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Epigenetic mechanisms and Transgenerational plasticity in *Bombyx mori*: Implications for stress adaptation and sustainable sericulture

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Abstract

Epigenetic regulation has emerged as a pivotal mechanism in shaping phenotypic plasticity and adaptive resilience in insects. In the silkworm *Bombyx mori*, a model organism of both scientific and economic value, DNA methylation, histone modifications, and non-coding RNAs collectively orchestrate development, immunity, and stress responses without altering the underlying genetic sequence. Recent studies demonstrate that environmental stressors, including thermal fluctuations, pathogen invasion, nutritional shifts, and chemical exposures induce dynamic epigenetic reprogramming, fine-tuning gene expression for survival under fluctuating rearing conditions. Increasingly, evidence also points toward transgenerational plasticity, where parental experiences influence offspring physiology through heritable epigenetic signatures mediated by DNA methylation and small RNAs. Such processes hold profound implications for sericulture, enabling the development of stress-resilient, disease-tolerant, and high-yield silkworm strains. This review synthesizes current advances in *Bombyx mori* epigenetics, explores mechanisms of transgenerational inheritance, and highlights their potential applications in sustainable sericulture. By integrating mechanistic insights with applied strategies, epigenetic research in silkworms offers a transformative path toward resilient silk production in the face of climate change and ecological challenges.

Keywords: Epigenetic regulation, DNA methylation, phenotypic plasticity, histone modifications, non-coding RNAs

1. Introduction

In recent years, epigenetics has emerged as a central theme in insect biology, reshaping our understanding of how organisms adapt to fluctuating environments. Epigenetic modifications including DNA methylation, histone modifications, and non-coding RNAs do not alter the underlying genetic code but instead regulate gene expression, often in a reversible and environmentally responsive manner [1, 2]. Such mechanisms provide insects with a flexible toolkit to fine-tune development, immunity, and metabolism in response to stressors. Increasingly, these epigenetic changes are being studied not only as within-generation regulators but also as transgenerational signals, where environmentally induced states are passed on to subsequent generations [3, 4]. This phenomenon of transgenerational epigenetic inheritance is now considered a major driver of phenotypic plasticity in insects, raising important questions about adaptation, resilience, and long-term ecological fitness.

Within this field, the silkworm, *Bombyx mori*, has gained special attention as both an applied and model organism. Beyond its economic role in silk production, *Bombyx mori* offers unique advantages for epigenetic studies: its genome is well-sequenced, molecular tools are available, and it exhibits clear, measurable traits linked to development and productivity. Recent genome-wide methylation studies highlight that *Bombyx mori* responds dynamically to environmental stress. For example, heat and humidity stress reshaped the methylome and altered transcriptional regulation of stress-related genes [5]. Similarly, DNA methylation was shown to suppress chitin degradation and promote normal wing development, underscoring its developmental importance [6]. Epigenetic reprogramming is also evident under disease pressure BmCPV infection induced histone modifications (H3K9me3/H3K9ac), which may influence host defense pathways [7].

Collectively, these studies confirm that epigenetic responses are not peripheral but integral to how silkworms manage environmental and biological challenges.

In parallel, the silkworm germline provides insights into transgenerational pathways, particularly through the piRNA and Argonaute machinery. Key regulators such as Gtsf paralogs have been shown to activate PIWI proteins and coordinate small-RNA mediated silencing [8], while extended Tudor domains in proteins like Vreteno help maintain piRNA protein interactions essential for germline integrity [9]. These findings suggest that *Bombyx mori* harbors well-conserved pathways capable of mediating heritable epigenetic states, even if direct evidence for stable transgenerational inheritance remains limited compared to models like Drosophila. Nonetheless, emerging reports of multigenerational effects of diet and stress in *Bombyx mori* highlight its potential as a system to probe adaptive inheritance under applied conditions [10]. Figure 1 provides a schematic overview of the main epigenetic layers like the DNA methylation, histone modifications, and non-coding RNAs and how they collectively regulate gene expression, stress responses, and potential transgenerational inheritance.

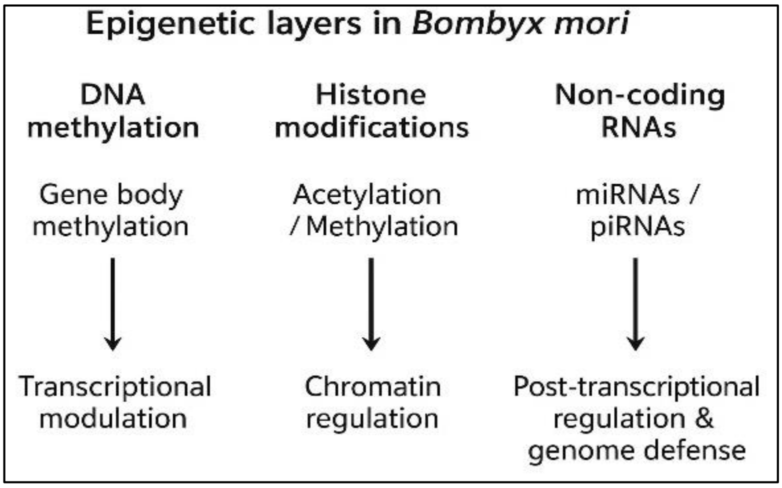

Fig 1: Epigenetic layers in Bombyx mori. DNA methylation, histone modifications, and non-coding RNAs coordinate gene expression and genome stability, providing a framework for development, stress response, and potential transgenerational inheritance.

With rising pressures from climate change, pathogens, and variable mulberry quality, the capacity of silkworms to mount epigenetic stress responses and potentially transmit them across generations has clear implications for sericulture sustainability. This review synthesizes current knowledge on epigenetic regulation in *Bombyx mori*, evaluates evidence for transgenerational plasticity, and explores how these insights could guide breeding, stress-resilient strains, and sustainable silk production. By linking mechanisms to applications, we position *Bombyx mori* as both a model for inheritance studies and a platform for sericultural innovation.

2. Epigenetic Mechanisms in *Bombyx mori*

Epigenetic regulation in *Bombyx mori* involves multiple, interacting layers-DNA methylation, histone modifications, and non-coding RNAs that collectively influence development, immunity, and responses to environmental stressors. Together, these mechanisms provide molecular plasticity that underlies the silkworm's ability to adapt to dynamic rearing conditions.

2.1 DNA methylation

Although *Bombyx mori* exhibits a relatively sparse methylome compared to vertebrates, DNA methylation remains an important regulatory mechanism. Reduced Representation Bisulfite Sequencing (RRBS) and whole-genome analyses reveal that methylation is enriched within gene bodies rather than promoter regions, shaping transcriptional activity [11, 12]. Experimental evidence shows that environmental stressors can modulate these methylation patterns: for instance, thermal stress induces differentially methylated regions that correlate with altered expression of heat shock proteins and metabolic genes [5, 13]. Such dynamic regulation indicates that even limited DNA methylation capacity in *Bombyx mori* contributes significantly to phenotypic plasticity.

2.2 Histone modifications

Chromatin remodeling through histone marks adds a further regulatory dimension. During viral infection with *Bombyx mori* cytoplasmic polyhedrosis virus (BmCPV), genome-wide analyses demonstrate shifts in H3K9 methylation and acetylation. These changes coincide with differential expression of host genes tied to metabolism and immunity, suggesting that histone modifications actively reprogram the transcriptional landscape in response to infection [7, 14]. Similarly, *Bombyx mori* nucleopolyhedrovirus (BmNPV) infection triggers redistribution of the activating histone mark H3K4me3, reinforcing the idea that chromatin is highly responsive to viral entry [15, 16]. Developmental studies further confirm that histone marks act as key modulators during hormone-driven transitions. Treatment of embryonic cells with the steroid hormone ecdysone (20-hydroxyecdysone, 20E) induces dynamic enhancer activity through changes in H3K4me1 and H3K27ac, thereby orchestrating stage-specific gene activation central to metamorphosis [17]. These findings highlight histone modifications as both developmental switches and rapid responders to stress.

2.3 Small RNAs

Non-coding RNAs, particularly microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs), form a critical epigenetic layer in *Bombyx mori* that modulates both short-term responses and longer-term developmental programs.

Genome-wide profiling has identified numerous miRNAs that regulate processes such as immunity, molting, and silk-gland function [18, 19]. Several miRNAs have been functionally linked to antiviral and immune pathways: for example, virus-responsive miRNAs can target viral or host transcripts to alter infection outcomes. Host miRNAs such as bmo-miR-278-3p, which modulates IBP2 expression, and miR-274-3p, which affects BmCPV NS5 levels, have been shown to influence viral replication in *Bombyx mori* [20, 21]. miR-2739, which was shown to regulate vitellogenin receptor expression, exemplifies how miRNAs also coordinate reproductive and developmental timing, linking physiology to gene regulation [5]. Beyond immunity and development, miRNAs participate in broader host physiology: integrated miRNA-microbiome analyses indicate that certain miRNAs respond to gut bacterial changes and metabolic cues, suggesting roles in nutritional adaptation and gut homeostasis [22].

The piRNA pathway complements these post-transcriptional mechanisms by protecting genome integrity in the germline and by regulating non-transposon targets with developmental or sex-determination roles. Molecular dissection of the silkworm piRNA machinery has revealed specialized factors (e.g., Gtsf paralogs, Tudor-domain proteins) that ensure robust piRNA biogenesis and PIWI-mediated target cleavage [8, 9]. Recent mechanistic work on piRNA maturation, such as characterization of the pre-piRNA trimming enzyme underscores how precise processing steps are essential for piRNA stability and function, with direct consequences for gametogenesis and offspring genome protection. Collectively, miRNAs and piRNAs provide versatile routes for rapid response to infection and environmental fluctuation, while also establishing mechanisms that could mediate intergenerational transmission of regulatory states [23, 24, 25].

3. Environmental Stress and Epigenetic Responses in *Bombyx mori*

Bombyx mori, a well-established model organism in entomology, exhibits remarkable physiological and molecular plasticity in response to various environmental stressors, including thermal extremes, pathogen exposure, and nutritional or chemical challenges. These complex adaptive responses are mediated through intricate epigenetic mechanisms, such as DNA methylation, histone modifications, and non-coding RNA regulation, which modulate gene expression without altering the underlying DNA sequence [26, 27, 28]. Such mechanisms allow silkworms to fine-tune gene activity rapidly, enhancing survival under fluctuating environmental conditions.

Figure 2 provides a schematic overview of how specific stressors, such as heat, pathogens, and chemical challenges, shape the epigenetic landscape of *Bombyx mori*. Table 1 further summarizes these environmental stressors, the epigenetic mechanisms they influence, their molecular effects, and the resulting phenotypic outcomes.

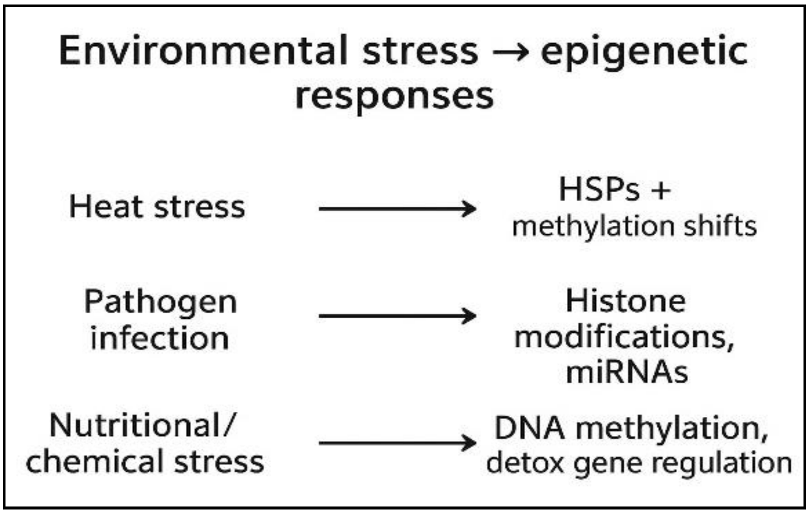

Fig 2: Conceptual diagram illustrating the link between environmental stressors and their epigenetic responses in Bombyx mori. Heat stress induces heat shock proteins (HSPs) and methylation shifts, pathogen infection triggers histone modifications and miRNA activity, while nutritional or chemical stress alters DNA methylation and regulates detoxification genes**.**

3.1 Heat and Climate Factors

Thermal stress exerts profound impacts on the physiological, developmental, and metabolic processes of *Bombyx mori*. Studies have shown that exposure to elevated temperatures triggers robust induction of heat shock proteins (HSPs), which are crucial in maintaining cellular integrity by preventing protein misfolding and stabilizing essential cellular structures [26, 29, 30]. For instance, the overexpression of the Bmsn gene enhances thermotolerance by reducing reactive oxygen species (ROS) accumulation, thereby minimizing oxidative stress-induced cellular damage and mitigating DNA damage in silkworms [31]. Additionally, transcriptomic analyses have revealed that heat stress induces widespread remodeling of gene expression, influencing pathways related to metabolism, stress response, development, and apoptosis, which collectively facilitate organismal adaptation to extreme thermal conditions [32].

Furthermore, epigenetic modifications such as DNA methylation and histone modifications serve as crucial regulators of the silkworm's response to heat stress. A comparative genome-wide DNA methylation analysis revealed dynamic epigenomic reprogramming in response to heat-humidity stress in *Bombyx mori*, indicating that DNA methylation patterns are highly responsive to thermal cues [5, 33]. These modifications can lead to the selective activation or repression of stress-responsive genes, thereby enhancing the organism's capacity to withstand fluctuating environmental temperatures and maintain homeostasis.

3.2 Pathogen Challenges (Viral and Bacterial)

Exposure to pathogens such as viruses and bacteria triggers multilayered immune responses in *Bombyx mori*, frequently involving epigenetic regulation. DNA methylation patterns are altered in response to *Bombyx mori* cypovirus infection, suggesting that methylation-mediated gene regulation is a key component of immune defense [7, 33]. Furthermore, small non-coding RNAs, particularly microRNAs, play critical roles in mediating antiviral responses. Specific microRNAs are upregulated during viral infections to modulate host gene expression, enhancing antiviral defense and preventing the proliferation of pathogens [34, 35].

In addition, histone modifications such as H3K9me3 and H3K9ac have been implicated in the silkworm's immune response to viral infections. Research has demonstrated that changes in gene expression driven by these histone marks are associated with *Bombyx mori* cypovirus (BmCPV) infection, indicating that chromatin remodeling via histone modifications regulates immune-related gene networks during pathogen challenges [7, 33]. This dynamic epigenetic modulation ensures rapid and efficient activation of defense mechanisms, thereby increasing the silkworm's survival under infectious stress.

3.3 Nutritional and Chemical Stressors

Nutritional deficiencies and exposure to environmental chemical pollutants also trigger profound epigenetic responses in *Bombyx mori*. Transcriptomic analyses have shown that exposure to hydrogen sulfide induces substantial changes in gene expression patterns related to detoxification, antioxidant defense, and general stress response pathways [26, 36, 37]. Moreover, studies examining mercury exposure demonstrate that silkworms undergo physiological and molecular adjustments, including altered gene expression profiles, reflecting the activation of detoxification and stress-response mechanisms [36, 38].

In addition to chemical stressors, dietary components significantly influence the epigenetic landscape of *Bombyx mori*. A systematic review of the nutritional composition of mulberry silkworm pupae indicated that variations in diet can lead to epigenetic modifications, resulting in changes in gene expression that affect metabolism, development, and overall physiological state [10, 39]. This evidence underscores that nutritional intake acts as an environmental cue, shaping epigenetic programs that support growth, development, and stress resilience in silkworms.

Overall, these studies highlight that epigenetic mechanisms provide a versatile framework for *Bombyx mori* to respond adaptively to diverse environmental stressors [28]. By integrating DNA methylation, histone modifications, and non-coding RNA regulation, silkworms can fine-tune gene expression in real time, enabling survival and optimal functioning under changing environmental conditions.

4. Transgenerational Plasticity and Adaptation

Transgenerational plasticity describes situations where an environmental experience of parents (or earlier generations) alters the phenotype of descendants without changes to DNA sequence. Figure 3 illustrates the key pathways by which parental stress can induce heritable epigenetic modifications, influencing offspring phenotypes. In insects, this can occur through maternal provisioning, altered hormonal signals, and heritable epigenetic marks (DNA methylation, chromatin states, and small RNAs) that persist into the germline or are re-established during early development [40, 41]. In *Bombyx mori*, growing evidence indicates that dietary inputs, thermal stress, and pathogen exposure can leave heritable epigenetic signatures mediated through mechanisms such as small RNAs and DNA methylation that contribute to adaptive phenotypes in subsequent generations. Table 2 highlights examples of transgenerational epigenetic inheritance in *Bombyx mori*, illustrating how parental exposure to environmental stressors can induce heritable epigenetic changes that influence offspring phenotypes.

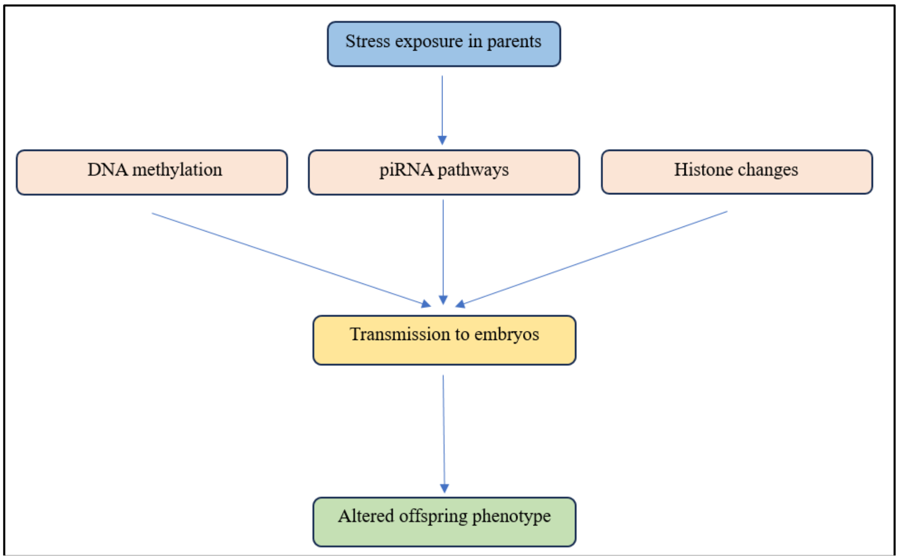

Fig 3: Transgenerational Epigenetic Inheritance in Bombyx mori. Parental stress induces germline epigenetic changes that alter offspring phenotypes.

4.1 Evidence of heritable epigenetic marks in *Bombyx mori*

Direct demonstrations of molecular changes in the germline that could mediate inheritance in *Bombyx mori* are emerging. Several studies document environment-associated changes in DNA methylation and small-RNA populations in parental tissues that plausibly influence offspring. For example, genome-scale methylome analyses show that thermal and pathogenic stresses induce differential methylation in somatic tissues and in eggs/early embryos, with correlated shifts in gene expression relevant to stress physiology [5, 42]. Mechanistic dissection of the piRNA pathway in silkworms has identified specialized germline factors (Gtsf paralogs, Tudor proteins) that ensure robust piRNA production and PIWI-mediated target cleavage molecular machinery that in other taxa is known to transmit regulatory states through gametes [8, 9]. Together, these observations establish plausible molecular routes, altered methylation landscapes, reprogrammed histone marks, and modified small-RNA repertoires, through which parental experiences could affect progeny phenotypes in *Bombyx mori*.

Although direct demonstrations of transgenerational inheritance in *Bombyx mori* remain limited, accumulating evidence from insect epigenetics strongly suggests that similar mechanisms operate in silkworms. Studies in other lepidopterans and model insects have shown that environmentally induced epigenetic modifications, such as DNA methylation, histone remodeling, and small RNA dynamics, can be transmitted across generations and shape adaptive traits [40, 41, 43]. In *Bombyx mori*, recent observations linking parental stress exposure to altered offspring performance, such as changes in immunity, fecundity, and stress tolerance support the plausibility of epigenetically mediated inheritance [1].

4.2 Multigenerational effects and resilience traits

Emerging studies in *Bombyx mori* indicate that epigenetically mediated changes can persist across generations, contributing to adaptive resilience in diverse contexts. For instance, dietary interventions have been shown to influence offspring physiology, where moderate dietary restriction not only enhances antioxidant activity but also modulates the expression of stress-response and longevity-associated genes in subsequent generations [10]. Similarly, long-term nutritional manipulations and selection experiments demonstrate that altered rearing diets can induce heritable changes in silk gland metabolism and gene expression, resulting in shifts in cocoon quality and silk yield [44, 45].

Beyond nutrition, environmental stressors such as pesticides and heavy metals have been reported to affect reproductive tissues and germline integrity, with measurable consequences for offspring development and fitness [46]. These findings collectively suggest that epigenetic inheritance in silkworms plays a role in shaping traits of both ecological and economic importance ranging from stress tolerance and disease resistance to silk production efficiency [47]. While mechanistic details remain under active investigation, the accumulating evidence highlights transgenerational plasticity as a significant contributor to resilience in sericulture systems.

5. Applications of Epigenetic Insights in Sericulture

Recent epigenetic discoveries in *Bombyx mori* are paving the way for practical applications that could transform sericulture through improved stress tolerance, disease resistance, and silk yield. One promising avenue lies in the identification of epigenetic biomarkers. For instance, DNA methylation profiles have been linked to stress resilience. Heat-humidity stress induces specific methylation changes in key stress-related genes, suggesting the potential for methylation-based biomarkers to select heat-resilient silkworm strains [5, 48].

Similarly, histone modification signatures show promise as early indicators of infection. During *Bombyx mori* cytoplasmic polyhedrosis virus (BmCPV) challenge, shifts in H3K9 methylation and acetylation correlate with altered immune gene expression. This finding proposes that chromatin marks could serve as pre-symptomatic markers of disease susceptibility [7]. Small RNAs, particularly miRNAs, also show diagnostic potential. Non-coding RNA profiling during viral infections uncovers miRNAs associated with antiviral defense, offering a minimally invasive indicator for pathogen exposure or resistance [35].

Beyond diagnostics, epigenetic-informed breeding presents another major application. Sustained dietary regimes influence fibroin gene expression across generations, linking epigenetic regulation to silk protein synthesis and cocoon quality. This highlights the possibility of using epigenomic guidance in breeding programs [45]. Likewise, CRISPR-based functional studies are identifying epigenetic regulators such as BmEcKL1, which plays a central role in silk-gland development. Such findings pave the way for the targeted enhancement of silk traits through epigenome editing [31, 49].

Integrating epigenetic insights with conventional resilience strategies provides a holistic opportunity for silk production. Combining molecular markers with established breeding and husbandry practices, such as dietary optimization and infection preconditioning could enable the development of adaptive, environment-aware production systems [48, 50]. This integrative approach holds promise for building a more resilient, climate-adapted sericulture sector capable of withstanding environmental and pathogenic stresses.

6. Future Prospects and Research Directions

Future research in *Bombyx mori* epigenetics must evolve beyond correlation to establish causality and heritability**.** While DNA methylation, histone modifications, and small RNAs are implicated in resilience and silk traits, validating causal relationships requires multi-generational studies (F2/F3**)** with germline sampling and molecular assays to confirm enduring transgenerational effects rather than temporary parental carry-over [41]. Additionally, life-stage and tissue-specific profiling are vital epigenetic marks like ecdysone-response enhancers (e.g., H3K4me1, H3K27ac) vary across developmental stages and tissues such as silk glands or ovaries. High-resolution temporal and spatial sampling will be essential to understand context-specific regulation [15, 17].

Technological advances also promise to accelerate progress. High-throughput, precision epigenomic techniques, such as CUT & Tag for histone marks, targeted bisulfite panels, and small-RNA sequencing can streamline identification and validation of candidate biomarkers for breeding [51]. Functional validation through CRISPR-based epigenome editing offers powerful causal tests: fusing deactivated Cas9 (dCas9) to epigenetic “writers” or “erasers” enables direct manipulation of candidate marks to assess effects on silk production, immune response, or stress tolerance [49, 52]. Looking ahead, integrating multi-omic data, like methylome, transcriptome, small RNAs with environmental and microbiome information can enable predictive, adaptive frameworks for precision sericulture management [5, 35, 42].

7. Conclusion

Epigenetic mechanisms in *Bombyx mori* represent a versatile and dynamic framework through which silkworms adapt to diverse environmental and biological stressors. DNA methylation, histone modifications, and non-coding RNAs not only regulate within-generation responses but also provide molecular pathways for potential inheritance of adaptive traits across generations. Evidence of transgenerational plasticity shaped by diet, thermal stress, pathogen exposure, and pollutants underscores the role of epigenetics in enhancing resilience traits critical for sericulture. While mechanistic understanding of stable inheritance remains incomplete, emerging tools such as high-throughput sequencing, multi-omic integration, and CRISPR-based epigenome editing are set to unravel causal relationships and enable targeted manipulation of epigenetic states. Importantly, translating these insights into practical applications ranging from epigenetic biomarkers for stress and disease resistance to guided breeding of superior strains can significantly strengthen sericulture sustainability. By bridging fundamental epigenetic research with applied innovation, *Bombyx mori* can serve as both a model for inheritance biology and a cornerstone for building climate-resilient, economically robust silk production systems.

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