***Review Article***

**20-Hydroxyecdysone in Silkworm Biology: Molecular Mechanisms of Biosynthesis, Development, and Biotechnological Applications**

**ABSTRACT**

20-Hydroxyecdysone (20E), a crucial steroid hormone in insects, plays a multifaceted role in regulating the developmental, physiological, and metabolic processes of the silkworm *Bombyx mori*. This review comprehensively covers its function across distinct developmental stages—embryonic, larval, and pupal. During embryogenesis and larval development, 20E biosynthesis depends on the maternal 3-epimerization pathway and key enzymes such as ecdysone oxidase (BmEO) and 3DE-3β-reductase. Functional studies show that disruption of these genes results in reduced ecdysone levels, developmental arrest, and decreased hatching rates. Notably, noppera-bo (nobo-Bm), a glutathione S-transferase gene involved in ecdysteroidogenesis, shows over 10-fold higher expression in the ovary, and its loss-of-function mutations cause larval lethality due to impaired sterol utilization. Beyond development, 20E plays a vital role in immune modulation by inducing antimicrobial peptides *via* the Broad-Complex Z2 (Br-C Z2) transcription factor and facilitates genital disc differentiation through the MAPK signalling pathway. In applied sericulture, the expression of the baculovirus-derived EGT gene in transgenic silkworms suppresses 20E levels, leading to prolonged larval feeding, inhibition of pupation, and a significant increase in cocoon shell ratio, thereby enhancing silk yield. Collectively, these insights highlight the central regulatory role of 20E across silkworm development and physiology, and underscore its biotechnological potential for improving sericultural productivity.

**Key words:** Diapause, silkworm, biosynthesis, maternal, 20-Hydroxyecdysone, ecdysteroids

1. **INTRODUCTION**

Hormones are the chemical messengers secreted by endocrine system. They play crucial role in regulating various physiological processes in insects (Lafont and Koolman1984). Ecdysteroids are the arthropod steroid hormones, where steroid refers to organic compounds having four fused carbon rings that are produced in the body. The steroids which act as hormones are called steroid hormone. They may be from animal origin (zooecdysteroids), plant origin (phytoecdysteroids) or fungi origin (mycoecdysteroids) (Baruah, 2020). Ecdysteroids in insects regulate various activities like development, moulting, metamorphosis and to some extent reproduction (Niu *et al*., 2025). In insects two important ecdysteroidogenic hormones necessary for growth and development are ecdysone(E) and 20-hydroxyecdysone (20E) (Makka, 2002). Ecdysone most commonly known as moulting hormone or alpha-ecdysone (α-Ecdysone) is the prohormone or precursor for the synthesis of its biologically active form 20E. Hydroxylation at carbon-20 yields 20E or beta-ecdysone (β-Ecdysone) which acts on the genes and gene factors responsible for development and metamorphosis. (Baruah, 2020). The 20-hydroxyecdysone (20E) steroid hormone plays critical role in insect development and physiology. 20E is important for inducing ecdysis and metamorphosis in Odonata (Okude *et al*., 2025). It regulates the formation and growth of imaginal discs such as wing and genital discs, that are essential for adult structure formation. Additionally, 20E is involved in controlling of diapause and embryogenesis, ensuring proper developmental transitions (Tawfik *et al.*, 2002). The silk gland undergoes programmed cell death after spinning. During pupation, the silk gland, which is no longer needed, undergoes programmed cell death and autophagy. The epithelial cells of the gland begin to degrade due to hormonal cues, particularly the action of 20-hydroxyecdysone, which promotes calcium mobilization and triggers apoptosis through caspase activation pathways (Wang et al., 2016). During pupal-adult metamorphosis, 20E facilitates the degradation of larval tissues, making way for adult organ development. In *Chilo suppressalis* 20E regulates pupal fatty acid metabolism thereby maintaining the lipid metabolic homeostasis (Gong *et al*., 2021). “Beyond development, 20E also modulates the innate immune response by stimulating the production of various antimicrobial peptides (AMPs). These include lysozymes, which hydrolyze peptidoglycan in bacterial cell walls; gloverins, which inhibit bacterial outer membrane synthesis; and cecropins, which are effective against a broad spectrum of microbes including gram-positive and gram-negative bacteria as well as fungi” (Wang *et al.*, 2008).

1. **Biosynthesis pathway of ecdysteroids**

The head of insects consist of neurosecretory cells (NSCs) present in either half of the brain called intercerebralis. NSCs upon receiving stimulus from environment or the body in turn stimulate corpora cardiaca to secrete a neuropeptide, prothoracicotropic hormone (PTTH). PTTH act on prothoracic glands and regulate synthesis of ecdysone. The dietry sterols are incorporated into ecdysteroidogenic organs like prothoracic gland and ovaries and undergo series of hydroxylation reactions (Nagasawa *et al.,* 1986, Mizuguchi *et al.,* 1987, Sakurai *et al.,* 1989).



**Fig.1: Ecdysteroidogenesis Pathway and Mechanism of 20-Hydroxyecdysone Biosynthesis in Insects**

The ecdysteroids biosynthesis pathway in prothoracic glands is well documented. It starts with conversion of cholesterol to 7‐dehydrocholesterrol (7dC). This step is mediated by *Rieske oxygenase* enzyme, designated Neverland (*Nvd*). Between 7dC and first upstream compound there is highly characteristic ecdysteroid structure, diketol, which lays the vital “black box.” Black box includes series of uncharacterized and reactions that ultimately result in oxidation of 7dC to diketol. Differential expression in *B. mori* demonstrated involvement of *CYP307A1* (*Spo*) in the black box, which appears to the rate‐limiting enzyme (Marchal *et al.,* 2011). Non‐molting glossy (*Nm‐g* in *B. mori*) was reported to be involved in the initial steps of ecdysteroid biosynthesis. Hydroxylation at C25, C22 and C2 is mediated by *CYP306A1* (*Phm*), *CYP302A1* (*Dib*) and *CYP315A1* (Sad), respectively (Spindler *et al.,* 2017) and the final hydroxylation is catalyzed by fourth hydroxylase, *CYP314A1* (*Shd*) or ecdysone 20‐hydroxylase (*E20OHase*) from ecdysone to 20‐hydroxyecdysone (20E) in the peripheral target tissues of *B. mori*. Subsequently, 20E binds with the ecdysone receptor (*EcR*), that forms a dimer with Ultraspiracle (*USP*) (Lammerding-Köppel *et al.,* 1998). “This complex further binds to ecdysone response element (*EcRE*) and regulate transcription of downstream target genes. Three levels of transcription occur. First, transcription of early genes, such as *E75*, *E74* and broad-complex (*Br‐C*). Early‐late genes, such as hormone receptor 39 (*HR39*) and 3 (*HR3*) are then expressed” (Huet *et al.,* 1995). “Subsequently, these early and early‐late genes induce transcription of late genes, such as fushi tarazu transcription factor 1 (*FTZ‐F1*)” (Gu *et al.,* 2021).

1. **20E biosynthesis during embryonic stage and larval development**

Ecdysteroids are mostly synthesized from prothoracic glands (PGs). However, studies have found the early embryonic development in insects was regulated independently by PGs (Rees, 1995). In addition, embryonic active ecdysteroids are present before PGs formation indicating that those ecdysteroids are of maternal origin and titre of which is important for the normal development of embryo, suggesting that there must be redundancy in synthesis of ecdysteroids in mother moth (Rubenstein, 1982). Therefore, various pathways are mediating synthesis of maternal ecdysteroids, of which 3-epimerisation pathway is among the important pathway (Hoffmann and Lagueux, 1985).

 20E is firstly converted into 3-dehydroecdysone (*3D20E* or *3DE*) by gene ecdysone oxidase (*EO*) then *3DE-3α-reductase* catalyze 3DE to 3-epiecdysone. This intermediate product, 3DE (or 3D20E) also be reduced reversibly to active ecdysone by *3DE-3β-reductase.* Previous studies shown that most of the lepidopteran insects can store large amount of 3DE in their prothoracic glands at immature stages (Kiriishi, 1990). When insects need, 3DE is released and reduced to ecdysone rapidly (Tan *et al*., 2008). “The function of the silkworm *BmEO* and *3DE-3β-reductase* and pathway involvedin strain N4 was demonstrated how the silkworm utilizes this pathway to regulate the ecdysone titer of the embryo” (Milner, 1985).

“Temporal expression patterns of genes involved in pathway showed that, on day one of pupal stage, the expression of *BmEO* gene was detected. The transcription level gradually increased with the development and reached peak at 8th Day after pupation, when ecdysone concentration became almost undetectable indicating that *BmEO* may play key role in converting ecdysone to 3DE during pupal stage. However, 3DE-3α-reductase gene level was low throughout the pupal stage” (Wang *et al*., 2018).

“The spatial expression profiles of *BmEO* gene are different in male or female tissues of the pupa at the 7th Day” (Kiguchi *et al.,* 1981). “The transcription signals of the gene were detected in fat body, hemocyte, gonad and weak signals in the pupal head. Furthermore, no sex bias was observed for *BmEO* gene expression in fat body, hemocyte and head” (Richards *et al.,* 1987). “However, *BmEO* gene was predominantly expressed in the female ovary and the expression level of the gene in ovary was almost 10 times higher than that in testis. The expression divergence of *BmEO* gene between sexes indicates that the gene might take part in female biological process” (Hanaoka and Ohnishi 1974). Overall, the *3DE-3β-reductase* gene had much higher expression level than other two genes (*EO* and *3DE-3α-reductase*) and it was expressed from 1st Day after oviposition and reached a peak at 3rd Day after oviposition. Then, the expression level decreased gradually to the basal level as the embryonic development continued. Interestingly, the gene is just expressed ahead of the increase of ecdysone titer. This timing ensures conversion of stored 3DE into active ecdysone right when embryos need it for key processes like segmentation and organogenesis (Yamada and Sonobe 2003, Shen *et al.,* 2018).

1. **The function of *BmEO* gene during the pupal stage of the silkworm**

*“BmEO* gene was prominently expressed in the pupal ovary. Knocking-down of mRNAs levels of *BmEO* gene (Day 7 of pupa) showed phenotypic variation examined between control and RNAi pupa” (Fakuda *et al*., 1940). “*BmEO*-dsRNA injected silkworms grew and developed normally like EGFP-dsRNA injected silkworms. *EO* can convert ecdysone into 3DE. Therefore, the fluctuation of 3DE was examined in the ovary of the female silkworm after RNAi experiment. The concentration of the 3DE extracted from RNAi silkworm was significantly reduced, indicating *EO* could take part in the accumulation of 3DE in the silkworm eggs” (Tsuchida *et al.,* 1987; Wang et al. 2018).

“In addition, the development of eggs laid by *EO* RNAi moths was arrested at organogenesis stage. Additionally, the product of *EO* is 3DE which also can be synthesized into ecdysone” (Okamoto *et al*., 2007). “Thus, 3DE was injected to rescue the *BmEO* RNAi eggs. The newly laid eggs of the *BmEO*-RNAi adults were collected and then 3DE was injected into the eggs. Injection with 3DE significantly elevated the hatching rate. Meanwhile, injection with 3DE could increase the 20E level at the 2nd Day after treatment. Taken together, the results indicated that maternal *BmEO* is important for the biosynthesis of 20E during the embryonic development” (Takeuchi *et al.,* 2000, Kiguchi *et al.,* 1981, Sonobe *et al.,* 2001)

1. **The function of *3DE-3β-reductase* gene during the embryonic development**

“3DE can be directly converted into ecdysone by *3DE-3β-reductase*. Two days after RNAi injection, the transcription level of the gene significantly decreased. Interestingly, similar to the maternal *BmEO* RNAi experiments the hatching rate of the eggs injected with *3DE-3β-reductase* dsRNA was also significantly lower. In addition, the down-regulated expression of the *3DE-3β-reductase* gene resulted in the significantly declined 20E level compared with the control at embryonic stages.Furthermore, the decreased 20E titer significantly affect the normal development of embryos. Development of about 75% embryos after RNAi treatment was arrested at the stage 20 (segmentation of head and thorax) or stage 21 (early blastokinesis). The stages were determined based on a previous study” (Miya, 1984). “Furthermore, 20E treatment could rescue the abnormal development. Taken together the results revealed that ecdysone 3-epimerization pathway might have been involved in the synthesis of molting hormone during embryonic development of the silkworm” (Weirich *et al*., 1993).

1. **Role of *glutathione S-transferase gene* *noppera-bo***

*Noppera-bo* (*nobo*)is a gene involved in incorporation of dietary sterols form food to ecdysteroidogenic tissue and utilization of cholesterol metabolite product (Nagata *et al.,* 1987, 1992). *nobo* gene encodes glutathione S-transferase (*GSTs*). *GSTs* catalyse the conjugation of glutathione to a wide variety of endogenous and exogenous electrophilic compounds thereby protecting macromolecules from oxidative stress (Marchal *et al.,* 2012). “Phylogenetic analysis suggests that GST genes belonging to the *nobo* family are also found in other dipteran and lepidopteran species; like *GSTe8* in the mosquito Anopheles gambiae and GSTe7 in the silkworm *B. mori”* (Yu *et al.,* 2008). “To avoid using a confusing numbering system to represent the orthologous GST genes of different insect species, a unique subfamily name, *noppera-bo* (*nobo*) was proposed, for these orthologs”(Enya *et al.,* 2014).

“The qRT-PCR experiments revealed that *nobo-Bm* in w1 pnd strain was highly expressed in the PGs and weaker expression in rest other tissues. Temporally, changes in the *nobo-Bm* expression level in the PG closely correlated with changes in the ecdysteroid titer of the hemolymph during 4th and 5th instar larval development which was similar to the expression pattern of other ecdysteroidogenic genes in *B. mori”* (Namiki *et al.*, 2005; Ono *et al.*, 2006; Yoshiyama *et al.*, 2006). These results support hypothesis that *nobo-Bm* is involved in the regulation of ecdysteroid biosynthesis in the PG in *B. mori* (Ebihara and Niwa 2023). To determine whether the *nobo-Bm* gene plays an essential role in *B. mori* development, a *nobo-Bm* mutant strain was generated using TALEN-mediated gene targeting technology (Cermak *et al.,* 2011). “The mRNAs encoding the TALEN pairs were microinjected into the embryos to induce a double-strand break within the 2nd exon. Eventually a *nobo-Bm* mutant strain was isolated that lacked 87 bp and had also exogenously added 2 bp in the 2nd exon and 2nd intron of the wild type *nobo-Bm* locus. This deletion allele was called *nobo-Bm****Δ****85*, lacks the exon - intron junction between the 2nd exon and the 2nd intron. The predicted protein encoded by the *nobo-Bm****Δ****85* allele lacks half of the GST N-terminal domain and the entire GST C-terminal domain, suggesting that the *nobo-Bm****Δ****85* allele is functionally null” (Takasu, 2013).

“Under regular rearing conditions, *nobo-Bm****Δ****85* homozygous mutants died at the 2nd instar stage; they never grew into the 3rd instar stage or beyond, while wild type and *nobo-Bm****Δ****85* heterozygous animals exhibited no developmental phenotypes. Interestingly, the cuticle of *nobo-Bm****Δ****85* homozygous mutants eventually became glossier than that of the control animals. This glossy cuticle phenotype is reminiscent of the classical *B. mori* mutant non-molting glossy (nmg), which is loss-of-function allele of the ecdysteroidogenic gene nm-g/shroud” (Enya *et al*., 2015, Ayres *et al.,* 2013, Nagata *et al.,* 1987).

“The sterol levels were examined in the PG cells of *nobo-Bm*D85 homozygotes. Mass spectrometric analysis revealed that the PGs from *nobo-Bm*D85 homozygotes second instar larvae accumulated significantly higher levels of 7dC, a cholesterol metabolite, than the PGs from control animals (wild type and *nobo-Bm*D85 heterozygotes). There were no statistically significant differences in the levels of cholesterol and b-sitosterol between the control and *nobo-Bm*D85 homozygous PG cells. These results suggest that nobo, *nobo-Bm* is essential for development and for the regulation of 7dC utilization in the PGs of *B. mori”* (Enya *et al*., 2015, Nagata *et al.,* 1992)*.*

1. **20-Hydroxyecdysone regulates lysozyme transcription through *Br-C Z2* gene**

“In insects, the Broad-Complex (*Br-C*) gene regulates the growth and metamorphosis. Most of the *Br-C* isoforms contain two highly conserved domains including a BTB (bric-a-brac, tramtrack and Broad-Complex) domain for protein–protein interaction and a pair of C2H2 zinc fingers. The developmental role of Br-C gene has been well studied in different insect species. In *Drosophila melanogaster* it controls the production of ecdysone-inducible genes” (Karim *et al.*, 1993). “It stimulates the formation of both wing disc and epidermis in *Manduca sexta* pupae” (Zhou *et al.*, 2001). “While, in *B. mori Br-C Z2* has been found to induce the expression of WCP10 in wing disc” (Ma *et al.,* 2019)

*“BmBR-C Z2* was expressed high in the testis, head and hemocytes were higher compared with that in other tissues. 20E was administered into 5th instar silkworm larvae to determine its effect on the expression of *BmBR-C Z2*. Hemocyte samples collected at different time points revealed that *BmBR-C Z2* mRNA level was remarkably high at 3, 6, 12, 24, 48 hours; however, it was greatest after 6 h of 20E administration. Furthermore, approximately similar *BmBR-C Z2* expression patterns were observed in the BmE cells upon 20E treatment suggesting that 20E regulates expression of *BmBR-C Z2.* To determine whether or not the activation of *BmBR-C Z2* can increase the production of AMPs, over-expression of *BmBR-C Z2* in BmE cells was performed using pSL1180-A4-SV40 DsRed-BmBr-C-Z2 vector. Localization of *BmBR-C Z2* protein in the nucleus of the normal cultured and transfected cells. Total RNA analysis revealed that several AMPs expression was up-regulated in the transfected cells compared with the control group. This implies that *BmBR-C Z2* is part of the immune regulatory pathway, possibly preparing the insect for immune challenges during hormonally sensitive periods (like molting, when cuticle defenses are weakened)” (Zhang *et al*., 2017).

1. **20E significance in sex expression**

“The effects 20E on genital disk growth was evaluated using male disks, because they were bigger in size and therefore easier to collect without damage than female disks. The male genital disk develops into inner and outer sexual organs” (Koyama *et al*., 2013 Nijhout and Callier, 2015, Nijhout *et al*., 2007). The upper region in develops into the external genitalia (penis), while the lower region into the internal genitalia (ejaculatory duct, seminal vesicle and accessory gland) (Swevers *et al.,* 2003, Suzuki *et al.*, 2005). During pupal-adult development, the ejaculatory duct and accessory gland elongate and this elongation can be induced *ex vivo* by 20E (Moriyama *et al.,* 2016). “Therefore, the length of the disk was used as an index of disk development. 20E induced remarkable elongation of the disks, especially at the presumptive ejaculatory duct and accessory gland. Interestingly, 20E also promoted protein synthesis” (Shinbo *et al.,* 1989).

“To explore the signaling pathway involved in the induction of disk elongation, the MAPK pathway hypothesized to plays a key role, as this pathway is widely used in the regulation of cell proliferation and differentiation” (Elmogy *et al.*, 2006; Manaboon *et al.*, 2009). “Thus, when MEK inhibitor, U0126, used, it inhibited both 20E-induced elongation and protein synthesis of the genital disks in a dose-dependent manner, suggesting that 20E induces the development of the genital disk through the MAPK pathway” (Fujinaga *et al*., 2017).

1. **20E expression andcocoon trait**

“Ecdysteroid UDP glucosyltransferase (EGT) is baculovirus-encoded protein, secreted by alfalfa looper, *Autographa californica* nuclear polyhedrosis virus (AcMNPV) and transfers UDP-glucoside to the hydroxyl group of C-22 in 20E to generate 20E 22-β-D-pyran glucoside” (Evans and Reilly 1998, Shikata *et al.,* 1998). “The EGT secreted by the virus upon infection can inactivate 20E in the host, thereby hindering normal moulting/eclosion. The GAL4/UAS system, used to express EGT from the LP3 promoter in fat body of last-instar silkworm. The EGT protein secreted into the silkworm haemolymph maintained 20E at low level, allowing to investigate role of 20E in the utilization of available nutrients for silk production. "UAS" stands for "Upstream Activating Sequence," that is a cis-acting regulatory element that is targeted by Gal4 for activating the expression of downstream genes” (Chaturvedi *et.al.,* 2011).

Two genetic vectors were developed and inserted into silkworms. When hybrid offspring (E3G and E4G lines) were generated, they showed longer feeding and cocoon-spinning periods, resulting in higher silk output (especially cocoon shell weight and ratio). However, EGT overexpression also blocked metamorphosis, preventing larvae from turning into pupae. In E4G, female larvae mostly failed to spin cocoons and died after feeding, leading to lower cocooning success compared to E3G. Wang *et al.,* (2010) showed that “the treatment of 20E decreases the consumption of food in silkworm larvae. ELISA tests showed that 20E levels in E3G/E4G larvae were consistently lower than in normal silkworms, confirming that EGT reduced 20E. E4G silkworms had more EGT and lower 20E than E3G. When 20E was fed to these silkworms, cocooning improved in females, but the overall silk ratio in males dropped. Earlier studies have reported that JH can inhibit 20E secretion during the early days of *B. mori* last (fifth)-instar larvae and further extend the duration of feeding and silk yields” (Akai *et al.,* 1985, 1971, Sakurai *et al.,* 1989).

Overall, reducing 20E through EGT expression extended larval feeding time, blocked pupation and increased silk production. This opens up possibilities for developing genetically modified silkworms that produce more silk and don’t need pupal killing, as they never reach the pupal stage (Gu and Chow, 1993). It can be inferred that expression of baculovirus-derived EGT 20E, delay metamorphosis and extend the larval feeding duration and also cocoon-spinning, thereby increasing silk yield. Excessive EGT expression however, especially in females (as seen in E4G), block the cocooning causing death, showing inverse relation between silk production and survival. This strategy genetically offers potential for further development of high yielding, non-pupating silkworm strains, thereby reducing the need for pupal killing in sericulture (Sun *et al.,* 2016).

1. **CONCLUSION**

20-hydroxyecdysone (20E) is a key hormone in *B. mori*, regulating embryonic development, molting, metamorphosis, immunity, sex differentiation and silk production. During early embryogenesis, maternally derived 20E is synthesized via the ecdysone 3-epimerization pathway involving enzymes like ecdysone oxidase and *3DE-3β-reductase*, essential for proper development and hatching. The *noppera-bo* (*nobo-Bm*) gene also supports ecdysteroid biosynthesis by aiding sterol metabolism in prothoracic gland. 20E additionally enhances immune response by inducing antimicrobial peptide genes through the *Br-C Z2* transcription factor and promotes genital disk growth via the MAPK pathway. Importantly, genetic suppression of 20E using a viral *EGT* gene in transgenic silkworms prolongs larval feeding, blocks pupation and increases silk yield especially cocoon shell ratio offering new potential to enhance sericulture productivity and eliminate the need for pupal killing.

**Disclaimer (Artificial intelligence)**

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

1. **REFERENCES:**
2. Akai, H., Kiguchi, K., & Mori, K. (1971). Increased accumulation of silk protein accompanies JH-induced prolongation of larval life in *Bombyx mori* L. Applied Entomology and Zoology, 6, 218–220.
3. Akai, H., Kimura, K., Kiuchi, M., & Shibukawa, A. (1985). Increase of silk production by repeated treatments with a juvenile hormone analogue. Journal of Sericultural Science of Japan, 54, 297–299.
4. Ayres, C., Müller, P., Dyer, N., Wilding, C., Rigden, D., & Donnelly, M. (2011). Comparative genomics of the anopheline glutathione S-transferase epsilon cluster. PLoS One, 6, 29237.
5. Baruah, J. P. (2020). Neuroendocrine system and its hormonal activities in silkworm *Bombyx mori* L. Journal of Academia and Industrial Research*,* 8(10), 170–172.
6. Cermak, T., Doyle, E., & Christian, M. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Research, 39, 82.
7. Chaturvedi, P., Misra, P., & Tuli, R. (2011). Sterol glycosyltransferases – The enzymes that modify sterols. Applied Biochemistry and Biotechnology, 165, 47–68.
8. Enya, S., Ameku, T., Igarashi, F., Iga, M., Kataoka, H., Shinoda, T., & Niwa, R. (2014). A Halloween gene *noppera-bo* encodes a glutathione S-transferase essential for ecdysteroid biosynthesis via regulating the behaviour of cholesterol in *Drosophila* melanogaster. Scientific Reports, 4, 6586–6592.
9. Ebihara, K., & Niwa, R. (2023). Compounds Inhibiting Noppera-bo, a Glutathione *S*-transferase Involved in Insect Ecdysteroid Biosynthesis: Novel Insect Growth Regulators. Biomolecules, 13(3), 461. <https://doi.org/10.3390/biom13030461>
10. Enya, S., Daimon, T., Igarashi, F., Kataoka, H., Uchibori, M., Sezutsu, H., Shinoda, T., & Niwa, R. (2015). The silkworm glutathione S-transferase gene *noppera-bo* is required for ecdysteroid biosynthesis and larval development. Insect Biochemistry and Molecular Biology, 61, 1–7.
11. Evans, O. P., & Reilly, D. R. (1998). Purification and kinetic analysis of a baculovirus ecdysteroid UDP-glucosyltransferase. Biochemical Journal, 332, 807–808.
12. Fujinaga, D., Kohmura, Y., Okamoto, N., Kataoka, H., & Mizoguchi, A. (2017). Insulin-like growth factor (IGF)-like peptide and 20-hydroxyecdysone regulate the growth and development of the male genital disk through different mechanisms in the silkmoth, *Bombyx mori* L. Insect Biochemistry and Molecular Biology, 87, 35–44.
13. Fukuda, S. (1940). Hormonal control of molting and pupation in the silkworm. Proceedings of the Imperial Academy, 8, 417–420.
14. Gong, Y. W., Sun, Y. J., Kuang, S. J., Ding, W. B., He, H. L., Gao, Q., *et al*. (2021). 20-Hydroxyecdysone regulates pupal fatty acid metabolism, maintaining lipid metabolic homeostasis in *Chilo suppressalis*. 2, 983-986
15. Gu, S. H., & Chow, Y. S. (1993). Role of low ecdysteroid levels in the early last larval instar of *Bombyx mori*. Experientia, 49, 806–809.
16. Gu, S., Chein, C., & Lin, P. (2021). Changes in expressions of ecdysteroidogenic enzyme and ecdysteroid signaling genes in relation to *Bombyx mori* L. embryonic development. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 335(5), 477–488.
17. Hanaoka, K., & Ohnishi, E. (1974). Changes in ecdysone titre during pupal-adult development in the silkworm, *Bombyx mori*. Journal of Insect Physiology, 20, 2375–2384.
18. Hoffmann, J. A., & Lagueux, M. (1985). Endocrine aspects of embryonic development in insects. Comprehensive Insect Physiology, Biochemistry and Pharmacology, 1, 435–460.
19. Huet, F., Ruiz, C., & Richards, G. (1995). Sequential gene activation by ecdysone in *Drosophila melanogaster*: The hierarchical equivalence of early and early late genes. Development, 121(4), 1195–1204.
20. Karim, F. D., Guild, G. M., & Thummel, C. S. (1993). The *Drosophila* Broad-Complex plays a key role in controlling ecdysone-regulated gene expression at the onset of metamorphosis. Development, 118, 977–988.
21. Kiguchi, K., & Agui, N. (1981). Ecdysteroid levels and developmental events during larval moulting in the silkworm, *Bombyx mori*. Journal of Insect Physiology, 27, 805–812.
22. Kiriishi, S., Rountree, D., Sakurai, S., & Gilbert, L. (1990). Prothoracic gland synthesis of 3-dehydroecdysone and its hemolymph 3β-reductase mediated conversion to ecdysone in representative insects. Cellular and Molecular Life Sciences, 46, 716–721.
23. Koyama, T., Mendes, C., & Mirth, C. K. (2013). Mechanisms regulating nutrition-dependent developmental plasticity through organ-specific effects in insects. Frontiers in Physiology, 4, 12.
24. Lafont, R., & Koolman, J. (1984). Ecdysone metabolism. Biosynthesis, metabolism and mode of action of invertebrate hormones. Springer, Berlin, Heidelberg, pp. 196–226
25. Lammerding-Köppel, M., Spindler-Barth, M., Steiner, E., Lezzi, M., Drews, U., & Spindler, K. D. (1998). Immunohistochemical localisation of ecdysteroid receptor and ultraspiracle in the epithelial cell line from *Chironomus tentans* (Insecta, Diptera). Tissue and Cell, 30, 187–194.
26. Ma, H., Abbas, N. M., Zhang, K., Hu, X., Xu, H., Liang, H., Kausar, S., Yang, L., & Cui, H. (2019). 20-Hydroxyecdysone regulates the transcription of the lysozyme via Broad-Complex Z2 gene in silkworm, *Bombyx mori* L. Developmental and Comparative Immunology, 94, 66–72.
27. Ma, H., Abbas, N. M., Zhang, K., Hu, X., Xu, H., Liang, H., Kausar, S., Yang, L., & Cui, H.
28. Makka, T., Seino, A., Tomita, S., Fujiwara, H., & Sonobe, H. (2002). A possible role of 20‐hydroxyecdysone in embryonic development of the silkworm *Bombyx mori*. Archives of Insect Biochemistry and Physiology, 51, 111–120.
29. Marchal, E., Badisco, L., Verlinden, H., Vandersmissen, T., Van Soest, S., Van Wielendaele, P., & Vanden Broeck, J. (2011). Role of the Halloween genes, *Spook* and *Phantom*, in ecdysteroidogenesis in the desert locust, *Schistocerca gregaria*. Journal of Insect Physiology, 57, 1240–1248.
30. Marchal, E., Verlinden, H., Badisco, L., Van Wielendaele, P., & Vanden Broeck, J. (2012). RNAi-mediated knockdown of *Shade* negatively affects ecdysone-20-hydroxylation in the desert locust, *Schistocerca gregaria*. Journal of Insect Physiology, 58, 890–896.
31. Milner, N., & Rees, H. (1985). Involvement of 3-dehydroecdysone in the 3-epimerization of ecdysone. Biochemical Journal, *231*, 369–374.
32. Miya, K. (1984). Early embryogenesis of embryonic lethal, kidney-shaped egg in *Bombyx mori* L. Zoological Science, *1*, 953–958.
33. Mizoguchi, A., Ishizaki, H., Nagasawa, H., Kataoka, H., Isogai, A., Tamura, S., Suzuki, A., Fujino, M., & Kitada, C. (1987). A monoclonal-antibody against a synthetic fragment of bombyxin (4k-prothoracicotropic hormone) from the silkmoth, *Bombyx mori* L.: Characterization and immunohistochemistry. Molecular and Cellular Endocrinology*, 51*, 227–235.
34. Moriyama, M., Osanai, K., Ohyoshi, T., Wang, H. B., Iwanaga, M., & Kawasaki, H. (2016). Ecdysteroid promotes cell cycle progression in the *Bombyx* wing disc through activation of c-Myc. Insect Biochemistry and Molecular Biology*, 70*, 1–9.
35. Nagasawa, H., Kataoka, H., Isogai, A., Tamura, S., Suzuki, A., Mizoguchi, A., Fujiwara, Y., Takahashi, S. Y., & Ishizaki, H. (1986). Amino acid sequence of a prothoracicotropic hormone of the silkworm *Bombyx mori*. Proceedings of the National Academy of Sciences, 83, 5840–5843.
36. Nagasawa, H., Kataoka, H., Isogai, A., Tamura, S., Suzuki, A., Mizoguchi, A., Fujiwara, Y., Takahashi, S. Y., & Ishizaki, H. (1986). Amino acid sequence of a prothoracicotropic hormone of the silkworm *Bombyx mori*. Proceedings of the National Academy of Sciences, 83, 5840–5843.
37. Nagata, M., Tsuchida, K., Orikasa, C., & Suzuki, A. (1992). Ecdysteroid secretion in the non-molting larvae of the *nm-g* mutant of the silkworm, *Bombyx mori*. Journal of Sericultural Science of Japan, 61, 400–406.
38. Nagata, M., Tsuchida, K., Shimizu, K., & Yoshitake, N. (1987). Physiological aspects of *nm-g* mutant: An ecdysteroid-deficient mutant of the silkworm, *Bombyx mori*. Journal of Insect Physiology, 33, 723–727.
39. Namiki, T., Niwa, R., Sakudoh, T., Shirai, K. I., Takeuchi, H., & Kataoka, H. (2005). Cytochrome P450 CYP307A1/Spook: A regulator for ecdysone synthesis in insects. Biochemical and Biophysical Research Communications, 337, 367–374.
40. Nijhout, H. F., & Callier, V. (2015). Developmental mechanisms of body size and wing–body scaling in insects. In Berenbaum, M. R. (Ed.), Annual Review of Entomology, 60, 141–156.
41. Nijhout, H. F., Smith, W. A., Schachar, I., Subramanian, S., Tobler, A., & Grunert, L. W. (2007). The control of growth and differentiation of the wing imaginal disks of *Manduca sexta*. Developmental Biology, 302, 569–576.
42. Niu, Y., Zhang, S., Shi, F., Zhao, Y., Li, M., Zong, S., & Tao, J. (2025). Transcriptome analysis identifies key genes in juvenile hormone and ecdysteroid signaling pathways and their roles in regulating reproductive system development of adult *Monochamus saltuarius*. International Journal of Biological Macromolecules, 295, 139634. [10.1016/j.ijbiomac.2025.139634](https://doi.org/10.1016/j.ijbiomac.2025.139634)
43. Okamoto, N., Yamanaka, N., Satake, H., Saegusa, H., Kataoka, H., & Mizoguchi, A. (2009). An ecdysteroid-inducible insulin-like growth factor-like peptide regulates adult development of the silkmoth, *Bombyx mori* L. FEBS Journal, 276, 1221–1232.
44. Okude, G., Ogihara, M. H., Moriyama, M., Yamagishi, T., Yamamoto, H., Fukatsu, T., & Futahashi, R. (2025). Identification of ecdysteroids and ecdysteroidogenic genes in dragonflies and damselflies. Scientific Reports, 15, 21971
45. Ono, H., Rewitz, K. F., Shinoda, T., Itoyama, K., Petryk, A., Rybczynski, R., Jarcho, M., Warren, J. T., Marques, G., Shimell, M. J., Gilbert, L. I., & O'Connor, M. B. (2006). *Spook* and *Spookier* code for stage-specific components of the ecdysone biosynthetic pathway in Diptera. Developmental Biology, 298, 555–570.
46. Qiao, L., Xiong, G., Wang, R. X., He, S. Z., Chen, J., Tong, X. L., *et al.* (2014). Mutation of a cuticular protein, BmorCPR2, alters larval body shape and adaptability in silkworm, *Bombyx mori* L. Genetics, 196, 1103–1115.
47. Rees, H. (1995). Ecdysteroid biosynthesis and inactivation in relation to function. European Journal of Entomology, *92*, 9–39.
48. Richards, G. (1978). The relative biological activities of α- and β-ecdysone and their 3-dehydro derivatives in the chromosome puffing assay. Journal of Insect Physiology, 24, 329–335.
49. Rubenstein, E. C., Kelly, T. J., Schwartz, M. B., & Woods, C. W. (1982). In vitro synthesis and secretion of ecdysteroids by *Drosophila melanogaster* ovaries. Journal of Experimental Zoology, 223, 305–308.
50. Sakurai, S., Okuda, M., & Ohtaki, T. (1989). Juvenile hormone inhibits ecdysone secretion and responsiveness to prothoracicotropic hormone in prothoracic glands of *Bombyx mori*. General and Comparative Endocrinology, 75, 222–230.
51. Sakurai, S., Warren, J. T., & Gilbert, L. I. (1989). Mediation of ecdysone synthesis in *Manduca sexta* by a hemolymph enzyme. Archives of Insect Biochemistry and Physiology, 10, 179–197.
52. Shikata M., Shibata, H., Sakurai, M., Sano, Y., Hashimoto, Y. & Matsumoto, T. (1998). The ecdysteroid UDP-glucosyltransferase gene of *Autographa californica* nucleopolyhedrovirus alters the moulting and metamorphosis of a non-target insect, the silkworm, *Bombyx mori* (Lepidoptera, Bombycidae). Journal of General Virology, 79(6), 1547–1551.
53. Sonobe, H., & Yamada, R. (2004). Ecdysteroids during early embryonic development in silkworm *Bombyx mori*: Metabolism and functions. Zoological Science, 21, 503–516.
54. Spindler, K. D., Koolman, J., Mosora, F., & Emmerich, H. (1977). Catalytical oxidation of ecdysteroids to 3-dehydro products and their biological activities. Journal of Insect Physiology, 23, 441–444.
55. Sun, W., Shen, Y. H., Zhou, L. X., & Zhang, Z. (2016). Ecdysone titer determined by 3DE-3β-reductase enhances the immune response in the silkworm. Journal of Immunology, 196, 1646–1654.
56. Swevers, L., & Iatrou, K. (2003). The ecdysone regulatory cascade and ovarian development in lepidopteran insects: Insights from the silkmoth paradigm. Insect Biochemistry and Molecular Biology, 33, 1285–1297.
57. Takasu, Y., Sajwan, S., Daimon, T., Osanai-Futahashi, M., Uchino, K., Sezutsu, H., Tamura, T., & Zurovec, M. (2013). Efficient TALEN construction for *Bombyx mori* gene targeting. PLoS One, 8, 73458.
58. Takeuchi, H., Chen, J. H., O'Reilly, D. R., Rees, H. H., & Turner, P. C. (2000). Regulation of ecdysteroid signalling: Molecular cloning, characterization and expression of 3-dehydroecdysone 3 alpha-reductase, a novel eukaryotic member of the short-chain dehydrogenases/reductases superfamily from the cotton leafworm, *Spodoptera littoralis*. Biochemical Journal, 349, 239–245.
59. Tan, A., & Palli, S. R. (2008). Ecdysone receptor isoforms play distinct roles in controlling molting and metamorphosis in the red flour beetle, *Tribolium castaneum*. Molecular and Cellular Endocrinology, 291(2), 42–49.
60. Tawfik, A. I., Tanaka, Y., & Tanaka, S. (2002). Possible involvement of ecdysteroids in embryonic diapause of *Locusta migratoria*. Journal of Insect Physiology, 48, 743–749.
61. Tsuchida, K., Nagata, M., & Suzuki, A. (1987). Hormonal control of ovarian development in the silkworm, *Bombyx mori*. Archives of Insect Biochemistry and Physiology, 5, 167–177.
62. Wang, D., Pei, X. Y., Zhao, W. L., & Zhao, X. F. (2016). Steroid hormone 20-hydroxyecdysone promotes higher calcium mobilization to induce apoptosis. Cell Calcium, 60(1), 1-12.
63. Wang, S., Liu, S., Liu, H., Wang, J., Zhou, S., Jiang, R. J. *et al.* (2010). 20-hydroxyecdysone reduces insect food consumption resulting in fat body lipolysis during moulting and pupation. Journal of Molecular Cell Biology, 3, 128–138.
64. Weirich, G. F., Feldlaufer, M. F., & Svoboda, J. A. (1993). Ecdysone oxidase and 3-oxoecdysteroid reductases in *Manduca sexta*: Developmental changes and tissue distribution. Archives of Insect Biochemistry and Physiology, 23, 199–211.
65. Yamada, R., & Sonobe, H. (2003). A novel enzyme ecdysteroid-phosphate phosphatase: Purification, kinetic characterization and molecular cloning. Journal of Biological Chemistry, 278, 26365–26373.
66. Zhang, X., & Zheng, S. (2017). 20-hydroxyecdysone enhances the expression of the chitinase 5 via Broad-Complex Zinc-Finger 4 during metamorphosis in silkworm, *Bombyx mori*. Insect Molecular Biology, 26(2), 243–253.
67. Wang CF, Zhang Z, Sun W. Ecdysone oxidase and 3-dehydroecdysone-3β-reductase contribute to the synthesis of ecdysone during early embryonic development of the silkworm. Int J Biol Sci 2018; 14(11):1472-1482. doi:10.7150/ijbs.26227. <https://www.ijbs.com/v14p1472.htm>