The *Aquilegia* genome reveals a hybrid origin of core eudicots

Gökçe Aköz[1,2] and Magnus Nordborg[1,*]
[1]Gregor Mendel Institute, Austrian Academy of Sciences, Vienna Biocenter, Vienna, Austria
[2]Vienna Graduate School of Population Genetics, Vienna, Austria
[*]magnus.nordborg@gmi.oeaw.ac.at

Abstract

Background: Whole-genome duplications (WGD) have dominated the evolutionary history of plants. One consequence of WGD is a dramatic restructuring of the genome as it undergoes diploidization, a process under which deletions and rearrangements of various sizes scramble the genetic material, leading to a repacking of the genome and eventual return to diploidy. Here, we investigate the history of WGD in the columbine genus *Aquilegia*, a basal eudicot, and use it to illuminate the origins of the core eudicots.
Results: Within-genome synteny confirms that columbines are ancient tetraploids, and comparison with the grape genome reveals that this tetraploidy is shared with the core eudicots. Thus, the ancient *gamma* hexaploidy found in all core eudicots must have involved a two-step process: first tetraploidy in the ancestry of all eudicots, then hexaploidy in the ancestry of core eudicots. Furthermore, the precise pattern of synteny sharing suggests that the latter involved allopolyploidization, and that core eudicots thus have a hybrid origin.
Conclusions: Novel analyses of synteny sharing together with the well-preserved structure of the columbine genome reveal that the *gamma* hexaploidy at the root of core eudicots is likely a result of hybridization between a tetraploid and a diploid species.

Background

Whole-genome duplication (WGD) is common in the evolutionary history of plants [reviewed in 1,2]. All flowering plants are descended from a polyploid ancestor, which in turn shows evidence of an even older WGD shared by all seed plants [3]. These repeated cycles of polyploidy dramatically restructure plant genomes. Presumably driven by the "diploidization" process, whereby genomes are returned to an effectively diploid state, chromosomes are scrambled via fusions and fissions, lose both repetitive and genic sequences, or are lost entirely [4–11]. Intriguingly, gene loss after WGD is non-random: not only is there a bias against the retention of certain genes [12,13], but also against the retention of one of the WGD-derived paralog chromosomes [6,9,14–16].

We investigated the history of WGDs in the columbine genus *Aquilegia* for two reasons. The first is related to its phylogenetic position: as a basal eudicot, columbines are members of the very earliest diverging branch of the eudicots [17,18]. This matters

because our understanding of eudicot karyotype evolution is limited to the heavily sampled core eudicots. Using the recently published *Aquilegia coerulea* genome [19], we are able to address key questions about the history of polyploidization in all eudicots. Second, we traced the origins of the columbine chromosomes with a particular focus on chromosome 4, which, compared to the rest of the genome: harbors more genetic polymorphism, has a higher transposable element density, has a lower gene density and reduced gene expression, shows less population structure worldwide, appears more permeable to gene flow, and carries the rDNA clusters [19].

Results

Within genome synteny confirms columbine paleotetraploidy

Ancient WGDs have been commonly inferred from the distribution of divergences between gene duplicates. The simultaneous generation of gene duplicates via WGD is expected to produce a peak in the age distribution relative to the background age distribution of single gene duplicates [20–22]. Such a spike of ancient gene birth was the first evidence of paleotetraploidy in columbines [23], and was later supported by gene count-based modelling [24].

Given an assembled genome, a more direct method to infer ancient polyploidy is to look for regions with conserved gene order [25,26]. Such conservation (a.k.a., synteny) decreases over time due to gene loss and rearrangements, but will still be detectable unless the extent of change is extreme. We detected a total of 121 synteny block pairs harboring at least five paralogous gene pairs within the columbine genome. The distribution of these blocks across the seven columbine chromosomes indicates pairwise synteny between large genomic regions (Fig. 1). This 1:1 relationship suggests a single round of WGD in columbine, and is further supported by similar levels of divergence between synteny pairs (Figs. S1 and S2).

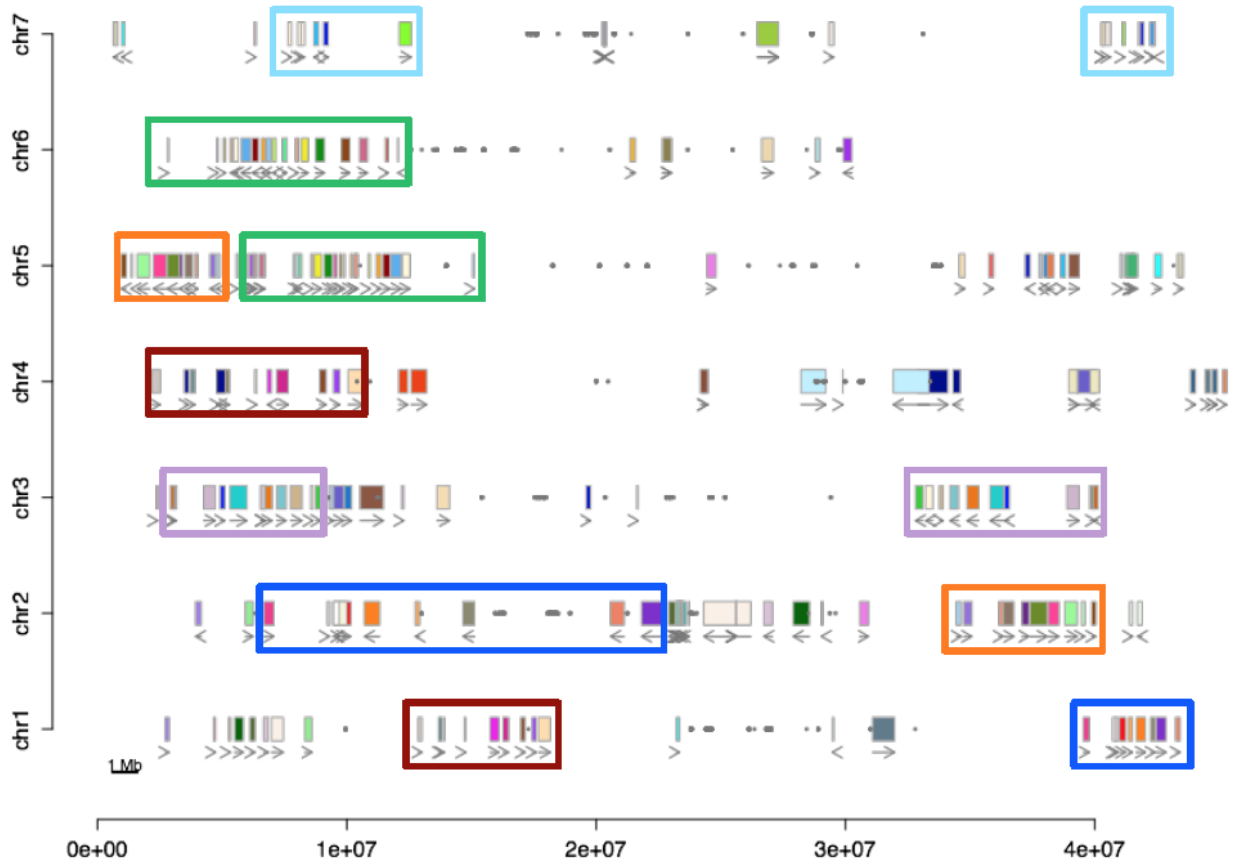


Fig. 1: Intragenomic synteny blocks in the columbine genome. Pairs of synteny blocks are denoted as uniquely colored small rectangles. Larger rectangles of the same color outline large regions of synteny. Arrows under the synteny blocks show the orientation of the alignment between collinear genes. Grey dots highlight BLAST hits of a 329 bp centromeric repeat monomer [19,27].

Columbines share ancient tetraploidy with core eudicots

All sequenced core eudicots appear to share a triplicate genome structure due to paleohexaploidy postdating the separation of monocots and eudicots [9,28–32, and Supplementary Note 5 in 33]. The tetraploidy in columbines, a basal eudicot, might be independent of this ancient "*gamma*" hexaploidy (Scenarios 1 and 2 in Fig. 2) or might be a remnant of a WGD at the base of all eudicots, which formed the first step of the *gamma* hexaploidy in core eudicots (Scenario 3 in Fig.2).

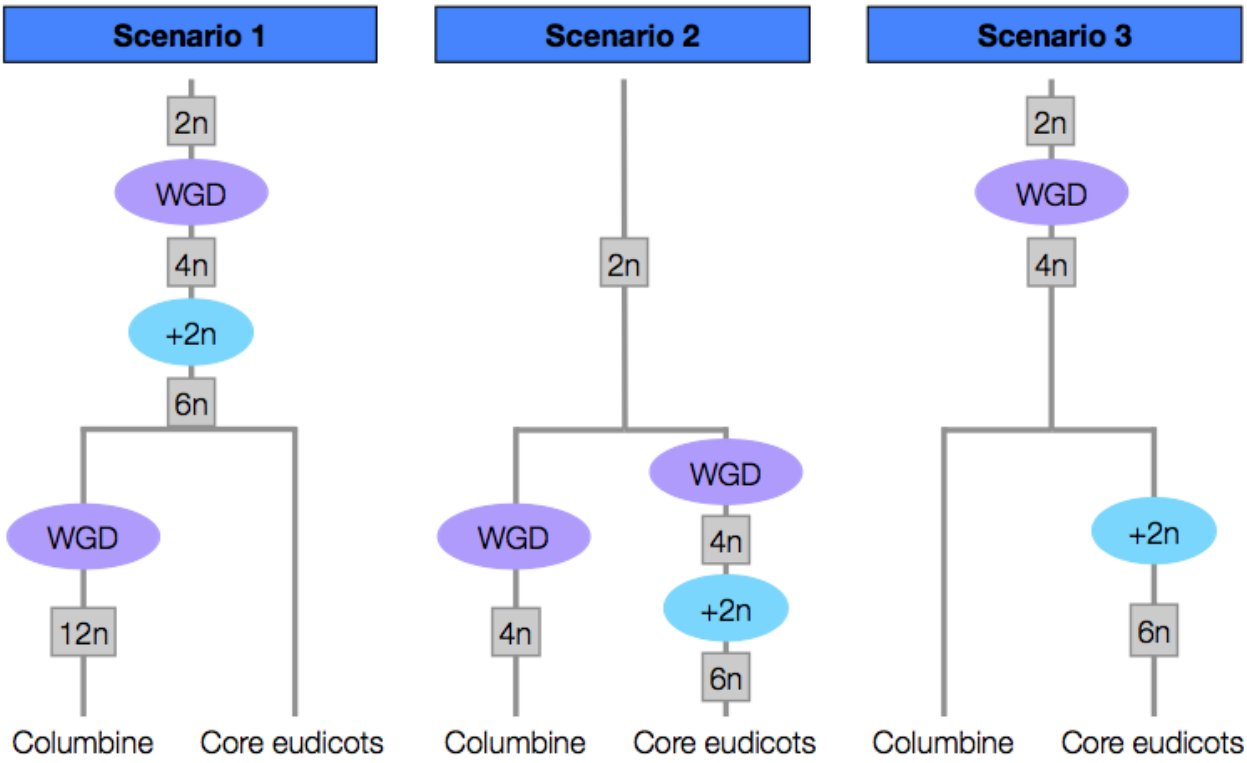


Fig. 2: Three scenarios for the relationship between columbine tetraploidy and core eudicot "*gamma*" hexaploidy. The *gamma* hexaploidy is a two-step process: a single round of WGD creates tetraploids (4n) whose unreduced gametes then fuse with diploid gametes (+2n). **Scenario 1**: *gamma* hexaploidy precedes the split between columbine and core eudicots, with the former undergoing an additional tetraploidy. **Scenario 2**: Both *gamma* hexaploidy and columbine tetraploidy occur after the split between columbines and core eudicots. **Scenario 3**: Columbine tetraploidy is derived from the ancient tetraploidy that was the first step of the process leading to *gamma* hexaploidy.

We used the grape (*Vitis vinifera*) genome as a representative of the core eudicots to distinguish between the three scenarios in Fig. 2. Grape has experienced the least number of chromosomal rearrangements post-*gamma* and thus strongly resembles the ancestral pre-hexaploid genome [34]. Given the ploidy level of columbine under each scenario, we can predict the synteny relationship between the homologous chromosomes of grape and columbine, which is simply the ratio of haploid chromosome set in grape to that of in columbine. If tetraploidy in columbines is lineage-specific and superimposed on the *gamma* hexaploidy (Scenario 1), we would expect to find a 3:6 ratio of grape and columbine synteny blocks. Instead, we observe a 3:2 relationship (Figs. 3 and S3) as expected under Scenarios 2 or 3. A similar 3:2 pattern is found in comparisons between grape and sacred lotus [35]. This strongly suggests that basal

eudicots do not share the triplicate genome structure of core eudicots, ruling out Scenario 1.

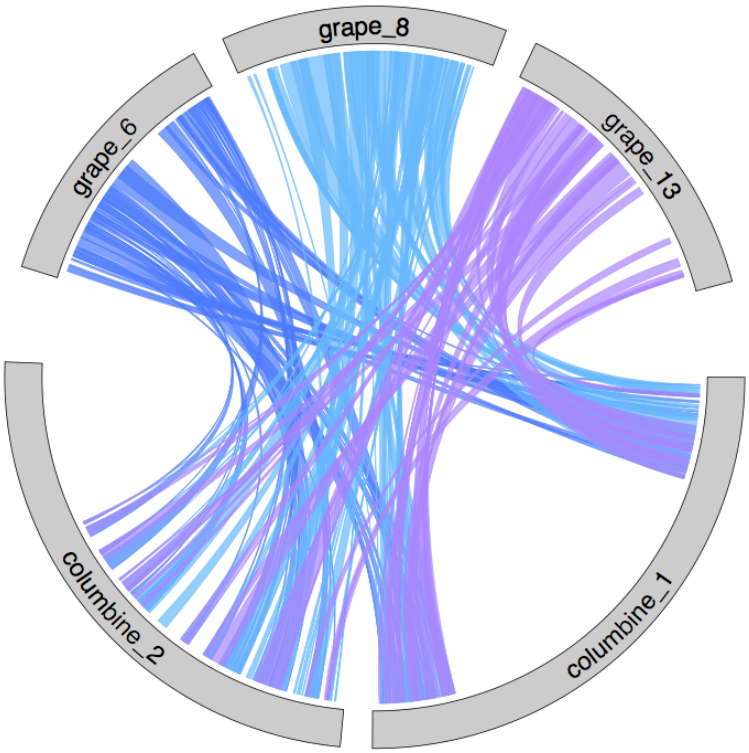


Fig. 3: Synteny between the homologous regions of columbine and grape. The results are shown here only for the columbine chromosomes 1,2 and the grape chromosomes 6,8 and 13 but reflect the overall synteny relationship of 3:2 between grape:columbine chromosomes (see Fig. S3 for the genome-wide synteny). This pattern argues against Scenario 1, but is consistent with either Scenario 2 or Scenario 3.

To distinguish between the two remaining scenarios, we compared the divergence at synonymous sites (Ks) between columbine paralogs, grape paralogs and columbine-grape homologs. In agreement with the analysis of Jiao et al. [36], the Ks distribution for grape paralogs shows two major peaks, as expected under the two-step model for gamma hexaploidy (Fig. 4). However, columbine paralogs and columbine-grape homologs each show a single peak of divergence — and the peaks overlap each other and the "older" divergence peak of grape paralogs. This suggests that columbine tetraploidy is derived from the tetraploidy that eventually led to *gamma* hexaploidy in core eudicots (Scenario 3).

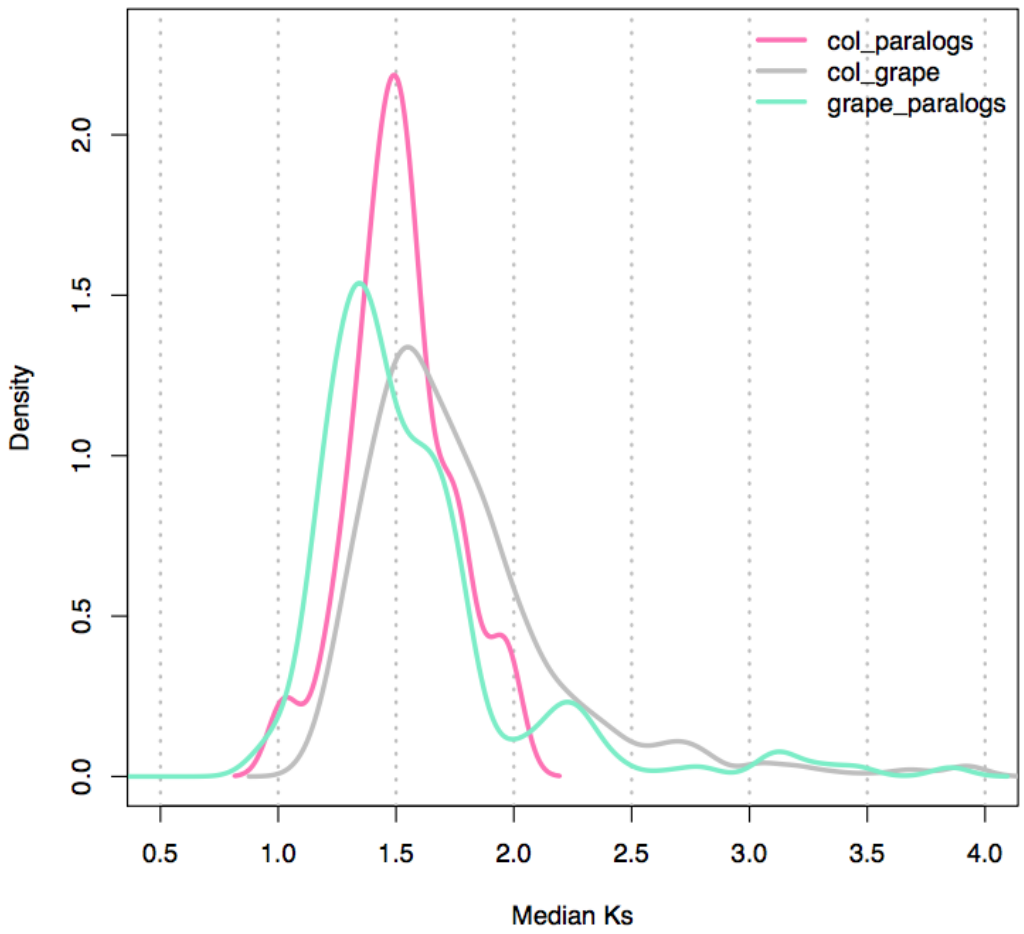
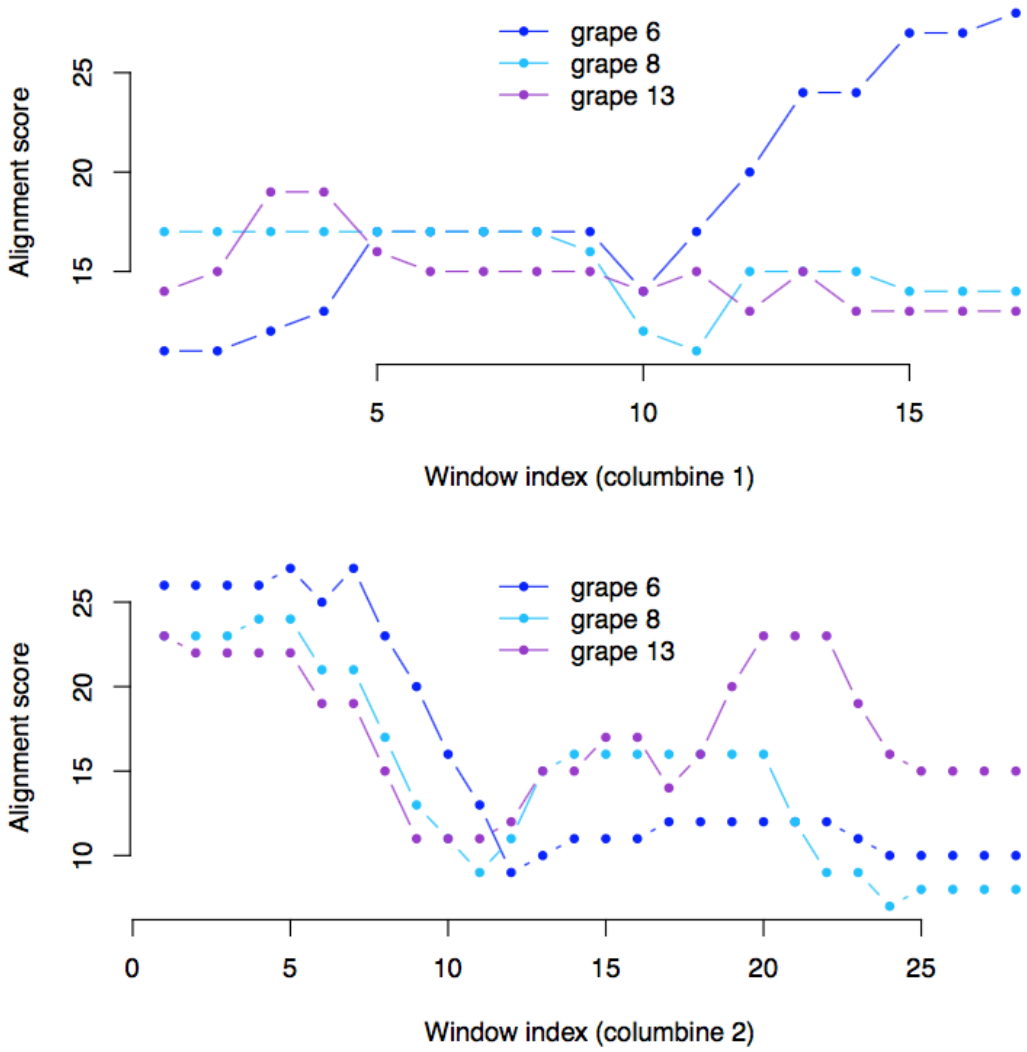


Fig. 4: The distribution of the median Ks across syntenic regions. Synteny blocks are identified within columbine (col_paralogs), between columbine and grape (col_grape) and within grape (grape_paralogs). Note that only the putative WGD-derived blocks (median Ks=1-2) are kept in columbine (Fig. S2).

To further explore the hypothesis of a shared WGD by all eudicots, we focused on the gene order similarity between the homologous regions of columbine and grape. If columbine and grape have descended from a common tetraploid ancestor, they should have inherited diploidization-driven differential gene order on the paralogous chromosomes of the ancestor (Fig. S5). As a result, we expect to see the alternative paralogous gene orders to be uniquely shared between two different pairs of columbine and grape chromosomes. To detect this, we first searched for at least three consecutive genes aligning between a pair of columbine and grape chromosomes and then looked at the distribution of these genes on all the columbine and grape chromosomes. This way of reconstructing chromosomes clearly shows that each of the paralogous chromosome pairs in columbine has a match to a single grape chromosome, with respect to its gene order (Fig. S6). This result was corroborated by a second approach where we quantify

146 the similarity between columbine and grape chromosomes. When we performed a
147 pairwise alignment between each sliding window of genes on a columbine chromosome
148 and all the genes on a grape homolog, we again see that each member of columbine
149 paralogs gets the best hit to a single grape chromosome (Figs. 5 and S7-9). Reshuffling
150 genes on grape chromosomes further indicates that this pattern of clustering is highly
151 unlikely to be produced by chance alone (p=0-0.05).
152



153
154
155 **Fig. 5: Examples of gene order similarity between the homologous regions**
156 **of columbine and grape.** For successive windows of genes within a given columbine
157 chromosomal region, the best alignment score with respect to each of the three grape
158 chromosomes harboring homologous regions, is given. For example, columbine
159 chromosomes 1 and 2 share a paralogous region homologous to grape chromosomes 6,
160 8, and 13 (Figs. 7 and S3). The chromosome 1 region **(top panel)** appears to be most
161 closely related to grape chromosome 6, whereas its paralogous counterpart on
162 chromosome 2 **(bottom panel)** appears to be most closely related to grape

chromosome 13. See Fig. S6 for the correspondence between gene orders and scores, which peak towards the end of each columbine region. Note that the results presented here are shown for a window size of 12 genes but remain significant for all the window sizes tested (p=0-0.05).

An eudicot-wide WGD is further supported by the observation that a chromosomal fusion, presumably experienced by the common tetraploid ancestor, is still detectable in the genomes of columbine and grape despite their separation of around 125 million years [37]. The first hint comes from the composition of the chromosomes: columbine chromosome 5 and grape chromosome 7 share the two chromosomal origins (Fig. 7). If these fused chromosomes were created by a single fusion event in the common tetraploid ancestor of eudicots, they should match each other with respect to gene order on each of the two homologous portions ("orange" and "green" portions in Fig. 6). This is what we see: columbine chromosome 5 and grape chromosome 7 cluster together with respect to their gene order on the "orange" portion (Fig. S7). For the "green" portion, columbine chromosome 5 matches grape chromosome 4 (Fig. S8), which used to be fused to grape chromosome 7 [38]. Additional support for shared ancestral fusion comes from the cacao (*Theobroma cacao*) genome [39]. The first chromosome of cacao does not only show a similar pattern of chromosomal ancestry [38,39], but also shares the gene order exclusively with the grape chromosomes 4 and 7 on the corresponding homologous portions (Fig. S10). In summary, the columbine fusion clusters with that of grape, which, in turn, clusters with that of cacao, strongly favoring a common origin of the fusion between "orange" and "green" ancestral chromosomes (Fig. 7).

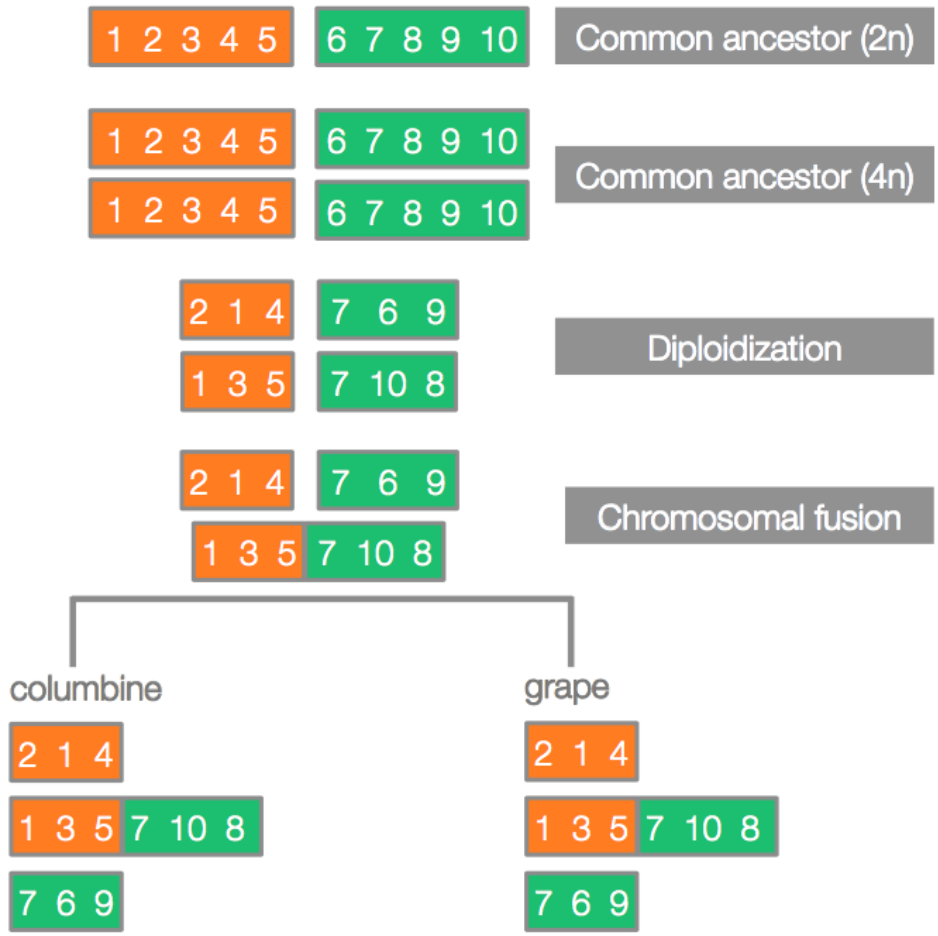


Fig. 6: Schematic of predicted synteny patterns in the case of shared ancestral fusion. Two ancestral chromosomes (orange and green rectangles, with genes depicted as numbers) undergo WGD. Paralogous chromosome pairs diverge as a part of the diploidization process. A fusion joins one version of the "orange" chromosome ('1, 3, 5') with one version of the "green" chromosome ('7, 10, 8'). If this took place in the common tetraploid ancestor of eudicots, the fused chromosomes in columbine and grape should also carry these versions on their "orange" and "green" portions. In the hypothetical example here, diploidization precedes the fusion event but may well happen afterwards with no effect on the predicted synteny patterns.

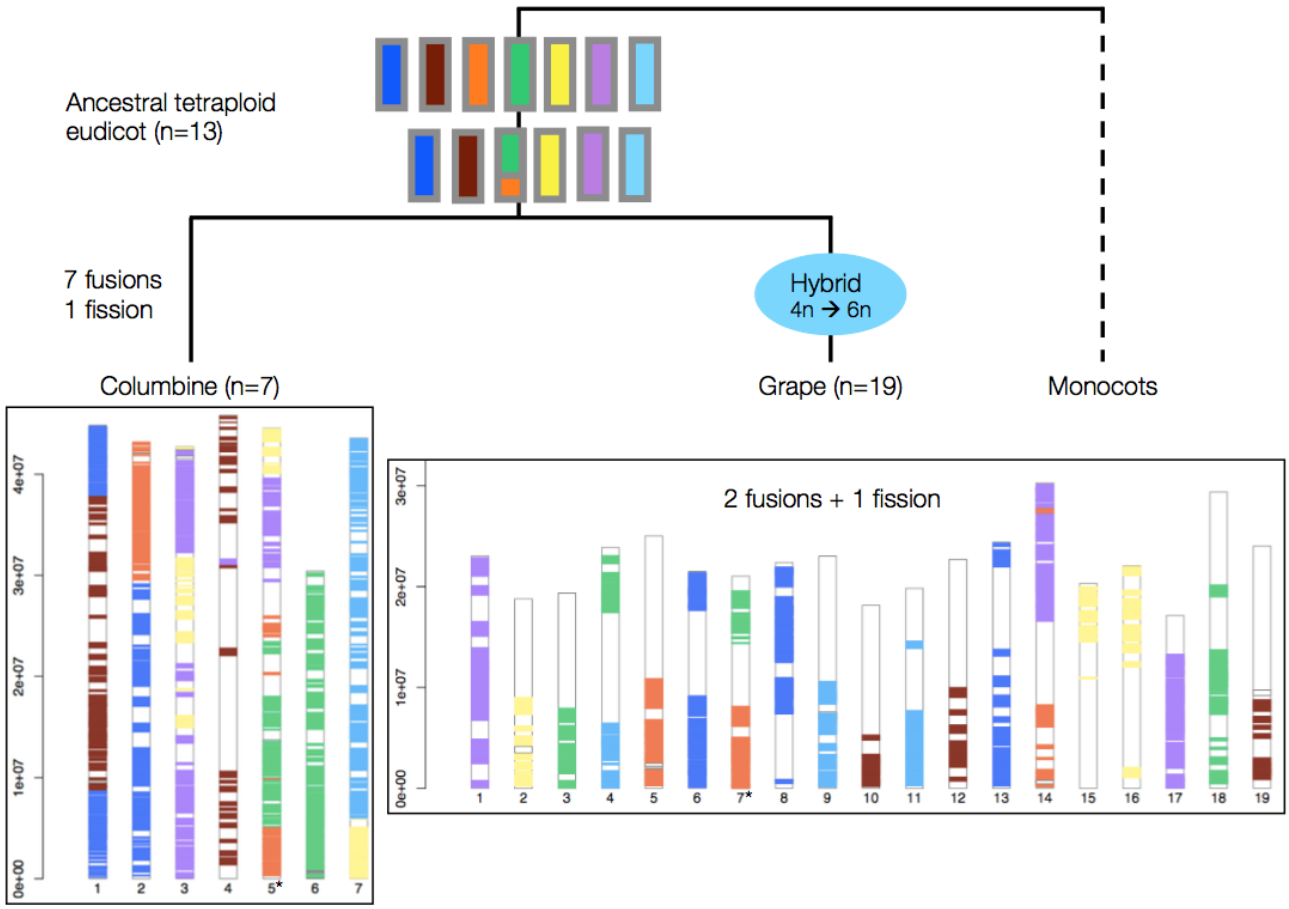


Fig. 7: Tracing the genome reshuffling in columbine following tetraploidy. Grape chromosomes **(bottom right)** are colored by within-genome synteny. Seven distinct colors represent the haploid set of seven ancestral chromosomes before the eudicot-wide WGD. Each color is shared by three grape chromosomes reflecting the triplicate genome structure of core eudicots. The only exception is the "green" chromosome which is shared by four grape chromosomes due to a fission event [38]. Columbine chromosomes **(bottom left)** are colored by their synteny to grape chromosomes. Each color is generally shared by two chromosomes, reflecting columbine paleotetraploidy. As few as 7 fusions and a single fission are enough to explain the current structure of the columbine genome. Of these 7 fusions, 5 are between different chromosomes while 2 are between WGD-derived paralogous chromosomes. Columbine chromosomes 3 and 7 are examples of the latter (Figs. 1 and S4). Note that chromosome 5 of columbine and chromosome 7 of grape (*) both have the colors "orange" and "green" (cf. Fig. 6).

The core eudicots have a hybrid origin

Our inference of shared tetraploidy between basal and core eudicots makes use of the signals presumably generated by diplodization (Figs. 6 and S5). However, hybridization of unreduced gametes from two divergent diploid genomes, "allotetraploidy", would also lead to gene order-based clustering between two different pairs of grape and columbine chromosomes (Figs. S11-12). In this case, the alternative paralogous gene orders of the tetraploid ancestor reflect the gene orders on progenitor chromosomes. Thus, the clustering pattern does not depend on whether the eudicot tetraploid genome evolved via "auto-" or "allopolyploidy". The same is not true for the second of the process leading to hexaploidy. Only allohexaploidy would lead to one of the three paralogous grape chromosomes being an "outlier" to the two grape-columbine pairing (Figs. S11-12) — which is what we see in our data (light blue lines in Figs. 5 and S7-9).

If our interpretation is correct and all core eudicots have a hybrid origin, the pattern of gene order-based clustering should be conserved. That is, we should be able to identify the hexaploidy-derived "outlier" chromosomes in other core eudicot genomes as well. To check this expectation, we again used the cacao genome, one of the most conserved genomes after grape [9,39]. Pairwise alignment between the homologous regions of columbine and cacao confirms our expectation: each member of columbine paralogs pairs up with a single cacao chromosome, leaving one of the cacao paralogs as an outlier (Figs. S13-14). Furthermore, as shown in Fig. 8 (see also Fig. S10), the cacao regions putatively derived from tetraploidy and hexaploidy, respectively, show a very clear one-to-one match to those in grape (detected in the grape-columbine comparison). As expected, the putatively orthologous pairs of cacao and grape regions show similar levels of synteny conservation with their paralogous counterparts, with the "outlier" regions being the most divergent [38]. Thus, the cacao genome provides an independent line of evidence for a hybrid origin, and highlights the key role of the columbine genome in unravelling the history of the eudicot genome.

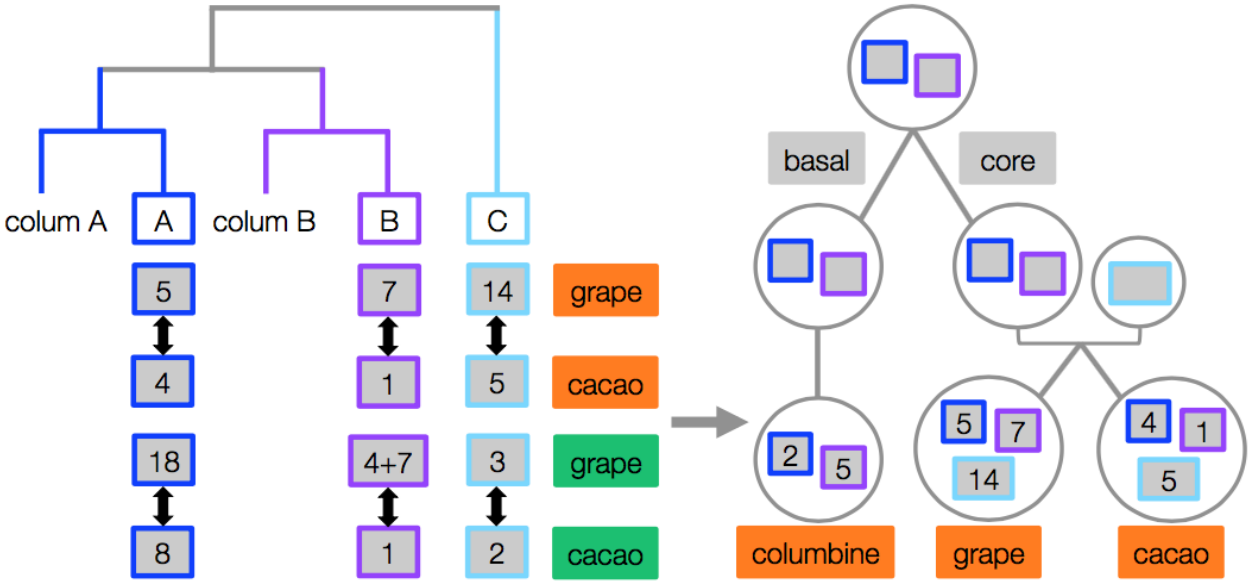


Fig. 8: The shared history of chromosomes in columbine, grape and cacao. Gene order-based clustering results **(left panel)** are summarized here for the chromosomes harboring the "orange" and "green" homologous portions. The former corresponds to 5, 7, 14 in grape and 1, 4, 5 in cacao. The latter corresponds to 3, 4+7 (products of a fission), 18 in grape and 1, 2, 8 in cacao. In columbine, the "orange" portions are on chromosomes 2 and 5 while the "green" portions are on chromosomes 6 and 5, each pair of which being denoted as *colum A* and *colum B*, respectively. Both grape- and cacao-columbine pairing distinguish tetraploidy-derived regions (blue and purple rectangles) from hybridization-derived ones (light blue rectangles), defining the orthologous sets of regions across the three eudicot genomes **(right panel)**. The conservation of gene order exclusively between the putatively orthologous regions of grape and cacao (black arrows, Fig. S10) further strengthens our columbine-based inference of orthology.

Current columbine chromosomes have mostly been generated via fusions

It is widely accepted that genome shuffling post-WGD has shaped the present-day karyotypes of all plant genomes [34]. Nevertheless, the extent of genome shuffling as a part of the "re-diploidization" process seems to vary widely: only 3 chromosomal rearrangements post-*gamma* are enough to explain the current structure of the grape genome (Fig. 7) while almost 150 chromosomal rearrangements were necessary for the sunflower genome to reach its current karyotype after several rounds of WGD [11]. To check where columbine falls in this spectrum, we identified chromosomal rearrangements likely to have happened after the tetraploidy shared by all eudicots: if the pre-WGD ancestral eudicot karyotype had a haploid number of 7 chromosomes [28], only seven columbine-specific fusions and a single fission are enough to explain the

reduction in columbine chromosome number from n=13 to n=7 after the ancestral fusion event (Fig. 7). These rearrangements involve all the chromosomes in columbine apart from chromosomes 4 and 6, the former of which paradoxically shows the greatest erosion of synteny with grape chromosomes (Figs. 7 and S3). Given all the evidence suggesting a "decaying" nature of columbine chromosome 4 [19], we repeated the analysis of grape-columbine synteny detection with relaxed parameter settings. We did this by decreasing the minimum number of aligned gene pairs within a block (from 5 to 3) and increasing the maximum genic distance between matches (from 20 to 30). This allowed us to extend the synteny blocks towards more proximal regions (Fig. S15). Further zooming into the synteny relationship between grape chromosomes that are homologous to columbine chromosome 4 confirmed that there is no evidence of a fusion event (Fig. 9).

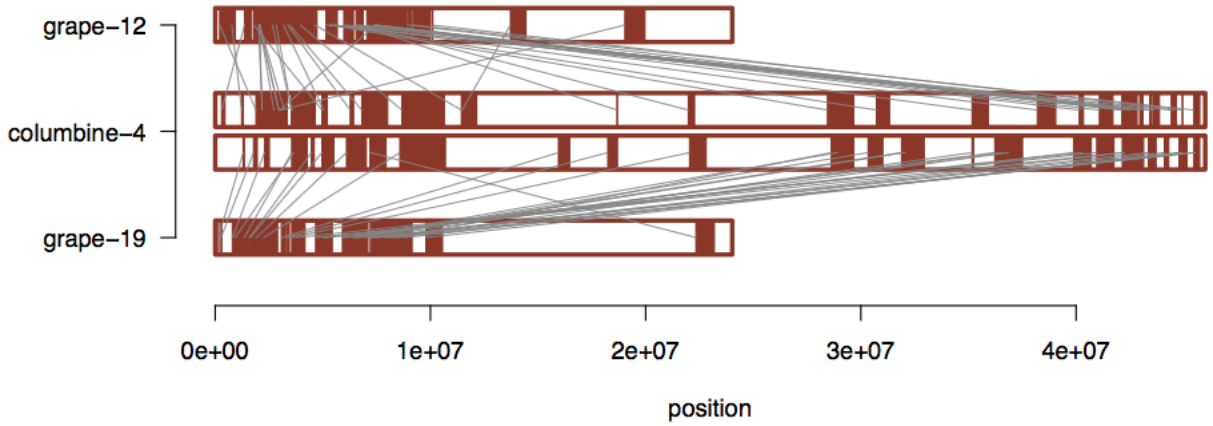


Fig. 9: Synteny between columbine chromosome 4 and grape chromosomes 12 and 19. Much smaller grape chromosomes look like the compact versions of columbine chromosome 4. Note that this result is generated with the most relaxed parameter combination in Fig. S15, but holds true for a less relaxed combination of parameters as well (Fig. S16).

The lack of a fusion event on columbine chromosome 6 might explain the fact that it is the smallest chromosome of columbine (Fig. 7). However, chromosome 4 is comparable in size to the remaining chromosomes, all of which are products of ancient fusion events. The observations that chromosome 4 has a higher proportion of genes in tandem duplicates (0.37 versus genome-wide mean of 0.22) and a greater extent of intra-chromosomal synteny (indicative of segmental duplications) (Fig. S17) suggest that chromosome 4 has reached a comparable size partly due to numerous tandem and segmental duplications and partly due to an expansion of repetitive DNA [19]. These results reinforce the idea that chromosome 4 has followed a distinct evolutionary path from the rest of genome.

Fusion-dominated genome shuffling [34] is not the only facet of diploidization [40]. Following WGDs, gene duplicates get lost and this happens in a non-random manner. Genes involved in connected molecular functions like kinases, transcription factors and ribosomal proteins are retained in pairs [41–45] potentially due to dosage-related constraints [46]: losing or duplicating some, but not all of these dosage-sensitive genes might upset the stoichiometric relationship between their protein products [47–49]. Consistent with this dosage balance hypothesis, columbine genes potentially retained post-WGD (1302 genes across 76 syntenic regions; Supplementary Data 1) are enriched for the GO categories "structural constituent of ribosome", "transcription factor activity", "translation" ($p<0.001$) and "protein tyrosine kinase activity" ($p<0.01$). Tandemly duplicated genes (n=6972), on the other hand, are depleted for the GO categories "structural constituent of ribosome", and "translation" ($p=10^{-17}$), reflecting the role of dosage-related purifying selection.

Discussion

The evolutionary history of plants is characterized by multiple WGDs and columbine is no exception. The alignment between chromosomal regions in a 1:1 ratio (Fig. 1) confirms a single round of WGD in columbine [23,24,50]. Furthermore, we demonstrate that this tetraploidy is shared with all eudicots, refuting a lineage-specific polyploidization in columbine [23,50], in favor of an eudicot-wide WGD [26,35,36,51]. Unlike previous attempts based on genetic distance, our approach simply relies on gene order conservation. It also takes advantage of the well-preserved genome structure of columbines: free from recent WGDs, the columbine genome carries only the traces of the ancient tetraploidy.

This approach also helps us shed light on the nature of the *gamma* hexaploidy found in all core eudicots [9,28–32, and Supplementary Note 5 in 33]. WGDs have often been discussed as if they were "events", ignoring the process by which they originated. We show here that core eudicot hexaploidy is the result of two processes: an ancient tetraploidization shared by all eudicots, followed by allopolyploidization leading to core eudicots. In other words, all core eudicots have a hybrid origin. An allohexaploid origin has indeed been previously suggested by Murat et al. [9], who identified the three subgenomes of grape using differential patterns of gene loss on "dominant" versus "sensitive" subgenomes. Their classification assumes that the most recently added set of paralogous chromosomes will be "dominant", because they have spent a shorter amount of time in the polyploid genome and thus experienced fewer gene losses. Contrary to this, our results suggest that the most recently added grape chromosomes (chromosomes 3, 8, 9 and 14) largely corresponds to the "sensitive" grape chromosomes identified by Murat et al. [9]. Instead, we argue that the extensive gene loss in the "youngest" subgenome reflects its divergence from the other two subgenomes at the time of hexaploid formation, perhaps similar to the situation in the allotetraploid

Arabidopsis suecica, which is a hybrid between the more ancestral (n=8) genome of *A. arenosa*, and the heavily reduced (n=5) genome of *A. thaliana* [52]. Another example is hexaploid wheat, which is a hybrid between tetraploid emmer wheat and a wild diploid grass, *Aegilops tauschii* [53 and references therein].

Conclusions

Our findings help us understand the hybrid structure of core eudicot genomes and will hopefully encourage larger scale analyses to understand what hybridization has meant for core eudicots — a group which comprises more than 70% of all living flowering plants [54]. What are the hybridization-coupled changes that has led to the current patterns of gene expression, methylation, transposable element density/distribution? All these questions call for additional genomes from basal eudicots which — as this study illustrates — have great values as outgroup to the core eudicots.

Materials and Methods

Synteny detection

We performed all genes (CDS)-against-all genes (CDS) BLAST for the latest version of *Aquilegia coerulea* reference genome (v3.1) using SynMap tool [29] in the online CoGe portal [55]. We also looked at the synteny within *Vitis vinifera* (v12) and between *V. vinifera* and *A. coerulea* using both default and more relaxed parameter combinations in DAGChainer. We filtered the raw output files for both within grape and grape-to-columbine synteny. For the former, we only kept the blocks that are syntenic between the polyploidy-derived paralogous chromosomes of grape as identified by Jaillon et al. [28]. For the latter, we required that a given columbine chromosome is overall syntenic to all the three paralogous chromosomes of grape. So, for a given pair of columbine and grape chromosomes, we only kept the blocks if the columbine chromosome also matches to the other members of paralogous grape chromosomes.

The raw output files can be regenerated at the CoGe portal [55] using the id numbers provided below for each species (Availability of data and material) and changing the default parameter combination in DAGChainer (D:A=20:5) when needed. D and A specify the maximum genic distance between two matches and the minimum number of aligned gene pairs, respectively, to form a collinear syntenic block.

Estimating the divergence between synteny block pairs

We used Ks (the number of synonymous substitutions per synonymous site) values provided for each duplicate gene pair by the CoGe portal [55]. We estimated the median

377 Ks of all gene duplicates in a syntenic block after filtering duplicates with Ks>10 due to
378 saturation effect [56].

379 **Quantifying gene order similarity**

380 We first detected three consecutive genes aligning between a pair of columbine and
381 grape chromosomes harboring homologous regions (D:A=0:3). We particularly chose
382 three genes since it is the most stringent value we could use to detect homologous
383 synteny blocks; we detected almost nothing when we required 4 consecutive genes
384 (D:A=0:4). We then looked at the distribution of these genes on a given pair of
385 columbine and grape chromosomes and also on their paralogous counterparts
386 (D:A=0:1). Once we had the gene order for each chromosome, we assigned a unique
387 word to each synteny block and the genes forming the block to be able to use the text
388 alignment provided by the R package *align_local* [57]. Having each chromosome
389 represented by a sentence, we quantified the gene ("word") similarity as such: for an
390 initial N number of words on a columbine chromosome (N=window size), we did a
391 pairwise alignment between these N words and a grape chromosome (match=4, gap=-
392 1). We repeated the same analysis with the inverted order of N words and picked the
393 maximum alignment score. We repeated these steps by sliding the window by one word
394 and keeping the N constant to get a distribution of scores as in Fig. 5. We used different
395 N values ranging from 4 to 15. Note that we excluded columbine chromosomes 3 and 4
396 from this analysis since both have a complex history of lineage-specific chromosomal
397 reshuffling events: fusions and a fission gave rise to columbine chromosome 3 (Figs. 1
398 and 7) while duplications have shaped the current structure of columbine chromosome
399 4 (Fig. S17).
400
401 We applied the same stringent criteria (D:A=0:3) to detect the homologous regions
402 between grape and cacao (*Theobroma cacao*, v1). The same criteria led to very few
403 homologous regions between columbine and cacao. So, we relaxed the parameters for
404 the synteny detection between these two genomes (D:A=0:2) and quantified the gene
405 order similarity with greater window sizes (N=20, 30, 35, 40 and 50). Note that we
406 focused on the triplicated regions distributed across 3 different cacao chromosomes
407 (Figs. 8, S13-14), which are rather unaffected by lineage-specific shuffling [38].

408 **Statistical testing of gene order similarity**

409 Given the gene order similarity between the two different pairs of columbine and grape
410 chromosomes harboring homologous regions, we performed permutation tests to
411 estimate the probability of observing such a clustering just by chance. To do so, we first
412 combined all the grape genes and sampled the same number of genes ("words") as we
413 observe to reconstruct each of the paralogous grape chromosome. We repeated the
414 quantification step as above to get a permuted distribution of alignment score between a

pair of columbine and grape chromosomes. We used Wilcoxon rank sum test (W-statistic) to quantify the shift in the distribution of alignment scores between one of the members of columbine paralogous chromosomes and its best grape hit when combined with the alignment scores between the same columbine chromosome and other grape chromosomes. We repeated the same analysis for the other member of columbine paralogous chromosomes as well. Having these *observed* W-statistics, we counted the number of cases (out of 100) where the permuted distributions generate W-statistics as high as or higher than the observed ones. Note that for columbine chromosome 7, whose structure has been greatly shaped by the fusion of WGD-derived paralog chromosomes (Fig. S4), we created two paralogous chromosomes using the observed distribution of alignment scores (Fig. S9). Columbine chromosome 7 matches best to grape chromosome 11 for the first 14 "words" and to grape chromosome 4 for most of the remaining "words", which define the putative boundaries of columbine paralogous chromosomes before the fusion event. We ran permutation tests for the columbine-cacao pairing as well (Figs. S13-14).

GO enrichment analysis

We used gene annotations provided by JGI [19] to test the null hypothesis that the property for a gene to be retained post-WGD and to belong to a given GO category are independent. We created a 2x2 contingency table as shown below and applied Fisher's exact test for each GO category independently. We repeated the same analysis for tandem gene duplicates as identified by SynMap [29,55]; this time testing the null hypothesis that the property for a gene to be tandemly duplicated and to belong to a given GO category are independent. We excluded genes on scaffolds and reported enriched/depleted categories if they remain significant ($p < 0.05$) after multiple test correction (fdr).

Table 1: 2x2 contingency table obtained by classifying genes into 2 categorical variables. The letters denote the number of genes for a given category (e.g. "a" denotes the number of retained genes annotated with the tested GO category).

	GO	**not-GO**	**SUM**
retained	a	b	a+b*
not-retained	c	d	c+d
SUM	a+c	b+d	N=total number of genes =29550 (across 7 chromosomes)

*equal to 1302 and 6972 for candidate WGD-derived paralogs and tandem gene duplicates, respectively.

454 # List of abbreviations

455 WGD: whole genome duplication; Ks: the number of synonymous substitutions per
456 synonymous site; GO: Gene Ontology.
457

458 # Declarations

459 ## Availability of data and material

460 The columbine, grape and cacao genomes are available at the CoGE portal for the
461 synteny analyses with the id numbers 28620, 19990 and 25287, respectively [55].

462 ## Competing interests

463 The authors declare no competing interests.

464 ## Funding

465 G.A. was supported by the Vienna Graduate School of Population Genetics (Austrian
466 Science Fund, FWF: DK W1225-B20).

467 ## Authors' contributions

468 G.A. performed all analyses. G.A. and M.N. wrote the manuscript.

469 ## Acknowledgements

470 We thank Robin Burns and Claus Vogl for their comments on the manuscript; Daniel
471 Gómez Sánchez and Benjamin Jaegle for the fruitful discussions.
472

473 # References

474 1. Wendel JF. The wondrous cycles of polyploidy in plants. Am J Bot. 2015;102:1753–6.

475 2. Van de Peer Y, Mizrachi E, Marchal K. The evolutionary significance of polyploidy.
476 Nat Rev Genet. 2017;18:411–24.

477 3. Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, et al.
478 Ancestral polyploidy in seed plants and angiosperms. Nature. 2011;473:97–100.

479 4. Leitch IJ, Bennett MD. Genome downsizing in polyploid plants. Biol J Linn Soc Lond.
480 Oxford University Press; 2004;82:651–63.

481 5. Comai L. The advantages and disadvantages of being polyploid. Nat Rev Genet.
482 2005;6:836–46.

483 6. Thomas BC, Pedersen B, Freeling M. Following tetraploidy in an Arabidopsis
484 ancestor, genes were removed preferentially from one homeolog leaving clusters
485 enriched in dose-sensitive genes. Genome Res. 2006;16:934–46.

486 7. Otto SP. The evolutionary consequences of polyploidy. Cell. 2007;131:452–62.

487 8. Renny-Byfield S, Chester M, Kovařík A, Le Comber SC, Grandbastien M-A, Deloger
488 M, et al. Next generation sequencing reveals genome downsizing in allotetraploid
489 Nicotiana tabacum, predominantly through the elimination of paternally derived
490 repetitive DNAs. Mol Biol Evol. 2011;28:2843–54.

491 9. Murat F, Zhang R, Guizard S, Gavranović H, Flores R, Steinbach D, et al. Karyotype
492 and gene order evolution from reconstructed extinct ancestors highlight contrasts in
493 genome plasticity of modern rosid crops. Genome Biol Evol. 2015;7:735–49.

494 10. Murat F, Armero A, Pont C, Klopp C, Salse J. Reconstructing the genome of the most
495 recent common ancestor of flowering plants. Nat Genet. 2017;49:490–6.

496 11. Badouin H, Gouzy J, Grassa CJ, Murat F, Staton SE, Cottret L, et al. The sunflower
497 genome provides insights into oil metabolism, flowering and Asterid evolution. Nature.
498 2017;546:148–52.

499 12. Blanc G, Wolfe KH. Functional divergence of duplicated genes formed by polyploidy
500 during Arabidopsis evolution. Plant Cell. 2004;16:1679–91.

501 13. De Smet R, Adams KL, Vandepoele K, Van Montagu MCE, Maere S, Van de Peer Y.
502 Convergent gene loss following gene and genome duplications creates single-copy
503 families in flowering plants. Proc Natl Acad Sci U S A. 2013;110:2898–903.

504 14. Woodhouse MR, Schnable JC, Pedersen BS, Lyons E, Lisch D, Subramaniam S, et al.
505 Following Tetraploidy in Maize, a Short Deletion Mechanism Removed Genes
506 Preferentially from One of the Two Homeologs. PLoS Biol. Public Library of Science;
507 2010;8:e1000409.

508 15. Schnable JC, Springer NM, Freeling M. Differentiation of the maize subgenomes by
509 genome dominance and both ancient and ongoing gene loss. Proc Natl Acad Sci U S A.
510 2011;108:4069–74.

511 16. Tang H, Woodhouse MR, Cheng F, Schnable JC, Pedersen BS, Conant G, et al.
512 Altered patterns of fractionation and exon deletions in Brassica rapa support a two-step
513 model of paleohexaploidy. Genetics. 2012;190:1563–74.

514 17. Hodges SA, Kramer EM. Columbines. Curr Biol. 2007;17:R992–4.

515 18. Soltis DE, Smith SA, Cellinese N, Wurdack KJ, Tank DC, Brockington SF, et al.
516 Angiosperm phylogeny: 17 genes, 640 taxa. Am J Bot. 2011;98:704–30.

517 19. Filiault D, Ballerini E, Mandakova T, Akoz G, Derieg N. The Aquilegia genome:
518 adaptive radiation and an extraordinarily polymorphic chromosome with a unique

519 history. bioRxiv [Internet]. biorxiv.org; 2018; Available from:
520 https://www.biorxiv.org/content/early/2018/02/12/264101.abstract

521 20. Lynch M, Conery JS. The evolutionary fate and consequences of duplicate genes.
522 Science. 2000;290:1151–5.

523 21. Blanc G, Wolfe KH. Widespread paleopolyploidy in model plant species inferred
524 from age distributions of duplicate genes. Plant Cell. 2004;16:1667–78.

525 22. Cui L, Wall PK, Leebens-Mack JH, Lindsay BG, Soltis DE, Doyle JJ, et al.
526 Widespread genome duplications throughout the history of flowering plants. Genome
527 Res. 2006;16:738–49.

528 23. Vanneste K, Baele G, Maere S, Van de Peer Y. Analysis of 41 plant genomes supports
529 a wave of successful genome duplications in association with the Cretaceous–Paleogene
530 boundary. Genome Res. 2014;24:1334–47.

531 24. Tiley GP, Ané C, Burleigh JG. Evaluating and Characterizing Ancient Whole-
532 Genome Duplications in Plants with Gene Count Data. Genome Biol Evol. 2016;8:1023–
533 37.

534 25. Doyle JJ, Egan AN. Dating the origins of polyploidy events. New Phytol.
535 2010;186:73–85.

536 26. Jiao Y, Paterson AH. Polyploidy-associated genome modifications during land plant
537 evolution. Philos Trans R Soc Lond B Biol Sci [Internet]. 2014;369. Available from:
538 http://dx.doi.org/10.1098/rstb.2013.0355

539 27. Melters DP, Bradnam KR, Young HA, Telis N, May MR, Ruby JG, et al. Comparative
540 analysis of tandem repeats from hundreds of species reveals unique insights into
541 centromere evolution. Genome Biol. 2013;14:R10.

542 28. Jaillon O, Aury J-M, Noel B, Policriti A, Clepet C, Casagrande A, et al. The grapevine
543 genome sequence suggests ancestral hexaploidization in major angiosperm phyla.
544 Nature. 2007;449:463–7.

545 29. Lyons E, Pedersen B, Kane J, Freeling M. The Value of Nonmodel Genomes and an
546 Example Using SynMap Within CoGe to Dissect the Hexaploidy that Predates the
547 Rosids. Trop Plant Biol. 2008;1:181–90.

548 30. Potato Genome Sequencing Consortium, Xu X, Pan S, Cheng S, Zhang B, Mu D, et
549 al. Genome sequence and analysis of the tuber crop potato. Nature. 2011;475:189–95.

550 31. Truco MJ, Ashrafi H, Kozik A, van Leeuwen H, Bowers J, Wo SRC, et al. An Ultra-
551 High-Density, Transcript-Based, Genetic Map of Lettuce. G3 . 2013;3:617–31.

552 32. Denoeud F, Carretero-Paulet L, Dereeper A, Droc G, Guyot R, Pietrella M, et al. The
553 coffee genome provides insight into the convergent evolution of caffeine biosynthesis.
554 Science. 2014;345:1181–4.

555 33. Bombarely A, Moser M, Amrad A, Bao M, Bapaume L, Barry CS, et al. Insight into
556 the evolution of the Solanaceae from the parental genomes of Petunia hybrida. Nat
557 Plants. 2016;2:16074.

558 34. Salse J. Ancestors of modern plant crops. Curr Opin Plant Biol. 2016;30:134–42.

559 35. Ming R, VanBuren R, Liu Y, Yang M, Han Y, Li L-T, et al. Genome of the long-living
560 sacred lotus (Nelumbo nucifera Gaertn.). Genome Biol. 2013;14:R41.

561 36. Jiao Y, Leebens-Mack J, Ayyampalayam S, Bowers JE, McKain MR, McNeal J, et al.
562 A genome triplication associated with early diversification of the core eudicots. Genome
563 Biol. 2012;13:R3.

564 37. Zeng L, Zhang Q, Sun R, Kong H, Zhang N, Ma H. Resolution of deep angiosperm
565 phylogeny using conserved nuclear genes and estimates of early divergence times. Nat
566 Commun. 2014;5:4956.

567 38. Zheng C, Chen E, Albert VA, Lyons E, Sankoff D. Ancient eudicot hexaploidy meets
568 ancestral eurosid gene order. BMC Genomics. 2013;14 Suppl 7:S3.

569 39. Argout X, Salse J, Aury J-M, Guiltinan MJ, Droc G, Gouzy J, et al. The genome of
570 Theobroma cacao. Nat Genet. 2011;43:101–8.

571 40. Soltis DE, Visger CJ, Marchant DB, Soltis PS. Polyploidy: Pitfalls and paths to a
572 paradigm. Am J Bot. 2016;103:1146–66.

573 41. Seoighe C, Wolfe KH. Yeast genome evolution in the post-genome era. Curr Opin
574 Microbiol. 1999;2:548–54.

575 42. Tian C-G, Xiong Y-Q, Liu T-Y, Sun S-H, Chen L-B, Chen M-S. Evidence for an
576 ancient whole-genome duplication event in rice and other cereals. Yi Chuan Xue Bao.
577 2005;32:519–27.

578 43. Aury J-M, Jaillon O, Duret L, Noel B, Jubin C, Porcel BM, et al. Global trends of
579 whole-genome duplications revealed by the ciliate Paramecium tetraurelia. Nature.
580 2006;444:171–8.

581 44. Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, et al. The B73 maize
582 genome: complexity, diversity, and dynamics. Science. 2009;326:1112–5.

583 45. Rodgers-Melnick E, Mane SP, Dharmawardhana P, Slavov GT, Crasta OR, Strauss
584 SH, et al. Contrasting patterns of evolution following whole genome versus tandem
585 duplication events in Populus. Genome Res. 2012;22:95–105.

586 46. Birchler JA, Bhadra U, Bhadra MP, Auger DL. Dosage-dependent gene regulation in
587 multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes,
588 and quantitative traits. Dev Biol. 2001;234:275–88.

589 47. Papp B, Pál C, Hurst LD. Dosage sensitivity and the evolution of gene families in

590 yeast. Nature. 2003;424:194–7.

591 48. Freeling M, Thomas BC. Gene-balanced duplications, like tetraploidy, provide
592 predictable drive to increase morphological complexity. Genome Res. 2006;16:805–14.

593 49. Birchler JA, Veitia RA. Gene balance hypothesis: connecting issues of dosage
594 sensitivity across biological disciplines. Proc Natl Acad Sci USA. 2012;109:14746–53.

595 50. Guo L, Winzer T, Yang X, Li Y, Ning Z, He Z, et al. The opium poppy genome and
596 morphinan production. Science [Internet]. 2018; Available from:
597 http://dx.doi.org/10.1126/science.aat4096

598 51. Malacarne G, Perazzolli M, Cestaro A, Sterck L, Fontana P, Van de Peer Y, et al.
599 Deconstruction of the (paleo)polyploid grapevine genome based on the analysis of
600 transposition events involving NBS resistance genes. PLoS One. 2012;7:e29762.

601 52. O'Kane SL, Schaal BA, Al-Shehbaz IA. The Origins of Arabidopsis suecica
602 (Brassicaceae) as Indicated by Nuclear rDNA Sequences. Syst Bot. American Society of
603 Plant Taxonomists; 1996;21:559–66.

604 53. Matsuoka Y. Evolution of polyploid triticum wheats under cultivation: the role of
605 domestication, natural hybridization and allopolyploid speciation in their
606 diversification. Plant Cell Physiol. 2011;52:750–64.

607 54. Friis EM, Pedersen KR, Crane PR. The emergence of core eudicots: new floral
608 evidence from the earliest Late Cretaceous. Proc Biol Sci [Internet]. 2016;283. Available
609 from: http://dx.doi.org/10.1098/rspb.2016.1325

610 55. Lyons E, Freeling M. How to usefully compare homologous plant genes and
611 chromosomes as DNA sequences. Plant J. 2008;53:661–73.

612 56. Blanc G, Hokamp K, Wolfe KH. A recent polyploidy superimposed on older large-
613 scale duplications in the Arabidopsis genome. Genome Res. 2003;13:137–44.

614 57. Smith DA, Cordell R, Dillon EM. Infectious texts: Modeling text reuse in nineteenth-
615 century newspapers. 2013 IEEE International Conference on Big Data. 2013. p. 86–94.

616