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Chromosome-scale genome assembly of kiwifruit Actinidia eriantha with single-molecule sequencing and chromatin conformation capture

--Manuscript Draft--

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Abstract:	Background: Kiwifruit (Actinidia spp.) is a dioecious plant with fruits containing abundant vitamin C and minerals. A handful of kiwifruit species have been domesticated, among which the A. eriantha is increasingly favored in breeding due to its superior commercial traits. Recently, elite cultivars from A. eriantha have been successfully selected and further studies on their biology and breeding potential require genomic information which is currently unavailable. Findings: Here, we assembled a chromosome-scale genome sequence of A. eriantha cv. White using single-molecular sequencing and chromatin conformation capture. The assembly has a total size of 690.6 Mb and an N50 of 21.7 Mb. Approximately 99% of the assembly were in 29 pseudomolecules corresponding to the 29 kiwifruit chromosomes. Forty-three percent of the A. eriantha genome are repetitive sequences, and the non-repetitive part encodes 42,850 protein-coding genes, of which 39,075 have homologues from other plant species or contain protein domains. The divergence time between A. eriantha and its close relative A. chinensis is estimated to be 3.3 million years, and after diversification, 1,740 and 1,345 gene families are expanded or contracted in A. eriantha, respectively. Conclusions: We generate a high-quality reference genome of kiwifruit A. eriantha. This chromosome-scale genome assembly is substantially better than two published kiwifruit assemblies from A. chinensis in terms of genome contiguity and completeness. The availability of A. eriantha genome provides a valuable resource for facilitating kiwifruit breeding and the studies of kiwifruit biology.
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Response to Reviewers:	Note: The response letter was also submitted as a supplementary file. Editor: One point raised related to the reproducibility and methodological detail, and one way to address this is to include the protocols in protocols.io. Response: Thanks. We have uploaded the detailed protocols of computational analyses to protocols.io: http://dx.doi.org/10.17504/protocols.io.vgse3we. This link has been added to the revised manuscript (Line 295-296). Reviewer #1: In the paper "Chromosome scale genome assembly of Kiwifruit (Actinidia eriantha) with single molecule sequencing and chromatin conformation capture "the authors present data on a large scale long read assembly of a new kiwifruit species (A. eriantha). This new construction has led to a significant improvement in the amount of sequence that is assembled. Overall the paper is well written with a good quality English. Major concerns: 1.The authors present the new genome data in the context of the already published data, they state that the new long read construction is more complete and high level of "macro collinearity". Yet the alignment figure (3b) suggests that there are some major differences in the construction (it is hard to tell from the figure which chromosomes) but it appears that chromosome 16 has significant rearrangements, there is a translocation from chr23 to chr19 and a region that is different on chr27. I feel that the wording in the paper does not address these differences. -Two of these differences have been highlighted in S1. For this paper it needs to be checked to see if this is a species difference (ie a true rearrangement) or whether the red5 or eriantha construction is wrongly assembled. Usually a mapping approach would facilitate this, my recollection was that the original 'hongyang' genome used eriantha interspecific map to anchor the chromosomes. Could this be used? Response: We thank the reviewer for the suggestion. We have checked the inconsistent regions between the A. eriantha ‘White’ and A. Chinensis ‘red5’ assemblies using the two genetic maps described in Zhang et al. (2015). We found that the genetic maps supported all these regions in the ‘White’ assembly but not the ‘red5’ assembly (Please see Supplementary Figure S2). This confirms the high quality of the ‘White’ assembly and indicates the potential assembly errors in the ‘red5’ assembly. We have modified the related text in the revised manuscript (Line 190-194). -The use of the word anchor in Table 1 needs to be changed as the eriantha genome was aligned to the chromosomes but the authors did not anchor it with a genetic map (or if they did they have not detailed this). Response: As indicated in our original manuscript, we used Hi-C chromatin interaction maps to anchor assembled contigs. Using Hi-C data to anchor assembled contigs and the term “anchor” under this context have been widely used in the recently published high-quality genomes. 2.I feel it is important to have some consistency with the naming of gene models found in Actinidia species, as this will ultimately facilitate cross comparisons across species. Indeed this paper is an ideal opportunity to start an Actinidia pan genome gene set. To this end I think it is important that there is a consistent naming convention. In many species the gene locations are given along chromosomes, yet when there is genome

divergence, this labelling becomes impossible across species. The manual annotated kiwifruit genome, the genes were given a unique number which bypasses this issue. I was unable to access supplemental data 3 and 4, so apologies if requests below has been done. (maybe some examples in the main body of the paper on the gene models would be of interest).
-We need consistency in the literature. To this end the authors need to name genes in a similar manner to the already published genomes (maybe call them AerXXXXX). And if they could have an orthologue they should be given a number corresponding to the Acc genes already published. New genes should then be given new numbers
Response: We politely disagree with the reviewer regarding his comments here. In 'White', we named the genes based on their chromosome location, which is a nomenclature that has been widely adopted in plant (and animal) genomes, e.g., Arabidopsis, rice, tomato etc. This conventional gene nomenclature (that includes chromosome information) can provide intuitive information such as candidate gene location in QTL mapping, tandemly duplicated genes etc. Actually, this is the nomenclature we should follow, instead of abandon. This is also the reason that in the version 2 of the 'Hongyang' genes, we changed gene names by adopting this nomenclature.
Regarding the orthology suggested by the reviewer, we have to point out that this would be very challenging and sometimes confusing due to gene changes in different genomes during their evolution such as gene loss and gain, tandem and segment duplications. This is why the plant community didn't use the approach suggested by the reviewer, e.g., Arabidopsis (thaliana and lyrata), tomato (lycopersicum and pennellii), rice (indica and japonica).

-The authors need to identify whether the new genes are unique to the eriantha genome and do a quality measure on these to establish whether they are true genes or computational artifacts.
Response: As shown in Table S4, 90.9% of the predicted genes have at least one annotation from the seven databases searched, including NR protein, InterPro, KEGG, GO, Pfam, SMART and PANTHER databases. In addition, 71.5% of the remaining genes (or genes without database support) have FPKM values ≥ 1. Therefore, the majority of the predicted models are true genes instead of computational artifacts.

-It would be good to have an idea whether the genes were new to eriantha or just missed in the A. chinensis manual annotation process.
Response: To address this question, we use orthology analysis (Fig 4) as an example. There are 727 orthogroups contain genes from A. eriantha (980 genes) and other species but not from A. chinensis 'red5'. Blast search of these genes against red5 genome didn't result valid alignments, suggesting that these genes could be absent in 'red5' or not assembled. In addition, we found 661 A. eriantha genes that were not predicted in red5 but had valid alignments against its genome. Among these genes, 428 were expressed and 188 had annotations. Therefore, we believe at least some of them were not annotated in the red5 genome during the manual process.

Minor concerns
1.P3 Line 24. I am not sure that any Actinidia species have been "domesticated" there is no A. domestica. I would say "commercialised"
Response: Only very few domesticated crops are named with 'domestica' (e.g., Malus domestica). No 'domestica' does not mean there is no domestication (e.g., rice, maize, tomato…). Actually, "domestication" has been widely used for kiwifruit. For example, please check the paper by Hongwen Huang and Ross Ferguson: Genetic Resources of Kiwifruit: Domestication and Breeding (https://onlinelibrary.wiley.com/doi/10.1002/9780470168011.ch1).

Reviewer #2:
This manuscript provides a high-quality chromosome-scale genome assembly and related resources for an important kiwifruit species. We saw significant improvement in the continuity of genome assembly reported over the previous ones. The genome assembly and related resources will be valuable for kiwifruit breeding and fruti science studies.

The manuscript is generally in good quality, and could be accepted after revision.

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Comments

1. A general process was reported for genome assembly, quality assessment, annotation and comparision, while details lost, such as settings, important parameters used for a specific software. More information is needed for the whole analytical process described. The availability of the full analytical pipeline is like a rule required by GigaScience, I think.
Response: Please see our response to the editor's comment.

2. Genome estimatiion on K-mer size is lacking. Other than assembly, K-mer distribution provide another set of information, from which genome size, redundancy, heterozygosity, sequencing error rate could be derived independently to assembly. Please add this.
Response: We agree with reviewer that k-mer statistics provide useful information on genome features. We have generated a graph for k-mer distribution, and based on this we estimated the heterozygosity level of the 'White' genome. However, due to the heterozygous nature of the genome, our attempts on genome size estimation using k-mer were not successful. Nonetheless, we estimated the genome size of 'White' by flow cytometry analyses. Both k-mer and flow cytometry analysis results have been added in the revised manuscript (Line 125-143).

3. Details are lacking for Evolutionary and comparative analysis. How did you generate a phylogenetic tree? What (software, algorithm, data) you used to generated this tree? How many genes? How did you get those genes? How did you do molecular dating? Divergence time (> 200 mya) seems inconsistency to generally reported divergence time (around 120 mya) for monocots and dicots. It would be better to share the gene alignment used for phylogenetic reconstruction and the generated tree as supplementary files.
Response: Thanks for pointing this out. We have corrected the chronogram and elaborated our methods in the protocol.io (http://dx.doi.org/10.17504/protocols.io.vgse3we). Protein alignments for the phylogeny has been deposited to GigaDB during our initial submission of the manuscript.

4. Citation to published papers or online databases and tools, is lacking for some instances, such as, citations to publised genomes are needed in Table 1. The NCBI nr protein database, TAIR, Swiss-Prot and TrEMBL, PANTHER, Pfam, SMART, and PROSITE databases, they all need citations in a proper way. Please also check supplementary tables, for citation and footnote. Full names are needed to be affiliated with abbreviates, such as SINEs, LINEs, LTRs et al., in the supplementary tables.
Response: Thanks for pointing these out. We have revised these accordingly.

5. Page 9, lines 47-48. "In addition, variations between the two kiwifruit species could also contribute to this difference." "variation" may not be the good word. It is better to use "divergence" to describe the genetic difference among species. Please improve it. I am not native speaker, I am open for different tendency/ideas.
Response: Done. Thanks.

6. How did you generate mapping for genomic and RNA-seq data, when you did "Evaluation of the genome assembly"? "high mapping rates, ranging from 98.6% to 98.8%, and the properly paired read mapping rates were between 76.9% and 90.4%." these ranges are not exact enough for detailed examination. The exact values could be presented in the supplementary tables, such as Table S1, and discussed specifically. Also, it would be interesting to present all the details on the inconsistence revealed by mapping of mate-paired reads to the assembly, in addition to simply the mapping rate values.
Response: We thank the reviewer for pointing this out. We mapped genomic data with BWA with default parameters. Based on the k-mer analysis, we did find that the quality of 220-bp and 500-bp paired-end libraries is not high, therefore sequences from these two libraries were not appropriate for assembly evaluation. Reads from these two libraries were only used for base correction after stringent filtering on the alignments (i.e., uniquely mapped and properly paired). We have recalculated mapping statistics using sequences from the 180-bp library focusing only on anchored chromosomes and the proportion of properly mapped paired reads is 92%. We revised the text

	accordingly (Line 183-185) Moreover, we have carefully examined inconsistent chromosomal regions with the two genetic maps described in Zhang et al. (2015) (Please see Figure S2). Almost all these regions in the 'White' assembly were supported by the genetic maps. 7. Personally, I would like to know whether the authors could set out to present the functional annotation of Vitamin C biosynthesis pathway or mineral processing pathway, given the pathways are important for kiwifruit community and fruit science. Comparsion among kiwifruit species on genetic composition of such pathways may also interest broader range of readers. Response: Our manuscript was submitted as a "Data Note". Adding the functional annotation and comparative analysis of Vitamin C biosynthesis pathway or mineral processing pathway could distract the focus of the manuscript. We checked a number of genomes published recently as "Data Note" in GigaScience, none have described specific interesting pathways. However, if the editor and the reviewer still think this is necessary, we are happy to comply. 8. The absence of consecutive line numbers is making harder this review process. Please improved it in the revised version. Response: Added.
Additional Information:	
Question	**Response**
Are you submitting this manuscript to a special series or article collection?	No
Experimental design and statistics Full details of the experimental design and statistical methods used should be given in the Methods section, as detailed in our Minimum Standards Reporting Checklist. Information essential to interpreting the data presented should be made available in the figure legends. Have you included all the information requested in your manuscript?	Yes
Resources A description of all resources used, including antibodies, cell lines, animals and software tools, with enough information to allow them to be uniquely identified, should be included in the Methods section. Authors are strongly encouraged to cite Research Resource Identifiers (RRIDs) for antibodies, model organisms and tools, where possible. Have you included the information	Yes

requested as detailed in our Minimum Standards Reporting Checklist?	
Availability of data and materials All datasets and code on which the conclusions of the paper rely must be either included in your submission or deposited in publicly available repositories (where available and ethically appropriate), referencing such data using a unique identifier in the references and in the “Availability of Data and Materials” section of your manuscript. Have you have met the above requirement as detailed in our Minimum Standards Reporting Checklist?	Yes

Chromosome-scale genome assembly of kiwifruit *Actinidia eriantha* with single-molecule sequencing and chromatin interaction mapping

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Abstract

Background: Kiwifruit (*Actinidia* spp.) is a dioecious plant with fruits containing abundant vitamin C and minerals. A handful of kiwifruit species have been domesticated, among which the *A. eriantha* is increasingly favored in breeding due to its superior commercial traits. Recently, elite cultivars from *A. eriantha* have been successfully selected and further studies on their biology and breeding potential require genomic information which is currently unavailable.

Findings: Here, we assembled a chromosome-scale genome sequence of *A. eriantha* cv. 'White' using single-molecular sequencing and chromatin interaction map based scaffolding. The assembly has a total size of 690.6 Mb and an N50 of 21.7 Mb. Approximately 99% of the assembly were in 29 pseudomolecules corresponding to the 29 kiwifruit chromosomes. Forty-three percent of the *A. eriantha* genome are repetitive sequences, and the non-repetitive part encodes 42,988 protein-coding genes, of which 39,075 have homologues from other plant species or protein domains. The divergence time between *A. eriantha* and its close relative *A. chinensis* is estimated to be 3.3 million years, and after diversification, 1,727 and 1,506 gene families are expanded or contracted in *A. eriantha*, respectively.

Conclusions: We provide a high-quality reference genome for kiwifruit *A. eriantha*. This chromosome-scale genome assembly is substantially better than two published kiwifruit assemblies from *A. chinensis* in terms of genome contiguity and completeness. The availability of *A. eriantha* genome provides a valuable resource for facilitating kiwifruit breeding and the studies of kiwifruit biology.

Key words: Kiwifruit; *Actinidia eriantha*; Genome assembly; single molecular sequencing; Hi-C

Data description

Introduction

Kiwifruit is well known as the king of fruits due to its remarkably high vitamin C content and abundant minerals [1, 2]. Native to China, kiwifruit belongs to the genus *Actinidia* which contains 54 species and 75 taxa [3]. All species in this genus are perennial, deciduous and dioecious plants with a climbing or scrambling growth habit, and they also have many common morphological features including the characteristic radiating arrangement of styles of female flower and the structure of the fruit [4]. Despite rich germplasm resources in kiwifruit, only a few *Actinidia* species have been domesticated, such as *A. chinensis* var. chinensis, *A. chinensis* var. deliciosa and *A. eriantha*, whose fruit size are close to commercial standard [5-7].

Owing to its strong resistance to *Pseudomonas syringae* pv. *Actinidiae,* long shelf-life, enriched ascorbic acid and peelable skin [7-11], the *A. eriantha* (2n=58) has been favored in kiwifruit breeding. Recently, new cultivars have been selected either from the wild germplasm of *A. eriantha* such as 'White' (Fig. 1) or from the interspecific hybridization between *A. eriantha* (♂) and *A. chinensis* (♀) such as 'Jinyan' [7, 12]. The 'White' has particularly large fruits (96 g on average) with green flesh and favorable flavor and has been widely cultivated in China [7].

Actinidia eriantha has also been used for genetic and genomic studies thanks to its high efficiency in genetic transformation and relatively short phase of juvenility [13]. The flowering and fruiting of *A. eriantha* can be accomplished within two years in green house conditions with a low requirement for winter chilling [13]. In addition, roots of *A. eriantha* which contain many bioactive compounds such as triterpenes and polysaccharides are employed as a traditional Chinese medicine for the treatment of gastric carcinoma, nasopharyngeal carcinoma, breast carcinoma, and hepatitis [12, 14].

Previously, two kiwifruit genomes were published and both were from *A. chinensis* ('Hongyang' and 'Red 5') [15, 16]. These short-read based assemblies are very fragmented, possibly due to the high complexity and heterozygosity of the kiwifruit genomes as well as technical limitations. Here, we used single-molecular sequencing combined with the high-throughput chromosome conformation capture (Hi-C) technology to assemble the genome of the elite kiwifruit cultivar 'White' of *A. eriantha*. The availability of this high-quality chromosome-scale genome sequence not only provides fundamental knowledge regarding kiwifruit biology but also presents a valuable resource for kiwifruit breeding programs.

Sample collection and genome sequencing

Fresh young leaves were collected from a female individual of *A. eriantha* cv. 'White'. High molecular weight (HMW) genomic DNA was extracted using the CTAB method as described in the protocol (https://www.pacb.com/wp-content/uploads/2015/09/Shared-Protocol-Preparing-Arabidopsis-DNA-for-20-kb-SMRTbell-Libraries.pdf). To construct genomic libraries (SMRTbell libraries) for PacBio long-read sequencing, HMW genomic DNA was sheared into fragments of approximately 20 kb using a Covaris g-Tube (KBiosciences p/n520079), enzymatically repaired and converted to SMRTbell template following the Manufacturer's instruction (DNA Template Prep Kit 1.0, PacBio p/n 100-259-100). The templates were size-selected using a BluePippin (SageScience, Inc.) to enrich large DNA fragments (> 10 kb) and then sequenced on a PacBio Sequel system. A total of 9 SMRT cells were sequenced, yielding 3,889,480 million reads with a mean and median length of 10,065 and 15,661 bp, respectively, and a total of 39.1 Gb sequences, about 52.5× coverage of the kiwifruit genome with an estimated size of 745.3 Mb based on the flow cytometry analysis (Fig. S1; Table S1).

Three paired-end Illumina libraries with insert sizes of 180, 220 and 500 bp, and seven mate-pair libraries with insert sizes of 3, 4, 5, 8, 10, 15, 17 kb, were prepared using Illumina's Genomic DNA Sample Preparation kit and the Nextera Mate Pair Sample Preparation kit (Illumina, San Diego, CA), respectively. All libraries were sequenced on an Illumina HiSeq 2500 system, which yielded about 80.1 and 97.3 Gb of raw sequence data for paired-end and mate-pair libraries, respectively (Table S1). The raw Illumina paired-end reads were processed to remove duplications, adaptors and low-quality bases using Super-Deduper [17] and Trimmomatic [18] (v0.35), and the mate-pair reads were cleaned using NextClip [19] (v1.3.1) with default parameters. Finally, we obtained 76.6 and 46.2 Gb high-quality cleaned sequences for paired-end and mate-pair libraries, respectively (Table S1).

To construct the Hi-C library, 'White' plants were grown in a greenhouse, and approximately 4~6 grams young leaves were then harvested and subsequently fixed in the formaldehyde (1% v/v) for 10 min at room temperature. The fixation was terminated by adding glycine to a final concentration of 0.125M. The fixed samples were ground into powder in liquid nitrogen and then lysed with the addition of Triton X-100 to a concentration of 1% (v/v). The nuclei were isolated and prepared for Hi-C library construction according to a previously published protocol [20].

Transcriptome sequencing

To improve gene prediction, we generated transcriptome sequences from a pool of mixed tissues of 'White' including root, stem, leaf, flower, and fruits at 7, 30, 60, 90 and 120 days after anthesis. Total RNA was extracted from these tissues using an RNA extraction kit (BIOFIT, China), treated with DNase I and further purified with RNA clean kit (Promega, USA). RNA-Seq libraries were

constructed with the NEBNext® Ultra™ RNA Library Prep Kit (Illumina, USA), and sequenced on an Illumina HiSeq 2500 system using paired-end mode. A total of ~19.5 million raw read pairs were obtained, which were processed with Trimmomatic to remove adaptors. The cleaned reads were assembled *de novo* with Trinity [21] (version 2.4.0). Additionally, we also generated genome-guided assemblies with both Trinity and StringTie [22]. Different transcriptome assemblies were eventually integrated by PASA [23] (version 2.3.3) and used as transcript evidence during gene prediction process. Mapping of RNA-Seq reads to the genome assembly was performed with STAR [24] (version 020201), and read counting on the coding regions was performed with HTSeq [25] (version 0.6.0.).

Chromosome-scale assembly of the A. eriantha genome

Actinidia eriantha is a diecious plant with a heterozygous diploid genome. We estimated the heterozygosity level through the k-mer spectrum analysis with GenomeScope [26] using sequences from the paired-end library with the insert size of 180 bp. The depth distribution of the derived 17-mers clearly showed two separate peaks, based on which we estimated the heterozygosity level of the *A. eriantha* cv. 'White' genome to be 1.21% (Fig. S1).

We then estimated the genome size of *A. eriantha* 'White' using the flow cytometry analysis, with tomato (*Solanum lycopersicum* cv. Ailsa Craig) used as the reference. We also performed flow cytometry analysis on *A. chinensis* cv. Hongyang. Approximately 1 g of young leaves were washed twice in distilled water and then chopped in ice-cold lysis buffer A (10 mmol/L MgSO4, 50 mmol/L KCl, 3.5 mmol/L HEPES pH 7.5, 0.3% (v/v) Triton x-100, 2% PVP 30 (W/V)). After 5 minutes, the crude lysate was passed through a 75-μm pore size nylon mesh to remove large cellular debris. The filtrate (1 ml) was transferred to a 1.5 ml plastic tube and

centrifuged at 1000 rpm for 5 minutes. The supernatant was discard, and the nuclei were then resuspended with lysis buffer B (10 mmol/L MgSO4, 50 mmol/L KCl, 3.5 mmol/L HEPES pH 7.5, 0.3% (v/v) Triton x-100, 0.4 mg/ml Propidium Iodide, 0.04 mg/ml RNase). After 15 minutes, samples were analyzed using a FACS Vantage SE flow cytometer (Becton-Dickinson, San José, USA). Four biological replicates were performed. Based on the 950-Mb genome of tomato, the genome size of 'White' was estimated to be 745.3±7.9 Mb, similar to the genome size of *A. chinensis* (Fig. S1) and consistent with that in a previous report (758 Mb; [27]).

We employed a strategy which took into account the unique advantage of different assemblers to construct the 'White' genome using PacBio long reads. First, PacBio long reads were corrected and assembled using the Canu program [28] (v1.7), which is a modularized pipeline consisting of three primary stages - read correction, trimming and assembly. The Canu-corrected reads were also assembled independently with the wtdbg program (https://github.com/ruanjue/wtdbg), a fast assembler for long noisy reads. Subsequently, the two independent assemblies (one with Canu and another with wtdbg) were merged by Quickmerge [29] (v0.2) to improve the contiguity. The merged assembly was further processed to correct errors using Pilon [30] (version 1.22) with high-quality cleaned Illumina reads from all paired-end and mate-pair libraries representing a total genome coverage of 171× (Table S1). This yielded 2,818,370 nucleotides, 2,495,388 insertions and 1,691,495 deletions being corrected. The resulting final assembled *A. eriantha* cv. 'White' genome contained 4,076 contigs with a N50 length of 539,246 bp and a cumulative size of 690,376,929 bp (Table 1). The contiguity and completeness of this assembly far exceeds that of two published kiwifruit *A. chinensis* genomes (Table 1).

Table 1 Assembly statistics

	A. eriantha	*A. chinensis*	
	White	**Hongyang**	**red5**
Contigs			
Total contig number (#)	4,076	26,721	39,868
Total contig length (Mb)	690.4	604.2	
Contig N50 (kb)	539.2	58.9	
Contig N90 (kb)	50.7	11.6	
Longest contig length (kb)	3,260.20	423.5	
Scaffolds			
Total scaffold number (#)	1,735	7,698	3,887
Total scaffold length (Mb)	690.6	616.1	550.5
Scaffold N50 (kb)	23,583.9	646.8	623.8
Scaffold N90 (kb)	20,112.1	122.7	140.7
Longest scaffold length (Mb)	28.6	3.4	4.43
Anchored to chromosome (Mb/%)	682.4 / 98.84	452.4 / 73.4	547.9 / 98.9
Anchored with order and orientation (Mb/%)	634,4 / 91.90	333.6 / 54.1	

To scaffold the contigs based on chromatin interaction maps inferred from the Hi-C data, we first used HiC-Pro [31] to evaluate and filter the cleaned Hi-C reads. The Hi-C data usually contains a considerable part of invalid interaction read pairs which are non-informative and need to be filtered out beforehand. Among the 51 million read pairs that were uniquely aligned to the *A. eriantha* assembly, 33 million (64.1%) were valid interaction pairs and their insertion size spanned predominantly from dozens to hundreds of kilobases, therefore providing efficient information for scaffolding. As a part of error correction of the assembly, we used valid Hi-C reads to identify misassembled contigs. In principle, a genuine contig should display a continuous Hi-C interaction map whereas the discrete distribution of an interaction map likely indicates a misassembly. We examined the interaction map for each contig and broke 51 that were possibly misassembled. Subsequently, the corrected PacBio assembly was used for scaffolding with the LACHESIS program [32] and parameters "CLUSTER_MIN_RE_SITES=48, CLUSTER_MAX_LINK_DENSITY=2, CLUSTER_NONINFORMATIVE_RATIO=2, ORDER_MIN_N_RES_IN_TRUN=14, ORDER_MIN_N_RES_IN_SHREDS=15". LACHESIS

assigned 3,666 contigs with a total size of 682,355,494 bp (98.84% of the assembly) into 29 groups corresponding to the 29 kiwifruit chromosomes (Fig. 2 and 3a), among which 634,430,648 bp (91.90%) had defined order and orientation (Table 1 and S2). The final chromosome-scale assembly had a total length of 690,781,529 bp and an N50 of 23,583,865 bp.

Evaluation of the genome assembly

We first evaluated the quality of the assembled *A. eriantha* 'White' genome by mapping Illumina genomic and RNA-Seq reads to the assembly. Reads from the paired-end genomic library (with insert size of 180 bp) had very high mapping rate (98.7%), and the properly paired read mapping rate was 92.0%. For the RNA-Seq reads, 91.7% could be mapped to the genome and 87.1% were uniquely mapped. The high mapping ratio of both genomic and RNA-Seq reads suggest a high quality of the *A. eriantha* 'White' assembly.

We then identified synteny between the *A. eriantha* 'White' assembly and the assembly of *A. chinensis* 'red5' using MUMMER [33] (version 4.0.0beta2). In general, the two assemblies showed a high macro-collinearity, with only a few inconsistencies (Fig. 3b). Detailed check of the major inconsistent regions using genetic maps [34] and mate-pair read alignments confirmed the high quality of the *A. eriantha* 'White' genome assembly, and particularly enabled us to discover that in the 'red5' genome a ~8-Mb region was possibly misassembled into chromosome 23 (Fig. S2).

Repeat annotation

Repeats were annotated following a protocol described in Campbell et al [35]. The customized repeat library was built to include both known and novel repeat families. We first searched the

assembly for miniature inverted transposable elements (MITEs) using MITE-Hunter [36] with default parameters. The long terminal repeat (LTR) retrotransposons were then identified from the *A. eriantha* 'White' genome using LTRharvest and LTRdigest wrapped in the GenomeTools package [37]. The LTR identification pipeline was run iteratively to collect both recent (sequence similarity ≥99%) and old (sequence similarity ≥85%) LTR retrotransposons. Candidates from each run were filtered based on the elements typically encoded by LTR retrotransposons. The default parameters (-minlenltr 100 -maxlenltr 6000 -mindistltr 1500 -maxdistltr 25000 -mintsd 5 -maxtsd 5 -motif tgca) were used in LTR calling according to Campbell *et al*. [35]. An initial repeat masking of *A. eriantha* 'White' genome was performed with the repeat library derived by combining the identified MITEs and LTR transposons. The repeat masked genome was fed to RepeatModeler (http://www.repeatmasker.org/RepeatModeler/) to identify novel repeat families. Finally, all identified repeat sequences were combined and searched against a plant protein database where transposon encoding proteins were excluded. Elements with significant similarity to plant genes were removed. The final repeat library contained 1,670 families, and 526 of them were potentially novel repeat families. We used this species-specific repeat library to mask the *A. eriantha* 'white' genome. Approximately 43.3% of the *A. eriantha* 'White' genome was masked, and the largest family of repeats was LTR transposons (Table S3). Repeat content identified in *A. eriantha* 'White' was much higher than that in *A. chinensis* [e.g. 36% in Hongyang [15]], and this difference may be largely due to the improvement of the repeat region assembly with PacBio long reads. In addition, divergence between the two kiwifruit species could also contribute to this difference.

Prediction and functional annotation of protein-coding genes

Protein-coding genes were predicted from the repeat-masked *A. eriantha* 'White' genome with the MAKER-P program [35] (version 2.31.10), which integrates evidence from protein homology, transcripts and *ab initio* predictions. The homology-based evidence was derived by aligning proteomes from 20 plant species to the 'White' genome assembly with exonerate (v2.26.1; https://www.ebi.ac.uk/about/vertebrate-genomics/software/exonerate). SNAP [38], AUGUSTUS [39] (version 3.3), and GeneMark-ES [40] (version 4.35) were used for *ab initio* gene predictions. RNA-Seq data generated in this study were assembled *de novo* with Trinity and the assembled contigs were aligned to the 'White' genome assembly to provide transcript evidence. Predictions supported by the three different sources of evidence were finally integrated by MAKER-P, which resulted in a total of 52,514 primitive gene models. We then filtered and polished these gene models by two steps. First, we combined our RNA-Seq data with others collected from a previous study [41], and mapped the reads to the 'White' genome using the STAR program [24], and a total of 266 million read pairs were mapped. Based on the mapping, raw count for each predicted gene model was derived and then normalized to CPM (counts per million mapped read pairs). Gene models with ultra-low expression (CPM < 0.1) were less likely to be real genes. Furthermore, we found that these lowly expressed genes had relatively high annotation edit distance (AED) score, an indication of low-confidence as defined by MAKER-P program. Therefore, for gene models with CPM < 0.1, we only kept those containing both pfam domains and homologous sequences in the NCBI nr protein database. After this filtering process 42,751 gene models were kept. Second, the predicted protein-coding genes of kiwifruit *A. chinensis* 'red5' have been manually curated [16], and therefore these gene models should have relatively higher accuracy and could be used to modify *A. eriantha* 'White' gene models whose predictions were not consistently supported by the different types of evidence. To this end, we performed another two *ab initio* predictions using

BRAKER [42] and GeMoMa [43] (version 1.5.2) with 'red5' proteome as the sole evidence. These two predictions were compared with the gene models predicted by MAKER-P. Consequently, a total of 237 gene models not predicted by MAKER-P were added and another 415 gene models which had better predictions by BRAKER2 or GeMoMa were used to replace the corresponding gene models predicted by MAKER-P. Finally, we obtained a total of 42,988 protein-coding genes in the *A. eriantha* 'White' genome, with a mean coding sequence (CDS) size of 1,004 bp and containing an average of five exons.

The predicted genes were functionally annotated by blasting their protein sequences against TAIR [44], Swiss-Prot [45] and TrEMBL [46] databases with an E-value cutoff of 1e-5. Functional descriptions of the protein hits were assembled with the AHRD program (https://github.com/groupschoof/AHRD) and transferred to *A. eriantha* genes. Protein domains were identified using InterProScan [47] (version 5.29-68.0) by searching the protein sequences against domain databases including PANTHER [48], Pfam [49], SMART [50], and PROSITE [51]. The Gene Ontology (GO) terms were assigned to the *A. eriantha* 'White' predicted genes using the Blast2GO program [52] with entries from NCBI protein database and InterProScan. Collectively, 90.9% (N=39,075) of the predicted genes contain at least one annotation from the above databases (Table S4).

Evolutionary and comparative genomic analysis

To infer the divergence time between *A. eriantha* and *A. chinensis*, we identified gene orthology between the two species using MCScanX [53] and calculated synonymous substitution rate (Ks) between each orthologous pair. Three additional species, cultivated tomato (*Solanum lycopersicum*), wild tomato (*S. penellii*) and potato (*S. tuberosum*), were also included in the

analysis. The Ks distribution (Fig. 4a) suggested that the divergence between the two kiwifruit species was earlier than that between the two tomato species. We dated the divergence by assuming a strict molecular clock [54], and the time when *A. eriantha* and *A. chinensis* separated was estimated to be ~3.3 million years ago (Mya), compared to ~1.9 Mya between *S. lycopersicum* and *S. penellii* and ~6.0 Mya between *S. lycopersicum* and *S. tuberosum*. Gene family evolution was analyzed by comparing genomes of *A. eriantha*, *A. chinensis*, *S. lycopersicum*, *S. tuberosum*, *Vitis vinifera*, *Arabidopsis thaliana* and *Oryza sativa*. A total of 17,593 orthogroups were defined by OrthoFinder [55] (version 2.2.6) and among which 1,246 were single-copy gene families (Fig. 4b). The single-copy family genes were aligned and concatenated to build a species phylogenetic tree using IQ-TREE [56] (version 1.5.5) with a best-fitting model (Fig. 4c). Gene family expansion/contraction along the branches of the phylogenic tree was analyzed by CAFÉ [57] (version 4.1). Finally, a total of 1,727 and 1,506 gene families were found apparently expanded and contracted, respectively, in *A. eriantha* (Fig. 4c).

Conclusion

Here, we report a high-quality reference genome of kiwifruit *A. eriantha* cv. 'White'. The assembly from single-molecular sequencing combined with Hi-C scaffolding yielded a highly continuous and complete genome than the two previously published kiwifruit genomes. This genome will provide a valuable source for exploration of genetic basis of unique traits in kiwifruit and also facilitate the studying of sexual determination loci in the dioecious plants.

Availability of supporting data

This Whole Genome Shotgun project has been deposited at DBJ/ENA/GenBank under the accession QOVS00000000. The version described in this paper is version QOVS01000000. Raw sequencing reads have been deposited in the Sequence Read Archive (SRA) database under the accession number SRP155011. The *Actinidia eriantha* 'White' genome sequence and the annotation are also available at Kiwifruit Information Resource (http://bdg.hfut.edu.cn/kir/). Detailed protocols of computational analyses have been deposited in protocols.io: http://dx.doi.org/10.17504/protocols.io.vgse3we.

Competing interests

The authors have no competing interests to declare.

Abbreviation

Blast: Basic Local Alignment Search Tool;

CTAB: Cetyl trimethylammonium bromide;

NCBI: National Center for Biotechnology Information;

RNA-Seq: RNA sequencing;

PacBio: Pacific Biosciences;

SMRT: Single Molecule Real-Time;

Mb: megabase;

Gb: gigabase

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Author contribution

W.T., X.S. and J.Y. contributed equally to this work. W.T., J.Y., X.T., Y.Y., X.N., M.M., D.Z., S.H., W.S., C.F. and M.L. collected plant samples, extracted DNA/RNA, and performed transcriptome sequencing and gene expression analyses; W.T., X.S., J.Y., X.T., C.J., Z.F. and Y.L. performed DNA sequencing, genome assembly, gene annotation, evolution and comparative genomic analyses, and website construction; X.S., W.T., Z.F. and Y.L. wrote and revised the manuscript; Y.L. and Z.F. conceived strategies, designed experiments and managed projects. All authors read and approved the manuscript.

Figure legends

Figure 1. Tree and fruits of *A. eriantha* cv. 'White'.

Figure 2. Chromatin interaction map of *A. eriantha* derived from Hi-C data. Each group represents an individual chromosome.

Figure 3. Genome of *A. eriantha* and synteny between the two kiwifruit species. (a) Genome landscape of *A. eriantha* cv. 'White'. Track A: gene density, Track B: repeat density, Track C: GC content; all were calculated in a 500-kb window; (b) Genome synteny between *A. eriantha* cv. 'White' and *A. chinensis* 'red5'.

Figure 4. Evolutionary and comparative genomic analyses. (a) Distribution of synonymous substitution rate (Ks) between *A. eriantha* and *A. chinensis*, *S. lycopersicum* and *S. penellii*, and *S. lycopersicum* and *S. tuberosum*; (b) Orthogroups shared by selected species; (c) Species phylogenetic tree and gene family evolution. Numbers on the branch indicate counts of gene family that under either expansion (red) or contraction (green).

Figure S1. Genome characteristics of *A. eriantha and A. chinensis.* (a) Flow cytometry analyses of *A. eriantha* cv. White and *A. chinensis* cv. Hongyang. The main peak (I) indicates G0/G1 cells and the secondary peak (II) represents G2/M cells. (b) Flow cytometry analyses of *A. eriantha* 'White' and *Solanum lycopersicum* cv. Ailsa Craig. Peaks a and b represent the G0/G1 cells of "White" and 'Ailsa Craig', respectively. The genome size of 'White' was estimated to be 745.3±7.9 Mb using 'Ailsa Craig' as the reference. (c) 17-mer distribution of 'White' genomic reads (180bp paired-end library).

Figure S2. Examination of assembly inconsistencies between *A. eriantha* cv. 'White' and *A. chinensis* cv. 'red5'. (a) Validation of genome assembly of 'White' using genetic maps. Horizontal lines within "White" chromosomes indicate gapped regions and lines between chromosomes of

two assemblies indicate syntenic regions. (b) A chromosomal segment assembled into the Chr23 in *A. chinenesis* "red5", is syntenic to the region located at the terminus of Chr19 in *A. eriantha* cv. 'White'. (c) Snapshots of Illumina mate-pair reads mapped to the junctions of the break point as well as nearby regions supporting the assembly of 'White'.

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Figure 1

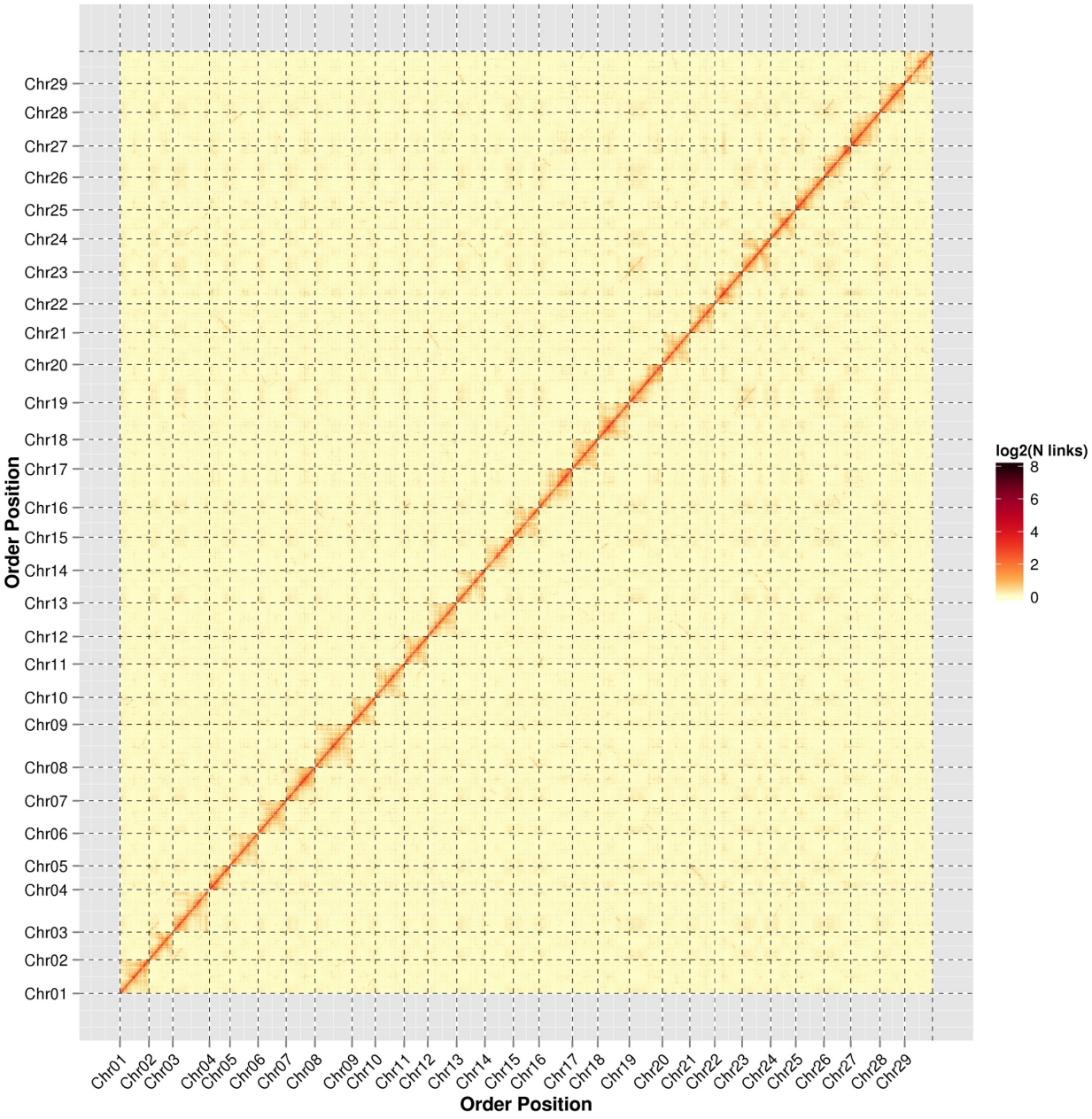
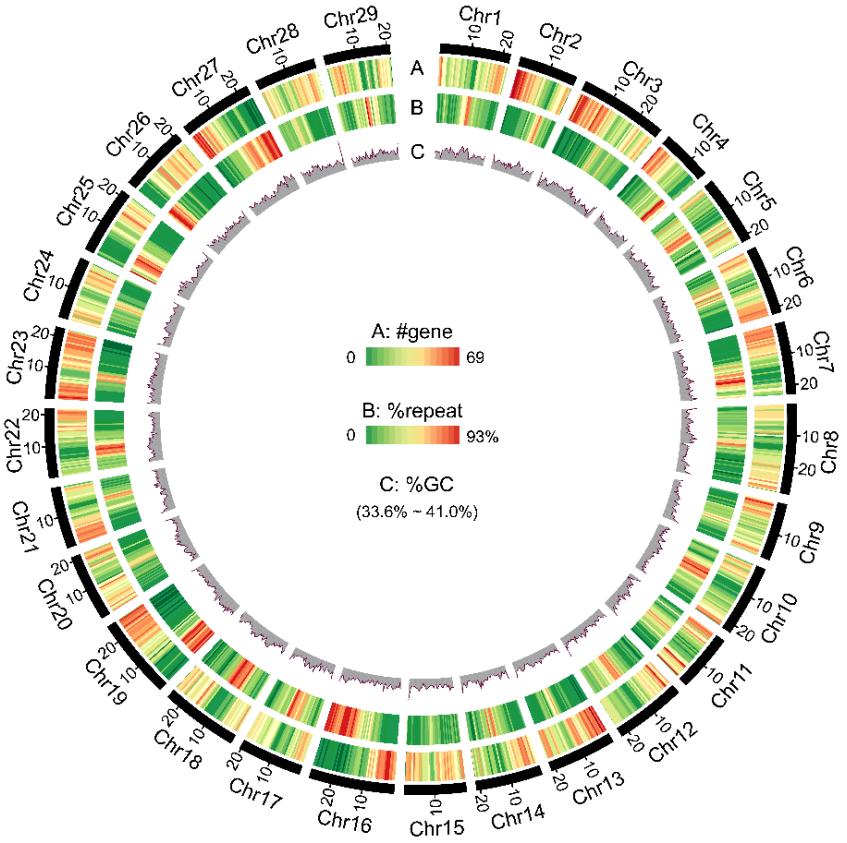


Figure 2.

a



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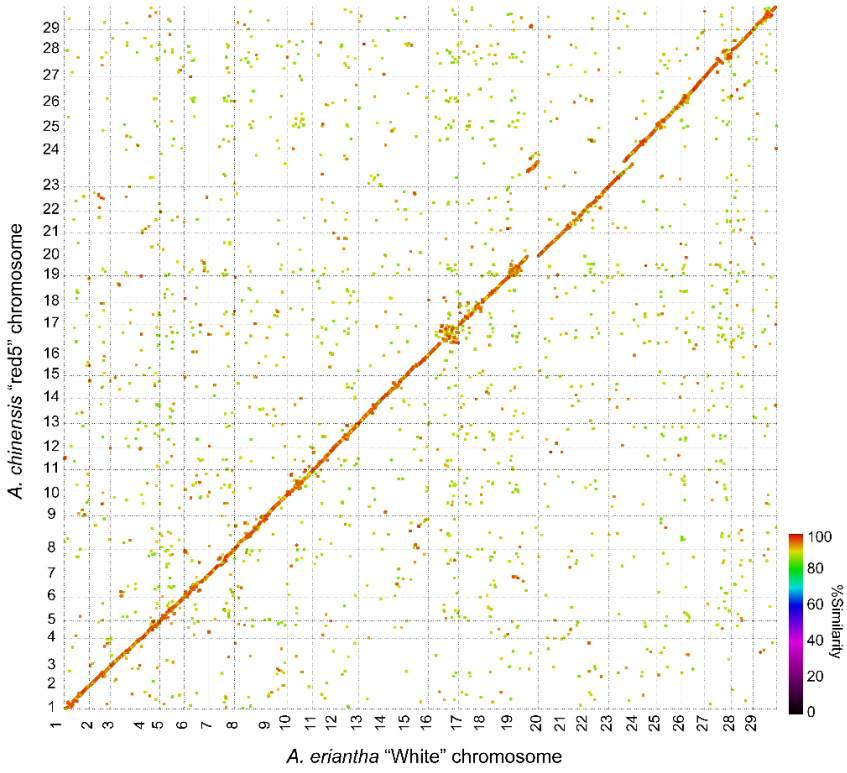
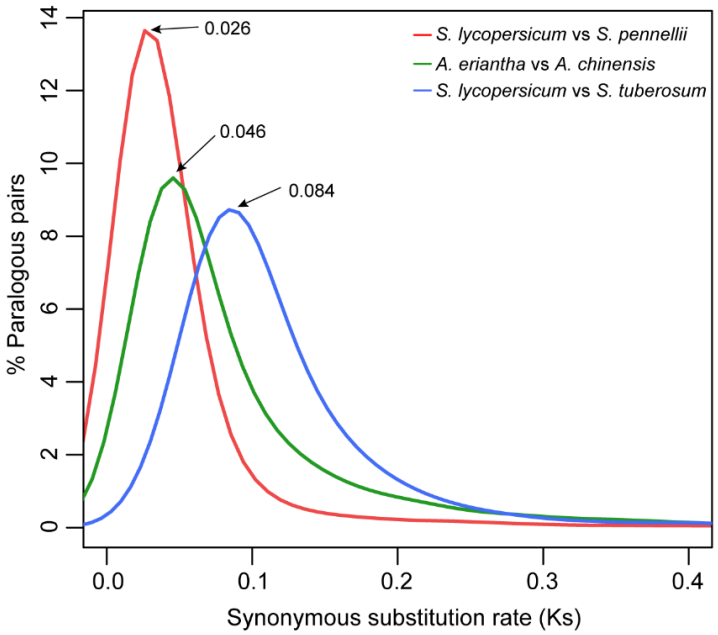


Figure 3

a



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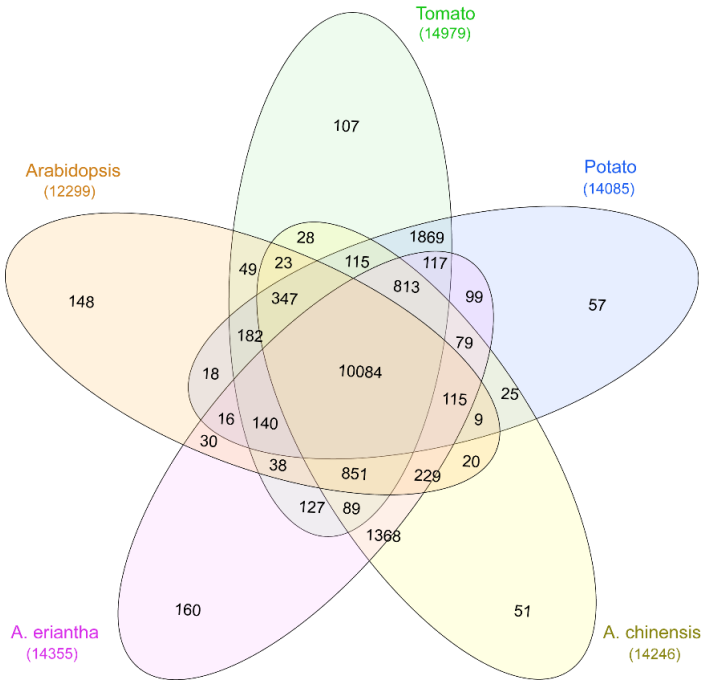

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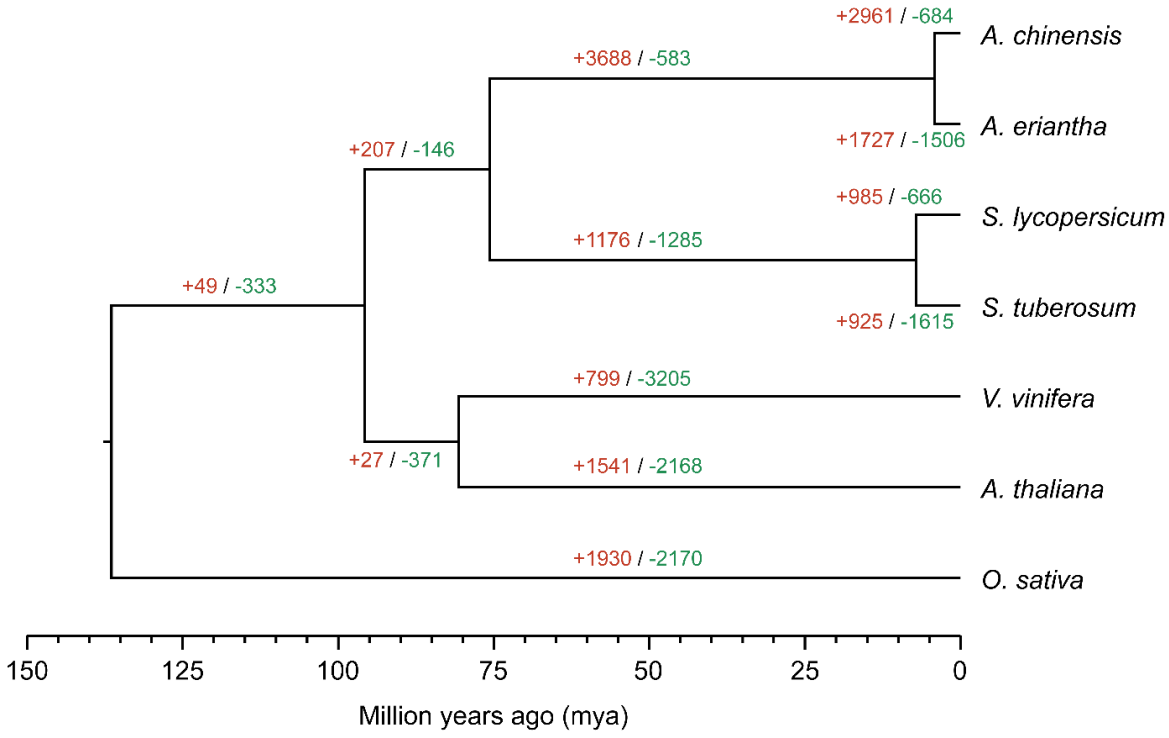

Figure 4

Supplementary Figures

Supplementary Tables

November 12, 2018

Dear Dr. Hongling Zhou

Thank you for your time and effort in handling our manuscript titled “Chromosome-scale genome assembly of kiwifruit *Actinidia eriantha* with single-molecule sequencing and chromatin conformation capture” (manuscript reference number: GIGA-D-18-00282). We have revised our manuscript in light of the detailed and helpful comments of the reviewers. We highlighted major changes in the revised manuscript with red text.

We have attached our detailed responses to the specific comments of the reviewers. We hope that we have addressed the reviewers’ concerns and ask that you please consider this revised manuscript for publication in GigaScience.

Sincerely,

Zhangjun Fei