Apidologie 33 (2002) 289–294

DOI: 10.1051/apido:2002019

Original article

Defense posture in the dwarf honeybee, *Apis florea*

Christian W.W. PIRK[a]*, Randall HEPBURN[a], Sarah E. RADLOFF[b], Johan ERLANDSSON[a]

[a] Department of Zoology and Entomology, Rhodes University, 6140 Grahamstown, Republic of South Africa
[b] Department of Statistics, Rhodes University, 6140 Grahamstown, Republic of South Africa

(Received 12 October 2001; revised 15 January 2002; accepted 16 January 2002)

Abstract – The defense postures of colonies of *Apis florea* were analysed sequentially before and after applications of smoke as an alarm stimulus. Significant changes in body posture and orientation occur following an alerting stimulus: the bees expose their abdomens and pack more closely together on the comb.

***Apis florea* / defence / alarm / behaviour**

1. INTRODUCTION

That honeybees readily defend their nests is known to all; less well known is the range of different behaviours associated with such defence. On the initial disturbance of a colony of honeybees, reactions may include wing shimmering and hissing; and, often there are also apparently unprovoked massive attacks. These behaviours vary widely among honeybee species and with the nature of the alarming stimulus (Butler, 1974). Colonies of the dwarf honeybee, *A. florea* Fabr., are very "gentle" compared to other honeybee species bees but nonetheless exhibit clear-cut changes of behaviour when disturbed or alarmed. Even the close presence of an observer/intruder induces those bees of the outermost layer of the bee curtain covering their single small comb to react. The bees were noted to flex their wings, shimmer, often hiss, and seemed to become more densely packed before eventually flying out at the source of disturbance (Akratanakul, 1977). Recent photographic and acoustical analyses critically confirm that *A. florea* do indeed shimmer and hiss when disturbed (Fuchs et al., 2001). Here we further document changes in behavioural posture with respect to

* Correspondence and reprints
E-mail: C.Pirk@ru.ac.za

neighbour-neighbour packing order on disturbance with smoke.

2. MATERIALS AND METHODS

2.1. Measurement

We simulated threats to five colonies of the dwarf bee, *A. florea*, at Chulalongkorn University, Bangkok by smoking them with smouldering palm fronds near their nests for about 10 seconds.

Although we cannot quantify the stimulus as such, a systematic approach was followed. A dead frond of a coconut palm was set alight at its base about 10 cm broad, the flame was then extinguished and the frond slowly smouldered. The smouldering frond was held about 20 cm just below the comb and the smoke allowed to waft upwards on to the bees. The smoke was gentle and much less than that generated by a bee-smoker. The smoking interval was the same for each colony and not changed with reaction of the bees.

The reactions of each colony of bees to this stimulus were photographed in sequence at a rate of three photographs per colony: the first picture was taken just before smoking the bees, the second 30 seconds later and the final one 15 minutes after smoking had been discontinued. The photographs were taken with a hand-held camera and were then corrected to the same real scale of 9 × 6 cm areas in the middle area of the combs. These areas were then subdivided into 9 rectangles of 3 × 2 cm each. Each individual rectangle was used as an independent sample of different groups of bees on different parts of the comb. Then the numbers of thoraxes and abdomens visible in each rectangle were counted to obtain colony mean values. To determine relative bee density per unit area, all bees (whatever portion of the bodies were in view) were counted and subsamples used to obtain the total number of bees. In addition to changes in density, the orientations of the bees with respect to the vertical axis of gravity (angular deviation of the longitudinal axis of the whole bee from the vertical) were also measured directly from the photographs (9 × 6 cm area) to further analyse packing order.

2.2. Data analysis

Four separate univariate ANOVA repeated measures procedures were carried out with (1) numbers of visible thoraxes per 6 cm^2, (2) numbers of visible abdomens per 6 cm^2, (3) densities per 6 cm^2 and (4) individual angles of orientation as the response variables observed before the disturbance, immediately after the disturbance and 15 minutes after the disturbance and colonies as the factor term. Kolmogorov-Smirnov and Levene's tests were used to test for normality and homogeneity of the variances of the response variables, respectively. In repeated measures ANOVA the sphericity assumption of the variance-covariance matrices is a necessary and sufficient condition for the F-test to be valid (Johnson, 1998). The sphericity assumption of the variance-covariance matrix was tested using Mauchly's W criterion (Statistica, 1995). If the sphericity assumption was violated then MANOVA procedures were used. These procedures included Wilks' lambda and Box M Statistics for multivariate comparisons of means and variance-covariance matrices.

3. RESULTS

Three response variables (numbers of visible thoraxes and abdomens and total bee density) passed tests of normality (Kolmogorov-Smirnov $d < 0.1546$, $P > 0.05$) and tests of homogeneity of the variances (Levene's $F < 2.96$, with 4,40 d.f., $P > 0.01$). The variance-covariance matrices passed Mauchey's sphericity tests (thorax:

W = 0.88, $P = 0.0808$; abdomen: W = 0.87, $P = 0.0652$; density: W = 0.99, $P = 0.9186$) and hence the univariate ANOVA procedure outcomes could be considered as reliable for these response variables. It was necessary to log (x + 1) transform the angle of orientation variable in order to pass the assumptions of normality and homogeneity of the variances. The variance-covariance matrix failed Mauchey's sphericity test (angle of orientation: W = 0.92, $P < 0.01$) and hence only the MANOVA results are considered.

3.1. Number of visible thoraxes

The results of the repeated measures ANOVA test revealed that the mean number of visible thoraxes per 6 cm^2 differed significantly between the five colonies (F = 13.3 with (4,40) d.f., $P < 0.0001$). The mean number of visible thoraxes for four colonies, however, all decreased (not necessarily by the same amount) from the period before smoking the bees to the period 30 seconds later and then all increased (not necessarily by the same amount) to the period 15 minutes after smoking had been discontinued. This showed that although the number of visible thoraxes differed between colonies the reaction of the bees in four colonies was the same. A significant difference in the mean number of visible thoraxes was found over the three periods observed (F = 6.86 with (2,80) d.f., $P = 0.0018$; Fig. 1). Tukey's multiple comparison tests for equal sample sizes indicated that the mean number of visible thoraxes taken at the disturbance period was significantly lower from that at the before and after disturbance periods (Tukey: before $P = 0.0115$; after: $P = 0.0056$). No significant difference was found between the before and after periods (Tukey: $P = 0.9678$). A significant interaction effect was found between the colonies and the periods of observations (F = 3.44 with 8,80 d.f., $P = 0.0019$).

3.2. Number of visible abdomens

A significant difference was found in the mean number of visible abdomens per 6 cm^2 between the five colonies (F = 4.52 with 4,40 d.f., $P = 0.0041$). Again although the number of visible abdomens differed between colonies the reaction of the bees for three colonies between the periods of observation was the same. The results of the ANOVA test showed that the mean number of visible abdomens increased significantly during the disturbance period (F = 6.57 with 2,80 d.f., $P = 0.0023$; Tukey: before $P = 0.0473$; after $P = 0.0023$). A significant interaction effect was also found between the colonies and the periods of observations (F = 7.53 with 8,80 d.f., $P < 0.0001$). It was also confirmed that the number of visible abdomens was significantly greater than the number of visible thoraxes following the smoke stimulus.

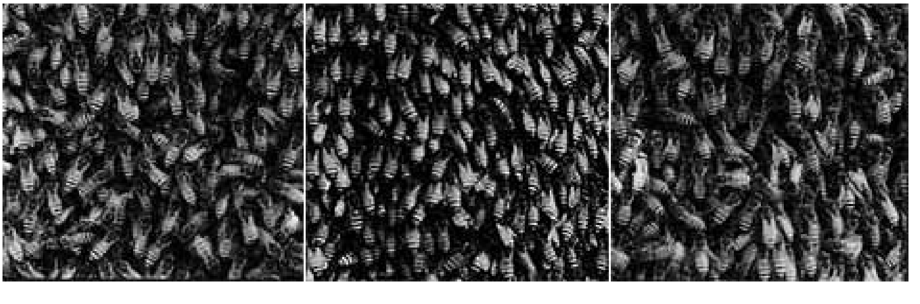

Figure 1. Postural changes in *A. florea* colony following stimulus with smoke: (a) before stimulation, (b) during and (c) after. Quantitative differences given in Table I.

3.3. Relative density of bees

No significant difference was found in the mean density of bees per 6 cm^2 between the five colonies ($F = 1.73$ with 4,40 d.f., $P = 0.1627$) so that density changes were the same for all colonies. A significant difference was observed in the mean density between the periods of observation, namely before and after smoking the bees (number of bees per unit area of measurement). It was least before the smoke stimulus, significantly higher shortly after smoking and then returned to pre-stimulus levels ($F = 57.99$, with 2,80 d.f., $P < 0.0001$; Tukey: before $P = 0.0001$, after $P = 0.0001$). Although the densities per 6 cm^2 for all five colonies increased from the period before smoking the bees to the period 30 seconds later and then all decreased to the period 15 minutes after smoking had been discontinued, a significant interaction effect was established between the colonies and the periods of observations ($F = 5.34$ with 8,80 d.f., $P < 0.0001$). This was because that the amounts of increase or decrease in densities were not necessarily the same for all colonies between the periods of observation.

3.4. Packing order of bees

Even though the relative density of honeybees per unit area for each of the 9 photographic subsamples for each individual comb did not differ significantly, there were highly significant differences in the mean relative packing order of the dwarf bees immediately following disturbance with smoke (Fig. 1). Prior to disturbance, the usual activities of the bees moving to and fro showed a mean angular deviation from the vertical of 23.43°±8.95°. After disturbance general activities switched to a defense-alarm mode of posture (Fig. 1) in which the bees were closely aligned side-by-side with a decrease in angular displacement from the vertical axis to only 15.14°±3.64°. As the stimulus abated and the bees returned to normal activities, angular displacement reverted to 16.09°±3.94°. The results of the MANOVA test showed that the mean angular deviation from the vertical decreased significantly during the disturbance period (Wilks' lambda $\Lambda = 0.75$, $P < 0.0001$; Box $M = 680.22$, $P < 0.0001$; Tukey: before $P = 0.00002$; after $P = 0.0057$).

4. DISCUSSION

The means and standard deviations of the number of visible thoraxes and abdomens, the density of bees per unit area (6 cm^2) and angular deviation of the bees are given in Table I and are also vividly illustrated in the photographic sequences (Fig. 1). The obvious visual differences discernible in the photographic sequences are confirmed by complex statistical analysis. The numerical results and the photographs

Table I. Means and standard deviations of the number of visible thoraxes and abdomens, density of bees per unit area of 6 cm^2 (where nine subsamples for each colony were measured) and angular deviation from the vertical (in degrees), observed for five colonies of *A. florea* before, during and after disturbance with smoke. Values within rows having different letters are significantly different.

	Periods		
	Before	Disturbance	After
Thorax	13.04 ± 2.19^a	11.98 ± 1.76^b	13.13 ± 2.41^a
Abdomen	16.89 ± 3.02^a	17.76 ± 2.82^b	16.20 ± 2.34^a
Density	19.28 ± 2.49^a	22.84 ± 2.48^b	18.80 ± 2.15^a
Angular deviation	23.43 ± 8.95^a	15.14 ± 3.64^b	16.09 ± 3.94^b

clearly demonstrate the sequence of postural changes associated with disturbing a colony of the dwarf honeybee, *A. florea* with smoke. To consider thoraxes and abdomens first, it is very evident that, on disturbance of the dwarf bees, they adopt slanted positions from the horizontal in which now erect abdomens dominate the field. This appears in two ways: firstly, although there are more abdomens than thoraxes visible in any of the frames at any point in the alarm sequence, there is a concomitant and significant reduction in visible thoraxes on disturbance. This demonstrates a significant change in posture.

Some might argue that honeybees commonly engorge on nectar when smoked and that this could explain increased abdomens and decreased thoraxes. But that this is not so is evident from the fact that the curtain of bees covering the single comb is 3–4 bees thick (about 10 mm) and the head and thorax of only one bee about 4.5 mm, so if an abdomen is visible at the surface of the curtain then there is not enough bee-length of bee to actually reach cells of nectar and still have the abdomen visible to the outside.

Not only do the bees tilt their bodies to present a cluster of abdomens (hence stings), but they simultaneously change their neighbour-neighbour spacing patterns from unordered (Fig. 1a) to a significantly more condensed and ordered pattern (Fig. 1b). The bees subsequently revert to a less ordered state once reaction to the stimulus has subsided (Fig. 1c). The combined changes in posture, position and packing of *A. florea* on disturbance with smoke is a behavioural analogue of the "testudo" approach of the ancient Romans.

ACKNOWLEDGEMENTS

We thank Kirsten Hepburn (San Francisco) for the photographic sequences and Siriwat Wongsiri (Bangkok) for access to his colonies of *A. florea*.

Résumé – Posture de défense chez l'abeille naine *Apis florea*. Bien que les colonies d'*Apis florea* Fabr. soient douces comparées à celles des autres races, elles présentent néanmoins de nettes modifications du comportement lorsqu'elles sont perturbées. Ces modifications comprennent le sifflement et le miroitement des ailes, c'est-à-dire qu'elles modifient leur position sur le rayon de façon à faire briller leurs ailes à la lumière. Nous avons analysé ces changements de posture, bien repérables visuellement, chez des colonies d'abeilles naines lorsqu'elles sont perturbées par la fumée. Nous avons quantifié les comportements et analysé statistiquement les données. Cinq colonies situées à Bangkok ont été légèrement enfumées durant 10 secondes environ. Leur comportement a été photographié juste avant l'enfumage, 30 secondes plus tard, puis 15 minutes plus tard (Fig. 1).

Les photographies ont été subdivisées en 9 rectangles de 3 × 2 cm chacun et le nombre de thorax et d'abdomens visibles dans chaque rectangle a été comptabilisé afin d'obtenir des valeurs moyennes pour la colonie. Le nombre de thorax visibles a varié significativement au cours des trois périodes analysées (F = 6,86 avec (2,80) d.f., *P* = 0,0018) et, immédiatement après l'enfumage, les abeilles ont augmenté leur densité de regroupement par unité de surface du rayon (Tab. I).

Avant l'enfumage, les abeilles présentaient les activités habituelles de déplacement sur le rayon avec une déviation angulaire par rapport à la verticale d'environ 23 degrés. Après l'enfumage, la plupart des activités normales ont été arrêtées et les abeilles sont passées à une posture de défense, c'est-à-dire qu'elles se sont alignées côte à côte avec une déviation angulaire moyenne d'environ 15 degrés (Tab. I).

Les séquences photographiques montrent qu'en cas de perturbation les abeilles adoptent une position inclinée si bien que les abdomens sont dominants dans le champ photographique (Fig. 1b). En outre les abeilles se serrent au plus près en se rapprochant

de la verticale de façon à produire un formation très ordonnée (comparer les Figs. 1a et 1b). Une fois l'effet du stimulus passé, les abeilles reviennent à un état moins ordonné (Fig. 1c). La combinaison des changements dans la posture, la position et le regroupement est comparable à la formation de défense des anciens Romains appelée « testudo ».

***Apis florea* / comportement d'alarme / comportement de défense**

Zusammenfassung – Verteidigungshaltung bei der Zwerghonigbiene *Apis florea.* *Apis florea* Fabr. Kolonien reagieren im Allgemeinen weniger aggressiv als Völker anderer Honigbienenarten. Es zeigt sich jedoch eine deutliche Veränderung im Verhalten einer Kolonie, wenn diese gestört wird. Die Bienen geben Zischlaute ab und verändern ihre Position in der Art und Weise, dass die Flügel im Licht schimmern.

Mit Hilfe einer Sequenz von Photographien wurden die Verhaltensänderungen durch Rauch gestörter Völker der Zwerghonigbiene festgehalten und statistisch ausgewertet. Fünf *Apis florea* Kolonien aus Bangkok störten wir 10 Sekunden lang mit etwas Rauch. Die daraus resultierenden Verhaltensveränderungen wurden in einer Sequenz von Bildern festgehalten, wobei das erste Foto unmittelbar vor der Störung gemacht wurde, das zweite 30 Sekunden nach der Störung und das dritte und letzte weitere 15 Minuten später (Abb. 1).

Zur statistischen Auswertung erfolgte eine Unterteilung der Fotos in neun Rechtecke, jedes einzelne 3 × 2 cm groß. In jedem dieser Rechtecke wurde die Anzahl von sichtbaren Bienen, Abdomen und Thoraces gezählt. Ferner wurde der Winkel jeder einzelnen Biene zur Vertikalen bestimmt. Während der drei Testperioden war die Anzahl der sichtbaren Bienenthoraces signifikant verschieden ($F = 6{,}86$ mit (2,80) d.f., $P = 0{,}0018$), wobei die Anzahl der Bienen pro Flächeneinheit direkt nach der Störung durch den Rauch anstieg (Tab. I).

Vor der Störung zeigten die Zwerghonigbienen ihre üblichen Verhaltensweisen und die Position der Bienen wich um durchschnittlich 22 Grad von der Vertikalen ab. Nach der Störung wurden alle normalen Aktivitäten größtenteils eingestellt und die Bienen formierten sich in einer Verteidigungshaltung, d.h., die Bienen rückten Seite an Seite zusammen, erkennbar in einer Abnahme auf weniger als 15 Grad der durchschnittlichen Abweichung von der Vertikalen (Tab. I).

Die Bildersequenz veranschaulicht deutlich, dass die gestörten Bienen eine geneigte Position auf der Wabe einnehmen, so dass Abdomen das Bild dominieren (Abb. 1b).

Ferner rücken die Bienen näher zusammen und richten sich mehrheitlich zur Vertikalen aus, hin zu einer mehr geordneten Formation (vergleiche Abb. 1a und 1b). Nach Ende des Reizeffektes kehren die Bienen zu ihren Aktivitäten zurück (Abb. 1c).

Diese Kombination von Änderungen in der Position der Haltung und das Zusammenrücken der Bienen als Antwort auf eine Störung mit Rauch kann mit einer „Testudo" Verteidigungsformation verglichen werden.

Alarm / *Apis florea* / Verhalten / Verteidigung

REFERENCES

Akratanakul P. (1977) The natural history of the dwarf honey bee, *Apis florea* F. in Thailand, Ph.D. thesis, Cornell University, New York.

Butler C.G. (1974) The World of the Honeybee, Collins, London.

Fuchs S., Sen Sarma M., Werber C., Tautz J. (2001) The cry of the honeybee *Apis florea*, Proc. 2001 meeting of the European sections of the Int. Union for the Study of Social Insects (IUSSI), September 25–29, Berlin, p. 164.

Johnson D.E. (1998) Applied Multivariate Methods for Data Analysts, Brooks/Cole Publishing Company, Pacific Groove.

Statistica for Windows (1995) General Conventions and Statistics I, Volume 1, 2nd Edition, StatSoft, Inc., Tulsa OK.