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Original article

Differences between queen piping temporal structures of two honeybee species, *Apis cerana* and *Apis mellifera*

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Abstract – In swarming season, honeybee queens emit two kinds of queen piping signals: tooting and quacking. These signals are considered to be important for swarming, and honeybees distinguish between the two signals through differences in their temporal structures. In this study, we revealed that the piping signals of two honeybee species, *Apis cerana* and *Apis mellifera*, had different temporal structures. The tooting of *A. cerana* consisted of almost one long syllable, while that of *A. mellifera* was constructed from several syllables. The quacking of both species comprised around 50 short syllables, but the duration and period of the syllables of *A. cerana* were about half those of *A. mellifera*. The findings provide new insights that reveal the mechanisms of signal discrimination and the functions of the signals.

Apis cerana **/ Queen piping / Communication / Swarming / Vibroacoustic**

1. INTRODUCTION

Social insects such as bees and ants use vibrations and airborne sounds as methods of communication (Kirchner 1997; Hunt and Richard 2013; Hepburn et al. 2014). These vibroacoustic communications are very important, especially for cavity-nesting honeybees such as *Apis cerana* and *Apis mellifera*, which live in dark hollows. Worker bees use many kinds of vibroacoustic communication related to foraging and swarming (Kircher 1993; Seeley and Tautz 2001; Schneider and Lewis 2004; Hrncir et al. 2005; Nieh 2010; Seeley et al. 2012; Tan et al. 2016). Queen bees also perform vibroacoustic communications, called queen piping, in the swarming season when new queens emerge, that were first described more than 200 years ago (Huber 1792). Although the mechanisms for triggering the preparation of swarming have not been fully described, several factors are known to induce a colony to prepare to swarm (Winston 1987; Grozinger et al. 2014), for example, colony size (comb area, worker population), brood nest congestion, worker age distribution (high population of young workers), reduced transmission of queen pheromones, and resource abundance (nectar, pollen). When a colony begins to prepare for swarming, worker bees construct multiple (5–20) queen cells (specially shaped, downward-facing cells for rearing queens) and rear new queens (Winston 1987; Grozinger et al. 2014; Smith et al. 2014). Before a new queen emerges from her cell, many workers (about half of all workers) and the mother queen leave the nest in a "prime swarm." On the same day or a few days later, a new queen emerges, and several days after that, some of the remaining workers may leave the nest with a newly emerged virgin queen

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in a secondary swarm (an "afterswarm"). This swarming behavior involving virgin queens may be repeated several times (Winston 1987; Seeley and Tautz. 2001; Grozinger et al. 2014). After that, the emerged queen, who remains in the nest, kills the other queens in the cells. If another queen emerges, the remaining queen and the newly emerged queen fight until one dies (Winston 1987; Gilley and Tarpy 2005), and the surviving queen takes over the parental colony. Queen piping is performed from after the prime swarm to the end of the final afterswarm (Michelsen et al. 1986; Grozinger et al. 2014). These phenomena, swarming and emitting queen pipings, occur in both species, *A. mellifera* and *A. cerana*.

Young queens produce two kinds of queen piping signals (Huber, 1792): tooting and quacking. Tooting is emitted by a queen who has newly emerged from her cell, whereas quacking is used by queens who are confined to their cells. The production, transmission, and biological significance of the signals have been studied in *A. mellifera*. Queens generate the piping signals by vibrating their flight muscles (Kirchner 1997; Hrncir et al. 2005); the signals are transmitted through the air and substrates in the nest and can be observed as substrate vibrations and airborne sounds (Kirchner 1997; Hrncir et al. 2005; Hunt and Richard 2013). The following phenomena and hypotheses of queen piping have been reported. The tooting signal frequently induces the quacking signal (Michelsen et al. 1986; Kirchner 1997); it also freezes worker movements and causes a delay in the emergence of confined queens (Fletcher 1978; Bruinsma et al. 1981; Grooters 1987; Gilley 2001). Furthermore, queens who emit tooting very frequently tend to kill many more rivals and survive longer (Schneider et al. 2001; Schneider and DeGrandi-Hoffman 2003; Long et al. 2017). A confined queen emits quacking and recruits workers to protect her cell from a tooter (Kirchner 1993). Moreover, Visscher (1993) argued that a tooter may use quacking to evaluate the number and strength of her competitors and, thus, the risk of fighting and taking over the colony instead of leaving with an afterswarm. These studies showed that queen piping may have an important biological role in swarming, but this has not yet been fully clarified.

The acoustical properties of queen piping signals of *A. mellifera* have also been studied for many years. Woods (1956) developed a sound recording system and found that piping consisted of a series of trains, and the fundamental piping frequency was approximately 350 Hz. Wenner (1964) analyzed piping signals using a sound spectrograph, which indicated that the tooting signal begins with a long syllable and several shorter syllables with a fundamental frequency of approximately 500 Hz, and the quacking signal is a series of short pulses with a lower fundamental frequency than tooting. Michelsen et al. (1986) measured the vibrations from tooting and quacking in the substrate of an observation hive using a laser vibrometer and, for the first time, revealed quantitative aspects of their characteristics. Tooting and quacking signals comprise a train of pulses (syllables). The first syllable of tooting lasts more than 1 s and is followed by a variable number of syllables lasting around 0.25 s each; the tooting syllables also show long rise times. Quacking comprises several syllables, each of which is less than 0.2 s in duration. The frequencies of both piping signals comprise one fundamental and several harmonic components. The fundamental frequency of tooting is between 350 and 500 Hz, and a large frequency sweep is present at the beginning of each syllable. Conversely, the fundamental frequency of quacking is between 200 and 350 Hz and is nearly constant.

Compared with *A. mellifera*, it is much more difficult to investigate or even record the piping signals of *A. cerana* because *A. cerana* can easily escape from an observation hive.

Otis et al. (1995) recorded tooting sounds from an opened hive and published the spectrograms. Tooting comprised only one syllable of 4.3 ± 2.1 s, and there were only three frequencies of around 2.7 kHz, 3.7 kHz, and 4.5 kHz in the spectrogram. These frequencies were very high compared with the fundamental frequency of *A. mellifera* tooting, and they did not show the time waveforms of tooting. Furthermore, the researchers did not record any quacking. Therefore, the acoustic characteristics of queen piping of *A. cerana* are still uncertain.

The purpose of this study was to quantitatively characterize *A. cerana* queen piping signals and to compare them with those of *A. mellifera*. We

developed a long-term observation method through which the queen piping signals of *A. cerana* could be recorded with a high signal-to-noise ratio in near-natural hive conditions. Honeybees respond to tooting and quacking with different behaviors (see above paragraph), although the two signals have similar fundamental frequencies. In addition, Michelsen et al. (1986) found that an artificial tooting signal without a frequency sweep induced quacking. Honeybees mainly distinguish between the two kinds of queen piping signals by making use of differences in the temporal structures, which include syllables with unique durations and intervals (Kirchner 1997; Hepburn et al., 2014). Therefore, the differences in the temporal structures of the piping signals used by the two species should be clarified so that the mechanisms of signal discrimination and the functions of queen piping can be investigated.

2. MATERIALS AND METHODS

2.1. Colonies

We used one swarm for each of the two species: *A. cerana japonica* and *A. mellifera ligustica*. In general, *A. cerana japonica* swarms between March and June in Japan. The experimental swarm of *A. cerana*, which left the parental nest in the observation field (Kawachinagano, Japan) on March 28, 2014, was trapped and kept in an empty hive box (without frames) and then the box was hung under the roof of a house. After 20 days, all walls and the floor of the box were removed, that is, only the lid was left under the roof. Using this procedure, an "artificial open nest" was built, which allowed us to make detailed observations of the "inhive" behavior of the honeybees. The observation field was in a rural area with many flowering plants and trees. Workers could forage freely, and the number of workers in the nest increased daily. Subsequently, queen cells were built and workers eventually swarmed with the mother queen at 11:00 on May 7, 2014. Figure 1a shows the artificial open nest 1 day after swarming. Altogether, there were three queen cells (C1, C2, and C3) in the nest. The queen in cell C1 emerged on May 10 and the virgin queen from C1 left with an afterswarm at 09:30 on May 14. The queen in C2 emerged on May 14. Cell C3 was destroyed, and the queen in C3 was killed. The *A. mellifera* nest was installed in a Langstroth-type hive at the apiculture company (Akitayahonten Co. Ltd., Gifu, Japan) and set in the observation field of the School of Human Science and Environment, University of Hyogo (Himeji, Japan), in March 2018. The observation field contained many flowering plants. The number of workers in the nest increased and several queen cells were built. Workers swarmed with the mother queen, after which the workers and virgin queens left the nest in several afterswarms (not recorded). Eventually, one surviving queen took over the parental nest.

2.2. Sound recording

The queen piping signals of *A. cerana* were recorded using a 10-mm diameter FM wireless condenser microphone (NT-7, EK Japan, Dazaifu, Japan) and a resin pipe (10-mm inner diameter and 60-mm length) to connect the microphone to the hive. The microphone was installed about 90 mm under queen cell C2. The distance between the cell and the top of the pipe was 30 mm. FM radio waves were received by a commercial radio receiver, and the audio signals were digitalized by a personal computer (p6320jp, HP Japan, Tokyo, Japan) and stored on hard disk. The piping signals of *A. mellifera* were recorded using a 10-mm diameter condenser microphone (ME52W, Olympus, Tokyo, Japan) through a resin pipe (10-mm inner diameter and 20-mm length), which was installed at a meshed ventilation window of the hive box (unlike with *A. cerana*, the microphone was not placed near the queen cell). The hive sounds were recorded using an IC recorder (ICD-PX470F, Sony, Tokyo, Japan).

2.3. Data analysis

We analyzed the sounds made by *A. cerana* recorded from the day when the queen emerged from cell C1 (May 10, 2014) to the day when she left the nest (May 14, 2014). Ten tooting (emitted by the virgin queen that emerged from C1) and 10

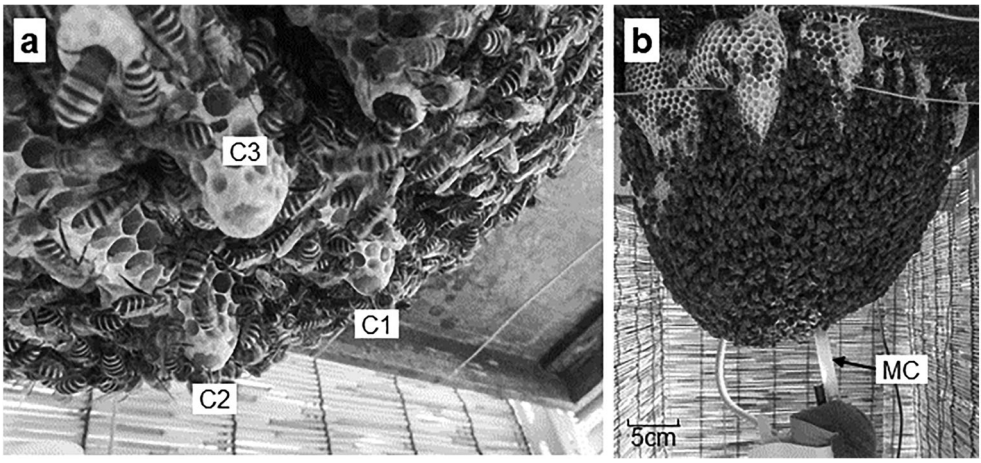

Figure 1. An artificial open nest. **a** There were three queen cells (C1-C3) in total on May 8, 2014, the day after mother queen swarming, and there were few worker bees because half of the worker bees had left with the mother queen at 11:00 on May 7. The queen in cell C1 emerged on May 10 and left the nest at 09:30 on May 14 in the second swarming. **b** View of the whole artificial open nest on May 12. The number of worker bees increased from May 8. *MC* microphone with a resin pipe connecting it to the hive

quacking signals (emitted by the confined queen in C2) were randomly sampled from a period of frequently emitted sounds. A 150-Hz to 5-kHz band-pass filter was applied to each signal. The beginning and termination times of the tooting and quacking syllables were measured using the sound editing software Sound Organizer 1.6 (Sony). The following parameters were calculated for the tooting signals: the duration of a whole tooting, the number of syllables in a tooting, the duration of each syllable, the percentage duration of the first syllable to the tooting period (the duration of the first syllable divided by the duration of the whole tooting signal), and the intervals between successive syllables. The quacking signal is a train of syllables of similar duration and interval. Therefore, the following parameters were calculated for quacking: the duration of an entire quacking period, the number of syllables in a quacking, the period of the syllables (the duration of an entire quacking period divided by the number of syllables), and the duration of each syllable (calculated using one randomly sampled quacking signal). The frequencies were analyzed using FFT software, RH1FFT 3.01 (RH1 Laboratory, Japan). RH1FFT 3.01 was also used to draw the time waveforms and spectrograms.

Similarly to *A. cerana* , we analyzed the sounds made by *A. mellifera* recorded between May 30 and June 1, 2018 (after the prime swarm). Ten tooting and ten quacking signals were randomly sampled (the tooter and quacker could not be identified). These piping signals had a lot of background noise, such as buzzing, owing to the distance between the microphone and the piping queen. Therefore, a 700-Hz to 5-kHz band-pass filter was applied to the signals to increase the signal-to-noise ratio. After confirming that the filter did not affect the temporal structure by comparing two *A. cerana* sound waveforms through the 700-Hz to 5-kHz and the 150-Hz to 5-kHz band-pass filters, the beginning and termination times of the *A. mellifera* syllables were measured. Then, the temporal parameters described above were calculated using the same methods for the piping signals of *A. cerana* .

2.4. Statistical analyses

Before performing statistical analyses, normality testing of the data was performed using the Jarque–Bera test for all temporal parameters. Because the number of tooting syllables did not follow a normal distribution, the Wilcoxon rank sum test was used. For other temporal parameters

that followed a normal distribution, the Welch's *t*-test was used.

3. RESULTS

3.1. Tooting signal

Using the surveying data for *A. cerana*, we determined that tooting began about 1 day before swarming, the occurrence of which increased to around 10 times every 10 min for approximately 6 h before swarming and stopped after swarming. The tooting signal began with a very long syllable followed by extremely short syllables with small amplitudes (Figure 2a). The long syllable had about 0.5 s of rising time. All tooting signals had the same temporal structure, although the number of syllables (including null) and the duration were slightly varied (details are described later). In comparison, the tooting signal of *A. mellifera* comprised a long syllable followed by short syllables (Figure 2b), the difference between the first syllable and the following syllables was smaller than in *A. cerana*. Each syllable had a rising time. All the tooting signals of *A. mellifera* also had the temporal structure shown in Figure 2b.

The temporal structures of the two species' signals were quantitatively compared. There was no significant difference between the two species (two-tailed *t*-test, $p = 0.077$) with regard to the duration of a whole tooting: *A. cerana* lasted 4.30 ± 0.83 s and *A. mellifera* lasted 4.98 ± 0.79 s (mean ± SD) (Figure 3a). However, there were significantly fewer syllables in the tooting of *A. cerana* (median 2.0) (Wilcoxon signed-rank test, $p < 0.001$) compared with that of *A. mellifera* (7.5 syllables) (Figure 3b). Furthermore, the first syllable in *A. cerana* was very long (4.04 ± 0.67 s) and the others were extremely short (0.06 ± 0.03 s) (mean ± SD after the second syllable) (Figure 3c), while the first syllable in *A. mellifera* was 2.10 ± 0.34 s and the following syllables had durations from 0.43 ± 0.09 s (mean ± SD in the second syllable) to 0.15 ± 0.06 s (mean ± SD in the ninth syllable) (Figure 3c). The first syllable in *A. cerana* was significantly longer than that of *A. mellifera*, by more than 1.6 times (one-tailed *t*-test, $p < 0.012$). The difference in the percentage duration of the first syllable to the entire tooting period showed even greater variation between the two species than was seen for the difference between the duration of the first syllables. The first syllable of *A. cerana* (94.7 ± 4.5%) was more than 1.7 times the length of that of *A. mellifera* (42.9 ± 8.4%) (one-tailed *t*-test, $p < 0.001$). The interval between successive syllables was also investigated. The intervals of *A. cerana*, 0.08 ± 0.05 s, were significantly shorter than those of *A. mellifera*, 0.18 ± 0.04, (mean ± SD) (one-tailed *t*-test, $p < 0.002$) (Figure 3d).

Michelsen et al. (1986) reported that a large frequency sweep was present at the beginning of the tooting syllables of *A. mellifera* and was characteristic of the tooting signal. A sweep from 375 to 410 Hz in the fundamental frequency was also present in the first 1 s of the first syllable of *A. cerana* (Figure 4b, black bar).

3.2. Quacking signal

Quacking of *A. cerana* also began around 1 day before swarming; the occurrence increased to around 10 times per 10 min for approximately 6 h before swarming, and quacking stopped after swarming. Not all, but many, quacking signals were induced by tooting. All quacking signals had the same temporal structure as is shown in Figure 5a, in which short syllables continued at a constant period. All quacking signals of *A. mellifera* also comprised many short syllables with a constant period (Figure 5b).

We compared the temporal parameters, and although the number of syllables varied widely, there was no significant difference between *A. cerana* (46.9 ± 15.4) and *A. mellifera* (49.3 ± 15.3) (Figure 6a, two-tailed *t*-test, $p = 0.73$). However, the duration of an entire quacking period of *A. cerana* (7.85 ± 2.39 s) was significantly shorter than that of *A. mellifera* (19.2 ± 6.12 s) (Figure 6b, one-tailed *t*-test, $p < 0.0001$). The period of the syllables in *A. cerana* (0.17 ± 0.01 s) was less than half of that in *A. mellifera* (0.39 ± 0.01 s) (Figure 6c, one-tailed *t*-test, $p < 0.0001$). The durations of each syllable in *A. cerana* and *A. mellifera* were 0.07 ± 0.01 s ($n = 61$) and 0.17

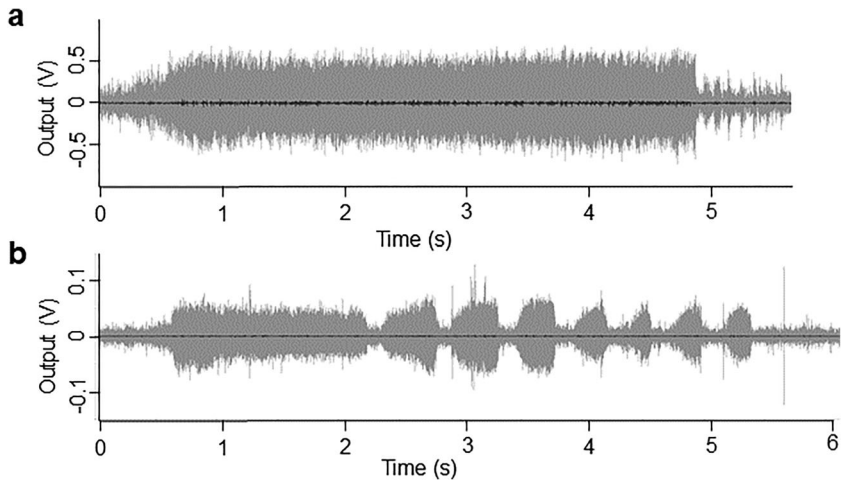

Figure 2. Time waveform of representative tooting. **a** Tooting signal of *A. cerana* after passing through the 150-Hz to 5-kHz band-pass filter; the signal comprised a long syllable of around 4 s and some smaller syllables shorter than 0.1 s. The long syllable had an approximately 0.5 s rise time. **b** Tooting signal of *A. mellifera* after passing through the 700-Hz to 5-kHz bandpass filter; the signal comprised a long syllable of around 2 s followed by short syllables. Each syllable had a rising time

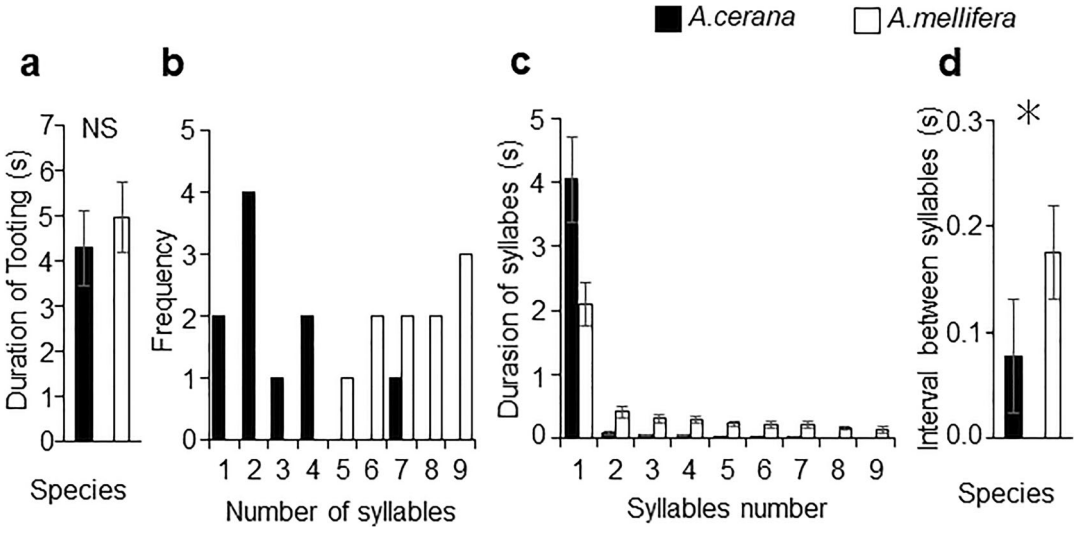

Figure 3. Temporal parameters of tooting in the two species. The black and white bars indicate *A. cerana* and *A. mellifera*, respectively. **a** Duration of a whole tooting. *A. cerana* lasted 4.30 ± 0.83 s, and *A. mellifera* lasted 4.98 ± 0.79 s (mean ± SD). There was no significant difference between the two species. **b** Histogram of the number of syllables; the median syllable numbers were 2.0 for *A. cerana* and 7.5 for *A. mellifera*. **c** Duration of each syllable; the mean duration (seconds ± SD) of the first syllable of *A. cerana* (4.04 ± 0.67) was longer than of *A. mellifera* (2.10 ± 0.34); the mean percentage (± SD) duration of the first syllable to the tooting period of *A. cerana* (94.7 ± 4.5%) was much longer than that of *A. mellifera* (42.9 ± 8.4%). **d** Interval between successive syllables; *A. cerana*, 0.08 ± 0.05 s, were significantly shorter than those of *A. mellifera*, 0.18 ± 0.04, (mean ± SD). Asterisk: significant difference ($p < 0.002$), *NS* no significant difference

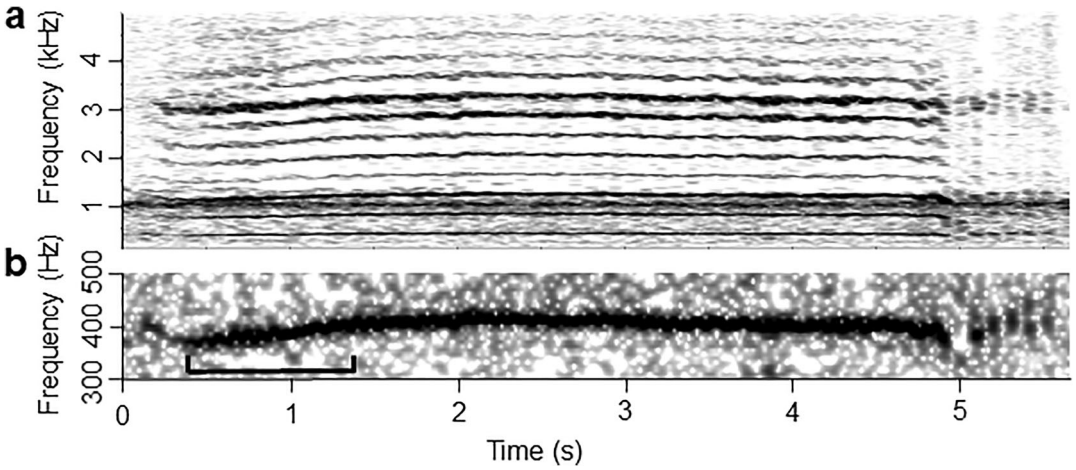

Figure 4. Spectrogram of representative tooting of *A. cerana*. **a** The tooting sound comprised a fundamental frequency (around 400 Hz) and many harmonics. **b** Expanded spectrogram between 300 and 500 Hz; a frequency sweep from 375 to 410 Hz is present during the first 1 s of the first syllable (black bar)

± 0.03 s (n = 31), respectively (Figure 6d). The duration of each syllable of *A. cerana* was also less than half of that of *A. mellifera* (one-tailed t-test, $p < 0.0001$). Consequently, the duration of an entire quacking period of *A. cerana* was less than half of that of *A. mellifera* because the

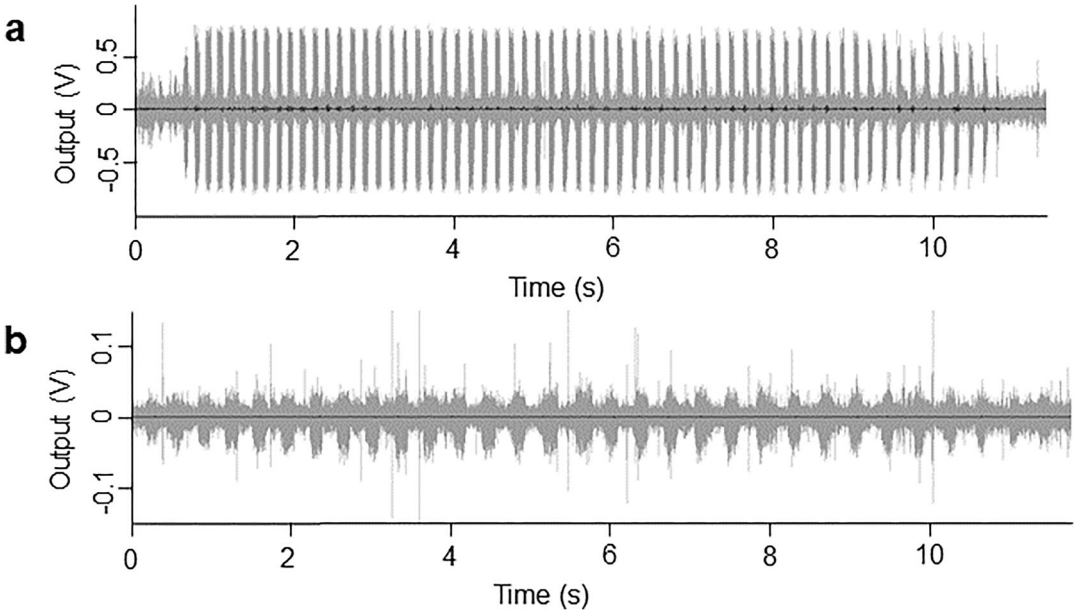

Figure 5. Time waveform of representative quacking. **a** Quacking signal of *A. cerana* after passing through the 150-Hz to 5-kHz band-pass filter; the signal comprised short syllables with a constant period. **b** Quacking signal of *A. mellifera* after passing through the 700-Hz to 5-kHz band-pass filter. The signals of both species comprised many short syllables with a constant period, but the tempo of *A. cerana* quacking was faster than that of *A. mellifera*

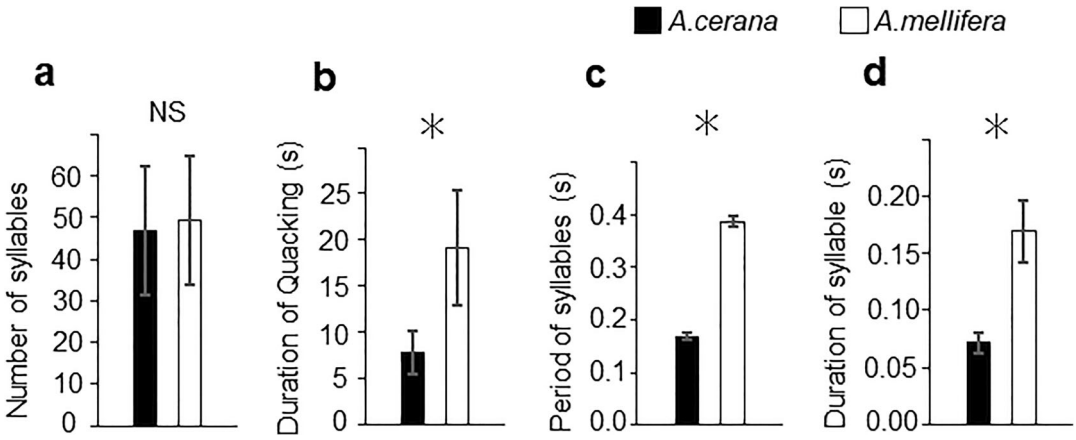

Figure 6. Temporal parameters of quacking in the two species. The black and white bars indicate *A. cerana* and *A. mellifera*, respectively. **a** Number of syllables; there was no significant difference between the two species. Error bars indicate standard deviations. **b** Duration of an entire quacking period; the mean duration (seconds ± SD) of *A. cerana* (19.2 ± 6.12) was significantly shorter than that of *A. mellifera* (7.85 ± 2.39), that is, less than half. **c** Period of syllables; the mean period (seconds ± SD) of *A. cerana* (0.17 ± 0.01) was significantly shorter than that of *A. mellifera*. (0.39 ± 0.01), that is, less than half. **d** Duration of a single syllable; the mean duration (seconds ± SD) of *A. cerana* (0.07 ± 0.01, $n = 61$) was also significantly shorter than that of *A. mellifera* (0.17 ± 0.03, $n = 31$), that is, less than half. Asterisk: significant difference ($p < 0.0001$), *NS* no significant difference

syllable periods and durations of *A. cerana* quacking were less than half of those for *A. mellifera*, although the numbers of syllables were comparable.

4. DISCUSSION

Michelsen et al. (1986) and Kirchner (1993) measured the piping signals of *A. mellifera carnica* queens using a laser vibrometer. They reported that the tooting signal comprised a first syllable lasting over 1 s and other syllables of around 0.25 s, and the signal was characterized by a frequency sweep and a rise in amplitude at the beginning of each syllable. In the present study, we used another subspecies, *A. mellifera ligustica*, and found that its tooting signals had the same temporal structures and characteristics (a frequency sweep was confirmed by listening to the sounds) as those of *A. mellifera carnica* (Fig. 2b). The tooting signal of *A. cerana* certainly had the same characteristics (a frequency sweep and a rise in amplitude at the beginning of the syllable) as *A. mellifera carnica*, but its temporal structures differed (Fig. 2). There were very few syllables in the tooting of *A. cerana* (Figure 3b). Furthermore, the percentage duration of the first syllable to the tooting period was much higher than that of *A. mellifera ligustica* (Figure 3c).

Otis et al. (1995) reported that the tooting of *A. cerana* comprised only one syllable of 4.3 ± 2.1 s (mean ± SD) and that it had three frequencies of approximately 2.7 kHz, 3.7 kHz, and 4.5 kHz. The measured sounds may have had only one syllable and very high frequencies for the following possible reasons: (1) it is difficult to measure the succeeding syllables because the amplitudes are very small and the duration extremely short (Figure 2a) and 20% of tooting has only one syllable (Figure 3b) and (2) it is difficult to measure the lower frequencies because the higher frequency sounds are amplified more than the lower frequency sounds in the hive (Michelsen et al. 1986).

Michelsen et al. (1986) and Kirchner (1993) also reported that the quacking signal of *A. mellifera carnica* comprised several syllables that were shorter than 0.2 s. The quacking signals of *A. mellifera ligustica* and *A. cerana* had the same temporal structures, but the tempo of

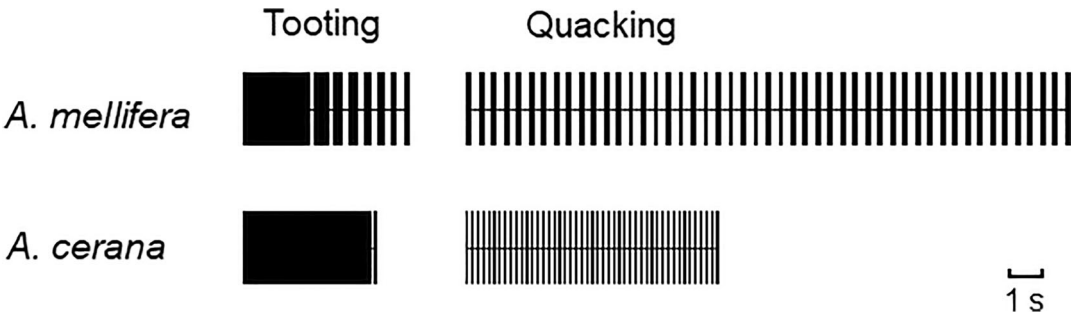

Figure 7. Schematic of the temporal patterns of tooting and quacking of *A. mellifera* and *A. cerana* from our results. The amplitudes of all syllables were set to 1 for convenience. The definitive difference between tooting and quacking is the length of the first syllable. Indeed, the temporal structure of *A. mellifera* tooting after the second syllable is similar to the temporal structure of *A. mellifera* quacking. However, the second and subsequent syllables of *A. cerana* tooting are extremely short

A. cerana was different. The period and the duration of the syllables were much less than half those of *A. mellifera* (Figure 6c, d).

The temporal structure of a signal has an important role in sound and vibration communications in many animals. Courtship and calling songs have different temporal structures in stink bugs, *Drosophila*, and Ensifera, (Ewing 1983; Bennet-Clark 1989; Čokl 2008). In both *A. mellifera* and *A. cerana*, the definitive difference between tooting and quacking is the length of the first syllable, which is a great deal longer in tooting than in quacking (Figure 7). However, Michelsen et al. (1986) found that an artificial tooting signal, which had a similar temporal structure to *A. mellifera* tooting after the second syllable, that is, six syllables with an approximate duration of 0.25 s and period of 0.5 s, induced quacking. The period of *A. mellifera*'s tooting after the second syllable was 0.43 ± 0.06 s, which is not significantly different from the period of *A. mellifera* quacking, that is, 0.39 ± 0.01 s (two-tailed t-test, $p = 0.072$). In addition, when one queen starts quacking, other queens will gradually join the "quacking concert" (Michelsen et al. 1986). Therefore, queens who emit quacking in response to an artificial signal might perceive the signal to be quacking. It would be interesting to conduct an experiment to verify if one long syllable of a tooting signal induces quacking in the two species.

The queen quacks when she is in her cell, and she changes her signal to tooting after emerging. She produces a tooting signal by activating the wing muscles without wing movement while grasping and pressing her thorax against a substrate (Kirchner 1993, Hunt and Richard 2013). When she is in her cell, it is likely that she cannot grasp a substrate and press her thorax against it, as a result, she activates her muscles in a pulsating manner and produces a train of pulsing

Table I. Comparison of piping temporal parameters of *A. cerana*, *A. koschevnikovi*, and *A. mellifera* (mean ± SD)

Species	Duration of the first syllable in a tooting (s)	Percentage duration of the first syllable to the tooting period (%)	Period of syllables in a quacking (s)
A. cerana	4.04 ± 0.67	94.7 ± 4.8	0.17 ± 0.01
A. koschevnikovi *	4.3 ± 0.6	100	0.23
A. mellifera	2.04 ± 0.34	42.9 ± 8.4	0.39 ± 0.01

* The parameters of *A. koschevnikovi* are from Otis et al. (1995)

vibrations, that is, quacking, because of the difficulty of continuous activation without using her legs to support her body. This hypothesis could be tested by observing whether an emerged queen reverts to emitting quacking signals when she is re-confined within a queen cell. Furthermore, in our measurements, the frequency of occurrence of short syllables after the first long syllable in *A. cerana* tooting tended to decrease with time after emergence. From this, we postulated that these short syllables might be a relic of quacking.

A quantitative comparison of the temporal parameters should provide some insights into the evolution of queen piping. Some possible key parameters (the duration of the first syllable of tooting, the percentage duration of the first syllable to the tooting period, and the period of syllables in quacking) of three cavity-nesting honeybees *A. cerana*, *A. koschevnikovi*, and *A. mellifera* are shown in Table I. When a phylogenic tree was constructed using mitochondria DNA, it showed that *A. mellifera* is distant from both *A. cerana* and *A. koschevnikovi* (Arias and Sheppard 2005). The temporal parameters of *A. cerana* and *A. koschevnikovi* are very similar, while those of *A. mellifera* differ from the others. Moreover, in *A. cerana* and *A. koschevnikovi*, tooting is clearly different from quacking; however, *A. mellifera* tooting is similar to its quacking, that is, the first syllable of tooting is short and the subsequent syllables after the second syllable are similar to quacking (Figure 7). Queens and workers distinguish between the two kinds of signals mainly via differences in the temporal structure (Kirchner 1997; Hepburn et al. 2014). Therefore, it would be interesting to investigate why these differences evolved between *A. mellifera* and the group containing *A. cerana* and *A. koschevnikovi*.

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AUTHOR CONTRIBUTION

TY, MS, RO, and HI conceived this research and wrote the paper. TY designed and performed the experiments and analyzed all data. TY, MS, RO, and HI interpreted the data. TY, RO, and HI read and approved the final manuscript.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPLIANCE WITH ETHICAL STANDARDS

Competing interests The authors have no conflicts of interests to declare that are relevant to the content of this article.

Ethical approval Not applicable.

Différences entre les structures temporelles du chant des reines de deux espèces d'abeille, *Apis cerana* et *Apis mellifera*.

***Apis cerana* / reine / chant / communication / essaimage / vibroacoustique.**

Unterschiede im zeitlichen Ablauf beim Tüten und Quaken von Königinnen bei zwei Arten von Honigbienen, *Apis cerana* und *Apis mellifera*.

***Apis cerana* / Königin / Tüten / Quaken / Kommunikation / Schwärmen / Vibrationsakustik.**

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