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Environmental sensing, circadian regulation, and neuroendocrine programming of embryonic diapause in the mulberry silkworm *Bombyx mori* (Lepidoptera: Bombycidae): An integrative review

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Abstract
Embryonic diapause in the domesticated mulberry silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), represents a remarkable example of environmentally regulated developmental plasticity and transgenerational biological programming. Unlike many insect diapause systems in which environmental cues are directly perceived by the dormant organism, diapause in *B. mori* is maternally determined, whereby photoperiodic and thermal information experienced by the mother is transmitted through physiological pathways that ultimately program the developmental fate of the progeny embryo. This review provides an integrative synthesis of the current understanding of the regulatory framework underlying embryonic diapause induction in *B. mori*, with particular emphasis on the interactions among environmental perception, circadian timekeeping, and neuroendocrine regulation. The article examines the genetic and physiological foundations of diapause competence, including voltinism-associated variation, major diapause-related loci, and the contribution of circadian clock genes to seasonal adaptation. The mechanisms through which photoperiod, temperature, and nutritional status are perceived via photoreceptive and thermosensory pathways—including carotenoid-dependent photoreception and BmTRPA1-mediated thermal signalling—are discussed in relation to their downstream effects on hormonal regulation. Emphasis is given to the circadian clock network and its functional integration with GABAergic signalling and diapause hormone (DH) secretion from the suboesophageal ganglion, which together constitute the key neuroendocrine pathway governing maternal diapause determination. Recent advances in genome editing, pan-genomic analysis, transcriptomics, and molecular signalling approaches have substantially reshaped our understanding of this complex regulatory system. These studies reveal that diapause is not merely the consequence of a single hormonal trigger but rather an emergent phenotype resulting from the coordinated integration and processing of environmental information across multiple physiological and molecular networks. By integrating classical physiological discoveries with contemporary molecular insights, this review highlights *B. mori* as a powerful model for understanding seasonal developmental regulation and establishes a conceptual framework for future studies aimed at manipulating diapause responses for sustainable and climate-resilient sericulture.

Keywords: Insect diapause, *Bombyx mori,* diapause hormone, photoperiodism, BmTRPA1, Boceropsin, maternal effect

Introduction

Diapause is a genetically programmed, hormonally regulated state of developmental arrest and metabolic suppression that enables insects to synchronise their life cycle with favourable environmental conditions [1-6]. It is a critical adaptive strategy that enhances survival during adverse climatic periods such as extreme temperatures, drought, or scarcity of food resources. Diapause is a pre-programmed process initiated in anticipation of adverse conditions, triggered by environmental cues—most commonly photoperiod or temperature—that reliably predict the approaching unfavourable season [1-6]. It is observed across virtually all major insect orders and can occur at any developmental stage—embryonic, larval, pupal, or adult—though each insect species is typically committed to diapause at only one stage [1-6]. The process involves profound physiological, biochemical, and molecular changes that minimise metabolic activity and preserve energy reserves until suitable environmental conditions for growth and reproduction return [1-6].

Diapause is now considered as a central driver of insect evolutionary success as it has enabled insects to colonise virtually every terrestrial and freshwater habitat on Earth. Understanding the mechanisms underlying diapause is therefore of considerable interest, both for basic insect physiology and for its practical applications in agriculture, pest management, sericulture, and pollinator conservation [1-6].

Among the many insect species that undergo diapause, the domesticated silkworm, *Bombyx mori* Linnaeus, 1758 (Lepidoptera: Bombycidae), occupies a uniquely privileged position—as both a major economic species and an important model system [7]. In *B. mori,* diapause occurs at the embryonic stage and serves as a natural mechanism to prevent hatching of eggs during unfavorable seasons. The diapause trait is maternally controlled and is induced by the neuroendocrine system of the mother silk moth in response to environmental cues such as temperature and photoperiod [5, 7-8]. In the 1950s, scientists identified the suboesophageal ganglion (SOG) of *B. mori* as the source of a protein, later purified and termed diapause hormone (DH), whose release by the maternal nervous system programmes the eggs for developmental arrest. These findings, accumulated over decades of careful physiological investigation, established *B. mori* as the definitive model for maternal-effect embryonic diapause and laid the conceptual groundwork for all subsequent molecular research [5, 7-8].

Sericulture—the rearing of silkworms for silk production—is one of humanity's oldest continuous agricultural practices, with origins in China dating back at least 5,000 years. *B. mori* is widely regarded as the most thoroughly domesticated insect species, having lost, through thousands of years of artificial selection, the capacity for sustained flight, functional cryptic colouration, and survival in the absence of human-managed mulberry (*Morus* spp.) cultivation [7-10]. This extraordinarily long history of domestication has produced thousands of genetically characterized strains with diverse voltinism (the number of generations completed per year), pigmentation, and diapause phenotypes, constituting a natural genetic resource of exceptional breadth [7-10]. The economic importance of the species is considerable: the global silk market was valued approximately 17-21 billion USD in 2024, with China and India collectively accounting for over 90% of world raw silk output. India, the world's second-largest silk producer, produced more than 38,000 metric tonnes of raw silk in 2023-24. The sector sustains the livelihoods of over 7 million people in rural and semi-urban areas, providing employment particularly to women and small-scale farmers and thereby contributing substantially to rural development and socioeconomic upliftment [11-14]. The diapause trait occupies a central position in sericulture production calendars: it determines how many silkworm cohorts can be raised per year and when disease-free seed stock is available, giving diapause research direct translational relevance. Artificial manipulation of diapause, through hormonal treatments, temperature control, or genetic approaches, enables year-round silkworm rearing and improves the efficiency and predictability of cocoon production [7-10].

Beyond its economic importance, *B. mori* offers a suite of biological advantages that make it an exceptional experimental model. Its complete genome was first drafted in 2004 and subsequently refined by the International Silkworm Genome Consortium in 2008, revealing approximately 432 Mb distributed across 28 chromosomes (2n = 56) encoding some 14,600-18,250 protein-coding genes. The assembly has since been resolved to chromosome scale and, most recently, to a T2T configuration, alongside a high-resolution pan-genome constructed from more than 500 wild and domesticated strains [15-19]. Most strikingly for diapause research, *B. mori* exhibits a maternal-effect embryonic diapause. It is the mother's photoperiodic and thermal experience during her own embryonic and early larval stages that determines whether her eggs will enter diapause, a transgenerational programming phenomenon mediated by DH released from the maternal SOG [5, 7-8]. This developmental dissociation - between the organism that perceives the environmental signal and the organism that executes the diapause programme – is experimentally advantageous. It makes *B. mori* an ideal system for dissecting each step in the complete chain of events from environmental cue perception through neuroendocrine transduction to metabolic arrest, independently and in defined sequence.

The DH of *B. mori* is a 24-amino acid, C-terminally amidated neuropeptide of the FXPRLamide family. Notably, it is co-encoded on the same precursor transcript as pheromone biosynthesis-activating neuropeptide (PBAN), a remarkable arrangement in which two functionally distinct neuropeptides governing reproduction and diapause share a common gene. The cloning of the DH-PBAN gene in the 1990s and the identification of its cognate G-protein-coupled receptor opened the molecular era of diapause research in *B. mori* [5, 7-8, 20-23]. The completion of the genome sequence in 2008 accelerated discovery substantially, enabling genome-wide screens for diapause-regulated genes, transcription factor binding sites, and epigenetic marks [15-19]. The most consequential recent advance has been the application of programmable nuclease technologies: TALEN and CRISPR/Cas9 [24-33]. These tools developed for the silkworm have made it possible to generate precise null mutants for DH, its receptor, circadian clock components, and neurotransmitter transporters, converting correlational evidence accumulated over decades into robust causal proof. CRISPR studies published have confirmed the indispensability of the circadian clock genes period (per), timeless (tim), Clock, cycle, and cryptochrome-1 (cry1) for photoperiodic diapause induction. These studies have also identified the GABAergic-corazonin-DH neuronal axis as the proximal neuroendocrine circuit governing DH release, and have established Boceropsin - a candidate long-wavelength opsin - as the probable mediator of photoperiodic light input to the brain [7, 18, 24-33]. These advances, together with progress in signalling pathway analysis, metabolomics, epigenomics, and novel diapause termination technologies, have substantially advanced mechanistic understanding and created new opportunities for applied intervention in sericulture.

Although several comprehensive reviews have addressed insect diapause broadly and embryonic diapause in *Bombyx mori* specifically, substantial conceptual and technological advances have emerged since the publication of these authoritative reviews. Recent applications of CRISPR/Cas9-mediated genome editing, telomere-to-telomere (T2T) genome assembly, high-resolution transcriptomics, metabolomics, epigenomics, and integrative systems biology approaches have significantly expanded our understanding of diapause regulation in *B. mori*, revealing its complex molecular, physiological, and developmental architecture [20-32]. However, these rapidly expanding discoveries remain dispersed across multiple disciplines, and an updated integrative synthesis encompassing environmental regulation, molecular pathways, neuroendocrine control, developmental physiology, and implications for sericulture is still lacking.

The present review aims to address this knowledge gap by critically synthesizing recent advances in the regulation of embryonic diapause in *B. mori* through integration of perspectives from physiology, biochemistry, cell biology, developmental biology, molecular genetics, and neuroendocrinology. This review specifically focuses on the regulatory processes governing the developmental decision to enter diapause. The review discusses the genetic architecture underlying voltinism and diapause competence; environmental cues, including photoperiod, temperature, and nutritional conditions, that influence maternal determination of diapause fate; photoperiodic perception mechanisms, including the proposed roles of Boceropsin and carotenoid-dependent pathways; the circadian clock network responsible for temporal processing and integration of environmental information; and the neuroendocrine cascade involving diapause hormone (DH), through which environmental signals are translated into developmental commitment to diapause.

Recent advances have highlighted the complex coordination among environmental sensing systems, circadian oscillators, and neuroendocrine regulators in determining whether embryos undergo diapause. Nevertheless, several fundamental questions remain unresolved, including the precise molecular mechanisms linking environmental perception to maternal determination of diapause fate, the integration of multiple external cues, and the downstream signaling events responsible for establishing diapause competence. The biochemical establishment, maintenance, termination, and applied manipulation of embryonic diapause within the *B. mori* egg will be discussed separately in another review.

The present article is therefore focused on the upstream regulatory architecture governing diapause initiation, including interactions between environmental signals and endocrine pathways, genetic and molecular networks determining diapause competence, mechanisms of photoperiodic perception, circadian clock-mediated temporal integration, and the neuroendocrine cascade culminating in diapause hormone receptor-mediated signaling. The forthcoming second review will address how this developmental decision is subsequently executed, maintained, and reversed at the biochemical and physiological levels within the egg, as well as how these insights may be translated into improved strategies for sustainable and climate-resilient sericulture.

Biological Background of *Bombyx mori*

The domesticated mulberry silkworm, *Bombyx mori* (Lepidoptera: Bombycidae), is a globally important insect model organism and the foundation of sericulture, representing a remarkable example of prolonged human-mediated domestication. Its highly specialised life cycle, developmental plasticity, and environmentally regulated embryonic diapause provide valuable insights into insect physiology, neuroendocrine regulation, and adaptive evolution. This section explores the biological foundations of *B. mori*, encompassing its taxonomy, life cycle characteristics, voltinism patterns, maternal regulation of diapause, circadian control mechanisms, genetic determinants, race-specific photoperiodic responses, and the complex genomic architecture contributing to diapause variation.

1. Taxonomy, Life Cycle, and Voltinism

The mulberry silkworm, *Bombyx mori* (Linnaeus, 1758), is a fully domesticated lepidopteran insect belonging to the order Lepidoptera, family Bombycidae, and genus *Bombyx*. It is believed to have diverged from its wild ancestor, *Bombyx mandarina,* through thousands of years of human-mediated selection and domestication. *B. mori* serves as an important model organism for studying insect physiology, neuroendocrinology, and developmental biology, owing to its well-characterised life cycle, ease of laboratory rearing, and substantial economic importance [7, 10].

Bombyx mori exhibits holometabolous development encompassing four distinct stages — egg, larva, pupa, and adult moth. The egg stage may undergo diapause or proceed through direct development depending upon maternal neuroendocrine programming and environmental cues. Upon hatching, the larva progresses through five instars, feeding exclusively on mulberry *(Morus* spp.) leaves, which provide the essential nutrients required for growth and silk synthesis. The fifth instar represents the most metabolically active larval stage, during which the silk glands enlarge substantially and have been estimated to account for approximately 25%–30% of total larval body weight. After completing larval development, the silkworm constructs a proteinaceous silk cocoon within which pupation occurs, and metamorphosis culminates in the emergence of the adult silk moth — flightless, incapable of feeding, and primarily adapted for reproduction [7,10, 22-23, 34-35].

One of the most ecologically and commercially significant expressions of diapause regulation in the silkworm is voltinism — the number of reproductive generations completed within a single calendar year. Strains of *B. mori* are broadly classified into three voltinism categories: univoltine, bivoltine, and multivoltine [34-35]. Univoltine strains produce one generation annually, obligatorily entering embryonic diapause and synchronising their developmental cycle with temperate seasonal rhythms; cocoons from these strains typically yield silk filaments of superior quality and lustre. Bivoltine strains complete two generations per year, exhibiting facultative diapause or non-diapause behaviour depending on prevailing environmental conditions; they display shorter larval periods, moderate tolerance of high temperatures and humidity, and produce silk of commercially acceptable quality. Multivoltine strains, predominantly distributed across tropical and subtropical regions such as India, Sri Lanka, and southern China, complete multiple generations per year under normal tropical conditions with greatly reduced or absent diapause induction, enabling continuous silk crop cycles throughout the year. However, despite their superior environmental resilience and greater tolerance of pathogen pressure compared to bivoltine strains, the quantity and overall quality of silk they produce are comparatively lower. Nonetheless, multivoltine strains remain invaluable genetic reserves for improving the characteristics of commercial bivoltine breeds and developing improved hybrid strains of *B. mori* with enhanced adaptability [34-35].

This fundamental trade-off between silk quality and environmental hardiness has made voltinism a central target in sericulture breeding programmes worldwide, and the genetic management of diapause has accordingly become a practical priority in applied silkworm research. The geographic distribution of voltinism types broadly reflects regional climatic adaptation. Univoltine races are predominantly found in the colder temperate regions of northern Japan and the Himalayan foothills. Bivoltine races thrive in the temperate-to-subtropical zones of central and southern Japan, the Yangtze River basin in China, and parts of the Korean Peninsula. Multivoltine races are native to the humid tropical climates of South and Southeast Asia, including India, Sri Lanka, southern China, and the Indonesian archipelago. In India, bivoltine hybrids have gained prominence owing to their superior silk quality and compatibility with tropical rearing conditions when crossed with locally adapted multivoltine strains. These differences in voltinism are genetically determined and are of profound significance to sericulture, as they influence cocoon quality, silk yield, and the degree of adaptability to prevailing regional climatic conditions [34-35].

2. Transgenerational (Maternal) Determination of Diapause in *Bombyx mori:* A Developmentally Displaced Regulatory Decision

The most biologically distinctive feature of *B. mori* diapause is its transgenerational, maternally controlled determination — a mechanism in which it is not the embryo destined for dormancy, but rather the mother in the preceding

generation, that perceives and integrates the environmental signals governing developmental arrest [5, 7-9, 22]. This regulatory architecture fundamentally distinguishes the silkworm from most insect diapause systems, in which the organism destined for dormancy directly perceives the environmental cues that trigger it. The temporal displacement is fully transgenerational: the photoperiodic and thermal experience of one generation determines the developmental fate of the next, and the progeny themselves play no direct role in this determination [5, 7-9, 22].

In bivoltine strains of *B. mori*, a facultative diapause phenotype is observed, determined by environmental conditions — principally photoperiod and temperature, and to a lesser extent nutritional status — experienced during the mother's embryonic and larval development [5, 7-9, 22]. Diapause egg production in bivoltine strains is predominantly induced by long-day photoperiods combined with elevated temperatures experienced during maternal embryonic development, which collectively stimulate the neuroendocrine cascade culminating in diapause hormone (DH) secretion. If the mother receives these environmental cues early in life, she will later produce and release DH as an adult. DH acts on the follicle cells of developing oocytes, where its cognate G-protein-coupled diapause hormone receptor (DHR) is expressed, and through downstream intracellular signalling events initiates the metabolic and cellular reprogramming that commits the eggs to diapause. Critically, when the gene encoding DH or that encoding DHR is individually disrupted using TALEN-based gene editing, females produce exclusively non-diapause eggs, demonstrating that intact DH–DHR signalling is both necessary and sufficient for diapause induction [5, 7-9, 22, 36-38].

At the mechanistic level, the maternal brain — specifically the suboesophageal ganglion (SOG) — synthesises and releases DH into the hemolymph during the pupal-to-adult transition. DH secretion in bivoltine strains is primarily governed by the long-day photoperiods and elevated temperatures experienced during the mother's embryonic and larval stages, with thermal conditions during the embryonic stage having a particularly potent diapause-inducing effect. The thermal memory acquired during embryonic development is retained across the larval moults and subsequently retrieved to modulate DH secretion at the pupal stage. This retention is conveyed at least in part through the thermosensitive transient receptor potential channel BmTRPA1 and represents a remarkable instance of physiological information storage across developmental stage boundaries [37-40]. The molecular mechanisms by which this environmental information is encoded and stably maintained across these developmental transitions to programme diapause in the next generation remain to be fully elucidated. Recent genome-wide transcriptomic screening efforts have begun to identify differentially expressed genes in the larval brains of diapause-egg producers versus non-diapause-egg producers, providing candidate molecular mediators of this intergenerational signalling cascade [39-40].

3. Genetic Architecture of Diapause: Heritable Variation, Domestication, and the Circadian Gene Repertoire

A comprehensive population genomic study resequencing 137 domestic and wild silkworm strains demonstrated that circadian clock genes are significantly associated with the domestication process of the silkworm through daily and seasonal adaptation [10]. Notably, domestication did not merely reshape genes directly involved in silk synthesis but also remodelled the circadian machinery that underpins the photoperiodic measurement essential for diapause programming [10].

The molecular clock in *B. mori* operates through interlocking transcription-translation feedback loops (TTFLs) composed of a set of well-characterised core clock genes. To date, six core clock genes have been identified in the domestic silkworm: period (per), timeless (tim), Clock (Clk), cycle (cyc), cryptochrome1 (cry1), and cryptochrome2 (cry2). Their expression exhibits circadian temporal oscillations during embryonic development under both light/dark cycle and constant darkness conditions [7, 27-31, 41-42]. The functional roles of CRY1 and CRY2 in *B. mori* are distinct and must not be conflated with the single-cryptochrome system of *Drosophila melanogaster*. CRY1 in *B. mori* is a light-sensitive, *Drosophila*-type photoreceptive cryptochrome that receives photic input and transmits it to the core clock machinery. CRY2, by contrast, is a mammalian-type transcriptional repressor that functions as a negative element within the core TTFL — repressing Clock/cycle-driven transcriptional activation in a light-independent manner. This dual-cryptochrome architecture, shared with the majority of non-drosophilid insects, gives *B. mori* a clock organisation more structurally similar to the vertebrate clock than to that of *Drosophila* with respect to the mechanism of transcriptional repression [7, 27-31, 41-42].

Genetic disruption of these clock genes has profound consequences for diapause determination. Knockout of both the negative elements (per and tim) and the positive elements (Clk and cyc) of the core TTFL consistently abolishes photoperiodic diapause induction. This demonstrates that the circadian clock operates as an integrated functional unit rather than through the isolated action of individual gene products [7, 27-31, 41-42]. Loss of per impairs the core feedback loop and leads to upregulation of GRD — the ionotropic GABA receptor gene — through altered cycle gene expression. This, in turn, elevates GABAergic signalling in the brain-suboesophageal ganglion complex, delays and suppresses DH release into the hemolymph, and ultimately results in non-diapause egg production [33]. Extending these findings, per, tim, Clk, cyc, and cry2 were shown to regulate temperature-induced diapause by acting upstream of cerebral GABAergic signalling and DH release pathways [42]. Furthermore, a CRISPR/Cas9-based study demonstrated that cry1 — functioning specifically as a circadian photoreceptor rather than a transcriptional repressor — also contributes to photoperiodic diapause induction. Females with cry1 knockout failed to induce diapause in eggs in response to diapause-inducing photoperiodic conditions, and in a cry1/tim double-knockout strain, photoperiodic diapause induction was completely abolished, establishing that photic information received by CRY1 is relayed into the core TTFL and thence to the DH-secretion pathway [29, 31, 42]. Together, these findings establish that the circadian clock is not merely a passive timekeeping mechanism but an essential upstream integrator that transduces both photoperiodic and thermoperiodic cues into the neuroendocrine cascade controlling DH release.

Beyond circadian genes, broader genomic approaches have further illuminated the genetic architecture of diapause

variation. A high-resolution pan-genome analysis of 545 wild and domesticated silkworms identified 7,308 new genes, 4,260 core genes, and over 3.4 million non-redundant structural variants — revealing hundreds of genes and structural variants potentially contributing to artificial selection, domestication, and breeding, including genes responsible for embryonic diapause competence [16]. Crucially, this study molecularly resolved the long-standing pnd (pigmented non-diapausing) locus, in which a 747 bp deletion in the 3′-untranslated region of BmTret1-like was identified in pnd homozygotes, significantly reducing its expression during the early embryonic stage. CRISPR/Cas9-mediated knockout of BmTret1-like in the Chinese bivoltine strain Dazao confirmed that loss of this gene results in a non-diapause phenotype. BmTret1-like is a facilitative trehalose transporter gene belonging to the Major Facilitator Superfamily whose principal substrate in insects is trehalose, the dominant disaccharide of the hemolymph, and which is functionally distinct from glucose transporters of the GLUT family. These findings demonstrate that diapause competence is contingent on intact BmTret1-like function at the embryonic level [7, 16, 42]. Earlier physiological characterisations of the pnd mutant showed that the mutant embryos never enter diapause regardless of maternal hormonal exposure. Taken together with the genomic findings stated above, these data demonstrate that diapause competence requires functional integrity at multiple levels: from hormone production in the SOG and DH–DHR signalling at the ovarian follicle cells, to downstream metabolic execution within the embryo itself [7, 16, 43].

4. Race-Specific Differences in Photoperiodic Response

Not all silkworm races perceive and respond to photoperiod identically, and the differences in photoperiodic response curves (PRCs) between geographic races are both ecologically meaningful and practically significant for sericulture management. Broadly, univoltine silkworms adapted to cold temperate regions obligatorily produce diapausing eggs, while multivoltine silkworms adapted to tropical regions produce non-diapausing eggs under normal ambient conditions. These race-specific differences reflect local adaptation to the prevailing day-length regimes and seasonal temperature profiles of their respective regions of origin [7, 38, 43-45].

Japanese bivoltine races, such as Kosetsu and the Japanese Daizo race, typically display a clearly defined long-day response during the larval stage, in which long-day conditions promote and short-day conditions suppress diapause egg production. In those bivoltine races mothers experiencing long days under high temperature produce diapause eggs in which embryonic development is arrested, while those experiencing short days at low temperature produce non-diapause eggs in which development proceeds to hatching. The Chinese bivoltine strain Dazao — a genetically distinct line from the Japanese Daizo race — shows broadly comparable photoperiodic sensitivity but may differ in the precise critical photoperiod threshold and in the relative weighting of temperature versus day-length signals during the sensitive period of embryonic development [7, 38, 43-45]. In the bivoltine Daizo race, the determination of seasonal developmental morph is governed by photoperiod and temperature experienced during embryonic and larval stages. Adults that developed under short-day conditions at low temperature were classified as summer morphs producing only non-diapause eggs, whereas those developing under long-day conditions at high temperature were classified as autumn morphs producing predominantly diapause eggs. Furthermore, the diapause-inductive response is not a fixed threshold response but is refined through the cumulative integration of environmental signals received throughout successive developmental stages [7, 38, 43-45].

Multivoltine races occupy the opposite end of the photoperiodic response spectrum. In such races, elevated temperature during incubation has been shown to exert limited diapause-inducing effects, in sharp contrast to temperate bivoltine races [43], suggesting that the functional linkage between thermal inputs and DH secretion has been attenuated or decoupled during tropical adaptation. The variation in PRCs among Japanese, Chinese, and tropical races therefore reflects not merely quantitative differences of degree but fundamentally different calibrations of the same underlying molecular clock–DH neuroendocrine axis. These calibrations have been shaped by both millennia of local natural selection and centuries of artificial selection during the history of sericulture. The difference in the photoperiodic response mode between silkworm races may be attributable to variation in GABAergic control arising from genetic variation within the circadian system. Understanding and quantifying these race-specific differences have direct applied implications [7, 38, 43-45]. They inform decisions about which races can be safely stored as diapause eggs for sericulture scheduling, which crosses between races might yield commercially useful hybrids with controllable diapause incidence, and how climate change-driven shifts in temperature and photoperiod may have differential effects on distinct regional strains.

5. QTL Mapping and Complex Inheritance of the Diapause Trait

The genetic architecture of embryonic diapause in *Bombyx mori* operates at multiple hierarchical levels. These range from major Mendelian loci that determine voltinism class, through intermediate-effect diapause-competence loci that regulate embryonic responsiveness to diapause hormone (DH), to a polygenic background of modifier loci that quantitatively influence photoperiodic sensitivity, critical temperature thresholds, and the calibration of the circadian–neuroendocrine axis. Understanding this multilayered architecture is both a fundamental scientific priority and a practical necessity for silkworm breeding programmes [46-48].

The most historically prominent component of this architecture is the Voltinism (*V*) locus. Classical genetic studies refined through decades of crossing experiments established that this locus comprises three multiallelic variants, V^1, V^2, and V^3, mapped to a single chromosomal region on chromosome 6. These alleles govern uni-, bi-, and polyvoltine phenotypes, respectively, in a hierarchical dominant–recessive relationship ($V^1 > V^2 > V^3$) [46]. Under this allelic system, V^1 homozygotes or heterozygotes obligatorily produce diapause eggs regardless of environmental conditions, representing the constitutive diapause phenotype of univoltine strains. In contrast, V^2 strains exhibit facultative, environmentally responsive diapause in which maternal temperature and photoperiod regulate DH release, resulting in either diapause or non-diapause eggs. Homozygous V^3 individuals produce non-diapause eggs constitutively under tropical conditions, as observed in multivoltine strains [46].

The molecular resolution of this classical *V* locus represents a major advance in diapause genetics. By integrating population genetic analysis of SNP variation across 109 silkworm strains using a silkworm pan-genome dataset with classical linkage mapping, the authors identified four candidate genes within the *V*-locus region. Of these, two genes, *BmSV2A* and *BmSV2B*, encode synaptic vesicle glycoproteins 2A and 2B, respectively, which are critically involved in vesicular neurotransmitter transport, including GABA transport. Functional validation through CRISPR/Cas9-mediated knockout experiments demonstrated that both genes participate in voltinism regulation by modulating the expression of GABAergic neuron-related genes. This discovery provides the first direct molecular mechanism linking allelic variation at the classical *V* locus to the GABAergic–corazonin–DH neuroendocrine axis. It further establishes that the *V* locus influences voltinism by modulating the efficiency of GABA transport and release within the circadian–diapause neuronal circuit [46-48].

A second major Mendelian locus with profound implications for diapause genetics is the *pnd* (*pigmented non-diapausing egg*) locus on chromosome 11. Yamamoto *et al*. (1978) genetically characterised this locus as a single recessive mutation producing embryos that are completely refractory to diapause induction. Such embryos never enter G2 cell-cycle arrest, even when the maternal suboesophageal ganglion (SOG) produces and releases DH normally and the developing eggs are exposed to diapause-inducing signals during oogenesis. These findings demonstrated that the *pnd* gene functions downstream of DH–DHR signalling and is essential for establishing diapause competence at the embryonic level [49-53].

The molecular identity of the *pnd* locus was subsequently resolved by the high-resolution pan-genome study of Tong *et al*. (2022). The authors identified a 747-bp deletion in the 3′-untranslated region (3′-UTR) of the trehalose transporter gene *BmTret1-like* (*KWMTBOMO06872*) at chromosomal position 11–55.89 cM in *pnd* homozygotes. This deletion significantly reduced *BmTret1-like* expression during early embryogenesis. Subsequent CRISPR/Cas9-mediated knockout of *BmTret1-like* in the bivoltine strain Dazao confirmed that disruption of this facilitative trehalose transporter produces a non-diapause phenotype. These results established that embryonic diapause competence requires intact trehalose transport capacity within the developing embryo and is not solely determined by maternal hormonal signalling [50-52].

More recently, a closely related locus designated *pnd-2* has been characterised. Functional analyses using dsRNA-mediated interference demonstrated that this locus is likewise required for the initiation of embryonic diapause. Unlike genes acting in the maternal neuroendocrine pathway, *pnd-2* functions within the embryo itself and contributes directly to the molecular processes underlying diapause establishment [52].

Taken together, the *V* locus, which regulates DH secretion propensity through GABAergic neurotransmitter transport, the *pnd* locus, which governs embryonic diapause competence through trehalose transport, and the *pnd-2* locus constitute a set of major-effect Mendelian determinants. These loci define whether a moth belongs to a diapause-capable class and whether its embryos are capable of responding to maternal DH. They therefore form the foundational genetic framework upon which quantitative variation is subsequently layered.

Beyond these discrete Mendelian loci, variation in diapause incidence within bivoltine strains exhibits a clear polygenic basis. This variation is expressed as continuous differences in the proportion of eggs entering diapause under a given photoperiodic or thermal regime. However, the genetic dissection of this variation through conventional quantitative trait locus (QTL) mapping has been severely constrained by a distinctive feature of Lepidopteran genetics: female achiasmy [44, 53, 54].

Silkworm females, like all studied Lepidoptera, undergo meiosis without chiasma formation and therefore do not experience meiotic crossing-over. Consequently, all genetic recombination in mapping populations originates from male parents [53]. This biological characteristic imposes a major methodological limitation on QTL analysis. In standard F_2 and backcross designs used in most organisms, approximately half of the recombination information derives from females. Female achiasmy therefore reduces effective mapping resolution and complicates the statistical models required to estimate QTL positions and effects. Conventional QTL mapping software developed for species with recombination in both sexes is formally inappropriate for silkworm populations unless modified to account for sex-specific recombination patterns. Failure to do so can result in biased QTL location estimates and inflated estimates of explained variance [44, 53, 54].

Li *et al*. (2015) addressed this methodological challenge by proposing a composite mapping strategy that combined rational population design with analytical methods based exclusively on male-informative recombination. Their approach explicitly modelled female achiasmy and sex-specific effects and demonstrated strong performance when applied to cocoon shell weight as a model quantitative trait. Similarly, the Markov chain Monte Carlo (MCMC) framework developed by Xu *et al*. (2011) incorporated sex-specific recombination parameters into the simultaneous estimation of QTL effects and QTL × sex interactions [54]. These methodological advances are directly applicable to diapause-trait mapping and constitute essential prerequisites for future high-resolution analyses of modifier loci controlling photoperiodic response variation.

Although no dedicated genome-wide QTL study has yet specifically targeted diapause as a quantitative trait in *B. mori*, population genomic investigations have begun to identify promising candidate loci. Xiang *et al*. (2018), through resequencing of 137 domestic and wild silkworm strains, demonstrated that several circadian clock genes, including *per*, *tim*, *Clock*, *cycle*, *cry1*, and *cry2*, are significantly enriched among genomic regions showing signatures of positive selection during domestication. These signals were associated with daily and seasonal adaptation, strongly suggesting that allelic variation in circadian clock genes contributes to differences in photoperiodic responses among Japanese, Chinese, and tropical silkworm races [10].

Additional evidence comes from SNP-based analyses of natural variation in *sorbitol dehydrogenase 2 (BmSdh2)*. These studies revealed consistent strain-specific sequence motifs associated with diapause status. Diapause strains, including univoltine and bivoltine races, possess a "TTGCC" motif at the first five positions of a discriminatory sequence region, whereas non-diapause polyvoltine strains display an "ACGTT" motif. This

observation suggests that allelic variation in *BmSdh2* may serve as a useful molecular marker for diapause competence across voltinism classes [55].

Integrated genetic linkage maps developed for *B. mori* using SSR, AFLP, RAPD, and SNP markers now provide chromosome-scale reference frameworks encompassing the *V* locus on chromosome 6, the *pnd* locus on chromosome 11, and the chromosomal locations of major circadian clock genes. When combined with the silkworm pan-genome catalogue containing approximately 3.4 million non-redundant structural variants and a dense SNP resource, these genomic tools are expected to facilitate genome-wide association studies of quantitative diapause variation in appropriately designed populations [44, 48, 56].

Genome-wide transcriptomic investigations have also identified potential regulators of diapause programming. These include the microarray study and the CAGE-based analysis, which identified genes such as *Jhamt*, *Kr-h1*, and *CYP18A1* in the larval brain. However, these genes represent functional candidates identified through expression profiling rather than loci detected through formal QTL mapping [39-40, 57-58].

A major scientific gap nevertheless remains. No chromosome-wide QTL scan has yet been conducted to dissect quantitative variation in diapause incidence across environmental gradients. Such a study would ideally compare bivoltine strains exhibiting distinct photoperiodic response curves, critical day-length thresholds, and temperature–diapause coupling coefficients. Implementation in a high-density SNP-genotyped BC_1 or F_2 population based on male-informative recombination, coupled with rigorous environmental control, would, for the first time, decompose the genetic variance underlying diapause responsiveness into its constituent QTL. It would further estimate chromosomal positions relative to known candidate genes, characterise additive, dominant, epistatic, and QTL-by-environment effects, and quantify genotype-dependent variation in diapause response curves.

The practical implications of such a QTL map would be significant. It would enable marker-assisted selection for optimal critical photoperiod thresholds in commercial bivoltine breeds adapted to specific latitudes, facilitate the introgression of diapause-regulating alleles from heat-tolerant multivoltine strains into productive bivoltine genetic backgrounds, and provide a genetic framework for CRISPR-based precision engineering of diapause phenotypes in commercially valuable strains that currently remain difficult to manipulate because of their obligate diapause characteristics.

Environmental Cues Governing Diapause Induction in *Bombyx mori*

The environmental information that governs embryonic diapause fate in *Bombyx mori* is not perceived by the embryo that will ultimately enter dormancy, but is instead integrated through the interplay of light, temperature, and nutritional signals into the developing nervous system of the mother during her own embryonic and larval stages. This fundamentally retrospective character of diapause programming makes the study of environmental cues in *B. mori* not merely a description of sensory biology, but an inquiry into how organisms encode the memory of their environment and transmit it forward to their progeny.

1. Photoperiod

The earliest systematic investigation of photoperiodic control of silkworm diapause was conducted by Makita Kogure, who established the first photoperiodic response curves (PRCs) for *Bombyx mori*. His work demonstrated that *B. mori* exhibits a clear and measurable photoperiodic response in embryonic diapause induction, and the identity and localization of the photoperiodic photoreceptor have since been investigated at levels ranging from the whole organ to the molecular scale [7, 42].

In bivoltine strains of *B. mori,* it is not simply short days or long days that uniformly drive diapause determination, but rather a developmental stage-specific switching between two qualitatively different photoperiodic response modes - a feature of considerable ecological sophistication. In certain bivoltine strains of *B. mori*, photoperiodic sensitivity changes qualitatively across development, with early larval stages showing a long-day-type response and later larval stages exhibiting a distinct, sometimes reversed photoperiodic response [7, 42]. During the embryonic and early larval stages - specifically the 1st through 3rd instars – some strains of *B. mori* exhibits a long-day (LD) response, in which long photoperiods promote and short photoperiods suppress diapause egg production. Within this sensitive window, constant light (LL) conditions experienced during the early larval stage induced significantly more diapause-egg producers than constant darkness (DD) conditions, as assessed by scoring at the egg stage. However, during the late larval stage (5th instar), the photoperiodic sign reverses: LL conditions induced significantly more non-diapause producers than DD conditions, confirming that this constitutes a critical sensitive period for a qualitatively distinct late-larval photoperiodic response. This developmental reversal of photoperiodic sign is ecologically logical and parallels a similar reversal in the temperature response. It allows the insect to integrate photoperiodic information gathered across its entire developmental trajectory, cross-referencing early-season cues with late-season ones, and arriving at a diapause commitment only after receiving a coherent, seasonally consistent set of signals across successive developmental windows [7, 42].

It has additionally been demonstrated that the photoperiodic response during the larval period is temperature-conditional, being expressed only when incubation temperatures fall below 20°C. Although the PRCs reported by different investigators vary in shape and critical day length depending on the strain and diet used [7, 42], the critical day length itself - the threshold photoperiod separating diapause-inducing from non-diapause-inducing regimes - does not shift with temperature. Instead, sufficiently high temperatures (≥ 25°C) can override photoperiodic control altogether through a temperature-dominant pathway [7, 42].

At the molecular level, the capacity to measure day length depends critically on the circadian clock. Females of the cry1 knockout strain failed to regulate photoperiodic diapause induction during the embryonic and larval stages, producing a diapause phenotype that mirrored that of wild-type individuals reared under constant darkness, indicating that *B. mori* CRY1 contributes to photoperiodic time measurement specifically in its capacity as a circadian photoreceptor [7, 27, 29, 42]. CRY1 in *B. mori* functions as a light-sensitive, *Drosophila*-type cryptochrome photoreceptor. This study represented the first evidence of cry1 involvement in insect photoperiodism, and established

that photic information received by CRY1 is relayed to the core circadian clock through TIMELESS (TIM), as demonstrated by the finding that a cry1/tim double-knockout strain completely abolished photoperiodic diapause induction during the larval stage [7, 27, 29, 42]. The photoperiodic photoreceptor is localized to extraocular cells within the brain, and a brain opsin termed Boceropsin has been identified as a likely candidate for the photoperiodic pigment involved in this process.

2. Temperature

Temperature acts on diapause induction in *B. mori* at multiple developmental windows and in ways that are neither simply monotonic in direction nor additively straightforward in their interaction with photoperiod. The most powerful single thermal signal for diapause induction is that experienced by the mother insect during her embryonic stage. When bivoltine *Bombyx mori* eggs (the embryonic stage of the maternal generation) are incubated at 25°C or above, particularly under continuous darkness, thermal signals reprogram the developing silkworm's neuroendocrine system. Consequently, when these embryos hatch, develop into adult female moths, and mate, these females lay almost 100% diapause eggs. In contrast, when the original eggs are incubated at a low temperature, such as 15°C, the female moths that develop from these embryos subsequently lay non-diapause eggs, which hatch immediately without entering diapause [7, 42, 59-61].

When bivoltine races are incubated at temperatures above 25°C during the egg stage and subsequently reared on mulberry leaves, they typically become diapause-egg producers regardless of the photoperiod experienced during the larval period [7, 36, 42]. This strong override effect of high embryonic temperature effectively renders photoperiodic measurement redundant when thermal conditions during incubation have already determined developmental fate - a phenomenon mechanistically connected to the BmTRPA1 channel [7, 42].

The molecular basis of thermoresponsive diapause induction has been substantially clarified in recent years. The thermosensitive transient receptor potential channel BmTRPA1, which in *B. mori* is activated at temperatures exceeding approximately 21°C, promotes diapause hormone (DH) release when activated. A loss-of-function mutation in BmTrpA1 reduces diapause intensity in offspring by reshaping the temporal pattern of DH secretion. It was further demonstrated that *BmTrpA1* transduces temperature cues to regulate diapause via the insulin-like peptide (ILP)/AKT/FOXO signalling pathway. Inhibition of FOXO reversed diapause, whereas AKT knockdown by RNAi was sufficient to induce diapause by activating FOXO through dephosphorylation, establishing the centrality of this signalling axis [7, 36, 42, 59-61].

The relative contributions of thermal and photoperiodic signals to diapause programming are not fixed but differ between strains and are further refined through cumulative signals received throughout successive developmental stages. In the Kosetsu strain, for instance, thermal signals dominate during embryonic development while photoperiodic signals assume greater importance during the larval stage - a hierarchical weighting that reflects the distinct adaptive history of this strain and differs from other bivoltine races [7, 36, 42, 59-61].

Perhaps the most conceptually striking aspect of temperature's role in diapause programming is a developmental reversal that closely parallels the photoperiodic sign reversal described before, suggesting a unified developmental logic underlying both phenomena. During the early larval stages (1st - 3rd instar), high temperature induced significantly more diapause-egg producers than low temperature. However, during the 4th and 5th instars, high temperature induced significantly more non-diapause individuals than low temperature, demonstrating that the temperature response governing diapause induction reverses direction during the mid-larval period. This reversal is ecologically coherent: a larva growing through warm early instars under summer conditions would be appropriately programmed for diapause. In contrast, a larva experiencing warm late instars - as might occur during a second-brood rearing in late summer in bivoltine systems - would be redirected away from diapause, preventing inappropriate dormancy entry late in the favourable season [7, 36, 42, 59-61].

Dopaminergic signaling provides an additional, parallel thermochemical channel linking thermal input to DH expression and diapause induction. Experimental studies demonstrated that high-temperature embryonic incubation elevates dopamine levels in the hemolymph and in the brain-suboesophageal ganglion complex, with this elevation persisting through the larval and into the early pupal stage, directly stimulating DH gene expression. Critically, exogenous dopamine administration was shown to convert adult moths otherwise destined to produce non-diapause eggs into diapause-egg producers at frequencies approaching 70%. This dopaminergic pathway represents a parallel thermochemical channel operating alongside the BmTRPA1-DH neuroendocrine axis [7, 36, 42, 59-61].

3. Nutrition

The nutritional state of the developing silkworm can profoundly modulate - and in some cases completely override - the photoperiodic and thermoperiodic responses governing *B. mori* diapause induction. The nature and extent of this nutritional modulation are best illustrated through what may be described here as "the mulberry diet paradox" a striking dissociation between the diapause-inducing consequences of thermal and photoperiodic experience in larvae fed natural mulberry versus those reared on artificial diets.

The high-temperature embryonic incubation that reliably converts mulberry-fed bivoltine silkworms into diapause-egg producers has little or no comparable effect when larvae are subsequently reared on artificial diet. When bivoltine races were provided with artificial diets in place of mulberry leaves, they exhibited a robust long-day (LD) response throughout the entire larval period, irrespective of thermal incubation conditions. This LD response was observed regardless of whether the artificial diets contained mulberry leaf powder or not [7, 62]. This striking observation implies that mulberry leaves contain one or more bioactive compounds that are either essential for temperature-dependent diapause programming, or the compounds themselves act as diapause-promoting signals. The persistence of this effect even when mulberry leaf powder is included in artificial diets suggests that the responsible compound(s) are labile - lost or inactivated during diet

preparation through autoclaving or dehydration - rather than simply absent from artificial diet formulations [7, 62].

Two principal nutritional axes have been identified as likely mediators of this phenomenon: the polyunsaturated fatty acid (PUFA) composition of the diet, and the carotenoid-derived vitamin A pathway.

Polyunsaturated Fatty Acids (PUFAs) and Membrane Composition

An elevated proportion of polyunsaturated fatty acids (PUFAs) in membrane lipids is widely regarded as an adaptive feature of overwintering diapause in insects, serving to maintain membrane fluidity at low temperatures [5, 7-8]. In *B. mori,* rearing under the low-temperature, long-day conditions associated with diapause induction generally increases the PUFA ratio in membrane lipids. By contrast, silkworm pupae reared on artificial diet showed higher proportions of monounsaturated fatty acids (MUFAs) and the lowest proportions of PUFAs compared to mulberry-fed counterparts. These findings demonstrate that artificial diet rearing alters the composition of fatty acid classes by reducing PUFAs and increasing MUFAs and saturated fatty acids (SFAs) [7, 63-65].

According to a hypothesis, changes in the PUFA composition of neuronal membranes alter the thermosensitivity of BmTRPA1-expressing cells, since neuronal PUFA composition has been shown to modulate temperature preference behaviour and neuronal activity in other systems. Under this hypothesis, the PUFA depletion characteristic of artificial diet-reared larvae would effectively attenuate BmTRPA1 thermal responsiveness. This would blunt the conversion of high embryonic temperature into a DH-secretion signal explaining why these larvae fail to enter diapause despite thermal conditions that reliably induce it in mulberry-fed animals. Consistent with this model, diapause and non-diapause eggs differ in their PUFA compositions, with diapause eggs generally containing higher proportions of unsaturated fatty acids - an adaptation likely serving to maintain membrane integrity and metabolic competence during extended developmental arrest [7, 63-66].

Carotenoids, Vitamin A, and Photoperiodic Photoreception

The second major nutritional axis involves the carotenoid pathway and its derivative, vitamin A (as retinal). Carotenoids are the dietary precursors of retinal, the chromophore of visual photopigments in insect brains. Mulberry leaves (*Morus* spp.) are rich in multiple carotenoids — primarily lutein, β-carotene, and α-carotene — supplying larvae fed on natural foliage with a steady provision of retinal precursors. Larvae fed on standard artificial diets may receive sub-optimal carotenoid amounts depending on formulation [7, 67-69].

When silkworms were reared on a vitamin A-deprived artificial diet, the resulting larvae exhibited loss of phototaxis and loss of the electroretinogram (ERG) response in their stemmata. Furthermore, larvae reared on the vitamin A-deprived diet lost the photoperiodic response during the larval stage. Crucially, however, dietary supplementation with β-carotene (provitamin A) restored the photoperiodic response in these animals [7, 67-69]. The effects of carotenoid and vitamin A deprivation were detectable only at low light intensities of approximately 1 lux, and were absent at higher intensities exceeding 5 lux [7, 67-69]. These results imply that this nutritional dependency is specifically associated with the photoreception stage of the photoperiodic pathway rather than with downstream diapause induction mechanisms. This dependency on low-light conditions is physiologically meaningful. The extraocular cerebral photoreceptors responsible for photoperiodic time measurement are expected to function at light intensities far lower than those saturating the stemmata or adult compound eyes, making photopigment chromophore supply - and thus dietary carotenoid availability - a genuine limiting factor for photoperiodic sensitivity under naturalistic light conditions.

The carotenoid requirement for photoperiodism extends transgenerationally. Larvae hatching from eggs laid by β-carotene-deficient moths also exhibited loss of the phototactic response. However, successive rearing with dietary β-carotene or vitamin A supplementation re-established this response [7, 67-69]. This demonstrates that the carotenoid requirement for photoperiodism reflects a genuine, heritable nutritional dependency that is expressed transgenerationally and correctable by dietary restoration, rather than merely an acute pharmacological effect of carotenoid deprivation in the experimental generation.

Taken together, the PUFA and carotenoid evidence suggest that mulberry leaves function not simply as a food source but as an "ecological permissive signal", a dietary environment in which the full suite of photoperiodic and thermoperiodic responses required for accurate seasonal programming of diapause can be expressed. The depletion of these specific dietary components in artificial diet formulations appears to selectively impair two distinct arms of the diapause-sensing machinery. PUFA depletion attenuates thermosensory transduction through membrane-level modulation of BmTRPA1, while carotenoid depletion impairs photoperiodic photoreception by limiting retinal availability at the cerebral photoreceptors [7, 63-69].

The Photoperiodic Photoreception Mechanism in *Bombyx mori*

Photoperic regulation of diapause in *Bombyx mori* depends on the precise perception and transduction of environmental light signals into neuroendocrine responses that ultimately determine embryonic developmental fate. The following sections examine the current understanding of the localization of the photoperiodic photoreceptor, the proposed role of Boceropsin as a candidate photoperiodic pigment, and the vitamin A/carotenoid-dependent processes implicated in photoperiodic signal perception and diapause induction.

1. Localization of the Photoperiodic Photoreceptor

A fundamental question in the biology of insect photoperiodism concerns where in the body the animal actually perceives the photoperiodic signal. In *Bombyx mori*, various experiments clearly demonstrated that the photoreceptor responsible for diapause induction is located in the larval head at an extraocular location, and not in the stemmata (the simple eyes of the larva) [62, 67, 69-73]. This ruled out peripheral photoreceptive structures as the primary site of photoperiodic light perception, pointing instead toward the central nervous system.

Compelling confirmation of a brain-centred photoreception system came from the landmark *in vitro* experiments of Hasegawa and Shimizu (1987). They demonstrated that an isolated brain (Br)–suboesophageal ganglion (SOG) complex, when cultured together with the corpora cardiaca–

corpora allata (CC-CA) complex, retained the ability to be programmed photoperiodically — that is, the isolated neuroendocrine tissue could distinguish long-day from short-day conditions and respond accordingly. This *in vitro* reprogramming of the Br-SOG complex firmly established that the entire machinery for photoperiodic photoreception and decision-making resides within the larval brain, operating independently of the peripheral tissues [72]. This finding was highly significant because it not only localised the photoreceptor but also established a direct functional connection between light perception in the brain and the neuroendocrine cascade controlling diapause hormone (DH) secretion from the SOG.

2. Boceropsin — The Candidate Photoperiodic Pigment

With the brain established as the seat of photoperiodic photoreception, attention turned to identifying the specific light-absorbing molecule — the photopigment — mediating this response. The molecular cloning of a novel opsin from the larval brain of *B. mori* represented a pivotal advance; this protein was designated Boceropsin [71]. Opsins are G-protein-coupled receptors (GPCRs) characterised by seven transmembrane segments; they bind a light-sensitive chromophore to form the functional photopigment. The deduced amino acid sequence of Boceropsin comprises 381 residues, and the protein retains the key residues conserved across insect visual pigments, including the critical lysine residue involved in chromophore binding [71]. Phylogenetic analysis placed Boceropsin within the green-sensitive opsin group; its predicted absorption peak of approximately 520 nm is consistent with sensitivity in the blue-green region of the visible spectrum [71].

Immunohistochemical analyses revealed that Boceropsin protein is expressed bilaterally in a small, defined cluster of neurons in the dorsal-anterior protocerebrum (DAP) and ventral-anterior protocerebrum (VAP) of the larval brain - regions long associated with neuroendocrine control in insects — but not in the SOG or thoracic ganglia [71]. This brain-restricted, spatially precise expression pattern strongly supports the hypothesis that Boceropsin serves as the photoperiodic photopigment in the silkworm. The photoperiodic action spectrum studies further corroborated Boceropsin's candidacy, showing that light in the blue-green wavelength range (approximately 411–547 nm) is most effective in inducing diapause, whereas red light is virtually ineffective [72-73]. This spectral sensitivity profile is consistent with the predicted absorption characteristics of Boceropsin, reinforcing its candidacy as the principal photoperiodic photopigment.

3. The Vitamin A / Carotenoid-Dependent Process (CDP)

All known opsin photopigments require a vitamin A-derived aldehyde — specifically retinal or a closely related retinoid aldehyde — as their light-absorbing chromophore; without this chromophore, opsins cannot form a functional photopigment and cannot transduce light signals. Consistent with this, Shimizu and Kato (1984) demonstrated that *B. mori* larvae raised on carotenoid-depleted artificial diets completely lost their photoperiodic response — they failed to mount appropriate diapause or non-diapause responses based on day length — while restoration of vitamin A to the diet fully rescued the photoperiodic response [73]. In further support of this opsin-chromophore mechanism, high-performance liquid chromatography (HPLC) analysis of brain tissue from the photoperiodic photoreceptor region of the silkworm revealed the presence of both retinal and 3-hydroxyretinal, the chromophores characteristic of insect visual pigments, indicating the existence of functional retinoid–protein complexes in the brain. This overall dependency of photoperiodic competence on dietary carotenoids and vitamin A constitutes what is referred to throughout this review as the carotenoid-dependent process (CDP) [7].

In addition to supplying the chromophore, the vitamin A-dependent photopigment is now thought to participate directly in the molecular timing mechanism underlying photoperiodic time measurement in *B. mori*. Insect opsins form thermally bistable photopigments: upon absorbing a photon, the rhodopsin form (Rh) is photoconverted to the active metarhodopsin form (Mrh); crucially, Mrh is not irreversibly degraded but can instead be directly photoreconverted back to Rh by absorption of a second photon of a different wavelength [74-75]. This bistable Rh–Mrh photoconversion system — in which Rh and Mrh have distinct and partially overlapping absorption spectra — means that the ratio of the two pigment states at any given moment is determined by the spectral composition, intensity, and duration of the prevailing light environment. The transition from Rh (inactive) to Mrh (active) upon light exposure, and the slow thermal or photochemical reversion of Mrh back to Rh in darkness, constitutes a plausible molecular hourglass mechanism for measuring night length [7]. Bivoltine *B. mori* displays a long-day response where short nights (long days) induce embryonic diapause, the duration of the dark period determines the residual level of active pigment. A short night prevents the complete thermal reversion of Mrh to Rh, leaving an incomplete reversion characterized by adequate levels of active Mrh at dawn which is transduced by the brain's neuroendocrine circuitry to stimulate diapause induction [7]. Conversely, prolonged darkness during long nights allows Mrh to revert fully to inactive Rh, signaling a short-day environment that results in non-diapause development. In this way, this bistable photochemical system provides a plausible molecular basis for the hourglass-type time measurement long inferred from the photoperiodic response characteristics of *B. mori* and related long-day responding insects [75-76].

The CDP thus links the nutritional environment of the larva to its capacity for accurate photoperiodic timing, with important implications for both the mechanistic understanding of seasonal biology and the practical management of sericulture operations.

The Circadian Clock in Photoperiodic Time Measurement

Diapause induction in *Bombyx mori* is widely believed to depend on the interaction between environmental light–dark cycles and endogenous circadian timing mechanisms. According to the circadian basis of photoperiodism, organisms measure seasonal day length through internal molecular oscillators that regulate downstream neuroendocrine and developmental responses. The following sections discuss the molecular architecture of the circadian clock network in *B. mori,* recent functional studies validating the roles of specific clock genes, the interaction between the thermosensitive channel BmTRPA1 and

circadian regulation, current models of photoperiodic time measurement, and the emerging role of miRNA-mediated modulation in circadian and diapause regulation.

1. The Core Clock Gene Network

The circadian clock — the internal timekeeping machinery that generates approximately 24-hour biological rhythms — is now understood to be deeply interwoven with the photoperiodic diapause decision in *Bombyx mori*. At the heart of this machinery lies a self-sustaining transcription–translation feedback loop (TTFL) built around a set of conserved clock genes. The positive arm of this loop consists of two transcription proteins — CLOCK (encoded by the *Clock gene*) and CYCLE (encoded by the *cycle gene*) — which form a heterodimeric complex (CLK/CYC) and drive the expression of the negative regulators. The negative arm is made up of *timeless* (*tim*), and the mammalian-type cryptochrome *cry2 genes*, whose protein products accumulate, eventually inhibit the CLK/CYC complex, and thereby suppress their own transcription, before being degraded to allow the transcriptional cycle to restart [42, 77]. The protein product of the *period* (*per*) gene is associated with stabilization of the CRY2 protein. In *B. mori*, a *Drosophila*-type cryptochrome, *cry1*, encodes a light-sensitive protein that functions in photoentrainment of the clock — that is, it helps reset the clock's phase in response to light — rather than acting as a negative transcriptional repressor, as it does in some other insects [27, 29]. Together, these six clock gene products — PER, TIM, and CRY2 (negative elements), CLK and CYC (positive transcriptional activators) and CRY1 (photoreceptive entrainment factor) — constitute the core oscillator of the silkworm circadian clock.

Long-day conditions, which are the primary inducers of diapause in bivoltine *B. mori*, alter the expression dynamics of these clock genes. Under diapause-inducing photoperiods, the temporal profiles of *per*, *tim*, and related clock genes shift in ways that are thought to coordinate downstream neuroendocrine signalling toward diapause hormone (DH) secretion [7, 76]. This photoperiodic modulation of the TTFL is thought to underlie the silk moth's capacity to measure night length and transduce that measurement into an appropriate hormonal response — diapause or non-diapause egg production — in the offspring [7, 42, 76=79].

2. Genome-Editing Approaches: TALEN and CRISPR/Cas9 Knockout Studies

Over the past decade, understanding of how the circadian clock contributes to diapause induction in *B. mori* has been substantially transformed, driven primarily by the application of genome-editing technologies — first TALEN (Transcription Activator-Like Effector Nuclease) and then CRISPR/Cas9 — to generate loss-of-function mutants in specific clock genes.

The first landmark study used TALEN-mediated genome editing to knock out *per* gene in the bivoltine Kosetsu strain [31]. The resulting *per* knockout strain lost circadian rhythmicity in both eclosion and egg hatching under constant darkness, confirming the gene's role in clock function. The wild-type bivoltine Kosetsu strain showed a clear long-day response for induction of embryonic diapause — producing diapause eggs under long-day conditions and non-diapause eggs under short-day conditions. By contrast, the *per* knockout strain lost sensitivity to photoperiod and laid non-diapause eggs under both conditions [31]. This pivotal finding was independently reproduced by a second group in the Dazao strain [33]. The impaired TTFL resulting from the absence of *per* gene caused direct upregulation of *Grd*, encoding a γ-aminobutyric acid (GABA) receptor, through altered expression of *cycle gene*. This in turn increased GABA synthesis in the brain–suboesophageal ganglion (SOG) complex, continuously promoting GABAergic signalling, ultimately inhibiting DH release into the hemolymph and attenuating the diapause-inducing effect of DH [27-33]. These independent knockouts in different *B. mori* strains thus unanimously demonstrated that the *per gene* is essential for photoperiodic diapause induction, acting through the GABA–DH neuroendocrine axis.

Tobita and Kiuchi (2022) used CRISPR/Cas9 to knock out negative (the *per* and the *tim genes*) and positive elements (the *Clock* and the *cycle genes*) in p50T, a bivoltine strain exhibiting photoperiodic diapause induction during both embryonic and larval stages. The temporal expression patterns of clock genes were altered in each core clock gene knockout strain, and female silk moths in all knockout strains lost the ability to mount photoperiod-dependent diapause responses during both embryonic and larval stages [27]. In parallel, Homma *et al*. (2022) confirmed that *per*, *tim*, *Clk*, *cyc*, and *cry2* regulated temperature-induced diapause by acting upstream of cerebral GABAergic and diapause hormone signalling pathways [42]. Together, these studies establish that it is the entire circadian clock machinery — and not the action of any single gene in isolation — that is integrated into the photoperiodic diapause mechanism.

In *B. mori,* CRY1, the *Drosophila*-type light-sensitive cryptochrome, is directly involved in photoperiodic time measurement. Using CRISPR/Cas9, Tobita and Kiuchi (2024) established a *cry1* knockout strain that exhibited arrhythmic eclosion at the population level. In addition, loss of *cry1* disrupted photoperiodic diapause induction, indicating that CRY1 contributes to photoperiodic photoreception. Females of a *cry1/tim* double-knockout strain produced only non-diapause eggs regardless of larval photoperiod, indicating that CRY1 protein acts as a photoreceptive input to the clock rather than as an intrinsic oscillator component [27, 29]. This finding provided direct evidence that the *cry1* gene participates in photoperiodism in *B. mori,* specifically in diapause induction, and it suggests that CRY1 protein relays light information into the core clock rather than functioning merely as a clock component.

3. BmTRPA1 as a Thermosensor Interacting with the Circadian Clock

Temperature is the second major environmental cue — alongside photoperiod — that determines diapause in bivoltine silkworms. The *Bombyx* TRPA1 ortholog (*BmTrpA1*) encodes a thermosensitive transient receptor potential (TRP) channel that is activated at temperatures above approximately 21°C and promotes diapause induction in progeny; embryonic RNAi of *BmTrpA1* affects diapause hormone release during pupal–adult development [37]. Importantly, knockout of *BmTrpA1* disrupted the rhythmic expression of core clock genes during the embryonic temperature-sensitive period, suggesting that the BmTRPA1-activated thermosensory pathway is functionally linked to the circadian clock oscillator [42]. Cerebral GABAergic and corazonin signalling pathways modulate

DH release, with the plasma membrane GABA transporter (GAT) implicated in temperature-dependent regulation of this process [59], and the BmTRPA1 signal appears to feed into the circadian clock machinery, which hierarchically regulates the downstream GABAergic pathway and subsequently results in the release of DH. Zhang *et al.* (2026) further demonstrated that *BmTrpA1* transduces temperature cues to regulate diapause in *Bombyx mori* via the ILP/AKT/FOXO signalling pathway. Loss of *BmTrpA1* function abolishes the transient peak of DH concentration in the hemolymph [55], adding an insulin signalling dimension to the thermosensory–clock–diapause axis.

4. Proposed Photoperiodic Time Measurement Model

How exactly does the circadian clock translate day-length information into a binary diapause decision? While experimentally unconfirmed, Shimizu (2024) has proposed a theoretical framework for photoperiodic time measurement in *B. mori* based on the External Coincidence Model (ECM) originally formulated by Pittendrigh and Minis (1964) [79]. It assumes the existence of two independent components that interact to determine the diapause outcome. The first is the circadian clock itself, which generates a rhythmic "photoperiodic gate" — a specific phase of high light-sensitivity that recurs once per circadian cycle. The second is a carotenoid-dependent hourglass-like process (CDP) driven by the bistable Rh–Mrh interconversion of the opsin-based photopigment. Diapause induction occurs when light coincides with the sensitive phase of the circadian oscillation, whereas the non-diapause state results when light fails to coincide with it; the CDP modulates the photoinductive threshold in both cases [7].

A key molecular node connecting the circadian clock to the downstream neuroendocrine pathway is the GABA transporter gene *GAT*. Temperature-dependent transcriptional changes of the plasma membrane GABA transporter gene in the pupal brain–suboesophageal ganglion complex are implicated in embryonic temperature-dependent diapause induction [80]. In Shimizu's (2024) ECM-based model, the clock-regulated transcription of *GAT* represents the most likely candidate for the so-called diapause-inducing event (DIE) — the specific molecular output of the clock that determines whether the neuroendocrine axis shifts toward DH secretion and diapause, or toward suppression of DH and the non-diapause state. In this framework, the circadian clock functions hierarchically upstream of GABAergic signaling, which in turn controls DH release from the SOG; the released DH ultimately acts on the ovary to determine the diapause fate of the progeny eggs [7, 42].

5. miRNA-Mediated Post-transcriptional Regulation of Clock Gene Expression

An emerging and increasingly important layer of regulation involves small non-coding RNAs — specifically microRNAs (miRNAs) — that act as post-transcriptional modulators of clock gene expression during diapause induction. Liu *et al*. (2021) compared differentially expressed miRNAs (DEmiRs) in bivoltine silkworm embryos incubated at diapause-inducing (25°C) and non-diapause-inducing (15°C) temperatures during the blastokinesis (BK) and head pigmentation (HP) phases using transcriptome sequencing [81]. This analysis identified 411 known and 71 novel miRNAs across the two developmental phases, with 108 and 74 DEmiRs in the BK and HP groups, respectively. Among the temperature-responsive miRNAs, one microRNA — bmo-miR-6497-3p — emerged as a particularly strong candidate regulator of the clock-diapause axis. A dual luciferase reporter assay demonstrated that bmo-miR-6497-3p directly regulated *Bmcycle* (the *B. mori* homolog of the *Drosophila cycle gene*) and subsequently regulated the expression of circadian genes. Since *Bmcycle* is a positive transcriptional activator in the core TTFL, its post-transcriptional suppression by bmo-miR-6497-3p at diapause-inducing temperatures (25°C) would be expected to dampen the amplitude of the core clock, potentially biasing the circadian output toward the neuroendocrine configuration that promotes diapause programming. These results imply that microRNAs, as key post-transcriptional regulators, respond to different temperatures and participate in diapause induction [7, 81].

This miRNA-mediated layer of regulation adds an important dimension to the already complex picture of diapause control in *B. mori*. The same environmental signal (temperature) that acts through BmTRPA1 to transduce thermosensory input into the circadian clock at the ion channel and protein level also appears to reshape clock gene expression post-transcriptionally via specific miRNAs. The net result is a multi-layered, temperature- and photoperiod-responsive regulatory hierarchy in which the circadian clock operates as the central integrating hub. It receives environmental inputs via multiple routes (CRY1-mediated photic signaling, BmTRPA1-mediated thermosensation, and miRNA-mediated post-transcriptional modulation) and converts these into a coherent, seasonally appropriate neuroendocrine output controlling embryonic diapause.

Neuroendocrine Control: Diapause Hormone (DH)

The induction of embryonic diapause in *Bombyx mori* is ultimately mediated through a highly coordinated neuroendocrine regulatory system centered on the diapause hormone (DH). Recent advances in neurobiology, molecular endocrinology, and functional genomics have revealed the complexity of DH regulation, including higher brain control, neurotransmitter-mediated modulation, and intracellular signaling cascades triggered through the diapause hormone receptor. The following sections examine the current understanding of DH biosynthesis and secretion, neural and GABAergic regulation of its release, and the downstream molecular mechanisms through which DH orchestrates diapause induction in *B. mori*.

1. Structure and Biosynthesis of DH

The regulation of embryonic diapause in *Bombyx mori* is primarily orchestrated by a 24-amino acid neuropeptide known as Diapause Hormone (DH). DH is a C-terminally amidated peptide belonging to the Phe-X-Pro-Arg-Leu amide (FXPRLa) family — a group of structurally related insect neuropeptides that share a conserved pentapeptide motif at their C-terminus. The C-terminal amidation is indispensable for receptor recognition. At the genetic level, DH is not encoded by a standalone gene. Instead, it is co-encoded along with the Pheromone Biosynthesis-Activating Neuropeptide (PBAN) and three suboesophageal ganglion neuropeptides (α-, β-, and γ-SGNPs) within a single polyprotein precursor, processed from a common mRNA transcript of the DH-PBAN gene [7-8, 33, 59, 80, 82].

Among all the FXPRLa peptides encoded by the DH-PBAN locus, DH is the only one with demonstrated embryonic diapause-inducing activity in *B. mori*. The expression of the DH-PBAN gene is tightly regulated by developmental stage and environmental temperature. High-temperature (25°C) incubation of eggs promotes strong expression during five different stages in the life cycle, while low-temperature (15°C) incubation markedly restricts expression to the late pharate stage only — closely mirroring the diapause incidence observed in the progeny generation [7-8, 33, 59, 80, 82].

2. Site of Production, Transport, and Secretion of DH

DH-producing neurosecretory cells are localized in the posterior cluster of the suboesophageal ganglion (SOG), a neuroendocrine center situated just below the brain in the *B. mori* head. Immunocytochemical studies have precisely mapped three clusters of FXPRLa-immunoreactive neurosecretory cells along the ventral midline of the SOG: four mandibular cells, six maxillary cells, two labial cells (superior lateral branch cells or SLb cells), and four lateral cells. Functional differentiation between these clusters has been established, with the SLb cells being specifically responsible for releasing DH to induce embryonic diapause in response to brain innervation. The axonal projections of the posterior cells pass through the brain and enter the nervi corporis cardiaci 3 (NCC3), distributing varicose terminal branches within the corpora cardiaca (CC) — the principal neurohemal release site [7-8, 33, 59, 80, 82].

The temporal dynamics of DH accumulation and release are tightly correlated with the developmental stage and diapause fate of the individual. DH levels in the SOG gradually build up during larval development and reach a peak at the early pupal stage. A critical divergence then occurs between the two developmental fates. In diapause-destined eggs producing individuals, DH levels in the SOG decline sharply at the mid-pupal stage owing to the active secretion of DH into the hemolymph, where it reaches the developing ovaries and triggers glycogen synthesis in the maturing eggs. In non-diapause-destined eggs producing individuals, this secretory pulse does not occur, and DH remains sequestered within the SOG [7-8, 33, 59, 80, 82].

3. Brain Control of DH Secretion

In diapause-producing silkworms, the protocerebrum of the brain exerts a stimulatory influence on the SOG, promoting DH release. In contrast, in non-diapause-producing individuals, the brain exerts an inhibitory influence on SOG, suppressing DH release into the hemolymph. Later immunochemical and electrophysiological studies demonstrated that brain innervation differentially modulates the firing activity of SLb neurosecretory cells in diapause- versus non-diapause-destined eggs producing silk moths. Taken together, these findings establish the protocerebrum as the principal upstream regulator of DH secretion, whose opposing stimulatory and inhibitory outputs ultimately converge on the SLb neurosecretory cells of the SOG — the final neuroendocrine effectors in the brain–SOG axis that translates environmental diapause cues, processed through the molecular and circadian framework, into the hormonal signal controlling embryonic diapause fate [7-8, 33, 59, 80, 82].

4. GABAergic Control — A Key Regulatory Node

Scientists have revealed the molecular identity of a critical upstream regulatory layer governing DH secretion: a GABAergic signaling axis involving corazonin (Crz) interneurons in the brain–suboesophageal ganglion (Br-SOG) complex, which acts as the pivotal switch between diapause and non-diapause developmental outcomes. This discovery established that γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter, acts on Crz-containing interneurons to suppress their activity, thereby reducing Crz-mediated stimulation of DH secretion from SOG neurons SOG [7-8, 33, 59, 80, 82].

Central to this regulatory switch is the plasma membrane GABA transporter (GAT), which actively removes GABA from the synaptic cleft back into presynaptic neurons and glial cells, thereby attenuating GABAergic inhibition. Tsuchiya *et al*. (2021) demonstrated that GAT expression in the Br-SOG complex was 10–100-fold higher in diapause-producing (25°C-reared) silkworms compared to non-diapause-producing (15°C-reared) individuals. Critically, CRISPR-Cas9-based knockout (KO) mutants of *GAT* laid mostly non-diapause eggs even under conditions normally conducive to diapause, confirming the indispensable role of GAT in diapause determination [7, 33, 59, 80, 82].

The mechanistic model emerging from these studies is as follows. Under diapause-inducing conditions (LD, 25°C), elevated GAT expression efficiently clears GABA from the synaptic cleft, relieving inhibition of Crz interneurons; released Crz then stimulates DH secretion from SOG neurons, and DH acts on developing ovaries to trigger diapause egg production. Conversely, under non-diapause conditions (SD, 15°C), GAT expression is suppressed, GABA accumulates in the synaptic cleft, Crz neuron activity is tonically inhibited, DH is not released, and non-diapause eggs result. Further mechanistic insight was provided by Cui *et al*. (2021) and Homma *et al*. (2022), who demonstrated that the circadian clock system is hierarchically upstream of this GABAergic–DH axis. Knockout of the core clock gene *Period* (*Per*) in *B. mori* impairs the feedback loop of the transcription-translation oscillator, resulting in upregulation of the GABA receptor subunit GRD — mediated via the clock protein CYCLE (CYC). This increases GABA synthesis, and reduces GAT expression, collectively promoting sustained GABAergic inhibition and thereby blocking DH release. Similarly, knockouts of the core clock genes *timeless*, *Clock*, and *cycle* disrupted normal photoperiod-dependent diapause induction, firmly establishing the circadian clock as hierarchically upstream of the GABAergic–DH signaling axis [7, 33, 42, 59, 80, 82].

5. Diapause Hormone Receptor and Downstream Intracellular Signaling

The physiological actions of DH are mediated through a specific G-protein-coupled receptor (GPCR), designated the Diapause Hormone Receptor (DHR). DHR possesses the seven-transmembrane (7-TM) domain architecture characteristic of class-A GPCRs. Receptor expression studies confirm that DHR is localized to the developing ovaries during pupal-adult development, where DH acts to commit the maturing eggs to diapause [7, 33, 42, 59, 80, 82].

Pharmacological characterization of DHR-mediated signaling by Jiang *et al*. (2016) revealed that upon DH binding, DHR couples to the Gq protein, triggering a phospholipase C (PLC) – protein kinase C (PKC) cascade that culminates in the phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2). This Gq-

dependence was confirmed pharmacologically: ERK1/2 phosphorylation was abolished by the selective Gq inhibitor UBO-QIC, the PLC inhibitor U73122, and PKC inhibitors, and was attenuated by chelation of Ca^{2+} with EGTA. Notably, EGFR transactivation was not involved in this pathway, distinguishing DHR from some other GPCRs that recruit receptor tyrosine kinase-mediated signaling. Downstream of the Gq–PLC–PKC cascade, DH signaling in the ovarian target tissue activates trehalase — the enzyme responsible for converting trehalose to glucose. Glucose is then converted to glycogen by glycogen synthase, resulting in massive glycogen accumulation in the maturing eggs. This glycogen-rich biochemical state is a hallmark and prerequisite of the diapause egg phenotype in *B. mori*. While new protein synthesis is not required for this trehalase induction, a Ca^{2+}-dependent protein kinase consistent with PKC has been implicated as a key mediator [7, 33, 42, 59, 80, 82].

Structure-activity relationship studies using alanine-scanning mutagenesis and N-terminally truncated analogs of DH have identified the C-terminal residues Arg23 and Leu24 as essential for DHR binding and activation, while Trp19 and Phe20 also contribute significantly to functional potency. Homology modeling and molecular dynamics simulations have further identified residues Glu89, Phe172, Phe194, and Tyr299 within DHR as critical contact points for DH binding [83].

The *in vivo* essentiality of the DH–DHR signaling axis was definitively established by Shiomi *et al*. (2015) through TALEN (Transcription Activator-Like Effector Nuclease)-based genome editing in *B. mori* [32]. Null mutant silkworms in which the *DH-PBAN* gene or the *DHR* gene was disrupted were fully viable and exhibited normal developmental timing, ecdysis, and metamorphosis. However, female adults of both mutant lines unfailingly produced non-diapause eggs even under environmental conditions that universally induce diapause in wild-type individuals. This genetic experiment demonstrated that DH signaling is both necessary and sufficient for diapause induction in *B. mori*, and that the high selectivity of DH for DHR — shaped by ligand–receptor coevolution — is the molecular foundation of diapause commitment in the silkworm [7, 32-33, 42, 59, 80, 82]. Collectively, the elucidation of the DH–DHR–Gq–PLC–PKC–ERK1/2 signaling cascade clarifies how an environmental signal, sensed during maternal embryogenesis, is ultimately transduced into a biochemical programme of developmental arrest in the progeny.

Future Perspectives and Research Gaps

Despite the remarkable progress made over the past two decades in dissecting the molecular architecture of diapause in *Bombyx mori*, numerous unresolved questions remain, and several emerging research frontiers demand focused attention. Each of these gaps not only represents a conceptual puzzle but also carries practical implications for silkworm biology, sericulture management, and entomology more broadly.

1. **The Identity and Sufficiency of the Molecular Thermosensor**

Perhaps the most debated issue in the field concerns whether BmTRPA1 is the definitive molecular thermosensor that integrates temperature with the neuroendocrine diapause pathway, or merely one of several contributing molecular components. Sato *et al*. (2014) demonstrated that BmTRPA1, a thermosensitive transient receptor potential (TRP) channel, is activated at temperatures above 21°C during the embryonic stage of the maternal generation, and that its disruption via RNAi dramatically reduces the proportion of diapause-destined eggs — strongly implicating it as a molecular switch for this transgenerational maternal effect [37]. More recently, Zhang *et al*. (2026) showed that *BmTrpA1* knockout abolishes the characteristic pulse of diapause hormone (DH) in pupal hemolymph and disrupts downstream insulin-like peptide (ILP)/AKT/FOXO signalling pathway, establishing a causal mechanistic link between thermosensation and the metabolic programming of diapause [55]. Nevertheless, significant ambiguity persists. The thermosensitive period in *B. mori* requires sustained exposure to high temperatures across an extended period of embryonic development, a property that distinguishes BmTRPA1 from other TRPA1 orthologues, which typically elicit rapid, acute responses. Whether BmTRPA1 acts alone, functions cooperatively with other TRP-family channels, or is itself regulated by upstream thermal integrators remains to be established. Furthermore, the precise embryonic cell types in which BmTRPA1 exerts its decisive action — and the identity of the downstream signal transduction cascade that connects calcium influx to long-lasting epigenetic reprogramming of the neuroendocrine axis — are still incompletely characterised. Resolving this debate will require conditional, cell-type-specific knockouts, live calcium imaging in intact embryos, and proteomics of BmTRPA1-interacting networks.

2. **Neuroanatomical Mapping of the Photoperiodic–Hormonal Relay**

A second major frontier concerns the precise neuroanatomical wiring that connects photic signals, received by extraocular *Boceropsin*-expressing cells in the larval brain, to the ultimate effectors that control DH secretion. Shimizu *et al*. (2001) cloned *Boceropsin* — a green-sensitive opsin expressed bilaterally in defined cells of the *B. mori* larval brain — and proposed that these dorsal anterior protocerebral (DAP) cells serve as the principal photoperiodic photoreceptors [71]. Tsuchiya *et al*. (2021) subsequently demonstrated that a GABAergic–corazonin (Crz) signalling cascade operates upstream of DH release, with ionotropic GABA receptor blockade dramatically inducing diapause egg production [80]. Yet the complete synaptic circuit linking *Boceropsin*-positive DAP neurons to GABAergic interneurons, thence to Crz-positive protocerebral neurons, and ultimately to the DH-producing labial neurosecretory cells of the suboesophageal ganglion (SOG), remains to be mapped at cellular resolution. A comprehensive neuroanatomical connectome of this relay — achievable through modern tools such as single-cell RNA sequencing, expansion microscopy, genetic sparse labelling with fluorescent reporters, and optogenetic circuit dissection — represents a research priority. Establishing the precise point at which circadian clock outputs converge with the photoperiodic photoreceptive signals in this circuit would be a particularly significant advance.

3. **Opsin as a Potential Dual Light-and-Temperature Sensor**

An emerging hypothesis is that *Boceropsin* and related extraocular opsins in the silkworm brain might function not

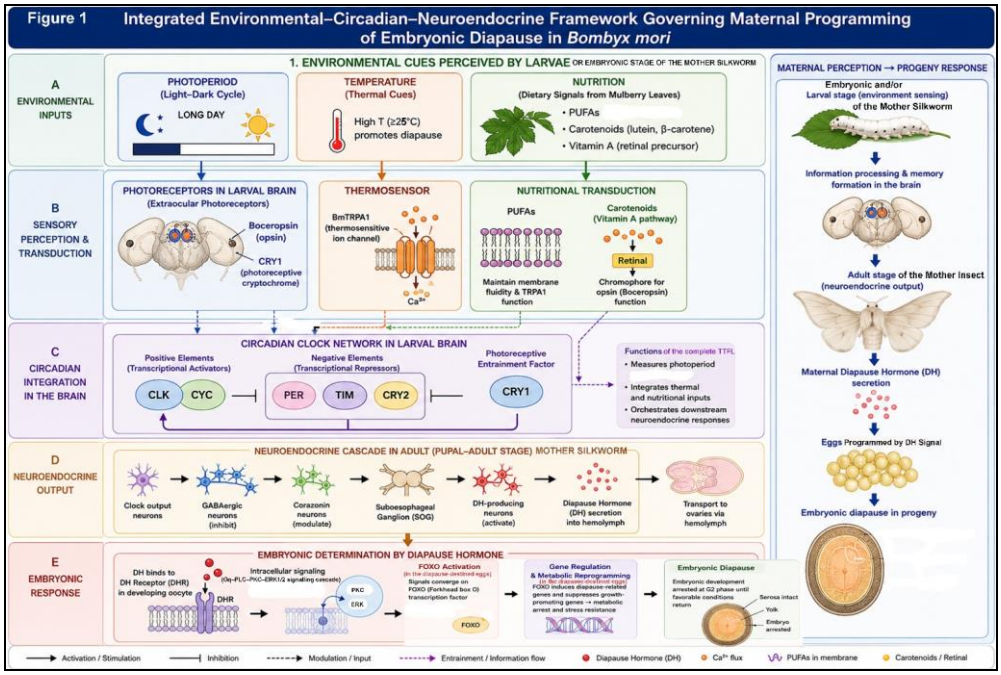
solely as photoreceptors but also as thermoreceptors. This idea is supported by recent discoveries in other invertebrates, where rhodopsin-family proteins have been shown to respond to both light and thermal stimuli. Yokoyama *et al*. (2021), in their comparative study of domestic *B. mori* and wild *B. mandarina*, found that while the DH signalling pathway and TRPA1 thermal sensitivity are conserved between species, photoperiod-dependent diapause induction predominates in *B. mandarina* even though its TRPA1 is functional — raising the possibility of an alternative photoreceptive pathway capable of integrating thermal information independently of TRPA1 [38]. Shimizu (2024) reviewed evidence from multiple insect species suggesting that the dorsal protocerebrum harbours photopigments that may be responsive to a range of environmental stimuli [7]. Empirically testing whether *Boceropsin* or other brain opsins in *B. mori* can detect thermal stimuli — through heterologous expression, patch-clamp electrophysiology, and behavioural thermal-response assays in opsin-knockout backgrounds — represents a promising and experimentally tractable research direction.

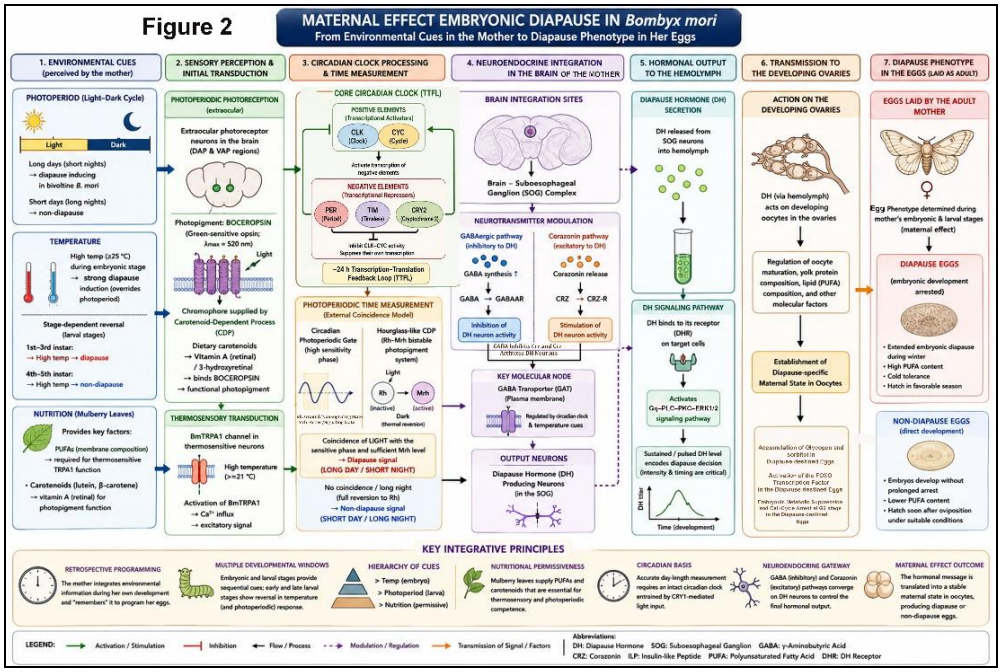
4. The Photoperiodic Counter: Accumulation of Light/Dark Cycle Information

One of the most fundamental unresolved questions in insect photoperiodism generally, and in *B. mori* specifically, is the identity and mechanism of the "photoperiodic counter" — the cellular or molecular entity that accumulates information about repeated long-day or short-day cycles across multiple developmental stages (embryonic and larval) and translates this cumulative signal into a binary diapause decision. Knockout studies of core circadian clock genes (*per*, *tim*, *Clk*, *cyc*, *cry2*) have collectively shown that all are required for normal photoperiodic diapause induction in *B. mori*, acting upstream of the GABAergic–DH pathway [27, 29, 31]. Tobita and Kiuchi (2024) further demonstrated that cryptochrome 1 (*cry1*) functions as a photoreceptor that relays photic information to the clock, with *cry1* knockout females failing to integrate photoperiodic information during both embryonic and larval stages [29]. Hasebe *et al*. (2024) showed that the *period* gene is required for the photoperiodic response during larval development and for diapause egg production [84]. Despite these advances, the molecular counter that integrates information from repeated light–dark cycles remains elusive. Whether it is an epigenetic mark that accumulates with each photoperiodic cycle, a gradual change in neuropeptide titre, a quantitative shift in gene expression thresholds, or a network-level ratcheting mechanism within the circadian feedback loop is entirely unknown. Identifying this counter remains the most important conceptual question in the field.

5. Upstream–Downstream Signalling Map in Embryonic Diapause

While the upstream neuroendocrine cascade culminating in DH release has been substantially elucidated, the downstream intracellular signalling map — from DH binding to its receptor (DHR) in the developing ovary through to G2 cell cycle arrest — remains incompletely understood. Transcriptomic profiling of DH-stimulated ovaries identified early differentially expressed genes enriched in ribosomal, epigenetic, metabolic, and immune functional categories, but how these transcriptional events are causally ordered to enforce cell cycle arrest is unclear [85]. The discovery that sorbitol dehydrogenase 2 (*BmSdh2*) regulates diapause in a dosage-dependent manner, with its complete loss abolishing diapause and its partial reduction leaving diapause intact, reveals an unexpected layer of metabolic gating in the diapause decision [28]. Homozygous null mutants (*BmSdh2*$^{-/-}$) completely failed to enter diapause, as they were unable to accumulate sorbitol — the central metabolic intermediate of the diapause programme. Metabolomic and lipidomic analyses in *BmSdh2* mutants have demonstrated that sorbitol accumulation and lipid remodelling are mechanistically linked to diapause maintenance, and that these metabolic shifts are intimately coupled to diapause depth and duration. A comprehensive, causal signalling map — from corazonin (Crz) neuroendocrine output through DH, DHR, second messengers, kinase cascades, cell cycle checkpoints, and metabolic reprogramming — constructed through systematic genetic epistasis and phosphoproteomics, is a priority for the field.





Conclusion

The developmental fate of a *Bombyx mori* egg—whether it enters embryonic diapause or proceeds through direct development—is not determined by the embryo itself but is maternally programmed during the mother's embryonic and larval development through an environmentally regulated, genetically encoded developmental decision-making process. The evidence synthesised in this review traces this process from the initial perception of seasonal cues to the point at which diapause commitment becomes fixed within the maternal neuroendocrine system.

Environmental signals represent the first layer of this regulatory hierarchy. In domesticated bivoltine strains, temperature experienced during the embryonic stage of the maternal generation serves as the primary environmental cue regulating diapause induction. Thermal information is detected through the thermosensitive TRP channel BmTRPA1, which becomes activated at temperatures above approximately 21°C and initiates a signalling cascade that ultimately influences whether maternally produced eggs enter diapause or undergo direct development [37]. Photoperiod functions as an additional essential seasonal cue. Photoperiodic information is detected through extraocular photoreception involving Boceropsin-positive neurons in the larval brain, with its regulatory influence varying across larval developmental stages. This stage-dependent reversal of photoperiodic responsiveness, together with corresponding changes in thermal sensitivity, enables the insect to integrate early- and late-season environmental information before committing to an appropriate developmental pathway. Nutritional status further modifies this regulatory process by influencing dietary polyunsaturated fatty acid composition and carotenoid-dependent retinal availability. These dietary changes alter photoperiodic sensitivity and may, under certain conditions, override the response to environmental cues, as observed in comparisons between mulberry-fed and artificial-diet-reared larvae (Figure 1 and Figure 2).

These environmental inputs are not interpreted independently but are integrated through the circadian clock system. The core transcription–translation feedback loop, comprising CLOCK and CYCLE as positive regulators and PERIOD, TIMELESS, and CRYPTOCHROME-2 as negative regulators, together with CRYPTOCHROME-1 as a photoreceptive entrainment factor, constitutes the temporal framework of the circadian system. This network enables measurement of photoperiodic information during diapause-sensitive developmental windows and contributes to the physiological integration of thermal history and seasonal timing. Functional analyses, including genome-editing studies across different genetic backgrounds, indicate that diapause induction depends on the coordinated operation of the circadian clock network rather than on the activity of any single clock component alone (Figure 1 and Figure 2).

Signals emerging from this sensory and circadian integration converge on the GABAergic–corazonin–diapause hormone (DH) neuroendocrine axis, which functions as the central neuroendocrine output pathway regulating diapause induction. GABAergic neurons modulate the activity of corazonin-producing protocerebral neurons, which subsequently regulate DH-producing labial neurosecretory cells located in the suboesophageal ganglion (SOG). The temperature- and photoperiod-sensitive expression of the plasma membrane GABA transporter (GAT) acts as a molecular rheostat, gating the inhibitory tone exerted on corazonin-secreting interneurons and thereby regulating whether diapause hormone is released from the labial neurosecretory cells of the suboesophageal ganglion. The abundance of GAT, itself regulated in a temperature-dependent manner through BmTRPA1-linked pathways, fine-tunes the inhibitory tone of GABAergic signalling (Figure 1 and Figure 2). The temperature- and photoperiod-dependent regulation of GAT thereby adjusts DH secretion according to the thermal and photoperiodic history of the mother insect. Through this mechanism, environmental history is converted into an endocrine signal that determines the developmental fate of the next generation [80].

During mid-pupal development, DH released from the corpus cardiacum enters the hemolymph and acts on diapause hormone receptors (DHR) expressed in developing ovarian tissues of the mother insect. DHR activation in

developing ovarian follicle cells of the mother silkworm initiates intracellular signalling through the canonical Gq–PLC–PKC–ERK1/2 signalling cascade, thereby reprogramming ovarian and oocyte developmental processes towards a diapause-destined fate. This signaling cascade, among many events, leads to trehalase activation and glycogen accumulation in diapause-destined eggs. In parallel, the ILP/AKT/FOXO axis, operating downstream of BmTRPA1 thermosensory input in the maternal organism, modulates the neuroendocrine propensity for DH secretion and contributes convergently to the establishment of the diapause-competent state. These two signalling axes — one within the maternal nervous and endocrine system (BmTRPA1–mediated ILP/AKT/FOXO signaling pathway), the other within the developing ovary (DH–DHR–Gq–PKC–ERK) — represent distinct but complementary regulatory layers in the hierarchy of diapause induction (Figure 1 and Figure 2). This event represents the molecular transition from environmental sensing to transgenerational developmental commitment. The cellular and molecular consequences of this maternal hormonal signal for the embryo are extensive.

Following maternal commitment, diapause-destined embryos undergo a prolonged metabolic arrest characterized by suppression of oxidative phosphorylation, activation of the FOXO transcription factor, autophagy induction, glycogen-to-sorbitol conversion, and extensive lipid remodelling — collectively reducing metabolic activity to a fraction of the level observed in actively developing, non-diapause embryos. Simultaneously, cell cycle progression is arrested at the G2 checkpoint, associated with suppression of Cdc2 kinase activity, ensuring that embryonic cells remain in a precisely defined, recoverable state until diapause-terminating signals — principally chilling and subsequent warming — reactivate development [28, 86]. TALEN-based knockouts of either DH or DHR abolish diapause production entirely, establishing that this signalling axis is both necessary and sufficient for the maternal control of embryonic diapause in *Bombyx mori.*

Collectively, the findings reviewed here reveal a hierarchical and highly coordinated signal-integration system controlling embryonic diapause in *Bombyx mori*. BmTRPA1 and Boceropsin sense temperature and light respectively, the circadian clock times and integrates these environmental signals across developmental windows, the GABAergic–corazonin pathway converts these signals into a binary neuroendocrine output, and DH–DHR signalling ultimately establishes the developmental fate of the eggs before they are even laid by the mother insect. Despite substantial progress, several fundamental questions remain unresolved, including the basis of the molecular mechanism responsible for photoperiodic counting, the precise neural connections linking Boceropsin-positive photoreceptors with the GABAergic–corazonin–DH pathway, and the possibility that Boceropsin or related opsins serve a dual sensory role as thermoreceptors as well as photoreceptors. Addressing these questions will require emerging approaches, including single-cell transcriptomics, circuit-level neuronal mapping, sparse genetic labelling, optogenetic manipulation, and integrative multi-omics analyses.

In summary, the embryonic diapause in *B. mori* represents a molecularly tractable regulatory programme whose dissection illuminates fundamental principles of seasonal biology, environmental sensing, circadian regulation, transgenerational epigenetic inheritance, neuroendocrine integration, and metabolic plasticity. The simplified regulatory cascade from environmental cue to diapause execution — BmTRPA1/*Boceropsin* → BmTRPA1-mediated regulation of the ILP/AKT/FOXO signaling pathway (operating in the maternal organism to modulate DH release) → circadian clock machinery → GABAergic neurons → GABA inhibits corazonin (Crz) interneurons → Crz interneurons (when active) stimulates DH-producing cells in SOG → DH release by the mother insect→ DHR activation in the ovarian follicle cells and the oocytes of the mother → Gq–PLC–PKC–ERK1/2 signaling cascade → various metabolic changes inside the eggs (accumulation of glycogen/sorbitol inside the diapause-destined eggs) → dephosphorylation and subsequent activation of FOXO in the diapause-destined eggs → embryonic metabolic suppression and cell-cycle arrest at G2 stage — represents one of the most well-characterized examples of environmental control of developmental fate in insects (Figure 1 and Figure 2). As the research community fills in the remaining gaps identified in this review — completing the neuronal circuit map, identifying the photoperiodic counter, resolving the dual sensory roles of opsins, building multi-omics atlases, and validating next-generation diapause management technologies — the science of silkworm diapause is poised to deliver both fundamental biological insight and valuable applications for a more sustainable and climate-resilient global sericulture industry.

The biochemical execution of diapause commitment in *Bombyx mori* described here will be addressed in a separate review which will address how DH receptor activation leads to glycogen-to-sorbitol conversion, cell-cycle arrest, and metabolic and proteomic adaptations that allow embryos to survive prolonged dormancy. The natural and artificial means by which this arrested embryonic state is later terminated, the mechanisms underlying diapause termination and the practical applications of this knowledge to sericulture will also be discussed in that review. Together, these complementary review articles aim to provide a comprehensive understanding of the entire embryonic diapause process in *Bombyx mori*, from environmental perception to embryonic survival and eventual larval emergence, representing one of the best-characterized seasonal adaptations known in invertebrates.

References

1. Denlinger DL. Regulation of diapause. Annual Review of Entomology,2002:47:93–122.
2. Gill HK, Goyal G, Chahil G. Insect diapause: a review. Journal of Agricultural Science and Technology A,2017:7:454–473.
3. Hahn DA, Denlinger DL. Energetics of insect diapause. Annual Review of Entomology,2011:56:103–121.
4. Denlinger DL. Insect diapause: from a rich history to an exciting future. Journal of Experimental Biology,2023:226(4):jeb245329. https://doi.org/10.1242/jeb.245329
5. Shome S. Decoding insect diapause: insights into physiology, environment, and ecology. International Journal of Entomology Research,2025:10(6):65–74.
6. Koštál V. Eco-physiological phases of insect diapause. Journal of Insect Physiology,2006:52:113–127.

7. Shimizu I. Photoperiodism of diapause induction in silkworm *Bombyx mori* L. Zoological Science,2024:41(2):141–158.
8. Shome S. Molecular regulation of diapause in insects: integration of signaling pathways, genes, enzymes, hormones, and epigenetic mechanisms. International Journal of Entomology Research,2025:10(10):105–120.
9. Goldsmith MR, Shimada T, Abe H. The genetics and genomics of the silkworm, *Bombyx mori*. Annual Review of Entomology,2005:50:71–100.
10. Xiang H, *et al*. The evolutionary road from wild moth to domestic silkworm. Nature Ecology & Evolution,2018:2:1268–1279.
11. Custom Market Insights. Global silk market size, trends, share, forecast 2033. CMI Market Research Report, 2024. https://www.custommarketinsights.com/report/silk-market/
12. International Sericultural Commission. Statistics. ISC Online, 2024. https://inserco.org/en/statistics
13. Ministry of Textiles, Government of India. India's silk industry — Annual Report 2023–24. Central Silk Board, Bangalore, 2024.
14. Market Data Forecast. Global silk market size, analysis, share & growth report 2033. Market Data Forecast, 2025. https://www.marketdataforecast.com/market-reports/silk-market
15. Xia Q, *et al*. A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). Science,2004:306:1937–1940.
16. Tong X, *et al*. High-resolution silkworm pan-genome provides genetic insights into artificial selection and ecological adaptation. Nature Communications,2022:13:5619. https://doi.org/10.1038/s41467-022-33366-x
17. International Silkworm Genome Consortium. The genome of a lepidopteran model insect, the silkworm *Bombyx mori*. Insect Biochemistry and Molecular Biology,2008:38:1036–1045.
18. Li WS, Xiao YD, Liu JQ, Li SL, Chen Y, Xu YJ, *et al*. The T2T genome of the domesticated silkworm *Bombyx mori*. International Journal of Molecular Sciences,2024:25(22):12341. https://doi.org/10.3390/ijms252212341
19. Lee J, Fujimoto T, Sahara K, Toyoda A, Shimada T. Comprehensive genome annotation of *Bombyx mori* p50ma strain, a newly developed standard strain. Scientific Data,2025:12:359. https://doi.org/10.1038/s41597-025-04679-5
20. Yamashita O. Diapause hormone of the silkworm, *Bombyx mori*: structure, gene expression and function. Journal of Insect Physiology,1996:42:669–679.
21. Izadi H. Endocrine and enzymatic shifts during insect diapause: a review of regulatory mechanisms. Frontiers in Physiology,2025:16:1544198. https://doi.org/10.3389/fphys.2025.1544198
22. Kankanawadi PR, Veeresh GKP, Ananda Rao A, Rajan RK. Recent advances in elucidating the mechanism of embryonic diapause and its management in silkworm, *Bombyx mori*. Journal of Advances in Biology & Biotechnology,2025:28(7):1612–1622.
23. Amarnatha B, *et al*. Recent developments in endocrinology of silkworm *Bombyx mori*. Archives of Current Research International,2024:24(7):163–174.
24. Takasu Y, *et al*. Efficient TALEN construction for *Bombyx mori* gene targeting. PLoS ONE,2010:5:e14286.
25. Daimon T, *et al*. CRISPR/Cas9-based generation of knock-out and knock-in silkworms. Methods in Molecular Biology,2019:1869:1–14.
26. Ma S, Smagghe G, Xia Q. Genome editing in *Bombyx mori*: new opportunities for silkworm functional genomics and the sericulture industry. Insect Science,2019:26(6):964–972. https://doi.org/10.1111/1744-7917.12693
27. Tobita H, Kiuchi T. Knockouts of positive and negative elements of the circadian clock disrupt photoperiodic diapause induction in the silkworm, *Bombyx mori*. Insect Biochemistry and Molecular Biology,2022:149:103842.
28. Chen D, Yang D, Fu X, Bian H, Huang Y, Liu Y, *et al*. Dosage-dependent regulation of embryonic diapause by sorbitol dehydrogenase in the silkworm, *Bombyx mori*. PLOS Genetics,2025:21(10):e1011933.
29. Tobita H, Kiuchi T. Knockout of cryptochrome 1 disrupts circadian rhythm and photoperiodic diapause induction in the silkworm, *Bombyx mori*. Insect Biochemistry and Molecular Biology,2024:172:104153.
30. Wu J, He C, Qin X, Wang M, Li Z, Cao Y, *et al*. Upf2-mediated nonsense-mediated degradation pathway involved in genetic compensation of TrpA1 knockout mutant silkworm (*Bombyx mori*). Insects,2024:15(5):313.
31. Ikeda K, Daimon T, Shiomi K, Udaka H, Numata H. Involvement of the clock gene period in the photoperiodism of the silkmoth *Bombyx mori*. Zoological Science,2021:38(6):523–530.
32. Shiomi K, Takasu Y, Kunii M, Tsuchiya R, Mukaida M, Kobayashi M, *et al*. Disruption of diapause induction by TALEN-based gene mutagenesis in relation to a unique neuropeptide signaling pathway in *Bombyx*. Scientific Reports,2015:5:15566.
33. Cui WZ, Qiu JF, Dai TM, Chen Z, Li JL, Xu Y. Circadian clock gene period contributes to diapause via GABAergic-diapause hormone pathway in *Bombyx mori*. Biology,2021:10(9):842.
34. Mishra PK, *et al*. Atypical voltinism in mulberry silkworm *Bombyx mori* L., 1758 (Lepidoptera: Bombycidae) races Barapat and Barapolu. Entomological Communications, 2021.
35. Awasthi AK, Kar PK, Srivastava PP, Rawat N, Vijayan K, Pradeep AR, *et al*. Molecular evaluation of bivoltine, polyvoltine and mutant silkworm (*Bombyx mori* L.) with RAPD, ISSR and RFLP-STS markers. Indian Journal of Biotechnology,2008:7:188–194.
36. Zhang Y, Xu H, Yin Y, Wang Y. BmTRPA1 regulates diapause induction through the insulin-like peptide/AKT/FOXO signalling pathway in the silkworm *Bombyx mori*. Insect Molecular Biology,2020:29(6):602–614.
37. Sato A, Sokabe T, Kashio M, Yasukochi Y, Tominaga M, Shiomi K. Embryonic thermosensitive TRPA1 determines transgenerational diapause phenotype of the silkworm, *Bombyx mori*. Proceedings of the National Academy of Sciences USA,2014:111(15):E1249–E1255.

38. Yokoyama T, Saito S, Shimoda M, *et al*. Comparisons in temperature and photoperiodic-dependent diapause induction between domestic and wild mulberry silkworms. Scientific Reports,2021:11:8052.
39. Egi Y, Sakamoto K. Genome-wide screening of genes involved in programming diapause in the next generation in silkworm, *Bombyx mori*. European Journal of Entomology,2022:119:405–412.
40. Akitomo S, Egi Y, Fujii T, Banno Y, Sakamoto K. Genome-wide microarray screening for *Bombyx mori* genes related to transmitting the determination outcome of whether to produce diapause or nondiapause eggs. Insect Science,2017:24(2):187–196.
41. Nartey MA, Sun X, Qin S, Hou CX, Li MW. CRISPR/Cas9-based knockout reveals that the clock gene timeless is indispensable for regulating circadian behavioral rhythms in *Bombyx mori*. Insect Science,2021:28(5):1414–1425.
42. Homma S, Murata A, Ikegami M, Kobayashi M, Yamazaki M, Ikeda K, *et al*. Circadian clock genes regulate temperature-dependent diapause induction in silkworm *Bombyx mori*. Frontiers in Physiology,2022:13:863380.
43. Kobayashi M, Oshiki T, Hasegawa K. Studies on the environmental regulation of diapause in the silkworm, *Bombyx mori*. Journal of Sericultural Science of Japan,1986:55:1–10.
44. Yasukochi Y, Ashakumary LA, Wu C, Yoshido A, Nohata J, Mita K. Genome-wide analysis of microsatellite polymorphism in the silkworm, *Bombyx mori*. Genetics,2006:173(1):125–138. https://doi.org/10.1534/genetics.105.053876
45. Ikeno T, Katagiri C, Numata H. GABAergic regulation of photoperiodic diapause induction in the silkworm, *Bombyx mori*. Journal of Experimental Biology,2010:213:1983–1988. https://doi.org/10.1242/jeb.040105
46. Liu Z, Idris NFB, Liu L, Hou C, Yang C, Zhan C, *et al*. BmSV2A and BmSV2B are involved in regulating GABAergic neuron-related gene expression in the silkworm, *Bombyx mori*. Insects,2025:16(3):251. https://doi.org/10.3390/insects16030251
47. Kikuchi K, Kato M. Genetic analysis of voltinism in the silkworm, *Bombyx mori*. Japanese Journal of Genetics,1978:53:1–10.
48. Yasukochi Y. A second-generation linkage map of the silkworm, *Bombyx mori*, covering all chromosomes based on microsatellite markers. Genetics,1998:150(4):1513–1525.
49. Su ZH, Mita K, Shimada T, Kusuda R, Yamamoto M, Yamashita O. Expression analysis and functional identification of several genes related to diapause in *Bombyx mori*. Archives of Insect Biochemistry and Physiology,2019:100(4):e21537. https://doi.org/10.1002/arch.21537
50. Sonobe H, Maotani K, Nakajima H. Studies on embryonic diapause in the pnd mutant of the silkworm, *Bombyx mori*: genetic control of embryogenesis. Journal of Insect Physiology,1986:32(3):215–220. https://doi.org/10.1016/0022-1910(86)90061-2
51. Yamamoto T, Gamo T, Hirobe T. Genetical studies of the pigmented and non-diapausing egg mutant in *Bombyx mori*. Journal of Sericultural Science of Japan,1978:47(3):181–185.
52. Yamada H, Kihara S, Niimi T, Kadono-Okuda K, Yaginuma T. Expression dynamics and functional characterization of the pigmented and non-diapausing egg gene (pnd) and pnd-2, responsible for the initiation of embryonic diapause in the silkworm, *Bombyx mori*. bioRxiv, 2025. https://doi.org/10.1101/2025.08.17.667111
53. Li Z, Dai F, Xiang Z. Genetic mapping and analysis of the silkworm, *Bombyx mori*: from classical linkage maps to genome-based approaches. Insect Science,2015:22(3):327–340. https://doi.org/10.1111/1744-7917.12174
54. Xu S, Hu Z, Zhang Q. A Bayesian approach to QTL mapping with sex-specific recombination rates. Genetics,2011:187(3):977–989.
55. Zhang Y, She H, Wang S, Cui Q, Zhang W, Tang S, *et al*. BmTrpA1-mediated ILP/AKT/FOXO signaling underlies temperature-dependent diapause in silkworms, *Bombyx mori*. Journal of Insect Physiology,2026:169:104939. https://doi.org/10.1016/j.jinsphys.2026.104939
56. Yamamoto K, Nohata J, Kadono-Okuda K, Narukawa J, Sasanuma M, Sasanuma S, *et al*. A BAC-based integrated linkage map of the silkworm *Bombyx mori*. Genome Biology,2008:9:R21. https://doi.org/10.1186/gb-2008-9-1-r21
57. Takahashi KH, Takahashi K. Molecular mechanisms underlying the hormonal regulation of diapause in the silkworm, *Bombyx mori*. Journal of Insect Physiology,2013:59(4):377–384.
58. Sasibhushan S, Rao CGP, Ponnuvel KM. Genome-wide microarray-based expression profiles during early embryogenesis in diapause-induced and non-diapause eggs of polyvoltine silkworm *Bombyx mori*. Genomics,2013:102(4):379–387. https://doi.org/10.1016/j.ygeno.2013.07.007
59. Tsuchiya R, Yamamoto K, Shiotsuki T. Dopaminergic regulation of diapause hormone expression in the silkworm, *Bombyx mori*. Insect Biochemistry and Molecular Biology,2020:120:103351. https://doi.org/10.1016/j.ibmb.2020.103351
60. Yamamoto K, Fujii T, Shiotsuki T, Ishikawa Y. Embryonic thermosensitive TRPA1 determines transgenerational diapause phenotype of the silkworm, *Bombyx mori*. Proceedings of the National Academy of Sciences of the United States of America,2014:111(51):E5578–E5583. https://doi.org/10.1073/pnas.1414518111
61. Yamamoto K, Zhang P, Miura K, Shiotsuki T. Temperature-dependent regulation of diapause through dopamine signalling in the silkworm, *Bombyx mori*. Journal of Experimental Biology,2016:219:363–371. https://doi.org/10.1242/jeb.130252
62. Shimizu I. Photoperiodic induction in the silkworm, *Bombyx mori*, reared on artificial diet: evidence for extraretinal photoreception. Journal of Insect Physiology,1982:28(10):841–846. https://doi.org/10.1016/0022-1910(82)90096-8
63. Michaud MR, Denlinger DL. Shifts in the carbohydrate, polyol, and lipid metabolism associated with rapid cold-

hardening and diapause in flesh flies. Journal of Comparative Physiology B,2007:177:753–763. https://doi.org/10.1007/s00360-007-0174-0
64. Ito Y, Kobayashi I, Yamashita O. Changes in lipid composition during diapause and development of the silkworm, *Bombyx mori*. Journal of Insect Physiology,2012:58(7):987–994.
65. Ohta K, Saito T, Takeda M. Effects of artificial diet rearing on lipid metabolism and fatty acid composition in the silkworm, *Bombyx mori*. Journal of Insect Biotechnology and Sericology,2015:84:1–10.
66. Kang K, Pulver SR, Panzano VC, Chang EC, Griffith LC, Theobald DL, *et al*. Analysis of *Drosophila* TRPA1 reveals an ancient origin for human chemical and cold sensing. Nature,2010:464:597–600. https://doi.org/10.1038/nature08848
67. Shimizu I, Hasegawa K, Fujii T. Vitamin A deficiency affects photoreception and photoperiodic response in the silkworm, *Bombyx mori*. Journal of Insect Physiology,1988:34(7):563–568.
68. Shimizu I, Hasegawa K, Yamamoto Y. Transgenerational effects of carotenoid deficiency on photoreception and photoperiodic response in the silkworm, *Bombyx mori*. Journal of Insect Physiology,1992:38(9):675–681.
69. Tsuchida K, Yoshitake N. Effect of different artificial diets on diapause induction under controlled temperature and photoperiod in the silkworm, *Bombyx mori* L. Physiological Entomology,1983:8:333–338.
70. Shimizu I, Hasegawa K. Photoperiodic induction of diapause in the silkworm, *Bombyx mori*: location of the photoreceptor using a chemiluminescent paint. Physiological Entomology,1988:13:81–88.
71. Shimizu I, *et al*. Molecular cloning of *Bombyx* cerebral opsin (Boceropsin) and cellular localization of its expression in the silkworm brain. Biochemical and Biophysical Research Communications,2001:287:27–34.
72. Hasegawa K, Shimizu I. *In vivo* and *in vitro* photoperiodic induction of diapause using isolated brain–suboesophageal ganglion complexes of the silkworm, *Bombyx mori*. Journal of Insect Physiology,1987:33:959–966.
73. Shimizu I, Kato M. Carotenoid function in the photoperiodic induction of the silkworm, *Bombyx mori*. Photobiochemistry and Photobiophysics,1984:7:47–52.
74. Minke B. The history of the *Drosophila* TRP channel: the birth of a new channel superfamily. Journal of Neurogenetics,2012:24(4):216–233. https://doi.org/10.3109/01677063.2010.514369
75. Tsukamoto H. Thermodynamic evaluation of bistable pigment photoconversion in invertebrate visual photoreception. Biophysics,2014:10:45–54. https://doi.org/10.2142/biophysics.10.45
76. Saunders DS. Dormancy, diapause, and the role of the circadian system in insect photoperiodism. Annual Review of Entomology,2020:65:373–389. https://doi.org/10.1146/annurev-ento-011019-025116
77. Kawamoto T, Shimajiri Y, Shoji K, Kiuchi T. Genome-wide discovery of clock gene expression in *Bombyx mori*. PLOS ONE,2019:14(12):e0216048. https://doi.org/10.1371/journal.pone.0216048
78. Hidalgo S, Chiu JC. Integration of photoperiodic and temperature cues by the circadian clock to regulate insect seasonal adaptations. Journal of Comparative Physiology A,2024:210(4):585–599.
79. Pittendrigh CS, Minis DH. The entrainment of circadian oscillations by light and their role as photoperiodic clocks. American Naturalist,1964:98(902):261–294.
80. Tsuchiya R, Kaneshima A, Kobayashi M, Yamazaki M, Takasu Y, Sezutsu H, *et al*. Maternal GABAergic and GnRH/corazonin pathway modulates egg diapause phenotype of the silkworm *Bombyx mori*. Proceedings of the National Academy of Sciences of the United States of America,2021:118(1):e2020028118.
81. Liu L, Zhang P, Gao Q, Feng X, Han L, Zhang F, *et al*. Comparative transcriptome analysis reveals bmo-miR-6497-3p regulates circadian clock genes during the embryonic diapause induction process in bivoltine silkworm. Insects,2021:12(8):739.
82. Jiang X, Yang JW, Shen ZF, Chen YJ, Shi LG, Zhou NM. Agonist-mediated activation of *Bombyx mori* diapause hormone receptor signals to extracellular signal-regulated kinases 1 and 2 through Gq-PLC-PKC-dependent cascade. Insect Biochemistry and Molecular Biology,2016:75:78–88.
83. Shen Z, Jiang X, Yan L, Chen Y, Wang W, Shi Y, *et al*. Structural basis for the interaction of diapause hormone with its receptor in the silkworm, *Bombyx mori*. The FASEB Journal,2018:32(3):1338–1353.
84. Hasebe M, Sato M, Ushioda S, Kusuhara W, Kominato K, Shiga S. Significance of the clock gene period in photoperiodism in larval development and production of diapause eggs in the silkworm *Bombyx mori*. Journal of Insect Physiology,2024:153:104615.
85. Chen L, Zhang Z, Chen K, Yu Y, Hu B, Song H, *et al*. Transcriptional dynamics induced by diapause hormone in the silkworm, *Bombyx mori*. Biology,2022:11(9):1313.
86. Nakagaki M, Takei R, Nagashima E, Yaginuma T. Cell cycles in embryos of the silkworm, *Bombyx mori*: G2-arrest at diapause. Roux's Archives of Developmental Biology,1991:200:223–229.