

Research



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Towards acoustic monitoring of bees: wingbeat sounds are related to species and individual traits

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Global pollinator decline urgently requires effective methods to assess their trends, distribution and behaviour. Passive acoustics is a non-invasive and cost-efficient monitoring tool increasingly employed for monitoring animal communities. However, insect sounds remain highly unexplored, hindering the application of this technique for pollinators. To overcome this shortfall and support future developments, we recorded and characterized wingbeat sounds of a variety of Iberian domestic and wild bees and tested their relationship with taxonomic, morphological, behavioural and environmental traits at inter- and intra-specific levels. Using directional microphones and machine learning, we shed light on the acoustic signature of bee wingbeat sounds and their potential to be used for species identification and monitoring. Our results revealed that frequency of wingbeat sounds is negatively related with body size and environmental temperature (between-species analysis), while it is positively related with experimentally induced stress conditions (within-individual analysis). We also found a characteristic acoustic signature in the European honeybee that supported automated classification of this bee from a pool of wild bees, paving the way for passive acoustic monitoring of pollinators. Overall, these findings confirm that insect sounds during flight activity can provide insights on individual and species traits, and hence suggest novel and promising applications for this endangered animal group.

This article is part of the theme issue 'Towards a toolkit for global insect biodiversity monitoring'.

1. Introduction

Bees (Hymenoptera: Apoidea) are considered the most important group of pollinators [1,2]. With over 20 000 species worldwide, these flying insects play a crucial role in ecosystem services, food security and sustainable development [3]. However, surveys in North America and Europe have reported negative trends in the population of bees and other relevant pollinators (e.g. syrphid flies) during the past decades [4,5], associated with different anthropogenic drivers, such as habitat loss and fragmentation, climate change or agricultural intensification [6–8]. This global decline calls for developing scalable, inexpensive and efficient methods to monitor behaviour and trends of bee communities.

Commonly studied in a variety of vertebrate and invertebrate species, animal sounds are a well-established source of ecological information [9] that can reveal species presence, abundance, position, body size or behavioural status [10]. Thus, passive acoustic monitoring (PAM) has become an emerging technique that is used to record, store and analyse animal sounds in an automated, non-invasive way [11]. In combination with computational tools, PAM has proven to efficiently record animal activity for a wide variety of subjects, e.g. population density [12,13] and distribution [14], conservation [15] or climate change research [16,17]. PAM is typically aimed to record well-known species-specific acoustic signals that are emitted by specialized sound-producing structures and play a role in mating, resource defence or navigation [18].

In comparison with birds, bats or anurans (animal groups extensively studied with PAM; [11]), bees do not possess specialized sound-producing structures but emit incidental sounds that arise as a by-product of activities such as moving or eating [19], e.g. a diverse suit of pitches generated by rhythmic thoracic oscillations (see review by [20]). Some of the most well-known examples of bee sounds are emitted during different behaviours of social species, such as honeybees, bumblebees or some stingless bees [20–23], and they are usually exclusive to certain families, genus or species (e.g. buzz pollination, see [24]). There is, however, one sound type found across all bee species: the flight buzz.

Bees' flight and its associated sounds have been theoretically studied since the 1960s [25–27]. More recently, applied studies have shown that wingbeat frequency and pattern during flight may be species-specific, and thus these could serve as a 'fingerprint' for automatic species classification [28,29]. However, most studies on this topic have been performed in laboratory settings, with a narrow number of species (e.g. only *Bombus sp.* or *Apis mellifera*), or without considering morphological (e.g. body size) and environmental (e.g. temperature) factors [30,31].

For PAM to be a reliable tool to remotely monitor bee communities, it is paramount to detect which are the most relevant factors affecting the variability of wingbeat sounds between and within species. Here we recorded and characterized wingbeat sounds of a variety of Iberian bees under field conditions and tested their relationship with species and individual traits. Particularly, we investigate four sources of inter- and intra-specific variation in sound frequency (taxonomy, morphology, temperature and behaviour) and hypothesize that: (i) each taxa shows a specific wingbeat acoustic signature; (ii) frequency of wingbeat sounds is negatively correlated with body size, and positively correlated with (iii) air temperature and (iv) induced stress. Thereby, we examine the information that can be retrieved from bees' wingbeat sounds of and assess the applicability of PAM to investigate bee diversity, morphology and behaviour through their sounds.

2. Material and methods

(a) Study area

The study was conducted in three farmlands located in Central Spain (Torrelaguna and La Cabrera, Madrid), between 600 and 1200 metres a.s.l., at the southern side of the mountain range Sierra de Guadarrama (see electronic supplementary material, figure S1 for farm pictures). These areas belong to the mesomediterranean bioclimatic layer, characterized by highly variable temperatures (mean annual temperatures between 12°C and 17°C) and moderate rainfall (annual precipitation of 500 mm; [32,33]).

(b) Sound recording and insect sampling

To record their sounds, we actively searched for bees in the three farmlands from May to July 2019, during a total of 8 sampling days. Fieldwork was conducted between 9:30 am and 1:30 pm in warm days, with clear sky, little wind and no precipitation. To increase the diversity of sampled species, the active search was oriented to maximize the selection of specimens with different characteristics, by *in-situ* observation of the size, colour and shape of the flying insects. Additionally, we focused on individuals of the European honeybee (*Apis mellifera*), the most abundant bee species in the study farmlands, to explore the intra-specific variability of their sounds. Each specimen was recorded under two treatments: (i) in natural conditions (NC) during free-ranging flight manoeuvres between flowers before capture; and (ii) subjected to an experimentally induced stress (IS) after capture, while being confined inside the entomological net.

We recorded wingbeat sounds of the focal bees with a directional microphone (ME-66 and module K6; Sennheiser) connected to a portable digital audio recorder (PMD-660 Marantz Professional). During recordings, the tip of the microphone was placed at a distance of 2–5 cm above the thorax of the insect to correctly register the sound without disrupting its behaviour ([34]; see electronic supplementary material, figure S2 for a graphic visualization of the recording protocol). To increase the signal-to-noise ratio, the audio gain of the recorder was manually regulated while continuously tracking sound amplitude. We used a shock-mount to avoid stand-borne noise, a windshield to reduce excessive pressure from wind and headphones to properly perceive the sound source. The recordings were stored as uncompressed .wav files and digitalized at a sampling rate of 48 kHz and a depth of 16 bits. All sound files were deposited in the Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales (MNCN-CSIC, Madrid, Spain).

During sampling, air temperature was measured with a datalogger (HOBO U23 Pro V2, ONSET), located at 20–30 cm from the floor in a shaded area. Every specimen was euthanized in a small plastic vial (33 cm³) with 70% alcohol to ensure their proper conservation. Individuals were then carefully dried and labelled for subsequent taxonomic identification and morphological measurement.

(c) Acoustic analysis

We created and visualized spectrograms of the recorded sounds using the Raven Pro v. 1.5.0. software (Cornell Lab of Ornithology; figure 1; electronic supplementary material, figure S3). An optimal spectrogram configuration was applied to all the recordings (window size: 4098; Overlap: 90%; window type: Hann), providing a high resolution of wingbeat sound frequencies. For each individual and treatment, a single audio with the best signal-to-noise ratio was selected and three acoustic parameters measured for both the fundamental harmonic (i.e. the lowest frequency of a periodic wave; [35]) and the second harmonic. These parameters were: duration (s), dominant frequency (Hz) and maximum energy (dB). To best characterize between- and within-bee variation in sound frequency throughout the individual displacement, we also measured dominant frequency and maximum energy in a short segment at the onset, centre

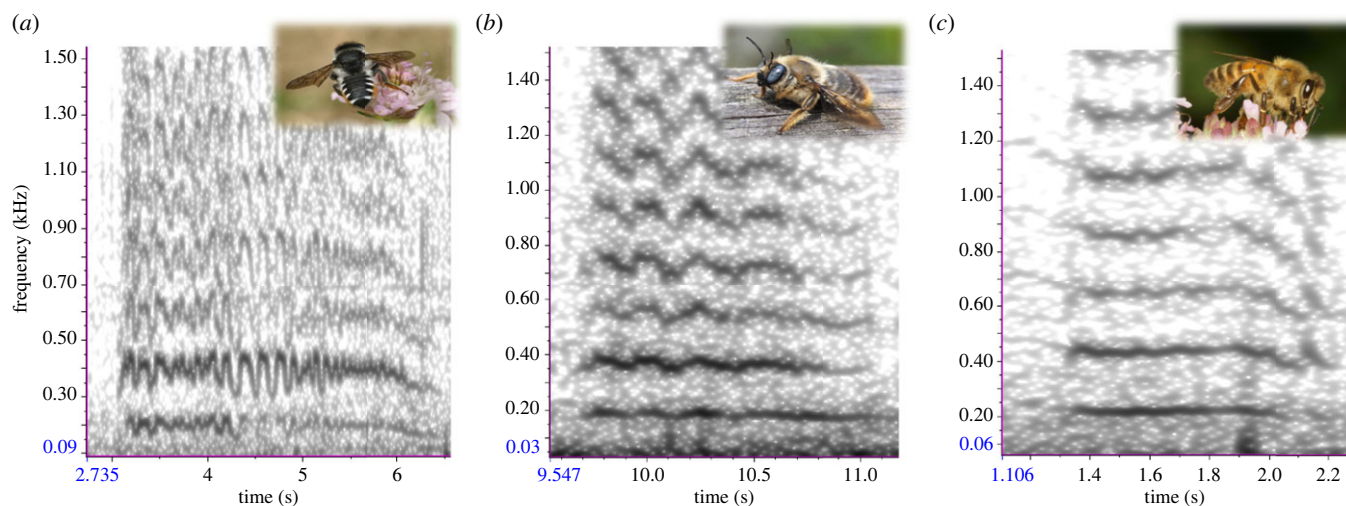


Figure 1. Spectrogram visualization of three collected individuals from different species: (a) *Megachile (Megachile) octosignata*, (b) *Xyllocopa cantabrita* and (c) *Apis mellifera*. Each spectrogram shows an example of (a) sharp, (b) moderate and (c) restricted frequency modulation. The spectral component observed at a frequency of 5 Hz in the third spectrogram corresponded to environmental noise.

and end of the selected audio (electronic supplementary material, figure S2). The duration of each segment corresponds to *ca* 10% of the duration of the entire audio. Based on these measurements, we calculated how often sound energy was higher at the fundamental harmonic than at the second one. By pairwise comparisons of energy in harmonics of the same segment, we thus estimated a within-bee percentage of audio segments with dominant frequency in the fundamental harmonic.

(d) Trait measurements

We measured two morphological traits from the focal specimens: the intertegular distance (ITD), defined as the minimum linear distance between both tegulae, measured over the thoracic dorsum [36], and the average forewings length (WL; electronic supplementary material, figure S4). Both morphological traits are considered good proxies for body size [37,38]. For these measurements, photographs of every specimen were taken over a millimetre scale, using a digital camera Canon EOS M10 (Canon, Tokyo, Japan) adjusted to an optical microscope Leica MZ6 (Leica, Wetzlar, Germany), ranging between 0.8x and 4x magnifications. The length in millimetres was measured with the software *Image J* [39]. When one of the two wings was deteriorated or absent, the average between two measurements of the other wing was calculated.

(e) Regression models

To examine factors that influence inter- and intra-specific variation in the dominant frequency of wingbeat sounds, we used general linear mixed-effect models (LMM), with Gaussian error structure, including dominant frequency as response variable, and morphological, environmental and behavioural factors as explanatory variables. Specifically, we fitted two models: (i) a LMM at inter-specific level, using all recorded individuals, and (ii) a LMM at intra-specific level, with only specimens from the most abundant species, the domestic bee (*A. mellifera*). In each model, we included seven fixed factors: WL (mm), ITD (mm), temperature ($^{\circ}\text{C}$), linear and quadratic terms, to account for potential linear and curvilinear relationships), experimental treatment (NC versus IS), audio segment (4 levels; entire, onset, centre and end), harmonic type (fundamental versus second) and family (Andrenidae, Apidae, Halictidae and Megachilidae). Additionally, we added individual identity as a random factor in both LMMs to account for repeated measures within individuals, and species and genus as random factors in the inter-specific model to evaluate the amount of variation explained by these factors. As ITD and WL were correlated at inter-specific level, we first fitted a linear regression with both variables at inter-specific level (\log_{10} -transformed to achieve linearity) and used WL and statistically controlled ITD (residuals of the regression) as explanatory variables in the LMM model to avoid collinearity. All continuous variables (WL, ITD and temperature) were centred and scaled (mean of 0 and standard deviation of 1) to facilitate model fitting.

We checked the assumptions of normality and homogeneity of the residuals by visually inspecting a quantile–quantile plot and the residuals against the fitted values, both indicating no deviation from these assumptions. We inspected model stability by excluding data points one at a time from the data. We derived variance inflation factors [40] using the function *vif* of the R-package *car* (v. 2.1-4; [41]) and they did not indicate collinearity between fixed effects to be an issue. We conducted all analyses in R using the *lmer* function of the R-package *lme4* (v. 3.1-139).

(f) Classification analysis

To visualize and test for inter-specific differences in wingbeat sounds, we created density scatter plots with dominant frequency and flight time as axes, and taxonomic entities as grouped variables. Among all measurements of dominant frequency, we chose the fundamental frequency at the centre of the recorded audio to avoid the Doppler effect (compared with onset and ending segments). Acoustic segregation between species, genus or families was then explored based on sound properties.

Additionally, we applied a machine learning framework to test if wingbeat sounds encode species-specific acoustic signatures that can be used for automated acoustic species identification. As a first approach, we focused the identification test on discriminating the wingbeat sounds produced by a domestic bee (*A. mellifera*) from those of wild bees (17 species). For this purpose, we only selected recordings of the first treatment (under natural conditions, NC) and with moderate and high signal to noise ratio ($\text{SNR} \geq 15$ dB). This led to a filtered database, including 18 bee species and 42 audio recordings, with a balanced distribution of classes (22 and 20 recordings from domestic and wild bees, respectively).

First, we characterized the audio samples in the spectral domain by computing the power spectrum. This spectral representation allowed us to compare visually the spectral differences between classes. To have tight representation of the spectral domain, we then

computed mel-frequency cepstral coefficients (MFCC). MFCCs have been used as the dominant features used for audio classification because they have the ability to deliver a compact representation of sounds with high harmonic content, such as speech and music [42]. In particular, we used 20 coefficients extracted between the frequency band where the wingbeat sounds and the harmonics were predominant (i.e. 0.1 and 5.0 kHz). The final predictor matrix included 20 MFCC coefficients and 42 observations. We measured binary classification performance using a Random Forest classifier (number of trees = 300, maximum features = 6) and computing the balanced accuracy metric in a stratified 10-fold cross-validation scheme. To assess whether the accuracy of the machine learning model was significantly better than expected by chance, we computed baseline accuracies using two dummy classifier strategies: majority and random. The majority strategy simulates a classifier always returning the most frequent class, which, in our case, is *Apis mellifera*. The random strategy generates predictions uniformly at random. The automated classification analyses were performed in Python 3, using the package *librosa* [43] for audio characterization, and the package *scikit-learn* [44] for fitting and evaluation of statistical classifiers.

3. Results

(a) Taxonomic and morphological traits

Overall, 73 bees were registered and captured, and 65 of them identified at species level (electronic supplementary material, table S1). The taxonomic identification revealed 27 species, belonging to 4 families: Apidae (42 individuals; 57%), Andrenidae (15; 21%) Halictidae (9; 12%) and Megachilidae (7; 10%). European honeybee (*Apis mellifera*) was the most commonly collected species, with 26 registered individuals (36%). Our dataset included 16 species and one subgenus, and one genus was represented by a single specimen. The average WL was 8.84 mm (± 2.39) and varied between the 2.73 and 17.73 mm, while the average ITD was 2.93 mm (± 0.97) and varied between 0.76 and 6.52 mm (electronic supplementary material, table S1). Temperature was 24.04°C (± 10.63) and ranged between 16.25 and 41.39°C.

(b) Bee sounds

Wingbeat sounds were analysed in 69 individuals under NC treatment and 71 individuals under IS treatment. These sounds were highly variable and characterized by a large number of harmonics (1–12), with sharp, moderate or restricted frequency modulation (figure 1). Sound energy of the harmonics typically decreases with frequency (i.e. lower harmonics having higher energy), although some individuals did not follow that pattern. Duration of bee flying between flowers ranged from 1.9 s (± 1.3) in wild bees to 2.1 s (± 0.8) in the European honeybee. The fundamental frequencies of sounds produced by this domestic bee (at the centre of the audio segment) were on average 222.4 Hz (± 21.4) and 251.3 Hz (± 15.9) under NC and IS treatments, respectively. In wild bees, the fundamental frequencies were on average 180.3 Hz (± 50.9) and 195.5 Hz (± 95.7). The fundamental harmonic was higher in energy than the second harmonic for 80% of the time for all observations, and 90% of the time for the IS treatment (electronic supplementary material, table S2). In general, the dominant frequency and patterns of frequency modulation were distinct across specimens and might be good candidate parameters for acoustic species identification (see electronic supplementary material, figure S5). The greatest intensity of background noise occurred between 0 and 70 Hz.

(c) Determinants of wingbeat sounds at inter- and intra-specific level

Using the full dataset ($n = 138$ individuals, 27 species), the first LMM model revealed that frequency of bee sounds was related to wing length bound to harmonic, as well as experimental treatment (NC versus IS) and air temperature (electronic supplementary material, table S3; figures 2 and 3). Lower frequencies were emitted by larger-sized bees and individuals subjected to higher temperatures, while stress was associated with high-pitched sounds. We also found significant differences in dominant frequency among the bee families and the audio segments within the recording (electronic supplementary material, table S3). Using the subset for the European honeybee ($n = 26$ individuals), the second LMM model indicated that the frequency of wingbeat sounds of this species was also associated with its behavioural status, with higher-pitched sounds under stress conditions (electronic supplementary material, table S4). We also found significant differences in dominant frequency among the harmonics and the audio segments within the recording (electronic supplementary material, table S4, figure 2).

(d) Inter- and intra-specific acoustic signatures

The power spectrum of wingbeat sounds of the European honeybee showed multiple consistent peaks at low frequencies (figure 4a) that were only masked by the background noise at higher frequencies. Conversely, the mixed set of wingbeat sounds of wild bee species were highly variable and did not show a clear signal (figure 4b). Our statistical analyses on the discrimination between these sounds showed that the Random Forest classifier had an average balanced accuracy of 0.77 ± 0.12 , over 10 cross-validation runs. The baseline accuracies obtained were 0.50 (± 0) for the majority strategy and 0.47 (± 0.21) for the random strategy, showing that our model's accuracy is significantly better than chance. The main classification errors (false positives and false negatives) were observed on the noisier samples. This suggests that the wingbeat sound of the European honeybee has a specific acoustic signature and machine learning could be used to automatically discriminate between domestic and wild bee species.

The recorded sounds were distinct in frequency and duration across genus and families, with some level of overlap between groups (figure 5). We found no significant differences in dominant frequency at the centre of the audio segment ($F = 0.11$; $df = 2$; $p = 0.89$) among the three taxonomic levels, calculated by the mean differences in frequency between pairs of species (mean \pm standard deviation: 57.6 Hz \pm 43.6), genus (59.9 Hz \pm 44.3) and families (58.3 \pm 43.8 Hz).

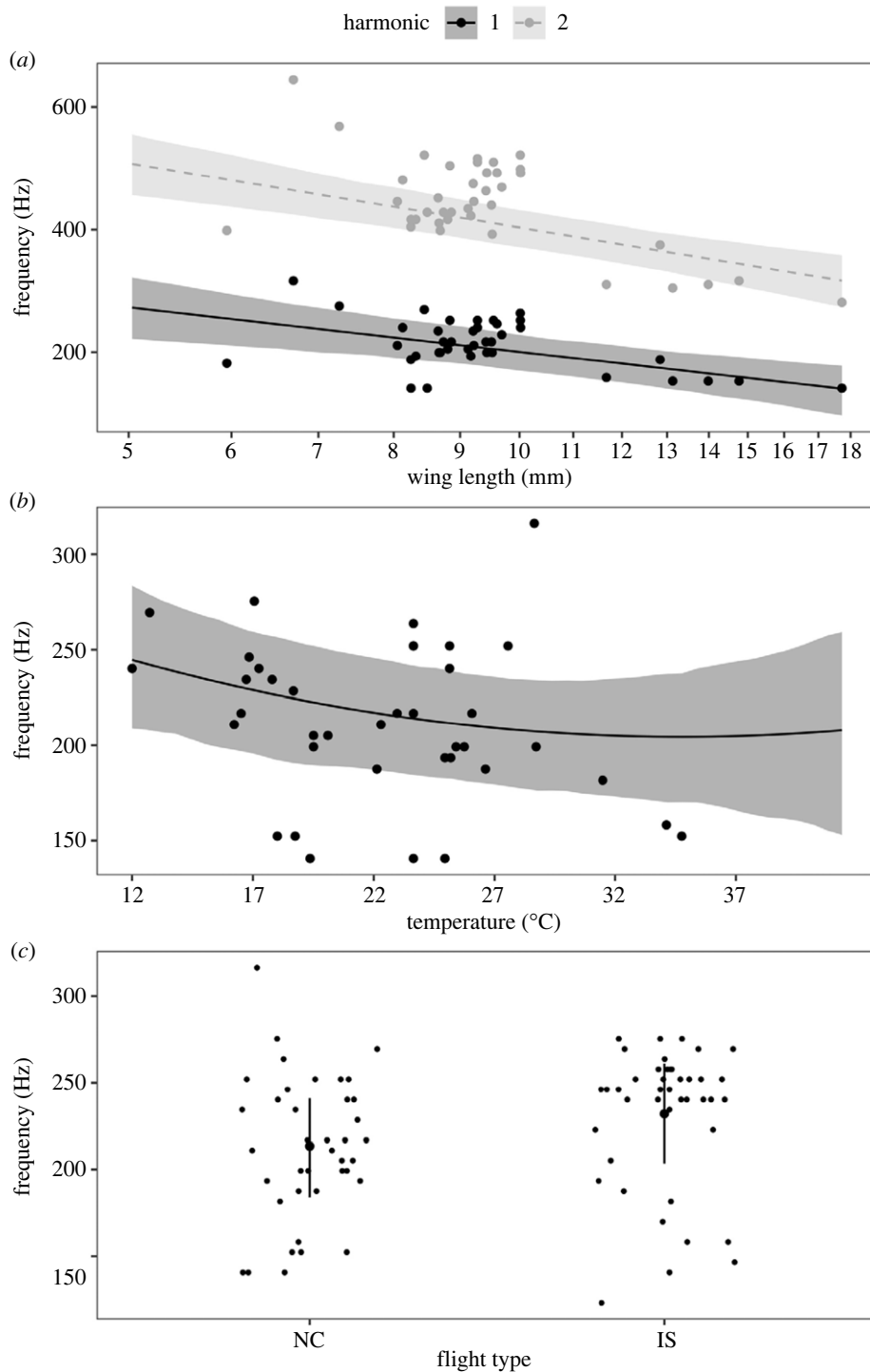


Figure 2. The effects of wing length, environmental temperature and treatment (NC versus IS) on the wingbeat frequency of all bees.

4. Discussion

Animal sounds have the potential to provide a large amount of inter- and intra-specific information and to assist researchers in monitoring species activity and diversity [9–11]. Bee sounds have as yet received little attention compared to other groups, despite their diversity and potential for aiding species' automatic classification [20,45]. At the inter-specific level, we found that variations in the frequencies of wingbeat sounds emerged from taxonomic, morphological, environmental and behavioural differences of a pool of 27 domestic and wild bee species. First, we confirmed a negative relationship between the dominant frequency of these sounds and the wing length of the recorded specimens, in agreement with the general allometric pattern that predicts lower sound frequencies in larger-sized animals. Second, we found a negative relationship between dominant frequency and environmental temperature. Finally, we demonstrated that bee behaviour also influences wingbeat sounds, with bees under experimentally induced stress conditions (in a net trap) emitting higher-pitched sounds than bees under natural conditions (in free-ranging movements). At the intra-specific level, wingbeat sounds of the European honeybee exhibited a consistent acoustic signature and limited variations: only behaviour was a significant determinant of sound frequency.

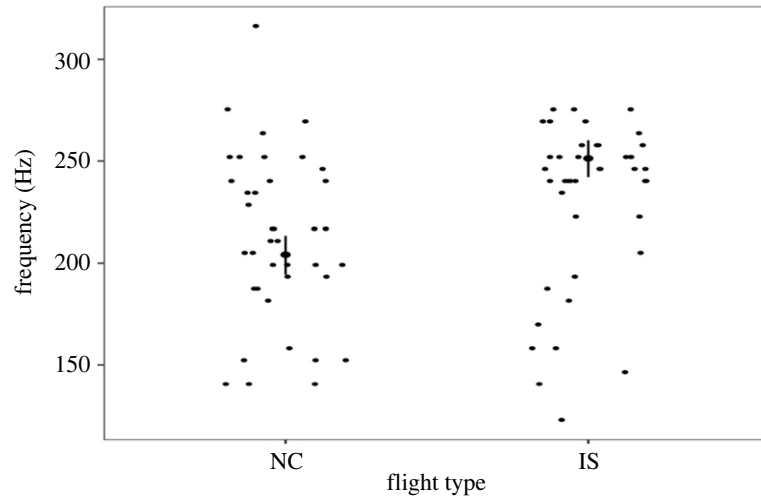


Figure 3. The dominant frequency of wingbeat sounds of the European honeybee (*Apis mellifera*) under two experimental treatments: flight between flowers under natural conditions (NC) and flight inside a net under experimentally induced stress (IS).

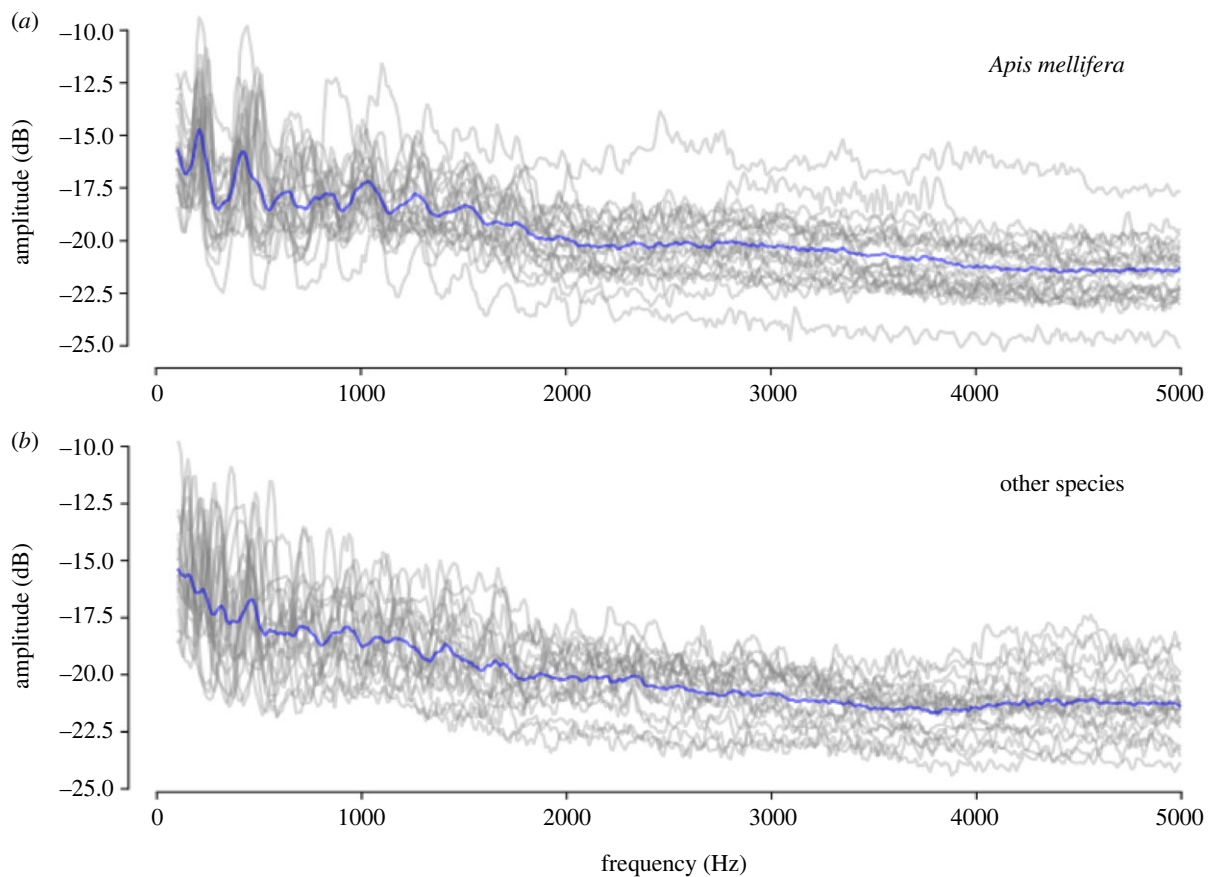


Figure 4. Spectral characteristics of wingbeat sounds of (a) the European honeybee (*Apis mellifera*) and (b) a mixed set of 17 wild bee species. The sounds of the domestic bee show consistent regularities at low frequencies, while the sounds of wild bee species are often noisy with no clear signal.

(a) Wingbeat frequency

The scientific community's interest in studying the wingbeat sounds produced by flying insects is increasing owing to their potential to support species identification, with applications in pest control or biodiversity monitoring [38,46]. In this study, we characterized wingbeat sounds of domestic and wild bees with acoustic methods under field conditions. The fundamental frequency of the sounds produced by the European honeybee (*Apis mellifera*) was on average 222.4 ± 21.4 Hz (\pm standard deviation; $n = 26$ specimens), which is similar to the frequency observed in previous studies with smaller samples (e.g. 235.2 ± 7.5 Hz, $n = 10$, [47]; 238.2 ± 4.57 Hz, $n = 10$, [48]), while the wingbeat sounds of wild bees were on average lower-pitched (180.3 Hz \pm 50.9).

It is worth mentioning that wingbeat sound frequencies could significantly vary depending on the insect flight pattern: whether it is sustained (hovering flight) or it goes in a specific direction [49]. Apart from these flying patterns, other factors such as temperature or the load imposed on the motor system could be influencing the vibrational system [25,50]. The sound produced is even trickier to study in certain taxa such as flies, where the wings do not only move from up and down, but also undergo

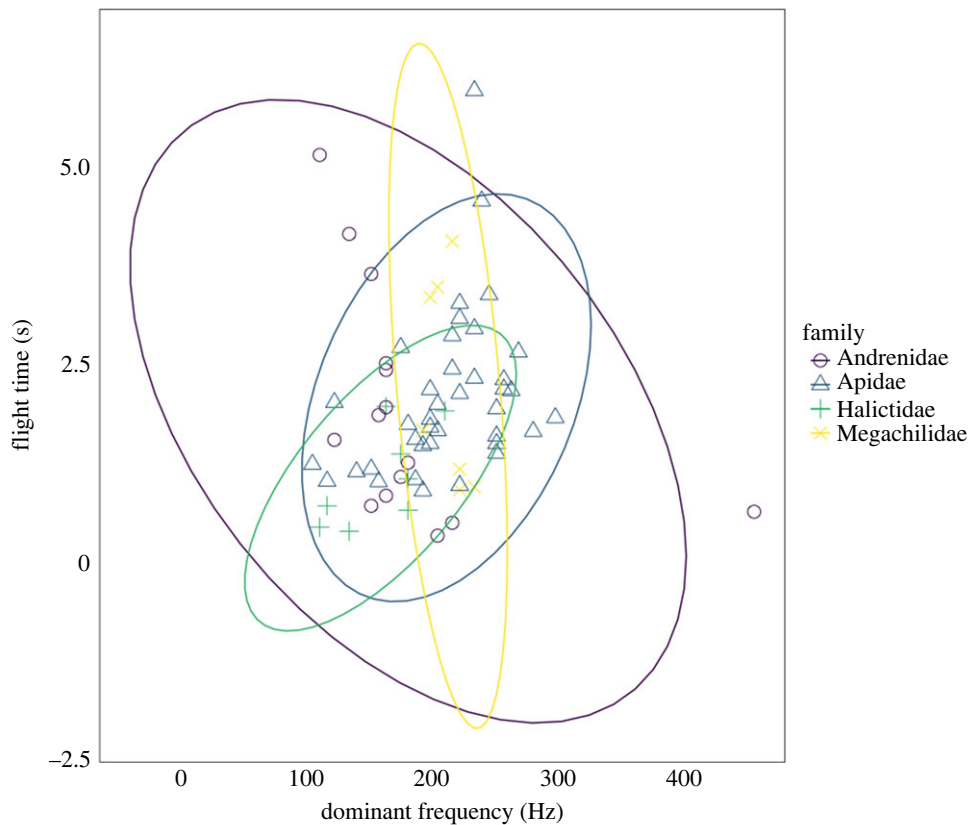


Figure 5. Dominant frequency of the wingbeat sounds (Hz) and flight time between flowers (s) of domestic and wild bees under NC treatment. Each dot indicates an individual ($n = 67$), grouped by family.

deformations, rotations and speed variations [51,52]. Thus, larger databases are needed to better determine the spectral patterns of wingbeat sounds in flying insects.

(b) Morphology and bee sounds

As in other animal groups, there is a certain consensus that the frequency of sounds emitted by insects (also wingbeat sounds) is negatively correlated with their body size [34,53–55]. A larger-sized individual typically has larger wings and hence produces higher forces, which end up reducing the number of wingbeats they need to fly [38]. This is also consistent with previous studies in individuals of the Euglossini tribe, bumblebees and orchid bees [55–57], but opposite to some studies in mosquitos (e.g. [58]). Our results, which include several solitary species as well as social bees, are still in line with this allometric pattern, supporting the belief that bees with longer wing length emit lower sound frequencies. In contrast with wing length, ITD did not show a clear relationship with wingbeat sounds, suggesting that the former morphological trait could be a better predictor of body size, and thus wingbeat sounds, than the latter one.

At the intra-specific level, neither of the two functional traits were related to the sound frequency emitted by the European honeybee, contrary to what was expected according to our hypothesis. This, however, aligns with Kendall *et al.* [59], because they found no significant correlations between ITD and dry weight (an alternative indicator of body size) in this species.

(c) Environmental temperature

Temperature is a pervasive factor influencing physiology and behaviour of ectotherms, such as flying insects [60]. Previous studies often found that increases in wingbeat fundamental frequency were correlated with increases in environmental temperature, e.g. in various bees and flies [26,61] or in mosquitos [58]. However, this is not always the case. In larger bees, air temperature can be negatively correlated with wingbeat frequency. As bees significantly differ in body size, ranging from a few mm to more than 20 mm, body temperature in larger-sized insects could substantially deviate from environmental temperature, hence attenuating such a relationship [26,31]. Actually, we found a negative correlation between wingbeat frequency and temperature at both the inter- and intra-specific levels, opposite to the effect that is most commonly observed. Other studies have also shown that social bees such as *Apis mellifera* are capable of controlling their wingbeat frequency regardless of external temperature [31]. Even, Sotavalta [61] did not find differences in *Bombus pascuorum* wingbeat frequency over a wide temperature range, while Spangler & Buchmann [50] did not consider temperature as an important factor affecting wingbeat frequency in social and non-social bees. Other factors, such as individuals' sex, can also be playing a role in wingbeat frequency [62]. Our results are aligned with this diversity of relationships between temperature and wingbeat frequency that are reported in the literature, and suggest that the effect of temperature on wingbeat frequency may be taxa-specific and that generalizations on this link are still elusive owing to the scarce amount of data available so far.

(d) Under stress conditions

When the European honeybees' flight was recorded under induced stress conditions (within an entomological net), the dominant frequency of their wingbeat sounds increased by 25 Hz in comparison with those under natural conditions. This shift may indicate an effect of stress on the frequency of these flight sounds, as a behavioural response to the context. Sounds emitted from bees' defensive behaviours, alarm signals or other intra-specific communication mechanisms have been previously reported in literature [20,21,24,63]. For instance, the African stingless bee *Axestotrigona ferruginea* produces frequent guarding signals to alarm nestmates [64]. However, during these behaviours, insects are typically lying on a surface and their wings are folded over the thorax, uncoupled from the indirect flight muscles. Thus, the vibrational response of these non-flying individuals is different (e.g. higher-pitched) from that of the flying individuals recorded in this study [21,65]. To the best of our knowledge, no other study has shown stress-related sounds in flying individuals. Our findings suggest that there is plenty of room to explore the diversity of bee sounds associated with different flying and non-flying behaviours.

(e) Acoustic species-level identification

Our Random Forest-based models proved effective at classifying wingbeat sounds between domestic (*Apis mellifera*) and wild bee species, evidencing the potential of bee flight sounds to support automated acoustic identification. Our results are in line with previous studies using machine learning approaches in other insect groups. For instance, Kawakita & Ichikawa [29] successfully identified three species of bees (*Apis mellifera*, *Bombus ardens* and *Tetralonia nipponensis*), and a hornet (*Vespa simillima xanthoptera*) using the fundamental frequency of their flight as a variable [29]. Li *et al.* [66] managed to classify five species of mosquitoes based on their sounds, with a success rate of 73%. Similarly, Yin *et al.*, [67] successfully detected and classified several mosquito species with wingbeat sounds using computational techniques. Folliot *et al.* [68] also monitored pollination by insects and tree use by woodpeckers with acoustics methods and artificial intelligence. Other studies that did not rely on wingbeat sounds but on measuring wingbeat frequency with laser sensors have also demonstrated the good potential of this related parameter for taxonomic identification of insect pests [28,69].

According to our results, acoustic classification of bees based on their wingbeat sound entities seems to be possible in some cases, but might be hindered by species overlap. The wingbeat sounds are quiet and thus difficult to record. Our findings show that improving signal-to-noise ratio of audio samples may further increase the accuracy of the automated classification. The design of techniques to better capture such sounds in the field and increasing the sample size of training datasets for statistical classifiers will likely contribute to the efforts of developing new methods for monitoring pollinating species in a non-intrusively and efficient way [70]. It is also important to highlight that our study, which aimed to distinguish *Apis mellifera* from other wild bee species, employed handcrafted features owing to the availability of samples. While our Random Forest-based models demonstrated commendable classification performance, we acknowledge the existence of more advanced methods, particularly the use of deep learning models for classifying bee species based on wingbeat sounds [45,71]. These advanced methods have demonstrated highly accurate classification results, especially when provided with a larger dataset.

Next steps should also be oriented towards: (i) the documentation of acoustic diversity of these sounds, including a fine analysis of their determinants, which will overcome the current lack of knowledge, (ii) the creation of sound libraries that support the future development of species classification algorithms, and (iii) testing of alternative machine learning techniques for the automated analysis of wingbeat sounds in flying insects.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. All sound files were deposited in the Fonoteca Zoológica of the Museo Nacional de Ciencias Naturales (MNCN-CSIC, Madrid, Spain) <https://www.fonozoo.com/>.

The data are provided in electronic supplementary material [72].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. A.R.B.: data curation, formal analysis, investigation, methodology, visualization, writing—original draft; C.D.: data curation, methodology, writing—review and editing; V.H.: conceptualization, funding acquisition, supervision, writing—review and editing; M.G.-L.: conceptualization, methodology, writing—review and editing; J.S.U.: formal analysis, methodology, visualization, writing—review and editing; D.L.: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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