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REVIEW ARTICLE

Juvenile hormone: Production, regulation, current application in vector control and its future applications

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ABSTRACT

Juvenile hormone is an exclusive hormone found in insects which involves regulating various insect physiology. A total of eight juvenile hormones have been identified in insects which include JH 0, JH I, JH II, JH III, 4-methyl JH I (Iso- JH 0), JHB III, JHSB III, and MF. Corpora allata are the glands responsible for the production and synthesis of these hormones. They are involved in moulting, reproduction, polyethism, and behavioural regulations in different orders of insects. Factors such as diet temperatures, photoperiods, and plant compounds affect the biosynthesis and regulation of juvenile hormones. Juvenile hormones analogue is usually used to disrupt normal regulation of JH and this analogue is categorized as insect-growth regulators (IGRs) and is widely used in pest control as an alternative to chemical insecticides. Other applications of biosynthesis activities of this hormone have not been explored in the area of JHs. In this review, current applications of JHs with an addition of their future application will be discussed.

Keywords: Juvenile hormones; corpora allata; insect brain; insect physiology; pest control.

INTRODUCTION

In 1936, an insect "inhibitory hormone" or currently known as juvenile hormones (JHs) was first discovered by Sir V. B. Wigglesworth in *Rhondius prolixus* (Hemiptera: Triatominae). In this early discovery, JH is classified as a sesquiterpenoid hormones found in insect. It is secreted and synthesis by a pair of endocrine glands located near the tritocerebrum area of an insect brains, known as corpora allata (CA). Secretion and biosynthesis of JHs by the CA regulate various physiological roles in insects such as reproduction, development, behaviour, and caste determination. Nevertheless, JHs are well known in their roles in metamorphosis and reproduction where inhibition of adult moulting and yolk deposition in eggs are regulated according to the level of JHs in the haemolymph (Gullan & Cranston, 2010). To date, eight different types of JHs have been identified in insects, namely JH 0, JH I, JH II, JH III, 4-methyl JH I, bis-epoxide JH III (JHB III), skipped bis-epoxide (JHSB III), and methyl farnesoate (MF). JH homologs present in various insects are summarised in Table 1.

Usually, mixtures of JHs are found in insects with a homologous function in different insect orders (Gullan & Cranston, 2010). These hormones are usually found in a mixture of different JHs but the most common is JH III and it can be found in Lepidoptera, Diptera, and Hymenoptera. A mixture of JH 0, JH I, and JH III are found in Lepidoptera only (i.e., *Manduca sexta, Hyalophora cecropia*). However, during the embryonic stage of *M. sexta,* only a mixture of JH 0 and JH I were found and there were no titres of JH III observed (Bergot *et al*., 1980). This may be related to different biosynthesis and regulation of JH during early stage of insect development. Occurrence of JH I were rarely reported in insects compared to JH III but in 2004, JH III and JH I were identified in the Mediterranean field cricket, *Gryllus bimaculatus* (Orthoptera: Gryllidae). In the same study, JH I, JH II, and JH III were found concurrently in the fifth instar larvae of the fall armyworm, *Spodoptera frugiperda* (Noctuidae) (Westerlund & Hoffmann, 2004).

In Diptera, one exclusive JH homologs known as bisepoxide JH III (JHB III) was discovered in *Drosophila melanogaster* (Drosophilidae), *Phormia regina* (Calliphoridae), *Aedes aegypti* (Culicidae), *Neobellieria bullata* (Sarcophagidae), *Calliphora vomitoria* (Calliphoridae), and *Lucilia cuprina* (Calliphopridae). This discovery was reviewed by Yin (1994) where JHB III were identified with two epoxy groups in JH

structure instead of one epoxy group like other JH homologs. A higher rate of JHB III biosynthesis compared to JH III was found in Diptera, indicating that JH III is not the sole JH in this Order (Bylemans *et al*., 1998). Nevertheless, the specific roles of this JH homolog are still unknown.

JHs are classified as a true hormone in insects, but the discovery of methyl farnesoate (MF), which is a hormone found in crustaceans, is believed to be grouped together with JHs because of their function. However, some believed that MF is not related to JHs. In crustaceans, MF serve as hormones in development and reproduction regulation similar to JH in insects. In a recent study by Di-Wen *et al*. (2015), MF is reported to act as an immediate precursor and hormone in the regulation of metamorphosis in *D. melanogaster*. In terms of the structure, MF is also classified as sesquiterpenoid compounds, but lacking the epoxide group. In order to prove the identity of MF, further structural and functional studies need to be conducted for a definite classification.

This review will briefly discuss about JH production, regulation, and their applications of JH is pest and vector control, developmental studies as well as future applications.

Juvenile hormone regulation by corpora allata and accessory glands

JH is known to be produced and synthesised by a pair of glands named CA. This gland can be categorized into five difference morphological types according to their positions which are: (1) lateralized; (2) distally lateralized; (3) semi centralized; (4) centralized; and (5) annular types (Goodman & Cusson, 2012).

However, the role of CA as the only organ to regulate the secretion and synthesis of JH is proven to be inaccurate by various researchers (Tobe *et al*., 1985; Bylemans *et al*., 1998). A concise review on the regulation of CA in JH synthesis and regulation has been done by K. Hartfelder (2000). Based on a study by Wu *et al*. (1987), decapitation of *D. melanogaster* brain has resulted in the activation of CA to secrete JH. The absent of the brain significantly affect JH production in this species. Also, the absent of the brain affect the hormonal control in the reproduction of *D. melanogaster* where yolk peptides failed to be synthesized. This finding is further supported by the radiochemical assay (RCA) performed on CA attached to the brain (Bylemans *et al*., 1998). Attachment of the brain to the CA, known as Br-CA complex, was observed to be more productive in synthesizing JH than the one without the brain.

In the absent of the brain, allatotropin will be produced by accessory glands, fat body, ovaries, and the gut. Allatotropin is a peptide that stimulates the secretion of JH to regulate the hormonal control. The effect of allatotropin on CA has been demonstrated by the application of synthetic allatotropin of *M. sexta* to the CA of *Lacanobia oleracea* (Lepidoptera: Noctuidae) where a stimulation by 37% was observed in the CA of *L. oleracea* (Audsley *et al*., 1999). In the same study, a peptide that inhibit CA activity known as allatostatin was also applied on the same insect and a maximum inhibition by 54% was recorded. However, applications of synthetic allatostatin and allatotropin did not show the same affect among insect orders, for example, cockroach's allatostatin, Dip- allatostatin-2, had no significant affect on JH synthesis on *L. oleracea* when compared to *M. sexta*. These findings suggested that a diversity of allotostatin and allatotropin may be present among the orders of insect. Nonetheless, the effect of allatostatin in JH synthesis remains inconclusive because of the constant low quantity of JHs being detected in insects (Audsley *et al*., 1999). In normal JH regulation, degradation of JHs is mediated by three enzymes: – juvenile hormone esterase (JHE), juvenile hormone epoxide hydrolase (JHEH), and juvenile hormone diol kinase (JHDK) (Yang *et al*., 2016). Yang *et al*. (2016) reported gene expressions of BdJHEH2 and BdJHDK in *Bacterocera dorsalis* (Diptera: Tephritidae) during larval-pupal transition and the genes suggested that JH was degraded in the preparation to pupate because low JH was required for successful larval-pupal development (Yang *et al*., 2016). Requirement of low JH during larval-larval moulting from early to late third instar larva followed by high demand JH degradation, which high expression of BdJHEH3 will be needed for this moulting to be successfully occurred. Adults of 7-days-old and 10- days-old *B. dorsalis* exhibited high expression of these three genes indicating that JH degradation was required for sexual maturation (Yang *et al*., 2016). In summary, the abundance of these genes found in the fat body and Malpighian tubules of *B. dorsalis* suggested that excretion of degraded JH was part of JH regulation.

Juvenile hormone biosynthetic pathway

Juvenile hormones are identified based on their chain length of 16-19 carbon atoms (Gullan & Cranston, 2010). JH is synthesized in CA through the mevalonate pathway (MVAP) in the early stage and undergo JH branching in the later stage of biosynthetic pathway. This pathway was reviewed by Noriega (2014) where the synthesis of JH in CA comprises of 13 enzymatic reactions which involves eight enzymes in early MVAP and five enzymes in the JH branch (Noriega, 2014; Huang *et al*., 2015). The early step of MVAP includes the conversion of Acetyl-CoA to farnesyl pyrophosphate (FPP). Next, oxidation of FPP to farnesoic acid (FA) occurred and later transformed into a final product namely JH. The last step in the JH branching was mediated by two enzymes genes, juvenile hormone acid O-methyltransferase (JHAMT) and methyl farnesoate epoxidase (CYP 15A1) (Marchal *et al*., 2011). JHAMT catalysed the FA and JH acid to the methyl ester forms which are JH and MF. Epoxidation of MF and JH is encoded by P450 enzyme with the expression of CYP 15A1. However, the late stage of MVAP is dependent on the order of an insects (Marchal *et al*., 2011). The late stage can precede with epoxidation followed by methylation in Lepidoptera or vice versa in Diptera, Coleoptera, Orthroptera, or Dictyoptera (Huang *et al*., 2015). Details on the pathway of JH biosynthesis (Figure 1) was reviewed by Noriega (2014) and includes the complete JH pathway and branching. However, Di Wen (2015) reported that the JH synthesis pathway of *Drosophila* is more complicated compared to other insects because of the presence of JHB III. The common precursor of JH present in *Drosophila* is believed to be farnesoic acid rather than methyl farnesoate. Methyl farnesoate is also reported to have a dual role as a hormone and precursor of JHBIII in *Drosophila*. The juvenile hormone acid methyltransferase (JHAMT) is known to be involved in JHB III biosynthesis only but not in JH III (Di-Wen *et al*., 2015). However, the complete pathway of JH biosynthesis is still unknown. Previous studies (Richards *et al*., 1989; Bylemans *et al*.,1998; Shiga *et al*., 2003) revealed a possibility of different biosynthesis in the type of JH and among different orders of insect. In other words, JH 0, JH I, and JH II may have different biosynthesis pathways that have yet to be explored.

Juvenile hormone control and regulation in insects

JH has been identified to control and regulate various functions in insects from all order of insects. In this section, the control and regulation will be discussed among the different insect orders. The control of reproduction and development seem to be the most important one, as it the

Table 1. Types of Juvenile Hormone (JH) identified in various insects

Order: Family	Species of insects	JH homologs						
		JH0	JH I	4-methyl JH I	JH II	JH III	JHB III	Methyl farnesoate (MF)
Hymenopetra: Apidae	*Apis mellifera*					/(Huang & Robinson, 1995)		
Lepidoptera: Sphingidae	*Manduca sexta*	/(eggs)(Bergot *et al.*, 1980)	/(eggs)(Bergot *et al.*, 1980)					
Lepidoptera: Noctuidae	*Lacanobia oleracea*		(Audsley *et al.*, 1999)		/(Audsley *et al.*, 1999)	/(Very low; Audsley *et al.*, 1999)		
Blattodea: Termosidae	*Hodotermopsis sjostedti*					/(Cornette *et al.*, 2008)		
Diptera: Culicidae	*Aedes aegpti*					/(Shapiro *et al.*, 1986)		
Diptera: Sarcophagidae	*Sarcophaga (Neobellieria) bullata*					/(Bylemans *et al.*, 1998)	/(Bylemans *et al.*, 1998)	/(Bylemans *et al.*, 1998)
Diptera: Calliphoridae	*Lucillia cuprina*					/(Lefevere *et al.*, 1993)	/(Lefevere *et al.*, 1993)	/(Lefevere *et al.*, 1993)
Diptera: Calliphoridae	*Phormia regina*							
Diptera: Calliphoridae	*Calliphora vomitoria*					/(Very low; Duve *et al.*, 1992)	/(Duve *et al.*, 1992)	
Diptera: Calliphoridae	*Protophormia terraenovae*						/(Shiga *et al.*, 2003)	
Diptera: Drosophilidae	*Drasophila melanogaster*					/(Bownes & Rembold, 1987)	/	
Diptera: Tephritidae	*Anastrepha suspense*					/(Teal *et al.*, 2000)	/(Teal *et al.*, 2000)	
Orthoptera: Acrididae	*Locusta migratoria*		/(Minderhoud *et al.*, 1980)		/(Minderhoud *et al.*, 1980)	/(Minderhoud *et al.*, 1980)		

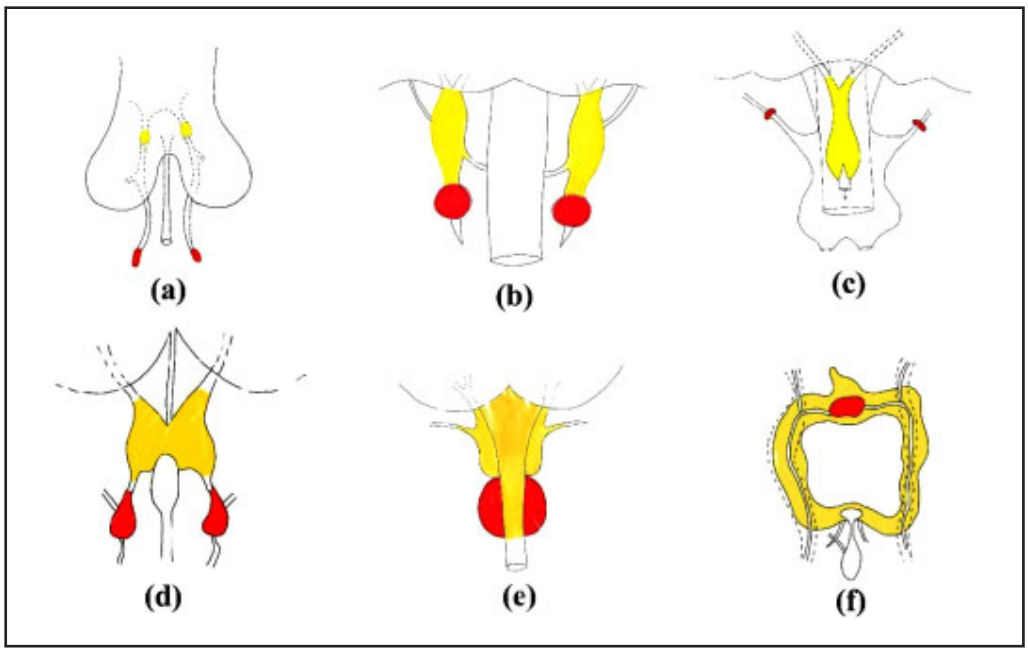

Figure 1. Morphological position of the retrocerebral glands in insects. (a) Lateralized types, (b) distal lateralized type, (c) ventral type, (d) semicentralized type, (e) centralized type, and (f) annular type. Red area represented corpora allata and yellow area represent corpora cardiaca. Adapted from Cassier (1979).

actively being studied in the past few years involving various insect species.

Despite the various forms of JH homologs, they shared the same control and regulations. In Diptera, three homologs were present in the extract of *Musca domestica* (Muscidae) and *Sarcophaga bullata* (Sarcophagidae) namely JH I, JH II, and JH III (Lefevere *et al*., 1993). However, further studies disputed the presence of JH II in *M. domestica* and *S. bullata* (Trumann, 1974; Girard *et al*., 1976; Schooley *et al*., 1976). Following this, a confirmation was made that JH III is the only principal JH found in cyclorrhapha Diptera. This JH is found in *Drosophila hydei, D. melanogaster, Calliphora vomitoria* (Calliphoridae), and *Phormia regina* (Calliphoridae) (Bownes & Rembold, 1987; Duve *et al*., 1992)

In reproduction, not only JHs control the vitellogenesis and oogenesis but also regulate the reproductive behaviour and pheromone production in adult insects (Raushenbach *et al*., 2004). JH titre is reported to rise immediately after the emergence of both male and female *D. melanogaster*: – 6.9 pmol/g of wet weight in females and 5.3 pmol/g wet weight in males (Nijhout & Williams, 1974). The increased concentration of JH in haemolymph immediately after emergence involves an initiation of the transcription and translation machinery in female's fat body for the induction of the first oogenesis cycle (reviewed by Wyatt & Davey, 1996). This result is consistent with other studies where there was high titre of JH in the females of *A. aegypti* (Diptera: Culicidae) and both females and males of *Neobellieria* (*Sarcophaga*) *bullata* (Diptera: Sarcophagidae) immediately after eclosion but rapidly declined thereafter. Teal *et al*. (2000) proposed that the increase of JH titre five days after eclosion in males *N. bullata* and *D. melanogaster* were associated with reproductive maturity and sexual signalling. However, females have lower titres compared to the males of the same age and these differences were related to the presence of an allostatic-like compound found in the ovaries of both *D. melanogaster* and *N. bullata* females (Bownes & Rembold, 1987; Bylemans *et al*., 1998). This allostatic-like compound may inhibit the biosynthesis of JH III and JHB III present in both insects which explained the rapid decline of these hormone titres in females. In males, the titre increases with mating behaviour as reported in *Anastrepha suspense* (Diptera: Tephritidae), where a 3-fold increase of JH concentration were observed in mated young males compared to the virgin males (Teal *et al*., 2000).

JH also plays a part in the production of eggs, where a high concentration of JH is correlated with high productions of eggs and a low concentration of JH is accompanied by low eggs production (Yamamoto *et al*., 2013). Low JH production is due to the knockout of CA (CAKO) in females of *D. melanogaster,* which is the site of JH production and synthesis. Even though the correlation existed between JH and egg production, Yamamoto *et al*. found that some CAKO females produced a similar quantity of eggs in comparison to wild strain flies. This situation may be due to sufficient amounts of JH being produced before the knockout and hence the egg production is similar to the wild strains. As mentioned previously, the brain may play a part in sending the signal to the other accessory gland to increase JH synthesis before the CA is shutting down. In addition, JH was observed to be involved in oogenesis when a sharp decline of JH titres in *A. aegypti* were observed immediately after the blood meal and the rise of JH took place between 36 to 48 hours post-feeding (Shapiro *et al*., 1986). The decline of JH in early blood meals after eclosion create an opportunity for 20-hydroecdysone (20E) to release a neurosecretory hormone for egg development (Shapiro *et al*., 1986). A decline of JH level may be due to the action of juvenile hormone esterase, excretion, or the activities of its CA (Shapiro *et al*., 1986). The second blood meal in mosquitoes trigger the development of second follicles which will cause the rise of JH and a new cycle of egg development. In flesh fly, *N. bullata*, the increase of JH titres was more rapid when compared to *A. aegypti* which took an additional one day in both males and females following a liver and blood meal (Bylemans *et al*., 1998). However, the decline of JH in *N. bullata* females a few days

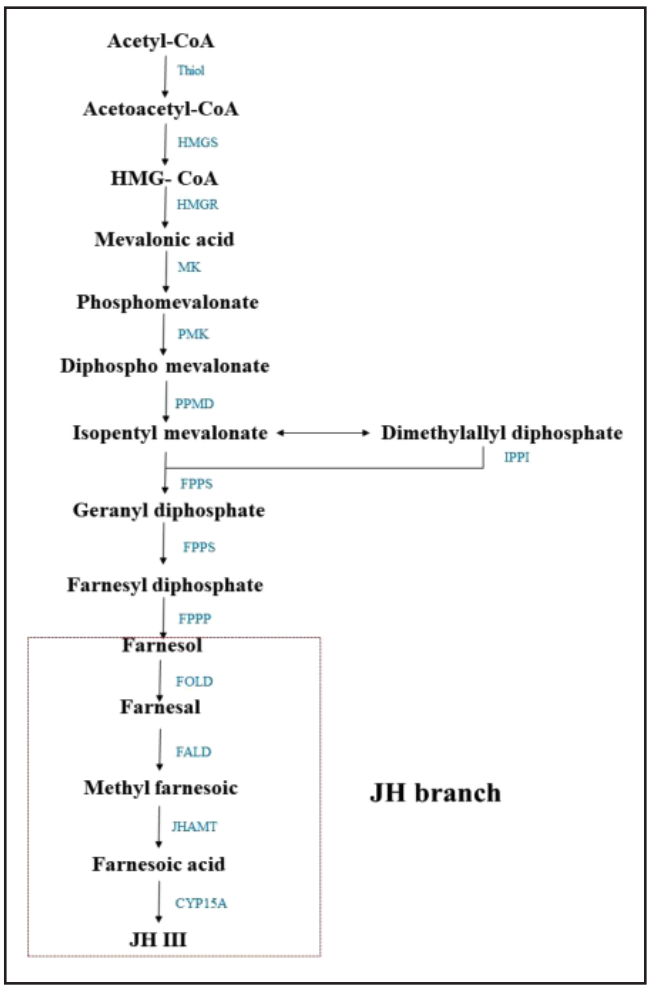

Figure 2. JH III biosynthesis pathway adapted from Noriega (2014). JH branch in the biosynthesis is in the red box. The enzymes involved are in blue. Abbreviations: Thiol= Acetoacetyl- CoA thiolase; HMGS= HMG-CoA synthase; HMGR= HMG-CoA reductase; MK= Mevalonate kinase; PMK= Phosphomevalonate kinase; PPMD= Diphosphomevalonate kinase; IIPI= Isophentenyl diphosphate isomerase; FPPS= Farnesyl diphosphate isomerase synthase; FPPP= Farnesyl diphosphate synthase; FOLD= Farnesol dehydrogenase; FALD= Farnesal dehydrogenase; JHAMT= Juvenile hormone acid methyl transferase; CYP15A= Methyl farnesoate epoxidase.

after the liver meal showed a similar trend with *A. aegypti,* demonstrating the involvement of JH in vitellogenesis (Bylemans *et al*., 1998).

In *D. melanogaster,* fluctuating patterns of JH titres were demonstrated throughout larval development until the prepupal stage (Bownes & Rembold, 1987). First and second instar of *D. melanogaster* larvae showed high titres of JH (1.3 pmol/g fresh weight) (Bownes & Rembold, 1987). However, third instar and prepupa showed low titre and pupae did not show any detectable titre of JH. Reappearance of JH in the prepupal stage is required to prevent precocious metamorphosis in some tissues such as the eyes, the optic lobe, and the ventral diaphragm (Riddiford, 2012). Larval-pupal development was interrupted when the secretion of prothoracicotrophic hormone (PTTH) was inhibited by the brain in the presence of high JH concentration (Riddiford, 2012). As such, elimination of JH in haemolymph was required for the secretion of PTTH from the brain (Nijhout & Williams, 1974; Riddiford, 2012).

JH regulation in moulting is further described at the molecular level when high Krüppel-homolog 1(*Kr-h1*) gene expression is required in larval-larval moulting of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Drosophila* (Riddiford, 2012; Jindra, 2019). Expression of *Kr-h1* was high in both *Tribolium* and *Drosophila* throughout larval stages. Cessation of feeding and the wandering phase were triggered by the low gene expression which subsequently led to larval-pupal moulting (Riddiford, 2012). Suppression of *Kr-h1* is regulated by histone deacetylase 1(HDAC 1) (George *et al*., 2019) with maximum mRNA expression of HDAC 1 was detected after 24 hours of entering the pupal stage. George *et al*. (2019) suggested that expression of *Kr-h1* was related to JH concentration. In burying beetles, *Nicropharus orbicollis* (Coleoptera: Silphidae), JH did not seem to be involved in ovarian maturation but involved in behavioural regulation instead (Trumbo, 1994). The spiking of JH titres was detected in *Ni. orbicollis* upon the discovery of rat carcasses. The peak value of JH upon the discovery of carcasses were accompanied by four behaviour changes including palpating, lifting, walking around carcasses, and making forays into surrounding soils (Trumbo *et al*., 1994). However, the action of JH in the behavioural regulation is still not fully understood.

In social insects, JH has a different role compared to non-social insects. JH has been known to be involved in the polyethism (i.e., age polyethism and caste polyethism) and also behavioural changes. Polyethism is defined as functional specialization in different members of a colony of social insects, which lead to a division of labour (i.e., organisation on insect society that consists of different activities performed simultaneously by a specialized group of individuals). Polyethism can be categorized into age polyethism and caste polyethism. For example, honey bees, *Apis mellifera* (Hymenoptera: Apidae) are one of the social insects that undergo age polyethism, which is related to changes of division of labour as they age (Huang & Robinson, 1995). For instance, the foragers have high JH titre compared to young *Ap. mellifera*. Huang and Robison (1995) also reported that the titres of JH are directly proportional with the age of honey bees, although low titre of JH was reported in inactive foragers and young *Ap. mellifera*. However, foraging activity was not totally dependent on the high titre of JH as active foragers in cold weather had a low titre than the active forager in warm weather. When compared with the young bees (which are non-foragers), the foraging bees in the cold weather exhibited a higher JH. This showed that JH titre increases directly with the age of honey bees and also environmental temperature. Note that JH response towards temperature alone will not affect honey bees division of labour (Huang & Robinson, 1995).

In addition, JH is playing a part in the pre-vitellogenic events of termites, *Hodotermopsis sjostedti* (Blattodea: Termosidae), *Reticulitermes speratus* (Blattodea: Rhinotermitidae), and *Cryptotermes secundus* (Blattodea: Kalotermitidae). High titre of JH during pre-vitellogenic events activates the vitellogenin synthesis (Cornette *et al*., 2008; Santos *et al*., 2018). The relationship between JH and polyethism was demonstrated in caste determination of damp wood termites, *H. sjostedti*. During moulting, high JH titre was associated with pre-soldier moulting while the moulting of nymph to the alate form showed low JH titres (Cornette *et al*., 2008). However, the titres started to fluctuate when soldiers reach maturity, in spite of these changes, titres in soldier termites were still higher than pseudergates termites (worker caste termites that unable to become a winged imago termites) (Cornette *et al*., 2008). This high titre may be linked to an increased volume of corpora allata in the soldiers. Yet, moulting of nymphs into pseudergates instead of alates due to the increase of JH titres may be due

to the aggressive conspecific interaction in termites (Cornette *et al*., 2008). In a recent study by Oguchi *et al*. (2020), the differentiation of pseudergates to neotenic forms was depending on the presence of neotenics in the colony and this was regulated by the concentration of JH. Juvenile hormone was the key player in controlling the caste differentiation during the inter-molt of termites which will later determine the fates of the caste. Based on the experiment, the presence of neotenics in the colony will accelerate the differentiation of the opposite sex pseudergates to neotenics. However, the presence of male neotenics were able to maintain a low level of JH that led to the moulting of pseudergates to neotenics. In contrast, the presence of female neotenics, have showed high level of JH in the first three days, and then a gradual decrease, eventually reached the lowest level of JH on day seven. This differentiation may be caused by the urgency of a male to copulate due to the intraspecific competition (Oguchi *et al*., 2020).

In a recent study, behavioural roles of JH in honey bee larvae were studied by exposure to juvenile hormone analogue (JHA), pyriproxyfen, which subsequently showed an effect on behavioural change in the resulting adults (Fourrier, 2015). The pyriproxyfen-exposed adults did not perform many social tasks (i.e., brood care and ventilation) but rather performed more non-social tasks (i.e., self-grooming, inactive, and walking instead of flying). Exposure of high JHA concentration may affect brain development and cognitive abilities of the honey bee and subsequently alter social performance that eventually led to its rejection by nestmates (Fourrier, 2015).

Factors affecting JH activities

As previously mentioned, JHs regulate many physiological activities in insects such as development, reproduction, polyethism, and behaviour. JHs are known to be declining in older larvae until reaching adulthood under normal regulation (Bownes & Rembold, 1987). However, some factors may cause interruptions in the regulation and lead to the changes in physiological activities. Common effects such as prolong phagoperiod in larvae and delayed pupation occur due to rising JH titres in the haemolymph (Slama & Williams, 1965; Saunders *et al*., 1990). Albeit low titres of JHs can also be a consequence due to environmental stress faced by insects.

One of the factors that affect JH activities is plant extracts, specifically balsam fir (*Abies balsamea*) that can be found in most American newspapers and some paper towels. This discovery was reported by Slama and Williams in 1965, when linden bug (*Pyrrhocoris apterus*) showed high titre of JH after being exposed to the plant extract. Following the high titre of JH, larvae failed to moult into adults and most of the larvae died without reaching adulthood. Slama and Williams (1965) also reported that CA was not involved in the sensitivity of *P. apterus* towards the plant extract. Involvement of CA was not observed because the plant extract was applied directly to the larvae rather than injecting it into the haemolymph. Application of the extract to the other species of Hemiptera such as *Oncopeltus fasciatus* and *Rhodnius prolixus* did not show the same effect as in *P. apterus*. This indicates that the balsam fir extract cause changes of JH concentration in certain insect species only. This suggests that the plant extract acts as JH agonists (JHAs) to stimulate JH receptors.

Nevertheless, plant compounds can also act as JH antagonists (JHANs) that inhibit the binding of JH to its receptor, known as Methoprene-tolerant (Met). Diterpene from two plant extracts, the fruits of *Lindera erythrocarpa* and the roots of *Solidago serotine,* showed relatively strong and mild activity in the regulation of JH in *A. aegypti,* respectively. Both diterpene LE3B (Kanakugiol) and SS5A (Kingidiol) caused mortality in third instars, disrupted ovarian follicles development, and also cause ovarian retardation in females of *A. aegypti*. Retardation of *A. aegypti* female ovaries may be caused by precocious development by LE3B applied on the second instar during metamorphosis (Lee *et al*., 2015). Another factor that affects the titres of JH is seasonal changes. Photoperiod and temperature changes in each season effect the titres of JH in many insects. Saunders *et al*. (1990) reported that short-day photoperiod affected JH biosynthesis in *D. melanogaster*. Ovarian diapause was induced in short-day photoperiod that was regulated by JH titres in the haemolymph. The relation of JH titres in the haemolymph and photoperiod was measured by using the uptake of yolk proteins (YPs) by the ovaries of female *D. melanogaster*. The uptakes of YPs were low in a short-day photoperiod. This is because the synthesis of JH may not occur after a certain period of diapause (Saunders *et al*., 1990). The YPs tend to accumulate in the haemolymph and cannot be taken up by the ovaries (Saunders *et al*., 1990). Vitellogenesis is not complete in this situation. Vitellogenin synthesis is resumed in *D. melanogaster* by transferring the colony into a higher temperature (25°C) and 12:12 light:dark photoperiod in order to break the ovarian diapause. However, the colony that were reared in short-day photoperiod for a long period (i.e., a month) did not recover from ovarian diapause following the method stated above. Another method that was applied to break the diapause was the topical application of JH (Saunders *et al*., 1990). Increasing JH in the haemolymph will increase the uptake of YPs by the ovaries. The correlation of JH and ovarian diapause was observed in non-diapausing flies that produced four times more JH than the diapausing flies (Saunders *et al*., 1990). The flies that produced more JH in the haemolymph were likely to undergo vitellogenesis (Saunders *et al*., 1990). Diapause-induced *Pr. terraenovae* also showed a low JH synthesis similar to diapause-induced *D. melanogaster* (Shiga *et al*., 2003).

It is known that JH titres are correlated with temperature. To further understand the effect of temperature on JH titres, foraging bees with high JH titres were placed into a cold room during summer (Huang *et al*., 1995). Eight days after the placement, *A. mellifera* showed a decline in the JH titres. Damp wood termites, *Hodotermopsis sjostedti,* also displayed temperature effects on JH but it is only affecting a certain caste which is the pseudergates termites (Cornette *et al*., 2008). Pseudergates termites experienced a dropped of JH titres during winter but the JH titres in soldier termites were similar to the warm weather despite the drop in temperature (Cornette *et al*., 2008).

Diet may also affect the production of JH. Duve *et al*. (1992) reported that unrestricted diet in blow flies, *C. vomitoria* showed high production of JH and oocytes development in the first gonadotrophic cycle. In the laboratory, colonies where a restricted diet (i.e., sugar, water, meat) was given, the JH produced was less than 50%. Bylemans *et al*. (1998) reported that the diet only affects biosynthesis of JH in female flesh flies, *N. bullata,* and not the males. This is based on an immediate increase of JH after a liver meal compared to males that showed a slower increase (Bylemans *et al*., 2015).

Starvation of fruit flies, *B. dorsalis,* demonstrated that the low expression of BdJHEH gene was responsible for the degradation of JH. Starvation can cause low degradation of JH in their haemolymph which contradicted with previous studies by Duve *et al.* (1992). Thus, high JH in the haemolymph causes slower larval-larval moult. However, BdJHDK gene

was highly expressed during insect starvation and caused precocious metamorphosis. In the adult females of *Drosophila virilis* (Diptera: Drosophilidae), starvation caused low production of eggs and a delay in oviposition (Raushenbach *et al*., 2004). This is caused by the low degeneration of JH which affect the production of 20-hydroxyecdysone (20E) that is important for the late stages of oogenesis.

Detection and quantification of juvenile hormone

Identification and quantification of JH had been performed in various method, ranging from classical method, bioassay, or physicochemical assay. In the early research, parabiosis and CA implantation was employed to study the JH synthesis (Villalobos-Sambucaro *et al*., 2020). Radiochemical assays, radioimmunoassay, and physicochemical methods which include gas chromatography (GC), liquid chromatography (LC), and spectrophotometry are the methods that being applied in JH studies to identify and quantify the hormone.

Radiochemical assay (RCA) is one of the common methods to analyse the rate of biosynthesis of JH in corpora allata. This assay is performed by tagging the compound with a radioactive isotope and incubation of the tissue (Bylemans *et al*., 1998). Observations were made in RCA by the incubation of brain-corpora cardiaca-corpora allata (Br-CC-CA) and corpora cardiaca-copora allata (CC-CA). The rate of biosynthesis between these two complexes was then compared and the result showed higher biosynthesis in Br-CC-CA than the CC-CA incubated without the brain. The synthesis is proven by the implantation of an active CA from young larvae into older larvae of *M. sexta* with less active CA. This implantation resulted in an increase of JH level in the haemolymph (Huibregtse-Minderhoud *et al*., 1980). *In vitro* incubation of CA in radiochemical assay (RCA) also showed JH synthesis by the CA. A study on locusts, *Locusta migratoria* by Huibregtse-Minderhoud *et al*. (1980) used capillary GC to detect three JH homologs; JH I, JH II, and JH III. A concentration of 2.3 ng/g haemolymph of JH III was detected in 0-24 hours fifth instar larvae compared to 24-48 hours larvae at minimum of 0.9 ng/g haemolymph which is much lower compared to the young larvae. After 18 days, upon reaching adult stage, the reading of JH III concentration is higher with 36.0-74 ng/g haemolymph. Whereas, JH I and JH II have low concentrations, less than 0.3-2.5 ng/g haemolymph, and cannot be detected in some samples.

In 1981, Bergot *et al*. developed a rapid, specific, and sensitive method for the extraction, purification, and quantitative determination of four known JHs which are JH 0, JH I, JH II, and JH III. This method involved the application of Gas Chromatography Mass-Spectrometry (GC-MS) in two lepidopteran species;*M. sexta* and *Heliothis virescens.* The method developed by Bergot *et al*. has simplified and shortened the purification process of the sample and only a small sample size is required for the analysis. Furthermore, higher sensitivities can be achieved using this method. Adapting the method, Tobe *et al*. (1985) detected 46-740 ng/g of JH III in the whole-body extract of *Diploptera punctata* for each developmental stage (n = 12-15 individuals).

In the recent advancement of technology, simultaneous identification and quantification of JH can be performed in a single run in LC Ramirez *et al*. (2020). This method allowed the identification and quantification of JH I, JH II, JH III, JHB III, and Juvenile Hormone Skipped-Bisepoxide (JHSB III). Employment of LC coupled to electrospray tandem mass spectrometry (LC-MS/MS) permitted the simultaneous identification and quantification based on JH structural features. This developed protocol could detect a femtomole range of the JH concentrations, allowing analysis of an individual insect. Previous methods developed by Rivera-Perez *et al*. (2012) are also able to detect a femtomole range of JH titres but this result is obtained by tagging the JH to the fluorescent tag prior to the High-Performance Liquid Chromatography (HPLC). This procedure can be applied to three type of samples which are the haemolymph, whole tissue, and CA-CC samples.

Application of JH in vector control and its future applications

The application of JH is mainly centred on the agricultural and vector control industries especially on the development of insecticides or insect growth regulators (IGRs). The use of pesticides renders significant negative impacts to human and environmental health. IGRs do not deliver a rapid termination on the insect lives but it affects the biology of insects with long term effects. In general, JH manipulation in insect pest and vector control was executed in the form of JH mimicry or JH analogues. These analogues include methoprene, pyriproxyfen, and hydroprene. The detailed management and potential use of IGRs was reviewed by Mondal and Parween (2001).

Despite of its effectiveness, tolerance and resistance towards IGRs by some insects such as mosquitoes, house flies, and flour beetles were reported (Cornel *et al*., 2002). Resistance towards methoprene maybe caused by (i) reduced penetration of the substance into the system; (ii) increased excretions, and (iii) insensitivity of the target site. While, in *D. melanogaster,* the resistance was caused by mutagenesis of the *Met* genes which is the receptor for JH (Minkoff & Wilson, 1992). In a review by Parthasarathy and Palli (2021), the tolerance of the JHA is simply depended on the specific gene in the targeted insects. A study on *Tribolium castaneum* (Coleoptera: Tenebrionidae) showed three different outcomes from the same treatment: – (i) failure of *T. castaneum* to emerge as adult; (ii) successfully emerged as adult but produced no offspring, and (iii) emerged as adult and produced normal offspring (Wijayaratne *et al*., 2012). Following these outcomes, males *T. castaneum* were more susceptible to the treatment compared to females. Treatment applied to females *T. castaneum* did not show significant differences in the production of normal offspring compared to untreated females. In contrast, untreated females mated with treated males showed differences in the production of eggs (e.g., failure to hatch). This indicated that males were more susceptible than females against treatment. Susceptibility of males may be caused by the disruption in the process of spermatogenesis by methoprene during the developmental cycle (Wijayaratne *et al*., 2012). Thus, disruption in reproductive development in insects reduces the progeny of insect population to achieve the purpose of pest control (Shapiro *et al*., 1986; Wijayaratne *et al*., 2012).

One of the most potent JH analogues used in insect population control is pyriproxyfen or 2 [I-methyl-2-(4 phenoxypheoxy) ethoxy] pyridine (Ishaaya & Horowitz, 1992). They reported the effect of pyriproxyfen on the development of sweet potato white flies, *Bemisia tabaci* (Hemiptera: Aleyrididae) which is known to infest cotton, vegetables, and ornamental plants. Pyriproxyfen did show a positive response in the suppression of the development of *B. tabaci* in every developmental stage. Inviable eggs were produced from adult females treated with the substance. The direct effect of pyriproxyfen to eggs was only seen in the 0-1 day-old eggs and not on the older eggs. The same was observed in the larval stages. However, the effects were only seen in the later stages when the pupae of the treated larvae failed to emerge as adults. Pyriproxyfen also showed localized effect on the area of interest, where eggs laid on specific

targeted area of the leaves failed to hatch (Ishaaya & Horowitz, 1992).

JH analogues (JHAs) has been investigated in insect vectors such as mosquitoes and triatomid bugs. Mosquito is a known vector of various tropical diseases such as dengue fever, malaria, and Zika. While triatomes are vectors of Chagas disease and both of these insects required a blood meat for oocytes productions (Ramos *et al*., 2020). Associations of these insects with human is concerning as they will spread diseases among human populations and therefore vector control programs are paramount important in this context. Methoprene, also known as isopropyl (2E, 4E)-11-methoxy-3,7,11-trimethyl-2-4-dodecadienoate, is the most common JH analogue used to control mosquitoes and fruit flies (Wilson & Turner, 1992). Shapiro *et al*. (1986) reported a five time increase in the amount of JH in unfed *A. aegypti* females when methoprene was applied topically (100 pg). The application of JHA causes vitellogenesis to occur in female *A. aegypti* and most of the females successfully oviposited. However, most of the eggs produced were not viable, which suggested that high levels of JH caused failure in embryogenesis (Shapiro *et al*., 1986).

JHA is also known to cause defect and death in mosquito larvae (Parthasarathy & Palli, 2021). It may produce a larva-pupal intermediate during the larval-pupal critical period. Continuous exposure to JH after the larval-pupal critical period will cause mortality during the prepupal stage (Braga *et al*., 2005). Methoprene has been reported to control *A. aegypti* successfully (Braga *et al*., 2005a, 2005b). While pyriproxyfen is able to inhibit adult emergence of *A. vigilax* and *A. japonicus* (Webb *et al*., 2012; Tuten *et al*., 2016). Furthermore, methoprene is able to control horn flies and house flies (Beadles *et al*., 1975; Miller *et al*., 1977; Breeden *et al*., 1980). However, it is interesting to note that no JH homologs was found in ticks (Acari). The JHA that has been studied thus far in insect vector is summarised in Table 2.

In a recent study, Chang *et al*. (2021) shows that the increase of JH in an adult *A. aegypti* reduces their immunity and that they are more susceptible to an infection. This study revealed that the disruption of JH signalling not only affect reproduction but also the insect immunity. In triatomid bugs, there is no reports on the JHA used to control this insect vector as the JH that is specific to this organism was just discovered recently (Villalobos-Sambucaro *et al*., 2020). The JH discovered is thoroughly studies for a development of specific targeted JHA (Villalobos-Sambucaro *et al*., 2020). Some JHAs do showed high specificity to a specific insect order, one of the JHAs acting this way is methyl ester of 3,7,11-trimethyl-7,11-dichloro-2-dodecenicacid or "dihydrochloride" of methyl farnesoate (Masner *et al*., 1968). This substance showed potency in sterilization of the linden bug, *Pyrrhocoris apterus*. Female *P. apterus* showed high sensitivity to this substance as low as 0.1 µg which is sufficient to cause partial viability of the eggs produced (Masner *et al*., 1968). However, no effect was observed in the developmental of first and fourth instars of *P. apterus*. This indicated that the effect was taking place during the embryogenesis. Yet, in some other species such as Lepidoptera, Coleoptera, Diptera, and some Heteroptera, it did not show any effect, suggesting high specificity of this substance (Masner *et al*., 1968).

Juvenile hormone chemistry, biosynthesis, and pathway have been extensively study for better understanding on its regulations in insects for a few decades. However, most of the studies were centred on JH application in the insect pest and vector control industry in the form of IGRs. JH analog are the most commonly used to regulate the JH titres in pest insects. High JH during embryogenesis caused non-viable egg production (Shapiro *et al*., 1986) which is efficient in controlling the insect pest and vector population. Altering the JH titre in larval stages is a useful strategy in vector population control because inducing JH titre in the old larvae stops adult transformation and resulted in the discontinuation of its life cycle (Bownes & Rembold, 1987).

Declining trends of JHs during fly larval development may be useful in the determination of the age of the larvae. Larval age is used for the determination of minimum post-mortem interval (mPMI) in forensic investigations (Goff, 1993), especially to differentiate the age of third instar and post feeding stages. It is known that the larvae of blow flies are common insect evidence found on decomposing remains (Lee *et al*., 2004). Furthermore, beetle larvae can also be used in the estimation of PMI at a later stage of body decomposition. We hypothesized that the levels of JH determined from fly larvae or beetle larvae during the discovery of a body can be used to determine PMI. Furthermore, the peak concentration of JH in carrion beetles during the discovery of the carcasses could potentially be useful in determining the time of the beetle arrival. Yet, to apply this knowledge in forensic entomology, extensive studies are required to fully understand the relationship of JH titres changes and the time of beetle arrival to the decomposing remains. Analysis of biological changes in each insect found at the crime scene can improve mPMI estimations. Although, a study focusing on the trends of JH in the development of a blowfly need to be performed to explore their application in forensic because of their value in mPMI estimation compared to beetles. Nevertheless, a baseline data on the JH changes in each insect species involved in crime scene investigation need to be constructed for its application in the real setting.

The other potential application of JH is in the recovery of an important social insects by studying evolution of JH in the regulation of insect behaviour. This may be advantageous in the recovery of a social insect colony. Instability of JH within the colony of a social insect may cause aggressiveness

Table 2. Application of JHA in controlling vector of diseases

JHA	Vector	Life stages	Authors
Methoprene	*Ochlerotatus nigromaculis* (Diptera: Culicidae)	Larvae	Cornel *et al*., 2002
	Aedes aegypti (Diptera: Culicidae)	Larvae	Braga *et al*., 2005a, 2005b; Wu *et al*., 2006
Pyriproxyfen	*Aedes vigilax* (Diptera: Culicidae)	Larvae	Webb *et al*., 2012
Methoprene (added to the chicken feed)	*Musca domestica* (Diptera: Muscidae)	Adults	Breeden *et al*., 1980
Methoprene (added to water system)	*Haematobia irritans* (Diptera: Muscidae)	Adults	Beadles *et al*., 1975; Miller *et al*., 1977.

and rejection behaviours by their nest mates which can lead to the collapse of a colony. These aggressive behaviours may be corrected by the application of JH. Acceptance rates by nestmates may be increases by manipulating the level of JH to induce social or non-social behaviour in social insects.

CONCLUSION

JH clearly have a complex and unique biosynthesis in each order of insects. Eight different JH homologs have been identified thus far and presented in a different combination for each insect order. These hormones are synthesized by the gland namely corpora allata (CA) which is situated near the brain.

JHs are known to regulate various physiological changes such as moulting, reproduction, polyethism, and behavioural changes. Due to the complexity of JH pathways and regulation, this has opened new research interests among researchers to fully understand the impact of JHs on every insect species. The relationship of JH titre which change with various abiotic factors such as temperature, photoperiod and also diet is an interesting area to be explored.

In addition, synthetic JH compounds have been widely applied in insect population control such as IGRs in the pest control industry. Due to its potency and environmental benefits compared to chemical pesticides, synthetic JH products have been widely used in the industry worldwide. Yet, a continuous effort should be conducted to understand the nature of each JH of every insect in molecular level for the production of effective vectors control compound. Nevertheless, the dynamics of JH titres in fly larval development on decomposing bodies might potentially be useful in the field of forensic entomology. As discussed in this review, extensive entomo-biochemistry studies are needed before the data can be applied in real forensic settings.

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Conflict of Interest

The author declares that there is no conflict of interest.

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