Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2021) **19**, pp. 2206–2220 doi: 10.1111/pbi.13648

Insights into the effect of human civilization on *Malus* evolution and domestication

Pengxiang Chen[1,†], Zhongxing Li[1,†], Dehui Zhang[1,†], Wenyun Shen[1,†], Yinpeng Xie[1], Jing Zhang[1], Lijuan Jiang[1], Xuewei Li[1], Xiaoxia Shen[1], Dali Geng[1], Liping Wang[1], Chundong Niu[1], Chana Bao[1], Mingjia Yan[1], Haiyan Li[1], Cuiying Li[1], Yan Yan[1], Yangjun Zou[1], Diego Micheletti[2], Emily Koot[3], Fengwang Ma[1,*] and Qingmei Guan[1,*]

[1]*State Key Laboratory of Crop Stress Biology for Arid Areas, College of Horticulture, Northwest A&F University, Yangling, China*
[2]*Research and Innovation Centre, Fondazione Edmund Mach, Trento, Italy*
[3]*The New Zealand Institute for Plant and Food Research Limited, Palmerston North, New Zealand*

Received 1 April 2021;
revised 2 June 2021;
accepted 5 June 2021.
**Correspondence* (Tel +86-029-87082648; fax +86-029-87082648; email fwm64@nwafu.edu.cn (F.M.) or Tel +86-029-87082427; fax +86-029-87082427; email qguan@nwafu.edu.cn (Q.G.)).
[†]These authors contributed equally to this work.

Keywords: *Malus*, apple, sequencing, evolution, domestication.

Abstract

The evolutionary history of the *Malus* genus has not been well studied. In the current study, we presented genetic evidence on the origin of the *Malus* genus based on genome sequencing of 297 *Malus* accessions, revealing the genetic relationship between wild species and cultivated apples. Our results demonstrated that North American and East Asian wild species are closer to the outgroup (pear) than Central Asian species, and hybrid species including natural (separated before the Pleistocene, about 2.5 Mya) and artificial hybrids (including ornamental trees and rootstocks) are between East and Central Asian wild species. Introgressions from *M. sylvestris* in cultivated apples appeared to be more extensive than those from *M. sieversii*, whose genetic background flowed westward across Eurasia and eastward to wild species including *M. prunifolia*, *M.* × *asiatica*, *M.* × *micromalus,* and *M.* × *robust*. Our results suggested that the loss of ancestral gene flow from *M. sieversii* in cultivated apples accompanied the movement of European traders around the world since the Age of Discovery. Natural SNP variations showed that cultivated apples had higher nucleotide diversity than wild species and more unique SNPs than other apple groups. An apple ERECTA-like gene that underwent selection during domestication on 15th chromosome was identified as a likely major determinant of fruit length and diameter, and an *NB-ARC* domain-containing gene was found to strongly affect anthocyanin accumulation using a genome-wide association approach. Our results provide new insights into the origin and domestication of apples and will be useful in new breeding programmes and efforts to increase fruit crop productivity.

Introduction

The genus *Malus* belongs to the family Rosaceae and comprises ~30 species of deciduous trees and shrubs native to the temperate regions of the northern hemisphere. Given the diversity of the *Malus* genus in the wild and cultivation (Robinson *et al*., 2001; Rohrer *et al*., 1994), it is unsurprising that its evolutionary origins are controversial. The current mainstream view is that the genus originated in the so-called Chuan-Dian Palaeoland, corresponding to Southern China, Northern Vietnam, and Northern Laos. This is partly because this region is the geographic centre for over two-thirds of extant wild *Malus* species, including the oldest species such as *M. yunnanensis*, *M. sikkimensis*, *M. kansuensis*, *M. prattii*, *M. sieboldii,* and *M. hupehensis* (Jiang, 1986). It is widely believed that these progenitor species spread across Eurasia in the aftermath of the Ice Ages as the glaciers melted and temperatures increased (Langenfeld, 1991). However, some scientists have suggested that the genus *Malus* had multiple genetic centres (Ferree and Warrington, 2003; Li, 1989). The wild species *M. sieversii*is distributed within the Tian Shan Mountains; its distribution has remained largely unchanged from antiquity to the present day (Li, 1989). Europe, North America, and the Far East have also been considered as genetic centres for *Malus* (Ferree and Warrington, 2003; Li, 1989) due to their distinct native species. These studies have sought to clarify the origin of the genus *Malus* by considering its morphology, geography, and cytology. While their results have largely been inconclusive, these investigations have yielded preliminary insights into the relationships between wild species from different regions. Studies on allelic variants can provide genetic evidence regarding a species' origins and facilitate understanding of its evolutionary history. This approach is particularly powerful when paired with genomic approaches using second-generation sequencing technologies that enable high-throughput genotyping and large-scale surveys of genetic variation (Weigel and Mott, 2009).

The cultivated apple (*Malus* × *domestica* Borkh) has greatly influenced human history. Its fruit is widely consumed and is regarded as a symbol of wisdom and love (Juniper and Mabberley, 2006). Previous studies on the biogeography, isozyme polymorphisms, cytological markers, and simple sequence repeat markers of cultivated apples (Cornille *et al*., 2014; Gross *et al*., 2014) have suggested that cultivated apples were first domesticated in Central Asia from *M. sieversii* and were brought to

Europe about 3000 years ago via migration and trade (Cornille *et al*., 2014; Harris *et al*., 2002; Juniper and Mabberley, 2006; Velasco *et al*., 2010). Analyses of microsatellite markers showed that the European species *M. sylvestris* and *M. orientalis* contributed to the genetic make-up of domesticated apples (Cornille *et al*., 2012; Harrison and Harrison, 2011). A re-sequencing of 117 diverse *Malus* accessions (Duan *et al*., 2017) indicated that cultivated apples were domesticated from *M. sieversii* and received a strong introgression from *M. sylvestris*. Genomic analysis of 49 cultivars revealed that *M. sieversii* and *M. sylvestris* contributed significantly to these cultivars (Sun *et al*., 2020). In addition, a recent study indicated that dessert and cider apples may have independent domestication events (Liao *et al*., 2021). However, the genetic flow and dispersal routes of these two wild apple species in worldwide cultivars remain unknown.

Apple domestication involved many changes in fruit morphology and biochemistry. The most pronounced morphological difference between cultivated and wild species is that the former produce much larger fruit, probably because of selection for consumption. In addition, most modern cultivars have skin that is deep red in colour, as opposed to the green or yellow skin of many wild species. Colour is an important variable in modern breeding programmes because it strongly affects consumer appeal (King and Cliff, 2002). The main contributors to red skin, anthocyanins, have strong antioxidant activity (Eberhardt *et al*., 2000; Wolfe *et al*., 2003) and are popularly believed to benefit human health (Butelli *et al*., 2008; Toufektsian *et al*., 2008). In addition to fruit size and colour, selected attributes include fruit firmness, flavour, acid, and sugar content. The major organic acid in cultivated apples is malic acid (Beruter, 2004; Ma *et al*., 2015), while their main soluble sugars are fructose and sucrose (Ma *et al*., 2015).

Genetic loci controlling domestication traits can be identified in populations through genome-wide association studies (GWAS). GWAS involve saturating the genome with molecular markers, typically SNPs. They were employed to study traits in agronomic crops (Chen *et al*., 2014; Cui *et al*., 2016; Tieman *et al*., 2017) and have recently been applied to fruit trees (Cao *et al*., 2016; Mariette *et al*., 2016). Previous GWAS on apple trees has used SNP arrays or re-sequencing (Bianco *et al*., 2016; Duan *et al*., 2017; Farneti *et al*., 2017). Other studies have made use of genotyping-by-sequencing using restriction endonucleases (Amyotte *et al*., 2017; McClure *et al*., 2018; Migicovsky *et al*., 2016; Migicovsky *et al*., 2017). These investigations provided valuable information on genes that may affect fruit quality traits. However, genes associated with other valuable agronomic traits such as sugar and organic acid content were not identified, and there is still a need for experimental verification of the relationship between the identified genes and fruit quality traits.

Here, we analyse whole-genome genetic variation from 297 *Malus* accessions using high-throughput re-sequencing technology and use these data to evaluate the geographic origins of the *Malus* genus and domesticated apples. With a total of ~4.4 million high-quality SNPs, our results uncover the relationships among wild species of the genus *Malus* through population structure, and introgressions from the wild progenitors, *M. sieversii* and *M. sylvestris*, to domesticated apples from different continents. In addition, we apply these data in a GWAS focusing on fruit size and fruit flesh colour. Our findings provide genetic evidence regarding the origins of apple trees and will facilitate trait improvement through breeding in this important crop.

Results

Genetic diversity and structure

To study *Malus* evolution and domestication, we sequenced 297 *Malus* accessions, including 20 species from East Asia (38 accessions), four species from Central Asia (43 accessions), four species from North America (four accessions), two species from Europe (two accessions), 12 hybrid species (57 accessions), dwarfing rootstock cultivars (20 accessions), and commercial cultivated apples (128 accessions) (Table S1a). Sequencing was performed using the Illumina HiSeq 4000 system to a mean depth of ~11×, generating >two-Tb high-quality data. The genome coverage was over 70% across all chromosomes for most accessions, several accession of artificial hybrid and East Asia showed comparatively low coverage (>40%) (Figure S1). A total of ~4.4 million high-quality SNPs (Table S2) were identified after mapping against an apple reference genome (Daccord *et al*., 2017).

We used this whole-genome SNP data to assess phylogenetic relationships among the accessions (Figure 1a and Figure S2). A few accessions were grouped into other clusters of different genetic backgrounds, suggesting that some accessions may have been misclassified or that there may be previously unrecognized genetic differences between some accessions. Three wild species from North America (*M. angustifolia*, *M. ioensis* and *M. fusca*) and one wild European species (*M. florentina*) were genetically proximal to East Asian species and closer to the outgroup (pear) than East Asian species. The data set included several accessions representing hybrid species including both natural and artificial hybrids. The included species *M. robusta, M. asiatica, M. micromalus*, and *M. prunifolia*, which separated before the Pleistocene, about 2.5 Mya (Kumar *et al*., 2017), have been identified as natural hybrids. *M.* × *domestica* subsp. Chinensis was hybridized mainly from *M. prunifolia* and *M.* × *asiatica* in ancient China over 2000 years ago (Luo, 2014). These accessions are also considered natural hybrids and clustered with *M. prunifolia* and *M.* × *asiatica*. Artificial hybrids include ornamental trees (such as *M. hybrid* cv. Prairie Fire and *M. hybrid* cv. Robinson) and dwarfing rootstocks (such as *M.* × *domestica* cv. M26 and *M.* × *domestica* cv. Budagovsky57-233). Ornamental trees have been grown in orchards and gardens in North America since the beginning of the 19th century, when North American local species were hybridized with wild East Asian species by American botanists to obtain ornamental trees with flowers having a more desirable appearance (Jefferson *et al*., 1970). Dwarfing rootstocks were bred from wild apples for dwarfing or resistance to environmental stresses. These hybrid species can be traced to wild parent species and clustered with wild species from East Asia, North America, and Central Asia. *M. sieversii* from Central Asia clustered close to *M.* × *domestica*, implying that, as previously thought, cultivated apples originated from *M. sieversii*. Twelve red-fleshed cultivated accessions were clustered with *M. Niedzwetzkyana*, in keeping with the genetic evidence that a presumed natural form of *M. sieversii* native to central Asia ('Niedzwetzkyana') is a major contributor to most red-fleshed apples (van Nocker *et al*., 2011). Based on the evolutionary tree derived from our genomic data and the available information on each accession's region of origin (Table S1a) (Li, 2001), we divided the accessions into four groups for subsequent analysis: East Asia, Natural Hybrids, Central Asia, and Domestica (Table S1a). Artificial hybrid species were ignored because they provide no useful information in the context of evolutionary analysis.

A survey of identified SNPs and InDels in the apple genome revealed that all its chromosomes have similar levels of SNP diversity and similar numbers of InDels (Figure 1b). An evaluation of global nucleotide diversity (π) across all *Malus* accessions revealed diverse genomic regions that were correlated to SNP density. Broadly speaking, we found that cultivated apples had higher nucleotide diversity than wild species (Figure 1b and Table S3) and more unique SNPs (12 721) than other apple groups (Figure S3). Principal component analysis (PCA) demonstrated that the East Asia, Central Asia, and Domestica groups clustered together, while the Natural Hybrids group was dispersed among the other groups (Figure 1c). Population structure analysis suggested that the similarity of the genetic backgrounds of the Domestica and Central Asia groups was higher than that for any other pair of groups ($K = 2$, Figure 1d). Together with the phylogenetic analysis (Figure 1a,c), these results suggest that domesticated apples originated from *M. sieversii* in Central Asia (Juniper and Mabberley, 2006; Velasco *et al*., 2010). The Domestica and Natural Hybrids groups exhibited clear patterns of relatively high genetic heterogeneity ($K = 3$, Figure 1d and Figure S4). Cultivated apples developed genetic polymorphism as a result of extensive human hybridization from wild apples, which was then maintained through asexual propagation. However, at higher K values ($K \geq 4$) some natural hybrids (including *M.* × *robusta*, *M.* × *asiatica*, *M.* × *micromalus,* and *M. prunifolia*) exhibited a similar genetic background and level of genetic diversity to the Domestica group (Figure S4), implying that there was considerable gene flow between these natural hybrids and the Domestica group during apple domestication.

To further analyse the contributions of different *Malus* species and cultivars and the relationships between them, various *Malus* species were divided into 22 different groups according to the varieties and regions (Table S1b). We inferred patterns of population splits and mixtures from our genomic data and previously reported re-sequencing data (Duan *et al*., 2017) for the major *Malus* accession groups using TreeMix (Pickrell and Pritchard, 2012). Using pear as the root of the likelihood tree, some native species (*M. kansuensis*, *M. Fusca, M.toringgoides*, *M. florentina*, *M. angustifolia*, *M. ioensis* and *M. coronaria*) demonstrated low levels of genetic drift relative to *M. sieversii* and *M.* × *domestica* (Figure 2a). The *M.* × *domestica* cultivars were close to *M. sylvestris* and *M. sieversii* in the tree (Figure 2a), indicating that the latter two species are closely related to domesticated apples. Interestingly, *M. fusca* from North America clustered with East Asian species rather than other North American species (Figure 2a). It was observed that an admixture event assigning 0.347357% of the ancestry of *M. sieversii* to *M.* × *micromalus* by TreeMix analysis (Figure 2a). *M.* × *micromalus* traced about 0.447285% of their ancestry to a population prior to the divergence of *M.* × *asiatica*. In addition, the analysis revealed frequent gene flow between wild species (*M. prunifolia*, *M.* × *micromalus*, *M.* × *asiatica,* and *M. sieversii*). Model-based analyses of population admixture ($K = 3$, Figure 2b**)** revealed that the *M.* × *domestica* had a more similar genetic background to *M. sylvestris* than to any other wild species. Population admixture analysis also suggested that *M. sieversii* had a similar genetic background to some of the *M.* × *domestica* and wild species (*M.* × *asiatica*, *M. prunifolia, M.* × *micromalus,* and *M. baccata*). This genetic background existed in *M.* × *domestica* from Europe and America but was rare in *M.* × *domestica* from Australia and New Zealand and absent in *M.* × *domestica* from Japan ($K \leq 6$; Figure 2b). Higher K values ($K \geq 7$; Figure 2b) reduced the similarity of the genetic background of *M.* × *domestica* to those of *M. sylvestris* and *M. sieversii;* this similarity was detectable in *M. domestica* from Europe and America but not in *M. domestica* from Australia, New Zealand, and Japan.

Selection signals of domestication

To identify signals of artificial selection during apple domestication (identified by comparing the Central Asia and Domestica groups) and improvement (identified by comparing the East Asia and Domestica groups), a 100-kb sliding window with 10-kb step approach was applied to quantify F_{ST} and $\theta\pi$, and the cross top 10% of two values was selected as selective signals. A total of 28 domestication-selective sweeps and 51 improvement-selective sweeps were detected (Tables S4 and S5). All chromosomes underwent selection, especially Chromosome 17 (Figure S6). Genes located in the selective sweep regions included those encoding disease resistance proteins, protein kinases, and transcription factors. GO enrichment (FDR $\leq$ 0.01) demonstrated that genes in domestication-selected sweep regions were enriched mainly for the terms 'cellulose biosynthetic and metabolic process' and 'glucosyltransferase activities', both of which are potentially relevant to fruit development. Enriched genes in improvement-selective sweep regions were mainly related to molecular functions, including 'transmembrane', 'signaling receptor activities', 'tetrapyrrole', 'iron ion binding', and 'response to biotic stimulus' (Table S6).

GWAS for agronomic traits

In order to identify the genes that associate with important agronomic traits of apple, we measured fruit weight, length, diameter, firmness, and flesh colour, as well as the soluble solid content (SSC), and contents of fructose, sucrose, and malic acid (Table S7), and performed a GWAS analysis. Fifteen to twenty mature fruits from more than two trees in 2015 and 2016 were collected based on Starch-Iodine Index (Blanpied and Silsby, 1992). Only accessions showing consistent data for each trait in the two years were used for GWAS analysis. An obvious trait that is likely to have been selected during domestication and improvement is (increased) fruit size. Wild *Malus* species and cultivated apples show striking differences in fruit size. For example, the fruit of East Asian species tends to be <20 mm in length and diameter, whereas the fruit of *M.* × *domestica* is typically >50 mm on both measures (Figure 3e and Table S7). A significant GWAS signal (the threshold for GWAS was $-\log_{10}P = 5$) related to fruit length and diameter was located in a 400-kb region of Chromosome 15 (Figure 3a). This region showed extensive polymorphisms between wild species and cultivated apples and overlapped with the improvement-selective sweeps (Figure 3b and Table S8). Within this region, strong GWAS signals were apparent for several SNPs within a gene designated *MD15G1049300*, which encodes a leucine-rich receptor-like protein kinase family protein homologous to *Arabidopsis ERECTA* (Figure 3c). This finding suggests that there was strong artificial selection acting on this gene during apple domestication. This was further supported by sequence analysis of *ERECTA* from F_1 hybrids of *M. prunifolia* (small fruit) and *M.* × *domestica* (large fruit), which segregated for fruit size (fruit diameter and length). Two SNPs (Chr15:3358841 and Chr15:3359594) located in the intron of *ERECTA* strongly associated with fruit size showed the same genotype in both F_1 hybrids and sequenced accessions

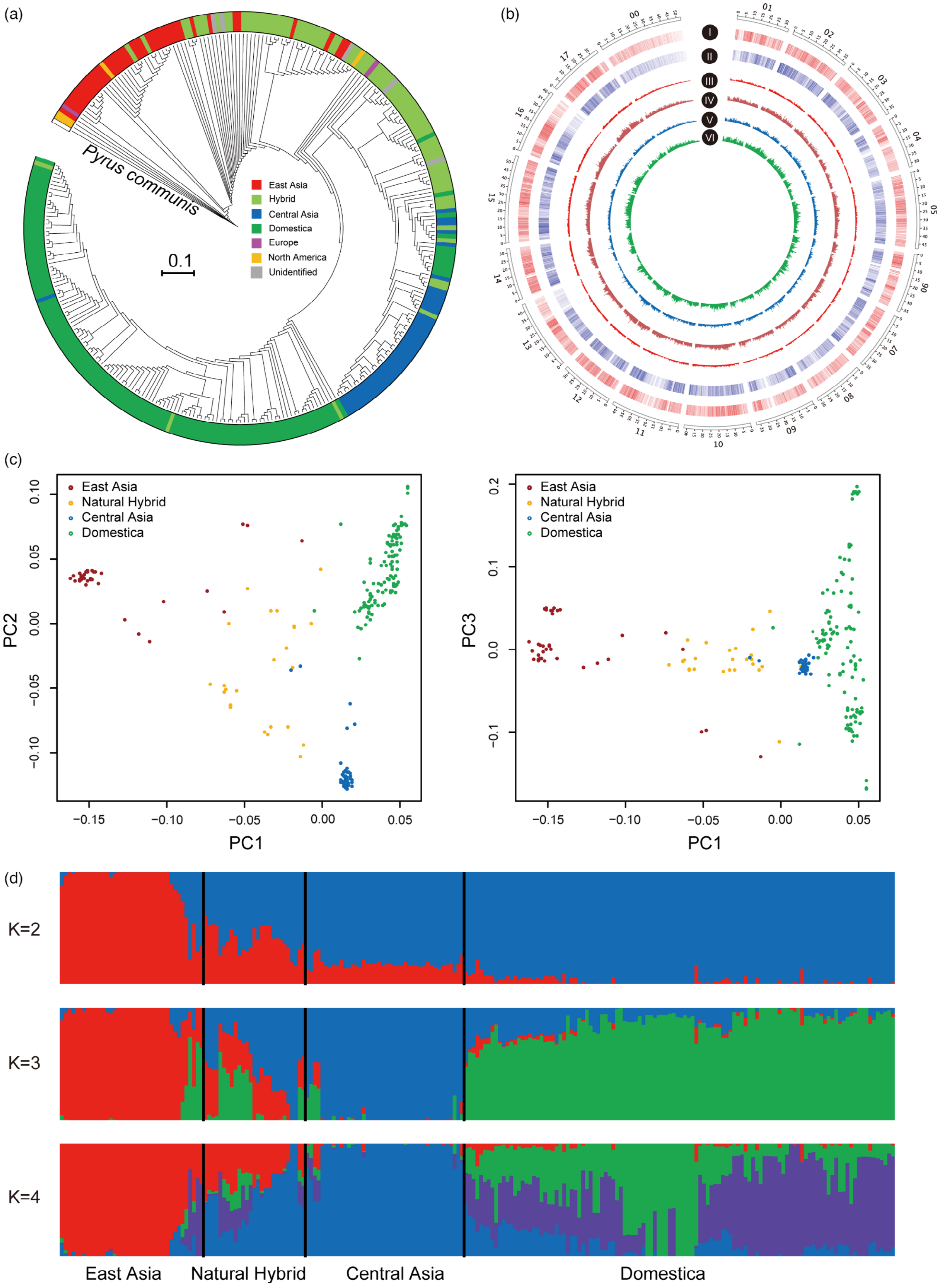

(a)
Pyrus communis
0.1
East Asia
Hybrid
Central Asia
Domestica
Europe
North America
Unidentified
(b)
I
II
III
IV
V
VI
00
01
02
03
04
05
06
07
08
09
10
11
12
13
14
15
16
17
(c)
East Asia
Natural Hybrid
Central Asia
Domestica
PC2
PC1
PC3
0.10
0.05
0.00
−0.05
−0.10
0.2
0.1
0.0
−0.1
−0.15
−0.10
−0.05
0.00
0.05
(d)
K=2
K=3
K=4
East Asia
Natural Hybrid
Central Asia
Domestica

Figure 1 Overview of population genetic analysis of *Malus* accessions. (a) Phylogenetic tree (neighbour-joining) of all accessions constructed using whole-genome SNPs. *Pyrus communis* (cultivated pear) is included as an outgroup. Four main groups are: East Asia (red), Hybrids (light green), Central Asia (blue), and Domestica (dark green). Other samples: Europe (purple), North America (yellow), and Unidentified (grey). (b) A Circos image representing the diversity of different components: heat map view of SNPs (I) and InDels (II). Total genetic diversity of the East Asia (III), Natural Hybrids (IV), Central Asia (V), and Domestica (VI) groups. The outer rings indicate individual chromosomes. (c) PCA plots of the first three components for four groups: East Asia (brown), Natural Hybrids (yellow), Central Asia (blue), and Domestica (green). (d) Population structure of four groups inferred using ADMIXTURE for an assumed number of groups (*K*) from 2 to 4. Each colour represents one ancestral population. Each group is indicated by a vertical bar.

(Figure 3d,e). To further verify whether *ERECTA* is a key gene controlling fruit size, we overexpressed apple *ERECTA* gene in tomato (*Solanum lycopersicum* cv. 'Ailsa Craig'). The results showed that overexpression of *MdERECTA* could significantly increase the size and weight of tomato fruits (Figure 3f–h). In addition to *ERECTA*, 23 genes associated with fruit size and fruit

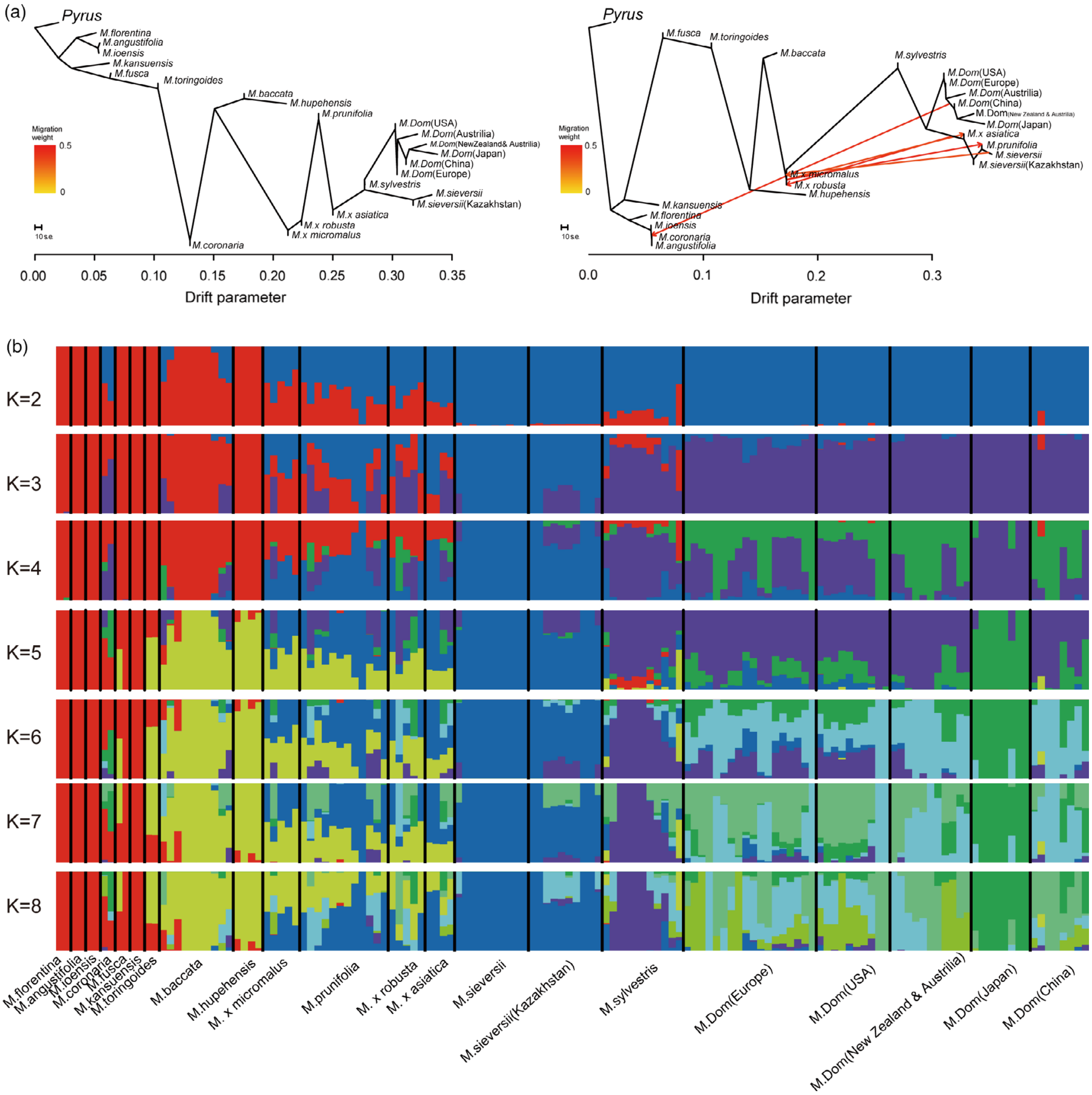
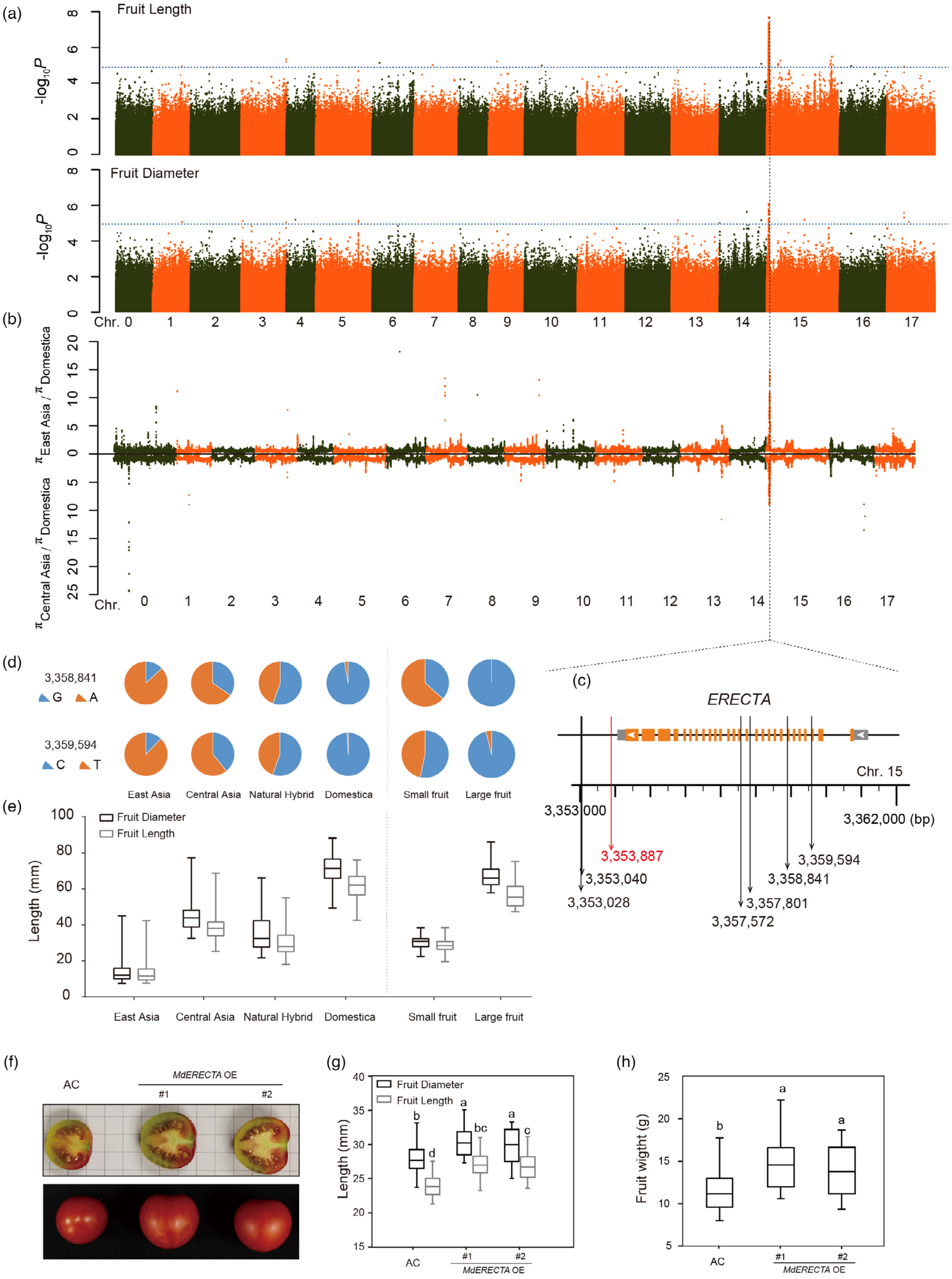


Figure 2 Introgression and structure of *Malus* accessions. (a) Maximum-likelihood (ML) tree of *Malus* species with inferred migration edges. Arrows on the graph represent admixture events between different *Malus* species. (b) Population structure of *Malus* species inferred using ADMIXTURE for an assumed number of groups (*K*) from 2 to 8.



weight were identified in a selective sweep region from 2.68 to 3.93 Mb in Chromosome 15 (Table S8). These results strongly suggest that these selected regions contributed to an increase in fruit size and weight during domestication. Other significant GWAS signals overlapping with selective sweep regions for improvement were also predicted to affect the traits of fruit

Figure 3 *ERECTA* was selected in artificial selection during apple domestication and affected fruit size. (a) GWAS analysis for apple fruit length and diameter. The dotted blue line represents the Bonferroni-corrected significant threshold for GWAS ($-\log_{10}P = 5$). (b) The region showed extensive polymorphisms between wild species and cultivated apples. (c) Location of *ERECTA* associated with fruit size GWAS and selection signals. The red arrow indicates the most significant SNP associated with fruit length and diameter. Black arrows indicate SNPs associated with fruit length. (d) Genotype of two SNPs associated with fruit length in *ERECTA* in both sequenced apple accessions and F_1 hybrids. (e) Fruit size of sequenced apple accessions and F_1 hybrids. (f) Phenotypes of transgenic tomato overexpressing *MdERECTA* (OE). (g) and (h) fruit size (g) and weight (h) of transgenic tomato overexpressing *MdERECTA*. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

length, diameter, weight, firmness, SSC, and contents of fructose and malic acid (Tables S6 and S9).

The GWAS analysis also identified several loci associated with fruit flesh colour located on Chromosome 9. A significant SNP signal was found in the promoter region of the *MD09G1278600* gene, which encodes an R2R3 MYB transcription factor designated MdMYB10 (Figure 4a and Table S10). *MdMYB10* was previously detected in a QTL region in the red-fleshed apple genome (Chagne *et al*., 2007). Ectopic activation of *MdMYB10* in apple induces anthocyanin accumulation in the fruit flesh and foliage (Allan *et al*., 2008; Espley *et al*., 2007), together with the transportation of anthocyanins into vacuoles (Hu *et al*., 2016). A rearrangement in the promoter region of *MdMYB10* enhances the gene's activation, leading to the phenotype of red foliage and red fruit flesh (Espley *et al*., 2009). MYB10 also strongly affects fruit colour in peach (Rahim *et al*., 2014), pear (Kusano *et al*., 2015), and strawberry (Medina-Puche *et al*., 2014). A gene on Chromosome 1 encoding a UDP-glycosyltransferase superfamily protein (UGT) was also detected in the GWAS analysis. This gene regulates the biosynthesis of flavonoids, thereby influencing pigmentation (Bowles *et al*., 2006; Caputi *et al*., 2012). However, the most significant SNP was located in a gene (*MD09G1272500*) encoding an NB-ARC domain-containing disease resistance protein (we named this gene *NB-ARC*) about 20-kb upstream of the *MdMYB10* gene (Figure 4a). This gene was expressed significantly more strongly in red-fleshed apples than typical white-fleshed apples (Figure 4b). To investigate the function of this *NB-ARC* gene in fruit coloration, we introduced a genomic copy of the *NB-ARC* sequence into *M.* × *domestica* cv. Granny Smith by transient expression analysis. This resulted in the accumulation of red coloration in the fruit flesh and a change in petiole colour from green to red (Figure 4c,d). Moreover, high-performance liquid chromatography (HPLC) analysis confirmed that the red components were anthocyanins (Figure 4e). In addition, we obtained transgenic plants overexpressing *MdNB-ARC* in GL-3. Under the treatment of 6% sucrose, anthocyanin accumulation was induced (Liu *et al*., 2017), We found that *MdNB-ARC* OE transgenic apple plants exhibited greater anthocyanin accumulation than the wild-type (GL-3) plants when 6% sucrose was added to the medium (Figure 4f,g). These results indicated that increased expression of *NB-ARC* led to anthocyanin accumulation.

The flavour of apples is strongly dependent on their malic acid content (Etienne *et al*., 2013). An aluminium-activated malate transporter family protein (MD14G1135700) and a NAD-dependent malic enzyme (MD07G1129000) related to malic acid metabolism were found in the GWAS results (Figure S7). Both genes have been reported to play key roles in malic acid biosynthesis (Bai *et al*., 2015; Etienne *et al*., 2013). The most significant SNP was located on Chromosome 10 and was associated with an integrin-linked protein kinase family gene (ILK). ILKs transduce multiple ion-mediated signalling pathways (Brauer *et al*., 2016; Popescu *et al*., 2017), including the potassium pathway, which influences plant photosynthesis and other metabolic processes affecting malic acid synthesis (Wang and Wu, 2017).

Strong GWAS signals for sucrose content were seen on multiple chromosomes (Figure S8). The most significant signal was associated with an F-box family protein (MD09G1064500) located on Chromosome 9. GC-MS analyses revealed two isomers of fructose in apples (fructose1 and fructose2) that had very similar GWAS patterns (Figure S9). *MD04G1247700*, which encodes an AP2-like ethylene-responsive transcription factor, was associated with the accumulation of both fructose1 and fructose2. The association of this gene with fructose (Figure S9), and with plant growth and fruit ripening (Gu *et al*., 2017; Phukan *et al*., 2017), may explain why ripe fruits accumulate more sucrose than immature ones and are thus sweeter (Ackermann *et al*., 1992). *MD17G1149000*, a gene encoding a synaptobrevin family protein, overlapped domestication-selective sweep signals located on Chromosome 17, suggesting that increased fructose production was selected during apple domestication (Table S7). SSC is another quality trait that influences apple flavour. The most significant SNPs for SSC were within a gene (*MD17G1286100*) of unknown function on Chromosome 17 and a gene (*MD05G1300000*) encoding a cyclic nucleotide-gated channel 1 protein on Chromosome 5 (Figure S10). Three significant SNPs were related to a gene encoding a sodium/calcium exchanger family protein (MD15G1340300) on Chromosome 15 and two genes encoding a Got1/Sft2-like vesicle transport protein (MD01G1219600) (Figure S10). The two most significant SNPs for fruit firmness were located between the genes *MD07G1071800* and *MD07G1071900*, which encode a Demeter-like protein and a nucleotide diphospho sugar transferases superfamily protein, respectively. This suggests that fruit firmness may be linked to glycosylation (Figure S11). Taken together, these results indicate that genes associated with the agronomic traits of apples may participate in a complex regulatory network affecting sugar metabolism, biosynthesis, and other metabolic pathways.

Discussion

The comprehensive evaluation and utilization of large-scale genomic data in this work has provided new insights into the origin of the *Malus* genus and the domestication history of cultivated apples. Our results indicate that the Central Asian species are genetically distinct from the East Asian species. Remarkably, *M. angustifolia* and *M. ioensis* from North America and *M. florentina* from Europe were more closely related to pears than to East Asian species. Unlike mammals, there is little fossil evidence available to support the hypothesis about the evolution of *Malus*, and it is difficult to define common ancestry (Li, 2001). In our study, wild *Malus* species from different continents were found to have very different genetic backgrounds, even those in Eurasia. We therefore conclude that wild *Malus* species from

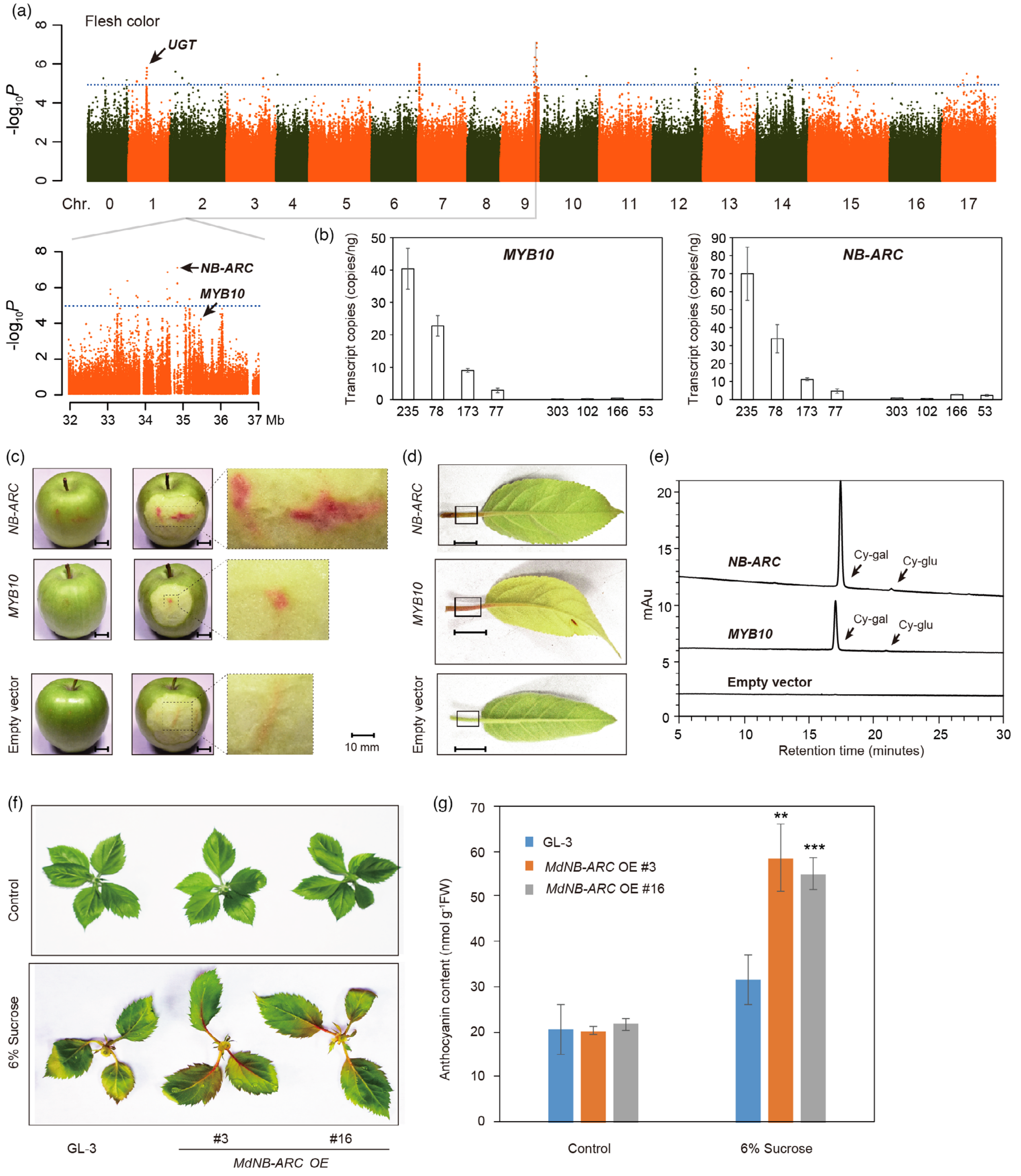


Figure 4 Identification and functional analyses of genes identified through GWAS analysis of flesh colour. (a) Manhattan plot showing the results of GWAS analysis of flesh colour and genomic locations of significant SNPs located around representative genes for flesh colour. The dotted blue line represents the Bonferroni-corrected significant threshold for GWAS ($-\log_{10}P = 5$). (b) Absolute quantification of *MdMYB10* and *MdNB-ARC* (*MD09G1272500*) expression in red- and white- fleshed accessions. 235, 78, 77, 173 are red-fleshed apple accessions; 303, 102, 166, 53 are white-fleshed accessions. (c) and (d) Phenotypes of apple fruit flesh (c) and foliage (d) transiently expressing *35S:MdMYB10* or *35S:MdNB-ARC*. Bars = 10 mm. (e) Flesh anthocyanin accumulation determined by HPLC analysis. Fruits were transiently transformed with *35S:MdMYB10* or *35S:MdNB-ARC*. Peaks were identified from HPLC traces at 520 nm. (f) Phenotypes of *MdNB-ARC* overexpression (OE) plants and GL-3 (the wild type) under sucrose treatment. (g) Anthocyanin content of the plants shown in (f). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Student's *t*-test. $^{**}P < 0.01$, $^{***}P < 0.001$.

different continents may have origins that are independent of their current geographical distribution.

As a self-incompatible perennial plant that has undergone frequent intraspecific and interspecific hybridization and adaptation to vegetative propagation, *Malus* has acquired an indistinct genetic background. Unlike many crops (such as wheat and rice), apples have not experienced domestication bottlenecks. The wild ancestors of wheat and rice have higher SNP diversity than cultivated species (Cavanagh *et al*., 2013; Pont *et al*., 2019; Wang *et al*., 2018; Wang *et al*., 2017a). This is because cultivated species have experienced domestication bottleneck in the process of artificial selection and have lost much ancestral SNP diversity. On the contrary, due to self-incompatibility, apple trees need to be crossed to set offspring, resulting in higher SNP diversity. Wild apples, for instance, *M. sieversii,* maintain a natural community until today in Tianshan Mountain, resulting in lower SNP diversity by crossing within the population. Rice has experienced the doctrine of single origin or multiple origins (Huang *et al*., 2012; Wang *et al*., 2014). In recent years, it has been gradually confirmed that indica and japonica originated separately (van Andel *et al*., 2016; Zhao *et al*., 2018). Cultivated apples have also experienced the debate about whether they originated from European *M. sylvestris* or Central Asian *M. sieversii*. Present study suggests that cultivated apples were domesticated from *M. sieversii* in Central Asia and brought to Europe, where they obtained introgressions from *M. sylvestris* (Cornille *et al*., 2013; Cornille *et al*., 2014; Cornille *et al*., 2012; Juniper *et al*., 1998; Velasco *et al*., 2010). Our data indicate that the genetic background of *M. sieversii* has similarities with those of wild species including *M. prunifolia*, *M.* × *asiatica*, *M.* × *micromalus* and *M.* × *robust*, and with domesticated apples (Figure 2). This genetic background was present to a significant degree in European and American cultivars, to a lesser degree in cultivars from Oceania and completely absent in Japanese cultivars (Figure 2). We thus infer that the genetic background of *M. sieversii* flowed eastward to wild species including *M. prunifolia*, *M.* × *asiatica*, *M.* × *micromalus* and *M.* × *robust*, and westward across Eurasia to cultivated apples (Figure 2). Moreover, the genetic background of cultivated apples is more similar to that of *M. sylvestris* than *M. sieversii*, indicating that *M. sylvestris* contributed more to cultivated apples than *M. sieversii* (Figure 2). Although confidence in this conclusion is limited by the sizes of the available samples, the genetic backgrounds of *M. sieversii* and *M. sylvestris*, which are ancestors of cultivated apples, were present to varying degrees in cultivars from different continents. Interestingly, although Japan is very close to Eurasia in geographical terms, we found no evidence of gene flow between *M. sieversii* and cultivated apples from Japan (Figure 2). Our results further clarify how wild apples affect cultivated apples through extensive sequencing and how did the European cultivar apples spread to the world with the genetic background of the ancestors.

Interestingly, the genetic background of wild ancestor apples shows a trend of gradual loss along with the trajectory of human activities. Maritime trade between Europe, America, and Oceania has been ongoing since the so-called Age of Discovery, which began in the 15th century (Diamond, 1997), whereas Europe's maritime exchanges with China and Japan took place after the 18th century (Diamond, 1997). Before the rise of maritime trade, China mainly communicated with Europe *via* the Silk Road, which became an important trading route 2000 years ago. It is thus possible that the gene pool of *M. sieversii* first spread along the Silk Road (Juniper and Mabberley, 2006) and then spread around the world with the advent of maritime trade. We therefore infer that after western traders began trading with Asia by the sea in the 18th century, nearly 300 varieties were introduced into Japan by 1910 (Yi, 1989), prompting extensive hybrid breeding. The genetic background of cultivated apples in Japan was thus established much later than that of cultivars in Europe, America, and Oceania, resulting in the complete loss of the *M. sieversii* background. A roadmap illustrating the spread of ancestral species' genetic backgrounds in modern apple cultivars is presented in Figure 5. The spread of food crops is considered to be an important symbol of the transition from nomadic to agricultural civilization. Dogs were domesticated by humans for hunting 16 000 years ago, and chickens and pigs were domesticated by farmers 7000 years ago (Kiple, 2007). Europe became the centre of civilization after the completion of the industrial revolution, which enabled marine trade and the spread of cultivated apples. This might be the reason why the gene flow from *M. sieversii* has declined gradually, while that from *M. sylvestris* has retained its dominant position during apple domestication. Our analysis indicates that cultivated apples traced the paths of human civilization from the 15th century to 19th century (Figure 5). In addition, humans have domesticated the genus *Malus* as ornamental plants, independently of its use as a source of food.

By combining GWAS with a selective sweep analysis, a signal related to fruit size was detected at the *ERECTA* gene locus on Chromosome 15 (Figure 3), indicating that *ERECTA* has important effects on fruit size and was subjected to artificial selection during apple domestication. In order to verify the GWAS signal of fruit size, we detected the genotypes of *ERECTA* from F_1 hybrids of 'Fuji' x *M. prunifolia*, which showed a segregation of large and small fruits. Our results showed that *ERECTA* does have a preference genotype (Figure 3). *ERECTA* and its counterparts from other plants are critical modulators of growth and development (Cai *et al*., 2017; Shpak, 2013) that regulate cell elongation (Bundy *et al*., 2012; Uchida *et al*., 2012) and have particularly strong effects on plant architecture and flower and fruit morphology (Torii *et al*., 1996). In rice, increased expression of *ERECTA* is associated with increased grain weight (Shen *et al*., 2015). Manipulation of this gene may thus be a powerful tool for increasing crop productivity.

We also identified a previously unknown NB-ARC domain-containing gene in anthocyanin accumulation (Figure 4). It has been reported that pathogen infection often causes the fruits to ripen and turn red. The ripening process of grapes has been accelerated upon *Botrytis cinerea* infection (Blanco-Ulate *et al*., 2015). Virus-infected grapes exhibit uneven ripening and red spots (Blanco-Ulate *et al*., 2017). *Alternaria alternata* infection can cause 'red ring', a typical phenotype of pathogenic infection on the jujube fruit (Yuan *et al*., 2019). Previous studies have shown that the NB-ARC domain is important for NLRs (nucleotide-binding, leucine-rich repeat proteins) function as immune receptors of plants to confer resistance against pathogens through regulating activation of NLRs self-association (Li *et al*., 2019). The genes containing the NB-ARC domain play a molecular switch in plant resistance to pathogen infection. This domain acts as an ATPase module that can hydrolyse ATP, thus triggering defence signalling and unravelling many new proteins of the plant immune system (Tameling *et al*., 2006). NLRs can interact with WRKY proteins such as WRKY1/2, WRKY45,

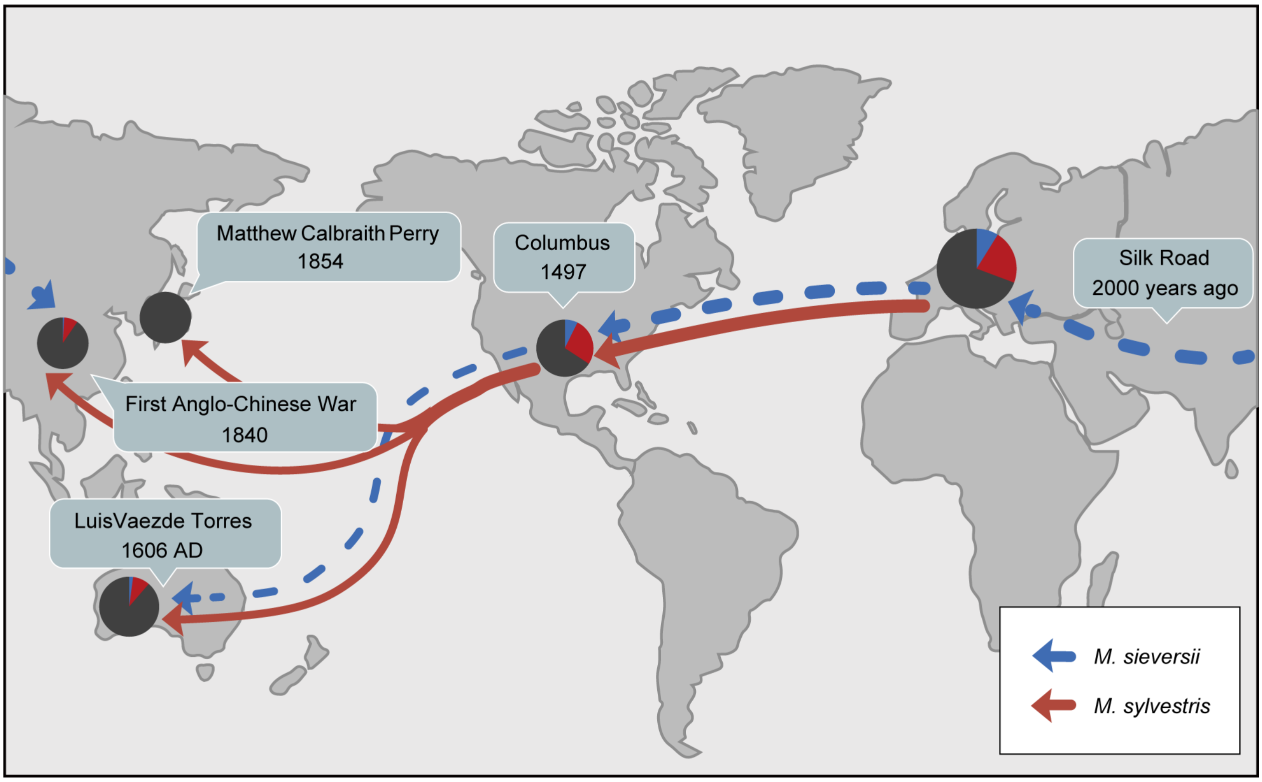


Figure 5 A roadmap illustrating distribution of genetic backgrounds of ancestral species in modern apple cultivars. The proportional pie charts showed the genetic backgrounds of *M. sieversii* (blue), *M. sylvestris* (red), and others (black) were defined by the ADMIXTURE analysis ($K = 6$). The areas of pie charts were proportional to the number of accessions. Arrows indicated potential spreading direction.

WRKY46, and WRKY72 to affect the expression of defence genes (Hu *et al*., 2017; Inoue *et al*., 2013; Shen *et al*., 2007). CaRGA (a CC-NB-ARC-LRR protein) interacts with WRKY64 that stimulates *EDS1* transcription in chickpea in response to *Fusarium* wilt infection (Chakraborty *et al*., 2018). Interestingly, WRKY family proteins play a role not only in plant disease resistance but also in anthocyanin accumulation. HpWRKY44 transcriptionally activates *HpCytP450-like1* in red pitaya fruit (Cheng *et al*., 2017). SlWRKYs is involved in colour change during tomato fruit ripening (Wang *et al*., 2017b). Apple MdWRKY40 promotes anthocyanin biosynthesis by interacting with MdMYB1 (An *et al*., 2019). In our study, overexpression of *MdNB-ARC* involved in plant defence signalling pathway leading to red apple fruit maybe through the WRYK family. However, this needs to be studied further. Taken together, our results will facilitate the breeding of new apple cultivars and future studies on various aspects of apple biology.

Methods

Sample collection and sequencing

Young leaves and fruits of 297 *Malus* accessions were collected from plants maintained at the Horticulture Experimental Station of Northwest A&F University (34°20′N, 108°24′E), Yangling, China, except that *M. baccata* cv. 'Dong Bei Shan Ding Zi' was provided by China National Fruit Germplasm Repository, Huludao, China. The origin of all the germplasms is shown in Table S1a. About five young leaves were collected in June 2015 and preserved in liquid nitrogen. Fifteen to twenty fruits at mature stage were collected from at least two trees of each accession. Fruit maturity was determined using the Starch-Iodine Index (Blanpied and Silsby, 1992). The fruits were collected in two years (2015 and 2016), from June to November each year. Fruits of F_1 hybrids of *M. prunifolia* and *M.* × *domestica* were collected at an experimental field located 7.9 km east of the Horticulture Experimental Station. Genomic DNA was extracted using the CTAB method (Murray and Thompson, 1980). At least 5 μg of genomic DNA from each sample was used to construct a sequencing library using NEBNext Ultra DNA Library Prep Kit (NEB Inc., America) in accordance with the manufacturer's instructions. Libraries with an insert size of approximately 500 bp were sequenced on an Illumina HiSeq 4000 sequencer by NowBio Company (Kunming) using Illumina TruSeq reagents and the paired-end protocol.

Mapping

Paired-end reads containing adapter sequences or low-quality reads (reads with >30% bases having Phred quality $\leq$ 25) were removed using NGSQCToolkit_v2.3.3 (Patel and Jain, 2012). In addition, 5 bp were trimmed off the 5′ end of each read and 15 bp were trimmed from the 3′ end (parameters: -l 5 -r 15). All reads from each line were mapped to a *Malus* × *domestica* reference genome (GDDH13 (Daccord *et al*., 2017)) using BWA (version: 0.7.10-r789) (Li and Durbin, 2009), allowing for mismatches of 4 bp in a single read (parameters: -t 10 –A 1 –B 4). The mapping results were then grouped by chromosome and sorted according to mapping coordinates. Only mapped paired-end reads were used for variant detection. Duplicated reads were filtered with the Picard package (picard.sourceforge.net, version: 2.1.1).

SNP variant calling

Variants were detected using HaplotypeCaller from GATK (version 3.3-0-g37228af) (Mckenna *et al.*, 2010) with parameters '-T HaplotypeCaller -stand_call_conf 30.0 -stand_emit_conf 10.0'. The process was as follows: (i) after BWA alignment, the reads around InDels were realigned. Realignment was performed with GATK in two steps. The first step used the RealignerTargetCreator package to identify regions where realignment was needed, and the second step used IndelRealigner to realign the regions found in the first step, which produced a realigned BAM file for each accession. (ii) Variations were called at a population level with HaplotypeCaller of GATK. The HaplotypeCaller parameter -stand_call_conf was set to 30 and -stand_emit_conf was set to 10. (iii) After variation calling, the SNPs and InDels were extracted from the HaplotypeCaller results, respectively. The SNPs were filtered using the GATK criteria with parameters 'QD < 20.0 II ReadPosRankSum < −8.0 II FS > 10.0 II QUAL < 6446.82'. SNPs with allele frequencies less than 5% and the proportion of missing data greater than 30% were ignored. SNPs were annotated against the apple genome (Daccord *et al.*, 2017) using the package ANNOVAR (version: 2015-12-14) (Wang *et al.*, 2010b). SNPs were categorized into exonic regions, splicing sites, 5′UTRs or 3′UTRs, intronic regions, upstream and downstream regions (within a 1-kb region upstream or downstream from the transcription start site), or intergenic regions. SNPs in coding exons were further categorized as synonymous, nonsynonymous, stop-gain or stop-loss.

Population analysis

SNPs within single-copy orthologous gene regions between pear (Chagné *et al.*, 2014) and apple were used to construct the phylogenetic tree. First, OrthoMCL (version 1.4) (Li *et al.*, 2003) was used to determine single-copy orthologous genes between pear and apple genomes. Then, multiple single-copy genes were aligned using Clustal W (version 2.1) (Larkin *et al.*, 2007). Finally, SNPs within these regions of the pear genome were extracted from the corresponding positions and concatenated into a supergene to provide outgroup information. A neighbour-joining tree was constructed using PHYLIP 3.696 (http://evolution.genetics.washington.edu/phylip.html) on the basis of a distance matrix. The bootstrap values on the tree are based on 1,000 replicates. The nucleotide diversity (π) was calculated using VCFtools (v0.1.13) (Danecek *et al.*, 2011) with 100-kb sliding windows and a 10-kb step size. Population structure was investigated using ADMIXTURE (version: 1.3) (Alexander *et al.*, 2009), which is a model-based clustering method for inferring population structure assuming different numbers of clusters (*K*). The statistic 'delta *K*', that is, the change in likelihood upon varying the number of assumed groups, was calculated and used to assess the most likely number of populations. Principal component analysis (PCA) was performed using GCTA (version: 1.25.3) software (Yang *et al.*, 2011), and the first three eigenvectors were plotted.

Conjoint analysis with Duan's samples

To characterize the gene flow between wild *Malus* species and cultivated apples, SNP variant calling was applied to the sequencing data for the apple accessions in Duan's analysis and our data using the previously discussed GATK method (Table S1b). In this case, the filtering criteria for GATT were set to 'QD < 20.0 II ReadPosRankSum < −8.0 II FS > 10.0 II QUAL < 5187.41'. After filtering SNPs with allele frequencies less than 5% and the proportion of missing data greater than 30%, a total of 134,781 SNPs were identified. These SNPs were used to determine population structure using ADMIXTURE (Alexander *et al.*, 2009) and to infer patterns of population splits and mixtures among wild *Malus* species and *M.* × *domestica* from different continents using TreeMix (Pickrell and Pritchard, 2012).

Selected sweeps

To minimize the influence of genetic drift, we combined the three cultivated groups into a single cultivated gene pool for the analysis. Genetic differentiation (F_{ST}) and polymorphism level ($\theta\pi$, a variable reflecting pairwise nucleotide variation as a measure of variability)-based cross approaches were used to investigate selection signals across the whole genome. F_{ST} and $\theta\pi$ were quantified with a 100-kb sliding window with 10-kb step approach using VCFtools software (v0.1.13) (Danecek *et al.*, 2011), and the cross top 10% of two values were selected as selective signals. GO enrichment was performed using Ontologizer (http://ontologizer.de) with default parameters.

Evaluation of agronomic traits

Levels of malic acid, sucrose and fructose were measured using the protocol of Lisec and Wang (Lisec *et al.*, 2006; Wang *et al.*, 2010a). Briefly, freeze-dried fruit powder (about 0.1 g) was extracted in 1.4 ml 75% methanol with ribitol as an internal standard. After adding chloroform to fractionated non-polar metabolites, 5 ul of supernatant was transferred into a 1.5-ml tube for drying under vacuum. After derivatization, metabolites were analysed by GC-MS using a Trace GC ULTRA/ISQ MS detector (Thermo Scientific™, Waltham, MA, USA). Fruit weight, SSC, and firmness were measured sequentially using a balance (TP-A500, HUAZHI®, Beijing, China), a refractometer (PAL-5, ATAGO®, Tokyo, Japan) and a Fruit Hardness Tester (FHM-5, Takemura Techno Works Co., Ltd., Tokyo, Japan), respectively. Fruit length and diameter were measured using a vernier calliper (MNT-150, Shanghai, China).

Genome-wide association study

GWAS was carried out using Genomic Association and Prediction Integrated Tool (GAPIT, version: 2016.03.01) (Lipka *et al.*, 2012) with the previously determined population parameters and the first three principal components (PCs) and Kinship (*K*) matrixes as covariates. $-\log_{10}P > 5$ was used to identify significant associations.

Transient expression in apple fruits, leaves and petioles

The coding sequence of *MdMYB10* or *MD09G1272500*(*MdNB-ARC*) was amplified from cDNA of 'Golden Delicious' and cloned into the pEarlygate203 vector under the control of 35S promoter, resulting in plasmids *35S:MdMYB10* and *35S:MD09G1272500*. Then, *35S:MdMYB10*, *35S:MD09G1272500* or the empty vector was transformed into *Agrobacterium* strain C58C1 and coinfiltrated with *35S:p19* (p19 is an RNA-silencing repressor protein from *Tomato bushy stunt virus*) in *Agrobacterium* strain C58C1 into 130 DAF (days after flowering) apple fruit (*M.* × *domestica* cv. Granny Smith). The injected apple fruits were placed in PVC bags and kept in the darkness for 12 h at room temperature and then transferred to a light growth chamber without the bags at 17 °C for 4–7 days (Li *et al.*, 2012) before observation. The apple leaves and petioles were transiently transformed with the same infiltration method.

Absolute quantitative PCR

Total RNA was extracted from apple fruits or leaves using Wolact®Plant RNA Isolation Kit (Hong Kong, China), following the manufacturer's protocol. Total RNA was subjected to reverse transcription using the RevertAid RT Reverse Transcription Kit (Thermo Scientific) following the manufacturer's protocol. Then, the cDNA was used for PCR or absolute quantitative PCR. The absolute quantification assay was performed as described (Shirima *et al*., 2017) with minor modifications. Briefly, specific primers were used to amplify the genes to create a standard curve after gel purification. Copy numbers (copies/ng) were then calculated based on the standard curve by SYBR Green Real-Time PCR.

Generation of transgenic plants

To generate transgenic tomato plants, coding region of MdERECTA from *Malus domestica* cv. 'Golden Delicious' was cloned into pGWB414 vector by Gateway® recombinant cloning technology. The resulting plasmid was transferred into *Agrobacterium tumefaciens* GV3101. The genetic transformation of tomato plants was performed as described previously (Jones *et al*., 2002). Homozygous T_2 plants were grown in a growth chamber for 30–40 days and then transplanted to a greenhouse. Fruits from 10 plants of each genotype were used to analysed the fruit length, diameter and fruit weight.

The coding region of MdNB-ARC was cloned into the vector of pRI101 and the resulting plasmid was transformed into *Agrobacterium tumefaciens* GV3101. The stably transgenic apple plants were obtained as described previously (Chen *et al*., 2020). To analyse the anthocyanin accumulation of *MdNB-ARC* transgenic plants, the shoot segments of tissue-cultured plants were transferred to Murashige and Skoog (MS) media containing 3% (normal concentration) and 6% (high concentration) sucrose with a long day photoperiod (light:dark, 14 h:10 h) for 30 days.

Measurement of anthocyanin

Measurement of anthocyanin from transiently transformed apple fruits, leaves and petioles were carried out as described (Wang *et al*., 2010a). Briefly, fruit samples were ground into powder under liquid nitrogen using the extraction buffer (methanol: formic acid, 70%:2%) and then centrifuged at 12 500 g for 20 min. The upper aqueous phase was then filtered through 0.45-μm syringe filters prior to high-performance liquid chromatography (HPLC) analysis using a HP1200 Liquid Chromatography with a diode array detector (Agilent technologies, Santa Clara, CA, USA).

The fresh leaf samples from transgenic plants and wild-type GL-3 plants were ground into fine powder which was then resuspended in methanol-HCl (1% v/v) buffer and transferred to 4°C for overnight. After centrifugation, supernatant was determined at 530, 620 and 650 nm. The concentration of anthocyanin was calculated according to the formula:

$$\mathrm{OD} = (\mathrm{A}530 - \mathrm{A}620) - 0.1 \times (\mathrm{A}650 - \mathrm{A}620).$$

Acknowledgements

We thank Dr. Kun Wang (China National Fruit Germplasm Repository, Huludao) for providing apple material (*M. baccata* cv. 'Dong Bei Shan Ding Zi') and Rui Zhai for helping with transient expression assays in apple fruit. We are very grateful to Dr. Pengmin Li for anthocyanin measurement.

Funding

This work was supported by the Key S&T Special Projects of Shaanxi Province, China (2020zdzx03-01-02), and the earmarked fund for the China Agriculture Research System (CARS-27).

Authors' contributions

Q.G. and F.M. designed the project; P.C., D.Z., P.X., J.Z., L.J. X.L., X.S., D. G., L.W., C.N., C.B., M.Y., H.L., C.L., Y.Y. and Y.Z. collected samples and performed phenotyping; P.C., D.M., E.K. and Z.L. analysed data; P.C., D.Z and W.S. performed experiments; P.C., Z.L., D.Z., W.S., and Q.G. wrote the manuscript; all authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Data Availability

The WGRS data set generated and analysed in the current study is available from NCBI under BioProject accession PRJNA728537.

References

Ackermann, J., Fischer, M. and Amado, R. (1992) Changes in sugars, acids, and amino acids during ripening and storage of apples (cv. Glockenapfel). *J. Agric. Food Chem.* **40**, 1131–1134.

Alexander, D.H., Novembre, J. and Lange, K. (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19**, 1655.

Allan, A.C., Hellens, R.P. and Laing, W.A. (2008) MYB transcription factors that colour our fruit. *Trends Plant Sci.* **13**, 99–102.

Amyotte, B., Bowen, A.J., Banks, T., Rajcan, I. and Somers, D.J. (2017) Mapping the sensory perception of apple using descriptive sensory evaluation in a genome wide association study. *PLoS One*, **12**, e0171710.

An, J.P., Zhang, X.W., You, C.X., Bi, S.Q., Wang, X.F. and Hao, Y.J. (2019) MdWRKY40 promotes wounding-induced anthocyanin biosynthesis in association with MdMYB1 and undergoes MdBT2-mediated degradation. *New Phytol.* **224**, 380–395.

van Andel, T.R., Meyer, R.S., Aflitos, S.A., Carney, J.A., Veltman, M.A., Copetti, D., Flowers, J.M. *et al*. (2016) Tracing ancestor rice of Suriname Maroons back to its African origin. *Nat. Plants*, **2**, 16149.

Bai, Y., Dougherty, L., Cheng, L., Zhong, G.Y. and Xu, K. (2015) Uncovering co-expression gene network modules regulating fruit acidity in diverse apples. *BMC Genom.* **16**, 612.

Beruter, J. (2004) Carbohydrate metabolism in two apple genotypes that differ in malate accumulation. *J. Plant Physiol.* **161**, 1011–1029.

Bianco, L., Cestaro, A., Linsmith, G., Muranty, H., Denance, C., Theron, A., Poncet, C. *et al*. (2016) Development and validation of the Axiom®Apple480K SNP genotyping array. *Plant J.* **86**, 62–74.

Blanco-Ulate, B., Amrine, K.C., Collins, T.S., Rivero, R.M., Vicente, A.R., Morales-Cruz, A., Doyle, C.L. *et al*. (2015) Developmental and metabolic plasticity of white-skinned grape berries in response to *Botrytis cinerea* during noble rot. *Plant Physiol.* **169**, 2422–2443.

Blanco-Ulate, B., Hopfer, H., Figueroa-Balderas, R., Ye, Z., Rivero, R.M., Albacete, A., Perez-Alfocea, F. *et al*. (2017) Red blotch disease alters grape berry development and metabolism by interfering with the transcriptional and hormonal regulation of ripening. *J. Exp. Bot.* **68**, 1225–1238.

Blanpied, G.D. and Silsby, K.J. (1992) Predicting harvest date windows for apples. In *Cornell University Cooperative Extension Bulletin 221*. New York: CCE Publications. https://ecommons.cornell.edu/handle/1813/3299

Bowles, D., Lim, E.K., Poppenberger, B. and Vaistij, F.E. (2006) Glycosyltransferases of lipophilic small molecules. *Annu. Rev. Plant Biol.* **57**, 567–597.

Brauer, E.K., Ahsan, N., Dale, R., Kato, N., Coluccio, A.E., Pineros, M.A., Kochian, L.V. *et al*. (2016) The Raf-like kinase *ILK1* and the high affinity K$^+$ transporter *HAK5* are required for innate immunity and abiotic stress response. *Plant Physiol.* **171**, 1470–1484.

Bundy, M.G.R., Thompson, O.A., Sieger, M.T. and Shpak, E.D. (2012) Patterns of cell division, cell differentiation and cell elongation in epidermis and cortex of *Arabidopsis* pedicels in the wild type and in erecta. *PLoS One*, **7**, e46262.

Butelli, E., Titta, L., Giorgio, M., Mock, H.-P., Matros, A., Peterek, S., Schijlen, E.G.W.M. *et al*. (2008) Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nat. Biotechnol.* **26**, 1301–1308.

Cai, H., Zhao, L., Wang, L., Zhang, M., Su, Z., Cheng, Y., Zhao, H. *et al*. (2017) ERECTA signaling controls *Arabidopsis* inflorescence architecture through chromatin-mediated activation of *PRE1* expression. *New Phytol.* **214**, 1579–1596.

Cao, K., Zhou, Z., Wang, Q., Guo, J., Zhao, P., Zhu, G., Fang, W. *et al*. (2016) Genome-wide association study of 12 agronomic traits in peach. *Nat Commun.* **7**, 13246.

Caputi, L., Malnoy, M., Goremykin, V., Nikiforova, S. and Martens, S. (2012) A genome-wide phylogenetic reconstruction of family 1 UDP-glycosyltransferases revealed the expansion of the family during the adaptation of plants to life on land. *Plant J.* **69**, 1030–1042.

Cavanagh, C.R., Chao, S., Wang, S., Huang, B.E., Stephen, S., Kiani, S., Forrest, K. *et al*. (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proc. Natl Acad. Sci. USA*, **110**, 8057–8062.

Chagne, D., Carlisle, C.M., Blond, C., Volz, R.K., Whitworth, C.J., Oraguzie, N.C., Crowhurst, R.N. *et al*. (2007) Mapping a candidate gene (MdMYB10) for red flesh and foliage colour in apple. *BMC Genom.* **8**, 212.

Chagné, D., Crowhurst, R.N., Pindo, M., Thrimawithana, A., Deng, C., Ireland, H., Fiers, M. *et al*. (2014) The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'). *PLoS One*, **9**, e92644.

Chakraborty, J., Priya, P., Dastidar, S.G. and Das, S. (2018) Physical interaction between nuclear accumulated CC-NB-ARC-LRR protein and WRKY64 promotes EDS1 dependent *Fusarium* wilt resistance in chickpea. *Plant Sci.* **276**, 111–133.

Chen, P., Yan, M., Li, L., He, J., Zhou, S., Li, Z., Niu, C. *et al*. (2020) The apple DNA-binding one zinc-finger protein MdDof54 promotes drought resistance. *Hortic Res.* **7**, 195.

Chen, W., Gao, Y., Xie, W., Gong, L., Lu, K., Wang, W., Li, Y. *et al*. (2014) Genome-wide association analyses provide genetic and biochemical insights into natural variation in rice metabolism. *Nat Genet.* **46**, 714–721.

Cheng, M.N., Huang, Z.J., Hua, Q.Z., Shan, W., Kuang, J.F., Lu, W.J., Qin, Y.H. *et al*. (2017) The WRKY transcription factor HpWRKY44 regulates *CytP450-like1* expression in red pitaya fruit (*Hylocereus polyrhizus*). *Hortic. Res.* **4**, 17039.

Cornille, A., Giraud, T., Bellard, C., Tellier, A., Le Cam, B., Smulders, M.J., Kleinschmit, J. *et al*. (2013) Postglacial recolonization history of the European crabapple (*Malus sylvestris* Mill.), a wild contributor to the domesticated apple. *Mol. Ecol.* **22**, 2249–2263.

Cornille, A., Giraud, T., Smulders, M.J., Roldan-Ruiz, I. and Gladieux, P. (2014) The domestication and evolutionary ecology of apples. *Trends Genet.* **30**, 57–65.

Cornille, A., Gladieux, P., Smulders, M.J., Roldan-Ruiz, I., Laurens, F., Le Cam, B., Nersesyan, A. *et al*. (2012) New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. *PLoS Genet.*, **8**, e1002703.

Cui, Z., Luo, J., Qi, C., Ruan, Y., Li, J., Zhang, A., Yang, X. *et al*. (2016) Genome-wide association study (GWAS) reveals the genetic architecture of four husk traits in maize. *BMC Genom.* **17**, 946.

Daccord, N., Celton, J.M., Linsmith, G., Becker, C., Choisne, N., Schijlen, E., van de Geest, H. *et al*. (2017) High-quality *de novo* assembly of the apple genome and methylome dynamics of early fruit development. *Nat. Genet.* **49**, 1099–1106.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., Depristo, M.A., Handsaker, R.E. *et al*. (2011) The variant call format and VCFtools. *Bioinformatics*, **27**, 2156.

Diamond, J. (1997) *Guns, germs, and steel: the fates of human societies*. New York, NY: W. W. Norton.

Duan, N., Bai, Y., Sun, H., Wang, N., Ma, Y., Li, M., Wang, X. *et al*. (2017) Genome re-sequencing reveals the history of apple and supports a two-stage model for fruit enlargement. *Nat. Commun.* **8**, 249.

Eberhardt, M.V., Lee, C.Y. and Liu, R.H. (2000) Nutrition: antioxidant activity of fresh apples. *Nature*, **405**, 903–904.

Espley, R.V., Brendolise, C., Chagne, D., Kutty-Amma, S., Green, S., Volz, R., Putterill, J. *et al*. (2009) Multiple repeats of a promoter segment causes transcription factor autoregulation in red apples. *Plant Cell*, **21**, 168–183.

Espley, R.V., Hellens, R.P., Putterill, J., Stevenson, D.E., Kutty-Amma, S. and Allan, A.C. (2007) Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. *Plant J.* **49**, 414–427.

Etienne, A., Génard, M., Lobit, P., Mbeguié-A-Mbéguié, D. and Bugaud, C. (2013) What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *J. Exp. Bot.* **64**, 1451–1469.

Farneti, B., Di Guardo, M., Khomenko, I., Cappellin, L., Biasioli, F., Velasco, R. and Costa, F. (2017) Genome-wide association study unravels the genetic control of the apple volatilome and its interplay with fruit texture. *J Exp Bot.* **68**, 1467–1478.

Ferree, D.C. and Warrington, I.J. (2003) *Apples: Botany, Production and Uses*. Wallingford, UK: CABI Publishing.

Gross, B.L., Henk, A.D., Richards, C.M., Fazio, G. and Volk, G.M. (2014) Genetic diversity in *Malus* x *domestica* (Rosaceae) through time in response to domestication. *Am. J. Bot.* **101**, 1770–1779.

Gu, C., Guo, Z.-H., Hao, P.-P., Wang, G.-M., Jin, Z.-M. and Zhang, S.-L. (2017) Multiple regulatory roles of AP2/ERF transcription factor in angiosperm. *Bot. Stud.* **58**, 6.

Harris, S.A., Robinson, J.P. and Juniper, B.E. (2002) Genetic clues to the origin of the apple. *Trends Genet.* **18**, 426–430.

Harrison, N. and Harrison, R.J. (2011) On the evolutionary history of the domesticated apple. *Nat. Genet.* **43**, 1043–1044. author reply 1044–1045.

Hu, D.G., Sun, C.H., Ma, Q.J., You, C.X., Cheng, L. and Hao, Y.J. (2016) MdMYB1 regulates anthocyanin and malate accumulation by directly facilitating their transport into vacuoles in apples. *Plant Physiol.* **170**, 1315–1330.

Hu, L., Wu, Y., Wu, D., Rao, W., Guo, J., Ma, Y., Wang, Z. *et al*. (2017) The coiled-coil and nucleotide binding domains of BROWN PLANTHOPPER RESISTANCE14 function in signaling and resistance against planthopper in rice. *Plant Cell*, **29**, 3157–3185.

Huang, X., Kurata, N., Wei, X., Wang, Z.X., Wang, A., Zhao, Q., Zhao, Y. *et al*. (2012) A map of rice genome variation reveals the origin of cultivated rice. *Nature*, **490**, 497–501.

Inoue, H., Hayashi, N., Matsushita, A., Xinqiong, L., Nakayama, A., Sugano, S., Jiang, C.J. *et al*. (2013) Blast resistance of CC-NB-LRR protein Pb1 is mediated by WRKY45 through protein-protein interaction. *Proc. Natl Acad. Sci. USA*, **110**, 9577–9582.

Jefferson, R.M., Division, C.R. and Sevice, A.R. (1970) *History, Progeny, and Locations of Crabapples of Documented Authentic Origin*. Washington, DC: U.S. Department of Agriculture.

Jiang, N. (1986) A preliminary research on the original centre of genus *Malus Miller*. *J. Southwest Agric. Univ.* **1**, 94–97.

Jones, B., Frasse, P., Olmos, E., Zegzouti, H., Li, Z.G., Latché, A., Pech, J.C. *et al*. (2002) Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. *Plant J.* **32**, 603–613.

Juniper, B.E. and Mabberley, D.J. (2006) *The story of the apple*. Portland, OR: Timber Press.

Juniper, B.E., Watkins, R. and Harris, S.A. (1998) The origin of the apple. *Eucarpia Symp. Fruit Breed Genet*, **484**, 27–34.

King, M.C. and Cliff, M.A. (2002) Development of a model for prediction of consumer liking from visual attributes of new and established apple cultivars. *J. Amer. Pomol. Soc.* **56**, 223–229.

Kiple, K.F. (2007) *A Movable Feast: Ten Millennia of Food Globalization*. Cambridge, UK: Cambridge University Press.

Kumar, S., Stecher, G., Suleski, M. and Hedges, S.B. (2017) TimeTree: a resource for timelines, timetrees, and divergence times. *Mol. Biol. Evol.* **34**, 1812–1819.

Kusano, M., Yang, Z., Okazaki, Y., Nakabayashi, R., Fukushima, A. and Saito, K. (2015) Using metabolomic approaches to explore chemical diversity in rice. *Mol. Plant* **8**, 58–67.

Langenfeld, T.V. (1991) *Apple-tree. Morphology, Evolution, Phylogeny, Geography, Taxonomy*. Riga: Zinatne.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F. *et al*. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, **23**, 2947–2948.

Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, **25**, 1754–1760.

Li, J., Huang, H., Zhu, M., Huang, S., Zhang, W., Dinesh-Kumar, S.P. and Tao, X. (2019) A plant immune receptor adopts a two-step recognition mechanism to enhance viral effector perception. *Mol. Plant*, **12**, 248–262.

Li, L., Stoeckert, C.J. and Roos, D.S. (2003) OrthoMCL: identification of ortholog groups for eukaryotic genomes. *Genome Res.* **13**, 2178–2189.

Li, Y. (1989) An investigation of the genetic centre of *M. Pumila* and *Malus* in the world. *Acta Hortic Sinica*, **16**, 101–108.

Li, Y. (2001) *Researches of Germplasm Resources of Malus Mill*. Beijing, China: China Agriculture Press.

Li, Y.Y., Mao, K., Zhao, C., Zhao, X.Y., Zhang, H.L., Shu, H.R. and Hao, Y.J. (2012) MdCOP1 ubiquitin E3 ligases interact with MdMYB1 to regulate light-induced anthocyanin biosynthesis and red fruit coloration in apple. *Plant Physiol.* **160**, 1011–1022.

Liao, L., Zhang, W., Zhang, B., Fang, T., Wang, X.-F., Cai, Y., Ogutu, C. *et al*. (2021) Unraveling a genetic roadmap for improved taste in the domesticated apple. *Mol Plant.* S1674-2052(21)00179-9. https://www.sciencedirect.com/science/article/pii/S1674205221001799

Lipka, A.E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P.J., Gore, M.A. *et al*. (2012) GAPIT: genome association and prediction integrated tool. *Bioinformatics*, **28**, 2397–2399.

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L. and Fernie, A.R. (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. *Nat Protoc.* **1**, 387–396.

Liu, X.J., An, X.H., Liu, X., Hu, D.G., Wang, X.F., You, C.X. and Hao, Y.J. (2017) MdSnRK1.1 interacts with MdJAZ18 to regulate sucrose-induced anthocyanin and proanthocyanidin accumulation in apple. *J. Exp. Bot.* **68**, 2977–2990.

Luo, G. (2014) The cultivation history of apple in China. *J Beijing For. Univ.* **13**, 15–25.

Ma, B., Chen, J., Zheng, H., Fang, T., Ogutu, C., Li, S., Han, Y. *et al*. (2015) Comparative assessment of sugar and malic acid composition in cultivated and wild apples. *Food Chem.* **172**, 86–91.

Mariette, S., Wong Jun Tai, F., Roch, G., Barre, A., Chague, A., Decroocq, S., Groppi, A. *et al*. (2016) Genome-wide association links candidate genes to resistance to *Plum Pox Virus* in apricot (*Prunus armeniaca*). *New Phytol.* **209**, 773–784.

McClure, K.A., Gardner, K.M., Douglas, G.M., Song, J., Forney, C.F., DeLong, J., Fan, L. *et al*. (2018) A genome-wide association study of apple quality and scab resistance. *Plant Genome*, **11**, 170075.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K. *et al*. (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303.

Medina-Puche, L., Cumplido-Laso, G., Amil-Ruiz, F., Hoffmann, T., Ring, L., Rodŕguez-Franco, A., Caballero, J.L. *et al*. (2014) MYB10 plays a major role in the regulation of flavonoid/phenylpropanoid metabolism during ripening of *Fragaria × ananassa* fruits. *J. Exp. Bot.* **65**, 401–417.

Migicovsky, Z., Gardner, K.M., Money, D., Sawler, J., Bloom, J.S., Moffett, P., Chao, C.T. *et al*. (2016) Genome to phenome mapping in apple using historical data. *Plant Genome*, **9**, plantgenome2015.11.0113.

Migicovsky, Z., Li, M., Chitwood, D.H. and Myles, S. (2017) Morphometrics reveals complex and heritable apple leaf shapes. *Front. Plant Sci.* **8**, 2185.

Murray, M.G. and Thompson, W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **8**, 4321–4325.

van Nocker, S., Berry, G., Najdowski, J., Michelutti, R., Luffman, M., Forsline, P., Alsmairat, N. *et al*. (2011) Genetic diversity of red-fleshed apples (*Malus*). *Euphytica*, **185**, 281–293.

Patel, R.K. and Jain, M. (2012) NGS QC Toolkit: a toolkit for quality control of next generation sequencing data. *PLoS One*, **7**, e30619.

Phukan, U.J., Jeena, G.S., Tripathi, V. and Shukla, R.K. (2017) Regulation of Apetala2/ethylene response factors in plants. *Front. Plant Sci.* **8**, 150.

Pickrell, J.K. and Pritchard, J.K. (2012) Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genet.* **8**, e1002967.

Pont, C., Leroy, T., Seidel, M., Tondelli, A., Duchemin, W., Armisen, D., Lang, D. *et al*. (2019) Tracing the ancestry of modern bread wheats. *Nat. Genet.* **51**, 905–911.

Popescu, S.C., Brauer, E.K., Dimlioglu, G. and Popescu, G.V. (2017) Insights into the structure, function, and ion-mediated signaling pathways transduced by plant integrin-linked kinases. *Front. Plant Sci.*, **8**, 376.

Rahim, M.A., Busatto, N. and Trainotti, L. (2014) Regulation of anthocyanin biosynthesis in peach fruits. *Planta*, **240**, 913–929.

Robinson, J.P., Harris, S.A. and Juniper, B.E. (2001) Taxonomy of the genus *Malus Mill*. (Rosaceae) with emphasis on the cultivated apple. *Malus domestica* Borkh. *Plant Syst. Evol.* **226**, 35–58.

Rohrer, J.R., Robertson, K.R. and Phipps, J.B. (1994) Floral morphology of Maloideae (Rosaceae) and its systematic relevance. *Am. J. Bot.* **81**, 574–581.

Shen, H., Zhong, X., Zhao, F., Wang, Y., Yan, B., Li, Q., Chen, G. *et al*. (2015) Overexpression of receptor-like kinase *ERECTA* improves thermotolerance in rice and tomato. *Nat Biotechnol.* **33**, 996–1003.

Shen, Q.-H., Saijo, Y., Mauch, S., Biskup, C., Bieri, S., Keller, B., Seki, H. *et al*. (2007) Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science* **315**, 1098.

Shirima, R.R., Maeda, D.G., Kanju, E., Ceasar, G., Tibazarwa, F.I. and Legg, J.P. (2017) Absolute quantification of cassava brown streak virus mRNA by real-time qPCR. *J. Virol. Methods* **245**, 5–13.

Shpak, E.D. (2013) Diverse roles of *ERECTA* family genes in plant development. *J. Integr. Plant Biol.* **55**, 1238–1250.

Sun, X., Jiao, C., Schwaninger, H., Chao, C.T., Ma, Y., Duan, N., Khan, A. *et al*. (2020) Phased diploid genome assemblies and pan-genomes provide insights into the genetic history of apple domestication. *Nat. Genet.* **52**, 1423–1432.

Tameling, W.I., Vossen, J.H., Albrecht, M., Lengauer, T., Berden, J.A., Haring, M.A., Cornelissen, B.J. and *et al*. (2006) Mutations in the NB-ARC domain of I-2 that impair ATP hydrolysis cause autoactivation. *Plant Physiol.* **140**, 1233–1245.

Tieman, D., Zhu, G., Resende, M.F. Jr, Lin, T., Nguyen, C., Bies, D., Rambla, J.L. *et al*. (2017) A chemical genetic roadmap to improved tomato flavor. *Science*, **355**, 391–394.

Torii, K.U., Mitsukawa, N., Oosumi, T., Matsuura, Y., Yokoyama, R., Whittier, R.F. and Komeda, Y. (1996) The *Arabidopsis ERECTA* gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. *Plant Cell*, **8**, 735–746.

Toufektsian, M.-C., de Lorgeril, M., Nagy, N., Salen, P., Donati, M.B., Giordano, L., Mock, H.-P. *et al*. (2008) Chronic dietary intake of plant-derived anthocyanins protects the rat heart against ischemia-reperfusion injury. *J. Nutr.* **138**, 747–752.

Uchida, N., Lee, J.S., Horst, R.J., Lai, H.-H., Kajita, R., Kakimoto, T., Tasaka, M. *et al*. (2012) Regulation of inflorescence architecture by intertissue layer ligand–receptor communication between endodermis and phloem. *Proc. Natl Acad. Sci. USA*, **109**, 6337–6342.

Velasco, R., Zharkikh, A., Affourtit, J., Dhingra, A., Cestaro, A., Kalyanaraman, A., Fontana, P. *et al*. (2010) The genome of the domesticated apple (*Malus* x *domestica* Borkh.). *Nat. Genet.* **42**, 833–839.

Wang, H., Ma, F. and Cheng, L. (2010a) Metabolism of organic acids, nitrogen and amino acids in chlorotic leaves of 'Honeycrisp' apple (*Malus domestica* Borkh) with excessive accumulation of carbohydrates. *Planta*, **232**, 511–522.

Wang, H., Vieira, F.G., Crawford, J.E., Chu, C. and Nielsen, R. (2017a) Asian wild rice is a hybrid swarm with extensive gene flow and feralization from domesticated rice. *Genome Res.* **27**, 1029–1038.

Wang, K., Li, M. and Hakonarson, H. (2010b) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* **38**, e164.

Wang, L., Zhang, X.L., Wang, L., Tian, Y., Jia, N., Chen, S., Shi, N.B. *et al*. (2017b) Regulation of ethylene-responsive *SlWRKY*s involved in color change during tomato fruit ripening. *Sci Rep.* **7**, 16674.

Wang, M., Yu, Y., Haberer, G., Marri, P.R., Fan, C., Goicoechea, J.L., Zuccolo, A. *et al*. (2014) The genome sequence of African rice (*Oryza glaberrima*) and evidence for independent domestication. *Nat. Genet.* **46**, 982–988.

Wang, W., Mauleon, R., Hu, Z., Chebotarov, D., Tai, S., Wu, Z., Li, M. *et al*. (2018) Genomic variation in 3,010 diverse accessions of Asian cultivated rice. *Nature*, **557**, 43–49.

Wang, Y. and Wu, W.H. (2017) Regulation of potassium transport and signaling in plants. *Curr. Opin. Plant Biol.* **39**, 123–128.

Weigel, D. and Mott, R. (2009) The 1001 genomes project for *Arabidopsis thaliana*. *Genome Biol.* **10**, 107.

Wolfe, K., Wu, X. and Liu, R.H. (2003) Antioxidant activity of apple peels. *J. Agric. Food Chem.* **51**, 609–614.

Yang, J., Lee, S.H., Goddard, M.E. and Visscher, P.M. (2011) GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* **88**, 76.

Yi, Z. (1989) The historical changes, composition, and trends of Japanese cultivated apples. *Deciduous Fruits*, **s1**, 7–10.

Yuan, S., Yan, J., Wang, M., Ding, X., Zhang, Y., Li, W., Cao, J. *et al*. (2019) Transcriptomic and metabolic profiling reveals 'Green Ring' and 'Red Ring' on jujube fruit upon postharvest *Alternaria alternata* infection. *Plant Cell Physiol.* **60**, 844–861.

Zhao, Q., Feng, Q., Lu, H., Li, Y., Wang, A., Tian, Q., Zhan, Q. *et al*. (2018) Pan-genome analysis highlights the extent of genomic variation in cultivated and wild rice. *Nat. Genet.* **50**, 278–284.

Supporting information

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

Figure S1. Heat map of the coverage of each accession across each chromosome of the apple genome. Artificial Hybrid, Central Asia, East Asia, and Other refer to the groups in Table S1a.

Figure S2. Unrooted phylogenetic tree of all accessions inferred from whole-genome re-sequencing, with *Pyrus* as an outgroup.

Figure S3. Venn diagram of SNPs among four groups.

Figure S4. Population structure of four groups inferred using ADMIXTURE. (a) The standard estimate of the cross-validation (CV) error (b) ADMIXTURE analyses for an assumed number of subpopulations (*K*) from 5 to 9. Each colour represents one ancestral population. Each group is indicated by a vertical bar.

Figure S5. The standard estimate of the cross-validation (CV) error of population structure of *Malus* species (Fig. 2B).

Figure S6. Genome-wide distribution of F_{ST}.

Figure S7. Manhattan plot showing the GWAS results for malic acid content. Gene abbreviations: ALMT, Aluminium-activated malate transporter family protein; ILK, Integrin-linked protein kinase family; NME, NAD-dependent malic enzyme 2. The dotted grey line represents the significant threshold for GWAS ($–\log_{10}P = 5$).

Figure S8. Manhattan plot showing the GWAS results of content for sucrose content. Gene abbreviations: FBX, F-box family protein. The dotted gray line represents the significant threshold for GWAS ($–\log_{10}P = 5$).

Figure S9. Manhattan plot showing the GWAS results for fructose content. Gene abbreviations: AP2L, AP2-like ethylene-responsive transcription factor; SFP, Synaptobrevin family protein. The dotted gray line represents the significant threshold for GWAS ($–\log_{10}P = 5$).

Figure S10. Manhattan plot showing the GWAS results for soluble solid content. Gene abbreviations: CNGC1, cyclic nucleotide-gated channel 1; Eu1035, Other Eukaryotes-1035; GVTP, Got1/Sft2-like vescicle transport protein family; SEFP, sodium/calcium exchanger family protein / calcium-binding EF hand family protein. The dotted gray line represents the significant threshold for GWAS ($–\log_{10}P = 5$).

Figure S11. Manhattan plot showing the GWAS results for fruit firmness. Gene abbreviations: DML1, demeter-like 1; NTSP, Nucleotide-diphospho-sugar transferases superfamily protein. The dotted gray line represents the significant threshold for GWAS ($–\log_{10}P = 5$).

Figure S12. Manhattan plot showing the GWAS results for fruit weight. The dotted gray line represents the significant threshold for GWAS ($–\log_{10}P = 5$).

Table S1. Details of all accessions and group information. (a) Details of 297 sequencing accessions for this study. (b) Details of accessions for Malus species analyses.

Table S2. Whole-genome distribution of SNPs and InDels.

Table S3. Summary SNP statistics in different apple groups and species.

Table S4. Regions significantly associated with domestication-selective sweeps and potential genes.

Table S5. Regions significantly associated with improvement-selective sweeps and potential genes.

Table S6. Enrichment of genes from selective sweeps.

Table S7. Agronomic traits and phenotyping data used for GWAS.

Table S8. GWAS-significant genes overlapping with improvement-selective sweep signals.

Table S9. GWAS-significant genes overlapping with domestication-selective sweep signals.

Table S10. Information of the significant SNPs and associated genes from the GWAS.