Exploring the role of insulin-like peptides in the Western tarnished plant bug *Lygus hesperus*

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Introduction

- Insulin-like peptides (ILPs) are involved in growth, reproduction and development, among other functions, in several organisms, including insects. (Zhu et al., 2024).
- Certain ILPs have been identified in several organisms as growth factors and are often referred to as "insulin-like growth factors" (IGFs).
- Three putative ILPs have been identified from a *L. hesperus* transcriptome. One of these (LhILP3) has structural features similar to IGFs, notably a truncated C-peptide.

LhILP1

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MPSQSIYVFLSLSALMCNALSSYPAEAFGKRSHQRYCGRDINEVLKLICHGVYNSMFTNKRNGGDQ
EIRDFGEYQVFPNERREDLTRPADMDAFVAAIQNLRYPFASKATSLTMLPRTNGIYPREKRGVYDE
CCKKTCSINELASYCGVN
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LhILP2

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MVSQGSKTSTMVLFVLAALFLILSFVDCVVPQDDWRSRLVKRQFKLCGPQIDQALNILCEAHASPH
PEDDTEEPQKRSFGMINYVPPVDSIRLLPYFHKKHAALSMLGNGHFSRVRRGDRIADECCYKGCTI
DELSSYCAVERINARSRFGA
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LhILP3

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MLGGRKLASSSHLLLLISMTIAPITPGTAVQFCGNDLSDAIALVCAETGYNMPHLFHSDSVDGSSD
RRYPREAGERHKRGIVDECCRSPCGWATIETYCRPQPSSTSSENLQIQKRSDPPSDAAFKEQVEAY
SISRTVDQGHGSRHQKRRLFSQAEDAVVIKNNRQFHSVVEDQRRPEVTITSNKVKRNDKRKSLGEE
HKDHKTTLHNTTDTEEKKVEELKEKFRLRELQYLQIRHHRANKSHHKDLKKGKSAQVGTVSPYYFG
KFLVSPKARRLPY
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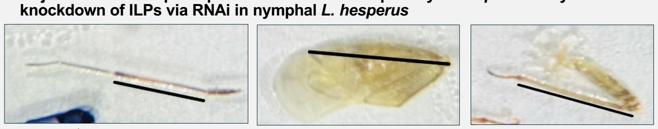
Figure 1. Amino acid sequences of *L. hesperus* ILPs. Three putative ILPs were identified from a *L. hesperus* transcriptome. Each has structural features consistent with ILPs including an B-chain (blue highlight), A-chain (orange highlight),C-peptide (residues between B- and A-chains), consistently spaced cysteine residues (bold blue), and dibasic cleavage sites (bold red).

Hypothesis

We hypothesized that one or more ILPs may function as a growth factor, and that RNAi knockdown of ILPs in the nymphal stages will result in smaller adults. We also predicted that ILPs are involved with *L. hesperus* reproduction, and that knockdown will disrupt egg laying.

Methods

- **Objectives: Develop morphometric methods to quantify *L. hesperus* body size and knockdown of ILPs via RNAi in nymphal *L. hesperus***



2nd antennal segment | Wing length | Tibia (leg)

Figure 2. Morphometric quantification. *L. hesperus* morphological characteristics used to quantify body size. As some body part sizes can change according to feeding or desiccation status, black lines indicate the features measured which represent sturdier parts of these structures not subject to size change after death.

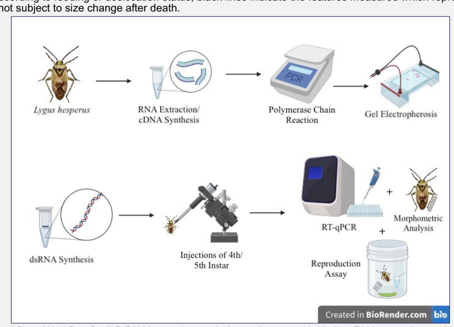


Figure 3. Methods Workflow. Workflow for ILP RNAi experiments in *Lygus hesperus*, including RNA extraction and cDNA synthesis, PCR verification, dsRNA synthesis, injections into 4th/5th instar nymphs, and downstream analyses (RT-qPCR, morphometrics, and reproduction assays).

Results

Impacts of ILP RNAi knockdown

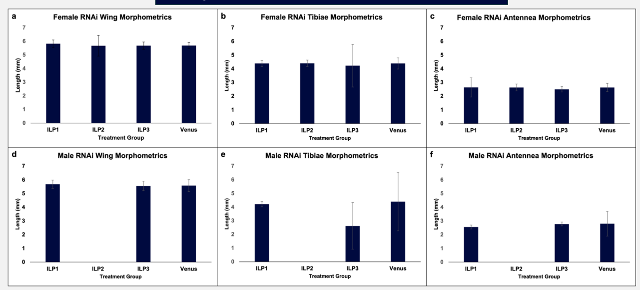


Figure 4. Morphometric effects of ILP RNAi on *Lygus hesperus*. Mean wing, tibia, and antenna lengths (± SEM) were measured in adults after RNAi knockdown of ILP1, ILP2, or ILP3, compared with Venus RNAi controls for females (a–c) and males (d–e). Bars represent mean structure length (mm) per treatment group (± SEM).

Mortality

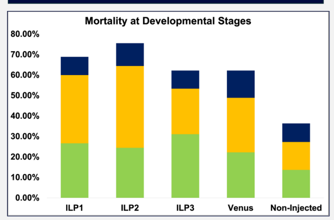


Figure 5. Mortality across developmental stages following ILP dsRNA treatment in *Lygus hesperus*. Percent mortality observed at the 4th instar (light green), 5th instar (yellow), and adult stage (dark blue) for insects treated with dsILP1, dsILP2, or dsILP3, compared with Venus-injected and non-injected controls.

Molting Abnormalities

Figure 6. Molting defects following ILP knockdown in *Lygus hesperus*. Representative *L. hesperus* individuals injected with dsRNA targeting ILP2 (left panel) or ILP3 (right panel) exhibit severe molting abnormalities during the transition from the 5th instar to adult.

Compared to a normal, non-injected adult (top), ILP-knockdown insects appear unable to free themselves from their larval cuticle, among other structural deformities.

Reproduction

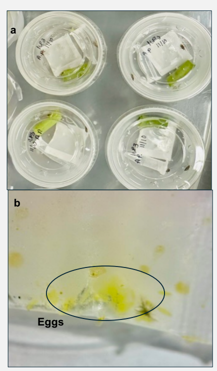


Figure 7. Reproduction Cage Setup and *L. hesperus* Egg-laying. (a) Individual reproduction chambers containing an adult female RNAi *L. hesperus*, a green bean, egg packet for oviposition, water tube, and two non-treated male *L.hesperus*. (b) Eggs deposited inside the egg packet (agar block contained in parafilm) visible as yellow ovoids. The trial was run for total of 7 days.

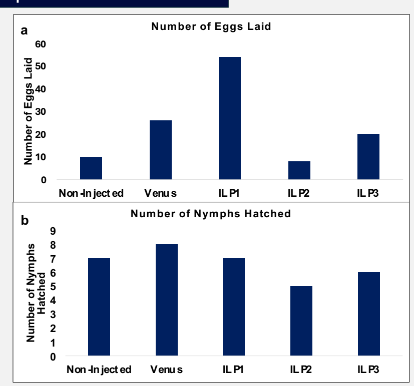


Figure 8. Reproductive output of *Lygus hesperus* females across ILP RNAi treatments. (a) Number of eggs laid by females in dsILP1 (*n* = 3 egg packets), dsILP2 (*n* = 2), dsILP3 (*n* = 3), Venus (*n* = 3), and non-injected (*n* = 2) groups. (b) Number of nymphs hatched from eggs from females subjected to ILP RNAi (ILP1, ILP2,or ILP3), including non-injected control, Venus control controls. Bars represent the total number of nymphs produced per treatment group.

Conclusions

Morphometrics

- Male *L. hesperus* were consistently smaller than females across all treatment groups.
- Measurements revealed no obvious differences in size of morphological features in dsILP knockdowns compared to controls (Venus) (Fig. 4).

Mortality Rate

- High mortality was observed in all treatments (Fig. 5), particularly as nymphs transitioned from 4th to 5th instar, or from 5th to adults (Fig. 6).
- ILP2 RNAi incurred the highest mortality.
- Mortality was observed in insects of all treatments, possibly due to mechanical injury from injection, yet even non-injected insects suffered some mortality, and could be a result of insect handling.

Reproduction

- Number of eggs laid was highly variable across RNAi treatment groups.
- Egg laying in non-injected insects was unexpectedly low, possibly due to low sample size.
- Egg laying was highest in ILP1 RNAi treatments.
- Number of eggs hatched was relatively consistent across treatment groups, despite a large difference in eggs laid.
- The large number of eggs laid in the ILP1 treatment may be non-viable or have a delayed development time before hatch.

Future Work

- Increased replication and sample size of RNAi treatments is needed for more comprehensive morphometric analysis, as well as improved handling procedures to reduce non-treatment mortality.
- Future work will include RT-qPCR to confirm gene knockdown from RNAi-mediated knockdown insects throughout development.
- dsRNA ILP treatments in earlier larval instars (1st or 2nd) may have a more evident impact on eventual adult morphology.
- Disruption of ILP signaling to prevent normal molting may be a target to pursue for development of future *L. hesperus* pest management strategies. Again, further replication will determine whether these effects are consistent.
- The involvement of ILPs in reproduction of *L.hesperus* will be further explored. Preliminary experiments presented in this work hint at increased egg production in some dsRNA ILP treatments, such as ILP1.
- Replication of the reproduction ILP knockdown experiment may confirm a role of ILPs in *L. hesperus* reproduction and may also serve as new pest management target.

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References

Zhu, R., & Chin-Sang, I. D. (2024). *C. elegans* insulin-like peptides. *Molecular and Cellular Endocrinology*, **585**, 112173. https://doi.org/10.1016/j.mce.2024.112173