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Sex Determination Pathways in Dioecious Plants: Insights from *Eucommia ulmoides*

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Abstract *Eucommia ulmoides*, a dioecious plant endemic to China, holds significant medicinal and economic value. This study, utilizing high-quality chromosome-level genome sequences and transcriptome data, reveals the mechanisms of sex determination in *E. ulmoides*. Key genes associated with sex differentiation, such as *EuAP3* and *EuAG* within the MADS-box transcription factor family, were identified. The results demonstrate that these genes exhibit significant sex-biased expression in male and female individuals, highlighting their crucial roles in sex determination. Additionally, several sex-linked molecular markers were discovered, which can be used for early sex identification, thereby optimizing breeding strategies. The study not only provides new insights into the mechanisms of sex determination in *E. ulmoides* but also offers theoretical support for the improvement of other economically important dioecious crops. The findings are of great significance for enhancing the breeding efficiency of *E. ulmoides* and other dioecious plants.

Keywords *Eucommia ulmoides*; Sex determination; Dioecious plants; Genome assembly; Transcriptome analysis; MADS-box genes; Sex-biased genes; Breeding

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1 Introduction

Dioecious plants, which have distinct male and female individuals, represent a fascinating minority within the plant kingdom, accounting for approximately 5%-10% of flowering plant species (Kersten et al., 2017; Leite Montalvão et al., 2021; Razumova et al., 2023). This separation of sexes provides a unique opportunity to study the mechanisms of sex determination and the evolution of sex chromosomes. Unlike hermaphroditic plants, which possess both male and female reproductive organs within the same flower, dioecious plants must rely on genetic and environmental cues to determine sex, making them an excellent model for understanding the complexities of sex expression and differentiation (Heikrujam et al., 2014; Pannell, 2017; Leite Montalvão et al., 2021).

Understanding the mechanisms of sex determination in dioecious plants is crucial for several reasons. Firstly, it provides insights into the evolutionary processes that lead to the development of separate sexes from hermaphroditic ancestors (Wang et al., 1999; Pannell, 2017; Henry et al., 2018; Leite Montalvão et al., 2021). Secondly, it aids in the identification of sex-determining regions (SDRs) and sex chromosomes, which are essential for breeding programs and the cultivation of economically important dioecious crops (HIrish and Nelson, 1989; eikrujam et al., 2014; Razumova et al., 2023). Advances in genome sequencing and gene editing have facilitated the discovery of sex-determining genes and pathways, revealing that similar genetic mechanisms may be employed across different species to achieve dioecy (Wang and Zhang, 2017; Leite Montalvão et al., 2021; Zhang et al., 2022).

Eucommia ulmoides, commonly known as the hardy rubber tree, is a dioecious plant endemic to China with significant medicinal and economic value (Du et al., 2023; Zhang et al., 2023). It is renowned for its production of α-linolenic acid and gutta, a natural rubber-like substance, making it a valuable resource for both pharmaceutical and industrial applications (Wang and Zhang, 2017; Du et al., 2023). The availability of high-quality genome sequences for both male and female *E. ulmoides* individuals provides a robust framework for studying the genetic basis of sex determination in this species (Du et al., 2023). Comparative transcriptome analyses have identified differentially expressed genes (DEGs) between male and female plants, highlighting potential sex-associated genes and pathways (Wang and Zhang, 2017; Zhang et al., 2023). These unique characteristics make *E. ulmoides* an ideal model for investigating the molecular mechanisms underlying sex differentiation in dioecious plants.

This study delves into the genetic and molecular mechanisms of sex determination in *E. ulmoides* using high-quality genome sequences and transcriptome data, identifying key genes and pathways that regulate sexual dimorphism in this species. The study elucidates the evolutionary patterns of dioecious plants and clarifies the role of specific genes, such as MADS-box transcription factors, in sex differentiation. This study provides new insights into the mechanisms of sex determination in dioecious plants and offers a scientific basis for the development of improved breeding strategies for economically important dioecious crops.

2 Mechanisms of Sex Determination in Dioecious Plants

2.1 Genetic basis of sex determination

In dioecious plants, sex determination often involves complex chromosomal mechanisms (Kazama et al., 2023). For instance, *Eucommia ulmoides* has been shown to possess distinct sex chromosomes, with the female genome assembly consisting of 17 pseudochromosomes and the male genome showing significant differences in size and gene content (Du et al., 2023) (Table 1). The evolution of sex chromosomes in dioecious plants can follow various pathways, including the classical model where two sex-determining genes become linked in a sex-determining region (SDR), leading to recombination suppression and chromosome differentiation. However, recent studies suggest that single genes can also act as master regulators of sex determination, as seen in species like *Populus* and *Diospyros* (Renner and Müller, 2021).

Table 1 Summary of the *Eucommia ulmoides* genome (Adopted from Du et al., 2023)

Type	Female V1	Male V2	Male V1
Genome size	1.01 Gb	1.24 Gb	1.18 Gb
Contig N50	1.33 Mb	17.06 Kb	17.06 Kb
Scaffold N50	5.31 Mb	1.03 Mb	1.03 Mb
SuperScaffold N50	51.89 Mb	48.30 Mb	1.88Mb
GC content	35.14%	35.19%	90%
Complete BUSCOs	93.2%	92.1%	26 732
Protein coding genes	31 665	37 998	-
Mean gene length	6 273 bp	6 199 bp	1 001
Mean coding sequence length	1 086 bp	1 007 bp	4.74
Mean number of exons per gene	5.18	4.45	211 bp
Mean exon length	209 bp	226 bp	61.24%
Total repetitive sequence	68.26%	62.25%	3 201
Total non-coding RNAs	2 488	2 865	Male V1

Gene expression and regulation play crucial roles in the sex determination of dioecious plants. In *Eucommia ulmoides*, transcriptome analysis has identified key genes such as *EuAP3* and *EuAG* that are involved in regulating sex differentiation (Du et al., 2023). MADS-box transcription factors, particularly B-class genes like *EuMADS39*, have been found to exhibit sex-biased expression, indicating their significant role in the genetic regulation of sex in *E. ulmoides* (Zhang et al., 2023). Additionally, comparative transcriptome analyses have revealed differentially expressed genes (DEGs) between male and female individuals, further highlighting the importance of gene regulation in sex determination (Wang and Zhang, 2017). In persimmon, sex determination is controlled by a pair of genes called *OGI* and *MeGI* (Figure 1), *OGI* is a Y-specific sex determination gene that produces non-coding hairpin formation Rnas and ultimately smRNA molecules that target homologous *MeGI* genes. *MeGI* is an autosomal gene that encodes homeodomain transcription factors that regulate flower development and anther fertility in a dose-dependent manner (Akagi et al., 2014).

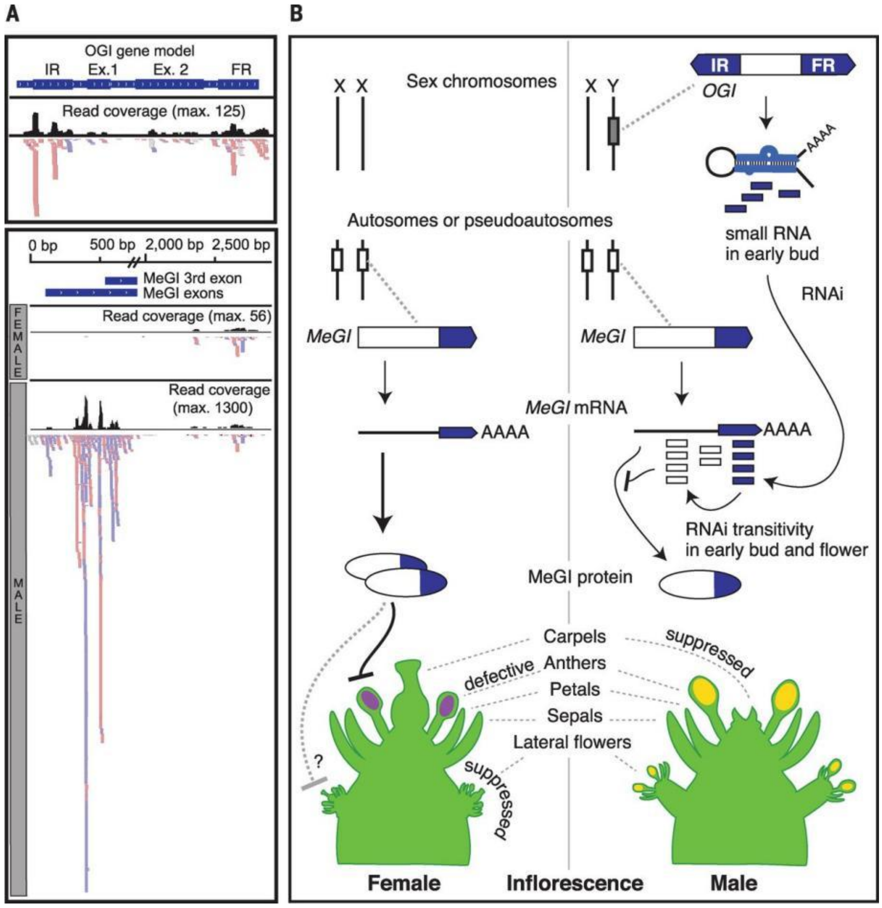


Figure 1 Functional analysis of *OGI* and *MeGI* (Adopted from Akagi et al., 2014)

2.2 Environmental factors influencing sex determination

Climate change drivers can influence sex determination in some intersex plants. For example, biomass accumulation, photosynthetic capacity and water and nitrogen utilization rates of female aspen trees increase significantly under high temperature conditions, while the positive effects of high CO_2 concentration on male aspen trees are higher than that of female aspen trees, which may affect the distribution and population dynamics of dioecious plants in the future (Chen et al., 2020). It is known that environmental factors, including temperature and CO_2, can affect the expression of sex-determining genes in other plant species. This suggests that similar mechanisms might be at play in *E. ulmoides* and other dioecious plants.

Nutritional and hormonal factors also play a role in sex determination. Plant hormones, such as cytokinins, have been implicated in the regulation of sex expression in several dioecious species (Leite Montalvão et al., 2021). Regulation of gibberellin can lead to high differentiation of male flowers in pomegranate during growth (Engin and Gökbayrak, 2019). In *Eucommia ulmoides*, the presence of phytohormone-response cis-elements in MADS-box genes suggests that hormonal regulation could be a critical factor in sex determination (Zhang et al., 2023). Additionally, nutritional status can influence the development and differentiation of male and female flowers, although specific studies on *E. ulmoides* are needed to confirm this (Heikrujam et al., 2014).

2.3 Epigenetic regulation in sex determination

Epigenetic mechanisms, such as DNA methylationexhibit 5' cytosine methylation and inhibition of *MeGI* expression in the bud that forms the male flower, but not in the rest of the plant body or in the bud that forms the female flower, and when treated with methylation inhibitors the developing male flower causes its sex reversal to female flower (Akagi et al., 2014). DNA methylation patterns can regulate the expression of sex-determining genes, thereby affecting the development of male and female flowers. In *Eucommia ulmoides*, further research is required to elucidate the specific DNA methylation patterns associated with sex determination.

Histone modifications and chromatin remodeling are other epigenetic factors that can impact sex determination. For example, histone methylation/acetylation may affect *OGI* expression, and these histone modifications are often related to the conditions of DNA methylation on transposons (Qian et al., 2012; Du et al., 2015; Zhang et al., 2018). In melons and cucumbers, *WIP* recruits the corepressor TOPLESS to the *CRC* promoter to inhibit its expression through histone deacetylation, thereby promoting male flower development (Zhang et al., 2022). These processes can alter the accessibility of sex-determining genes to the transcriptional machinery, thereby influencing their expression (Heikrujam et al., 2014). While specific studies on histone modifications in *Eucommia ulmoides* are lacking, it is likely that similar epigenetic mechanisms are involved in regulating sex determination in this species. The mechanisms of sex determination in dioecious plants like *Eucommia ulmoides* involve a complex interplay of genetic, environmental, and epigenetic factors. Understanding these mechanisms can provide valuable insights into the evolution and regulation of sex differentiation in plants.

3 Genetic Basis of Sex Determination in *Eucommia ulmoides*

3.1 Chromosome-level genome analysis

The high-quality chromosome-level genome of female *Eucommia ulmoides* was obtained using PacBio and Hi-C technologies. The assembly size of the female genome was 1.01 Gb, organized into 17 pseudo-chromosomes, and included 31 665 protein-coding genes. This comprehensive genomic resource provides a solid foundation for understanding the genetic mechanisms underlying sex determination and other biological processes in *E. ulmoides*.

The male genome of *E. ulmoides* was reassembled using Hi-C technology, resulting in a genome size of 1.24 Gb with a super scaffold N50 of 48.30 Mb, which is a significant improvement over previous assemblies. The number of predicted genes in the male genome increased by 11 266 compared to earlier versions. Comparative analysis between the male and female genomes revealed that *E. ulmoides* has undergone two whole-genome duplication events, including a recent duplication approximately 27.3 million years ago. These findings highlight the evolutionary complexity and provide insights into the genetic basis of sex differentiation in this species (Du et al., 2023).

3.2 Transcriptome analysis

Comparative transcriptome analyses of male and female *E. ulmoides* individuals identified 116 differentially expressed genes (DEGs), with 73 male-biased and 43 female-biased genes. These DEGs include genes related to sexually dimorphic traits, such as gutta content in females and floral organ identity in males. The identification of these sex-biased genes provides valuable insights into the molecular mechanisms driving sexual dimorphism in *E. ulmoides* (Wang and Zhang, 2017).

Key genes involved in sex differentiation in *E. ulmoides* include *EuAP3* and *EuAG*, which are part of the MADS-box gene family. *EuAP3*, a B-class floral organ identity gene, was identified as a male-biased DEG and is likely involved in male flower development. These genes play crucial roles in the regulation of sex differentiation, and their expression patterns provide a deeper understanding of the genetic control of sexual development in *E. ulmoides* (Wang and Zhang, 2017; Du et al., 2023; Zhang et al., 2023).

3.3 Molecular markers for sex identification

Random Amplified Polymorphic DNA (RAPD) and Sequence Characterized Amplified Region (SCAR) markers have been utilized for early sex identification in *E. ulmoides*. These markers are valuable tools for distinguishing male and female plants at an early developmental stage, facilitating breeding programs and the cultivation of desired sex types (Figure 2). Double-digest Restriction-site Associated DNA (ddRAD) markers have been developed to identify male-specific loci in *E. ulmoides*. In terms of sex differentiation of *Eucommia eucommia*, ddRA has been used to identify an ideal sex-linked site, MSL4, which can distinguish male and female *Eucommia* seedlings (Wang et al., 2020). These markers enhance the precision of sex identification and provide a robust method for genetic studies and breeding efforts aimed at improving the species.

Amplified Fragment Length Polymorphism (AFLP) and SCAR markers have also been employed for early sexual identification in *E. ulmoides*. These molecular markers offer reliable and efficient means to determine the sex of plants, which is crucial for optimizing breeding strategies and ensuring the production of high-quality plant material (Wang et al., 2011; Razumova et al., 2023).

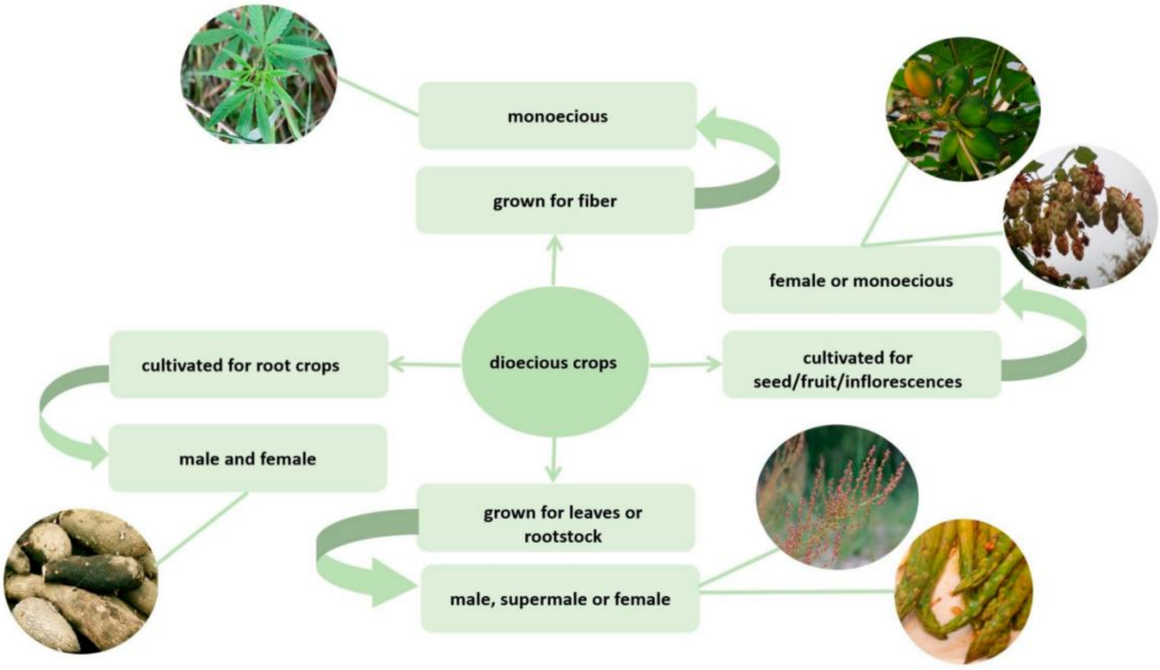


Figure 2 The use of dioecious plants depending on its sex (Adopted from Razumova et al., 2023)

4 Comparative Analysis of Sex Determination Pathways

4.1 Common genetic markers across dioecious species

Sex determination in dioecious plants often involves conserved sex-linked genes that play crucial roles across different species. For instance, in *Eucommia ulmoides*, genes such as *EuAP3* and *EuAG* have been identified as key regulators of sex differentiation (Du et al., 2023). Similarly, MADS-box genes, particularly B-class genes, have been implicated in the sex determination pathways of various dioecious plants, suggesting a convergent mechanism where distinct sex-determining genes regulate these floral genes (Mai et al., 2022; Zhang et al., 2022). Additionally, the presence of sex-linked molecular markers, such as the MSL4 locus in *E. ulmoides*, further underscores the conservation of sex-linked genes across dioecious species (Wang et al., 2020).

Marker-assisted selection (MAS) is a powerful tool in breeding programs for dioecious plants. In *E. ulmoides*, the identification of sex-linked markers like MSL4 enables early sex identification, which is crucial for breeding and commercial production (Wang et al., 2020). The use of molecular markers has also shown advantages in other dioecious crops, such as papaya, whose molecular marker W11 can identify male/hermaphrodite sex in papaya seedling populations with 100% accuracy (Thanh et al., 2024). These markers help to verify sex before phenotypic expression, thus simplifying the breeding process (Heikrujam et al., 2014).

4.2 Differences in sex determination pathways between species

While there are conserved elements in sex determination pathways, significant divergence exists between species. For example, the sex determination system in *E. ulmoides* involves specific MADS-box transcription factors that exhibit sex-biased expression, with certain genes being almost exclusively expressed in male or female tissues (Zhang et al., 2023). In contrast, in other species such as kiwifruit, the Y-encoded SyGI and FrBy determine sex differentiation (Akagi et al., 2019). Poplar employs a single-gene mechanism for sex determination, with the feminizing factor popARR17 acting as the master regulator, indicating that its sex determination pathway is simpler compared to the complex gene network in *Eucommia ulmoides* (Cronk and Müller, 2020).

Species-specific adaptations in sex determination pathways are evident in the unique genetic and epigenetic mechanisms employed by different dioecious plants. For instance, the high expression of ω-3 fatty acid desaturase coding gene *EU0103017* in *E. ulmoides* is linked to its high α-linolenic acid content, showcasing a metabolic adaptation intertwined with its sex determination pathway (Du et al., 2023). Additionally, the role of cytokinin signaling in sex determination has been highlighted in several species, indicating a common yet adaptable pathway across different dioecious plants (Leite Montalvão et al., 2021).

4.3 Implications for breeding and genetic engineering

Genetic engineering offers promising approaches to manipulate sex determination pathways in dioecious plants. The use of CRISPR-Cas9 to knock out sex-determining genes, as demonstrated in poplar, provides a potential method to control sex expression and improve breeding efficiency (Cronk and Müller, 2020). In *E. ulmoides*, the identification of key sex-linked genes and markers opens avenues for targeted genetic modifications to enhance desirable traits and streamline breeding programs (Wang et al., 2020; Du et al., 2023).

Breeding strategies for dioecious plants can be significantly enhanced by understanding their sex determination pathways. The use of marker-assisted selection allows for early sex identification, reducing the time and resources required for breeding (Wang et al., 2020). Additionally, insights into the genetic regulation of sex differentiation, such as the role of MADS-box genes in *E. ulmoides*, can inform selective breeding practices to develop superior varieties with desired traits (Zhang et al., 2023). The integration of molecular and conventional breeding techniques will be crucial in optimizing breeding strategies for dioecious crops (Heikrujam et al., 2014).

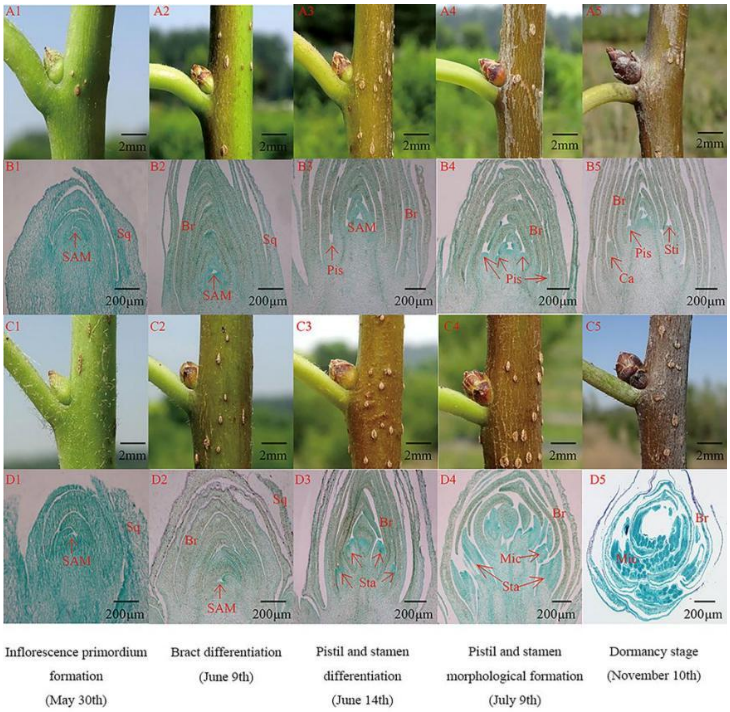
The comparative analysis of sex determination pathways in dioecious plants like *Eucommia ulmoides* reveals both conserved and divergent mechanisms. Understanding these pathways not only provides insights into the evolution of plant dioecy but also has practical implications for breeding and genetic engineering, ultimately contributing to the development of superior plant varieties.

5 Case Studies

5.1 Sex determination in *Eucommia ulmoides*

Eucommia ulmoides, commonly known as the hardy rubber tree, is a dioecious plant with significant medicinal and economic value (Figure 3). Recent genomic studies have provided valuable insights into its sex determination mechanisms. A high-quality chromosome-level genome assembly for both male and female *E. ulmoides* has been achieved using PacBio and Hi-C technologies. The female genome assembly is 1.01 Gb with 17 pseudochromosomes and 31,665 protein-coding genes, while the reassembled male genome is 1.24 Gb with a superscaffold N50 of 48.30 Mb and 42,931 predicted genes. Transcriptome analysis has identified key genes such as *EuAP3* and *EuAG* that may regulate sex differentiation in *E. ulmoides* (Du et al., 2023) (Figure 4).

Additionally, double-digest restriction site-associated DNA sequencing (ddRAD-seq) has been employed to identify sex-linked molecular markers. This study identified five candidate male-specific loci, with one ideal sex-linked locus, MSL4, being highly conserved in all male individuals. This marker is valuable for rapid breeding and commercial production (Wang et al., 2020). Comparative transcriptome analyses have further identified 116 differentially expressed genes (DEGs) between male and female *E. ulmoides*, including genes related to sexually dimorphic traits and potential sex-associated genes (Wang and Zhang, 2017).



Inflorescence primordium formation (May 30th)

Bract differentiation (June 9th)

Pistil and stamen differentiation (June 14th)

Pistil and stamen morphological formation (July 9th)

Dormancy stage (November 10th)

Figure 3 Internal anatomical structure and external morphological characteristics of male and female flower buds at different differentiation stages (Adopted from Du et al., 2023)

Image caption: Br, Bract; Ca, Carp; Mic, Microsporocyte; Pis, Pistil, SAM, Stem Apical Meristem; Sq, Squama; Sta, Stamen; Sti, Stigma. (A1-A5): The external morphology structure of female flower bud; (B1-B5): The anatomic structure of female flower bud; (C1-C5): The external morphology structure of male flower bud; (D1-D5): The anatomic structure of male flower bud (Adopted from Du et al., 2023)

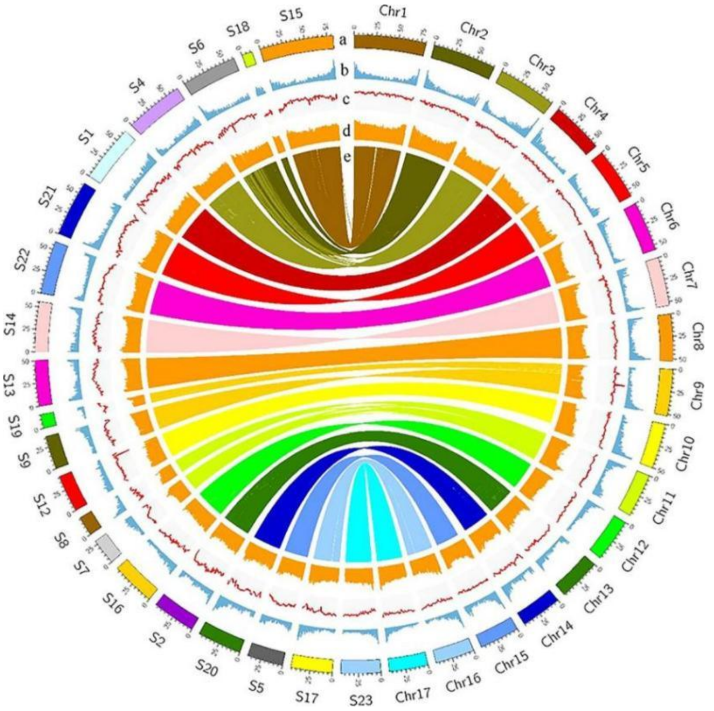


Figure 4 Landscape of Female V1 and Male V2 genome (Adopted from Du et al., 2023)

Image caption: The circle from outside to inside represents, (A) Chromosomes of Female V1 and Superscaffolds of Male V2, (B) gene density, (C) GC content, (D) repeat abundance, (E) synteny information. All distributions were drawn in a window size of 1 Mb (Adopted from Du et al., 2023)

5.2 Genetic markers in other dioecious plants

The study of sex determination in dioecious plants extends beyond *E. ulmoides* to other species with significant agricultural and economic importance. For instance, in asparagus and kiwifruit, two sex-determining genes within the non-recombining sex-determining regions (SDRs) have been identified, supporting the classical model of dioecy evolution from hermaphroditism via gynodioecy. In spinach, using BSA combined with SLAF-seq technology and a high-density genetic map, the sex-determining genes X/Y were located on linkage group (LG) 4, which corresponds to the ideal region for sex determination genes (Wei et al., 2017). In contrast, single-factor sex determination has been observed in species like persimmon and poplar, where genome sequencing and gene editing have played crucial roles in identifying sex-determining genes (Leite Montalvão et al., 2021).

In agricultural plants, the mechanisms of sex determination can range from single-gene control to complex interactions involving heteromorphic sex chromosomes. These mechanisms can directly influence sex differentiation or participate in the balance determination of sex, such as the ratio of X chromosomes to autosomes. The development of sequencing techniques and bioinformatics has significantly advanced our understanding of these processes, particularly in economically valuable dioecious crops (Scharmann et al., 2019; Razumova et al., 2023).

5.3 Comparative outcomes of genetic interventions

Comparative studies of genetic interventions in dioecious plants reveal diverse outcomes depending on the species and the genetic mechanisms involved. In *E. ulmoides*, the identification of sex-linked markers like MSL4 has facilitated early sex identification, aiding in breeding and commercial production (Wang et al., 2020). Similarly, the identification of differentially expressed genes and sex-associated genes through transcriptome analyses has provided deeper insights into the genetic basis of sex determination (Wang and Zhang, 2017).

In other dioecious plants, genetic interventions have led to the discovery of sex-determining genes and pathways that are often conserved across species. For example, the cytokinin signaling pathway has been implicated in sex determination in several species, regardless of the underlying genetic system. Tapetum-related genes frequently act as male-promoting factors in species with two-gene sex determination systems (Leite Montalvão et al., 2021). These findings suggest that while the specific genes and mechanisms may vary, there are common pathways and genetic networks that are repeatedly employed in the evolution of dioecy across different plant species.

6 Environmental Influences on Sex Determination in *Eucommia ulmoides*

6.1 Climatic factors affecting sex ratios

Climatic factors play a significant role in influencing the sex ratios of *Eucommia ulmoides*. Research has shown that environmental stressors such as temperature and precipitation can impact the frequency of female individuals in populations of sexually dimorphic plants. For instance, studies have indicated that increased environmental stress, particularly temperature-related stress, is positively correlated with higher female frequencies in certain plant species (Varga and Soulsbury, 2020). Additionally, phenotypic variation in traits such as leaf, fruit, and seed size among different populations of *E. ulmoides* has been linked to climatic factors, with average annual temperature and rainfall accounting for most of the observed variation (Wang et al., 2023). These findings suggest that climatic conditions can significantly influence the sex expression and distribution of *E. ulmoides* populations.

6.2 Soil and nutrient impacts on sex expression

Soil quality and nutrient availability are crucial factors that can affect sex expression in *Eucommia ulmoides*. Although specific studies on *E. ulmoides* are limited, general research on dioecious plants indicates that soil fertility and nutrient levels can influence the growth and reproductive success of male and female plants differently. For example, nutrient-rich soils may favor the growth of female plants, which often require more resources for seed production, while nutrient-poor conditions might be more favorable for male plants (Munné-Bosch, 2015). Understanding the specific soil and nutrient requirements for *E. ulmoides* can help in managing and optimizing the growth conditions to achieve desired sex ratios in cultivated populations.

6.3 Adaptive strategies of *Eucommia ulmoides*

Eucommia ulmoides has developed several adaptive strategies to cope with varying environmental conditions, which in turn influence its sex determination mechanisms. The species exhibits significant phenotypic variation within and among populations, which is a key adaptive trait. This variation allows *E. ulmoides* to thrive in diverse climatic and geographic conditions (Wang et al., 2023). Additionally, the genetic mechanisms underlying sex determination in *E. ulmoides* involve complex interactions between multiple genes and environmental factors. For instance, the expression of certain MADS-box transcription factors, which are involved in floral organ development, is differentially regulated in male and female plants, suggesting a sophisticated genetic control of sex differentiation that can respond to environmental cues (Zhang et al., 2023). These adaptive strategies enable *E. ulmoides* to maintain balanced sex ratios and reproductive success across different habitats.

7 Molecular and Genetic Tools in Sex Determination Research

7.1 Advances in molecular markers for sex determination

Recent advancements in molecular markers have significantly enhanced our understanding of sex determination in dioecious plants, including *Eucommia ulmoides*. Techniques such as Random Amplified Polymorphic DNA (RAPD) and Sequence Characterized Amplified Region (SCAR) markers have been employed to identify sex-specific markers. For instance, a 569 bp RAPD marker linked to sex determination in *E. ulmoides* was identified and subsequently converted into a SCAR marker, which can be used to screen plants for gender before they reach reproductive maturity, thus saving time and resources (Xu et al., 2004). Additionally, double-digest restriction site-associated DNA sequencing (ddRAD-seq) has been utilized to screen sex-linked molecular markers, leading to the identification of a male-specific locus, MSL4, which is highly conserved and can reliably distinguish male from female seedlings (Wang et al., 2020). These molecular markers are invaluable for early sex identification and breeding programs.

7.2 Application of CRISPR/Cas9 in sex determination studies

The CRISPR/Cas9 genome editing technology has revolutionized genetic research, including studies on sex determination in plants. Although specific applications of CRISPR/Cas9 in *E. ulmoides* have not been extensively reported, the technology holds great potential for manipulating sex-determining genes. For example, CRISPR/Cas9 can be used to knock out or modify genes involved in sex differentiation pathways, such as the MADS-box genes, which play a crucial role in the development of unisexual flowers in *E. ulmoides* (Zhang et al., 2023). By targeting these genes, researchers can potentially control the sex of the plants, which would be highly beneficial for breeding and commercial production.

7.3 Future directions for genetic research

Future research in the genetic mechanisms of sex determination in dioecious plants like *E. ulmoides* should focus on several key areas. There is a need for comprehensive genome-wide association studies (GWAS) to identify additional sex-linked genes and regulatory elements. The high-quality chromosome-level genome assemblies of both female and male *E. ulmoides* provide a valuable resource for such studies. Integrating transcriptome analysis with advanced bioinformatics tools can help elucidate the complex regulatory networks involved in sex differentiation. For instance, genes such as *EuAP3* and *EuAG* have been identified as key regulators in *E. ulmoides*, and further functional studies are needed to understand their roles (Du et al., 2023).

Furthermore, the application of CRISPR/Cas9 and other gene-editing technologies should be expanded to validate the functions of candidate sex-determining genes and to develop new strategies for sex control in breeding programs. By addressing these areas, researchers can gain deeper insights into the molecular basis of sex determination and improve the efficiency of breeding dioecious plants. The integration of advanced molecular markers, genome editing technologies, and comprehensive genetic studies will pave the way for significant advancements in understanding and manipulating sex determination in dioecious plants like *Eucommia ulmoides*.

8 Applications in Breeding and Conservation

8.1 Breeding strategies for dioecious plants

Breeding strategies for dioecious plants, such as *Eucommia ulmoides*, require a nuanced understanding of their sex determination mechanisms. The identification of sex-linked markers is crucial for early sex identification, which can significantly enhance breeding efficiency. For instance, the use of RAPD and SCAR markers has been shown to effectively distinguish between male and female *E. ulmoides* plants before they reach reproductive maturity, saving both time and resources (Xu et al., 2004). Additionally, the application of ddRAD-seq has identified stable and reproducible sex-linked loci, such as MSL4, which can be used to discriminate male from female seedlings, facilitating rapid breeding practices (Wang et al., 2020). These molecular tools are invaluable for developing superior varieties with desired traits, such as higher α-linolenic acid content, which is economically beneficial (Du et al., 2023).

8.2 Sex determination in conservation programs

In conservation programs, understanding the sex determination pathways of dioecious plants is essential for maintaining genetic diversity and ensuring the survival of both male and female populations. The genetic mechanisms underlying sex determination in *E. ulmoides*, including the role of MADS-box genes and other sex-biased genes, provide insights that can be applied to conservation efforts (Wang and Zhang, 2017; Zhang et al., 2023). By utilizing molecular markers and transcriptome analyses, conservationists can monitor and manage the sex ratios within populations, ensuring that both sexes are adequately represented. This is particularly important for species like *E. ulmoides*, where the pistillate plants have higher economic value (Xu et al., 2004). Moreover, the identification of sex-determining regions (SDRs) and sex chromosomes in various dioecious plants can inform conservation strategies across different species (Leite Montalvão et al., 2021; Razumova et al., 2023).

8.3 Breeding practices in *Eucommia ulmoides*

Eucommia ulmoides, a dioecious tree with significant medicinal and economic value, serves as an excellent case study for breeding practices. The high-quality chromosome-level genome assemblies of both female and male *E. ulmoides* have provided a valuable resource for understanding sex differentiation and facilitating breeding programs. Transcriptome analyses have identified key genes, such as *EuAP3* and *EuAG*, that regulate sex differentiation, which can be targeted in breeding strategies to develop superior varieties (Du et al., 2023). Additionally, the use of molecular markers, such as the 569 bp SCAR marker specific to pistillate plants, allows for early sex identification, streamlining the breeding process (Xu et al., 2004). The integration of these genetic tools into breeding programs can enhance the production of high-value pistillate plants, contributing to the economic viability of *E. ulmoides* cultivation (Wang et al., 2020; You et al., 2023; Zhang et al., 2023).

9 Concluding Remarks

Research on sex determination pathways in *Eucommia ulmoides* has yielded significant insights into the genetic and molecular mechanisms underlying sexual differentiation in this dioecious plant. The high-quality chromosome-level genome assemblies for both female and male *E. ulmoides* have provided a valuable resource for understanding sex differentiation. Key genes such as *EuAP3* and *EuAG* have been identified as crucial regulators of sex differentiation. Additionally, molecular markers like MSL4 and SCARmr have been developed for early sex identification, which is essential for breeding and commercial production. Comparative transcriptome analyses have revealed differentially expressed genes between male and female plants, further elucidating the genetic basis of sexual dimorphism.

The findings from these studies have several practical applications in agriculture and industry. The ability to identify the sex of *E. ulmoides* plants at an early stage using molecular markers can significantly enhance breeding programs by allowing for the selection of desired traits before the plants reach reproductive maturity. This can lead to more efficient cultivation practices and improved yields. Additionally, understanding the genetic mechanisms of sex differentiation can aid in the development of superior varieties with enhanced medicinal and economic properties, such as higher α-linolenic acid content. The identification of sex-specific genes and markers also opens up possibilities for genetic engineering to further optimize the traits of *E. ulmoides* for industrial use.

Future research in sex determination in *E. ulmoides* should focus on further elucidating the complex genetic and epigenetic mechanisms involved in sexual differentiation. Advanced sequencing technologies and bioinformatics tools can be employed to identify additional sex-linked genes and regulatory pathways. Moreover, exploring the role of environmental factors and plant hormones in sex expression could provide a more comprehensive understanding of the sex determination process. Collaborative efforts integrating genomics, transcriptomics, and proteomics will be crucial in developing a holistic view of sex determination in *E. ulmoides*. These insights can then be applied to other dioecious plants, potentially benefiting a wide range of agricultural and industrial applications.

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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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