Observation of Defensive Behaviour and Characterization of *sting-2* gene and EST1 Apis cerana in West Java, Indonesia

Rika Raffiudin[*], Tri Atmowidi, Taruni Sri Prawasti, Utut Widyastuti, Achmad Farajallah, Apriani, Ramsi Eka Putra, Nden Rissa Hadikusumah, Mita Aditama, Ruth Martha Winnie

Department of Biology, Faculty of Mathematics and Natural Sciences, Darmaga Campus, Bogor Agricultural University, Bogor 16680, Indonesia.

[*]Correspondence author: rika_r@cbn.net.id

Abstract

Apis cerana is the native honey bee of Asia, keep by people in the rural or forest margin area. The main problem to keep *A. cerana* is its agressive behavior and tending to abscond. Hence, this study objective was to observe the defensive behaviour of *A. cerana* in two lowlands areas, Parung Panjang (PP) and Lebak (LB) West Java, Indonesia. The observations were carried out for six times in a day (6 am-4 pm). We used eight colonies of *A. cerana* for each location studied. Twenty μl of isopentyl acetate (IPA) as the alarm pheromone was pipetted to the black cotton ball hanging 10 cm from the bee hive entrance. Result showed that there were five sequentials behaviour of *A. cerana* defensive behaviour in respond to the stimulus i.e. alert with nestmate recruitment, fly surrounding the stimulus, hanging on the stimulus, and balling behaviour at the nest entrance and balling at the stimulus. *A. cerana* in LB tend to form balling directly around the stimulus (attack the stimulus); however *A. cerana* in PP tend to form ball in front of their nest. Hence, *A. cerana* from LB showed more aggressive behaviour compared to that of bees from PP.

Introduction

Beekeeping In Indonesia comprises of two honey bee species, those are the native Asia honey bee *Apis cerana* and the imported European honey bee, *Apis mellifera*. Currently, *Apis mellifera* is more attractive to be used as the honey bee in the beekeeping because of the high honey production. However, *Apis mellifera* has several drawbacks, such as its susceptibility to the Asian bee *Varrhoa* mites pest. *Apis cerana* is the native honey bee in Asia, hence rural or forest margin community could keep the bees as one of their income source. As a native bee, this species is adaptive to the *Varrhoa* mite.

The main problem to keep A. cerana is the bees perform aggressive behavior. In A. mellifera, there are colonies perform defensivie behavior as well as gentle colony. Both gentle and defensive behavior have been extensively studied in A. mellifera. The defensive *A.mellifera* showed their behavior while defense the nest. *A. mellifera* colony defense comprises of two distinct behaviours: guardians and stinging (Hunt 1998). For *A. mellifera*, guards are workers of mean age of 15 days that patrol the entrance of the hive in search of animal that approaches the nest. Stinging are *A. mellifera* bees of the mean age of 19 days that response to major disturbances of the colony by flying out, stinging and pursuing intruders. However, there is a lack study for *A. cerana* behavior and genetics, including the defensive behavior. So far, Indonesia beekeeper mentioned that *A. cerana* as defensive bees. There is no information of variation of sting and guard behavior. Since A. cerana is suitable for Indonesia rural bee keeper, we need to improve the *A. cerana* management; one way is to find the less aggressive defensive *A. cerana*. To achive the goal to obtain a gentle non-aggressive *A. cerana* race need a multiyear researches. This proposed research is the first year and is a basic research. The output of the research first is the type of defensive behavior in

A. cerana. By selecting that non-defensive type, we can obtain the better perform A. cerana in beekeeping management.

In this study we want to examine the mechanism of defensive of *A. cerana* in lowland such as in Parung Panjang (PP) and Lebak (LB), Indonesia. For the guardian behavior, questions were: how many guardians bee perform in each nest? How long do they guard? What time do they guard? Is there any gentle *Apis cerana* in the population? Here based on this study, we found that *A. cerana* from PP showed less aggressive at the five sequential steps of defensive behaviour compare to *A. cerana* from LB.

MATERIALS AND METHOD

Metode

Research station

Eight colonies of feral *A. cerana* were collected from local area i.e. from Parung Panjang (PP) and Lebak (LB), West Java, Indonesia. Those colonies were collected at the 110 m and 105 asl, for PP and LB, respectively. *A. cerana* was kept in the hive box each for 40 x 20 x 30 cm. Each colony consisted of four combs. Prior to the experiment, the colonies were kept in the hive box at least for two weeks for adaptation purpose.

DNA was extracted by using CTAB extraction method. Putative genes and EST1 QTL *sting-2* was amplified by primer designed based on cDNA of *Apis mellifera* (Lobo *et al.* 2003). BLASTN and MegaBLAST were performed to obtain the homology value with those of Genbank published data.



***A. cerana* Defensive behaviour (Abrol 2006)**

Observations of A. cerana behavior

Observations were carried out in August 2007. Two parts of behavior observations: (1) A. cerana flying activity, i.e. numbers of *A. cerana* flying out from the nest were counted in 5 minutes; (2) defensive behavior (Wojke 1992). Those behaviours were carried out to examine the correlation between the flying activity and the defensive behavior. Both were observed at six times experiments, i.e. 06.00, 08.00, 10.00, 12.00, 14.00, dan 16.00 (two replicates). In observations of *A. cerana* defensive behavior, we used a 1 meter stick with a black cotton ball hanging at one end. Twenty µl isopentil asetat (IPA) 98% as the alarm pheromone was pipette to the cotton ball in each experiment (Arechavaleta-Velasco et al. 2003). The ball was horizontally hanging 10 cm in front of the hive within five minutes. During the experiments, humidity, temperature and light intensity were recorded.

Data analysis. Data were analyzed by using ULEAD Video and Cyberlink Power DVD. Number of guardians, bees hanging at the cotton ball and bees flying around the cotton ball were counted (Hunt *et al.* 1998). Five *A. cerana* defensive behavior categories were determined based on bees numbers, i.e.: 1, 2, 3, 4, and 5 each for numbers of bees ranging from 1-25, 26-50, 51-75 and 76-100.

RESULTS AND DISCUSSION

Descriptions of A. cerana Defensive Behaviour

This study confirmed that the five characteristics of five characters of *A. cerana* were detected (Table 1). *Guarding* is the first outdoor task perform by a worker bees. They inspect the organisms coming towards to the colony, based on the olfactory, visual, and tactile (Hunt 2007). Alert is the first behavior detected, produced a recruitment of the colony nest mate (Figure 1a). Then the bees fly surrounding the stimulus (cotton ball). The next response was constructing a balling behaviour at the nest entrance (Figure 1b) and balling behaviour at the cotton ball stimulus (Figure 1c). This behavior sequences were agree with that of Breed (2004) mentioned that *A. mellifera* defensive behavior was perception, orientation, identification, alert, recruitment, flying toward to stimulus, and attack.

***A.cerana* defensive behavior: Step 1. Alert and Recruitment behaviour**

The guardian recruited other bees from inside the nest (Breed et al. 2004.). Hence, the number of the bees in front of the entrance hole increased rapidly within 1-15 sec. Most of the guardian bees performed the recruitment (93%, N=183) (Table 2). The recruitment mean time was 25.51 ± 22.07 and 24. 84 ± 21.19 sec, respectively for *A. cerana* from PP and LB. In other study, *A. cerana* tend to rush entering the nest when a predator approach to the nest (Seeley 1982).

***A.cerana* defensive behaviour: Step 2. Fly surrounding to the cotton ball behaviour**

Most *A. cerana* (99%, N=187) fly surrounding to the cotton ball and the mean of time bees fly surrounding the ball was 32.87 ± 33.59 and 23.06 ± 17.9 sec, respectively for PP and LB. This showed that *A. cerana* in LB gave faster response to the alarm pheromone, compare to the A. cerana from PP.

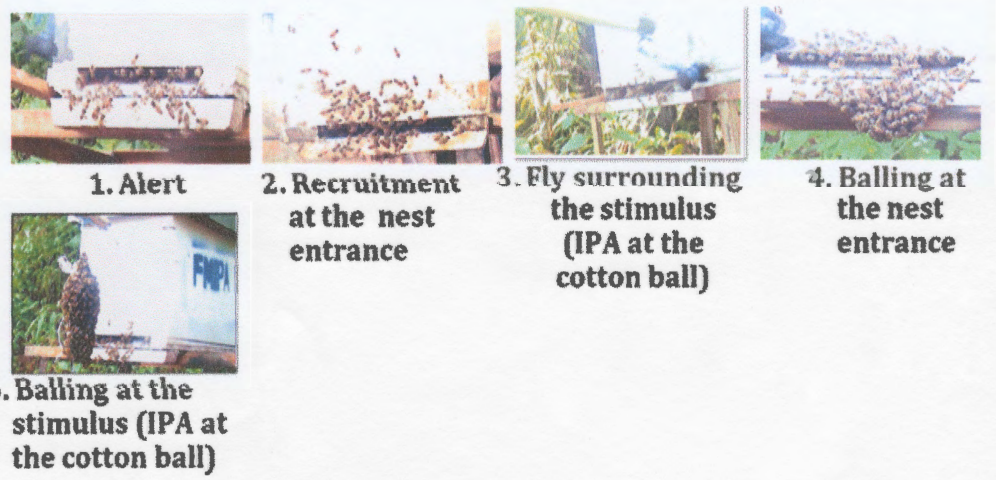
***A.cerana* defensive behavior: Step 3. Hanging at the cotton ball**

A number of 60.3 % observations of A. cerana performed hanging to the stimulus (cotton ball) observations (N=114). This behavior was seen at the 98.23 ± 53.52 and 77.14 ± 54.34 sec, respectively for *A. cerana* from PP and LB (Table 4). Hanging at the ball would be a secondary perception and discrimination to the stimulus (Breed et al. 2004).

***A. cerana* defensive behavior: Step 4 and 5. Balling behaviour**

The next two defensive behaviors were balling behavior that the bees performed a ball structure. *A. cerana* from PP tend to perform balling behaviour in front of the nest, whereas *A. cerana* from LB tend to be more aggressive as performed balling behavior directly towards the target. This behaviour was similar occurred in the natural situation that basically, *A. cerana* from LB tend to be more aggressive, they frequently attack the people that open the nest/hive.

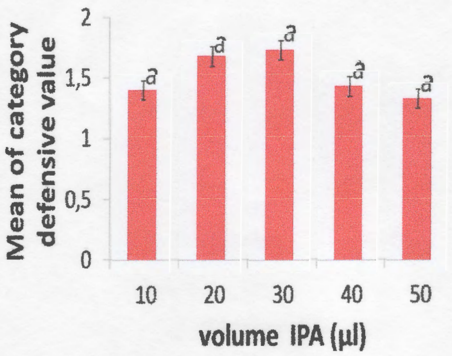
The balling behavior at the nest entrance in *A. cerana* started at the second – third minute from the stimulus given and the mean was 165.93 ± 58.08 sec and 160 ± 27.92 sec for A. cerana from PP and LB. Balling at the stimulus (cotton black ball) occurred at 145.31 ± 61.66 sec , whereas 90 ± 42.42 sec in *A. cerana* LB. In natural, the bees will attack the predator such as *Vespa* and it was killed by the layered of balling bees (Abrol 2006). In *A. mellifera* behaviour experiment by using the isopentyl acetate, the bees stung the ball and do not perform the balling behavior (Guzman-Novoa et al. 2002).

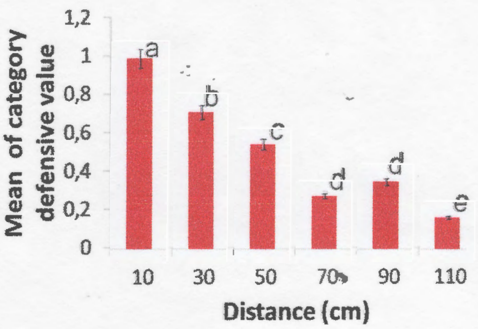


Five sequential of *A. cerana* defensive behaviour in response to the stimulus IPA (alarm pheromone)

***A. cerana* defensive behaviour during 6 am- 4 pm in response to 20 ul IPA**

Time	Category of defensive behaviour: Recruitment at the nest entrance PP	Recruitment at the nest entrance BS	Balling PP	Balling BS	Fly surrounding the stimulus PP	Fly surrounding the stimulus BS
6	0.943a	0.914a	0.277a	0.604a	0.875a	0.807a
8	0.943a	1.027a	0.283a	0.539a	0.929a	0.908a
10	1.054a	1.196a	0.327a	0.568a	0.768a	0.926a
12	1.113a	1.324a	0.56a	0.607a	0.857a	1.068a
14	1.536a	1.28a	0.5a	0.69a	0.875a	1.021a
16	1.31a	1.17a	0.357a	0.604a	1.021a	0.985a





Mean of *A. cerana* defensive behaviour in response to several IPA volume and IPA distance

We could not determine as yet, factor(s) influenced the different defensive response between the *A. cerana* from PP and LB (although the response for all step was not significantly difference P<0.01, see Table 8). Both environment data, i.e. humidity, elevation, light intensity, and temperature were similar between those two locations (data not shown). The flying activities showed the same pattern for those two areas as well (Figure 2). We understand that defensive behaviour of honey bee was influenced by the environment, genetics and the interactions between those factors (Lenoir et al. 2006). Lenoir *et al.* (2006) found variations of number of sting in *A. mellifera* colony. Several studies also showed that the defensive behaviour was influenced by the paternal lineage and the haplo-diploid system (Hunt 2007). Hence, defensive mechanism studies of *A. cerana* need to be more explored, one direction is to examine the gene(s) responding this defensive behavior.

***A. cerana* putative *sting-2* and EST1 Characterization**

We amplified *Apis cerana* putative genes and EST1 based on published *Apis mellifera* QTL *sting-2* i.e 36L17.15,36L17.14, EST1, which had 97%, 93%, and 81% homology with *Apis mellifera,* respectively

Alignment *A. cerana* putative gene 36L17.15 with *A. mellifera* 36L17.15 (Lobo *et al.*2003)

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Query  2      CTCGACGAGACGACCAACTTGGAGAGCCCCGCAATGCGTTCATTTCATTAGACGGTGGAT  61
              |||||||||||||||||||| | ||||||||||| |||||||||||||||||||||||||
Sbjct  46433  CTCGACGAGACGACCAACTT-G-GAGCCCCGCAACGCGTTCATTTCATTAGACGGTGGAT  46490

Query  62     ACTTAGTGAAATTAGTATTAACACTCGTAACGAGTACGGCCGCTCTACACTCTCGAGCCA  121
              ||||||||||||||||||||||||||| |||||| ||||||||||| |||||||||||||
Sbjct  46491  ACTTAGTGAAATTAGTATTAACACTCG-AACGAG-ACGGCCGCTCT-CACTCTCGAGCCA  46547

Query  122    TTGTGGCAAATGTTTTCCGGAAAAGAAAGCTCTTCGCATTGCCTCGCGAAAACTGCCGCG  181
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  46548  TTGTGGCAAATGTTTTCCGGAAAAGAAAGCTCTTCGCATTGCCTCGCGAAAACTGCCGCG  46607

Query  182    CCAACCAGAGTATCGCGAGTGTTAC  206
              |||||||||||||||||||||||||
Sbjct  46608  CCAACCAGAGTATCGCGAGTGTTAC  46632
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Alignment *A. cerana* EST1 with *A. mellifera* (Lobo *et al.* 2003)

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Query  1      GAGCGCGATATACCGAGTGTTGCTCGAGCCGGCACTTGCAACCTATAATTTACAGGTTAG  60
              ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  81128  GAGCGCGATATACCGAGTGTTGCTCGAGCCGCCACTTGCAACCTATAATTTACAGGTTAG  81069
Query  61     TCGGAAGAAA-GGGGGAAAAAATCAAGGGGATC---T-T-AATCGATTTACGGGGT-ATA  113
              |||||||||| ||||||||||||||||||||||   | | |||||||||||||||| |||
Sbjct  81068  TCGGAAGAAAAGGGGGAAAAAATCAAGGGGATCGGATCTTAATCGATTTACGGGGTTATA  81009
Query  114    TTCTCCTCTTATTAAAATATCACTTGACCAATTTACCCCCGACTCGATATCGTTCGAATA  173
              ||||| |||||||||||||||||||||| ||||||| |  || | |    |||| |||||
Sbjct  81008  TTCTCTTCTTATTAAAATATCACTTGACTAATTTACTC--GA-T-G----CGTTGGAATA  80957
Query  174    tttttttCTCTTCCCCTCTCTCGCTCCCTTTCTT-CGATTTATCTTCGATCGATCTTCAA  232
              || |||| | ||| | ||||| | | | ||| || ||||  |||||| ||   | || ||
Sbjct  80956  TTCTTTTTT-TTCTC-TCTCTTGGTTC-TTTATTTCGATCGATCTTCAAT---T-TTAAA  80904
Query  233    T-ATT---GRAACGATTCACGATTTCTTTTAAT----T-CGATTCGATGAAAATATA-AC  282
              | |||   | |||||||||  ||||||||||||    | |||| |||||||||||||| | 
Sbjct  80903  TGATTATCG-AACGATTCA--ATTTCTTTTAATCGAATTCGATCCGATGAAAATATATAA  80847
Query  283    -GTTTTGTGTCTTTAATCGGTATGATCGGTGGAATTATC----------G-ATGAACAA-  329
               |||| ||||||||||||||||||||||||||||||||||           | |||||||| 
Sbjct  80846  TGTTTCGTGTCTTTAATCGGTATGATCGGTGGAATTATATATATATATTGGATGAACAAA  80787
Query  330    TTGAAACAAATCTTCTTGTTA-CATTATTGATTTTCGTTCTCGAACGTGTATTATTATTT  388
              || |||||||||| |||||||| || |||| |||||||      ||||||||||||| |  
Sbjct  80786  TT-AAACAAATCTCCTTGTTAACAATATTAATTTTCG------AACGTGTATTATT-T--  80737
Query  389    CCCTTCTCTTCTTCTCTCGAATCGAACGAATCGTTTCTGTGATCRATGATCAACGGCGCT  448
              |||||| ||||  || ||   || | |||||||||||| ||||| ||||||| |  || |
Sbjct  80736  CCCTTCCCTTCC-CT-TC---TC-A-CGAATCGTTTCTATGATCGATGATCAGCA-CGAT  80685
Query  449    CGTTTAATCACGAATGCAATTCCAATCCGAGTCATCGATCGAC  491
              |||||||||||||||||||||||||||||||||||||||||||
Sbjct  80684  CGTTTAATCACGAATGCAATTCCAATCCGAGTCATCGATCGAC  80642
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(GenBank Acc *Number* NW_001253303): query shows the sequence of *A. cerana* EST1 region. Sbjct shows the sequence of *A. mellifera* genome

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