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Pan-Genomics and CRISPR genome editing for silk biosynthesis improvement in *Bombyx mori*: A Review

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Abstract

Sericulture based on the mulberry silkworm *Bombyx mori* is an important agro-industry, but improvement of silk yield and quality is often limited by genetic and environmental constraints. Recent pan-genomic studies have revealed extensive genetic variation, novel genes, and regulatory elements associated with silk biosynthesis and cocoon traits. These genomic resources provide deeper insight into the molecular basis of fibroin and sericin production. The emergence of CRISPR-based genome editing enables precise modification and functional validation of genes involved in silk production. Integrating pan-genomics with CRISPR technologies offers new possibilities for improving silk yield, fibre quality, and stress tolerance. Such approaches are expected to support precision breeding and sustainable advancement of modern sericulture.

Keywords: Silkworm genomics, genome editing, silk gland biology, structural genomic variation, precision sericulture

Introduction

Sericulture, the agricultural practice centered on the mulberry silkworm, *Bombyx mori*, has been a globally significant industry for millennia, playing a critical role in textile production and supporting numerous livelihoods, particularly across Asia (Bora *et al.*, 2025) [3]. Silk, often referred to as "The Queen of Textiles," remains a highly sought-after natural fibre due to its exceptional quality, durability, and aesthetic appeal, factors that make its yield and intrinsic properties economically critical (Pavithra *et al.*, 2024; Sree *et al.*, 2024) [27]. The global sericulture industry, particularly in China, is valued at tens of billions of CNY annually, highlighting its substantial economic impact (Lu *et al.*, 2026) [36].

Traditionally, enhancing silk yield and quality has relied heavily on conventional breeding methods. However, these methods face considerable limitations, including susceptibility to climate variability, outbreaks of pathogens, and inherent genetic constraints within domesticated silkworm strains (Bora *et al.*, 2025) [3]. For instance, silkworm health is frequently compromised by a range of pathogens, such as viruses, bacteria, fungi, and protozoa, which can lead to significant productivity losses. The *Bombyx mori* nucleopolyhedrovirus (BmNPV) alone is a major concern, often accounting for more than 60% of total losses from silkworm diseases (Kaushik *et al.*, 2026) [13]. Furthermore, environmental stressors like temperature fluctuations and pesticide exposure can directly impact cocoon production and silk quality, posing ongoing challenges to the industry (Lu *et al.*, 2026; Kaushik *et al.*, 2026) [13]. The laborious task of separating male and female pupae for egg production in grainage also highlights a practical limitation in conventional sericulture, especially given the typically lower fecundity of sex-limited breeds (Kasa *et al.*, 2023) [12].

The emergence of advanced molecular biology tools, specifically pan-genomics and genome editing technologies like CRISPR-Cas systems, has revolutionized silkworm research, offering unprecedented opportunities for genetic improvement and sustainable silk production (Bora *et al.*, 2025; Kalpana *et al.*, 2024) [3, 35]. *Bombyx mori* is a critical lepidopteran model organism, not only for sericulture research but also for broader biological studies, owing to its well-characterized biology and significant economic value (Lu *et al.*, 2023; Tong *et al.*, 2023) [43]. Its long history of domestication has resulted in extreme domestication bottlenecks, leading to substantial structural and presence-absence variation (PAV) within its populations (Tong *et al.*, 2023) [43]. This considerable genetic diversity

necessitates pan-genomic approaches to comprehensively capture the species genomic repertoire, moving beyond single reference genomes to understand the full spectrum of genetic variation across different strains (Lu *et al.*, 2023; Tong *et al.*, 2022)^[43, 22]. Recent efforts have led to the assembly of high-resolution pan-genomes for *B. mori* using long-read sequencing technologies, which have revealed thousands of novel genes and regulatory elements previously absent from earlier reference genomes (Tong *et al.*, 2022)^[43]. For example, the SilkMeta platform provides a comprehensive database integrating pan-genomic and multi-omic data from 1082 short-read and 546 long-read assembled genomes, serving as a valuable resource for genotype-phenotype association studies (Lu *et al.*, 2023)^[22]. This extensive genomic information, combined with the precision of CRISPR-Cas9 genome editing, facilitates targeted modifications to key genes involved in silk biosynthesis and other economically important traits (Bora *et al.*, 2025; Pavithra *et al.*, 2024)^[3, 27]. For instance, the *BmSur8* gene, which encodes a scaffold protein, has been identified as a positive regulator of the RAS-MAPK signaling cascade, significantly influencing cocoon silk production (Zhou *et al.*, 2025)^[53]. The application of nutrient supplements, such as spermidine, has also shown promise in improving fecundity and economic traits in bivoltine cocoon color sex-limited breeds, addressing previous limitations in these strains (Sree *et al.*, 2024; Kasa *et al.*, 2023)^[12, 36].

CRISPR-Cas systems represent a powerful genome editing technology that enables precise alterations to the genetic code of living organisms (Bora *et al.*, 2025)^[3]. In *Bombyx mori*, this technology is being applied to enhance various aspects of sericulture, including improving silk yield and quality, increasing disease resistance, and facilitating sex control for optimized production (Bora *et al.*, 2025; Pavithra *et al.*, 2024)^[3, 27]. The integration of these biotechnological advancements, ranging from comprehensive genomic understanding to precise genetic engineering, signifies a paradigm shift towards functionally guided engineering of silk traits, offering new hope for addressing the persistent challenges faced by the sericulture industry (Bora *et al.*, 2025; Kalpana *et al.*, 2024)^[3, 35].

1. Silk biosynthesis in *Bombyx mori*: Silk biosynthesis in *Bombyx mori* occurs in the larval silk gland, a specialized organ responsible for producing silk proteins during the final larval stage (Xia *et al.*, 2004). The major structural component of silk, fibroin, is synthesized in the posterior silk gland and consists mainly of fibroin heavy chain, light chain, and P25 proteins that form a stable complex (Inoue *et al.*, 2000)^[11]. Another important protein, sericin, is produced in the middle silk gland and acts as a binding material that coats fibroin fibers during cocoon formation (Kawasaki *et al.*, 2007). The coordinated expression of silk protein genes and their secretion through the silk gland enable the formation of strong and continuous silk filaments used to construct the cocoon (Xia *et al.*, 2004)^[44].

Table 1: Important genes involved in silk production (Inoue *et al.*, 2000; Zhao *et al.*, 2019; Zhou *et al.*, 2025)^[11, 52, 53].

Gene	Function	Role in Silk Production
Fib-H	Fibroin heavy chain	Major structural component of silk fibre
Fib-L	Fibroin light chain	Stabilizes fibroin complex
P25	Glycoprotein	Maintains stability of fibroin complex
BmVps13d	Cellular metabolism regulator	Influences silk gland development
BmSur8	Signalling protein	Regulates silk yield through MAPK pathway

2. Pan-Genomics of *Bombyx mori*

Pan-genome studies in silkworm are redefining how silk yield and quality genes are discovered, especially by revealing structural variation and dispensable genes missed

by single references. These genomic resources now directly connect to CRISPR-based functional validation and breeding for improved silk production.

Table 2: Major omics approaches used in silkworm research (Xia *et al.*, 2024; Tong *et al.*, 2022; Sun *et al.*, 2023)^[38, 42, 44]

Omics Approach	Purpose	Application in Silkworm Research
Genomics	Study of entire genome	Identification of genes controlling silk production
Transcriptomics	Analysis of gene expression	Understanding silk gland gene regulation
Proteomics	Study of protein expression	Identification of fibroin and sericin proteins
Metabolomics	Study of metabolic pathways	Understanding nutrient utilization during silk synthesis
Pan-genomics	Comparative analysis of multiple genomes	Identification of structural variations affecting silk traits

2.1 Concept of Pan-Genome in insects

In insects, the pan-genome includes all genes present across a species, partitioned into core genes (present in almost all individuals) and dispensable/variable genes (present in subsets, often linked to adaptation) (Tong *et al.* 2022; li *et al.*, 2024; Lu *et al.*, 2025; Tong *et al.*, 2021)^[42]. In *B. mori*, long-read assemblies of 545 genomes identified 7,308 new gene families, of which 4,260 (22%) are core, alongside ~3.4 million nonredundant structural variants (SVs), including insertions, deletions, CNVs and presence/absence variants (PAVs) (Tong *et al.*, 2021; Tong *et al.*, 2022)^[42]. Comparable insect pan-genomes (*Drosophila*, butterflies, *Apis cerana*) show that SV-rich dispensable genomes

strongly affect ecological adaptation and complex traits (Tong *et al.*, 2023; li *et al.*, 2024; Tong *et al.*, 2021)^[43].

2.2 Comparative genomics of wild vs domesticated silkworm

Population-scale genomics show strong genetic bottlenecks during domestication and subsequent improvement, with commercial lines having reduced genetic diversity compared with wild *B. mandarina* and local breeds (Tong *et al.*, 2022; Tong *et al.*, 2021; Fang *et al.*, 2020)^[7]. Pan-genome scans identify hundreds of genes and SVs associated with domestication and breeding, including loci affecting behaviour, diapause, coloration, and silk traits

(Tong *et al.*, 2022; Tong *et al.*, 2021)^[42]. QTL mapping in crosses between *B. mori* and *B. mandarina* detected 11 cocoon yield QTLs, including one yield-enhancing allele contributed by the wild species and candidate genes (e.g., KWMTBOMO04917, KWMTBOMO12906) expressed in silk gland (Fang *et al.*, 2020)^[7].

2.3 Structural variations influencing silk traits

Structural variations (SVs) are pivotal drivers of phenotypic diversity in *Bombyx mori*, profoundly influencing silk traits through mechanisms such as copy number variations (CNVs), presence/absence variations (PAVs), and gene duplications within critical fibroin gene clusters (Tong *et al.*, 2022; Fang *et al.*, 2020)^[7]. CNVs, which involve gains or losses of DNA segments, alter gene dosage and expression, thereby affecting the quantity of silk protein produced (Luan *et al.*, 2021)^[25]. Similarly, PAVs represent the complete presence or absence of genes or genomic regions, contributing unique physiological or biochemical pathways that directly impact silk quality and quantity across different silkworm strains (Tong *et al.*, 2022)^[42]. Gene duplications, particularly within the Fibroin Heavy (Fib-H), Fibroin Light (Fib-L), and P25 clusters, are directly correlated with increased silk yield and enhanced Fiber properties by augmenting the templates available for silk protein synthesis (Luan *et al.*, 2021)^[25]. The comprehensive functional annotation of novel pan-genes, identified through high-resolution pan-genomic studies, further elucidates the genetic architecture underlying silk production (Tong *et al.*, 2022)^[42]. Many of these pan genes are associated with traits targeted during artificial selection, particularly silk yield. Candidate genes such as *Vps13d*, which plays an important role in silk production, along with other genes involved in protein secretion, chaperone assisted protein folding, and transcriptional regulation of silk gland development, contribute significantly to variations in silk productivity. (Luan *et al.*, 2021)^[25]. Beyond protein-coding genes, the diversity in non-coding regulatory sequences, including promoters, enhancers, and microRNAs like *let-7*, plays a crucial role in fine-tuning gene expression, thus modulating the timing, location, and magnitude of gene activity that ultimately impacts silk production efficiency (Tong *et al* 2022; li *et al.*, 2024)^[42].

2.4 Functional Annotation of Novel Pan-Genes

Pan-genomic analysis in *Bombyx mori* has identified over 7,000 novel genes and millions of structural variants, many under strong artificial selection for high silk yield and improved fibre fineness, thereby defining a rich pool of silk-associated pan-genes (Tong *et al.*, 2022)^[42]. Multi-omics and SNP-chip studies further refine this space, linking silk

gland size, metabolism and cocoon traits to specific genes such as BmOCIAD1, BmCOPB2, Vps13d, BmSur8 and others, whose CRISPR perturbation substantially alters cocoon shell weight and silk output (Zhou *et al.*, 2025; Sun *et al.*, 2023; Li *et al.*, 2025; Zhou *et al.*, 2022; Luan *et al.*, 2018)^[26, 38, 53]. At the regulatory level, genome-wide maps of open chromatin and transcription factor (TF) binding in the silk gland identify tens of thousands of OCRs and hundreds of TF motifs, including CORE clusters that synergistically control silk-protein loci (Zhang *et al.*, 2018; Zhang *et al.*, 2019). Spatial Hi-C and FAIRE-seq analyses in middle and posterior silk gland compartments reveal dynamic topologically associating domains and region-specific TF networks (for example POU-M2) that underlie sharp spatial differences in silk gene expression and quality (Zhang *et al.*, 2023; Zhang *et al.*, 2018). Together, these functional annotations of novel pan-genes and cis-regulatory landscapes provide a mechanistic blueprint for CRISPR-mediated rewiring of silk biosynthesis, enabling targeted editing of both coding and regulatory variants to boost yield while tuning Fiber properties in *B. mori* (Tong *et al.*, 2022; Zhang *et al.*, 2023; Sun *et al.*, 2023; Zhang *et al.*, 2019; Li *et al.*, 2025; Luan *et al.*, 2018)^[26, 38, 42, 48-51].

3 CRISPR-Based genome engineering in Bombyx mori

Pan-genome and SNP-array resources in *Bombyx mori* are now systematically annotated using silk-gland transcriptomes, QTL mapping, and association analyses to define candidate pan-genes for silk yield and quality (Kawamoto *et al.*, 2019; Lu *et al.*, 2021)^[15]. Key yield-related loci include genes detected in cocoon yield QTLs and high-density SNP-array studies, for which CRISPR/Cas9 knockouts have validated causal roles in cocoon shell ratio and silk output (Zhang *et al.*, 2018; Li *et al.*, 2022). Functional studies highlight regulators such as BmVps13d and signalling components whose disruption reduces silk yield by altering energy and material metabolism in the silk gland (Zhao *et al.*, 2019)^[52]. Beyond structural silk-protein loci, genome editing of regulatory network genes like the circadian regulator Clock (Clk) has increased silk production, illustrating that systemic regulators outside fibroin clusters can be engineered for productivity gains (Xu *et al.*, 2020)^[45]. Regulatory sequence diversity is being captured with ATAC-seq and small-RNA profiling in *B. mori* and its wild ancestor *B. mandarina*, providing maps of open chromatin and small-RNA-regulated regions that guide precise CRISPR-mediated editing of promoters and enhancers for silk biosynthesis rewiring (Su *et al.*, 2021).

Table 3: Applications of CRISPR genome editing in silkworm research (Ma *et al.*, 2017; Xu *et al.*, 2020; Taning *et al.*, 2017)^[41, 45, 30]

Application	Description	Outcome
Gene knockout	Removal of target gene	Identification of silk-related gene functions
Gene insertion	Introduction of foreign gene	Production of modified silk proteins
Gene regulation	Editing regulatory regions	Improved silk production
Disease resistance	Editing immune genes	Increased resistance to silkworm pathogens

Table 3.1: CRISPR-edited genes affecting silk production

Gene	Function	Editing Outcome	Reference
BmVps13d	Silk gland metabolism	Reduced silk yield when disrupted	Zhao <i>et al.</i> , 2019 ^[52]
BmSur8	RAS-MAPK signaling pathway	Influences cocoon silk production	Zhou <i>et al.</i> , 2025 ^[53]
Clock (Clk)	Circadian rhythm regulator	Increased silk production when edited	Xu <i>et al.</i> , 2020 ^[45]
BmPDI	Protein folding enzyme	Affects mechanical strength of silk	Yang <i>et al.</i> , 2025 ^[46]

4. Rewiring silk biosynthetic pathways

Rewiring silk biosynthetic pathways in *Bombyx mori* will rely first on gene regulatory network mapping, where large-scale RNA-seq and single-cell datasets are used to construct co-expression networks and identify transcriptional hubs and spatially restricted regulators that coordinate silk protein gene expression in distinct silk gland regions (Masuoka *et al.*, 2022; Ma *et al.*, 2022; Zhang *et al.*, 2023) [32]. These empirical networks can then be integrated into systems biology models, including pathway reconstruction for nitrogen and amino acid mobilization and genome-scale representations of silk gland metabolism, enabling in silico perturbation and metabolic flux analysis to predict how changes in key enzymes or transporters alter fibroin/sericin synthesis and gland physiology (Yi *et al.*, 2025; Pio *et al.*, 2021; Grimes *et al.*, 2019) [8, 34]. Building on these quantitative frameworks, synthetic biology approaches can rationally reprogram silk output and material properties by CRISPR-mediated editing of regulators such as *BmPDI*, *BmSur8*, and other hub factors that affect protein folding, gland growth, and energy metabolism, and by introducing recombinant or modified silk proteins whose post-translational processing and amino-acid composition are optimized for enhanced mechanical performance or new functionalities (Yang *et al.*, 2025; Zhou *et al.*, 2025; Bashor and Collins, 2018) [2, 46, 53].

5. Integrating Pan-Genomics with CRISPR for Trait Improvement:

High-resolution silkworm pan-genomes now provide a catalogue of genes, structural variants, and domestication sweeps associated with silk yield, fineness, diapause, and other adaptive traits, enabling identification of high-value targets (e.g., *BmChit* β -GlcNAcase, silk-yield and fineness loci) for functional validation and editing (Tong *et al.*, 2022) [42]. These pan-genomic signals, together with QTLs and GWAS hits discovered using tools such as the Silk_40K SNP array, prioritize candidate genes like *BmOCIAD1* and *BmCOPB2* that have been experimentally confirmed to increase cocoon shell weight and shell ratio when disrupted by CRISPR (Li *et al.*, 2025). Once targets are defined, precision editing of elite commercial lines using optimized and broadened CRISPR platforms (integrated SpCas9/SaCas9/Cas12a systems, PAM-flexible SpCas9-NG/xCas9 variants, and improved delivery workflows) allows direct introduction of beneficial alleles—such as edits in *BmEcKL1*, *let-7*, or *Clock* that enlarge silk glands, extend growth, or shift physiology to increase silk output without losing complex backgrounds that underpin field performance (Ma *et al.*, 2017; Sun *et al.*, 2024; Li *et al.*, 2024; Brady *et al.*, 2025) [4, 39].

Finally, marker-assisted selection and genomic selection can be tightly integrated with genome editing: pan-genome-anchored SNP chips support genomic prediction of complex traits and tracking of edited alleles across breeding cycles, creating a feedback loop where MAS/GS identify superior haplotypes, CRISPR recreates or refines them in elite germplasm, and subsequent genomic evaluation quantifies gains in silk yield, quality, and resilience (Li *et al.*, 2025; Bora *et al.*, 2025; Ma *et al.*, 2018) [3].

6. Biosafety, Ethical and Regulatory Considerations

Rewiring silk biosynthesis in *Bombyx mori* with pan-genomics and CRISPR demands a rigorous treatment of biosafety, ethics and regulation, beginning with off-target

effects at both the molecular and ecological levels. At the genome-scale, unintended mutations, chromosomal rearrangements, and large on-target indels remain key concerns, requiring careful sgRNA design, in silico off-target prediction, and whole-genome sequencing-based validation to minimize and monitor genomic irregularities in edited silkworm lines (Manghwar *et al.*, 2020; Guo *et al.*, 2023; Kawall *et al.*, 2020) [9, 14, 31]. Beyond individual animals, ecological risk assessment must consider accidental escape of edited strains and, in future, gene-drive-based sericulture strategies, where self-propagating constructs might spread into wild *Bombyx* relatives, alter food webs, or affect non-target species through horizontal transfer or trophic interactions (Devos *et al.*, 2021; Devos *et al.*, 2021; Hoepers *et al.*, 2024; Komal *et al.*, 2023; Kumari *et al.*, 2024) [10, 16, 17]. Lessons from gene-drive work in mosquitoes and conceptual models for pest control emphasize phased testing, confinement, robust problem formulation, and pre-release modelling combined with post-release monitoring to manage uncertainty and avoid irreversible ecological impacts (Tajudeen *et al.*, 2023; Aslam *et al.*, 2025; Naegeli *et al.*, 2020; Devos *et al.*, 2021) [1, 40].

Finally, regulatory frameworks for edited insects are still evolving: in most jurisdictions, CRISPR-modified insects (with or without transgenes) fall under GMO or “new breeding technique” rules, but current guidance largely developed for plants or conventional GM animals has been judged adequate yet insufficient for insects containing engineered gene drives, prompting calls in the EU and elsewhere for expanded molecular characterization requirements, broader environmental risk assessment, and adaptive, stepwise authorization schemes that explicitly integrate ethical analysis, stakeholder engagement, and comparison with alternative pest- and disease-control options (Zhang *et al.*, 2020; Kawall *et al.*, 2020; Naegeli *et al.*, 2020; Devos *et al.*, 2021; Taning *et al.*, 2017) [14, 41].

7. Future Perspectives

Future research on pan-genomics and CRISPR-mediated rewiring of silk biosynthesis in *Bombyx mori* will focus on identifying novel alleles and regulatory genes associated with silk production and environmental adaptability. Integration of these genomic insights with AI-guided genome design may help predict key targets for precise genome editing. CRISPR technologies could also facilitate the development of climate-resilient silkworm strains capable of maintaining productivity under changing environmental conditions. In addition, digital sericulture platforms supported by data analytics and smart monitoring systems may link genomic innovations with improved rearing and management practices.

9. Conclusions

The integration of pan-genomics and CRISPR-mediated genome engineering is transforming research and innovation in *Bombyx mori*. Pan-genomic studies have revealed extensive structural variation, novel genes, and regulatory elements associated with silk production, providing a comprehensive view of the genetic architecture underlying economically important traits. Coupled with CRISPR-based functional validation, these genomic resources enable precise modification of key genes and regulatory networks controlling silk biosynthesis, cocoon yield, and fibre quality. Advances in systems biology and synthetic biology further

support the rational rewiring of metabolic and regulatory pathways within the silk gland. Together, these approaches establish a powerful framework for accelerating genetic improvement in silkworms. The continued integration of genomic data, genome editing, and advanced breeding strategies will play a critical role in enhancing silk productivity, resilience, and sustainability, ultimately driving the modernization of the global sericulture industry.

Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Mode (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

Competing interests

Authors have declared that no competing interests exist.

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