

## Research Insight

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## Characterization of Chloroplast Genome Structure in *Eucommia ulmoides*

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**Abstract** *Eucommia ulmoides*, a significant medicinal and industrial plant endemic to China, has been the subject of various genomic studies due to its unique properties and evolutionary significance. This study focuses on the characterization of the chloroplast genome structure of *E. ulmoides*. The complete chloroplast genome of *E. ulmoides* was sequenced and analyzed, revealing a typical quadripartite structure with a total length of 163 586 bp, including a large single-copy region, a small single-copy region, and two inverted repeat regions. The genome contains 135 genes, comprising 89 protein-coding genes, 38 transfer RNA genes, and 8 ribosomal RNA genes. Comparative genomic analysis indicated high structural consistency and size variation due to DNA repeat variations. Phylogenetic analysis confirmed the close relationship between *E. ulmoides* and *Aucuba japonica*. This comprehensive characterization provides valuable insights into the genetic makeup and evolutionary history of *E. ulmoides*, facilitating future conservation and utilization efforts.

**Keywords** *Eucommia ulmoides*; Chloroplast genome; Phylogenetic analysis; Genomic structure; Conservation genomics

### 1 Introduction

Chloroplasts are essential organelles in plants, algae, and cyanobacteria, primarily responsible for photosynthesis, which converts light energy into chemical energy stored in organic compounds. Besides, chloroplasts are involved in the biosynthesis of fatty acids, amino acids, hormones, vitamins, nucleotides, and secondary metabolites (Dobrogojski et al., 2020). The chloroplast genome, or plastome, is highly conserved in terms of gene order and content, making it a valuable tool for phylogenetic studies and evolutionary inferences. The nonrecombinant and uniparentally inherited nature of chloroplast genomes further enhances their utility in evolutionary studies, as they retain signatures of their age better than nuclear genomes (Provan et al., 2000; Ravi et al., 2008). The increasing availability of complete chloroplast genome sequences has facilitated detailed comparative analyses, revealing dynamic patterns of evolution and aiding in the resolution of phylogenetic relationships among plant species (Bayly et al., 2013; Zhou et al., 2021).

*Eucommia ulmoides*, commonly known as the hardy rubber tree, is a significant traditional medicinal plant in China, widely used for orthopedic treatments (Zhu et al., 2020). It is the sole species of the family Eucommiaceae and is considered a Tertiary relic plant endemic to China (Rogers et al., 2007). Despite its medicinal and industrial importance, the population genetics of *E. ulmoides* has been underexplored due to limited genomic data (Wang et al., 2017). Recent studies have begun to address this gap by generating and analyzing the complete chloroplast genome of *E. ulmoides*, providing insights into its genomic structure and evolutionary relationships (Wang et al., 2017; Zhu et al., 2020). The chloroplast genome of *E. ulmoides* exhibits a typical quadripartite structure, with a large single-copy (LSC) region, a small single-copy (SSC) region, and two inverted repeat (IR) regions (Zhu et al., 2020). Comparative analyses have shown that the chloroplast genome of *E. ulmoides* is highly consistent in structure, with variations primarily resulting from DNA repeat variations (Wang et al., 2017).

This study provides a comprehensive characterization of the chloroplast genome structure of *E. ulmoides*. By analyzing the complete chloroplast genome, it clarifies genomic features such as genome size, gene content, and repetitive sequences, and identifies potential molecular markers for population genetics research. Additionally, it

explores the phylogenetic relationships between *E. ulmoides* and related species, aiding in the understanding of its evolutionary history. These findings not only enhance the genomic resources of *E. ulmoides* but also offer valuable insights for its conservation and utilization in medicinal and industrial applications. By leveraging detailed chloroplast genome data, this research establishes a foundation for future studies on the genetic diversity and evolutionary dynamics of this important medicinal plant.

## 2 Characterization of *Eucommia ulmoides* Chloroplast Genome

### 2.1 Genome size and content

The chloroplast genome of *Eucommia ulmoides*, a significant traditional medicinal plant in China, has been thoroughly analyzed and reported to be 163 586 base pairs (bp) in length. This genome exhibits the typical quadripartite structure, comprising a LSC region of 86 773 bp, a SSC region of 14 167 bp, and two IR regions, each 31 323 bp in length. The overall GC content of the chloroplast genome is 38.4%, with the remaining nucleotides being adenine (A) and thymine (T) at 30.8% each, and cytosine (C) and guanine (G) at 19.2% each (Zhu et al., 2020).

### 2.2 Gene organization and coding regions

The chloroplast genome of *E. ulmoides* contains a total of 135 genes. These include 89 protein-coding genes, 38 transfer *RNA* (*tRNA*) genes, and 8 ribosomal *RNA* (*rRNA*) genes. This gene composition is consistent with other angiosperm chloroplast genomes, which typically feature a similar number of genes and organization. The presence of these genes supports the essential functions of the chloroplast, including photosynthesis and various biosynthetic pathways (Zhu et al., 2020; Li et al., 2021).

### 2.3 Repetitive sequences and inverted repeats

Repetitive sequences play a crucial role in the structural variation and evolution of chloroplast genomes. In the case of *E. ulmoides*, the chloroplast genome contains several simple sequence repeats (SSRs), which are predominantly composed of single nucleotides (A) *n* and (T) *n*, reflecting an A/T bias. These SSRs are mostly 10~12 bp in length. Additionally, the IR regions in the *E. ulmoides* chloroplast genome are significant, each being 31 323 bp long. These IR regions contribute to the stability of the chloroplast genome and are involved in the regulation of gene expression (Wang et al., 2018; Zhu et al., 2020; Li et al., 2021). The detailed characterization of the chloroplast genome of *E. ulmoides* provides valuable insights into its genetic structure and evolutionary relationships, which are essential for further studies on its conservation and utilization in medicinal and industrial applications.

## 3 Comparative Genomics

### 3.1 Intraspecific comparisons

The chloroplast genomes of *Eucommia ulmoides* exhibit notable consistency in their overall structure. However, variations in genome size have been observed, which are primarily attributed to differences in DNA repeat sequences. A comprehensive comparison of two *E. ulmoides* chloroplast genomes revealed that the structure remains highly conserved, but sequence divergence patterns vary across different regions. Specifically, most SNPs are located in gene regions, while indels are predominantly found in intergenic spacers. Additionally, all detected coding-region SNPs were synonymous mutations, indicating a high level of functional conservation (Yang et al., 2020).

The chloroplast genome of *E. ulmoides* is characterized by a typical quadripartite structure, consisting of a LSC region, a SSC region, and two IR regions. The genome size is approximately 163 586 bp, with a GC content of 38.4%. This structure includes 135 genes, comprising 89 protein-coding genes, 38 *tRNA* genes, and 8 *rRNA* genes. Despite the overall structural consistency, variations in genome size are influenced by the presence of DNA repeats and other sequence elements (Zhu et al., 2020).

### 3.2 Interspecific comparisons

Phylogenetic analyses have shown that *Eucommia ulmoides* shared a close evolutionary relationship with *Aucuba japonica*, both belonging to the order *Garryales*. This relationship is supported by chloroplast comparative

genome, which revealed significant similarities in genome structure and gene content between the two species. Such comparisons help elucidate the evolutionary pathways and divergence events that have shaped the chloroplast genomes of these related species (Figure 1) (Wang et al., 2018; Hu et al., 2023).

The phylogenetic relationship between *E. ulmoides* and *Aucuba japonica* has been confirmed through chloroplast phylogenomic analyses. It indicated a sisterly relationship between them, suggesting a relatively recent common ancestor. The divergence between these species is marked by specific genomic rearrangements and sequence variations that have occurred over evolutionary time scales. This phylogenetic insight is crucial for understanding the evolutionary history and genetic diversity within the Eucommiaceae family and related groups.

### 3.3 Evolutionary insights

The evolution of chloroplast genomes in the Eucommiaceae family, particularly in *Eucommia ulmoides*, is characterized by a high degree of structural conservation and functional stability. The presence of synonymous mutations in coding regions and the distribution of indels in intergenic spacers suggested that selective pressures have maintained the integrity of essential genes while allowing for variability in non-coding regions. This pattern of evolution highlights the balance between conservation and adaptation in chloroplast genomes (Green, 2011; Zhu et al., 2020).

Genome rearrangements and duplications have played a significant role in the evolution of chloroplast genomes in *E. ulmoides* and related species. The identification of a new whole-genome duplication event, superimposed on an earlier paleohexaploidization event, underscores the complexity of genomic evolution in this lineage. These duplications have contributed to the expansion of gene families and the diversification of metabolic pathways, such as those involved in rubber and chlorogenic acid biosynthesis. Understanding these genomic rearrangements provides valuable insights into the adaptive strategies and evolutionary dynamics of chloroplast genomes in the Eucommiaceae family (Jin et al., 2020).

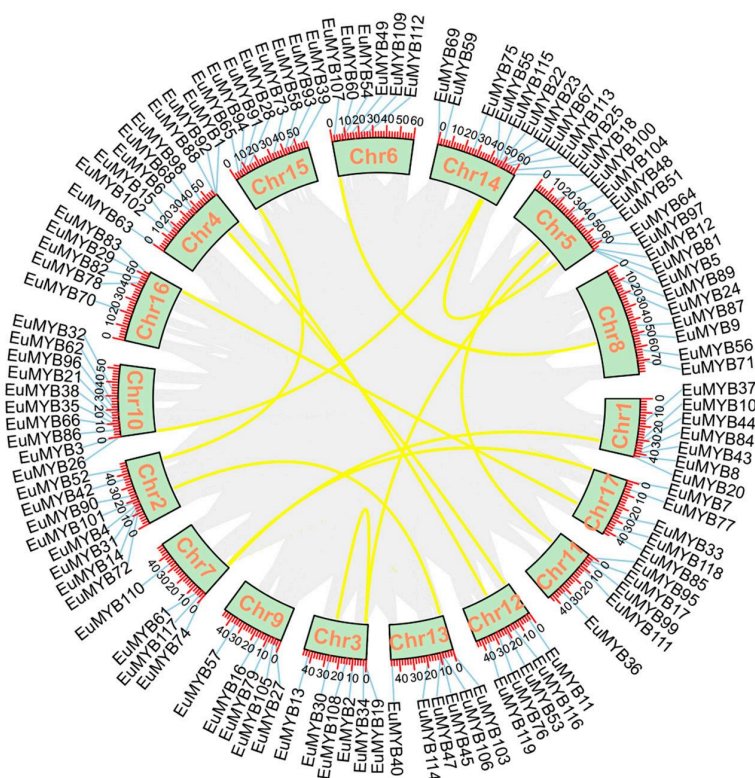


Figure 1 Collinearity analyses of the MYB gene family in *E. ulmoides* (Adopted from Hu et al., 2023)

Image caption: The Circos plot provides a visual representation of the genomic relationships and collinearity of the MYB gene family in *Eucommia ulmoides*. It highlights the extensive duplication and distribution of these genes across multiple chromosomes, which likely plays a significant role in the plant's adaptability and functional diversity (Adopted from Hu et al., 2023)

## 4 Functional Implications of Chloroplast Genome Structure

### 4.1 Impact on photosynthetic efficiency

The chloroplast genome plays a crucial role in the photosynthetic efficiency of *Eucommia ulmoides*. The structure and organization of genes within the chloroplast genome can influence the expression of key enzymes involved in the photosynthetic process. For instance, the identification of 35 *Eu4CL* genes, which were involved in phenylpropanoid metabolism, has suggested that they may also impact the biosynthesis of compounds that protected the photosynthetic machinery under stress conditions (Zhong et al., 2020). Additionally, the high-quality genome assembly of *E. ulmoides* has revealed the presence of genes that are preferentially expressed in leaves, which are the primary sites of photosynthesis. This indicates that the chloroplast genome structure is optimized to support efficient photosynthesis by ensuring the availability of necessary enzymes and regulatory elements (Li et al., 2020).

### 4.2 Role in adaptation to environmental conditions

The chloroplast genome structure of *Eucommia ulmoides* also plays a significant role in plant adaptation to various environmental conditions. The presence of numerous cis-elements related to stress and plant hormone responses in the promoter regions of *Eu4CL* genes suggests that the chloroplast genome is equipped to modulate gene expression in response to biotic and abiotic stresses. This adaptive capability is further supported by the differential expression patterns of *Eu4CL* genes under cold, methyl jasmonate, and ethylene treatments, indicating that the chloroplast genome can dynamically respond to environmental changes to maintain cellular homeostasis and protect the plant (Figure 2) (Xiao et al., 2023).

### 4.3 Potential applications in breeding programs

Understanding the chloroplast genome structure of *Eucommia ulmoides* has significant implications for breeding programs aimed at improving the species for industrial and medicinal uses. The high-quality genome assembly provides a comprehensive framework for identifying genes associated with desirable traits, such as enhanced rubber biosynthesis and stress tolerance. By leveraging this genomic information, breeders can develop strategies to introduce or enhance specific chloroplast genes that contribute to these traits. For example, genes involved in the methylerythritol-phosphate pathway, which is crucial for rubber biosynthesis, can be targeted for genetic engineering to increase rubber yield. Additionally, the knowledge of stress-responsive elements in the chloroplast genome can be used to breed varieties with improved resilience to environmental stresses, thereby ensuring sustainable production (Li et al., 2020; Zhong et al., 2020).

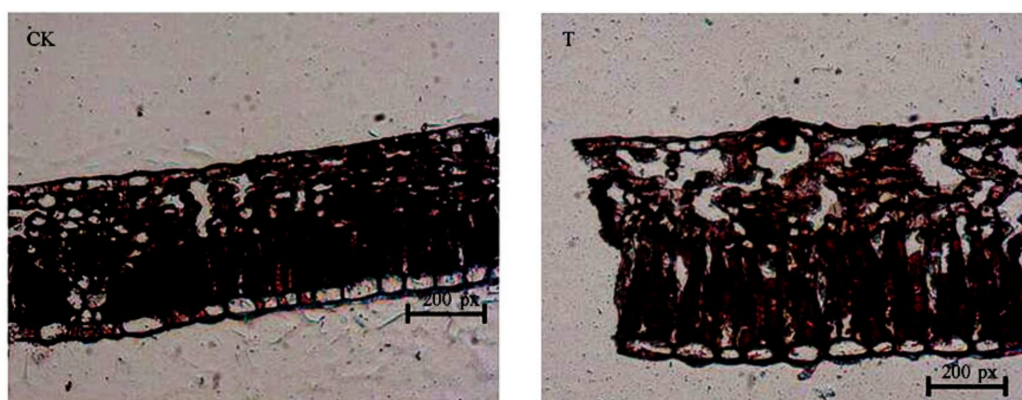


Figure 2 Anatomical characteristics of *E. ulmoides*; CK: anatomical characteristics of leaves under natural light (bar = 200 px); T: anatomical characteristics of leaves after UV-B treatment (bar = 200 px) (Adopted from Xiao et al., 2023)

Image caption: The figure shows that after UV-B treatment, the palisade tissue cells in *Eucommia ulmoides* leaves significantly elongated, the spaces between cells increased, and the spongy tissue cells underwent morphological changes and became more dispersed. These anatomical changes indicate that UV-B radiation induced significant structural adjustments in the cells, which may contribute to enhanced photosynthetic efficiency and support better leaf growth and development. This result confirms the positive impact of supplementary UV-B radiation on the structure and function of *Eucommia ulmoides* leaves (Adapted from Xiao et al., 2023)



## 5 Case Studies

### 5.1 Adaptive evolution in *Eucommia ulmoides*

*Eucommia ulmoides*, a Tertiary relic plant endemic to China, has shown significant adaptive evolution in its chloroplast genome. A comprehensive comparison of two complete chloroplast genomes of *E. ulmoides* revealed heterogeneous sequence divergence patterns across different regions. Notably, most SNPs were located in gene regions, while indels were predominantly found in intergenic spacers. Interestingly, all detected SNPs in coding regions were synonymous mutations, indicating a potential mechanism for maintaining protein function while allowing genetic diversity (Figure 3) (Du et al., 2023). This adaptive evolution is crucial for the conservation and future genetic studies of this endangered species.

### 5.2 Chloroplast genome variation under different environmental conditions

The chloroplast genome of *Eucommia ulmoides* has been characterized to understand its variation under different environmental conditions. The chloroplast genome, with a length of 163 586 bp, exhibits a typical quadripartite structure, including LSC and SSC regions, separated by two IRs. The genome contains 135 genes, including 89 protein-coding genes, 38 transfer RNA genes, and 8 ribosomal RNA genes. The overall GC content is 38.4%, which is consistent with other chloroplast genomes. Phylogenetic analysis has shown that *E. ulmoides* clusters closely with *Aucuba japonica*, suggesting a shared evolutionary history (Zhu et al., 2020). This detailed characterization provides a foundation for studying how environmental factors may influence chloroplast genome variation in *E. ulmoides*.

### 5.3 Comparison of case studies with other species

Comparing the chloroplast genome of *Eucommia ulmoides* with other species, several unique features and commonalities emerge. For instance, the chloroplast genome of *E. ulmoides* shows a high degree of structural consistency and sequence divergence patterns similar to those observed in other plant species. However, the presence of a significant number of synonymous SNPs in *E. ulmoides* suggests a unique adaptive strategy to maintain protein function while allowing genetic diversity (Wang et al., 2018). Additionally, the phylogenetic relationship between *E. ulmoides* and *Aucuba japonica* highlights a close evolutionary link, which is also observed in other species within the *Garryales* order (Turmel et al., 2009). These comparisons underscore the importance of chloroplast genome studies in understanding the evolutionary dynamics and adaptive mechanisms in different plant species.

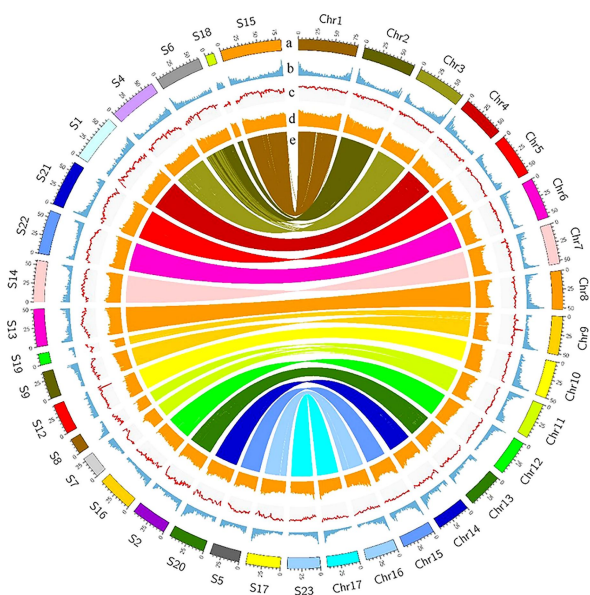


Figure 3 Landscape of female V1 and male V2 genome (Adopted from Du et al., 2023)

Image caption: This Circos plot provides a comprehensive overview of the comparative genomic landscape between Female V1 and Male V2. It highlights both the similarities and differences at the chromosomal level, offering insights into structural variations, evolutionary relationships, and potential functional implications of the observed genomic patterns (Adopted from Du et al., 2023)

## 6 Methodologies for Chloroplast Genome Analysis

### 6.1 Techniques for chloroplast DNA extraction and sequencing

Chloroplast DNA (cpDNA) extraction and sequencing are critical steps in the characterization of chloroplast genomes. Various methods have been developed to optimize the yield and purity of cpDNA. Traditional methods often struggle to balance quality and yield, but recent advancements have improved these processes significantly. For example, a modified protocol based on sucrose gradients has been shown to efficiently isolate cpDNA from angiosperms, achieving 40%-50% purity, which is sufficient for subsequent genome assembly using Illumina sequencing technology (Shi et al., 2012). Additionally, the use of single molecule, real-time (SMRT) DNA sequencing technology, such as the circular consensus sequencing (CCS) strategy, allows for high-accuracy de novo assembly and SNP detection of chloroplast genomes without the need for a reference genome (Li et al., 2014; Freudenthal et al., 2019). Multiplex sequencing-by-synthesis (MSBS) using the Illumina Genome Analyzer is another effective method, enabling the simultaneous sequencing of multiple chloroplast genomes with high coverage and accuracy (Cronn et al., 2008).

### 6.2 Bioinformatics tools for genome assembly and annotation

The assembly and annotation of chloroplast genomes require specialized bioinformatics tools. A systematic comparison of various chloroplast genome assembly tools has revealed significant differences in their performance and computational requirements. These tools are capable of successfully assembling chloroplast genomes in more than 60% of known real data sets, leading to the assembly of novel chloroplast genomes (Freudenthal et al., 2019). Docker images for each tested tool have been created to ensure reproducibility and facilitate large-scale screening of genomic data for chloroplast genomes. Additionally, standard practices for DNA extraction, sequencing library preparation, and bioinformatics analyses, including assembly, verification, annotation, and sequence comparisons, are recommended to ensure high-quality chloroplast genome sequencing reports (Heinze, 2021).

### 6.3 Challenges in chloroplast genome analysis

Despite advancements in chloroplast genome analysis, several challenges remain. One major issue is the conservative nature of chloroplast gene and genome evolution, which can limit phylogenetic resolution and statistical power in evolutionary and population genetic studies (Cronn et al., 2008). Moreover, the presence of mononucleotide repeats can interrupt contig assembly, with increasing repeat length posing further difficulties. Another challenge is the need for cost-effective high-throughput cpDNA extraction methods, as conventional methods often fail to achieve the necessary balance between quality and yield (Shi et al., 2012). Furthermore, the analysis of large multi-gene data sets can be complicated by systematic biases and conflicting signals, making it difficult to resolve ancient divergences accurately (Fučíková et al., 2016). These challenges highlight the need for continued development and refinement of methodologies and tools in chloroplast genome analysis.

## 7 Challenges and Limitations

### 7.1 Technical challenges

The characterization of the chloroplast genome in *Eucommia ulmoides* faces several technical challenges. One significant issue is the complexity of the chloroplast genome itself, which includes a LSC region, a SSC region, and twoIR regions. This structure can complicate the assembly and annotation processes, as seen in the study where the chloroplast genome length was determined to be 163 586 bp with a typical quadripartite structure (Zhu et al., 2020; Zhong et al., 2022). Additionally, the presence of repetitive sequences and DNA repeat variations can lead to difficulties in achieving high-quality genome assemblies. For instance, the high-quality haploid genome assembly of *E. ulmoides* required advanced technologies like PacBio and Hi-C to improve the scaffold N50 significantly and reduce the number of gaps (Li et al., 2020).

### 7.2 Data availability

Another major limitation is the scarcity of genomic data available for *E. ulmoides*. The lack of comprehensive genomic datasets hinders the ability to perform extensive comparative analyses and population genetics studies. For example, the study on the chloroplast genome of *E. ulmoides* highlighted the limited availability of complete

chloroplast genomes, which restricts the scope of comparative genomics and the identification of mutation hotspots (Mo et al., 2020). Moreover, the limited data availability also affects the development of molecular markers, which are crucial for conservation and breeding programs. The identification of polymorphic cpDNA fragments and cpSSR loci in *E. ulmoides* is a step forward, but more extensive datasets are needed for robust marker development (Wang et al., 2018; Yang et al., 2022).

### 7.3 Interpretation of results

Interpreting the results of chloroplast genome studies in *E. ulmoides* is also fraught with challenges. The heterogeneous sequence divergence patterns observed in different regions of the chloroplast genome complicate the understanding of evolutionary relationships and genetic diversity. It found that most SNPs were located in gene regions, while indels were primarily in intergenic spacers, with all coding-region-located SNPs being synonymous mutations (Wang et al., 2018; Qing et al., 2021). This variability makes it difficult to draw definitive conclusions about the evolutionary pressures and functional implications of these mutations. The phylogenetic analyses, which confirmed the sisterly relationship between *E. ulmoides* and *Aucuba japonica*, require careful interpretation to avoid overestimating the evolutionary significance of observed genetic similarities (Wang et al., 2018; Zhu et al., 2020). The complexity of the chloroplast genome structure and the presence of multiple rearrangement events further add to the challenges in interpreting the genomic data accurately (Lian et al., 2019; Liu and Melton, 2021).

## 8 Future Research Directions

### 8.1 Unexplored aspects of chloroplast genome structure

The chloroplast genome of *Eucommia ulmoides* has been partially characterized, revealing a typical quadripartite structure and a significant number of protein-coding genes, tRNAs, and rRNAs (Zhu et al., 2020). However, there remain several unexplored aspects that warrant further investigation. For instance, the role of DNA repeat variations in genome size variation and their impact on the overall genomic stability and function is not fully understood (Wang et al., 2018). And the heterogeneous sequence divergence patterns observed in different regions of the chloroplast genome suggest that some regions may be more prone to mutations than others, which could have implications for the plant's adaptability and evolution (Wang et al., 2018). Future studies should aim to map these variations more comprehensively and understand their functional consequences.

### 8.2 Potential for genetic modification based on chloroplast genome

The chloroplast genome of *Eucommia ulmoides* offers promising avenues for genetic modification, particularly for enhancing its medicinal and industrial applications. The identification of polymorphic cpDNA fragments and cpSSR loci provides a valuable resource for genetic engineering (Wang et al., 2018). Moreover, the high-quality haploid genome assembly of *E. ulmoides*, which includes a detailed map of protein-coding genes, can facilitate targeted genetic modifications aimed at improving rubber biosynthesis and other desirable traits. Genetic modifications could focus on optimizing the methylerythritol-phosphate pathway, which is crucial for the plant's rubber biosynthesis, to enhance yield and quality (Li et al., 2020). Additionally, the chlorogenic acid biosynthesis pathway, predominantly active in leaves, could be targeted to increase the medicinal value of the plant.

### 8.3 Integration of chloroplast genome data with other genomic studies

Integrating chloroplast genome data with nuclear and mitochondrial genomic studies can provide a more holistic understanding of *Eucommia ulmoides*' biology and evolution. The high-quality de novo assembly of the haploid genome, which includes insights into genome structure, gene mapping, and epigenetic analysis, offers a robust framework for such integrative studies (Li et al., 2020). Phylogenomic analyses have already confirmed the sister relationship between *E. ulmoides* and *Aucuba japonica*, highlighting the potential for comparative genomic studies to uncover evolutionary patterns and relationships (Wang et al., 2018; Zhu et al., 2020). Future research should focus on combining chloroplast genome data with other genomic datasets to explore gene-environment interactions, adaptive evolution, and the genetic basis of important traits. This integrative approach could also aid in the conservation efforts of this endangered species by providing a comprehensive genetic blueprint for its preservation and sustainable use.

## 9 Concluding Remarks

The study on the chloroplast genome structure of *Eucommia ulmoides* has yielded several significant insights. The chloroplast genome of *E. ulmoides* was found to be highly consistent in structure, with variations in genome size attributed to DNA repeat variations. The genome exhibited a typical quadripartite structure, including a LSC region, a SSC region, and two IR regions. The study identified 135 genes within the chloroplast genome, including 89 protein-coding genes, 38 tRNA genes, and 8 rRNA genes. Additionally, the research revealed heterogeneous sequence divergence patterns, with most SNPs located in gene regions and most indels in intergenic spacers. Phylogenetic analysis confirmed the sister relationship between *E. ulmoides* and *Aucuba japonica*.

The findings from this study have several implications for future research. The identification of 71 polymorphic cpDNA fragments and the development of eight polymorphic cpSSR loci provide valuable molecular markers for subsequent population genetics studies of *E. ulmoides*. These markers can be instrumental in conservation genomics, aiding in the preservation of this endangered species. Furthermore, the comprehensive chloroplast genome data can facilitate comparative genomic studies, enhancing our understanding of the evolutionary relationships within the *Garryales* order. Future research could also explore the functional genomics of the identified genes, particularly those involved in the biosynthesis of medicinal compounds, to improve the industrial and medicinal applications of *E. ulmoides*.

The characterization of the chloroplast genome in *Eucommia ulmoides* is a significant milestone in the genomic study of this medicinally and industrially important species. The detailed genomic information not only enhances our understanding of the genetic structure and evolutionary history of *E. ulmoides* but also provides essential tools for its conservation and improvement. The insights gained from this study underscore the importance of chloroplast genome research in uncovering the genetic basis of key traits and in supporting the sustainable utilization of *E. ulmoides* in various applications. As we continue to explore the genetic intricacies of this species, the knowledge generated will undoubtedly contribute to its preservation and the optimization of its valuable properties.

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## Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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