection preferentially target paleologs? One possible explanation is that these genes may harbor more genetic diversity owing to their paralogous history over the past 20 Myr. To test this hypothesis, we used three different approaches to examine the nucleotide diversity per gene across the *B. rapa* genome. First, we estimated nucleotide diversity in VCFtools using only uniquely mapped reads from TOPHAT to minimize error from incorrectly mapped reads. We found that the genes derived from the mesohexaploidy were significantly more diverse than the other genes in the genome regardless of how we identified paleologs (Fig. 5). Paleologs identified by the *B. rapa* only

approach were significantly more diverse than non-paleologs ($t = 43.721$, $df = 6535$, $P < 2.2 \times 10^{-16}$ for Student's t -test; $W = 70\,733\,526$ and $P < 2.2 \times 10^{-16}$ for Mann–Whitney U -test), and had four-fold greater mean and an order of magnitude greater median nucleotide diversity (mean $\pi = 0.408 \times 10^{-3}$, median $\pi = 0.170 \times 10^{-3}$) than the non-paleolog fraction of the genome (mean $\pi = 0.111 \times 10^{-3}$, median $\pi = 0.124 \times 10^{-4}$). This was consistent when considering only synonymous or non-synonymous substitutions (Fig. S5). POINT paleologs also were significantly more diverse than non-paleologs ($t = 14.828$, $df = 25\,101$, $P < 2.2 \times 10^{-16}$ for Student's t -test; $W = 128\,146\,882$ and $P < 2.2 \times 10^{-16}$ for Mann–Whitney U -test), with a more modest difference in mean paleolog nucleotide diversity yet still an order of magnitude higher median nucleotide diversity (mean $\pi = 0.433 \times 10^{-3}$, median $\pi = 0.217 \times 10^{-3}$) compared to non-paleologs (mean $\pi = 0.335 \times 10^{-3}$, median $\pi = 0.713 \times 10^{-4}$). All categories of retained POINT paleologs – single, double and triple retained copies – were significantly more diverse than the non-paleologs (Fig. 5).

In order to further validate these results, we re-mapped reads with STAR (Dobin *et al.*, 2013) and calculated nucleotide diversity with ANGSD (Korneliussen *et al.*, 2014). Although the empirical estimates of π were different because of differences in how π is calculated in these approaches, the ANGSD results recapitulated those from TOPHAT and VCFtools (Fig. S6). Focussing on the paleologs inferred with the *B. rapa* synteny + K_s approach, paleologs were again found to have significantly higher levels of diversity than non-paleologs (Fig. S6; $t = 48.997$, $df = 25\,842$, $P < 2.2 \times 10^{-16}$ for Student's t -test; $W = 238\,210\,946$ and $P < 2.2 \times 10^{-16}$ for Mann–Whitney U -test). Importantly, these results do not differ when filtering for only uniquely mapping reads ($t = 44.11$, $df = 26\,044$, and $P < 2.2 \times 10^{-16}$ for Student's t -test; $W = 232\,582\,598$ and $P < 2.2 \times 10^{-16}$ for Mann–Whitney U -test), demonstrating that our results are unlikely to have been affected by read mismapping. The higher genetic diversity of paleologs in *B. rapa* may have been important for the rapid response of these plants to artificial selection during domestication.

Discussion

Our results establish a connection between ancient polyploidy and contemporary diversity and domestication in crop varieties of *Brassica rapa*. Although polyploidy has long been hypothesized to be important for crop domestication, phylogenetic analyses have only recently confirmed that it is a key factor (Salman-Minkov *et al.*, 2016). However, the mechanisms linking polyploidy and domestication itself have remained unresolved. Our results provide support for at least one genetic mechanism linking polyploidy and domestication. Paleologs, genes retained from an ancient polyploidy, were enriched in our candidate gene lists for crops of *B. rapa*. They were enriched in all three of our lists from genome scans using different statistical approaches, as well as a list developed from fine mapping and other genetic studies in the literature. In the case of *B. rapa*, the polyploid event occurred 9–28 Ma (Lukens *et al.*, 2004; Lysak *et al.*, 2005; Beilstein *et al.*, 2010; Wang *et al.*, 2011; Arias *et al.*, 2014; Cheng *et al.*, 2014;

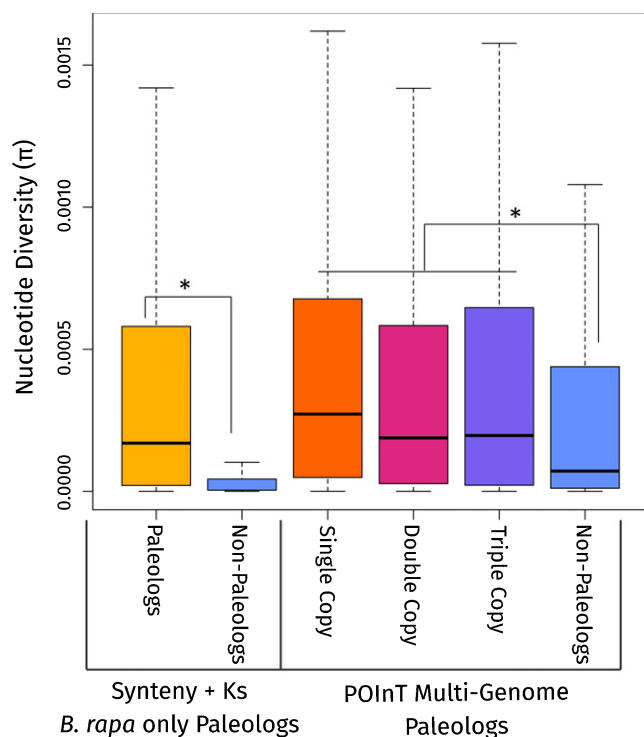


Fig. 5 Comparison of nucleotide diversity (π) across *Brassica rapa* paleologs and non-paleologs. The bottom and top of each box represents the first and third quartiles, and the band inside each box is the median. Asterisks indicate significant differences between categories with a Mann–Whitney *U*-test ($P < 0.05$).

Franzke *et al.*, 2016), long before the crop varieties were domesticated by humans. Many of these crop varieties were domesticated only in the last few thousand years (Qi *et al.*, 2017), but we still find that paleologs are over-represented in a diverse range of candidate gene lists. This suggests that even ancient polyploidy may contribute to domestication and potentially adaptation long after genomes have diploidized.

We also found that paleolog retention patterns were important during domestication. Genes that are now singletons or paleologs retained in duplicate were enriched in candidate gene lists that involved significant changes in protein evolution and differential gene expression. By contrast, paleologs retained in triplicate from the Brassiceae mesohexaploidy were enriched only in candidate gene lists with significant differential expression changes, but not in those with significant protein changes during domestication. Notably, the patterns of retention among our paleologs were consistent with those from a global analysis of plant genomes (Tasdigian *et al.*, 2017). These results are consistent with the dosage-balance hypothesis. Genes that retain all copies to maintain dosage balance are expected to be more constrained to continue making the same protein, whereas genes that are not retained to maintain dosage balance are expected to have less constraint on their protein coding sequences (Freeling, 2009; Jiang *et al.*, 2013; Conant *et al.*, 2014; Blischak *et al.*, 2016; Defoort *et al.*, 2019). Although analyses find that some genes returning to single-copy following whole genome duplication (WGD) may be highly constrained (De Smet *et al.*, 2013; Li *et al.*, 2016), we

found that genes from these lists, in particular Li *et al.* (2016), were classified mostly as paleologs and were enriched in our candidate gene lists. This is likely because many of these ‘core single copy’ genes are still present in multiple copy in *B. rapa*. The gradient of paleolog retention categories following the Brassiceae mesohexaploidy – single, double or triple retained genes – may produce more variation in the resolution of genes that would only be able to return to single copy following a less complex WGD. The paleologs retained in triplicate, in particular, provide a strong case to test the biology of genes under apparently strong dosage balance. Overall, the patterns of paleolog retention and their roles in the domestication of *B. rapa* appear to follow the rules of the dosage-balance hypothesis.

Why are paleologs over-represented among the candidate genes for *B. rapa* domestication and crop improvement? Our results suggest that it may be because this class of genes harbors greater genetic diversity than other genes in the genome. Across all of our analyses, paleologs consistently had significantly more nucleotide diversity than non-paleologs in *B. rapa*. Considering that many of these genes have been maintained in duplicate or triplicate for many generations since the mesohexaploidy that occurred 9–28 Ma, relaxed selection may yield elevated diversity at these genes as observed in other studies of paralog evolution (Lynch & Conery, 2000; Kondrashov *et al.*, 2002; Conant & Wagner, 2003; Brunet *et al.*, 2006; Aagaard *et al.*, 2006; Scannell & Wolfe, 2008; Shan *et al.*, 2009; Viaene *et al.*, 2009; Innan & Kondrashov, 2010; Lee & Irish, 2011; Ascencio *et al.*, 2017). Consistent with relaxed selection, we found that nucleotide diversity was elevated for both synonymous and nonsynonymous substitutions in *B. rapa* paleologs compared to non-paleologs. Notably, nucleotide diversity was higher across all categories of paleologs in our analyses, including genes duplicated in the hexaploidy but are now retained in single copy. This suggests that the higher diversity could be a legacy of past duplication rather than a product of ongoing masking at some paleolog loci. It also may be that non-paleologs are under greater constraint than paleologs. However, we still observed a significant difference in diversity even in our POINT analysis where nearly two-thirds Irish, 2011; Ascencio *et al.*, 2011 thirds of the genome was classified as non-paleologs, suggesting that the difference probably is not entirely due to extraordinary constraint and purifying selection on non-paleologs. Further research is needed to better understand why the paleologs and genes that returned to single copy after the hexaploidy have elevated nucleotide diversity compared to the non-paleologs. The elevated diversity in the paleologs does not appear to be an artifact of read mapping error given that we recovered the same pattern with two different read mapping approaches and observed no difference when using only uniquely mapped reads. Relatively long Illumina read lengths (150 bp) and the nearly 30% synonymous divergence of paleologs from the Brassiceae mesohexaploidy may have limited read mapping errors caused by paralogy. Notably, the most recent paralogs were classified as ‘non-paleologs’ together with other genes derived from duplication events other than polyploidy. Read mapping error rates would be expected to be the highest among these recent paralogs, but we consistently observed that estimates of

nucleotide diversity were significantly lower among non-paleologs than the paleologs. Regardless of the cause of the increased variation, it does not appear to be an artifact of read mapping and may explain why these genes are over-represented in the candidate gene lists. Increased genetic diversity is expected to be associated with greater phenotypic variation that could be selected during domestication. Paleologs may be over-represented in our candidate gene lists simply because they contain greater genetic diversity than non-paleologs in *B. rapa*. Although many paleopolyploid plant genomes have been analyzed, this difference in genetic diversity has not yet been observed to the best of our knowledge.

The presence of elevated genetic variation in *B. rapa* paleologs suggests that domestication and crop improvement may have proceeded largely from standing variation. Rapid responses to selection, such as the response to selection in domestication and crop improvement, will proceed much faster if standing genetic diversity is high enough to facilitate simultaneous selection at multiple sites (Barrett & Schluter, 2008; Messer & Petrov, 2013; Matuszewski *et al.*, 2015). It is an open question how much domestication has proceeded from standing genetic diversity, but a recent analysis suggests that maize could have been domesticated from standing variation in teosinte (Yang *et al.*, 2019). There also are examples of candidate genes associated with crops, including in *Brassica oleracea* (Purugganan *et al.*, 2000), that appear to be selected from standing genetic diversity. Given the elevated diversity of paleologs in *B. rapa*, we may expect domestication in these crops to be dominated by soft sweeps (Messer & Petrov, 2013; Hermisson & Pennings, 2017). A complicating factor in characterizing hard and soft sweeps in *B. rapa* at the moment is the significant difference in genetic variation between paleologs and non-paleologs that may skew such diversity-based analyses. More sophisticated approaches that leverage recent advances in deep learning (Schridder & Kern, 2018; Kern & Schridder, 2018; Flagel *et al.*, 2019) that are trained to account for differences in variation because of gene origins will likely overcome these issues. Ultimately, this will allow us to characterize the genetics of domestication in the diverse crop varieties of *B. rapa* while providing new insight into how paleopolyploidy may influence the architecture of adaptation in plant genomes.

The ancient hexaploidy in the Brassiceae has been hypothesized to be the source of the outstanding diversity of *Brassica* crop varieties (Lukens *et al.*, 2004; Cheng *et al.*, 2014, 2016). Our results support this hypothesis by finding that candidate genes for domestication of *B. rapa* crop varieties are enriched in regions of the genome duplicated in the mesohexaploidy. It remains to be seen if paleologs also are significantly enriched in the candidate domestication genes in the crops of other *Brassica* species. Other types of genetic variation, such as structural variants like presence/absence and copy number variants, also may be influenced by post-polyploid genome evolution and play a role in domestication (Golicz *et al.*, 2016; Song *et al.*, 2020; Gabur *et al.*, 2020). Developing pan-genome resources (Bayer *et al.*, 2020) for *B. rapa* would allow us to better assess diversity across the crop varieties, and test how different types of genetic diversity have contributed to domestication. *Brassica* are known as the dogs of the plant

world for the incredible morphological diversity and number of different domesticated crops compared to other plants (Cheng *et al.*, 2014; Liu *et al.*, 2014; An *et al.*, 2019). Experimental evolution studies in *B. rapa* also have been able to rapidly select for new pollination syndromes (Gervasi & Schiestl, 2017; Schiestl *et al.*, 2018). The higher genetic diversity of paleologs in *Brassica* may play a significant role in this morphological diversity and the rapid responses to selection. However, paleopolyploidy is common among flowering plants with the average species experiencing nearly five rounds of WGD in its ancestry (One Thousand Plant Transcriptomes Initiative, 2019; Li & Barker, 2020). If elevated variation in paleologs is a general phenomenon, then we would expect it to be important in the domestication of many other crops and it may not completely explain the outstanding diversity of *Brassica* crops. Further analyses of paleolog variation and enrichment in other crops are needed to understand the generality of our findings and the implications for the domestication of diverse crops like in *Brassica*. Presently, our results provide a testable hypothesis to explain the observed correlation of polyploidy and crop domestication (Salman-Minkov *et al.*, 2016).

More broadly, our results suggest that paleopolyploidy may leave behind a legacy of elevated genetic diversity across the duplicated remnants of diploidized genomes. Although most models and studies of polyploid evolution compare diploids and polyploids (Otto & Whitton, 2000; Ramsey & Schemske, 2002; Otto, 2007; Selmecki *et al.*, 2015; Laport & Ng, 2017; Paape *et al.*, 2018; Han *et al.*, 2019; Monnahan *et al.*, 2019; Baniaga *et al.*, 2020), our comparison of paleologs and non-paleologs within a diploidized paleopolyploid uncovered evidence for similar dynamics ongoing within plant genomes even millions of years after WGD. The extensive genome duplication history of plants may result in genomes with different levels of diversity based on the mechanisms of gene origin and retention. Although this remains to be broadly tested, it raises a few testable predictions. If this is a general phenomenon in plants, then we may expect that diploid plants with more paleologs will have higher genetic diversity than those with fewer paleologs. As paleologs are lost over time as a consequence of fractionation and gene turnover, we also would expect genetic diversity in diploid plants to be correlated with the time since their most recent paleopolyploidization. The long-term effects of paleopolyploidy on genetic diversity observed here in *B. rapa* also may help explain a broader phenomenon: the lag time of paleopolyploidy and diversification (Schranz *et al.*, 2012). Recent research has found that paleopolyploidy in plants is associated with diversification rate increases, but these rate increases often occur many millions of years following WGDs (Landis *et al.*, 2018). If relatively high genetic diversity is important for the adaptation and expansion that leads to macroevolutionary signatures of net diversification rate increases, then the observed lag between diversification and polyploidy observed in many studies could be explained by the lasting increase in diversity at paleologs. Although our analyses are centered on how ancient polyploidy contributed to the diversity of *B. rapa*, additional analyses in other plants will provide crucial data to test the new hypotheses described above. Given the distribution of polyploidy throughout the history of flowering plants (One Thousand

Plant Transcriptomes Initiative, 2019), our results suggest that the genetic legacy of these WGDs could contribute to the diversity and adaptation of plants millions of years later.









Acknowledgements

We thank A. Baniaga, G. Finch, Z. Li, B. Sutherland and two anonymous reviewers for feedback and comments that improved this manuscript. Hosting infrastructure and services provided by the Biotechnology Computing Facility (BCF) at the University of Arizona. This research was supported by NSF-IOS-1339156 to MSB, GCC and JCP. The authors declare no competing financial interests.

Author contributions

HA and JCP conducted the RNA experiments; XQ conducted gene age distribution, candidate gene surveys and all related statistics; XQ, YH, MTWM and GCC inferred paleologs; XQ and TEH conducted the differential gene expression analyses; XQ, CD, MTWM and PDB conducted the gene diversity analyses; XQ and MSB designed the experiments and wrote the manuscript with input from all co-authors. All authors read and approved the manuscript.

ORCID

Hong An  <https://orcid.org/0000-0002-2367-5508>
 Michael S. Barker  <https://orcid.org/0000-0001-7173-1319>
 Paul D. Blischak  <https://orcid.org/0000-0001-9177-8958>
 Gavin C. Conant  <https://orcid.org/0000-0002-8677-4933>
 Yue Hao  <https://orcid.org/0000-0002-5065-8906>
 Michael T. W. McKibben  <https://orcid.org/0000-0002-1342-0085>
 J. Chris Pires  <https://orcid.org/0000-0001-9682-2639>
 Xinshuai Qi  <https://orcid.org/0000-0003-3883-0387>

Data availability

All the datasets generated during the current study are available in the NCBI Sequence Read Archive (SRA) under accession number SRP072186 (<http://www.ncbi.nlm.nih.gov/sra/SRP072186>). The accession numbers are summarized in Table S1.

References

Aagaard JE, Willis JH, Phillips PC. 2006. Relaxed selection among duplicate floral regulatory genes in Lamiales. *Journal of Molecular Evolution* 63: 493–503.
 An H, Qi X, Gaynor ML, Hao Y, Gebken SC, Mabry ME, McAlvay AC, Teakle GR, Conant GC, Barker MS *et al.* 2019. Transcriptome and organellar sequencing highlights the complex origin and diversification of allotetraploid *Brassica napus*. *Nature Communications* 10: 2878.
 Anderson E. 1969. *Plants, man and life*. Berkeley, CA, USA: University of California Press.
 Arias T, Beilstein MA, Tang M, McKain MR, Pires JC. 2014. Diversification times among Brassica (Brassicaceae) crops suggest hybrid formation after 20 million years of divergence. *American Journal of Botany* 101: 86–91.

Arrigo N, Barker MS. 2012. Rarely successful polyploids and their legacy in plant genomes. *Current Opinion in Plant Biology* 15: 140–146.
 Ascencio D, Ochoa S, Delaye L, DeLuna A. 2017. Increased rates of protein evolution and asymmetric deceleration after the whole-genome duplication in yeasts. *BMC Evolutionary Biology* 17: 40.
 Baduel P, Bray S, Vallejo-Marín M, Kolár F, Yant L. 2018. The 'Polyploid Hop': shifting challenges and opportunities over the evolutionary lifespan of genome duplications. *Frontiers in Ecology and the Environment* 6: 117.
 Baniaga AE, Marx HE, Arrigo N, Barker MS. 2020. Polyploid plants have faster rates of multivariate niche differentiation than their diploid relatives. *Ecology Letters* 23: 68–78.
 Barker MS, Dlugosch KM, Dinh L, Challa RS, Kane NC, King MG, Rieseberg LH. 2010. EvoPipes.net: bioinformatic tools for ecological and evolutionary genomics. *Evolutionary Bioinformatics* 6: 143.
 Barker MS, Kane NC, Matvienko M, Kozik A, Michelmore RW, Knapp SJ, Rieseberg LH. 2008. Multiple paleopolyploidizations during the evolution of the Compositae reveal parallel patterns of duplicate gene retention after millions of years. *Molecular Biology and Evolution* 25: 2445–2455.
 Barker MS, Vogel H, Schranz ME. 2009. Paleopolyploidy in the Brassicales: analyses of the *Cleome* transcriptome elucidate the history of genome duplications in *Arabidopsis* and other Brassicales. *Genome Biology and Evolution* 1: 391–399.
 Barrett RDH, Schluter D. 2008. Adaptation from standing genetic variation. *Trends in Ecology & Evolution* 23: 38–44.
 Bayer PE, Golitz AA, Scheben A, Batley J, Edwards D. 2020. Plant pan-genomes are the new reference. *Nature Plants* 6: 914–920.
 Beilstein MA, Nagalingum NS, Clements MD, Manchester SR, Mathews S. 2010. Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 107: 18724–18728.
 Blischak PD, Mabry ME, Conant GC, Chris PJ. 2016. Integrating networks, phylogenomics, and population genomics for the study of polyploidy. *Annual Review of Ecology, Evolution, and Systematics* 49: 253–278.
 Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.
 Brunet FG, Roest Crollius H, Paris M, Aury J-M, Gibert P, Jaillon O, Laudet V, Robinson-Rechavi M. 2006. Gene loss and evolutionary rates following whole-genome duplication in teleost fishes. *Molecular Biology and Evolution* 23: 1808–1816.
 Cheng F, Mandáková T, Wu J, Xie Q, Lysak MA, Wang X. 2013. Deciphering the diploid ancestral genome of the Mesohexaploid *Brassica rapa*. *The Plant Cell* 25: 1541–1554.
 Cheng F, Sun R, Hou X, Zheng H, Zhang F, Zhang Y, Liu B, Liang J, Zhuang M, Liu Y *et al.* 2016. Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in *Brassica rapa* and *Brassica oleracea*. *Nature Genetics* 48: 1218–1224.
 Cheng F, Wu J, Fang L, Sun S, Liu B, Lin K, Bonnema G, Wang X. 2012. Biased gene fractionation and dominant gene expression among the subgenomes of *Brassica rapa*. *PLoS ONE* 7: e36442.
 Cheng F, Wu J, Wang X. 2014. Genome triplication drove the diversification of *Brassica* plants. *Horticulture Research* 1: 14024.
 Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly* 6: 80–92.
 Conant GC, Birchler JA, Pires JC. 2014. Dosage, duplication, and diploidization: clarifying the interplay of multiple models for duplicate gene evolution over time. *Current Opinion in Plant Biology* 19: 91–98.
 Conant GC, Wagner A. 2003. Asymmetric sequence divergence of duplicate genes. *Genome Research* 13: 2052–2058.
 Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST *et al.* 2011. The variant call format and VCFtools. *Bioinformatics* 27: 2156–2158.
 De Smet R, Adams KL, Vandepoele K, Van Montagu MCE, Maere S, Van de Peer Y. 2013. Convergent gene loss following gene and genome duplications creates single-copy families in flowering plants. *Proceedings of the National Academy of Sciences, USA* 110: 2898–2903.

- Defoort J, Van de Peer Y, Carretero-Paulet L. 2019. The evolution of gene duplicates in angiosperms and the impact of protein-protein interactions and the mechanism of duplication. *Genome Biology and Evolution* 11: 2292–2305.
- Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29: 15–21.
- Flagel L, Brandvain Y, Schrider DR. 2019. The unreasonable effectiveness of convolutional neural networks in population genetic inference. *Molecular Biology and Evolution* 36: 220–238.
- Fränke A, Koch MA, Mummenhoff K. 2016. Turnip time travels: age estimates in brassicaceae. *Trends in Plant Science* 21: 554–561.
- Freeling M. 2009. Bias in plant gene content following different sorts of duplication: tandem, whole-genome, segmental, or by transposition. *Annual Review of Plant Biology* 60: 433–453.
- Fu W, Ye X, Ren J, Li Q, Du J, Hou A, Mei F, Feng H, Liu Z. 2019. Fine mapping of lclm1, a gene conferring chlorophyll-deficient golden leaf in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). *Molecular Breeding: New Strategies in Plant Improvement* 39: 52.
- Gabur I, Chawla HS, Lopisso DT, von Tiedemann A, Snowdon RJ, Obermeier C. 2020. Gene presence-absence variation associates with quantitative *Verticillium longisporum* disease resistance in *Brassica napus*. *Scientific Reports* 10: 4131.
- Gervasi DDL, Schiestl FP. 2017. Real-time divergent evolution in plants driven by pollinators. *Nature Communications* 8: 14691.
- Goff L, Trapnell C, Kelley D. 2012. cummeRbund: analysis, exploration, manipulation, and visualization of Cufflinks high-throughput sequencing data. R package v.2.7.2 [WWW document] <https://rdrr.io/bioc/cummeRbund/>.
- Golicz AA, Bayer PE, Barker GC, Edger PP, Kim H, Martinez PA, Chan CKK, Severn-Ellis A, McCombie WR, Parkin IAP *et al.* 2016. The pangenome of an agronomically important crop plant *Brassica oleracea*. *Nature Communications* 7: 13390.
- Han T-S, Zheng Q-J, Onstein RE, Rojas-Andrés BM, Hauenschild F, Muellner-Riehl AN, Xing Y-W. 2019. Polyploidy promotes species diversification of *Allium* through ecological shifts. *New Phytologist* 225: 571–583.
- Hao Y, Mabry ME, Edger P, Freeling M, Zheng C, Jin L, VanBuren R, Colle M, An H, Shawn Abrahams *Ret al.* 2020. The contributions of the allopolyploid parents of the mesopolyploid brassicaceae are evolutionarily distinct but functionally compatible. *bioRxiv* 2020.08.10.245258
- Heiser CB. 1990. *Seed to civilization: the story of food*. Cambridge, MA, USA: Harvard University Press.
- Hermisson J, Pennings PS. 2017. Soft sweeps and beyond: understanding the patterns and probabilities of selection footprints under rapid adaptation (J Kelley, Ed.). *Methods in Ecology and Evolution* 8: 700–716.
- Hilu KW. 1993. Polyploidy and the evolution of domesticated plants. *American Journal of Botany* 80: 1494–1499.
- Huang Z, Peng G, Gossen BD, Yu F. 2019. Fine mapping of a clubroot resistance gene from turnip using SNP markers identified from bulked segregant RNA-Seq. *Molecular Breeding: New Strategies in Plant Improvement* 39: 131.
- Innan H, Kondrashov F. 2010. The evolution of gene duplications: classifying and distinguishing between models. *Nature Reviews Genetics* 11: 97–108.
- Jiang W-K, Liu Y-L, Xia E-H, Gao L-Z. 2013. Prevalent role of gene features in determining evolutionary fates of whole-genome duplication duplicated genes in flowering plants. *Plant Physiology* 161: 1844–1861.
- Kato T, Morita MT, Fukaki H, Yamauchi Y, Uehara M, Niihama M, Tasaka M. 2002. SGR2, a phospholipase-like protein, and ZIG/SGR4, a SNARE, are involved in the shoot gravitropism of *Arabidopsis*. *The Plant Cell* 14: 33–46.
- Kern AD, Schrider DR. 2018. diploS/HIC: an updated approach to classifying selective sweeps. *G3: Genes, Genomes, Genetics* 8: 1959–1970.
- Kondrashov FA, Rogozin IB, Wolf YI, Koonin EV. 2002. Selection in the evolution of gene duplications. *Genome Biology* 3: RESEARCH0008.
- Korneliussen TS, Albrechtsen A, Nielsen R. 2014. ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics* 15: 356.
- Korneliussen TS, Moltke I, Albrechtsen A, Nielsen R. 2013. Calculation of Tajima's D and other neutrality test statistics from low depth next-generation sequencing data. *BMC Bioinformatics* 14: 289.
- Landis JB, Soltis DE, Li Z, Marx HE, Barker MS, Tank DC, Soltis PS. 2018. Impact of whole-genome duplication events on diversification rates in angiosperms. *American Journal of Botany* 105: 348–363.
- Laport RG, Ng J. 2017. Out of one, many: The biodiversity considerations of polyploidy. *American Journal of Botany* 104: 1119–1121.
- Lee H-L, Irish VF. 2011. Gene duplication and loss in a MADS box gene transcription factor circuit. *Molecular Biology and Evolution* 28: 3367–3380.
- Levin DA. 1983. Polyploidy and novelty in flowering plants. *The American Naturalist* 122: 1–25.
- Lewis WH. 1980. Polyploidy in species populations. In: Lewis WH, ed. *Basic life sciences. Polyploidy: biological relevance*. Boston, MA, USA: Springer US, 103–144.
- Li H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 27: 2987–2993.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics* 25: 2078–2079.
- Li J, Liu B, Cheng F, Wang X, Aarts MGM, Wu J. 2014. Expression profiling reveals functionally redundant multiple-copy genes related to zinc, iron and cadmium responses in *Brassica rapa*. *New Phytologist* 203: 182–194.
- Li Z, Barker MS. 2020. Inferring putative ancient whole-genome duplications in the 1000 Plants (1KP) initiative: access to gene family phylogenies and age distributions. *GigaScience* 9: gaa004.
- Li Z, Defoort J, Tasdighian S, Maere S, Van de Peer Y, De Smet R. 2016. Gene duplicability of core genes is highly consistent across all angiosperms. *The Plant Cell* 28: 326–344.
- Liu S, Liu Y, Yang X, Tong C, Edwards D, Parkin IAP, Zhao M, Ma J, Yu J, Huang S *et al.* 2014. The *Brassica oleracea* genome reveals the asymmetrical evolution of polyploid genomes. *Nature Communications* 5: 3930.
- Lukens LN, Quijada PA, Udall J, Pires JC, Schranz ME, Osborn TC. 2004. Genome redundancy and plasticity within ancient and recent *Brassica* crop species: BRASSICA GENOME STRUCTURE AND PLASTICITY. *Biological Journal of the Linnean Society* 82: 665–674.
- Lynch M, Conery JS. 2000. The evolutionary fate and consequences of duplicate genes. *Science* 290: 1151–1155.
- Lysak MA, Koch MA, Pecinka A, Schubert I. 2005. Chromosome triplication found across the tribe Brassicaceae. *Genome Research* 15: 516–525.
- Mano E, Horiguchi G, Tsukaya H. 2006. Gravitropism in leaves of *Arabidopsis thaliana* (L.) Heynh. *Plant & Cell Physiology* 47: 217–223.
- Matuszewski S, Hermisson J, Kopp M. 2015. Catch Me if you can: adaptation from standing genetic variation to a moving phenotypic optimum. *Genetics* 200: 1255–1274.
- McDonald JH, Kreitman M. 1991. Adaptive protein evolution at the *Adh* locus in *Drosophila*. *Nature* 351: 652–654.
- McLachlan GJ, Peel D, Basford KE, Adams P. 1999. The EMMIX software for the fitting of mixtures of normal and t-components. *Journal of Statistical Software* 4: 1–14.
- Messer PW, Petrov DA. 2013. Population genomics of rapid adaptation by soft selective sweeps. *Trends in Ecology & Evolution* 28: 659–669.
- Meyer RS, DuVal AE, Jensen HR. 2012. Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytologist* 196: 29–48.
- Monnahan P, Kolář F, Baduel P, Sailer C, Koch J, Horvath R, Laenen B, Schmickl R, Paajanen P, Šrámková G *et al.* 2019. Pervasive population genomic consequences of genome duplication in *Arabidopsis arenosa*. *Nature Ecology & Evolution* 3: 457–468.
- Nielsen R, Korneliussen T, Albrechtsen A, Li Y, Wang J. 2012. SNP calling, genotype calling, and sample allele frequency estimation from New-Generation Sequencing data. *PLoS ONE* 7: e37558.
- One Thousand Plant Transcriptomes Initiative. 2019. One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* 574: 679–685.
- Otto SP. 2007. The evolutionary consequences of polyploidy. *Cell* 131: 452–462.
- Otto SP, Whitton J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* 34: 401–437.

- Paape T, Briskine RV, Halstead-Nussloch G, Lischer HEL, Shimizu-Inatsugi R, Hatakeyama M, Tanaka K, Nishiyama T, Sabirov R, Sese J *et al.* 2018. Patterns of polymorphism and selection in the subgenomes of the allopolyploid *Arabidopsis kamchatica*. *Nature Communications* 9: 3909.
- Paterson AH. 2005. Polyploidy, evolutionary opportunity, and crop adaptation. *Genetica* 123: 191–196.
- Pavlidis P, Živkovic D, Stamatakis A, Alachiotis N. 2013. SweeD: likelihood-based detection of selective sweeps in thousands of genomes. *Molecular Biology and Evolution* 30: 2224–2234.
- Purugganan MD, Boyles AL, Suddith JI. 2000. Variation and selection at the CAULIFLOWER floral homeotic gene accompanying the evolution of domesticated *Brassica oleracea*. *Genetics* 155: 855–862.
- Qi X, An H, Ragsdale A, Hall TE, Gutenkunst RN, Pires JC, Barker MS. 2017. Genomic inferences of domestication events are corroborated by written records in *Brassica rapa*. *Molecular Ecology* 26: 3373–3388.
- R Core Team. 2019. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Ramsey J, Schenck DW. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* 33: 589–639.
- Renny-Byfield S, Wendel JF. 2014. Doubling down on genomes: polyploidy and crop plants. *American Journal of Botany* 101: 1711–1725.
- Salman-Minkov A, Sabath N, Mayrose I. 2016. Whole-genome duplication as a key factor in crop domestication. *Nature Plants* 2: 16115.
- Scannell DR, Wolfe KH. 2008. A burst of protein sequence evolution and a prolonged period of asymmetric evolution follow gene duplication in yeast. *Genome Research* 18: 137–147.
- Schiestl FP, Balmer A, Gervasi DD. 2018. Real-time evolution supports a unique trajectory for generalized pollination. *Evolution* 72: 2653–2668.
- Schranz ME, Mohammadin S, Edger PP. 2012. Ancient whole genome duplications, novelty and diversification: the WGD Radiation Lag-Time Model. *Current Opinion in Plant Biology* 15: 147–153.
- Schrider DR, Kern AD. 2018. Supervised machine learning for population genetics: a new paradigm. *Trends in Genetics* 34: 301–312.
- Selmecki AM, Maruvka YE, Richmond PA, Guillet M, Shores N, Sorenson AL, De S, Kishony R, Michor F, Dowell R *et al.* 2015. Polyploidy can drive rapid adaptation in yeast. *Nature* 519: 349–352.
- Shan H, Zahn L, Guindon S, Wall PK, Kong H, Ma H, DePamphilis CW, Leebens-Mack J. 2009. Evolution of plant MAD5 box transcription factors: evidence for shifts in selection associated with early angiosperm diversification and concerted gene duplications. *Molecular Biology and Evolution* 26: 2229–2244.
- Shimizu-Inatsugi R, Terada A, Hirose K, Kudoh H, Sese J, Shimizu KK. 2017. Plant adaptive radiation mediated by polyploid plasticity in transcriptomes. *Molecular Ecology* 26: 193–207.
- Song J-M, Guan Z, Hu J, Guo C, Yang Z, Wang S, Liu D, Wang B, Lu S, Zhou R *et al.* 2020. Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of *Brassica napus*. *Nature Plants* 6: 34–45.
- Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P *et al.* 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research* 47: D607–D613.
- Tang H, Lyons E, Pedersen B, Schnable JC, Paterson AH, Freeling M. 2011. Screening synteny blocks in pairwise genome comparisons through integer programming. *BMC Bioinformatics* 12: 102.
- Tasdigian S, Van Bel M, Li Z, Van de Peer Y, Carretero-Paulet L, Maere S. 2017. Reciprocally retained genes in the angiosperm lineage show the hallmarks of dosage balance sensitivity. *The Plant Cell* 29: 2766–2785.
- Tiley GP, Barker MS, Burleigh JG. 2018. Assessing the performance of Ks plots for detecting ancient whole genome duplications. *Genome Biology and Evolution* 10: 2882–2898.
- Trapnell C, Hendrickson DG, Sauvageau M, Goff L, Rinn JL, Pachter L. 2013. Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nature Biotechnology* 31: 46–53.
- Trapnell C, Pachter L, Salzberg SL. 2009. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25: 1105–1111.
- Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L. 2012. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nature Protocols* 7: 562–578.
- Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L. 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology* 28: 511–515.
- Tuteja N, Singh S, Tuteja R. 2012. Helicases in improving abiotic stress tolerance in crop plants. In: Tuteja N, Gill SS, Tiburcio AF, Tuteja R, eds. *Improving crop resistance to abiotic stress*. Weinheim, Germany: Wiley-VCH, 435–449.
- Udall JA, Wendel JF. 2006. Polyploidy and crop improvement. *Crop Science* 46: S3–S14.
- UniProt Consortium T. 2018. UniProt: the universal protein knowledgebase. *Nucleic Acids Research* 46: 2699.
- Viaene T, Vekemans D, Irish VF, Geeraerts A, Huysmans S, Janssens S, Smets E, Geuten K. 2009. *Pistillata*-duplications as a mode for floral diversification in (basal) asterids. *Molecular Biology and Evolution* 26: 2627–2645.
- Wang C, Li H, Li Y, Meng Q, Xie F, Xu Y, Wan Z. 2019. Genetic characterization and fine mapping BrCER4 in involved cuticular wax formation in purple cai-tai (*Brassica rapa* L. var. purpurea). *Molecular Breeding: New Strategies in Plant Improvement* 39: 12.
- Wang C, Li Y, Xie F, Kuang H, Wan Z. 2017. Cloning of the BrCER1 gene involved in cuticular wax production in a glossy mutant of non-heading Chinese cabbage (*Brassica rapa* L. var. communis). *Molecular Breeding: New Strategies in Plant Improvement* 37: 142.
- Wang X, Wang H, Wang J, Sun R, Wu J, Liu S, Bai Y, Mun J-H, Bancroft I, Cheng F *et al.* 2011. The genome of the mesopolyploid crop species *Brassica rapa*. *Nature Genetics* 43: 1035–1039.
- Yang CJ, Samayoa LF, Bradbury PJ, Olukolu BA, Xue W, York AM, Tuholski MR, Wang W, Daskalska LL, Neumeyer MA *et al.* 2019. The genetic architecture of teosinte catalyzed and constrained maize domestication. *Proceedings of the National Academy of Sciences, USA* 116: 5643–5652.
- Yu F, Zhang X, Huang Z, Chu M, Song T, Falk KC, Deora A, Chen Q, Zhang Y, McGregor L *et al.* 2016. Identification of genome-wide variants and discovery of variants associated with *Brassica rapa* clubroot resistance gene *Rcr1* through bulked segregant RNA sequencing. *PLoS ONE* 11: e0153218.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Comparison of the POINt inferred paleologs with Tasdigian *et al.* (2017).

Fig. S2 Manhattan plots of the SWEED analyses.

Fig. S3 Circos plot of SWEED and GDE gene density across the *B. rapa* genome.

Fig. S4 Heatmaps of expression level of genes with significantly different expression in four *B. rapa* crop varieties compared to a control group.

Fig. S5 Comparison of π across *Brassica rapa* paleologs and non-paleologs from the *B. rapa* only synteny + K_s paleolog inference approach.

Fig. S6 ANGSD read mapping.

Table S1 Sample information for the 102 *B. rapa* accessions used in this study.

Table S2 *Brassica rapa* gene IDs for genes identified as paleologs and non-paleologs in our analyses.

Table S3 Comparisons of our paleolog lists and candidate genes with the 'core single copy' genes from Li *et al.* (2016).

Table S4 *Brassica rapa* gene IDs for candidate genes identified by our analyses for each crop variety.

Table S5 Total number of candidate genes identified in our analyses and their distribution on the ten chromosomes of *B. rapa*.

Table S6 Detailed information of the five *B. rapa* domestication candidate genes identified in all three of our genome scan approaches.

Table S7 Breakdown of POINT paleolog enrichment in candidate gene lists and diversity.

Table S8 Summary of the *B. rapa* candidate genes identified from published mapping studies.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.



About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Foundation, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews and Tansley insights.
- Regular papers, Letters, Viewpoints, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**