# Supplementary Materials: Venom of Parasitoid Pteromalus puparum Impairs Host Humoral Antimicrobial Activity by Decreasing Host Cecropin and Lysozyme Gene Expression 

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#### Abstract

(A) cgcgggaccattcaacaagcagcacgcttcacaagttgttagtgacaaaggaagaaagaaaaataaaaaATGAAT [M N TTCGGAAAATTGTTTTTGTTCGTCTTCGCATGTGTCTTGGCTITGAGCTCGGTGTCGGCGGCGCCAAAATGGAAG F G K L F L F V F A C V L A L S S V S A A P] K K K ATTTTCAAGAAAATTGAACATATGGGCCAAAACATTCGTGATGGTCTCATTAAAGCTGGTCCGGCAGTTCAGGTA I F K K I E H M G Q N I R D G L I K A G P A V $\quad$ R V GTAGGCCAAGCTGCCACCATCTATAAAGGAAAATAGaaaagctagaatttcagctagaagccataccattaacgg $V G \quad Q \quad A \quad A \quad I \quad Y \quad K \quad K \quad$ * ttatttgaattaagaatccaagaccaaaccttctgatacaaatattatcaattcaatcggaaaaaaactaatgta cctatatctgacattactttagaaaatattaactttcgcaaccacctacattttgtatcactgccgctactcttt gttattcccaatcattgactcataatttgctgaaatatagaaaagcctgtataccaataatcatgacgagcgatt atgctattctgatggaaatgctggaatttcctactatctattattgaaataggctttacgcagcaagcctgcaga atgtttaaactatctctactcttgagtttggttattggttctattgtaattatttactctgtacttactgcctca aatacccgaattaatttatttgcttattaagatattaggattatttggtaataaatatttaagacgaaaaaaa aaaaaaaaaaaaa


(B)


Figure S1. Nucleotide and deduced amino acid sequences of the Pr-cec gene. (A) Pr-cec cDNA (above) and amino acid (below) sequences are shown with a predicted signal peptide (shown in a pair of brackets), and the predicted cleavage site is indicated by a black triangle. Positively charged amino acid residues, arginine $(\mathrm{K})$ and lysine $(\mathrm{R})$, which bind to the negatively charged bacterial cell membrane, are shaded. Polyadenylation signal sequence is double underlined. Initial and stop codons are single underlined. (B) Multiple comparison of the amino acid sequence of Pr-cec with typical lepidopteran cecropin precursors (containing the signal peptide of each cecropin). The sequences that were used are listed in Supplementary Table S1. The boxes with the same color indicate the same amino acid residues in those species. Spots indicate gaps to optimize the sequence alignment. The target sequence is boxed in red and is the same in the other supplementary figures.


Figure S2. Phylogenetic analysis between Pr-cec and other cecropins. Construction was performed based on homology sequences that were calculated from the complete amino acid sequences of cecropin (cec) using the UPGMA method. Sequences were selected from NCBI databases (listed in Supplementary Table S1), and the sequence of the nematode Ascaris cecropins (AscecP1-4) was used as the out-group. The values in the tree are bootstrap support values.


#### Abstract

(A)

1 gcggggatacgcctttggtcaagagtagagaacacaactATGAAGTTAGCAGTATTCATTTTTGCACTTGCTGCT 76 CTGTTCGGAGCAGAAGCCGTTACGTTTACAAGATGCCAATTGGTGCGCGAATTAAGGAATCAAGGCTTTCCAGAA L F G A E A]IV T F T R C Q L V R E L R N Q G F P E 151 ACTAAAATGAGAGATTGGGTATGTCTCGTTGAAAACGAGAGTAGCCATAACACAGCCAAAGTGGGAAAAGTGAAC T K M R D W V C L V E N E S S H N T A K V G K V N 226 AAGAATGGTTCCAGAGACTACGGTCTCTTCCAGATCAACGACAAGTACTGGTGCAGCAATACTAACACTGCCGGA K N G S R D Y G L F Q I N D K Y W C S N T N T A G 301 AAAGACTGCAATGTCACATGTGCGCAGGTGACAACGGACGACATCACAAAAGCTGCAACCTGTGCTAAAAAGATC K D C N V T C A Q V T T D D I T K A A T C A K K I 376 TTTAAGCGCCATGGATTCAATGCTTGGTATGGTTGGAAGAACCACTGCCAAGGCTCTCTCCCTGACATAAGTTCT F K R H G F N A W Y G W K N H C O G S L P D I S S 451 TGTTAAaagctctactctagttaaacaatattatataaaacactacgatcctatacagaatattgtgaaataata C.*

526 tatctatgtaattagttatttcacaaatgttctcaaataaaaataattaaatccaaaaaaaaaaaaaaaaa


## (B)



Figure S3. Nucleotide and deduced amino acid sequences of the Pr-lys gene. (A) Pr-lys cDNA (above) and amino acid (below) sequences are shown with a predicted signal peptide (shown in a pair of brackets), and the predicted cleavage site is indicated by a black triangle. The polyadenylation signal sequence is doubly underlined. Initial and stop codons are single underlined. (B) Multiple comparison of the amino acid sequence of Pr-lys with typical lepidopteran lysozyme precursors (containing the signal peptide of each lysozyme). The sequences that were used are listed in Supplementary Table S1. The boxes with the same color indicate the same amino acid residues in those species. Spots indicate gaps to optimize the sequence alignment.


Figure S4. Phylogenetic analysis between Pr-lys and other lysozymes. Construction was performed based on homology sequences that were calculated from the complete amino acid sequences of lysozyme (lys) using the UPGMA method. Sequences were selected from NCBI databases (listed in Supplementary Table S1). The sequence of the lysozyme of Gallus gallus (Chicken) (Gg-lys) was used as the out-group. The values in the tree are bootstrap support values.

Table S1. Deduced amino acid sequences of cecropin and lysozyme, which were used in the multiple sequences alignments and phylogenetic tree constructions.

| Sequences of Cecropin |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Name | Accession No. | Species | Name | Accession No. | Species |
| Pr-cec | - | Pieris rapae | Bm-cecCBM1 | NP_001037030 | Bombyx mori |
| Ar-hinII | AAT94287 | Artogeia rapae | Bm-cecCBM2 | NP_001037031 | Bombyx mori |
| As-cecP1 | BAD89085 | Ascaris suum | Bm-cecCBM2-2 | NP_001037032 | Bombyx mori |
| As-cecP2 | BAD89086 | Ascaris suum | Bm-cecD | NP_001036833 | Bombyx mori |
| As-cecP3 | BAD89091 | Ascaris suum | Bm-cecE | NP_001037392 | Bombyx mori |
| As-cecP4 | BAD89092 | Ascaris suum | Dm-cecA1 | NP_524588 | Drosophila melanogaster |
| Bm-cecA | NP_001037462 | Bombyx mori | Dm-cecA2 | NP_524589 | Drosophila melanogaster |
| Bm-cecB | NP_001037460 | Bombyx mori | Dm-cecB | BAA28722 | Drosophila melanogaster |
| Hce-cecA | P01507 | Hyalophora cecropia | Dm-cecC | AAB82507 | Drosophila melanogaster |
| Hce-cecB | P01508 | Hyalophora cecropia | Md-cec1 | AAL08023 | Musca domestica |
| Hce-cecD | P01510 | Hyalophora cecropia | Ms-cec6 | CAL25128 | Manduca sexta |
| Tn-cecA | P50724 | Trichoplusia ni | Pi-cecA | AAR99379 | Pseudoplusia includens |
| Tn-cecB | ABV68872 | Trichoplusia ni | Px-cecA | BAF64473 | Plutella xylostella |
| Tn-cecD | ABV68873 | Trichoplusia ni | Px-cecE | BAF36816 | Plutella xylostella |
| Hcu-cecA | P50720 | Hyphantria cunea | Hcu-cecA2 | P50722 | Hyphantria cunea |
| Hcu-cecA1 | P50721 | Hyphantria cunea | Hcu-cecA3 | P50723 | Hyphantria cunea |
| На-cec | AAX51304 | Helicoverpa armigera | - | - | - |
| Sequences of lysozyme |  |  |  |  |  |
| Name | Accession No. | Species | Name | Accession No. | Species |
| Pr-lys | - | Pieris rapae | Ha-lys | ABF51015 | Helicoverpa armigera |
| Hcu-lys | AAA84747 | Hyphantria cunea | Ac-lys | AAN87265 | Agrius convolvuli |
| Ct-lys | ACJ64375 | Culex tarsalis | Aae-lys | AAU09087 | Aedes aegypti |
| Ag-lys | AAC47326 | Anopheles gambiae | Hz -lys pre | ACL51928 | Helicoverpa zea |
| Ap-lys | ABC73705 | Antheraea pernyi | Hce-lys | P05105 | Hyalophora cecropia |
| Ar-lysII | AAT94286 | Artogeia rapae | Hv-lys | AAD00078 | Heliothis virescens |
| Bm-lys | NP_001037448 | Bombyx mori | Ms-lys | AAB31190 | Manduca sexta |
| Gg-lys | NP_990612 | Gallus gallus (Chicken) | Of-lys pre | ABN54797 | Ostrinia furnacalis |
| Cq-lys | XP_001847112 | Culex quinquefasciatus | Pi-lys | AAS48094 | Pseudoplusia includens |
| Aal-lys | AAM11885 | Aedes albopictus | Scr-lys | BAB20806 | Samia cynthia ricini |
| Ag-lys c-2 | AAT51797 | Anopheles gambiae | Se-lys | AAP03061 | Spodoptera exigua |
| Ag-lys c-8 | AAY21241 | Anopheles gambiae | Sl-lys | ACI16106 | Spodoptera litura |
| As-sal lys | AAO74844 | Anopheles stephensi | Aal-sal lys | AAV90643 | Aedes albopictus |
| Ad-lys | ACI30031 | Anopheles darlingi | Ag-lys c-3 | AAT51798 | Anopheles gambiae |
| Ag-lys c-1 | AAY24699 | Anopheles gambiae | - | - | - |

