Parasitology Research (2025) 124:36
https://doi.org/10.1007/s00436-025-08479-z

RESEARCH

Krüppel homolog 1, a juvenile hormone transcription factor, regulates the reproduction and development of *Aedes albopictus*(Skuse) (Diptera: Culicidae)

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Received: 12 November 2024 / Accepted: 5 March 2025 / Published online: 22 March 2025

Abstract

The control of *Aedes albopictus* (Skuse) (Diptera: Culicidae), a vector for several important viral diseases, is crucial for mitigating mosquito-borne diseases. This study focused on the *Krüppel homolog 1 (Kr-h1)* gene, a transcription factor in juvenile hormone (JH) signaling, which plays a pivotal role in inhibiting metamorphosis and promoting adult reproduction. We characterized *Aedes albopictus Kr-h1*(*AalbKr-h1*), identified its eight zinc finger domains, and confirmed its orthology among insects through phylogenetic analysis. Expression profiling across life stages showed high level of expression in eggs, late larvae, and adults, with minimal expression in pupae. In adults, *AalbKr-h1* was most active in the fat body and ovaries. Exposing larvae to a JH analogue significantly upregulated *AalbKr-h1* expression in both larval and adult stages. RNAi-mediated knockdown of Kr-h1 protein reduced egg reproduction, survival rate and gene expression levels. These results provide a solid foundation for further exploration of the function of the *AalbKr-h1* gene and the potential development of novel strategies for mosquito control and prevention of mosquito-borne diseases.

Section Editor: Alessia Cappelli

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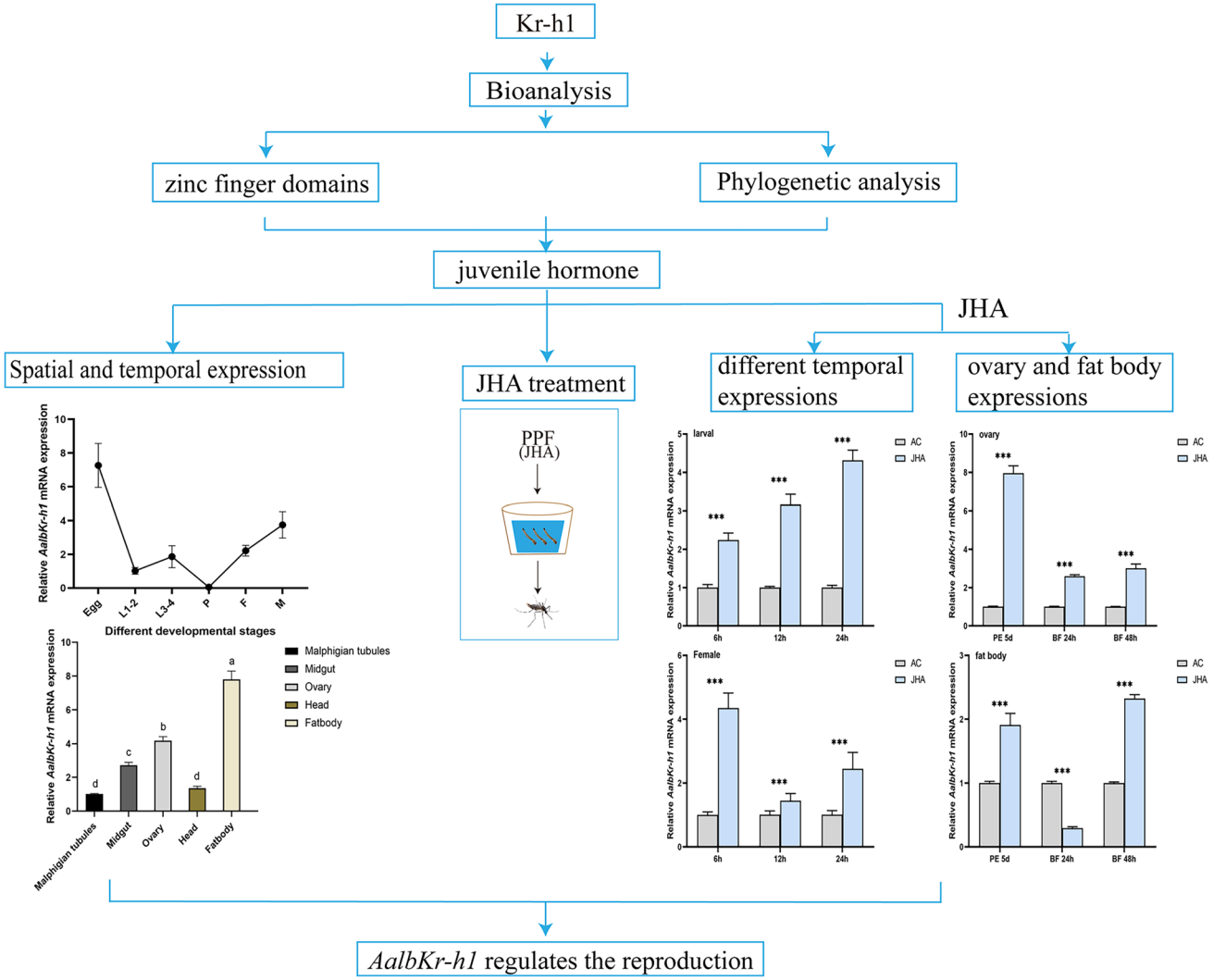
Graphical Abstract



Keywords *Aedes albopictus* · *Krüppel homolog 1* · Zinc finger domains · Juvenile hormone · Juvenile hormone analogue

Introduction

Aedes albopictus (Skuse) (Diptera: Culicidae) (*Ae. albopictus*) is an important vector of the Zika virus, dengue fever, yellow fever, and chikungunya, posing a substantial threat to global human health (Weaver and Lecuit 2015). Dengue fever is one of the most rapidly spreading mosquito-borne infectious diseases in the world, with approximately 390 million people in 128 countries or regions at risk, and 96 million experiencing varying degrees of clinical symptoms (Turtle and Solomon 2018). Currently, effective vaccines and specific antiviral therapies for many mosquito-borne diseases remain unavailable (Onen et al. 2023). Chemical control remains the primary method for managing *Ae. albopictus* populations and reducing the risk of dengue fever. Pyriproxyfen, a juvenile hormone analogue (JHA), is an insect growth regulator widely used in mosquito control applications due to its environmental friendliness and target specificity (Sullivan and Goh 2008).

The complex life cycle of insects, including changes in morphology, physiology, and behavior, is closely linked to two hormonal systems: the juvenile hormone (JH) and 20-hydroxyecdysone (20E). These hormones play crucial roles during both the embryonic period and late embryogenesis (He and Zhang 2022). JH and 20E also regulate various aspects of reproduction, including follicular development, folliculogenesis and oogenesis (Santos et al. 2019; Swevers 2019; Swevers and Iatrou 2009). Vitellogenesis is crucial for female reproduction, where vitellogenin (Vg) is synthesized in the fat body, secreted into the hemolymph, and subsequently absorbed by developing oocytes through receptor-mediated endocytosis (Arrese and Soulages 2010).

Krüppel homolog 1 (*Kr-h1*) contains the DNA-binding domain of the C2H2-type zinc finger and is a key transcription factor for JH signaling, which plays a critical role in insect metamorphosis, development, and adult reproduction (Truman 2019; Roy et al. 2018; Belles 2020). JH prevents metamorphosis at the ultimate pre-immature stage. It disappears in the final larval instar, allowing metamorphic molting to transform the larvae either directly (hemimetaboly) or through the pupal stage (holometaboly) into adults (Truman 2019; Jindra 2019; Belles 2019). The dynamic expression pattern of *Kr-h1* is closely related to its various functions throughout the insect's life (He and Zhang 2022).

The function of *Kr-h1* in female reproduction varies among different insect species. In species such as *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Zhang et al. 2018a, b), *Locusta migratoria* (Orthoptera: Acrididae) (Song et al. 2014), *Aedes aegypti* (Diptera: Culicidae) (Ojani et al. 2018), *Bactrocera dorsalis* (Diptera: Tephritidae) (Yue et al. 2018), and *Sogatella furcifera* (Hemiptera: Delphacidae) (Hu et al. 2020), *Kr-h1* silencing impedes JH-regulated Vg expression, oocyte maturation, and ovarian development. In *Locusta migratoria* (Song et al. 2014) and *Colaphellus bowringi* (Coleoptera: Chrysomelidae) (Liu et al. 2019), JHA administration enhances the transcription of *Met* and *Kr-h1*, promoting the expression of vitellogenin receptor (VgR) genes associated with reproduction. However, *Met* depletion, while inhibiting Vg expression in the fat body, does not affect Vg expression when *Kr-h1* is silenced in *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae) (Smykal et al. 2014) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Parthasarathy et al. 2010).

Additionally, *Kr-h1* is implicated in neuronal morphogenesis, sexual behavior maturation, embryogenesis, and metabolic homeostasis regulation in insects (He and Zhang 2022). *Kr-h1* plays a crucial role in the metamorphosis of insects. Currently, there are limited studies on the physicochemical properties, molecular structure and induced reproductive development in *Ae. albopictus*. Therefore, our research focuses on the effects of *Kr-h1* on the development and tissues of *Ae. albopictus* at various life stages. This is achieved through the analysis of sequencing results and the expression analysis of JHA-induced *Ae. albopictus*. RNAi experiments indicated that AalbKr-h1 played an important role in the growth and egg production. These studies aim to provide new insights into the biological function and potential biological control of *Kr-h1* in the metamorphosis of *Ae. albopictus*.

Materials and methods

Mosquito maintenance and sample preparation

Ae. albopictus of the Foshan (FS) strain was maintained at 26 ± 2 °C and 70 ± 10% relative humidity, with a light–dark cycle of 16: 8 h (L: D). The larvae were fed turtle food in 15 × 10.5 × 5.5 cm plastic pans. Pupae were collected daily and transferred to a water-filled cup within 50 × 50 × 50 cm adult rearing cage. Adults were provided with a 10% (wt/vol) sucrose solution daily using cotton pads. Female mosquitoes were fed on commercial defibrinated sheep blood (Hongquan Biotechnology Company) to facilitate egg laying. To investigate the developmental expression profiles of *AalbKr-h1*, individuals were collected from egg (50 individuals / replicate), larvae (1–2-instar; 3–4-instar; 20 individuals / replicate), pupae (20 individuals / replicate) and adult (5 days post-eclosion; 15 individuals / replicate) stages. Females and males were prepared at 2-day intervals. Tissues from adults (25 individuals / replicate), including the malpighian tubules, midgut, ovary, head and fat body were dissected in PBS. Each sample was performed in three biological replicates.

Kr-h1 gene sequence analysis

The *Kr-h1* gene sequence for *Ae. albopictus* (XP_062699652.1) was obtained from the NCBI database. The gene was compared to the *Ae. albopictus* protein database in NCBI using the BLASTp program (30 insect species are shown in Dataset S2). The length of amino acids, molecular weights, and isoelectric points of the sequences were predicted using the ExPASy Proteomics tool (https://web.expasy.org/protparam/) (Gasteiger et al. 2005). Protein structural domains were predicted using SMART (http://smart.emblheidelberg.de/). Phosphorylation site prediction and glycosylation analysis were performed using NetPhos-3.1 (https://services.healthtech.dtu.dk/services/NetPhos-3.1/) and NetGlycate-1.0 (https://services.healthtech.dtu.dk/services/NetGlycate-1.0/. Kr-h1 protein sequences of Diptera were aligned using GeneDoc software with the ClustalW alignment function. Conserved motifs of Kr-h1 proteins from 30 insect species were analyzed using MEME (https://prosite.expasy.org/scanprosite/). The files obtained from MEME predictions were stored in XML format, imported into Tbtool software, and the conserved motifs were mapped. Finally, the Kr-h1 phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA 7.0 software the (Kumar et al. 2016).

Pyriproxyfen treatment

To further investigate the induction response of *AalbKr-h1* to the hormone, larvae were soaked in the pyriproxyfen (JHA) solution at 3–4 instar stage. Based on our laboratory testing, exogenous sublethal doses of the JH analog (HA) pyriproxyfen were diluted to a concentration of 5 μg / μL with acetone (AC) (Lin et al. 2024). For the experimental groups, 25 3-rd to 4-th instar *Ae. albopictus* larvae were added to 99 mL of

dechlorinated tap water and 1 mL of 5 µg / µL pyriproxyfen solution. The control group consisted of 25 3-rd to 4-th instar *Ae. albopictus* larvae placed in 99 mL of dechlorinated tap water with 1 mL of AC. Surviving pupae from the treatment were collected and transferred into adult cages. Adults were provided 10% sucrose solution and females were fed on commercial defibrinated sheep blood 5 days after post-eclosion (PE 5d). Fully engorged mosquitoes were kept for subsequent experiments. To examine the effect of exogenous hormone application on *AalbKr-h1* transcription in *Ae. albopictus*, the expression level of *AalbKr-h1* was measured at different treatment times. Larvae (20 individuals) and females (15 individuals) were collected at 6, 12, and 24 h. Females (25 individuals) were dissected under the microscope at 24 h after blood-feeding (BF 24 h), 48 h after blood-feeding (BF 48 h), and PE 5d after treatment and the corresponding ovaries and fat bodies were collected. Furthermore, the role of *AalbKr-h1* in the ovary and fat body of female mosquitoes were investigated following blood feeding. All experiments were performed in triplicate, with each experiment being repeated at least three times.

RNAi experiment

The dsRNA was prepared using the T7 RiboMAX™ Express RNAi System Kit according to the instructions. The synthesized dsRNA was dissolved with RNase-free water to get a final concentration of 3000 ng / µL. For the microinjection, surviving female mosquitoes 5 days post-eclosion were selected, anesthetized with CO_2, and subjected to thoracic injection under a body microscope (Nikon Company), in which 0.5 µL of dsRNA was injected into the thoracic cavity of adult mosquitoes using a microinjection needle (BF100, Sutte Company). The dsRNA of the Green fluorescent protein (GFP) gene was used as negative control. At least 200 surviving female mosquitoes were injected with dsRNA and then reared with the a 10% (wt/vol) sucrose solution and used in the observation of their survival rate and development. Samples were collected 1 and 2 days after injection (15 individuals / replicate). RNA was extracted from the injected mosquitoes and the expression of Kr-h1 gene was analyzed by qRT-PCR. In addition, survival rates were recorded two days after injection (15 individuals / replicate). The survival rate(15 individuals / replicate) and egg production (30 individuals) of female mosquitoes after the blood meal were counted. And the hatching rate was counted after 7 days of incubation. Each sample was performed in three biological replicates. Primers are shown in Dataset S1.

Quantitative real-time PCR

Mosquito samples were homogenized using a motor-driven pellet pestle mixer and lysed with TRIzol reagent (Aidlab). Total RNA was extracted following the protocol (Hou et al. 2015). Reverse transcription was performed using the NovoScript® Plus All-in-one 1st Strand cDNA Synthesis SuperMix Kit (Novoprotein). qRT-PCR was performed using the NovoStart® SYBR qPCR SuperMix Plus (Novoprotein) under the following conditions: 95 °C for 1 min, followed by 40 cycles at 95 °C for 20 s, and 60 °C for 20 s. Three independent replicates were performed for each experiment. Template concentrations were normalized to endogenous reference *β-Actin*, were calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen 2001). Primers are shown in Dataset S1.

Statistical analysis

Data were presented as means ± standard error (SE). Significant differences were identified using a one-tailed Student's *t*-test or one-way analysis of variance (ANOVA), followed by a least significant difference (LSD) test for multiple comparisons. All statistical analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA), and the results were plotted by GraphPad Prism version 9.0 (San Diego, CA, USA).

Results

Identification of the *Kr-h1* gene

The *AalbKr-h1* gene encodes a protein consisting of 718 amino acids, with a molecular weight of approximately 80 kDa and an isoelectric point (PI) of 8.49. Using NetPhos, we predicted that the most abundant amino acids in *Ae. albopictus* are tryptophan, threonine, and tyrosine. BLASTp analysis revealed that these amino acids are also abundant in other Diptera species such as *Aedes aegypti*, *Culex pipiens pallens* (Diptera: Culicidae), *Anopheles gambiae* (Diptera: Culicidae), *Sitodiplosis mosellana* (Diptera: Cecidomyiidae), and *Drosophila melanogaster* (Diptera: Drosophilidae). The corresponding physicochemical properties are shown in Table 1. Sequence analysis revealed that *AalbKr-h1* contains eight putative C2H2-type zinc finger domains, which share high similarity with their homologues from Diptera species (Fig. 1).

Protein conserved motif and phylogenetic analysis of *Kr-h1*

For most insects of the Kr-h1 proteins share a similar motif composition. Specifically, motifs 1–9 were present in nearly all the examined insect orders except for *Gryllus bimaculatus* (Orthoptera: Gryllidae) of Orthoptera (Fig. 2A). Notably, motif 10 appeared exclusively

Table 1 Information on *Kr-h1* genes in Diptera

Species	Gene symbol	Transcript ID in NCBI	Length of amino acid (aa)	Molecular weight (kD)	Isoelectric point
Aedes albopictus	*AalbKr-h1*	XP_062699652.1	718	80.49	8.49
Aedes aegypti	*AaKr-h1*	XP_021701249.1	702	78.42	8.4
Culex pipiens pallens	*CppKr-h1*	XP_039448473.1	739	81.32	8.47
Anopheles gambiae	*AgKr-h1*	XP_061511231.1	956	102.15	6.56
Sitodiplosis mosellana	*SmKr-h1*	XP_055300622.1	652	72.01	8.66
Drosophila melanogaster	*DmKr-h1*	NP_477467.1	791	85.58	8.15

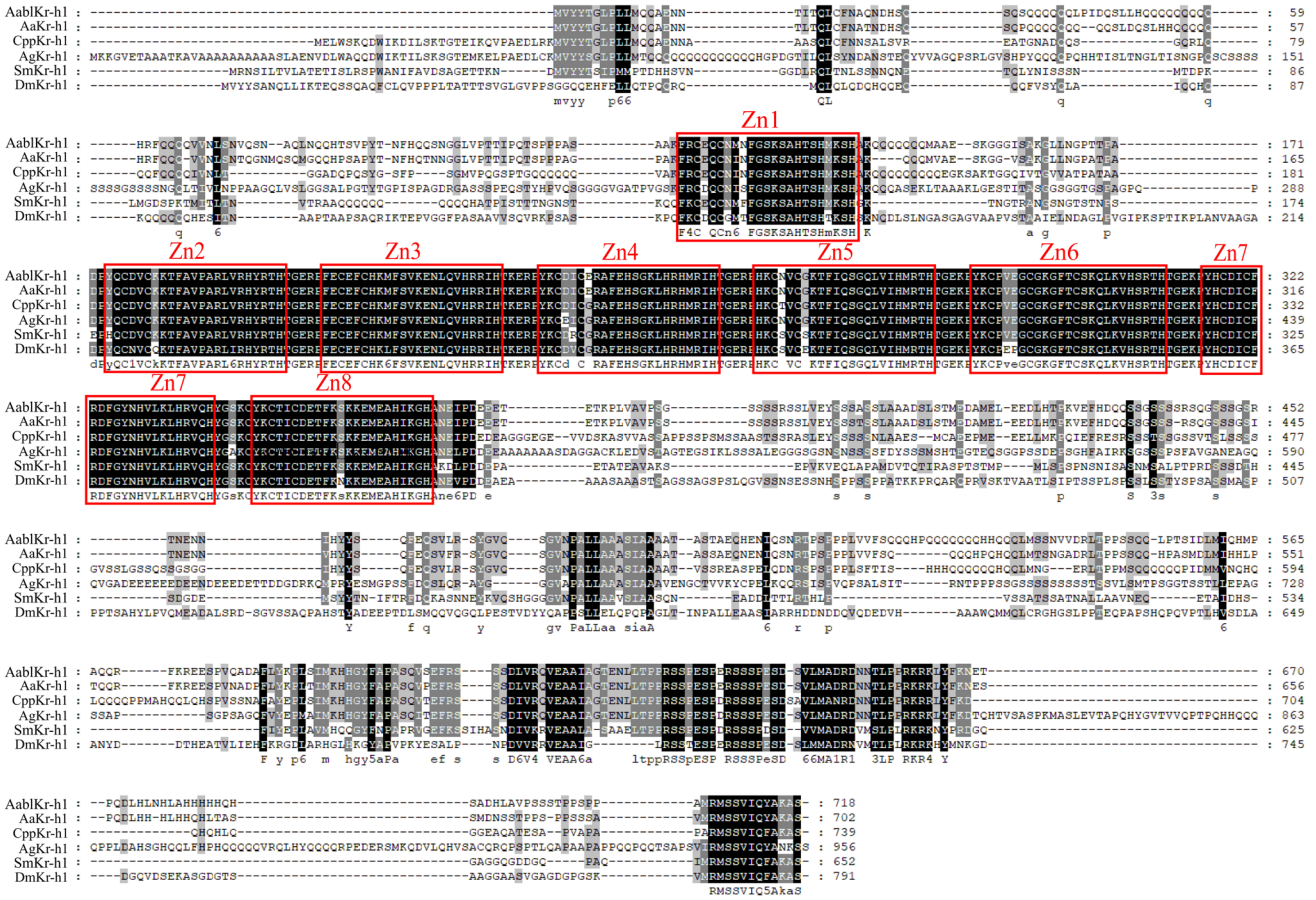


Fig. 1 Gene identification of *AalbKr-h1*. Alignment of the amino acid sequences of *AalbKr-h1* with those of its homologues in *Aedes albopictus* (Ae), *Aedes aegypti* (Aa), *Culex pipiens pallens* (Cpp), *Anopheles gambiae* (Ag), *Sitodiplosis mosellana* (Sm), and *Drosophila melanogaster* (Dm). The zinc finger domains are indicated by the red boxes

in Diptera, Lepidoptera, Coleoptera, and Hymenoptera, suggesting unique evolutionary pressures have shaped these motifs over time (Fig. 2A). Phylogenetic analysis clustered AalbKr-h1 with homologues from Diptera into a distinct group, including *Aedes aegypti*, *Culex pipiens pallens*, *Anopheles gambiae*, *Sitodiplosis mosellana*, and *Drosophila melanogaster*. Further analysis demonstrated that *Kr-h1* sequences from both Diptera and Lepidoptera formed a primary cluster, indicating a closer evolutionary relationship between these orders compared to others (Fig. 2B).

The spatial and temporal expression of *AalbKr-h1*

The results of different developmental stages of *Ae. albopictus AalbKr-h1* gene expression patterns indicated differential expression across all developmental stages and tissues. The highest expression was observed during the

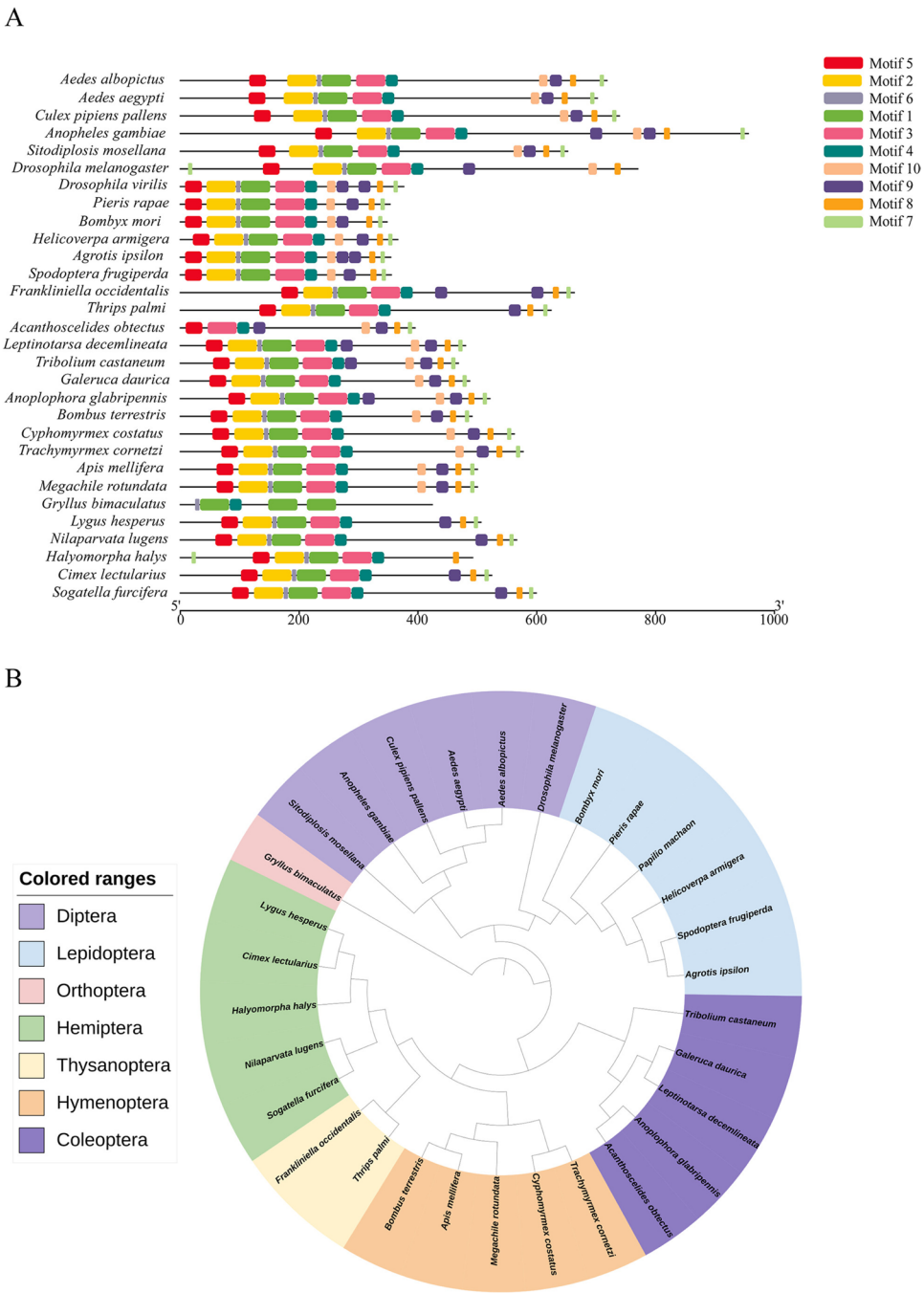


Fig. 2 Conserved motifs and Phylogenetic tree in insect Kr-h1 protein. (**A**) Ten conserved motifs are displayed using different colors. (**B**) Phylogenetic relationships of Kr-h1 from different species. Diptera, Lepidoptera, Orthoptera, Hemiptera, Thysanoptera, Hymenoptera, and Coleoptera are indicated using seven different colors

egg stage, followed by adult mosquitoes, whereas larval and pupal stages exhibited relatively low expression levels (Fig. 3A; $F = 122.229$; $df = 5,48$; $P < 0.001$). In females, *AalbKr-h1* mRNA levels significantly increased by 1.38- to 1.63-fold at day 3 to day 9 compared to day 1 (Fig. 3B; $F = 29.792$; $df = 4,40$; $P < 0.001$). In males, the highest expression level occurred on day 5, being 3.12 times that of day 1, followed by a gradual decrease (Fig. 3C; $F = 108.983$; $df = 4,40$; $P < 0.001$). Tissue-specific expression of *AalbKr-h1* transcripts, confirmed via qRT-PCR, revealed the highest transcription level in the fat body, followed by the female ovaries, with lower expression in the malphigian tubules, head, and midgut (Fig. 3D; $F = 110.876$; $df = 4,40$; $P < 0.001$).

Expression of *AalbKr-h1* is regulated by JHA

Pyriproxyfen-treated samples showed a significant increase in the relative expression levels of larval and adult mosquitoes observed after 24 h of treatment, which showed a 4.3-fold increase compared to the control group; Pyriproxyfen-treated mosquitoes at 6 h and 12 h displayed 2.24- and

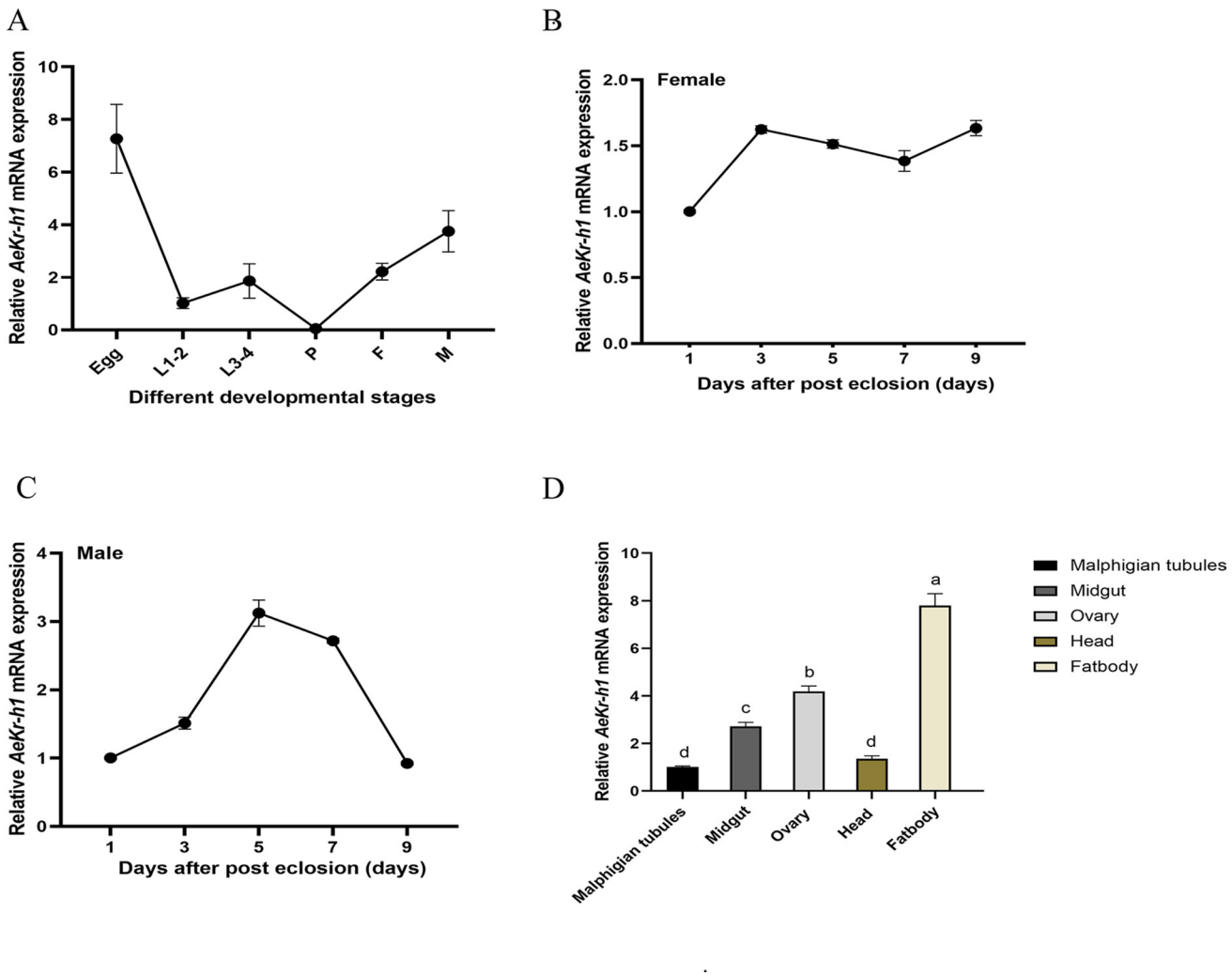


Fig. 3 Different spatial and temporal expression profiles of *AalbKr-h1*. (**A**) Relative expression of *AalbKr-h1* in eggs, larval (L1-2: larval 1–2-instar; L3-4: larval 3–4-instar), pupal (P), and adults (F: female; M: male) and were normalized to L1-2. Expression levels of *AalbKr-h1* in the female (**B**) and male adults (**C**) and were normalized to PE 1d. (**D**) The relative expression of *AalbKr-h1* in various tissues and were normalized to malphigian tubules. Expression levels of *AalbKr-h1* at different stages of the females. Transcript abundance was normalized to that of the *β-Actin* gene. Data are shown as means ± standard error (SE) of three biological replicates. Different letters above the bars indicate significant differences ($P < 0.05$)

3.17-fold upregulation, respectively, compared to the control (Fig. 4A). In females, the expression increase was most significant after 6 h of treatment, with a 4.35-fold increase compared to the control group; treatments at 12 h and 24 h resulted in 1.14 and 2.45-fold increases, respectively, compared to the control (Fig. 4B). Furthermore, in ovary tissues, JHA treatment demonstrated significantly higher expression in PE 5d, BF 24 h and BF 48 h compared to the control group (Fig. 4C). Conversely, the relative expression levels in the ovary were down-regulated after blood feeding (Fig. 4D; AC: $F = 836.038$; $df = 2,24$; $P < 0.001$; JHA: $F = 1119.353$; $df = 2,24$; $P < 0.001$). In fat body tissues, JHA treatment showed significantly higher expression in PE 5d and BF 48 h compared to the control group, while BF 24 h showed a decrease (Fig. 4E). Additionally, we observed that the up- and down-regulation of fat body expression by different treatments of AC and JHA was inconsistent (Fig. 4F; AC: $F = 267.957$; $df = 2,24$; $P < 0.001$; JHA: $F = 268.816$; $df = 2,24$; $P < 0.001$). Based on these results, we can infer that *Ae. albopictus* is regulated by JH during both larval and adult stages, and that external stimuli from JHA impact the development of its ovary and fat body, suggesting potential effects on these tissues. Statistical results were presented in Table 2.

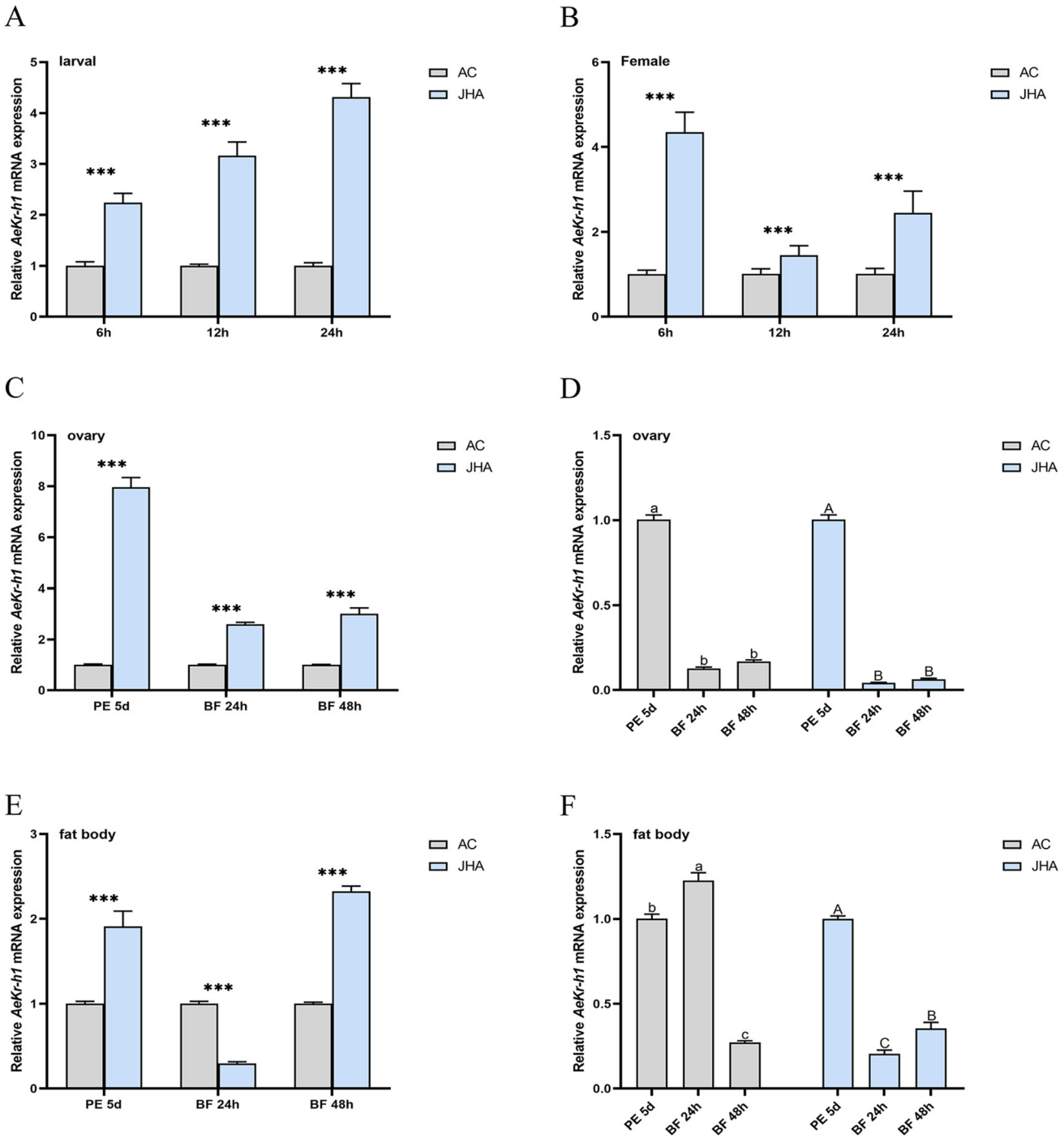


Fig. 4 Expression of *AalbKr-h1* gene by JHA treatment. qRT-PCR to measure the expression level of *AalbKr-h1* in the larval (**A**) and female mosquitoes post-eclosion (**B**) at 6 h, 12 h and 24 h. The relative abundances at different time points were normalized to AC treatment. The relative expression of *AalbKr-h1* in various tissues. The relative abundances at PE and BF time points were normalized to AC treatment (**C**), and were normalized to PE 5d (**D**). The relative expression of *AalbKr-h1* in fat body tissues. The relative abundances at PE and BF time points were normalized to AC treatment (**E**), and were normalized to PE 5d (**F**). Transcript abundance was normalized to that of the *β-Actin* gene. Data are shown as means ± standard error (SE) of three biological replicates. Student t-test was performed (***$P < 0.001$). Different letters above the bars indicate significant differences ($P < 0.05$). PE 5d: five days after post-eclosion; BF 24 h: 24 h after blood feeding; BF 48 h: 48 h after blood feeding

Table 2 Statistical analysis of JHA treatment

Symbol	Time of exposure to JHA (pyriproxyfen)	Statistical significance (Student's t-test)
larval	6 h	t = 18.903; df = 16; P < 0.001
	12 h	t = 24.215; df = 16; P < 0.001
	24 h	t = 36.476; df = 16; P < 0.001
female	6 h	t = 21.029; df = 16; P < 0.001
	12 h	t = 5.202; df = 16; P < 0.001
	24 h	t = 8.248; df = 16; P < 0.001
ovary	PE 5d	t = 18.443; df = 16; P < 0.001
	BF 24 h	t = 22.920; df = 16; P < 0.001
	BF 48 h	t = 9.058; df = 16; P < 0.001
fat body	PE 5d	t = 4.998; df = 16; P < 0.001
	BF 24 h	t = 22.430; df = 16; P < 0.001
	BF 48 h	t = 20.792; df = 16; P < 0.001

Kr-h1 regulates the reproductive development in *Ae. albopictus*

To examine the role of *AablKr-h1* in reproductive development, the expression of *AablKr-h1* was knocked down using RNAi. The successful knockdown of *AablKr-h1* was confirmed by qRT-PCR, with the expression level of the ds*Kr-h1* group in 1d and 2d reduced signifcantly to 24.9% and 59.8%, compared with the dsGFP control (Fig. 5A). Survival rate was recorded at 2 days post-injection and decreased to 68.9% (Fig. 5B). One day after injection, the uninjected, dsGFP and dsKr-h1-injected surviving mosquitoes were blood-fed. Manual counting of the number of eggs laid by each female mosquito 3 days after the blood meal. The result indicated that the dsKr-h1-injected mosquitoes produced 30.0% fewer eggs(Fig. 5C), compared with the dsGFP

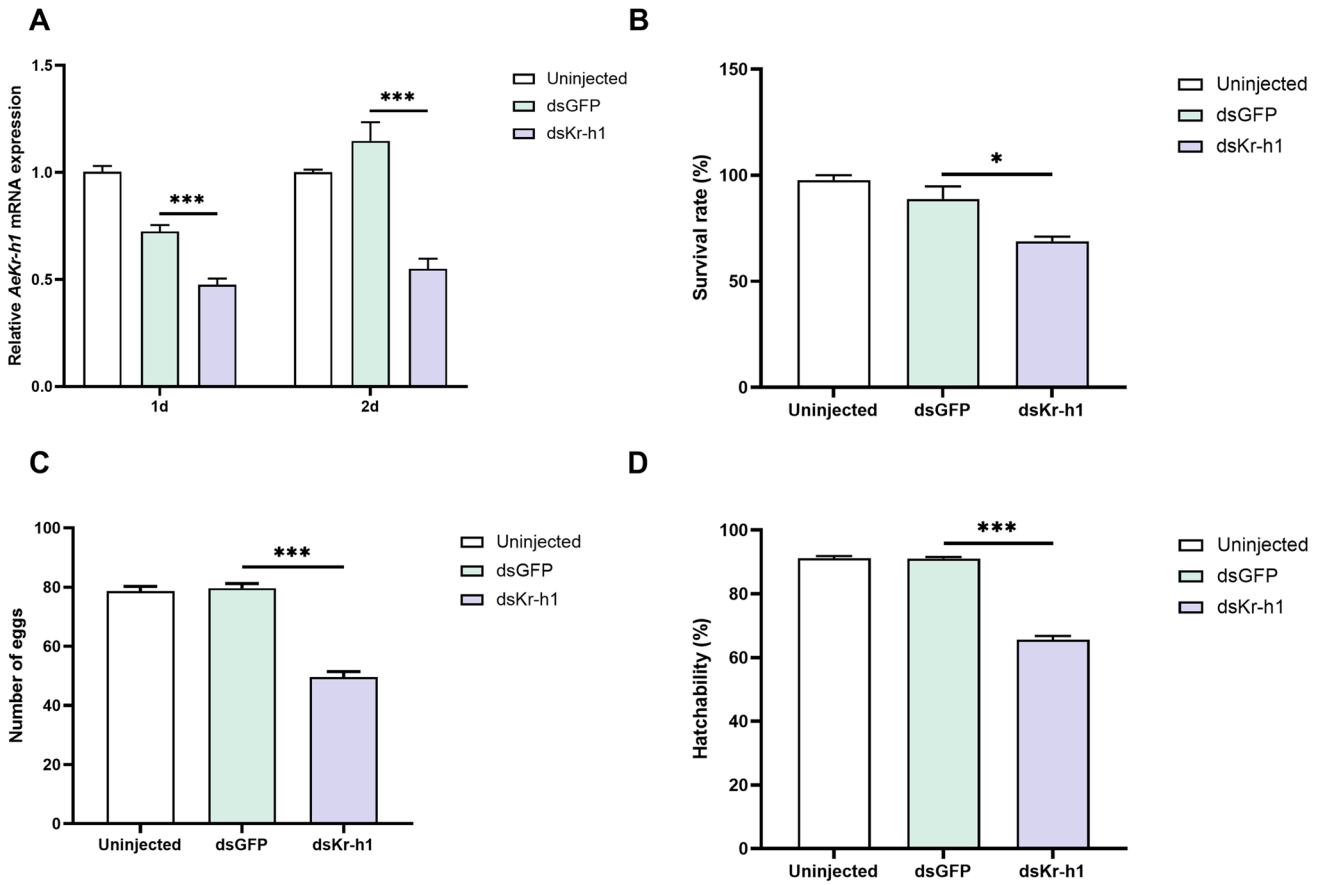


Fig. 5 *Kr-h1* regulates the reproductive development in *Ae. albopictus*. Female mosquitoes were injected with dsRNA. (**A**) qRT-PCR to measure the expression level of *AalbKr-h1* at one and two days post-injection and the relative abundances at different time points were normalized to uninjected. Results are the means ± standard error (SE) of three replicates. Statistical analysis was conducted by Student t-test (***$P < 0.001$). (**B**) Survival rate of mosquitoes was calculated two days after injection and were normalized to dsGFP group. Each bar represents means ± standard error (SE) of three independent measurements from 15 mosquitoes in each group. Statistical analysis was conducted by Student t-test (*$P < 0.05$). (**C**) Egg production after blood feeding in the *AalbKr-h1* RNAi mosquitoes. The number of eggs of individual mosquitoes was counted 4 days after blood feeding. (**D**) Hatching rate of female mosquitoes in different treatments. The eggs were allowed to incubate for 7 days and the hatchability was recorded. Data are shown as means ± standard error (SE) and Student t-test was performed (*$P < 0.05$;**$P < 0.01$;***$P < 0.001$). 1d: 1 day after injection; 2d: 2 days after injection

control. Moreover, the hatch rate decreased from 91.1% for the dsGFP-injected group to 65.6% for the dsKr-h1-injected group (Fig. 5D). These results showed that *AablKr-h1* plays an essential role in the reproductive development.

Discussion

Recognized as a vector for over 20 types of arboviruses and one of the most severe invasive species in the world (Goubert et al. 2016; Guo et al. 2022), *Ae. albopictus* plays a significant role in the outbreaks of arbovirus-related diseases in the field of public health. Currently, controlling the population size of *Ae. albopictus* is the primary strategy for reducing arbovirus infections.

Kr-h1, an early JH-inducible gene, conserves C2H2-type zinc finger transcription factors. In our study, the *AalbKr-h1* gene was compared with the sequences of other insect species and was identified as containing 8 putative zinc finger structural domains. These structural domains were very similar to those reported in many other insect species (Yue et al. 2018), but unlike mammalian Kruppel-like factors (KLFs), which contain three zinc finger DNA-binding domains (Bieker 2001).

Our gene structure analysis showed that the *Kr-h1* gene was relatively conserved in throughout the long-term evolutionary process. Most insects contained the same 10 conserved motifs. It was hypothesized that the encoded proteins have little structural variation and similar roles in different insects. There may be other protein structural domains in addition to these zinc finger structural domain included in the protein conserved motifs (Li et al. 2021). Combined with the phylogenetic tree, it was found that changes in the gene structure of *Kr-h1* were correlated with the living habits of insects belonging to the same monophyletic group in the phylogenetic tree. The gene structures were also more similar, suggesting that the evolution of the genetic structure of *Kr-h1* accompanied the evolution of insect living habits. The change in genetic structure could be related to environmental adaptation options, such as *Ae. albopictus*, which was mainly found in small and large aquatic environments (Cui et al.2021), while *Gryllus bimaculatus* prefered fields and grasses (Odhiambo et al. 2022).

JH plays an important role in the transition of fully metamorphosed or semi-metamorphosed insects from the immature stage to the adult stage (Truman and Riddiford 2019). JH and 20E regulate various physiological processes, including insect development, metamorphosis, and reproduction (Jindra et al. 2013). During the larvae stage, JH initiates its signaling pathway by binding to the Met receptor, inhibiting metamorphosis until the larval reach an appropriate size and developmental stage. At the final stage, a decrease in JH titers and an increase in 20E levels induce larval-pupal metamorphosis (Kayukawa et al. 2017).

In holometabolous insects, such as *Drosophila melanogaster* (Beck, Pecasse, and Richards 2004) and *Tribolium castaneum* (Minakuchi et al. 2009), *Kr-h1* gene is more active during embryonic development and larval molting and is almost absent at the pupal stage. Our study found that *AalbKr-h1* showed high expression during the egg and adult stages, with lower expression during the larval to pupal stages, indicating a role in preventing precocious metamorphosis and stimulating adult reproduction (Wu et al. 2021). The expression level of *AalbKr-h1* in female adults increased post-eclosion, peaking at 3 days of age, correlating with ovarian maturation (Yu et al. 2020). In male mosquitoes, the highest expression level of *AalbKr-h1* was observed on the fifth day post-eclosion, suggesting a link to sexual maturation and attraction to sex pheromones, which are known to be JH-dependent in the *noctuid moth* and *Agrotis ipsilon* (He and Zhang 2022). Furthermore, the high expression of *AalbKr-h1* in the fat body and ovaries of females implies a role in energy metabolism, vitellogenesis, and the reproductive maturation process (Song et al. 2014; Smykal et al. 2014).

JH, serving as an insect growth regulator (IGR), is a targeted alternative to environmentally harmful chemical insecticides. It does not produce rapid contact toxicity in insects but can have long-term physiological impacts and interfere with insect life activities (Nur Aliah et al. 2021). As a biological insecticide, JHA has characteristics of biological safety, no environmental pollution, low drug resistance, and extremely low toxicity to non-target organisms (Parthasarathy and Palli 2021). Pyriproxyfen (JHA) is currently widely used in the following areas: granules, ultra-low volume spray technology, and automatic propagation (Hustedt et al. 2020). *Kr-h1* is involved in regulating insect JH signal transmission, affecting metamorphosis and reproductive physiology. JHA can modulate the expression level of *AeKr-hl*, maintaining larval morphology or inhibiting development (Feyereisen and Jindra 2012).

In insects such as *Drosophila melanogaster* and *Bombyx*, JHA treatment promotes the transcriptional expression of *Kr-hl*, delaying larval pupation and decreasing 20E titers (Zhang et al. 2018a, b; Minakuchi et al. 2008). Our results showed that *AalbKr-h1* is induced by JH in both larval and adult stages when JHA is applied externally to the larval, but its physiological functions require further investigation. During mosquito reproduction, JH regulates early follicular development and vitellogenin synthesis in female mosquitoes, with lipid mobilization from the fat body to the ovary increasing after blood feeding to support follicular development. Knockdown of *Kr-h1* expression in adult mosquitoes using RNAi showed a significant decrease in mosquito survival and egg production.

In *Aedes aegypti* (Ahmed et al. 2020), Kr-h1 was significantly overexpressed in both the ovary and fat body from 120 h PE to BF 24 h after Pyriproxyfen exposure. Our study indicates that JHA enhances *AalbKr-h1* expression at PE 5d and after blood feeding; JHA may promote lipid mobilization from the fat body to the ovary for follicular development. Our findings not only contribute to the fundamental understanding of mosquito development and reproduction but also provide a foundation for the developing novel strategies for mosquito control. The innovative approach of targeting the *Kr-h1* gene and its response to JH signaling offers a promising avenue for the prevention of mosquito-borne diseases.

Conclusions

The *AalbKr-h1* gene contains eight putative C2H2-type zinc finger domains, which are highly conserved among Diptera insects. Analysis of expression patterns revealed a strong correlation between *AalbKr-h1* expression levels and developmental stages. Specifically, *AalbKr-h1* is highly expressed during the egg stage, shows low expression during the larval and pupal stages, and significantly increase in the adult stage. High expression in the fat body and ovary tissues of *Ae. albopictus* suggests its crucial role in reproductive maturation. Additionally, *AalbKr-h1* expression is induced by JHA in both larval and adult stages, suggesting its involvement in regulating metamorphosis and adult reproduction. RNAi showed that the reproductive development of *Ae. albopictus* was regulated by *Kr-h1*. These findings provide a solid foundation for developing novel pest control agents targeting genes in the JH signaling pathway.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-025-08479-z.

Acknowledgements The authors gratefully acknowledge the generous donation of the sensitive *Ae. albopictus* FS population from Professor Xiaoguang Chen's group at Southern Medical University.

Author contributions All authors contributed significantly to this study. LHX and FH conceived the study and coordinated its implementation. YHC, YYX, and XTH performed the experiments. YHC and CW analyzed the data and drafted the manuscript, which was critically revised by LHX and YHZ. All authors read and approved the final manuscript.

Funding This research was supported by Natural Science Foundation of Fujian Province (2023J01543), the Startup Project for High-level Talents of Fujian Medical University (XRCZX2020016), and the National Nature Science Foundation of China (82402662), the Fujian Medical University Qihang Fund (2021QH1005), and the Education and Scientific Research Project for Young and Middle-aged Teachers in Fujian Province (JAT210116).

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate No specific permits were required for our experiments. This study did not involve endangered or protected species.

Competing interests The authors declare no competing interests.

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