Domesticated bacterial origin genes regulate homeostasis of the insect gut microbiome and empower adaptation

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Research Background

Sap-sucking insects: highly diverse, with unique foraging habits and life histories

- Play multiple ecological roles as prey, pollinators, and pathogen vectors, with major implications for biodiversity and agricultural security

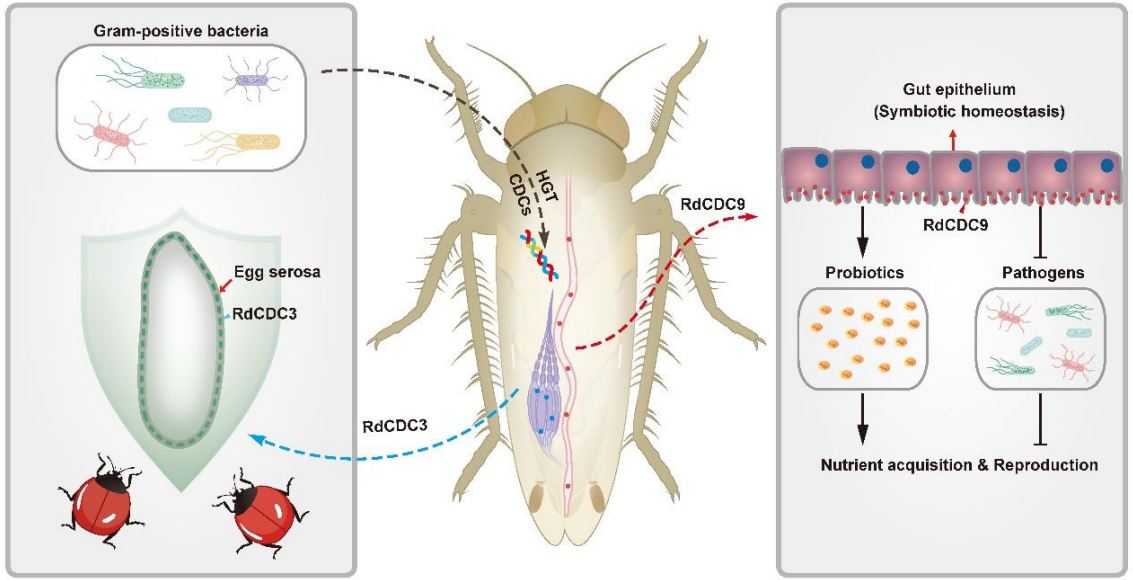
- Family Cicadellidae: **>20,000 described species**
- Major agricultural pests and important insect vectors of plant pathogens
- An ideal system for studying complex interactions among insects, symbiotic microbes, plants, and pathogens

- Study system: three representative rice-associated leafhoppers—***Recilia dorsalis***, ***Nephotettix cincticeps***, **and** ***Nephotettix nigropictus***
- Integrating comparative genomics, metagenomics, bioinformatics, molecular biology, biochemistry, and behavioral assays

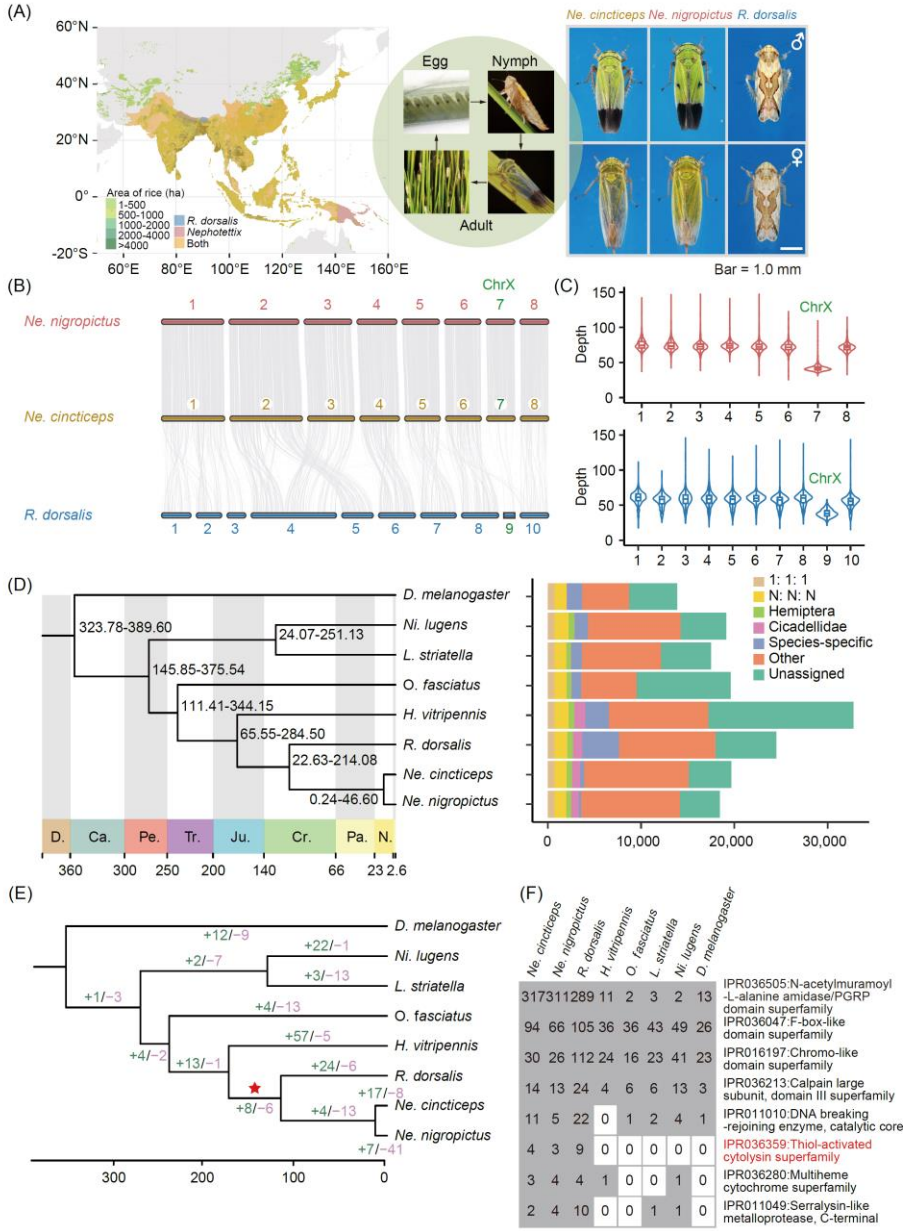
Highlights

Key Findings

- Leafhoppers acquired **cholesterol-dependent cytolysin (*CDC*)** genes from Gram-positive bacteria through **horizontal gene transfer (HGT)**
- Conserved toxic *CDCs* are specifically expressed in the **serosa of eggs**, acting as a defensive weapon **against predators**
- Non-toxic *CDCs* acquired new functions: **gut-specific** expression may contribute to **gut microbiome homeostasis and regulate leafhopper growth and development**
- Domestication and functional diversification of exogenous genes **enhance leafhopper fitness** through multiple mechanisms



Leafhopper Genome Architecture and Cross-Species Comparison



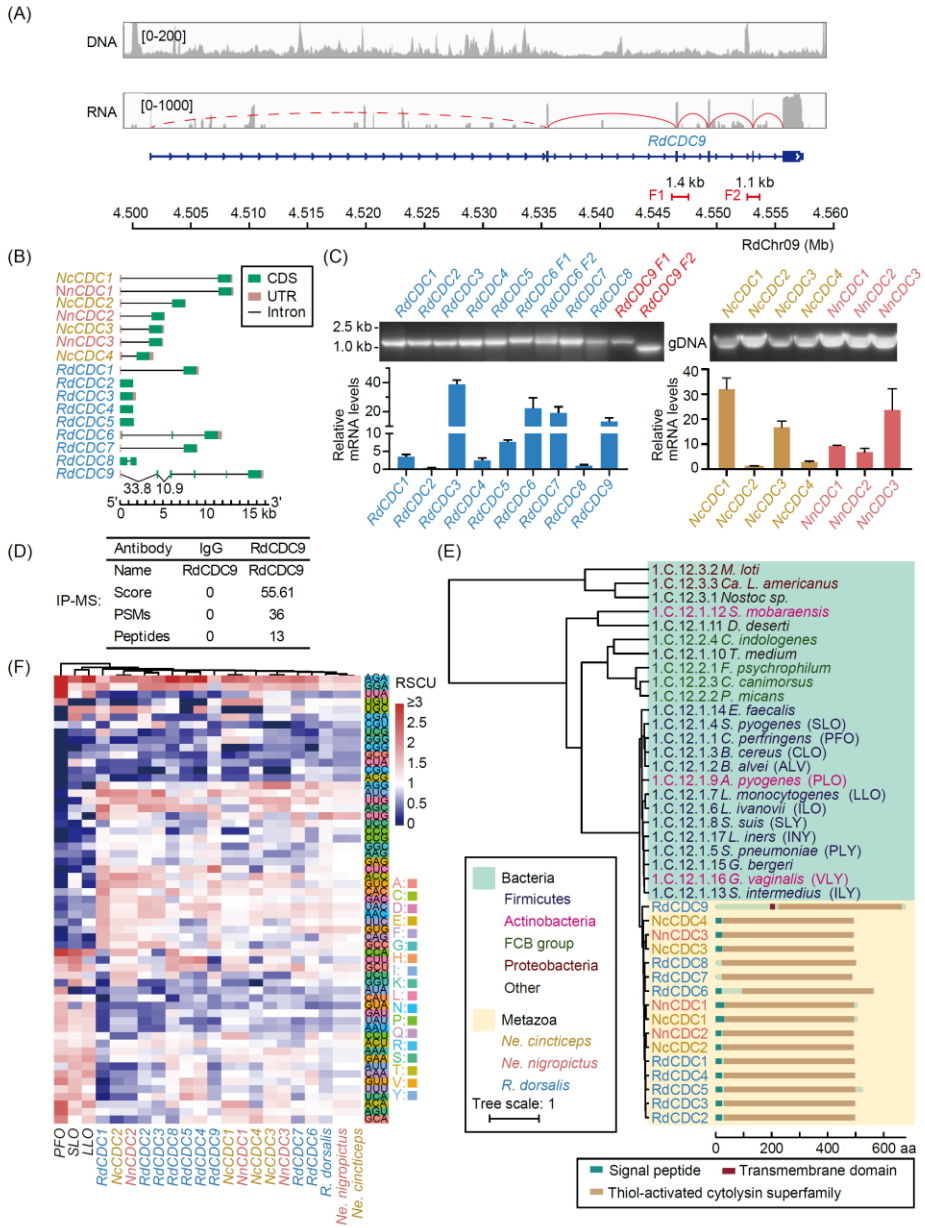
Chromosome-level genomes reveal the presence of *CDC* genes in leafhoppers

- PacBio + Illumina + Hi-C assemblies: *Nephotettix cincticeps*, 788 Mb; *Nephotettix nigropictus*, 770 Mb; *Recilia dorsalis*, 1223 Mb

- Sex chromosomes are relatively conserved

- The **cholesterol-dependent cytolysin (CDC) superfamily** is found only in leafhoppers
— **Not previously identified in any eukaryotic genome**
- Hypothesis: CDC genes were acquired from bacteria through **horizontal gene transfer**

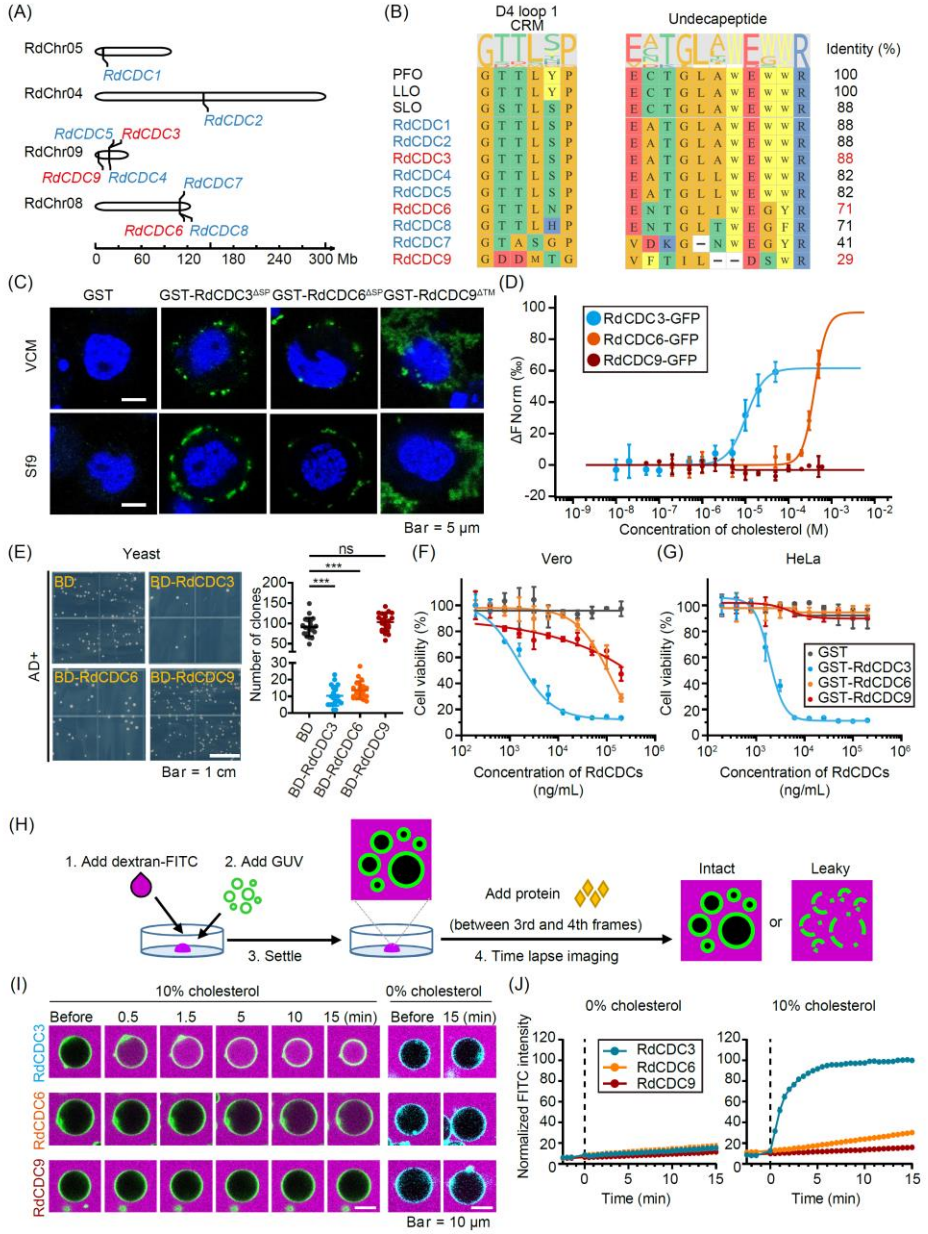
CDC Genes Originated from Bacterial Horizontal Gene Transfer



Phylogeny, gene structure, and protein sequences support an HGT origin

- Gene structures were validated by molecular cloning, PCR, and proteomic analysis
- Phylogeny: leafhopper *CDCs* **cluster with classical *CDCs* from Gram-positive bacteria**
- Present in all examined species of the subfamily **Deltocephalinae**
- Contain **introns** and show codon usage similar to **leafhopper genes**
- Some copies show signatures of **positive selection** and mutations

Functional Divergence in the Cytotoxicity of RdCDC Proteins

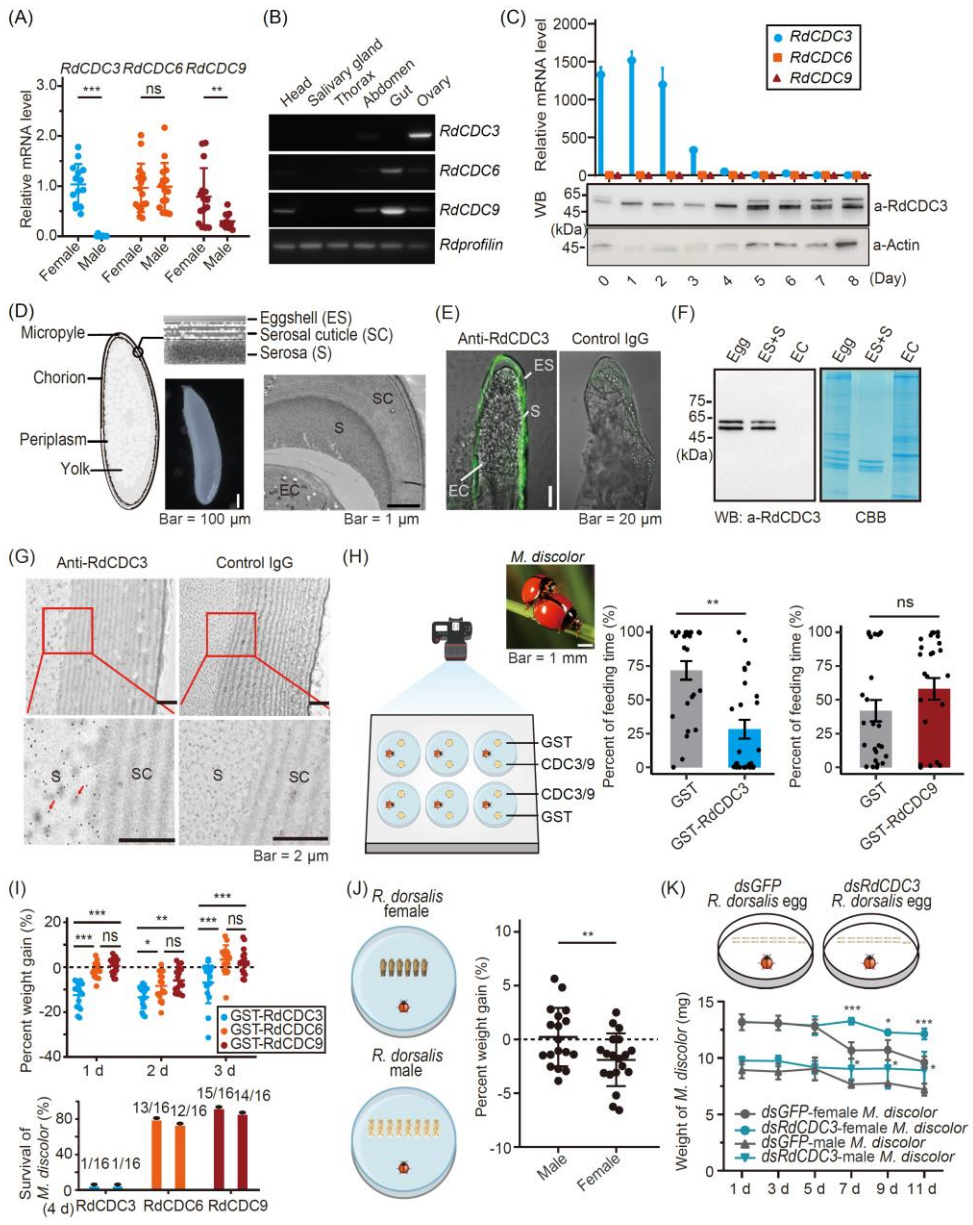


RdCDC3: strong cholesterol binding and high cytotoxicity

- Three representative genes were selected: ***RdCDC3***, ***RdCDC6***, **and *RdCDC9***
- RdCDC3 strongly binds cholesterol (Kd: 6.8 μM) and shows **high cytotoxicity** (IC50: 1–3 μg/mL)
- RdCDC6 shows weaker cytotoxicity; RdCDC9 has completely lost both activities

- Giant unilamellar vesicle (GUV) assays: pore-forming activity strictly **depends on membrane cholesterol**
- RdCDC3 exhibits stronger pore-forming activity than the bacterial CDC

RdCDC3 Protects Leafhopper Eggs against Predators



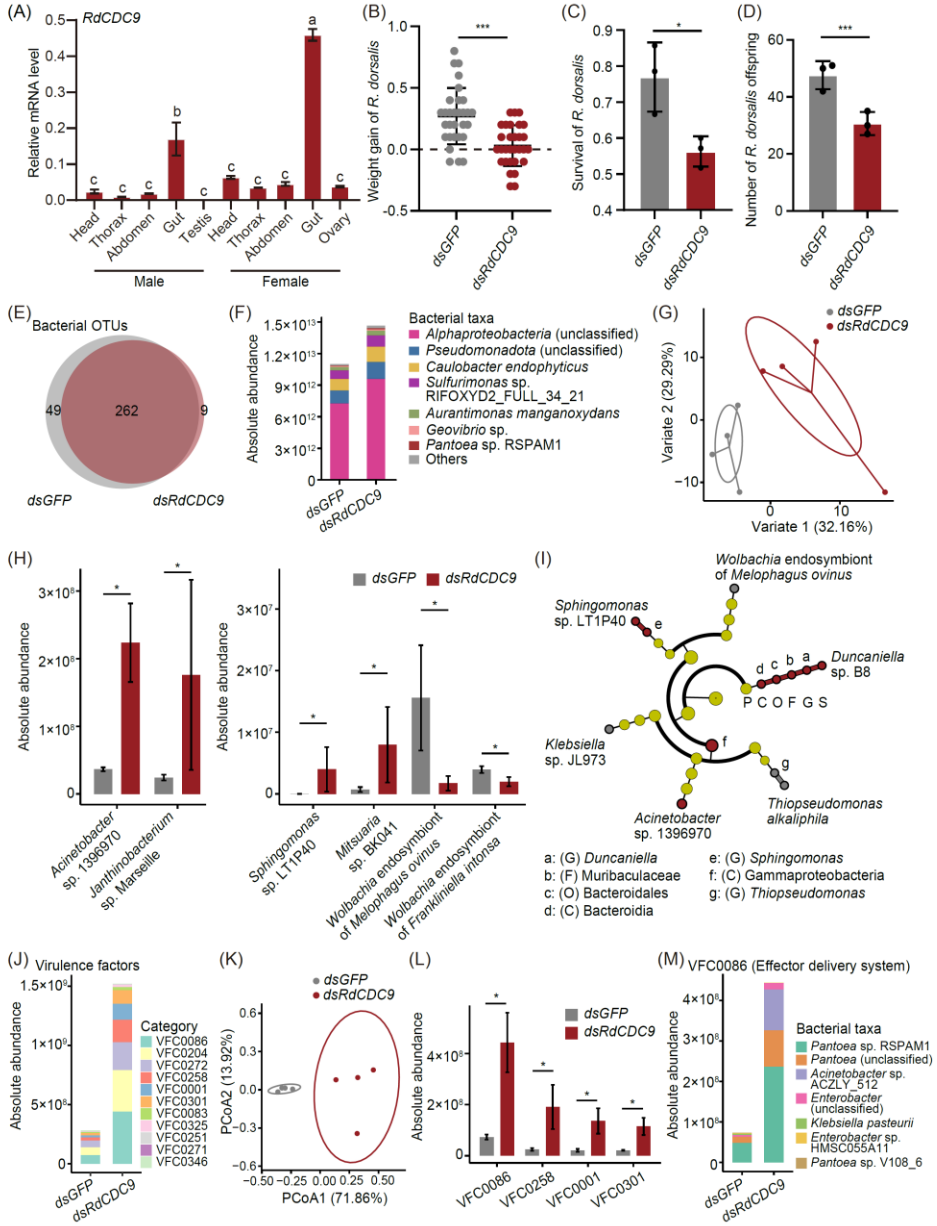
RdCDC3 is expressed in the eggshell and effectively reduces predation

- *RdCDC3* is predominantly expressed in **females, especially in ovaries and eggs**
- IF and WB demonstrate specific expression in the **eggshell**
- Immunogold labeling further confirms localization in the serosa

- Feeding assays: **predators show significantly shorter feeding times** on food containing RdCDC3
- Microinjection of RdCDC3 reduces predator survival and body-weight gain

- Precise regulation: non-toxic to the host but **toxic to predators**—protecting offspring

RdCDC9 Maintains Gut Microbiome Homeostasis



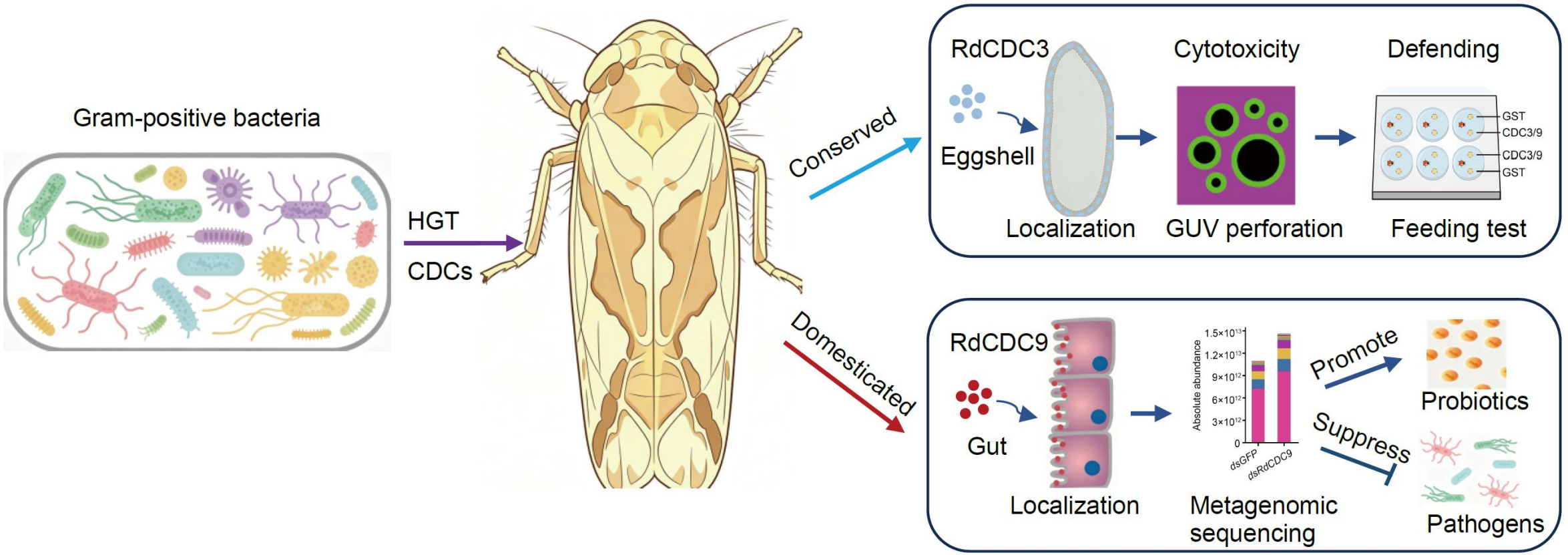
RdCDC9 Regulates Leafhopper Growth and Development

- *RdCDC9* is specifically expressed in the **gut**
- Silencing *RdCDC9* significantly **reduces body weight, survival, and fecundity**

- Metagenomics: the core microbiota remains relatively stable
- **PLS-DA clearly separates the two groups**

- Silencing *RdCDC9* significantly increases the absolute abundance of potential pathogens and **virulence factors**

Model of Exogenous Gene Domestication and Functional Diversification



Conserved toxic RdCDC3 → Egg defense

Domesticated RdCDC9 → Gut microbiome homeostasis

Conclusions and Perspectives

Key Conclusions

- Leafhoppers acquired ***CDC* genes** from bacteria through **HGT**, followed by **domestication and functional diversification**
- Toxic *CDCs*: **serosa expression and defensive function**; non-toxic *CDCs*: **gut expression and novel molecular and physiological functions**
- Insects can use **exogenous genes to generate evolutionary innovations** and gain fitness advantages
- Systematically tracing the evolutionary history and functional diversification of exogenous genes within the same recipient genome
- Provides new opportunities for sustainable pest management

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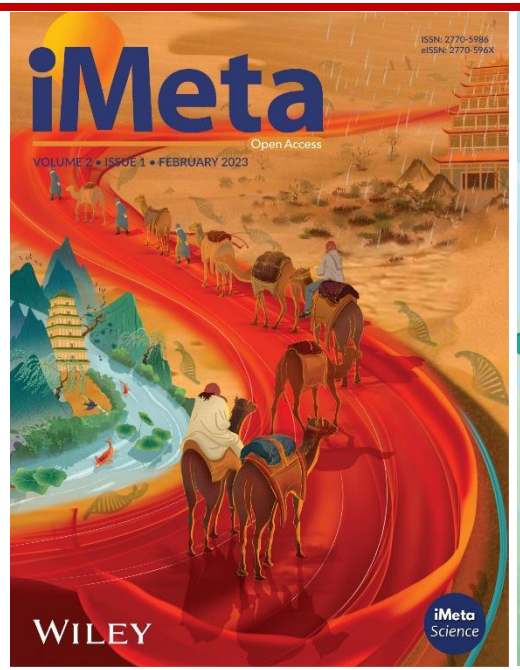

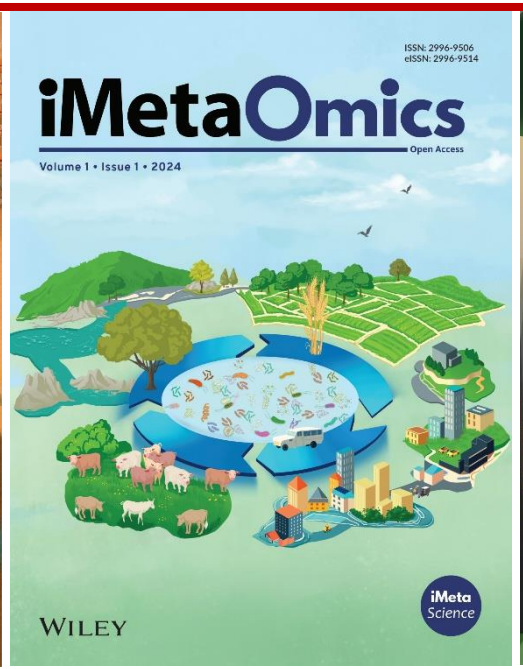

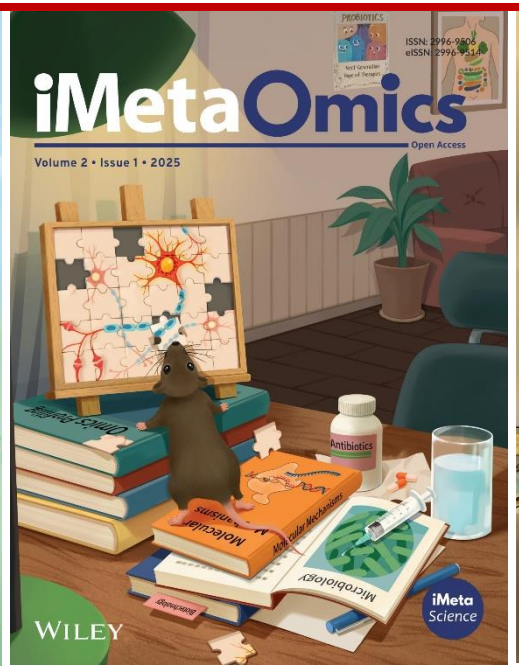

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