

The genome of the domesticated apple (*Malus × domestica* Borkh.)

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We report a high-quality draft genome sequence of the domesticated apple (*Malus × domestica*). We show that a relatively recent (>50 million years ago) genome-wide duplication (GWD) has resulted in the transition from nine ancestral chromosomes to 17 chromosomes in the Pyrae. Traces of older GWDs partly support the monophyly of the ancestral paleohexaploidy of eudicots. Phylogenetic reconstruction of Pyrae and the genus *Malus*, relative to major Rosaceae taxa, identified the progenitor of the cultivated apple as *M. sieversii*. Expansion of gene families reported to be involved in fruit development may explain formation of the pome, a Pyrae-specific false fruit that develops by proliferation of the basal part of the sepals, the receptacle. In apple, a subclade of *MADS-box* genes, normally involved in flower and fruit development, is expanded to include 15 members, as are other gene families involved in Rosaceae-specific metabolism, such as transport and assimilation of sorbitol.

The domesticated apple (*Malus × domestica* Borkh., family Rosaceae, tribe Pyrae) is the main fruit crop of temperate regions of the world. Here we describe a high-quality draft genome sequence of the diploid apple cultivar ‘Golden Delicious’. Domesticated apple genotypes are all highly heterozygous, imposing

technical challenges in genome sequencing and assembly¹ while allowing identification of a very large set of SNPs².

Rosaceae belong to the rosids, which include one-third of all flowering plants³. Whereas the haploid (*x*) chromosome numbers of most Rosaceae are 7, 8 or 9, Pyrae have a distinctive *x* = 17. Pyrae have

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Received 19 November 2009; accepted 3 August 2010; published online 29 August 2010; doi:10.1038/ng.654



Table 1 Summary of genome assembly of the apple variety ‘Golden Delicious’

		Size (Mb)	No.	N_{50}	L_{50}	% of sequence assembled	% of sequence anchored
Contigs	All	603.9^a	122,146	16,171	13.4	81.3	
	Singletons	26.6	19,070	5,327	3.4		
	In metacontigs	577.3	103,076	13,031	15.2		
	Anchored	550.3	95,716	12,149	15.4		
Metacontigs	All	598.3	1,629	102	1,542.7		71.2
	Anchored	528.3	439	80	1,971.0		
Repetitive Sequences	Assembled	362.3 (48.8%)				72.3	
	Not assembled	138.4 (18.6%)					
	Total	500.7 (67.4%)					
Estimated genome size ^b		742.3					
No. of genes ^c			95,216/57,386				90.2
Anchoring markers			1,643				

N_{50} , minimum number of contigs required to represent 50% of the genome. L_{50} , length of the smallest N_{50} contig.
^aThe value reported was reduced to take into account the haplotype overlapping and hemizygous DNA. See details in **Supplementary Note**. ^bCalculated as the sum of 603.9 and 138.4 Mb (see **Supplementary Note**). ^cBefore/after excluding transposable element-related genes, alleles, short predictions and low-level functional annotate genes.

long been considered an example of allopolyploidization between species related to extant Spiraeoideae ($x = 9$) and Amygdaleoideae ($x = 8$), although a within-lineage polyploidization event has also been hypothesized⁴.

In addition, we examine the genetic variability in Rosaceae and related taxa, comparing Pyraeae species, Rosaceae tribes and two rosid families. Gene content and order of the assembled chromosomes indicate that both recent and old GWDs have occurred. We provide a model describing the evolution of the Pyraeae genome, including *Malus*, and offer insights into the origin of the domesticated apple.

RESULTS

Sequencing, assembling and anchoring the apple genome

Sequencing and assembly of the ‘Golden Delicious’ apple genome followed the whole-genome shotgun approach. Of the 16.9-fold genome coverage, 26% was provided by Sanger dye primer sequencing of paired reads, and the remaining 74% was from 454 sequencing by synthesis of paired and unpaired reads (**Supplementary Table 1** and **Supplementary Note**). An iterative assembly approach, previously used to assemble the highly heterozygous grape genome¹, produced 122,146 contigs, 103,076 of which were assembled into 1,629 metacontigs (**Table 1**, **Supplementary Fig. 1** and **Supplementary Note**). The total contig length (603.9 Mb) covers about 81.3% of the apple genome (**Table 1** and **Supplementary Note**). Anchoring of metacontigs (598.3 Mbp, or 71.2% of genome) was based on the high-quality genetic map with 1,643 markers (**Supplementary Table 2** and **Supplementary Note**). In total, 17 linkage groups, or chromosomes, were reconstructed. In the genome, repetitive elements correspond to 500.7 Mb (67%; **Supplementary Note**). The unassembled part of the genome is 98% repetitive (138.4 Mb), and the estimated genome size is 742.3 Mb (**Table 1** and **Supplementary Note**). We compared repetitive elements among ten plant species (**Supplementary Tables 3–6**). Information on relevant genes and genome parameters is provided in **Tables 1** and **2**, **Supplementary Figures 2–5** and **Supplementary Tables 7–19**. Comparing

gene families among ten sequenced plant species revealed apple-specific subclades of genes encoding MADS-box transcription factors and overrepresented sorbitol-related genes, which may contribute to specific aspects of apple development and carbohydrate metabolism (**Table 2** and **Supplementary Table 7**, and see Discussion). The 71.2% of the genomic sequences that were anchored represent the gene-rich part of the genome, which covers as many as 90.2% of the genes assigned to the chromosomes. The distribution of transposable elements and predicted genes along the linkage groups is reported in **Supplementary Figure 6**. The total number of genes predicted for the apple genome (57,386, including some genes that may be present only in one of the two chromosomes of a pair) is the highest reported among plants so far (**Supplementary Note**).

Genome-wide duplications and the origin of the Pyraeae

Pairwise comparison of 17 apple chromosomes highlighted strong collinearity between large segments of chromosomes 3 and 11, 5 and 10, 9 and 17, and 13 and 16, and between shorter segments of chromosomes 1 and 7, 2 and 7, 2 and 15, 4 and 12, 12 and 14, 6 and 14, and 8 and 15 (**Fig. 1a**). The distribution of synonymous substitution rates (K_S)—an indication of the relative age of duplication, based on the number of synonymous substitutions in the coding sequences—peaked around 0.2 for recently duplicated genes (**Fig. 1b**), indicating that a (recent) GWD has shaped the genome of the domesticated apple.

Dating of this GWD (**Supplementary Note**) was based on the construction of penalized likelihood trees, as described previously⁵. Given a node of grape to rosids fixed at 115 million years ago (Mya), the GWD has been dated to between 30 and 45 Mya⁵. If similar rates of protein evolution are assumed for apple and poplar (**Fig. 1c**), the recent apple GWD may be as old as that of poplar, about 60 to 65 Mya⁶.

Remnants of older large-scale gene duplications or GWDs were also evident (**Supplementary Fig. 7a,b**). Genes in these duplicated regions had average K_S values around 1.6, as expected for paleo-duplication events (**Fig. 1b**). Most remnants of these older duplications

Table 2 Comparison of the apple genome to other sequenced plant genomes

	Genes/gene density ^a	Transposable elements ^b	Transcription factors ^c	miRNAs ^d	Resistance genes ^e	Biosynthetic genes ^f
Apple	57,386/0.78	42.4	4,021	178	992 (58, 27)	1,246
Cucumber	26,682/0.73	14.8	ND	ND (171)	61 (ND)	ND
Soybean	46,430/0.42	50.3	5,671	41 (85)	392 (61, 32)	958
Poplar	45,654/0.94	35.0	2,758	174 (234)	402 (59, 20)	1,034
<i>Arabidopsis</i>	27,228/2.2	18.5	2,437	89 (199)	178 (32, 52)	719
Grape	33,514/0.66	21.5	2,080	130 (137)	341 (57, 11)	1,121
Rice	40,577/0.97	39.5	2,798	140 (447)	535 (89, 0)	910
<i>Brachypodium</i>	25,532/0.94	28.1	2,187	62 (129)	238 (89, 0)	390
Sorghum	34,496/0.47	62.0	2,312	116 (148)	245 (75, 0)	555
Maize	32,540/0.15	84.2	5,246	153 (170)	129 (74, 0)	457

Only statistics showing major or important differences among species are reported (ND, not determined). Data were taken from web sites listed in the ‘URLs’ section in the Online Methods.

^aGene density expressed in number of genes per 10 kb. ^bExpressed in % of total genome size. For details, see **Supplementary Table 7**. ^cTotal number of transcription-factor genes identified in plant genomes. For details, see **Supplementary Tables 7 and 8**.

^dNumber of microRNAs (miRNAs) from miRNA families present in apple is given outside brackets; total number of miRNAs found in the literature is in brackets. For details, see **Supplementary Tables 13–16**. ^eTotal number of NBS resistance genes (numbers in brackets respectively indicate % NBS-LRR, % TIR-NBS-LRR). For details see **Supplementary Tables 9 and 10**.

^fTotal number of genes involved in volatile, aromatic-compound, pigment, antioxidant and sorbitol biosynthetic pathways. For details see **Supplementary Table 7**.

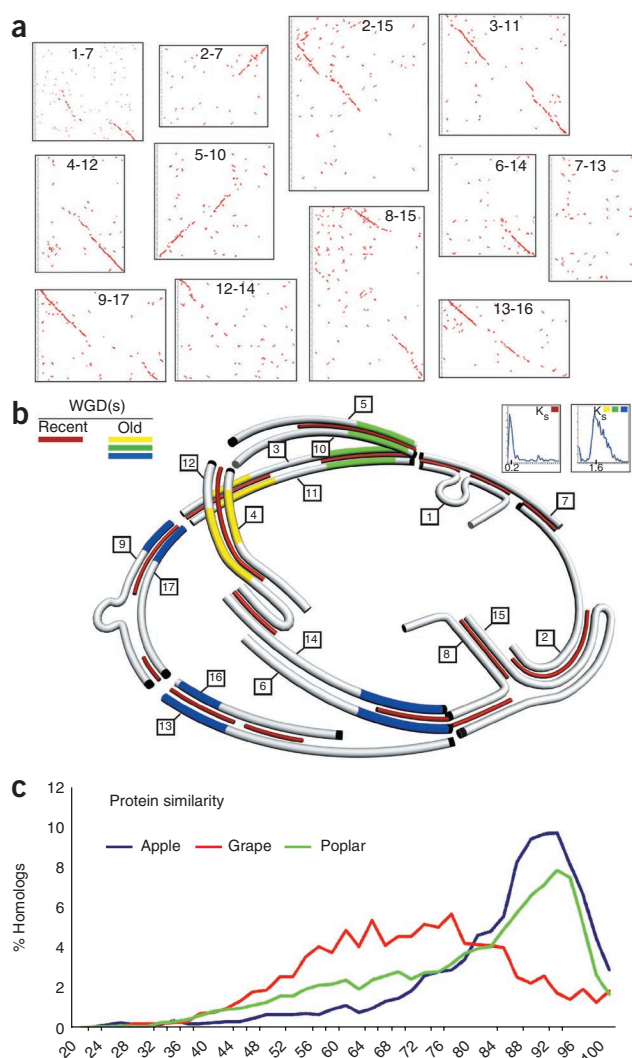


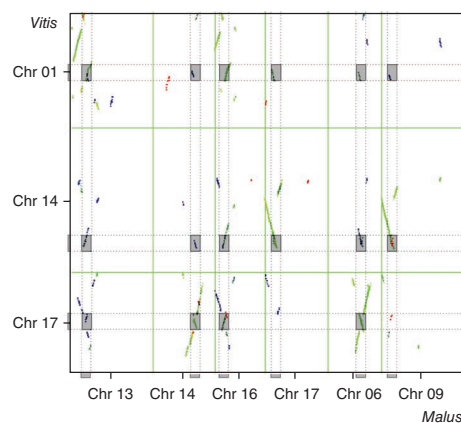
Figure 1 Genome-wide duplications in the apple genome. (a) Alignment of apple chromosomes shown by pairwise dot plots based on gene homology. Strong collinearity of members of chromosome doublets, or of large chromosome segments, indicates a recent GWD (red dots and bars in **a** and **b**, respectively). Unrelated chromosomes 7 and 13 were compared as a negative control. (b) Reconstruction of the relationships among apple chromosomes based on the most recent and the older GWD. The model derives from data in **a** for the recent GWD and from data in **Supplementary Figure 6b** for the oldest GWD. The chromosomes ends represented at bottom right corners in **a** are marked in black in **b**. Red bars, regions of synteny that support the recent GWD. Size of chromosomes is proportional to their DNA content in megabases. Segments of chromosomes 1, 5, 6, 8, 10, 13, 14 and 15 have no syntenic counterparts. Chromosome segments predicted to be the outcome of the older duplication are highlighted with blue, green and orange. Chromosomes 1, 2, 7, 8 and 15 do not show obvious signs of the older duplications, although they may contain short blocks of genes that reveal old paleopolyploid events. Inset graphs show that K_s from the comparisons between paralogous genes has a peak at 0.2 when the recent duplication is considered, and between 1.4 and 1.6 for the older paleopolyploid events. (c) Distributions of protein similarities for duplicated genes in duplicated segments compared with grape (red), poplar (green) and apple (blue).

can also be interpreted as remains of a paleohexaploid state of the eudicot progenitor on the basis of dot-plot comparisons among other grape and apple chromosomes (**Supplementary Fig. 8a,b**). This provides further evidence for a paleohexaploid state shared by most eudicots^{8,9}.

The chromosome homologies derived from the recent GWD allow inference of the cytological events that have led to the number and composition of the extant apple chromosomes, starting from a putative nine-chromosome ancestor (**Fig. 3**). Each doublet of the eight apple chromosomes (3-11, 5-10, 9-17 and 13-16) is derived principally from one ancestor, although minor interchromosomal rearrangements have occurred (**Supplementary Fig. 9a-k**). Chromosomes 4, 6, 12 and 14 originate from duplications of the ancient chromosomes V and VI, followed by a translocation and a deletion event. Similar events have generated chromosomes 1, 2, 7, 8 and 15 from chromosomes VII, VIII and IX. Chromosome 15 could have been produced from the translocation of an entire copy of chromosome IX into the centromeric region of chromosome VIII, following a model of dysploidy (reduction of chromosome number) common in cereals¹⁰. The second copy of ancient chromosome VIII has evolved into the extant chromosome 8. A conservative estimate of the number of large chromosome rearrangements since the divergence of the Pyreae subtribe, corresponding to the recent chromosome duplication, includes one chromosome fusion (extant chromosome 15), three translocations

are found between chromosomes 5 and 10 and chromosomes 3 and 11, between chromosomes 3 and 11 and chromosomes 4 and 12, and between chromosomes 6 and 14, 13 and 16, and 9 and 17 (**Fig. 1a,b**). Chromosomes 1, 2, 7, 8 and 15 seem relatively devoid of older duplicated blocks; however, short blocks of genes showing old polyploidy events were found on all chromosomes. One region in the apple genome with an approximate size of 4 to 7 Mbp seems to be clearly present in six copies (regions in blue, **Fig. 1a,b**). Remapping those to the ancestral state reveals a triplicate structure among parts of chromosomes 9 and 17, 6 and 14 and 13 and 16. Notably, we found that these regions are collinear with chromosomes 1, 14 and 17 of grape (**Fig. 2**), which have been demonstrated to be homologous because of an ancient hexaploidy⁷. Additional chromosomal fragments that we found to be duplicated in apple (green and yellow bars in **Fig. 1b**)

Figure 2 Dot-plot comparisons between apple and grape chromosomes. Dot plots are based on gene homology. The apple chromosomes are those with the segment triplication deriving from an old GWD (shown in blue in **Fig. 1b**). Grape chromosomes 1, 14 and 17 constitute a triplet having the same ancestor in common⁷. Chromosome segments with homologous genes common both to grape and apple (16 of a total of 18 comparisons) are indicated by gray boxes connected with dashed lines. Green, red and blue dots indicate increasing K_s values, in that order. Perpendicular lines on the x and y axes mark the middle of each chromosome. Green grid separates chromosomes.



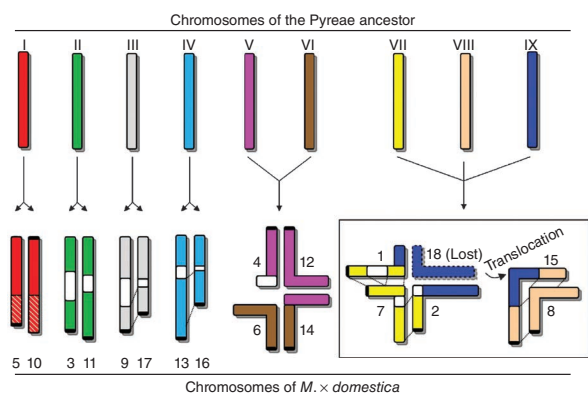


Figure 3 A model explaining the evolution from a 9-chromosome ancestor to the 17-chromosome karyotype of extant Pyrae, including the genus *Malus*. A GWD followed by a parsimony model of chromosome rearrangements is postulated. Shared colors indicate homology between extant chromosomes. White fragments of chromosomes indicate lack of a duplicated counterpart. The white-hatched portions of chromosomes 5 and 10 indicate partial homology (see also **Supplementary Fig. 9**). Black marks at chromosome ends correspond to those in **Figure 1b**.

(involving extant chromosomes 1, 2 and 14), six deletions defined by telomeres that are not currently duplicated (chromosomes 4, 6, 8, 10, 11 and 13), one intrachromosome deletion (within chromosome 7, according to the chromosome 1–chromosome 7 comparison) and a deletion of a centromere (from ancient chromosome IX).

Molecular distances, taxonomy and phylogeny of Rosaceae

Available Rosaceae molecular data allow intrafamily comparisons of apple with pear and of a consensus of apple and pear with peach. Further comparisons with grape—a species basal to rosids but belonging to the Vitaceae, a strictly different, although related, family—introduce the possibility of comparing interfamily molecular distances. DNA sequences used in this molecular phylogeny consist of those from EST databases and, for apple, the genomic data as described in detail in the **Supplementary Note**. Data from a three-way sequence alignment between predicted gene space in apple (~84 Mb) and experimentally derived EST data from pear (~14.9 Mb) and peach (~18 Mb), performed as in ref. 11, indicates that the genetic distance, based on DNA sequence divergence per base pair between members of Rosaceae, increases from apple to pear to peach (**Supplementary Table 20**). When predicted gene spaces of apple and pear were compared, a value of 96.35% nucleotide identity was calculated between these two species of the tribe Pyrae. The estimate for nucleotide identity between the tribes Pyrae and Amygdaleae (apple and peach) was 90.64%. When grape was compared with apple and pear, nucleotide identity was estimated at 85.31%. When the frequency of transitions and transversions was considered (**Fig. 4**), the ratio R (transitions/transversions) was similar for apple-specific and pear-specific mutations. For peach-specific mutations, the R value is more difficult to interpret, as it is probably biased by the existence of recent GWD in apple and pear. The comparison of apple and pear with grape showed that although transitions were only 20% more frequent than transversions, T-to-G transversions represented 12% of the total number of mutations observed (**Fig. 4d**), implying that Vitaceae is strongly divergent taxonomically from core members of the Rosaceae.

The granule-bound starch synthase (*gbss*) genes, also known as waxy (*Wx*) genes (divided in two groups, *Wx1* and *Wx2*), were also used⁴ as a tool to study molecular taxonomy of Rosaceae (**Supplementary**

Table 21). We identified six *Wx* genes in the apple genome, located on chromosomes 7, 9 and 16 (*Wx1* type) and 8, 6 and 14 (*Wx2* type) (**Supplementary Fig. 10**). After counting *Wx* genes of apple, including putative gene losses in syntenic chromosomal segments, we were able to identify eight two-by-two syntenic regions containing or expected to contain *Wx* loci. If *Wx1-1* on chromosome 7 is not considered (because neither a syntenic *Wx1-1* region nor a paralogous *Wx1-1* copy was found), four *Wx* loci should have been present in the nine-chromosome Pyrae ancestor, a result that is consistent with an ancestral paleopolyploid state. When the genomic *Wx* gene sequences were integrated in the phylogenetic analysis based on sequences present in the Rosaceae database¹², the three *Wx-1* and the three *Wx-2* genes were mapped to two separate clades, both of which also included *Wx* genes of *Gillenia* (**Supplementary Fig. 11**). However, *Prunus* and *Spiraea* sequences clustered in separate clades, supporting the conclusions that the tight relationships between apple and *Gillenia* *Wx1* genes, as well as between apple and *Gillenia* *Wx2* genes, were probably generated by the recent GWD (the Pyrae event)—the founding step of the Pyrae genome—and that *Prunus*- and *Spiraea*-related species are less likely to have contributed to the Pyrae genome. Hence, we tested the Rosaceae molecular taxonomy¹² by Bayesian analysis of the sequences of seven nuclear and chloroplast genes. A major clade with the maximum statistical support included all Pyrae ($x = 17$) as well as *Gillenia* ($x = 9$) (**Supplementary Fig. 12**). Notably, the genera *Spiraea* ($x = 9$) and *Prunus* ($x = 8$) were not included in this clade.

Apple domestication

Although *M. sieversii* has been considered to be the ancestor of the domesticated apple¹³, this has been challenged by the identification of molecular similarities between domestic apple and *M. sylvestris*¹⁴. To test these two hypotheses, we surveyed molecular differences at 23 genes across the genus *Malus* (**Supplementary Table 22**). The 74 accessions we considered included 12 *M. × domestica* cultivars, 10 *M. sieversii*, 21 *M. sylvestris*, all major wild apple species and two *Pyrus* species (**Supplementary Table 23**). For *M. × domestica*, we included the cultivars ‘Cox’s Orange Pippin’, ‘Golden Delicious’, ‘McIntosh’, ‘Red Delicious’ and ‘Jonathan’, the most important ‘founders’ of

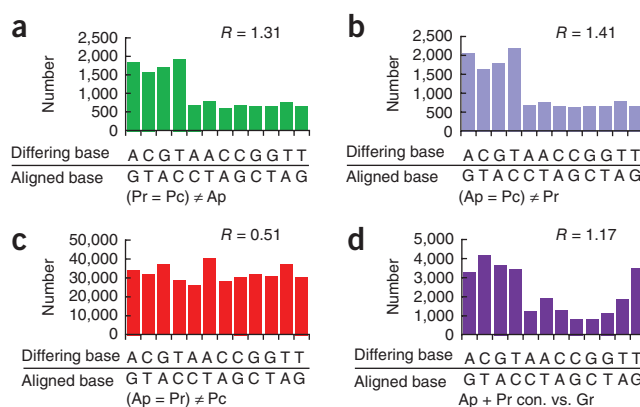


Figure 4 Molecular distances among Rosaceae species and their comparison with grape. (**a–c**) Mutations identified in a three-way comparison of apple, pear and peach. Numbers of transitions and transversions where apple (Ap) differs from pear (Pr) and peach (Pc) (**a**; 12,273 total), where pear differs from apple and peach (**b**; 13,124 total), and where peach differs from apple and pear (**c**; 381,619 total). (**d**) Number of transitions and transversions in a two-way alignment of grape DNA (Gr) to a consensus sequence (con.) of apple and pear (26,693 total). Note the high rate of T-to-G transversions. R is the transitions/transversions ratio. Methods and computer calculations were similar to those in ref. 11 (**Supplementary Note**).

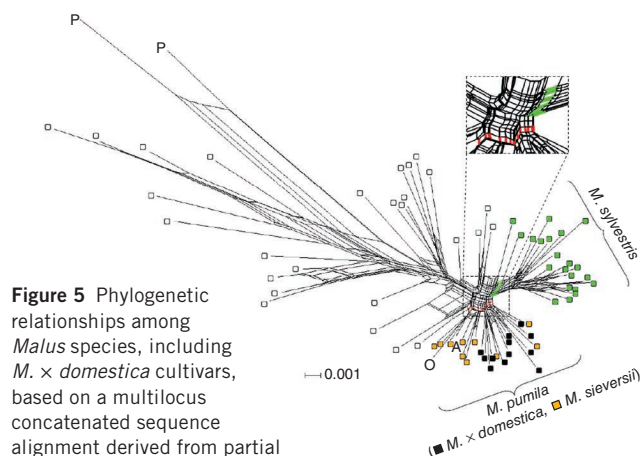


Figure 5 Phylogenetic relationships among *Malus* species, including *M. × domestica* cultivars, based on a multilocus concatenated sequence alignment derived from partial resequencing of 23 apple genetic loci. Black, orange and green, accessions of *M. × domestica* cultivars, *M. sieversii* and *M. sylvestris*, respectively; A, O and P, accessions of *M. × asiatica*, *M. orientalis* and *Pyrus*, respectively; squares, accessions of all other wild species. Full information on accessions is provided in **Supplementary Table 23** and **Supplementary Figure 13**. The split separating the *M. × domestica*–*M. sieversii*–*M. orientalis*–*M. × asiatica* complex from other species is highlighted in red, and the split separating *M. sylvestris* is highlighted in green. Genetic distances were obtained as Hamming distances, with pairwise alignment of nucleotide positions. The planar graph was constructed with Splits-Tree 4.10.

modern apple breeding¹⁵ (**Supplementary Note**). For each gene and accession, a PCR amplicon was resequenced and the data were analyzed as a concatenated data set with a total length of ~11,300 bp, with 1,507 polymorphic informative sites. A neighbor-net planar graph¹⁶ was constructed from the molecular differences among accessions (**Fig. 5** and **Supplementary Fig. 13**). Although the clade containing *M. sylvestris* was well separated from the clade with *M. × domestica*, *M. sieversii* and *M. × domestica* genotypes shared a large common clade that also included accessions of *M. orientalis* and *M. × asiatica*. The average polymorphism rate within the domestic cultivars was 4.8 SNPs per kb, with 5.7 SNPs per kb between ‘Golden Delicious’ and *M. sieversii*, and 9.6 SNPs per kb between and *M. sylvestris* (**Supplementary Table 24**). The genetic differentiation was categorized as ‘moderate’ between *M. × domestica* and *M. sieversii* ($F_{st} = 0.14$), and ‘great’ between *M. × domestica* and *M. sylvestris* and between *M. sieversii* and *M. sylvestris* ($F_{st} = 0.17$ and $F_{st} = 0.21$, respectively)¹⁷. The mean numbers of haplotypes per gene were 6.4, 5.8 and 10.0 for *M. × domestica*, *M. sieversii* and *M. sylvestris*, respectively (**Supplementary Table 25**).

DISCUSSION

The putative gene content in apple (57,386 putative genes plus 31,678 transposable element–related ORFs) is high compared to *Arabidopsis thaliana* (27,228), poplar (45,654), papaya (28,027), *Brachypodium distachyon* (25,532), grape (33,514), rice (40,577), sorghum (34,496), cucumber (26,682), soybean (46,430) and maize (32,540). Putative apple-specific genes, identified as described in **Supplementary Note**, totaled 11,444. The gene density in apple (**Table 2**) is within the range of those in poplar and grape, but lower than those in *Arabidopsis*, *Brachypodium* and rice. The existence of hemizygous DNA in the heterozygous variety ‘Golden Delicious’ may have contributed to this gene number, as has also been noted for grape².

The apple genome has a relatively high number of repeated sequences, which are difficult to assemble or anchor. As seen in grape

and cereals, retrotransposons represent the most abundant transposable-element fraction, comprising 38% of the total genome and 89% of all transposable elements (**Table 2** and **Supplementary Table 7**). In contrast, apple has the lowest content of DNA transposons (including the CACTA superfamily) among the reported plant genomes.

The number of transcription factors identified (4,021; **Supplementary Table 7**) was among the highest of the sequenced plant genomes (**Table 2**), although the allocation of transcription factor genes to gene families was similar to other sequenced plant species (**Supplementary Fig. 3**). Partial exceptions were the families C2H2, CCAAT and NAC, which were notably more represented in apple.

The fraction of nucleotide-binding site–leucine-rich repeat (NBS–LRR) resistance genes is considerably higher in eurosids II (apple, poplar and grape) than in eurosids I (*Arabidopsis*). In monocotyledons (rice), this class of genes predominates. The content of Toll/interleukin region (TIR)–NBS–LRR genes is highest in *Arabidopsis* (52%), lower in other eurosids (11–32%) and absent in monocots (**Table 2**). In addition to NBS genes, the apple genome contains 575 LRR–kinase genes.

As seen in other genomes, different classes of apple genes differ greatly in their degree of duplication (**Supplementary Table 11** and **Supplementary Fig. 4**). Across the ten genomes considered, there are gene families with either low or high numbers of paralogous copies. This is particularly evident for genes likely to be involved in metabolism of anthocyanins and flavonoids, isoflavones and isoflavonones, and terpenes (**Supplementary Table 7**). Relevant cases in each pathway are flavonone 3-hydroxylase (2–13 copies in nine plant genomes) and isoflavone reductase (3–19 copies) compared to isoflavone synthase (54–151 copies); squalene synthase (13 copies) compared to squalene monooxygenase (1–27 copies). It seems that, for some gene classes, the number of paralogous copies may already have been established in the genome of common progenitor(s) of higher plants.

An intriguing aspect of the apple’s biology concerns its characteristic fruit, the pome, which is found only in the Pyreae tribe¹². This indicates that the pome probably evolved after a relatively recent Pyreae-specific GWD, a polyploidization step that we hypothesize has contributed to the apple’s developmental and metabolic specificity (**Supplementary Table 7**). Pome fruit is derived by enlargement of the receptacle, which is the region below the whorl of sepals in the apple flower. *MADS-box* genes may regulate pome development, as they determine the eventual fate of floral tissues in all plant species analyzed so far¹⁸. For example, it has recently been shown that an apple *MADS-box* gene that is a member of the *API* clade, common to all flowering plants¹⁹ and closely related to *Arabidopsis FRUITFULL* (*FUL*), is differentially expressed during pome development²⁰. In addition, a substantial number of apple type II *MADS-box* genes belong, phylogenetically, to the *StMADS11* subclade, a group named for its first reported member, which was isolated from potato (**Supplementary Fig. 14a**)²¹. This subclade includes only two *Arabidopsis* genes, *SVP* and *AGL24*. Ectopic overexpression of *SVP* and related genes in *Arabidopsis* leads to foliose sepal syndrome—that is, the formation of large sepals²². In apple, this specific subclade not only includes two genes expressed in the pome but is also expanded to include 15 other genes.

Carbohydrate metabolism is another important aspect of fruit composition. In Rosaceae, photosynthesis-derived carbohydrates are transported mainly as sorbitol^{23,24}. Compared with other plant genomes, apple has considerably more copies of key genes related to sorbitol metabolism. These include aldose 6-P reductase (*A6PR*), which is rate-limiting for sorbitol biosynthesis, sorbitol-dehydrogenase (*SDH*), which converts sorbitol to fructose in the fruit²⁵, and sorbitol transporter *PcSOT2*, which is specific to Rosaceae fruit^{26,27}. In total,

there are 71 sorbitol metabolism genes in apple; in other species, the number ranges between 9 and 43 (**Supplementary Tables 7 and 26**, and **Supplementary Fig. 14b–d**). In the Rosaceae, an evolutionary trend toward fruit organ specialization may have been partially based on gene duplication, which has created large families of specific paralogous genes (particularly evident for *SDH*; **Supplementary Fig. 14c**). Gene families expanded in apple, such as *StMADS11*-like and *SDH*-like, have yet to be tested functionally for their involvement in fruit characteristics.

A number of models have been proposed to explain the uniquely high number of chromosomes in Pyraeae, the most popular being the ‘wide-hybridization’ hypothesis based on an allopolyploidization event between spireoid ($x = 9$) and amygdaloid ($x = 8$) ancestors^{28,29}. More recent molecular phylogeny studies point to the possibility that Pyraeae originated by autopolyploidization or by hybridization between two sister taxa with $x = 9$ (similar to extant *Gillenia*), followed by diploidization and aneuploidization⁴ to $x = 17$. This hypothesis takes into account that *Gillenia* and related taxa are New World species and that the earliest fossil evidence of specimens belonging to extant genera of Pyraeae are from North America.

Our results support the autopolyploidization hypothesis⁴, as the derivation from a *Gillenia*-like taxon best fits the available data. First, the apple genome derives from a relatively recent duplication. Relationships between its homologous chromosomes based on genome sequence extend observations based on synteny and collinearity of molecular markers^{30,31}. The timing of such a GWD, as estimated from our genomic data (**Fig. 1c** and **Supplementary Figs. 15 and 16**), agrees with archeobotanical dates of 48–50 Mya³².

Second, molecular phylogeny of *Wx* genes in the apple genome confirms the close relationship of *Gillenia* ($x = 9$) with the Pyraeae ($x = 17$) lineage, as the *Wx* gene sequences of *Prunus*, *Spiraea* and other Rosaceae genera belong to a different phylogenetic cluster (**Supplementary Fig. 11**). The monophyletic origin of Pyraeae and *Gillenia* was confirmed by a molecular phylogeny of a broader set of genes (**Supplementary Fig. 12**).

In addition, a simple and parsimonious pattern of chromosome breakage and fusion explains the derivation of the current $x = 17$ Pyraeae karyotype from a polyploidization event of two $x = 9$ genomes (**Fig. 3**). The rate of chromosome rearrangements after polyploidization (12 chromosome events in 60 My) is similar to that for poplar (~16 events in 60 My)⁶ and lower than in maize (at least 17 chromosome fusion events in 5 My)³³ or in artificial neopolyploids³⁴. In this sense, molecular clocks of perennial woody species seem slower than those of annual species, in terms of both nucleotide substitutions and chromosome rearrangements⁹. For the genus *Helianthus*, a similar observation that only some of the ancestor chromosomes are rearranged in the extant chromosomes has been discussed in detail. In this genus, such rearrangement was associated with chromosomal differences between two sister species contributing to a GWD allopolyploid event³⁵.

Similarly, the collinearity between *Pyrus* and *Malus* genetic maps^{31,36} suggests that the Pyraeae genome reorganization occurred before the divergence of the two genera. A rapid genome rearrangement after polyploidization is expected in species lacking the *Ph1*-like function that prevents the pairing of homologous chromosomes in wheat³⁷.

It has been proposed that central Asia is the center of origin of domesticated apple³⁸. Between 25 and 47 different *Malus* species, including *M. × domestica*, are currently recognized³⁹. As asiatic *M. × asiatica*, *M. baccata*, *M. micromalus*, *M. orientalis*, *M. prunifolia* and *M. sieversii*, and European *M. sylvestris*, are the species taxonomically closest to *M. × domestica*³⁹, they are considered to have contributed, to differing

extents, to the domestic gene pool. *M. sieversii*, common in the Tian Shan region of central Asia, is the only wild species sharing all the qualities of the domesticated apple in terms of fruit and tree morphology⁴⁰.

Apples are known to have been gathered in the Neolithic and Bronze Age in the Near East and Europe, and all archaeological findings indicate a fruit size compatible with those of the wild *M. sylvestris*⁴¹, a species bearing small astringent and acidulate fruits. Sweet apples corresponding to extant domestic apples appeared in the Near East around 4,000 years ago⁴¹, at the time when the grafting technology used to propagate the highly heterozygous and self-incompatible apple was becoming available. From the Middle East, the domesticated apple passed to the Greeks and Romans, who spread fruit cultivation across Europe^{13,41}.

On the basis of our molecular results, *M. × domestica* cultivars appear more closely related to accessions of the wild species *M. sieversii* and less closely related to accessions of *M. sylvestris*, *M. baccata*, *M. micromalus* and *M. prunifolia*. The already known^{42,43} genetic similarity of *M. sieversii* to *M. orientalis* and to *M. × asiatica* (a Chinese cultivated apple form) is also confirmed by our data.

The data support the formation of the *M. × domestica* gene pool from *M. sieversii*. Once grafting was introduced, the crop passed through a process described as ‘instant domestication’⁴⁴. This could explain apple’s lack of domestication syndrome, which is the loss of sexual reproduction, seed dispersion and seed dormancy. Despite evidence of intrageneric hybridizations^{14,45}, the possibility of substantial genetic contributions to the domestic gene pool of other wild *Malus* species, such as *M. sylvestris*¹⁴, was rejected in our analysis.

Our study also fully supports the proposal that *M. × domestica* and *M. sieversii* are the same species, for which the more appropriate nomenclature of *M. pumila* Mill. could be adopted^{13,46}.

A practical goal of sequencing the complex heterozygous apple genome is to accelerate the breeding of this economically important perennial crop species. Many genes related to disease resistance, aroma and taste, plant development and reaction to the environment have been identified and mapped to the chromosomes. In addition, SNP molecular markers have been made available at a frequency of 4.4 SNPs per kb. These markers are currently being used in advanced breeding programs and comparative genetic studies³¹ that should speed cultivar development. The anchored sequence of the apple genome will be a tool to initiate a new era in the breeding of this crop. The availability of nearly all apple gene sequences should benefit apple researchers by enabling genome-wide functional studies and accelerating establishment of gene-trait relationships.

URLs. *Arabidopsis thaliana* (TAIR Release 8.0), <ftp://ftp.arabidopsis.org>; *Carica papaya*, <ftp://asgpb.mhpc.hawaii.edu/papaya/annotation/>; *Populus trichocarpa* (assembly release v1.0, annotation v1.1.), http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html; *Vitis vinifera* (assembly release v1.0, annotation v2.0), <http://genomics.research.iasma.it>; *Oryza sativa* (MSU Rice Genome Annotation Project Release 6.0 assembly), <http://rice.plantbiology.msu.edu/index.shtml>; *Sorghum bicolor* (assembly release v1.0, annotation v1.4), <http://www.phytozome.net/sorghum>; *Cucumis sativus* (assembly release v1.0, annotation v1.0), <http://cucumber.genomics.org.cn/page/cucumber/index.jsp>; *Glycine max* (assembly release Glyma1, annotation Glyma1.0), <http://genome.jgi-psf.org/soybean/soybean.home.html>; *Zea mays* (assembly release B73 RefGen_v1), http://www.maizesequence.org/Zea_mays/Info/Index; *Brachypodium distachyon* (assembly release v1.0, annotation v1.0), <http://www.brachybase.org>; integrated genetic map, <http://genomics.research.iasma.it; RepBase14.01>, <http://www.girinst.org>.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturegenetics/>.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

The Italian apple genome project was supported by the research office of the Provincia Autonoma di Trento. The US apple genome project was supported by Washington State University Agriculture Research Center, Washington Tree Fruit Research Commission and US Department of Agriculture National Research Initiative (USDA-NRI) grant 2008–35300-04676 to A.D., A.K. and R.E.B. V.K. and C.W. received support from the USDA-NRI grant. S. Schaeffer and T.K. were supported by the US National Institutes of Health Protein Biotechnology Training Program and an Achievement Rewards for College Scientists fellowship. A.C.A., V.B., D.C., A.P.G., S.E.G., R.P.H. and R.N.C. were partially supported by the New Zealand Foundation for Research Science and Technology, contract no. C06X0812. We thank S. Attiya, E. Buglione and C. Celone from 454 Life Sciences-Roche Company as well as E. Stefani, A. Castelli and E. Potenza for technical support and V. Sgarbetta for critical reading of the manuscript. Fosmid and shotgun libraries were prepared following the method developed by R. Meilan (Oregon State University).

AUTHOR CONTRIBUTIONS

R. Velasco, A.D., A.K., R.E.B., M.S., M.E., F.S. and R. Viola managed the project. R. Velasco, A.Z., J.A., A.D., A.C., A.K., P.F., S.K.B., M.T., D.P., S. Salvi, J.L., A.G., R.E.B., S.E.G., M.S., M.E., Y.V.d.P. and F.S. designed the analyses. J.A., S.K.B., D.P., M. Pindo, G.C., D.M., G.E., L.M.F., N.G., T.M., J.T.M., J.R., B.W., C.K., Z.C., B.D., F.N., M. Palmer, T.K., D.J., S. Schaeffer, V.T.C., S.T.K., J.V., Q.T., A.M., A. Stormo, K.S. and R.B. performed DNA preparation and sequencing. R. Velasco, A.Z., A.D., A.C., A.K., P.F., M.T., D.P., P.B., V.C., A.D.R., M.K., P.M., D.M., P.L., Y.L., V.B., D.C., R.N.C., S.T., C.-E.D., A.G., R.E.B. and S.E.G. contributed to sequence assembly and anchoring to chromosomes. A.Z., A.D., A.C., A.K., P.F., M.T., S. Salvi, M. Pindo, S.C., M.C., F.C., V.G., S.L., G.M., M. Malnoy, D.M., M. Moretto, M. Perazzolli, A.S.-A., S.V., E.Z., V.K., C.W., D.E., A. Stella, A.V., M.M.K., S.M., A.C.A., R.N.C., A.P.G., E.L., J.A.F., S.P., P.R., L.S., S.T., B.L., R.P.H., Y.V.d.P. and F.S. contributed to automatic and manual genome annotation, genome structure and evolution analyses. R. Velasco, A.Z., J.A., A.D., A.K., P.F., M.T., S. Salvi, F.C., M. Malnoy, A.S.-A., S.V., R.E.B., A.V., S.M., J.A.F., L.S., S.T., B.L. and F.S. wrote the paper. M.M.K., A.C.A., R.N.C., A.P.G., R.P.H., C.-E.D., A.G., R.E.B., S.E.G., M.S., Y.V.d.P. and R. Viola revised the paper. All authors read and approved the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Plant material. The DNA of *Malus × domestica*, variety 'Golden Delicious', was extracted from young leaves of a two-year-old plant grown in the greenhouse at Fondazione Edmund Mac-Istituto Agrario di San Michele all'Adige. The dihaploid 'Golden Delicious' derivative genotype used at Washington State University and the University of Washington to produce 1.5× of 454 sequence was developed by the French National Institute for Agricultural Research⁴⁷ after a spontaneous duplication of a haploid individual selected in the progeny of a selfed derivative from 'Golden Delicious'⁴⁷.

'Golden Delicious' was chosen for genome sequencing because of its extensive use in apple breeding programs worldwide. Its heterozygous status did not hamper the genome assembly, thanks to expertise gained in heterozygous grape sequencing¹. Indeed, it allowed the inference of both haplotypes, thus giving access to both allelic versions for further genomic projects, and the development of SNP markers. The dihaploid genotype was important for a more accurate haplotype phase determination.

Bacterial artificial chromosomes, shotgun libraries and Sanger sequencing.

The apple bacterial artificial chromosome (BAC) library was from high-molecular weight genomic DNA (Amplicon Express), prepared as described⁴⁸. The fosmid and shotgun libraries were from genomic DNA provided by R. Meilan (Oregon State University). The shotgun libraries were from DNA sheared with a Gene Machines Hydroshear device. The DNA was size-selected for inserts from 2 to 12 kb to produce libraries of 2, 3, 6, 9 and 11 kb (average sizes). DNA was amplified with the Templiphi kit (GE Healthcare) and sequenced with the Sanger method.

Libraries and 454 pyrosequencing. Two random shotgun genomic libraries were created by fragmentation of 10 µg of genomic DNA with the GS FLX Titanium library preparation kit (454 Life Sciences). Sequencing was performed with the GS FLX instrument (454 Life Sciences). Further details on library construction and pyrosequencing are in the **Supplementary Note**.

Genome assembly and anchoring. From 27 libraries, 39.2 million reads (11.6 billion Q20 bases) were produced by Sanger sequencing and sequencing by synthesis (**Supplementary Table 1**). Chloroplast and mitochondrial sequences were identified with 847× and 168× coverage, respectively. Chloroplast (160,068 bp) and mitochondrial (396,947 bp) genomes were used to assess sequence quality and clone size in each library. Preliminary estimates of one to two SNPs per 1,000 bp were adopted in the assembly process. The actual SNP rate (4.4 SNPs per 1,000 bp) indicates that the preliminary value was conservative. Metacontigs were constructed on the basis of paired reads matching to nonrepetitive parts of contigs. Merging of contigs into metacontigs accepted a maximum total average coverage of 20×. Fifteen BAC clones were sequenced and individually assembled for quality assessment of sequencing accuracy and genome assembly.

Genetic maps used in metacontig anchoring were derived from six F₁ populations totaling 720 individuals (**Supplementary Note**). Simple sequence repeat primer sequences^{49–52} enabled detection of 196 polymorphic markers. Thirty-four SNP-based markers were from apple EST sequences, and 1,489 from genomic electronic SNPs, deduced by genomic sequence comparison between the two haplotypes present in the heterozygous genotype of 'Golden Delicious'. The consensus genetic maps for the six populations were used to generate an integrated genetic map (**Supplementary Fig. 1**) with TMAP⁵³ and a minimum logarithmic odds of 10.

Repetitive elements. The highest-coverage sequences were characterized as repetitive elements. Identified elements were iteratively masked, and the remaining sequences were searched for the next highest-coverage sequence. For each type, members were searched (BLASTN and BLASTX) against RepBase14.01, the NCBI databases and the Uniprot database^{54,55}.

Gene prediction and annotation. FgenesH⁵⁶, Twinscan⁵⁷, GlimmerHMM⁵⁸ and GeneWise⁵⁹ were used. The predicted protein sequences were searched with BLAST against Uniprot, protein domain data banks and plant protein databases annotated with GO terms. The GO terms were extracted by Argot⁶⁰ and InterproScan⁶¹. Unique genes were searched against proteins from rice, poplar, papaya, barrel medic, sorghum, *Arabidopsis* and grape by BLAST with an *e*-value cutoff of *e*^{−10}.

All-versus-all BLAST. Protein sequences from apple, poplar⁶ and grape² were extracted from a BLAST database, and pairwise similarities between all genes was obtained by BLASTP *e*-value (cutoff *e*^{−5}; 500 hits)⁶².

Gene families. Tribe-MCL⁶³ was adopted, with parameter *I* set to 2 and parameter 'scheme' to 4; other parameters were at default values.

Detection of collinearity. Metacontig anchoring generated lists of apple genes, from which transposable element-related sequences were removed. Poplar and grape gene lists were as described². Collinearity in the gene order was detected with i-ADHoRe 2.4 (ref. 64), with the following parameters: family blast type; alignment method, gg; gap size, 30; cluster gap, 35; *q* value, 0.9; prob cutoff, 0.0001; anchor points, 4; level 2 only, false.

K_s dating. Homologous genes were aligned with CLUSTALW⁶⁵. K_s dating was based on codeml⁶⁶ with the following parameters: verbose, 0; noisy, 0; runmode, −2; seqtype, 1; model, 0; NSsites, 0; icode, 0; fix_alpha, 0; fix_kappa, 0; RateAncestor, 0.

Molecular distances, taxonomy and phylogeny. Molecular distances were analyzed with EST data sets. A two-way alignment between apple and pear contigs (cDNA sequences, data not shown) was first generated. Sequences from apple and pear were combined with the peach sequence (EST databases) in three-way alignments. Phylogenetic analysis of the Wx genes included *gbss1* (Wx1) and *gbss2* (Wx2) sequences from the apple genome and from ref. 12. Sequences were aligned by T-coffee⁶⁷, and phylogenesis was by a Bayesian inference approach (MrBayes program). Phylogeny of Rosaceae was based on four chloroplast DNA sequences and on the nuclear internal transcribed spacer region. The data set included 6,308 positions in 85 operational taxonomic units, each representing one genus, aligned by a Bayesian method⁶⁸.

Apple domestication. A set of 74 *Malus* accessions, including 12 accessions of *M. × domestica* cultivars¹⁵, 10 of *M. sieversii* and 21 of *M. sylvestris*, was assembled. This included 31 of 34 recognized *Malus* species⁶⁹. Twenty-three genes were resequenced and, after alignment⁶⁷, a concatenated 11,300-bp multi-locus sequence was generated for each accession. Genetic relationships analysis used Splits-Tree v4.10 (ref. 16) and Hamming distance per pair of accessions. Haplotypes were computed with Phase v2.1 (ref. 70). Nucleotide diversity (π), H_e , H_o and F_{st} values¹⁷ were computed with Arlequin 3.1 (ref. 71).

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