

# The giant genome of lily provides insights into the hybridization of cultivated lilies

Corresponding Author: Professor Liangsheng Zhang

**This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.**

**Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.**

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

In this manuscript, the authors presented two Liliales genomes, *Lilium sargentiae* (lily, 47.09G) and *Gloriosa superba* (flame lily, 5.15 Gb) in the Liliaceae and Colchicaceae family, respectively. As expected, the extra-large size of *L. sargentiae* is mainly due to the substantial activity of LTR-RTs. The authors suggest lily and flame lily each experienced a WGD event after differentiation. Using transcriptome sequencing of many 51 wild *Lilium* species and 34 lily cultivars, the authors investigated the phylogeny of *Lilium* species and origins of lily cultivars. They also compared genome and transcriptome of lily and flame lily to analyze the genes involved in the formation of bulbs and rhizomes, flower development and growth transition of bulbs. Finally, the authors discussed the origin, evolution, and function of colchicine in flame lily.

I read the paper with interest and have some concerns/comments that, if addressed, would strengthen the manuscript.

1. The authors should provide direct evidence or reference for the genome sizes of the two species in Figure 1D and Supplementary Data 5 which are 35.66 Gb and 5.15 Gb, respectively.

2. I am a little concerned about the quality of the two genomes. Both HiFi and Hi-C sequencing depths of lily are low, only 16.88X (602.05/35.66) and 30X (1070/35.66), respectively. The assembled contig N50 is only 0.93 Mb. In addition, the heterozygosity of this species is very high (3.4%). With high level of heterozygosity, inadequate sequencing depth will lead to low integrity and base quality of the final genome assembly. Similarly, the contig N50 of flame lily (HiFi reads, 82.50/5.15=16X) is only 0.48 Mb. And there is no evaluation of genome integrity and accuracy in the manuscript. It is recommended to supplement sufficient sequencing depth to improve genome integrity and accuracy.

3. The lily assembly is 47.09 Gb, which is 1.32 times of 35.66 Gb. This is most likely because the assembly contains two haplotypes. Although the authors performed "sequence dehybridization" (L721), the effect was obviously not good.

4. L143. "A phylogenomic analysis of 26 flowering plant species from 18 orders, including 9 monocot orders, revealed that *L. sargentiae* and *G. superba* from Liliales are sisters to the Asparagales species (Figure 1A)."

L752. "The species phylogenetic tree of 26 plant species including *L. sargentiae* and *G. superba* was constructed by 893 single-copy homologous genes obtained by BUSCO (Benchmarking Universal Single-Copy Orthologs) (Simão et al., 2015)."

The description of the phylogenetic tree results and methods is too brief and there is no discussion and comparison with previous phylogenetic analysis in literature.

5. The author inferred from the results of the Ks and self-synteny dotplot that lily had two WGDs, one of which was the  $\tau$  event ( $Ks=0.92$ ) and the other was the recent  $Ks=0.73$ . However, as mentioned above, it is very likely that a large part of the assembled genome is diploid, i.e. duplication and this duplication have a low similarity due to high heterozygosity, resulting in  $Ks=0.73$ . This can also be inferred from the self-synteny diagram of the two species that although the Ks peak (0.55) corresponding to the most recent WGD of flame lily is smaller than that of lily, the blocks of lily are more complete. And the

blocks that marked to be 1 of lily all span at least one complete scaffold, which is most likely because diploid chromosomes or scaffolds were assembled.

6. L228, “orthologous” should be paralogous.

7. Figure S3, the results of inter-species Ks distribution show that the Ks distribution between Gsu, *Gloriosa superba*; Lsa, *Lilium sargentiae*; Egu, *Elaeis guineensis* is bimodal, indicating that the three species experienced a common WGD event before divergence, namely the  $\tau$  event, where the corresponding Ks=1.5, and the divergence peak should be the recent peak, ie. Lsa-Gsu: Ks=1.0, Egu-Lsa: Ks=1.2 (Figure S3, Ata should be Aco). The species divergence peak Ks (1.0 or 1.2) calculated by the authors is greater than the Ks peak value of the  $\tau$  event (0.92) inferred by the previous paralogous gene pairs, and this needs to be corrected by the evolutionary rate (<https://doi.org/10.1093/bioinformatics/btab602>).

8. Figure S7, the legends A and B are reversed.

9. L191, “The average intron lengths in *L. sargentiae* and *G. superba* reached 8.8 kb and 4.7 kb, respectively, both are substantially larger than that (0.5 kb) in most angiosperms which have smaller genomes (Niu et al., 2022) (Tables S7 and S8 and Supplementary Data 4)”.

The referenced Tables S7 and S8 should be Tables S5 and S6, and Supplementary Data 4 does not contain the average intron length data.

10. L217, “Interestingly, the ratios of the number of solo-LTRs to that of intact-LTRs in *L. sargentiae* (2.6) and *G. superba* (3.9) were both higher than those in other angiosperms, but similar to those in the examined gymnosperms with expansion of ancient LTR-RT bursts (10-30 Mya), suggesting that the LTR removal rates are higher in large genomes driven by LTR-RT expansion (Figure 1D and Supplementary Data 5)”.

The Solo LTR number/intact LTR number of conifers in supplementary Data 5 ranges from 0.13 to 0.27, which does not support the conclusion. And those data were missing in Figure 1D with no explanation.

11. On the transcriptome phylogenomic analysis of wild species and cultivars, the authors stated in the abstract L48 that “numerous wild lilies have not contributed to cultivated lily breeding”. This seems a true but useless statement for the breeding in most plant and animal groups. And the presented data can’t support this statement because only transcriptome data rather than whole genome data have been analyzed.

“B” is missing in L285 for legend of Figure 2.

For Figure 2B, the heatmap should be symmetrical along the diagonal line. But it’s not for many parts. Why?

The signal between *L. brownii* with almost all wild species and cultivars seem very unbelievable. Please check this out.

12. In Figure 3G and Figure S19, the cartoons for the models are over-simplified. At least, functional roles of those important genes should be indicated in the drawings.

13. L463, please add citations of genes related to colchicine synthesis.

14. L464, Figure 4C should be Figure 5C.

15. The Figure S27 is not easy to understand. How do we interpret the “Traits” in the module-trait analysis? Why was the magenta module selected for network display? Is there statistical test to confirm confidence of the modules? Similarly, the figure S23 should also be improved.

16. The total ion chromatograms (TICs) of lily in Figure 5A are inconsistent with those in Figure S31.

## Reviewer #2

### (Remarks to the Author)

The manuscript titled “The giant genome of lily provides insights into the hybridization of cultivated lilies” by Liang et al. presents the first genome assemblies and analyses from the Liliales order, focusing on *Lilium sargentiae* and *Gloriosa superba*. This study fills in an essential evolutionary gap by providing significant genomic data to in depth understanding the formation and evolution of eukaryotic giant genomes, given that this manuscript explored the genome architecture, compare the differences in genome sizes between two Liliales species, and elucidated the reasons for genome enlargement. Based on genomic data, the phylogenetic relationships, putative hybridization events between wild and cultivated *Lilium* species were investigated. Additionally, the genetic mechanisms underling the formation of lineage-specific morphological traits of the genus *Lilium* were elucidated, and the evolutionary trajectory of biosynthetic pathway of colchicine in *Gloriosa superba* was explored. Overall, the findings of this study provide empirical evidence for the formation and evolution of giant eukaryotic genomes. Additionally, this study provides essential genomic resources for the exploration of the germplasms of *Lilium* species as well as for expedition of breeding efforts. Accordingly, the manuscript represents a significant study. Further detailed information and analyses are necessary to strengthen the impact and reproducibility of this study. Below are

my specific comments and suggestions:

#### Major concerns

1. Did the stem lineage ancestor of extant monocots undergo whole genome duplication events? Please clarify this issue based on syntenic analysis and Ks data.
2. The manuscript would benefit from details regarding the quality evaluation of genome assembly and annotation for each species.
3. Given the previous findings indicating potential recent radiative speciation in the genus *Lilium* since the Neocene, it is plausible that the similarity of transcripts among extant species could be attributed to incomplete lineage sorting of polyphyletic alleles rather than gene flow. Accordingly, I would like to suggest the author to carry out reticulate evolution analysis to identify potential hybridization events among *Lilium* species, using transcriptomic data.
4. Regarding the genome part, the genome size of *L. sargentiae* is 9 times that of the *G. superba* (L132-L133), but such a huge difference is not shown in the WGD events and LTR parts. Although it demonstrates that the LTR burst event led to gene enlargement, the proportion of gene enlargement is around 5 times (L139). Is the genome size here the actual size of *L. sargentiae*?
5. In the analysis of the *Lilium* genus, the authors reconstructed the phylogenetic tree using transcriptome data from 85 transcriptomes of the *Lilium* genus. How were the 360 genes (L239) chosen here? If WGS data is used here, can the same classification be observed? In routine analysis, we often use WGS to analyze gene flow between different subspecies. In this part, an excellent analysis shows the genetic diversity of hybrid commercial varieties (Figure 2C), but the description here is only one sentence (L274-275). Please describe the conclusions and analysis methods in detail.
6. Language improvements are necessary throughout the manuscript and some grammatical errors should be corrected, to improve the readability and grammatical accuracy of the manuscript.
7. Detailed information should be provided within the section "Methods" to enhance the repeatability of this study.
8. More details should be provided in the methods. For example, I don't see the methods and cutoff of GO enrichment analyses.

#### Special comments:

Line 64 : In relation to the possession of extensive genomes in *Lilium* species, it is essential to included pertinent references in this sentence.

Line 128: The genomic heterozygosity of the two species were 3.4% to 0.7%. It is necessary to describe the methodology for evaluation this genomic traits within the section "methods".

Line 394-395: The assertion that the species of Liliales exhibit undifferentiated petals and sepals is problematic, as some families within the Liliales order, such as Melanthiaceae, possess distinctive sepals and petals.

Line 524: The subterranean organ of *Salvia miltiorrhiza* is a taproot, rather than rhizome or bulb.

Line 537-677: Within the references, the botanic names of all genu- and species-level taxonomic units should be italicized.

Line 684-689: It is critical to associate the individual used for sequencing (especially those used for RNA-seq) with a voucher specimen deposited in a recognized herbarium. This practice ensures that the plant material can be accurately identified and verified in future studies.

Line 693-697: For PacBio sequencing, the size of fragments selected for sequencing should be described in detail.

Line 698-706: Please provide details on the number of PCR cycles for the preparation of the Hi-C library.

Line 710-716: Please specify which organs have been sample for constructing the cDNA library for each species.

Although Figure 2 is very informative, it needs to be further adjusted. For example, in Figure 2C, the different lily varieties can be labeled more clearly, and figure resolution should be improved to meet publishable standards. Additionally, I would suggest the authors to improve the readability of the majority supplementary figures.

#### Reviewer #3

##### (Remarks to the Author)

In this manuscript, the authors determined the genome sequences of lily and gloriosa. They analyzed the phylogeny of *Lilium* species and lily cultivars. Also, they compared the structure and expression of the genes related to bulb/rhizome formation, flower development, and growth transition of bulbs. Although there are many interesting data in this manuscript but the authors did not discuss well in the discussion part. In the flower development section, their results are not sufficient and there are many mistakes. Therefore, they need to address all the comments before publication.

#### Major comments

L.393-409:

The authors analyzed the expression of floral organ identity genes in lily and gloriosa here. These genes have been already isolated and characterized from *Lilium* species as below. So, after adding the reported lily genes for their phylogenetic tree, the authors should compare their results and the results previously reported. Also, the authors should show the developmental stage of the flower they used in this study, because the expression patterns of floral organ identity genes change during flower development.

##### 1) Class A gene

The authors used AGL6-like genes for class A genes, but this is wrong. AGL6-like genes are related to SEP-like genes (class E). Class A genes are AP1-like genes.

##### 2) Class B genes

Lily has two type of PI-like genes but the C-terminal region of these genes are very different; one type has PI-motif but the others does not (Journal of Experimental Botany 63:941–961, 2012). Since gene duplication of lily PI-like genes occurred after divergence between lily and tulip, LsaPIa-GsuPIa and LsaPIb-GsuPIb are not orthologs, respectively. The authors should compare the structures of PI-like genes and discuss about it.

### 3) Class C genes

In general, class C genes are called as AGAMOUS-like genes. There are two types of AG-like genes in lily and Arabidopsis; one is AG/PLE-like (class C) and the other is STK-like (class D). These AG-like genes have different C-terminal region at the end of the protein and the expression patterns of these are different (Plant Sci. 241:266–276, 2015). In figure S21, the authors showed the phylogenetic relationship of AG-like genes from lily and gloriosa. This result showed that two lily AG genes are far related but two gloriosa AG genes are closely related. This indicates that two lily AG genes seem to belong to different clade but two gloriosa AG genes do to the same clade. The authors should show the relationship of lily and gloriosa AG genes and discuss about the expression patterns of these genes.

### 4) Class E genes

Lily has two type of SEP-like (class E) genes. SEP-like genes were previously reported as AGL2 genes and two lily SEP-like genes has been already isolated and characterized (Plant Physiol 133:1091-1101, 2003). These two lily SEP-like genes are far related in the previous report, while the two lily genes in this manuscript is closely related (figure S21). This is likely that the authors analyzed one type of SEP-like genes in lily. The authors should show the relationship of lily and gloriosa SEP-like genes and discuss about the expression patterns of these genes.

### 5) A new ABCE model

Lily has two whorls of petaloid organs and this is explained by the expansion of class B gene expression. This new floral model is called as modified ABC model (Flower. Newslett. 16: 33–38, 1993; Plant Mol. Biol. 52: 831–841, 2003) or modified ABCE model (Hortic. J. 85:8–22, 2016; Trend. Plant Sci. 22: 8-10, 2017). The important point is the expression of class B genes in whorls 1 and 2 because this can show the expansion of class B genes in whorl 1. The authors mentioned that “Based on these results, we propose a new ABCE model applicable to the petaloid Liliales” (L. 408-409), but their results did not support the expansion of class B genes because they mixed the expression of these genes in whorls 1 and 2. If the authors want to say this sentence, they should divide outer and inner tepals for expression analysis.

L.507-534, Discussion:

Discussion part seems to be summary. The authors need to go into more detail and depth in discussion.

### Minor comments

L.90-91, 394-395:

Tepal is one of the component parts of the perianth. When the perianth is divided into two whorls of unequal appearance, we call sepal and petal instead of outer tepal and inner tepal, respectively.

L.110:

Here the authors mentioned the reason why they used gloriosa. Since they compared lily and gloriosa genome in this manuscript, they should add the reason from a systematic point of view.

L.252:

Figure 2A, D -> Figure 2A? Figure 2D is missing.

L.252-255:

“The results indicate that these wild lilies in Clade 2-1 might not have been widely used in the past lily breeding programs, highlighting a previously untapped genetic reservoir, which may offer immense potential for future breeding endeavors.”

->Are there any reason why Clade2-1 were not used for lily breeding to date? If any, the authors should discuss about that in discussion.

L.285:

(B) is missing in this legend. Add (B) just before “Heatmap”.

Figure 3G:

YABB1 -> YABBY1

L.397:

the floral identity genes -> the floral organ identity genes

L.397-407:

“MADS” is not a gene name and does not need to be italicized.

L.464:

Figure 4C -> Figure 5C

L.536: References

There are so many mistakes.

For example,

Adam, P., Du, Y.-p., Wei, C., Wang, Z.-x., Li, S., He, H.-b., and Jia, G.-x. (2014). *Lilium* spp. pollen in China (Liliaceae): Taxonomic and Phylogenetic Implications and Pollen Evolution Related to Environmental Conditions. PLoS ONE 9.

->Author is wrong. Adam is editor's name and this paper is Du et al.

->Upper and lower case letters are mixed in the title.

->Pages are missing.

Cantalapiedra et al. also include the editor name in the authors.  
The style of journal abbreviations is not unified.  
The authors should check all the references very carefully.

#### Reviewer #4

##### (Remarks to the Author)

The manuscript "The giant genome of lily provides insights into the hybridization of 2 cultivated lilies", by Liang et al. provides long-awaited data about lily genomes. Key results include insights into the structure and the evolution of the lily genomes, into the genetic composition of lily hybrids and important data related to the molecular control of major mechanisms, such as bulblet initiation and floral transition. This research brings about the availability of reference genome sequences for two lily species, providing essential molecular tools in lily, which were clearly lacking. This paper can be considered as a pioneer work, with capital significance in the field. The availability of genomic reference sequences in lily will enable further in-depth studies in this genus, hopefully leading to the elucidation of important mechanisms. These data will greatly contribute to advances not only in lily research, breeding and utilization, but also in other geophytes. The bioinformatic data are outside of my scope, so I will refer to other aspects of the manuscript.

##### Specific comments

1. Please refer also to the remarks within the text (attached file)
2. *L. longiflorum* was not part of this study and was not mentioned in any way, despite of its importance in the industry and breeding. Can the authors comment on this?
3. For such an integrative and important study, the discussion is rather limited. It also totally lacks reference to the part about colchicine part, which constitutes a significant portion of the study. The quality of the manuscript would be much improved by a more structured and in-depth discussion, which could include sub-sections for clarity.
4. For the qRT-PCR analyses, please give more details about the tissues from which RNA was extracted and about the and the developmental stage of the plant at sampling time.

#### Reviewer #5

##### (Remarks to the Author)

This manuscript entitled "The giant genome of lily provides insights into the hybridization of cultivated lilies" by Liang et al. reports the reference genome sequences of *Lilium sargentiae* and *Gloriosa superba*. The expansion of LTR-RTs was found to be responsible for the giant genome of lilies. However, it is plausible that genes related to sucrose and starch metabolism play important roles in the bulblet initiation and expansion as well as rhizome development. The detailed concerns are listed as followings.

##### Main concerns

This implies that the expansion of LTR-RTs—besides genome size expansion—might also have influenced the evolution and structure of genes underlying crucial bulb-related traits, including bulb formation as well as growth transition. This conclusion was solely based on a GO-term associated with bulblet initiation and development, which was detected for *L. sargentiae* and *G. superba*. However, the storage and propagation organs of *G. superba* are rhizomes, rather than bulbs. Thus, it is unsure if this conclusion is reasonable.

Along with their proliferation, LTR-RTs are also actively removed through ectopic homologous recombination, which also generates solo-LTRs. Is there any evidence to support this finding?

Lines 304-306, a literature (Li et al., 2014) was cited to indicate that genes involved in starch and sucrose metabolism play important roles in the initiation and expansion of storage organs. However, this citation is not correct because the main purpose of the literature is to investigate gene expression profiles during bulblet formation based on transcriptome analysis. The results of the literature cannot point out any roles of starch/sucrose related genes in bulb initiation and expansion. Therefore, subsequent analyses are not scientific at all. And the related finding that the XTH genes play crucial roles in the bulblet initiation and expansion lacks solid evidence.

Weighted gene co-expression network analysis (WGCNA) was used to identify key genes responsible for bulblet development. The method can be conducted, but may be not suitable in high-ranked journals. Of course, the results are not reliable and more evidence such as genetic evidence must be provided to validate the results. Lily bulb size varies greatly and candidate gene association analysis can be conducted to validate the role of candidate genes in bulb formation.

It is well known that GBSS genes are responsible for amylose synthesis. However, *GsuGBSS1* was assumed to regulate rhizome development. This finding is unusual. More solid evidence such as genetic results should be provided.

Figure 3G, too many genes are listed here and the results are not reliable because these genes are mainly identified solely based on bioinformatics analysis.

Line 408, we propose a new ABCE model applicable to the petaloid Liliales. I don't think there is any new findings related to flower development in this manuscript because all the genes were estimated based on the previously reported ABCE models

in model plants.

For the evolution of colchicine in flame lily, it is nuclear which genes are responsible for the biosynthesis of colchicine although many kinds of OMT genes are described in the manuscript. Is there any explanation why the keys responsible for colchicine biosynthesis have been lost in *L. sargentiae*.

Others

Line 60, colchicine was not mentioned in the abstract.

Assembly of the *G. superba* genome resulted in 11 chromosomes, while 35 scaffolds were generated for the *L. sargentiae*. Is *G. superba* a diploid species? What is the ploidy level for *L. sargentiae*?

Lines 204-206, it is difficult to understand this sentence.

Line 265, Wild lilies of *Sinomatagon* 5b and 5a wild lilies. "wild lilies" appears twice, correct?

Figure 2, this picture is very fuzzy and the legend for figure 2B could not be found as well.

Line 464, change figure 4c to figure 5c.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have properly answered most of my previous queries. However, there are still several points that require further improvement.

1. K-mer analysis provided an estimation of genome size. But usually, plant tissue is analyzed by flow-cytometry to directly measure the nuclear DNA content of the plant cells. It would be more convincing if the authors could provide those data. And I also noticed that the DNA content (1C) of *L. sargentiae* has been estimated to be 60 pg by flow cytometry (Du, Yun-peng, et al. 2017), which is much larger than the size estimated based on the k-mer method in this manuscript. (Du Y, Bi Y, Zhang M, et al. Genome size diversity in *Lilium* (Liliaceae) is correlated with karyotype and environmental traits[J]. *Frontiers in plant science*, 2017, 8: 1303.)

2. It is also routine to provide Hi-C interaction heatmap when the assembled contigs were anchored to the chromosomes. This data could show the quality of the chromosome level assembly.

3. BUSCO is used to assess the completeness of the coding parts of the genome. However, a direct assessment of the whole genome quality would be Merqury's evaluation of QV and completeness.

4. Figure S2. The sizes of the scaffolds in the figure do not correspond to those in Table S2. For example, Scaffold3 and Scaffold15 are 3.55 Gb and 3.79 Gb, respectively, but Scaffold3 in Figure S2 is only half the size of Scaffold15.

Reviewer #2

(Remarks to the Author)

The revised manuscript has been undergone a comprehensive review, and it is evident that the concerns previously raised by both myself and other two reviewers have been satisfactorily addressed and improved. I do not have further comments.

Reviewer #3

(Remarks to the Author)

This manuscript is the revised version. The authors addressed many of my comments, but I still have two comments about floral organ identity genes.

P.432-434, Fig. S22C: AG-like genes

The authors mentioned only class C genes here. The phylogenetic tree showed that two lily AG-like genes were a little far related. Since there are two types of AG-like genes, i.e., class C and class D genes, in lily, they may be class C and class D genes. Differences between class C and class D genes in lily were well analyzed in a previous study (J. Hort. Sci. Biotech. 83:453-461, 2008), the authors should add lily class C/D genes in their phylogenetic analysis. Also, monocot class D genes have "MD motif" at the C-terminal region, so it is not difficult to distinguish class C and class D genes.

Figure 4: Expression of floral ABCE genes

Although the expression patterns of class B genes cannot explain the expansion of class B gene expression in outer tepals, the original figures (fig. 4 A and B) which showed the expression patterns of floral ABCE genes was very helpful to understand. I recommend to add these figures in the main manuscript or in a supplemental file.

Reviewer #4

(Remarks to the Author)  
[see attachment]

Reviewer #5

(Remarks to the Author)  
Lily bulb size varies greatly. It will be much better if candidate gene association analysis is conducted to validate the role of candidate genes in bulb formation, such as XTH and GBSS1.

GsuGBSS1 was assumed to play an important role in rhizome development. Why not include in figure 3G?

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)  
The authors have largely addressed my concerns. I have no more question.

Reviewer #3

(Remarks to the Author)  
This manuscript is the revised version. I found one mistake and I still have one comment about AG-like gene in lily.

L.434

We identified a total of four class A, three class B, two class C, and three class E MADS genes in *L. sargentiae*, and two class A, three class B, two class C, and two class E MADS genes in *G. superba* (Figures S22).

-> The authors isolated two AG genes from lily. The results of the gene phylogenetic analysis indicate that LsaAGb is very homologous to LMADS10, which has been isolated previously, but it is questionable whether LsaAGa is homologous to AG genes from other Asparagales plants. Expression results for this gene (Fig. S23A) showed that its expression have not been detected in any organs of lily. Based on these results, it is doubtful that the LsaAGa gene is a Class C gene in lily. Do the authors have any other evidence to show that LsaAGa is lily AG gene?

L.455

LsaAGa in lily and two AG genes in flame lily almost exhibited no expressions in tepals, but those showed high levels of expression in the reproductive organs (Figure S23).

-> It seems that LsaAGa is a mistake for LsaAGb.

Reviewer #5

(Remarks to the Author)  
This revision is suitable for publication.

Version 3:

Reviewer comments:

Reviewer #3

(Remarks to the Author)  
The authors have addressed my concerns.  
This revision is suitable for publication.

**Open Access** This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

Here are point-to-point responses to the reviewers' comments.

### Point to point responses for reviewer #1

**Comment 1.** The authors should provide direct evidence or reference for the genome sizes of the two species in Figure 1D and Supplementary Data 5 which are 35.66 Gb and 5.15 Gb, respectively.

**Response:** Thank you for this comment. We have now added the results (**L127-130, Figure S1**) and methods (**L951-958**) of the *K*-mer analysis to provide direct evidence for the estimated genome sizes of lily and flame lily.

**L127-130:** The genome size of *L. sargentiae* and *G. superba* were estimated to be ~35.66 Gb and ~5.09 Gb, respectively, with heterozygosity calculated as 3.35% and 0.70%, respectively (Figure S1).

### **L951-958:** Genome size and heterozygosity estimation

Genome size and heterozygosity were estimated by *K*-mer frequency distribution analysis. Initially, the short reads were filtered using fastp (v0.19.4) with default settings. *K*-mers were then counted using Jellyfish (v2.2.10) with the parameters “-C -m 17 -s 1G -t 56”<sup>67, 68</sup>. The resulting output file was subsequently used as input for GenomeScope<sup>67</sup>, which estimated the genome size and heterozygosity using default parameters. The 17-mer frequency distribution was analyzed based on genome characteristics, considering the pattern of Poisson distribution.

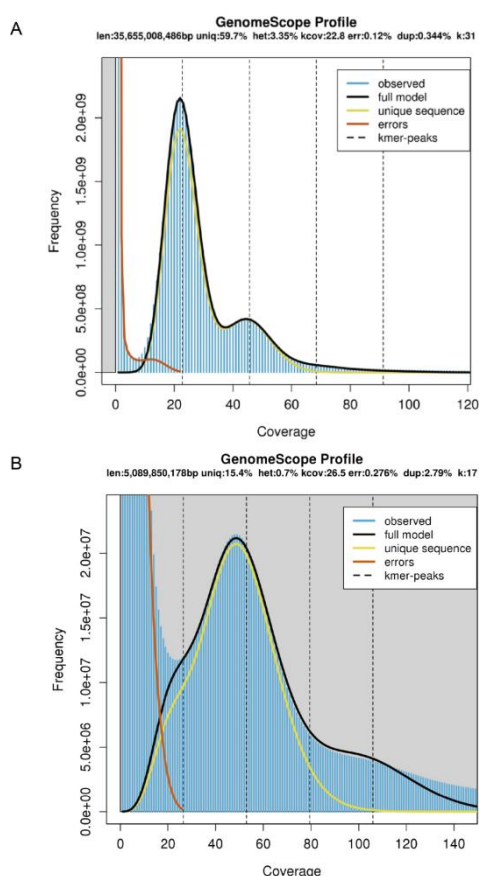


Figure S1. *K*-mer analysis of the *Lilium sargentiae* (A) and *Gloriosa superba* genome (B).

**Comment 2.** I am a little concerned about the quality of the two genomes. Both HiFi and

Hi-C sequencing depths of lily are low, only 16.88X (602.05/35.66) and 30X (1070/35.66), respectively. The assembled contig N50 is only 0.93 Mb. In addition, the heterozygosity of this species is very high (3.4%). With high level of heterozygosity, inadequate sequencing depth will lead to low integrity and base quality of the final genome assembly. Similarly, the contig N50 of flame lily (HiFi reads, 82.50/5.15=16X) is only 0.48 Mb. And there is no evaluation of genome integrity and accuracy in the manuscript. It is recommended to supplement sufficient sequencing depth to improve genome integrity and accuracy.

**Response:** Thanks a lot for this comment. We initially conducted quality assessment of the two genomes, but the article was very content-heavy. As a result, we streamlined it and removed the quality assessment section in the original manuscript. However, we've actually obtained the datasets sufficient for achieving high-quality genome assemblies. The mapping rates of WGS and RNA-seq data for the lily genome reached 97.2 % and 96.6%, respectively (Supplementary Data 1). Moreover, the mapping rate of RNA-seq data for the flame lily genome was 90.48 % (Supplementary Data 2). Additionally, our BUSCO analysis results could also support the high quality of our genomes.

In the revised version, we included the results of BUSCO analyses (**L139-143**), which support the high quality of our genome assemblies: **BUSCO <sup>17</sup> analysis of the *L. sargentiae* and *G. superba* genome revealed the complete gene information at 90.8% and 91.8%, respectively; BUSCO analysis of the *L. sargentiae* and *G. superba* protein sets revealed the complete gene information at 88.3% and 85.9%, respectively (Tables S5 and S6).** These mentioned limitations are mostly related to continuity. For biological questions examined in this study, genome completeness (e.g., BUSCO) was more critical than continuity metrics (e.g., N50). The gene contents of both genomes are still largely complete and thus provide a solid foundation for current and future investigations.

**TableS5. BUSCO evaluation of the genomic completeness of *Lilium sargentiae*.**

Type	Genome	%	Protein	%
Complete BUSCOs (C)	1465	90.77%	1425	88.30%
Complete and single-copy BUSCOs (S)	865	53.59%	682	42.30%
Complete and duplicated BUSCOs (D)	600	37.17%	743	46.00%
Fragmented BUSCOs (F)	41	2.54%	56	3.50%
Missing BUSCOs (M)	108	6.69%	133	8.20%
Total BUSCO groups searched	1614		1614	

**TableS6. BUSCO evaluation of the genomic completeness of *Gloriosa superba*.**

Type	Genome	%	Protein	%
Complete BUSCOs (C)	1481	91.76%	1387	85.90%
Complete and single-copy BUSCOs (S)	1411	87.42%	1309	81.10%
Complete and duplicated BUSCOs (D)	70	4.34%	78	4.80%
Fragmented BUSCOs (F)	52	3.22%	107	6.60%
Missing BUSCOs (M)	81	5.02%	120	7.50%
Total BUSCO groups searched	1614		1614	

HiFi data (CCS reads) is known for its high-quality sequencing, and typically, even 5x

to 10x coverages are sufficient for genome assembly. For instance, a recent study achieved a well-assembled draft diploid goatgrass (DD genome) using CCS reads at 6.6x coverage. The diploid goatgrass assembly consisted of 36,983 contigs with a contig N50 of 0.164 Mb. A total of 316,038 structural variants (SVs) were identified, including 162,784 deletions and 153,254 insertions. It was found that 91.76% of deletions and 81.71% of insertions in the SV truth set were confirmed by the assembly-based SV call set (Zhang et al, 2024). Thus, given our 16x coverage, we are confident that we have obtained a high-quality genome with a contig N50 approaching 1 Mb.

Our work goes beyond merely decoding and describing two large plant genomes. We aim to leverage the genomic data to explore several critical biological questions within the context of both genomic and transcriptomic information. We utilized these two genome assemblies as a powerful tool to investigate biological questions and provided valuable resources for the future studies of Liliales plants, ensuring that the quantity of data and the quality of the genomes did not compromise the integrity of our results.

The exceptionally large genome of the lily posed significant challenges. Additionally, we sequenced the flame lily genome, and together, the combined genome size exceeded 40 Gb, further driving up the expenses. Despite the economic constraints, we made every effort to enhance the quality of the genome assembly with the available data.

#### Reference:

Zhang Z, Zhang J, Kang L, et al. Structural variation discovery in wheat using PacBio high-fidelity sequencing. *Plant J* Published online September 6, 2024. doi:10.1111/tpj.17011

**Comment 3.** The lily assembly is 47.09 Gb, which is 1.32 times of 35.66 Gb. This is most likely because the assembly contains two haplotypes. Although the authors performed “sequence dehybridization” (L721), the effect was obviously not good.

**Response:** Thank you for your comment. Removing redundancy in a 5 Gb genome is challenging but still achievable. However, for genomes larger than 20 Gb, it becomes nearly impossible with the current technologies. We have tested numerous software tools, but none could run due to excessive memory requirements. Additionally, we believe that the genome's redundancy significantly impact neither gene discovery nor data mining analyses.

As described in our manuscript, the final assembly result was indeed larger than what we have expected. During the initial assembly, we used Purge\_dups to filter out heterozygous regions, resulting in a genome size of 65.49 Gb (contig N50: 897.77 KB). We then utilized the Hi-C data for clustering and retained the clearly defined chromosome regions, ultimately obtaining a genome size of 47.09 Gb. This represents the most complete genome assembly we could achieve with minimal redundancy. Using the available data and the latest analytical tools, we have made our best efforts to enhance the quality of these two Liliales genomes. The largely complete gene repertoires presented in this study have significantly advanced our understanding of the evolution and development of the important ornamental lily and flame lily species. Moreover, the two genomes hold great potential to accelerate their molecular breeding programs and

pharmaceutical applications.

**Comment 4.** L143. “A phylogenomic analysis of 26 flowering plant species from 18 orders, including 9 monocot orders, revealed that *L. sargentiae* and *G. superba* from Liliales are sisters to the Asparagales species (Figure 1A).” L752. “The species phylogenetic tree of 26 plant species including *L. sargentiae* and *G. superba* was constructed by 893 single-copy homologous genes obtained by BUSCO (Benchmarking Universal Single-Copy Orthologs) (Simão et al., 2015).” The description of the phylogenetic tree results and methods is too brief and there is no discussion and comparison with previous phylogenetic analysis in literature.

**Response:** Thanks a lot for this comment. We removed the results from content-heavy original manuscript. In the revised version, we have added more information on the phylogenetic tree results (L149-160) and methods (L998-1004) as well as detailed comparison of our results with previous findings (L149-160).

**L149-160:** *Although the genomes of 8 monocot plant orders have been sequenced, the order Liliales—which includes economically significant crops such as lilies and tulips—remains unsequenced. As the first two sequenced genomes from Liliales, our assemblies of lily and flame lily provide an invaluable opportunity to explore the phylogenetic position of the Liliales order within the monocots. We conducted a phylogenomic analysis of 26 flowering plant species from 18 orders, including 9 of the 12 monocot orders. The resulting phylogenetic tree revealed consistent evolutionary positions for the 8 monocot orders examined (Figure 1A)<sup>18, 19, 20</sup>. We found that *L. sargentiae* and *G. superba* form a monophyletic clade, which is sister to the Asparagales species, consistent with the previous findings (Figure 1A)<sup>18, 21</sup>. We also confirmed that the Liliales and Asparagales species constitute a distinct clade, which is sister to the clade comprising Arecales, Zingiberales, and Poales species, consistent with previous reports (Figure 1A)<sup>21, 22</sup>.*

**L998-1004:** *The species phylogenetic tree of 26 plant species including *L. sargentiae* and *G. superba* was constructed by 893 single-copy orthologous genes obtained by BUSCO (Benchmarking Universal Single-Copy Orthologs)<sup>17</sup>. Protein sequences from these single-copy homologous genes were aligned using MAFFT (v 7.467) with default settings, followed by trimming with default parameters of trimAL (v 1.4.1)<sup>80, 81</sup>. The maximum likelihood tree was then constructed using FastTree default settings<sup>82</sup> and visualized with MEGA (v 7.0.26)<sup>83</sup>.*

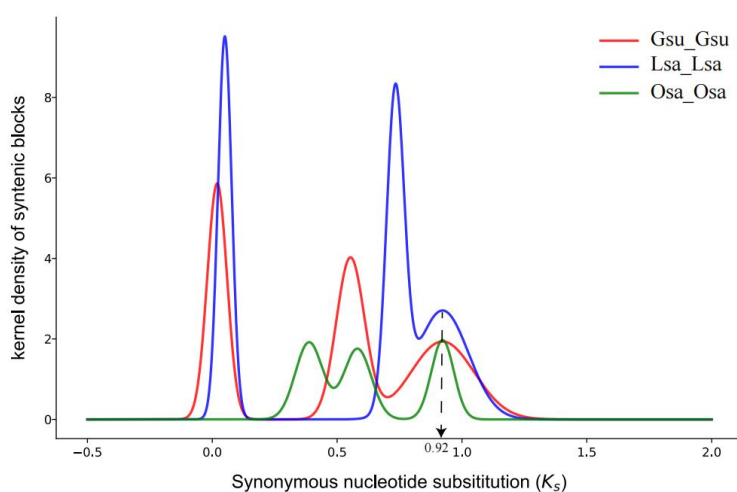
**Comment 5.** The author inferred from the results of the Ks and self-synteny dotplot that lily had two WGDs, one of which was the  $\tau$  event (Ks=0.92) and the other was the recent Ks=0.73. However, as mentioned above, it is very likely that a large part of the assembled genome is diploid, i.e. duplication and this duplication have a low similarity due to high heterozygosity, resulting in Ks=0.73. This can also be inferred from the self-synteny diagram of the two species that although the Ks peak (0.55) corresponding to the most recent WGD of flame lily is smaller than that of lily, the blocks of lily are more complete. And the blocks that marked to be 1 of lily all span at least one complete scaffold, which is most likely because diploid chromosomes or scaffolds were assembled.

**Response:** Thanks a lot for this comment. During our analysis, we did observe a heterozygous peak of the  $K_s$  distribution curve within the lily genome when the  $K_s$  values were close to 0 (**See the  $K_s$  distribution plot within species before correlation below**). We initially removed the heterozygous peak, as well as peaks caused by redundant fragments. In the  $K_s$  density distribution plot presented in our manuscript, we have corrected the  $K_s$  values of the recent WGD using shared duplication methods and excluded the influence of the heterozygous peak (**Figure 1B**). After the correction, the  $K_s$  distribution curve within the lily genome shows a bimodal structure, with the right-hand peak corresponding to the  $\tau$  event and the left-hand peak representing the recent WGD event, and the  $K_s$  peak value of lily's recent WGD event is approximately 0.73 (**Figure 1B**).

Due to significant differences in molecular sequence evolution rates between species, the inferred evolutionary relationships between different species can vary considerably (Baer et al., 2007). Therefore, while intraspecific synteny dotplots can reflect the recursive polyploidization events experienced by a species, they cannot be used as a standard for estimating the timing of polyploidization events between species.

#### Reference:

Baer, CF, Miyamoto, MM, Denver, DR. Mutation rate variation in multicellular eukaryotes: causes and consequences. *Nat Rev Genet* 8, 619-631 (2007).



$K_s$  distribution plot within species before correlation (Gsu, *Gloriosa superba*; Lsa, *Lilium sargentiae*; Osa, *Oryza sativa*)

**Comment 6.** L228, “orthologous” should be paralogous.

**Response:** Thanks a lot for this comment. We have revised the text and changed “orthologous” to “paralogous”. (**L245**)

**L245:** (B)  $K_s$  distributions of paralogous gene pairs within species.

**Comment 7.** Figure S3, the results of inter-species  $K_s$  distribution show that the  $K_s$  distribution between Gsu, *Gloriosa superba*; Lsa, *Lilium sargentiae*; Egu, *Elaeis guineensis* is bimodal, indicating that the three species experienced a common WGD

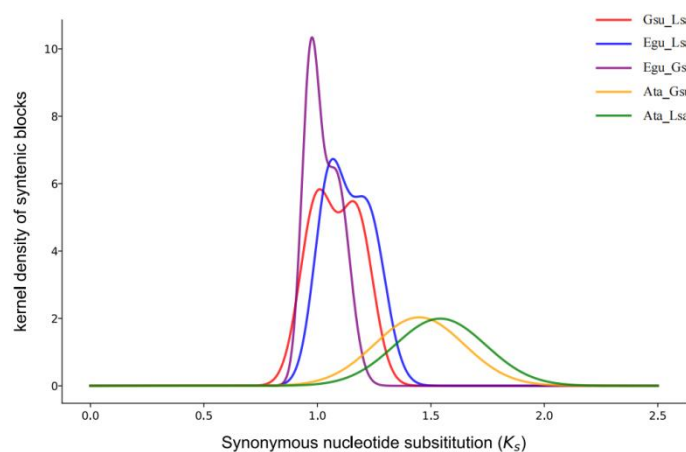
event before divergence, namely the  $\tau$  event, where the corresponding  $K_s=1.5$ , and the divergence peak should be the recent peak, ie. Lsa-Gsu:  $K_s=1.0$ , Egu-Lsa:  $K_s=1.2$  (Figure S3, Ata should be Aco). The species divergence peak  $K_s$  (1.0 or 1.2) calculated by the authors is greater than the  $K_s$  peak value of the  $\tau$  event (0.92) inferred by the previous paralogous gene pairs, and this needs to be corrected by the evolutionary rate (<https://doi.org/10.1093/bioinformatics/btab602>).

**Response:** Thanks a lot for this comment. We have corrected the results of our inter-species  $K_s$  distribution analysis (**See the updated Figure S4 attached below**).

In the inter-species  $K_s$  distribution plot before correction (**See  $K_s$  distributions of orthologous gene pairs between species before correction attached below**), all the  $K_s$  distribution results between *Elaeis guineensis* (Egu) and *Gloriosa superba* (Gsu), Egu and *Lilium sargentiae* (Lsa), and Gsu and Lsa all show a bimodal pattern, suggesting that these three species underwent a shared whole-genome duplication (WGD) event prior to their divergence, known as the  $\tau$  event, with corresponding  $K_s$  values ranging between 1.0 and 1.2. The recent peaks reflect the species divergence, with  $K_s$  values of Lsa-Gsu, Egu-Lsa, and Egu-Gsu at 0.99, 1.05, and 0.97, respectively.

Given this shared  $\tau$  event, we applied a  $K_s$  correction method as follows: ***If species A and B experienced the same duplication event, it should have occurred simultaneously in both, meaning their  $K_s$  peaks should be equal (i.e.,  $K_{sA} = K_{sB}$ ). However, due to different evolutionary rates among species, the actual  $K_{sA}$  and  $K_{sB}$  are not identical. Assuming that after the duplication event, species A and B evolved at rates  $V_A$  and  $V_B$ , respectively, and that the divergence point between them,  $O$ , reflects an ancestral evolutionary rate of  $V$ , the correction factor for species A is  $\lambda_A = V/V_A$ , and for species B is  $\lambda_B = V/V_B$ . Thus, the corrected  $K_s$  value between species A and B is  $K_{sAB\text{-correction}} = K_{sAB} * \lambda_A * \lambda_B$ . The correction for key evolutionary events within species A and B are  $K_{sA\text{-correction}} = K_{sA} * \lambda_A * \lambda_A$  and  $K_{sB\text{-correction}} = K_{sB} * \lambda_B * \lambda_B$ .*** And we have added this method in the “Synteny analysis” part of methods (L1009-1019).

Ultimately, the corrected  $K_s$  value for the shared  $\tau$  event is approximately 0.92, with the divergence peaks being Lsa-Gsu at 0.78, Egu-Lsa at 0.79, and Egu-Gsu at 0.82 (**See the updated Figure S4**). Additionally, only a single peak representing species divergence was identified between *A. tatarinowii* (Ata) and Gsu, as well as between Ata and Lsa, respectively. This suggests that *A. tatarinowii* did not undergo the  $\tau$  WGD event.



Ks distributions of orthologous gene pairs between species before correction

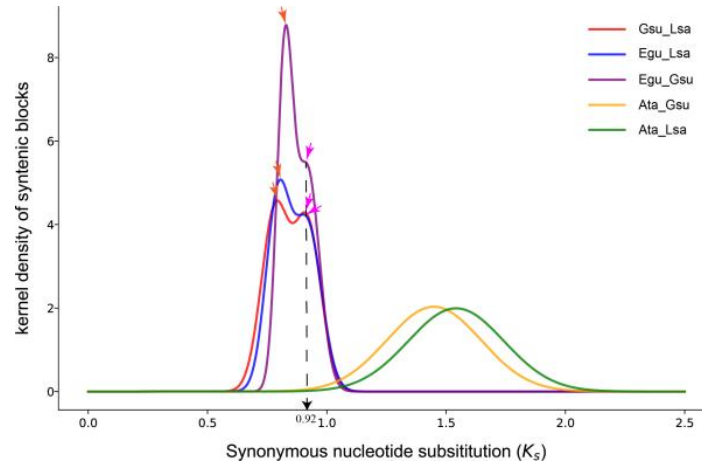


Figure S4. Ks distributions of orthologous gene pairs between species after correction.

**Comment 8.** Figure S7, the legends A and B are reversed.

**Response:** Thanks a lot for this comment. **Figure S7** corresponds to **Figure S8** in the new version. We have changed the order of figure legends of A and B.

**Figure legend of Figure S8 is as follows:**

**Figure S8. Statistics of genomic features in different genomes.** (A) Statistics of the ratio of total intron length to total exon length. All the data were used to calculate the Pearson correlation coefficient ( $R^2$ ). The ratio for other species was obtained from (Niu et al., 2022). (B) Statistics of lengths of intergenic regions.

**Comment 9.** L191, “The average intron lengths in *L. sargentiae* and *G. superba* reached 8.8 kb and 4.7 kb, respectively, both are substantially larger than that (0.5 kb) in most angiosperms which have smaller genomes (Niu et al., 2022) (Tables S7 and S8 and Supplementary Data 4)”. The referenced Tables S7 and S8 should be Tables S5 and S6, and Supplementary Data 4 does not contain the average intron length data.

**Response:** Thanks a lot for your comment. We checked the referenced tables. The referenced tables here are “**Tables S7 and S8**” now (**L210**, because we added two new supplementary tables (Table S5 and Table S6) in the revised version. Additionally, we have included the relevant information on average intron length in **Supplementary Data 4**.

**Comment 10.** L217, “Interestingly, the ratios of the number of solo-LTRs to that of intact-LTRs in *L. sargentiae* (2.6) and *G. superba* (3.9) were both higher than those in other angiosperms, but similar to those in the examined gymnosperms with expansion of ancient LTR-RT bursts (10-30 Mya), suggesting that the LTR removal rates are higher in large genomes driven by LTR-RT expansion (Figure 1D and Supplementary Data 5)”. The Solo LTR number/intact LTR number of conifers in supplementary Data 5 ranges from 0.13 to 0.27, which does not support the conclusion. And those data were missing in Figure 1D with no explanation.

**Response:** Thanks a lot for this comment. Your point about Figure 1D is crucial. We have added the information of the five conifer species to **Figure 1D**. And additionally, we

revised our conclusion (L234-240). We observed that the four coniferous plants have lower ratios compared to several other gymnosperms included, which may be attributed to specific events. However, we did not address or discuss this in our study, because it is not directly relevant to the scope of the paper.

**L234-240:** Interestingly, the ratios of the number of solo-LTRs to that of intact-LTRs in *L. sargentiae* (2.6) and *G. superba* (3.9) were both higher than those in other angiosperms, but similar to the ratios found in the three gymnosperms studied (*Torreya grandis*, *Ginkgo biloba*, and *Welwitschia mirabilis*), which have all undergone expansions of ancient LTR-RT bursts (10-30 Mya) <sup>24</sup>. This suggests that LTR removal rates are higher in these above-mentioned large genomes driven by LTR-RT expansion (Figure 1D and Supplementary Data 5).

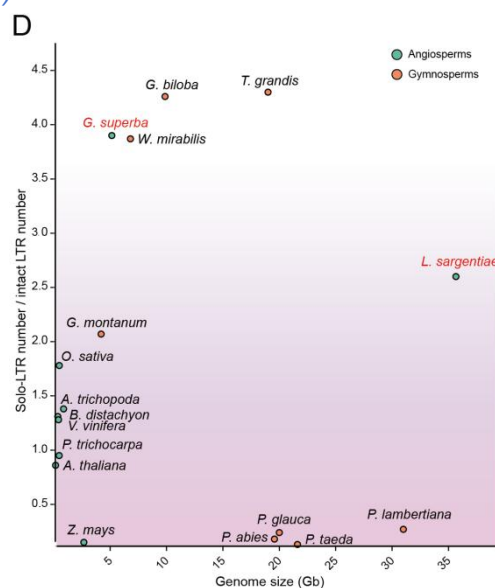


Figure 1D. Statistics of the ratio of the solo-LTR number to the intact-LTR number is plotted against their genome sizes

**Comment 11.** On the transcriptome phylogenomic analysis of wild species and cultivars, the authors stated in the abstract L48 that “numerous wild lilies have not contributed to cultivated lily breeding”. This seems a true but useless statement for the breeding in most plant and animal groups. And the presented data can’t support this statement because only transcriptome data rather than whole genome data have been analyzed.

**Response:** Thanks a lot for this comment. We have deleted the statement that “numerous wild lilies have not contributed to cultivated lily breeding” from the Abstract. In conventional population genetic analyses of the same species, such as maize, rice, soybeans, and buckwheat, methods like gene flow analysis through DNA sequencing can clarify the origin, domestication, and differentiation history of different subgroups in the same species (Huang et al., 2012; Liu et al., 2023; Zhang et al., 2021; Cao et al., 2023). However, in the case of lilies, we cannot use traditional population genetic methods because we are analyzing different species within a single genus, where the DNA differences between species are quite large.

We have attempted whole-genome sequencing (WGS) data for population genetic analysis, but the mapping rate was only 60% to 80%. Additionally, many crop cultivars

share a common ancestor, having been domesticated only once. This is the case for crops like peanuts, maize, wheat, and rice, resulting in minimal differences between cultivars (Huang et al., 2012; Liu et al., 2023; Zhang et al., 2021; Cao et al., 2023). However, lily cultivars lack a known ancestor because they were domesticated from different wild species. This leads to significant variation between cultivars, representing more challenging genome-based comparisons and analyses more challenging.

Therefore, we turned to transcriptomics and transcript identification. Transcript analysis is a common approach for species-level studies (Zhang et al. 2020). By directly analyzing proteins, we clarified the relationships among different species and commercial varieties of lilies. We developed a protein similarity matrix (**L1033-1038**), which was employed to visually demonstrate the relationships between different lily subgroups and their commercial varieties. This is the first time such relationships have been discovered in lily research.

### References:

- Zhang L, Chen F, Zhang X, et al. The water lily genome and the early evolution of flowering plants. *Nature* **577**, 79-84 (2020).
- Huang X, et al. A map of rice genome variation reveals the origin of cultivated rice. *Nature* **490**, 497-501 (2012).
- Liu Y, et al. Genetic basis of geographical differentiation and breeding selection for wheat plant architecture traits. *Genome Biol* **24**, 114 (2023).
- Zhang K, et al. Resequencing of global Tartary buckwheat accessions reveals multiple domestication events and key loci associated with agronomic traits. *Genome Biol* **22**, 1-17 (2021).
- Chao J, et al. Genomic insight into domestication of rubber tree. *Nat Commun* **14**, 4651 (2023).

### Comment 12. “B” is missing in L285 for legend of Figure 2.

**Response:** Thanks a lot for this comment. We have added the “(B)” in the legend of Figure 2. (**L312**)

**L312:** (B) Heatmap showing shared genomic haplotype patterns among different lily species.

**Comment 13.** For Figure 2B, the heatmap should be symmetrical along the diagonal line. But it's not for many parts. Why?

**Response:** Thanks a lot for this comment. As it has been described in the figure legend of **Figure 2B**, the heatmap shows shared genomic haplotype patterns among different lily species. Each cell represents the ratio of the total number of transcripts with the highest gene similarity (**TNTHGS**) (identity > 95%) between two species to the total number of transcripts (**TNT**) within the corresponding species in that row (excluding self-aligning transcripts) (**L313-315**). Therefore, when calculating the ratios represented by different cells in the same row, the denominator is consistent. However, for the ratios in each column, the denominators used in the calculation are different. For example, in the comparison between A and B, the two relevant cells would have values of

TNTHGS/TNT\_A and TNTHGS/TNT\_B, which are different when TNT\_A != TNT\_B. Thus, this heatmap is not symmetrical along the diagonal line.

**Comment 14.** The signal between *L. brownii* with almost all wild species and cultivars seem very unbelievable. Please check this out.

**Response:** Thank you very much for this comment. *Lilium brownii*, which is widely distributed in 17 provinces across China, exhibits a significantly higher proportion of shared genomic haplotypes with other lilies (Dhiman et al., 2021). In contrast, lilies with more restricted regional distributions, such as *L. formosanum*, *L. formosanum* var. *pricei*, *L. callosum*, and others, rarely share genomic haplotypes with other lilies (Dhiman et al., 2021). This suggests that lilies with a broader geographic distribution tend to share more genomic haplotypes with other species.

#### Reference:

Dhiman MR, Sharma P, Bhargava B. *Lilium*: Conservation, characterization, and evaluation. *Floriculture and Ornamental Plant* 1-36 (2021)

**Comment 15.** In Figure 3G and Figure S19, the cartoons for the models are over-simplified. At least, functional roles of those important genes should be indicated in the drawings.

**Response:** Thank you very much for this comment. We have revised **Figure 3G** and **Figure S19 (corresponding to Figure S21 now)** by adding related gene functions to these figures. Additionally, a comprehensive understanding of the functions of genes of **Figure 3G** can be obtained by referring to their functional information in **Supplementary Data 6**.

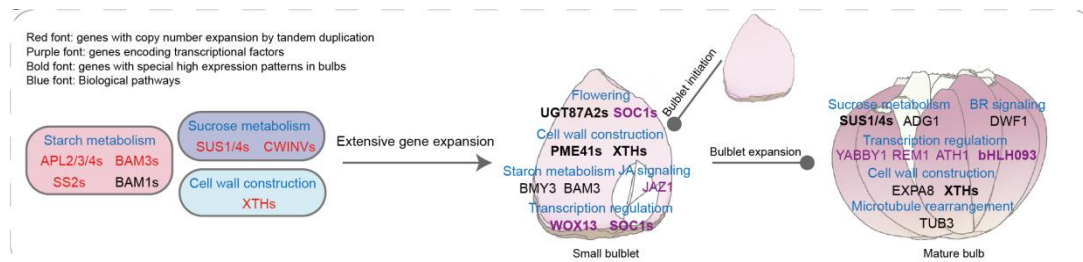


Figure 3G. A proposed model of the evolutionary and genetic mechanisms of bulblet initiation and bulblet expansion in *Lilium sargentiae*.

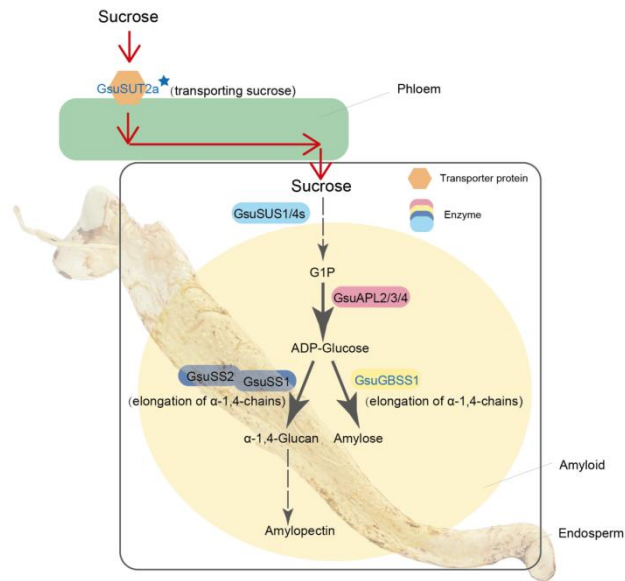


Figure S21. A proposed model of rhizome evolution and formation in *Gloriosa superba*.

**Comment 16.** L463, please add citations of genes related to colchicine synthesis.

**Response:** Thanks a lot for this comment. We have added two references for these genes (L510-512).

**L510-512:** We identified multiple homologues of the previously identified colchicine biosynthesis genes<sup>39, 40</sup>.

**The cited articles are as follows:**

Nett RS, Sattely ES. Total biosynthesis of the tubulin-binding alkaloid colchicine. *J. Am. Chem. Soc.* **143**, 19454-19465 (2021).

Nett RS, Lau W, Sattely ES. Discovery and engineering of colchicine alkaloid biosynthesis. *Nature* **584**, 148-153 (2020)

**Comment 17.** L464, Figure 4C should be Figure 5C.

**Response:** Thanks a lot for this comment. We have revised the text and changed “Figure 4C” to “Figure 5C”. (L512)

**L510-512:** We identified multiple homologues of the previously identified colchicine biosynthesis genes<sup>39, 40</sup> (Figure 5C and Figures S28-30).

**Comment 18.** The Figure S27 is not easy to understand. How do we interpret the “Traits” in the module-trait analysis? Why was the magenta module selected for network display? Is there statistical test to confirm confidence of the modules? Similarly, the figure S23 should also be improved.

**Response:** Thank you very much for your comment. It was extremely helpful in improving the readability of these two figures. For both **Figure S23 (corresponding to Figure S26 now)** and **Figure S27 (corresponding to Figure S31 now)**, we have enhanced the figure legends by including more detailed descriptions, such as information on each trait used in the Weighted gene co-expression network analysis (WGCNA). Pearson's correlation coefficient  $r$  (used to evaluate the degree of correlation between gene modules and external traits) and the  $P$ -value (used to confirm statistical significance) are key measures

for assessing the relationship between gene expression profiles and traits.

In **Figure S26**, we added an explanation for selecting the turquoise module: it was considered dormancy-related due to its significant positive correlation ( $r = 1$ ,  $P < 0.01$ ) with dormancy-related bulbs. For **Figure S31**, our focus was on identifying colchicine-related modules and exploring the co-expression patterns among genes involved in colchicine biosynthesis. The selection of the magenta module was not primarily based on correlation and  $P$ -value parameters; rather, it was chosen because it contained multiple potential key genes related to colchicine, such as the homolog of CYP75A110. This provided a valuable opportunity to investigate the co-expression patterns of crucial colchicine-related genes. We have included this reasoning in the legend of **Figure S31** as well.

**Figure legend of Figure S26 is as follows:**

**Figure S26. Weighted gene co-expression network analysis (WGCNA) of bulb dormancy in lily.** (A) Relationship between modules and traits, including dormancy bulb (DB) and dormancy-released bulb (DRB). Each column corresponds to a tissue, and each row corresponds to a gene module. Each cell contains the corresponding Pearson's correlation efficient  $r$  and  $P$ -value. The cell is color-coded by correlation efficient according to the color legend on the right of the heatmap. (B) Venn diagram of genes of the turquoise module generated by WGCNA and differentially expressed genes (DEGs) between DB and DRB. (C) GO enrichment of genes of the turquoise module. GO terms related to cell cycle are in red fonts. The turquoise module was considered to be dormancy-related because it showed a significant ( $P < 0.01$ ) positive correlation ( $r = 1$ ) with DRB. (D) Heatmap of expression patterns of candidate genes involved in lily bulb dormancy.

**Figure legend of Figure S31 is as follows:**

**Figure S31. Weighted gene co-expression network analysis (WGCNA) of colchicine biosynthesis in flame lily.** (A) Clustering of RNA-seq samples used in WGCNA. (B) Hierarchical cluster dendrogram using dynamic tree cut, revealing 20 modules generated by WGCNA. The different colors under the dendrogram show co-expressed modules identified using WGCNA. (C) The co-expression network of the magenta module generated by WGCNA where multiple colchicine-related genes are co-expressed, which shows the correlation of colchicine-related genes. (D) Relationship between modules and traits. The traits includes different flame lily tissues across development as well as the relative colchicine content in the rhizome of flame lily of 80 days after sprouting (DAS). Each column corresponds to a tissue, and each row corresponds to a gene module. Each cell contains the corresponding Pearson's correlation efficient  $r$  and  $P$ -value. The cell is color-coded by correlation according to the color legend on the right of the heatmap. The used tissues (from left to right of the horizontal axis of the heatmap) include the leaves from flame lilies of DAS, flowers on the bud stage (Bud), initial opening stage (Ini), turning stage (Tur), and mature stage (Mat) from flame lilies of 60 DAS, and rhizomes of 20, 40, 60, and 80 DAS.

**Comment 19.** The total ion chromatograms (TICs) of lily in Figure 5A are inconsistent with those in Figure S31.

**Response:** Thank you for the comment. All panels in **Figures 5A and S31** display the extracted ion chromatogram (EIC) of the theoretical  $m/z$  value (400.1755,  $[M + H]^+$ ) for colchicine within the total ion chromatograms (TICs). The EIC on the Y-axis specifically represents the response for colchicine alone. The difference in EIC values between The differences between **Figures 5A and S31** in the lily-related images are due to the use of different lily tissues for colchicine detection, with bulbs used in **Figure 5A** and buds in **Figure S31**. The images of tissues analyzed of **Figure 5A** are labeled in figures of **Figure 5A**. To make it clearer, we add the information on the types of tissues in the legend of **Figure 5A (L551-553)**. The used tissues are also labeled in **Figure S23** as well as its legend. In **Figure 5A**, lily bulbs were analyzed, whereas in **Figure S31**, lily buds were used. Although colchicine itself was not detected in either tissue of lily bulb or bud, different colchicine isomers were identified in both tissues, leading to the observed differences in EIC values. In summary, the variation in EIC values between these figures is attributed to the use of different tissues in the analysis.

**L551-553:** (A) The extracted ion chromatogram (EIC) of the theoretical  $m/z$  value (400.1755,  $[M + H]^+$ ) of colchicine in the total ion chromatograms (TICs) of the colchicine standard, the rhizome of *Gloriosa superba*, and the bulb of *Lilium sargentiae*.

## Point to point responses for reviewer #2

**Comment 1.** Did the stem lineage ancestor of extant monocots undergo whole genome duplication events? Please clarify this issue based on syntenic analysis and Ks data.

**Response:** Thank you for your comment. The stem lineage ancestor of *Lilium sargentiae* (Lsa), *Gloriosa superba* (Gsu), and *Oryza sativa* (Osa) underwent a whole-genome duplication event (WGD), known as the  $\tau$  event, which is depicted in both **Figure 1A and 1B**. In **Figure 1B**, we conducted an intra-species Ks distribution analysis, revealing that the genomes of *L. sargentiae*, *G. superba*, and *O. sativa* all exhibited a peak at 0.92, corresponding to the monocot-shared  $\tau$  WGD event that occurred 120-130 million years ago.

In **Figure S4**, the Ks distribution results between *Elaeis guineensis* (Egu) and Gsu, Egu and Lsa, and Gsu and Lsa all show a bimodal pattern, indicating that *L. sargentiae*, *G. superba*, and *E. guineensis* experienced a common  $\tau$  WGD event before divergence, which is the WGD event that the stem lineage ancestor of extant monocots has experienced. This is represented by the right-hand peaks pointed by pink arrows. Additionally, only a single peak representing species divergence was identified between *A. tatarinowii* (Ata) and Gsu, as well as between Ata and Lsa. This suggests that *A. tatarinowii* did not undergo the  $\tau$  WGD event.

**Figures S2 and S3** present syntenic analyses of Lsa with Lsa (**Figure S2A**), Gsu with Gsu (**Figure S2B**), Lsa with Gsu (**Figure S3A**), and Gsu with *Acorus calamus* (Aca) (**Figure S3B**), all of which show evidence of the  $\tau$  WGD event, represented by the green triangles labeled as 2. In conclusion, the ancestor of *L. sargentiae*, *G. superba*, *O. sativa*, and *A. calamus* experienced the  $\tau$  WGD event.

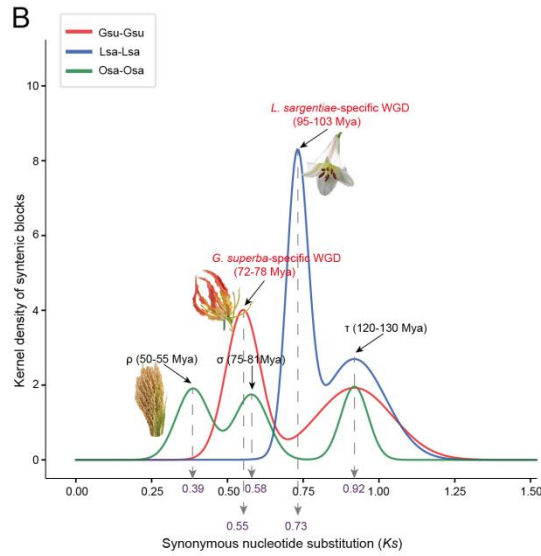


Figure 1B. Ks distributions of paralogous gene pairs within species.

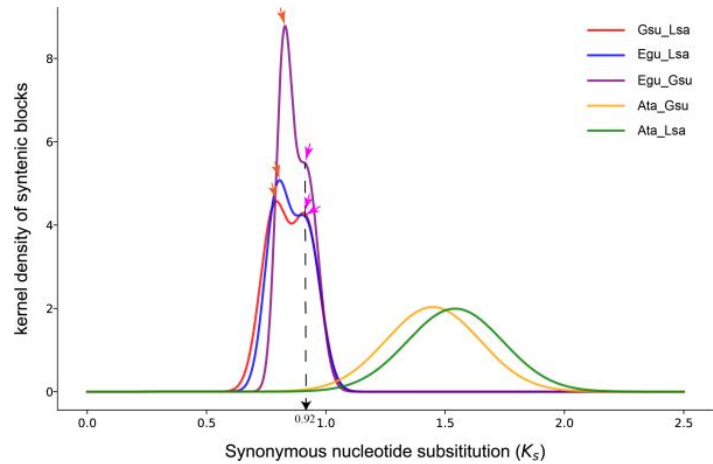


Figure S4. Ks distributions of orthologous gene pairs between species after correlation.

**Comment 2.** The manuscript would benefit from details regarding the quality evaluation of genome assembly and annotation for each species.

**Response:** Thanks a lot for this comment. In the revised version, we included the results of BUSCO analyses (L139-143), which support the high quality of our genome assemblies and annotation: *BUSCO*<sup>17</sup> analysis of the *L. sargentiae* and *G. superba* genome revealed the complete gene information at 90.8% and 91.8%, respectively; *BUSCO* analysis of the *L. sargentiae* and *G. superba* protein sets revealed the complete gene information at 88.3% and 85.9%, respectively (Tables S5 and S6).

**TableS5. BUSCO evaluation of the genomic completeness of *Lilium sargentiae*.**

Type	Genome	%	Protein	%
Complete BUSCOs (C)	1465	90.77%	1425	88.30%
Complete and single-copy BUSCOs (S)	865	53.59%	682	42.30%
Complete and duplicated BUSCOs (D)	600	37.17%	743	46.00%
Fragmented BUSCOs (F)	41	2.54%	56	3.50%
Missing BUSCOs (M)	108	6.69%	133	8.20%

Total BUSCO groups searched	1614	1614
-----------------------------	------	------

**TableS6. BUSCO evaluation of the genomic completeness of *Gloriosa superba*.**

Type	Genome	%	Protein	%
Complete BUSCOs (C)	1481	91.76%	1387	85.90%
Complete and single-copy BUSCOs (S)	1411	87.42%	1309	81.10%
Complete and duplicated BUSCOs (D)	70	4.34%	78	4.80%
Fragmented BUSCOs (F)	52	3.22%	107	6.60%
Missing BUSCOs (M)	81	5.02%	120	7.50%
Total BUSCO groups searched	1614		1614	

**Comment 3.** Given the previous findings indicating potential recent radiative speciation in the genus *Lilium* since the Neocene, it is plausible that the similarity of transcripts among extant species could be attributed to incomplete lineage sorting of polyphyletic alleles rather than gene flow. Accordingly, I would like to suggest the author to carry out reticulate evolution analysis to identify potential hybridization events among *Lilium* species, using transcriptomic data.

**Response:** Thanks a lot for this comment. We agree that the similarity of transcripts among species could be attributed to incomplete lineage sorting rather than gene flow. Currently, there is ongoing debate about whether the polyphyly in the genus *Lilium* is due to incomplete lineage sorting or inter-sectional hybridization causing gene flows among *Lilium* sections (Gao et al., 2013; Gong et al., 2017). In our study, we focused solely on the transcript similarity among different lilies by calculating core-gene sequence similarities, without addressing the underlying causes of these transcript similarities. We have removed the inaccurate references to “**gene flow**” in our manuscript and replaced them with “**shared genomic haplotypes**” instead, to clarify our findings.

#### References:

Gao YD, Harris AJ, Zhou SD, He XJ. Evolutionary events in *Lilium* (including *Nomocharis*, Liliaceae) are temporally correlated with orogenies of the Q-T plateau and the Hengduan Mountains. *Mol. Phylogenet. Evol.* **68**, 443-460 (2013)

Gong X, Hung KH, Ting YW, et al. Frequent gene flow blurred taxonomic boundaries of sections in *Lilium* L. (Liliaceae). *PLoS One* **12**, e0183209 (2017)

**Comment 4.** Regarding the genome part, the genome size of *L. sargentiae* is 9 times that of the *G. superba* (L132-L133), but such a huge difference is not shown in the WGD events and LTR parts. Although it demonstrates that the LTR burst event led to gene enlargement, the proportion of gene enlargement is around 5 times (L139). Is the genome size here the actual size of *L. sargentiae*?

**Response:** Thanks a lot for this comment. The genomes size here is the assembled genome size. The genome size of *L. sargentiae* is 35 Gb, while our assembly result is 47 Gb, which includes heterozygous segments that could not be filtered out. Therefore, the *L. sargentiae* genome is six times larger than the *G. superba* genome, and the proportion of

gene expansion is roughly comparable.

**Comment 5.** In the analysis of the *Lilium* genus, the authors reconstructed the phylogenetic tree using transcriptome data from 85 transcriptomes of the *Lilium* genus. How were the 360 genes (L239) chosen here? If WGS data is used here, can the same classification be observed? In routine analysis, we often use WGS to analyze gene flow between different subspecies. In this part, an excellent analysis shows the genetic diversity of hybrid commercial varieties (Figure 2C), but the description here is only one sentence (L274-275). Please describe the conclusions and analysis methods in detail.

**Response:** We have provided a more detailed description of Figure 2C (L290-L305). The 360 genes were chosen as described: *Trinity*<sup>88</sup> was used for transcriptome assembly, yielding the longest transcript. TransDecoder v5.5 (<https://www.msi.umn.edu/sw/transdecoder>) was then used for opening reading frame (ORF) prediction, resulting in the extraction of the protein sequences from the longest transcripts. Orthofinder<sup>89</sup> was then used to identify single-copy genes, resulting in the detection of 360 single-copy orthologous genes (L1028-L1033). During the analysis phase of the manuscript, we had access to WGS data. However, upon performing alignment-based analysis, we observed that due to the high variation within the *Lilium* genus, the mapping rate for second-generation sequencing was only around 60% to 80%. We were concerned that continuing to use DNA for our analysis might introduce a bias, so we opted to use transcriptomic data instead, focusing on gene-level transcript similarity analysis.

**L290-305:** *For lilies in Clade 1, we used transcriptome assemblies from different lilies and the longest transcripts of the L. sargentiae genome assembly to calculate the number of gene clusters for each species (Figure 2C). Among the lilies from Clades 1-1 and 1-2, three cultivated lilies ('Regale Album', 'Red Morning', and 'Judith Saffigna') from Clade 1-1, and four ('Black Beauty', 'Palazzo', 'Conca d'Or', and 'Zelmira') from Clade 1-2 exhibited a higher number of clusters with multi-copy transcripts (two or three copies) compared to the number of clusters with single-copy transcripts, indicating the presence of genetic materials from more than one parental line (Figure 2C). For instance, 'Red Morning' showed the highest number of gene clusters with two-copy transcripts (Figure 2C). In contrast, wild lilies, and some other cultivated lilies ('Beijing Moon', 'Siberia', 'Marlon', 'Sorbonne', 'Isabella', and 'Brasilia') predominantly showed single-copy transcripts (Figure 2C). This implies that the seven cultivated lilies with a higher number of clusters with multi-copy transcripts likely underwent hybridization, as also evidenced by their positions at the outermost branches of clades (Figure 2A, 2C). While the other cultivated lilies with the highest number of clusters with single-copy genes are likely bred through alternative methods, such as domestication and mutation breeding (Figure 2C).*

**Comment 6.** Language improvements are necessary throughout the manuscript and some grammatical errors should be corrected, to improve the readability and grammatical accuracy of the manuscript.

**Response:** Thanks a lot for this comment. We appreciate your attention to detail. We have thoroughly reviewed the manuscript and addressed the language and grammatical

issues.

**Comment 7.** Detailed information should be provided within the section “Methods” to enhance the repeatability of this study.

**Response:** Thanks a lot for this comment. We have provided a more detailed description of the methods used in our study.

**Comment 8.** More details should be provided in the methods. For example, I don't see the methods and cutoff of GO enrichment analyses.

**Response:** Thanks a lot for this comment. We have added more detailed description of the methods, including GO enrichment analysis (L992-996).

**L992-996:** *The GO annotation information on genes were obtained from the results of eggNOG-Mapper. TBtools (v 1.098696) was used to conduct GO enrichment analysis of genes in proximity (within 2 kb) to Tork and Tekay LTR-RTs or containing these LTR-RTs in their introns in the lily genome <sup>79</sup>. The P-value cutoff for measuring whether the enrichment of the GO term is significant is 0.05.*

**Comment 9.** Line 64: In relation to the possession of extensive genomes in *Lilium* species, it is essential to included pertinent references in this sentence.

**Response:** Thanks a lot for this comment. We have cited two references here (L65). The cited articles are as follows:

Leitch IJ, Beaulieu JM, Cheung K, Hanson L, Lysak MA, Fay MF. Punctuated genome size evolution in Liliaceae. *J. Evol. Biol.* **20**, 2296-2308 (2007).

Du YP, Bi Y, Zhang MF, Yang FP, Jia GX, Zhang XH. Genome size diversity in *Lilium* (Liliaceae) Is correlated with karyotype and environmental traits. *Front. Plant Sci.* **8**, 1303 (2017).

**Comment 10.** Line 128: The genomic heterozygosity of the two species were 3.4% to 0.7%. It is necessary to describe the methodology for evaluation this genomic traits within the section “methods”.

**Response:** Thanks a lot for this comment. We have added the related method in the revised manuscript. (L951-958)

**L951-958:** *Genome size and heterozygosity estimation*

*Genome size and heterozygosity were estimated by K-mer frequency distribution analysis. Initially, the short reads were filtered using fastp (v0.19.4) with default settings. K-mers were then counted using Jellyfish (v2.2.10) with the parameters “-C -m 17 -s 1G -t 56” <sup>67, 68</sup>. The resulting output file was subsequently used as input for GenomeScope <sup>69</sup>, which estimated the genome size and heterozygosity using default parameters. The 17-mer frequency distribution was analyzed based on genome characteristics, considering the pattern of Poisson distribution.*

**Comment 11.** Line 394-395: The assertion that the species of Liliales exhibit undifferentiated petals and sepals is problematic, as some families within the Liliales order, such as Melanthiaceae, possess distinctive sepals and petals.

**Response:** Thanks a lot for this comment. We have revised this sentence to “The lilies and flame lilies feature flower structures with petaloid organs called tepals, which contribute significantly to their ornamental value.” (L428-429)

**Comment 12.** Line 524: The subterranean organ of *Salvia miltiorrhiza* is a taproot, rather than rhizome or bulb.

**Response:** Thank you for your comment. It has greatly enhanced the accuracy of our manuscript. We have removed the reference to *Salvia miltiorrhiza* from the discussion section, as it does not belong to the plant species with bulbs and rhizomes that are the focus of our manuscript. (L614-615)

**L614-615:** *To date, while several genomes of bulbous and rhizomatous plants have been previously documented<sup>21, 22, 58, 59</sup>, our understanding of these unique plant structures remains limited.*

**Comment 13.** Line 537-677: Within the references, the botanic names of all genus- and species-level taxonomic units should be italicized.

**Response:** Thank you for your comment. We have revised all the corresponding names to italicized names.

**Comment 14.** Line 684-689: It is critical to associate the individual used for sequencing (especially those used for RNA-seq) with a voucher specimen deposited in a recognized herbarium. This practice ensures that the plant material can be accurately identified and verified in future studies.

**Response:** We have included the specimen IDs for plant materials used in our study in **Supplementary Data 1**. These specimen IDs are sourced from the Genetic Resources Center at the Institute of Grassland, Flowers, and Ecology, Beijing Academy of Agriculture and Forestry Sciences, China. For further information or to request related materials, individuals can contact the institute via email. Additionally, the available specimen IDs for the wild Liliales species (obtained from <https://www.cvh.ac.cn/index.php>) are also provided in **Supplementary Data 1**.

**Comment 15.** Line 693-697: For PacBio sequencing, the size of fragments selected for sequencing should be described in detail.

**Response:** Thanks a lot for this comment. We have described the size of fragments selected for sequencing in detail. (L924-925)

**L924-925:** *The size of fragments selected for sequencing was approximately 20 kb.*

**Comment 16.** Line 698-706: Please provide details on the number of PCR cycles for the preparation of the Hi-C library.

**Response:** Thanks a lot for this comment. We have described the setting conditions in detail. (L932-L938)

**L932-L938:** *The cross-linked long-distance physical connections were next processed into chimeric fragments, followed by reverse cross-linking, purification, and PCR amplification, and were subsequently used to create paired-end sequencing libraries. For*

the PCR amplification reaction, the cycling conditions are set as follows: (1) initial denaturation: 95°C for 3 minutes for (1 cycle); (2) denaturation: 98°C for 20 seconds (10 cycles); (3) annealing: 60°C for 15 seconds (10 cycles); (3) extension: 72°C for 30 seconds(10 cycles); (4) final extension: 72°C for 5 minutes (1 cycle); (5) hold: 4°C.

**Comment 17.** Line 710-716: Please specify which organs have been sample for constructing the cDNA library for each species.

**Response:** Thanks a lot for this comment. We have specified the organs used in the construction of cDNA libraries of lily and flame lily, respectively. (L943-945)

**L943-945:** For transcriptome sequencing, cDNA libraries were constructed from the integrated and high-quality RNA of the mixture of bulbs, stems, leaves, and flowers from lilies as well as rhizomes, leaves, and flowers from flame lily.

**Comment 18.** Although Figure 2 is very informative, it needs to be further adjusted. For example, in Figure 2C, the different lily varieties can be labeled more clearly, and figure resolution should be improved to meet publishable standards. Additionally, I would suggest the authors to improve the readability of the majority supplementary figures.

**Response:** Thanks a lot for this comment. We have simplified Figure 2 for its clarity. Additionally, the supplementary figures including their legends have been improved.

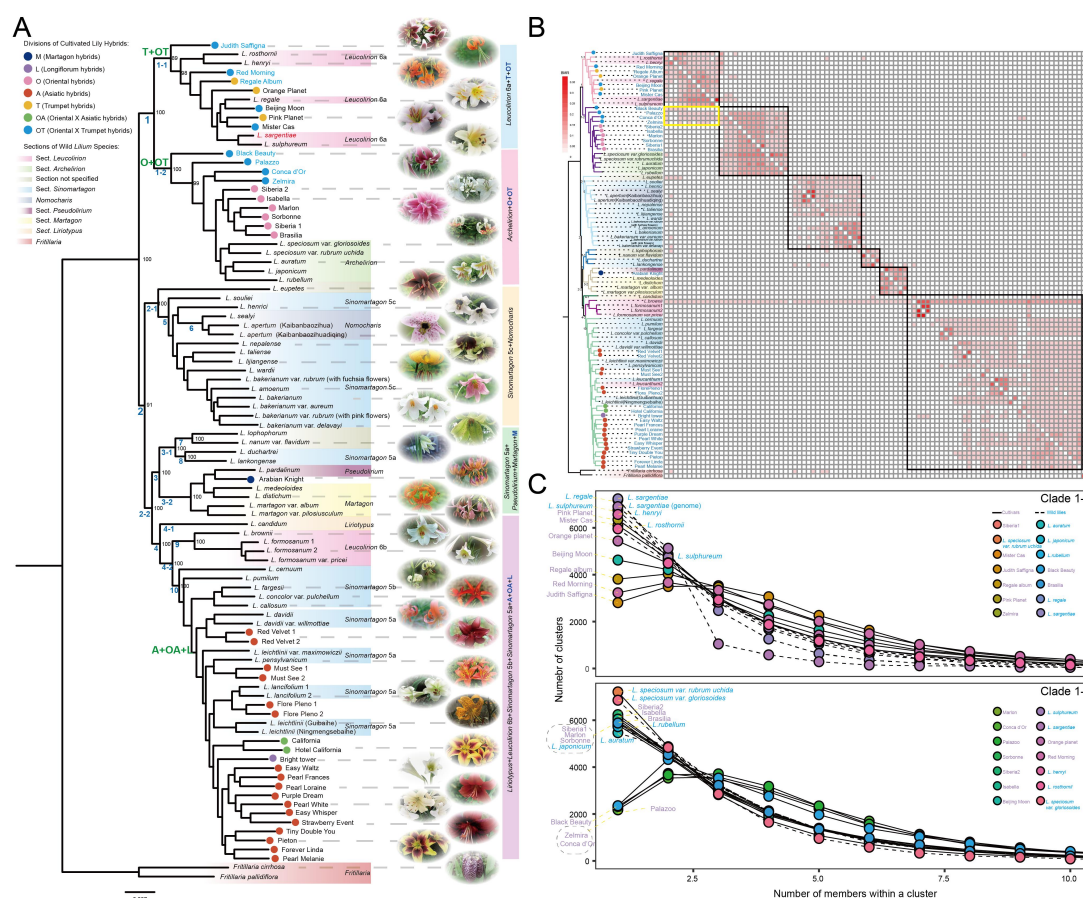


Figure 2. Phylogeny and hybridization patterns of *Lilium*.

**Point to point responses for reviewer #3**

**Comment 1.** L.393-409: The authors analyzed the expression of floral organ identity genes in lily and gloriosa here. These genes have been already isolated and characterized from *Lilium* species as below. So, after adding the reported lily genes for their phylogenetic tree, the authors should compare their results and the results previously reported. Also, the authors should show the developmental stage of the flower they used in this study, because the expression patterns of floral organ identity genes change during flower development.

**Response:** Thank you very much for providing those useful comments regarding floral development genes. We have incorporated the previously reported lily ABCE MADS genes into our analysis and compared our findings with the earlier results. Some of our findings are consistent with previous studies, while we also uncovered novel insights. For example, we discovered that although both lily and flame lily possess two copies of *PI-like* genes, these genes underwent independent duplication events after the divergence of the two species. Additionally, we identified new copies of lily ABCE genes, such as the class E *LsaAGL6* gene. And the expression pattern of *LsaAGL6* suggested its A-class function in lily. Furthermore, we have clarified the developmental stages of the flower tissues used in both lily and flame lily. (L444-447)

**L444-447:** For *L. sargentiae*, the expression profiles of various floral tissues, including tepals, stamens, filaments, ovaries, and stigmas, were analyzed across five consecutive stages (S1–S5) from the bud stage to full maturity. For *G. superba*, tepals, stamens, and carpels were analyzed at the bud stage (Figure S23).

**Comment 2.** 1) Class A gene The authors used AGL6-like genes for class A genes, but this is wrong. AGL6-like genes are related to SEP-like genes (class E). Class A genes are AP1-like genes.

**Response:** Thanks a lot for this comment. We have refined our classification of class A genes. We identified a total of four and two class A genes in *L. sargentiae* and *G. superba*, respectively (Figure S22A) (L432-434).

**L432-434:** We identified a total of four class A, three class B, two class C, and three class E MADS genes in *L. sargentiae*, and two class A, three class B, two class C, and two class E MADS genes in *G. superba* (Figures S22).

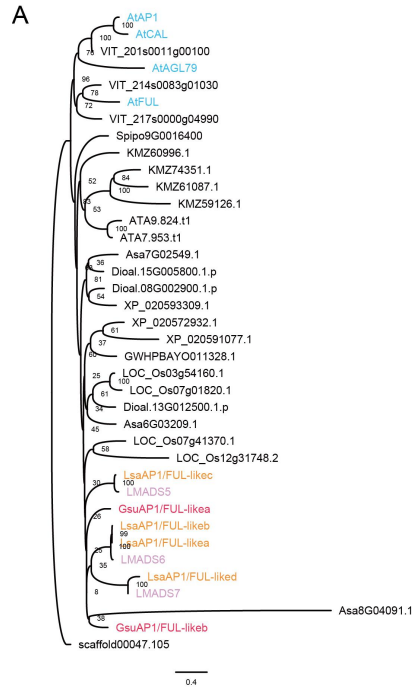


Figure S22A. Phylogenetic analysis of class A genes in *Lilium sargentiae*, *Gloriosa superba*, and other species.

**Comment 3.** 2) Class B genes Lily has two type of PI-like genes but the C-terminal region of these genes are very different; one type has PI-motif (LMADS8) but the others does not (LMADS9) (Journal of Experimental Botany 63:941–961, 2012). Since gene duplication of lily PI-like genes occurred after divergence between lily and tulip, LsaPIa-GsuPIa and LsaPIb-GsuPIb are not orthologs, respectively. The authors should compare the structures of PI-like genes and discuss about it.

**Response:** Thanks a lot for this comment. We agreed that *LsaPIa-GsuPIa* and *LsaPIb-GsuPIb* are not orthologs, respectively. We have compared the structures of *PI-like* genes and discussed about their structures (Figure S24) (L436-440).

**L436-440:** Additionally, both *PI-like* genes (*GsuPIa* and *GsuPIb*) in *G. superba* contain the *PI-motif*. In contrast, only one *PI-like* gene (*LsaPIa*) in *L. sargentiae* possesses the *PI-motif*, while the other (*LsaPIb*) does not (Figure S24A). This finding is in agreement with the previous reports that there are two types of *PI-like* genes in lilies with one containing the *PI motif*<sup>35</sup>.

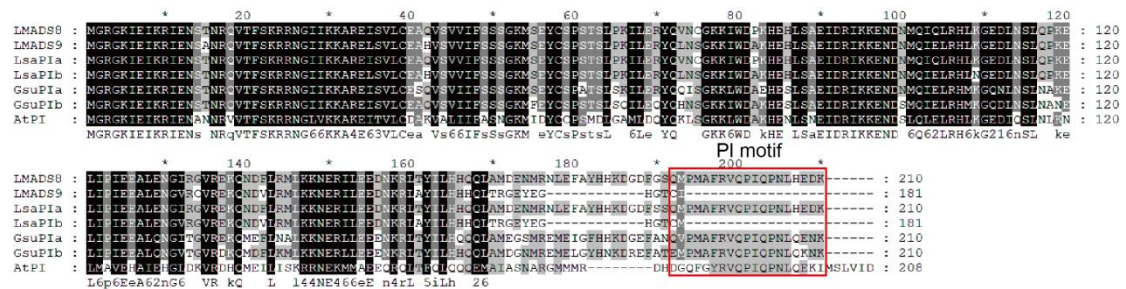


Figure S24. Comparison of C-terminal regions of PI-like MADS-box proteins.

**Comment 4.** 3) Class C genes In general, class C genes are called as AGAMOUS-like genes. There are two types of AG-like genes in lily and Arabidopsis; one is AG/PLE-like (class C) and the other is STK-like (class D). These AG-like genes have different C-terminal region at the end of the protein and the expression patterns of these are different (Plant Sci. 241:266–276, 2015) In figure S21, the authors showed the phylogenetic relationship of AG-like genes from lily and gloriosa. This result showed that two lily AG genes are far related but two gloriosa AG genes are closely related. This indicates that two lily AG genes seem to belong to different clade but two gloriosa AG genes does to the same clade. The authors should show the relationship of lily and gloriosa AG genes and discuss about the expression patterns of these genes.

**Response:** Thanks a lot for this comment. Our study primarily focused on class C AG/PLE-like genes. To enhance the accuracy of our phylogenetic analysis, we used IQ-TREE, which selected the most appropriate evolutionary model based on the data, to construct the phylogenetic tree. We identified two AG-like genes in both *L. sargentiae* and *G. superba*, respectively. Interestingly, while the two AG genes from each species are not closely related, the four genes cluster together in a single clade (**Figure S22C**). Additionally, we compared the expression patterns of these class C AG/PLE-like genes (**L452-455, Figure S23**).

**L452-455:** For class C AG/PLE-like genes, *LsaAGa* was not expressed in all the examined tissues (Figure S23). *LsaAGa* in lily and two AG genes in flame lily almost exhibited no expressions in tepals, but those showed high levels of expression in the reproductive organs (Figure S23).

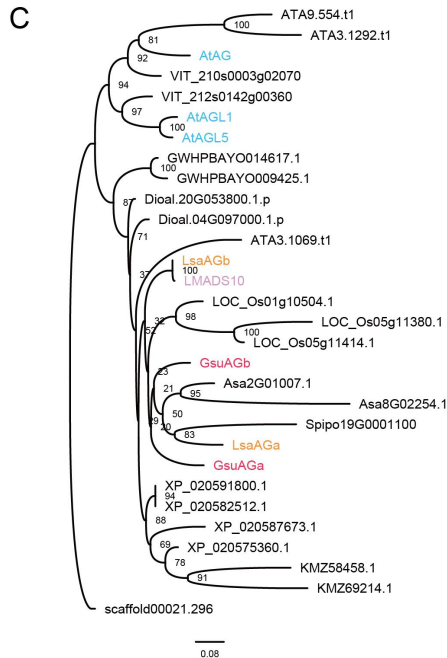
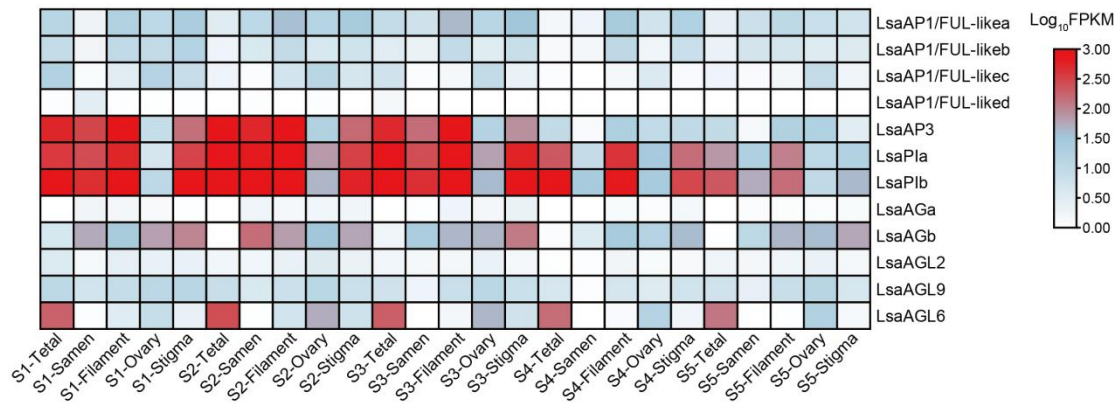


Figure S22C. Phylogenetic analysis of class C genes in *Lilium sargentiae*, *Gloriosa superba*, and other species.

A



B

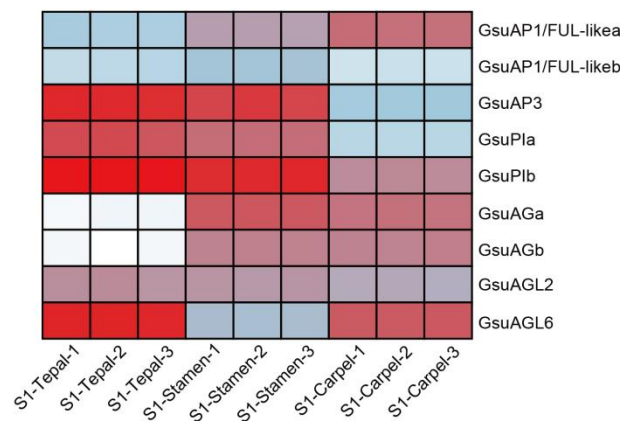


Figure S23. (A) and (B) showed gene expression patterns of ABCE MADS-box genes from various organs of *Lilium sargentiae* and *Gloriosa superba*, respectively. Expression values were scaled by  $\log_{10}$ FPKM, in which FPKM was fragments per kilobase of exon per million mapped reads. For *L. sargentiae*, the expression profiles of flower tissues, including tepals, stamens, filaments, ovaries, and stigmas at five consecutive stages (S1-S5) from the bud stage to the mature stage were analyzed. For *G. superba*, tepals, stamens, and carpels at the bud stage (S1) were analyzed.

**Comment 5.** 4) Class E genes Lily has two type of SEP-like (class E) genes. SEP-like genes were previously reported as AGL2 genes and two lily SEP-like genes has been already isolated and characterized (Plant Physiol 133:1091-1101, 2003). These two lily SEP-like genes are far related in the previous report, while the two lily genes in this manuscript is closely related (figure S21). This is likely that the authors analyzed one type of SEP-like genes in lily. The authors should show the relationship of lily and gloriosa SEP-like genes and discuss about the expression patterns of these genes.

**Response:** Thanks a lot for this comment. We have updated our phylogenetic analysis result of AGL-like genes (Figure S22D; L440-443). In the revised version, we identified two AGL-like genes in lily, each situated in different clades of the phylogenetic tree. Additionally, we compared the expression patterns of these AGL-like genes (L455-459).

**L440-443:** As for the AGL2 homologues in the class E MADS genes, the two identified AGL2 homologues (LsaAGL9 and LsaAGL6) in lily are distributed across two separate clades: one clustering with AtAGL9 and another grouping more closely with AtAGL2/3/4

(Figure S22) <sup>36</sup>.

**L455-459:** For class E genes, *GsuAGL2* and *GsuAGL6* showed high expression levels in all examined flower tissues at the bud stage (Figure S23). While in lily, class E *LsaAGL2* showed lower expressions than the other two class E genes, *LsaAGL9* and *LsaAGL6*. *LsaAGL9* was expressed in all tissues, while *LsaAGL6* was not expressed in stamens (Figure S23).

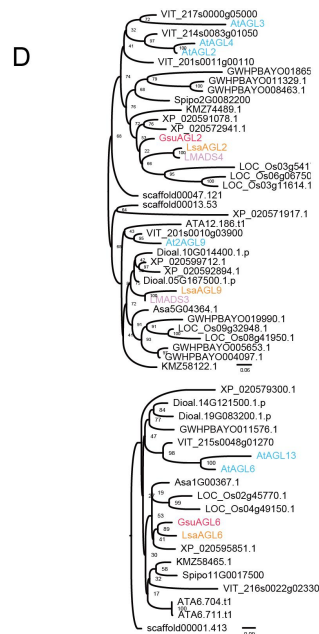


Figure S22E. Phylogenetic analysis of class E genes in *Lilium sargentiae*, *Gloriosa superba*, and other species.

**Comment 6.** 5) A new ABCE model Lily has two whorls of petaloid organs and this is explained by the expansion of class B gene expression. This new floral model is called as modified ABC model (Flower. Newslett. 16: 33–38, 1993; Plant Mol. Biol. 52: 831–841, 2003) or modified ABCE model (Hortic. J. 85:8–22, 2016; Trend. Plant Sci. 22: 8-10, 2017). The important point is the expression of class B genes in whorls 1 and 2 because this can show the expansion of class B genes in whorl 1. The authors mentioned that “Based on these results, we propose a new ABCE model applicable to the petaloid Liliales” (L. 408-409), but their results did not support the expansion of class B genes because they mixed the expression of these genes in whorls 1 and 2. If the authors want to say this sentence, they should divide outer and inner tepals for expression analysis.

**Response:** Thanks for the clear explanation. Due to the mixed sampling of whorls 1 and 2 in lily, we were unable to distinguish the expression patterns of floral identity genes in each whorl separately. As a result, we have removed the proposed models from the revised manuscript.

**Comment 7.** L.507-534, Discussion: Discussion part seems to be summary. The authors need to go into more detail and depth in discussion.

**Response:** Thanks a lot for this comment. We have revised the discussion part into a thorough and detailed discussion. (L564-669)

**L564-669:**

*Unveiling the Gigantic Genome of *L. sargentiae**

*In this study, we unveil the first reference genome assemblies for both *L. sargentiae* (47.09 Gb) and *G. superba* (5.15 Gb) of Liliales. Notably, *L. sargentiae* represents the largest sequenced eukaryotic genome published to date, surpassing even that of the Chinese pine genome (*Pinus tabulaeformis*, 25 Gb), as well as the lungfish genome (*Neoceratodus forsteri*, 37 Gb)<sup>43</sup>. The extraordinary size of *L. sargentiae* is mainly attributed to the substantial activity of LTR-RTs, which are recurrent factors in the evolution of large plant genomes<sup>23, 44, 45</sup>. While the genomes of most monocot orders have been sequenced, genomic information for the order Liliales remains sparse. The large genome sizes in Liliales, with an exceptionally high mean 1C value of 39.26 pg (38.39 Gb) for monocots<sup>46</sup>, have evidently increased the challenge of resolving their genomes. The genome size diversity among Liliaceae, Melanthiaceae, and Colchicaceae has been evaluated<sup>1, 47, 48</sup>. For example, *Lilium* species exhibit a wide range of estimated genome sizes, from 44.88 pg (43.89 Gb) in *L. souliei* to 167.58 pg (163.93 Gb) in *L. grayi*<sup>2</sup>, which offers a valuable opportunity to compare the underlying evolutionary mechanisms driving genome size diversity in plants. We will gain more detailed insights into genome size and diversity of Liliales as more genomes within Liliales are sequenced in the future.*

*A Revised Phylogeny of *Lilium**

*Several phylogenetic studies have utilized plastome (the chloroplast genome) sequences to infer relationships among species in *Lilium*<sup>49, 50, 51, 52, 53, 54, 55</sup>. However, relying solely in plastome sequences has limitations that could compromise the reliability of the resulting phylogeny. A main limitation is that plastid gene sequences only reflect maternal lineages, making them insufficient for detecting hybridization events<sup>55</sup>. Furthermore, the limited taxon in these previous studies may have further reduced the accuracy of the evolutionary conclusions from phylogenetic analyses<sup>49</sup>. In our study, nuclear sequences from 85 lilies were analyzed, resulting in the most comprehensive and up-to-date phylogenetic study of the *Lilium* genus. Unlike previous reports, our findings revealed that *Lilium* was divided into two major clades: one consisting solely of Asian species (the *Leucolirion* 6a + *Archelirion* species) and the other comprising species from Asia, Europe, and North America<sup>49, 50, 51, 55</sup>. Notably, we found that *Sinomartagon* is polyphyletic, with species distributed across three different clades, and that *Leucolirion* 6a and 6b are independent sections rather than subsections, consistent with earlier findings<sup>49, 50, 51, 54</sup>. Additionally, we further demonstrated that *L. henryi* and *L. rosthornii* belonged to *Leucolirion* 6a, while *L. brownii* was part of *Leucolirion* 6b, respectively<sup>49, 50, 56</sup>. However, the composition of sections within the three *Sinomartagon* clades differs from the previous reports<sup>49, 50</sup>. Our results also confirmed that the *Nomocharis* genus is closely related to *Sinomartagon* 5c<sup>49, 54</sup>. Our results confirmed that *Martagon* was monophyletic and sister to *Sinomartagon* 5a, rather than derived from *Sinomartagon*<sup>49, 50</sup>. We also validated that numerous wild lilies belonging to *Sinomartagon* 5c and *Nomocharis* have not undergone hybridization with the dominant cultivated lily species. This highlights the existence of untapped genetic reservoirs and offers immense potential for future lily breeding endeavors. For the first*

time, we detected shared genome haplotypes among wild and cultivated lily species. While significant advancements in lily breeding have been made in the past 50 years, particularly through interspecific hybridization<sup>57</sup>, we found that some modern lilies may have been developed through methods other than hybridization, such as domestication and breeding. Our classification of the *Lilium* genus, the origin of cultivated lilies, and hybridization patterns at the nuclear gene level provide extensive insights into the relationships among major wild and cultivated lily species and offer new directions for future breeding efforts.

#### Genomic Insights into Bulb and Rhizome Formation

To date, while several genomes of bulbous and rhizomatous plants have been previously documented<sup>21, 22, 58, 59</sup>, our understanding of these unique plant structures remains limited. In our proposed module for lily bulb development, we identified biological pathways similar to those found during bulb formation in onion, which are known to play crucial roles in storage organ formation<sup>22</sup>. Notably, we discovered that multiple gene families involved in sucrose and starch metabolism, key processes in the initiation and expansion of storage organs<sup>13, 25, 26, 27, 28, 29</sup>, have undergone significantly tandem duplication (TD) in lily. Furthermore, members of the XTH gene family in lily are also significantly expanded due to TD; these genes have been previously reported to exhibit differential expressions during storage organ development<sup>33</sup>. Importantly, we provide the first experimental evidence of the role of XTH in bulb formation in plants. The research on rhizome development in flame lilies is more fragmentary compared to that of lily bulb formation<sup>60</sup>. In this study, we proposed a model for rhizome formation in flame lily, highlighting the potential importance of sucrose metabolism/transport and starch biosynthesis pathways in this process. On the basis of the crucial roles of SUT and GBSS genes in potato tuber development<sup>61, 62, 63</sup>, we propose that the rhizome specific *GsuSUT2* and *GsuGBSS1* genes may be vital for flame lily rhizome formation. Specifically, in the flame lily rhizome, *GsuSUT2* likely facilitates the transport of sucrose for subsequent starch biosynthesis in amyloid, while *GsuGBSS1* is involved in the following amylose synthesis process. Additionally, the two significantly expanded gene families in lily and flame lily are different, providing a valuable opportunity to understand the formation of distinct modified stem structures in these two species.

#### Evolution of Colchicine Biosynthesis in Flame Lily

Although a near-complete colchicine biosynthesis pathway in *G. superba* has recently been elucidated, the genomic foundation of this pathway remains largely unexplored<sup>39, 40</sup>. We identified gene clusters responsible for colchicine biosynthesis that are unique to flame lily. Notably, the critical gene clusters associated with colchicine biosynthesis and colchicine-related metabolites, are absent in *L. sargentiae*, suggesting that this species lacks the complete pathway to synthesize colchicine. This finding is consistent with the previous report that colchicine production is restricted to the Colchicaceae family<sup>16</sup>, supporting the hypothesis of an independent origin of colchicine biosynthesis within this family. Within the syntenic genomic region of the colchicine-related CYP71DA12 tandem array in flame lily, the lily genome also contains a CYP71DA12 tandem array. However, only two of the lily CYP71DA12 genes are syntenic homologues of the flame lily CYP71DA12 genes. Interestingly, each of these two lily CYP71DA12 genes forms multiple

syntenic gene pairs with the flame lily CYP71DA12s, particularly LsaCYP71DA12a (lili00G285310). This suggests that after the divergence of the lily and flame lily, their ancestral CYP71DA12 genes underwent TD in flame lily, leading to functional divergence of these genes. Subsequently, the CYP71DA12 genes in flame lily have evolved to encode enzymes for colchicine biosynthesis. However, it remains to be verified whether the CYP71DA12 genes in lily exhibit similar function to the CYP71DA12s of flame lily. Furthermore, in the phylogenetic analysis of CYP75A genes, we identified one GsCYP75A109 homologue, and multiple GsCYP75A110 and GsCYP75A109/110-like homologues in flame lily. In contrast, we identified only one CYP75A gene in both the lily genome and the transcriptome assemblies of various lilies, which was found to occupy the most basal branches relative to the homologues of GsCYP75A109 and GsCYP75A110 (Figure S29C). Considering that GsCYP75A109 and GsCYP75A110 play distinct roles within colchicine biosynthesis—specifically in the phenethylisoquinoline scaffold hydroxylation and phenol ring coupling, respectively<sup>39, 40</sup>, we propose that the CYP75A109 and CYP75A110 genes in flame lily may have undergone functional divergence following the formation of multiple copies and have acquired novel functions. In contrast, the single CYP75A gene in lily is not likely to contain these specialized functionalities.

In summary, our work provides some novel insights into the evolution of expansive plant genomes, which offers valuable genomic resources for the future study of Liliales plants and acceleration of the selection and breeding of lilies and flame lilies.

**Comment 8.** L.90-91, 394-395: Tepal is one of the component parts of the perianth. When the perianth is divided into two whorls of unequal appearance, we call sepal and petal instead of outer tepal and inner tepal, respectively.

**Response:** Thank you for your comment. We have revised and improved the relevant description in the corresponding part of our manuscript. (L86-88; L428-429)

**L86-88:** Lily species exhibit distinctive flower structures characterized by six petaloid tepals (three outer tepals and three inner tepals), the constituent parts of the undifferentiated perianth.

**L428-429:** The lilies and flame lilies feature flower structures with petaloid organs called tepals, which contribute significantly to their ornamental values.

**Comment 9.** L.110: Here the authors mentioned the reason why they used gloriosa. Since they compared lily and gloriosa genome in this manuscript, they should add the reason from a systematic point of view.

**Response:** Thanks a lot for this comment. We have added the reason why both lily and flame lily's genomes are chosen and used in our study in the introduction part from a systematic point of view. (L111-117)

**L111-117:** Lily and flame lily are amongst the most popular ornamental plants and cutting flowers in the Liliales order. They share similar floral structures but differ in their stem modifications: lily has bulbs while flame lily has rhizomes, ideal for the comparative study of organ development in Liliales. While only the Colchicaceae family can synthesize colchicine<sup>16</sup>, a comparison of the genomes of lily and flame lily offers insights into the

origin and evolution of colchicine. Additionally, their contrasting genome sizes will enable the exploration of the underlying evolutionary mechanisms of the giant lily genome.

**Comment 10.** L.252: Figure 2A, D -> Figure 2A? Figure 2D is missing.

**Response:** Thanks a lot for this comment. We have revised “Figure 2A, D” to “Figure 2A”. (L257)

**L255-257:** We took advantage of the first lily genome and transcriptome datasets of a wide range of lilies to conduct the most comprehensive to-date phylogenomic analysis of lily (Figure 2A).

**Comment 11.** L.252-255: “The results indicate that these wild lilies in Clade 2-1 might not have been widely used in the past lily breeding programs, highlighting a previously untapped genetic reservoir, which may offer immense potential for future breeding endeavors.”->Are there any reason why Clade2-1 were not used for lily breeding to date? If any, the authors should discuss about that in discussion.

**Response:** Thanks a lot for this comment. Although lily breeding dates back over 200 years, significant breakthroughs have occurred only in the past 50 years. We are confident that these valuable lily genomic and germplasm resources will be utilized in the future. Additionally, the lily species in Clade 2-1, which naturally grow in high plateau, have not adapted well to current domestication efforts in greenhouse conditions in lowland areas.

**Comment 12.** L.285: (B) is missing in this legend. Add (B) just before “Heatmap”.

**Response:** Thanks a lot for this comment. We have added “(B)” before “Heatmap”. (L312)

**L312:** (B) Heatmap showing shared genomic haplotype patterns among different lily species.

**Comment 13.** Figure 3G:YABB1 -> YABBY1

**Response:** Thanks a lot for this comment. We have revised “YABB1” to “YABBY1”.

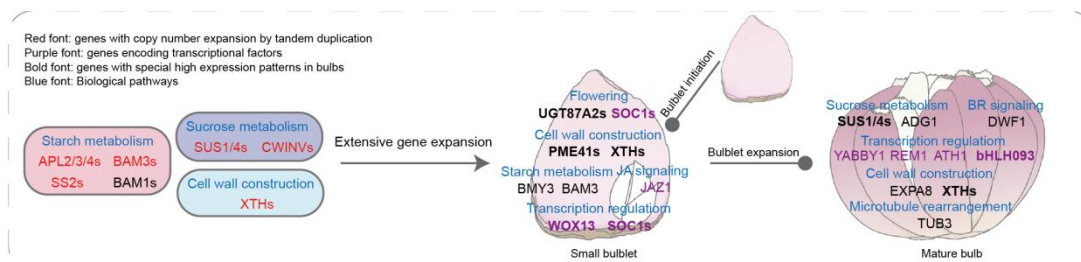


Figure 3G. A proposed model of the evolutionary and genetic mechanisms of bulblet initiation and bulblet expansion in *L. sargentiae*.

**Comment 14.** L.397: the floral identity genes -> the floral organ identity genes

**Response:** Thanks a lot for this comment. We have revised “floral identity genes” to “floral organ identity genes”. (L431)

**L428-431:** Here we elucidate the molecular basis of the floral morphology of *L. sargentiae* and *G. superba* by identifying the floral organ identity genes (including class A, B, C, and E MADS-box genes) and analyzing their expression patterns (Figure S22, 23).

**Comment 15.** L.397-407: “MADS” is not a gene name and does not need to be italicized.

**Response:** Thanks a lot for this comment. We have changed the italics of “MADS” to non-italics.

**Comment 16.** L.464: Figure 4C -> Figure 5C

**Response:** Thanks a lot for this comment. We have revised “Figure 4C” to “Figure 5C”. (L512)

**L510-512:** We identified multiple homologues of the previously identified colchicine biosynthesis genes<sup>39, 40</sup> (Figure 5C and Figures S28-30).

**Comment 17.** L.536: References There are so many mistakes. For example, Adam, P., Du, Y.-p., Wei, C., Wang, Z.-x., Li, S., He, H.-b., and Jia, G.-x. (2014). *Lilium* spp. pollen in China (Liliaceae): Taxonomic and Phylogenetic Implications and Pollen Evolution Related to Environmental Conditions. PLoS ONE 9.

->Author is wrong. Adam is editor's name and this paper is Du et al.

->Upper and lower case letters are mixed in the title.

->Pages are missing.

Cantalapiedra et al. also include the editor name in the authors.

The style of journal abbreviations is not unified.

The authors should check all the references very carefully.

**Response:** Thanks a lot for your comment. We have checked the references and rectified related mistakes. The two references you mentioned are also revised as follows:

Du YP, Wei C, Wang ZX, Li S, He HB, Jia GX. *Lilium* spp. pollen in China (Liliaceae): taxonomic and phylogenetic implications and pollen evolution related to environmental conditions. *PloS one* **9**, e87841 (2014).

Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. eggNOG-mapper v2: Functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol Biol Evol* **38**, 5825-5829 (2021).

#### Point to point responses for reviewer #4

**Comment 1.** Please refer also to the remarks within the text (attached file)

**Response:** Thanks a lot for this comment. We have cited two articles to the first remark.

**The two cited articles are as follows:**

Pan W, et al. Epigenetic silencing of callose synthase by VIL1 promotes bud-growth transition in lily bulbs. *Nat. Plants* **9**, 1451-1467 (2023).

Lazare S, Zaccai M. Flowering pathway is regulated by bulb size in *Lilium longiflorum* (Easter lily). *Plant Biol.* **18**, 577-584 (2016).

We have cited three articles to the second remark. **The three cited articles are as follows:**

Pan W, et al. Epigenetic silencing of callose synthase by VIL1 promotes bud-growth transition in lily bulbs. *Nat. Plants* **9**, 1451-1467 (2023).

Li X, et al. Transcriptome analysis of carbohydrate metabolism during bulblet formation and development in *Lilium davidii* var. unicolor. *BMC Plant Biol.* **14**, 358 (2014).

Xin Y, et al. Auxin regulates bulbil initiation by mediating sucrose metabolism in *Lilium lancifolium*. *Hortic. Res.* **11**, uhae054 (2024)

**Comment 2.** *L. longiflorum* was not part of this study and was not mentioned in any way, despite of its importance in the industry and breeding. Can the authors comment on this?

**Response:** Thanks a lot for this comment. *L. longiflorum* is closely related to *L. formosanum*. In our study, we used *L. formosanum* and *L. formosanum* var. *pricei* as representatives of this group of lilies. Indeed, *L. longiflorum* is an important species for lilies that is widely used in molecular biology research. However, there are relatively few genes mined from *L. longiflorum*, which is why it is cited less frequently. We chose *L. sargentiae* for specific reasons: it is a close relative of lilies such as *Lilium regale* and exhibits high resistance. Additionally, *L. sargentiae* produces bulbils that can be removed and propagated into new plants, representing a significant breakthrough for the rapid reproduction of lilies for the horticultural industry in the future.

**Comment 3.** For such an integrative and important study, the discussion is rather limited. It also totally lacks reference to the part about colchicine part, which constitutes a significant portion of the study. The quality of the manuscript would be much improved by a more structured and in-depth discussion, which could include sub-sections for clarity.

**Response:** Thanks a lot for this comment. We had an in-depth Discussion in the previous versions, but it was removed in the original manuscript due to the overall length of the article exceeded the word limit. We have conducted comprehensive and in-depth revisions to the discussion section, including the discussion on colchicine (**L564-669**).

#### **L564-669:**

##### *Unveiling the Gigantic Genome of *L. sargentiae**

*In this study, we unveil the first reference genome assemblies for both *L. sargentiae* (47.09 Gb) and *G. superba* (5.15 Gb) of Liliales. Notably, *L. sargentiae* represents the largest sequenced eukaryotic genome published to date, surpassing even that of the Chinese pine genome (*Pinus tabulaeformis*, 25 Gb), as well as the lungfish genome (*Neoceratodus forsteri*, 37 Gb) <sup>43</sup>. The extraordinary size of *L. sargentiae* is mainly attributed to the substantial activity of LTR-RTs, which are recurrent factors in the evolution of large plant genomes <sup>23, 44, 45</sup>. While the genomes of most monocot orders have been sequenced, genomic information for the order Liliales remains sparse. The large genome sizes in Liliales, with an exceptionally high mean 1C value of 39.26 pg (38.39 Gb) for monocots <sup>46</sup>, have evidently increased the challenge of resolving their genomes. The genome size diversity among Liliaceae, Melanthiaceae, and Colchicaceae has been evaluated <sup>1, 47, 48</sup>. For example, *Lilium* species exhibit a wide range of estimated genome sizes, from 44.88 pg (43.89 Gb) in *L. souliei* to 167.58 pg (163.93 Gb) in *L. grayi* <sup>2</sup>, which offers a valuable opportunity to compare the underlying evolutionary mechanisms driving genome size diversity in plants. We will gain more detailed insights into genome*

size and diversity of Liliales as more genomes within Liliales are sequenced in the future.

#### *A Revised Phylogeny of Lilium*

Several phylogenetic studies have utilized plastome (the chloroplast genome) sequences to infer relationships among species in *Lilium* <sup>49, 50, 51, 52, 53, 54, 55</sup>. However, relying solely in plastome sequences has limitations that could compromise the reliability of the resulting phylogeny. A main limitation is that plastid gene sequences only reflect maternal lineages, making them insufficient for detecting hybridization events <sup>55</sup>. Furthermore, the limited taxon in these previous studies may have further reduced the accuracy of the evolutionary conclusions from phylogenetic analyses <sup>49</sup>. In our study, nuclear sequences from 85 lilies were analyzed, resulting in the most comprehensive and up-to-date phylogenetic study of the *Lilium* genus. Unlike previous reports, our findings revealed that *Lilium* was divided into two major clades: one consisting solely of Asian species (the *Leucolirion* 6a + *Archelirion* species) and the other comprising species from Asia, Europe, and North America <sup>49, 50, 51, 55</sup>. Notably, we found that *Sinomartagon* is polyphyletic, with species distributed across three different clades, and that *Leucolirion* 6a and 6b are independent sections rather than subsections, consistent with earlier findings <sup>49, 50, 51, 54</sup>. Additionally, we further demonstrated that *L. henryi* and *L. rosthornii* belonged to *Leucolirion* 6a, while *L. brownii* was part of *Leucolirion* 6b, respectively <sup>49, 50, 56</sup>. However, the composition of sections within the three *Sinomartagon* clades differs from the previous reports <sup>49, 50</sup>. Our results also confirmed that the *Nomocharis* genus is closely related to *Sinomartagon* 5c <sup>49, 54</sup>. Our results confirmed that *Martagon* was monophyletic and sister to *Sinomartagon* 5a, rather than derived from *Sinomartagon* <sup>49, 50</sup>. We also validated that numerous wild lilies belonging to *Sinomartagon* 5c and *Nomocharis* have not undergone hybridization with the dominant cultivated lily species. This highlights the existence of untapped genetic reservoirs and offers immense potential for future lily breeding endeavors. For the first time, we detected shared genome haplotypes among wild and cultivated lily species. While significant advancements in lily breeding have been made in the past 50 years, particularly through interspecific hybridization <sup>57</sup>, we found that some modern lilies may have been developed through methods other than hybridization, such as domestication and breeding. Our classification of the *Lilium* genus, the origin of cultivated lilies, and hybridization patterns at the nuclear gene level provide extensive insights into the relationships among major wild and cultivated lily species and offer new directions for future breeding efforts.

#### *Genomic Insights into Bulb and Rhizome Formation*

To date, while several genomes of bulbous and rhizomatous plants have been previously documented <sup>21, 22, 58, 59</sup>, our understanding of these unique plant structures remains limited. In our proposed module for lily bulb development, we identified biological pathways similar to those found during bulb formation in onion, which are known to play crucial roles in storage organ formation <sup>22</sup>. Notably, we discovered that multiple gene families involved in sucrose and starch metabolism, key processes in the initiation and expansion of storage organs <sup>13, 25, 26, 27, 28, 29</sup>, have undergone significantly tandem duplication (TD) in lily. Furthermore, members of the XTH gene family in lily are also significantly expanded due to TD; these genes have been previously reported to exhibit differential expressions during storage organ development <sup>33</sup>. Importantly, we provide the first experimental

evidence of the role of XTH in bulb formation in plants. The research on rhizome development in flame lilies is more fragmentary compared to that of lily bulb formation<sup>60</sup>. In this study, we proposed a model for rhizome formation in flame lily, highlighting the potential importance of sucrose metabolism/transport and starch biosynthesis pathways in this process. On the basis of the crucial roles of SUT and GBSS genes in potato tuber development<sup>61, 62, 63</sup>. We propose that the rhizome specific GsuSUT2 and GsuGBSS1 genes may be vital for flame lily rhizome formation. Specifically, in the flame lily rhizome, GsuSUT2 likely facilitates the transport of sucrose for subsequent starch biosynthesis in amyloid, while GsuGBSS1 is involved in the following amylose synthesis process. Additionally, the two significantly expanded gene families in lily and flame lily are different, providing a valuable opportunity to understand the formation of distinct modified stem structures in these two species.

#### *Evolution of Colchicine Biosynthesis in Flame Lily*

Although a near-complete colchicine biosynthesis pathway in *G. superba* has recently been elucidated, the genomic foundation of this pathway remains largely unexplored<sup>39, 40</sup>. We identified gene clusters responsible for colchicine biosynthesis that are unique to flame lily. Notably, the critical gene clusters associated with colchicine biosynthesis and colchicine-related metabolites, are absent in *L. sargentiae*, suggesting that this species lacks the complete pathway to synthesize colchicine. This finding is consistent with the previous report that colchicine production is restricted to the Colchicaceae family<sup>16</sup>, supporting the hypothesis of an independent origin of colchicine biosynthesis within this family. Within the syntenic genomic region of the colchicine-related CYP71DA12 tandem array in flame lily, the lily genome also contains a CYP71DA12 tandem array. However, only two of the lily CYP71DA12 genes are syntenic homologues of the flame lily CYP71DA12 genes. Interestingly, each of these two lily CYP71DA12 genes forms multiple syntenic gene pairs with the flame lily CYP71DA12s, particularly LsaCYP71DA12a (lili00G285310). This suggests that after the divergence of the lily and flame lily, their ancestral CYP71DA12 genes underwent TD in flame lily, leading to functional divergence of these genes. Subsequently, the CYP71DA12 genes in flame lily have evolved to encode enzymes for colchicine biosynthesis. However, it remains to be verified whether the CYP71DA12 genes in lily exhibit similar function to the CYP71DA12s of flame lily. Furthermore, in the phylogenetic analysis of CYP75A genes, we identified one GsCYP75A109 homologue, and multiple GsCYP75A110 and GsCYP75A109/110-like homologues in flame lily. In contrast, we identified only one CYP75A gene in both the lily genome and the transcriptome assemblies of various lilies, which was found to occupy the most basal branches relative to the homologues of GsCYP75A109 and GsCYP75A110 (Figure S29C). Considering that GsCYP75A109 and GsCYP75A110 play distinct roles within colchicine biosynthesis—specifically in the phenethylisoquinoline scaffold hydroxylation and phenol ring coupling, respectively<sup>39, 40</sup>, we propose that the CYP75A109 and CYP75A110 genes in flame lily may have undergone functional divergence following the formation of multiple copies and have acquired novel functions. In contrast, the single CYP75A gene in lily is not likely to contain these specialized functionalities.

*In summary, our work provides some novel insights into the evolution of expansive plant genomes, which offers valuable genomic resources for the future study of Liliales plants and acceleration of the selection and breeding of lilies and flame lilies.*

**Comment 4.** For the qRT-PCR analyses, please give more details about the tissues from which RNA was extracted and about the and the developmental stage of the plant at sampling time.

**Response:** Thanks a lot for this comment. We have added the description of the used tissues. (L1108-1111)

**L1108-1111:** *Total RNA was extracted from SAMs of lily cultivars ‘Siberia’ under two conditions: 4 ° C from 0 to 8 weeks, and 12 ° C bud-forcing treatment from 3 to 15 days. Additionally, RNA was extracted from tissue located 2 mm above the base of the lily scales in pTRV2, pTRV2-LaXTH, EV, and OE-LaXTH lines at the S1 stage.*

### **Point to point responses for reviewer #5**

**Comment 1.** This implies that the expansion of LTR-RTs—besides genome size expansion—might also have influenced the evolution and structure of genes underlying crucial bulb-related traits, including bulb formation as well as growth transition. This conclusion was solely based on a GO-term associated with bulblet initiation and development, which was detected for *L. sargentiae* and *G. superba*. However, the storage and propagation organs of *G. superba* are rhizomes, rather than bulbs. Thus, it is unsure if this conclusion is reasonable.

**Response:** Thank you for your comment. In this part of the manuscript, we did not refer to the rhizomes of *G. superba*. We only conducted GO enrichment analysis of genes in the lily genome. Since LTRs caused significant expansion of the lily genome, and Tork (43.2%) and Tekay (17.1%). The most abundant LTRs in lily are *Copia* and *Gypsy*. We conducted GO enrichment analysis of genes located within 2 kb of Tork and Tekay LTR-RTs, or containing these LTR-RTs within their introns in the lily genome, to explore the potential functional contributions of these LTRs in the formation of the giant lily genome.

To clarify this result, we have revised the text from “By analyzing genes in proximity (within 2 kb) to Tork and Tekay LTR-RTs or containing these LTR-RTs in their introns” to “**Through GO enrichment analysis of genes located within 2 kb of Tork and Tekay LTR-RTs, or containing these LTR-RTs within their introns in the lily genome**” (L190-192) to highlight that our analysis is exclusively related to lily.

**Comment 2.** Along with their proliferation, LTR-RTs are also actively removed through ectopic homologous recombination, which also generates solo-LTRs. Is there any evidence to support this finding?

**Response:** Thanks a lot for this comment. We have cited a reference (L234) here to support this known mechanism of LTR-RT removal.

**Reference:**

Cossu RM, Casola C, Giacomello S, Vidalis A, Scofield DG, Zuccolo A. LTR retrotransposons show low levels of unequal recombination and high rates of intraelement gene conversion in large plant genomes. *Genome Biol. Evol.* **9**, 3449-3462 (2017).

**Comment 3.** Lines 304-306, a literature (Li et al., 2014) was cited to indicate that genes involved in starch and sucrose metabolism play important roles in the initiation and expansion of storage organs. However, this citation is not correct because the main purpose of the literature is to investigate gene expression profiles during bulblet formation based on transcriptome analysis. The results of the literature cannot point out any roles of starch/sucrose related genes in bulb initiation and expansion. Therefore, subsequent analyses are not scientific at all. And the related finding that the XTH genes play crucial roles in the bulblet initiation and expansion lacks solid evidence.

**Response:** Thank you for your valuable suggestion. Starch and sugar metabolism are indeed fundamental for providing the material and energy necessary for the development of storage organs (Zierer et al., 2021). These processes play a crucial role in the initiation and expansion of these organs, as highlighted by Zierer et al. (2021). Recent studies further demonstrate that the application of sucrose or glucose promotes the initiation and development of bulbs or bulbils (Liang et al., 2023; Xin et al., 2024; Hao et al., 2024). It was shown that starch biosynthesis genes regulate the development of stem-modified organs, as observed in corm development in *Gladiolus* (Seng et al., 2016, 2017). Consequently, we have added these four references and others that could support the important roles of sucrose/starch metabolism in storage organ development in **Line 336**.

*XTH* genes belong to the xyloglucan endotransglucosylase/hydrolase (*XTH*) family and play a pivotal role in cell wall plasticity by cleaving and reconnecting xyloglucan molecules. This process is essential for cell wall loosening, a critical step in plant cell expansion—a vital process in the development of *Allium* bulbs (Hao et al., 2023; Ishida et al., 2022). We did not study *XTHs* because they are involved in sucrose and starch metabolism; in fact, these genes are not involved in these processes. We focused on *XTHs* because we have identified multiple co-expressed *XTHs* during lily bulb development through WGCNA, aiming to discover additional genes potentially related to bulblet development in lily. Based on the reported functions of *XTHs* (**L365-366**), we hypothesized that *XTH* genes are important for bulb development in lilies.

To test this hypothesis, we selected a representative *XTH* gene (*LaXTH*) and conducted gene silencing and overexpression experiments in lily scales to investigate its role in bulblet development. Our results demonstrated a positive correlation between bulb induction (initiation and expansion of bulblets) and *LaXTH* expression, supporting the significant role of *XTH* genes in bulb development in lilies (**L370-380; Figure 3E and F**).

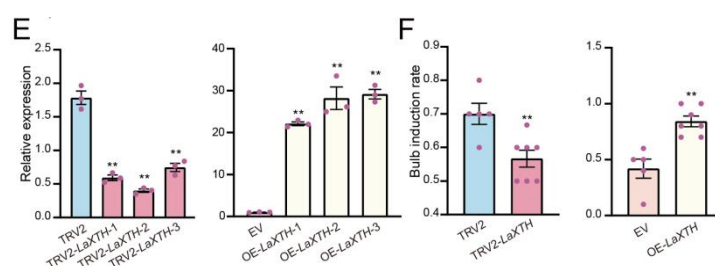


Figure 3E and 3F. Expression of *LaXTH* in TRV2 control lines and TRV2-*LaXTH* silenced lines as well as EV control lines and OE-*LaXTH* overexpressed lines, respectively (3E). The statistics of bulb induction

rate (bulblet initiation rate plus bulblet expansion rate) of the TRV2 ( $n = 5$ ) and TRV2-*LaXTH* ( $n = 7$ ) lines as well as EV ( $n = 5$ ) and OE-*LaXTH* ( $n = 7$ ) lines, respectively (3F).

#### References:

- Zierer W, Ruscher D, Sonnewald U, Sonnewald S. Tuber and tuberous root development. *Annu. Rev. Plant Biol.* **72**, 551-580 (2021).
- Liang J, et al. Cytokinins influence bulblet formation by modulating sugar metabolism and endogenous hormones in Asiatic hybrid lily. *Ornam. Plant Res.* **3**, 19 (2023).
- Xin Y, et al. Auxin regulates bulbil initiation by mediating sucrose metabolism in *Lilium lancifolium*. *Hortic. Res.* **11**, uhae054 (2024).
- Hao C, et al. Sucrose function on the bulbil formation of *Lilium lancifolium*. *Sci. Hortic.* **323**, 112538 (2024).
- Seng S, et al. ADP-glucose pyrophosphorylase gene plays a key role in the quality of corm and yield of cormels in gladiolus. *Biochem Bioph Res Co* **474**, 206-212 (2016).
- Seng S, et al. Silencing *GhAGPL1* reduces the quality and quantity of corms and cormels in gladiolus. *J Am Soc Hortic Sci* **142**, 119-125 (2017).
- Hao F, et al. Chromosome-level genomes of three key *Allium* crops and their trait evolution. *Nat Genet* **55**, 1976-1986 (2023).
- Ishida K, Yokoyama R. Reconsidering the function of the xyloglucan endotransglucosylase/hydrolase family. *J Plant Res* **135**, 145-156 (2022).

**Comment 4.** Weighted gene co-expression network analysis (WGCNA) was used to identify key genes responsible for bulblet development. The method can be conducted but may be not suitable in high-ranked journals. Of course, the results are not reliable and more evidence such as genetic evidence must be provided to validate the results. Lily bulb size varies greatly and candidate gene association analysis can be conducted to validate the role of candidate genes in bulb formation.

**Response:** Thanks a lot for this comment. Regarding our results of bulb development, we not only present WGCNA results but also integrate homologous gene comparisons and existing functional studies to draw the relevant conclusions. Given that the lily genome is reported for the first time here, a comprehensive analysis using WGCNA is essential.

WGCNA has been widely adopted in numerous high-impact studies across various fields, including genomics and transcriptomics, due to its robustness in identifying gene co-expression modules and its biological relevance. Since the WGCNA method was published in 2008 ([https://scholar.google.com/scholar?hl=en&as\\_sdt=0%2C5&q=WGCNA%3A+an+R+package+for+weighted+correlation+network+analysis&btnG=](https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=WGCNA%3A+an+R+package+for+weighted+correlation+network+analysis&btnG=)), it has been cited for almost **20,000** times, including papers on high-ranking journals (e.g., **Science**, **Nature Genetics**, **Nature Communications**) (See the quantity and quality of citations of WGCNA on Google Scholar below). The method has been validated in many publications in top-tier journals. Therefore, we believe it provides significant insights into the biological questions we are addressing. Here, we provided some examples of high-quality articles using WGCNA for your reference:

Ramírez-González RH, et al. The transcriptional landscape of polyploid wheat. *Science*

**361**, eaar6089 (2018).

Liu N, Lyu X, Zhang X, et al. Reference genome sequence and population genomic analysis of peas provide insights into the genetic basis of Mendelian and other agronomic traits. *Nat. Genet.* **56**,1964–1974 (2024).

Zhang F, Qiu F, Zeng J, et al. Revealing evolution of tropane alkaloid biosynthesis by analyzing two genomes in the Solanaceae family. *Nat. Commun.* **14**, 1446 (2023).

Song A, Su J, Wang H, et al. Analyses of a chromosome-scale genome assembly reveal the origin and evolution of cultivated chrysanthemum. *Nat. Commun.* **14**, 2021 (2023).

Johnson ECB., Carter EK, Dammer EB, et al. Large-scale deep multi-layer analysis of Alzheimer's disease brain reveals strong proteomic disease-related changes not observed at the RNA level. *Nat. Neurosci.* **25**, 213–225 (2022).

In this study, WGCNA was specifically chosen because it aligns well with our goal of detecting co-expression patterns and relating these patterns to phenotypic traits, including bulb development and growth transition. We identified multiple potential genes involved in bulb development using WGCNA and proposed a potential model for lily bulb development, according to their previously reported roles in storage organ development. Their established functional roles in other plants might indicate their involvement in lily bulb development (the relevant references were cited at **Line 336 and 385**). The functions of these candidate genes were labeled in Figure 3G, and the detailed functions of them could be found in Supplementary Data 6. Additionally, we discussed our results of lily bulb development in the Discussion part (**L613-635**). We hope that the corresponding discussion will enhance the credibility of our results and provide a clearer understanding of lily bulb development.

More importantly, based on the candidate genes identified by WGCNA, we performed functional verification of *XTH* and *AP1/FUL-like* genes in lily. The results all showed that these two genes are related to the specific traits (bulb development and floral growth transition, respectively) in lily. These findings further demonstrate that WGCNA is a suitable and scientifically valuable analytical method, which has helped us obtain relatively reliable and valuable results, laying the foundation for future research on lilies.

Thus, using WGCNA to identify potential key genes responsible for bulblet development are still valuable for the future study.

Among the candidate genes in bulb formation, we selected a representative *XTH* gene and verified its function during lily bulb development. We found the positive correlation between bulb induction and *LaXTH* expression, indicating *XTH*'s role in lily bulb development.

#### **L613-635:**

##### *Genomic Insights into Bulb and Rhizome Formation*

*To date, while several genomes of bulbous and rhizomatous plants have been previously documented<sup>21, 22, 58, 59</sup>, our understanding of these unique plant structures remains limited. In our proposed module for lily bulb development, we identified biological pathways similar to those found during bulb formation in onion, which are known to play crucial roles in storage organ formation<sup>22</sup>. Notably, we discovered that multiple gene families involved in sucrose and starch metabolism, key processes in the initiation and expansion of storage organs<sup>13, 25, 26, 27, 28, 29</sup>, have undergone significantly tandem duplication (TD) in lily.*



the significance of the *GBSS* gene function in the starch biosynthesis during tuber development in potatoes has been demonstrated through numerous published studies (van der Steege et al., 1992; Andersson et al., 2017). Based on this knowledge, we hypothesize that *GsuGBSS1* may be involved in rhizome development in flame lily. Additionally, we discussed *GsuGBSS1*'s potential functions in flame lily rhizome development in the Discussion part (**L629-632**). We hope that the corresponding discussion will enhance the credibility of our results and provide a clearer understanding of *GsuGBSS1*'s role.

Considering that no genetic transformation system of flame lily has been established, we were not able to verify the function of *GsuGBSS1*. However, based on the expression data, we proposed that in flame lily, *GsuGBSS1* may have both of the conserved function in starch biosynthesis and potential new function in the regulation of rhizome development.

**L629-632:** *We propose that the rhizome specific GsuSUT2 and GsuGBSS1 genes may be vital for flame lily rhizome formation. Specifically, in the flame lily rhizome, GsuSUT2 likely facilitates the transport of sucrose for subsequent starch biosynthesis in amyloid, while GsuGBSS1 is involved in the following amylose synthesis process.*

#### References:

- Zierer W, Ruscher D, Sonnewald U, Sonnewald S. Tuber and tuberous root development. *Annu. Rev. Plant Biol.* **72**, 551-580 (2021).
- Seng S, et al. ADP-glucose pyrophosphorylase gene plays a key role in the quality of corm and yield of cormels in gladiolus. *Biochem. Biophys. Res. Commun.* **474**, 206-212 (2016).
- Seng S, et al. Silencing *GhAGPL1* reduces the quality and quantity of corms and cormels in gladiolus. *J. Am. Soc. Hortic. Sci.* **142**, 119-125 (2017).
- Li X, et al. Transcriptome analysis of carbohydrate metabolism during bulblet formation and development in *Lilium davidii* var. unicolor. *BMC Plant Biol.* **14**, 358 (2014).
- van der Steege G, Nieboer M, Swaving J, Tempelaar MJ. Potato granule-bound starch synthase promoter-controlled GUS expression: regulation of expression after transient and stable transformation. *Plant Mol. Biol.* **20**, 19-30 (1992).
- Andersson M, Turesson H, Nicolia A, Falt AS, Samuelsson M, Hofvander P. Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. *Plant Cell Rep.* **36**, 117-128 (2017).

**Comment 6.** Figure 3G, too many genes are listed here and the results are not reliable because these genes are mainly identified solely based on bioinformatics analysis.

**Response:** Thanks a lot for this comment. These genes were identified using WGCNA, whose robustness in identifying potential functional genes has been validated in many publications in top-tier journals as well as in our study. We performed functional verification of *XTH* and *AP1/FUL-like* genes identified using WGCNA in lily, and the results showed that these two genes are related to the specific traits in lily. Additionally, when we constructed the potential model of lily bulb development, we referred to the established functional roles of these related genes, and corresponding references were

cited at **Line 336 and 385** to support our hypothesis. We have revised Figure 3G by adding related gene functions to it. A comprehensive understanding of genes' functions of Figure 3G can be obtained by referring to their functional information in Supplementary Data 6.

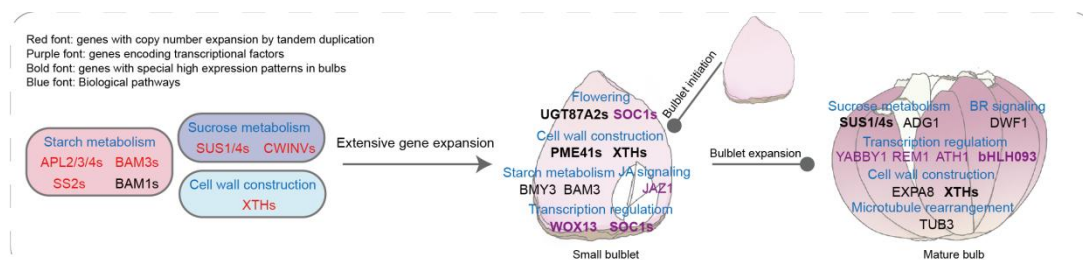


Figure 3G. A proposed model of the evolutionary and genetic mechanisms of bulblet initiation and bulblet expansion in *L. sargentiae*.

**Comment 7.** Line 408, we propose a new ABCE model applicable to the petaloid Liliales. I don't think there is any new findings related to flower development in this manuscript because all the genes were estimated based on the previously reported ABCE models in model plants.

**Response:** Thanks a lot for this comment. Although we referred to the known ABCE genes, we believe our work still offers significant value. In the revised version, we have removed the proposed models. Instead, we incorporated the previously reported lily ABCE MADS genes into our analysis and compared our findings with the earlier results. Some of our findings are consistent with previous studies, while we also uncovered novel insights. For example, we discovered that although both lily and flame lily possess two copies of *PI-like* genes, these genes underwent independent duplication events after the divergence of the two species. Additionally, we identified new copies of lily ABCE genes, such as the class E *LsaAGL6* gene. And the expression pattern of *LsaAGL6* suggested its A-class function in lily. Moreover, there has been no prior study on the floral identity genes in flame lily, a popular high-end cut flower with a distinctive flame-like corolla. Therefore, our study provides a foundation for future research on the flower development of flame lily. (L427-459)

**L427-459:**

#### **Floral organ identity genes in lily and flame lily**

The lilies and flame lilies feature flower structures with petaloid organs called tepals, which contribute significantly to their ornamental values. Here we elucidate the molecular basis of the floral morphology of *L. sargentiae* and *G. superba* by identifying the floral organ identity genes (including class A, B, C, and E MADS-box genes) and analyzing their expression patterns (Figure S22, 23). We identified a total of four class A, three class B, two class C, and three class E MADS genes in *L. sargentiae*, and two class A, three class B, two class C, and two class E MADS genes in *G. superba* (Figures S22). Regarding the class B *PI-like* genes, both *PI-like* genes in *L. sargentiae* and *G. superba* underwent independent duplication events after their divergence (Figure S22). Additionally, both *PI-like* genes (*GsuPIa* and *GsuPIb*) in *G. superba* contain the *PI*-motif. In contrast, only one *PI-like* gene (*LsaPIa*) in *L. sargentiae* possesses the *PI*-motif, while the other (*LsaPIb*)

does not (Figure S24A). This finding is in agreement with the previous reports that there are two types of PI-like genes in lilies with one containing the PI motif<sup>35</sup>. As for the AGL2 homologues in the class E MADS genes, the two identified AGL2 homologues (LsaAGL9 and LsaAGL6) in lily are distributed across two separate clades: one clustering with AtAGL9 and another grouping more closely with AtAGL2/3/4 (Figure S22)<sup>36</sup>.

For *L. sargentiae*, the expression profiles of various floral tissues, including tepals, stamens, filaments, ovaries, and stigmas, were analyzed across five consecutive stages (S1–S5) from the bud stage to full maturity. For *G. superba*, tepals, stamens, and carpels were analyzed at the bud stage (Figure S23). We found that the class A and B MADSs in *L. sargentiae* and *G. superba* exhibited broader expression in floral organs than their counterparts in eudicot model systems (Figure S23). Compared to class A MADS genes, both LsaAGL6 and GsuAGL6 exhibited significantly higher expression levels in tepals, indicating their A-class function, similar to the roles of AGL6s reported in other plants, e.g., rice<sup>37, 38</sup> (Figure S23). For class C AG/PLE-like genes, LsaAGa was not expressed in all the examined tissues (Figure S23). LsaAGa in lily and two AG genes in flame lily almost exhibited no expressions in tepals, but those showed high levels of expression in the reproductive organs (Figure S23). For class E genes, GsuAGL2 and GsuAGL6 showed high expression levels in all examined flower tissues at the bud stage (Figure S23). While in lily, class E LsaAGL2 showed lower expressions than the other two class E genes, LsaAGL9 and LsaAGL6. LsaAGL9 was expressed in all tissues, while LsaAGL6 was not expressed in stamens (Figure S23).

**Comment 8.** For the evolution of colchicine in flame lily, it is nuclear (most likely “unclear”?) which genes are responsible for the biosynthesis of colchicine although many kinds of OMT genes are described in the manuscript. Is there any explanation why the keys responsible for colchicine biosynthesis have been lost in *L. sargentiae*.

**Response:** Thank you for this comment. We have introduced the OMT genes responsible for the biosynthesis of colchicine in our manuscript as follows: **While plant OMTs constitute a large family of enzymes that methylate the oxygen atom of a variety of secondary metabolites, only GsOMT1, GsOMT2, GsOMT3, and GsOMT4 in flame lily are reported to function in colchicine biosynthesis<sup>40, 42</sup> (L529-532).** The reasons for the loss of key colchicine biosynthesis genes in lily are not yet clear. We speculate that flame lilies independently evolved these OMT genes following the divergence with lilies. Further genomic information from other Liliales species and functional analysis will be necessary to better understand the origin and evolution of colchicine synthesis and to elucidate why these genes are absent in *L. sargentiae*.

**Comment 9.** Line 60, colchicine was not mentioned in the abstract.

**Response:** Thanks a lot for this comment. We have added the result related to colchicine in our abstract. (L54-57)

**L54-57:** We further identify clusters of colchicine biosynthetic genes in the flame lily genome and demonstrate that these clusters are absent in the lily genome, which supports the independent origin of colchicine production within Colchicaceae.

**Comment 10.** Assembly of the *G. superba* genome resulted in 11 chromosomes, while 35 scaffolds were generated for the *L. sargentiae*. Is *G. superba* a diploid species? What is the ploidy level for *L. sargentiae*?

**Response:** Thanks a lot for this comment. Our lily genome assembly is at the scaffold level, rather than chromosome level, whereas the flame lily genome is assembled at the chromosome level. As a result, the *G. superba* genome is organized into 11 chromosomes, while the *L. sargentiae* genome comprises 35 scaffolds (which has 12 chromosomes in fact). Both the genome survey and early flow cytometry analysis confirm that the *G. superba* and *L. sargentiae* are diploid. (Du et al., 2017). Other species within the *Lilium* genus are not necessarily diploid, but so far, all wild species, except for *Lilium lancifolium*, have been found to be diploid.

**Reference:**

Du YP, Bi Y, Zhang MF, Yang FP, Jia GX, Zhan g XH. Genome size diversity in *Lilium* (Liliaceae) Is correlated with karyotype and environmental traits. *Front. Plant Sci.* **8**, 1303 (2017).

**Comment 11.** Lines 204-206, it is difficulty to understand this sentence.

**Response:** Thanks a lot for this comment. We have revised the original sentence. (L220-223)

**L220-223:** Both *L. sargentiae* and *G. superba* genomes showed a clear peak around 100 bp in LTR length (Figure S7B). Additionally, *L. sargentiae* had a noticeable peak near 10 kb, while *G. superba* exhibited another peak around 3.2 kb in LTR length (Figure S7B).

**Comment 12.** Line 265, Wild lilies of Sinomartagon 5b and 5a wild lilies. “wild lilies” appears twice, correct?

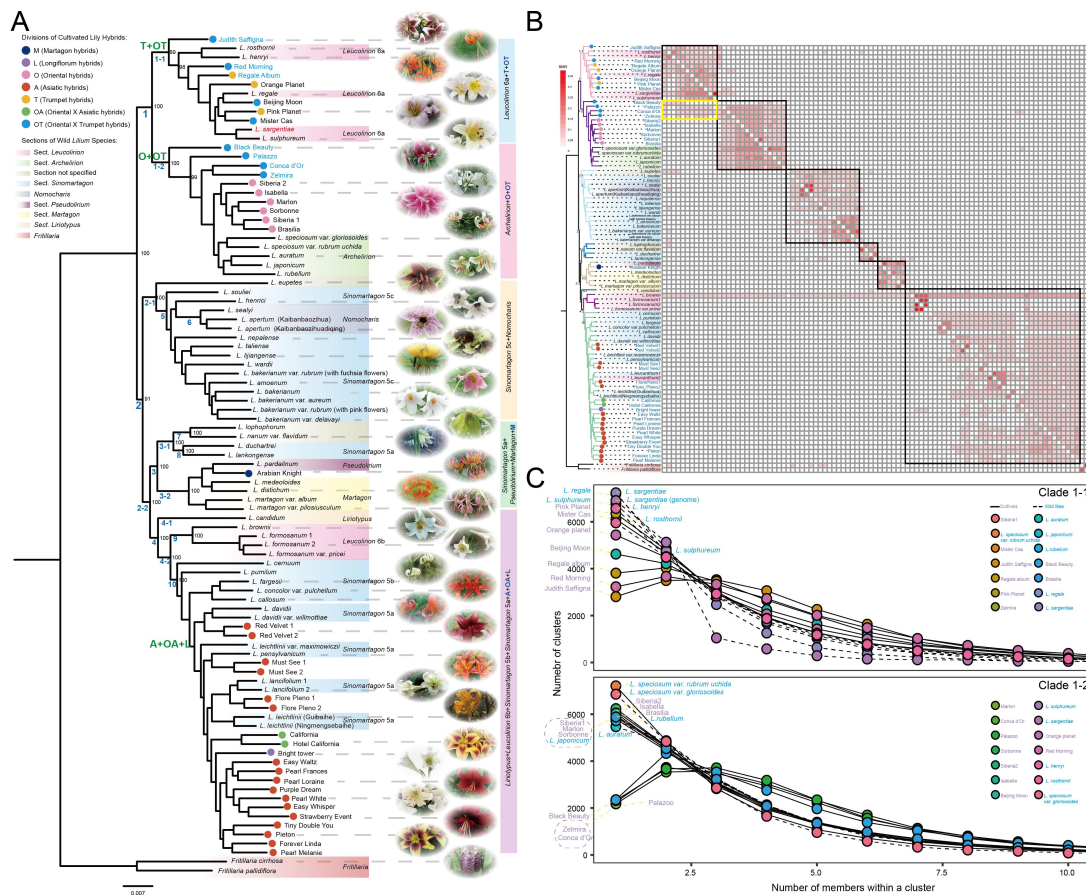
**Response:** Thanks a lot for this comment. We have deleted the second “wild” in the sentence. (L283-285)

**L283-285:** Wild lilies of Sinomartagon 5b and 5a lilies are potential parents for all the three types (A, OA, and L) of lily hybrids (Figure 2B).

**Comment 13.** Figure 2, this picture is very fuzzy and the legend for figure 2B could not be found as well.

**Response:** Thanks a lot. We have revised the figure and its legend, and improved its clarity. The new figure legend of Figure 2B is as

follows:



**Figure 2. Phylogeny and hybridization patterns of *Lilium*.**

(A) The phylogenetic tree of the wild *Lilium* species and cultivated lilies was inferred from combined RNA-seq data of wild *Lilium* species and *Lilium* cultivars. The blue numbers labeled on the tree represent the classified clades. Bootstrap values for important branches were labeled above branches.

(B) Heatmap showing shared genomic haplotype patterns among different lily species. Each cell represents the ratio of the number of transcripts with the highest gene similarity (identity > 95%) between two species to the total number of transcripts within the corresponding species in that row (excluding self-aligning transcripts). The intensity of the box color reflects the proportion of gene similarity (best match ratio, BMR), with redder shades indicating higher similarity. The yellow rectangle highlights shared genomic haplotypes between clades, while boxes of species within the same clade are delineated by black rectangles. The order of species represented vertically (from top to bottom) and horizontally (from left to right) in the heatmap corresponds to the order of species in the phylogenetic tree represented in (A).

(C) The ratios of the number of gene clusters obtained through Orthofinder to the number of genes within a cluster were depicted for different lilies within Clade 1-1 (upper panel) and Clade 1-2 (lower panel).

**Comment 14.** Line 464, change figure 4c to figure 5c.

**Response:** Thanks a lot for this comment. We have revised “Figure 4C” to “Figure 5C”.  
(L510-512)

**L510-512:** *We identified multiple homologues of the previously identified colchicine biosynthesis genes* <sup>40, 41</sup> *(Figure 5C and Figures S28-30).*

**Here are point-to-point responses to the reviewers' comments.**

**Point to point responses for reviewer #1**

**Comment 1.** K-mer analysis provided an estimation of genome size. But usually, plant tissue is analyzed by flow-cytometry to directly measure the nuclear DNA content of the plant cells. It would be more convincing if the authors could provide those data. And I also noticed that the DNA content (1C) of *L. sargentiae* has been estimated to be 60 pg by flow cytometry (Du, Yun-peng, et al. 2017), which is much larger than the size estimated based on the k-mer method in this manuscript. (Du Y, Bi Y, Zhang M, et al. Genome size diversity in *Lilium* (Liliaceae) is correlated with karyotype and environmental traits[J]. *Frontiers in plant science*, 2017, 8: 1303.)

**Response:** Thanks for this important comment. We have followed the reviewer's suggestion to add the results of genome size estimation by flow cytometry analysis in the revised manuscript (**L131-133; L960-963; Figure S1B and 1D**). For the flow cytometry analysis of *L. sargentiae*, ten replicates were conducted, yielding an average **2C** value of 63.60 pg (**refer to the table attached below**). Among these, Sample 5, which was closest to the average value, was selected as the representative sample for the manuscript. Flow cytometry is commonly used for initial estimations of genome sizes, particularly for smaller genomes with fewer repetitive sequences. For large and complex genomes with highly repetitive sequences, *k*-mer analysis (**1C**) is more efficient.

In the reference (Du et al., 2017) you mentioned, the DNA content (**2C**) of *L. sargentiae* was estimated to be 60 pg by flow cytometry with a **1C value of 30 pg**, using wheat (*Triticum aestivum* L.) cultivar 'Chinese Spring' as an internal standard (**2C = 30.9 pg, genome size = 15 Gb**). The relevant description in the reference (Du et al., 2017) you mentioned is as follows: "*Triticum aestivum* L. 'Chinese Spring' was used as an internal standard (**2C = 30.9 pg**, 43.7% GC) (Marie and Brown, 1993). To estimate the GS of the investigated taxa, at least five individuals per species were analyzed. The analysis was repeated twice for each sample. A DNA content of 5,000-10,000 stained nuclei was determined for each sample. Based on the peak of internal standard and *Lilium* species, experimental GS were calculated following equation (Figure S2):  $2C = (\text{sample G1 peak mean} / \text{standard G1 peak mean}) \times \text{standard 2C genome size (pg DNA)}$  (Du et al., 2017)."

**L131-133:** The genome sizes of *L. sargentiae* and *G. superba* were estimated to be ~31.8 Gb and 5.7 Gb, respectively, by flow cytometry analysis (Figure S1B, 1D).

**L960-963:** The genome sizes of *L. sargentiae* and *G. superba* were also estimated by flow cytometry using DNA from fresh leaves. *Zea mays* (2C = 4.7 pg) and *Triticum aestivum* (2C = 30.9 pg) were used as an internal standard for the evaluation of the genome sizes of *G. superba* and *L. sargentiae*, respectively.

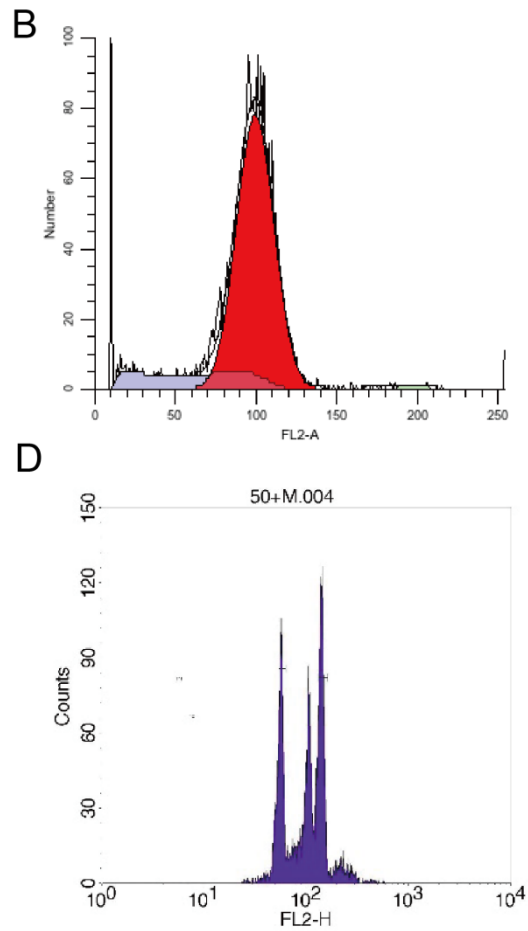


Figure S1. Genome size estimation of *Lilium sargentiae* (B) and *Gloriosa superba* (D) genomes.

Statistics of lily genome size assessment results by flow cytometry analysis.

Sample	G1	Control	G1/Control	Control genome size (pg, 2C)	Sample genome size (D * E, 2C)
1	106.73	48.21	2.21	30.90	68.41
2	94.68	48.21	1.96	30.90	60.68
3	104.25	48.21	2.16	30.90	66.82
4	94.09	48.21	1.95	30.90	60.31
<b>5</b>	<b>99.31</b>	<b>48.21</b>	<b>2.06</b>	<b>30.90</b>	<b>63.65</b>
6	103.42	48.21	2.15	30.90	66.29
7	96.62	48.21	2.00	30.90	61.93
8	92.64	48.21	1.92	30.90	59.38
9	91.06	48.21	1.89	30.90	58.36
10	109.45	48.21	2.27	30.90	70.15
Average	94.59	48.21	1.96	30.90	63.60

**Comment 2.** It is also routine to provide Hi-C interaction heatmap when the assembled contigs were anchored to the chromosomes. This data could show the quality of the chromosome level assembly.

**Response:** Thanks a lot for this comment. We have added the Hi-C interaction heatmap of the flame lily (*Gloriosa superba*) genome in the revised version (**Figure S1E**).

Due to the large genome size of lilies, we have generated 1 terabyte of Hi-C data, which can be used to connect contigs to generate scaffolds approaching the chromosomal level. However, this amount of data is insufficient for generating Hi-C interaction heatmaps.

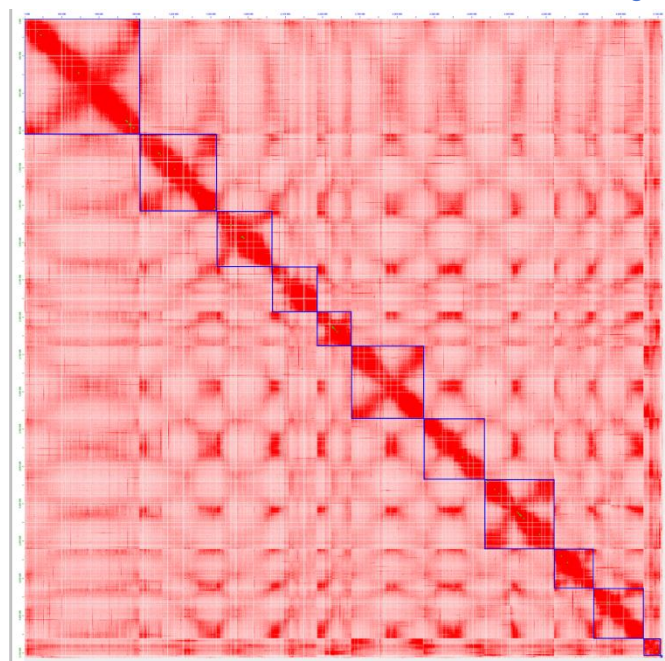


Figure S1E. Hi-C heatmap of the *G. superba* genome.

**Comment 3.** BUSCO is used to assess the completeness of the coding parts of the genome. However, a direct assessment of the whole genome quality would be Merquy's evaluation of QV and completeness.

**Response:** Thanks a lot for this comment. We have added the Merquy's evaluation of quality value (QV) and completeness of the *Lilium sargentiae* (completeness = 80.32) and *Gloriosa superba* (completeness = 79.66) genome using Mercury, yielding QV of 18.89 and 37.63, respectively.

In the article titled "Merquy: reference-free quality, completeness, and phasing assessment for genome assemblies" by Rhie et al., it is noted that "*The assembly QV will be underestimated if the read set does not contain all true k-mers of the genome due to low or heavily biased sequencing coverage.*" This suggests that biased sequencing coverage can lead to an underestimation of QV. For a genome of around 5 Gb, removing redundancy is challenging but feasible. However, for genomes exceeding 20 Gb, current technology makes redundancy reduction nearly impossible. After testing numerous software tools, we obtained a lily genome assembly with a size of 47.09 Gb, representing the most complete genome we could achieve with minimal redundancy. This final assembly inevitably includes both haplotypes. When we evaluated the lily genome's QV and

completeness using Merquy with second-generation sequencing data, the sequencing depth was distributed across the two haplotypes, resulting in a relatively modest QV score.

Given the size and complexity of the genomes, we believe we have achieved reasonable completeness and accuracy for both the coding and non-coding regions. BUSCO, widely recognized as a universal method for assessing genome completeness, has been the primary criterion in numerous genome publications (Zhang et al., 2020; Niu et al., 2022; Song et al., 2023). Our high BUSCO scores further confirm the completeness of the coding regions, which serve as the foundation for many of our biological insights and conclusions.

#### References:

Zhang, L., Chen, F., Zhang, X. *et al.* The water lily genome and the early evolution of flowering plants. *Nature* **577**, 79–84 (2020).  
Niu S, Li J, Bo W, et al. The Chinese pine genome and methylome unveil key features of conifer evolution. *Cell* **185**, 204-217.e14 (2022).  
Song, A., Su, J., Wang, H. et al. Analyses of a chromosome-scale genome assembly reveal the origin and evolution of cultivated chrysanthemum. *Nat Commun* **14**, 2021 (2023).  
Rhie, A., Walenz, B.P., Koren, S., A.M. Phillippy . Merquy: reference-free quality, completeness, and phasing assessment for genome assemblies. *Genome Biol* **21**, 245 (2020).

**Comment 4.** Figure S2. The sizes of the scaffolds in the figure do not correspond to those in Table S2. For example, Scaffold3 and Scaffold15 are 3.55 Gb and 3.79 Gb, respectively, but Scaffold3 in Figure S2 is only half the size of Scaffold15.

**Response:** Thanks a lot for this comment. In Figure S2, the homologous gene dotplot was generated through syntenic analysis using WGDl; the length of each cell corresponds to the number of genes located on the respective scaffold or chromosome instead of the full sequence length. **Scaffold3** contains **3,588 genes**, while **Scaffold15** contains **5,958 genes**. Therefore, size of Scaffold3 in Figure S2 is roughly half the size of Scaffold15, despite their similar sequence lengths, resulting in the cell representing Scaffold3 being roughly half the length of the cell representing Scaffold15 (Figure S2). We have examined that there are indeed many more transposons on Scaffold 3 than on Scaffold 5.

#### Point to point responses for reviewer #3

**Comment 1.** The authors mentioned only class C genes here. The phylogenetic tree showed that two lily AG-like genes were a little far related. Since there are two types of AG-like genes, i.e., class C and class D genes, in lily, they may be class C and class D genes. Differences between class C and class D genes in lily were well analyzed in a previous study (J. Hort. Sci. Biotech. 83:453-461, 2008), the authors should add lily class C/D genes in their phylogenetic analysis. Also, monocot class D genes have “MD motif” at the C-terminal region, so it is not difficult to distinguish class C and class D genes.

**Response:** Thanks for the comment. In the revised version, we have included the phylogenetic analysis of the lily class D gene (**Figure S22C**). The analysis showed that *Lilium sargentiae* has two class C genes (*LsaAGa* and *LsaAGb*) and one class D gene

(*LsaAGL11*) (**Figure S22C**). Although the two class C genes in *L. sargentiae* are not closely clustered, they both are grouped with the Arabidopsis class C genes (**Figure S22C**). Similarly, the *L. sargentiae* class D gene clusters with Arabidopsis class D gene (**Figure S22C**). Furthermore, the comparison of the C-terminal regions of these class C MADS-box proteins revealed that *LsaAGa* and *LsaAGb* share the same motifs (AG motif I and II) with other class C proteins, such as LMADS10 (**Figure S24B**). This further supports the classification of *LsaAGa* and *LsaAGb* as class C MADS-box genes .

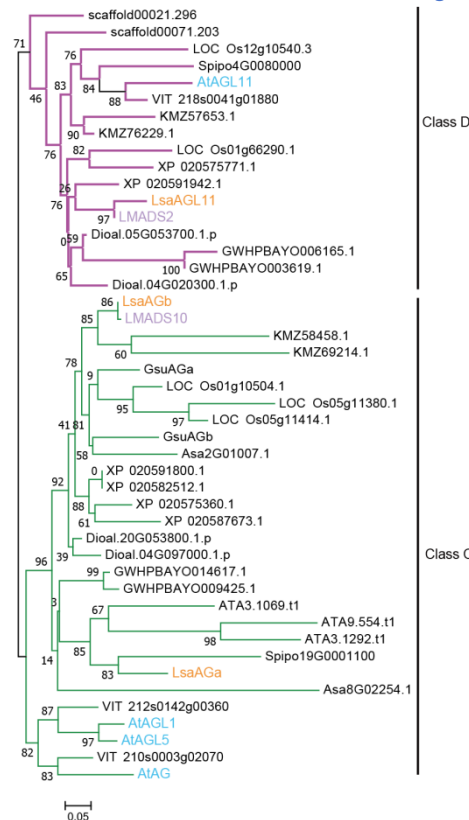


Figure S22C. Phylogenetic analysis of class C and D genes in *Lilium sargentiae*, *Gloriosa superba*, and other species.

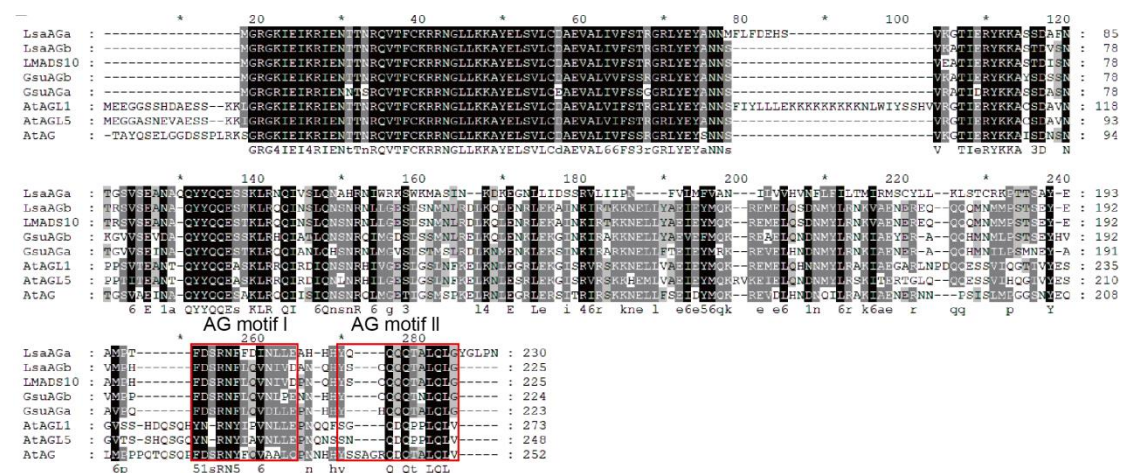


Figure S24B. Comparison of C-terminal regions of AG-like MADS-box proteins.

**Comment 2.** Although the expression patterns of class B genes cannot explain the

expansion of class B gene expression in outer tepals, the original figures (fig. 4 A and B) which showed the expression patterns of floral ABCE genes was very helpful to understand. I recommend to add these figures in the main manuscript or in a supplemental file.

**Response:** Thanks a lot for this comment. We have added the figures showing the expression patterns of floral ABCE genes in lily (**Figure S23C**) and flame lily (**Figure S23D**) to Figure S23 in the revised manuscript.

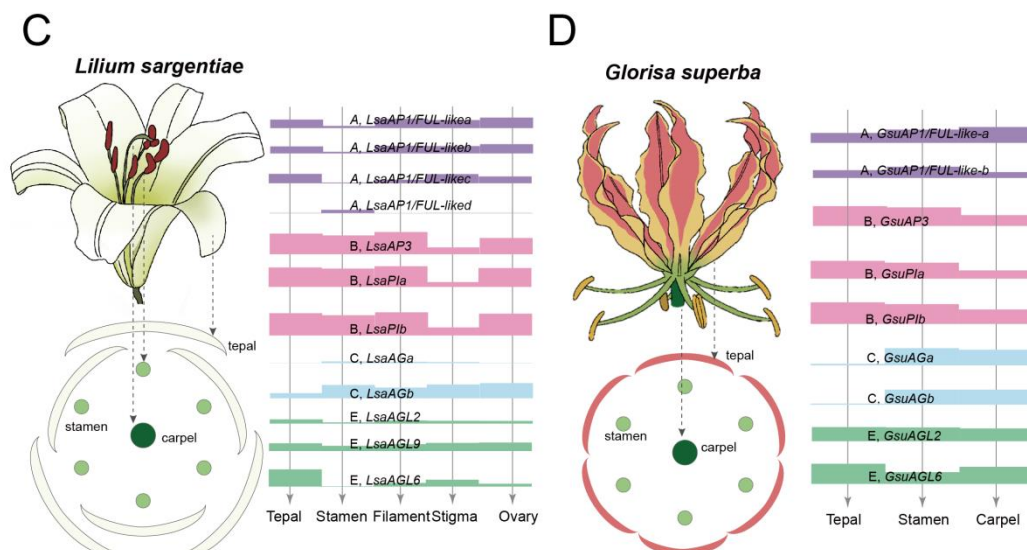


Figure S23. The proposed flowering ABCE models that specified floral organs in lily (C) and flame lily (D).

## Point to point responses for reviewer #5

**Comment 1.** Lily bulb size varies greatly. It will be much better if candidate gene association analysis is conducted to validate the role of candidate genes in bulb formation, such as XTH and GBSS1.

**Response:** Thanks a lot for this comment. In our study, we employed Weighted Gene Co-Expression Network Analysis (WGCNA) to explore the association relationships between gene modules and key traits, particularly focusing on biological questions such as bulb development in lily. WGCNA is a systems biology approach used to identify clusters (modules) of highly associated genes across multiple samples (Langfelder et al., 2008). These modules often represent genes with similar functions from the same or different gene families, which are then correlated with phenotypic traits (Langfelder et al., 2008). To assess these relationships, Pearson's correlation coefficient ( $r$ ) is used to measure the degree of association between gene modules and traits, while  $P$ -values confirm the statistical significance (Langfelder et al., 2008). The strength of these correlations is visually represented in heatmaps, where the color of the cells reflects the magnitude of the  $r$  values.

Through WGCNA, we identified several candidate genes that may play a role in lily bulb development. Specifically, we focused on a representative *XTH* gene and conducted gene silencing and overexpression experiments in lily scales to validate its role during lily bulb formation. Our results indicated a positive correlation between bulb induction (both initiation and expansion of bulblets) and *XTH* expression, highlighting the significant role

of *XTH* genes in lily bulb development (Figure 3E and F). Therefore, our findings provide strong functional evidence of the *XTH* function in lily bulb development and we plan to conduct functional experiments on more candidate genes in the near future as suggested by Reviewer #5.

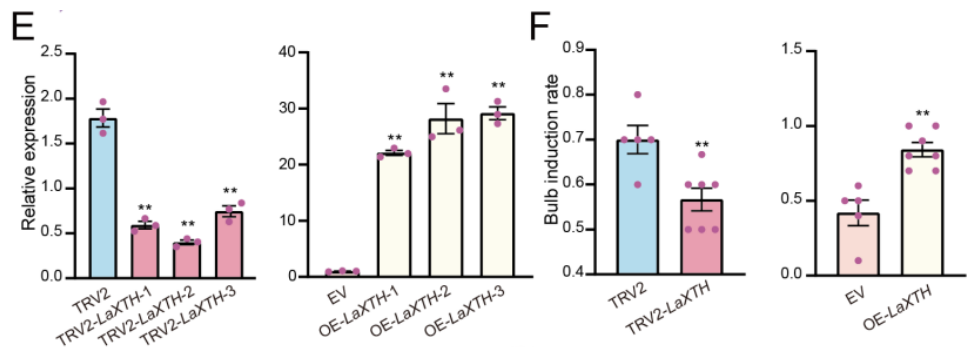


Figure 3. Expression of *LaXTH* in TRV2 control lines and TRV2-*LaXTH* silenced lines as well as EV control lines and OE-*LaXTH* overexpressed lines, respectively (E). The statistics of bulb induction rate (bulblet initiation rate plus bulblet expansion rate) of the TRV2 (n = 5) and TRV2-*LaXTH* (n = 7) lines as well as EV (n = 5) and OE-*LaXTH* (n = 7) lines, respectively (F).

**Reference:**

Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis[J]. BMC Bioinformatics, 2008, 9: 1-13.

**Comment 2.** *GsuGBSS1* was assumed to play an important role in rhizome development. Why not include in figure 3G?

**Response:** Thanks a lot for this comment. In our study, *GsuGBSS1* was identified as a potential key gene in rhizome development of the flame lily (*Gloriosa superba*), rather than as a candidate gene for bulb development in the lily (*Lilium sargentiae*). These two species have distinct types of modified stems: rhizomes in *G. superba* and bulbs in *L. sargentiae*. Figure 3G presents a proposed model for bulb development in *L. sargentiae*, focusing exclusively on candidate genes associated with this species. As *GsuGBSS1* is presumed to function in rhizome development in *G. superba* rather than in bulb development in *L. sargentiae*, it was excluded from Figure 3G. However, it is included in Figure S21, alongside other potential genes implicated in rhizome development in *G. superba*.

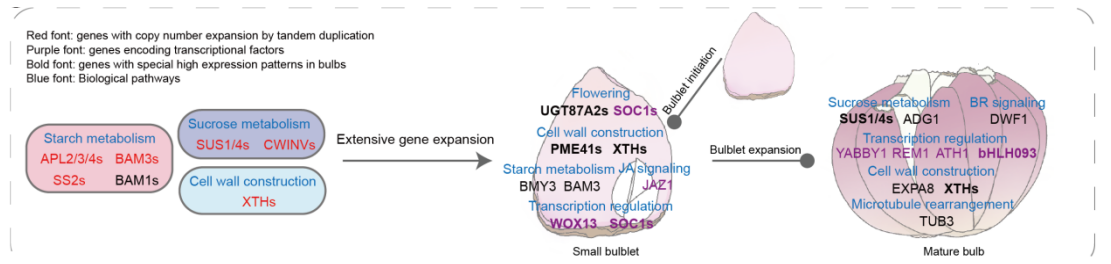


Figure 3G. A proposed model of the evolutionary and genetic mechanisms of bulblet initiation and bulblet expansion in *Lilium sargentiae*.

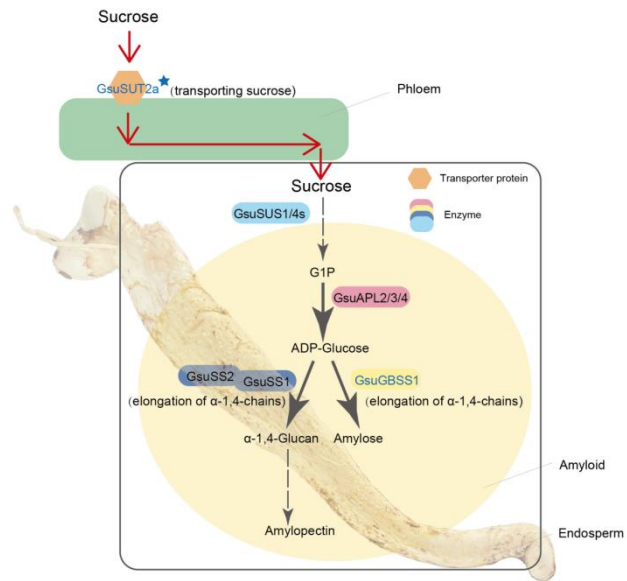


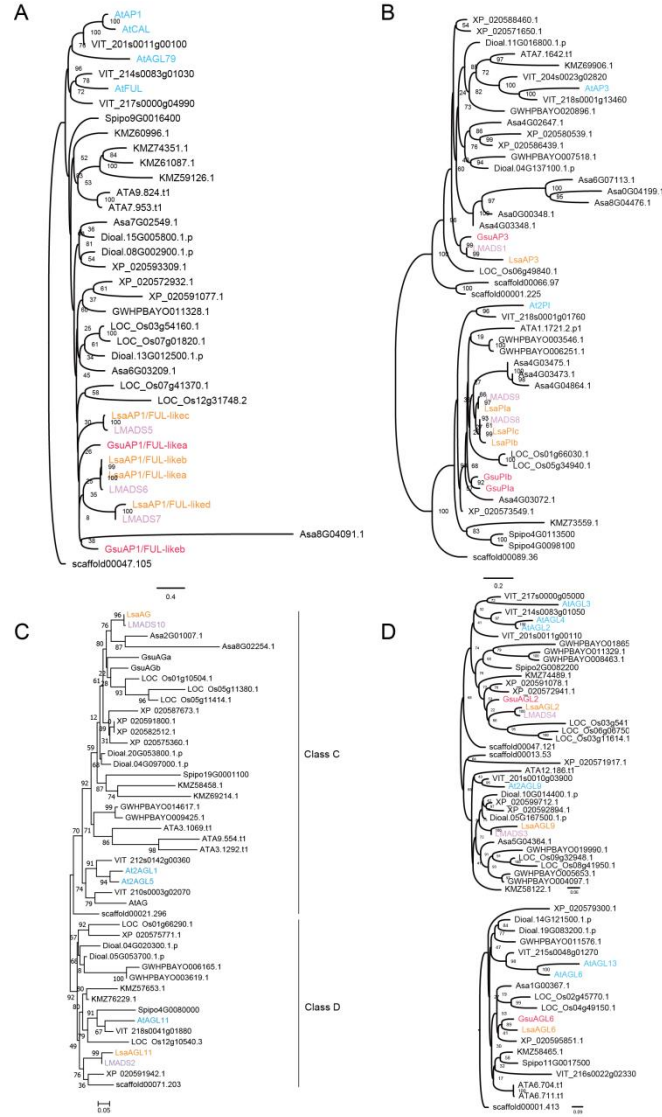
Figure S21. A proposed model of rhizome evolution and formation in *Gloriosa superba*.

### Point to point responses for reviewer #3

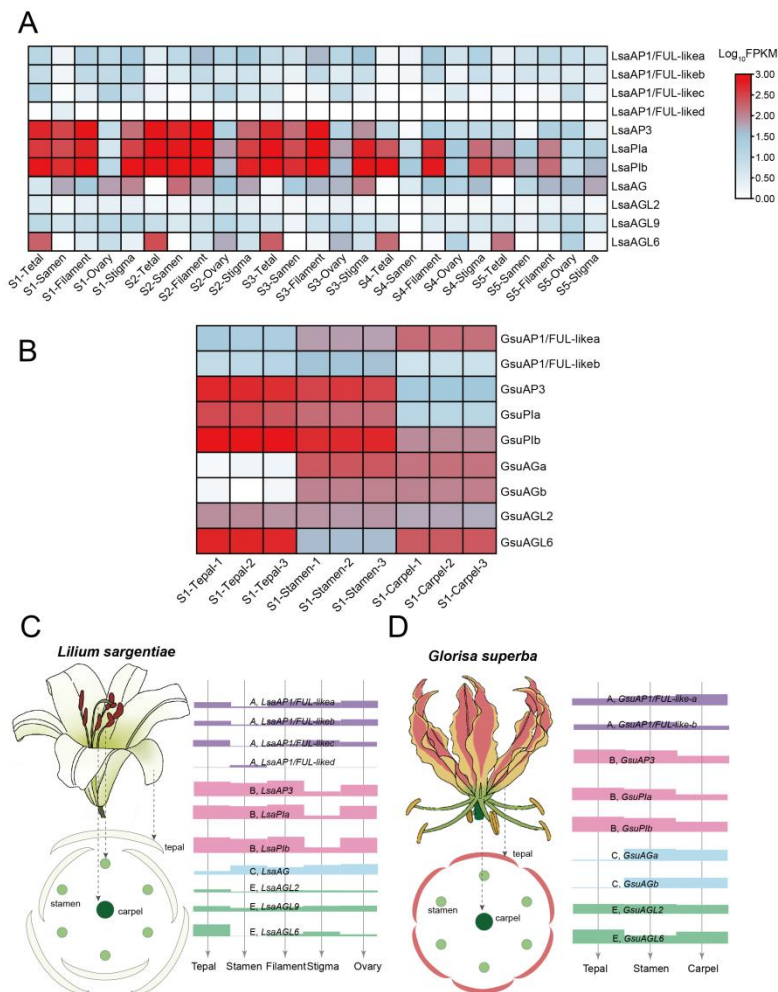
**Comment 1.** L.434 We identified a total of four class A, three class B, two class C, and three class E MADS genes in *L. sargentiae*, and two class A, three class B, two class C, and two class E MADS genes in *G. superba* (Figures S22).

-> The authors isolated two AG genes from lily. The results of the gene phylogenetic analysis indicate that *LsaAGb* is very homologous to *LMADS10*, which has been isolated previously, but it is questionable whether *LsaAGa* is homologous to AG genes from other Asparagales plants. Expression results for this gene (Fig. S23A) showed that its expression have not been detected in any organs of lily. Based on these results, it is doubtful that the *LsaAGa* gene is a Class C gene in lily. Do the authors have any other evidence to show that *LsaAGa* is lily AG gene?

**Response:** Thank you for this valuable comment. We currently lack sufficient evidence to support *LsaAGa* as a class C gene. Therefore, based on its expression profile and phylogenetic position, we have decided to remove it from the identified class C lily genes for accuracy. After revision, the corresponding manuscript content is as follows: *We identified a total of four class A, three class B, one class C, and three class E MADS genes in L. sargentiae, and two class A, three class B, two class C, and two class E MADS genes in G. superba (Figures S22) (L434-436).* Consequently, the original Figures S22-S24 have been revised as follows:



**Figure S22. Phylogenetic analysis of floral identity genes in *Lilium sargentiae*, *Gloriosa superba*, and other species.** (A) Class A, (B) class B, (C) class C and D, and (D) class E genes. Genes in *L. sargentiae*, *G. superba*, and *Arabidopsis thaliana* are presented by orange, red, and blue fonts, respectively. And the previously cloned MADS genes from *L. longiflorum* are in light purple fonts.



**Figure S23. Expression profiles of ABCE MADS-box genes.** (A) and (B) showed gene expression patterns of ABCE MADS-box genes from various organs of *Lilium sargentiae* and *Gloriosa superba*, respectively. Expression values were scaled by  $\log_{10}$ FPKM, in which FPKM was fragments per kilobase of exon per million mapped reads. For *L. sargentiae*, the expression profiles of flower tissues, including tepals, stamens, filaments, ovaries, and stigmas at five consecutive stages (S1-S5) from the bud stage to the mature stage were analyzed. For *G. superba*, tepals, stamens, and carpels at the bud stage (S1) were analyzed. (C) and (D) are the proposed flowering ABCE models that specified floral organs in *L. sargentiae* and *G. superba*, respectively, which were based on the  $\log_{10}(\text{FPKM}+1)$  format of FPKMs. FPKM, fragments per kilobase of exon per million mapped reads.

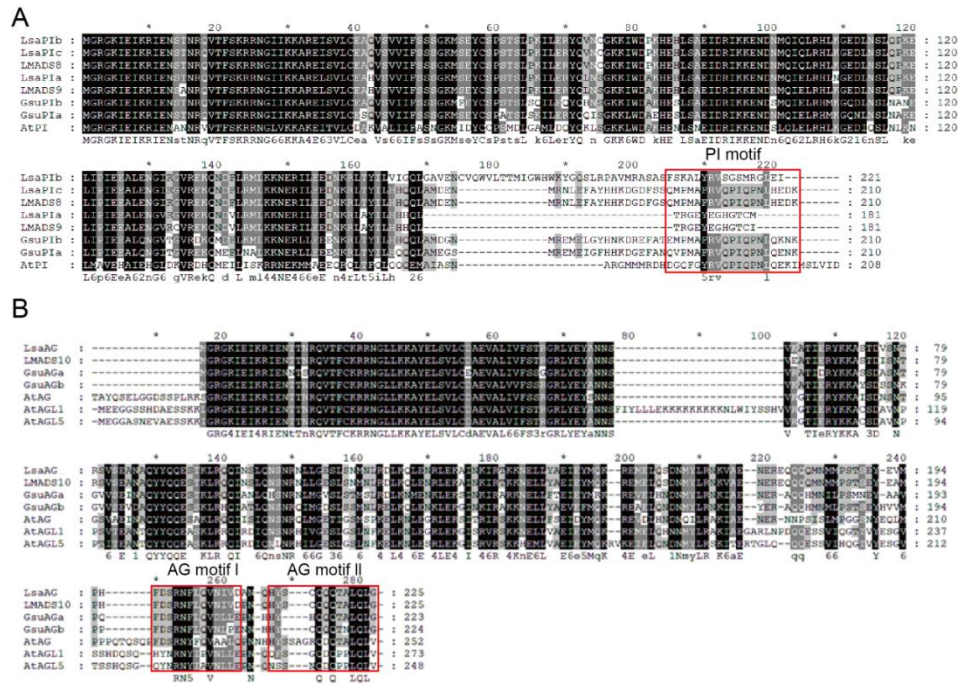


Figure S24. Comparison of C-terminal regions of PI-like MADS-box proteins (A) and AG-like MADS-box proteins (B).

**Comment 2.** L455 *LsaAGa* in lily and two AG genes in flame lily almost exhibited no expressions in tepals, but those showed high levels of expression in the reproductive organs (Figure S23).

-> It seems that *LsaAGa* is a mistake for *LsaAGb*.

**Response:** Thank you for this insightful comment. In the original manuscript at Line 455, We mistakenly wrote *LsaAGb* as *LsaAGa*. In the revised manuscript, we have removed *LsaAGa* (lili00G301020) from the identified class C lily genes for accuracy. Consequently, the original *LsaAGb* (lili00G421300) has been renamed to *LsaAG* in the revised version (L454-456).

**L454-456:** *LsaAG* in lily and two AG genes in flame lily almost exhibited no expressions in tepals, but those showed high levels of expression in the reproductive organs (Figure S23).

Reviewer 4 attachment

As previously stated, the bioinformatic data are outside of my scope. Concerning other aspects of the manuscript, the authors have addressed all comments in a satisfactory way.