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Cloning and spatio-temporal expression of *CsKr-h1* encoding the juvenile hormone response gene in *Coccinella septempunctata* L

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Research Paper

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Abstract

The gene encoding juvenile hormone response (Krüppel homolog1, *Kr-hl*) in *Coccinella septempunctata* was investigated by cloning and analysing expression profiles in different developmental stages and tissues by quantitative real-time polymerase chain reaction (PCR). *C. septempunctata Kr-hl* (*CsKr-hl*) encoded a 1338 bp open reading frame (ORF) with a predicted protein product of 445 amino acids; the latter showed high similarity to orthologs in other species and contained eight highly-conserved Zn-finger motifs for DNA-binding. *CsKr-hl* was expressed in different developmental stages of *C. septempunctata*. The expression levels of *CsKr-hl* in eggs, 2nd, 3rd, 4th instar larvae, and pupa were 3.31, 2.30, 7.09, 0.58, and 7.48 times the number of 1st instar larvae, respectively. *CsKr-hl* expression levels in female adults gradually increased at 25–30 days and were significantly higher than expression at 1–20 days. *CsKr-hl* expression in 20–30 days-old male adults was significantly higher than males aged 1–15 days. *CsKr-hl* expression levels in heads of male and female adults were significantly higher than expression levels in the thorax, adipose, and reproductive system. Interestingly, *CsKr-hl* expression levels in the adipose and reproductive system of female adults were significantly higher than in adult male corresponding organs, which suggest that *CsKr-hl* plays an important role in regulating reproductive development in *C. septempunctata*.

Introduction

Juvenile hormone (JH) is secreted by the insect corpora allata (Hang *et al.*, 2004), which maintains larval characteristics and the prothoracic gland, promotes ovarian maturation, and regulates insect development, metamorphosis, and reproduction (Shelby *et al.*, 2007; Riddiford *et al.*, 2010; Kayukawa *et al.*, 2012; Hiruma and Kaneko, 2013; Hu *et al.*, 2020). JH regulates the downstream response gene krüppel homolog1 (*Kr-hl*) through the JH receptor methoprene-tolerant (*Met*) gene, thus leading to corresponding physiological responses (Jin and Lin, 2014). The regulatory mode of JH-Met-Kr-h1 is complex, and research on *Kr-h1* function gene has primarily focused on its role in regulating insect growth and development, neuronal cell formation, foraging behaviour, and sexual maturation (Jin and Lin, 2014; Gassias *et al.*, 2021).

Coccinella septempunctata L. (Coleoptera: Coccinellidae) is an important predatory insect that can effectively control aphids, white flies, jassids, and small lepidoptera larvae in the field (Zhou *et al.*, 2017; Chatha and Naz, 2020; Hakeem *et al.*, 2021; Bajracharya *et al.*, 2023). Due to climate change and the application of pesticides, field population of *C. septempunctata* have decreased (Sun and Wan, 1999; Zhang *et al.*, 2014), and it is now necessary to artificially rear *C. septempunctata*. The cost of feeding of *C. septempunctata* with an aphid diet is high, so it is necessary to develop artificial food for ladybird; unfortunately, artificial diets are generally inferior to aphid diets with regard to weight gain, pupation rates, and eclosion rates as compared with aphid diets (Sarwar and Saqib, 2010; Yazdani and Zarabi, 2011; Cheng *et al.*, 2022, 2023). A diet of artificial food impacts the normal secretory activities of *C. septempunctata* neurosecretory cells and the pharyngeal lateral body. Low levels of endogenous hormones inhibit the synthesis and release of vitellogenin protein, thus affecting ovary development and oocyte maturation, resulting in more female individuals entering reproductive diapause (Fu and Chen, 1984). The impact on gonadal development and semen proteins has not been reported yet.

In the early stages of optimising the ladybird artificial diet, we observed that the addition of JH to the diet resulted in adult egg production and hatching rates that were respectively four- and three-fold higher than diets lacking JH. However, amendment of the diet with JH significantly prolonged the duration of larval development and reduced the pupation rate (Cheng *et al.*, 2022, 2023). The underlying mechanism by which JH regulates the expression of *Met* and *Kr-hl* genes in *C. septempunctata* remains unclear.

Our group recently cloned and monitored spatiotemporal expression of the *C. septempunctata Met* (*CsMet*) gene; this was expressed in different developmental stages and was most highly expressed in 3rd instar larvae (unpublished). *CsMet* expression levels gradually increased in 20- to 30-d-old female adults. With the exception of 20-d-old female adults, the expression level of *CsMet* was higher in females as compared to male adults. *CsMet* expression levels in male and female adult adipose tissue were significantly higher than expression in the head, thorax, and reproductive systems.

In this study, we evaluate whether the expression of *Kr-hl* in *C. septempunctata* is similar to *CsMet*. The transcriptome database of *C. septempunctata* (Cheng *et al*., 2020) was used to clone the cDNA sequence of *Kr-h1,* and its expression was characterised in different developmental stages and tissues to evaluate potential improvement of the artificial ladybird diet.

Materials and methods

Insects

C. septempunctata were collected from Jinzhu Town, Huaxi District, Guiyang City, Guizhou Province. Ladybugs were reared indoors on *Aphis craccivora* Koch (Hemiptera: Aphididae) for over 20 generations at the Institute of Plant Protection, Guizhou Academy of Agricultural Sciences. Experiments were performed in environmental chambers at 25 ± 1°C, 70 ± 5% RH with a 16: 8 h light : dark photoperiod.

RNA extraction and cDNA synthesis

Ten-day-old female adults were grinded to a powder in liquid nitrogen and then transferred to 1.5 mL RNase-free microcentrifuge tubes. Trizol (1 ml) was added, and the mixture was incubated for 5 min at room temperature; trichloromethane (200 μl) was then added, gently mixed, incubated at room temperature for 3 min, and then centrifuged at 12,000 × g for 15 min at 4°C. A 600 μl volume of the supernatant was transferred into a new microcentrifuge tube, 500 μl of 100% isopropanol was added, and the mixture was incubated at room temperature for 10 min. The suspension was then centrifuged at 12,000 × g at 4°C for 10 min; the supernatant was then removed and 75% ethanol was added, gently inverted eight times, and centrifuged at 7500 × g for 5 min at 4°C. Ethanol was then removed and RNA pellets were allowed to air dry for 5–10 min. RNA (1 μg) was used as a template, and the first strand of cDNA was synthesised using the RevertAid First Strand cDNA Synthesis Kit (Fermentas Co.) and stored at −80°C for future use.

Cloning and sequencing of Kr-h1

Based on the transcriptome database of *C. septempunctata* constructed in our laboratory, the ORF encoding *Kr-h1* was selected for cloning. cDNA from 10-d-old female adults was used as a template; primers were designed using ClustalX and Primer Premier 5.0 and synthesised by Sangon Biotech Co. (Shanghai) (Table 1). Using the 3' and 5' first-strand cDNA as templates, full-length amplification of *CsKr-h1* was performed using the SMARTer RACE cDNA Amplification Kit (Clontech Co.) and the 5' RACE System for Rapid Amplification of cDNA Ends (Invitrogen Co.). PCR products were purified and recovered by 1% agarose gel electrophoresis, and then connected to pmd-18t carrier, transformed DH5a competent cells, and sequenced by Sangon Biotech Co. The ORF Finder program (https://www.ncbi.nlm. nih.gov/orffinder/) was used to identify the coding region for *Kr-h1*, and the ExPASy program (https://web.expasy.org/compute_pi/) was used to identify the isoelectric point and molecular weight of the *Kr-h1*-encoded protein. SMART 8 software (https://prosite.expasy.org/) was used to identify the structural domain of the encoded protein.

Table 1. Primers used in this study

Primer name	Primer sequence (5′ to 3′)	Usage
Kr-h1- 3'1	TCTCTAAGTCACCTTGCTCTTGGGA	RACE
Kr-h1 −3'2	AGGACGATAGGGACAGTTTCCACGA	
Kr-h1-5'1	GTCTCTTGAATAATGAAAACACTT	
Kr-h1-5'2	GAAGTAATAATAATTATCATTCG	
Kr-h1-QF	AACCTTTCGAGTGCCCTGAAT	Real-time PCR
Kr-h1-QR	ATGCCTCCTCCTGAACCTACT	
Actin-F	GATTCGCCATCCAGGACATCTC	
Actin -R	TCCTTGCTCAGCTTGTTGTAGTC	

BLAST and phylogenetic analyses of Kr-hl

The NCBI BLAST program (https://blast.ncbi.nlm.nih.gov/Blast) was used to analyse similarity between *C. septempunctata Kr-hl* (*CsKr-hl*) and orthologs in other insect species. To further investigate evolutionary relationships, deduced amino acids of insect *Kr-hl* sequences were downloaded from NCBI, and a phylogenetic tree was constructed using the neighbour-joining method by MEGA 6 software.

Spatio-temporal expression of CsKr-hl

Samples of different developmental stages (e.g., 2-d-old eggs, 1st −4th instar larvae, pupae, and 1-, 5-, 10-, 15-, 20-, 25- and 30-d-old female and male adults) were collected along with heads, chest, adipose, and reproductive systems of 10-d-old female and male adults. A single replicate consisted of the following: 60 eggs; 30, 1st instar larvae; 15, 2nd instar larvae; 10, 3rd instar larvae; four 4th instar larvae; four pupae; and four adults. The head, chest, adipose, and reproductive system were dissected from 6 10-d-old female and male adults, respectively, and considered as one replicate (Table 2). Each sample was replicated three times. Collected samples were immediately frozen in liquid nitrogen and stored at −80°C for future use.

Total RNA was extracted from each sample according to the instructions included with the Eastep® Super Total RNA

Table 2. Sample size for the spatio-temporal expression of *CsKr-hl*

Samples	Numbers	Samples	Numbers
2-days-old eggs	60	Adults	4
1st instar larvae	30	Head	6
2nd instar larvae	15	Chest	6
3rd instar larvae	10	Adipose	6
4th instar larvae	4	Ovary	6
Pupas	4	Testis	6

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Isolation Kit (Promega Co.). The IScript cDNA Synthesis Kit (Bio-Rad Co.) was used to reverse transcribe and synthesise cDNA, and samples were stored at −20°C for future use. The Kr-hl-specific primers, Kr-hl-QF/ Kr-hl-QR, and Actin-F/ Actin-R (internal standard, Liu *et al*., 2019) were used to measure expression in different developmental stages and tissues (Table 1). qPCR was conducted in a 20 μl volume containing the following: cDNA template, 2 μl; upstream and downstream primers, 2 μl each; Sso Advanced Universal SYBR Green Supermix, 10 μl; and ddH_2O, 4 μl. The qPCR reaction conditions included pre-denaturation at 95°C for 2 min; 95°C denaturation for 5 s, and 60°C annealing for 30 s for a total of 39 cycles. The relative expression level of *Kr-hl* was calculated using the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001).

Statistical analysis

One-way ANOVA was performed on the experimental data, and the multiple comparison LSD method was used to determine significance with DPS 17.0 software (Tang and Zhang, 2013).

Results

Cloning and sequence analysis of C. septempunctata Kr-hl

Based on our transcriptome data of *C. septempunctata*, cDNA from female adults was used as a template to clone *CsKr-hl* (GenBank accession no. OR183710). Sequence analysis showed that *CsKr-hl* cDNA was 2694 bp and encoded a 1338 bp ORF consisting of 445 predicted amino acids and 5' and 3' noncoding

```
1    ATGGAAATCAGTGTATTAAATAAATTAATAAGATTTCGAATGATAATTATTATTACTTCTTTTGTGATAAAA
1     M  E  I  S  V  L  N  K  L  I  R  F  R  M  I  I  I  I  T  S  F  V  I  K
73   AAGTGTTTTCATTATTCAAGAGACATGCGTGTGAGTGAGGGTGAGCAATTTGAGTTCGGATTACTGCACTAT
25    K  C  F  H  Y  S  R  D  M  R  V  S  E  G  E  Q  F  E  F  G  L  L  H  Y
145 ATCACAACATACTTTGGAATGGATTTTATTGAAATCCTAACTCCAAGGGAGATCAAGGTAGAACCAGATGAC
49    I  T  T  Y  F  G  M  D  F  I  E  I  L  T  P  R  E  I  K  V  E  P  D  D
217 CAAACGAATTTGAACAATTCGTTTTCCGCAGTTGGATTCCCATCGCCCTTCCCAAACCCAGCGATGTTCACT
73    Q  T  N  L  N  N  S  F  S  A  V  G  F  P  S  P  F  P  N  P  A  M  F  T
289 CCTTCCCAACTGCTTATGGCCAGTCAATTCATGGCAGCCTCAGGATTGGCCATCCCGCCAAACCCTGCATTC
97    P  S  Q  L  L  M  A  S  Q  F  M  A  A  S  G  L  A  I  P  P  N  P  A  F
361 TTCCATCCAAGTTTGCTGGGGCAGTTAACGTGGTCGAACACATCTCCACCATCTCCTCCAAACAGCAGCCAA
121   F  H  P  S  L  L  G  Q  L  T  W  S  N  T  S  P  P  S  P  P  N  S  S  Q
433 CAGTTATCTCCAGCAACGAAGGTTAGAAAGCTCAACATAAACAACAATAACAATATCGTGACAAGTAGTACA
145   Q  L  S  P  A  T  K  V  R  K  L  N  I  N  N  N  N  N  I  V  T  S  S  T
505 AATGAATTAAAGGCTGTTACGAAGAAGAGATGGAAGGAAGAAAGGGAAGCTGTATCACCCACCTCCAGTTCT
169   N  E  L  K  A  V  T  K  K  R  W  K  E  E  R  E  A  V  S  P  T  S  S  S
577 TCACCACCATCGAGCACTGACGTGAGCACCAAAGAAATCAACAGGGATAAACAGTTCACCTGCACCATTTGC
193   S  P  P  S  S  T  D  V  S  T  K  E  I  N  R  D  K  Q  F  T  C  T  I  C
649 AACAGGTCCTTCGGCTACAAACACGTGCTACAGAACCATGAGAGAACCCACACGGGGGAAAAACCTTTCGAG
217   N  R  S  F  G  Y  K  H  V  L  Q  N  H  E  R  T  H  T  G  E  K  P  F  E
721 TGCCCTGAATGCCATAAGAGATTCACTAGGGATCATCATTTGAAAACTCACATGCGTCTACATACAGGAGAA
241   C  P  E  C  H  K  R  F  T  R  D  H  H  L  K  T  H  M  R  L  H  T  G  E
793 AGACCGTATCATTGTGAACACTGTGATAGGCAATTCGTTCAAGTGGCGAATTTGAGGAGGCACCTACGGGTA
265   R  P  Y  H  C  E  H  C  D  R  Q  F  V  Q  V  A  N  L  R  R  H  L  R  V
865 CACACTGGGGAAAGACCATATGCTTGTGAGCACTGCAGCGCTAAATTCTCTGATTCAAATCAGCTCAAAGCG
289   H  T  G  E  R  P  Y  A  C  E  H  C  S  A  K  F  S  D  S  N  Q  L  K  A
937 CATCTTCTGATTCATACCAACGAGAAACCGTTTGATTGCGAAAAATGTCACAGTAGGTTCAGGAGGAGGCAT
313   H  L  L  I  H  T  N  E  K  P  F  D  C  E  K  C  H  S  R  F  R  R  R  H
1009 CACTTACTTCATCACAAGTGCGGGATGGACAAAGAAGTTAAGATAGAAGTCGATGAAATGGAGGAGATCGCT
337    H  L  L  H  H  K  C  G  M  D  K  E  V  K  I  E  V  D  E  M  E  E  I  A
1081 AGGACAAGACAAAGGATCTCACCACCGACACCAATGATTGTACAGTCATCTCCACTCATATCCCCCAAAACT
361   R  T  R  Q  R  I  S  P  P  T  P  M  I  V  Q  S  S  P  L  I  S  P  K  T
1153 CCAATTTTATCACTTCCTCTATCATCATCTTTACCAGAACAAACGGAGCCGGAAGATTTGTCCATGAGCACA
385   P  I  L  S  L  P  L  S  S  S  L  P  E  Q  T  E  P  E  D  L  S  M  S  T
1225 GGCATGCATTCTCCAGTCTCTAAGTCACCTTGCTCTTGGGAGGCTGAGGACGATAGGGACAGTTTCCACGAA
409   M  H  S  P  V  S  K  S  P  C  S  G  W  E  A  E  D  D  R  D  S  F  H  E
1297 GATCTTCAACCTATTGATCTTCGAGGTAAAACTAAATCTTAG↵
433   D  L  Q  P  I  D  L  R  G  K  T  K  S  *  ↵
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Figure 1. Nucelotide and deduced amino acid sequence of *CsKr-hl*.

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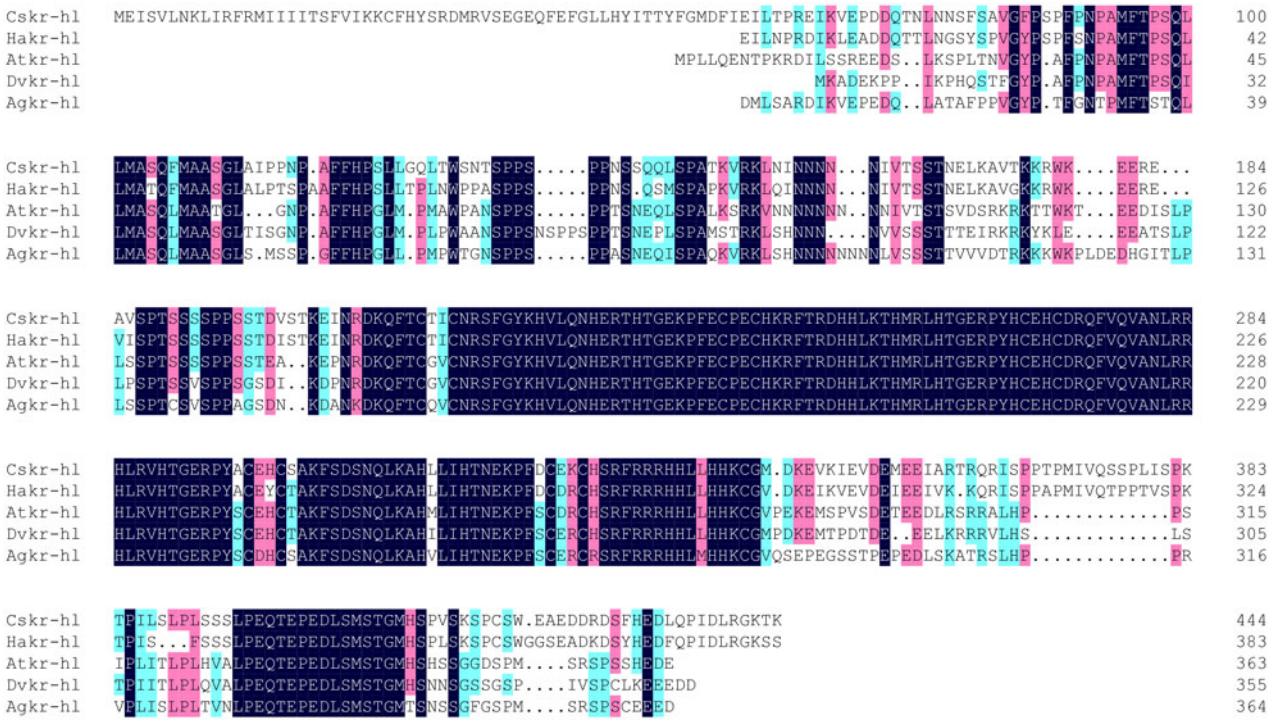

Figure 2. Multiple sequence alignments of *CsKr-hl* from *Coccinella septempunctata* and orthologs in other insect species. Abbreviations: Cskr-hl, *Coccinella septempunctata*; HaKr-hl, *Harmonia axyridis*; AtKr-hl, *Aethina tumida*; DvKr-h, *Diabrotica vir gifera*; and AgKr-hl, *Anthonomus grandis.*

regions of 126 and 1230 bp, respectively (fig. 1). The molecular weight of the predicted *CsKr-hl* protein was 51.01kD, and its isoelectric point was 8.50. There were eight conserved domains, namely ZINC_FINGER_C2H2_2, Zinc finger C2H2 type domain profile, ZINC_FINGER_C2H2_1, and Zinc finger C2H2 type domain signature repeat; these were located at amino acid residues 211–238, 239–266, 267–294, 295–322, and 213–233, 241–261, 269–289, 297–317, respectively.

BLAST comparison and phylogenetic analysis of CsKr-hl

BLAST analysis of *CsKr-hl* in NCBI revealed 83.29% similarity with *HmKr-hl* (*Harmonia axyridis)*, and then 63.09%, 65.07%, and 63.12% similarity with *AtKr-hl* (*Aethina tumida*), *DvKr-hl* (*Diabrotica virgifera*), and *AgKr-hl* (*Anthonomus grandis)*, respectively (fig. 2). Phylogenetic analysis indicated that *CsKr-hl* and *AtKr-hl* clustered together in one branch (fig. 3).

Spatiotemporal expression analysis of CsKr-hl

There were significant differences in *CsKr-hl* expression levels in the egg to pupal stages at different developmental stages ($F = 12.06$, $df = 5,10$, $P = 0.0006$) (fig. 4). Expression gradually increased in 1st to 3rd instar larvae, decreased in 4th instar larvae, and increased significantly in the pupal stage. The relative expression level of *CsKr-hl* in 1st instar larvae was set to 1. From egg to pupal stage, the expression level of 4th instar larvae was the lowest at 0.58 times the number of 1st instar larvae, while the expression level of pupa was the highest at 7.48 times the number of 1st instar larvae.

There were obvious differences in *CsKr-hl* expression levels when comparing female and male adults at different developmental stages (fig. 5). Expression levels in 1 to 15-d-old female and male adults were relatively low, and only *CsKr-hl* expression in1-d-old female adults was higher than male adults. The relative expression level of *CsKr-hl* in 1-d-old male adults was set to 1. The expression levels of *CsKr-hl* in 1, 5, 10, 15, and 20-d-old female adults were higher at 3.26, 1.28, 1.05, 1.35, and 2.00 times the number of 1-d-old male adults, respectively. The expression levels of *CsKr-hl* in 1, 5, 10, and 15-d-old male adults were 1, 1.36, 0.91, and 1.31 times the number of 1-d-old male adults, respectively. Expression levels gradually increased in 20-d-old adults and was significantly higher in males vs females at ages 20 d ($F = 35.79$, $df = 1,2$, $P = 0.0268$) and 25 d ($F = 54.38$, $df = 1,2$, $P = 0.0179$). The expression level of *CsKr-hl* in 30-d-old female adults was significantly higher than in males ($F = 37.36$, $df = 1,2$, $P = 0.0257$) (fig. 6).

The relative expression level of *CsKr-hl* in thorax was set to 1. The expression levels of *CsKr-hl* gene in female adults in head, adipose, and ovary were 25.96, 6.69, and 6.57 times the number of thorax, respectively. The expression levels of *CsKr-hl* gene in male adults in head, adipose, and ovary were 19.79, 2.86, and 2.75 times the number of thorax, respectively. The expression level of *CsKr-hl* in females and males was highest in the head. The relative expression level of *Cskr-hl* in the head ($F = 22.33$, $df = 1,2$, $P = 0.0420$), adipose ($F = 2226.19$, $df = 1,2$, $P = 0.0004$), and reproductive system ($F = 75.52$, $df = 1,2$, $P = 0.0130$) of females was significantly higher than males; however, there was no significant difference in *Cskr-hl* expression in the thorax of the two sexes ($F = 0.0369$, $df = 1,2$, $P = 0.8655$).

Discussion

JH is secreted by the corpora allata and forms a complex with carrier protein and ultimately reaches the nucleus through blood circulation. JH then binds specifically to *Met* or related complexes to

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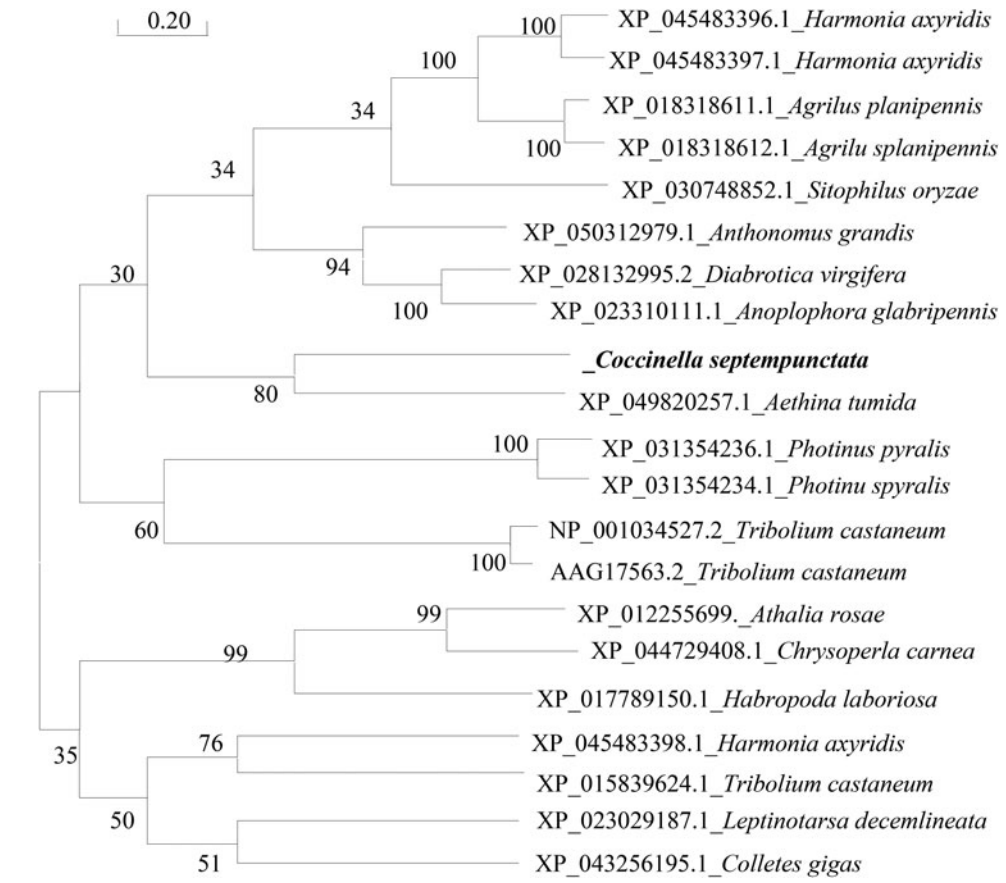

Figure 3. Phylogenetic tree of *CsKr-hl* from *Coccinella septempunctata* and orthologs in other insect species.

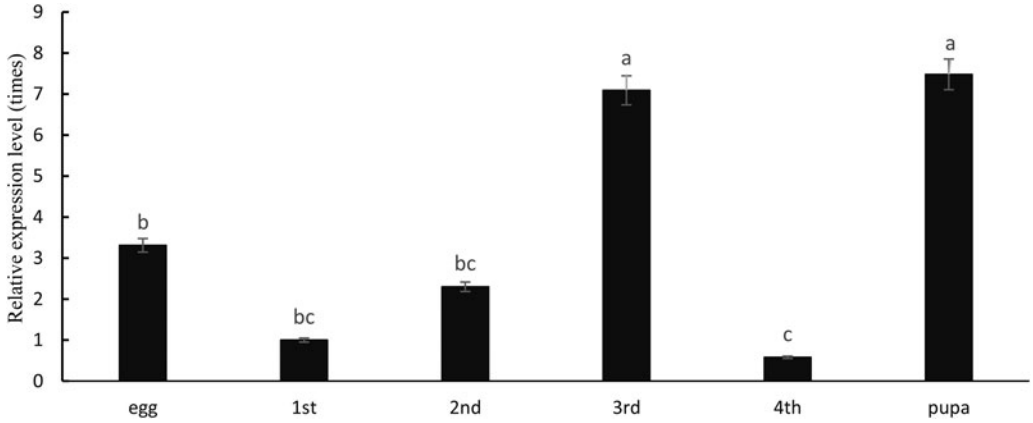

Figure 4. Relative expression levels of *CsKr-hl* in different developmental stages of *Coccinella septempunctata.* Columns labelled with different letters indicate significance at $P < 0.05$ using the LSD test.

convert hormonal signals and initiate *Kr-h1* transcription, which affects growth and development and causes morphological changes in insects (Jindra *et al.*, 2013). *Kr-h1* protein is a transcription factor containing C2H2 zinc fingers (Jin and Lin, 2014). *CsKr-h1* has eight zinc fingers, and phylogenetic analysis indicated that *CsKr-h1* is closely related to *Kr-h1* in *Aethina tumida*, which is also a member of the Coleoptera, which indicates a high level of evolutionary conservatism among members of the Coleoptera.

In this study, qPCR revealed that *CsKr-h1* is expressed in various developmental stages of *C. septempunctata*. In the larval stage, the expression of *CsKr-h1* was consistent with *CsMet*, and expression levels in 3rd instar larvae were highest; this may be caused by the rapid growth rate of 3rd instar larvae and the need for larger quantities of JH. *CsKr-h1* expression rapidly decreased as larvae entered the 4th instar stage. Furthermore, *Kr-h1* is regarded as an early-stage inducible gene that is responsible for the repression of metamorphosis (Kayukawa *et al.*, 2016). The 4th instar larvae

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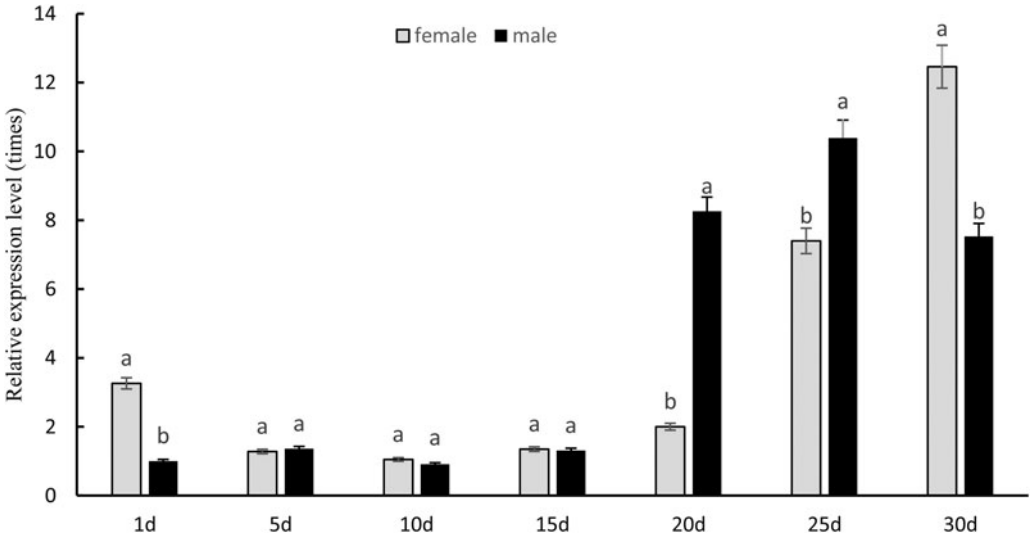

Figure 5. Relative expression levels of *CsKr-hl* in different ages of *Coccinella septempunctata* adults. Data are means ± SD. Columns labelled with different letters indicate significance at $P < 0.05$ using the LSD test.

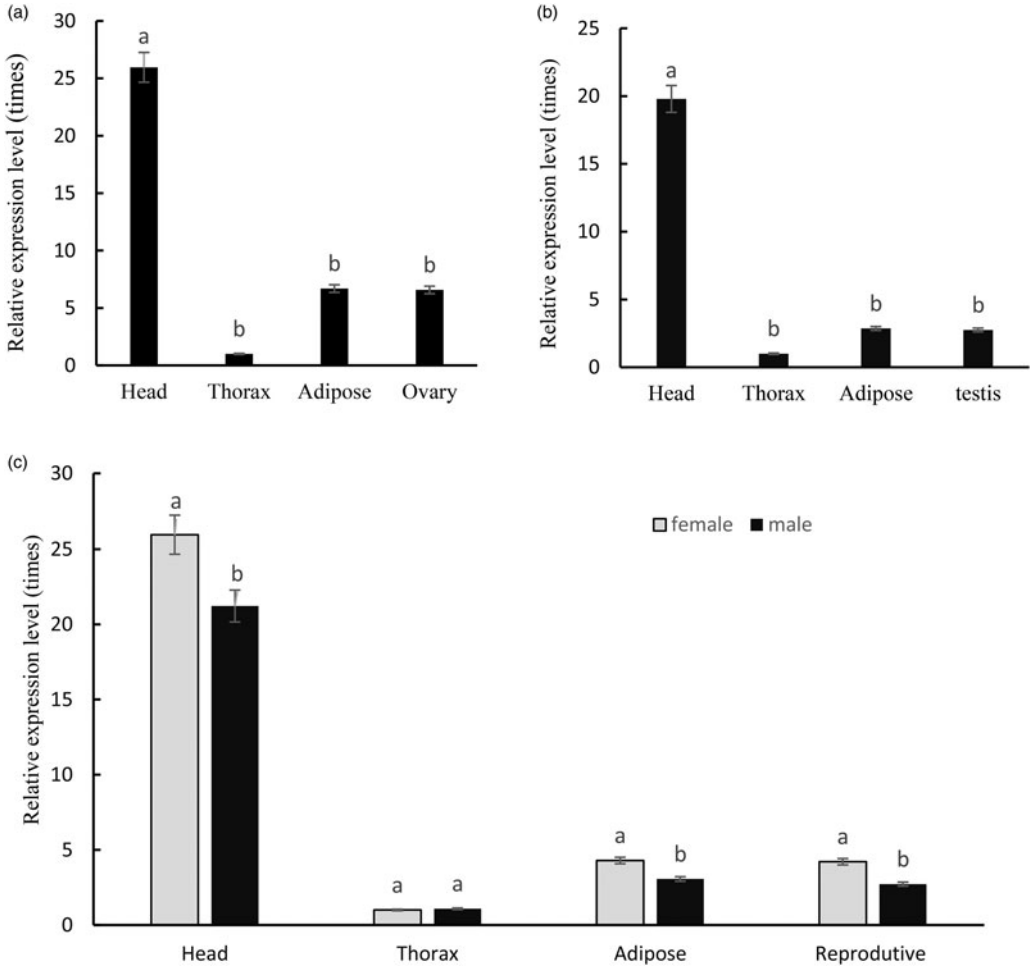

Figure 6. Relative expression levels of *CsKr-h1* in different tissues of *Coccinella septempunctata* adults. Panels: (a) female adults; (b) male adults; and (c) female and male adults. Data are mean ± SD. Columns labelled with different letters indicate significance at $P < 0.05$ using the LSD test.

of ladybirds metamorphose into pupal stage, and *CsKr-h1* may be reduced by regulation. When *C. septempunctata* entered the pupal stage, *CsKr-h1* expression levels were significantly upregulated, which prepares the insect for reproduction in the adult stage. Addition of JH to the artificial diet of the *C. septempunctata* prolonged the larval lifespan and decreased pupation rates (Cheng

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et al., 2022, 2023). Collectively, our results indicate that JH regulates insect metamorphosis via *CsKr-h1*; however, further investigation is needed to determine whether changes in JH levels impact the transcription of *CsKr-h1*.

The expression of *CsKr-h1* in female adults of *C. septempunctata* was lower from 1 to 15 days than 20 to 30 days after eclosion. During the peak period of egg deposition (20–30 days after eclosion), the expression levels of *CsKr-h1* gradually increased; this suggests that *CsKr-h1* is involved in the regulation of reproduction. Furthermore, *CsKr-h1* expression levels in males were significantly higher at 20–30 days post-emergence as compared to 1–15 days. Gassias *et al*. (2021) highlighted the involvement of the JH-Met-Kr-h1 signalling pathway in the development and secretory activity of male accessory glands in *Agrotis ipsilon*. We speculate that *Kr-h1* in *C. septempunctata* was also involved in the development and secretory activity of male accessory glands, and its peak secretion was consistent with the peak period of egg deposition by female adults, with both occurring 20–30 days after eclosion. Injection of JH-II into newly emerged *A. ipsilon* adult males induced the transcription of Met1, Met2, and Kr-h1 associated to an increase in the length and protein content of the male accessory glands (Gassias *et al*., 2021). In future studies, we will utilise gene silencing to verify whether the *CsKr-h1* is involved in the regulation of male accessory gland development and secretion activity. Analysis of tissue expression patterns showed that *CsKr-h1* was most highly expressed in adult ladybug heads, whereas *Kr-h1* was higher expressed in larval heads of *Dendroctonus armandi* (Sun *et al*., 2022). In *Drosophila photodetector* and *Agrotis ipsilon*, *Kr-h1* promotes neuronal cell formation in head (Duportets *et al*., 2012; Fichelson *et al*., 2012), which is also a possible function of *CsKr-h1* in *C. septempunctata*. *CsKr-h1* was highly expressed not only in the heads but also in the adipose tissue and reproductive system, while *Kr-h1* of *H. axisis* was highly expressed in wings, legs, and adipose tissue (Han *et al*., 2022).

In summary, we cloned the JH response gene *CsKr-h1* from *C. septempunctata* and analysed its transcription, thus providing a foundation for clarifying the role of JH in regulating reproductive development in ladybird beetles. In the future, JH will be added to artificial diets and *CsKr-h1* transcription will be assessed. Gene silencing will be used to analyse development of the reproductive system, egg production, and hatchability of *C. septempunctata*, and the regulatory effects of *CsKr-h1* on the growth and reproduction of ladybird beetles will be explored.

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