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Tracing the diploid ancestry of the cultivated octoploid strawberry

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Abstract

The commercial strawberry, *Fragaria × ananassa*, is a recent allo-octoploid that is cultivated worldwide. However, other than *F. vesca*, which is universally accepted one of its diploid ancestors, its other early diploid progenitors remain unclear. Here, we performed comparative analyses of the genomes of five diploid strawberries, *F. itinumae*, *F. vesca*, *F. nilgerrensis*, *F. nubicola*, and *F. viridis*, of which the latter three are newly sequenced. We found that the genomes of these species share highly conserved gene content and gene order. Using an alignment-based approach, we show that *F. itinumae* and *F. vesca* are the diploid progenitors to the octoploid *F. × ananassa*, whereas the other three diploids that we analyzed in this study are not parental species. We generated a fully resolved, dated phylogeny of *Fragaria* and determined that the genus arose ca. 6.37 million years ago. Our results effectively resolve conflicting hypotheses regarding the putative diploid progenitors of the cultivated strawberry, establish a reliable backbone phylogeny for the genus, and provide genetic resources for molecular breeding.

Key words: *Fragaria*, chromosome-level genome, diploid progenitors, gene tree discordance, ILS and hybridization, sppIDer

Introduction

The commercial strawberry, *Fragaria × ananassa*, is one of the most recently domesticated plants in the world and is among many economically important fruit crops of the Rosaceae plant family (Hummer and Hancock 2009). According to the Food and Agriculture Organization (FAO) of the United Nations, world production of strawberries has exceeded eight million tons since 2018 (FAO 2020). In addition to being visually appealing and tasty, strawberries provide a wide range of nutritional benefits because they are rich in vitamin C, phenolic compounds, and micronutrients (Giampieri et al. 2012).

The genus *Fragaria* is circumscribed with approximately 25 species, which represent five ploidy levels, ranging from diploid to decaploid with a base
chromosome number of seven (Folta and Davis 2006; Hummer and Hancock 2009; Lei et al. 2017). Species of *Fragaria* have a natural distribution in the Northern Hemisphere with their center of diversity being within China, where the most diploid (eight out of 12) and all five tetraploid species of the genus occur (Liston et al. 2014; Lei et al. 2017). Wild species of *Fragaria* are known to have small genomes (~200–300 Mbp for diploid species) and diverse breeding systems from self-compatibility to dioecy and, among species, barriers to crossing are low. Most species of *Fragaria* can be clonally propagated by stolons. Mature plants are usually small and this facilitates cultivation in enclosed, controlled conditions. These characteristics render *Fragaria* a uniquely powerful system for studies of sexual system evolution, polyploidization, and evolutionary genomics (Liston et al. 2014). Moreover, wild species are valuable in breeding programs aimed at broadening the gene pools for cultivated strawberries (Hancock 1999; Chambers et al. 2013).

The modern cultivated strawberry is a recent allo-octoploid (2n = 8x = 56) species, which is thought to have arisen via spontaneous hybridization between representatives of its two octoploid progenitor species, *F. chiloensis* and *F. virginiana*, in Europe in the mid-18th century (Darrow 1966). To elucidate the precursory diploid progenitors, numerous cytological and phylogenetic studies have been undertaken and have led to four contradictory hypotheses involving two to five plausible diploid progenitors for the octoploid genome (Fedorova 1946; Senanayake and Bringhurst 1967; Bringhurst 1990; Rousseau-Gueutin et al. 2009; DiMeglio et al. 2014; Tennessen et al. 2014; Sargent et al. 2016; Kamneva et al. 2017; Yang and Davis 2017). For example, Tennessen et al. (2014) hypothesized that the allo-octoploid cultivated strawberry *F. × ananassa* originated from a complex series of genetic contributions from *F. vesca*, *F. iinumae*, and two *F. iinumae*-like ancestors, based on evidence from linkage maps. In contrast, Yang and Davis (2017) proposed that genetic signatures of at least five diploid ancestors (*F. vesca*, *F. iinumae*, *F. bucharica*, *F. viridis*, and one with an unknown identity) are present in octoploid *Fragaria* species. Recently, Edger et al. (2019) proposed that four diploid species, *F. vesca*, *F. iinumae*, *F. viridis*, and *F. nipponica*, comprise the subgenomes of the octoploid strawberry, *F. ×
ananassa, based on a high-quality, sequenced genome of the commercial species and a tree-searching algorithm. They further suggested that the hexaploid species *F. moschata* may be evolutionary intermediate in the formation of the octoploid species. However, this hypothesis was rejected with a reanalysis of these data (Liston et al. 2020). Thus, although *F. vesca* has been universally accepted as a diploid ancestor in previous studies, the subgenomic composition of the octoploid strawberry is still under debate (Liston et al. 2020; Edger et al. 2020).

Understanding the phylogenetic relationships among species of *Fragaria* is critical for unraveling the diploid origins of this crop. However, the relationships within this genus remain recalcitrant to phylogenetic resolution (Liston et al. 2014), especially the position of three diploid species, *F. viridis*, *F. nilgerrensis*, and *F. iinumae*, even using sizable, multi-locus gene datasets (Qiao et al. 2016; Kamneva et al. 2017; Yang and Davis 2017). Inferring the correct species phylogeny for recently diverged lineages such as *Fragaria* is notoriously challenging because both incomplete lineage sorting (ILS) and hybridization often cause discordance between gene and species trees. Therefore, phylogenetic methods can potentially result in misleading conclusions. Moreover, hybrid species such as *F. × ananassa* specifically violate some fundamental assumptions of phylogenetic methods, so integrating these species into molecular phylogeny to determine their closeness to putative parental species may be problematic. To overcome this, spplIDer (Langdon et al. 2018) was recently developed for mapping short-read sequencing data to a composite reference genome constructed from potential progenitor species to determine their contributions to hybrid genomes. This method does not require the underlying assumptions of phylogenetic methods and can, therefore, mitigate their drawbacks. SpplIDer has been shown to have high accuracy in identifying the genomic origins of hybrid species (Langdon et al. 2018).

Here, we fully sequenced and assembled *de novo* the genomes of three wild, diploid strawberry species: *F. nilgerrensis*, *F. nubicola*, and *F. viridis*. Combined with the existing high-quality assemblies of *F. vesca* (Edger et al. 2017) and *F. iinumae* (Edger et al. 2020), we performed comparative genomic analyses that revealed a high
degree of conserved genomic composition across species and only a small number of species-specific genes. Using these five genomes, we reconstructed a phylogeny of *Fragaria* and inferred the crown age of the genus as ca. 6.37 Ma. The phylogeny exhibits high levels of gene tree discordance due to both extensive ILS and interspecific hybridization. Finally, using pplIDer, we clearly show that *F. iinumae* and *F. vesca* are the diploid progenitors of the octoploid *F. × ananassa*, whereas *F. viridis* is not a parental species. Our analyses provide new insights into the evolutionary history of *Fragaria* and resolve the origins of the commercial species.

**Results and Discussion**

**The conserved genome across the five diploid species**

We adopted PacBio (Pacific Biosystems) long-read sequencing (104–116 × coverage) and Hi-C (High-throughput Chromosome Conformation Capture) technologies to sequence and assemble chromosome-level genomes for three diploid species of *Fragaria* (*2n = 2x = 14*): *F. nilgerrensis*, *F. nubicola* and *F. viridis* (Fig. 1a; Fig. S1; Table S1). The length of the assemblies ranged from 214.6 to 272.0 Mb with N50 of contigs between 2.5 and 4.0 Mb, and 97.1–98.2% of contigs being anchored to seven pseudo-molecules (Table 1; Tables S2 and S3). We determined that the assemblies exhibited high levels of completeness and consistency through a series of assessments (Figs. S2–S4; Tables S4–S9; Supplementary Methods). Comparisons among assemblies and to the available genomes of *F. vesca* (Edger et al. 2017) and *F. iinumae* (Edger et al. 2020) revealed significant positive correlations between the proportion of transposable elements (TE) and assembly size, indicating that size variation in the diploid genomes is mainly driven by TE proliferation (Fig. 1b; Tables S3 and S9).

Using a combination of *de novo* identification, homology-based prediction, and RNA-Seq-based prediction, we identified 26,199–29,068 protein-coding genes in the newly assembled genomes (Table S10). These gene counts were similar to 28,588 in *F. vesca* (Edger et al. 2017), but slightly higher than 23,665 in *F. iinumae* (Edger et al. 2020). Further, pairwise comparisons among all five genomes revealed that 82.7–
91.4% of genes have conserved order, while rearrangements occurred for an average of 7.4% of genes (ranging from 1.4% to 13.5%) (Fig. 1a; Figs. S5 and S6; Table S1). Remarkably, *F. iinumae* has a species-specific inversion (located from 13.4 M to 32.7 M in Chr 3) that is nearly 20 Mb in length and bears over 1,000 orthologous genes (Fig. 1a; Fig. S6). Nevertheless, our results suggest highly conserved gene content and gene order across species.

**Young age of Fragaria**

The phylogenetic relationships in the genus *Fragaria* have remained controversial and unresolved. We estimated a species tree of *Fragaria* via the summary-coalescent method implemented in ASTRAL (Mirarab and Warnow 2015) using 1,476 single-copy orthologs, which we identified from the Rosaceae family. In this species tree, the phylogenetic relationships among the five diploid species of *Fragaria* were fully resolved with high support (Fig. 1c). The recovered topology of our species tree is similar to that inferred from 257 genes sequenced by target-capture in a prior study (Kamneva et al. 2017). The only difference is the position of *F. iinumae*. While *F. iinumae* was nested in a clade with *F. nubicola* in the prior study (Kamneva et al. 2017), we found it to be the first-diverging lineage among the five species.

The topology of our species tree was totally inconsistent with the phylogenetic relationships inferred from a concatenated data matrix of 276 single-copy genes from transcriptome sequencing in Qiao et al. (2016). In that study, the concatenated analysis likely resulted in incorrect phylogenetic relationships among species because phylogenetic reconstruction based on concatenated sequence data cannot account for gene tree heterogeneity (Maddison and Wiens 1997; Degnan and Rosenberg 2009). Given the prevalence of gene tree discordance in *Fragaria* (see below), our inference of a species tree using summary-coalescent methods likely represents a more robust phylogeny of the genus.

Using a well-resolved species tree representing Rosaceae, we dated the origin of the crown node of *Fragaria* to be 6.37 Ma (95% CI: 5.54–8.38 Ma; Fig. 1c). This age
is substantially older than that previously inferred with chloroplast genomic data (1.52–4.44 Ma; Njuguna et al. 2013). This difference may be because chloroplast genes evolve slowly (Wolfe et al. 1987). In addition, we used a more ancient fossil, *Prunus wutuensis* (age: Early Eocene, 55.0 Ma), to calibrate the stem node of *Prunus* (Xiang et al. 2017) compared to Njuguna et al. (2013), who used *Prunus cathybrownae* (age: late Early Eocene, 48.4 Ma; Benedic et al. 2011). While the 95% credibility interval from this study is largely overlapping with that estimated from transcriptomic data (Qiao et al. 2016), our estimate of the median age is slightly younger (i.e., compared with ca. 7.99 Ma). Nevertheless, these two dating analyses both suggest that *Fragaria* is a recently diverged lineage, and this may partly explain the conserved genomic structure across species.

**Widespread ILS and hybridization across diploid genomes**

To assess inherent conflicts between gene and species trees for *Fragaria*, we estimated both individual gene trees and a species tree based on 8,663 orthologs shared among the five available diploid genomes and the outgroup, *Potentilla micrantha* (Buti et al. 2018) (Fig. 2a). Only 5.48% of these gene trees (topo1) were consistent with the species tree, and these also coincided with the phylogenetic position of *F. iinumae* obtained using Quartet Sampling (QS) scores (Fig. 2a), albeit with weak support. The second and third most frequent topologies (topo2 and topo3, accounting for 4.62% and 2.54% of trees, respectively) show *F. iinumae* as sister to a clade of *F. nilgerrensis* and *F. nubicola*, and a clade of *F. viridis* and *F. vesca*, respectively (Fig. 2b; Table S12). Remarkably, we found genes of chromosomes (Chr) 1–4 more frequently yielded topo1, ranging 4.65% to 9.53%, while genes of Chr7 more often yielded topo2 (8.04%), and genes of Chr 5–6 more frequently resulted in topo3 (Fig. 2a, b, c; Table S12). These results not only demonstrate widespread gene tree discordance across the *Fragaria* genome but also suggest unique evolutionary histories for each chromosome.

To further dissect the cause of the phylogenetic discordance, we assessed the degree of incomplete lineage sorting (ILS) across the genus according to ASTRAL
quartet-scores (Mirarab et al. 2014). All branches except that subtending the crown node of *Fragaria* (QS2–4) have low major quartet scores (q1) of less than 0.5 (Fig. 2a), indicating high levels of ILS (Mirarab et al. 2014). Branches QS2 and QS3 received almost equal quartet scores for q1, q2 and q3 (Fig. 2a), suggesting that the gene trees yield random topologies with respect to the species tree, and levels of ILS are extremely high.

We also identified signals of hybridization by using a combination of the $D$-statistic (Durand et al. 2011), $D_{\text{FOIL}}$ (Pease et al. 2015), and PhyloNet (Wen et al. 2018) analyses. The $D$-statistic showed significant gene flow between *F. iinumae* and both *F. nilgerrensis* and *F. nubicola* at the whole genome level (Fig. 2d; Table S13), and this could explain the phylogenetic relationships of topo2, in which *F. iinumae* is sister to the clade of *F. nilgerrensis* and *F. nubicola* (Fig. 2b). Notably, we also detected numerous signals of gene flow between *F. iinumae* and both *F. viridis* and *F. vesca* based on four-taxon phylogenies constructed using genes of individual chromosomes (Fig. 2d; Table S13). Overall, the results indicate a complex pattern of gene flow among species of *Fragaria*.

We performed $D_{\text{FOIL}}$ analyses to evaluate two alternative topologies for each of the seven chromosomes of *Fragaria*. For Chr 5, 6, and 7, we found a strong signal for gene flow from *F. nilgerrensis* or the most recent common ancestors of *F. nilgerrensis* and *F. nubicola* to *F. vesca* (Fig. 2e; Table S14), and this is in agreement with the inconsistent topologies of Chr 5, 6, and 7 compared to other chromosomes. Similarly, PhyloNet identified extremely complicated and statistically significant signals for gene flow across the genus and showed that signals of introgression vary greatly among different chromosomes (Fig. 2f). Collectively, our results suggest that *Fragaria* is especially prone to hybridization. Our findings of the prevalence of both ILS and hybridization in the genus agree with those from other recently diverged lineages (Novikova et al. 2016; Liu et al. 2018; Wu et al. 2018) and highlight the roles of both of these mechanisms in shaping genomic and species evolution.

**Tracing the diploid ancestors of the cultivated strawberry**
Widespread ILS and hybridization across genomes in *Fragaria* make it difficult to trace the diploid origins of the octoploid strawberry. Previous work based on phylogenetic approaches has led to conflicting hypotheses (Rousseau-Gueutin et al. 2009; Tennessen et al. 2014; Sargent et al. 2016; Kamneva et al. 2017; Yang and Davis 2017; Edger et al. 2019; Edger et al. 2020; Liston et al. 2020). While *F. vesca* and *F. iinumae* have been commonly proposed as ancestors, which/whether additional subgenomes contributed to the octoploid genome remains under debate (Edger et al. 2019; Edger et al. 2020; Liston et al. 2020). Specifically, *F. viridis* and *F. nipponica*, were identified as two additional, putative ancestors based on a tree-searching algorithm (Edger et al. 2019). However, reanalysis of the same datasets using a chromosome-scale phylogenomic approach led Liston et al. (2020) to argue that unsampled or an extinct populations of *F. iinumae* comprise the progenitors of *F. × ananassa*.

To avoid the drawbacks arising from phylogenetic approaches, we applied a novel alignment-based approach, sppIDer (Langdon et al. 2018), which directly maps short-read sequence data to a composite reference genome constructed from potential progenitors to determine their contributions to hybrid genomes. Using this method, we mapped sequence data from 73 genomes of the octoploid strawberry, *F. × ananassa* (Table S15), to a composite of five diploid genomes of *Fragaria*. We found similar trends for each of the 73 octoploid genomes; namely that nearly one third of reads were mapped to *F. vesca* and *F. iinumae*, while only approximately 12%, 11%, and 11% of reads were mapped to *F. viridis*, *F. nilgerrensis*, and *F. nubicola*, respectively (Fig. 3a). Furthermore, we found that mapped reads of a representative genome of *F. × ananassa* across the composite genome was relatively consistent in coverage depth, with only a few loci containing aberrant coverage that could be consistent with introgression (Fig. 3b; Fig. S7). These results suggest that *F. vesca* and *F. iinumae*, rather than *F. viridis*, *F. nilgerrensis*, and *F. nubicola*, are the diploid progenitors of the octoploid commercial strawberry. This finding is in agreement with the results of Liston et al. (2020) but rejects the hypothesis of Edger et al. (2019, 2020), who found that *F. viridis* represented one of the four subgenomes of the cultivated strawberry.
However, at present, we cannot entirely rule out genomic contributions from other diploid species to the cultivated strawberry, and high quality genomes from additional diploid *Fragaria* are needed to fully confirm our hypothesis.

We further compared proteins from the cultivated octoploid strawberry to those in the five diploid species of *Fragaria*. We found that *F. iinumae* and *F. vesca* have only 1,726 and 2,383 genes, respectively, that have no orthologs in *F. × ananassa*, while the other three diploid species have a much larger number of genes absent from the commercial strawberry (3,929–4,466; Fig. 3c; Fig. S8). These proteomic data provide additional evidence that *F. iinumae* and *F. vesca*, not *F. viridis*, *F. nubicola* and/or *F. nilgerrensis*, are the diploid progenitors of *F. × ananassa*.

Notably, genes that exist in diploid species of *Fragaria* but are absent from cultivated strawberries largely comprise transcription factors (TFs), resistance (R) genes, protein kinases (PKs), and genes related to flowering time (such as FT) and fruit quality, including color, taste, texture, and aroma (Tables S16 and S17). Therefore, the diploid genomes of *Fragaria* provide an extremely valuable resource for identifying genes and alleles for potential genetic improvements to commercial strawberries.

**Materials and Methods**

**Genome sequencing, assembly and annotation**

For genome sequencing, we collected the fresh leaves and stolons from one mature individual each of *F. nilgerrensis*, *F. nubicola*, and *F. viridis* in Hubei (111°94'E, 31°76’N), Xizang (88°91'E, 27°71’N), and Xinjiang (83°42'E, 43°22’N) provinces of China, respectively. These germplasm accessions were maintained in the China National Germplasm Repository for Peach and Strawberry (Nanjing) at the Jiangsu Academy of Agricultural Sciences (Nanjing, China).

We assembled the three *Fragaria* genomes according to PacBio SMRT sequencing and Hi-C technology, followed by screened the repetitive sequences and predicted the protein-coding gene structure. Further we annotated the gene functions of the three *Fragaria* assemblies according to a serious of public databases and
identified tandemly repeated gene arrays using TD_identification (Feng et al. 2020).

**Syntenic analysis among five Fragaria species**

We identified syntenic blocks and generated dot plots for all pairs of the five diploid Fragaria species in MCScan (https://github.com/tanghaibao/jcvi/wiki/). Further, we displayed the links of the blocks with CIRCOS (Darzentas 2010).

**Orthogroup clustering, species tree construction and divergence time estimation**

We used OrthoFinder (Emms and Kelly 2015) to classify the proteins from the five diploid species of Fragaria and six other sequenced Rosales plants, including Potentilla micrantha (Buti et al. 2018), Rosa chinensis (Raymond et al. 2018), Rubus occidentalis (VanBuren et al. 2018), Malus domestica (Daccord et al. 2017), Prunus persica (Verde et al. 2013), and Morus notabilis (He et al. 2013).

We selected proteins of single-copy orthogroups present in M. notabilis and in at least 70% of the other ten plants, followed by aligned these proteins (MAFFT; Katoh and Standley 2013), performed CDS conversion (PAL2NAL; Suyama et al. 2006), applied them to reconstructing gene trees (IQ-TREE; Nguyen et al. 2015), and then obtained a species tree representing Rosaceae using ASTRAL (Mirarab and Warnow 2015). We inferred divergence times in r8s (Sanderson 2003), with two fossil and one secondary age calibrations.

**Discordance assessment and gene flow analyses**

We followed Yang and Smith (2014) to infer orthologs shared among the five diploid Fragaria species and P. micrantha. Then we produced a cloudogram with DensiTree (Bouckaert 2010) and evaluated discordance between gene trees and species tree across Fragaria using ASTRAL (Mirarab and Warnow 2015) and the Quartet Sampling method (Pease et al. 2018). We also classified the gene trees into different topologies and determined the frequency of each topology at the whole genome- and chromosomal-levels. In addition, we detected the signals for introgression across Fragaria for the whole genome and each chromosome using a
combination of $D$-statistics (Durand et al. 2011), $D_{FOIL}$ (Pease et al. 2015), and PhyloNet (Wen et al. 2018) analyses.

**Tracing the diploid ancestors of the cultivated strawberry**

We downloaded Illumina resequencing data of 73 cultivated octoploid strawberries ($F \times ananassa$) from the NCBI (BioProject accession No. PRJNA578384). We mapped short reads from each sample to the composite reference of the five diploid Fragaria genomes in sppIDer (Langdon et al. 2018) and calculated the percentage of read mappings to each of the five genomes.

We applied OrthoFinder (Emms and Kelly 2015) to classify the proteins from the five diploid species of Fragaria and the cultivated octoploid species. Then we compared and annotated the genes that exist in diploid Fragaria but lack homologs in the cultivated strawberry.

Detailed methods are included in the Supplementary Methods.

**Data availability**

Illumina sequences and PacBio reads from this study have been deposited in GenBank under BioProject PRJNA634576, and the genome assemblies and annotations of the three newly sequenced species of Fragaria have been submitted to the Genome Database for Rosaceae (GDR; www.rosaceae.org).

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**References**

Benedict JC, DeVore ML, Pigg KB. 2011. *Prunus and Oemleria* (Rosaceae) flowers from the late early Eocene Republic flora of northeastern Washington State, USA. Int. J. Plant Sci. 172: 948–958
Bouckaert RR. 2010. DensiTree: making sense of sets of phylogenetic trees. Bioinformatics 26: 1372-1373.

Bringhurst RS. 1990. Cytogenetics and evolution in American Fragaria. HortScience 25: 879–881.

Buti M, Moretto M, Barghini E, Mascagni F, Natali L, Brilli M, Lomsadze A, Sonigo P, Giongo L, Alonge M, et al. 2018. The genome sequence and transcriptome of Potentilla micrantha and their comparison to Fragaria vesca (the woodland strawberry). GigaScience 7, giy010.

Chambers A, Carle S, Njuguna W, Chamala S, Bassil NV, Whitaker VM, Barbazuk W, Folta KM. 2013. A genome-enabled, high-throughput, and multiplexed fingerprinting platform for strawberry (Fragaria L.). Mol Breed. 31: 615–629.

Daccord N, Celton JM, Linsmith G, Becker C, Choisne N, Schijlen E, van de Geest H, Bianco L, Micheletti D, Velasco R, et al. 2017. High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development. Nat Genet. 49: 1099–1106.

Darrow GM. 1966. The strawberry history, breeding and physiology. Holt, Rinehart and Winston, New York.

Darzentas N. 2010. Circoletto: visualizing sequence similarity with Circos. Bioinformatics 26: 2620–2621.

Degnan JH, Rosenberg NA. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol Evol. 24: 332–340.

DiMeglio LM, Staudt G, Yu H, Davis TM. 2014. A phylogenetic analysis of the genus Fragaria (strawberry) using intron-containing sequence from the ADH-1 gene. PLoS One 9: e102237.

Durand EY, Patterson N, Reich D, Slatkin M. 2011. Testing for ancient admixture between closely related populations. Mol Biol Evol. 28: 2239–2252.

Edger PP, McKain MR, Yocca AE, Knapp SJ, Qiao Q, and Zhang T. 2020. Reply to: Revisiting the origin of octoploid strawberry. Nat Genet. 52: 5–7.

Edger PP, Poorten TJ, VanBuren R, Hardigan MA, Colle M, McKain MR, Smith RD, Teresi SJ, Nelson ADL, Wai CM, et al. 2019. Origin and evolution of the
octoploid strawberry genome. Nat Genet. 51: 541–547.

Edger PP, VanBuren R, Colle M, Poorten TJ, Wai CM, Niederhuth CE, Alger EI, Ou S, Acharya CB, Wang J, et al. 2017. Single-molecule sequencing and optical mapping yields an improved genome of woodland strawberry (*Fragaria vesca*) with chromosome-scale contiguity. GigaScience 7, gix124.

Emms DM, Kelly S. 2015. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biol. 16: 157.

FAO. 2020. Food and Agriculture Organization of the United Nations. FAOSTAT. Available at: [http://www.fao.org/faostat/en/#data/QC accessed March 2020](http://www.fao.org/faostat/en/#data/QC accessed March 2020)

Fedorova NJ. 1946. Crossability and phylogenetic relations in the main European species of *Fragaria*. Compte Rend (Doklady) Acad Sci USSR. 52: 545–547.

Feng C, Wang J, Wu L, Kong H, Yang L, Feng C, Wang K, Rausher M, Kang M. 2020. The genome of a cave plant, *Primulina huaijiensis*, provides insights into adaptation to limestone karst habitats. New Phytol. 227: 1249–1263.

Folta KM, Davis TM. 2006. Strawberry genes and genomics. Crit Rev Plant Sci. 25: 399–415.

Giampieri F, Tulipani S, Alvarez-Suarez JM, Quiles JL, Mezzetti B, Battino M. 2012. The strawberry: Composition, nutritional quality, and impact on human health. Nutrition 28: 9–19.

Hancock JF. 1999. Strawberries. CAB International, Wallingford, UK.

He NJ, Zhang C, Qi XW, Zhao SC, Tao Y, Yang GJ, Lee TH, Wang XY, Cai QL, Li D, et al. 2013. Draft genome sequence of the mulberry tree *Morus notabilis*. Nat Commun. 4: 2445.

Hummer KE, Hancock J. 2009. Strawberry genomics: Botanical history, cultivation, traditional breeding, and new technologies. In: K. Folta and S. Gardiner, editors, Genetics and genomics of Rosaceae. Springer, New York.

Kamneva OK, Syring J, Liston A, Rosenberg NA. 2017. Evaluating allopolyploid origins in strawberries (*Fragaria*) using haplotypes generated from target capture sequencing. BMC Evol Biol. 17: 180.
Katoh K, Standley DM. 2013. MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30: 772–780.

Langdon QK, Peris D, Kyle B, Hittinger CT. 2018. sppIDer: a species identification tool to investigate hybrid genomes with high-throughput sequencing. Mol Biol Evol. 35: 2835–2849.

Lei JJ, Xue L, Guo RX, Dai HP. 2017. The *Fragaria* species native to China and their geographical distribution. Acta Hortic. 1156: 37–46.

Liston A, Cronn R, Ashman T-L. 2014. *Fragaria*: a genus with deep historical roots and ripe for evolutionary and ecological insights. Am J Bot. 101: 1686–1699.

Liston A, Wei N, Tennessen JA, Li J, Dong M, Ashman TL. 2020. Revisiting the origin of octoploid strawberry. Nat Genet. 52: 2–4.

Liu YP, Ren ZM, Harris AJ, Peterson PM, Wen J, Su X. 2018. Phylogeography of *Orinus* (Poaceae), a dominant grass genus on the Qinghai-Tibet Plateau. Bot J Linn Soc.186: 202–223.

Maddison WP, Wiens JJ. 1997. Gene trees in species trees. Syst Biol. 46: 523–536.

Mirarab S, Reaz R, Bayzid MS, Zimmermann T, Swenson MS, Warnow T. 2014. ASTRAL: genome-scale oalescent-based species tree estimation. Bioinformatics 30: i541–i548.

Mirarab S, Warnow T. 2015. ASTRAL-II: coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. Bioinformatics 31: 44–52.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32: 772–780.

Njuguna W, Liston A, Cronn R, Ashman TL, Bassil N. 2013. Insights into phylogeny, sex function and age of *Fragaria* based on whole chloroplast genome sequencing. Mol Phylogenet Evol. 66: 17–29.

Novikova PY, Hohmann N, Nizhynska V, Tsuchimatsu T, Ali J, Muir G, Guggisberg A, Paape T, Schmid K, Fedorenko OM, et al. 2016. Sequencing of the genus *Arabidopsis* identifies a complex history of nonbifurcating speciation and abundant trans-specific polymorphism. Nat Genet. 48: 1077–1082.
Pease JB, Hahn MW. 2015. Detection and polarization of introgression in a five-taxon phylogeny. Syst Biol. 64: 651–662.

Qiao Q, Xue L, Wang Q, Sun H, Zhong Y, Huang J, Lei J, Zhang T. 2016. Comparative transcriptomics of strawberries (Fragaria spp.) provides insights into evolutionary patterns. Front Plant Sci. 7: 1839.

Raymond O, Gouzy J, Just J, Badouin H, Verdenaud M, Lemainque A, Vergne P, Moja S, Choisne N, Pont C, et al. 2018. The Rosa genome provides new insights into the domestication of modern roses. Nat Genet. 50: 772–777.

Rousseau-Gueutin MA, Gaston A, Aïnouche A, Aïnouche ML, Olbricht K, Staudt G, Richard L, Denoyes-Rothan B. 2009. Tracking the evolutionary history of polyploidy in Fragaria L. (strawberry): new insights from phylogenetic analyses of low-copy nuclear genes. Mol Phylogenet Evol. 51: 515–530.

Sanderson MJ. 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19: 662–684.

Sargent DJ, Yang Y, Surbanovski N, Bianco L, Buti M, Velasco R, Giongo L, Davis TM. 2016. HaploSNP affinities and linkage map positions illuminate subgenome composition in the octoploid, cultivated strawberry (Fragaria × ananassa). Plant Sci. 242: 140–150.

Senanayake YDA, Bringhurst RS. 1967. Origin of Fragaria polyploids. I. Cytological analysis. Am J Bot. 54: 221–228.

Suyama M, Torrents D, Bork P. 2006. PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. Nucleic Acids Res. 34: W609–W612.

Tennessen JA, Govindaraju R, Ashman T-L, Liston A. 2014. Evolutionary origins and dynamics of octoploid strawberry subgenomes revealed by dense targeted capture linkage maps. Genome Biol Evol. 6: 3295–313.

VanBuren, R., Wai, C.M., Colle, M., Wang, J., Sullivan, S., Bushakra, J.M., Liachko, I., Vining, K.J., Dossett, M., Finn, C.E., et al. 2018. A near complete, chromosome-scale assembly of the black raspberry (Rubus occidentalis) genome. GigaScience
17: giy094.

Verde I, Abbott AG, Scalabrin S, Jung S, Shu S, Marroni F, Zhebentyayeva T, Dettori MT, Grimwood J, Cattonaro F, et al. 2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nat Genet. 45: 487–494.

Wen D, Yu Y, Zhu J, Nakhleh L. 2018. Inferring phylogenetic networks using PhyloNet, Syst Biol. 67: 735-740.

Wolfe KH, Li WH, Sharp PM. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast and nuclear DNA. Proc Natl Acad Sci USA. 84: 9054–9058.

Wu M, Kostyun JL, Hahn MW, Moyle LC. 2018. Dissecting the basis of novel trait evolution in a radiation with widespread phylogenetic discordance. Mol Ecol. 27: 3301–3316

Xiang Y, Huang CH, Hu Y, Wen J, Li S, Yi T, Chen H, Xiang J, Ma H. 2017. Evolution of Rosaceae fruit types based on nuclear phylogeny in the context of geological times and genome duplication. Mol Biol Evol. 34: 262-281.

Yang Y, Davis TM. 2017. A new perspective on polyploid *Fragaria* (strawberry) genome composition based on large-scale, multi-locus phylogenetic analysis. Genome Biol Evol. 9: 3433–3448.

Yang Y, Smith SA. 2014. Orthology inference in nonmodel organisms using transcriptomes and low-coverage genomes: improving accuracy and matrix occupancy for phylogenomics. Mol Biol Evol. 31: 3081–3092.
Supporting Information

Fig. S1. Kmer frequency distribution for three diploid species of *Fragaria*. Kmer length was set as 17.

Fig. S2. Heat map of chromosome interaction of *Fragaria nilgerrensis*.

Fig. S3. Heat map of chromosome interaction of *Fragaria nubicola*.

Fig. S4. Heat map of chromosome interaction of *Fragaria viridis*.

Fig. S5. Circos plots showing syntenic blocks for each of seven chromosomes. Colored links indicate regions containing over 100 genes that are consistent in gene order (a, Chr1, red; b, Chr2, orange; c, Chr3, yellow; d, Chr4, green; e, Chr5, cyan; f, Chr6, blue; g, Chr7, purple), while grey links represent rearrangement in regions bearing at least 100 genes.

Fig. S6. Dot plot graph showing syntenic blocks for comparisons of five species of *Fragaria*. a, *F. iinumae* vs *F. vesca*; b, *F. iinumae* vs *F. nilgerrensis*; c, *F. iinumae* vs *F. viridis*; d, *F. iinumae* vs *F. nubicola*; e, *F. vesca* vs *F. nilgerrensis*; f, *F. vesca* vs *F. viridis*; g, *F. vesca* vs *F. nubicola*; h, *F. nilgerrensis* vs *F. viridis*; i, *F. nilgerrensis* vs *F. nubicola*; j, *F. viridis* vs *F. nubicola*.

Fig. S7. Visual example of depth of mapped reads of a representative octoploid strawberry, FL_1 3C026p134 (Accession No.: SRR10312160 & SRR10312161) to a composite reference genome of five diploid species of *Fragaria*.

Fig. S8. Intersection diagram showing shared orthologous groups among cultivated octoploid strawberry and five diploid species of *Fragaria*. The pink columns indicate the groups that are absent in cultivated strawberry but present in diploid species.

Table S1. Statistics for genome sequencing data of three diploid species of *Fragaria*.

Table S2. Statistics for genome assemblies of the three diploid species of *Fragaria*.

Table S3. Comparisons of the lengths of pseudo-molecules in the genomic assemblies of five diploid species of *Fragaria*.

Table S4. Evaluation of the genome assemblies of the three diploid species of *Fragaria* using remapping of reads from short-insert libraries.

Table S5. Statistics from the RNA-Seq data representing different tissues and
developmental stages of strawberry.

**Table S6.** Evaluation of the genomic assemblies of the three diploid species of *Fragaria* using EST data.

**Table S7.** Evaluation of the genomic assemblies of the three diploid species of *Fragaria* using core Eukaryotic genes mapping approach (CEGMA).

**Table S8.** Evaluation of the genomic assemblies of the twelve species of Rosales using Benchmarking Universal Single-Copy Orthologs (BUSCO).

**Table S9.** Comparisons of transposable elements among the genomes of the five diploid species of *Fragaria*.

**Table S10.** Summary of gene structure of the genomes of the three diploid species of *Fragaria*.

**Table S11.** The proportion of genes with conserved gene order and rearrangements among ten pairwise comparisons of genomes of *Fragaria*.

**Table S12.** Topology weighting with each sample by pseudo-molecules for all the possible topologies from six taxa.

**Table S13.** Results of all possible D-statistic tests for topologies resulting from each pseudo-molecule for *Fragaria*.

**Table S14.** Results of $D_{\text{FOIL}}$ for two alternative phylogenetic topologies based on the pseudo-molecules for five species of *Fragaria*.

**Table S15.** Identities and percentage of reads from 73 *F. × ananassa* varieries (downloaded from NCBI PRJNA578384) to five diploid species of *Fragaria*.

**Table S16.** Summary of annotations of protein-coding genes in the genomes of the three newly sequenced diploid species of *Fragaria*.

**Table S17.** Functional categories for the genes detected in five diploid species of *Fragaria* that are absent in cultivated octoploid strawberry.

**Methods S1.** Supplemental Methods
Figure legends

Fig. 1 Features of five diploid *Fragaria* genomes and evolution across Rosaceae.

a, Multidimensional topography for *F. nilgerrensis* (*Fnil*), *F. nubicola* (*Fnub*), *F. viridis* (*Fvir*), *F. vesca* (*Fves*) and *F. iinumae* (*Fiin*) genomes. Circos plots as concentric circles from outermost to innermost show TE percentage (dark grey columns), gene density (Brown columns), density of tandem duplicates (red-brown columns), and inverted regions detected among the assemblies of the five genomes (colorful links). Details of syntenic blocks are provided in Fig. S5 and Fig. S6. Each column represents a 250-kb non-overlapping window, and every link contains at least 100 genes. b, Scatter plot and regression showing significant positive correlations between TE proportion and assembly size. c, Phylogenetic tree showing the topology, divergence time, and expansions/contractions of orthologous groups for ten species of Rosaceae. The species tree was constructed by ASTRAL using 1,476 single copy orthologous groups with *Morus notabilis* as the outgroup. The divergence time was estimated by r8s according to three node age calibrations (blue circles). Blue bars indicate the 95% confidence intervals (CI) of divergence times. Red and green numbers along the branches show expanded and contracted orthologous groups, respectively.

Fig. 2 Discordance between gene and species trees, incomplete lineage sorting (ILS), and gene flow in *Fragaria*. 
a, Cloudogram of gene trees constructed from 8,663 single copy genes. The topology of the species tree is displayed in bold black, while gene trees are plotted in grey. The bold black numbers along the branches indicate the percentage of gene trees that contain each of the subtrees of the species tree. The numbers in brackets indicate the topological weight, or the percentage of gene trees that are consistent with the species tree (i.e., topo 1). The brown and grey numbers in the table represent the Quartet Sampling (QS) scores and quartet scores from ASTRAL, respectively. b, Nine alternative topologies (topos 2–10) for the six sampled taxa, sorted by frequency of occurrence, as shown in brackets. c, Weights for the top ten possible topologies among the whole genome and each chromosome. The
black line represents topo 1, while the colored lines represent the corresponding topologies in Fig. 2b. **d,** Signals of gene flow in *Fragaria* for the whole genome and each chromosome inferred by the analysis using the $D$-statistic. Columns with different colors indicate the $D$-value for the corresponding four-taxon phylogeny with Z value higher than 3.0. Negative and positive $D$-values represent the strength of gene flow between P1 and P3 and between P2 and P3. The results of the $D$-statistic analysis for all combinations of a four-taxon phylogeny are shown in Table S13. **e,** Signals of gene flow in *Fragaria* inferred by $D_{FOIL}$ analyses. Details are provided in Table S14. **f,** Signals of gene flow in *Fragaria* among the whole genome and each chromosome inferred by Bayesian MCMC posterior estimation in PhyloNet. Olive, green, dark red, and blue lines indicate the optimal networks of hybrid nodes H1–4, respectively. Colored numbers next to the lines indicate inheritance probabilities for corresponding edges.

**Fig. 3** **Tracing the diploid ancestors of the cultivated octoploid strawberry. a,** Comparison of the percentage of reads from 73 octoploid strawberries, *F. × ananassa* genotype resequencing data (downloaded from NCBI PRJNA578384), mapped to the five diploid species of *Fragaria. b,** visual example of depth of mapped reads of a representative octoploid strawberry, FL_13C026p134 (Accession No.: SRR10312160 & SRR10312161), across the composite genomes of putative diploid progenitors. Details are provided in Fig. 8. **c,** Comparison of the number of genes that exist in diploid species of *Fragaria* but lack orthologs in *F. × ananassa.* The orange regions indicate the number of species-specific genes in five diploid species of *Fragaria,* while the blue regions represent the number of genes belonging to the orthologous groups shared in two or more diploid species of *Fragaria* but absent in cultivated strawberry.
Table 1. Summary statistics from the assembly and annotation of three diploid species of *Fragaria*.

| Assembly feature                        | F. nilgerrensis | F. nubicola | F. viridis |
|-----------------------------------------|----------------|-------------|------------|
| Genome-sequencing depth (×)             | 373            | 406         | 473        |
| Estimated genome size (Mb)              | 279            | 273         | 219        |
| Total length of scaffolds (Mb)          | 272.0          | 247.5       | 214.9      |
| N50 of scaffolds (Mb)                   | 37.5           | 35.0        | 29.2       |
| Total length of contigs (Mb)            | 271.9          | 247.2       | 214.6      |
| N50 of contigs (Mb)                     | 4.0            | 2.6         | 3.5        |
| Mapping rate of reads from short-insert libraries | 96.3% | 90.0% | 94.3% |
| CEGMA evaluation                        | 97.2%          | 92.3%       | 94.8%      |
| BUSCO evaluation                        | 93.7%          | 87.5%       | 88.5%      |
| LAI evaluation                          | 10.2           | 17.5        | 16.7       |
| EST evaluation                          | 92.5%          | 92.4%       | 93.2%      |
| RNA-Seq evaluation                      | 88.6–93.4%     | 81.0–84.4%  | 82.7–86.6% |

**Genome annotation**

| Percentage of TE                          | 43.60         | 43.07       | 38.67       |
| Percentage of LTRs                        | 35.29         | 32.87       | 26.92       |
| No. of predicted protein-coding genes     | 29,068        | 27,594      | 26,199      |
| No. of genes annotated to public database | 26,353        | 25,418      | 24,370      |
a

b

| Assembly size (Mb) | TE (% in assembly) |
|-------------------|--------------------|
| 210 - 220         | 36                 |
| 220 - 230         | 39                 |
| 230 - 240         | 42                 |
| 240 - 250         | 45                 |
| 250 - 260         |                    |
| 260 - 270         |                    |
| 270 - 280         |                    |

R^2 = 0.9333

Fragaria nilgerrensis
Fragaria nubicola
Fragaria iinumae
Fragaria vesca
Fragaria viridis
Rosales
Rosaceae
Rospideae
Potentilleae
Prunus persica
Malus domestica
Rubus occidentalis
Fragaria vesca
Fragaria nilgerrensis
Fragaria iinumae
Fragaria nubicola

Calibration points
Expansion/contraction Orthogroups

95% CI

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