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The miRNA plays a key role in the regulation of hormone signaling in insects. The pathways by which miRNAs affect hormone levels are unclear in the honeybee (*Apis mellifera*), an indispensable pollinator in nature. In this study, ame-miR-5119 was overexpressed and knocked down in larvae by feeding mimics and inhibitors, respectively, and we determined that ame-miR-5119 regulates hormone signaling through the target gene ecdysis triggering hormone (Eth), which affects the larval-pupal transition of workers. The results showed that ame-miR-5119 with a length of 19 nt targets six genes related to the hormone pathway. We focused on Eth and found that ame-miR-5119 and Eth exhibited reverse expression patterns during the transition from larval to pupal stages in workers. Dual luciferase assay confirmed the negative regulatory between ame-miR-5119 and Eth. Overexpression of ame-miR-5119 decreased the mRNA level of Eth, and the Eth receptor (Ethr) expression was not significantly affected, but the expression levels of juvenile hormone (JH) pathway related genes juvenile hormone acid methyltransferase (Jhamt) and Krüppel homolog 1 (Kr-h1) were significantly reduced. In contrast, knockdown of ame-miR-5119 increased the mRNA level of Eth, and the expression of Ethr, Jhamt and Kr-h1 was significantly upregulated. ame-miR-5119 did not affect larval body weight. The number of larvae overexpressing ame-miR-5119 survived in the prepupal stage was lower than that in the control group, and the number of pupations reduced at 11-day-old. The number of larvae that knocked down ame-miR-5119 survived in the prepupal stage was significantly higher than that in the control group, and the number of pupations increased at 11-day-old. These results indicated that ame-miR-5119 negatively regulates the expression of Eth, indirectly inhibits the expression of Ethr, Jhamt, and Kr-h1, and affects the JH biosynthesis, thereby preventing the metamorphic transition from larva to pupa in worker bees. These findings provide evidence that the miRNA regulation of hormone levels in honey bees.



Ame-miR-5119-Eth axis modulates larval-pupal transition of western honeybee worker

Jianfeng Qiu

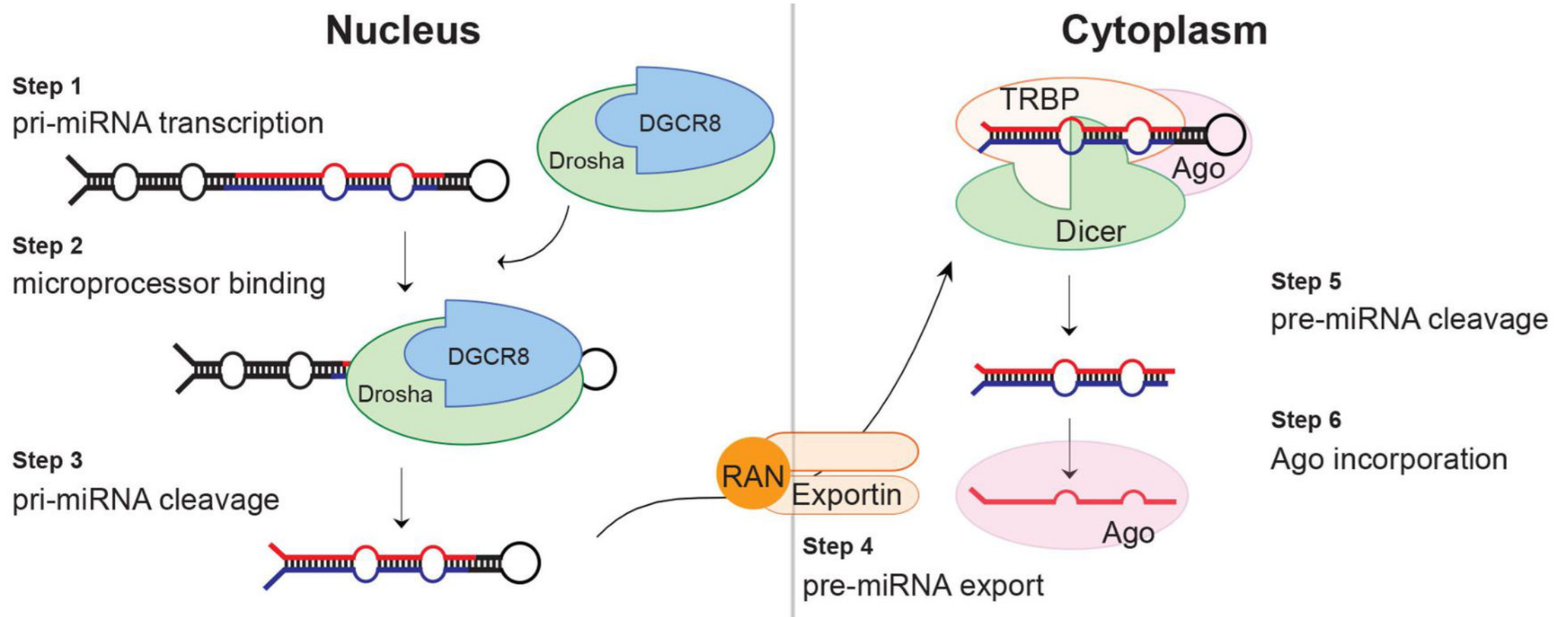
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National & Local United Engineering Laboratory of
Natural Biotoxin

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Copenhagen, Denmark 2025.09

Background



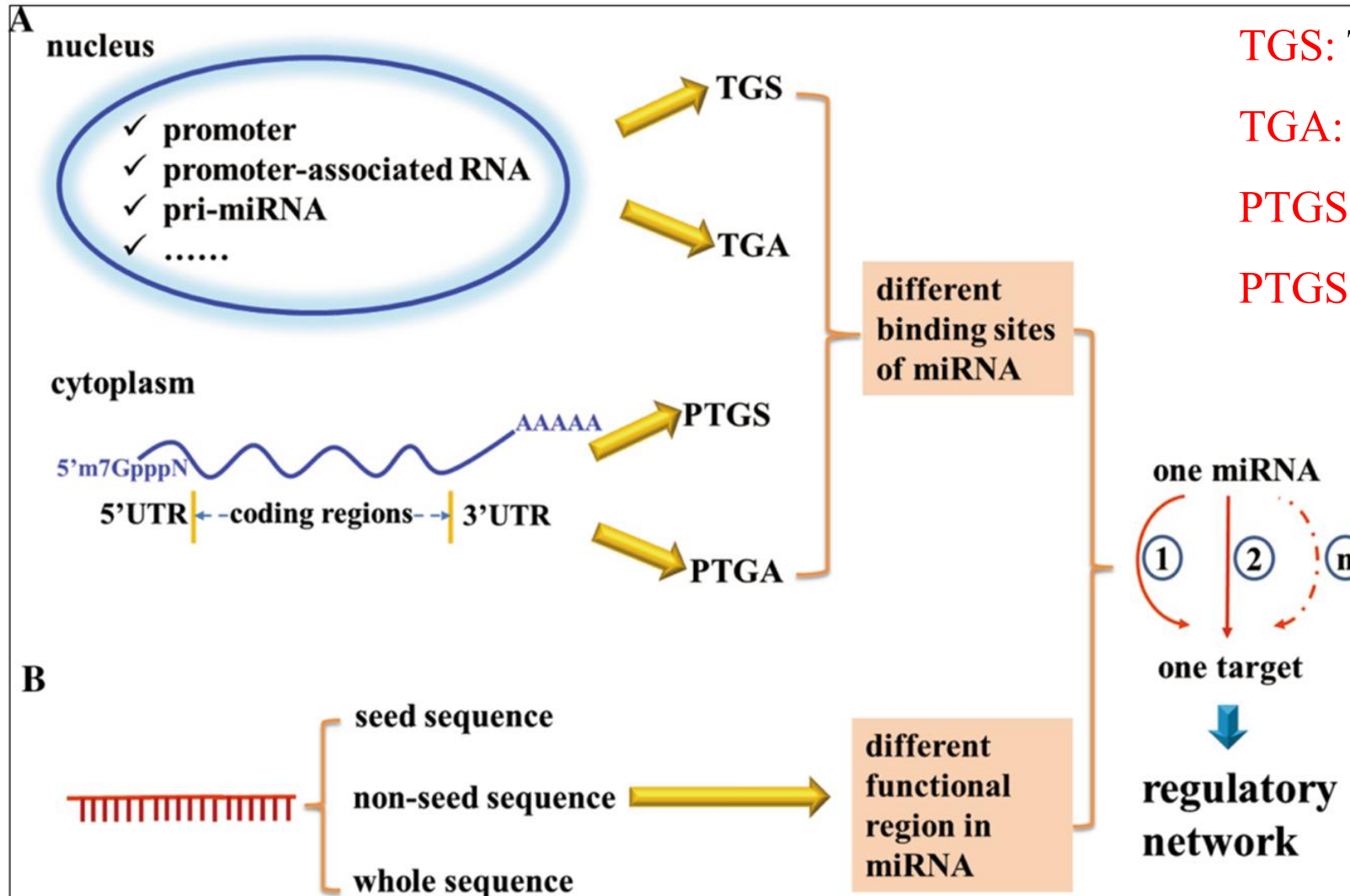
1. MicroRNAs biogenesis



Background



2. Dual regulation on a target gene by miRNA



TGS: Transcriptional gene silencing

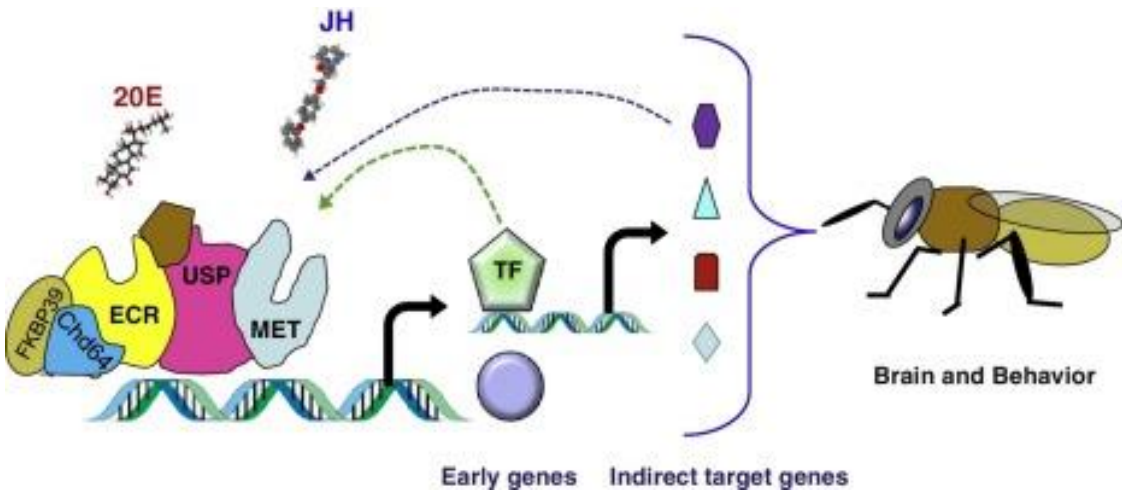
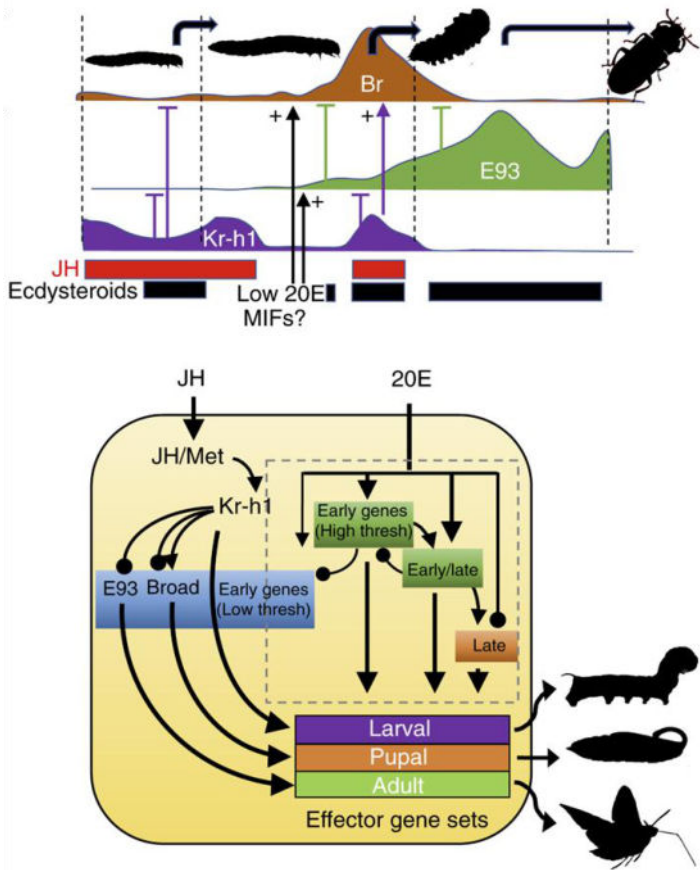
TGA: Transcriptional gene activating

PTGS: Post-transcriptional gene silencing

PTGS: Post-transcriptional gene silencing



3. The hormones and insect development



Bees and Hormone Signals

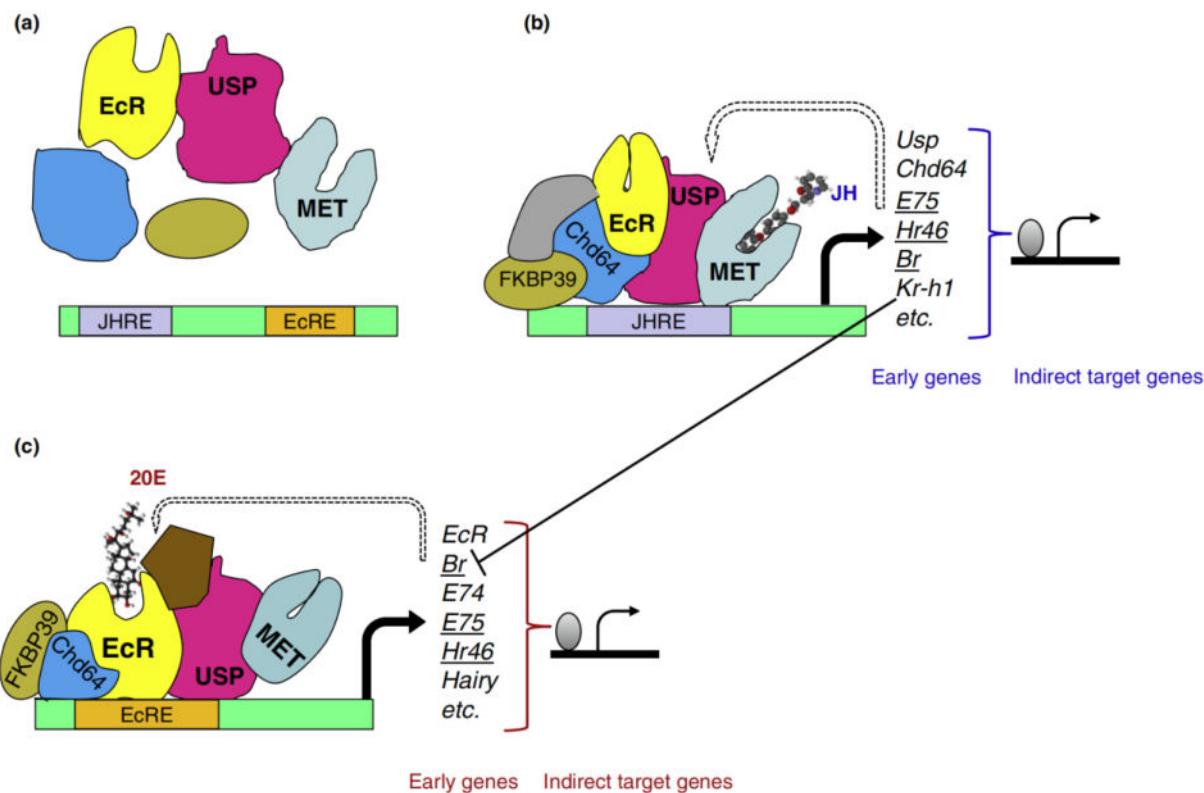
Pandey and Bloch, 2015. Curr Opin Insect Sci

The molting hormone (ecdysone, 20E) and the juvenile hormone (JH) jointly determine the development of insects.

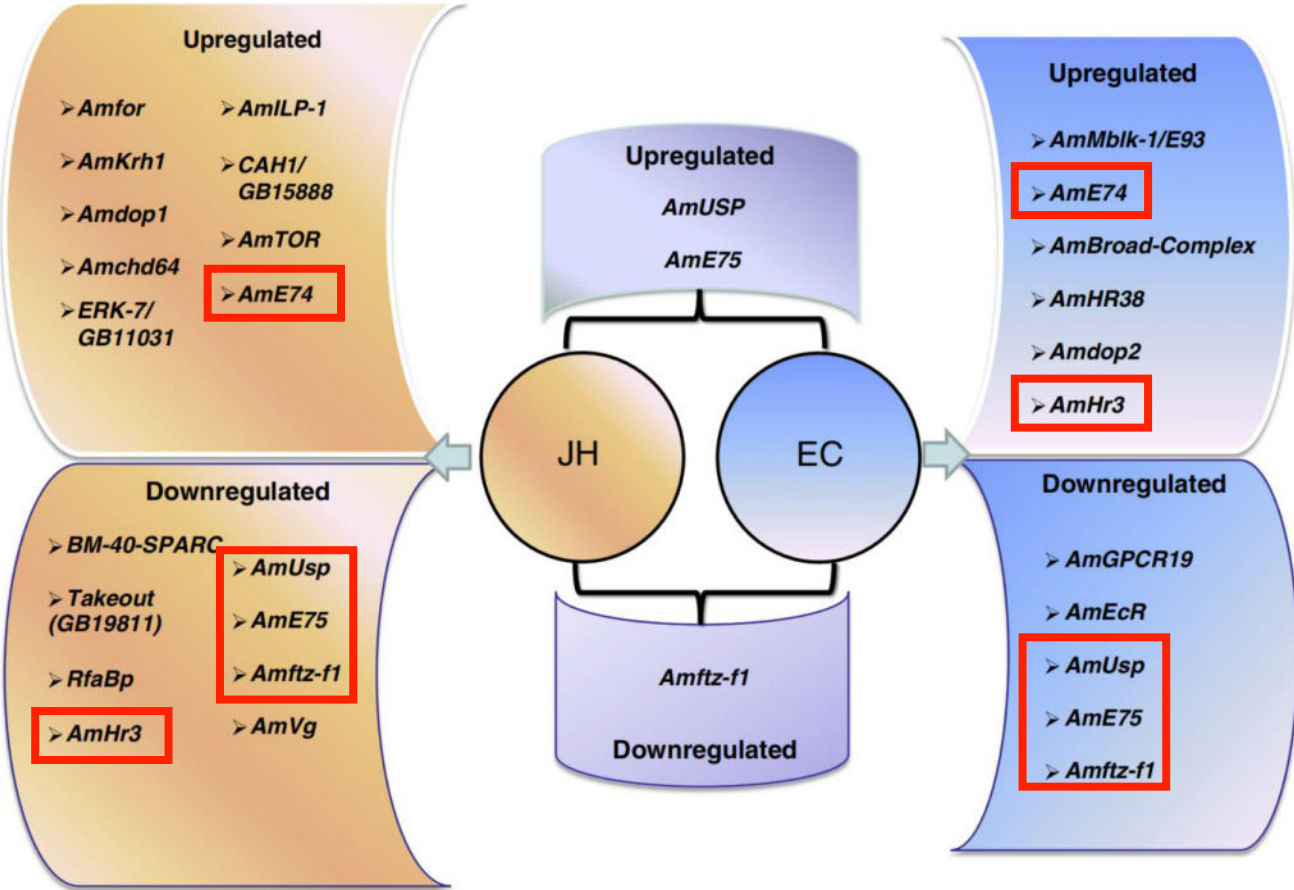
Truman, 2019. Curr Biol



3. The hormones and insect development

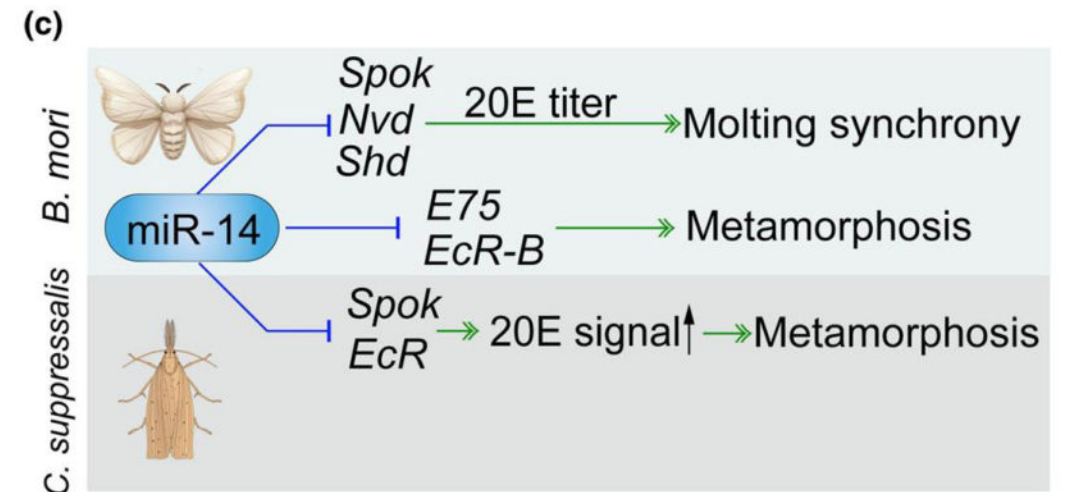
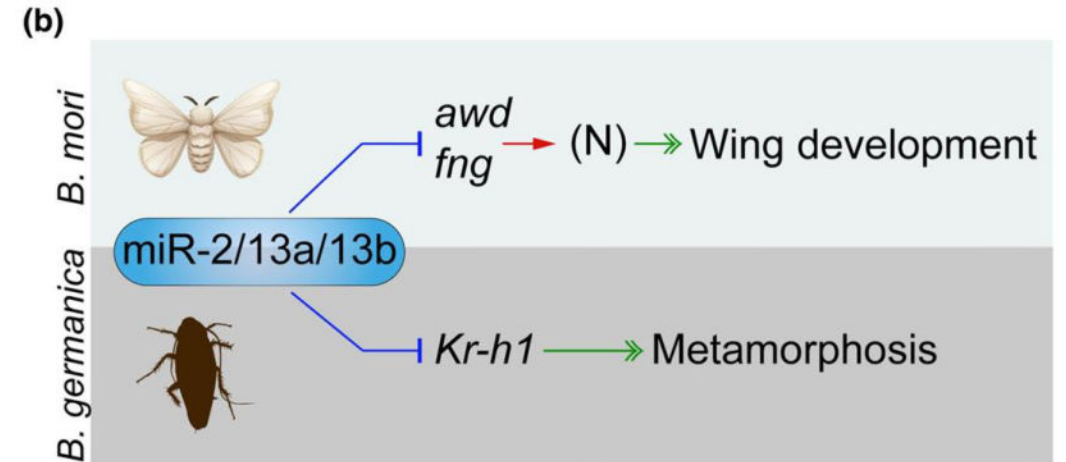
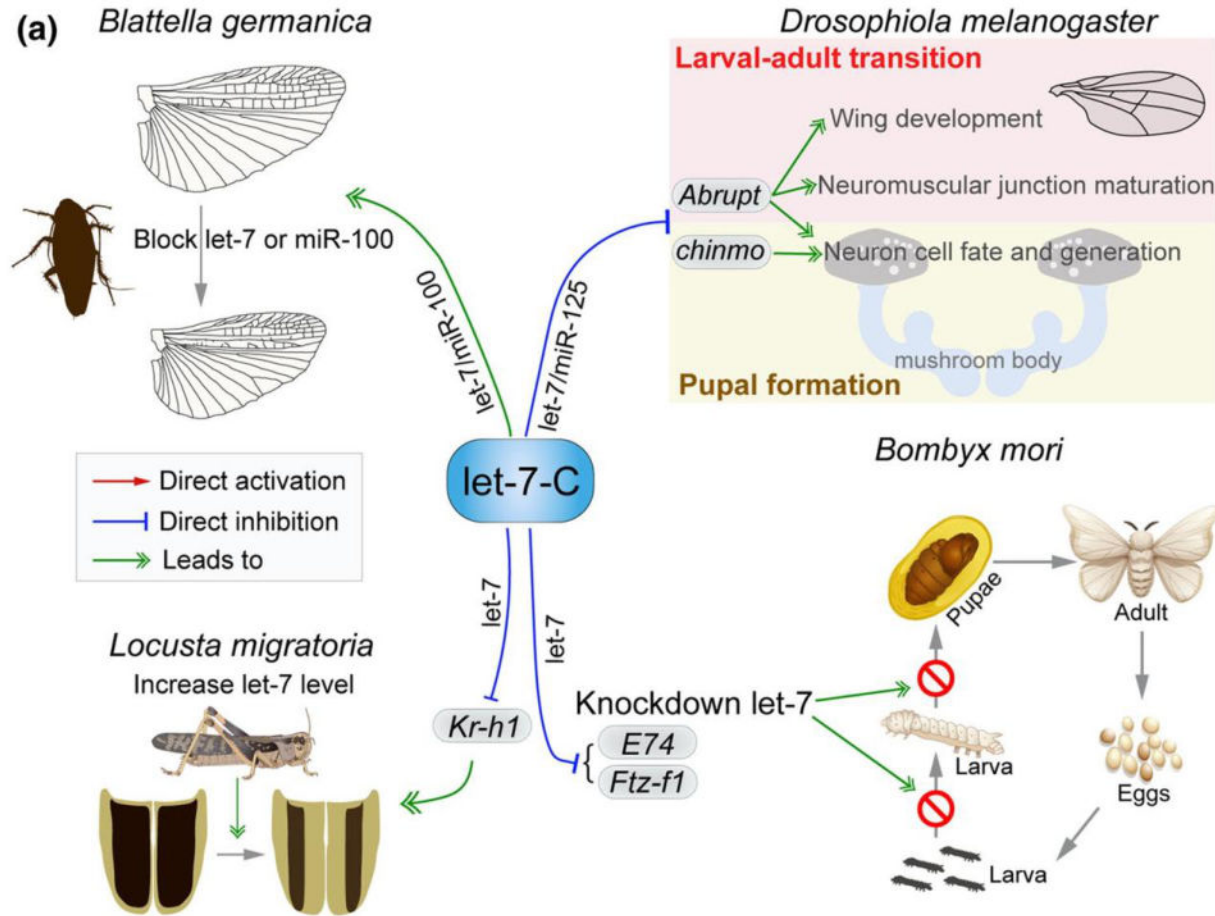


Schematic diagram model of mutual antagonism between JH and 20E



The regulatory effects of JH and 20E on signal pathway genes in bees

4. The regulation between miRNA, hormones and insect development



Background



5. Regulation networks of miRNAs involved in bee hormone

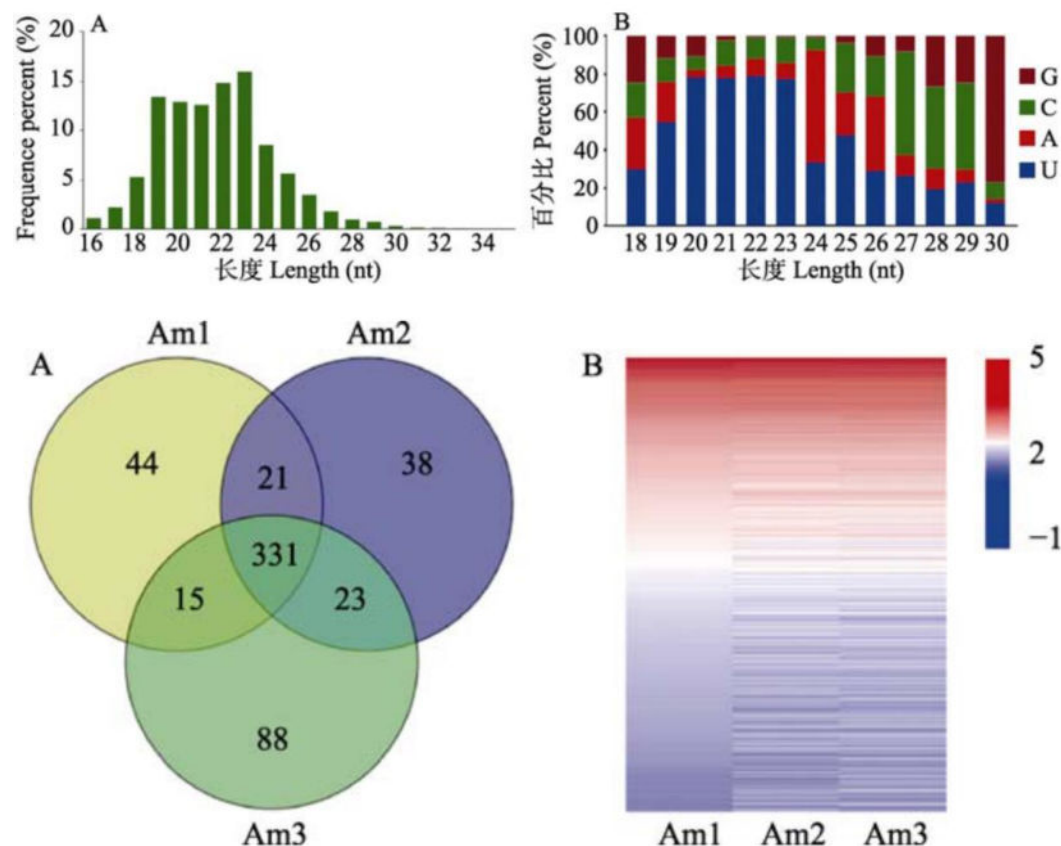


Fig.1 small RNA sequencing at 4 to 6-day-old larval gut

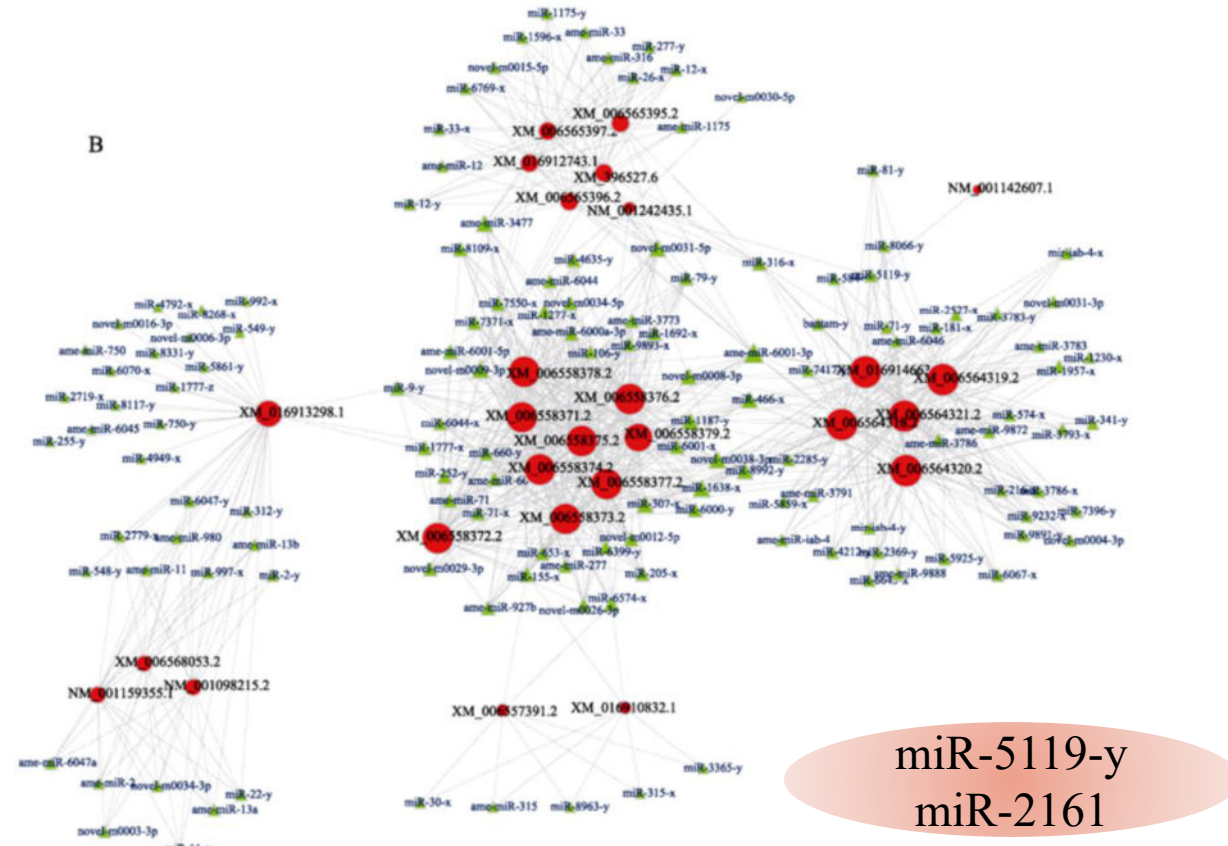


Fig.2 The regulatory network of miRNAs and hormone pathways

1. The target genes of Ame-miR-5119

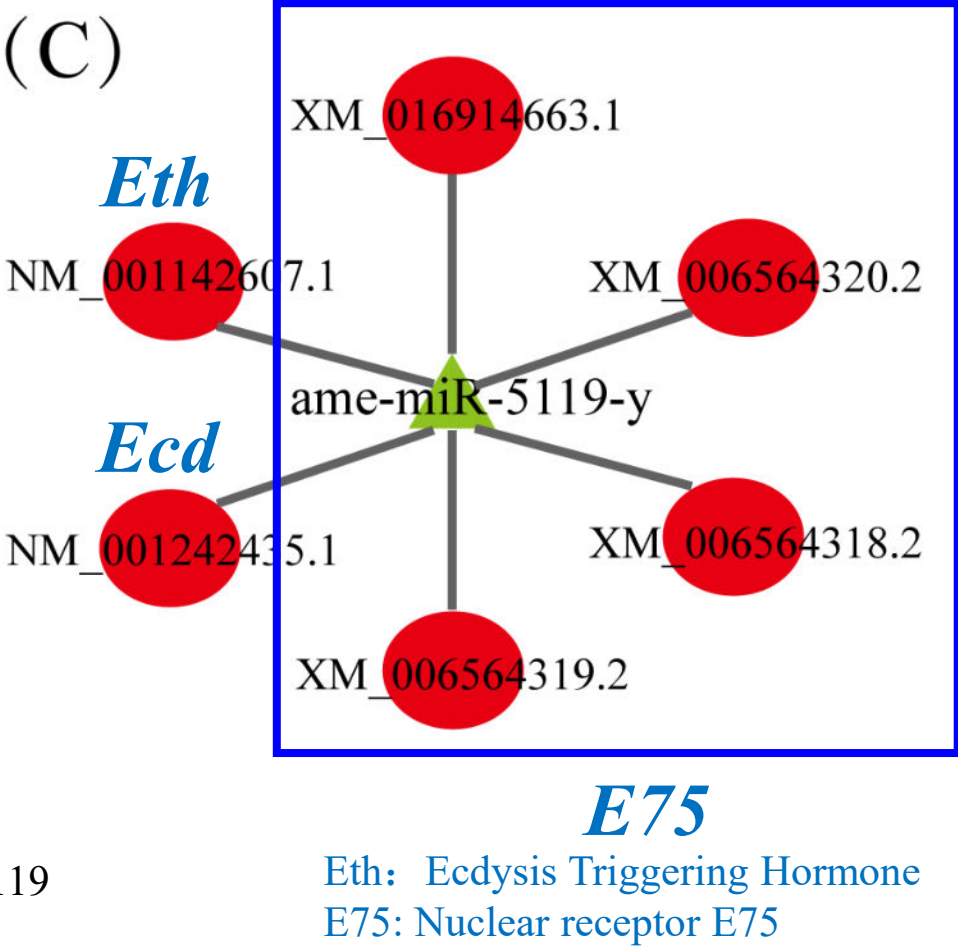
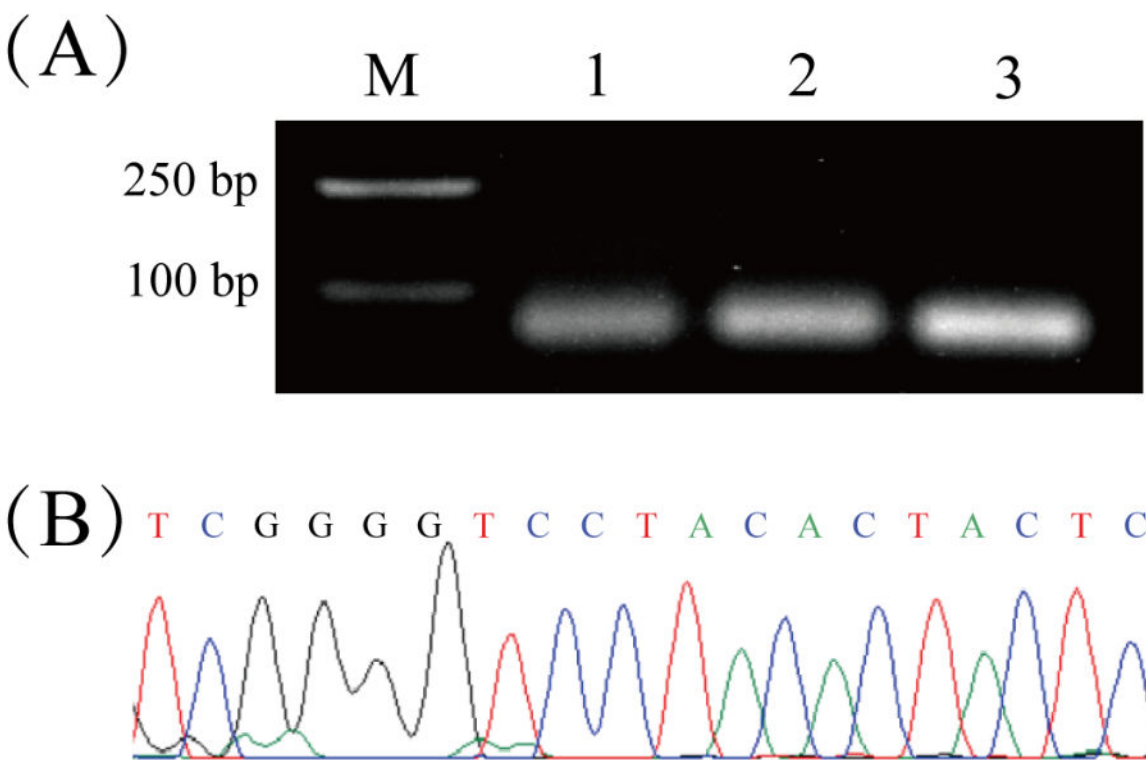


Fig.3 Molecular validation and regulatory network of ame-miR-5119

2. Ame-miR-5119 negatively regulates the expression of the target gene *Eth*

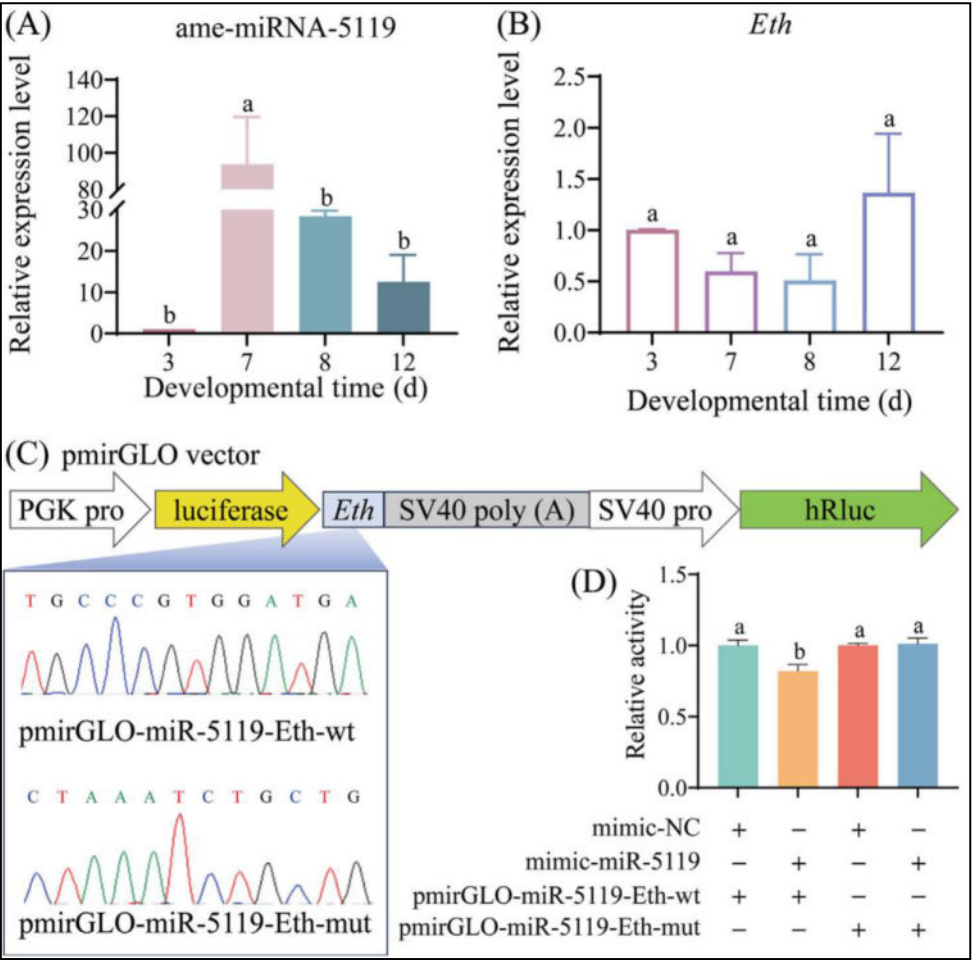


Fig.4 The target binding of ame-miR-5119 and *Eth*

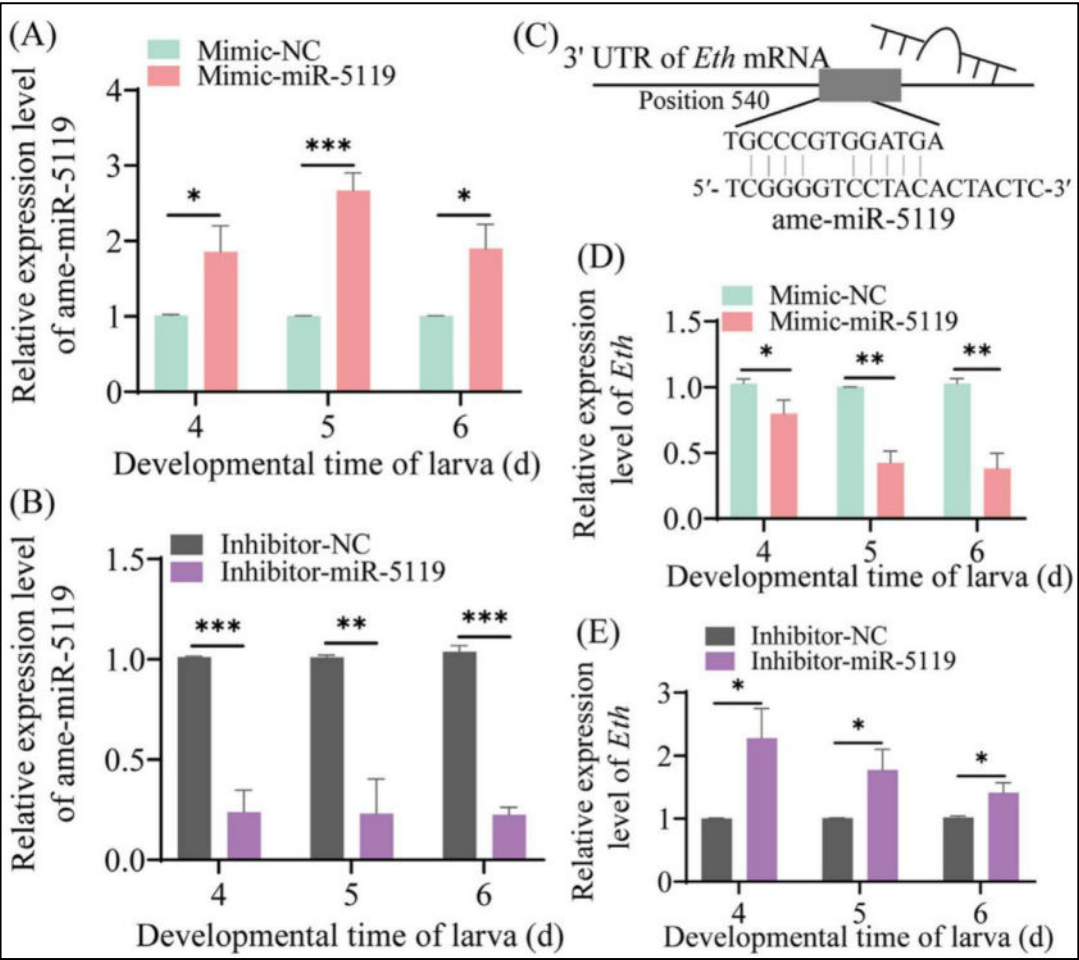


Fig.5 Ame-miR-5119 negatively regulates the *Eth* expression

3. Ame-miR-5119 affects JH hormone signaling through *Eth*

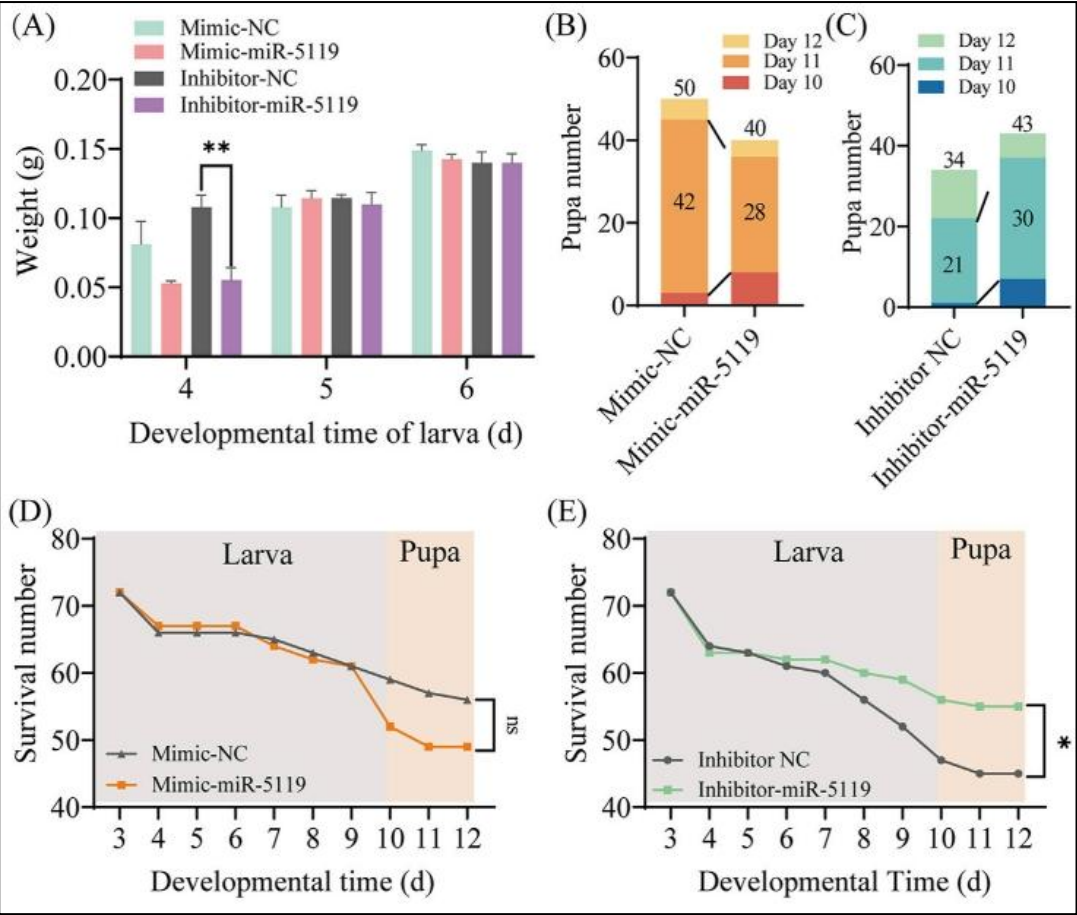


Fig.6 Effect of ame-miR-5119 on *A. mellifera* larval metamorphosis

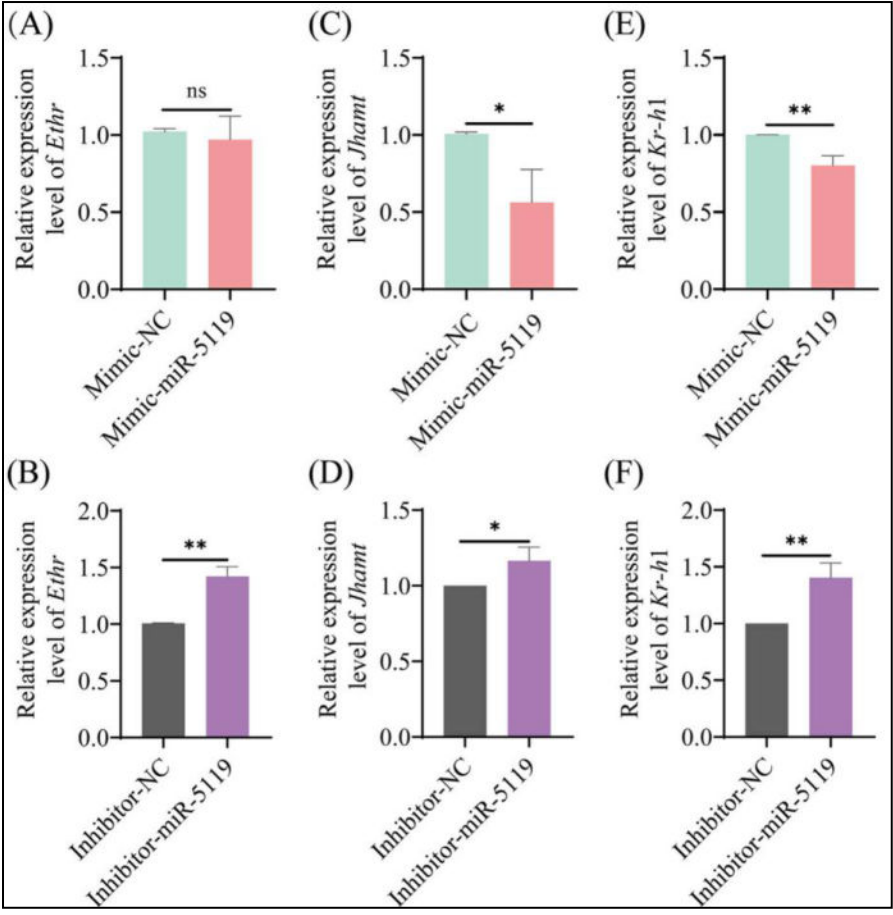


Fig.7 Effect of ame-miR-5119 on the JH signaling in bees

Conclusion

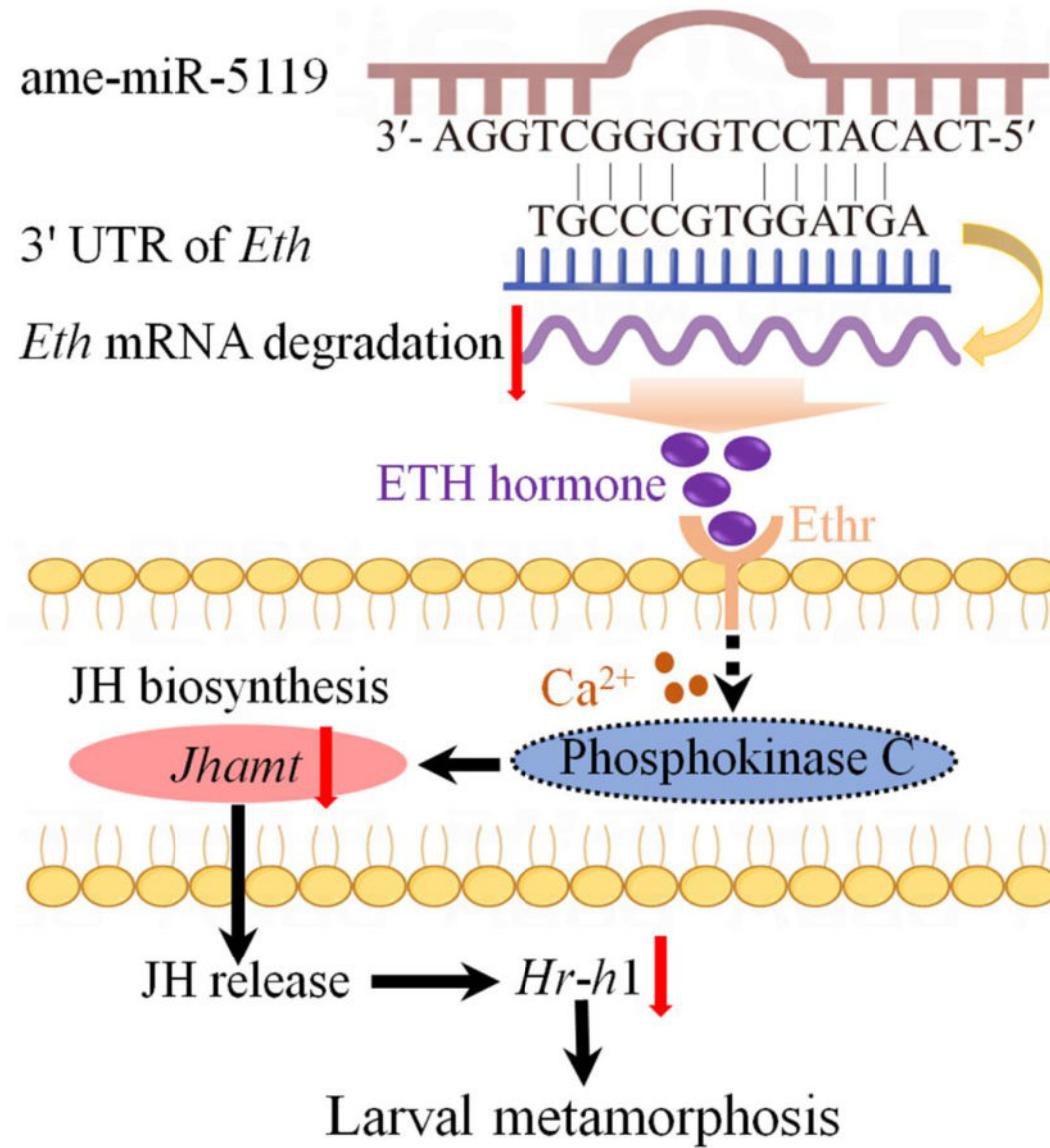


Fig.8 Speculated model of ame-miR-5119 regulating larval metamorphosis through Eth

1. The target genes of Ame-miR-2161

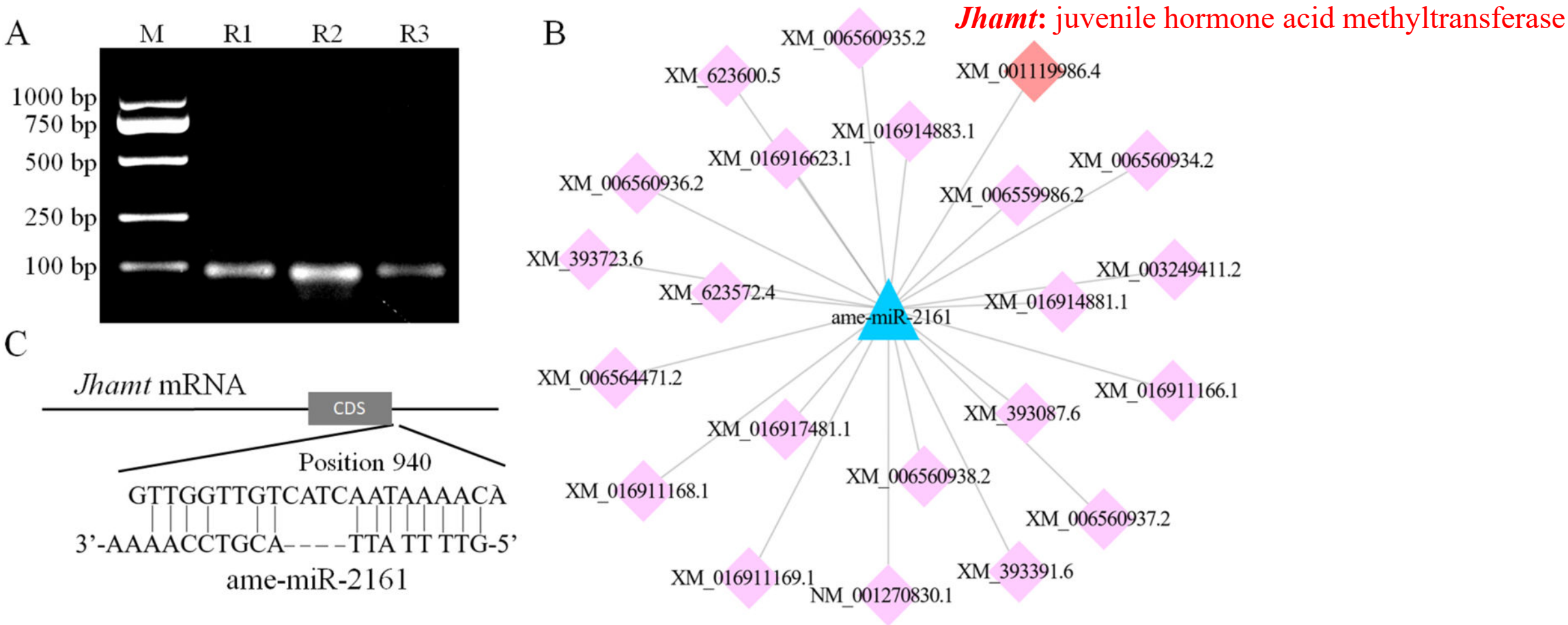


Fig.1 Molecular verification and regulatory network of ame-miR-2161 Song *et al.*, 2025. Insect Molecular Biology

2. Ame-miR-2161 positively regulates the expression of *Jhamt*

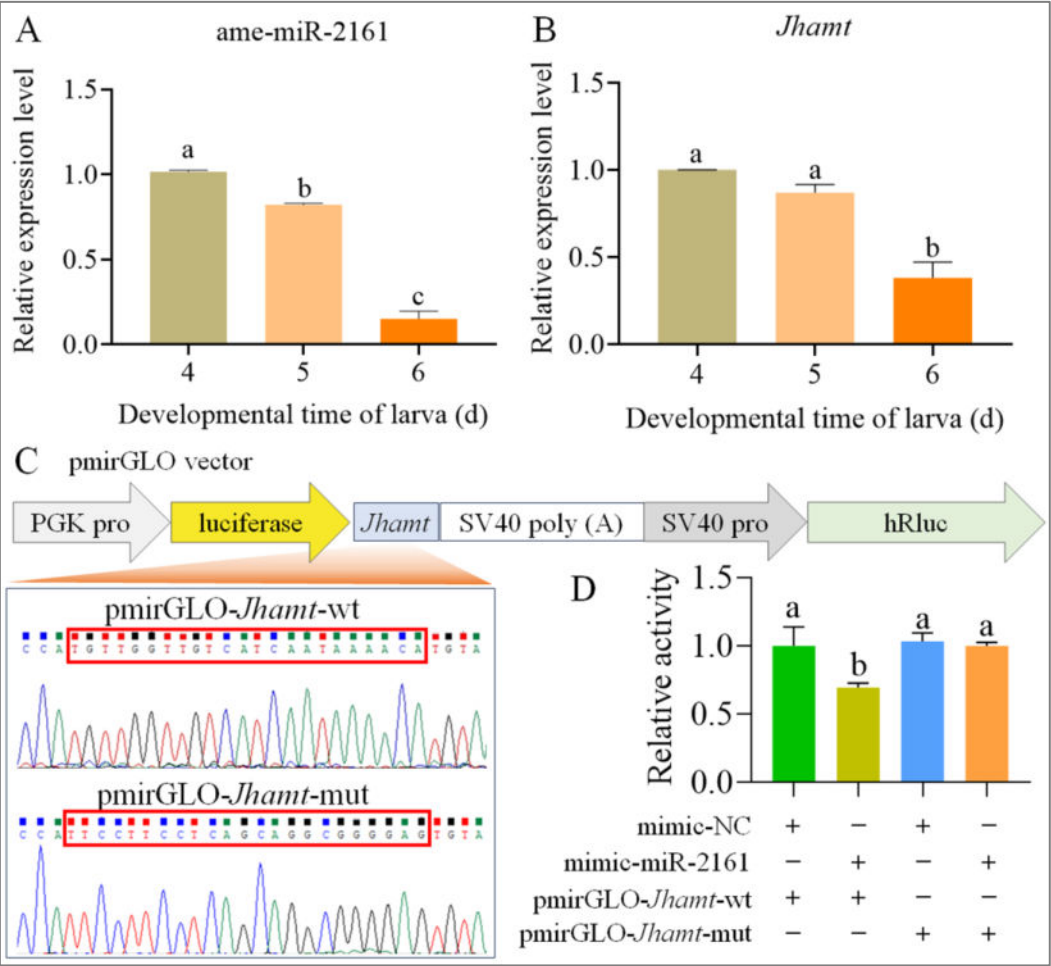


Fig.2 The target binding of ame-miR-2161 and *Jhamt*

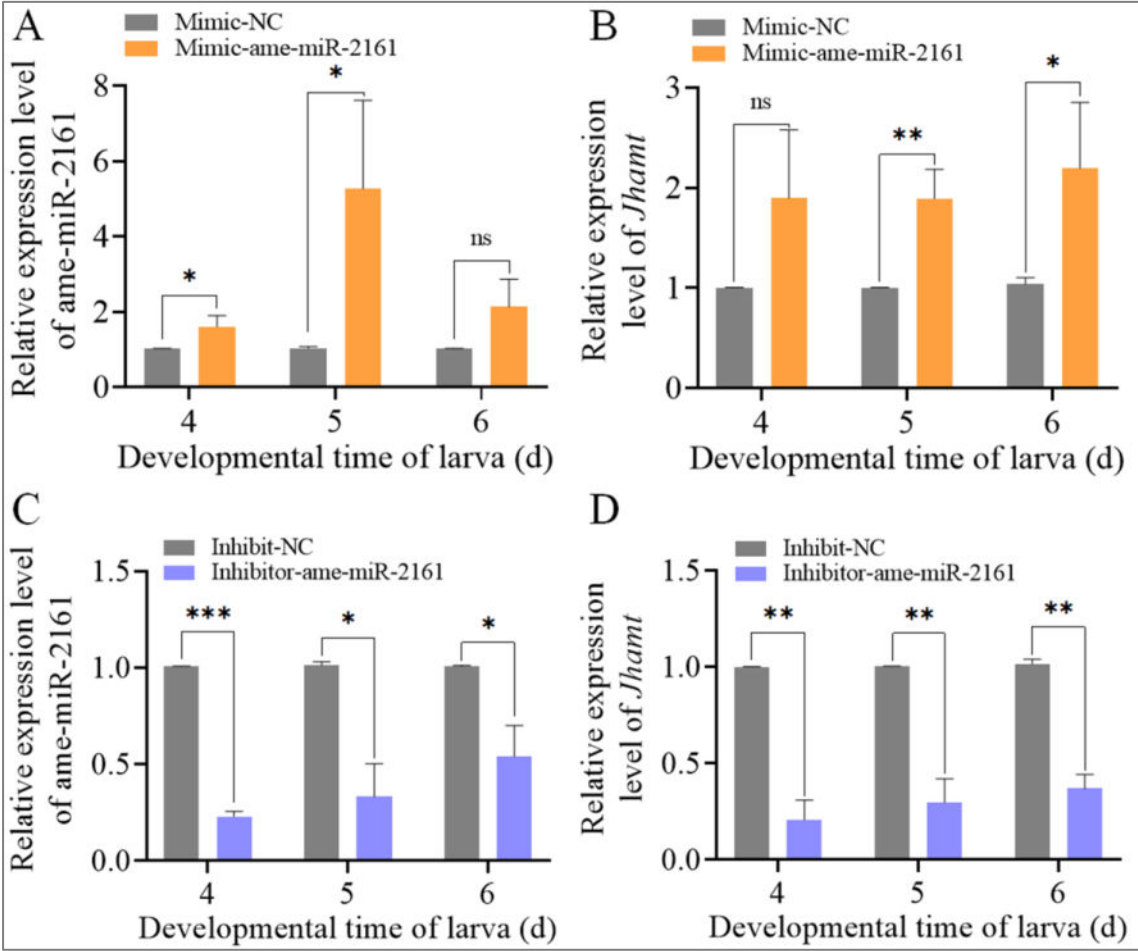


Fig.3 The effect of ame-miR-2161 on *Jhamt* expression

3. Ame-miR-2161 affects the JH signal through *Jhamt*

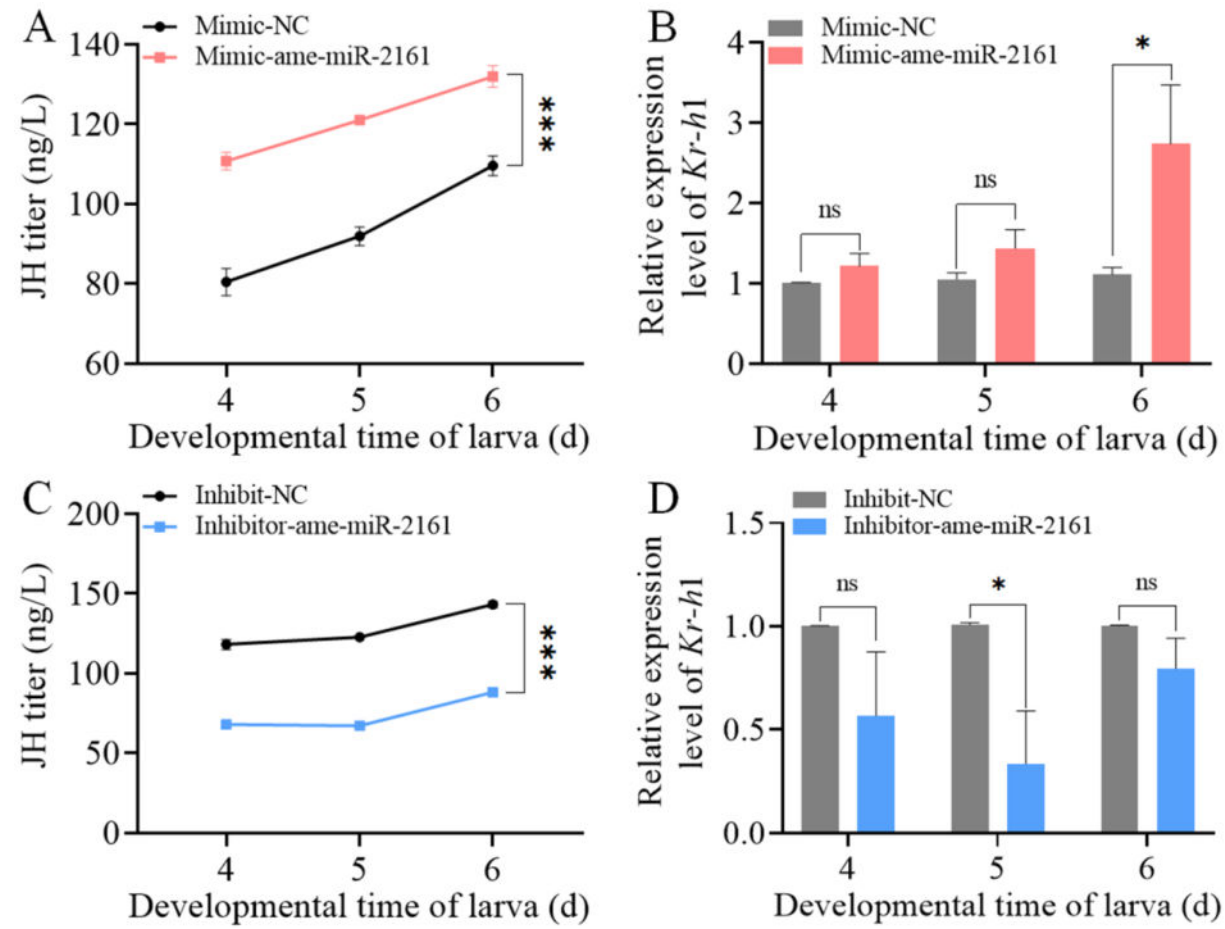


Fig.4 The effect of ame-miR-2161 on JH titer and *Kr-h1* expression in worker larvae

4. Ame-miR-2161 affects larval survival

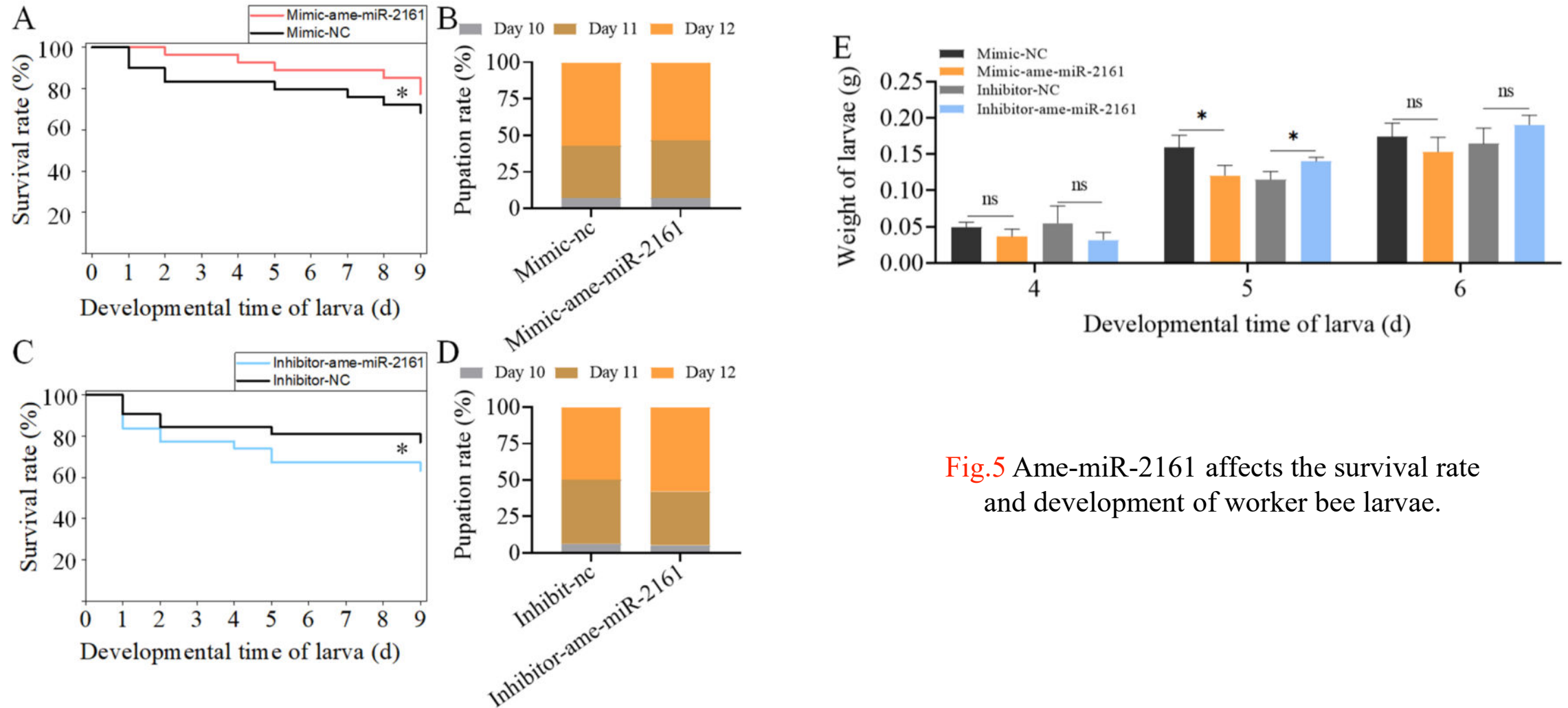


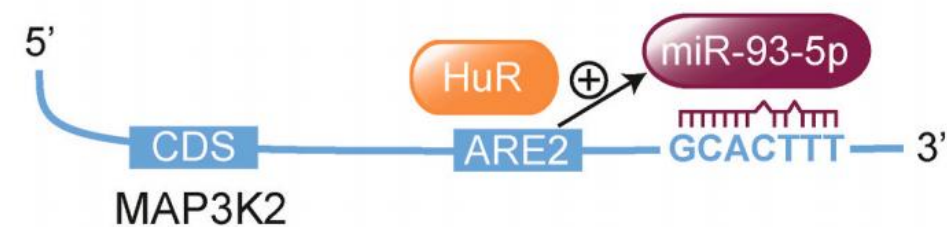
Fig.5 Ame-miR-2161 affects the survival rate and development of worker bee larvae.

Conclusion



AU-rich elements (AREs)

ARE-dependent RNA activation



miR-93-5p cooperates with an HuR-ARE2 complex to bind the MAP3K2 3'UTR and drive mRNA translation

Shi et al., 2024

Biochemical and Biophysical Research Communications

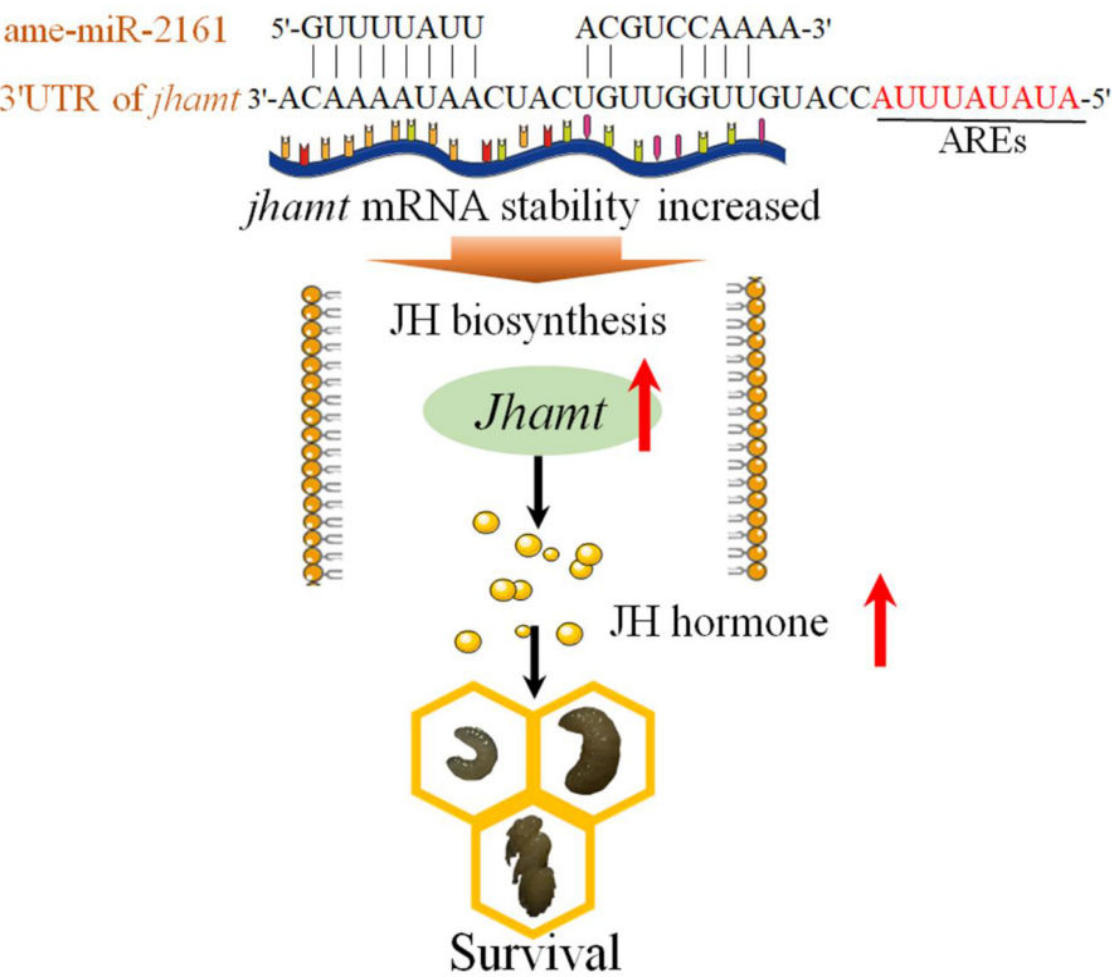


Fig.6 Schematic diagram of ame-miR-2161 affecting the development and survival of honeybee larvae through the JH signaling pathway

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