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Insights into angiosperm evolution, floral development and chemical biosynthesis from the Aristolochia fimbriata genome

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Aristolochia, a genus in the magnoliid order Piperales, has been famous for centuries for its highly specialized flowers and wide medicinal applications. Here, we present a new, high-quality genome sequence of Aristolochia fimbriata, a species that, similar to Amborella trichopoda, lacks further whole-genome duplications since the origin of extant angiosperms. As such, the A. fimbriata genome is an excellent reference for inferences of angiosperm genome evolution, enabling detection of two novel whole-genome duplications in Piperales and dating of previously reported whole-genome duplications in other magnoliids. Genomic comparisons between A. fimbriata and other angiosperms facilitated the identification of ancient genomic rearrangements suggesting the placement of magnoliids as sister to monocots, whereas phylogenetic inferences based on sequence data we compiled yielded ambiguous relationships. By identifying associated homologues and investigating their evolutionary histories and expression patterns, we revealed highly conserved floral developmental genes and their distinct downstream regulatory network that may contribute to the complex flower morphology in A. fimbriata. Finally, we elucidated the genetic basis underlying the biosynthesis of terpenoids and aristolochic acids in A. fimbriata.

Angiosperms, or flowering plants, are by far the largest group of land plants and comprise more than 350,000 living species (http://www.theplantlist.org/). Among extant angiosperms, Amborellales, Nymphaeales and Austrobaileyales (the so-called ANA grade) are followed by the rapid diversification of the remaining angiosperms or mesangiosperms1,2. The major mesangiosperm lineages are the eudicot, monocot and magnoliid clades, which make up approximately 75, 22 and 3% of angiosperm species diversity, respectively, and are the product of an ancient, rapid radiation1,3. Despite the availability of numerous sequenced nuclear genomes from eudicots and monocots, as well as the recently sequenced genome of Amborella trichopoda, lacks further whole-genome duplications since the origin of extant angiosperms. As such, the A. fimbriata genome is an excellent reference for inferences of angiosperm genome evolution, enabling detection of two novel whole-genome duplications in Piperales and dating of previously reported whole-genome duplications in other magnoliids. Genomic comparisons between A. fimbriata and other angiosperms facilitated the identification of ancient genomic rearrangements suggesting the placement of magnoliids as sister to monocots, whereas phylogenetic inferences based on sequence data we compiled yielded ambiguous relationships. By identifying associated homologues and investigating their evolutionary histories and expression patterns, we revealed highly conserved floral developmental genes and their distinct downstream regulatory network that may contribute to the complex flower morphology in A. fimbriata. Finally, we elucidated the genetic basis underlying the biosynthesis of terpenoids and aristolochic acids in A. fimbriata.

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simply (Amborellaceae; hereafter Amborella trichopoda) be the case is 32. The absence of WGDs and subsequent subgenome rearrangement make Aristolochia an exceptionally powerful evolutionary genomic resource that we use to improve understanding of WGDs in magnoliids and early angiosperm diversification and to decipher molecular developmental genetics underlying both flower development and natural products (terpenoids and AAs) biosynthesis.

Results
High-quality genome assembly and annotation of A. fimbriata.
The genome of A. fimbriata was sequenced and assembled using Oxford Nanopore Technologies, Bionano optical mapping and Hi-C sequencing (Fig. 1b). The final nuclear genome assembly is about 258 megabases (Mb) and consists of 283 scaffolds with an N50 of 12.9 Mb (Supplementary Tables 1.6 and 1.7). The assembled genome size is similar to the estimated genome size based on flow cytometry and k-mer analyses (Extended Data Fig. 2). Using the Hi-C contact information, these scaffolds were further anchored onto seven pseudochromosomes, which cover ~95% of the assembled sequences (Supplementary Note 1.3 and Supplementary Fig. 1.3).

Probably due to propagation via selfing over ~20 yr in cultivation, the sequenced A. fimbriata accession has extremely low heterozygosity (~0.07%) simplifying genome assembly (Fig. 1). The overall read-mapping rates for transcriptomes (for example, those from illumina reads, bionano data, nanopore contig, polished contig, nanopore raw data, juicer and 3d-dna, miniasm racon, pilon) make an exceptionally powerful evolutionarily and small genome size (~0.87 pg 2C value). Our most striking finding is that, unlike nearly all other ~200 angiosperm genomes sequenced to date, A. fimbriata has not undergone any whole-genome duplications (WGDs) beyond the ancestral WGD that predated diversification of all living angiosperm lineages31. The only other angiosperm for which this is known to be the case is Amborella trichopoda (Amborellaceae; hereafter simply Amborella), the sister to all other living angiosperms32. The absences of WGDs and subsequent subgenome rearrangement make Aristolochia an exceptionally powerful evolutionary genomic resource that we use to improve understanding of WGDs in magnoliids and early angiosperm diversification and to decipher molecular developmental genetics underlying both flower development and natural products (terpenoids and AAs) biosynthesis.

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leaves, flowers, roots and seedlings with and without stress treatments) and for genomic sequences exceeded 93 and 99%, respectively (Supplementary Tables 1.9 and 1.10). Moreover, 96.8% of the Plantae BUSCO (Benchmarking Universal Single-Copy Orthologs) genes were identified in the genome (Supplementary Table 1.11). The long terminal repeat (LTR) Assembly Index (LAI) of the genome assembly is ~21 (Fig. 1c and Extended Data Fig. 3b,d).

These results, as well as those from other genome quality assessments (Supplementary Note 1.4 and Extended Data Fig. 3), suggest that the *A. fimbriata* genome assembly is of high quality.

We annotated 21,751 protein-coding gene models from the *A. fimbriata* genome, 19,582 of which were classified as high-confidence genes on the basis of whether they have support from the aforementioned transcriptomes and whether they exhibit overlapping with TEs (Supplementary Note 2). Gene family classification and comparison showed that most of the commonly shared orthogroups comprise annotated *A. fimbriata* genes and that *A. fimbriata* has fewer species-specific orthogroups than many other flowering plants (Supplementary Fig. 2.3). Transposable elements (TEs) occupy ~52.1% of the *A. fimbriata* genome and the LTR retrotransposons represent 38.2% of the assembly (Supplementary Table 2.2). Ty3/Gypsy elements account for 21.3%, while the Ty1/Copia elements cover 4.6% of the genome (Supplementary Table 2.2). DNA transposons MULE-MuDR and CMC-EnSpm are enriched in centromeric regions but are absent from the rest of the genome (Fig. 1d). Notably, and clearly distinct from reports for the other published magnolid genomes, LINE/L1 elements have expanded substantially in *A. fimbriata*; these elements tend to be located outside of the centromeric regions and are especially evident in genic regions (Fig. 1d and Supplementary Fig. 2.1b,c). We also observed an elevation in the expression levels of genes with the insertion of LINE/L1 elements in the intron regions as compared to the much larger set of genes lacking such insertions (Supplementary Fig. 2.1d).

A genome sequence free of lineage-specific WGD. WGDs have occurred frequently throughout the evolutionary history of angiosperms and a genome sequence lacking lineage-specific WGD could facilitate the studies of genome evolution and inference of the WGD history in other species. Until now, only *Amborella* is known to lack any lineage-specific WGD; it only possesses evidence for a WGD that occurred in an ancestor of all extant flowering plants. It is therefore noteworthy that an intragenomic comparison of the genome of *A. fimbriata* revealed very sparse self-synteny blocks, indicating absence of any recent WGDs in *A. fimbriata* (Supplementary Fig. 3.1). We further conducted intergenomic comparisons against *Amborella* and also against a water lily (*Nymphaea colorata*) that has one lineage-specific WGD. The corresponding syntenic depth ratios are 1:1 and 1:2 (Fig. 2a and Supplementary Figs. 3.2 and 3.3), respectively, which strongly support the lack of further WGD in *A. fimbriata* since the earliest diversification of extant angiosperm lineages (Supplementary Note 3.1). Notably, *A. fimbriata* is thus only the second flowering plant species with a sequenced genome that has a genomic evolutionary history that is similar to that of *Amborella* in having no additional lineage-specific WGD.

Comparing the genomes of *Amborella* and *A. fimbriata*, we identified 450 intergenic syntenic blocks comprising 6,378 anchor genes in each genome, of which ten syntenic blocks have >50 anchor gene pairs (Supplementary Table 3.1). The longest syntenic block, which is between *A. fimbriata* chromosome 3 and *Amborella* chromosome 4, has 77 anchor gene pairs, suggestive of high conservation (Fig. 2a, Supplementary Fig. 3.2a and Supplementary Table 3.1). In contrast, we only detected three syntenic regions with >50 anchor gene pairs between *A. fimbriata* and *N. colorata* (Supplementary Table 3.1), which suggests extensive chromosomal rearrangements in *Nymphaea*, perhaps following WGD. These results suggest that the *A. fimbriata* genome could serve as another exceptional reference for evolutionary genomic studies of angiosperms.

Using the *A. fimbriata* genome as a reference, we were able to identify new WGDs in Piperales and clarify the timing of the previously proposed WGDs in Laurales and Magnoliales. By comparing the genome of *A. fimbriata* with that of black pepper (*Piper nigrum*; Piperales), we found one-to-eight well-preserved intergenomic syntenic blocks, suggesting three successive rounds of lineage-specific WGDs in black pepper (Fig. 2b,c and Supplementary Fig. 3.4). Further synonymous substitutions per site (Ks) analyses of the anchor gene pairs in the self-synteny blocks of black pepper also provide estimates of these same three duplication events (Ks peaks around 0.11, 0.69 and 0.91; we named them Pn-α, Pn-β and Pn-γ, respectively), all of which occurred after the divergence of black pepper and *A. fimbriata* (Supplementary Figs. 3.5 and 3.6). However, only the most recent lineage-specific WGD (Pn-α) was reported in the previous analysis of the black pepper genome.

In addition, we identified a 1:2 syntenic depth ratio between *A. fimbriata* and *Liriodendron chinense* (Magnoliaceae) and a 1:4 ratio between *A. fimbriata* and *Cinnamomum kanehira* (Lauraceae) (Fig. 2d and Supplementary Figs. 3.7 and 3.8), thereby confirming the previously reported single WGD in *L. chinense* and two rounds of WGD in *C. kanehira* since the divergence of magnolids. Ks-based analyses could possibly verify these WGDs; however, owing to the variable evolutionary rates of different species, it is hard to confidently conclude whether any of the WGDs were shared among magnolid species. Using integrated phylogenomic and synteny analyses, we found that, of the two WGDs identified in *C. kanehira*, the more ancient one was shared with *L. chinense* whereas the recent one was shared with *Persea americana* (Lauraceae) (Supplementary Note 3.3).

Structural variation and angiosperm phylogeny. Recently, several other genome sequencing and phylogenomic studies have proposed discordant phylogenetic relationships among the mesangiosperm clades of eudicots, monocots and magnolids, which is probably due in part to the different and sparse taxon sampling used, rapid diversification and true variation in the phylogenetic histories of nuclear genes and the plastid genome. A recent phylogenetic study based on genome-wide synteny network data suggested the magnolids as a sister lineage to monocots. Other phylogenetic studies, which combined nuclear genome sequences and transcriptomes from large-scale species sampling, recovered a sister relationship between magnolids and eudicots. Analyses using chloroplast genomes, however, seem to strongly support magnolids as a sister to the clade of monocots and eudicots. Here, we attempted to investigate these phylogenetic discrepancies through comparisons of genomic structural features.

Specifically, after comparing the *A. fimbriata* genome to those of the other angiosperms, we identified several large chromosomal rearrangements that probably occurred during the early evolution of angiosperms (Supplementary Note 3.4). Through intergenomic comparisons between the *A. fimbriata* genome and those of *Amborella* and *N. colorata* from the ANA grade, we found that regions of *A. fimbriata* chromosome 6 (A6f) are orthologous with segments of *Amborella* chromosomes 7 or 9 and *N. colorata* chromosomes 4 and 12 or chromosomes 2 and 9 (Supplementary Fig. 3.10). Similarly, we also found that chromosome 7 of *A. fimbriata* (A7f) has non-overlapped orthologous syntenic regions in *Amborella*, as well as in *N. colorata* (Supplementary Fig. 3.10). These structural comparisons indicate that chromosomes 6 and 7 of *A. fimbriata* might have formed via fusion events in an ancestor of *A. fimbriata*.

We further compared the *A. fimbriata* genome to those of representative magnolid, eudicot and monocot species to determine whether or not the associated genomic rearrangements are shared by two or all three mesangiosperm clades. Chromosome A6f has
Fig. 2 | Intergenomic comparisons revealed that *A. fimbriata* lacks any WGD after the shared WGD in the common ancestor of all angiosperms and identified two novel WGDs in *P. nigrum*. a, Syntenic comparison between *A. fimbriata* and *A. trichopoda* revealed a 1:1 ratio that suggests no lineage-specific WGD in *A. fimbriata* after its divergence from *A. trichopoda*. Syntenic blocks with more than ten genes are linked by grey lines; the largest ten syntenic blocks are highlighted in orange. b, Three rounds of WGDs in *P. nigrum* were identified via syntenic comparison to *P. nigrum*, *A. fimbriata* after its divergence from *A. trichopoda* lineage-specific WGD in *A. fimbriata*. Syntenic blocks with more than ten genes are linked by grey lines; the largest eight regions in ten syntenic blocks are highlighted in orange. c, Three rounds of WGDs in *P. nigrum* were identified via syntenic comparison to *P. nigrum*, *A. fimbriata* after its divergence from *A. trichopoda* lineage-specific WGD in *A. fimbriata*. Syntenic blocks with more than ten genes are linked by grey lines; the largest eight regions in ten syntenic blocks are highlighted in orange. d, Chromosome-level syntenic alignments of *A. fimbriata* to the *P. nigrum* genome of *A. fimbriata* are highlighted in orange. A. *fimbriata* and *P. nigrum* genome organization patterns that differ from those of the *A. fimbriata* genome, we propose that the separated genomic regions were ancestral and either a fusion event occurred before the divergence of monocots and magnoliids followed by a further fission event in the common ancestor of Laurales and Magnoliales (scenario I) or parallel evolution in the Piperales and monocots led to similar fusions (scenario II). Scenario I would support the magnoliids and monocots as sister clades and eudicots as their sister lineage, while the scenario II could not provide evidence for the phylogenetic placement of magnoliids.
Fig. 3 | Common genomic rearrangements present in magnoliids and monocots but absent from eudicots and the two representative species of the ANA grade. **a**, The local syntenic blocks identified between the A. fimbriata genome and the genomes of A. trichopoda, T. sinense, S. polyrhiza and L. chinense. The specific genomic regions associated with the A7 fusion were named regions of E, A1, A2, B1, B2, C1, C2, D, D1 and D2 as marked on top of the plot. The A1 region seems to be embedded in the E region, and is indicated as E(A1). Similarly, the D region seems to be embedded in the C2 region and is indicated as C2(D). Highlighted regions represent syntenic blocks among the compared genomes. The grey dotted lines in **a** indicate the fusion point in chromosome 7 of A. fimbriata. **b**, The connection patterns of the orthologous regions in the representative genomes of magnoliids, monocots and eudicots. There are two different connection patterns for the paralogous regions in the C. kanehirae and L. cubeba genomes and both patterns were presented. **c**, The inferred topology of angiosperms based on a common genomic exchange event shared by monocots and magnoliids. MRCA here represents the most recent common ancestor of extant angiosperms.

**Aristolochia fimbriata**
- E-A1-A2
- B1-B2
- C1-D1-D2

**Piper nigrum**
- E-A1-A2
- B1-B2
- C1-C2-D1-D2

**Cinnamomum kanehirae**
- C1-A1-A2-B1-B2-D1-D2-C2

**Litsea cubeba**
- A1-A2-B1-B2
- D1-D2

**Magnoliids**
- A1-A2-B1-B2
- C1-C2
- D1-D2

**Magnoliids**
- A1-A2-B1-B2
- C1-C2
- D1-D2

**Ananas comosus**
- A1-A2-B1-B2
- C1-C2
- D1-D2

**Elaeis guineensis**
- A1-A2-B1-B2
- C1-C2
- D1-D2

**Asparagus setaceus**
- A1-A2-B1-B2
- C1-C2
- D1-D2

**Spirodela polyrhiza**
- A1-A2-B1-B2
- C1-C2
- D1-D2

**Monocots**
- A1-A2-B1-B2
- C1-C2
- D1-D2

**Acer yangbiense**
- A1-A2-C1
- B1-B2
- D1-D2

**Vitis vinifera**
- A1-A2-C1
- B1-B2
- D1-D2

**Tetracentron sinense**
- A1-A2-C1
- B1-B2
- D1-D2

**Aquilegia coerulea**
- A1-A2-C1
- B1-B2
- D1-D2

**Eudicots**
- A1-A2-C1
- B1-B2
- D1-D2

**Nymphaea colorata**
- A1-A2-C1
- B1-B2
- D1-D2

**Amborella trichopoda**
- A1-A2-C1
- B1-B2
- D1-D2

**MRCA**
Data Figs. 4–6 and Supplementary Figs. 3.10–3.14 and 3.17–3.21). We also detected several lineages-specific structural changes, such as the Piperales-specific translocation of E region to the A1–A2, A. *fimbriata*-specific insertion of C2 into D1 and D2 and the separation of B1–B2 found in *Amborella* (Supplementary Note 3.4). After comprehensive examination of the connection pattern of these defined regions in the selected species, we reconstructed the most parsimonious ancestral patterns for the three major angiosperm clades, which are (A1–A2–B1–B2, C1–C2, D1–D2 and E) for magnoliids, (A1–A2–B1–B2, C1–C2, D1–D2 and E) for monocots and (A1–A2–C1, B1–B2, D1–D2–C2 and E) for eudicots (Fig. 3b). Together with the synteny patterns between *A. fimbriata* and the *Amborella* and *N. colorata* genomes, we predicted the structure of the homologous chromosome in the last common ancestor of extant angiosperms was (A1–A2–C1, B1–B2–C2, D1–D2 and E) (Fig. 3c). The reconstructions of ancestral chromosome structure imply a genomic exchange between regions of B1–B2 and C1 that occurred just before the divergence of monocots and magnoliids (Fig. 3c). This shared, derived (synapomorphic) chromosomal arrangement in magnoliids and monocots, but missing in eudicots, provides support for a magnolid + monocot clade with eudicots as their sister lineage (Fig. 3c).

We also performed phylogenetic analyses using different taxon sampling datasets to investigate the reasons for the discordant topologies of monocots, eudicots and magnoliids (Supplementary Note 4). We identified 98 strictly single-copy (SSC) and 535 mostly single-copy (MSC) gene families from 22 representative species and maximum likelihood trees were constructed (Fig. 4 and Supplementary Table 2.8). Notably, we found that most of the individual nuclear gene trees show weak or no resolution regarding the phylogenetic relationships of the magnoliids, monocots and eudicots (Fig. 4a,b and Supplementary Table 4.2). In fact, a polytomy null hypothesis could not be rejected (the node of magnoliids, eudicots and monocots is a polytomy) (Supplementary Table 4.2). Gene tree quartet frequencies of the 98 SSC datasets slightly supported T2 (magnoliids and monocots are sister clades; Fig. 4a), whereas the three topologies were almost equally supported from the 535 MSC datasets (Supplementary Note 4.1 and Extended Data Fig. 7), lending support for the polytomy hypothesis or rapid diversification with a high degree of incomplete lineage sorting (ILS) between successive bifurcations. Interestingly, strongly skewed quartet frequencies were recovered for one alternative tree (T2) relative to the other (T1) in ASTRAL analyses of the 535 MSC gene trees suggesting that processes other than ILS (for example, gene flow or gene duplication and loss of paralogous copies) may be contributing to gene tree discordance.

Analyses of concatenated nuclear gene alignments does not account for variation in gene histories due to ILS of ancestral sequence diversity but they can yield trees with identical branching orders if ILS is weak. The concatenation-based inferences using the various datasets of amino acid sequences and protein-coding sequences, as well as the partitioned codons, from the 98 SSC and 535 MSC gene families consistently supported magnoliids and eudicots as sister lineages (T2; Supplementary Note 4.1, Fig. 4a,b and Supplementary Fig. 4.1). Coalescent-based phylogenetic analyses of the 535 MSC nucleotide dataset also weakly supported T2 using ASTRAL and MP-EST (Supplementary Figs. 4.2a and 4.4a,c). However, if we used 535 MSC individual trees with collapsed nodes setting gradient bootstrap support (BS) values for coalescent analyses, the resulting topologies changed from T2 to T3, magnoliids as sister to monocots (Supplementary Fig. 4.2). Moreover, if we input the trees with nodes collapsed when their BS values were <50% to ASTRAL, the quartet frequency of T3 is much higher than the other two topologies (Fig. 4c). In addition, we used multigene tree summary methods ASTRAL-Pro and STAG with 22,563 gene families and the results both support magnoliids and eudicots as sister groups (T2) (Supplementary Fig. 4.8). Therefore, our results showed that most of the individual gene trees exhibited low resolution regarding the topology of monocots, magnoliids and eudicots, while the resolution of individual gene trees has a great effect on the inferred topology for coalescent-based analyses.

Combining the genome structural evidence and the phylogenetic results, we propose the T3 topology (magnoliids and monocots are sister clades) as a possible relationship worthy of further study and we further performed molecular dating (Supplementary Note 4.5 and Fig. 4d). The crown age of angiosperms was inferred to be 190–315 million years ago (Ma). The split between monocots and magnoliids was estimated at 138–241 Ma and the divergence time between the magnolid + monocot clade and eudicots was at 143–249 Ma. As noted in a previous study, the temporal proximity of the split among magnoliids, monocots and eudicots (within ~7 Ma) and broadly overlapping divergence time confidence intervals indicate that rapid divergence, is probably responsible for the great difficulty in reconstructing the relationship using a phylogenetic approach based on sequence data (Supplementary Note 4.5).

The genetic basis of unique floral features in *Aristolochia*. *Aristolochia* has a unique floral morphology that consists of a monosymmetric, trumpet-shaped, petaloid perianth and a gynostemium formed by the congenital fusion between stamens and the stigmatic region of the carpels (Fig. 1a and Extended Data Fig. 1). The *A. fimbriata* genome contains a relatively small number of floral regulatory genes (Supplementary Note 5.1, Fig. 5a, Supplementary Fig. 5.1 and Supplementary Table 5.2) and only one homologue for each of the eight classes of floral organ identity genes with high similarity to their corresponding orthologues in *Amborella* (Fig. 5b and Extended Data Fig. 8). Among the floral organ identity genes, *AfAP3* and *AfPIF* are highly expressed in the perianth, suggesting that the petaloidy of the perianth was caused by outward expansion of the expression domains of B-function genes and supporting the hypothesis of a sepal-derived perianth in *Aristolochia*. Also, both B-function genes and *AfAG* are expressed in the gynostemium, supporting the hypothesis that the gynostemium is a fused structure (Supplementary Note 5.3 and Fig. 5c). Two *CUP-SHAPED COTYLEDON* genes, *AfCUC1* and 2, whose orthologues in other species specify the boundaries between floral organs14,15, were also identified in *A. fimbriata* (Supplementary Note 5.4 and Supplementary Fig. 5.5). Consistent with the formation of the trumpet-shaped perianth and the fusion of stamens and the stigmatic region of the carpels, neither of these genes is expressed in the perianth or gynostemium (Fig. 5d). Notably, the *A. fimbriata* genome contains one *CYCLOIDEA* (*Cyc*) and three *CINCINNATA* (*Cin*) genes (Supplementary Fig. 5.6), which are orthologues of the flower symmetry establishment and leaf-like organs morphogenesis genes in other species46,47. While the expression levels of *AfCyc* are very low in all of the tissues examined, the three *Cin* genes (that is, *AfCIN1*, 2 and 3) show differential expression basipetally, with the highest expression being found in the limb region (Fig. 5c). This evidence, together with the observation of their expression profiles in *Aristolochia arborea* and *A. fimbriata*, strongly suggests that the *Cin* genes are responsible for the heterogeneous growth and morphological deformation of the perianth in *Aristolochia*. *Aristolochia* flowers often exhibit a dull, purple-brown colour in different parts of the perianth, probably related to pollinator attraction24. In the *A. fimbriata* genome, we identified 13 putative anthocyanin biosynthetic genes, consistent with the previously known pigmentation stages24, several key enzyme-encoding genes, such as *CHALCONE SYNTHASE* (*Chs*), *FLAVANONE 3-HYDROXYLASE* (*F3H*), *DIHYDROFLAVONOL 4-REDUCTASE* (*Dfr*) and *ANTHOCYANIDIN SYNTHASE* (*Ash*), showed relatively higher expression in the pre-anthetic flowers compared to anthetic flowers (Fig. 5f). It is very likely that the *A. fimbriata*
Fig. 4 | Challenges in using a phylogenomic approach to resolve relationships among the major angiosperm groups. a, Possible topologies among magnoliids, monocots and eudicots. b, The discordant topologies inferred from various taxon sampling using ASTRAL- and supermatrix-based approaches and individual gene trees. Numbers in the coloured boxes are the supporting values of the LPP or BS for the 'N2' nodes of the different topologies as shown in a. The bottom histogram shows the numbers of individual low-copy gene trees supporting the respective topologies. Species and clades are abbreviated as: A. fimbriata, Afi; C. kanehirae, Cka; L. chinense and Lch; magnoliids, Magno. c, Effect of gene tree resolution on the quartet frequencies of the 535 MSC gene families. Note that use of ML gene trees resulted in similar support levels for T2 and T3, whereas collapsing of nodes with BS values <50% in the ML trees resulted in strongest support for T3. Dashed lines show mean quartet frequencies at 0.33. d, The inferred phylogeny of representative angiosperms, shown with estimated divergence times. Blue bars at the nodes represent 95% confidence intervals of the estimated divergence time. WGD events are also shown on the species tree. The rapid divergence of eudicots, monocots and magnoliids at ~200 Ma is highlighted in grey.
flowers lack delphinidin-based anthocyanins because none of the identified candidate genes encode for flavonoid 3′-hydroxylase (F3′5′H), a key enzyme for the synthesis of delphinidin-based lilac to blue anthocyanins51,52. In addition, the B-function genes (AfAP3 and AfPI) are positively co-expressed with three structural genes (F3H, DFR and ANS) and a regulatory gene (TRANSPARENT TESTA 8, TT8), suggesting that they may regulate anthocyanin biosynthesis (Supplementary Note 5.5 and Fig. 5g). The observation that putative AP3/PI-specific binding motifs (CArG-box) can also be found in the promoter regions of the F3H, DFR, ANS and TT8 genes further supports this idea (Supplementary Table 5.5). Further analysis of anthocyanin biosynthesis in the flowers of *A. fimbriata* is warranted.

**Terpenoid and AA biosynthesis in *A. fimbriata*.** Because of their enriched secondary metabolites, *Aristolochia* species have long been used in traditional pharmacopeias5. In the *A. fimbriata* genome, 1,803 genes belonging to ~20 secondary metabolism pathways (including isoquinoline alkaloid biosynthesis, tyrosine metabolism and other alkaloid biosynthesis pathways) were annotated (Supplementary Table 6.1). Thirty-three metabolic biosynthetic gene clusters (BGCs), which were annotated as alkald-, polyketide-, saccharide- and terpene-related clusters, were also found (Supplementary Fig. 6.1 and Supplementary Table 6.2). The large proportion of the annotated terpene (14/33) and alkald-related (9/33) BGCs appears to associate with the enriched production of terpoid and alkaloid compounds in *A. fimbriata* (Fig. 6a)5,17,21,27,35,53.

Specifically, our GC–MS analyses detected complex volatile compounds, including fatty acid derivatives, benzenoids and two types of terpenoids (sesquiterpenoids and monoterpoids) (Fig. 6a) but no diterpenoids in the *A. fimbriata* flowers. In the *A. fimbriata* genome, 41 putative terpene synthase (TPS) genes were identified and phylogenetic analyses further classified them into TPS-a, TPS-b, TPS-c, TPS-e/f and TPS-g subfamilies (Fig. 6b). TPS-a genes often encode sesquiterpene synthases5. Notably, the *Af06G158900* locus from the TPS-a clade exhibited extremely high expression in the utricle of anthetic flowers (Fig. 6c), which is consistent with the abundant component of sesquiterpene detected in anthetic flower volatile (Fig. 6a). Because it was also annotated in the terpene-related gene cluster (BGC22; Fig. 6d), it is very likely that *Af06G158900* is a sesquiterpene synthase-coding gene in *A. fimbriata* (Supplementary Note 6.2 and Fig. 6a–d). The other gene that presents a similar case is *Af01G154900*, which codes for a monoterpene synthase (Fig. 6a–d). In contrast, the genes in the TPS-c and TPS-e/f clades, which are responsible for the biosynthesis of diterpenoids5,42,45, showed very low expression in both pre-anthec- and anthetic flowers (Fig. 6b,c). Presumably, it is the low expression of these genes that is responsible for the lack of diterpenoids in *A. fimbriata* flower volatile compounds.

Given the widely known toxicity problems with AAs—major toxic alkaloid compounds present in many popular medicinal plants of Aristolochiaceae55,56,57—we also explored the *A. fimbriata* genome assembly to yield some insights into AA biochemistry. After liquid chromatography–mass spectrometry (LC–MS)-based confirmation of the accumulation of an AA compound (AA1) in *A. fimbriata* tissues (Extended Data Fig. 9), we constructed the AA1 biosynthesis pathway on the basis of the previous studies (Supplementary Table 6.4) and identified the main enzymes involved (Supplementary Note 6.3 and Fig. 6e). Our extensive metabolic enzyme annotation, family phylogeny construction and key catalytic motif/residues investigations led to the putative identification of the main candidate genes encoding these associated enzymes (Supplementary Note 6.4). For example, norcoclaure synthase (NCS) is crucial for the biosynthesis of benzyisouquinoline alkaloids (BIAs) in Ranunculaceae, Papaveraceae, Berberidaceae and Nelumbonaceae56,57. Phylogenetic analysis found seven NCS genes that were grouped together with the known alkaloid biosynthetic genes of opium poppy (*Papaver somniferum*) in the NCSI clade (Supplementary Fig. 6.8). Six of them (*Af02G077000, Af02G077000, Af02G263900, Af02G264000, Af01G154600* and *Af05G030600*) were annotated in alkaloid-associated gene clusters (BGC1, 10, 24 and 25) (Supplementary Fig. 6.1 and Supplementary Table 6.2) and their amino acid sequences exhibit conserved catalytic residues (Supplementary Fig. 6.9). Notably, the expression levels of the two genes (*Af02G077000* and *Af01G154600*) were highly correlated with the concentration of AA1 in the examined tissues (Extended Data Fig. 10), suggesting their roles in encoding the main functional norcoclaure synthase in *A. fimbriata*.

**Discussion**

The tremendous diversification of angiosperms can be at least partially attributed to prevalent WGDs throughout their evolutionary history5,37,38,43. Previously, *Amborella* was considered the sole angiosperm genome lacking a lineage-specific WGD, possessing only the single WGD event characteristic of all extant angiosperms5. Our work establishes that *A. fimbriata* is the second among the several hundred sequenced flowering plant genomes to retain this ancient genomic condition; this genome sequence therefore offers exceptional opportunities for unravelling the WGD history and genomic changes of other lineages, especially other magnoliids. Moreover, genomic analysis anchored by *Amborella* and *A. fimbriata* can ultimately deepen our understanding of genome evolution across angiosperms46. The well-conserved synteny between *A. fimbriata* and *Amborella* also enables a more resolved reconstruction of the ancestral angiosperm genome and thus provides insights into the genomic features of the common ancestor of extant angiosperms.

The *A. fimbriata* genome may help to clarify early mesangiosperm diversification and the phylogenetic placement of magnoliids through analysis of the evolutionary history of genomic structural

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Fig. 5 | **Using the *A. fimbriata* genome to elucidate the molecular developmental genetics of a highly specialized flower.** a, Variation in the copy numbers of flowering-associated transcription factors during land plant evolution. *A. fimbriata* and *A. trichopoda* exhibit the lowest mean size for the investigated gene families. b, Phylogenetic inference of floral organ identity genes. Branches of the maximum likelihood tree were coloured on the basis of the species colour scheme (on the right). BS > 50% are shown. The numbers of floral organ identity genes are also shown and coloured according to the species colour scheme. c, Expression patterns of the floral organ identity genes. The numbers in the boxes are the TPM expression values for each gene at the pre-anthesis and anthesis stages. The relative expression levels were further normalized by calculating the ratio of their TPM expression values to that of the functionally conserved *AfAP3* gene in the gynostemium. The ratios were illustrated by four colour gradations representing 0.01–0.1, 0.1–0.25, 0.25–0.5 and >0.5. No colour was filled if the gene has no expression. The asterisks indicate genes with different relative expression levels between the two examined developmental stages; heatmap colours correspond to the relative expression levels in pre-anthetic flowers. d–f, Expression levels of the putative candidate genes involved in floral organ fusion (d), floral symmetry (e) and anthocyanin biosynthesis (f), in late pre-anthetic and anthetic flowers and leaves (LF). L, limb; T, tube; U, utricle; G, gynostemium; O, ovary; EBGs, early biosynthesis genes; LBGs, late biosynthesis genes; PRs, positive regulators; and NRs, negative regulators. g, Co-expression network reconstruction identified MADS-box B-class genes clustered with the genes involved in anthocyanin biosynthesis, as well as some trichome formation genes, suggesting that floral organ identity genes have expanded their regulatory networks.
variations, as we demonstrate here. Recent studies have used a phylogenetic approach to determine the relationship among the monocot, eudicot and magnolid clades \cite{11, 12, 20} but have often recovered different topologies (Supplementary Table 4.4). After comprehensively testing alternative taxon sampling and tree-constructing strategies, we also found it challenging to resolve with strong support the relationship among these three clades (Supplementary Note 4.2). Moreover, codon usage bias also affected the resolution of the tree as well as the topology (Supplementary Note 4.4 and Supplementary Figs. 4.12–4.15). The difficulty in resolving relationships among these clades may be due to the limited informative sequence divergence generated during their rapid diversification.

\[ \text{Anthocyanin biosynthesis pathway genes} \]

\[ \text{B-class genes} \]

\[ \text{Anthocyanin biosynthesis pathway genes} \]

\[ \text{Trichome formation pathway genes} \]

\[ \text{Involved in both anthocyanin biosynthesis and trichome formation pathway genes} \]
In such cases, it is plausible that some rare genomic changes, such as genomic structural changes, may potentially have occurred in a very compressed evolutionary window. Because rare genomic changes have more alternative states and may be less vulnerable to the high frequency of reversals or parallel substitutions in sequence evolution, they can offer valuable insights into the phylogenetic...
relationships as proposed previously. Although it remains hard to completely exclude the possibility of ancient hybridization, parallel evolution and ILS, the identified genome structural changes most parsimoniously imply a sister relationship between magnolids and monocots, a relationship that has also been recovered in another study. We stress however, that other key mesangiosperm lineages (Chloranthales and Ceratophyllales) are not included in these analyses and it will be crucial to investigate their patterns of genomic rearrangement.

The genome assembly of *A. fimbriata* also serves as a functional genomic resource for pinpointing the genetic bases for the origins and modifications of phenotypic traits, such as the highly modified flower and the enriched alkaloid chemistry of *A. fimbriata*. Gene duplication is considered to be a driving force for the evolution of phenotypical and functional novelty. Here, we found similar numbers of MADS-box genes, as well as other floral regulators, between *A. fimbriata* and *Amborella*, two species with dramatically different flower morphologies. We also noted that alternative splicing variant forms for these genes are very rare in *A. fimbriata* (Supplementary Note 5.2 and Supplementary Fig. 5.3). At minimum, these findings suggest that MADS-box gene repertoire has not expanded in *A. fimbriata*, excluding one of the possible mechanisms of flower diversification via gene duplication and neofunctionalization.

The expanded regulatory networks involving the floral organ identity genes and genes associated with other developmental features identified in this study can help at least partially explain the morphogenesis of the highly modified flowers of *A. fimbriata*. Further comparative analyses of expression profiling and chromatin immunoprecipitation followed by sequencing (ChIP–seq) of MADS-box genes in *A. fimbriata* and *Amborella* could be used to better understand the evolutionary developmental mechanism of the distinct flowers in *A. fimbriata*.

In conclusion, the *A. fimbriata* genome lacks any additional WGDs beyond that shared by all extant angiosperms. Thus, it provides an outstanding new evolutionary reference for comparative genomics and for inferring the ancestral angiosperm genome and patterns and processes of genome evolution in other angiosperms. The *A. fimbriata* genome also facilitated the identification of genomic structural changes, which is shared with other magnolids and with monocots, suggesting a sister relationship between magnolids and monocots, in contrast to many sequence-based analyses that have found monocots and eudicots to be sisters. Finally, the genome also provides insights into the genetic basis underlying both the highly specialized flower development and aristolochic acid biosynthesis. Given its low genetic redundancy and ease of large-scale cultivation, *A. fimbriata* could readily be developed into an important new genetic model species given its phylogenetic position as a member of the magnolid clade; the species affords opportunities for further functional genomic studies, serving as an excellent system for studies of floral biology, developmental genetics, biochemical pathways and development of synthetic chemicals.

### Methods

**Plant materials and DNA sequencing.** Fresh leaves were collected from the same individual of *A. fimbriata* plant for DNA extraction and sequencing. For Oxford Nanopore Technologies (ONT) sequencing, DNA was extracted from young leaves using QIAGEN Genomic Kits and libraries with an insert size of 20–40 kb were then prepared and sequenced on a GridION X5 instrument. For optical maps, DNA was extracted from young leaves according to a modified Bionano genomics protocol. The long high-quality DNA was labelled by enzyme Nt.BspQI and then loaded into the Saphyr chip for scanning. To collect sufficient material for Hi-C sequencing, we cultivated the seedlings by tissue culture using stem cuttings from the same individual used for the above sequencing. The samples were processed and the DNA was extracted and crosslinked using the standard protocol. The Hi-C libraries were then amplified and sequenced with 150-bp paired-end reads using Illumina HiSeq.

**Genome assembly and assessment.** ONT long reads were de novo assembled using minimap2 v2.15.914 (ref. 36) and miniasm v0.3.0 (ref. 37). Then, three rounds of polishing with racon and one round of polishing with Pilon were applied to the assembled contigs. Optical molecules with length > 180 kb or the molecule label number > 9 were used for optical map assembly using the Bionano Solve Pipeline v3.3 (https://bionanogenomics.com/support/software-downloads/) and hybrid scaffolds were generated by aligning the optical maps to ONT assembled genomic contigs using Bionano’s hybrid-scaffold software (https://bionanogenomics.com/ support/software-downloads/). The hybrid scaffolds with length > 100 kb were further anchored and oriented to seven pseudochromosomes on the basis of the Hi-C contact frequency using Juicebox Assembly Tools (IBAT v1.8.8).

The quality and completeness of the *A. fimbriata* genome assembly were assessed from four aspects. First, we evaluated the mapping rates of the clean raw reads from transcriptomes and genomic DNA by TopHat2 (ref. 38) and BWA-MEM (ref. 39) with default parameters, respectively. We further used the “--vcf” option in Pilon v1.12.3 (ref. 40) to call single nucleotide polymorphisms from the Illumina genomic reads. Second, we investigated the BUSCO genes from Embryophyta in the final assembly. Third, we used the LAI to infer the assembly continuity. Finally, we aligned Bionano molecules back to the final *A. fimbriata* genome assembly to check the consistency between Bionano molecules and the final genome assembly using the ReAligner tool (https://bionanogenomics.com/support/software-downloads/) with default parameters. In addition, we also checked the consistency of the Bionano assembly consensus genome maps (CMAIP) and the in-silico maps of the *A. fimbriata* genome assembly.

**Transcriptome sequencing.** Several organs and tissues were sampled for total RNAs extraction and transcriptome sequencing, including leaves, seedlings under normal and low temperature (4 °C) conditions, roots and five different floral organs at different developmental stages (stage 8 and anthesis flower) were separately extracted and processed using Trizol reagent (Invitrogen) following the manufacturer’s procedure. The paired-end complementary DNA libraries with insert size of 150 bp were constructed and sequenced using Illumina HiSeq4000 instrument. For full-length transcriptome sequencing, the samples from anthetic flowers, seedlings under normal growth conditions, seedlings treated with low temperature (4 °C) for 9 h and roots were collected and the extracted RNAs from the four samples were mixed together in equal amount to obtain transcriptomes from various plant tissues and treatments. The cDNA libraries were constructed using the SMARTer PCR cDNA Synthesis Kit. The full-length cDNA fragments were screened using a BluePippin instrument to construct cDNA libraries of different sizes (1–2, 2–3 and 3–6 kb) (Supplementary Fig. 2.2). The libraries were sequenced on a PacBio RS II instrument. In addition, we further collected and pooled the flower buds at different developmental stages (from stage 5 to anthesis) together in relatively equal amount to perform much deeper
Articles

Article 1: Protein-coding gene prediction and functional annotation. The protein-coding genes were predicted using the well-developed combination strategies of transcriptome, homology-based annotation and ab initio gene prediction. For the ab initio prediction, Ensembl and AUGUSTUS were run on the repeat-masked genes were predicted using the well-developed combination strategies of Protein-coding gene prediction and functional annotation. The protein-coding genes were then combined to obtain the final TE annotation. Results from these two runs of RepeatMasker were merged.

Article 2: Transcriptomic data analyses. RNA-seq raw reads were preprocessed using Trimmomatic to remove adapter sequences and low-quality reads. The clean
reads were then mapped to the reference genome using HISAT2 with default parameters. The expression abundance values were calculated using Stringtie\(^3\) and we averaged the abundance values from the three biological replicates of each sample to obtain levels of gene expression.

For the Iso-seq data of mixed tissues sequenced on PacBio RS II instrument, the raw reads were processed using SMRT Link 5.0 software. First, the circular consensus sequences (CCSs) were generated from the subreads BAM files with parameters of \(n=5\)–\(\minLength=300\)–\(\minPasses=1\)–\(\minPredictedAccuracy=90\). Next, all the CCSs were further classified into full-length non-chimeric (FLNC) and non-full-length (nFL) transcript sequences on the basis of the whether the 5’-primers, 3’-primers and poly(A) tail could be detected. To improve consensus accuracy, we clustered and polished the FL sequences using an isoform-level clustering algorithm, iterative clustering for error correction (ICE) and the Quiver algorithm,\(^4\) respectively. Then, we averaged the abundance values from the three biological replicates of each sample to obtain the mean expression value.

For the Iso-seq data of mixed flower buds sequenced on PacBio Sequel II platform, the raw sequence data were processed by SMRT Link v8.0 software (https://www.pacb.com/support/software-downloads/). First, CCSs were generated from the raw subreads BAM file to identify full-length (FL) reads using CCSs with parameters of \(n=\minPasses=1\)–\(\minLength=100\). Then, FLNC reads were identified if they had the 5’-primer, 3’-primer and poly(A) tail. Finally, FLNC reads from the same isoform were clustered and further polished using subreads.

The construction of co-expression networks. For the construction of co-expression networks, we used all RNA-seq data from 14 samples described above (tissues of flowers at anthesis and pre-anthesis, leaves and seedlings with 10°C min\(^{-1}\) heating rate) for all flowering stages and tissues. Three biological replicates were conducted for the GC–MC analysis. Triplicate experiments were performed for the GC–MC analysis.

Floral scent measurement. To investigate the floral volatile production of \(A.\) fimbriata, we collected the newly opened flowers for gas chromatography–mass spectrometry (GC–MS)\(^5\) analysis as described in Bliss et al.\(^6\) First, the samples were incubated at 40°C for 30 min. The volatiles were further extracted using SPEME fibre with 50/30 μm of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Co.). Finally, GC–MS analysis was conducted on an Agilent 7890B gas chromatograph coupled to a mass spectrometer (Agilent 7000D) with a fused silica capillary column (HP-5MS) coated with polydimethylsiloxane (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The oven temperature was programmed to start at 40°C for 3 min and then ramped to 130°C at a rate of 5°C min\(^{-1}\), followed by a second ramp to 156°C at a rate of 2°C min\(^{-1}\) and the final ramp to 280°C at a rate of 10°C min\(^{-1}\). Three biological replicates were conducted for the GC–MC analysis.

Aristolochic acid identification. We performed an LC–MS-based metabolomic analysis for the root, stem, leaf and fruit from one-year-old \(A.\) fimbriata flowers. A total 50 mg of each dried tissue were processed for the HPLC-AD-ESIMS/MS measurements. AAs were separated by UPLC (Waters, ACQUITY) equipped with an ACQUITY UPLC HSS T3 column (Waters) and detected by MS/MS using a Triple Quad Xevo TQ-S (Waters) mass spectrometer. The mobile phase consisted of buffer A (5 mM ammonium acetate and 0.1% formic acid) and buffer B (100% acetonitrile). AAs were qualified using the ion mass transitions of 150–160 ppm and 2–3 ppm for each AAs. Then, the samples were incubated at 40°C for 30 min. The volatiles were further extracted using SPME fibre with 50/30 μm of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Co.). Finally, GC–MS analysis was conducted on an Agilent 7890B gas chromatograph coupled to a mass spectrometer (Agilent 7000D) with a fused silica capillary column (HP-5MS) coated with polydimethylsiloxane (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The oven temperature was programmed to start at 40°C for 3 min and then ramped to 130°C at a rate of 5°C min\(^{-1}\), followed by a second ramp to 156°C at a rate of 2°C min\(^{-1}\) and the final ramp to 280°C at a rate of 10°C min\(^{-1}\). Three biological replicates were conducted for the GC–MC analysis.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.
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Extended Data Fig. 1 | Flower morphologies of eight other Aristolochia species. For each selected species, the front and side views and longitudinal section of flowers at anthesis, as well as the scanning electron micrographs of the inner epidermis of perianth, are shown.
**Extended Data Fig. 2 | Genome size estimation of *A. fimbriata*.** a, Genome size estimation for *A. fimbriata* (R2) based on flow cytometry using *A. thaliana* (R1, 125 Mb/2C) as an internal reference. The genome size of *A. fimbriata* was estimated to be approximately 289.50 Mb. b, 17-mer-based analysis of estimation of genome size. The total number of k-mer is ~20,853,344,487, and the peak of the k-mer depth is ~83; therefore, the estimated genome size is approximately 251 Mb.
Extended Data Fig. 3 | Genome assembly quality assessments. a, Mapping profile of the Nanopore clean reads to the final A. fimbriata assembly. b, Comparison of the length of contig N50 and the LTR Assembly Index (LAI) for the 105 published plant genome assemblies. c, Alignments of the Bionano molecules to the assembled chromosomes of A. fimbriata. d, Comparison of LAIs for several representative plant genomes. All stats indicate that the A. fimbriata assembly quality is outstanding.
Extended Data Fig. 4 | Genomic comparison of the *A. fimbriata* and *L. chinense*, with the *P. nigrum* and *M. biondii* genomes, respectively. **a**, Syntenic dotplot between the *A. fimbriata* and *P. nigrum* genomes. **b**, Syntenic dotplot between the *A. fimbriata* and *L. chinense* genomes. **c**, Syntenic dotplot between the *A. fimbriata* and *M. biondii* genomes. **d**, Syntenic dotplot between the *L. chinense* and *M. biondii* genomes. Given the orthologous D and E regions in *L. chinense* remain in ancestral status (not merged with C2 or A), we could use the *L. chinense* as another comparing reference to clearly infer the A1-A2/E and the D1-D2/C2 orthologous regions in other genomes. The names of these circled syntenic blocks in **d** were inferred based on the D and E genomic regions in *L. chinense* that exhibiting orthologous relationships to these defined D and E regions in *A. fimbriata* respectively.
Extended Data Fig. 5 | Genomic comparisons of the A. fimbriata and L. chinense with the S. polyrhzia and A. comosus genomes, respectively. a, Syntenic dotplot between the A. fimbriata and S. polyrhzia genomes. b, Syntenic dotplot between the L. chinense and S. polyrhzia genomes. c, Syntenic dotplot between the A. fimbriata and A. comosus genomes. d, Syntenic dotplot between the L. chinense and A. comosus genomes. The orthologous region of the D1-D2 and E in S. polyrhzia and A. comosus could be further verified by the syntenic relationship to the corresponding D1-D2 and E regions in L. chinense.
Extended Data Fig. 6 | Local syntenic relationships among the selected genomic regions that associated with the structural rearrangements of *A. fimbriata* chromosome 7. **a**, The local syntenic blocks identified between the *A. fimbriata* and *M. biondii* genomes, **b**, The local syntenic blocks identified between the *A. fimbriata* and *P. nigrum* genomes, **c**, The local syntenic blocks identified between the *A. fimbriata* and *C. kanehirae* genomes, **d**, The syntenic blocks identified between the *A. fimbriata* and *L. cubeba* genomes. Similar to the Fig. 3a, the specific genomic regions associated with the *A. fimbriata* chromosome 7 fusion were named regions of E, A1, A2, B1, B2, C1, C2, D, D1 and D2 as marked on top of the plot.
Extended Data Fig. 7 | Gene tree quartet frequencies of 535 MSC gene families for different topologies. Here we inputted individual genes trees (a-d), and also ran with collapsed trees if BS was less than 50% (e-h). The x-axis labels T1, T2, and T3 refer to the quartet support for the topologies of T1 (red), T2 (blue), and T3 (yellow) in Fig. 4a respectively. The dashed line refers to a proportion of 0.33.
Extended Data Fig. 8 | Comparisons of gene structure and exon sequence similarity of the floral organ identity genes in *A. fimbriata* and *A. trichopoda*. The *A. trichopoda* genes tend to have longer introns than that of *A. fimbriata*. 
Extended Data Fig. 9 | LC–MS analysis of aristolochic acid content in *A. fimbriata*. The purchased samples of AA I and AA II were used as standards, and the samples of fruit, stem, leaf, and root were analysed by LC–MS. Only AA I was detected in the investigated tissues of *A. fimbriata*. 
Extended Data Fig. 10 | Gene expression quantification by qRT-PCR for the seven NCSI genes in *A. fimbriata*. All data are presented as the means ± s.d. (n = 3 biological replicates, as shown in solid black dots). The NCSI gene expression levels in five other tissues (root, stem, leaf, flower, and fruit) were compared with that in seedlings, and the two-tailed t tests were used to analyse the statistical significance of their expression levels. * indicates a significant difference at P value < 0.05, and ** indicates a significant difference at P value < 0.01, P values are shown above each bar chart.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection: No software was used to collect the data.

Data analysis: A lot of softwares were used for data analysis in this paper.
- Nuclear genome size estimation: Summit v5.2, Jellyfish v2.2.10, GenomeScope v2.0.
- Genome assembly: miniasm v2.15-r914, miniasm v0.3, racon v1.3.3, bwa-mem v0.7.12-r1039, Pilon v1.22, Bionano Solve Pipeline v3.3, Juice v1.7.6, Juicebox v1.8.8.8 and 3D-DNA v180114.
- Chloroplast genome assembly: Canu v1.8, Trimmomatic v3.8, miniasm v2.16-r922, bowtie2 v2.3.4.1, samtools v1.9, SPAdes v3.11.1, Geneious v8.0.2, MITObim v1.9 and OGDRAW v1.3.1.
- Genome quality assessments: TopHat2, bwa-mem v0.7.12-r1039, Pilon v1.23, RefAligner in Bionano Solve Pipeline v3.3 and LTR Assembly Index (LAI).
- Genome annotation: Repbase v20.05, RepeatMasker v4.0.7, RepeatModeler v1.0.10, LTRharvest v1.5.10, LTR_FINDER v1.05, LTR_retriever v1.8.0, LoDeC v0.8, Cd-hit v4.0, Fgenesh v2.6, AUGUSTUS v3.3.1, GeneWise v2.4.0, GeMoMa v1.6.1, PASA v2.3.3, GMAP-2018-07-04, TransposonPSI (http://transposonpsi.sourceforge.net/) and EvidenceModeler v1.1.1, BLASTP v2.2.26, Blast2GO v5.2.5 and eggNOG mapper v22.
- Genome structural comparisons and polyploidization analysis: all-vs-all BLASTP within and between genomes, MCScanX and Ks estimated using the Nei–Gojobori approach.
- Phylogenetic analyses: OrthoMCL v2.0.9, MUSCLE v3.8.31, PAL2NAL v24, RaxML v8.2.12, ASTRAL-II v5.5.11, MP-EST v2.0, ASTRAL-Pro v1.1.5, STAG v1.0.0 and phypars v0.0.1.
- Codon usage bias analysis: CodonW v1.4.2.
- Molecular dating and gene family evolution analysis: MCMCTree and BASEML in the PAML v4.9e, Tracer v1.7 and CAFÉ v4.1.
- Gene family annotation: BLASTP v2.2.26, HHMER v3.3, InterProScan v5, MAFFT v7.312, PAL2NAL v14, trimAL v3 and RaxML v8.2.12.
- Transcriptomes analyses: Trimmomatic v0.36, HISAT2, Stringtie, SMRT Link v8.0 and SpliceGrapher v0.2.7.
The main custom scripts used for some of these analyses have been deposited in Github (https://github.com/yhenghui/Aristolochia_fimbriata_genome_analysis).

For manuscripts utilizing custom algorithms or software that are not central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. Github). See the Nature Research guidelines for submitting code & software for further information.

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The raw sequence reads, genome assembly and annotations of A. fimbriata have been deposited in NCBI under the BioProject accession numbers PRJNA656149. The genome assembly and annotations have also been deposited in the BIG Data Center (https://bigd.big.ac.cn/) as a BioProject PRJCA004207 and CoGe. The Amborella genome assembly and annotations used in this study are available from CoGe (https://genomevolution.org/coge/GenomeInfo.pl?gid=50948).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size  No statistical methods were used to predetermine sample size. The Aristolochia fimbriata used for sequencing has been propagated via selling for approximately 20 years in cultivation, and therefore has very low heterozygosity, one individual of them was selected for genome sequencing. For RNA-Seq, 34 different samples were collected and sequenced.

Data exclusions  For Nanopore long reads, the runs with the mean Q-scores less than 7 were removed, referring to the long reads quality control pipeline in Nanopore official website (https://nanoporetech.com/resource-centre/longqc-quality-control-tool-third-generation-sequencing-long-read-data; https://nanoporetech.com/resource-centre/minion-nanopore-sequencing-and-assembly-complete-human-papillomavirus-genome). For Illumina short reads, the following criteria were performed to filter the low quality reads: (1) leading and trailing low quality or N bases (quality below 20); (2) sliding window (4-base) with the average quality per base drops below 20; (3) reads below the 50 bases long.

Replication  The genome sequence was taken and sequenced with more than 1.20 fold coverage. No replication is needed for our genome report. For RNA-Seq, three biological replicates of each sample were used and the good correlation was confirmed, except one replicate of leaf with data pollution.

Randomization  No randomization in this manuscript as genomes assemblies were not allocated into experimental groups.

Blinding  The Aristolochia fimbriata genome were sequenced and assembled with no binding as the data were not allocated into groups.

Behavioural & social sciences study design

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Study description  Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

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Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.

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Describe the research sample (e.g. a group of tagged Passer domesticus, all Sterococerus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.

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Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer’s website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

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State the source of each cell line used.

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ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

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Flow Cytometry

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Confirm that:

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☒ All plots are contour plots with outliers or pseudocolor plots.
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Methodology

Sample preparation
Tissues of about 30mg were excised from freshly collected leaves, and placed in plastic petri dishes (35 mm x 10 mm) on ice, and sliced to fine pieces using a new razor blade in extraction buffer, and then filtered using 400T filter cloth.

Instrument
The data were collected the MoFlo XDP Cell Sorter (Beckman-Coulter)

Software
Summit v5.2

Cell population abundance
Flow cytometry was used for quantification and genome size estimation purposely only, and no post-sorting fraction was collected.

Gating strategy
Filter-675/76 was used in gating. The FL3-H/SSC-H gate method was used to eliminate debris, cell fragments, and dead cells. Single cell and double cells were discriminated by using FL3-H/FL3-A.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type
Indicate task or resting state; event-related or block design.

Design specifications
Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures
State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).
## Acquisition

**Imaging type(s)**
Specify: functional, structural, diffusion, perfusion.

**Field strength**
Specify in Tesla

**Sequence & Imaging parameters**
Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

**Area of acquisition**
State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

**Diffusion MRI**
- [ ] Used
- [ ] Not used

## Preprocessing

**Preprocessing software**
Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

**Normalization**
If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

**Normalization template**
Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

**Noise and artifact removal**
Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

**Volume censoring**
Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

## Statistical modeling & inference

**Model type and settings**
Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

**Effect(s) tested**
Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

**Specify type of analysis:**
- [ ] Whole brain
- [ ] ROI-based
- [ ] Both

**Statistic type for inference**
(See Eklund et al. 2016)
Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

**Correction**
Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

## Models & analysis

| n/a | Involved in the study |
|-----|------------------------|
|     | Functional and/or effective connectivity |
|     | Graph analysis |
|     | Multivariate modeling or predictive analysis |

**Functional and/or effective connectivity**
Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

**Graph analysis**
Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

**Multivariate modeling and predictive analysis**
Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.