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Syntenic Relationships and Chromosomal Evolution in the Legume Family

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Abstract This study explores the syntenic relationships and chromosomal evolution within the legume family, providing key insights into their genomic architecture and evolutionary history. The study highlights the conservation of receptor-like kinases (RLK) and receptor-like proteins (RLP) across various legume species, revealing highly conserved syntenic blocks that contribute to stress resistance. Comparative cytogenetic analyses in species like *Vigna angularis* and *Phaseolus vulgaris* demonstrate significant macrosynteny and chromosomal rearrangements, particularly on chromosomes 2 and 3. The study also discusses whole-genome duplication events and their impact on the diversification and adaptation of legumes. Additionally, the study underscores the importance of leveraging advanced genomic technologies and multi-omics approaches to further elucidate the genetic and evolutionary dynamics in legumes. The findings offer valuable genomic resources and a framework for breeding strategies aimed at improving legume crop traits, ultimately enhancing agricultural productivity and sustainability.

Keywords Synteny; Chromosomal evolution; Legumes; Whole-genome duplication; Comparative genomics

1 Introduction

Legumes have been integral to agricultural systems since the dawn of civilization, serving as a crucial source of food for humans and feed for animals. Their unique ability to symbiotically fix atmospheric nitrogen not only enhances soil fertility but also reduces the need for synthetic fertilizers, making them indispensable in sustainable agriculture (Smýkal et al., 2020). Economically, legumes are the second most important family of crop plants after grasses, contributing significantly to global food security by providing 33% of the dietary protein consumed by humans and playing a vital role in animal nutrition (Smýkal et al., 2015). The historical significance of legumes is further underscored by their role in the foundational work of Gregor Mendel, whose experiments with pea plants laid the groundwork for modern genetics.

Chromosomal evolution and synteny, the conservation of blocks of genes across different species, are critical areas of study in plant genomics. In legumes, these phenomena have been shaped by key events such as whole-genome duplications, which have significantly influenced their genetic architecture and evolutionary trajectory. For instance, a major whole-genome duplication event approximately 58 million years ago is shared by most agriculturally important legumes, while a more recent duplication occurred specifically in the soybean lineage. These duplications have led to gene fractionation and the emergence of new gene functions, contributing to the development of legume-specific traits and enhancing their adaptability and utility in agriculture (Young and Bharti, 2012).

This study aims to explore the patterns of syntenic relationships and chromosomal changes within the legume family. By examining the genetic and chromosomal evolution of legumes, it seeks to understand how these processes have contributed to their diversification and adaptation. The study will also highlight the implications of these evolutionary changes for legume breeding and genetic improvement, providing insights that could enhance the productivity and sustainability of legume crops. Through a comprehensive analysis of recent genomic studies, this study will elucidate the complex interplay between chromosomal evolution and synteny in shaping the genetic landscape of legumes, ultimately contributing to the advancement of legume genomics and agricultural practices.

2 Understanding Synteny and Chromosomal Evolution

2.1 Concepts of synteny and chromosomal rearrangements

Synteny refers to the conservation of blocks of genes on chromosomes across different species. This concept is crucial for understanding the evolutionary relationships between species and the mechanisms driving chromosomal evolution. Chromosomal rearrangements, such as inversions, translocations, fusions, and fissions, play a significant role in the diversification of genomes. For instance, in the legume family, studies have shown that *Vigna angularis* shares a high degree of macrosynteny with *Vigna unguiculata* and *Phaseolus vulgaris*, with conserved syntenic chromosomes and specific chromosomal rearrangements identified (Martins et al., 2021). Similarly, the genus *Cercis*, an early-diverging clade within legumes, provides insights into the ancestral legume genome, suggesting a progenitor with seven chromosomes (Stai et al., 2019).

2.2 Mechanisms driving chromosomal evolution in plants

Chromosomal evolution in plants is driven by various mechanisms, including whole-genome duplications, segmental duplications, and independent gene duplications or losses. These events lead to complex histories of genome rearrangements. For example, the reconstruction of an ancestral genome for papilionoid legumes revealed a common ancestor with nine chromosomes, which likely evolved from an earlier state of 14 chromosomes following a whole-genome duplication (Ren et al., 2019). Additionally, the study of plastome evolution in legumes has highlighted the role of inversions, expansions, contractions, and loss of the inverted repeat in driving plastome variability (Lee et al., 2021). In holocentric plants like *Carex*, chromosome rearrangements through fission and fusion are more likely to become fixed, contributing to high rates of chromosomal evolution.

2.3 Tools and techniques for studying synteny and chromosome structure

Several tools and techniques have been developed to study synteny and chromosome structure in plants. Fluorescence in situ hybridization (FISH) using bacterial artificial chromosome (BAC) and oligonucleotide (oligo) probes is a powerful method for mapping chromosomes and identifying syntenic relationships. For instance, BAC- and oligo-FISH mapping has been used to trace chromosome evolution in *Vigna* and *Phaseolus* species, revealing conserved syntenic chromosomes and specific rearrangements (Martins et al., 2021). Chromosome painting probes have also been developed for *Citrus* species, enabling the identification of individual chromosomes and comparative chromosome painting analysis across different species (He et al., 2020). Additionally, algorithms like syntR and syngraph have been implemented to identify regions of synteny and infer ancestral linkage groups and chromosomal rearrangements from genome data (Ostevik et al., 2020).

Understanding synteny and chromosomal evolution in the legume family involves studying the conservation of gene blocks, mechanisms driving chromosomal changes, and utilizing advanced tools and techniques for chromosome mapping and analysis. These studies provide valuable insights into the evolutionary history and genomic diversity of legumes.

3 Syntenic Relationships in Legumes

3.1 Overview of known syntenic blocks within the legume family

Synteny, the conservation of blocks of genes across different species, is a crucial aspect of understanding the evolutionary relationships within the legume family. Studies have shown that a significant proportion of genes in legumes are conserved in syntenic blocks. For instance, in the study of receptor-like kinases (RLK) and receptor-like proteins (RLP) across seven legume species, it was found that between 75% and 98% of these genes were present in syntenic blocks, indicating a high degree of conservation (Restrepo-Montoya et al., 2021). Similarly, the genetic map of *Bituminaria bituminosa* revealed highly conserved synteny with phaseoloid legumes, despite the divergence of these species millions of years ago (Nelson et al., 2020). These findings underscore the importance of syntenic blocks in maintaining genetic integrity and facilitating comparative genomics within the legume family.

3.2 Comparative analysis of synteny between model legume species and crops

Comparative synteny analysis between model legume species and crops has provided valuable insights into chromosomal evolution and gene conservation. For example, the genome of *Medicago truncatula*, a model legume, has been extensively studied to understand its syntenic relationships with other legumes. The improved genome assembly of *M. truncatula* cv. R108, which includes chromosome-length scaffolds, has enabled accurate synteny analysis and comprehensive genome-scale comparisons (Kaur et al., 2021). Additionally, the cytogenetic map of *Vigna angularis* was compared with those of *Vigna unguiculata* and *Phaseolus vulgaris*, revealing a high degree of macrosynteny and identifying specific chromosomal rearrangements that have occurred since their divergence (Martins et al., 2021). These comparative analyses highlight the evolutionary processes that have shaped the genomes of both model species and economically important legume crops.

3.3 Impact of synteny on gene conservation and divergence

The conservation of syntenic blocks has a profound impact on gene conservation and divergence within the legume family. Synteny helps preserve the functional integrity of gene clusters, which is crucial for maintaining essential biological processes. For instance, the study of glutamine synthetase (GS) and phosphoenolpyruvate carboxylase (PEPC) gene families in narrow-leafed lupin demonstrated that whole genome duplications have largely retained the function of these gene duplicates, contributing to the diversity and adaptability of legumes (Czyż et al., 2020). Furthermore, the identification of conserved syntenic blocks containing RLK and RLP genes across multiple legume species suggests that these genes play a critical role in stress responses and have been conserved through evolutionary pressures (Restrepo-Montoya et al., 2021). However, synteny also allows for divergence through chromosomal rearrangements and gene duplications, which can lead to the development of new traits and increased genetic diversity. The study of the pea genome, for example, revealed intense gene dynamics and genomic rearrangements that have contributed to its evolution and adaptation (Kreplak et al., 2019). These findings illustrate the dual role of synteny in both conserving essential genes and facilitating evolutionary innovation within the legume family.

4 Chromosomal Evolution in Legumes

4.1 Historical perspectives on chromosomal changes in legumes

The legume family, Fabaceae, has undergone significant chromosomal changes throughout its evolutionary history. Early studies have highlighted the role of whole-genome duplications, segmental duplications, and independent gene duplications or losses in shaping the genomes of various legume species (Ren et al., 2019). The genus *Cercis*, for instance, provides a model for an early evolutionary form of the legume genome, suggesting that the legume progenitor had seven chromosomes, which diversified into the various legume subfamilies (Stai et al., 2019). This early chromosomal configuration and subsequent duplications have been pivotal in the diversification and adaptation of legumes.

4.2 Significant chromosomal rearrangements and their functional implications

Several case studies have provided insights into the significant chromosomal rearrangements in legumes and their functional implications. For example, the cytogenetic mapping of *Vigna angularis*, *Vigna unguiculata*, and *Phaseolus vulgaris* revealed multiple chromosomal rearrangements, including reciprocal translocations and inversions, which have occurred after the divergence of these species (Martins et al., 2021). These rearrangements are believed to be hotspots for chromosomal evolution and de novo centromere formation.

In another study, the reference genome assembly for pea (*Pisum sativum*) highlighted intense gene dynamics and chromosomal rearrangements, which are associated with genome size expansion and the evolution of agronomically important traits (Kreplak et al., 2019). The orthology and synteny analysis of receptor-like kinases (RLK) and receptor-like proteins (RLP) in legumes further demonstrated the dynamic evolution of these genes, with a high proportion of RLK and RLP genes being conserved in syntenic blocks across different legume species (Restrepo-Montoya et al., 2021). For instance, the peanut genome illustrates significant chromosomal rearrangements, such as translocations between chromosomes A03 and B03, which highlight the functional implications of chromosomal evolution in legume species (Figure 1) (Zhuang et al., 2019).

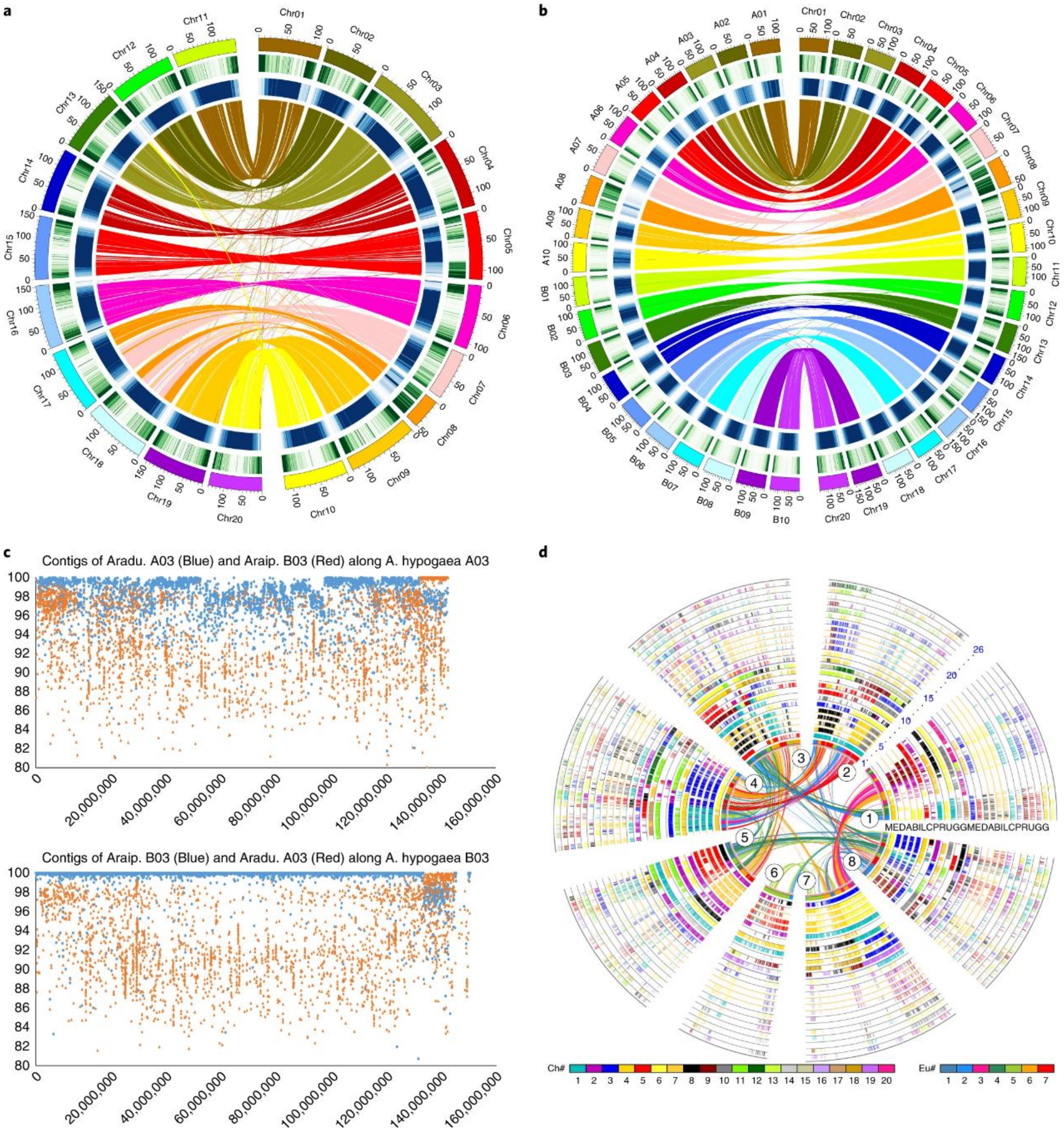

Figure 1 Characterization of the peanut genome and chromosomes (Adopted from Zhuang et al., 2019)

Image caption: a, Circos diagram depicting relationships of A and B subgenome chromosomal pseudomolecules. The scale for the chromosomes (outer bars) is megabases; colors represent the density of nonautonomous LTR retrotransposons and Ty3-gypsy elements (blue) and genes (green). Homeologous blocks of ≥30 gene pairs between Chr01-Chr10 and Chr11-Chr20 (A01-A10 and B01-B10, respectively) are connected with lines. b, Syntenic comparisons between peanut subgenomes and diploid A and B genomes. The outer three circles are chromosomes, density of genes and of Ty3-gypsy and nonautonomous LTR retrotransposons (as shown in a). Colored lines connect blocks with ≥30 orthologous gene pairs between the A and B subgenomes and *A. duranensis* and *A. ipaensis* genomes, respectively, based on BLASTN. c, Alignment of diploid peanut A03 and B03 contigs to corresponding tetraploid chromosomes, with parameters: -a 8 -p blastn -m 9-e 1e-10. The best hits with alignment length ≥2 000 bp were plotted. Translocation between chromosomes A03 and B03 is evident in cultivated peanut. d, Eleven-genome alignment using co-linear genes, each mapped onto the barrel medic chromosomes. A, *A. hypogaea* A; B, *A. hypogaea* B; C, *C. cajan*; D, *A. duranensis*; E, *C. arietinum*; G, *G. max*; I, *A. ipaensis*; L, *L. japonicus*; M, *M. truncatula*; P, *P. vulgaris*; R, *V. radiata*; U, *V. angularis* (Adopted from Zhuang et al., 2019)

4.3 Correlation between chromosomal changes and adaptive traits

Chromosomal changes in legumes have been closely linked to adaptive traits, particularly in response to biotic and abiotic stresses. The dynamic evolution of RLK and RLP genes, which are involved in stress responses, suggests that chromosomal rearrangements have played a crucial role in enhancing the adaptability and productivity of legumes (Restrepo-Montoya et al., 2021). Additionally, the loss of the inverted repeat (IR) in the plastomes of certain legume species, such as those in the IR-lacking clade (IRLC), has been associated with increased plastome variation and adaptive evolution (Lee et al., 2021).

The rapid evolution and pseudogenization of the ycf4 gene in the tribe Fabeae, which plays a role in photosystem I assembly, further exemplify the correlation between chromosomal changes and adaptive traits. The lineage-specific accelerated rate of evolution and gene loss in this tribe indicate that such chromosomal changes have been driven by selective pressures to enhance photosynthetic efficiency and adaptation to diverse environments (Moghaddam and Kazempour-Osaloo, 2020).

The chromosomal evolution in legumes has been marked by significant rearrangements and duplications, which have contributed to the diversification and adaptation of this important plant family. These changes have not only shaped the genomic architecture of legumes but also facilitated the development of key adaptive traits, enhancing their ecological and agricultural significance.

5 Genomic Insights from Syntenic Studies

5.1 Insights into gene function and regulation from syntenic alignments

Syntenic alignments have proven invaluable in elucidating gene function and regulation across various species. For instance, the study on narrow-leafed lupin (*Lupinus angustifolius* L.) utilized synteny-based approaches to analyze the glutamine synthetase (GS) and phosphoenolpyruvate carboxylase (PEPC) gene families. This analysis revealed that gene duplications have significantly contributed to the diversity of these gene families, while the function of the duplicates has largely been retained (Czyż et al., 2020). Similarly, synteny-guided phylogenies have been shown to provide more accurate reconstructions of gene trees, which are crucial for understanding the functional impact of whole genome duplications (WGDs) (Parey et al., 2020). These synteny-guided methods help in identifying orthologs and paralogs more reliably, thereby facilitating the exploration of functional consequences of WGDs.

5.2 Role of whole genome duplications in syntenic conservation

Whole genome duplications (WGDs) play a pivotal role in syntenic conservation and the evolution of gene families. The study on the oxytocin and vasotocin receptor gene family highlights how WGDs have shaped the vertebrate genome, suggesting that a single round of WGD is more consistent with the synteny and evolution of chromosomes where these receptors are found. In legumes, WGDs have been instrumental in the diversification of gene families, as evidenced by the evolutionary history of the legume family, where multiple independent WGDs have given rise to the various subfamilies (Stai et al., 2019). Additionally, the study on narrow-leafed lupin corroborates earlier findings that key WGD events have significantly impacted the genistoid lineage, contributing to the extant diversity of gene families (Czyż et al., 2020).

5.3 Contributions of syntenic studies to understanding evolutionary dynamics

Syntenic studies have significantly advanced our understanding of evolutionary dynamics by revealing patterns of chromosomal evolution and gene retention. For example, the comparative analysis of *Vigna angularis*, *Vigna unguiculata*, and *Phaseolus vulgaris* demonstrated high degrees of macrosynteny and identified specific chromosomal rearrangements that have occurred since their divergence (Martins et al., 2021). Furthermore, the study on metazoan chromosomes uncovered deeply conserved synteny among bilaterians, cnidarians, and sponges, providing insights into the processes of chromosome evolution from Precambrian progenitors (Simakov et al., 2022). These findings underscore the importance of syntenic studies in reconstructing ancestral chromosomes and understanding the evolutionary history of various taxa.

Syntenic studies offer profound insights into gene function and regulation, highlight the critical role of WGDs in syntenic conservation, and contribute to our understanding of evolutionary dynamics across different species. By leveraging syntenic alignments, researchers can more accurately reconstruct gene phylogenies, elucidate the functional impacts of gene duplications, and trace the evolutionary history of chromosomes.

6 Applications of Syntenic and Chromosomal Knowledge

6.1 Breeding strategies enhanced by syntenic information

Syntenic relationships and chromosomal evolution studies have significantly advanced breeding strategies in legumes. By understanding the genetic architecture and evolutionary history of legume species, breeders can identify and utilize conserved genomic regions to enhance crop traits. For instance, the identification of syntenic blocks containing resistance genes in legumes has facilitated the development of stress-resistant varieties. The orthology and synteny analysis of receptor-like kinases (RLK) and receptor-like proteins (RLP) in legumes revealed conserved syntenic blocks that include resistance genes, which can be targeted for breeding programs to improve stress tolerance (Restrepo-Montoya et al., 2021). Additionally, the use of whole genome sequencing and omics-level data has expanded the capacity to monitor genetic changes, uncovering breeding targets to develop modern cultivars with improved yield, quality, and stress tolerance (Bohra et al., 2022).

6.2 Biotechnological applications in crop improvement

Biotechnological applications leveraging syntenic and chromosomal knowledge have revolutionized crop improvement in legumes. The development of high-quality genome assemblies, such as the chromosome-length genome assembly for *Medicago truncatula*, has provided foundational resources for functional genomics and trait improvement (Kaur et al., 2021). These genomic resources enable precise gene editing and the identification of quantitative trait loci (QTLs) associated with desirable agronomic traits. For example, genome-wide association studies (GWAS) in *Medicago truncatula* have identified candidate genes regulating seed size and composition, which can be exploited in breeding programs to enhance seed nutritional quality and yield in other legume crops due to their high synteny (Chen et al., 2021). Furthermore, the integration of next-generation sequencing (NGS) and high-throughput genotyping methods has accelerated the delivery of genomic information, facilitating the development of improved legume cultivars (Afzal et al., 2019).

6.3 Conservation genetics based on chromosomal studies

Chromosomal studies have also played a crucial role in conservation genetics, aiding in the preservation of genetic diversity in legume species. The establishment of cytogenetic maps and the comparison of chromosomal structures among legume species have provided insights into chromosomal evolution and the identification of conserved syntenic regions (Martins et al., 2021). These studies help in tracing the evolutionary history and genetic relationships among species, which is essential for the conservation of genetic resources. Additionally, the use of chromosomal information in introgression breeding has enabled the transfer of beneficial traits from wild relatives to cultivated varieties, thereby broadening the genetic base and enhancing the resilience of legume crops (Pratap et al., 2021).

7 Case Studies: Chromosomal Evolution and Trait Development

7.1 Detailed analysis of chromosomal rearrangements affecting specific traits

Chromosomal rearrangements play a significant role in the evolution of legume genomes, impacting trait development and adaptation. For instance, in *Vigna angularis*, *Vigna unguiculata*, and *Phaseolus vulgaris*, BAC- and oligo-FISH mapping revealed several chromosomal rearrangements, including reciprocal translocations and inversions, which have occurred post-divergence of these species. These rearrangements, particularly on chromosomes 2 and 3, are proposed as hotspots for chromosomal changes and de novo centromere formation, which could influence trait development and speciation within these genera (Martins et al., 2021). Similarly, in *Medicago truncatula*, a chromosome-length genome assembly has identified a known chromosome 4/8 translocation, providing insights into synteny and trait mapping, which are crucial for functional genomics and crop improvement (Kaur et al., 2021).

7.2 Examples of evolutionary success facilitated by chromosomal changes

Chromosomal changes have facilitated evolutionary success in various legume species by enabling adaptation to different environments and improving agricultural traits. The reference genome for pea (*Pisum sativum*) highlights the role of genomic rearrangements and repetitive elements in genome size expansion and trait development. These changes have been pivotal in the evolution of the Fabeae tribe, contributing to the diversification and adaptation of pea species (Kreplak et al., 2019). Additionally, the genus *Cercis*, which lacks evidence of polyploidy, provides a model for understanding early legume genome evolution. The slow mutation rate and small genome size of *Cercis* suggest that chromosomal stability has been advantageous for its evolutionary success (Stai et al., 2019).

7.3 Comparative genomics and trait mapping in legumes

Comparative genomics has been instrumental in mapping traits and understanding chromosomal evolution in legumes. The reconstruction of an ancestral genome for papilionoid legumes, which inferred a common ancestor with nine chromosomes, aligns with chromosomal and phylogenetic histories. This reconstruction aids in understanding the chromosomal evolution and trait mapping across diverse legume species (Ren et al., 2019). Furthermore, the orthology and synteny analysis of receptor-like kinases (RLK) and receptor-like proteins (RLP) in legumes has revealed highly conserved syntenic blocks on multiple chromosomes. This conservation is crucial for identifying stress response genes and improving legume adaptability and productivity (Restrepo-Montoya et al., 2021). The study of plastome evolution in papilionoid legumes also underscores the role of chromosomal changes, such as the loss of the inverted repeat, in the diversification and adaptation of these species (Lee et al., 2021).

In this study, comparative genomics has been instrumental in mapping traits and understanding chromosomal evolution in legumes. For instance, the detailed characterization of the pea genome provides valuable insights into the chromosomal features, syntenic relationships, and the spatial organization of key genomic elements, which are critical for further advancing our understanding of legume genome evolution (Figure 2) (Kreplak et al., 2019).

8 Challenges and Limitations in Current Research

8.1 Technical and methodological challenges in chromosomal analysis

Chromosomal analysis in legumes faces several technical and methodological challenges. One significant issue is the complexity of legume genomes, which often include whole-genome duplications, segmental duplications, and independent gene duplications or losses. These complexities make it difficult to reconstruct ancestral genomes and understand chromosomal evolution accurately (Ren et al., 2019). Additionally, the presence of polyploidy in many legume species adds another layer of complexity, as it complicates the identification of orthologous and paralogous genes and the interpretation of syntenic relationships (Zhuang et al., 2019). The variability in plastome structures, such as inversions, expansions, contractions, and loss of the inverted repeat, further complicates the analysis of chromosomal evolution in legumes (Lee et al., 2021).

8.2 Limitations of current genomic resources for non-model legumes

While significant progress has been made in sequencing the genomes of model legume species, genomic resources for non-model legumes remain limited. This lack of comprehensive genomic data hinders comparative genomic studies and the identification of conserved syntenic blocks across different legume species. For instance, the first genetic map for *Bituminaria bituminosa* revealed highly conserved synteny with phaseoloid legumes, but the lack of extensive genomic resources for this species limits further research and breeding efforts (Nelson et al., 2020). Similarly, the genome of narrow-leafed lupin has provided insights into gene family evolution, but the limited genomic resources for this species constrain broader comparative analyses (Czyż et al., 2020).

8.3 Addressing complexities in polyploid legumes

Polyploidy is a common phenomenon in legumes, and it presents unique challenges for genomic research. The presence of multiple sets of chromosomes complicates the assembly and annotation of genomes, as well as the identification of functional genes and regulatory elements. For example, the tetraploid genome of cultivated peanut has provided insights into polyploid evolution and crop domestication, but the complexity of the polyploid genome poses challenges for mapping and candidate-gene discovery (Zhuang et al., 2019). Additionally, the slow rate of mutation accumulation in some polyploid legumes, such as *Cercis*, complicates the study of evolutionary processes and the identification of genomic changes associated with polyploidy (Stai et al., 2019). Addressing these complexities requires advanced genomic tools and methodologies, as well as comprehensive genomic resources for a wider range of legume species.

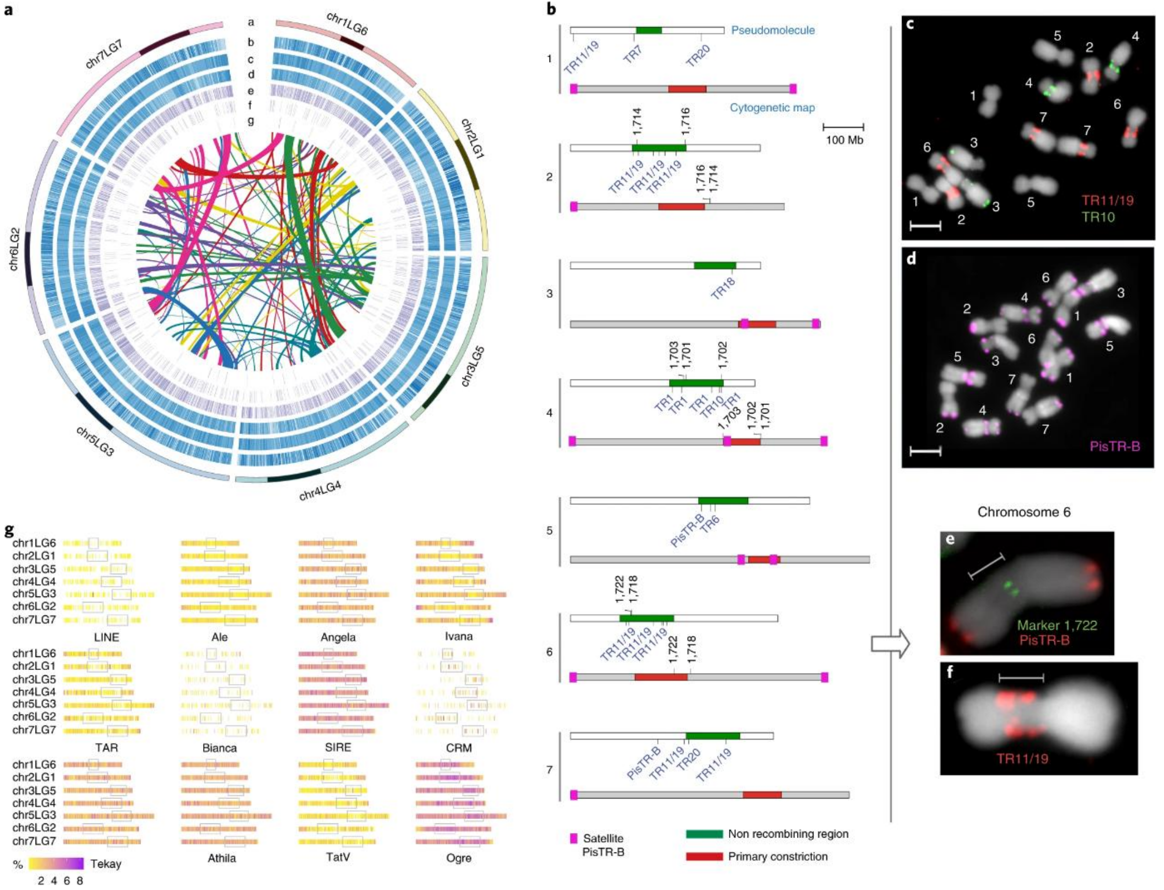

Figure 2 Pea genome features (Adopeted from Kreplak et al., 2019)

Image caption: a, Circos view of the pea genome. Pseudomolecule color-code is shaded at estimated centromere positions. Lanes depict circular representation of pseudomolecules (a) and the density of retrotransposons, transposons, genes, ncRNA, tRNA and miRNA coding sequences (b-g). Lines in the inner circle represent links between synteny-selected paralogs. b, Estimated positions of centromeres in the assembly and their comparison to pea cytogenetic map is schematically represented, with pseudomolecules as white bars and cytogenetic maps of pea chromosomes as gray bars. Non-recombining regions representing the centromeres are marked in green. Positions of centromeric single-copy FISH markers are indicated above the pseudomolecules in black and positions of arrays of centromeric satellites present in the assembly are shown below them in blue. Positions of primary constrictions on the cytogenetic maps are labeled in red. PisTR-B satellite loci used to discriminate individual chromosomes are shown in purple boxes on the gray bars. c, FISH localization of the satellite repeats TR11/19 (red) and TR10 (green) on metaphase chromosomes (gray). d, Discrimination of chromosomes within the pea karyotype using FISH with PisTR-B probe (purple). e, Example of FISH detection of the single-copy marker (1 722, green) in the centromere of chromosome 6. f, Chromosome 6 with labeled centromeric repeat TR11/19. g, The density of different TE lineages inferred from the detection of their protein-coding domains along pseudomolecules (Adopeted from Kreplak et al., 2019)

9 Future Research Directions

9.1 Emerging technologies and their potential impact on synteny and chromosome research

The advent of advanced cytogenomic techniques such as BAC-FISH and oligopainting has significantly enhanced our understanding of chromosome evolution and synteny in legumes. These technologies have enabled the creation of detailed cytogenetic maps and the identification of chromosomal rearrangements, as demonstrated in studies on *Vigna angularis*, *Vigna unguiculata*, and *Phaseolus vulgaris* (Martins et al., 2021). The use of Hi-C sequencing to achieve chromosome-length genome assemblies, as seen in *Medicago truncatula*, further underscores the potential of these technologies to refine our understanding of chromosomal architecture and synteny (Kaur et al., 2021). Future research should leverage these and other emerging technologies, such as CRISPR-based genome editing and single-cell sequencing, to explore chromosomal dynamics at unprecedented resolution.

9.2 Prospects for integrated multi-species comparative studies

Comparative genomics across multiple legume species has revealed significant insights into the evolutionary history and syntenic relationships within the family. For instance, the orthology and synteny analysis of receptor-like kinases and proteins across seven legume species has highlighted the conserved nature of these genes and their roles in stress responses (Restrepo-Montoya et al., 2021). Similarly, the reconstruction of ancestral genomes for papilionoid legumes has provided a framework for understanding chromosomal evolution in this subfamily. Future research should aim to integrate data from a broader range of legume species, including under-studied taxa like *Bituminaria bituminosa*, to build a more comprehensive picture of legume genome evolution (Nelson et al., 2020). Such studies will benefit from the development of high-quality reference genomes and the application of phylogenomic approaches to resolve deep evolutionary relationships (Koenen et al., 2019).

9.3 Funding and collaboration opportunities in legume genomic research

The complexity and scale of legume genomic research necessitate substantial funding and collaborative efforts. International consortia and funding bodies should prioritize projects that aim to generate high-quality reference genomes, as these resources are critical for advancing our understanding of legume biology and improving crop traits (Kreplak et al., 2019). Collaborative initiatives that bring together expertise in genomics, bioinformatics, and plant biology will be essential for tackling the challenges of reconstructing ancestral genomes and elucidating the mechanisms of chromosomal evolution (Ren et al., 2019). Additionally, partnerships with agricultural stakeholders can help translate genomic discoveries into practical applications, such as the development of stress-resistant legume varieties (Restrepo-Montoya et al., 2021). By fostering a collaborative and well-funded research environment, the legume genomics community can make significant strides in understanding and harnessing the genetic diversity of this important plant family.

10 Concluding Remarks

The study of syntenic relationships and chromosomal evolution in legumes has provided significant insights into the genomic architecture and evolutionary history of this plant family. A high proportion of receptor-like kinases (RLK) and receptor-like proteins (RLP) in legumes are part of orthologous clusters, with 66%~90% of RLKs and 83%~88% of RLPs falling into this category. These syntenic blocks are highly conserved among legume species, indicating a dynamic yet conserved evolutionary pattern. Comparative cytogenetic mapping has revealed that Vigna angularis shares a high degree of macrosynteny with Vigna unguiculata and Phaseolus vulgaris, with chromosomal rearrangements such as translocations and inversions, particularly on chromosomes 2 and 3, serving as hotspots for changes. The genome of narrow-leafed lupin has experienced a whole-genome triplication event about 20~30 million years ago, followed by multiple chromosomal rearrangements, yet still shows substantial genomic synteny with other legumes. *Cercis*, lacking evidence of polyploidy, serves as a model for early legume genome evolution, retaining a small genome with a slow mutation rate, suggesting an ancestral state. Phylogenomic analyses have resolved deep divergences in legume phylogeny, showing a near-simultaneous origin of all six subfamilies, challenging traditional views of their evolutionary relationships. Multiple whole-genome duplication events, particularly around the Cretaceous-Paleogene boundary, have likely driven the rapid diversification and evolutionary success of legumes.

This study advances plant genetics and breeding by identifying conserved syntenic blocks and orthologous gene clusters, providing valuable genomic markers for improving stress resistance and other traits in legumes. It offers a framework for understanding the genetic basis of important traits and their evolutionary history, aiding breeding strategies. Detailed genomic analyses of model legumes like Medicago truncatula and narrow-leafed lupin support functional genomics studies, enabling gene identification and manipulation in key physiological processes.

To further advance legume genomic research, the following strategies are recommended: expanding comparative genomic studies to include a broader range of legume species, particularly those with unique evolutionary histories or agronomic importance; integrating multi-omics approaches to gain a comprehensive understanding of gene function and regulation; prioritizing research on understudied legume species to uncover novel genetic and evolutionary insights; leveraging advanced genomic technologies to refine genome assemblies and improve the resolution of syntenic and chromosomal analyses; and promoting collaborative research efforts across institutions and countries to share resources, data, and expertise. Implementing these recommendations will help the legume research community continue to make significant strides in understanding and harnessing the genetic potential of this diverse and agriculturally important plant family.

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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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