

Supporting Information

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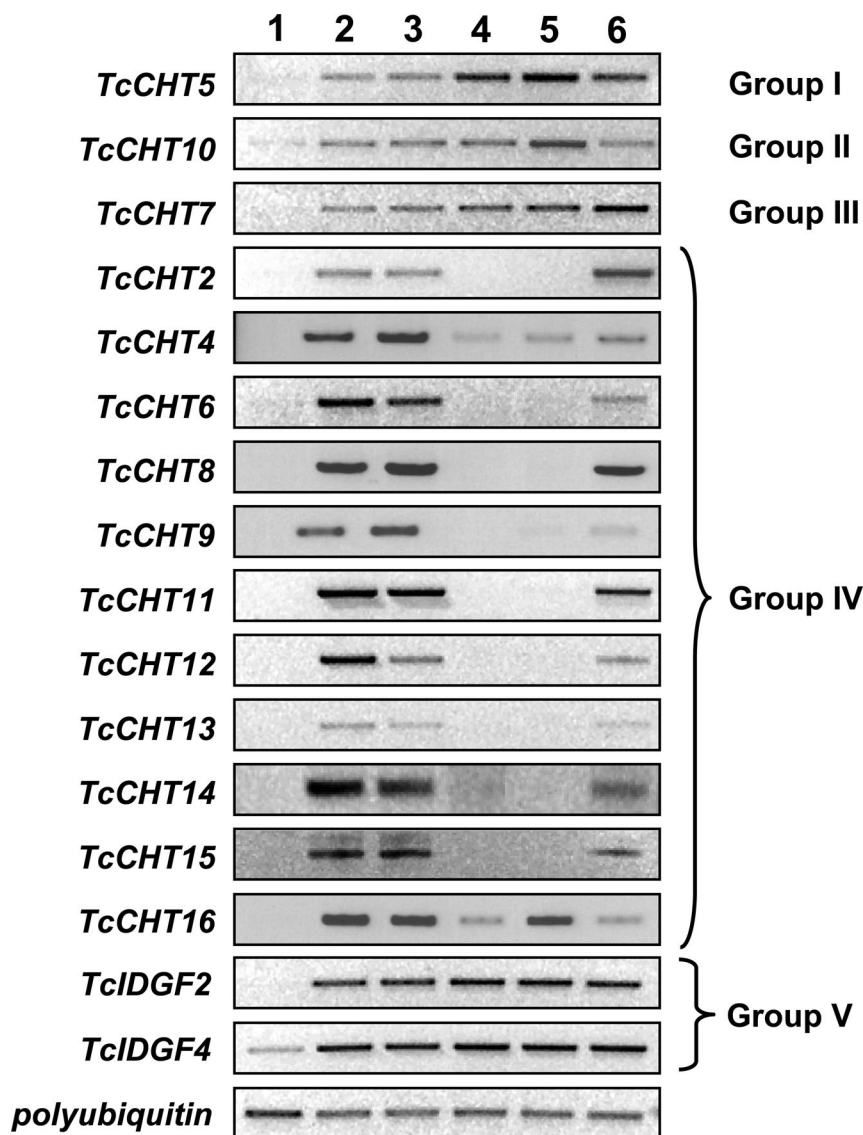


Fig. S1. Gene expression profiles and tissue specificity of chitinase gene family as determined by RT-PCR. Total RNA was prepared from ≈ 100 eggs (about 3.5 mg per 100 eggs). For larvae, pharate pupae, pupae, and adult stages, four insects were collected and used for preparation of total RNA. cDNAs synthesized from total RNAs using oligo-(dT)₂₀ primers and reverse transcriptase were used as templates for RT-PCR (25 cycles) using gene-specific primer pairs. RT-PCR product from a reaction using a pair of primers for *polyubiquitin* was used as an internal loading control (20 cycles). Lanes: 1, eggs; 2, penultimate instar larvae; 3, last instar larvae; 4, pharate pupae; 5, pupae; 6, adults. The expression profiles of seven additional members of this family that are not fully characterized are not shown.

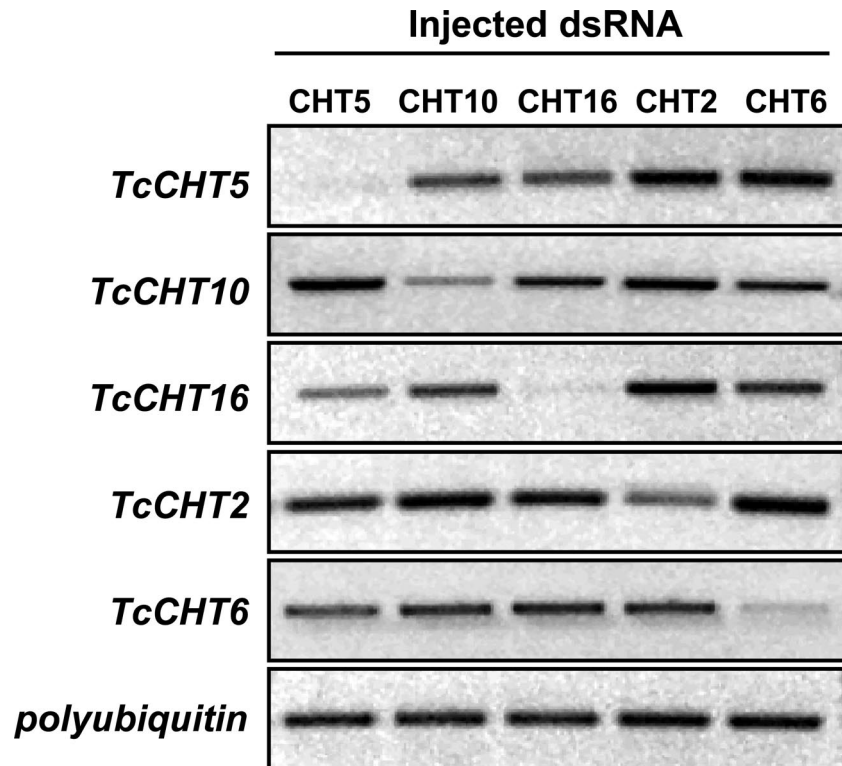


Fig. S2. Specificity of transcript down-regulation by dsRNAs for chitinase genes. dsRNAs for *TcCHT2*, *TcCHT5*, *TcCHT6*, *TcCHT10*, and *TcCHT16* (200 ng per insect) were injected into penultimate instar larvae. Three days after injections, four insects from each treatment were collected for total RNA preparation. cDNAs prepared from total RNA using oligo(dT)₂₀ primers and reverse transcriptase were used as templates for RT-PCR (25 cycles). Pairs of gene-specific primers were used to monitor the transcript levels of *TcCHT2*, *TcCHT5*, *TcCHT6*, *TcCHT10*, and *TcCHT16* in five separate reactions (Left). RT-PCR product with a pair of primers for polyubiquitin was used as the internal loading control (20 cycles).

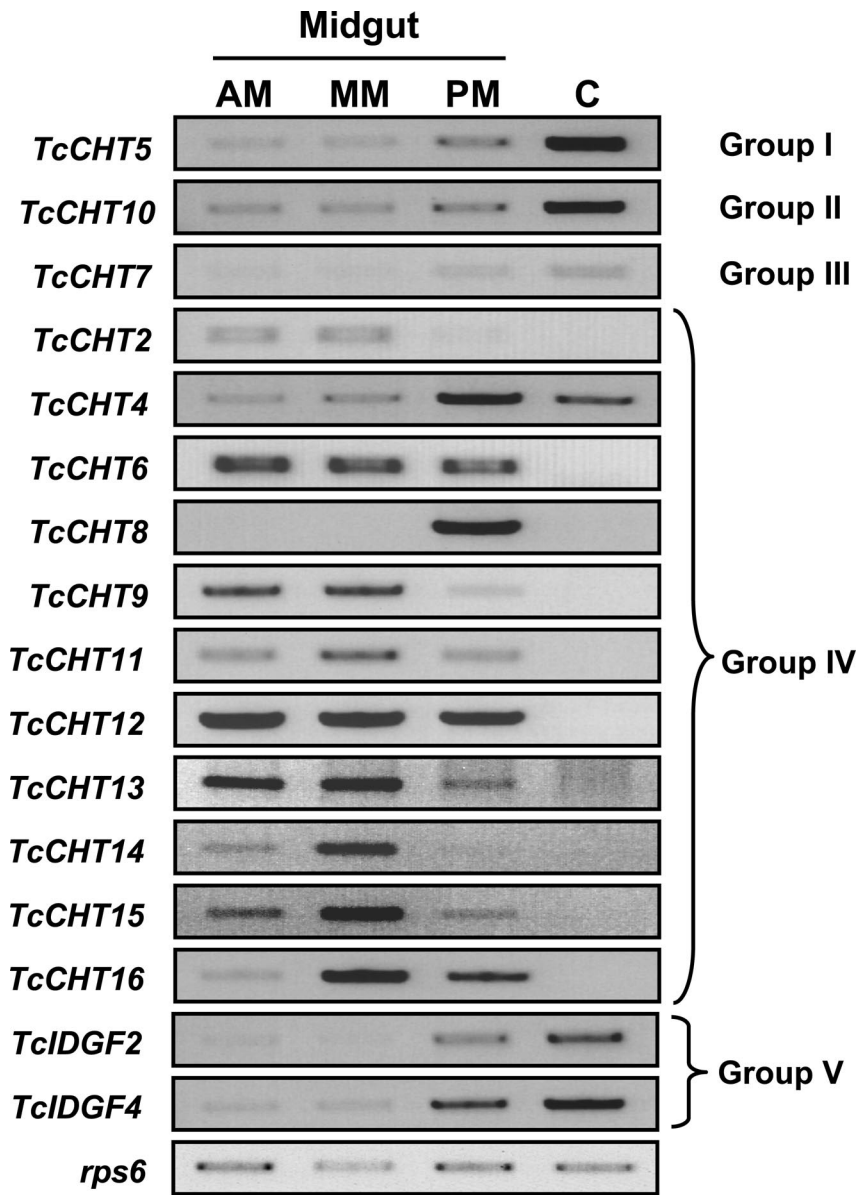


Fig. S3. Expression of chitinase genes in different parts of the midgut and carcass. Ten midguts were dissected from last instar larvae and then divided roughly equally into three parts, which were labeled anterior (AM), middle (MM), and posterior (PM) midguts. The carcass (whole body minus gut, head, and posterior tip) was also collected to analyze for expression of individual chitinase genes as described in the legend to Fig. S1. RT-PCR product amplified with a pair of primers for the ribosomal protein S6 (*rps6*) was used as an internal loading control. RT-PCR was carried out for 30 cycles for all genes except *rps6* (24 cycles).

Table S1. Primers used for checking gene expression profiles of Tc chitinase family

Primers	Primer sequences, 5' to 3'
<i>Tc</i> CHT2	
F	TCCTAGTTAAAGCAAACACCAAGA
R	GTTCAGCCTCCTATGCTGATT
<i>Tc</i> CHT4	
F	ACCAACCAGAAACTGTTGAC
R	AAGGAACAAAACCTCCATTG
<i>Tc</i> CHT5	
F	ATGTGTGGATGCCATTCATGT
R	GGTGGCGTTTGTGTATGGTC
<i>Tc</i> CHT6	
F	TGGGTACAATGAGTTGTGTGA
R	GCATGTAAATCGTCCGTTTCAA
<i>Tc</i> CHT7	
F	GAACAAAATGAAGTGGATCAAGGAT
R	GTCGTACATTTCCGGGCTTTTATC
<i>Tc</i> CHT8	
F	GAGGGTAGTCAAAGTTACTC
R	CTGACCAATTTACTTCCATC
<i>Tc</i> CHT9	
F	TTGGCTCTCCTTGCTGCTTC
R	CGCACTTTTGGCAACAGTTG
<i>Tc</i> CHT10	
F	GGTTTCCTAGCCTACTATGAGATTTG
R	GGCGTCCCTCTCAACTCATT
<i>Tc</i> CHT11	
F	GTCGCCTCGAGCAAACCTAG
R	TCGTTGAAACCCAAAACCTCC
<i>Tc</i> CHT12	
F	CCCGAATCTGAAAGTGTTGC
R	CATTCAAGCCATGGTCTCCT
<i>Tc</i> CHT13	
F	TAGTGCCGATTTGAGCCAAG
R	GTCTAGACCATGGTCATTC
<i>Tc</i> CHT14	
F	AGCAGCTGCTAACGATCCAG
R	GTCATTAAGCCCATGTTTGCTG
<i>Tc</i> CHT15	
F	TAGTGACCAGCGTTTTGAGC
R	GGCGTACAGTTCGGTGTTTG
<i>Tc</i> CHT16	
F	CTAGCAATCCTCGCCTTCGC
R	TGGATTAGCTGCCACTTGCG
<i>Tc</i> IDGF2	
F	GGCTCCTTCAAGCCACTTCT
R	TTTGATCGGATTTTCTTCG
<i>Tc</i> IDGF4	
F	ATGCCGAAACTTTCAAAGCA
R	TGGGGGTGTAGAAGTCGAAG

F, forward; R, reverse.

Table S2. Summary of properties of dsRNAs used for RNAi studies

Gene and dsRNA	Nucleotide positions	Length, bp
<i>TcCHT2</i>		
1	11–114	104
2	700–1149	450
<i>TcCHT16</i>		
1	225–402	178
2	125–410	286
<i>TcCHT5</i>		
1	426–797	372
2	21–200	180
<i>TcCHT6</i>		
1	1–104	104
<i>TcCHT7</i>		
1	2342–2818	476
2	2821–3329	508
<i>TcCHT10</i>		
1	7180–8012	833
2	8004–8116	113
<i>TcIDGF2</i>		
1	508–667	160
<i>TcIDGF4</i>		
1	489–648	160

The most divergent regions in individual genes were selected to synthesize dsRNAs.