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Original article

High frequency sounds produced by Cyprian honeybees *Apis mellifera cypria* when confronting their predator, the Oriental hornet *Vespa orientalis**

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Abstract – Honeybees face several predators and their ability to express collective defence behaviour is one of their major life traits that promote colony survival. We discovered that, while confronting attacks by the Oriental hornet *Vespa orientalis*, *Apis mellifera cypria* honeybees engage in a distinct acoustic behaviour: they produce a characteristic hissing sound of unexpectedly high frequency. When recording and analysing these hissing sounds during an extended sample of artificial attacks by hornets, we found that honeybees can produce sounds covering a wide frequency spectrum with a dominant frequency around 6 kHz. Notably, these acoustic emissions are distinct from the background noise of neighbouring flying bees. These results provide a detailed description of the sounds generated by *A. m. cypria* when defending their nest against hornets, and they could be used for future research to better understand the biological function of the acoustic behaviour in honeybees' colony defence.

hissing sound / colony defence / *Apis mellifera cypria* / *Vespa orientalis* / prey-predator contest

1. INTRODUCTION

Massive defence behaviour is a predominant life trait of social bees. Honeybees face several predators during their cycle. Hornets (Hymenoptera, Vespidae) are particularly known to induce serious damages to apiaries by killing many individual honeybees or even by destroying entire colonies and occupying the beehive, using its resources (honey, pollen, brood and adult honeybees) to feed their brood (Morse, 1978; Ono et al., 1987, 1995; Abrol, 1994; Ken et al., 2005). The recent invasion of the Asiatic hornet *Vespa velutina* Lepeletier in France and its rapid dispersal (Villemant et al., 2006) may represent a threat to bee colonies. Fine-tuning coevolving prey-predator relationships allow coexistence among sympatric species, but in the case of recent invasion, such relationships may not have the time to develop. However, given the relatively high number of *Apis mellifera* subspecies and their large geographic range and habitat characteristics, a wide variety of behavioural responses are expected among them (Ruttner, 1988), including defensive behaviour. Collective anti-predator behaviour, which promotes colony safety, may be characterized by specific displays such as the so-called "curtain phenomenon", the "heat-balling" strategy and in some other cases the stinging behaviour (Breed et al., 2004). During

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such aggressive interactions, bees are also able to release alarm pheromones (i.e. isoamyl acetate, 2-heptanone) and to produce typical sounds, namely "hissing" or "shimmering", which may serve as alarms to deter intruder attacks and recruit defending bees (Lindauer, 1956; Schneider and Kloft, 1971; Fuchs and Koeniger, 1974; Ohtani and Kamada, 1980; Seeley et al., 1982; Kastberger and Sharma, 2000; Sen-Sarma et al., 2002). These signals show a 0.3–0.7 kHz fundamental frequency with harmonic bands extending up to 5 kHz.

In the endemic honeybee of Cyprus *A. m. cypria*, a specific and effective defensive behaviour against its main native predator, the Oriental hornet *V. orientalis* has been demonstrated (Morse, 1978). This behaviour consists of coordinated, collective defence behaviour against the attacking hornet that involves shimmering and balling. During conflict and, especially, during balling, Cyprian honeybees can induce the death of the predator through asphyxiation (Papachristoforou et al., 2007) and produce a very distinct hissing sound (Butler, 1954). Yet, no systematic acoustic documentation deriving from a large sample of recordings of this acoustic behaviour has ever been attempted. In particular, no comparison has been conducted to estimate its variability and to test for potential acoustic differences within and between colonies. To better understand the interactions between honeybees and one of their main predator and thus to help estimating the risks for apiculture, but also for biodiversity since such predators may have an important impact on ecosystems (Abrol, 1994), we studied in detail the high-frequency sounds produced by *A. m. cypria* when attacked by *V. orientalis*.

2. MATERIALS AND METHODS

The sounds produced by *A. m. cypria* were recorded in nine colonies in Cyprus (Amiantos-N34°53', E32°56'; Alassa-N34°46', E32°57'). These sounds included both defence hisses produced by guards and buzzes due to flying activity of workers. Experiments were conducted between the end of September and beginning of October 2006, which is the period of the most extensive flying activity and predation for hornets. Recordings were made with a Sennheiser ME64 microphone (frequency response: ± 2.5 dB between 0.04 kHz and 20 kHz) connected to a Marantz PMD 670 digital recorder (16 bit/44.1 kHz sampling frequency). The microphone was set up at the beehive entrance, 5–20 cm from the defending bees engaged in conflicts. To simulate attacks, hornets were collected in the apiaries and were anaesthetized by CO_2 for 5 seconds and then tied at the petiole to a stick with a 10 cm nylon wire to a stick. When hornets fully recovered, they were brought to the beehive flight board and exposed to colonies guards.

Sounds emitted during visual and physical contacts with hornets were analysed using Avisoft (Specht, 2004) and Seewave (Sueur et al., in press). Hisses were detected by computing sliding short-term Fourier transforms (Hamming window duration = 0.023 s, 87.5% of overlap). Hiss duration was measured with a 0.001 s precision, and frequency properties were described by computing Fourier transform in the middle of the sound (frequency precision = 11 Hz). The resulting frequency spectrum allowed measuring the fundamental frequency f_0 (i.e. the lowest frequency band) and the dominant frequency (i.e. the frequency band with the highest energy). Because the fundamental frequency was difficult to detect, it was indirectly estimated by measuring two successive harmonic peaks f_n and f_{n+1} and computing $f_{n+1} - f_n = (n + 1)f_0 - nf_0 = f_0$. The fundamental frequency of the background sound due to flying bees was estimated on spectrum computed immediately before or after the hiss.

Mean, standard deviation and inter-quartile-range (IQR) were calculated for each variable (hiss duration, hiss dominant frequency, hiss and buzz fundamental frequency) and the normality Shapiro-Wilk test was run before conducting parametric or non-parametric tests (one-way ANOVAs and Kruskal-Wallis tests, respectively). To assess potential differences between colonies, the ratio CV_w/CV_b was calculated for each parameter, where CV_w is the within-colony variation and CV_b is the between-colony variation (Sokal and Rohlf, 1995). All statistics were run using R (R Development Core Team, 2004).

3. RESULTS

During conflict with hornets, honeybees exhibited intense collective defence by engulfing and trapping the intruder in balls of 50–120 defending guards. Before the beginning or during

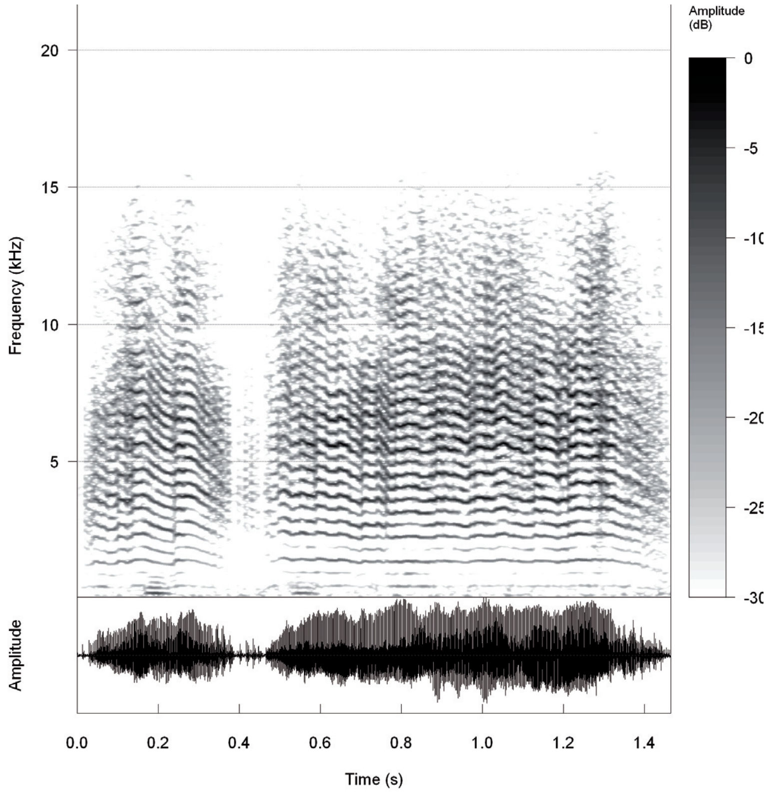

Figure 1. Two successive hisses produced during hornet attacks: spectrogram (frequency vs. time vs. retative amplitude in relative dB) and oscillogram (relative amplitude vs. time).

conflict, hissing was produced. The characteristic hiss was distinct from the background sound (buzz). A total of 93 hisses were studied with 10 ± 2.6 [7–15] hisses per colony (mean ± SD [min–max]). The sounds produced during hornets' attacks lasted 0.622 ± 0.708 s (IQR = 0.560) and were made of a harmonic series modulated in frequency (Fig. 1).

The fundamental frequency was 0.499 ± 0.067 kHz (IQR = 0.125) and the harmonic series included up to 30–35 harmonic bands. The frequency spectrum extended up to 16–17 kHz, with the 12th ± 4 (IQR = 4) harmonic band being the dominant frequency at 5.910 ± 1.428 kHz (IQR = 2.026) (Figs. 1, 2). Oscillograms of hisses showed regular succession of pulses while those of buzzes showed a sinusoidal-like waveform (Fig. 2). Sounds produced from nearby buzzing honeybees were continuous and not modulated in frequency. The dominant frequency was the fundamental frequency at 0.246 ± 0.024 kHz (IQR = 0.038, n = 80), a value significantly lower than the one observed for hisses (ANOVA, $F = 1029.1$, $P = 2.2 \cdot 10^{-16}$). Hisses were therefore remarkably different from the low-frequency buzzes (Fig. 2).

Multi-group tests revealed some acoustic heterogeneity among colonies (hiss duration: Kruskal-Wallis, $df = 8$, $\chi^2 = 20.18$, $P = 9.67 \cdot 10^{-3}$; hiss dominant frequency: ANOVA, $df = 8$, $F = 4.55$, $P = 1.24 \cdot 10^{-4}$; hiss fundamental frequency: ANOVA, $df = 8$, $F = 7.55$, $P = 1.5 \cdot 10^{-7}$; buzz fundamental frequency: ANOVA, $df = 8$, $F = 4.21$, $P = 3.58 \cdot 10^{-4}$; Fig. 3). However, the ratio CV_w/CV_b was always above one (1.82 for hiss duration, 1.49 for hiss dominant frequency, 1.26 for hiss fundamental frequency and 1.48 for buzz

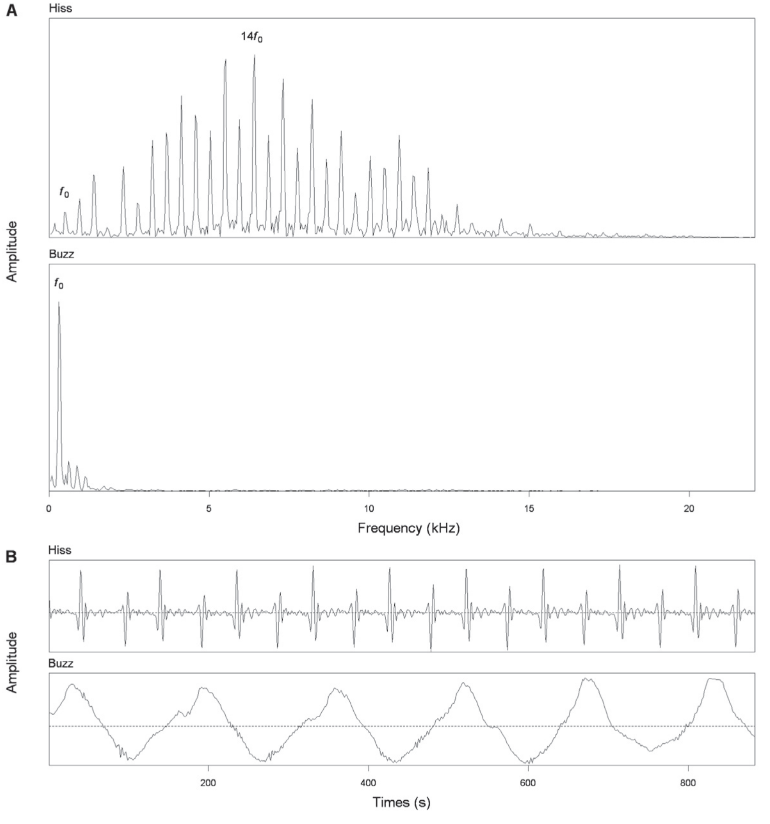

Figure 2. Comparison of hiss and buzz acoustic characteristics. (A) Typical spectrum (relative amplitude vs. frequency) of hiss and buzz For the hiss, the fundamental frequency (f_0) was at around 0.47 kHz and the dominant frequency (14 f_0) was at around 6.60 kHz. For the buzz, the fundamental frequency (f_0), which also was the dominant frequency was at around 0.3 kHz. Note that both spectra almost do not overlap, (B) temporal characteristics: hiss oscillogram show the regular succession of pulses while buzz oscillogram shows a sinusoid-like waveform. Both oscillograms are at the same time scale.

fundamental frequency) indicating the absence of a colony acoustic signature.

4. DISCUSSION

The dominant frequency of the hissing sounds emitted by *A. m. cypria* during attacks by *V. orientalis* appears to be unique amongst defence sounds previously described in social bees. When confronting *V. simillima* with *A. m. ligustica*, bee guards produce hissing sounds (Ohtani and Kamada, 1980) with a fundamental frequency of 0.5–0.7 kHz, associated with few, less prominent harmonics of around 1.5–2 kHz. Colonies of *A. cerana* (Schneider and Kloft, 1971; Fuchs and Koeniger, 1974) and *A. florea* (Seeley et al., 1982; Sen-Sarma et al., 2002) react to predators by respectively emitting sounds with a fundamental frequency of around 0.3 kHz and 0.5 kHz, and harmonics up to 3.6 kHz and 5 kHz. Foragers of *A. florea* react to potential predators by "pipping" at 0.33–0.48 kHz, triggering hissing by counterparts (fundamental frequency of 0.3 kHz and several harmonics in the kHz range) (Sen-Sarma et al., 2002). All the sounds previously described show frequency bands lower than the dominant frequency emitted by *A. m cypria* guards.

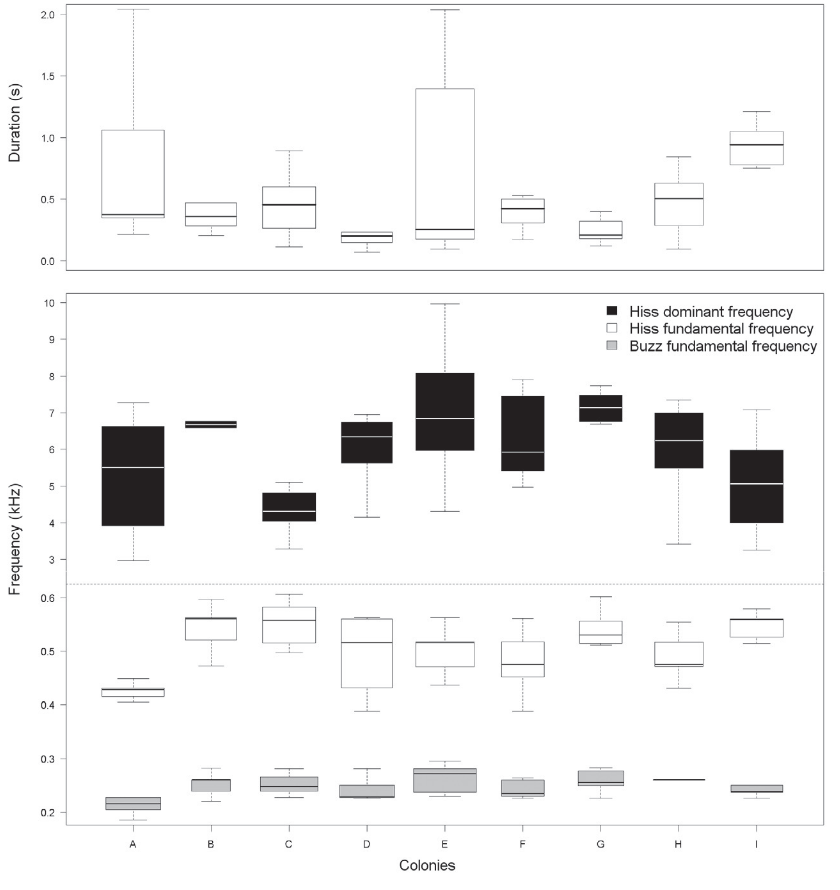

Figure 3. Comparison of acoustic parameters between 9 colonies (A-H: Amiantos, I: Alassa). Horizontal line = median, box length = inter-quantile-range, box width = proportional to the square-root of the number of observations, whiskers = range. Note the frequency scale break (dotted horizontal line).

Hissing sound might be a by-product of a stress-behaviour due to the intrusion of the hornet. In that case, it would not code for any information and could not be considered as a signal. However, the characteristics of the sound suggest some hypotheses about its function that could be experimentally tested in future research. First, the high frequency structure makes the sound very distinct from the background noise of the colony. As a result, the sound is transmitted through a "free channel" potentially avoiding any interference. Second, the frequency range of the sound is wide implying that various auditory systems tuned to different frequencies might detect and localize it. This is observed in distress, aposematic, and assembly alarm signals which cause dispersed receivers to move toward the sender (Bradbury and Vehrencamp, 1998). Third, the hissing emitters are defending honeybees usually standing on the flight board or at the hive entrance. What we recorded as airborne sound can actually be perceived as substrate-borne sound by the surrounding workers. Honeybees can receive airborne sound by detecting air-particle movements through the Johnston's organ located in the antenna pedicel (Dreller and Kirchner, 1993; Kirchner, 1994). A recent study has shown that this hearing system is only tuned to low frequency sound, around 250–300 Hz, corresponding to the frequency of the waggle dance sound (Tsujiuchi et al., 2007). However, honeybees can also receive sound through vibrations transmitted by the comb responding in that case to frequencies of up to 3 kHz (Michelsen et al., 1986). Such substrate-borne signals are detected by the sub-genual organ, a chordotonal sensor localized in the proximal part of the tibia of each leg, that shows a highest sensitivity around 500 Hz but might be stimulated by loud high frequency sound reaching 10 kHz (Kilpinen and Storm, 1997). All these data suggest that the comb vibratory channel could be a more suitable way to transmit hissing sound. As expected and observed in the alarm signals of several other animal groups (Bradbury and Vehrencamp, 1998), the hissing sound does not seem to be highly stereotyped and, in particular, is not colony specific. If directed to congeners, it can be received by all colony members.

The sound can also be sent to the hornet as an aposematic signal. Emission and reception of sounds, including high frequency sounds, play indeed an important role in the communication of hornets as it has been pointed out in extensive studies (Ishay, 1975; Ishay and Hochberg, 1979; Bergman and Ishay, 2007). Outstanding questions encompass the capacity of the putative receiver, the hornet, to receive the hissing sounds from a defensive colony, and in turn, the behaviour of the hornet to that signal.

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Les abeilles chypriotes, *Apis mellifera cypria*, émettent des sons à haute fréquence en présence de leur prédateur le frelon oriental, *Vespa orientalis*.

Apis mellifera cypria* / communication sonore / défense de la colonie / sifflement / relation prédateur-proie / *Vespa orientalis

Zusammenfassung – Von der zyprischen Honigbiene *Apis mellifera cypria* während der Abwehr gegen ihren Raubfeind, der orientalischen Hornisse *Vespa orientalis*, erzeugte hochfrequente Töne. Während der Abwehr von Angriffen durch die orientalische Hornisse *Vespa orientalis* zeigen die zyprischen Honigbienen *Apis mellifera cypria* ein spezifisches akustisches Verhalten, bei dem sie einen charakteristischen Zischlaut von unerwartet hoher Frequenz erzeugen. Dieser charakteristische Ton wird während der Annäherung des Räubers und während der Auseinandersetzung mit verteidigenden Bienen am Eingang der Kolonie hervorgebracht. Wir nahmen diese Zischlaute während einer ausgedehnten Serie künstlicher Angriffe von Hornissen auf und analysierten sie. Für die Aufnahmen verwendeten wir ein mit einem Marantz PMD 670 Digitalrekorder verbundenes Sennheiser ME64 Mikrofon. Die Aufnahmen wurden unter Verwendung der Avisoft und Seewave Software untersucht. Wir ermittelten, dass die Bienen ein weites Frequenzspektrum mit einer dominanten Frequenz um 6 kHz erzeugen können. Die Zischlaute zeigten ein bis zu 16–17 kHz ausgedehntes Spektrum, das bis zu 30–35 harmonische Bänder einschloss. Die Untersuchung von 93 an 9 Kolonien aufgenommenen verschiedenen Zischlauten ergab keine Hinweise auf akustische Kennzeichen der unterschiedlichen Kolonien. Insbesondere waren die akustischen Emissionen deutlich von den durch benachbarte fliegende Bienen hervorgebrachten Hintergrundgeräuschen abgesetzt. Diese Ergebnisse beinhalten eine detaillierte Beschreibung der von *A. m. cypria* bei der Verteidigung gegen Hornissen hervorgebrachten Töne und können in zukünftigen Untersuchungen zu einem besseren Verständnis der biologischen Bedeutung des akustischen Verhaltens bei der Nestverteidigung der Honigbienen beitragen.

Zischlaute / Kolonieverteidigung / *Apis mellifera cypria* / *Vespa orientalis* / Räuber-Beute-Beziehung

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