Original Article

Antibacterial Peptide Gene Expression in the Honey Bee (*Apis mellifera* Feb.) Parasitized by *Tropilaelaps* Mite

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Abstract

The mite, was originally confined to the wild honey bees in Asia, but now plagues the European honey bee, *Apis mellifera*. We amplified antimicrobial peptide cDNA genes (*Defensin, Abaecin, Royalicin,* and *Hymenoptaecin*) by RT-PCR in order to explore the transcriptional response to mite parasitism in *A. mellifera*, which differs in susceptibility to *T. clareae*, by comparing parasitized and non-parasitized full-sister adult bees (worker bees, drone bees) from the same hive. Differential gene expression in worker and drone bees induced by *Tropilaelaps* infestation was investigated by northern blotting. *Tropilaelaps* parasitism caused changes in the expression of genes related to sex distinction. Bees tolerant to viruses from *Tropilaelaps* were mainly characterized by differences in the expression of genes regulating antimicrobial gene expression. This finding provides a first step towards a better understanding of the molecular expression involved in this differential sex distinction host-parasite relationship. Therefore, this result demonstrated that *Tropilaelaps* were possible route of induction of antibacterial gene expression.

Key words: Antibacterial peptide, *Apis mellifera*, Abaecin, Hymenoptaecin, Defensin, Royalicin, *Tropilaelaps*, Bee virus

INTRODUCTION

Honey bees are important economic eusocial insects that are vital for pollination and for the production of several bee products. The ectoparasitic *Tropilaelaps* mite feeds on the body fluids of the larvae, pupae, and adults of the western honey bee (*Apis mellifera* L.) causing serious damage to bee colonies. As eusocial insects, honeybees have evolved both communal and individual traits that reduce the impact of their numerous parasites and pathogens. Honeybees eliminate harmful invading pathogens and parasites through an inducible defense system (Boman *et al*., 1991) that includes at least four antimicrobial

peptides (abaecin, defensin, apidaecin, and hymenoptaecin) (Casteels *et al*., 1989, 1993; Hoffmann *et al*., 1993). Following bacterial challenge, antibacterial peptides are produced in the fat body or hemocytes and then released into the hemolymph of insects. The following classes of insect antibacterial peptides have been proposed (Bulet *et al*.,1993; Casteels *et al*., 1993; Hultmark, 1993): (1) lysozymetype, (2) short α-helical, (3) short intra-disulfide linked, (4) short Arg-Pro-rich, and (5) long multi-domain. These antibacterial peptides function by deteriorating the bacterial cell envelope, with the exception of apidaecin, which is non-lytic (Casteels *et al*., 1994). In honeybees (*Apis mellifera*), the immune repertoire consists of four

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antibacterial peptides that are induced by bacterial infection and provide broad-spectrum antibacterial defense through complementarity (Casteels-Josson *et al*., 1994). Furthermore, induction of the entire antibacterial peptide repertoire in these bees can be triggered by infection with *Escherichia coli* alone (Casteels *et al*., 1989; Casteels-Josson *et al*., 1994). In the hemolymph of *A. mellifera* infected with *E.coli*, four different antibacterial peptides have been identified: apidaecin (Casteels *et al*., 1989), abaecin (Casteels *et al*., 1990), hymenoptaecin (Casteels *et al*., 1993) and defensin (Casteels-Jonsson *et al*., 1994). Recently, two structurally different defensin genes were cloned from *A. mellifera* (Klaudiny *et al*., 2005). It is believed that insects express a combination of antibacterial peptides in response to natural infection, leading to a broad spectrum of activity against microorganisms. Healthy animals can build up a variety of immune responses to microbial infections and septic wounds, and the rapid production of AMPs is one of the steps in this process.

AMPs are small bioactive molecules that are normally released in the hemolymph of infected invertebrate animals, such as honey bees. However, immune suppressed individuals will show greatly reduced responses. The AMP response is suppressed in *varroa*-parasitized bees, so that *varroa* infestation may suppress the bee immune system, resulting, for example, in increased titers of Deformed Wing Virus (DWV) and bees with visibly deformed wings. (Xiaolong and Cox-Foster, 2005).

We report here the detection of bee viruses and antibacterial gene products (abaecin, defensin, royalicin, and hymenopteacin) in *A. mellifera* based on sampling of adult worker and drone bees parasitized by *T. clareae*. We hypothesize that mites may suppress expression of genes that encode antimicrobial peptides. In addition, we have examined the differential expression between workers and drones. This research might help to explain how an ectoparasite can affect the immunity and pathology of its invertebrate host.

MATERIALS AND METHODS

Experimental animals

The honeybees (*Apis mellifera*) were collected from

apparently infected colonies from an experimental apiary in Suwon and were analyzed using the RT-PCR for identification of virus and antibacterial genes. The live worker and drone honey bees were stored at −80°C until processing.

cDNA construction, genomic DNA isolation, and PCR

The whole bodies of *A. mellifera* worker, drone bees and *Tropilaelaps* mites were ground to a fine powder in liquid nitrogen for generation of cDNAs from mRNA. A 0.03g sample of powder was used to extract total RNA using a total RNA extraction kit (Promega, Madison, WI). RT-PCR was performed using the Qiagen One Step RT-PCR kit (Qiagen, Germany) in 25μl reactions containing 200ng RNA templates and 2.5 pmol of each primer. Thermal reactions proceeded with an initial reverse transcription incubation at 42°C for 60 min then incubation at 95°C for 1 min. This was followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 46°C for 45 s, extension at 72°C for 1 min. A final extension step was performed at 72°C for 10 min. PCR products were electrophoresed on a 1.2% agarose gel containing 0.5μg/ml ethidium bromide and subsequently visualized by UV transillumination. PCR products were excised using the Qiaex II gel extraction kit (Qiagen, Germany) following the manufacturer's instructions and used as the templates for sequencing reactions. Genomic DNA was extracted from whole bodies of *A. mellifera* worker bees using the Wizard Genomic DNA Purification Kit (Promega, USA) and then used for Genomic PCR amplification with oligonucleotide primers designed from the cDNA sequences. Primers used were as follows: for abaecin, forward primer (5'-GATCTGCACA-CTCGAGGTCTG-3') and reverse primer (5'-GGGCGAATTGGGCCCGACTCGC-3'); for defensin, forward primer (5'-TGAGCTGTCGTTCAGACAC-3') and reverse primer (5'-TCGTTGAAT-CTTCATAATG-GCAC-3'); for hymenoptaecin, forward primer (5'-TTCCAAGATGAAATTGATCGTGTTAG-3') and reverse primer (5'-TGCTACGTAAATACGAATCGTA-ATTTATTG-3'); for royalicin, forward primer (5'-CTTTGTGCTGGGAAGATGAAGTTC-3') and reverse primer (5'- GGGCGAATTGGGCCCGACGTCG-3'),

based on the cDNA sequence. Genomic PCR was performed using 200ng RNA templates and 2.5 pmol of each primer. After 35 cycles of amplification (94°C for 30 s; 48°C for 40s; 72°C for 2 min), the PCR products were electrophoresed on a 1.2% agarose gel containing 0.5μg/ml ethidium bromide and subsequently visualized by UV transillumination. RT-PCR was performed using the Qiagen OneStep RT-PCR kit (Qiagen, Germany) in 25μl reactions containing 200ng RNA templates and 2.5 pmol each primer for detection of antibacterial peptides. Thermal reactions proceeded with an initial reverse transcription incubation at 50°C for 30 min then incubation at 95°C for 15 min. This was followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 1 min. A final extension step was performed at 72°C for 10 min. PCR products were electrophoresed on a 2% agarose gel containing 0.5μg/ml ethidium bromide and subsequently visualized by UV transillumination.

DNA sequence analysis, genomic DNA isolation, PCR of antibacterial peptide genes

The RT-PCR (cDNA) and genomic PCR products were sequenced using an automatic sequencer (Model 3100 Genetic Analyzer; Perkin Elmer Applied Biosystems, Foster City, CA). Sequence data were automatically collated and analyzed using the ABI sequencing analysis software and subsequently manually verified. Similarities between virus sequences and published sequences were determined using BLAST (Basic Local Alignment Search Tool) within the National Centre for Biotechnology Information (NCBI) database (http://www.//ncbi.nlm.nih.gov/BLAST). Genomic DNA was extracted from fat body tissues of *A. mellifera* using the Wizard Genomic DNA Purification Kit (Promega, USA) and then used for PCR amplification with oligonucleotide primers designed from the cDNA sequences. After a 35-cycle amplification (94°C for 30 s; 48°C for 40 s; 72°C for 2 min), the PCR products were cloned into the pGem-T vector for sequencing (Promega, USA) and were transformed into E. coli TOP10F' cells (Invitrogen, Carlsbad, CA, USA). The nucleotide sequence was determined using a BigDye Terminator cycle sequencing kit and an automated DNA sequencer as described above.

RNA isolation and Northern blotting analysis

A. mellifera workers and drones were collected and washed twice with phosphate-buffered saline (PBS; 140mM NaCl, 27mM KCl, 8mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.4). Total RNA was isolated from whole bee bodies using a Total RNA Extraction Kit (Promega, USA). Purified total RNA (1μg/lane) was separated on a 1.0% formaldehyde agarose gel, transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany), and hybridized at 46°C with a probe in hybridization buffer containing 5×SSC, 5×Denhardt's solution, 0.5% SDS, and 100μg/ml denatured salmon sperm DNA. The cDNA PCR products were labeled with $[\alpha-^{32}P]dCTP$ (Amersham, Arlington Heights, IL, USA) using a Prime-It Random Primer Labeling Kit (Stratagene, La Jolla, CA, USA) and used as a probe for hybridization. After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2×SSC at 65°C and then exposed to autoradiography film.

RESULTS AND DISCUSSION

Analysis antibacterial nucleotide sequencing and genomic DNA isolation and PCR

Four antibacterial peptide cDNAs (defensing, royalicin, hymenopteacin, abaecin,) were identified as having homology to previously reported antibacterial peptide genes. These cDNA clones, including the full-length open reading frames (ORFs), were sequenced and characterized (Fig. 1, 2, 3, and 4). We cloned *A. mellifera* defensin cDNA, a 398 bp long gene containing an ORF of 285 nuc-leotides capable of encoding a 95 amino acid polypeptide (Fig. 1A). The gene structure revealed that the *A. mellifera* defensin gene spans 2011 bp and contains two introns and three exons (Fig. 1B). From the sequence data, it appears that all insect defensins have a conserved region of six cysteine residues and share a common three-dimensional structure, made of an N-terminal loop, a central amphipathic α-helix, and a Cterminal antiparallel β-sheet
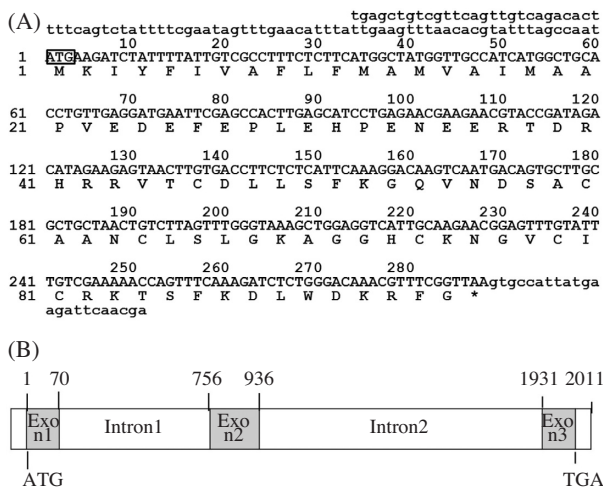
(A)
```
                                            tgagctgtcgttcagttgtcagacact
                                 10        20        30        40        50        60
tttcagtctattttcgaatagtttgaacatttattgaagtttaacacgtatttagccaat
  1 ATGAAGATCTATTTTATTGTCGCCTTTCTCTTCATGGCTATGGTTGCCATCATGGCTGCA
  1 M  K  I  Y  F  I  V  A  F  L  F  M  A  M  V  A  I  M  A  A

           70        80        90       100       110       120
 61 CCTGTTGAGGATGAATTCGAGCCACTTGAGCATCCTGAGAACGAAGAACGTACCGATAGA
 21 P  V  E  D  E  F  E  P  L  E  H  P  E  N  E  E  R  T  D  R

          130       140       150       160       170       180
121 CATAGAAGAGTAACTTGTGACCTTCTCTCATTCAAAGGACAAGTCAATGACAGTGCTTGC
 41 H  R  R  V  T  C  D  L  L  S  F  K  G  Q  V  N  D  S  A  C

          190       200       210       220       230       240
181 GCTGCTAACTGTCTTAGTTTGGGTAAAGCTGGAGGTCATTGCAAGAACGGAGTTTGTATT
 61 A  A  N  C  L  S  L  G  K  A  G  G  H  C  K  N  G  V  C  I

          250       260       270       280
241 TGTCGAAAAACCAGTTTCAAAGATCTCTGGGACAAACGTTTCGGTTAAgtgccattatga
 81 C  R  K  T  S  F  K  D  L  W  D  K  R  F  G  *
    agattcaacga
```

(B)
```
  1   70              756  936                      1931 2011
 ┌──┬───────────────┬────┬───────────────────────┬─────┐
 │Exo│   Intron1     │Exo │        Intron2        │ Exo │
 │n1 │               │n2  │                       │ n3  │
 └──┴───────────────┴────┴───────────────────────┴─────┘
 ATG                                              TGA
```

Fig. 1. cDNA sequence and structure of the *Am-def* gene. (A) Nucleotide sequence and predicated amino acid sequences of prepro-defencin cDNA clones numbers at the left indicate positions of the nucleotide and amino acid. The ATG start codon is boxed. The star symbol above the nucleotide sequence indicates the translational stop signal (TAA). (B) Genomic structure of the *Am-def* gene revealed by PCR amplification from *Apis mellifera* genomic DNA. Numbers indicate the position in the genomic sequences.

(A)
```
                                          ctttgtgctgggaag
                                 10        20        30        40        50        60
  1 ATGAAGTTCTTTGTACTTTTCGCGATTCTCGTCGCTATCGTTTACGCATCTTGCGCAAGT
  1 M  K  F  F  V  L  F  A  I  L  V  A  I  V  Y  A  S  C  A  S

           70        80        90       100       110       120
 61 GTTTCTCAAGTTGCTTACGATGAACCGATTTACGATTTGAGGCAAATTGAGGAGGAAAAT
 21 V  S  Q  V  A  Y  D  E  P  I  Y  D  L  R  Q  I  E  E  E  N

          130       140       150       160       170       180
121 ATAGAACCAGATACGGAATTGATGGATTCCAACGAACCGCTACTGCTTCTACGACATCGAAGG
 41 I  E  P  D  T  E  L  M  D  S  N  E  P  L  L  L  R  H  R  R

          190       200       210       220       230       240
181 GTAACGTGCGACGTTTTATCATGGCAATCAAAATGGCGTGAGCATTTATCATTCAGCCTGC
 61 V  T  C  D  V  L  S  W  Q  S  K  W  L  S  I  Y  H  S  A  C

          250       260       270       280       290       300
241 GCTATCAGATGTTTAGCTAAACGACGTAAAGGTGGCAGATGCAAAAATGGCGTGTGCATC
 81 A  I  R  C  L  A  K  R  R  K  G  G  R  C  K  N  G  V  C  I

          310
301 TGTCGAAAGTGAaacgattcgaatcgaattcccgcggccgccatggcggccgggagcatg
101 C  R  K  *
    cgacgtcgggcccaattcgccc
```

(B)
```
  1   70              791  982                      1958 1079
 ┌──┬───────────────┬────┬───────────────────────┬─────┐
 │Exo│   Intron1     │Exo │        Intron2        │ Exo │
 │n1 │               │n2  │                       │ n3  │
 └──┴───────────────┴────┴───────────────────────┴─────┘
 ATG                                              TAA
```
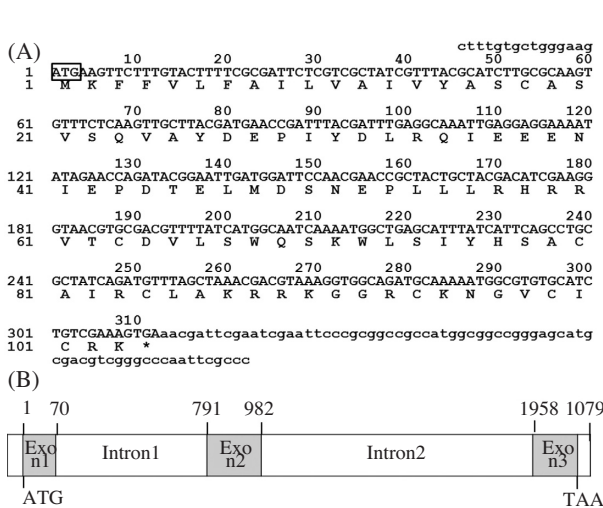
Fig. 2. cDNA sequence and structure of the *Am-royal* gene. (A) Nucleotide sequence and predicated amino acid sequences of prepro-royalisin cDNA clones numbers at the left indicate positions of the nucleotide and amino acid. The ATG start codon is boxed. The star symbol above the nucleotide sequence indicates the translational stop signal (TGA). (B) Genomic structure of the *Am-royal* gene revealed by PCR amplification from *Apis mellifera* genomic DNA. Numbers indicate the position in the genomic sequences.

(A)
```
                                          ttccaag
                                 10        20        30        40        50        60
  1 ATGAAATTGATCGTGTTAGTTGTCTTCGCGCTATTGCATACGTTTCTGCTCAAGCGGAA
  1 M  K  L  I  V  L  V  V  F  C  A  I  A  Y  V  S  A  Q  A  E

           70        80        90       100       110       120
 61 TTAGAACCTGAGGATTATATCCCGTCTCGTTTCCGACGACAGGAAAGAGGATCCATCGTC
 21 L  E  P  E  D  Y  I  P  S  R  F  R  R  Q  E  R  G  S  I  V

          130       140       150       160       170       180
121 ATTCAAGGAACTAAAGAGGGAAGAAATCGTCCATCCTTGGACATTGACTACAAACAACGT
 41 I  Q  G  T  K  E  G  R  N  R  P  S  L  D  I  D  Y  K  Q  R

          190       200       210       220       230       240
181 GTCTACGATAAGAATGGAATGACGGGAGATGCCTATGGTGGAGTAAACATTCGTCCTGGA
 61 V  Y  D  K  N  G  M  T  G  D  A  Y  G  G  V  N  I  R  P  G

          250       260       270       280       290       300
241 CAACCTGCTCGACAACATGCAGGCTTCGAGTTTGGGAAGGAATATAAGAATGGTTTCATC
 81 Q  P  A  R  Q  H  A  G  F  E  F  G  K  E  Y  K  N  G  F  I

          310       320       330       340       350       360
301 AGAGGACAAAGTGAAGTTCAACGTGGTCCTGGTGGCAGACTTTCACCATACGTTGGAATA
101 R  G  Q  S  E  V  Q  R  G  P  G  G  R  L  S  P  Y  V  G  I

          370       380
361 AATGGTGGATTTAGATTTTAAgatattttcaagtaacaattaacaataaaattacgattcg
121 N  G  G  F  R  F  *
    tatttacgtagca
```

(B)
```
  1    88       254        492               1024 1968
 ┌──┬────────┬──────────┬──────────────────┬─────┐
 │Exon1│Intron1│  Exon2  │     Intron2      │ Exo │
 │     │       │         │                  │ n3  │
 └──┴────────┴──────────┴──────────────────┴─────┘
                                            TAA
```

Fig. 3. cDNA sequence and structure of the *Am-hym* gene. (A) Nucleotide sequence and predicated amino acid sequences of prepro-hymenoptaecin cDNA clones numbers at the left indicate positions of the nucleotide and amino acid. The ATG start codon is boxed. The star symbol above the nucleotide sequence indicates the translational stop signal (TAA). (B) Genomic structure of the *Am-hym* gene revealed by PCR amplification from *Apis mellifera* genomic DNA. Numbers indicate the position in the genomic sequences.

(A)
```
                                         gatc
                                 10        20        30        40        50        60
tgcacactcgaggtctgtaccttcgacagttgcaaaaaaccaggaaaaaaagaatacgag
  1 ATGAAGGTAGTAATATTTATCTTCGCACTACTCGCCACGATATGCGCAGCGTTCGCATAT
  1 M  K  V  V  I  F  I  F  A  L  L  A  T  I  C  A  A  F  A  Y

           70        80        90       100       110       120
 61 GTACCATTACCAAATATACCACAACCAGGACGTCGTCCGTTTCCAACGTTTCCTGGTCAG
 21 V  P  L  P  N  I  P  Q  P  G  R  R  P  F  P  T  F  P  G  Q

          130       140       150       160
121 GGACCATTCAATCCGAAAATTAAATGGCCTCAAGGCTATTAAtcgtcaaatggacaaatc
 41 G  P  F  N  P  K  I  K  W  P  Q  G  Y  *
    ggaatacggaatactaaaatacttgcacggttcatgtgatgaatctgtggaatcgaattc
    ccgcggccgccatggcggccgggagcatgcgacgtcgggcccaattcgccc
```

Fig. 4. cDNA sequence and structure of the *Am-abae* gene. (A) Nucleotide sequence and predicated amino acid sequences of prepro-abaecin cDNA clones numbers at the left indicate positions of the nucleotide and amino acid. The ATG start codon is boxed. The star symbol above the nucleotide sequence indicates the translational stop signal (TAA).

α-helix domain (Casteels-Josson *et al*., 1994). Moreover, the ORF of *A. mellifera* defensin has one extra amino acid (Gly) at the C-terminus, which may be amidated as suggested in the mature antibacterial peptide of *A. mellifera* defensin (Casteels-Josson *et al*., 1994). The structural similarity suggests that *A. mellifera* defensin has antibacterial activity, which has activity against Gram positive and Gram-negative bacteria (Rees *et al*., 1997).

And, we cloned *A. mellifera* royalicin cDNA, a 397 bp long gene containing an ORF of 309 nucleotides capable of encoding a 103 amino acid polypeptide (Fig. 2A). The

(Bonmatin *et al*., 1992). In contrast to all other insect defensins, bee defensins have a 12 amino acid extension on their C-terminus, which encodes an additional C-terminal
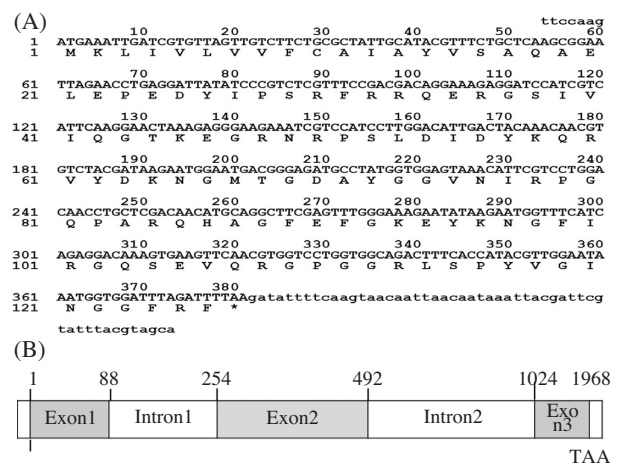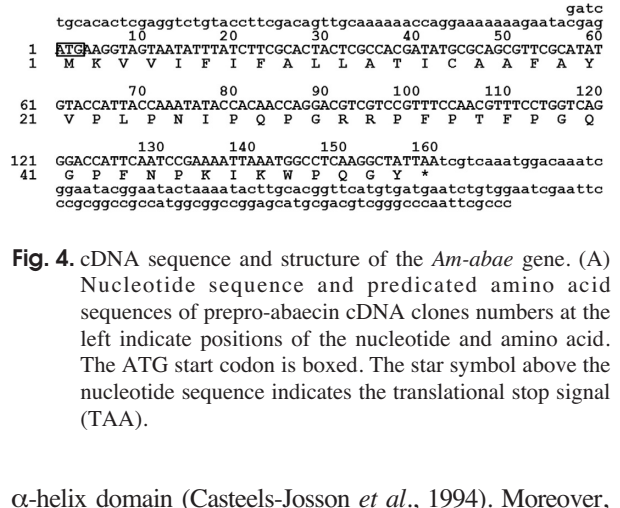
gene structure revealed that the *A. mellifera* royalicin gene spans 1079 bp and contains two introns and three exons (Fig. 2B). The royalicin has very similar function of antibacterial activity with defencin.

We also cloned the *A. mellifera* hymenoptaecin cDNA, a 440 bp sequence that contains an ORF of 378 nucleotides that encodes a 126 amino acid polypeptide (Fig. 3A). The gene structure revealed that the *A. mellifera* hymenoptaecin gene spans 1968 bp and contains two intron and three exons (Fig. 3B). The reported antibacterial effects of *A. mellifera* hymenoptaecin suggest that it inhibits the viability of Gram negative and Gram-positive bacteria and its lethal effects against *E. coli* are secondary to sequential permeabilization of the outer and inner membranes (Casteels *et al.*, 1993).

Finally, we also cloned *A. mellifera* abaecin cDNA according to results from searches of RT-PCR. The *A. mellifera* abecin cDNA is 354 bp long and contains an ORF of 157 nucleotides capable of encoding a 53 amino acid polypeptide. Comparisons of the amplicon size between the genomic DNA and cDNA sequences revealed that *A. mellifera* abaecin is an intron-less gene (Fig. 4). Comparative analysis suggests that the precursor molecule of *A. mellifera* abaecin is 53 amino acids long and contains only one possible processing site, resulting in a putative signal sequence of 19 amino acids (Casteels *et al.*, 1990; Casteels-Josson *et al.*, 1994; Rees *et al.*, 1997). The 39-residue abaecin is a proline-rich antibacterial peptide with activity against both Gram-negative and Gram-positive bacteria (Rees *et al.*, 1997).

Transcriptional expression profile of antibacterial peptide genes

The validity and quality of the PCR and northern hybridization results were further evaluated by examining the expression pattern of a selection of putative genes, based on their predicted involvement in mite infestation, by PCR and Northern hybridization (for detection of antimicrobial peptides). Differential expressions of four cDNAs from *A. mellifera* (for royalicin, defensin, abaecin, hymenopteacin) were compared between parasitized and normal bees (Fig. 5). The expressions of genes encoding four antimicrobial peptides (royalicin, defensin, abaecin
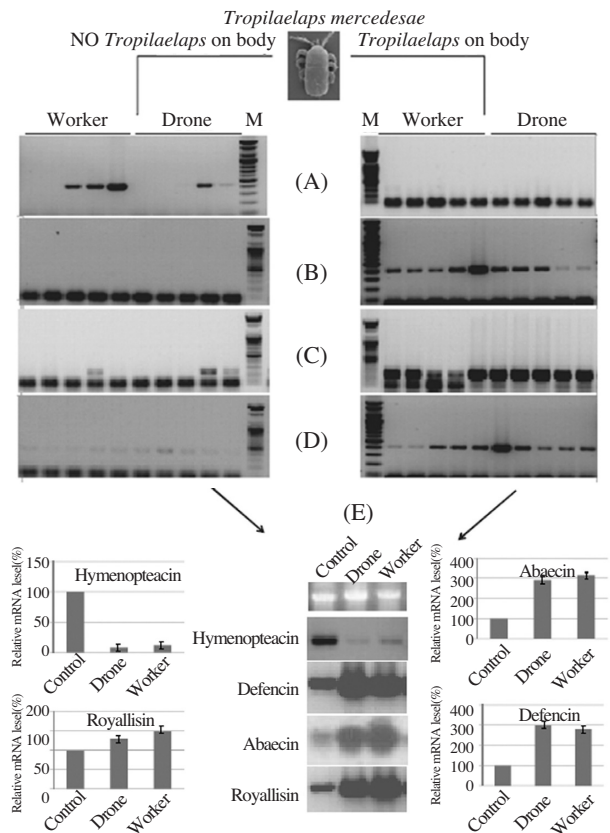

Fig. 5. Sex distinction of the antimicrobial gene mRNA by RT-PCR and Northern blot hybridization. Total RNA was isolated from the female and male bee, respectively. Induction of Abacin (A), Hymenopteacin (B), Defencin (C), and Royallisin (D) in female and male bee by and *T. clareae*. The template *cDNA* were diluted by nuclease free water. Transcriptional expression profile of antimicrobial peptide genes of sex specific induction by *T. clareae* (E). The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis, transferred onto a nylon membrane, and hybridized with radiolabelled Abacin, Hymenopteacin, Defencin, and Royallisin cDNA. Ethidium bromide stain and Northern blot of rRNA are shown as a loading control.

and, hymenopteacin) were used as markers to measure the difference in the immune response between parasitized and normal bees (Fig. 3). We found that the transcriptional induction of three antibacterial peptide genes in the *A. mellifera* larvae significantly increased by *Tropilaelaps* mite. The transcriptional expression profile of antimicrobial peptide genes were expressed following sex specific induction or suppression by *Tropilaelaps* mites. The drone larva was not expression of abaecin, defensin and royalicin more than worker larva by *Tropilaelaps* mite infestations. However, hymenopteacin is non up-regulated following by *Tropilaelaps* mite infestation compare with control

(normal worker larva). Interestingly, hymenopteacin gene expression was suppressed in *A. mellifera* worker and drone by *Tropilaelaps* mite (Fig. 5(E)). We demonstrated that this ectoparasite could immunosuppress its invertebrate host with the evidence that parasitization significantly suppressed expression of some immunityrelated antibacterial genes. Ticks are known to immunosuppress their vertebrate hosts (Xiaolong and CoxFoster, 2005), so our finding indicates that immunosuppression of hosts may be a common antibacterial peptide in the interaction and coevolution between ectoparasites and their vertebrate and invertebrate hosts. The expression of the genes encoding the three antibacterial peptides was significantly up-regulated by *Tropilaelaps* mite infestation. The worker bee was expressed antibacterial peptides more than drone bee. This work demonstrated that *Tropilaelaps* mite infestation suppressed hymenopteacin gene expression in honey bees. Not only the worker bees but also drone bees had suppressed hymenopteacin gene expression due to the ectoparasitization.

ACKNOWLEDGEMENTS

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