



HAL
open science

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

Alberto Rodríguez Ballesteros, Camille Desjonquères, Violeta Hevia, Marina García Llorente, Juan Ulloa, Diego Llusia

► To cite this version:

Alberto Rodríguez Ballesteros, Camille Desjonquères, Violeta Hevia, Marina García Llorente, Juan Ulloa, et al.. Towards acoustic monitoring of bees: wingbeat sounds are related to species and individual traits. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 2024, 379 (1904), 10.1098/rstb.2023.0111 . hal-04804883

HAL Id: hal-04804883

<https://hal.science/hal-04804883v1>

Submitted on 26 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title:** Towards acoustic monitoring of bees: wingbeat sounds are related to species and
2 individual traits

3

4 Alberto Rodríguez Ballesteros^{1,2}, Camille Desjonquères^{1,3}, Violeta Hevia^{2,4}, Marina
5 García Llorente^{2,4}, Juan Sebastián Ulloa⁵, Diego Llusia Genique^{1,4,6}

6

7 ¹ Terrestrial Ecology Group, Department of Ecology, Universidad Autónoma de Madrid,
8 Darwin 2, 28049, Madrid, Spain.

9 ² Social-ecological Systems Laboratory, Department of Ecology, Universidad Autónoma
10 de Madrid, Darwin 2, 28049, Madrid, Spain.

11 ³ Université Grenoble Alpes, Université Savoie Mont Blanc, CNRS, LECA, Grenoble,
12 France

13 ⁴ Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM), Universidad
14 Autónoma de Madrid, Darwin 2, 28049, Madrid, Spain.

15 ⁵ Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Avenida
16 Paseo Bolívar 16-20, Bogotá, Colombia

17 ⁶ Laboratório de Herpetologia e Comportamento Animal, Department of Ecology,
18 Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiás, Brazil

19

20 *Corresponding author: diego.llusia@uam.es

21

22 23 pages; 7073 words; 5 figures

23

24 **Abstract**

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

43

44 **Keywords**

45 *pollinators; body size; fundamental frequency; acoustic signature; species classification;*
46 *bioacoustics*

47

48 **Introduction**

49 Bees (Hymenoptera: Apoidea) are considered the most important group of pollinators
50 (Pashte & Kulkarni, 2015; Hung et al., 2018). With over 20,000 species worldwide, these
51 flying insects play a crucial role in ecosystem services, food security and sustainable
52 development (Patel et al., 2021). However, surveys in North America and Europe have
53 reported negative trends in bees and other relevant pollinators (e.g. syrphid flies) during
54 the past decades (National Research Council, 2007; Potts *et al.*, 2016), associated to
55 different anthropogenic drivers, such as habitat loss and fragmentation, climate change or
56 agricultural intensification (Winfree, 2010; Goulson et al., 2015; Wagner et al., 2021).
57 This global decline calls for developing scalable, inexpensive and efficient methods to
58 monitor behaviour and trends of bee communities.

59 Commonly studied in a variety of vertebrate and invertebrate species, animal
60 sounds are a well-established source of ecological information (Bradbury & Vehrencamp,
61 1998) that can reveal species presence, abundance, position, body size or behavioural
62 status (Obrist *et al.*, 2010). Thus, passive acoustic monitoring (PAM) has become an
63 emerging technique that is used to record, store and analyse animal sounds in an
64 automated, non-invasive way (Sugai et al., 2019a). In combination with computational
65 tools, PAM has proven to efficiently record animal activity for a wide variety of subjects,
66 e.g. population density (Marques *et al.*, 2013; Pérez-Granados & Traba, 2021) and
67 distribution (Ribeiro *et al.*, 2022), conservation (Laiolo, 2010) or climate change research
68 (Llusia et al., 2013; Desjonquères *et al.*, 2022). PAM is typically aimed to record well-
69 known species-specific acoustic signals that are emitted by specialized sound-producing
70 structures and play a role in mating, resource defence or navigation (Wilkins *et al.*, 2013).

71 In comparison with birds, bats or anurans (animal groups extensively studied with
72 PAM; Sugai et al., 2019a), bees do not possess specialized sound-producing structures
73 and emit incidental sounds that arise as a by-product of activities such as moving or eating
74 (Chesmore, 2008), e.g. a diverse a suit of pitches generated by rhythmic thoracic
75 oscillations (see review by Hrnčir et al., 2005). Some of the most well-known examples
76 of bee sounds are emitted during different behaviours of social species, such as
77 honeybees, bumblebees or some stingless bees (Hrnčir et al., 2005; Hrnčir et al., 2008;
78 Rittschof & Seeley, 2008; De Luca et al., 2014), and they are usually exclusive to certain
79 families, genus or species (e.g. buzz pollination, see Pritchard & Vallejo-Marín, 2020).
80 There is, however, one sound type found across all bee species: the flight buzz.

81 Bees' flight and its associated sounds have been theoretically studied since the 60s
82 (Esch & Wilson, 1967; Unwin & Corbet, 1984; Santoyo et al., 2016). More recently,
83 applied studies have shown that wingbeat frequency and pattern during flight may be
84 species-specific, and thus it could serve as a 'fingerprint' for automatic species
85 classification (Potamitis & Rigakis, 2015; Kawakita & Ichikawa, 2019). However, most
86 studies on this topic have been performed in laboratory settings, with a narrow number of
87 species (e.g. only *Bombus sp.* or *Apis mellifera*), or without considering morphological
88 (e.g. body size) and environmental (e.g. temperature) factors (Gradišek et al., 2017;
89 Parmezan et al., 2021).

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

102

103 **Material & Methods**

104 *Study area*

105 The study was conducted in three farmlands located in Central Spain (Torrelaguna and
106 La Cabrera, Madrid), between 600 and 1200 meters a.s.l., at the southern side of the
107 mountain range Sierra de Guadarrama (see Fig. S1 for farm pictures). These areas belong
108 to the mesomediterranean bioclimatic layer, characterized by highly variable
109 temperatures (mean annual temperatures between 12°C and 17°C) and moderate rainfall
110 (annual precipitation of 500 mm; Rivas-Martínez, 1983; AEMET, 2020; Girón *et. al.*,
111 2020).

112 *Sound recording and insect sampling*

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

124 We recorded wingbeat sounds of the focal bees with a directional microphone
125 (ME-66 and module K6; Sennheiser) connected to a portable digital audio recorder
126 (PMD-660 Marantz Professional). During recordings, the tip of the microphone was
127 placed at a distance of 2-5 cm above the thorax of the insect to correctly register the sound
128 without disrupting its behaviour (De Luca *et. al*, 2019; see Fig S2 for a graphic
129 visualization of the recording protocol). To increase the signal-to-noise ratio, the audio
130 gain of the recorder was manually regulated while continuously tracking sound
131 amplitude. We used a shock-mount to avoid stand-borne noise, a windshield to reduce
132 excessive pressure from wind, and headphones to properly perceive the sound source.
133 The recordings were stored as uncompressed .wav files and digitalised at a sampling rate
134 of 48kHz and a depth of 16 bits.

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

140

141 *Acoustic analysis*

142 We created and visualized spectrograms of the recorded sounds using the Raven Pro v.
143 1.5.0. software (Cornell Lab of Ornithology; Fig. 1; Fig. S3). An optimal spectrogram
144 configuration was applied to all the recordings (Window size: 4098; Overlap: 90%;
145 Window Type: Hann), providing a high resolution of wingbeat sound frequencies. For
146 each individual and treatment, a single audio with the best signal-to-noise ratio was
147 selected and three acoustic parameters measured for both the fundamental harmonic (i.e.
148 the lowest frequency of a periodic wave; Arthur *et. al*, 2014) and the second harmonic.
149 These parameters were: duration (s), dominant frequency (Hz) and maximum energy
150 (dB). To best characterise between- and within-bee variation in sound frequency
151 throughout the individual displacement, we also measured dominant frequency and
152 maximum energy in a short segment at the onset, centre and end of the selected audio
153 (Fig. S2). The duration of each segment corresponds to ca. 10% of the duration of the
154 entire audio. Based on these measurements, we calculated how often sound energy was
155 higher at the fundamental harmonic than at the second one. By pairwise comparisons of
156 energy in harmonics of the same segment, we thus estimated a within-bee percentage of
157 audio segments with dominant frequency in the fundamental harmonic.

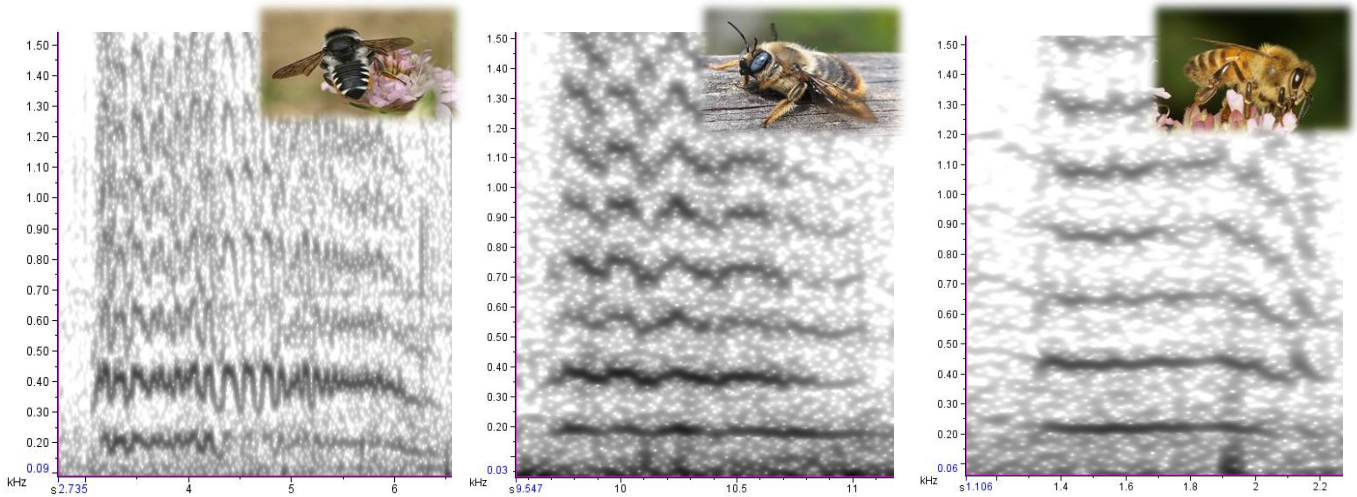
158

159 *Trait measurements*

160 We measured two morphological traits from the focal specimens: the intertegular distance
161 (ITD), defined as the minimum linear distance between both tegulae, measured over the
162 thoracic dorsum (Cane, 1987), and the average forewings length (WL; Fig. S4). Both
163 morphological traits are considered good proxies for body size (Hagen & Dupont, 2013;
164 van Roy *et. al*, 2014). For these measurements, photographs of every specimen were taken
165 over paper measured in millimetres with a digital camera Canon EOS M10 (Canon,
166 Tokyo, Japan) adjusted to an optical microscope Leica MZ6 (Leica, Wetzlar, Germany),
167 ranging between 0,8 and 4 magnifications. The length in millimetres was measured with
168 the software *Image J* (Abràmoff *et. al*, 2004). When one of the two wings was deteriorated
169 or absent, the average between two measurements of the other wing was calculated.

170

171



172 **Figure 1.** Spectrogram visualization of three collected individuals from different species: *Megachile*
 173 (*Megachile*) *octosignata*, *Xyllocopa cantabrita*, *Apis mellifera*. The spectral component observed at a
 174 frequency of 5 Hz in the third spectrogram corresponded to environmental noise.

175

176 *Regression models*

177 To examine factors that influence inter- and intra-specific variation in the dominant
 178 frequency of wingbeat sounds, we used general linear mixed-effect models (LMM), with
 179 Gaussian error structure, including dominant frequency as response variable, and
 180 morphological, environmental and behavioural factors as explanatory variables.
 181 Specifically, we fitted two models: (i) a LMM at inter-specific level, using all recorded
 182 individuals, and (ii) a LMM at intra-specific level, with only specimens from the most
 183 abundant species, the domestic bee (*A. mellifera*). In each model, we included seven fixed
 184 factors: WL (mm), ITD (mm), temperature ($^{\circ}\text{C}$, linear and quadratic terms, to account for
 185 potential linear and curvilinear relationships), experimental treatment (NC vs. IS), audio
 186 segment (4 levels; entire, onset, centre and end), harmonic type (fundamental vs. second)
 187 and family (Andrenidae, Apidae, Halictidae, and Megachilidae). Additionally, we added
 188 individual identity as a random factor in both LMMs to account for repeated measures
 189 within individuals, and species and genus as random factors in the inter-specific model to
 190 evaluate the amount of variation explained by these factors. As ITD and WL were
 191 correlated at inter-specific level, we first fitted a linear regression with both variables at
 192 inter-specific level (log10 transformed to achieve linearity) and used WL and statistically
 193 controlled ITD (residuals of the regression) as explanatory variables in the LMM model
 194 to avoid collinearity. All continuous variables (WL, ITD and temperature) were centred
 195 and scaled (mean of 0 and standard deviation of 1) to facilitate model fitting.

196 We checked the assumptions of normality and homogeneity of the residuals by
197 visually inspecting a quantile-quantile plot and the residuals against the fitted values, both
198 indicating no deviation from these assumptions. We inspected model stability by
199 excluding data points one at a time from the data. We derived variance inflation factors
200 (Field, 2009) using the function *vif* of the R-package *car* (version 2.1-4; Fox & Weisberg
201 2011) and they did not indicate collinearity between fixed effects to be an issue. We
202 conducted all analyses in R using the *lmer* function of the R-package *lme4* (v. 3.1-139;
203 Pinheiro et al. 2017).

204

205 *Classification analysis*

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

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

221 First, we characterized the audio samples in the spectral domain by computing the
222 power spectrum. This spectral representation allowed us to compare visually the spectral
223 differences between classes. To have tight representation of the spectral domain, we then
224 computed mel-frequency cepstral coefficients (MFCC). MFCCs have been the dominant
225 features used for audio classification since they have the ability to deliver a compact
226 representation of sounds with high harmonic content, such as speech and music (Rabiner
227 & Schafer, 2010). In particular, we used 20 coefficients extracted between the frequency

228 band where the wingbeat sounds and the harmonics were predominant (i.e., 0.1 and 5
229 kHz). The final predictor matrix included 20 MFCC coefficients and 42 observations. We
230 measured binary classification performance using a Random Forest classifier (number of
231 trees = 300, maximum features = 6) and computing the balanced accuracy metric in a
232 stratified 10-fold cross-validation scheme. To assess whether the accuracy of the machine
233 learning model was significantly better than expected by chance, we computed baseline
234 accuracies using two dummy classifier strategies: majority and random. The majority
235 strategy simulates a classifier always returning the most frequent class, which, in our case,
236 is *Apis mellifera*. The random strategy generates predictions uniformly at random. The
237 automated classification analyses were performed in Python 3, using the package *librosa*
238 (McFee *et al.*, 2015) for audio characterization, and the package *scikit-learn* (Pedregosa
239 *et al.*, 2011) for fitting and evaluation of statistical classifiers.

240

241 **Results**

242 *Taxonomic and morphological traits*

243 Overall, 73 bees were registered and captured, and 65 of them identified at species level
244 (Table S1). The taxonomic identification revealed 27 species, belonging to 4 families:
245 Apidae (42 individuals; 57%), Andrenidae (15; 21%) Halictidae (9; 12%) and
246 Megachilidae (7; 10%). European honeybee (*Apis mellifera*) was the most commonly
247 collected species, with 26 registered individuals (36%). A set of 16 species, one subgenus,
248 and one genus was represented by a single specimen. The average WL was 8.84 mm
249 (± 2.39) and varied between the 2.73 and 17.73 mm, while the average ITD was 2.93 mm
250 (± 0.97) and varied between 0.76 and 6.52 mm (Table S1). Temperature was 24.04 °C
251 (± 10.63) and ranged between 16.25 and 41.39 °C.

252

253 *Bee sounds*

254 Wingbeat sounds were analysed in 69 individuals under NC treatment and 71 individuals
255 under IS treatment. These sounds were highly variable and characterized by a large
256 number of harmonics (1-12), with sharp, moderate or restricted frequency modulation
257 (Fig. 1). Sound energy of the harmonics typically decreases with frequency (i.e. lower
258 harmonics having higher energy), although some individuals did not follow that pattern.

259 Duration of bee flying between flowers ranged from 1.9 s (\pm 1.3) in wild bees to 2.1 s (\pm
260 0.8) in the European honeybee. The fundamental frequency of sounds produced by this
261 domestic bee (at the centre of the audio segment) was on average 222.4 Hz (\pm 21.4) and
262 251.3 Hz (\pm 15.9) under NC and IS treatments, respectively. In wild bees, the fundamental
263 frequency was on average 180.3 Hz (\pm 50.9) and 195.5 Hz (\pm 95.7). The fundamental
264 harmonic was higher in energy than the second harmonic in 80% of the time for all
265 observations, and 90% of the time for the IS treatment (Table S2). In general, dominant
266 frequency and the patterns of frequency modulation were distinct across specimens and
267 might be good candidate parameters for acoustic species identification (see Figure S5).
268 The greatest intensity of background noise occurred between 0 and 70 Hz.

269

270 *Determinants of wingbeat sounds at inter- and intra-specific level*

271 Using the full dataset (n=138 individuals, 27 species), the first LMM model revealed that
272 frequency of bee sounds was related to wing length bound to harmonic, as well as
273 experimental treatment (NC vs. IS) and air temperature (Table S3; Figure 2). Lower
274 frequencies were emitted by larger-sized bees and individuals subjected to higher
275 temperatures, while stress was associated with high-pitched sounds. We also found
276 significant differences in dominant frequency among the bee families and the audio
277 segments within the recording (Table S3). Using the subset for the European honeybee
278 (n=26 individuals), the second LMM model pointed out that the frequency of wingbeat
279 sounds of this species was also associated to its behavioural status, with higher-pitched
280 sounds under stress conditions (Table S4). We also found significant differences in
281 dominant frequency among the harmonics and the audio segments within the recording
282 (Table S4, Figure 2).

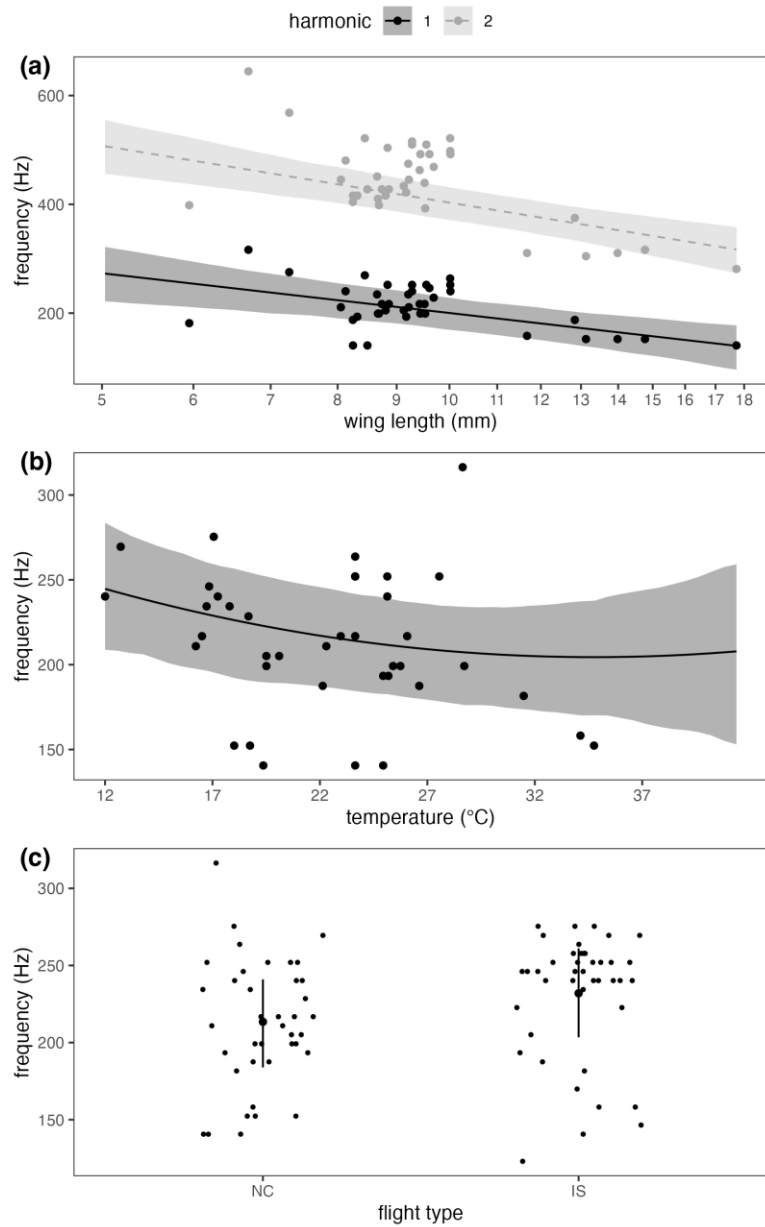
283

284

285

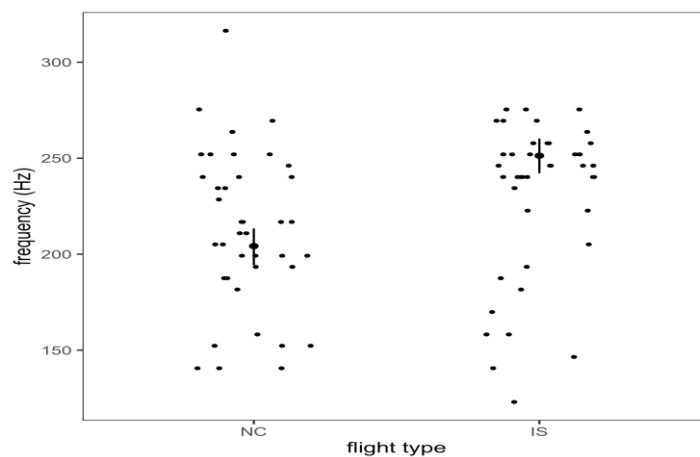
286

287



288

289 **Fig. 2.** The effects of wing length, environmental temperature and treatment (NC vs. IS) on the wingbeat
 290 frequency of all bees.

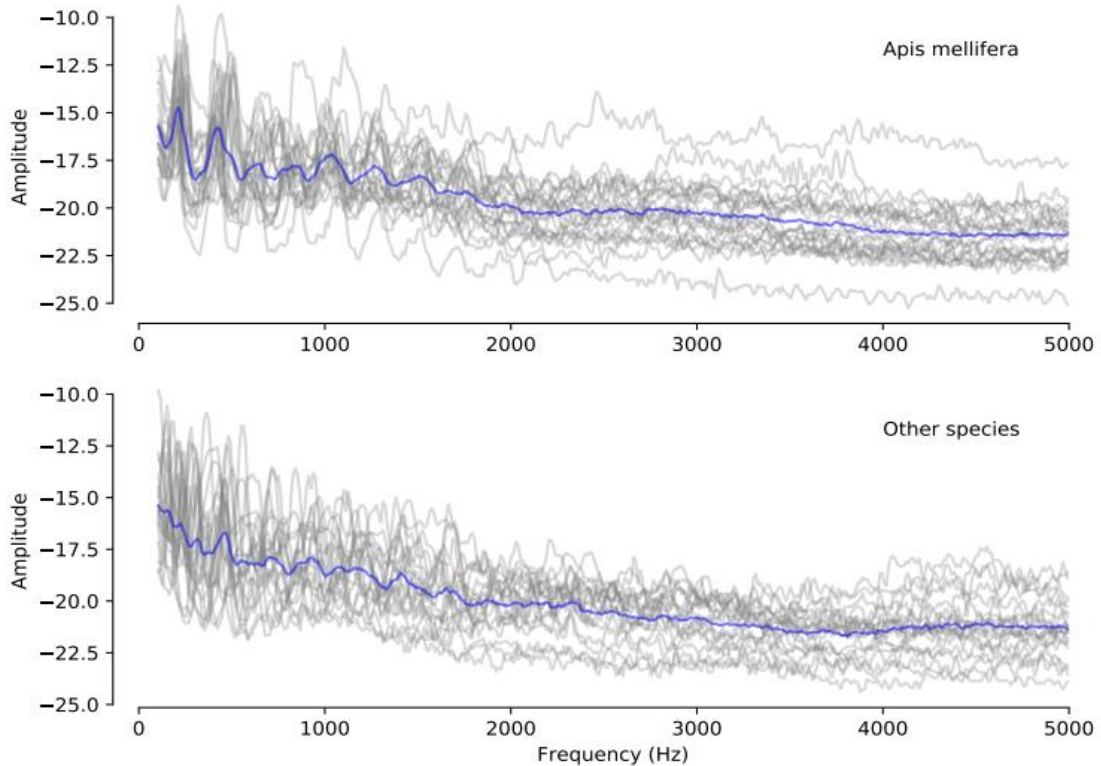


291

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

295 *Inter- and intra-specific acoustic signatures*

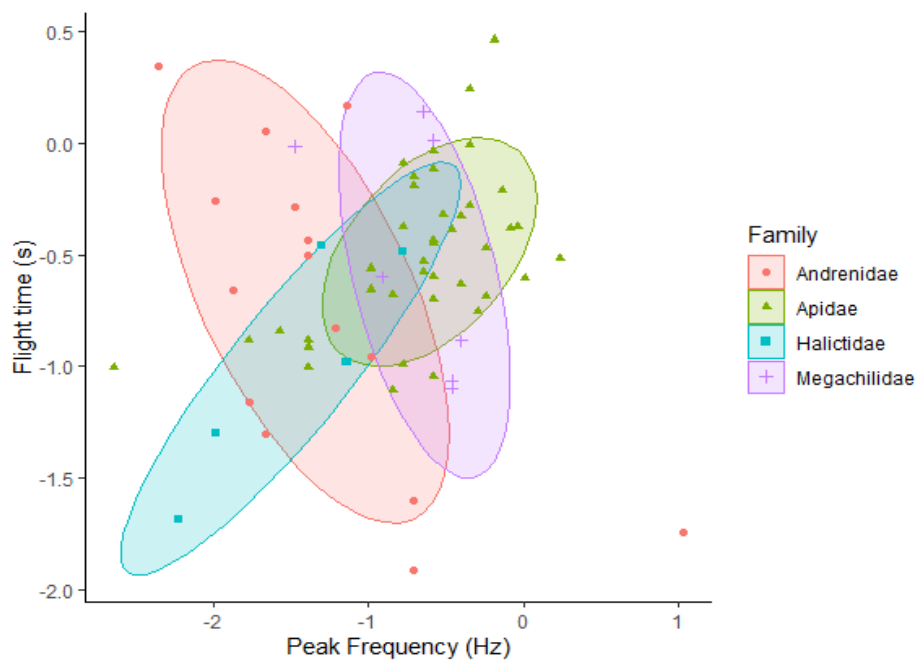
296 The power spectrum of wingbeat sounds of the European honeybee showed multiple
297 consistent peaks at low frequencies (Fig. 4) that were only masked by the background
298 noise at higher frequencies. On the contrary, the mixed set of wingbeat sounds of wild
299 bee species were highly variable and did not show a clear signal. Our statistical analyses
300 on the discrimination between these sounds showed that the Random Forest classifier had
301 an average balanced accuracy of 0.77 ± 0.12 , over 10 cross-validation runs. The baseline
302 accuracies obtained were $0.5 (\pm 0)$ for the majority strategy and $0.47 (\pm 0.21)$ for the
303 random strategy, showing that our model's accuracy is significantly better than chance.
304 The main classification errors (false positives and false negatives) were observed on the
305 noisier samples. This suggests that the wingbeat sound of the European honeybee has a
306 specific acoustic signature and machine learning could be used to automatically
307 discriminate between domestic and wild bee species.



308
309 **Fig. 4.** Spectral characteristics of wingbeat sounds of the European honeybee (*Apis mellifera*; top) and a
310 mixed set of 17 wild bee species (bottom). The sounds of the domestic bee show consistent regularities at
311 low frequencies, while the sounds of wild bee species are often noisy with no clear signal.

312

313 The recorded sounds were distinct in frequency and duration across genus and families,
314 with some level of overlap between groups (Figure 5). We found no significant
315 differences in dominant frequency at the centre of the audio segment ($F=0.11$; $df=2$;
316 $p=0.89$) amongst the three taxonomic levels, calculated by the mean differences in
317 frequency between pairs of species (mean \pm standard deviation: $57.6 \text{ Hz} \pm 43.6$), genus
318 ($59.9 \text{ Hz} \pm 44.3$) and families ($58.3 \pm 43.8 \text{ Hz}$).



319

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

322

323 Discussion

324 Animal sounds have the potential to provide a large amount of inter- and intra-specific
325 information and assist researchers to monitor species activity and diversity (Bradbury &
326 Vehrencamp, 1998; Obrist *et al.*, 2010; Sugai *et. al.*, 2019a). Bee sounds have received
327 still little attention compared to other groups, despite their diversity and potential for
328 species automatic classification (Hrncir *et al.*, 2005; Truong *et al.*, 2023). At the inter-
329 specific level, we found that variations in frequency of wingbeat sounds emerged from
330 taxonomic, morphological, environmental and behavioural differences of a pool of 27
331 domestic and wild bee species. First, we confirmed a negative relationship between
332 dominant frequency of these sounds and wing length of the recorded specimens, in

333 agreement with the general allometric pattern that predicts lower sound frequencies in
334 larger-sized animals. Second, we found a negative relationship between dominant
335 frequency and environmental temperature. Finally, we unveiled that bee behaviour also
336 influenced wingbeat sounds, with bees under induced experimentally-induced stress
337 conditions (in a net trap) emitting higher-pitched sounds than bees under natural
338 conditions (in free ranging movements). At the intra-specific level, wingbeat sounds of
339 the European honeybee exhibited a consistent acoustic signature and limited variations,
340 being only behaviour a significant determinant of sound frequency.

341

342 *Wingbeat frequency*

343 The interest of the scientific community to study wingbeat sounds produced by flying
344 insects is increasingly growing due to its potential to support species identification, with
345 applications in pest control or biodiversity monitoring (van Roy *et. al*, 2014; Zhang *et.*
346 *al*, 2017). In this study, we characterized wingbeat sounds of domestic and wild bees with
347 acoustic methods under field conditions. Fundamental frequency of the sounds produced
348 by the European honeybee (*Apis mellifera*) was on average (\pm standard deviation) 222.4
349 ± 21.4 Hz (n=26 specimens), a similar frequency to the one observed in previous studies
350 with smaller samples (e.g. 235.2 ± 7.5 Hz, n = 10, Goyal & Atwal, 1977; 238.2 ± 4.57
351 Hz, n = 10, Spangler, 1986), while wingbeat sounds of wild bees were on average lower-
352 pitched sounds (180.3 Hz ± 50.9).

353 It is worth mentioning that frequencies of wingbeat sounds could significantly
354 vary depending if the insect flight is sustained (hovering flight) or describes a specific
355 direction (Bae & Moon, 2008). Apart from these flying patterns, other factors such as
356 temperature or the load imposed on the motor system could be influencing the vibrational
357 system (Esch & Wilson, 1967; Spangler & Buchmann, 1991). The sound produced is
358 even more delicate to study in certain taxa such as flies, where wings do not only move
359 from top to bottom, but also undergo deformations, rotations and speed variations (Sueur
360 *et. al*, 2005; Geng *et. al*, 2017). Thus, further analysis based on larger databases would be
361 helpful to keep increasing the resolution of insect's wingbeats frequencies values.

362

363 *Morphology and bee sounds*

364 As in other animal groups, there is a certain consensus that frequency of sounds emitted
365 by insects (also wingbeat sounds) is negatively correlated with their body size (Moore *et.*
366 *al.*, 1986; Bennet-Clark, 1998; Darveau *et. al.*, 2005; De Luca *et al.*, 2019). A larger-sized
367 individual typically has larger wings and hence produces higher forces, which end up
368 reducing the number of wingbeats they need to fly (van Roy *et. al.*, 2014). This is also
369 consistent with previous studies in individuals of the Euglossini tribe, bumblebees and
370 orchid bees (Casey *et al.*, 1985; Joos *et. al.*, 1991; Darveau *et. al.*, 2005), whereas opposite
371 to some studies in mosquitos (e.g. Villareal *et al.*, 2017). Our results, which include
372 several solitary species as well as social bees, are still in line with this allometric pattern,
373 supporting that bees with longer wing length emit lower sound frequencies. In contrast
374 with wing length, ITD did not show a clear relationship with wingbeat sounds, suggesting
375 that the former morphological trait could be a better predictor of body size, and thus
376 wingbeat sounds, than the latter one.

377 At the intra-specific level, neither of the two functional traits were related to the
378 sound frequency emitted by the European honeybee, contrary to what was expected
379 according to our hypothesis. This, however, aligns with Kendall *et. al.*, (2019), as they
380 found no significant correlations between ITD and dry weight (an alternative indicator of
381 body size) in this species.

382

383 *Environmental temperature*

384 Temperature is a pervasive factor influencing physiology and behaviour of ectotherms,
385 such as flying insects (Belton, 1986). Previous studies often found that increases in
386 wingbeat fundamental frequency were correlated with increases in environmental
387 temperature, e.g. in various bees and flies (Sotavalta, 1947; Unwin & Corbet, 1984;) or
388 in mosquitos (Villarreal *et al.*, 2017). However, this is not always the case. In larger bees,
389 air temperature can be negatively correlated with wingbeat frequency. As bees
390 significantly differ in body size, ranging from a few mm to more than 20 mm, body
391 temperature in larger-sized insects could substantially deviate from environmental
392 temperature, hence attenuating such a relationship (Unwin & Corbet, 1984; Parmezan *et*
393 *al.*, 2021). Actually, we found a negative correlation between wingbeat frequency and
394 temperature at both the inter- and intra-specific levels, opposite to the most commonly

395 effect. Other studies have also shown that social bees such as *Apis mellifera* can be able
396 to control their wingbeat frequency regardless of external temperature (Parmezan et al.,
397 2021). Even, Sotavalta (1947) did not find differences in *Bombus pascuorum* wingbeat
398 frequency over a wide temperature range, while Spangler & Buchmann (1991) did not
399 consider temperature as an important factor affecting wingbeat frequency in social and
400 non-social bees. Other factors, such as sex of the individuals, can also be playing a role
401 in wingbeat frequency (Coelho, 1991). Our results are aligned with this diversity of
402 relationships reported in the literature between temperature and wingbeat frequency, and
403 suggest that the effect of temperature on wingbeat frequency may be taxa-specific and
404 that generalizations on this link are still elusive due to the scarce amount of
405 documentation available so far.

406

407 *Under stress conditions*

408 When the flight of European honeybees were recorded under induced stress conditions
409 (within an entomological net), dominant frequency of their wingbeat sounds significantly
410 increased (above 25 Hz) in comparison with those under natural conditions. This shift
411 may indicate an effect of stress in the frequency of these flight sounds, as a behavioural
412 response to the context. Sounds emitted from bees' defensive behaviours, alarming
413 signals or other intra-specific communication mechanisms have been previously reported
414 in literature (Hrncir et al., 2005; Hrncir *et al.*, 2008; Wehmann et al., 2015; Pritchard &
415 Vallejo-Marín, 2020). For instance, the African stingless bee *Axestotrigona ferruginea*
416 produces frequent guarding signals to alarm nestmates (Krausa et al., 2021). However,
417 during these behaviours, insects are typically laying on a surface and their wings are
418 folded over the thorax, uncoupled from the indirect flight muscles. Thus, the vibrational
419 response of these non-flying individuals is different (e. g. higher pitched) from the flying
420 individuals recorded in this study (King & Buchmann, 2003; Hrncir et al., 2008). To the
421 best of our knowledge, no other study has shown stress-related sounds in flying
422 individuals. Our findings suggest that there is plenty of room to explore the diversity of
423 bee sounds under different flying and non-flying behaviors.

424

425 *Acoustic species-level identification*

426 Our Random Forest-based models showed a good classification performance of wingbeat
427 sounds between domestic (*Apis mellifera*) and wild bee species, evidencing the potential
428 of bee flight sounds to support automated acoustic identification. Our results are in line
429 with previous studies using machine learning approaches in other insect groups. For
430 instance, Kawakita & Ichikawa (2019) successfully identified three species of bees (*Apis*
431 *mellifera*, *Bombus ardens* and *Tetralonia nipponensis*), and a hornet (*Vespa simillima*
432 *xanthoptera*) using the fundamental frequency of their flight as a variable (Kawakita &
433 Ichikawa, 2019). Li *et. al* (2005) managed to classify five species of mosquitoes based on
434 their sounds, with a success rate of 73%. Similarly, Yin *et al.*, (2023) successfully
435 detected and classified several mosquito species with wingbeat sounds using
436 computational techniques. Folliot *et al.* (2022) also monitored pollination by insects and
437 tree use by woodpecker with acoustics methods and artificial intelligence. Other studies
438 which did not rely on wingbeat sounds but on measuring wingbeat frequency with laser
439 sensors have also demonstrated a good potential of this related parameter for taxonomic
440 identification of insect pests (Moore & Miller, 2002; Potamitis & Rigakis, 2015).

441 According to our results, acoustic classification of bees based on their wingbeat
442 sounds entities seem to be possible in some cases, but might be hindered by species
443 overlap. The wingbeat sounds are weak and hence their recording is challenging. Our
444 findings showed that improving signal-to-noise ratio of audio samples may further
445 increase the accuracy of the automated classification. The design of techniques to better
446 capture such sounds in the field and increasing the sample size of training dataset for
447 statistical classifiers will likely contribute to the efforts of developing new methods for
448 monitor pollinating species in a non-intrusively and efficient way (Høye *et al.*, 2021). It
449 is also important to highlight that our study, which aimed to distinguish *Apis mellifera*
450 from other wild bee species, employed handcrafted features due to the availability of
451 samples. While our Random Forest-based models demonstrated commendable
452 classification performance, we acknowledge the existence of more advanced methods,
453 particularly the use of deep learning models for classifying bee species based on wingbeat
454 sounds (Truong *et al.*, 2023; Ferreira *et al.*, 2023). These advanced methods have
455 demonstrated highly accurate classification results, especially when provided with a
456 larger dataset.

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

463 **Acknowledgements**

464 We wanted to thank Irene Alcocer Bernal, Marianne Sarfati, Zé Vinicius Bernardy, Jorge
465 Ortega Marcos and Inés Gutiérrez Briceño, for volunteering to sample and store bees, and
466 to show our gratitude to Luis Óscar Aguado, entomologist specialized on bees, for the
467 taxonomic identification of the sampled specimens. We thank the two independent
468 reviewers for their valuable feedback on our manuscript. DL acknowledges a post-
469 doctoral grant (2020-T1/AMB-20636) provided by the Comunidad de Madrid (Atracción
470 de Talento Investigador, Spain) and a research project (PID2022-141923OB-I00) funded
471 by the Agencia Estatal de Investigación (MCIN/AEI/10.13039/501100011033,
472 Ministerio de Ciencia e Innovación, Spain) and the European Social Fund Plus.

473 **Bibliography**

- 474 AEMET (2020). Valores climatológicos normales. Comunidad de Madrid. Acceso el día
475 09 de junio de 2020, en:
476 [http://www.aemet.es/es/serviciosclimaticos/datosclimatologic](http://www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos?l=8096&k=clm)
477 [os/valoresclimatologicos?l=8096&k=clm](http://www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos?l=8096&k=clm)
- 478 Abràmoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ.
479 *Biophotonics international*, 11(7), 36-42.
- 480 Arthur, B. J., Emr, K. S., Wytttenbach, R. A., & Hoy, R. R. (2014). Mosquito (*Aedes*
481 *aegypti*) flight tones: Frequency, harmonicity, spherical spreading, and phase
482 relationships. *The Journal of the Acoustical Society of America*, 135(2), 933-941.
- 483 Bae, Y., & Moon, Y. J. (2008). Aerodynamic sound generation of flapping wing. *The*
484 *Journal of the Acoustical Society of America*, 124(1), 72-81.
- 485 Belton, P. (1986). Sounds of insects in flight. *Insect flight: Dispersal and migration*, 60-
486 70.
- 487 Bennet-Clark, H. C. (1998). Size and scale effects as constraints in insect sound
488 communication. *Philosophical Transactions of the Royal Society of London.*
489 *Series B: Biological Sciences*, 353(1367), 407-419.

- 490 Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of animal communication*.
- 491 Cane, J. H. (1987). Estimation of bee size using intertegular span (Apoidea). *Journal of*
492 *the Kansas Entomological Society*, 145-147.
- 493 Casey, T. M., May, M. L., & Morgan, K. R. (1985). Flight energetics of euglossine bees
494 in relation to morphology and wing stroke frequency. *Journal of Experimental*
495 *Biology*, 116(1), 271-289.
- 496 Chesmore, D. A. V. I. D. (2008). Automated bioacoustic identification of insects for
497 phytosanitary and ecological applications. *Computational bioacoustics for*
498 *assessing biodiversity*, 59
- 499 Coelho, J. R. (1991). The effect of thorax temperature on force production during tethered
500 flight in honeybee (*Apis mellifera*) drones, workers, and queens. *Physiological*
501 *Zoology*, 64(3), 823-835.
- 502 Darveau, C. A., Hochachka, P. W., Welch, K. C., Roubik, D. W., & Suarez, R. K. (2005).
503 Allometric scaling of flight energetics in Panamanian orchid bees: a comparative
504 phylogenetic approach. *Journal of Experimental Biology*, 208(18), 3581-3591.
- 505 De Luca, P. A., Cox, D. A., & Vallejo-Marín, M. (2014). Comparison of pollination and
506 defensive buzzes in bumblebees indicates species-specific and context-dependent
507 vibrations. *Naturwissenschaften*, 101, 331-338.
- 508 De Luca, P. A., Buchmann, S., Galen, C., Mason, A. C., & Vallejo-Marín, M. (2019).
509 Does body size predict the buzz-pollination frequencies used by bees? *Ecology*
510 *and evolution*, 9(8), 4875-4887.
- 511 Desjonquères, C., Villén-Pérez, S., De Marco, P., Márquez, R., Beltrán, J. F., & Llusia,
512 D. (2022). Acoustic species distribution models (aSDMs): A framework to
513 forecast shifts in calling behaviour under climate change. *Methods in Ecology and*
514 *Evolution*, 13(10), 2275-2288.
- 515 Esch, H., & Wilson, D. (1967). The sounds produced by flies and bees. *Zeitschrift für*
516 *vergleichende Physiologie*, 54(2), 256-267.
- 517 Ferreira, A. I. S., da Silva, N. F. F., Mesquita, F. N., Rosa, T. C., Monzón, V. H., &
518 Mesquita-Neto, J. N. (2023). Automatic acoustic recognition of pollinating bee
519 species can be highly improved by Deep Learning models accompanied by pre-
520 training and strong data augmentation. *Frontiers in Plant Science*, 14, 1081050.
- 521 Field, A. 2009. *Discovering statistics using SPSS*. Sage publications.
- 522 Folliot, A., Hauptert, S., Ducretet, M., Sèbe, F., & Sueur, J. (2022). Using acoustics and
523 artificial intelligence to monitor pollination by insects and tree use by
524 woodpeckers. *Science of the Total Environment*, 838, 155883.
- 525 Fox, J., and S. Weisberg. 2011. *An R companion to applied regression*. Second edition.
526 Sage, Thousand Oaks CA.

- 527 Geng, B., Xue, Q., Zheng, X., Liu, G., Ren, Y., & Dong, H. (2017). The effect of wing
528 flexibility on sound generation of flapping wings. *Bioinspiration & biomimetics*,
529 13(1), 016010.
- 530 Girón A. G., Amo de Paz G. y Martín C. M. (2020). Informe Botánico. Vulnerabilidad y
531 elementos potenciales de resiliencia frente al cambio climático en la diversidad
532 vegetal. *Biodiversia*. 98 pp.
- 533 Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by
534 combined stress from parasites, pesticides, and lack of flowers. *Science*,
535 347(6229), 1255957.
- 536 Goyal, N. P., & Atwal, A. S. (1977). Wing beat frequencies of *Apis cerana indica* and
537 *Apis mellifera*. *Journal of Apicultural Research*, 16(1), 47-48.
- 538 Gradišek, A., Slapničar, G., Šorn, J., Luštrek, M., Gams, M., & Grad, J. (2017). Predicting
539 species identity of bumblebees through analysis of flight buzzing sounds.
540 *Bioacoustics*, 26(1), 63-76.
- 541 Hagen, M., & Dupont, Y. L. (2013). Inter-tegular span and head width as estimators of
542 fresh and dry body mass in bumblebees (*Bombus* spp.). *Insectes Sociaux*, 60(2),
543 251-257.
- 544 Høye, T. T., Ärje, J., Bjerger, K., Hansen, O. L. P., Iosifidis, A., Leese, F., Mann, H. M.
545 R., Meissner, K., Melvad, C., & Raitoharju, J. (2021). Deep learning and computer
546 vision will transform entomology. *Proceedings of the National Academy of
547 Sciences*, 118(2), e2002545117.
- 548 Hrcir, M., Barth, F. G., & Tautz, J. (2005). 32 vibratory and airborne-sound signals in
549 bee communication (hymenoptera). *Insect sounds and communication:
550 physiology, behaviour, ecology, and evolution*, 421.
- 551 Hrcir, M., Gravel, A. I., Schorkopf, D. L. P., Schmidt, V. M., Zucchi, R., & Barth, F. G.
552 (2008). Thoracic vibrations in stingless bees (*Melipona seminigra*): resonances of
553 the thorax influence vibrations associated with flight but not those associated with
554 sound production. *Journal of Experimental Biology*, 211(5), 678-685.
- 555 Hung, K. L. J., Kingston, J. M., Albrecht, M., Holway, D. A., & Kohn, J. R. (2018). The
556 worldwide importance of honeybees as pollinators in natural habitats. *Proceedings
557 of the Royal Society B: Biological Sciences*, 285(1870), 20172140.
- 558 Joos, B., Young, P. A., & Casey, T. M. (1991). Wingstroke frequency of foraging and
559 hovering bumblebees in relation to morphology and temperature. *Physiological
560 entomology*, 16(2), 191-200.
- 561 Kawakita, S., & Ichikawa, K. (2019). Automated classification of bees and hornet using
562 acoustic analysis of their flight sounds. *Apidologie*, 50(1), 71-79.

- 563 Kendall, L. K., Rader, R., Gagic, V., Cariveau, D. P., Albrecht, M., Baldock, K. C., ... &
564 Morten, J. M. (2019). Pollinator size and its consequences: Robust estimates of
565 body size in pollinating insects. *Ecology and evolution*, 9(4), 1702-1714.
- 566 King, M. J., & Buchmann, S. L. (2003). Floral sonication by bees: mesosomal vibration
567 by *Bombus* and *Xylocopa*, but not *Apis* (Hymenoptera: Apidae), ejects pollen
568 from poricidal anthers. *Journal of the Kansas Entomological Society*, 295-305.
- 569 Krausa, K., Hager, F. A., & Kirchner, W. H. (2021). Guarding Vibrations—*Axestotrigona*
570 *ferruginea* Produces Vibrations When Encountering Non-Nestmates. *Insects*,
571 12(5), 395.
- 572 Laiolo, P. (2010). The emerging significance of bioacoustics in animal species
573 conservation. *Biological conservation*, 143(7), 1635-1645.
- 574 Li, Z., Zhou, Z., Shen, Z., & Yao, Q. (2005). Automated identification of mosquito
575 (Diptera: Culicidae) wingbeat waveform by artificial neural network. In IFIP
576 International Conference on Artificial Intelligence Applications and Innovations
577 (pp. 483-489). Springer, Boston, MA.
- 578 Llusia, D., Márquez, R., Beltrán, J. F., Benítez, M., & Do Amaral, J. P. (2013). Calling
579 behaviour under climate change: geographical and seasonal variation of calling
580 temperatures in ectotherms. *Global Change Biology*, 19(9), 2655-2674.
- 581 McFee, Brian, Colin Raffel, Dawen Liang, Daniel PW Ellis, Matt McVicar, Eric
582 Battenberg, and Oriol Nieto. "librosa: Audio and music signal analysis in python."
583 In Proceedings of the 14th python in science conference, pp. 18-25. 2015.
- 584 Marques, T. A., Thomas, L., Martin, S. W., Mellinger, D. K., Ward, J. A., Moretti, D. J.,
585 ... & Tyack, P. L. (2013). Estimating animal population density using passive
586 acoustics. *Biological reviews*, 88(2), 287-309.
- 587 Moore, A., Miller, J. R., Tabashnik, B. E., & Gage, S. H. (1986). Automated identification
588 of flying insects by analysis of wingbeat frequencies. *Journal of economic*
589 *entomology*, 79(6), 1703-1706.
- 590 Moore, A., & Miller, R. H. (2002). Automated identification of optically sensed aphid
591 (Homoptera: Aphidae) wingbeat waveforms. *Annals of the Entomological*
592 *Society of America*, 95(1), 1-8.
- 593 National Research Council (2007). Status of pollinators in North America. *National*
594 *Academies Press*.
- 595 Obrist, M. K., Pavan, G., Sueur, J., Riede, K., Llusia, D., & Márquez, R. (2010).
596 Bioacoustics approaches in biodiversity inventories. *Abc Taxa*, 8, 68-99.
- 597 Parmezan, A. R., Souza, V. M., Žliobaitė, I., & Batista, G. E. (2021). Changes in the
598 wing-beat frequency of bees and wasps depending on environmental conditions:
599 a study with optical sensors. *Apidologie*, 52(4), 731-748.

- 600 Pashte, V. V., & Kulkarni, S. R. (2015). Role of pollinators in qualitative fruit crop
601 production: a review. *Trends in Biosciences*, 8(15), 3743-9.
- 602 Patel, V., Pauli, N., Biggs, E., Barbour, L., & Boruff, B. (2021). Why bees are critical for
603 achieving sustainable development. *Ambio*, 50(1), 49-59.
- 604 Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel,
605 M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A.,
606 Cournapeau, D., Brucher, M., Perrot, M., & Duchesnay, É. (2011). Scikit-learn:
607 Machine Learning in Python. *Journal of Machine Learning Research*, 12(Oct),
608 2825–2830.
- 609 Pérez-Granados, C., & Traba, J. (2021). Estimating bird density using passive acoustic
610 monitoring: a review of methods and suggestions for further research. *Ibis*, 163(3),
611 765-783.
- 612 Potamitis, I., & Rigakis, I. (2015). Novel noise-robust optoacoustic sensors to identify
613 insects through wingbeats. *IEEE Sensors Journal*, 15(8), 4621-4631.
- 614 Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze,
615 T. D., ... & Vanbergen, A. J. (2016). Safeguarding pollinators and their values to
616 human well-being. *Nature*, 540(7632), 220-229.
- 617 Rabiner, L., & Schafer, R. (2010). *Theory and Applications of Digital Speech Processing*.
- 618 Ribeiro, Jr, J. W., Harmon, K., Leite, G. A., de Melo, T. N., LeBien, J., & Campos-
619 Cerqueira, M. (2022). Passive acoustic monitoring as a tool to investigate the
620 spatial distribution of invasive alien species. *Remote Sensing*, 14(18), 4565.
- 621 Rittschof, C. C., & Seeley, T. D. (2008). The buzz-run: how honeybees signal ‘Time to
622 go!’. *Animal Behaviour*, 75(1), 189-197.
- 623 Rivas-Martínez, S. (1983). Pisos bioclimáticos de España. *Lazaroa*, 5, 33-43.
- 624 van Roy, J., De Baerdemaeker, J., Saeys, W., & De Ketelaere, B. (2014). Optical
625 identification of bumblebee species: Effect of morphology on wingbeat
626 frequency. *Computers and electronics in agriculture*, 109, 94-100.
- 627 Santoyo, J., Azarcoya, W., Valencia, M., Torres, A., & Salas, J. (2016). Frequency
628 analysis of a bumblebee (*Bombus impatiens*) wingbeat. *Pattern Analysis and
629 Applications*, 19(2), 487-493.
- 630 Sotavalta, O. (1947). The Flight-tone (wing-stroke Frequency) of Insects:(contributions
631 to the Problem of Insect Flight I). *Suomen Hyönteistieteellinen Seura*.
- 632 Spangler, H. G. (1986). High-frequency sound production by honeybees. *Journal of
633 apicultural research*, 25(4), 213-219.

- 634 Spangler, H. G., & Buchmann, S. L. (1991). Effects of temperature on wingbeat
635 frequency in the solitary bee *Centris caesalpiniae* (Anthophoridae:
636 Hymenoptera). *Journal of the Kansas Entomological Society*, 107-109.
- 637 Sueur, J., Tuck, E. J., & Robert, D. (2005). Sound radiation around a flying fly. *The*
638 *Journal of the Acoustical Society of America*, 118(1), 530-538.
- 639 Sugai, L. S. M., Silva, T. S. F., Ribeiro Jr, J. W., & Llusia, D. (2019a). Terrestrial passive
640 acoustic monitoring: review and perspectives. *BioScience*, 69(1), 15-25.
- 641 Truong, T. H., Du Nguyen, H., Mai, T. Q. A., Nguyen, H. L., & Dang, T. N. M. (2023).
642 A deep learning-based approach for bee sound identification. *Ecological*
643 *Informatics*, 78, 102274.
- 644 Unwin, D. M., & Corbet, S. A. (1984). Wingbeat frequency, temperature and body size
645 in bees and flies. *Physiological Entomology*, 9(1), 115-121.
- 646 Vallejo-Marín, M. (2022). How and why do bees buzz? Implications for buzz pollination.
647 *Journal of Experimental Botany*, 73(4), 1080-1092.
- 648 Villarreal, S. M., Winokur, O., & Harrington, L. (2017). The impact of temperature and
649 body size on fundamental flight tone variation in the mosquito vector *Aedes*
650 *aegypti* (Diptera: Culicidae): implications for acoustic lures. *Journal of medical*
651 *entomology*, 54(5), 1116-1121.
- 652 Wagner, D. L., Grames, E. M., Forister, M. L., Berenbaum, M. R., & Stopak, D. (2021).
653 Insect decline in the Anthropocene: Death by a thousand cuts. *Proceedings of the*
654 *National Academy of Sciences*, 118(2), e2023989118.
- 655 Wehmann, H. N., Gustav, D., Kirkerud, N. H., & Galizia, C. G. (2015). The sound and
656 the fury—bees hiss when expecting danger. *PLoS One*, 10(3), e0118708.
- 657 Wilkins, M. R., Seddon, N., & Safran, R. J. (2013). Evolutionary divergence in acoustic
658 signals: causes and consequences. *Trends in ecology & evolution*, 28(3), 156-166.
- 659 Winfree, R. (2010). The conservation and restoration of wild bees. *Annals of the New*
660 *York academy of sciences*, 1195(1), 169-197.
- 661 Yin, M. S., Haddawy, P., Ziemer, T., Wetjen, F., Supratak, A., Chiamsakul, K., ... & Sa-
662 ngamuang, C. (2023). A deep learning-based pipeline for mosquito detection and
663 classification from wingbeat sounds. *Multimedia Tools and Applications*, 82(4),
664 5189-5205.
- 665 Zhang, C., Wang, P., Guo, H., Fan, G., Chen, K., & Kämäräinen, J. K. (2017). Turning
666 wingbeat sounds into spectrum images for acoustic insect classification.
667 *Electronics Letters*, 53(25), 1674-1676.