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# Identification of putative ecdysteroid and juvenile hormone pathway genes in the shrimp *Neocaridina denticulata*

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ABSTRACT

Although the sesquiterpenoid juvenile hormone (JH) and the steroidal ecdysteroids are of vital importance to the development and reproduction of insects, our understanding of the evolution of these crucial hormonal regulators in other arthropods is limited. To better understand arthropod hormone evolution and regulation, here we describe the hormonal pathway genes (e.g. those involved in hormone biosynthesis, degradation, regulation and signal transduction) of a new decapod model, the shrimp *Neocaridina denticulata*. The majority of known insect sesquiterpenoid and ecdysteroid pathway genes and their regulators are contained in the *N. denticulata* genome. In the sesquiterpenoid pathway, these include biosynthetic pathway components: juvenile hormone acid methyltransferase (JHAMT); hormone binding protein: juvenile hormone binding protein (JHBP); and degradation pathway components: juvenile hormone esterase (JHE), juvenile hormone esterase binding protein (JHEBP) and juvenile hormone epoxide hydrolase (JHEH), with the JHBP, JHEBP and JHEH genes being discovered in a crustacean for the first time here. Ecdysteroid biosynthetic pathway genes identified include *spook*, *phantom*, *disembodied*, *shadow* and *CYP18*. Potential hormonal regulators and signal transducers such as allatostatins (ASTs), Methoprene-tolerant (Met), Retinoid X receptor (RXR), Ecdysone receptor (EcR), calponin-like protein Chd64, FK509-binding protein (FKBP39), Broad-complex (Br-c), and crustacean hyperglycemic hormone/molt-inhibiting hormone/gonad-inhibiting hormone (CHH/MIH/GIH) genes are all present in the shrimp *N. denticulata*. To our knowledge, this is the first report of these hormonal pathways and their regulatory genes together in a single decapod, providing a vital resource for further research into development, reproduction, endocrinology and evolution of crustaceans, and arthropods in general.



## 1. Introduction

Recent investigations into arthropod phylogeny have nested the Insecta within the Crustacea, forming a clade known as the Pancrustacea (Glenner et al., 2006; Regier et al., 2005). This has led to renewed interest in the ancestral state of a range of characters previously regarded as insect-specific, whose origin may in fact lie within crustaceans. One prominent example of such an under-investigated trait is the endocrine system that controls molting and reproduction, which is regulated primarily by sesquiterpene hormones and ecdysteroids.

Sesquiterpene hormone production in crustaceans and insects shares a bilaterian-conserved mevalonate biosynthetic pathway (Kenny et al., 2013; Tobe and Bendena, 1999). However, different final products are generated in different animal groups; for example, juvenile hormone (JH) is produced in the corpora allata (CA) of insects, and methyl farnesoate (MF) and farnesoic acid (FA) in the mandibular organ of crustaceans (Fig. 1) (Laufer and Biggers, 2001). Both hormones are believed to serve similar functions in development, growth, molting and reproduction (Homola and Chang, 1997b). As yet, JH has not been conclusively identified in

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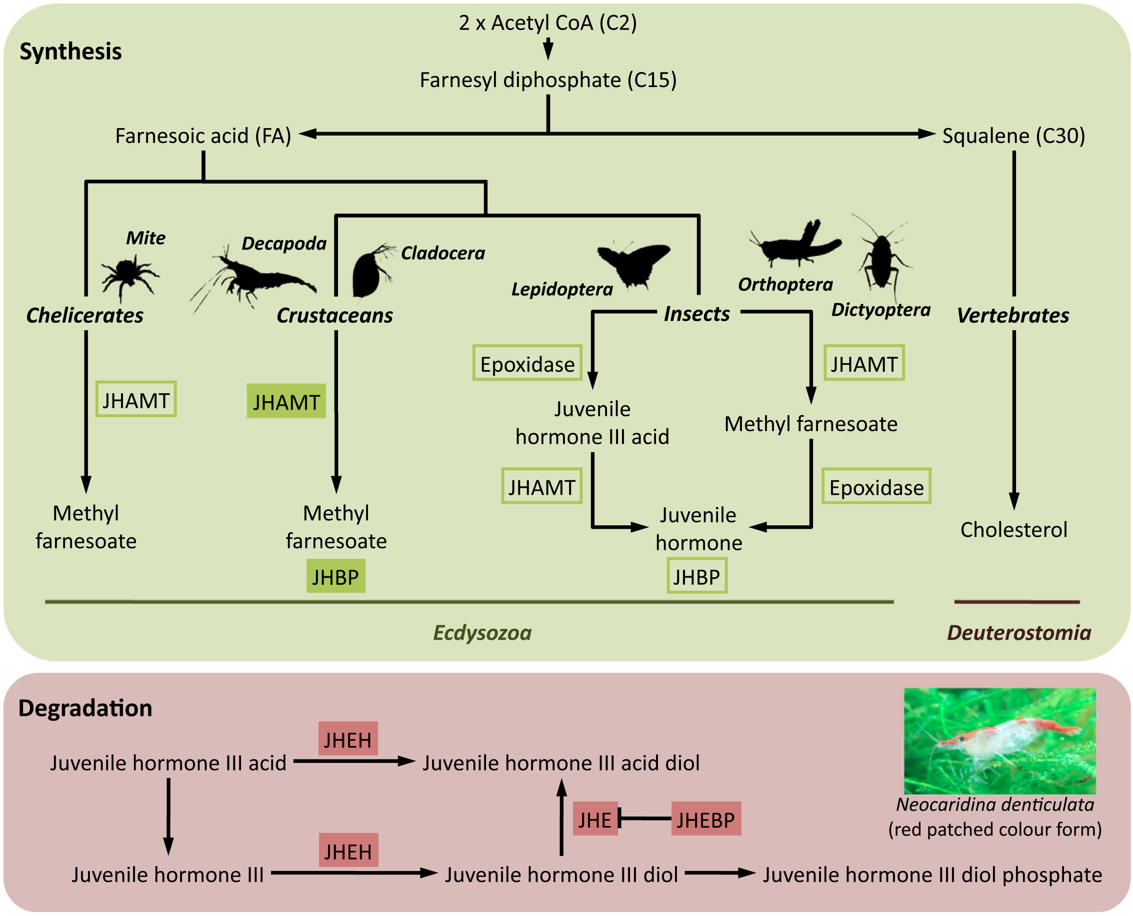


**Fig. 1.** Juvenile hormone pathway overview (biosynthesis and degradation). Comparative endocrinology of the mevalonate pathway in the Bilateria modified from Bellés et al. (2005), Hui et al. (2013), and Tobe and Bendena (1999). Genes identified in *Neocaridina denticulata* are shown in boxes with darker coloration (i.e. JHAMT, JHBP, JHEH, JHE and JHEBP). Note that the crustacean water flea *Daphnia pulex* (Hui et al., 2010), shrimp *Neocaridina denticulata* (this study), and chelicerate spider mite *Tetranychus urticae* (Grbić et al., 2011) contain JHAMT proteins in their genomes, but may not produce JH. For details, please refer to text.

crustaceans, and MF is generally thought to be the crustacean equivalent of JH, with JH historically considered an evolutionary derivative of MF unique to insects. However, in the early embryonic life of *Diploptera punctata*, more MF is biosynthesized and released than JH (Stay et al., 2002). The ability of the embryonic CA to convert MF to JH is acquired gradually in approximately 18–30 day old embryos. MF production in insects by the early embryonic CA suggests that MF as the final product of the sesquiterpenoid pathway could represent an ancestrally shared trait in arthropods.

In the last decades, one key rate-determining step in the biosynthesis of the juvenoid hormones has been thought to be the final conversion to JH or MF through an *S*-adenosyl-methyltransferase (SAM)-dependent methylation (Tobe and Bendena, 1999; Hui et al., 2010, 2013). In insects, juvenile hormone acid methyltransferase (JHAMT) is responsible for the methylation which converts juvenile hormone acid (JHA) to JH in the Lepidoptera, and farnesoic acid (FA) to MF in other insects (Fig. 1) (Defelipe et al., 2011). However, all insect JHAMTs are able to recognize both FA and JHA as substrates (Defelipe et al., 2011), and thus have the ability to convert FA to MF. The identification of *JHAMT* in the water flea *Daphnia pulex* (Hui et al., 2010) raises many questions as to the role and function of JHAMT in crustacean endocrinology, and the possible wider presence of the JH pathway across the crustaceans and other arthropods (Hui et al., 2010).

In both crustaceans and insects, development and reproduction are regulated by ecdysteroids or molting hormones, which are synthesized from dietary cholesterol by steroidogenic enzymes of the cytochrome P450 (CYP) family (Chávez et al., 2000; Mykles, 2011; Niwa et al., 2004; Petryk et al., 2003; Warren et al., 2002, 2004). The molting hormone 20-hydroxyecdysone (20E) is highly conserved in insects (Fig. 2) (Rewitz et al., 2007). The steroidogenic CYPs are encoded by the Halloween genes *phantom* (*CYP306A1*), *disembodied* (*CYP302A1*), *shadow* (*CYP315A1*) and *shade* (*CYP314A1*), which are collectively responsible for the last four hydroxylations in the pathway leading to 20E. Each of the Halloween enzymes is believed to mediate one specific enzymatic step in the biosynthesis of 20E, as mutations in these genes result in low ecdysteroid levels and embryonic death. Another CYP enzyme, *spook* (*CYP307A1*), is believed to mediate an uncharacterized step in the biosynthesis of 20E, as its mutation will also result in low 20E production (Rewitz et al., 2007). In crustaceans, orthologues of the Halloween genes have only been identified in a branchiopod, the water flea *D. pulex* (Rewitz and Gilbert, 2008), and the biosynthetic pathway of ecdysteroids remains largely undescribed in crustaceans outside the Insecta.

These gaps in our understanding can however now be addressed. Recent advances in genomic research have revolutionized comparative biology and allowed the investigation of trait evolution at the molecular level (Kenny et al., 2013), providing a