

## Expression of melittin gene in the venom gland of the Chinese honeybee, *Apis cerana cerana*<sup>1</sup>

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**Abstract** – Melittin is the principal component of bee venom. *Melittin* in *Apis cerana* (*Ac-melt*) is a single copy gene. A full length *Ac-melt* cDNA is 389 bp, with a single 191 bp intron in the genome. Its mRNA level was high during the first week of adult life and low during the rest of adult life. Melittin or its precursor could not be detected in the pupal stage. Melittin level increased rapidly to its maximum (about 95 µg per worker bee) during the first 8–10 days of adult age, and remained constant for about 20 days before starting to decline slowly. The expression of melittin is regulated at the transcriptional level.

**Melittin / gene expression / venom gland / *Apis cerana***

### 1. INTRODUCTION

Melittin is a major protein component of bee venom, comprising 50% of its dry weight in *Apis mellifera* L. (Habermann, 1972). It was first isolated from the venom of *A. mellifera* (Neumann et al., 1952). The homologous genes from venom of other species of the same genus were successively cloned, including *Apis cerana* (Q8LW54, P59260), *Apis dorsata* (Kreil, 1975) and *Apis florae* (Kreil, 1973). The amino acid sequences of melittin from different honeybee species are rather conservative, sharing 93% sequence identities. Recently, we cloned the *melittin* gene from other Hymenoptera species, including *Vespula maculifrons*, *Polistes hebraeus* and *Polistes* sp. (Shi et al., 2003a). To date, no homologue of melittin has been isolated from other insect species outside Hymenoptera.

The mRNA of *melittin* in the venom gland of *A. mellifera* is first translated into a pre-

promelittin of 70 amino acid residues, consisting of a signal peptide (21 residues), a proregion (22 residues) and a mature melittin (26 residues). Melittin has a wide range of pharmacological functions. It can interact strongly with lipid bilayer membranes and disrupt them (Papo and Shai, 2003; Higashino et al., 2001; Sessa et al., 1969). Other biological functions such as hemolysis (Hinch et al., 1996), anti-tumors (Winder et al., 1998), protection against irradiation (Ginsberg et al., 1968) and antibacterial properties (Fennell et al., 1968) are also well documented. Furthermore, melittin inhibits the activities of Na<sup>+</sup>-ATPase, K<sup>+</sup>-ATPase and Ca<sup>+</sup>-ATPase (Cuppoletti and Abbott, 1990; Raynor et al., 1991) using different mechanisms (Murtazina et al., 1997). Melittin can also significantly increase inward rectifier K<sup>+</sup> current in a concentration dependent manner, which contributes to shortening the action potential duration (Zhang et al., 2000). Melittin thus has many prospects for application in the

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medical field. Although melittin is widely used in medical and biological research, many of its important characteristics such as the gene copy number, transcription and regulation are unclear.

*A. cerana* is an economically important bee species, with about two million colonies being bred in China (Chen, 2001). But no venom utilization projects have been implemented yet, as these would require basic biological information about venom secretion. In our earlier studies, we have cloned the coding region of melittin cDNA by RT-PCR from the venom glands of *Apis cerana cerana* (Shi et al., 2003b), and expressed it in *E. coli*. (Shi et al., 2004). In this study, we demonstrated that it is a single gene coding for *A. cerana cerana* melittin. High expression of *Ac-melt* mainly occurred during the first week of adult stage. Its expression is regulated at the transcriptional level.

## 2. MATERIALS AND METHODS

### 2.1. Bees

Larvae, pupae and adults were collected from *A. cerana* colonies bred at the Experimental Apiary of Zhejiang University, Hangzhou, China. The developmental stages of workers were classified according to the criteria proposed by Rachinsky et al. (1990) and Michelette and Soares (1993). Bees of known ages were obtained by marking the newly emerged bees from a frame of capped broods kept in an incubator at 34 °C and about 80% relative humidity (Piulachs et al., 2003) and the bees were returned to the hive and collected when required.

### 2.2. Preparation of polyclonal antiserum

For adequate immunization of rabbits with melittin, the peptide was coupled with albumin. The conjugation was done using EDAC (1-Ethyl-3-Carbodiimide, Sigma). Briefly, 500 µL EDAC solution (5 mg/mL in 0.9% NaCl) and 2 mL albumin solution (1.25 mg/mL in 0.9% NaCl) were blended completely, then 1 mL melittin (Sigma, HPLC) solution (2 mg/mL in 0.9% NaCl) was added and agitated to ensure complete coupling; 0.5 mL of the coupled melittin was mixed thoroughly with 0.5 mL of Freund's complete adjuvant or Freund's incomplete adjuvant (Freund, 1947) and used for immunizing the rabbits. Albino rabbits weighing between 1.5 and 2.0 kg were used. Antiserum was collected by cen-

trifugation, decplemented at 56 °C for 30 min and stored at -70 °C after adding sodium azide to a final concentration of 0.2%.

### 2.3. Construction and sequencing of cDNA library

The honeybees were first anesthetized on ice and the venom glands were then dissected under a binocular microscope and were transferred into Trizol reagent. Total RNA was isolated from the venom glands according to the protocol recommended by the supplier (Invitrogen). mRNA was extracted using Poly (A) Quick® mRNA Isolation Kit (Stratagene). About 5 µg of venom gland mRNA was used to construct the cDNA library, using the ZAP-cDNA® Gigapack III Gold Cloning Kit (Stratagene). The library was titred and checked by PCR using the universal primers, M13F and M13R.

Seventy clones randomly selected were sequenced at Shanghai Sangon Bio-technology Corporation, using the dideoxynucleotide chain termination method in an ABI377 automated sequencer. The M13<sup>-</sup>, M13<sup>+</sup> universal primers were used for sequencing. The cDNA sequence reported here was submitted to GenBank with the accession number AF487907.

### 2.4. Amplification of genomic DNA corresponding to *Ac-melt* region

Genomic DNA was isolated from female worker bees according to Beye and Raeder (1993). Genomic DNA fragment encoding the melittin was amplified by PCR, using the primers based on the *Ac-melt* cDNA (Fig.1) follows:

Forward 1: 5'-GAATTAACAGCATTAACACAG-3',

Reverse 1: 5'- GATTTGATGAATGAAAATT-TATATTATC -3'.

Amplification conditions were 3 min at 94 °C, followed by 30 cycles of 50 s at 94 °C, 45 s at 52 °C and 50 s at 72 °C, and final extension for 10 min at 72 °C. The amplified fragments were purified using QIAquick PCR Purification Kit (Qiagen), cloned into the pGEM T easy Vector System (Promega, USA) and sequenced at Shanghai Sangon Bio-technology Corporation. The genomic DNA sequence reported here was submitted to EMBL database with the accession number AJ786346.

### 2.5. Southern blotting

*Ac-melt* coding region amplified from the cloned cDNA using the primer Forward 2: 5'- ATGAAAT-TCTTGGTCAACG-3' and Reverse 2: CTAAC-CCTGTTGCCTCTTAC was Dig-labeled using the

DIG High Prime DNA Labeling and Detection Starter Kit II (Roche) and used as hybridization probe. Honeybee genomic DNA digested with six restriction enzymes (*EcoRI*, *XhoI*, *PstI*, *Sall*, *XbaI* and *HindIII*, Takara) was blotted onto the Hybond-N+ membranes (Amersham) as described (Sambrook and Russeau, 2001). DNA was UV crosslinked with the membranes (TL-2000, UVP). Hybridization and detection were performed using the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche).

## 2.6. Northern blotting

Total RNA from venom glands of thirty bees of each required age was isolated with Trizol reagent (Invitrogen) according to the protocol recommended by the supplier. About 4 µg RNA samples (two bees equivalent) were separated on formaldehyde gels and then blotted onto the Hybond-N+ membranes (Amersham) for 8 h with 0.05 M NaOH as described (Sambrook and Russeau, 2001). The transferred RNA was crosslinked with the membranes in a UV-crosslinker (TL-2000, UVP). Prehybridization, hybridization and detection were performed according to manufacturer's instructions (Roche). The Dig-labeled probe (see Sect. 2.5) was also used here.

## 2.7. Venom protein preparation

Bees of known age were collected from the labeled workers (described in Sect. 2.1). The venom sac with venom glands were dissected under a microscope, homogenized by sonication in 200 µL PBS (pH 7.4), and centrifuged for 5 min at 14 000 g. The supernatant was collected and stored in -20 °C and used as the venom extracts. Each venom sample was prepared from one worker.

## 2.8. Enzyme-linked immunoassays (ELISA)

The 96-well microtiter plates with 5 µl of diluted venom extracts (five times) and 95 µl coat buffer (0.05M Na<sub>2</sub>CO<sub>3</sub>-NaHCO<sub>3</sub> buffer, pH 9.6) were incubated overnight at 4 °C. The plates were washed and then blocked with 200 µL of BSA solution (0.1% in PBS) for 1 h at room temperature. The rabbit anti-melittin polyclonal antiserum (diluted 1:3000 in PBST/BSA) was added and incubated for 1 h at room temperature. Horse Radish Peroxidase (HRP) conjugated goat anti-rabbit IgG (Sigma; diluted 1:1000 in PBS, containing 10 mg/mL BSA) was added and incubated for 1 h at room temperature. The plate was washed and then incubated with the substrate (O-phenylenediamine) for 30 min at room temperature. The reaction was terminated by adding 50 µL of 2M H<sub>2</sub>SO<sub>4</sub>. The absorbance values were read at

492 nm in a Microplate Reader (µQuant, Bio-TEK). Pure melittin (Sigma, HPLC purity) was used to construct a standard curve. The melittin concentrations in other wells were calculated according to the standard curve. Eight repeats for each age were performed and their average value and standard deviation were calculated.

## 2.9. Western blotting

The venom extract was fractioned on 15% tris-glycine SDS-polyacrylamide gels, transferred to nitrocellulose membranes using a Bio-Rad Trans-blot (Bio-Rad) as described in the protocol and immunoblotted using the anti-melittin polyclonal antiserum (1:3000) and Horse Radish Peroxidase (HRP) conjugated second antibody. The immunoreactive proteins were visualized in color development solution (2 mg diaminobenzidine in 10 mL 0.1M PBS, pH 6.4, 60 µL 10% H<sub>2</sub>O<sub>2</sub>).

## 3. RESULTS

### 3.1. cDNA sequence

Seventy clones from the cDNA library were randomly sequenced and compared with homologues in the GenBank/EMBL nr-database using advanced Blast Search Server (Altschul et al., 1997). Among these, five clones contained *Ac-melt* cDNA fragment. The full-length of *Ac-melt* cDNA was 389 bp, consisting of a 47 bp 5'-UTR (untranslated region), a 213 bp *Ac-melt* coding region and a 129 bp 3'-UTR (GenBank accession number: AF487907). The nucleotide sequence around the initial codon ATG was in agreement with the Kozak's sequence (Kozak, 2002) (Fig. 1). Analysis of the nucleotide sequence and their deduced amino acid sequence of the coding region confirmed the report of Shi et al. (2003b).

### 3.2. *Ac-melt* gene structure

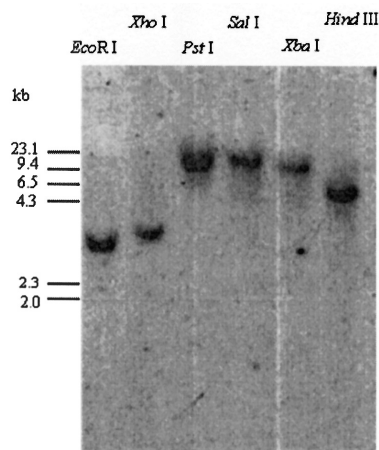
In order to know the genomic structure of *melittin* gene, the corresponding region of the genomic DNA was amplified by PCR using the primers designed based on the *Ac-melt* cDNA sequence. The amplified fragment from the genomic DNA of *A. cerana* was 557 bp in length. Comparison of the DNA sequence with the cDNA sequence revealed that the genomic sequence of *Ac-melt* consisted of two exons

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GAATTAACAGCATTAAACACAGGAAGGAAGGATCGGAGAAATC ATG AAA TTC TTG GTC AAC 65
M K F L V N
GTT GCC CTT GTT TTT ATG GTT GTA TAC ATT TCT TTC ATC TAT GCG GCC CCT GAA 119
V A L V F M V V Y I S F I Y A A P E
CCA GAA CCG GCA CCG GAG GCA GAG GCA GAG GCA GAC GCG GAG GCA^GAT CCG GAA 173
P E P A P E A E A E A D A E A D P E
GCA GGA ATT GGA GCA GTT CTC AAA GTA TTA ACC ACA GGA TTG CCT GCC CTT ATA 227
A G I G A V L K V L T T G L P A L I
GGT TGG ATT AAA CGT AAG AGG CAA CAG GGT TAG TCGATCCACGATGCCGATTTACGACCT 287
G W I K R K R Q Q G
ATCGAATGGTCGAAAAATTTGTGTAACCTTGAATTAACATGTATACATGTTGATAATATAAATTTTTTCATTC 358
ATCAAATCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 389

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**Figure 1.** Full cDNA sequence and deduced amino acid sequence of prepromelittin from *Apis cerana* showing the coding region of prepromelittin (bold). The nucleotides that corresponded to the Kozak's sequence are underlined.  $\Delta$  indicates the site where the 191 bp intron is inserted. The mature melittin sequence is set in italics.



**Figure 2.** Southern blot analysis using a Dig-labeled *Ac-melt* cDNA as the probe. DNA samples digested with different restriction enzymes are indicated above the corresponding lanes. Position of DNA molecular weight standards (in kilobases) are shown on the left.

with 164 bp and 202 bp respectively (Fig. 1), separated by an intron of 191 bp (EMBL accession number: AJ786346), which contained the consensus GT-AG borders (Breathnach and Chambon, 1981). The intron sequence from *A. cerana* was not homologous to that from *A. mellifera*, which is 1200 bp (AADG02008070) in length.

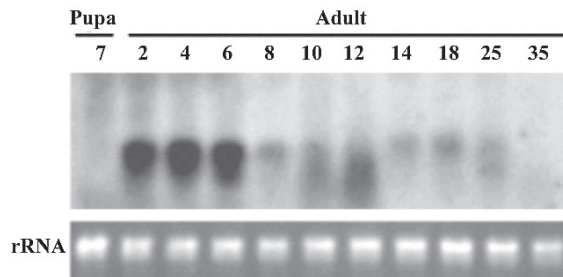
### 3.3. Gene copy number

Melittin was responsible for about 50% of the total dry venom proteins. It was the most abundantly expressed component in bee venom. In order to explore the relationship between gene copy number and the high expression of melittin, Southern blotting analysis of the genomic DNA of worker bee was performed. The results showed that only one discrete band

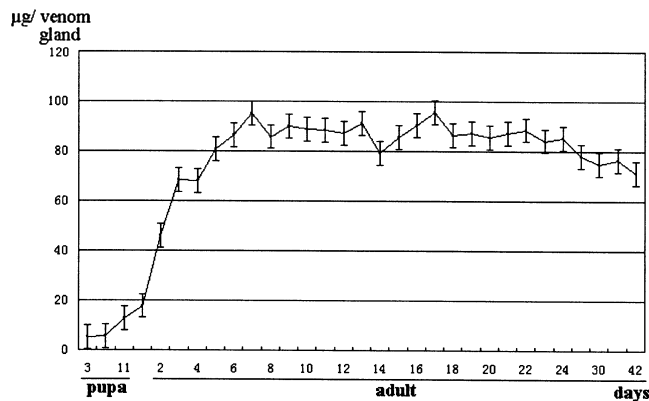
was detected in each of the 6 restriction enzyme (*EcoRI*, *XhoI*, *PstI*, *SalI*, *XbaI* and *HindIII*) digested-DNA samples, and the size of corresponding bands ranged from 3 kb to about 20 kb (Fig. 2). The result suggests that *Ac-melt* is a single copy gene in the genome.

### 3.4. Age-related expression

In order to investigate the transcriptional activity of *Ac-melt*, Northern blot was performed using a Dig-labeled *Ac-melt* cDNA as the probe to detect the mRNA level in total RNA prepared from the venom glands of different ages (Fig. 3). The transcription of approximate 400 bp corresponding to the predicted size of the sequenced cDNA was detected. Northern blot analysis showed that *Ac-melt* mRNA was not detectable in the pupal



**Figure 3.** Northern blot analysis using a Dig-labeled *Ac-melt* cDNA as the probe. Time points (development stage) are indicated above the corresponding lanes. Position of RNA molecular weight standards are shown on the left. RNA samples (4  $\mu$ g each) were taken from a pool of 30 bees per age cohort. The portion of the gel containing rRNA was stained with ethidium bromide to show equivalent sample loading.



**Figure 4.** Melittin or its precursor content in venom reservoir of different development stage of *A. cerana* as determined by ELISA. The content in bee pupae was at a low level, which increased sharply during the first week of adult life to their maximum (about 95  $\mu$ g per honeybee), then the content was maintained at a relatively steady level for about four weeks. For older honeybees, melittin content (about 70  $\mu$ g per honeybee) showed a slight decrease from their maximum.

stage (lane 1), but was abundant in adults less than one week old (lane 2, 3, 4), and abruptly declined to a very low level at day 8 (Fig. 3).

Melittin content detected by ELISA revealed that melittin or its precursor was barely detectable in the venom reservoir at the pupal stage. It was first detected at a low level in newly eclosed adult bees, and then quickly increased to its maximal level (about 95  $\mu$ g per individual) in the 8–10 days old adult bees. Melittin or its precursor was maintained at the maximum level for about 20 days followed by a slight decline to the level of about 70  $\mu$ g per individual and maintained at that level until death (Fig. 4).

These measurements were confirmed by Western blot analysis of melittin in venom sacs of different ages, the 7 days old pupae and the 2, 8, 12, 18, 22, 30 and 42 days old adults. Melittin was undetectable in pupal stage, only a weak signal was detected on the 2nd day of

adult age, and a signal of similar strength was continuously detected from the 8 days old adult to the rest of adult life.

#### 4. DISCUSSION

Gene expression can be regulated at several levels, including the gene copy number, transcription and translation. Southern blotting results showed that *Ac-melt* is a single copy in the genome of the Chinese honeybee. The ongoing honeybee genomic project also showed that only a single copy of *melt* gene was detected in the incompletely sequenced genome of the European honeybee, *A. mellifera* (AADG-02008070). Northern blot analysis showed that a high mRNA level of *Ac-melt* was maintained during the first week of adult life, followed by an abrupt decrease during the rest of adult life. Such results suggest that the main transcriptional activity of *Ac-melt* occurs during the first

week after eclosion. Synthesis of melittin or its precursor in the first week of the adult stage, as measured by ELISA and Western blot, was proportional with *Ac-melt* mRNA levels, suggesting that the transcribed mRNA was quickly translated. During the later adult life much less *Ac-melt* mRNA was detected, which apparently served to maintain the melittin protein level. The high level of mRNA during the first week of adult life can be due to high transcriptional efficiency and/or stability of mRNA (longevity). High activity of melittin transcription can be deduced from the high level of mRNA found shortly after eclosion. Two elements, TATAAA and CACAATCT, located 51 bp and 153 bp upstream of the transcription initiation site of the *melittin* gene of *A. mellifera* (AADG02008070) presumably belong to regulatory elements responsible for transcriptional control.

Results of ELISA showed that melittin was at low levels in the pupal stage, but increased sharply during the first week of adult life and were maintained a steady level for about 20 days. This expression pattern is not consistent with the results from *A. mellifera* reported by Owen and Pfaff (1995) based on the hemolytic activity of melittin, which showed a small melittin increase in the first week of adult life and a rapid increase in the second and third weeks of adult life. The difference in expression pattern could be methodological and related to the complicate post-translational processing of melittin. The primary translational product of *melittin* mRNA contains a signal peptide which targets the peptide to the exocytotic pathway. This signal peptide is removed after finishing the transport across the membrane. Once melittin precursor-promelittin (Kreil and Bachmayer, 1971) enters the venom reservoir it is further processed by dipeptidylpeptidase IV to physiologically active melittin (Kreil et al., 1980). In fact, the promelittin in the venom reservoir is gradually processed into melittin as the bees get older (Bachmayer et al., 1972). Because our polyclonal antiserum against melittin might cross react with promelittin, melittin measurements in our experiment by ELISA represent the total translation products including melittin and its precursor, while in Owen and Pfaff's experiments, only mature melittin was involved in hemolytic function and therefore measured.

Seasonal factors were not studied during our experiments, but must be concerned when measuring the venom content (Owen and Pfaff, 1995; Owen and Soley, 1988; Owen and Bridges, 1982). Samples used here were collected over a short time span from May to early June and might not have been affected by seasonal changes. Data from Owen and Pfaff show a reduction in the amount of melittin in the venom system of *A. mellifera* from June to August. Similar seasonal change of venom content in *A. cerana* is possible. Thus, bee samples collected in the highest melittin content season need further investigation.

Compared with *A. mellifera*, melittin level in *A. cerana* was only 70–95 µg, much less than the value of 250–500 µg in *A. mellifera*. Furthermore, the total venom content in *A. cerana* was only 43 µg/bee, much less than the value of 138–187 µg/bee in *A. mellifera* (Schmidt, 1995). The lower venom content in *A. cerana* may have a biological significance. The Chinese honeybees maintain small colonies and are prone to flee when confronted with adverse circumstances, which is a different survival strategy than that of *A. mellifera*. Thus a lower melittin and venom content in *A. cerana* seems reasonable.

As for the decreased melittin content in bees over 25 day old (Fig. 4), we speculate that it might be decomposed and reutilized to meet metabolic requirements, which could be in the interest of the whole colony. Reallocation of protein substrate has been reported in hypopharyngeal gland cells to utilize the vitellogenin secreted by the fat body for synthesizing MRJP (major royal jelly protein) in worker honeybees (Amdam et al., 2003). Thus a similar protein reallocating and metabolizing process in honeybees venom system was conceivable, which needs further work to confirm.

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**Résumé – Expression du gène de la mélittine dans la glande à venin de l’Abeille chinoise *Apis cerana cerana*.** La mélittine, le principal constituant du venin d’abeille, possède toute une gamme de fonctions pharmacologiques. *Apis cerana cerana* est l’une des espèces d’abeilles d’importance économique en Chine. La majeure partie de la recherche scientifique consacrée au venin d’abeille a été faite sur le venin de l’Abeille européenne, *Apis mellifera* L. et seules quelques études existent pour les autres espèces, dont *Apis cerana* Fabricius. En outre la connaissance de la biologie de la sécrétion du venin, que ce soit chez *A. mellifera* ou *A. cerana*, est très restreinte. Le but de cette étude est d’étudier l’expression protéique du gène *mélittine* par les analyses suivantes : estimation du nombre de copies du gène dans l’ADN génomique, abondance de l’ARNm de la mélittine transcrit à différents stades de développement et modifications du contenu protéique du réservoir à venin.

La longueur totale de l’ADNc de *Ac-melt* est de 389 pb avec un seul intron de 191 pb dans le génome (Fig. 1). Il n’y a qu’une seule copie du gène *mélittine* (*Ac-melt*) chez *A. cerana* (Fig. 2). Pendant la première semaine du stade adulte son niveau d’ARNm est élevé et il est faible durant le reste de la vie adulte (Fig. 3). La mélittine, ni son précurseur, n’ont pu être détectés au stade nymphal. Les niveaux de protéine de mélittine augmentent rapidement jusqu’à un maximum d’environ 95 µg/ouvrière au cours des 8 à 10 premiers jours de la vie adulte et restent constants durant 20 jours avant de commencer à décroître doucement (Fig. 4). Le niveau élevé d’ARNm pendant la première semaine de la vie adulte peut être dû à l’activité élevée de transcription et/ou à la stabilité de l’ARNm (longévité). On a pu déduire une activité élevée de transcription de la mélittine à partir du niveau élevé d’ARNm détecté juste après l’éclosion. La synthèse de la mélittine ou de son précurseur au cours de la première semaine de la vie adulte, mesurée par les techniques d’ELISA et de Western blot, a été proportionnelle aux niveaux d’ARN de *Ac-melt*, suggérant que l’ARNm transcrit est rapidement traduit. On pense donc que l’expression de la mélittine est principalement régulée au niveau de la transcription.

La différence observée dans le profil d’expression de la mélittine entre notre étude et celle de Owen et Pfaff (1995) pourrait être d’ordre méthodologique et liée au processus compliqué de post-traduction de la mélittine. Les teneurs en mélittine et en venin total d’*A. cerana* sont inférieures à celles observées chez *A. mellifera*, ce qui peut revêtir une signification biologique. Les ouvrières d’abeilles n’utilisent leur venin que pour défendre la colonie contre les prédateurs et les intrus, gros mammifères et autres vertébrés notamment. Les abeilles chinoises constituent de petites colonies et ont tendance à prendre la fuite lorsqu’elles sont confrontées à des conditions adverses. Cette stratégie de survie est différente de celle des abeilles européennes, qui défendent féroce ment leurs grosses colonies lorsqu’elles sont menacées.

***Apis cerana* / glande à venin / mélittine / expression génique**

**Zusammenfassung – Expression des Melittin-Gens in der Giftdrüse der chinesischen Honigbiene, *Apis cerana cerana*.** Melittin ist eine der wichtigsten Komponenten im Bienengift mit einer Reihe pharmakologischer Funktionen. *Apis cerana cerana* besitzt eine grosse ökonomische Bedeutung in China. Die meisten wissenschaftlichen Studien über Bienengift wurde an der europäischen Biene *Apis mellifera* durchgeführt, und nur wenige Studien waren dem Gift anderer Bienenarten gewidmet, hier insbesondere *A. cerana*. Insbesondere die Kenntnisse über die Biologie der Giftfreisetzung sind sowohl bei *A. mellifera* als auch bei *A. cerana* noch sehr beschränkt. Das Ziel der gegenwärtigen Studie war die Untersuchung der Expression von Proteinkomponenten des Gifts mittels folgender Analysen: eine Abschätzung der Kopienzahl des für Melittin kodierenden Gens, die Bestimmung der Abundanz der Melittin-Transkripte in verschiedenen Entwicklungsstadien und die Analyse der Veränderung im Gehalt der translatierten Peptide im Giftdrüsenreservoir.

Die komplette *Ac-melt* cDNA umfasst 389 bp, mit einem einzigen 191 bp Intron im Genom (Abb. 1). *Apis cerana* (*Ac-melt*) Melittin liegt nur in einer einzigen Kopie vor (Abb. 2). Die entsprechenden mRNA Gehalte sind in der ersten Woche nach dem Schlupf der Bienen vergleichsweise hoch und sinken dann für den Rest des adulten Lebens auf ein niedriges Niveau ab (Abb. 3). In der Puppenphase konnte weder Melittin-Protein noch seine Vorstufe gefunden werden. Die Melittin-Proteingehalte steigen jedoch innerhalb der ersten 8-10 Tage der Adultphase rasch an und erreichen ein Maximum von ca. 95 µg pro Arbeiterin. Danach bleiben die Melittin-Proteingehalte für etwa 20 Tage lang konstant und sinken erst dann langsam ab (Abb. 4). Der hohe mRNA-Gehalt während der ersten Woche der Adultphase kann möglicherweise auf eine hohe Transkriptionsaktivität und/oder hohe mRNA-Stabilität zurückgeführt werden. Auf eine hohe Melittin-Transkriptionsrate schliessen wir aufgrund der hohen mRNA-Gehalte, die bald nach der Eclosion zu finden waren. Die ELISA und Western Blot-Ergebnisse zeigten, dass sich die Syntheseraten von Melittin oder seiner Vorstufe in der ersten Woche der Adultphase proportional zum *Ac-melt*-Gehalt verhalten, was darauf hinweist, dass die transkribierte mRNA schnell translatiert wird. Die Expression von Melittin scheint deshalb primär über die Transkriptionsrate gesteuert zu sein.

Die Unterschiede im Muster der Melittin-Expression unserer Studie zu der von Owen und Pfaff (1995) könnte methodisch bedingt und auf die komplizierte posttranslationale Prozessierung von Melittin zurückzuführen sein. Sowohl der Melittin- als auch der Gesamtgift-Gehalt war bei *A. cerana* niedriger als bei *A. mellifera*, was eine biologische Bedeutung haben könnte. Arbeiterinnen benutzen ihr Gift nur

zur Kolonieverteidigung gegen Räuber und andere Eindringlinge, in der Regel grosse Säugetiere und andere Vertebraten. Die chinesische Honigbiene hingegen bildet nur relativ kleine Völker, die angesichts widriger Umstände dazu tendieren zu fliehen. Diese Überlebensstrategie unterscheidet sich von der der europäischen Bienen, die ihre Kolonien heftig gegen Bedrohungen verteidigen.

#### Melittin / Expression / Giftdrüse / *Apis cerana*

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