J Insect Behav (2008) 21:481–494
DOI 10.1007/s10905-008-9144-1

Comparison of Defense Body Movements of *Apis laboriosa*, *Apis dorsata dorsata* and *Apis dorsata breviligula* Honey Bees

Jerzy Woyke · Jerzy Wilde · Maria Wilde · Venkataramegowda Sivaram · Cleofas Cervancia · Narajanappa Nagaraja · Muniswamyreddy Reddy

Revised: 22 April 2008 / Accepted: 25 June 2008 /
Published online: 21 August 2008

Abstract Defense behavior of three, free living giant (*Megapis*) honey bee subspecies, *Apis laboriosa*, *A. dorsata dorsata* and *A. dorsata breviligula*, was compared. Disturbed worker bees responded with characteristic dorso-ventral defense body twisting (DBT). Workers of *A. laboriosa* twisted the thorax by 55°, and the two other *A. dorsata* subspecies by about 10° more. *A. laboriosa* workers raised the tip of the abdomen by 90° and workers of the two other bee subspecies by about 20° higher. Differences in those traits were highly significant between *A. laboriosa* and both *A. dorsata* subspecies, but were not significant between those two subspecies. The whole cycle of DBT was the most vigorous in *A. d. breviligula* (0.11 s), and it was twice as vigorous as in *A. d. dorsata* (0.26 s) and trice as in *A. laboriosa* (0.32 s). *A. laboriosa* twisted the body together with wings folded over the

Electronic supplementary material The online version of this article (doi:10.1007/s10905-008-9144-1) contains supplementary material, which is available to authorized users. Available also at Woyke www page

Hornet hunting near A. laboriosa nest, avi, 2.30 MB

Dorso-ventral Defense Body Twisting (DBT) of A. laboriosa

Circular Defense Waves (DW) of A. laboriosa, avi 2.3 MB

Defense Waves (DW) of A. d. breviligula provoked by dummy hornet

Defense body twisting (DBT) of A. d. breviligula, avi 2.4 MB

J. Woyke (✉)
Apiculture Division, Agricultural University, Warsaw, Poland
e-mail: jerzy_woyke@sggw.pl

J. Wilde
Apiculture Division, WM University, Olsztyn, Poland

M. Wilde
Dabur Apicultural Centre, Jugedi, Chitwan, Nepal

V. Sivaram
Division of Biodiversity and Apiculture, Department of Botany, Bangalore University, Bangalore, India

C. Cervancia
Institute of Biological Science, University of the Philippines, Los Baños, Philippines

N. Nagaraja · M. Reddy
Zoology Department, Bangalore University, Bangalore, India

abdomen, while the two *A. dorsata* subspecies raised the abdomen between spread wings. This supports the opinion to treat *A. laboriosa* as a separate species.

Keywords *Apis laboriosa* · *Apis dorsata* · defense behavior · Nepal · India · the Philippines

Introduction

Most honey produced in Nepal, India, and some other south-east Asian countries (70–80%) originates from the free living giant (*Megapis*) honey bees, *Apis laboriosa* and *Apis dorsata*. Nests of those bees are found beneath branches or overhangs and are composed of a single large comb covered by several layers of worker bees forming the curtain. The curtain presents head-insulation of the nest, and worker bees of the curtain protect the nest against predators. Bees of those colonies are known for their effective defense behavior. Therefore, honey from those colonies is harvested mostly at night. Roepke (1930) and Lindauer (1956), described the mass attack of *A. d. dorsata*. Morse and Laigo (1969) referred *A. d. breviligula* in the Philippines as the most ferocious insect on earth.

Butler (1954) reported that individual worker honey bees in South Asia exhibit a remarkable defensive shimmering behavior of shaking their bodies from side to side. Sakagami (1960); Schneider and Kloft (1971); and Koeniger and Fuchs (1975) described in details the side to side body movements in *A. cerana*. Kastberger et al. (1998), found defensive vertical abdominal shaking of individual *A. dorsata* worker bees. Defensive behavior of individual workers followed by neighboring bees, results in circular defensive waves on the surface of the curtain of worker bees covering the nests (Roepke 1930).

The knowledge of the defense behavior is highly essential to honey hunter and researchers who work with these bees.

Furthermore, doubt exists whether *A. laboriosa* is a separate species, or is it just a subspecies of *A. dorsata* (Ruttner 1992). The doubts are based on the identity or similarity of the endophallus of drones of *A. dorsata* and *A. laboriosa* (McEvoy and Underwood 1988). Therefore, the main purpose of our investigation was to find out whether detectable differences exist in the defense behavior of individual worker bees of *A. laboriosa* and the two other subspecies, *A. d. dorsata* and *A. d. breviligula*

Materials and Methods

Study Sites

Investigations on *A. laboriosa* were conducted in the Himalayas of Nepal in November and December 1999. *A. laboriosa* nests were observed on two cliff sites. One rock cliff with 17 nests was located on the slope of Annapurna mountain in Kyumi, near Landrung at Modi Khola river in Kasaki district (long. 83°50′ E., lat. 28°22′ N., alt. 1,200 m.). The other rock cliff with 53 nests was situated in Chale at Koshi river near the village of Chaku in Sindhupalchok district (long. 85°48′ E., lat.

28°09′ N., alt. 1,500 m) near the Tibetan border. All the colonies were observed from the other side of the rivers from a distance of 28 m. In Chaku, three additional colonies were observed to which we had direct access and their activities were recorded from a distance of 0.25–1.5 m.

The defense behavior of all the colonies was observed during several days continuously from 0800 to 1600 hours while investigating periodic mass flights (Woyke et al. 2003).

A. d. dorsata colonies were observed in Nepal during 5 months from January to May 1999 and during 8 months from October 1999 to June 2000. Five sites with a total of 141 nests were observed at the Tribhuvan University campus in Rampur, Chitwan district, southern Nepal (long. 84°21′ E, lat. 27°39′ N., alt. 190 m) and the vicinity. Fourteen full day observations were made at the University on three sites with 7, 19 and 65 *A. dorsata* nests while investigating periodic mass flights at weekly intervals (Woyke et al. 2004a). The nests were observed from a distance of 4–12 m. We had also direct access to several colonies where the bees could be observed and recorded from a close distance.

Colonies of *A. d. dorsata* were also observed in Bangalore, India. The investigations were conducted for 11 days from 3 to 13 March 2002. Greater congregations of *A. dorsata* colonies were observed in two nest sites, one consisting of 26 nests at the polytechnic building in the city and the other of 82 nests on a banyan tree (*Ficus bengalensis*) at the campus of Agricultural University. The nests were observed from a distance of 1.5 and 8 to 10 m. All the colonies were observed continuously from 0800 to 1800 hours. We had close access to four nests.

Close observation on two nests of *A. d. breviligula* were conducted in The Philippines in Los Baños and in Alfonso from 27 February to 6 March 2004.

Observations

Bee colonies located at inaccessible places were examined with the aid of 12×50 binoculars. The activities of workers were recorded with a video camera with 24× optical zoom. Close observations and video recording were made from a distance of 0.25 to 1.5 m.

Stimulation of Defense Body Movements

Defense behavior of individual worker bees was stimulated by free flying hornets, by killed hornet or dummy hornet fixed to a wire and also by different other objects. The disturbing objects were moved in front of the nests at a distance of 10 to 30 cm. In addition, the defense behavior was also observed while we were working directly with the colonies. We exchanged pieces of brood combs from *A. d. dorsata* and *A. mellifera* and *A. cerana* (Woyke et al. 2001), or we investigated hygienic behavior (Woyke et al. 2004b, c).

Temperature Measurements

The ambient temperatures were measured every hour while conducting other studies (Woyke et al. 2004d, 2006). In The Philippines, in addition to ambient temperature also temperature of the outer layer of the nest curtain was measured with the aid of a thermocouple inserted in the outer layer of the curtain.

Data Generation and Analysis

Defense body movements of individual worker bees was observed and analyzed from close up video recording. Close up video records of worker bees performing defensive body movements were made from lateral body side. The video was displayed at TV screen 43×56 cm where the length of the bee was 9 cm. The film was displayed at very slow mode (video frame after frame). The speed of successive frames was 1/25 s=0.04 s. The TV display could be stopped at any frame. The workers moved their body up and down. Depending upon the distance and the angle of video recording, some bees visible on the TV screen were blurred. However, bees recorded perpendicularly to their bodies were clear, and we were able to select many clear and sharp images of bees. When the up movement reached its maximum, it stopped before changing into the down direction. We were interested in the maximal inclination when the body stopped to move. When the right video frame was found, the display was stopped. We selected clear and sharp images of body position in its maximal deflection. We were interested in two traits, maximal body inclination and the duration of particular body movement phases. The inclination was measured in relation to the vertical. At this frame stop, the drawings were also made. For that purpose, a transparent paper was fixed to the TV screen and the drawing was made.

The duration of particular phases of body movement was measured after the number of video frames was counted in which the phenomenon occurred and next it was multiplied by 0.04 s (frame succession speed 1/25 s=0.04 s). Measurement of inclination or duration was repeated in each bee subspecies 30 or 45 times and all together 540 measurements were made. [Inclination: 30 repetitions × three species × three inclination measurements (thorax, abdomen, tip)=270. Duration: 45 repetitions × two duration (up and down) × three species=270. Together 270+270=540]. Altogether more than 1,000 video frames were individually checked.

Statistical Analysis

ANOVA was applied and LSD was used to determine the significant differences between the means.

Video Clips

Video clips recording hornet hunting near *A. laboriosa* nest, defense body movements of individual workers of the three bee subspecies and defense waving on curtain nests of the colonies are presented in the online version. Due to transformation from PAL to *. avi, format and due to small size, many details described by us are not visible at the clips. We made all observations and all measurement on the original images recorded at the tape in Pal format and displayed at TV screen.

Results

Giant Honey Bee Workers in the Outside Layer of the Nest Curtain

Undisturbed *A. laboriosa* and *A. dorsata* bees hang in the outside layer of the curtain covering the comb, head upwards. The anterior part of the body is inclined toward the comb by an angle of about 5° with respect to the vertical (Fig. 2a). The wings are spread apart. In *A. laboriosa* forewings were spread under an angle of about 55°, and the hind wings were spread more, under an angle of about 160° (Fig. 1a). In *A. dorsata,* forewings were spread more than in *A. laboriosa* under an angle of about 75°, however hind wings were spread less, under an angle of about 145° (Fig. 1a). Some *A. dorsata* workers in the curtain did not spread hind wings more than the forewings, but kept them spread under the forewings under an angle of only 80°. In this case, the forewings were spread under larger angle (about 95°) than in the other worker (75°) bees (Fig. 1b).

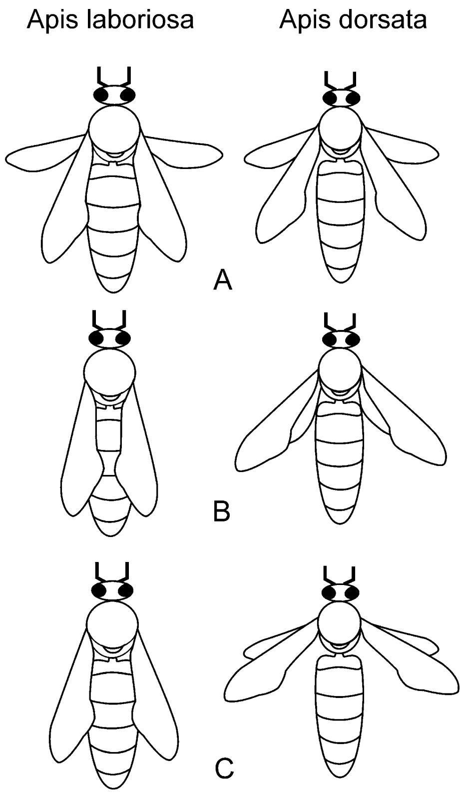

Fig. 1 Dorsal view of the position of wings during particular stages of defense body twists in two species of the giant honey bees *A. dorsata* and *A. laboriosa*. **a** Resting position, **b** alert position in *A. laboriosa* and resting position in *A. dorsata*, **c** position at maximal body twist.

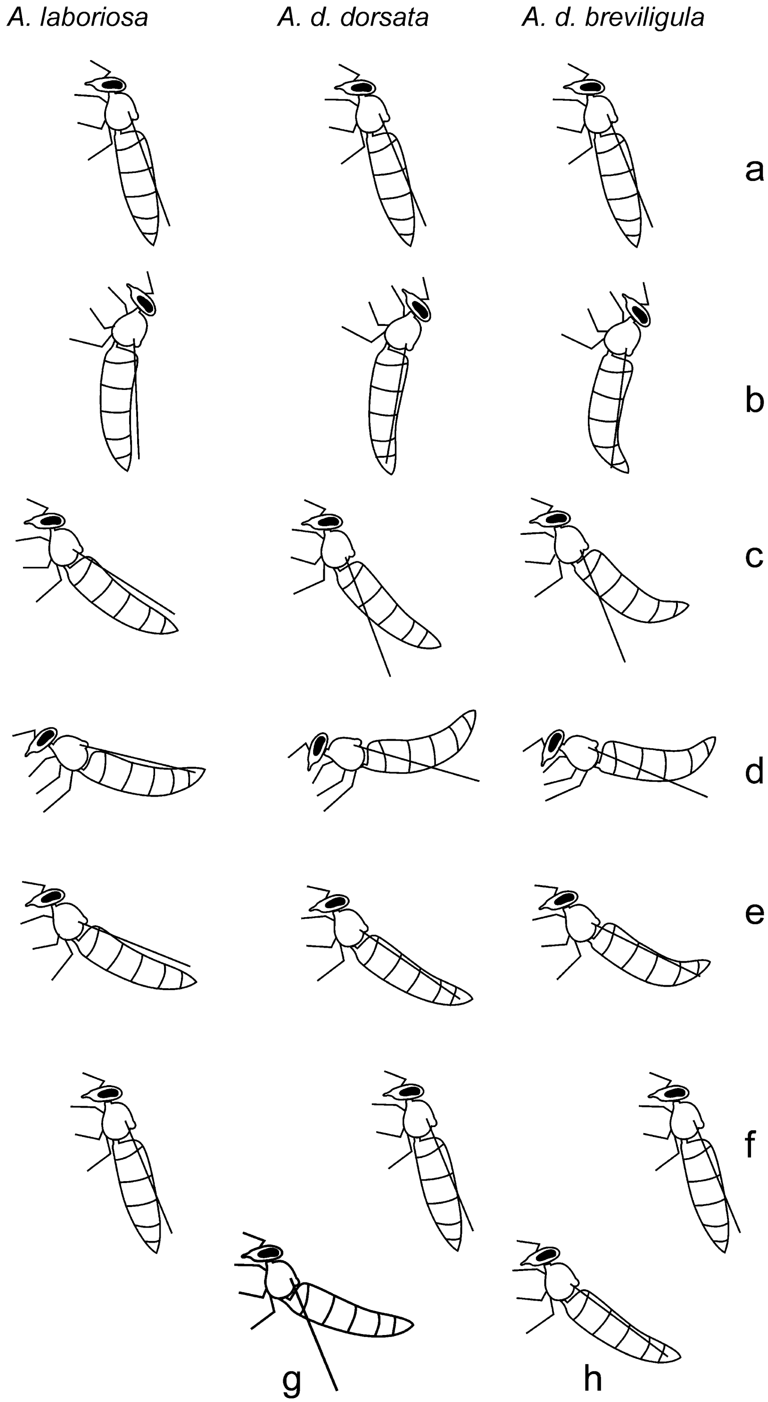

Fig. 2 Lateral view of body position during successive stages of defense body twist in three subspecies of the giant honey bees. **a** Resting position, **b** alert position, **c** defense body twisting, **d** position at maximal body twist, **e** start of returning to resting position, **f** resting position, **g**, **h** some unusual position in *A. dorsata*. The legs are not proportional to the body.

At lower temperature, the bees lift and lower the abdomen between spread wings, performing temperature dependent, dorso-ventral abdomen flipping (AF; Woyke et al. 2004d, 2006). No any thorax or wing movement occurs.

Defense Body Twisting of *Apis laboriosa*

When a hornet was hovering near the nest, some of the near by bees took an alert position. They turned their heads and thoraces toward the hornet. The inclination was about 10° to 15° with respect to the vertical (Fig. 2b). Contrary to the resting bees, the tip of the abdomen of alerted bees was deflected dorsally. All alerted bees folded the wings over the abdomen (Fig. 1b, alert position). Any movement of the hornet triggered defense body movement of the nearby worker bees. The bees turned their bodies in the vertical direction. The turning axis passes through the thorax. The thorax rotated by an angle of about 60°. While the thorax rotated, the head was lowered and the abdomen and the wings were lifted up (Fig. 2c,d). Thus, really, only the thorax rotated and as a result of this, the head was lowered and the abdomen, due to high speed, was trust up to about 75° in relation to the vertical (Fig. 2d). The wings remained all the time over the abdomen, however they sprayed a little more (angle about 45°, Fig. 1c), than during the alert position (Fig. 1b). At the maximal inclination of the abdomen, the wings of all the bees performing DBM were positioned above the abdomen (Fig. 2d). However, the deflected abdominal tips, of part of the bees, protrude over the wings. No any stroke down of wings occurred during the DBM. Next, the whole body turned back to the resting position (Fig. 2f). If the disturbance continued the DBM were repeated as long as the threat operated.

The term defense body movement does not specify the type of the movement. Therefore, we recommend using the term defense body twisting (DBT), which suggests (fort and beck) up and down turns of the body.

Defense Body Twist by *Apis dorsata*

When a hornet approached *A. dorsata* nest, then an alert position was also noticed, however it differed from that of *A. laboriosa*. The body position was similar (Fig. 2b). However, contrary to *A. laboriosa,* none *A. dorsata* worker bee folded the wings over abdomen. Wings of most of the bees remained extended. Many bees kept hind wings extended more than forewings (Fig. 1a). However, some bees extended hind wings less then forewings (Fig. 1b). Any disturbing movement of the hornet triggered DBT. However, significant differences occurred. At the beginning, *A. dorsata* bees lifted the tip of the abdomen between the extended wings. The tip was deflected more or less upwards. The anal part of the abdomen was lifted higher than the wings (Fig. 2c and d). Such phenomenon newer occurs in any *A. laboriosa* bee. Those few bees, which had the wings folded over the abdomen, extend them now. Next, the bee rotated the thorax by about 65°. At the same time, the head was lowered and the abdomen, due to high speed, was trusted up to about the horizon position and the tip was deflected more (Fig. 2d). While the thorax rotated, the wings were lifted up and were extended apart more, to an angle of about 120° (Fig. 1c). Extension of the wings varied. Sometimes forewings extended as much as they covered the hind wings. Thus the wings of all *A. dorsata* bees were extended at the maximum DBT much more than in *A. laboriosa* (Fig. 1c). All movements of the abdomen of *A. dorsata* were performed between spread wings. Contrary to *A. laboriosa*, the wings of all *A. dorsata* bees were lifted lower, than the dorsal surface of the abdomen. As a result, at the maximal inclination of the abdomen, the wings of all *A. laboriosa* were positioned above the abdomen, and of all *A. dorsata* below the dorsal surface of the abdomen (Fig. 2e). Next the bee turned the body back to the initial position (Fig. 2f). DBTs were repeated as long, as the threat operated.

Variations were found in DBT of *A. dorsata* worker bees. Few workers lifted only the abdomen, but did not turn the thorax. Wings of such bees were not lifted, but were kept down in the initial position, during the whole DBT (Fig. 2g). Such behavior was never detected in any *A. laboriosa* bee. Some other *A. dorsata* bees turned the thorax already at the beginning of the DBT and lifted the wings and the abdomen already at the initiation of the DBT (Fig. 2h). All this shows that raising and lowering the wings is closely related with twisting the thorax.

All this above shows that several considerable differences were found in the DBT between both species. The differences did not concern some bees of both species, but all or none.

Close observations did not reveal any distinct differences in DBT of individual worker bees to stimuli from free flying hornet, killed hornet, dummy hornet or other moving objects.

Comparison of Body Rotation and Inclination of Bees Performing Defense Body Twisting

Threatened worker bees responded with the characteristic defense body twist (DBT). While the thorax rotates, the head is lowered and the abdomen and wings are thrust upwards. At the maximal DBT (Fig. 2d), the thorax of *A. laboriosa* rotated on the average up to 55° with respect to the vertical (Table 1). Thoraces of both, *A. d. dorsata* and *A. d. breviligula* rotated significantly more by about 10°. The mean of maximal abdomen lift of *A. laboriosa* was about 75° and of both *A. dorsata* subspecies significantly about 15° and 8° higher respectively. At the summit phase of abdomen lifting, the last three to four abdominal segments were deflected more

Table 1 Rotation Angel of Thorax (°) and Inclination Angel of Abdomen at the Maximal up Turn of Defense Body Twisting in *A. laboriosa A. d. dorsata* and *A. d. breviligula*

Bee sub-species	Thorax		Abdomen[a]		Tip segments[b]	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
A. laboriosa	36.0–75.0	54.5±11.9 aA	65.0–95.0	75.7±13.1 cA	70.0–110.0	89.8±10.9 eA
A. d. dorsata	45.0–85.0	67.5±12.6 bB	84.0–100.0	90.0±2.7 eB	84.0–140.0	109.0±19.7 fB
A. d. breviligula	45.0–90.0	64.5±12.2 bB	65.0–100.0	83.5±10.6 dB	90.0–135.0	108.3±14.1 fB

Different small letters after means indicate significant differences between all of them $P<0.05$. Different capital letters after means indicate highly significant differences in the same trait between different bee subspecies (columns) $P<0.01$ (each mean, $n=30$ repetitions, together, $n=270$ measurements)
[a] Two to three oral abdominal segments
[b] Three to four terminal abdominal segments

than the two to three oral abdominal segments (Fig. 2d). The mean lift of the abdomen tip in the two *A. dorsata* subspecies (109° and 108°) was significantly about 20° higher, than in *A. laboriosa* (90°). The maximal inclination of abdominal tips reached in *A. laboriosa* and *A. dorsata* even 110° to 140° respectively. Highly significant differences were found in all three traits (thorax, abdomen, and tip) between *A. laboriosa* and the two *A. dorsata* subspecies (Table 1). However, no highly significant differences were detected between *A. d. dorsata* and *A. d. breviligula*. Thus, both *A. dorsata* subspecies twisted the thorax more and lifted the abdomen higher than *A. laboriosa*.

Duration of Defense Body Twist

Table 2 shows the duration of the up stage, the down stage and the total DBT cycles in the three bee subspecies. No significant differences were detected between the means of the three repetitions within any trait of any subspecies. The up phase of DBT in *A. dorsata* (0.12 s) was significantly shorter than the down phase (0.14 s). No significant differences were detected between up and down phase of DBT in the two other bee subspecies.

Highly significant differences were detected in all traits between the tree subspecies. The total cycle of DBT in *A. d. breviligula* was the most vigorous,

Table 2 Duration (s) of Different Phases of Defense Body Twisting (DBT) in *A. laboriosa*, *A. d. dorsata* and *A. d. breviligula* (Each Mean, n=15, Each Overall Mean, n=45, Each Total Overall (Bold) n=90, Together, n=270 measurements)

Bee species	Up movement Mean±SD/overall range	Down movement Mean±SD/overall range	Total DBT cycle Mean±SD/overall range
A. laboriosa			
1[a]	0.15±0.04 a	0.16±0.04 a	0.31±0.08 a
2	0.17±0.03 a	0.17±0.03 a	0.34±0.06 a
3	0.17±0.03 a	0.17±0.03 a	0.33±0.06 a
Overall	0.16±0.04 Aγ	0.17±0.03 Aγ	**0.33**±0.04 γ
Range	0.08–0.24	0.12–0.24	0.20–0.44
A. d. dorsata			
1	0.12±0.03 a	0.13±0.02 a	0.25±0.04 a
2	0.13±0.02 a	0.14±0.02 a	0.27±0.03 a
3	0.13±0.01 a	0.14±0.03 a	0.26±0.03 a
Overall	0.12±0.02 Aβ	0.14±0.02 Bβ	**0.26**±0.03 β
Range	0.08–0.16	0.08–0.16	0.20–0.32
A. d. breviligula			
1	0.06±0.02 a	0.06±0.02 a	0.12±0.03 a
2	0.06±0.02 a	0.05±0.02 a	0.11±0.03 a
3	0.06±0.02 a	0.06±0.02 a	0.11±0.02 a
Overall	0.06±0.02 Aα	0.06±0.02 Aα	**0.11**±0.03 α
Range	0.04–0.08	0.04–0.08	0.08–0.16

Different small letters after means indicate significant differences between repetitions within the same bee subspecies (three lines in the same column) $P<0.05$. Different capital letters indicate significant differences between up and down DBT within the same bee subspecies (different columns) $P<0.05$. Different Greek letters indicate highly significant differences in total overall of DBT between the three bee subspecies $P<0.001$.

[a] Repetitions

lasting only 0.11 s. The duration of DBT in *A. d. dorsata* (0.26 s) was twice as long and in *A. laboriosa* (0.33 s) three times as long. It was noticed that the duration of DBT was longer in those individual bees, which raised the abdomen higher.

Relationship Between Ambient Temperature and Defense Body Twisting

December 6, 1999 in Chaku (Himalayas near Tibet), the day when DBT of *A. laboriosa* was video recorded, ambient temperature (t_a), varied, between 1100 and 1500 hours, from 15 to 17°C. During proper video recording, 1100–1130 hours, the t_a was 15°C.

December 20, 1999 in Rampur (Chitwan, Nepal), when DBT of *A. d. dorsata* was recorded, t_a varied, between the same hours, from 18 to 26°C. During recording 1200–1230 hours, the t_a was 20°C.

March 1, 2004 in Los Baños (The Philippines), when DBT of *A. d. breviligula* was recorded, t_a varied, between the same hours, from 35 to 37°C. Here also temperature of the outer layer of the curtain (t_c) was recorded. It varied between the same hours from 34.9 to 35.4°C. The difference between minimum and maximum t_a was here 2°C while between t_c only 0.5°C. The low difference in t_c is due to the ability of the bees to regulate curtain temperature and to keep it quite stable. During proper recording, 1100–1200 hours, t_a varied from 35 to 36°C and t_c from 34.9 to 35.3°C.

Despite the low difference between t_a in Chaku and in Rampur (5°C), the angel of rotating the thorax and raising the abdomen by *A. laboriosa* and *A. d. dorsata* differed highly significantly (Table 1). Contrary, despite the high difference between t_a in Rampur and Los Baños (16°C), rotating the thorax and raising the abdomen by *A. d. dorsata* and *A. d. breviligula* did not differ highly significantly. All the results indicate that the differences in DBT between the three bee subspecies were not related to differences in t_a between the three places. This suggests that the differences in DBT behavior are characteristic for those subspecies.

Hornet Hunting and *Apis laboriosa* Defense Waving

During the 2 days of continuous close observations in Chaku, (5 and 6 December) the defense behavior of *A. laboriosa* workers against a hornet hunting near the nest was video recorded (presented in the online version). The hornet was flying at a distance of 5–20 cm from the curtain surface. It was hovering head outside the nests watching for returning foragers. Most of returning foragers changed the direction of flight and avoided of being caught. The hornet was most successful in catching returning bees during periodic mass flight (Woyke et al. 2003). However, early in the morning, when the temperature was low (7°C to 9°C) and the bees were not flying, the hornets picked the workers directly from the lower edge of the nest curtain.

At higher temperature the neighboring workers surrounding the center of the disturbance followed the DBT with a delay of 0.04 s. This resulted in the circular defense waves (DW) spreading over the curtain of the nest. We observed the DW behavior of *A. laboriosa* colonies several thousands times. One quick pass of an intruder like an insect, bird or even a falling leaf near *A. laboriosa* nest triggered normally only one defensive wave. A longer pass generated two or three waves.

However, hunting of a hornet near the nest differed essentially from other disturbances. The hornet was hovering near *A. laboriosa* nest for a dozen of minutes. As long as the hornet did not change its position, the bees did not make any DBT. However, any approach (backwards) of the hornet to the curtain, or change of the position in vertical or horizontal plane, triggered new DBT (presented online). As a result, we observed 40 DW per 1 min. Occasionally, when a second hornet approached the nest for hunting, the circular DW over the curtain expended from two different centers. As a result, the number of circular waves increased to 70 during 1 min. As soon as the hornet flew away, the workers stopped the defensive behavior. The hunting hornets disturbed the colonies almost continuously throughout the day.

We also provoked defense behavior many times by approaching a pencil, pocket knife, finger, hand, stick or photo- and video camera. Each approach of those objects triggered one DBT and one DW. Each movement resulted in a new DBT. The number of DBT did not depend upon the size of the disturbing object nor its nature, like live, dead or dummy hornet. As soon as the disturbing object was removed, no any more DWs were performed, although individual workers could make some DBT.

We conducted similar observations on *A. dorsata* nests in India. Our results were similar to various published already. However, we must add that in *A. dorsata* also as soon as the hornet left the colony, the bees of the curtain stopped to perform DW.

Discussion

We recorded defense behavior of the three *Apis* subspecies with the speed of 1/25= 0.04 s. If records were made with double speed, the time difference could differ maximally by 0.04 s and on average only by 0.02 s. However, the differences in the duration of the total DBT cycle between the three subspecies were 0.06, 0.15 or 0.21 s. Thus, higher speed record would not change statistically significant.

We recorded the defense behavior of the three bee subspecies in their typical environment. The question is does the defense behavior change at different ambient temperatures? To answer this it is important to note the following. It is known, that the nest temperature of *A. dorsata* is quite stable. This is despite environmental temperature variations (Burgett and Titayavan 1993). The bees are able to regulate their own temperatures and thus keep the nest temperature quite stable. Woyke et al. (2006) showed that the curtain temperature is also quite stable, even when the ambient temperature varies. Furthermore, Woyke et al. (2006) proved that the behavior of the bees in the curtain was mainly correlated with curtain temperature and not with ambient temperature.

Environmental conditions (30–35°C) as well as recording conditions differed during investigation conducted by Kastberger et al. (1998) and by us (20°C). Despite this, the duration of DBT presented by Kastberger et al. (1998) for *A. dorsata*, (cycle 120–200+residual moving 80–120, average=260 ms) was identical to that presented by us (0.26 s). All this above shows that due to bee ability to regulate and to keep curtain temperatures quite stable, the ambient temperature did not dominantly affect DBT behavior.

A film of defence strategies of giant honey bees exists (Kastberger 2000). However, that film was made from dorsal side of the bees, and therefore phenomena described by us are not visible at all or are unclear, because they must be recorded from lateral body side.

Defense waving Close observation of a hornet hunting near the nest was not described. According to Seeley et al. (1982, p. 57) "Whereas an *A. cerana* robber or a falling leaf triggers just one to three waves of shimmering, a *Vespa* wasp's close approach, even one lasting just a few seconds, elicits 50 or more waves of shimmering". This opinion is repeated in two Ruttner books (Ruttner 1988, p. 114, 1992, p. 316) and is generally accepted. However, our closes up observations showed that the cause of the differences is not the type of the intruders, but the number of repeated disturbances. When a disturbing insect, bird or any other intruder just pass or disturbed the nest for short time, this triggers just one or few DW. However, a hornet hunting near the nest is hovering there for a long time. Each change of its position triggers a new DW (see this behavior online). After the hornet flies away, no more DWs are performed. The reported continuation of DWs after the hornet left the nest are probably due to inability in seeing the hornet from long distance of 18 m to the observed nests.

Batra (1996) observing *A. laboriosa* nest from a distance of 40 m, reports that once clouds of bees suddenly flew simultaneously for a distance of 3.5 m around the nests for 4 min. She suggests that, perhaps a bee had been trampled by a hornet, releasing alarm pheromone. However, we know now that this was typical periodic mass flight of *A. laboriosa* described by Woyke et al. (2003).

At Kastberger film (2000), the hornet picks *A. dorsata* bees directly from the curtain. This happened with *A. laboriosa* only at lower temperature (7–9°C) when the bees were chilled. However, it is visible that both hornets are different species.

Shimmering in A. cerana Butler (1954, p. 14) writes that honeybees in South Asia "exhibit a remarkable behavior pattern when a hornet, ant, wax-moth or other intruder approaches their nests. They shake their bodies violently from side to side in concert, a behavior pattern which I have called the "shimmering" behavior". Schneider and Kloft (1971) and Koeniger and Fuchs (1975) showed that the amplitude of the abdominal tip reaches in *A. cerana* 1 cm to the right and 1 cm to the left. Koeniger and Fuchs (1975, p. 102) suggested that similar behavior occurs in *A. dorsata*. Although, we observed many times the side to side shakings called shimmering in *A. cerana*, we have never seen movements in this direction performed by *A. laboriosa or A. dorsata*.

Previous authors made close-up observations of *A. cerana* shaking their bodies from side to side. However, they observed *A. dorsata* from a distance of several meters. Koeniger and Fuchs (1975, p. 104) write "Eine entsprechend genaue Bewegungsanalyse liegt für *A. dorsata* und *A. florea* wegen technischer Schwierigkeiten nicht vor" Therefore, the authors probably presumed that *A. dorsata* was making movements similar to *A. cerana*.

Defense body movements in A. laboriosa and A. dorsata According to Kastberger at al. (1998, p. 30–31) disturbed individual bees respond "by trusting their abdomen at more than 90° in upward direction" They call this behavior "abdominal shaking". Abdominal shaking called shimmering is also reported by Schmelzer et al. (2004), who presented a poster on which only the abdomen is lifted. However, we found that disturbed bees do not raise only the abdomen but twist also the thorax and lower the head. Due to bee morphology, it is impossible for a worker bee to trust the abdomen up, by 90° or more, or deflect even more than 120° without twisting the thorax. Really, the whole body is twisted. Therefore, we propose to replace the term “abdominal shaking” by the term dorso ventral “defense body twisting” (DBT).

Shimmering and defense waving We would like to point out that two different defense reactions exist in Asian honey bees. *A. cerana* moves the body side to side in lateral direction, which is called “shimmering” (Butler 1954). But individuals of *A. laboriosa* and *A. dorsata* respond with defense body twisting in vertical direction. Thus the use of the term “shimmering” is not appropriate neither for DBT of individual bees or for DW in *A. laboriosa* and *A. dorsata*.

Defense body movements of A. laboriosa versus both subspecies of A. dorsata. A. d. dorsata and *A. d. breviligula* twisted the thorax and lifted the abdomen highly significantly more than *A. laboriosa*. However, no highly significant differences were detected between those two *A. dorsata* subspecies. *A. laboriosa* twisted the body together with wings folded over the abdomen, while both *A. dorsata* subspecies raised the abdomen between spread wings. Thus, *A. d. dorsata* and *A. d. breviligula* differ quantitatively in performing DBT. However, both subspecies differ from *A. laboriosa* qualitatively in performing DBT. Those differences support the opinion to treat *A. laboriosa* as a separate species.

Acknowledgements We would like to thank very much Mr K. Neupane from Tribhuvan University in Rampur, Nepal and dr A. Fajardo from the University in Los Baños, Philippines, for their help during the investigation.

References

Batra SWT (1996) Biology of *Apis laboriosa* Smith, a pollinator of apples at high altitude in the great Himalaya range of Garhwal, India, (Hymenoptera: Apidae). J Kansas Entomol Soc 69:177–181

Burgett M, Titayavan M (1993) Brood thermoregulation by the giant honey bee (*Apis dorsata* F.). Nat Hist Bull Siam Soc 41:93–98

Butler CG (1954) The world of the honeybee. Collins, London

Kastberger G (2000) Defence strategies of giant honeybees. Film, epofilm Wien-Graz

Kastberger G, Raspoting G, Biswas S, Winder O (1998) Evidence of Nasanov scenting in colony defence of the giant honeybee *Apis dorsata*. Ethology 104:27–37

Koeniger N, Fuchs S (1975) Zur Kolonieverteidigung der asiatischen Honigbienen. Z Tierpsychol 37:99–106

Lindauer M (1956) Über die Verständigung bei indischen Bienen. Z vergl Physiol 38:521–557

McEvoy MV, Underwood BA (1988) The drone and species status of the Himalayan honey bee, *Apis laboriosa* (Hymenoptera: Apidae). J Kans ent Soc 61:246–249

Morse RA, Laigo FM (1969) *Apis dorsata* in the Philippines. Monogr Phillip Assoc Entom 1:1–96

Roepke W (1930) Beobachtungen an Indischen Honigbienen, insbesondere an *Apis dorsata* F. Meded Landbouwhogesch Wageningen 34:1–28

Ruttner F (1988) Biogeography and taxonomy o honeybees. Springer, Berlin

Ruttner F (1992) Naturgeschichte der Honigbienen. Ehrenwirth, München

Sakagami S (1960) Preliminary report on the specific deference of behavior and other ecological characters between European and Japanese honeybees. Acta Hymenopterologica 1:172–198

Schmelzer E, Hartbauer M, Kastberger G (2004) Defence waving in *Apis dorsata*. Proc. First European Conf. Apidology, Udine, Italy (61+ poster)

Schneider P, Kloft W (1971) Beobachtungen zum Gruppenverteidigungsverhalten der östlichen Honigbiene *Apis cerana* Fabr. Z Tierpsychol 29:337–342

Seeley TD, Seeley RH, Akratanakul P (1982) Colony defense strategies of the honeybees in Thailand. Ecol Monogr 52:43–63

Woyke J, Wilde J, Wilde M (2001) Coexistence of *Apis mellifera* and *Apis dorsata* workers in the same colonies. Proceedings VII IBRA Conference on Tropical Bees, and V Asian Apicultural Association Conference, Chiang Mai, 19–25 March, 2000, pp 115–120

Woyke J, Wilde J, Wilde M (2003) Periodic mass flights of *Apis laboriosa* in Nepal. Apidologie 34:121–127

Woyke J, Kruk C, Wilde J, Wilde M (2004a) Periodic mass flights of the giant honey bee *Apis dorsata*. J apic Res 43:180–185

Woyke J, Wilde J, Reddy CC (2004b) Open-air-nesting honey bees *Apis dorsata* and *Apis laboriosa* differ from the cavity-nesting *A. mellifera* and *Apis cerana* in brood hygiene behavior. J Invert Path 86:1–6

Woyke J, Wilde J, Reddy CC (2004c) The gentleness of *Apis dorsata* verified while investigating brood cross-fostering and hygienic behavior. Proceedings of 7th Asian Apicultural Association Conference. Los Baños, Philippines, pp 87–91

Woyke J, Wilde J, Wilde M (2004d) Temperature correlated dorso-ventral abdomen flipping of *Apis laboriosa* and *Apis dorsata* worker bees. Apidologie 35:493–502

Woyke J, Wilde J, Wilde M, Cervancia C (2006) Abdomen flipping of *Apis dorsata breviligula* worker bees correlated with temperature of nest curtain surface. Apidologie 37:501–505